



Book of Abstracts

XIII Weurman Flavour Research Symposium
27-30 September 2011, Zaragoza, SPAIN



Laboratory for Aroma Analysis and Enology (LAAE)
University of Zaragoza

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Organisation

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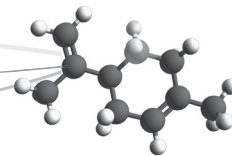
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XIII WEURMAN FLAVOUR RESEARCH SYMPOSIUM

Scientific Program

(Updated September the 14th)

Monday 26th September

17:00 Registration desk opens

20:00 Welcome party. Finger food, Jazz, beer and wine

Tuesday 27th September

9:00 Welcome and Official Opening

SESSION 1: EFFECTS, MEANING AND ROLE OF FLAVOUR ON NATURE

Chairs of the session: Andy Taylor and Loic Briand

9:10 Veronika Somoza: Bioactivity of volatile organic compounds

9:55 Andrea Buettner: Odorant-physiology interactions. In search of effects beyond smell perception

10:25 Flash Presentation (FP): Combined chemo-analytical and electrophysiological characterization of neurotropic activity of odorants. -*Artur Kessel*

10:35 Coffee Break and Posters

SESSION 2: PHYSIOLOGY OF FLAVOUR PERCEPTION

Chairs of the session: Loic Briand and Jerome Golebiowski

11:30 Hiroaki Matsunami: Functional variation of human odorant receptors

12:15 Pierre Chatellant: Deorphanization and characterization of human odorant receptors in heterologous cells

12:45 Kambiz Shekdar: Recent advances in cell based discovery of natural flavours

13:10 Lunch/Posters

SESSION 3: OPTIMIZATION OF TASTE

Chairs of the session: Don Mottram and Veronika Somoza

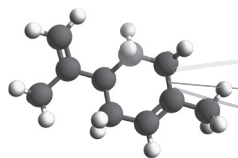
15:00 Gerrit Smit: Strategies for sodium reduction: translating theory to practice

15:30 Max Batenburg: Salt enhancement by aroma compounds

15:55 Lisa Methven: Taste and flavour enhancement using natural ingredients: the prediction and optimization of umami taste in real food systems

16:20 Coffee Break/Posters

XIII Weurman Flavour Research Symposium Zaragoza (Spain)



SESSIONS 4A AND 4B: PRACTICAL AND INDUSTRIAL ASPECTS

Chairs of the sessions: Gerrit Smit and Don Mottram

16:50 Juergen Schnabel: Labelling and safety issues in the flavour industry

4A Main Hall: D. Mottram	4B Workshop Hall, Flashes tastants/safety: Gerrit Smit
17:20 Karl H. Engel: Assessment of the intake of flavouring substances via consumption of flavoured teas -analysis of Earl Grey teas marketed in the European Union	17:20 (FP) The relationship between umami taste perception in humans and the experimentally determined persistence of umami inducing compounds in the mouth – <i>James Marshall</i>
17:45 Imre Blank: Optimization of caramel-flavour generation upon extrusion	17:27 (FP) Synergistic/suppressive effects of binary and ternary mixtures of sweeteners in semi-skimmed milk – <i>Christine Counet-Kersch</i>
	17:34 (FP) Correlations between aroma-active compounds and the food-borne toxicant styrene during wheat beer processing: formation pathways from selected precursors – <i>Michael Granvogl</i>
	17:39 (FP) Important aroma compounds in salami and correlations with biogenic amines – <i>Johanna Kreissl</i>

18:30 Visit to La Aljaferia, the Arabic Palace of old Zaragoza

20:30 Traditional tapas dinner and wine

Wednesday 28th September

SESSIONS 5A: BIOFLAVOUR; AND 6A: –FLAVOUR MODELING

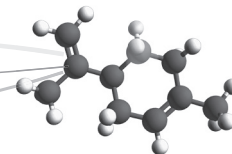
Chairs of the sessions: Ziya Gunata and Terry Acree

9:00 Gustavo de Billeberk: Systems Microbiology for flavours and fragrances production - BIOFLAVOUR Consortium - COST Action FA0907

9:30 (FP) Beer volatile compounds formation at different fermentation temperature using immobilised yeasts –*Daniela Smogrovicova*

5A Main Hall: Ziya Gunata	6A Workshop Hall: Terry Acree
9:40 (FP) Exploitation of orange peel for the production of flavour-active compounds with the use of a commercial wine strain – <i>Adamantini Paraskevopoulou</i>	9:40 (FP) Quantification of relevant flavour compounds in beef stocks and correlation to sensory results by "Reverse Metabolomics" – <i>Andreas Degenhart</i>
9:47 Mathias Wüst: Demonstration of sesquiterpene biosynthesis in grape berry exocarp by deuterium labelling studies	9:47 Joseph Kerler: Advanced analytical sensory correlation – towards a better molecular understanding of coffee flavour descriptors
10:12 Wilfred Schwab: Reaction mechanism of the strawberry enone oxidoreductase	10:12 Wender Bredie: Flavour pairing of foods – Facts and fiction

10:37 Coffee Break/Posters



SESSIONS 5B: BIOFLAVOUR; AND 6B: FLAVOUR INTERACTIONS,

Chairs of the sessions: Mathias Wüst and Wender Bredie

11:30 Detlef Ulrich: Towards the development of molecular markers for apple volatiles

11:55 Roland Mumm: A Combinatorial Metabolomics approach to decipher flavour traits in crops

5B Main Hall: Mathias Wüst	6B Workshop Hall: Wender Bredie
12:20 Rik De Vos: Metabolomics data fusion reveals specific glycoconjugation of phenylpropanoid volatiles as key to differential tomato flavour	12:20 (FP) Multiple time-intensity profiling (mTIP) as an advanced Evaluation toll for complex tastants – <i>Katja Obst</i>
12:45 (FP) Biosynthesis of vanillin: Enzyme involving the conversion of ferulic acid to vanillin in <i>Vanilla Planifolia</i> . - <i>Osamu Negishi</i>	12:27 (FP) The impact of vision on flavour perception – <i>Ann Kurtz</i>
	12:34 (FP) A standardised approach to in vivo measurement of volatile release: the effect of food matrix on release and intra- and inter-individual differences – <i>Damian Frank</i>
	12:41 (FP) Perceptual interactions in odour mixtures: The blending effect – <i>Charlotte Sinding</i>
	12:48 (FP) Influence of composition (CO ₂ and sugar) on aroma release and perception of mint-flavoured carbonated beverages – <i>Isabelle Déléris</i>

13:00 Lunch/Posters

14:30 Vendor Seminar: Gerstel

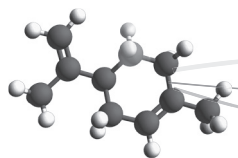
SESSION 7: SULFUR PRECURSOR SYSTEMS

Chairs of the session: Michael Qian and Russel Rouseff

15:00 Christian Starkenman: Enzyme- and microorganism-guided discovery of natural sulfur compound precursors

15:35 Remi Schneider: The contribution of organic synthesis and analytical chemistry to the knowledge of varietal thiols biogenesis in wines and their contribution to the wine aroma

16:10. Coffee Break/Posters



SESSIONS 8: MAILLARD SYSTEMS; AND 9: OXYGEN AND WINE

Main Hall	Workshop Hall
8 MAILLARD SYSTEMS <i>Chairs: Andy Taylor and Imre Blank</i>	9 OXYGEN AND WINE FLAVOUR <i>Chairs: Doris Rauhut and Antonio S. Ferreira</i>
16:40 Fien Van Lancker: Formation of pyrazines in Maillard model systems containing peptides	16:40 Michael Qian: Comparison of screw cap and cork closure on oxygen permeability and flavour development during post-bottle aging
17:05 Sara Martins: Enhancing meat flavour generation by increasing cysteine efficiency in H ₂ S liberation using a 2-step process: "pH/thermal" vs "enzymatic/thermal"	17:05 Maurizio Ugliano: Factors affecting accumulation of certain volatile sulfur compounds during bottle ageing of wines
17:30 Tomas Davidek: Generation of roast-smelling compounds upon extrusion cooking –first insight into formation pathways using the camola approach	17:30 Alexandre Pons: Origins of 3-methyl-2,4-nonanedione in red wines
17:55 (FP) Effect of lipid oxidation on the formation of pyrazines and on colour in complex Maillard systems with meaty-like flavour – <i>Guillaume Desclaux</i>	17:55 (FP) Nightmare problems in the analysis of VSCs and in the work with "oxygen-free" atmospheres – <i>Ernesto Franco</i>

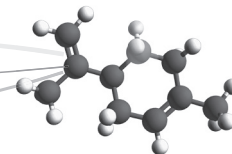
Free Evening

Thursday 29th September

SESSIONS 10: STRUCTURE-ACTIVITY; AND 11A: WINE FLAVOUR

Main Hall	Workshop Hall
10 TASTE-ACTIVE AND STRUCTURE-ACTIVITY <i>Chairs: Loic Briand and Patrick Dirink</i>	11A WINE FLAVOUR <i>Chairs: Philippe Darriet and Antonio S. Ferreira</i>
9:00 Barbara Suess: Discovery and Structure-Activity Studies of Maillard-Modified Guanosine 5'-Monophosphates using Human Sensory Studies and Cell-Based Taste Receptor Assays	9:00 Nadine Wollman. Decoding the taste of red wine by means of a sensomics approach
9:25 Devin Peterson: Identification of Thermally Generated Bitter Compounds in Whole Wheat Bread	9:25 M ^a Pilar Saenz-Navajas: Implication of red wine non-volatile composition in quality perception
9:50 Andrea Strube: Halogenated phenols and cresols: structure-odour relationships and occurrence in nature	9:50 Terry Acree: The perception of Riesling varietal character: the role of 2,2,1-trimethyldihydronaphthalene (TDN).
10:15 Johannes Polster: Structure/odour relationships in homologous series of polyfunctional thiols	10:15 Hans Georg Schmarr: Profiling analysis of volatile and non-volatile compounds in wine for a better understanding of wine quality?

10:40 Coffee Break/Posters



SESSIONS 12: FLAVOUR SYSTEMS; AND 11B: WINE FLAVOUR

<i>Main Hall</i>	<i>Workshop Hall</i>
12 FLAVOUR SYSTEMS <i>Chairs: Peter Schieberle and Imre Blank</i>	11B WINE FLAVOUR <i>Chairs: Ana Escudero and Georg H Schmarr</i>
11:30 Klaus Gassenmeier: A metallic solvent-like off-flavour in Hazelnuts: Identification of prenyl/Ethyl/Ether as a key flavour compound and formation studies	11:30 Josh Hixson: Determination of alternative precursors to Brettanomyces/Dekkera spp. derived off-Flavour
11:55 Martin Steinhaus: Molecular insights into the off-flavour formation during pineapple juice processing	11:55 (FP) Oak aroma precursors – <i>Davide Slaghenauhi</i>
12:20 Andreas Degenhardt: Studies on stability of citrus flavours and insights into degradation pathways of key aroma compounds	12:02 (FP) Oxidized guaienes and their relationship to the aroma compound rotundone – <i>Stacey Burrell</i>
12:45 Keith Cadwallader: Identification, characterization and relative aroma impact of 2,3-dehydro-1,8-cineol in lemon-lime flavoured carbonated beverages	12:09 (FP) Precursors to the potent odorant; wine lactone – <i>Joanne Giaccio</i>
	12:16 (FP) Influence of the manufacturing process on changes in the concentrations of selected key aroma compounds of Dornfelder red wine – <i>Stephanie Frank</i>
	12:23 (FP) Interest of online higher alcohol and ester determinations during winemaking fermentations – <i>Jean-Roch Mouret</i>
	12:30 (FP) Differences in chemical composition of aroma among red wines of different price category – <i>Felipe San Juan</i>
	12:37 (FP) Perceptive interactions on wine typical fruity aroma – <i>Georgia Lytra</i>
	12:44 (FP) Characterisation of dry Riesling wines aromatic typicality using sensory and instrumental analytical methods. A comparative approach – <i>Armin Schüttler</i>

13:10 Lunch/Posters

14:30 Vendor Seminar: Agilent

SESSION 13: TEMPORAL ASPECTS OF FLAVOUR PERCEPTION

Chairs: Elisabeth Guichard and Rob Linforth

15:00 Nathalie Martin: Dynamics of perception and key moments in consumer experience

15:35 Guillaume Blancher: Relevance and limitations of using in-nose aroma concentration to predict sensory dynamic perception during sequential eating of flavoured candies

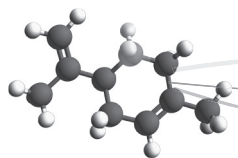
16:00 Andy Taylor: Measuring variation in odour delivery for sensory testing

16:25 Coffee Break/Posters

16:50 Kerstin Burseg: Taste enhancement by pulsatile stimulation meets cross-modal interaction: New insights into aroma-taste and texture-taste interaction

17:15 Peter De Kok: Flavour composition, temporal release and complexity determining food quality perception

19:30 Bus departure to Gala Dinner



Friday 30th September

SESSION 14: DYNAMIC ASPECTS OF FLAVOUR PERCEPTION

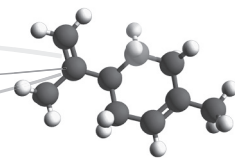
Chairs: Andy Taylor and Loic Briand

- 9:20 Jerome Golebiowsky: Molecular features underlying the chemoreception of odorant binding proteins and olfactory receptors. Insights from molecular modeling and biophysical data
- 9:50 Elisabeth Guichard: Understanding the dynamics of flavour compounds release during food mastication of cheese products in relation with perception
- 10:20 Rob Linforth: Modelling the effect of lipid on in-vivo flavour delivery
- 10:45 Coffee Break**

SESSION 15: INSTRUMENTAL ASPECTS AND TOOLS OF THE TRADE

Chairs: Keith Cadwallader and Ricardo López

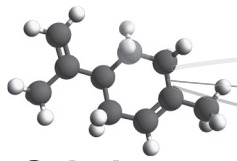
- 11:15 Alejandro Cifuentes: Foodomics: A new omics for a new food era
- 12:00 Ricard Boqué: Instrumental sensometry. A tool for food quality control
- 12:25 Alain Chaintreau: Rapid quantification technique without authentic samples and application to complex, unstable or unavailable compounds
- 12:50 Monica Flores: Selected ion flow tube mass spectrometry (sift-ms) for flavour evaluation of dry fermented sausages
- 13:15 Coffee Break**
- 13:25 Joao Rodrigues: Evaluation of beer deterioration by gas chromatography–mass spectrometry/ multivariate analysis: a rapid tool for assessing beer composition
- 13:50 Jonathan Beauchamp: On-line flavour analysis - novel techniques and applications
- 14:15 Conference Closing**
- 14:30 Farewell Lunch**



Session:

**Effects, meaning
and role of flavour
on nature**

Lectures



BIOACTIVITY OF FLAVOUR COMPOUNDS

VERONIKA SOMOZA

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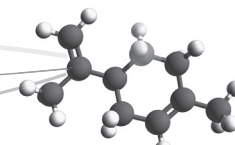
Keywords: Bioactivity of flavour compounds, inflammation, satiety, cancer, cardiovascular diseases

Flavour compounds may not only serve as important indicators of food quality. Flavour compounds are also gaining growing interest because of their potential health benefits. This review provides scientific evidence for effects on behaviour, mood, satiety, and also on the progression of several diseases such as irritable bowel syndrome, cancer, inflammation and cardiovascular diseases. Although most of the studies reported effects in rodents, there is considerable evidence that flavour compounds exert health beneficial effects in humans as well. Efficacy in humans has, for instance, been shown for a daily dose of 900 mg peppermint oil, which significantly reduced clinical symptoms of abdominal distension, bloating, abdominal pain, and diarrhoea in irritable bowel patients [1]. Of the most widely studied group of flavour compounds, the terpenes, limonene and alpha-terpineol have been found to reduce the progression of experimentally initiated skin cancer in rodents, and to exert anti-inflammatory effects in human buccal cells.

While human intervention trials and animal studies are suitable for providing data on health benefits *in vivo*, cell culture studies may only prove mechanisms of action. Pharmacokinetics, including intestinal degradation, absorption, transport and metabolic transformation in response to a given dose cannot be investigated in cell systems, but are mandatory for evaluating a compound's efficacy. Alpha-terpineol, for instance, exerts its anti-inflammatory activity prior to intestinal and metabolic transformation in buccal cells, but loses this activity after absorption into circulation. However, data clearly show that several flavour compounds are absorbed and exert health beneficial effects either prior to or after metabolic transformation.

References

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ODORANT - PHYSIOLOGY INTERACTIONS: IN SEARCH OF EFFECTS BEYOND SMELL PERCEPTION

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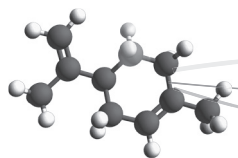
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Keywords: inhalation, ingestion, resorption, biotransformation, post-oral, post-inhalation, physiological action

The last decade has seen numerous studies on the complex mechanisms that are involved in immediate aroma perception, both when aromas are inhaled or ingested. Through such studies it has become more and more clear that odorants do not only migrate into and distribute within the oral and nasal cavities, but that they interact, e.g. with metabolising systems or mucosal tissues and can be resorbed or even newly generated from other food constituents (1, 2, 3). Aroma stimulation, on the other hand, has been shown to modulate human behavioural response, most specifically with respect to liking, and this in turn influences odorant distribution and their odour effects *in vivo* (2).

This awareness of the plasticity of odorant-physiology interaction has rapidly expanded in recent research, especially regarding considerations on odorant-physiology interactions that take place after the compounds have been inhaled or swallowed. These so-called “post-oral” or “post-inhalation” effects have attracted increasingly more attention, resulting in reports on neurotropic, anti-carcinogenic, and anti-inflammatory action, to name but a few (4, 5, 6, 7, 8, 9). This is most likely due to the fact that numerous studies demonstrate that food ingredients, among them odorants, can considerably affect our physiology and psychology or wellbeing in general. Also, there is evidence to indicate that these effects are not always explicable based only on pure odour perception (10, 11, 12).

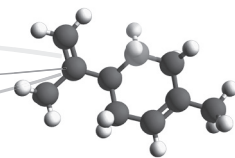
Until today, reports on post-oral and post-inhalation effects of food aroma compounds should be regarded as being predominantly anecdotal, with studies being carried out on isolated effects such as resorption model experiments on single substances in the gastro-intestinal tract (e.g. 13), or distinct physiological action of odorants investigated in highly specific assays (e.g. 4,6). Nevertheless, the whole series of underlying processes is hitherto barely understood. This comprises reactions and transformations taking place during the long passage of an odour molecule through the gastrointestinal tract, during resorptive uptake, and subsequent processes that take place until the odour molecule reaches its destination, e.g. a brain receptor cell. This presentation will provide an overview on current knowledge on odorant-physiology interactions other than pure smell perception, starting from uptake and resorption processes in the airways and the gastrointestinal tract, as well as distribution and transfer processes within the human body. It will further focus on metabolism and secretion, and on diverse aspects of physiological action of odorants. *In vivo* studies as well as insights



from *in vitro* and animal models will be discussed with an overview of up-to-date techniques on characterization of odorant-physiology interaction.

References

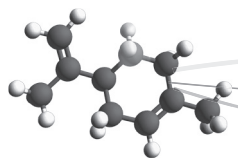
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Session:

**Physiology of
flavour
perception**

Lectures



02.1

FUNCTIONAL VARIATION OF HUMAN ODORANT RECEPTORS

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Keywords: olfactory, G-protein coupled receptor, single nucleotide polymorphism, functional expression, heterologous cells

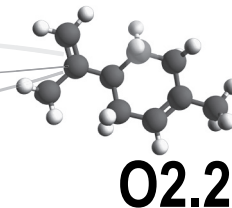
Humans have nearly 400 potentially functional OR genes, but among this set there are a large number of sequence variations between individuals. In some cases, these variations cause an OR to be nonfunctional in a subset of the population, and therefore likely underlie a portion of the inter-individual variation in olfactory perception. Human OR7D4 responds to androstenone, and genotypic variation in OR7D4 predicts variation in the perception of androstenone (Keller et al., 2007).

Although odour perception impacts food preferences, the effect of genotypic variation of odorant receptors (ORs) on the sensory perception of food is unclear. Since androstenone is naturally present in meat derived from male pigs, we asked whether OR7D4 genotype correlates with either the ability to detect androstenone or the evaluation of pork tainted with varying levels of androstenone within the naturally occurring range. When pork containing varying levels of androstenone was cooked and tested by sniffing and tasting, subjects with two copies of the functional RT variant tended to rate the androstenone-containing meat as less favourable than subjects carrying the non-functional WM variant. Our data is consistent with the idea that OR7D4 genotype predicts sensory perception of meat containing androstenone and that genetic variation in an odorant receptor can alter food preferences.

To understand the logic of odor coding in humans, it is fundamental to know which odorant activates which ORs. However, the vast majority of human ORs still remain orphan with only ~20 published human ORs that have active ligands. Large-scale deorphanization of ORs using tissue culture cells is an efficient strategy to decrease the number of orphan ORs (Saito et al., 2009). Once odor-OR pairs are identified, generating a list of OR variants and determining how their responses to odorants are altered is a critical step to address the question of how OR variation contributes perceptual variation. Understanding the links between OR variation and function will contribute to reveal the underlying logic of the human odor code and individual differences of odor perception.

References

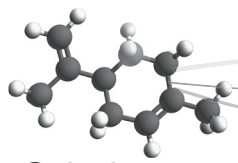
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DEORPHANIZATION AND CHARACTERIZATION OF HUMAN ODORANT RECEPTORS IN HETEROLOGOUS CELLS

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Olfaction plays an indispensable role in human and animals in self and environmental recognition as well as intra- and inter-specific communication. Following the discovery by Buck and Axel in 1991 of a family of odorant receptors (OR), it has been established that the sense of smell begins with the molecular recognition of a chemical odorant by one or more ORs expressed in the olfactory sensory neurons. Therefore characterization of the molecular interactions between odorant molecules and ORs is a key step in the elucidation of the general properties of the olfactory system and in the development of applications: design of new odorants, search for blockers,...The presentation will show the process putted in place in TecnoScent to deorphanize and to characterize the interaction between chemical odorants and ORs. The family of human ORS includes ~400 putatively functional ORs which are GPCRs. To date over 100 hORs have been deorphanized.



02.3

RECENT ADVANCES IN CELL BASED DISCOVERY OF NATURAL FLAVOR INGREDIENTS

KAMBIZ SHEKDAR

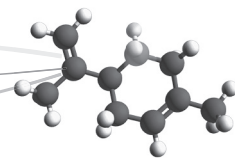
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Key words: salt, sweet, bitter, umami, sour, enhancers, blockers, natural, flavor, flavor, cell, receptor

Novelty: We have identified rare, naturally-occurring cells with the ability to mimic human taste and smell. In contrast to average laboratory cells where taste and odorant receptors are typically modified to enable their expression, the naturally-occurring systems allow for native receptor expression and function exactly as it exists *in vivo*. Data for cell based assays across taste modalities and their use in discovering new flavor ingredients is presented, including salt taste enhancers, bitter blockers and aroma and fragrance enhancers and blockers, all confirmed in human sensory studies.

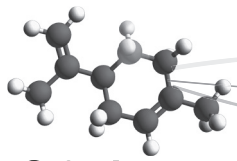
A new approach to cell based flavors discovery has resulted in the identification of a broad range of previously inaccessible flavor ingredients, including salt taste enhancers, bitter blockers, aroma enhancers that amplify aromatic notes of composite fragrances, and malodor blockers. Earlier cell based flavors discovery approaches modeled on drug discovery attempted to re-create human taste or smell using genetically engineered systems for over-expression of corresponding receptor genes. Often, receptor tagging or truncation was relied upon for maximal or appreciable expression levels. Our alternative approach identifies and selects naturally-occurring cells that naturally have the ability to mimic human taste or olfaction, even without the addition of any genes. When tested with known tastants and odorants, the functional response of the resulting cell based assays mimicked the corresponding reported human sensory data across several taste modalities. Cell based assays comprising these cells allow unmodified, full-length taste and odorant receptors to be used during screening, such that the receptors are provided as they exist in taste and olfactory cells, respectively, *in vivo*. These results demonstrate that the nature of the host cell environment relied upon in cell based assays provides essential context for human taste and smell biology as it occurs *in vivo* and that receptor genes by themselves or in mutagenized form are not sufficient. The increased reliability of the cell based assays in turn translates into more effective testing of many millions natural compounds and extracts derived from commonly-consumed fruits, herbs and vegetables. Moreover, with the increased reliability with which flavors cell based assays may now be screened, for the first time, combinations of commonly-consumed natural extracts that meet desired flavor profiles and that are at the same time supported by extensive prior history of human consumption may be identified.



Session:

**Optimization of
taste**

Lectures



03.1

STRATEGIES FOR SODIUM REDUCTION: TRANSLATING THEORY TO PRACTICE

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Keywords: salt reduction, salt receptors, multisensory perception, aroma-taste-texture interactions

Most of the sodium intake in the Western diet is consumed through processed and restaurant foods, and the average intake of salt is approximately 9-12 g per day. Since salt intake is well correlated with blood pressure and associated cardiovascular diseases (1), the World Health Organisation (WHO) advocates sodium reduction of foods to improve public health. They recommend a lowering of the salt intake to 5 g per day (2).

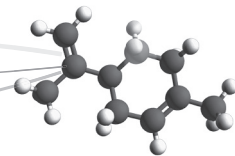
Salt fulfils a number of functionalities in food, such as preservation via regulating water activity, influencing fermentation rate (e.g. in bread) and increasing water-holding capacity (meat products) (3). But most importantly, the primary function of salt is to deliver a salty taste as well as to enhance the overall flavour perception. This means that these functions of salt need to be compensated when the level of salt is reduced, in order to maintain tasty products which consumers will re-purchase.

Different approaches have been identified for sodium reduction, and they will be discussed in this presentation: (i) Gradual reduction of salt in small steps, which is often a first approach that is implemented. If conducted cross-industry, consumers become gradually accustomed to lower levels of salt and this will alter their preference accordingly; (ii) The use of salt replacers such as KCl. This is widely used, however, it is less salty than NaCl and gives bitter and metallic off-tastes when applied at higher levels (4); (iii) The search for salt boosters, preferentially natural ingredients that enhance the sensitivity of the sodium receptor; (iv) Application of insights of (multi)sensory interactions, based on the knowledge that the brain integrates the signals of all senses to create an overall percept. There are many examples of interactions between the senses, see e.g. (5) for a review, and these insights provide new opportunities for practical applications in complex food products.

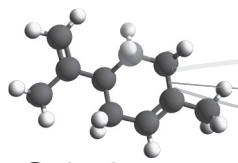
The industry is keen to apply insights in salt compensation strategies, but in reality the translation of the insights into products appears to be a relatively slow and difficult process. This has to do with the difficulty to translate basic insights into the reality of complex food matrices, oncost linked to the application of new technologies as well as the communication towards the consumer. These aspects will be discussed in this presentation.

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SALT ENHANCEMENT BY AROMA COMPOUNDS

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Keywords: Multi-sensory interaction, sodium reduction, salt enhancement, aroma-taste interaction

Introduction

High dietary sodium has been shown to cause hypertension and hence to increase the risk of developing cardiovascular disease. However, reduction of salt without affecting taste is a major challenge. Reducing the salt content in food products obviously leads to a loss of saltiness, but also to a decrease of overall flavour intensity.

One of the approaches to compensate the effects of low salt content could be the exploitation of sensory integration. For sweet flavour the enhancing effect of taste on aroma, and vice versa, has clearly been shown. Only two papers report an enhancement of saltiness by a 'congruent' aroma [1,2], and only studied model conditions. It is not clear if the findings can be exploited in real-life food products.

The present work [3] was aimed to show that this principle can be used in a very practical way: flavourings were selected that upon addition to salt-reduced bouillon or soup should compensate the effects of the low salt level without significantly changing the flavour profile of the product.

The second part is a systematic study of the impact of various congruent, savoury aroma compounds ('brothy', 'meaty', and 'roasted') in the complex flavour on perceived saltiness.

Results

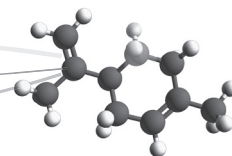
First results showed that addition of extra flavour to a salt-reduced bouillon delivers the effect we aimed for, demonstrating the saltiness enhancement of a complex savoury aroma, without changes in profile. For further unravelling of the effect of these cross-modal interactions we have made use of three categories of subjects: naïve panellists, trained panellists and consumers. Using the naïve panellists we found that several single compounds significantly enhanced salt perception, 'seasoning' or 'brothy' compounds having the largest impact. A quantitative approach indicated that these compounds explain a significant fraction of the salt enhancement of the complex flavour and hence should be regarded as key salt enhancing compounds.

The trained panel did not demonstrate a statistically significant effect on saltiness of these single compounds, in line with its analytical way of tasting and the unlearning during training of the associations on which multi-sensory interactions are based.

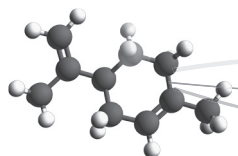
For consumers the effect of single aroma compounds could only be noticed when subjects were focused on saltiness perception by means of a forced comparison test. With open questions saltiness enhancement could be demonstrated, however, for a complex flavour.

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O3.3

TASTE AND FLAVOUR ENHANCEMENT USING NATURAL INGREDIENTS: THE PREDICTION AND OPTIMISATION OF UMAMI TASTE IN REAL FOOD SYSTEMS

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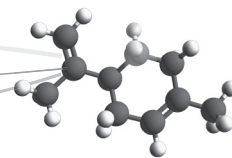
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Keywords: Umami, Equivalent Umami Concentration, Savoury, Suppression

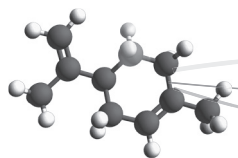
The savoury taste known as Umami is of great importance to the food industry, the chef and the consumer due to its role in achieving strong, balanced and preferred product flavour. An increased interest in umami in recent years is due to its arguable ability to enhance salt perception, the need for “additive-free” food labels, the identification of “new” umami taste compounds, and to the greater understanding of how this taste is perceived and can lead to cross-modal sensory interactions at a receptor and cognitive level (1,2). However, in order to predict the umami taste perceived in real food systems, and its enhancement of overall flavour, quantification of the umami taste amino acids and 5'-ribonucleotides may be insufficient. In model systems and simple food matrices Yamaguchi and colleagues developed a regression model to predict umami taste from the levels of glutamic acid, aspartic acid and four 5'-ribonucleotides, allowing the equivalent umami concentration of foods to be calculated in units of mg monosodium glutamate (MSG) per 100 g (3). This was used as the basis for this present work in which natural taste enhancers were added to real food systems, comprising cooked minced meat products, meat stocks or meat gravies. Ingredients used were yeast extracts, an extract of mycoprotein, fermented soya bean pastes (miso), soya sauce, shiitake mushroom extracts and concentrated tomato extracts. These were added to basic meat formulations with the aim of achieving an equivalent umami concentration of 0.5 % MSG, whilst maintaining final sodium of 0.16 %. Modified formulations were compared to control meat formulations and to meat plus 0.5% MSG. A trained sensory panel developed a vocabulary of 66 attributes and, for the minced meat products, found significant differences in 31 of these. Both umami and salt perception were significantly different between samples ($p=0.0009$) with the majority of modified formulations being significantly higher than the control. Increase in umami taste enhanced the perception of salt and flavour attributes of beef stock ($p=0.07$), yeast extract ($p<0.0001$) soy sauce ($p=0.03$) and mushroom ($p=0.0015$). However, not all ingredients rich in umami taste components resulted in an increased umami perception; and the calculated EUC did not reliably predict the extent of umami perception in the final product. In real food systems, contribution of other tastes, such as sweetness and acidity, which may suppress or modify the umami taste should be taken into consideration. The potential to predict umami taste and flavour enhancement in real foods will be discussed.

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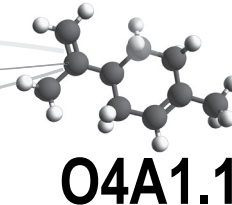


Session:

Practical and

industrial aspects

Lectures



THE DEVELOPMENT AND USE OF FLAVOUR MODIFIERS IN LIGHT OF RECENT REGULATORY CHANGES

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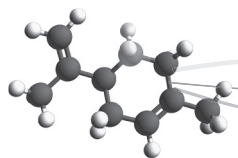
Key Words: Flavour Labeling, Flavour Registration, Flavour Modifiers, Flavour Regulations

Historically, flavour substances have been simple aromatic chemicals derived from foods registered and approved with a supporting safety assessment heavily based on low exposures. However, recent advances in molecular and receptor biology in the areas of taste and smell have allowed for the development of specific, non aromatic molecules that can modify flavour based on interactions with specific taste receptors. Although this is not unlike aromatic compounds that we know interact with olfactory receptors, the development of flavour modifiers has expanded the chemical space in which traditional flavourings have been developed. These powerful tools have allowed scientist to identify substances that alone have no aromatic flavour characteristics but can modify the flavour perception of other taste or flavour ingredients through interactions with taste receptors. Because of this receptor interaction, flavour modifiers may impact the sensory profile of a complex flavour in a food without introducing any specific flavour of its own. Examples of how flavour modifiers interact with taste receptors to modify flavour, how these substances are identified and how sensory evaluations are used to show true flavour modification at the consumer level will be discussed.

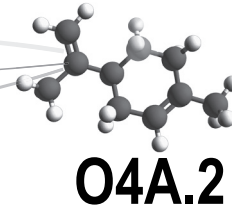
An explanation of the current regulatory landscape related to flavour labeling will also be presented. All authorities issue their regulations or guidelines for two main purposes. First and foremost, is to protect the consumer from harm. The second function is to prevent deception and fraud against the consumer. Currently, there are three internationally recognized authorities (FDA in the USA, EU Commission and the World Health Organization (Codex) that have established guidelines for the safe use and classification of flavourings and products containing flavourings: 3 years ago Codex Alimentarius has amended the definition of flavourings to include the concept of flavour modification (CAC GL 66-20). This concept was taken over by the EU Commission in the new flavour regulation in the EU (EC/1334/2008).

This is a significant improvement over the old regulations that took a less sophisticated approach to limiting flavourings to materials imparting flavour and/or taste.

In addition, the EU has modified almost all of their food regulations that impact everything from ingredient safety to consumer packaging. These changes are significantly impacting the flavour industry as the new regulations have established new registration requirements for new flavouring substances and certain complex flavouring materials. For the safety assessment of flavouring substances, globally most countries / regions including the USA and Europe follow the procedure applied by JECFA, which is accepted as the most updated and systematic one.



Nevertheless the approaches on how the JECFA methodology is applied by the various Committees differ in some important aspects, among others in the method of exposure assessment and the extent of using toxicity data of structurally related compounds. This and other significant differences between the previous and current regulations and the impact of these changes on the flavor industry will be discussed.



ASSESSMENT OF THE INTAKE OF FLAVOURING SUBSTANCES VIA CONSUMPTION OF FLAVOURED TEAS – ANALYSIS OF EARL GREY TEAS MARKETING IN THE EUROPEAN UNION

A.-M. Orth, L. Yu, J. Poplacean, K.-H. ENGEL

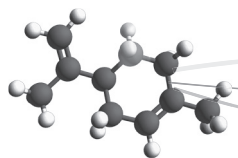
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Keywords: Intake assessment of flavourings substances, safety evaluation, frequency distributions

Regulation (EC) No 2232/96 of the European Parliament and the Council [1] laid down a procedure for the establishment of a list of flavouring substances, the use of which will be authorized to the exclusion of all others in the EU. The estimation of the intake of flavouring substances plays a central role in their safety evaluation. Various models, such as the “Maximized Survey-derived Daily Intake (MSDI)” approach, based on annual production volumes of flavouring substances or the “Modified Theoretical Added Maximum Daily Intake (mTAMDI)” approach, based on use levels and consumption data for certain food categories, are being employed. In order to assess uncertainties arising from the application of these models, analytical data on the actual concentrations of flavouring substances in flavoured foods are required.

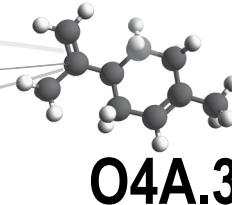
The first objective of this study was to determine distributions of the concentrations of flavouring substances in flavoured teas, using bergamot-flavoured Earl Grey tea as example. The flavour of this tea is dominated by the monoterpenes linalyl acetate, linalool, limonene, β -pinene and γ -terpinene, which represent more than 90 % of volatile substances of coldpressed bergamot oil [2]. The contents of these flavouring substances were determined in 90 Earl Grey teas purchased in ten countries of the European Union. In addition, enantiomeric compositions were determined for the chiral monoterpenes. Mean, median and 97.5 percentile contents of linalool and linalyl acetate were calculated and the frequency distributions of these flavouring substances in teas from the European market were determined. Statistically significant differences were assessed in the light of the country of purchase and of the typology of the products, i.e. factors such as international/national brands, private label brands or not assignable brands sold in speciality tea-shops. In addition, the contents were grouped according to criteria, such as sale of products in tea-bags or as loose leaves, and were differentiated depending on the enantiomeric compositions of the monoterpenes. In the second part of the study, transfer rates for linalool and linalyl acetate from tea leaves into the tea beverage in the course of the hot-water infusion were determined. The impact of this step on the actual concentrations of flavouring substances in the final tea product and on their enantiomeric compositions was followed. These data were also established for structurally related monoterpenes in order to allow more generalized conclusions regarding the behavior of these flavouring substances during the infusion process and to establish respective correction factors.



The data provide an exemplary approach to reveal and to compensate for uncertainties in the intake calculation of flavouring substances and demonstrate the usefulness of analytical determinations in the risk assessment process.

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OPTIMISATION OF CARAMEL-FLAVOUR GENERATION UPON EXTRUSION -A HOLISTIC APPROACH BASED ON EXPERIMENTAL DESIGN

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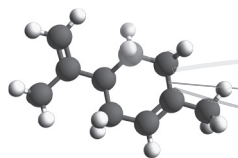
Keywords: Maillard reaction, flavour formation, caramel note, acrylamide, extrusion cooking, cereals, texture, colour, viscosity

Extrusion cooking has gained considerable importance in food industry for manufacturing numerous food items including cereal products. It permits in one processing step to perform several operations such as mixing transporting, cooking and texturing. However, desirable flavour characteristics associated with conventionally cooked cereals do not develop to the same extent during extrusion cooking. Several academic studies have recently been published concerning the effect of extrusion parameters on the volatile formation (1-3). Extrusion temperature, moisture, pH and composition of the feedstock were shown to have the highest effect on the volatiles formed. Unfortunately, no or very limited information is available on the formation of odour-active compounds or the organoleptic qualities of the extrudates.

The use of specific precursors and ingredients is of particular interest to achieve flavour modulation. However, knowledge has to be built-up concerning the effect of precursors and extrusion parameters on flavour development and on other key product attributes such as colour and texture, while mitigating the formation of undesirable compounds. This study is based on an experimental design approach targeting the formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone from rhamnose to impart caramel-type flavour notes upon extrusion. Result will be compared with those obtained in an aqueous system (4).

References:

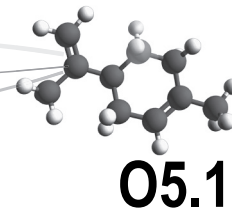
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Session:

Bioflavour

Lectures



SYSTEMS MICROBIOLOGY FOR FLAVOURS AND FRAGRANCES PRODUCTION BIOFLAVOUR CONSORTIUM - COST ACTION FA0907

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Keywords: bioflavour, systems microbiology, natural flavours and fragrances production,

Flavours and Fragrances (F&F) are highly important quality components in food, beverages, cosmetics, detergents and pharmaceutical products. Nowadays, most F&F molecules are produced by chemical synthesis or by extraction from plants. The need for environmentally friendly processes and the consumer's preference for natural products encourage research and development in alternative processes, such as the biotechnological production of F&F with microorganisms like yeast.

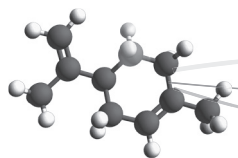
The fields of genomics, functional genomics, proteomics, protein and metabolic engineering of microbial and plant systems are currently making extraordinary steps forward. This knowledge will be the foundation of a truly sustainable biotechnology alternative for natural flavour production; an alternative that should have a strong impact in reorienting the agro-food and biotechnological industries to respond better to consumer needs.

The **BIOFLAVOUR** Consortium, also known as COST Action FA0907, has set up a “European Yeast Flavour Network” for integrated research and development in the field of natural F&F. The main objective of this network is to increase knowledge and competitiveness of the different partners by sharing their expertise in a common research space. This is also an exceptional opportunity to strengthen links between industrial and academic research centres across Europe and beyond. From a scientific and technological point of view, **BIOFLAVOUR** aims at developing a fully and integrated understanding of the biochemical and molecular mechanisms of flavour compounds biosynthesis by microbial systems, through cutting-edge technological methods collectively known as a Systems Microbiology approach.

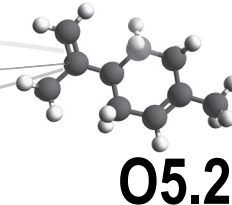
The expected chief outcomes of this Action are the development of new microbial and/or biomolecular catalysts; the creation of novel eco-efficient bioprocesses for the production of high added-value natural F&F compounds which will benefit the health of end consumers and the environment; and the reinforcement of the competitiveness of the biotechnological industry in this field.

The **BIOFLAVOUR** Consortium is organised into four interactive Working Groups:

- **WG1:** Biodiversity and High Throughput Screening for New Biocatalysts



- **WG2:** Functional Analysis of F&F Pathways by a Systems Biology Approach
- **WG3:** Engineering Strategies for F&F Production by Microbial Systems
- **WG4:** Tailored Bioprocesses for Natural F&F Production.



DEMONSTRATION OF SESQUITERPENE BIOSYNTHESIS IN GRAPE BERRY EXOCARP BY DEUTERIUM LABELING STUDIES

Bianca May, MATTHIAS WÜST

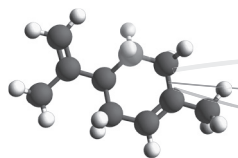
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Keywords: *Vitis vinifera*, DOXP/MEP-pathway, terpenes, wine aroma, SPME-GC/MS

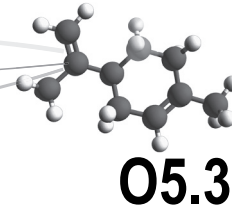
Until recently the contribution of sesquiterpenes to the overall aroma of wine has been completely overlooked. With the discovery of rotundone as an important aroma impact compound for the peppery characters in Shiraz red wine (1) there is now a growing interest to understand the biosynthesis of sesquiterpenes in grapevine. However, while numerous research studies are focused on the biochemical regulation and development of monoterpenes in grapevine, much less is known about sesquiterpenes which are generated from farnesyl diphosphate by various terpene synthases yielding different acyclic, mono- and polycyclic basic structures (2). Therefore, in this work the biosynthesis of sesquiterpenes has been investigated by using deuterium labeled precursors in an *in vivo*-approach. It could be shown that the sesquiterpene biosynthesis is mainly localized in the grape berry skin (exocarp) with only small or negligible biosynthetic activity in the berry flesh (mesocarp). Furthermore, a metabolic cross-talk between the plastidial DOXP/MEP-pathway and the classical cytosolic MVA-pathway could be demonstrated for sesquiterpene biosynthesis as opposed to monoterpene biosynthesis, which relies exclusively on the plastidial DOXP/MEP-pathway (3). Measurements with intact berries showed that sesquiterpenes are released selectively by the exocarp into the gas phase. The antimicrobial diterpene 13-*epi*-manoyl oxide was released as well. High amounts of this compound occurred at the stage of full ripeness and often coincided with an increase of the total sesquiterpene concentration. The dynamic allocation of biochemical resources to assure the biosynthesis of sesquiterpenes by a metabolic cross-talk, and the fact that sesquiterpenes are released through the grape skin confirm their function as a response to environmental or other external stimuli like pathogens and herbivores which is comparable with the induced biosynthesis of sesquiterpenes in foliage of *V. vinifera* (4).

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REACTION MECHANISM OF THE STRAWBERRY ENONE OXIDOREDUCTASE

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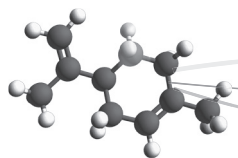
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Keywords: strawberry, Furaneol[®], quinone oxidoreductase, enone oxidoreductase

The flavor of strawberry (*Fragaria x ananassa*) fruit is dominated by an uncommon group of aroma compounds with a 2,5-dimethyl-3(5*H*)-furanone structure. Recently, we reported on the characterization of an enzyme involved in the biosynthesis of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF; Furaneol[®]), the key flavor compound in strawberries (1). *Fragaria x ananassa* enone oxidoreductase (FaEO), earlier putatively assigned as quinone oxidoreductase, is a ripening-induced, negatively auxin-regulated enzyme that catalyzes the formation of HDMF in strawberry fruit by the reduction of the α,β -unsaturated bond of the highly reactive precursor 4-hydroxy-5-methyl-2-methylene-3(2*H*)-furanone (HMMF). *In vitro*, the enzyme catalyzes the reduction of HMMF as well as 9,10-phenanthrenequinone (PQ; 2), however, with differing mechanisms: via two-electron and one-electron reduction, respectively. Using measurements of the stoichiometry of radicals and products, we were able to confirm that the reaction mechanism of FaEO hence depends on the availability of unsaturated hydroxyfuranone or (artificial) quinone substrates. Both reactions depend on NAD(P)H as a cosubstrate. Using the recombinant protein, which can be readily expressed in *E. coli*, we currently investigate the structure-function relationships of this interesting plant oxidoreductase. Also, X-ray structural studies are in progress to elucidate the stereoselective formation of the aroma metabolite HDMF.

References

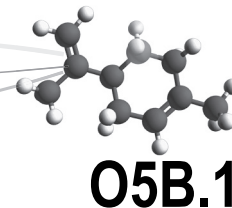
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Session:

Bioflavour

Lectures



TOWARDS THE DEVELOPMENT OF MOLECULAR MARKERS FOR APPLE VOLATILES

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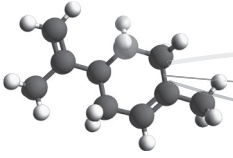
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Flavour is one of the key attributes of apple fruit quality. Consequently, actual cultivar breeding programs include sensory quality as an important but complex breeding goal. Although more than 350 volatile compounds have been found in the aroma profiles of apples, only a few appear to dominate the typical apple aroma. Among these compounds esters are quantitatively the most abundant volatiles in apple, contributing to more than 80 % of the aroma-relevant substances of an apple fruit. Additionally, most of the preferred cultivars on the market today are apples with an ester accentuated volatile pattern (Cox-type) in contrast to alcohol-types [1].

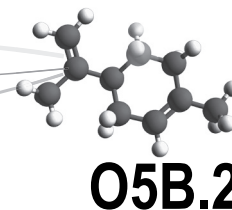
The aim of the presented research was a) to study the volatile patterns in an apple progeny derived from the cultivars 'Discovery' and 'Prima' (called C3), b) to use semi-quantitative data of volatile key compounds for QTL (quantitative trait locus) analyses and c) candidate gene mapping. The final goal is the development of functional markers to support molecular apple breeding and to enable a more efficient documentation of biodiversity of aroma patterns in *Malus* gene bank accessions, apple cultivars and breeding material.

QTLs for volatile compounds including important straight chain and branched chain esters were identified using the GC-data of the individuals of the apple C3 family and the genetic information of the two parental molecular linkage maps constructed for 'Discovery' and 'Prima' [2]. More than fifty QTLs for about 30 different apple fruit volatiles were detected through an interval mapping approach by using genotypic data of 150 F1 individuals of the C3 mapping population together with metabolic data obtained by HS-SPME-GC and nontargeted data treatment. In the candidate gene mapping approach, an alcohol acyltransferase (AAT) gene putatively involved in aroma-related volatile metabolism was mapped. The AAT gene inventory of apple was then characterized by bioinformatic mining the assembled 'Golden Delicious' genome. The gene *MdAAT1* located on chromosome 2 was selected as the putative candidate gene associated with QTLs for different acetate esters, and its allelic diversity was assessed by direct amplicon sequencing in a collection of 102 apple cultivars with available ester volatile profiles. Sequencing a 468 bp nucleotide sequence of the *MdAAT1* coding region resulted in the detection of four SNPs. Association analyses resulted in highly significant associations of both individual SNPs and distinct SNP patterns with the content of acetate esters including hexyl acetate, butyl acetate and 2-methyl-butyl acetate. The observed association suggests a putative causal functional relationship between *MdAAT1* and production of apple key esters [3].



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A COMBINATORIAL METABOLOMICS APPROACH TO DECIPHER FLAVOR TRAITS IN CROPS

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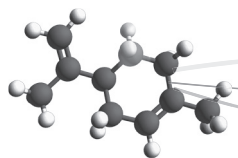
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Global food crops such as rice, melon, and tomato are of high economic importance. The quality of plant tissues determines their commercial value in relation to aspects of, for example, flavour, fragrance, shelf life, physical attributes, etc. Hence the quality crop plants is a direct function of their metabolite content (1). Each of these can be fully defined in terms of the metabolic profile of the material concerned at a particular time (2).

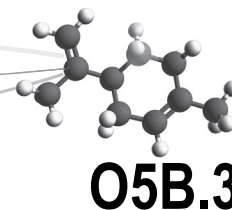
Within the framework of the EU-project METAPHOR we applied an unprecedented range of complementary metabolomic profiling platforms, including ¹H-NMR, liquid chromatography coupled to photodiode array and fluorescence detection (HPLC-PDA-FL), diverse GC-MS and LC-MS screenings, and a macro- and microelement screening by inductively coupled plasma mass spectrometry (ICP-MS) in order to study the spatial and developmental dynamics of metabolic profiles in plant tissues. The combined data were analysed using unsupervised multivariate analysis to highlight sample similarities and dissimilarities. Clustering and correlation network approaches were then applied to visualize inter-analyte relationships (3). In melon for example, the study design enabled the identification of so-called co-regulated hub metabolites in metabolic association networks and revealed links of primary and secondary metabolism to key mineral and volatile flavour complements. The



results illustrate the extent and complexity of metabolic interactions relevant to aroma production and demonstrate the power of such a multidisciplinary, multi-platform approach. These metabolomics technologies are thus enabling us to identify and home in on essential candidate metabolites which can become the subject of high-throughput screening approaches as part of targeted breeding programmes aimed at flavour and fragrance improvement in crop plants. The generic nature of such approaches entails broad future use for both food and ornamental crops.

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METABOLOMICS DATA FUSION REVEALS SPECIFIC GLYCOCONJUGATION OF PHENYLPROPANOID VOLATILES AS KEY TO DIFFERENTIAL TOMATO FLAVOUR

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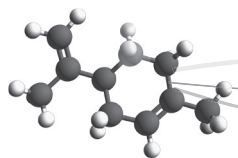
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Keywords: metabolomics, tomato flavour, glycoconjugation, volatiles, precursors

Organoleptic characteristics are main quality aspects in tomato and volatiles are considered as the major determinant in the flavour of ripe fruits. Several hundred volatile compounds have been described for tomato, but only a small part of this mixture is believed to contribute to the consumption quality of tomato fruits. In our research towards elucidating the molecular and biochemical mechanisms determining tomato fruit flavour and taste, we applied both sensory assessment and large-scale untargeted metabolomics profiling approaches of the same fruits as tool in identifying key compounds determining consumer quality of ripe tomato fruits.

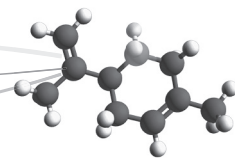
Large-scale GCMS-based untargeted metabolomics profiling of disrupted ripe fruits of nearly hundred commercial tomato varieties, representing the genetic diversity within tomatoes used for fresh consumption, combined with sensory profiling of the same fruits, methylsalicylate, guaiacol and eugenol were identified as key volatiles determining differential tomato flavour characteristics(1). Most volatiles from ripe fruit are released upon tissue disruption, such as cutting and chewing, as a result of enzymatic conversion of their non-volatile precursors. The same collection of tomatoes was therefore also analyzed for their non-volatile profiles by using untargeted LCMS-based metabolomics. Subsequent metabolomics data fusion and multivariate analyses revealed differential glycoconjugation as the main biochemical mechanism underlying differential flavour generation (2). While in certain cultivars the key volatiles accumulated as diglycoside conjugates, which were susceptible to rapid enzymatic cleavage upon disruption resulting in release of the corresponding volatile aglycons, in other cultivars the same volatiles were bound in higher conjugated complexes, i.e. triglycosides and malonyl-triglycosides, which could not be cleaved. This higher level of glycoconjugation is specifically induced upon ripening of the fruits.

This new principle of differential glycoconjugation of key volatiles can pave the way towards new strategies for controlling tomato fruit flavour and taste. Our results with tomato show that large-scale untargeted metabolic profiling combined with sensory and chemometric analysis is a powerful approach to get new insights into biochemical mechanisms underlying differential volatile generation and emission in plants and products derived thereof.

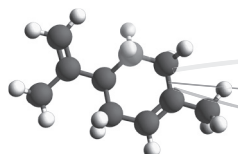


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Session: Flavour modeling Lectures



O6A.1

ADVANCED ANALYTICAL SENSORY CORRELATION – TOWARDS A BETTER MOLECULAR UNDERSTANDING OF COFFEE FLAVOR DESCRIPTORS

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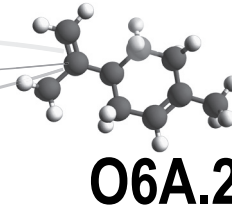
Keywords: Coffee, Flavor, Aroma, sensory-analytical correlation, predictive model

Coffee aroma is a complex mixture of more than 1000 volatile compounds. Aroma models which exhibit decent coffee aroma character have been developed based on the so-called ‘Odor Activity Value’ concept (1). The sensory evaluation of a ‘complete’ coffee aroma model vs. a model lacking in a single or a group of key odorants resulted in the identification of 23 character impact compounds, i.e. odorants having a major impact on the overall coffee character (2-3). However, an in-depth understanding of the link between coffee aroma sensory attributes and key aroma compounds is still missing. One reason for this is that omission experiments in aroma models cannot account for the full complexity of a coffee beverage, yet they do not, for example, involve possible interactive sensory effects (aroma-aroma, aroma-taste) and also no sensory profiling has been reported. An attempt to relate coffee volatiles to the sensory perception was made in a more recent study, where an untargeted approach was chosen to correlate analytical data (i.e. 16 ion traces) from headspace PTR-MS measurements with sensory descriptors. The results obtained allowed the development of a predictive model for coffee aroma (4).

In a similar, but targeted approach, the present study aims at a deeper understanding of the link of the character impact compounds mentioned above as well as additional coffee aroma and taste compounds to coffee aroma sensory attributes. In a set of 10 different espresso brews, 42 coffee odorants and 12 taste-active compounds were quantified using GC-MS and LC-MS techniques. The data obtained were then correlated with sensory descriptors from an expert panel, and an advanced predictive model was established and cross-validated. The results represent a significant progress in correlating sensory with instrumental data exemplified on one of the most complex aromas, i.e. coffee.

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FLAVOUR PAIRING OF FOODS – FACTS AND FICTION

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Keywords: Flavour pairing; odour mixture psychophysics; arousal; hedonic response

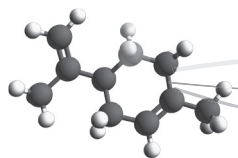
Flavour pairing has become a popular endeavour for renowned chefs and has also entered the food industry innovation and R&D laboratories. The flavour pairing theory, or rather postulate, was proposed by Heston Blumenthal and Francis Benzi, a chemist from Firmenich. They proposed that if two foods share a large proportion of the same flavour components, they will taste pleasant when eaten together. However, studies on odour or taste mixtures have shown more varied results for hedonic and intensity responses to odour mixtures (1).

In the present study the odours of 19 common foods and 53 binary odour pairs made from them were evaluated by a sensory panel of 37 subjects in two replicates. The foods were presented in such a way that the foods were physically separated but shared a common headspace for sensory evaluation. The Volatile Compounds in Foods database (2) was used to estimate the volatiles the experimental foods and the overlap in volatiles (as number and relative to the total) in the food pairs. The sensory responses were measured for the attributes intensity, pleasantness, complexity, harmony and novelty. The latter variables were selected to measure the level of arousal exhibited by the flavour pairs.

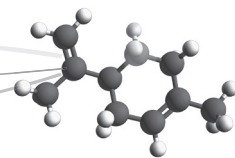
The perceived intensity of the odour pairs was in 91% of the cases lower than the constituent food odour with the highest intensity of the pair. Therefore, intensity of the food pairs was characterized by compromise and compensation. In 49% of the food odour pairs the hedonic response was reduced in comparison to the individual food odours. In 42% of the odour pairs the hedonic response was lower than the hedonic response to the most liked constituent food odour of the odour pair. The hedonic response in the food odour were therefore also predominantly characterised by compromise and compensation responses. In contrast, the arousal of the food odour pairs as measured by novelty was additive and in 34% of the cases hyper-additive. The correlation coefficient (R^2) of the hedonic responses to the estimated overlap of volatiles in the odours pairs was 0.046 when based on the number of overlapping volatiles and 0.0005 when based on the relative number of overlapping volatiles, respectively. The study indicated that flavour pairing of foods leads to increased arousal but compromised hedonic responses. Furthermore, more sophisticated analytical chemistry approaches are needed to evaluate the level by which the composition of aroma volatiles and taste components can predict the hedonic response food pairings.

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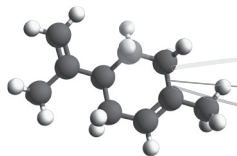
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Session:

**Sulfur precursor
systems**

Lectures



07.1

ENZYME- AND MICROORGANISM-GUIDED DISCOVERY OF NATURAL SULFUR COMPOUND PRECURSORS

CHRISTIAN STARKENMANN

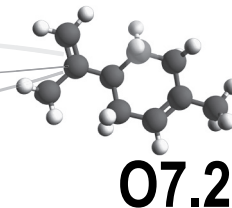
The implementation of gas chromatography coupled to mass spectrometry in the year 1970 permitted chemists to discover numerous new volatile organic compounds. Twenty years later a plateau was reached and it has become more difficult to discover new odor-active molecules. Sulfur compounds are often key odorants, but present at very low concentrations. An analytical breakthrough in 1994 was the use of affinity chromatography by a German group to enrich and isolate fractions containing thiols.

The next logical step was to develop an understanding of the precursor chemistry of these sulfur compounds. With the more recent development of mass spectrometers adapted to liquid chromatography, a new avenue for analyzing non volatile compounds was opened. The ionization patterns and chromatographic properties of non volatiles are not easily predictable as for volatile compounds, therefore discoveries guided by the use of enzymes or micro-organisms can help to discover new natural molecules.

This presentation discusses how we discovered the important role of cysteine-*S*-conjugates in the perception of onion flavor, via liquid chromatography and tasting (*in vivo* assay)(1). We then realized that this strategy could be applied to elucidate the precursors of other sulfur odorants with the help of enzymes or micro-organisms. An *in vitro* approach was used to discover [1-(2-hydroxyethyl)-1-methylbutyl]-(*L*)-cysteinylglycine (Cys-Gly-*S*-conjugate), the precursor of a key human axillary odorant, the (R/S)-3-methyl-3-sulfanylhexas-1-ol, using skin micro-organisms to ferment sterile human sweat (2). Our interest was then to show the diversity of the cysteine-*S*-conjugates in plants. This is illustrated by the analysis of bell peppers and the structure elucidation of *S*-(3-hydroxy-1-methylhexyl)-L-cysteine, *S*-(3-hydroxy-1-propylbutyl)-L-cysteine, *S*-(3-oxo-1-propylbutyl)-L-cysteine and (2R,2'R)-3,3'-(4-hydroxyheptane-2,6-diyl)bis(sulfanediyl)bis(2-aminopropanoic acid). The volatile organic sulfur compounds were released by a beta-lyase from an authentic sample as well as from bell peppers fractions (3).

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THE CONTRIBUTION OF ORGANIC SYNTHESIS AND ANALYTICAL CHEMISTRY TO THE KNOWLEDGE OF VARIETAL THIOLS BIOGENESIS IN WINES AND THEIR CONTRIBUTION TO THE WINE AROMA

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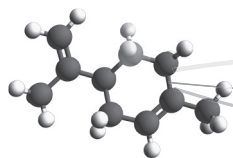
Keywords: varietal thiols, wine, aroma, stable isotope dilution assay, alcoholic fermentation

Aroma compounds are produced in grapes and/or throughout the wine making process. For a better understanding, they are classified according to their formation period:

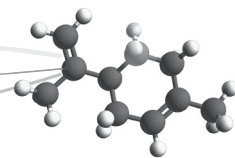
- Varietal aroma compounds are present in grapes, either in a free form, which means volatile and directly perceptible by the olfactory receptors, or in a bound form (aroma precursors) that is cleaved during winemaking. They are responsible for the varietal specificity of wine aroma.
- Pre-fermentation aromas appear between harvest and alcoholic fermentation, throughout enzymatic reactions occurring when berries are crushed.
- Fermentation aromas are secondary products of micro-organism metabolism (yeast or lactic acid bacteria), as ethyl esters and fusel alcohols, and are responsible for the vinous and fruity olfactive characteristics of the product.
- Finally, post-fermentation aroma compounds are formed during wines ageing and involved chemical or biochemical conversion of volatile compounds. They are responsible for the complexity of old wines.

Among these compounds, the 4-methyl-4-mercaptopentan-2-one (4MMP), the 3-mercaptohexylacetate (3MHA) and the 3-mercaptohexan-1-ol (3MH) have been identified as key molecules of young wines elaborated with many varieties. These varietal compounds result from the cleavage of odorless precursors present in grapes or musts by yeast during the alcoholic fermentation. The positive contribution of varietal thiols was firstly pointed out by Du Plessis and Augustyn (1980) who demonstrated that the guava aroma found in South African Sauvignon Blanc wines was mainly due to the occurrence of 4MMP. In contrast with light sulfur compounds such as carbon sulfide, ethanethiol, methanethiol and hydrogen sulfide (bp<90°C), which are mostly produced during the alcoholic fermentation, held to be responsible for olfactory defects and often present at high concentrations, varietal thiols occurred at very low levels in some *Vitis Vinifera* wines and exhibited pleasant odors such as blackcurrant bud, passion fruit and grapefruit.

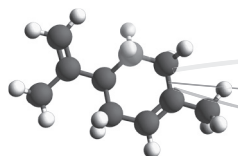
For twenty years ago, the interest for varietal thiols involvement in young wines has considerably grown, especially for wine industry. Most literature dealing with varietal thiols



in wine are focused on the aforementioned compounds and demonstrate the central role played by the different branches of chemistry in the understanding and control of biotechnological processes responsible for the release of these powerful odoriferous compounds. This key lecture will intend to describe the synthetic routes and their contribution to the biochemical transformation studies occurring during winemaking and their regulation. The analytical procedures developed in several papers will be also discussed as they contribute to the general knowledge of the enological and viticultural aspects impacting thiols levels in wines.



Session: Maillard systems Lectures



08.1

FORMATION OF PYRAZINES IN MAILLARD MODEL SYSTEMS CONTAINING PEPTIDES

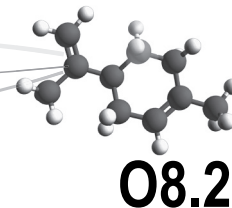
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Keywords: peptides, Maillard, pyrazines, flavor, model reactions, SBSE

The Maillard reaction or non-enzymatic browning is the reaction of carbohydrates with the amino function of free amino acids, peptides or proteins. Whereas most studies concerning the Maillard reaction have focused on free amino acids, little information is available on the impact of peptides and proteins on this important reaction in food chemistry. However, the amount of free amino acids in food is always very low as compared to the amounts of peptides and especially proteins. As a first step to extend the current knowledge on the reactivity of free amino acids, this study was undertaken to investigate the formation of flavor compounds from di- and tripeptides in Maillard model systems with glucose, methylglyoxal or glyoxal. The flavor compounds produced by the reactions of these peptides were analyzed by means of SBSE-GC-MS and compared with those obtained from the mixture of the corresponding free amino acids with the same carbonyl compound. At first, the reactions of eight dipeptides with lysine at the *N*-terminus were studied. The *C*-terminal amino acid was varied in order to determine the influence of the neighboring amino acid on the flavor production by the lysine residue, since theoretically, only the two amino groups of lysine are able to react. Pyrazines were the most important volatiles detected in case of reactions with the dipeptides. These volatiles are known to contribute significantly to the unique roasted aroma of many heated food products. The pyrazines described in this study generally have pleasant roasted, nutty flavor characteristics. Generally, the pyrazines were produced more in case of dipeptides as compared to free amino acids. For reactions with glucose and methylglyoxal, this difference was mainly caused by the large amounts of 2,5(6)-dimethylpyrazine and trimethylpyrazine produced from the reactions with dipeptides. For reactions with glyoxal, the difference in pyrazine production was rather small and mostly unsubstituted pyrazine was formed.

In another series of experiments with dipeptides, the *N*-terminal amino acid was varied, while glycine was always chosen as the *C*-terminal amino acid. Also for these peptides, pyrazines were the most important volatiles detected. In addition, flavor formation from Maillard model systems containing tripeptides was studied. A reaction mechanism for pyrazine formation from peptides was proposed and evaluated. This study clearly illustrates the capability of peptides to produce Maillard flavor compounds which can differ from those obtained from the corresponding reactions with free amino acids.



ENHANCING MEAT FLAVOUR GENERATION BY INCREASING CYSTEINE EFFICIENCY IN H₂S LIBERATION USING A 2-STEP PROCESS: “PH/THERMAL” VS “ENZYMATIC/THERMAL”

MARTINS, SARA, Batenburg, AM & Cambeen, A.

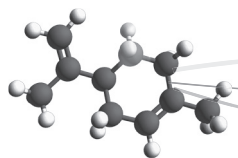
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Keywords: Maillard, meat flavour generation, two steps, bioconversion, H₂S, MFT, FFT

The aroma of cooked meat is provided by a complex mixture of volatile compounds produced during cooking (1, 2), where sulfur-containing compounds are considered to be particularly important. A major route to generate these compounds is the Maillard reaction between reducing sugars and the amino acid cysteine, a sulphur containing amino acid. However, cysteine efficiency is normally dependent on its ability to generate hydrogen sulphide (H₂S) and its pH dependent. Besides pH, the application of enzymes, more specifically C-S lyases, is also known to be able to generate H₂S from cysteine and possibly from cysteine containing peptides (3, 4). The success in generating H₂S more efficiently meant to produce more intense meat flavour, from the same starting material or the same flavour intensity in a shorter reaction time. As a result, the focus of the present study was to understand by using a two step approach if using a different pH and/or enzymes in the first step would enhance H₂S generation from cysteine and whereas meat flavour generated thereafter upon heating with xylose would also be enhanced.

In a first instance several sources of enzymes were screened and the best results were obtained with microbial lyases. Six strains were selected from the Unilever-Vlaardingen culture collection, representing the various classes of lactic acid bacteria and *Brevibacteria* reported to be rich in lyases. They were grown overnight in shake-flasks, harvested, washed and tipsonicated to release the intracellular enzymes. The cell lysates showed remarkable differences in activity, with the two *Brevibacteria* and especially one of the selected *L. fermentum* strains generating significant amounts of H₂S. Without enzyme, the thermal H₂S generation was found to be strongly favoured by higher pH, being pH 8 the best. When compared the two processes, the benefit of the enzymatic over the thermal process at pH 8 was a factor of 2.5. However, apparently due to product inhibition, the absolute H₂S concentration that can be generated by the enzyme was limited to ~5 mM, and hence at higher cysteine concentrations the advantage of the enzyme treatment was lost.

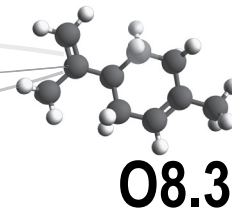
The ultimate target was to enhance meat flavour generation. For two key meat flavor compounds, 2-methyl-3-furanthiol (MFT) and 2-furfurylthiol (FFT), analysis methodology was therefore set up, based on stable isotope dilution analysis. The influence of pH on the Maillard reaction step was also studied, indicating an optimum production of MFT and FFT at pH 6. H₂S was best generated at pH 8, and best meat flavour was obtained upon shifting the pH to 6.0 while adding xylose, and subsequent heating. Under these optimised two-step conditions very similar levels of MFT and FFT were obtained for both processes, and in fact no clear relation was found with the amount of H₂S generated in the first step. In a



provisional sensory evaluation the product of the “all-thermal” process was preferred over the “enzymatic/thermal” process. Nevertheless, independently of the used process, pH/thermal or enzymatic/thermal, the two-step approach clearly lead to best flavour generation, then the traditional one-step process.

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GENERATION OF ROAST-SMELLING COMPOUNDS UPON EXTRUSION COOKING –FIRST INSIGHT INTO FORMATION PATHWAYS USING THE CAMOLA APPROACH

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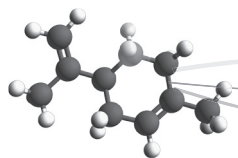
Keywords: Maillard reaction, flavour formation, popcorn, labelling experiments, extrusion cooking

Extrusion cooking is gaining increasing importance in food industry as a cost efficient way of manufacturing of numerous foods including cereal products. However, desirable flavour characteristics associated with conventionally cooked cereals do not develop to the same extent during extrusion cooking. Consequently, the extruded food products are generally inferior in flavour as compared to those obtained by conventional thermal processing. Currently, only very limited information is available concerning formation mechanisms leading to odour-active molecules by extrusion cooking. While the reaction pathways of some buttery smelling (2,3-butanedione, 2,3-pentanedione) and caramel smelling compounds (4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) and cyclotene) have recently been revealed (1), no such information exists concerning roast-smelling compounds. In contrast, several pathways were proposed for the formation of these compounds in model systems both under roasting and aqueous conditions (2-4). As the targeted roast-smelling compounds belong to key odorants of many thermally processed cereals, the knowledge of their formation mechanism upon extrusion would permit optimization of their concentration in the final product.

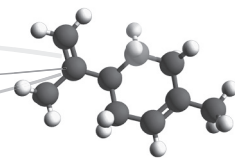
The aim of our study was therefore to gain a deeper insight into the reaction mechanisms of roast-smelling compounds upon extrusion cooking. The approach consists in CAMOLA experiments (5) with [U-13C6]-glucose applied to a rice containing extruded food system. The formation of several odorants will be discussed including 2-acetyl-1-pyrroline, 2-acetyl-1(or 3),4,5,6-tetrahydropyridine, 2-propionyl-1-pyrroline, and 2-propionyl-1(or 3),4,5,6-tetrahydropyridine. Among the flavor compounds studied, 2-propionyl-1(or 3),4,5,6-tetrahydropyridine have been detected for the first time in thermally processed foods.

References:

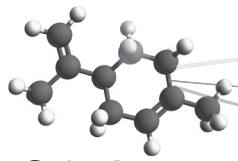
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Session: Oxygen and wine Lectures



09.1

COMPARISON OF SCREW CAP AND CORK CLOSURE ON OXYGEN PERMEABILITY AND FLAVOR DEVELOPMENT DURING POST-BOTTLE AGING

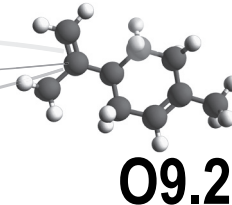
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Key words: screw cap, volatile sulfur compound, wine aroma

It is generally accepted that bottled wine is a dynamic system. A proper wine bottle closure will allow a dynamic and healthy gas exchange between the wine and the air. The amount of oxygen ingress through the wine closure can directly affect wine aging and flavor development. Natural cork (up and down), synthetic, and screw caps with Saran-Tin, Saranex and low density polyethylene (LDPE) lines were investigated on both Pinot noir and Chardonnay wines over three years storage. For Chardonnay wine, the LDPE screw cap gave the highest dissolved oxygen, lowest free SO₂, and highest absorbance at 420 nm. The Saran-Tin screw cap gave the lowest dissolved oxygen. Similar trends were observed for Pinot noir wines, with Saran-Tin screw cap gave the lowest dissolved O₂ and highest free SO₂ and total SO₂. Hydrogen sulfide, methanethiol (MeSH), dimethyl sulfide (DMS), methyl thioacetate (MeSOAc), dimethyldisulfide (DMDS) and dimethyltrisulfide (DMTS) were extracted using Carboxen/PDMS SPME fiber, and quantified by gas chromatography-pulsed flame photometric detection using methyl ethyl sulfide and isopropyl sulfide as internal standard. A reduction of H₂S, methanethiol and methyl thioacetate were observed during the three year aging process for both wines. The reduction of H₂S and MeSH was correlated well with oxygen permeability of the closure and dissolved oxygen, and their concentration decreased most in wines with LDPE screw cap and synthetic closures, while the decrease of methyl thioacetate was independent with closure types. For both wines, we did not detect any elevated sulfur compound for any screw cap closures, neither did we detect any DMDS or DMTS in any of the experimental wines. Potential volatile sulfur conversion was also investigated in synthetic wine at 4°C, 40°C and 50°C for four months. Eight sulfur containing compounds, MeSH, DMDS, DMS, MeSOAc, methionol, methionine, cysteine and glutathione, were studied. DMDS is very stable in synthetic wine during storage while DMS can be converted to MeSOAc and DMDS. MeSH can be easily converted to DMDS and DMTS. MeSOAc is stable at 4°C but some conversions to MeSH and DMDS was observed at elevated temperature. Methionol can be easily converted to DMDS. Cysteine can generate H₂S while glutathione can generate DMS. The development of other volatile compounds will also be discussed.



FACTORS AFFECTING ACCUMULATION OF CERTAIN VOLATILE SULFUR COMPOUNDS DURING BOTTLE AGEING OF WINES

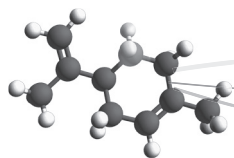
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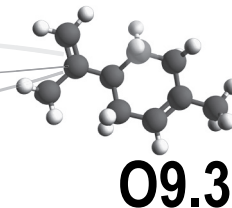
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During bottle storage of wines, excessive amounts of certain volatile sulfur compounds (VSCs) can develop, which has been associated with occurrence of the so-called ‘reductive’ off-odors (1). Reductive wines are characterized by odor attributes reminiscent of rotten egg, sewage, rubber, and cabbage, which can be highly detrimental to wine quality. Hydrogen sulfide (H_2S), methyl mercaptan (MeSH) and dimethyl sulfide (DMS) are among the VSCs most frequently associated with reductive off-odors. While DMS has been shown to arise from the degradation of S-methylmethionine, the origin of H_2S and MeSH during bottle storage remains unclear. In a complex matrix such as wine, several pathways have been proposed, including degradation of sulfur-containing amino acids and, in the case of MeSH, hydrolysis of methylthioacetate (MeSAc) produced by yeast during fermentation. Moreover, based on the observation that low exposure to oxygen favors accumulation of H_2S and MeSH, oxidation-related factors can also be implicated, including formation of MeSH from reduction of symmetric and asymmetric disulfides. In this study, we have followed the evolution of MeSH and H_2S during bottle ageing of different Shiraz and Sauvignon Blanc wines stored under variable regimes of oxygen. In Sauvignon Blanc, H_2S was found to increase during 12 months of bottle storage. A positive correlation was observed between H_2S concentration and occurrence of the antioxidant glutathione (GSH), suggesting presence of this antioxidant can promote accumulation of reductive VSCs. Surprisingly, the level of copper was also positively correlated with H_2S formation, in spite of the supposed ability of Cu^{2+} to bind –SH compounds. In Shiraz wines, H_2S was relatively stable, while a steady increase of MeSH was observed during ageing, regardless of the wine. This appeared to be not linked to the behavior of possible precursors such as MeSAc and dimethyldisulfide (DMDS). However, in all wines, it was found that the degree of oxygen exposure in the bottle affected accumulation of H_2S and MeSH, with higher oxygen exposure generally corresponding to lower concentrations at the end of ageing. While this highlights the key role of oxygen in the accumulation of MeSH during bottle storage, we could not establish a clear correlation between the behavior of DMDS and that of MeSH. Therefore, the factors linking oxygen exposure and MeSH accumulation during bottle ageing remain to be established. This study indicates that management of oxygen exposure in the bottle, which can be obtained by using closures with different oxygen permeability, allows controlling accumulation of VSCs during wine bottle ageing. For the first time, it is shown that the ability of a wine to develop H_2S can be positively correlated to its content in copper and GSH. These results highlight the need to reassess the currently common practices aimed at increasing copper and GSH content of bottled wines.

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ORIGINS OF 3-METHYL-2,4-NONANEDIONE IN RED WINES

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Keywords: red wine, oxidation, flavour, furan fatty acids

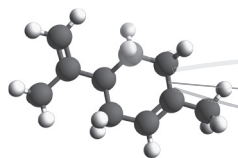
The reputation of famous red Bordeaux wines is strongly associated with their aging potential. Indeed, some of these wines keep the varietal flavor nuances of young wines while developing specific empyreumatic nuances during aging. However, this ideal aging does not occur in every wine. Premature-aging aroma phenomena reflect the oxidative aging of red wines. Prematurely-aged red wines develop several aromatic nuances reminiscent of prunes and figs. The presence of these overriding odors affects the quality and subtlety of the wine flavor and may shorten its shelf life.

Recently we identified a new compound in red wines: 3-methyl-2,4-nonanedione (MND) associated with red wines marked with prune odors (1). MND is present at very low concentrations in wines, the quantitative determination of this compound and the study of its organoleptic impact in wines require very accurate and sensitive analytical method. Liquid-liquid extraction followed by gas chromatography analysis coupled with chemical ionization mass spectrometry with methanol as liquid reactant was optimized. During validation, the method exhibited a high level of accuracy and repeatability (< 7 %) with limit of detection (LOD) suitable for the analysis of this compound below its perception threshold (16 ng/L) in red wines. The method was successfully applied for the analysis of 130 wines, including red, white, claret, dessert and fortified wines from several origins and vintages (from 1929 to 2008). Correlation between the concentration of 3-methyl-2,4-nonanedione and sensory analysis will be described. In addition, we report first results concerning the incidence of oxygen on its formation with the study of the impact of the closure (cork, screw caps, synthetic) or the packaging (glass, PET) on its formation during aging in bottle.

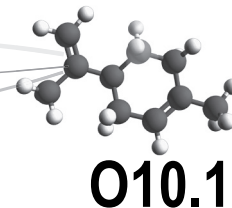
We also reported the identification of a family of precursors of this diketone in red wines: the furan fatty acids family. 11-(5-pentyl-3,4-dimethyl-2-furyl) undecanoic acid was the main furan fatty acid and was identified for the first time in wines. Details of this study and consequences of the level and the distribution of this furan fatty acid in wines on the formation of MND will be presented.

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Session: Structure-activity Lectures



DISCOVERY AND STRUCTURE-ACTIVITY STUDIES OF MAILLARD-MODIFIED GUANOSINE 5'- MONOPHOSPHATES USING HUMAN SENSORY STUDIES AND CELL-BASED TASTE RECEPTOR ASSAYS

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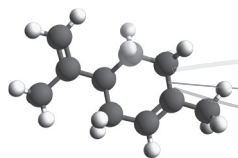
Key words: umami, 5'-GMP, taste enhancer, Maillard reaction, hTAS1R1/TAS1R3, structure/activity relations

Throughout the past decades the taste enhancing ribonucleotide guanosine 5'-monophosphate (5'-GMP) has been used in various foods to increase the palatability due to the pronounced synergistic behavior towards the umami taste of monosodium L-glutamate. Only recently, the identification of additional 5'-GMP derivatives such as, e.g. *N*²-lactoyl-5'-GMP (1), formed upon food processing has been successful.

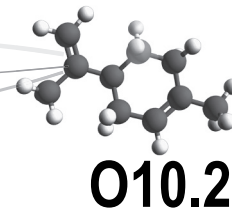
With the objective to identify novel taste enhancing ribonucleotides formed upon manufacturing of yeast autolysates, commercial yeast autolysates were fractionated by means of GPC and HPLC and the fractions obtained were analyzed by means of a comparative taste dilution analysis (2). HPLC-MS/MS analysis of these yeast autolysate fractions, followed by targeted Maillard-type model reactions involving 5'-GMP and reducing carbohydrates led to the discovery of a series of *N*-glycated 5'-GMP reaction products which were purified and identified by means of LC-MS/MS, LC-TOF-MS, 1D/2D-NMR, and CD spectroscopy. Systematic structure-activity studies using targeted Maillard-type transformation reactions of 5'-GMP led to a series of novel ribonucleotides such as, e.g. *N*²-(1-carboxyalkyl)-5'-GMP and *N*²-((1-alkylamino)carbonylalkyl)-5'-GMP derivatives. The umami modulating properties of these compounds were determined with a paired choice comparison test (3) resulting in the so called β -value, which describes the taste modifying potential of the target compound in reference to the taste enhancing capacity of the nucleotide inosine 5'-monophosphate (5'-IMP). Strongly depending on their chemical structure as well as on the stereochemistry of the *N*-substituent, the β -values of the novel compounds covered a broad range from nearly inactive 5'-GMP derivatives (β -value of 0.1) to highly active taste enhancers exhibiting β -values of more than 7.0. Finally, the results of the human sensory studies were verified by means of a cell-based *in vitro* assay based on the functional expression of the heterologues hTAS1R1/TAS1R3 receptor dimer.

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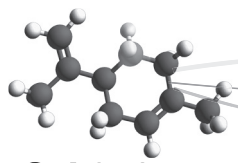


IDENTIFICATION OF THERMALLY GENERATED BITTER COMPOUNDS IN WHOLE WHEAT BREAD

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Bitterness in whole grain foods can lead to poor consumer acceptability. The objective of this study was to identify the primary bitter compounds in whole grain bread. Sensory-guided fractionation of commercial bread using liquid-liquid extraction, solid phase extraction, ultra-filtration and 2-D offline RPLC revealed multiple bitter compounds existed in the crust. The structures of the most intense bitter compounds were identified by HR-MS, MS/MS, 1D and 2D NMR spectroscopy as Acortatarins A, 5-(hydroxymethyl)furfural(HMF), N-(1-deoxy-D-fructos-1-yl)-L-tryptophan (tryptophan-Amadori compound), and 2-(2-formyl-5-(hydroxymethyl-1H-pyrrole-1-yl) butanoic acid. Based on these results, bitterness of whole grain bread was attributed the Maillard reaction during bread making rather than native compounds from the flour. This information provides a molecular basis to understand the negative flavor traits of whole grain bread and creates a foundation for flavor improvement.



O10.3

HALOGENATED PHENOLS AND CRESOLS: STRUCTURE – ODOR RELATIONSHIPS AND OCCURRENCE IN NATURE

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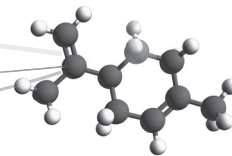
Keywords: chloro, iodo, bromo, trichloroanisole, wine, water, food

Halogenated phenols and anisols belong to another group of volatiles consistently reported as off-odorants. 2,4,6-Trichloroanisole (TCA), with its musty and corky character, was found to negatively affect various foods such as wine, cocoa powder, and drinking water (1,2,3). The high odour impact of TCA was proven in a study investigating the so-called hardish Rio-off-flavour in coffee (4,5). Although TCA was found by the authors to be only an ultra trace constituent (0.6 µg/L) in coffee brews affected with musty odour, the odour threshold (0.002 µg/L) was 300-fold lower than the analysed concentration.

Throughout the 1960s, Australian prawns frequently exhibited a disagreeable iodinelike odour, which was attributed at first to five bromophenols by GC/MS analysis. Sensory investigations elucidated 2,6-dibromophenol (DBP) as the dominant off-odorant. The compound was evaluated as the most odour-active amongst the bromophenols and the odour threshold of DBP was determined to be somewhat lower than TCA (3). Further examples of halogenated phenols that have been confirmed as off-flavour sources are 2-bromophenol in drinking water (6), 2,6-dichlorophenol in orange juice (7), 2-chloro-6-methylphenol in chicken and biscuits, 2-bromo-4-methylphenol in salted Gouda cheese, and 4-bromo-2-chlorophenol in melons (8). In most cases, microbiological activity was assumed to be the reason for generation of the halogenated compounds from phenols and halogen salts.

Regarding this widespread occurrence of halogenated aromatic compounds, it is remarkable that little information on – in particular – odour-active iodinated phenols is available in literature. Generation of mono-, di- and tri-iodinated phenols via reaction of iodine and phenol was confirmed in model studies (9). In particular, 2-iodophenol with a medicinal, phenol-like, and chemical odour was evaluated as an odour-active compound with high odour potency. A cake mix affected with an unpleasant medicinal odour was caused by an iodo-methylphenol isomer (10). As recently reported, we identified the medicinal flavor in mineral water to be caused by 2-iodo-4-methylphenol (11).

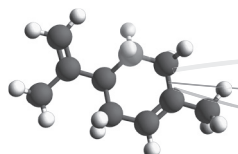
A common property of all of the phenols mentioned above is that they are substituted with a halogen atom in position 2. It seems that molecules with this substitution pattern interact strongly with human odour receptors, resulting in high odour activity. This assumption is supported by sensory literature data. For example, the odour thresholds of 2-chloro-, 2-bromo-, and 2-iodophenol were determined to range from 0.36 – 2 µg/L water (9,11,12). However, the odour activity increased significantly by insertion of a methyl group to 2-chlorophenol in position 4, as demonstrated for 2-chloro-4-methylphenol (12,13).



However, no comprehensive data are at hand showing the influence of interchanging iodo- or other halogen-moieties with methyl-groups and vice versa, or elimination of a substituent on odour impact and odour character of halogenated anisols and cresols. To gain a more systematic picture of the structure-activity relationships of halogenated phenols and anisols, comprehensive sensory investigations have been undertaken in our group e.g. utilizing one and two-dimensional high resolution gas chromatography-olfactometry. Structural relationships with specific odor characteristics will be presented, and discussed with regard to the natural occurrence of the respective compounds, and their sensory relevance in everyday life.

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O10.4

STRUCTURE/ODOUR RELATIONSHIPS IN HOMOLOGOUS SERIES OF POLYFUNCTIONAL THIOLS

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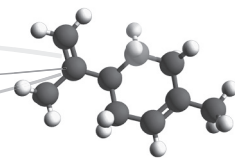
Keywords: Polyfunctional thiols, structure/odour relationship, odour threshold, QSAR

Up to now, more than 50 distinct polyfunctional thiols have been identified in foods and are still under on-going research. Although these compounds usually occur only in trace levels, polyfunctional thiols are well known as significant contributor to the overall aroma of many foods, due to their extreme low odour thresholds. Exemplary, 3-methyl-3-mercaptopentyl formate or 2-methyl-3-mercaptopentanol are among the most potent odorants identified up to now.

Despite the great importance of thiols in flavour chemistry, nearly no data are available on structure/odour relationships of polyfunctional thiols. Thus, the aims of the present study were i) to determine the odour thresholds of different homologues series of polyfunctional thiols, ii) to correlate the chemical structure with the thresholds obtained and, finally, iii) to create a 3D-QSAR-model, to predict the thresholds of so far unknown thiols.

Therefore, polyfunctional thiols (e.g. mercaptoalkanes, mercaptoalkanols, mercaptoalkyl esters) with differing carbon chain length (C3-C10) and differing positions of the functional groups were synthesized. The odour thresholds in air and the odour qualities at threshold level were determined by means of GC-Olfactometry and the results obtained were correlated to the chemical structure. The influence of chain length, position of the mercapto group within the carbon chain and the presence and position of additional functional groups in the molecule on the differences in odour thresholds and odour qualities were tested. Thereby, it was shown that, e.g., steric effects have a significant influence on the olfactory perception of thiols. To show the crucial influence of the SH-group in chemoreception, the odour thresholds of some mercaptans after *S*-acetylation were also determined and the data obtained were compared with the results for the corresponding mercaptoalkyl acetates.

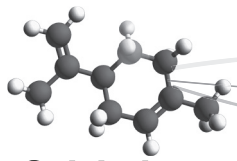
The presentation will summarise these data and will also show a first 3D-QSAR-model which was able to predict odour thresholds of some thiols in good accordance with the experimental results.



Session:

Wine flavour

Lectures



011.1

DECODING THE TASTE OF RED WINE BY MEANS OF A SENSOMICS APPROACH

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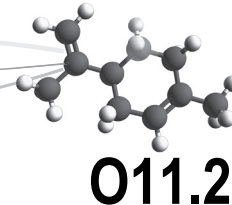
Keywords: taste, red wine, astringency, taste dilution analysis, high molecular weight compounds

Besides aroma and colour, the bitter taste as well as the astringent oral sensation are important contributors to the flavor quality of red wine. Although many attempts have been made to correlate analytical data on distinct wine components with the sensory data obtained from human subjects, the reports on the chemical species imparting the wine's bitterness and astringency are rather contradictory and the key drivers have not yet been comprehensively elucidated on a molecular level.

Aimed at molecularizing the key players driving the attractive taste of a red wine, the taste dilution analysis (TDA) was applied to a red wine in order to identify the key compounds exhibiting velvety astringency, puckering astringency, as well as bitterness (1). A total number of 26 orosensory-active non-volatiles were identified amongst which several hydroxybenzoic acids, hydroxycinnamic acids, flavon-3-ol glycosides, dihydroflavon-3-ol rhamnosides as well as a structurally undefined high molecular weight fraction (>5 kDa) were found as the important astringent compounds, and several flavan-3-ols as well as a series of hydroxybenzoic acid ethyl esters and hydroxycinnamic acid ethyl esters were found as bitter compounds in the red wine. Quantitative studies, followed by taste reconstitution and omission experiments revealed that besides the low-molecular weight compounds, in particular, the high molecular weight fraction was key for the overall puckering astringency but not for bitter taste (2). Gel permeation chromatography, alkaline treatment, acidic hydrolysis, and thiolytic depolymerization, respectively, followed by compositional analysis using LC-MS/MS and HPIC-PAD demonstrated these taste-active polymers to be made up by flavan-3-ols and diversely substituted derivatives, carbohydrates, phenylpropenoic acids, hydroxylated benzoic acids, and anthocyanins. Finally, model reactions were carried out in order to clarify possible linkages and to understand the formation of these astringent polymers on a molecular level (3).

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IMPLICATION OF RED WINE NONVOLATILE COMPOSITION IN QUALITY PERCEPTION

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Keywords: quality; nonvolatile composition; PLS; wine

One of the most determining parameter of wine as a product is that its acquired value highly depends on the sensory information provided during its consumption. Thus, the main challenge for winemakers is to translate the chemical composition of wine into sensory attributes that determine quality and consequently wine price. There is an increasing interest in finding the key chemical molecules responsible for the alluring sensory properties of wines and thus their quality (1). In this regard, there is a great deal of work aiming at understanding the volatile chemicals behind those perceptions. However, little is known about the real impact of nonvolatile molecules in quality perception of wines. Thus, the major goals of the present work were to predict sensory properties from non-volatile composition and then to evaluate the implication of fixed compounds in quality judgements of wine experts.

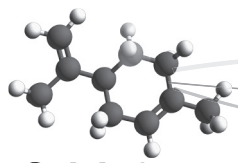
To accomplish such goal two sets of Spanish red wines were submitted to sensory descriptive analysis (taste, astringency, persistence and global intensity in mouth), their nonvolatile composition was analyzed by HPLC-DAD-MS and Fourier-transform Infrared (FTIR) spectroscopy. In parallel, a panel of experts evaluated the overall quality by a sorting task methodology.

Results revealed that, in both set of red wines, nonvolatiles such as flavonols, polimerized PAs, and tartrates of hydroxycinnamic acids are positively correlated to quality perception; however caffeic acid seems to reduce the scores of quality given by experts.

This study increases the information on the quantitative sensory and chemical profiles for two homogeneous quality set of wines present in the Spanish market and provides with concrete nonvolatile compounds involved in their sensory properties and responsible for an increase in quality.

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O11.3

**THE PERCEPTION OF RIESLING VARIETAL CHARACTER: THE ROLE OF
2,2,1-TRIMETHY-DIHYDRONAPHTHALENE (TDN)**

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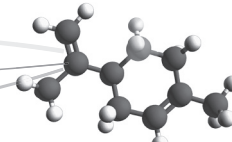
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Keywords: perception, configural, analytical, simulation

The perception of wine aroma is an excellent example of the human ability to experience multiple sensations as a single gestalt, i.e. a configural perception or as a top-down process. This behavior is relatively instantaneous (when compared to analysis) and results in reports like “That is a Riesling wine.” In contrast, humans can also smell a wine and analyze it, i.e. attend to a particular sensation while ignoring others. For example, the same wine recognized as Riesling could after some time spent on analysis yield “This Riesling has a moderate petrol aroma and a faint lemon smell”(1-2). Recent studies of binary mixtures of similar smelling and different smelling odorants showed as would be expected that only similar odors cross-adapt while dissimilar odors suppress each other in mixtures (3-5) indicating a possible explanation for the suppression of fruity/floral by 2,2,1-trimethyldihydronaphthalene (TDN). Additional studies using gas chromatography-pedestal olfactometry (GCPO) has indicated the powerful suppression or even masking of odorants by dissimilar smelling odorants in a dynamic presentation (6). This presentation will present results of experiments using puff-bottle, GCPO, and simulation experiments to demonstrate the behavior of TDN in informing the varietal nature of Riesling wine and the unusual sub-threshold effects of TDN on the other wine odorants.

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PROFILING ANALYSIS OF VOLATILE AND NON-VOLATILE COMPOUNDS IN WINE FOR A BETTER UNDERSTANDING OF WINE QUALITY?

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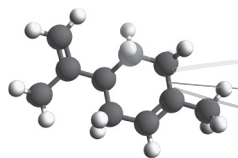
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Keywords: GCxGC-MS; Profiling analysis; NMR; Wine omics

Since wine aroma is an impressively complex matrix, comprising compounds from all chemical classes and concentration ranges from sub-ng/L to the g/L level, analytical chemists working in this field have always been challenged.

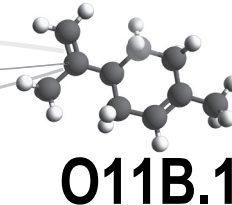
In the past, many questions in wine aroma research had been raised in the light of understanding off-flavors or the underlying differences between aroma of grape varieties, wine origin and the development of particular aroma compounds, respectively compound classes, during ripening. Today's interest moves more to global or comprehensive questions and particular, towards a better understanding on the viti- and vinicultural influences on wine composition. Considering such complex interactions, a classical single or even multi-component targeted analysis is no longer feasible. With the availability of the impressive separation power of comprehensive two-dimensional chromatography, such as GC × GC, profiling analysis of e.g. wine volatile compounds now allows tackling such challenges. Correlation of wine quality with chemical compositional data can now be investigated on a broader scope, applying ideas found in the field of “omics” research, such as metabolomics. In our approach, profiles of volatiles obtained by GC × GC analysis are treated as fingerprints and are studied by means of multivariate statistics in order to discriminate between enological variants or grape origin. Furthermore, incorporation of spectroscopic techniques, such as NMR, allows correlation of wine compositional data with its quality, based on data of non-volatiles. First results on vintages and origin will be presented.



Session:

Wine flavour

Lectures



**DETERMINATION OF ALTERNATIVE PRECURSORS TO
BRETTANOMYCES/DEKKERA SPP. DERIVED OFF-FLAVOUR**

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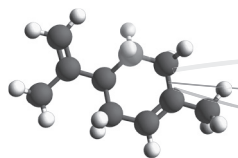
Keywords: *Brettanomyces*, *Dekkera*, Hydroxycinnamic acids, Brett taint, Precursors

‘Brett’ taint, or the accumulation of spoilage compounds due to the action of *Brettanomyces* and *Dekkera* yeasts is a serious issue that has been experienced in winemaking globally (1). This build-up of off-flavour is widely accepted to occur via the enzymatic breakdown of the hydroxycinnamic acids, *p*-coumaric and ferulic acids, to give 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol and 4-vinylguaiacol in varying quantities and ratios (2). However, in addition to the hydroxycinnamic acids, which are rarely found in their free form in the grape berry (3), esterified conjugates are also present in the form of both glucose and tartaric acid esters (4, 5). Not restricted to berry constituents, hydroxycinnamate conjugates can be formed during vinification, with the ethyl hydroxycinnamates being a product of the hydroxycinnamic acids and ethanol (6).

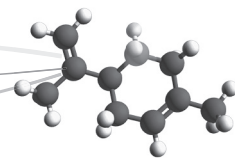
Furthermore, the nature of the grape berry allows for a UV catalysed photoisomerisation from the naturally occurring, and thermodynamically more stable, *trans*-acids to afford the *cis*-acids (7). These *cis*-isomers commonly contribute to around 20% of the non-anthocyanin bound hydroxycinnamic acid content of the grape berry (8, 9). With hydroxycinnamic acid conjugates present in grapes and wine, and possessing differing stereochemistry, it cannot be assumed that the free *trans*-acids are solely responsible for the evolution of Brett taint compounds. This study investigates the role of glucose, tartrate and ethyl esters of the hydroxycinnamic acids in the production of Brett taint, as well as determining the enzymatic activity of these yeasts towards hydroxycinnamic acids of opposing stereochemistry. *D. Bruxellensis* exhibited a substrate preference in metabolising the esters, which could help to explain the ‘Bordeaux ratio’ which is commonly seen in red wines affected by *Brettanomyces/Dekkera*. Fermentation experiments have shown that *D. bruxellensis* displays a strong stereospecificity in both decarboxylase and esterase activities. This means that the entire hydroxycinnamic acid content of the berry cannot be considered as the origin of Brett taint, but that only those possessing the right stereochemistry can contribute.

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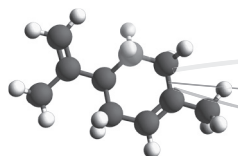
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Session:

Flavour systems

Lectures



012.1

**A METALLIC, SOLVENT-LIKE OFF-FLAVOR IN HAZELNUTS:
IDENTIFICATION OF PRENYL ETHYL ETHER AS A KEY FLAVOUR
COMPOUND AND FORMATION STUDIES**

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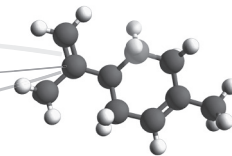
Keywords: hazel nut, off-flavour, prenyl ethyl ether

Hazelnut (*Corylus avellana* L.) is an important crop with a global production exceeding 800'000 metric tons accounting for an economical value of more than 800 million \$. The attractive and typical aroma of roasted hazelnuts has been intensively investigated and 5-methyl-(*E*)-2-hepten-4-one (filbertone) has been reported as a key compound for the hazelnut aroma (1-5). In an industrial production of ground hazelnuts a metallic, solvent-like off-note was detected, which affected the production of a major batch of ground hazelnuts. Rancidity of hazelnuts is commonly described as an off flavour in hazelnuts, which is caused by oxidation of lipids (6). Neither preliminary analytical investigations nor the sensory description of the observed metallic solvent like off flavour did match the known rancidity off-notes. The volatiles of a batch with off notes and a batch without off-notes were extracted using SPME and SDE techniques. Comparison of chromatograms did not reveal obvious differences. Based on GC-sniffing and instrumental analysis an odor active region was detected by several panelists, which showed a metallic solvent like aroma impression. GC/MS spectrum interpretation and comparison with reference data allowed identifying the compound as prenyl ethyl ether (7). The compound was quantified in several batches of ground hazelnut and market products. Furthermore its contribution to the metallic, solvent-like off-flavor was evaluated by spiking experiments and sensory evaluations.

The experiments showed that addition of prenyl ethyl ether to hazelnuts without off notes is evoking the typical off note. Additional investigations and model experiments were conducted targeting the possible source of this off note. The study included microbiological analysis of the sample. Systems were found, which generate prenyl ethyl ether. It is suggest that microbiological growth cause the formation of prenyl ethyl ether.

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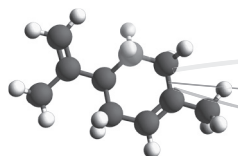


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012.2

MOLECULAR INSIGHTS INTO THE OFF-FLAVOUR FORMATION DURING PINEAPPLE JUICE PROCESSING

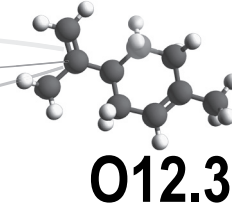
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Keywords: Pineapple juice, aroma extract dilution analysis, stable isotope dilution analysis, methional

Although the major part of the world's pineapple harvest is consumed as fresh fruit, a considerable share is processed to canned fruits and fruit juice. Fresh pineapple juice exhibits a pleasant fresh fruity-sweet aroma, but industrially manufactured pineapple juices generally show a characteristic undesirable "cooked" aroma. In order to gain insight into the molecular basis responsible for this aroma difference, a pineapple juice freshly prepared from fruits as well as a typical commercial pineapple juice purchased at the supermarket was screened for aroma-active compounds by application of a comparative aroma extract dilution analysis.

Results indicated a variety of different esters, undecapolyenes, lactones, and furanones as major aroma compounds in the fresh juice. In the supermarket sample methional was additionally detected among the most odour-active substances, suggesting a crucial role of this cooked potato-like smelling compound for the characteristic off-flavour of industrial pineapple juices. To corroborate this assumption, methional concentrations in different brands of commercial pineapple juice as well as in fresh juices obtained from fruits of different origin and harvest time were determined by stable isotope dilution analyses using [$^2\text{H}_3$]-methional as internal standard. Methional levels in the supermarket juices ranged from 20 to 70 $\mu\text{g}/\text{kg}$, which was well above the threshold value calculated as 0.4 $\mu\text{g}/\text{kg}$, while they were below 0.1 $\mu\text{g}/\text{kg}$ in the freshly prepared juices. The analysis of samples taken from one batch in a commercial pineapple juice plant confirmed the formation of methional during NFC processing and indicated heating as crucial parameter. This was further corroborated by lab-scale pasteurization experiments. Lab-scale pasteurization experiments also allowed identifying L-methionine as the precursor of methional. Based on these results, reducing the L-methionine content of the fruits, optimization of the heating parameters during processing and the application of non-thermal preservation methods are suggested as potential measures in order to reduce the off-flavour associated with high methional concentrations in processed pineapple juice.



STUDIES ON STABILITY OF CITRUS FLAVORS AND INSIGHTS INTO DEGRADATION PATHWAYS OF KEY AROMA COMPOUNDS

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Keywords: Citrus, aroma, stability, sensory

Freshness is a key attribute of modern citrus flavorings. Nowadays consumers expect that the freshness profile of beverage products is maintained for the full shelf life period. This requires a full understanding of the stability profile of aroma-intensive key molecules as well as potential degradation pathways.

Model studies have been performed with individual citrus related aroma compounds such as limonene, citral, myrcene and linalool, in order to de-couple the complexity of citrus flavors. Degradation pathways and transformation rates have been determined for the most relevant compounds. In the case of citral, the degradation proceeds via α - and γ -terpinene, pcymol, p-8-cymenol leading to 4-methyl acetophenone and para- α -dimethyl styrene (1,2).

Similar compounds can be detected in the model degradation reaction of limonene, however with lower conversion rates. The analytical results have been linked with odor activity values and correlated with sensory results. It has been confirmed that pH levels are an essential factor which influence the stability of the compounds.

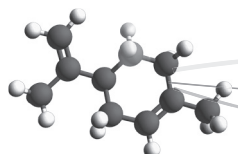
While compounds such as citral and limonene form degradation products with significant negative sensorial impact, the conversion of compounds such as α -terpineol enhance the earthy and lime-like notes which add a – sensory-wise acceptable - lime character to the citrus flavor.

Catechins (Flavan-3-ols) are often used as antioxidants for stabilization of flavor mixtures. The reactivity of catechins towards aldehydes is underestimated. In our study we demonstrate the formation of covalent catechin-citral adducts, a reaction which influences aroma stability of citrus flavors in the presence of catechins.

As a result, the freshness of citrus flavors and enhanced stability of key aroma-active substances can be based on the result of this study. Important products like for example clear lemon beverages directly benefit from new solutions for stability enhanced citrus flavors.

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012.4

IDENTIFICATION, CHARACTERIZATION AND RELATIVE AROMA IMPACT OF 2,3-DEHYDRO-1,8-CINEOL IN LEMON-LIME FLAVORED CARBONATED BEVERAGES

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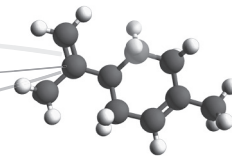
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Keywords: lemon-lime, carbonated beverage, 2,3-dehydro-1,8-cineol, gas chromatography-olfactometry, acid-catalyzed reaction

“Lemon-lime” is a universally recognizable and highly popular lemon oil-derived flavoring used in carbonated beverages. This paper characterizes the compound 2,3-dehydro-1,8-cineol (dehydrocineol) in terms of its relative importance in lemon-lime carbonated beverages. Also described are the convenient chemical synthesis of both the unlabeled and deuterium labeled standard compounds and the determination of the orthonasal detection limit. Finally, the possible pathway of the formation/degradation of dehydrocineol in a carbonated beverage matrix is examined using a model system.

Aroma extracts from three commercial brands of lemon-lime flavored carbonated beverages were prepared by continuous liquid-liquid solvent extraction/solvent-assisted flavor evaporation (SAFE), with special precautions taken to avoid volatile compound losses due to the decarbonation of the beverage. Potent odorants were determined using gas chromatography-olfactometry (GCO) and aroma extract dilution analysis (AEDA). Twenty eight compounds were detected by GCO with linalool (*floral, lavender*), octanal (*pungent orange*) and dehydrocineol (*minty*) determined to be predominant aroma compounds based on their high flavor dilution (FD) factors by aroma extract dilution analysis (AEDA). Other odorants detected in at least one brand included 1,8-cineol (*eucalyptus, minty*), nonanal (*orange*), decanal (*pungent, green, cilantro*), borneol (*camphorous*), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (*burnt sugar*), *p*-cresol (*stable, dung*), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (*curry*), benzoic acid (*sweet, candy*), and an unknown (*fresh, melony*).

Dehydrocineol was chosen for further study. This *minty, eucalyptus* smelling compound has been previously reported as a constituent of various essential oils and as a minor product of the acid-catalyzed degradation of neral/geranial (cital)(1,2); however, to our knowledge, dehydrocineol has not been regarded as a potent odorant in any particular product. The accurate quantification of dehydrocineol and other selected odorants was done by application of stable isotope dilution assay (SIDA)-GC-MS. The method of Bugarčić et al. (3), followed by the method of Nicolaou et al. (4), was adapted for the synthesis of dehydrocineol from α -terpineol or for the synthesis of $[10,10,10\text{-}^2\text{H}_3]$ -2,3-dehydro-1,8-cineol from $[10,10,10\text{-}^2\text{H}_3]$ - α -terpineol.

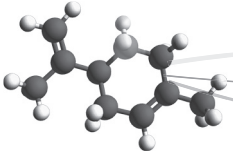


Based on calculated odor-activity values, the key odorants were found to be similar across brands and included (in decreasing order of OAV) decanal, octanal, 2,3-dehydro-1,8-cineol (*threshold* = 17 $\mu\text{g/L}$), linalool and 1,8-cineol.

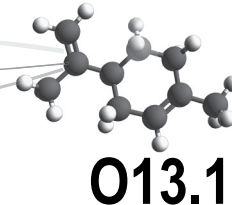
The stability and possible formation pathway of 2,3-dehydro-1,8-cineol via acid-catalyzed rearrangement (in particular, formation from neral/geranial) were examined using a model carbonated beverage system (0.12% w/v citric acid solution; 25 psi CO_2). High purity standards, including 2,3-dehydro-1,8-cineol, were individually added (100 $\mu\text{g/mL}$) to the matrix solution through a septum. Tridecane was used as internal standard. The solution was stirred continuously for 3 days in the dark, after which it was neutralized/de-carbonated with 2 M NaOH, and extracted with diethyl ether for subsequent analysis by GC-MS. Results confirmed previous studies that dehydrocineol can be formed from neral/geranial. Unexpectedly, it was determined that dehydrocineol was highly unstable under the acidic conditions of the model. Despite its apparent instability, the existence of dehydrocineol in lemon-lime beverages may be the result of a steady-state reached among reversible acid-catalyzed reactions.

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Session: Temporal aspects of flavour perception Lectures



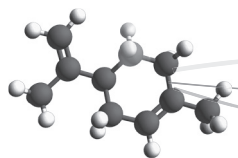
DYNAMICS OF PERCEPTION AND KEY MOMENTS IN CONSUMER EXPERIENCE

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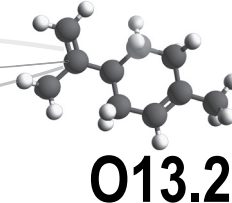
Sensory perceptions experienced when eating a food largely depend on the in-mouth food manipulation and transformation. This is a dynamic process in which the sensory attributes are continuously analysed by the oral and nasal sensory systems. This information allows a permanent readjustment of the eating behaviour not only to bring the food to a bolus suitable for swallowing but also to optimise pleasure. Both the food transformation and the perceptual response are time-dependent. The sensory changes are different according to the food structure, i.e. the food initial physical state and to the eating behaviour. They are probably key drivers of preference. However, the particular sequence of perceptual events that occur during food consumption has been scarcely studied. This is likely because classical sensory methods record the intensity of attributes in a single, time-averaged response integrating all the possible sensory changes that occur over the food oral breakdown process. Dynamic sensory methodologies are available to describe the changes of sensory perceptions over the eating process. The Time Intensity method monitors the intensity of a single attribute (1). Although mainly applied to describe flavour attributes (2, 3), time intensity has also been used to describe various texture perceptions (4). The main limitation of T-I is that the evaluation is limited to one or two sensory attributes at a time. The multidimensionality of the perceptual space over time is therefore not considered. For this reason, Temporal Dominance of Sensation (TDS) was developed (5) and started to be used (6, 7). TDS consists in assessing iteratively at each specific time until the sensations ends, which sensation is dominant. We used this method to better understand the sequence of perceptions associated to breakfast cereals' consumption and proposed the concept of sensory trajectory (8) as an extension to the model of Hutchings & Lillford (9) illustrating the physico-chemical breakdown pathway of the food during consumption. Then, we moved a step further and looked at the impact of the sensory changes on the level of liking throughout the consumption of a food. Even though Lee and Pangborn (10) reported that pleasantness was also a temporal phenomena that could be measured through a temporal procedure, liking for a food is usually measured through a single response. Very few attempts can be found in the literature for measuring the temporal evolution of liking during a consumption event (11,12,13).

The talk will first aim at showing how we monitored sensory perception during a short consumption event (a mouthful of breakfast cereals). Then we will present an exploratory study where we have tested and compared two methods to measure the dynamics of liking associated to the same event. Finally, we will open the discussion on how to extend the approach to provide a deeper understanding of preference formation and hopefully help us to better identify the key moments of the consumption experience. This should open new opportunities in the design of products that trigger specific sensory contrasts and consequently induce a temporal pattern of liking for optimised consumers' satisfaction.



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RELEVANCE AND LIMITATIONS OF USING IN-NOSE AROMA CONCENTRATION TO PREDICT SENSORY DYNAMIC PERCEPTION DURING SEQUENTIAL EATING OF FLAVORED CANDIES

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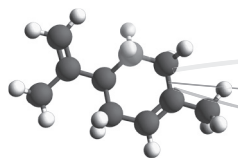
Keywords: In-nose concentration, carryover effect, time-intensity, perception, PTR-MS

Sensory perception of flavor intensity during food consumption admittedly results from a dynamic integration of various sensory stimuli generated in the orosensory system, namely taste, texture and aroma. The in-nose aroma concentration as released from the food matrix during consumption has been used to understand and predict the sensory time-intensity profile of simple model foods, in which aroma concentration is the main source of variation between samples (1, 2, 3). However, in reality food is usually eaten as a succession of bites during a meal lasting a few minutes, most often without rinsing in-between pieces. Therefore, it is very likely that carryover effects are substantial during a meal. In this context, one could question the relevance of monadic, sequential protocols with rinsing of the mouth often used in studies aiming at inferring sensory time-intensity profile based on the concentration of aroma compounds.

The objectives of this study were, first, to assess the magnitude of carryover effects on the sensory perception of aroma intensity over time in the case of sequential eating of given samples, and second, to evaluate to what extent the in-nose aroma concentration could predict sensory perception of aroma in such eating situations.

A group of 29 trained panelists recorded their aroma perception of iso-amyl acetate – flavored gummies (2 concentration levels, High (H) and Low (L) and 3 sequences of 2 samples, i.e. HH, HL and LH) with a Time-Intensity (TI) sensory data acquisition software, while breathing into the inlet of a Proton-Transfer Reaction Mass Spectrometer (PTR-MS). Sensory and PTR-MS TI data were smoothed and parameters were extracted from the individual curves: I_{max} , T_{max} , area under the curve (AUC), ascending (slope1) and descending (slope2) slopes, time at half cumulative AUC (t_{50}). These curve parameters were submitted to analysis of variance and to principal component analysis. As expected, both sensory and PTRMS measurements showed that L was significantly more intense than H. In addition, the sensory measurements were significantly affected by sequence effects ($p < 5\%$) for AUC, I_{max} , slope1 and slope2 and reveal adaptation and contrast effects, in accordance with the theory of Sequential Sensitivity Analysis (4,5). However, the sequence effects were either not observed for PTRMS or, in some cases, inverted compared to sensory measurements.

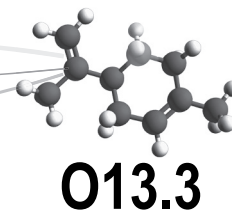
Overall, this study suggests that sensory specific phenomena, such as adaptation and contrast effects, cannot be accounted for by real time in-nose concentration, which is only *one* of the parameters influencing sensory perception over time. In addition, the data reinforced the important effect of recent past events (sequence of previous samples) on aroma perception via peripheral (olfactory receptors) and/or central (brain) phenomena during food consumption. In



conclusion, the presence of carryover effects stresses the need for development of new comprehensive predictive models of sensory dynamic perception.

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MEASURING VARIATION IN ODOUR DELIVERY FOR SENSORY TESTING

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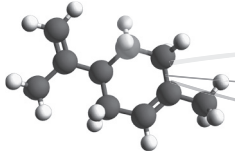
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Keywords: odour stimulus, threshold value, methodology, APCI

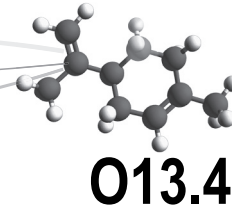
Sensory testing of odours has been carried out for many years to measure properties like odour threshold values (1), respiratory irritation (2) and the sensory quality of odour mixtures (3). In the former case, the odour threshold values obtained by different groups vary by many orders of magnitude (4) and it has been proposed (5) that some of the sensory variation is due to variation in the delivery of the odour stimulus. Schmidt & Cain (6) demonstrated that, by controlling odour stimulus delivery, the variation in odour thresholds could be reduced to lower levels. However, using large volumes of air to “flood” the face of panelists assessing odours to ensure consistent odour stimuli requires a considerable investment in time and cost to set up a suitable olfactometer device. For this reason, simpler alternatives like sniffing sticks (7, 8), squeezable sniff bottles or sniff pots have been used but no information seems to be available on the consistency of odour delivery from these devices nor whether other issues, like adsorption of odours to the plastic bottles used can cause significant changes in odour delivery and over what time scales. A systematic study was undertaken to measure odour delivery from the simpler systems and determine variability under typical usage conditions and during repeated usage. A simple odour mixture comprising a range of hydrophobicity values was used to assess consistency of odour delivery in the gas phase and potential binding to the materials used in the delivery apparatus. Odour concentration was measured using on-line APCI-MS which sampled the air entering the nose of subjects as they used the various delivery devices and measured potential absorption of odours to the apparatus via headspace measurements. Data on the variation and repeatability of the different devices will be presented.

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TASTE ENHANCEMENT BY PULSATILE STIMULATION MEETS CROSS-MODAL INTERACTION: NEW INSIGHTS INTO AROMA-TASTE AND TEXTURE-TASTE INTERACTION

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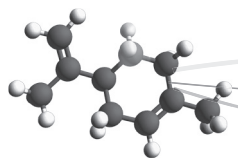
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Keywords: pulsatile stimulation, taste enhancement, aroma-taste interaction, texture-taste interaction

The intensity of a sensation is not only controlled by the stimulus concentration. The dynamics of stimulus intensity over time also play an important role. This was demonstrated for vision (1), olfaction (2) as well as gustation (3-4). On stimulation with discontinuously presented tastant concentrations (pulsatile stimulation) humans report higher average taste intensities than on continuous stimulation with the same average tastant concentration. As taste is known to be affected by stimuli within other sensory modalities, we hypothesized that the effect size of pulsation induced taste enhancement will change by manipulating other modalities and that the nature of this interaction can reveal the mechanism involved. This was tested for aroma-taste and texture-taste interactions. In Study 1 the effects of pulsatile delivery of taste and aroma on taste intensity was studied (5). Effects on taste perception were evaluated for aroma and taste pulsation and for the aroma pulse-taste pulse phase shift. High-concentration sucrose pulses were alternated with water rinses every 2.5s. Four different aroma (isoamyl acetate) versions were presented: a) no aroma, b) continuous aroma c) aroma pulses in-phase and d) aroma-pulses out-of-phase with taste pulses. Sweetness intensities of aroma-taste combinations were rated by a 15-member trained panel using time-intensity analysis. Results showed that sweetness intensity was enhanced by pulsatile stimulation of sucrose or isoamyl acetate. In addition, taste enhancement by aroma and tastant pulses was additive if both were presented out-of-phase which resulted a sweetness intensity enhancement by more than 35% compared to a continuous sucrose reference of the same net sucrose concentration. Aroma induced sweetness enhancement can be explained by cross-modal aroma-taste integration. Amplification of aroma-taste integration by pulsatile stimulation may be attributed to a potentiated afferent input of aroma and taste information prior to aroma-taste integration. Alternative mechanisms include the importance of swallowing on aroma-taste integration.

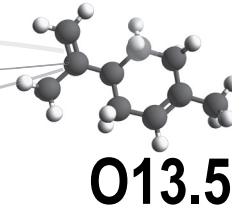
In Study 2 the combined effects of taste pulsation rate and viscosity on pulsation-induced taste enhancement were tested in apple juice (6). According to a tastant-kinetics hypothesis, less pulsation induced taste enhancement is expected at enhanced pulsation rates in the high viscous proximal stimulus compared to lower viscous stimuli. High concentration sucrose apple juice pulses and low concentration sucrose apple juice intervals were alternated at different pulsation periods every 2.5s (period length= 5s) or every 1.25s (period length= 2.5s).



Pulsed stimuli were presented at two viscosity levels by addition of pectin (0 and 10g/L). Sweetness intensities of pulsed stimuli were compared to a continuous reference of the same net but non-alternating sucrose concentration. Sweetness ratings were higher for pulsatile stimuli than for continuous stimuli. In low viscous stimuli, enhancement depended on the pulsation period and peaked at low pulsation rates (i.e. at 5s-periods). In high viscous stimuli, the same enhancement was observed for both pulsation periods. These results contradict a tastant kinetics hypothesis of viscosity induced taste suppression since impaired tastant kinetics by viscosity would predict the opposite: lower pulsation-induced taste enhancement for viscous stimuli, especially at higher pulsation rates. Instead, these observations favour an explanation based on perceptual texture-taste interactions which predicts the observed independence between viscosity and pulsation rate.

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FLAVOUR COMPOSITION, TEMPORAL RELEASE AND COMPLEXITY DETERMINING FOOD QUALITY PERCEPTION.

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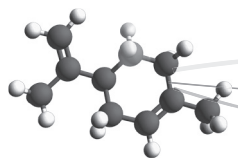
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Keywords: Cross-modal interaction, flavour complexity, ingredient-related aroma cues, satiation, indulgence, food quality.

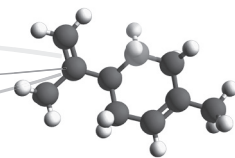
The phenomenon of cross modal interactions between sensory stimuli is already widely acknowledged to be a factor of importance when describing the overall flavour perception and quality of a food. E.g. aroma can affect the salty taste intensity perception of a food (1). While in most cases salt enhancement is found by testing aroma compounds with congruent modalities, olfactoscan (2) offers a novel technology to link this phenomenon to the compounds that cause the effect. An example will be given in which fractions of a food extract increased the perceived saltiness of a product. This experiment shows that salty perception is a hedonic interpretation of an amalgamate of sensory inputs affecting the state-of-mind of a consumer. Similarly it will be shown that well-chosen aromas can induce ingredient-related aroma cues, subconsciously translated into the expected energy content of a food delivering a modulated level of satiation. This study is in agreement with results reported earlier (3), which showed that prolonged aroma release profiles superimposed on a dairy drink also provoked enhanced levels of satiation compared to the native aroma release profiles of the same dairy drink. It was hypothesised that this form of flavour-induced sensory satiation was the result of a prolonged aroma release profile cueing for high fat product formulations or high viscosity matrices, both richer in their calorie contents compared to low fat or less viscous foods. Also comparably, the richness of a flavour was investigated. This presentation will show that, in an olfactometer-aided or *ad libitum* eating experimental design, comparing sweetened yogurt drinks with a single-component strawberry flavour with a multi-compound version, the complex aroma system generated enhanced perceived satiation. Whereas complexity has thought to cue for variation, these results indicate that within a food system, complexity cues for “richness”, which is linked to perceived calorie intake.

These observations indicate that the performance of a flavour system of a food, in terms of its release, complexity and composition, are being subconsciously translated by consumers to cue for food ingredient compositions. As these observations all relate to sensory satiation affecting the state-of-mind of consumers (and not to satiety which is largely based on physiological reactions) one can start to understand how consumers judge the richness and quality of a food and experience the pleasure from the eating expressing these using terms as creaminess, indulgence and ultimately the joy of eating. Implementing these principles will strongly affect the consumer acceptance of healthy foods. This holds for (novel) low-calorie and low-salt foods as well as for developing foods targeted at increasing food intake for elderly people and clinical applications.

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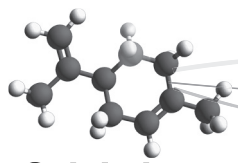
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Session:

**Dynamic aspects
of flavour
perception**

Lectures



014.1

**MOLECULAR FEATURES UNDERLYING THE CHEMORECEPTION OF
ODORANT BINDING PROTEINS AND OLFACTORY RECEPTORS. INSIGHTS
FROM MOLECULAR MODELING AND BIOPHYSICAL DATA**

JÉRÔME GOLEBIEWSKI, Landry Charlier

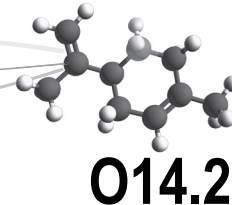
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Keywords: OBP, OR, structure, odorant, binding, molecular modeling

Odorant Binding Proteins (OBPs) and Olfactory Receptors (ORs) are the main molecular protagonists of the perception of smell. They are extensively studied but information on their atomic-level function remains scarce. With the use of state-of-the-art molecular modeling tools, we are now in good position to decipher the nature of both OBP / odorants and OR / odorants interactions. Here I present the use of molecular dynamics simulations to identifying the residues responsible for the affinity of both several OBPs and human OR1G1 for various odorants. The affinity predictions compare well with biophysical experiments (ITC for OBPs and calcium imaging for OR1G1).(1-3) In addition, for both proteins, the entry door for odorants together with the residues lining the way to the binding cavity are identified.(4)

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UNDERSTANDING THE DYNAMICS OF FLAVOUR COMPOUNDS RELEASE DURING FOOD MASTICATION OF CHEESE PRODUCTS IN RELATION WITH PERCEPTION

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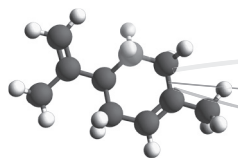
Keywords: aroma release and perception, food mastication, oral physiology, bolus rheology.

During food consumption, aroma compounds are released and then transferred to olfactory receptors in order to be perceived. This release highly depends on food composition and structure, but also on physiological characteristics of individuals (1, 2). The mastication process is known to adjust to different textural properties of foods, and high intra-individual variability occurs either in chewing behaviour (3) or for physiological parameters (4). However, the consequences of individual chewing strategies on bolus properties, aroma release and perception have not been explored yet.

This present paper proposed an integrated approach to better understand the role i) of the products' composition, ii) of different physiological parameters and iii) of the individual chewing behaviour, on bolus rheological properties and aroma release.

For that purpose 50 healthy consumers were recruited for their normality and repeatability regarding their dental status and saliva flows. Oral physiological parameters (salivary flows, respiratory flow, saliva composition, oral volume) were measured on these subjects. Six based cheese products varying in firmness (from spreadable to sliceable), fat content and texture (from pasty / high fat to gel/low fat,) were characterised by their rheological properties and flavoured with nonan-2-one (blue cheese) and ethyl propanoate (fruity). Masticatory behaviour was recorded by electromyography during cheese consumption. Subjects' boluses were collected at the swallowing time. Bolus rheological properties were measured by compression. Mouth coating was evaluated by fluorimetry. *In vivo* aroma release was followed by nose-space Atmospheric Pressure ionisation- Mass Spectrometry (API-MS) measurements (5). A group of 16 subjects representative of the 50 was asked to measure the intensity of 2 odour descriptors (blue cheese, fruity) after each swallowing event.

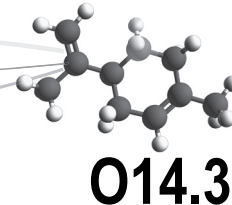
The main electromyographic parameter traducing a chewing behaviour adaptation to cheese characteristics is the total muscle work produced for food breakdown (sum of forces applied during mastication). Some subjects did not modify their total muscle work whatever the cheese eaten whereas others adapted their total muscle work to product characteristics. No direct relationship was found between masticatory behaviour and bolus rheological properties. *In vivo* aroma release was found to depend both on the hydrophobicity of aroma compound and on the texture/composition of the cheese. More ethyl propanoate and less nonan-2-one (more hydrophobic compound) were released at the higher fat level, and more of the two



aroma compounds were released from the firmest cheeses. Three groups of subjects differing by their aroma release profiles have been identified. The group with the highest release before swallowing presented a high masticatory activity whereas the group with the highest release after swallowing presented bolus with a high amount of saliva incorporated and large particles. Neither respiratory nor salivary flow rates explained the differences in aroma release profiles. A higher amount of cheese remained in the mouth after swallowing for high fat products. For the 16 subjects it was thus possible to better understand the perception of the two odorants during cheese consumption, in function of their *in-vivo* aroma release profiles and human physiology.

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MODELLING THE EFFECT OF LIPID ON IN-VIVO FLAVOUR DELIVERY

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Keywords: APCI, MS

Lipid is a major component of foods affecting flavor delivery, both in-vitro and in-vivo. The impact of lipid on the partitioning of aromas between the sample and headspace under static conditions is a function of the hydrophobicity of the aroma compounds and the oil fraction. Models describing volatile behavior under these conditions are well known (1).

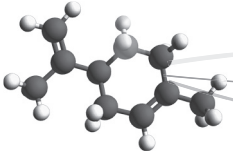
In-vivo, the dynamics of volatile partitioning during consumption limits the delivery of compounds with high air/product partition coefficients (2). Volatile delivery under these dynamic conditions is not explained by the static equilibrium model. It is however, still going to be affected by the lipid content of the samples and the hydrophobicity of the compounds. Since both of these factors affect the partition coefficient.

In the literature, there are several papers describing the in-vivo release of flavor from emulsions with different fat contents. These were used as an initial database for flavor release modeling (3). The objective was to model the effect on flavor release of changing from one lipid content to another. The factors used for the model were the hydrophobicity of the compound (Log P), and the fat content of the original and new samples. The initial model (based on the literature) showed that the data from different groups could be brought into one overall model.

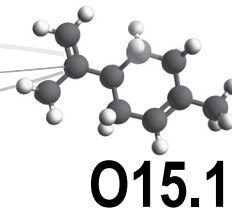
The model had limited data on the effect of higher fat content samples (up to 30%), with most data clustered around low fat content samples (0-5%). Additional data were generated to strengthen the model in both the fat content and Log P dimensions. This data was modelled along with the existing data from the literature (3). The final model can be used to predict the differences in flavor release between two emulsion systems, acting as a guide for flavor reformulation. In addition, the model also appeared to predict differences observed between high and low fat solid foods with reasonable accuracy. However, there was a limited amount of data available to validate this aspect of the model.

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Session: Instrumental aspects and tools of the trade Lectures



FOODOMICS: A NEW OMICS FOR A NEW FOOD ERA

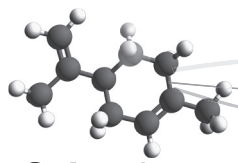
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One of the main goals in modern Food Science and Nutrition is to improve our limited understanding of the roles of nutritional compounds at molecular level (i.e., their interaction with genes and their subsequent effect on proteins and metabolites). This knowledge should allow the rational design of strategies to manipulate cell functions through diet, which is expected to have an extraordinary impact on our health. In order to achieve this goal, food science researchers are moving from classical methodologies to more advanced strategies, usually borrowing methods well established in medical, pharmacological and/or biotechnology research. Following this global trend, our group has recently coined the term Foodomics (1,2) to define a discipline that studies the food and nutrition domains through the application of advanced omics technologies in order to improve consumers well-being, health and confidence. In this context, the global objective of the current work will be to show some of the latest results from our group on Foodomics with special emphasis on the difficulty to achieve a global Metabolomic analysis of biological systems.

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O15.2

INSTRUMENTAL SENSOMETRY. A TOOL FOR FOOD QUALITY CONTROL

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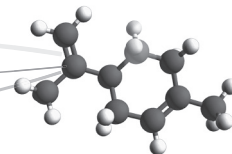
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Keywords: MS e-nose, mid-IR e-tongue, classification, multivariate calibration, LDA, PLS, data fusion, beer and wine characterization.

The organoleptic characteristics of a food commodity depend on the composition of the raw materials, and on compounds that are generated during processing and storage of the finished product. These compounds leave their chemical fingerprint in non-selective measures such as NIR, mid-IR, NMR or mass spectra. Correlating these information-rich measurements with the sensory properties identified by a panel of assessors is important in order to (a) perform sample screening before samples are submitted to the tasting panel and ultimately confirm a negative result of the panel, (b) replace the panel in the continuous monitoring of the sensory properties (e.g., ageing studies) and (c) understand better the characteristics of the marketed product, supplementing the sensory evaluation of the panels. The objective of instrumental sensometry is to define instrumental sensory specifications (of quality and/or origin) of food products by correlating the data from instrumental analytical techniques to the sensory evaluations of taste panels using multivariate chemometric techniques.

In this presentation, several applications of instrumental sensometry are shown. First, an electronic nose (a static headspace (HS) coupled with a mass spectrometer (MS)) and Linear Discriminant Analysis (LDA) were successfully applied to classify and characterize beers of the same brand according to their production site and their chemical composition (1). The samples were also subjected to sensory evaluation by a panel of experts. Both sensory evaluation and e-nose data revealed differences between factories. LDA showed that these sensory differences were related to the presence (and abundance) of certain ions of different compounds typically found in beer, previously detected by the HS-MS analysis and corroborated by GC-MS analysis.

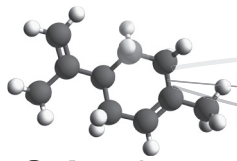
In a second example, an electronic tongue based on mid infrared (mid-IR) spectroscopy was applied to emulate the responses of a tasting panel for the gustative mouthfeel “tannin amount” in wines (2). The mid-IR spectra were modeled against the sensory responses of an standardized panel by means of partial least squares (PLS) regression models coupled with variable selection techniques, showing good prediction results.



Nowadays spectroscopic techniques such as mid-IR and NIR, together with multivariate analysis, are routinely used in the food industry. In this progression to achieve more and better information, the next step is to combine the results of several multivariate instrumental techniques, to increase the reliability of a classification or prediction regarding a single analytical technique. This is called *data fusion*. In the final example, different data fusion strategies are applied to the discrimination of beer samples of the same brand and commercialized as a same product, but brewed in four different factories (3). The data collected from three instrumental techniques (HS-MS, mid-IR and UV-visible spectroscopy) were fused and analyzed by LDA. The results showed an improvement in the ability of classification with respect to the individual use of the techniques.

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O15.3

**RAPID QUANTIFICATION TECHNIQUE WITHOUT AUTHENTIC SAMPLES AND
APPLICATION TO COMPLEX, UNSTABLE OR UNAVAILABLE COMPOUNDS**

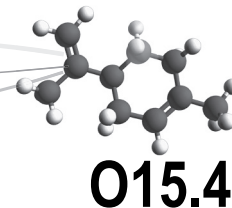
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In the scientific literature, the composition of complex mixtures such as the essential oils is determined by GC/FID and the so-called “semi-quantification” technique. It consists in the addition of an internal standard in the mixture and assumes that all response coefficients referring to this internal standard are equal to unity. We have shown that it is very inaccurate and can give rise to biases up to 40%.

Because a FID is based on the combustion of analytes, we have shown that a good correlation exists between the relative response factors (RRF) and the combustion enthalpies. The latter can themselves be predicted by ab-initio calculation, or only from the molecular formula. As a consequence, the RRFs can be simulated with a good accuracy for all volatile constituents of flavours and fragrances.

Such a prediction is particularly useful when the classical internal standardization technique is not applicable because of the lack of authentic standard compound. The technique is exemplified with the following cases. Few sesquiterpenic alcohols are commercially available and not easily synthesized, but they can be well quantified in essential oils using predicted RRFs. The availability of pure unstable standards is an issue, and measuring their purity is a challenge. This can also be overcome with the present technique. Last but not least, the full quantification of a multi-constituent mixture by internal standardization is very time consuming. The use of predicted RRFs allows to easily and quickly achieve this task



SELECTED ION FLOW TUBE MASS SPECTROMETRY (SIFT-MS) FOR FLAVOUR EVALUATION OF DRY FERMENTED SAUSAGES.

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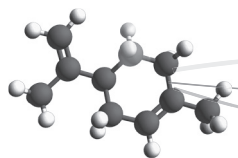
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Keywords: SIFT-MS, aroma, sausage, SPME-GC-MS.

Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) is a direct mass spectrometric technique based on the chemical ionization of a gas sample using selected precursor ions (1). This technique does not require sample preparation and quantification is achieved on the basis of chemical ionization reaction kinetics. This technique has been widely applied for online trace gas analysis in biology and medicine (2). However, its use for food flavour analyses is a very recent development (3, 4).

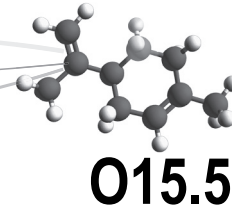
In the present work, SIFT-MS was applied to real time analysis of aroma compounds in the headspace of dry fermented sausages during processing. Several aroma-active volatile compounds were selected as previously detected by GC-O in dry fermented sausages (5). Then, in order to confirm the ability of SIFT-MS to detect the volatile compounds of interest, full scan SIFT-MS spectra were obtained using H_3O^+ , NO^+ and O_2^+ precursor ions. Three different mass spectra were obtained. The characteristic product ions were assigned on the basis of the kinetics library and a total of 31 aroma compounds were identified in the full scan spectra (6). For quantification, the multiple ion monitoring mode (MIM) was used to target specific aroma compounds: in this mode the mass spectrometer is switched between selected m/z values of both precursor and product ions. Data for each precursor ion were collected for a period of 200 seconds.

In order to compare the effectiveness of this technique, the same sausage samples were evaluated by SPME-GC-MS. Generally, both the GC-MS and SIFT-MS techniques detected differences in volatile compound concentrations and increases with ripening time. Significant and positive correlations were found between SIFT-MS and SPME-GC-MS measurements for the compounds pentanal, hexanal, 2-heptenal, octanal, 2-nonenal, 2-butanone, 2-pentanone, ethanol, acetic acid, and hexanoic acid. In the case of nonanal, 2,3-butanedione, 2-octanone, and 2-nonanone there were not observable differences during processing. This may be due to their HS concentration being close or below the quantification limit of the SIFT-MS (<10 ppbv). This study demonstrated the value of SIFT-MS for the real time monitoring of headspace volatile compounds. Thus, this technique could be used for a fast sensory quality control of dry fermented sausages.



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**EVALUATION OF BEER DETERIORATION BY GAS CHROMATOGRAPHY–
MASS SPECTROMETRY/ MULTIVARIATE ANALYSIS: A RAPID TOOL FOR
ASSESSING BEER COMPOSITION**

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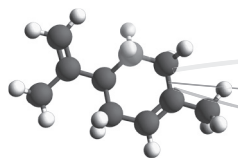
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Keywords: beer, forced aging, volatile signature, gas chromatography – mass spectrometry/ multivariate analysis (GC-MS/MVA), data fusion.

The ability of maintaining beer chemical and organoleptic properties, while achieving better understanding of beer chemistry, has been a major concern of the brewing industry (1). Typically, sensory data obtained using trained panels constitute the backbone of quality control of flavour stability at the industrial level. However, the quest for molecular markers extracted with analytical chemistry methods suited for online assessment of beer stability justifies the need for a high-throughput methodology. Gas chromatography coupled with mass spectrometry (GC-MS) has been extensively employed to identify and quantify relevant aroma/flavour components in several foodstuffs, including beer (2, 3). The application of multivariate analysis (MVA) to complex analytical datasets (e.g. GC-MS datasets) allows the extraction of information in an untargeted manner, considering the system as multi-dimensional and taking into account potential chemical and physical interactions between the different constituents present in the sample. In fact, GC-MS/MVA has been used with great success in several areas, such as food and nutrition research (4, 5).

This work describes a novel non-targeted methodology for monitoring the compositional changes occurring in beer exposed to deterioration (thermal treatment at 45°C during 18 days) using GC-MS in tandem with multivariate analysis (MVA). Faster chromatographic runs were developed, allowing a threefold reduction of the chromatographic time. In spite of compromising optimum chromatographic resolution, rapid GC-MS runs showed similar chromatographic profiles and semi-quantitative ability to characterize volatile compounds, when compared with conventional/longer GC runs. Principal Component Analysis of GC-MS data (GC-MS/PCA), a nonsupervised MVA method, enabled the assessment of the main profile changes as a function of beer deterioration, allowing for the identification of specific compounds of relevance. These included well established markers such as 5-hydroxymethylfurfural (5-HMF), furfural and diethyl succinate, as well as other newly detected compounds related to beer aging.

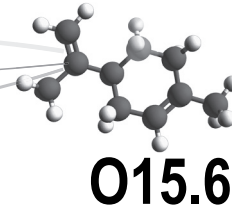
Furthermore, the use of this methodology, which reduces the GC-MS data matrix significantly, requires less computational power compared with conventional GC-MS data matrices. This enables inter-correlation assessment to be achieved between GC-MS and other



complementary analytical techniques (such as nuclear magnetic resonance (NMR) spectroscopy) thus contributing to a deeper chemical understanding of the beer aging process.

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ON-LINE FLAVOUR ANALYSIS - NOVEL TECHNIQUES AND APPLICATIONS

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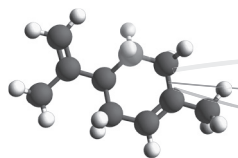
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Keywords: Real-time analysis, flavour compounds, PTR-MS, PTR-TOF, industrial applications, odour perception

Proton-transfer-reaction mass spectrometry (PTR-MS) is a soft chemical ionization technique in which volatile (flavour) compounds can be analysed directly in real-time, without the need for sample workup prior to analysis (1). This analytical tool is already well-established in food and flavour research and has been employed in diverse applications within this field for more than a decade (e.g. 2-4). Amongst these studies, PTR-MS has been used for *in vitro* measurements, for example for dynamic headspace analysis of food flavor release (5), prediction of food sensory profiles (6), or characterisation of food product origins (7), and for *in vivo* assessments of aromas, such as temporal volatile release profiles during mastication via nosespace analysis (8) or characterisation of intra-nasal odour perception (9). Thus it is a useful tool in many applications that require high time resolution measurements. Despite its widespread use, however, the instrument has some shortcomings, primarily relating to the low mass resolution of the quadrupole mass spectrometer and the potential complications arising when analysing hydrophilic or labile compounds (the latter being a problem that is not unique to PTR-MS).

Recent developments of this technology have attempted to address these issues and include the coupling of the PTR-MS reaction chamber to a time-of-flight (TOF) mass spectrometer, the so-called PTR-TOF (10), as well as the construction of a high temperature instrument for analysing problematic 'sticky' compounds (11). Furthermore, a novel liquid calibration unit (LCU) has been recently developed to generate defined gas-phase concentrations of compounds from aqueous solutions to enable determination of instrumental sensitivity to aroma compounds, which are typically not available within a gas standard matrix (12).

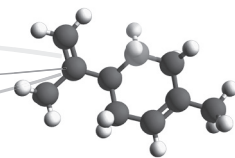
Although PTR-MS has been employed diversely for food/flavour measurements, we have recently exploited the on-line capabilities of the instrument for several novel applications that have hitherto been less widely investigated directly with PTR-MS. The first deals with real-time release, *in vitro* as well as *in vivo*, of flavour compounds from in-house developed food matrices, including foams and gels free of (or with lowered) sugar or fat. The resulting profiles clearly show a dependency of the release of different flavour compounds on the viscosity of the food matrix, as well as on other factors such as compound volatility and hydrophobicity (13). A second novel application of the PTR-MS technique was to conduct intra-nasal measurements of compound concentrations directly at the olfactory cleft. Although this particular application investigated odour perception according to sniff behaviour (9), it is equally applicable for assessing the degree to which aroma compounds are transferred to the olfactory epithelium during food consumption.



This talk will outline the most recent instrumental developments of the PTR-MS technique and discuss novel applications for food development and aroma perception characterisation. This will include consideration of the conventional and new PTR-MS systems. Finally, an outlook on the future direction of this technology in the food/flavour field will be presented.

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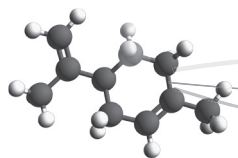
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Topic:

**Effects, meaning
and role of flavour
on Nature**

**Poster
Presentations**



1.01

COMBINED CHEMO-ANALYTICAL AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF NEUROTROPIC ACTIVITY OF ODORANTS

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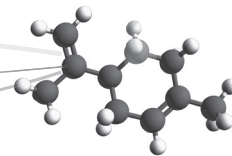
Keywords: physiological action, GABA_A, γ -aminobutyric acid, terpenoids, patch clamp, HEK293, lavender, sideritis

Apart from binding to odour receptors and consequential perception of smell, aroma substances can elicit various physiological effects. Because of their small molecular size and hydrophobicity, these volatile compounds are assumed to migrate through the blood brain barrier where they could bind to ligand-gated ion channels in the central nervous system (CNS). Amongst these neurotropic properties, modulation of the ionotropic GABA receptors (γ -aminobutyric acid receptors, GABA_{A/C}) is of special interest. The GABA_A receptors belong to the superfamily of Cys-loop receptors forming pentameric inhibitory neurotransmitter receptor complexes. The activation of these heteromeric ion channels can be modulated by a variety of different endo- and exogenous substances. Increased activation of this chloride channel leads to sedative and anxiolytic effects (1). Previous work has shown that some food odorants are able to enhance GABAergic currents in a *Xenopus* oocyte system expressing bovine GABA_A $\alpha 1\beta 1$ subunits (2).

The first objective of our study was to verify if specific aroma compounds are able to interact with the GABA_A receptor complexes expressed in mammalian cells. For this purpose, HEK293 cells were transfected with murine receptor cDNA to achieve a receptor composition of $\alpha 1\beta 2\gamma 2L$ subunits (Prof. Sieghart, Wien). Thus, GABA effects could be tested with the most common subunit composition (3). The cDNA stoichiometry was 1:1:2 ($\alpha 1\beta 2\gamma 2L$) to ensure the incorporation of gamma subunits into the receptor complex. Electrophysiological analysis of GABA_A receptor properties was performed using patch-clamp analysis in a whole cell configuration mode. Modulation of the signal was achieved by coapplication of the aroma substances together with the neurotransmitter GABA. To exclude unspecific effects, only reversible currents were taken into account.

First, some GABA-enhancing monoterpenoids were analyzed to validate the test system. Among these aroma substances, linalool, 1-octen-3-ol and geraniol showed the most pronounced effects with a potentiation of whole cell maximal currents up to 4-fold. Other substances like sabinene and α -phellandrene, which had not been tested previously, showed weaker, about 2-fold, enhancement.

In addition, the volatile fractions of aroma extracts from selected plant materials were prepared to screen for active components. For molecular characterization, the respective

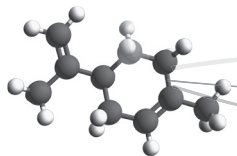


volatile compounds were solvent extracted and separated by solvent assisted flavour evaporation (SAFE) distillation. Electrophysiological analysis showed that extracts of *lavender* and *sideritis* (mountain tea) contain active constituents leading to potentiated GABA-mediated currents. Preliminary investigations indicate that the activity of *lavender* extracts resulted from the main compound linalool. However, the identification of potentiating components in *sideritis* tea is still in progress. For this reason, GC-MS screening was performed with crude extracts, to identify fragrance components and probe them via patchclamp analysis.

This presentation will exemplify how combined gas chromatographic and electrophysiological characterization of isolated compounds allows the detection of GABA_A modulators in foods and provides prospects for physiological effects *in vivo*.

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1.02

GENE-EXPRESSION ANALYSIS OF PRO-INFLAMMATORY GENES AFTER 1,8-CINEOLE TREATMENT IN PORCINE INTESTINAL CELLS

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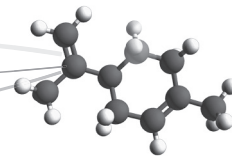
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Keywords: RT-qPCR, IPI-2I, IPEC-J2, immune response, TNF α , IL1b, IL5, IL6, IL8

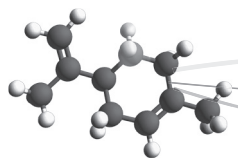
1,8-Cineole is a volatile aroma compound in various herbs of the daily cuisine (1, 2, 3, 4) but it is also an active agent used in oral medication to treat respiratory tract diseases (5, 6, 7, 8). Capsules containing cineole are enteric coated and then the first contact site of 1,8-cineole with the organism is the small intestinal epithelium. The chosen *in vitro* models were two porcine small intestinal epithelial cell lines, the jejunal IPEC-J2 and the ileal IPI-2I cells, based on the high similarity of the human and the porcine intestinal tract and the nutrient uptake capacity of these upper small intestinal segments (9). Along with food most of the time bacteria are ingested. Among these bacteria could be several pathogens and other problematic substances like toxins formed by bacteria and fungi. Accordingly, beside the nutrient uptake the gastrointestinal tract has an important immunologic role as protective barrier. The defence consists in the physical barrier of the epithelial cell layer itself, secretion of sticky mucus by goblet cells in which the pathogens get stuck and can not reach the cells, synthesis of cytokines and antibacterial agents like the peptide defensin, and response to exogenous chemokines (9, 10). In case of an activation of the innate immune defence, epithelial cells secrete the cytokines TNF α , IL1 α , IL1b, IL6 and IL8 (9, 11, 12). TNF α , IL1 α and IL1b are so-called pro-inflammatory cytokines which stimulate leukocyte proliferation, cytotoxicity, release of proteolytic enzymes and synthesis of prostaglandins and initiate the production and secretion of a cascade of secondary cytokines like IL6 and IL8 at the infection locus (13). In addition to these cytokines involved in the direct immune response, the cytokine IL5 was examined. It is responsible for differentiation and IgA production (11) and involved in allergic processes (14). According to our knowledge, this was the first study investigating the influence of the pharmacological agent and volatile aroma compound 1,8-cineole on the geneexpression in intestinal epithelial cells. To examine whether 1,8-cineole induces an immune reaction, the gene-expression of the listed cytokines was determined by quantitative real-time reverse transcription (RT) polymerase-chain-reaction (PCR) (RT-qPCR) after treatment of confluent cell layers with different pharmacological and physiological concentrations of 1,8-cineole (0.2 g/l, 0.12 g/l, 0.02 g/l, 0.012 g/l, 0.002 g/l, 0.0002 g/l) for varying exposure times. Data analysis revealed that cineole application did not influence the mRNA expression of the chosen cytokines. Hence consumption of drugs, herbs or tea containing 1,8-cineole was strongly supposed to have no adverse effect on the intestinal



system regarding the activation of an immune reaction. Solely the intake of high concentrations of 1,8-cineole (2 g/l) seemed to induce inflammation of the small intestinal epithelium as such treatment led to significant ($p < 0.001$) up-regulation of the examined genes and was shown to lead to rapid cell death. Concluding it could be said that the present study did elucidate the basal, not regulated expression levels of different pro-inflammatory genes post 1,8-cineole appliance. No measurable immune stimulatory effect in pharmacological and physiological concentrations could be ascertained.

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DETERMINATION OF CELL MORPHOLOGY UNDER 1,8-CINEOLE TREATMENT IN PORCINE INTESTINAL CELLS

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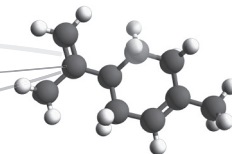
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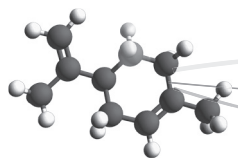
Keywords: ECIS, IPI-2I, IPEC-J2

1,8-Cineole is the main compound of eucalyptus oil and based on those formulation it is used in oral medication to treat respiratory tract diseases (1, 2, 3, 4). Other natural sources could be herbs used in the daily cuisine (5, 6, 7, 8). The first direct contact of this substance with the consuming organism *in vivo* depends on the administration form. If 1,8-cineole is consumed as pharmacological agent in enteric coated capsules, the small intestinal system, in particular the epithelia of the jejunum and ileum, are the first sites of contact as these segments mediate the nutrient uptake (9). Based on that fact, the chosen *in vitro* models were two porcine small intestinal epithelial cell lines, the jejunal IPEC-J2 and the ileal IPI-2I cells. The swine served as model organism because of the high similarity of the human and the porcine intestinal tract. According to our knowledge, this was the first study investigating the effect of the pharmacological agent and volatile aroma compound 1,8-cineole on the morphology of epithelial cells lining the intestine. The influence of the substance was examined by real-time tracking of cell attachment to the culture dish and proliferation under 1,8-cineole treatment with the electric cell substrate impedance sensing (ECIS) system (10). No morphological changes in terms of an altered attachment and spreading behaviour of the jejunal cells could be detected when seeded in cineole containing medium. Also the treatment of confluent cell layers of both cell lines with different pharmacological and physiological 1,8-cineole concentrations (2 g/l, 0.2 g/l, 0.12 g/l, 0.02 g/l, 0.012 g/l, 0.002 g/l, 0.0002 g/l) had no effect in comparison to untreated control cells. Another experiment, the wound healing assay (11), revealed that cineole treatment (1.2 g/l) did not influence the healing behaviour of the jejunal cells. After a pointed electrical wounding there were no growth rate changes, neither after 1,8-cineole pre-treatment, nor by culturing the cells in cineole containing medium during the healing process. But with both experimental setups it was possible to identify lethal 1,8-cineole concentrations. 1.2 g/l was found to be the maximal non-lethal-cineole concentration for the IPEC-J2 cells, for the IPI-2I cells 2 g/l cineole unveiled as lethal. So it could be summarized that morphologically the substance had no effect on jejunal and ileal porcine cells applied in pharmacological or physiological concentrations, neither on cells during attachment and spreading, nor on confluent cell layers. Solely the exposure to high concentrations of cineole adversely affected the cells and led to subsequent apoptosis. In the wound healing assay a potential protective or growth promoting effect by cineole could not be detected.



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1.04

MONITORING OF HOP AROMATIC COMPOUNDS STABILITY IN AN *IN VITRO* DIGESTION MODEL

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Keywords: gastrointestinal, bioavailability, terpenoids

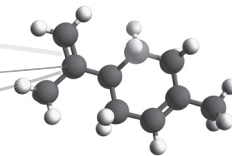
The hop plant (*Humulus lupulus* L.) is generally renowned for its use in the brewing industry, where its female inflorescences (strobili) are utilized for their flavouring and preserving properties. Yet it is also used in phytotherapy, where extracts of the strobili are known for their therapeutic benefit in the treatment of sleep disorders and mood disturbances such as restlessness and anxiety (1). While the hop constituents responsible for these sedative properties have not yet been conclusively clarified, the volatile fraction of hop extracts is suspected to be at least in part responsible for these effects. Some indications result from studies showing that e.g. the hop oil constituents linalool, geraniol and 1-octen-3-ol as well as hop oil itself elicit potentiation of the GABAA-receptor response (2).

On the other hand, stability along with bioavailability in the human body is an important factor when it comes to narrowing down the various hop constituents to bioactive ones. Apart from that, generation of constituents with potentially higher activity, e.g. due to biotransformation processes, needs to be taken into consideration. Regarding bioavailability, the first crucial step after oral ingestion is the passage of the respective volatiles through the gastrointestinal tract. There in particular the low pH of the stomach compartment possibly gives rise to transformations of acid degradable molecules.

Based on these considerations, several odor active compounds of hop essential oil were tested for their stability in an *in vitro* digestion model. Special focus was placed on those substances that had been previously described to be physiologically active. To the best of our knowledge, data on transformations occurring in aqueous acid solutions exist for only a few of the investigated compounds (3-8) and these data do not reflect physiological conditions due to higher temperatures, different pH-values and incubation times. The digestion model, based on the procedure introduced by Oomen et al. (9), mimics the fasted-state human gastrointestinal tract regarding composition and pH-values of gastric juices as well as transition times and temperature.

Monitoring of the transformation processes taking place in this digestion model was accomplished in our study utilizing gas chromatography-mass spectrometry / olfactometry with characterization of the target compounds at defined time intervals, with identification being based on the respective reference compounds.

While some aroma compounds showed high stability under simulated physiological conditions, others underwent transformations to a great extent. Furthermore, our data showed that some odorants were transformed into substances that had been themselves attributed previously with physiological activity. Based on these findings it is justifiable to speculate

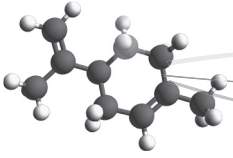


that gastrointestinal processes even might increase, or at least modulate the bioactivity of aroma compositions.

Accordingly, the presented data can be regarded as the basis for further investigations into food matrix effects that might possibly modulate the observed gastrointestinal reactions, as well as intestinal resorption studies to investigate the further fate of the formed compounds in the human physiology.

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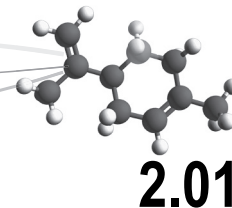
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Topic:

**Physiology of
flavour
perception**

**Poster
Presentations**



IN-MOUTH AIR CAVITY VOLUME CHANGES AND AROMA RELEASE DURING MINT CONSUMPTION IN HUMAN

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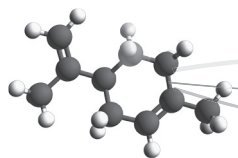
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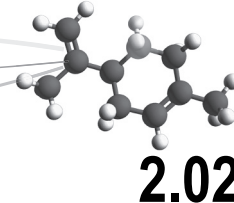
Keywords: Acoustic pharyngometer, in-mouth air cavity, tongue position, swallowing, aroma release
- API-MS - subject variability

Inter-individual variations regarding *in vivo* aroma release profiles in human have been observed widely in the literature. However the corresponding causes have not been clearly identified yet. In this context, inter-individual differences in motor activities during chewing and/or swallowing are thought to be the cause of the difference between the observed patterns of aromatized air passage through the velo-glossal isthmus. In particular, the In-Mouth Air Cavity (IMAC) participates to both jaw posture and speech and it may be involved in the transport of volatile aroma towards the olfactory mucosa during chewing and swallowing. This work aims at investigating the relationships between the level of aroma release in the nasal cavity and IMAC volume changes after deglutition. Twenty four subjects were selected from a panel of 100 subjects where the release of menthone was measured 3 times using Atmospheric Pressure Ionisation – Mass Spectrometer (API-MS) during mint consumption with an imposed chewing protocol. The 24 subjects were separated in two groups corresponding to two levels of menthone release (High or Low classes) adjusted to the amount of mint consumed (H class correspond to a ratio "% of mint degraded/area of aroma release" = 2.44 ± 1.18 , and low class to a ratio of 12.93 ± 6 ; $F = 34.2$, $P < 0.0001$). The IMAC mean volume was measured during empty deglutition and also after it at different times (1, 3, 9 and 27 seconds) using an acoustic pharyngometer device. Four repetitions were performed for each time.

The H class displayed low amplitude of IMAC volume change during deglutition ($7.56 \text{ ml} \pm 4$) and an almost constant IMAC volume after deglutition ($6.67 \text{ ml} \pm 5.93$) that corresponded to high tongue position. The L class displayed high amplitude of IMAC volume changes during deglutition ($13.7 \text{ ml} \pm 5.5$) and a progressive increase of IMAC (from $10.78 \text{ ml} \pm 6.7$ at 1 second to $15.67 \text{ ml} \pm 6.2$ at 27 second). It corresponded to a progressive lowering of the tongue while getting back to its resting position. One way ANOVA showed a significant difference between the two classes for the amplitude of IMAC volume changes during deglutition ($F=9.557$, $P=0.006$). Moreover, a repeated ANOVA measure showed a significant



difference between the two classes for the IMAC at 9s ($F=7.24$, $P=0.014$) and 27s ($F=9.762$, $P=0.005$). It is likely the H group released continuously aroma through the velo-glossal isthmus during mint consumption, whereas the L group trapped the aroma in the oral cavity before its release towards the nose cavity. These results show that the *in vivo* aroma release profile in human is highly dependent on the level and amplitude of IMAC volume changes during oral movements and particularly during deglutition.



IMPACT OF SWALLOWING ON THE DYNAMICS OF AROMA RELEASE AND PERCEPTION DURING THE CONSUMPTION OF ALCOHOLIC BEVERAGES

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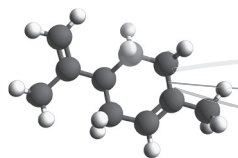
Keywords: swallowing, release kinetics, temporal perception, TDS method, PTR-MS

The overall perceived flavour of a food depends largely on the way in which volatile aroma compounds are released in the mouth and transported to the olfactory receptors in the nose during food consumption. Swallowing is particularly important in the drinking of beverages, which requires only limited oral manipulations, resulting in the product remaining in the mouth for only a short period (1-3). In the field of alcoholic beverages, expert panels often make use of specific tasting techniques and various protocols to evaluate sensory properties. In particular, products are often tasted without swallowing, to limit the effect of ethanol ingestion. Perceptions are thus evaluated in conditions that do not really represent actual consumption conditions of a real tasting. The importance of swallowing for perception raises questions about the possible influence of tasting conditions on perception of the sensory properties of the product. However, this question has never before been addressed.

The aim of this study was to quantify the impact of tasting protocol (with or without swallowing of the product) on aroma release and perception in the case of a commercial flavoured vodka. An integrated approach combining sensory analysis and physicochemistry was used to investigate the impact of swallowing on aroma release and perception.

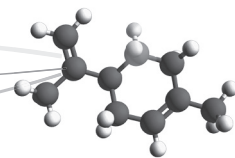
A panel of 10 persons evaluated the dynamics of aroma perception during the consumption of a commercial flavoured vodka, using two protocols (spitting out or swallowing of the product) and the temporal dominance of sensations method (TDS). Nosespace analysis was simultaneously carried out by proton transfer reaction mass spectrometry (PTR-MS) to evaluate aroma release in their nasal cavity.

Comparison of the results obtained with the two protocols highlighted significant differences in both the perception and the release of aroma: the swallowing of the product resulted in more complex perceptions, but decreased the dominance rates of aromatic attributes. Ethanol perception also had a high impact when the product was swallowed. These results may have implications for product formulation, depending on the way in which products are evaluated and/or consumed. Some relationships between sensory and physicochemical data have been established, particularly concerning the temporal dimension of sensory and release phenomena, but the lack of knowledge concerning the variety and complexity of mechanisms continues to limit our understanding of the link between aroma release and perception



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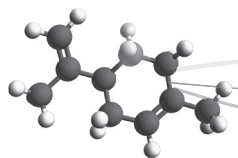
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Topic:

**Practical and
industrial
aspects**

**Poster
Presentations**



3.01

**THE RELATIONSHIP BETWEEN UMAMI TASTE PERCEPTION IN HUMANS
AND THE EXPERIMENTALLY DETERMINED PERSISTENCE OF UMAMI
INDUCING COMPOUNDS IN THE MOUTH**

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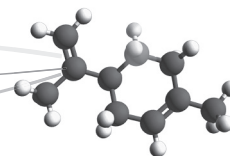
Orthonasal or ‘sniff’ testing of odours has been carried out for many years to measure properties like odour threshold values (1), and the sensory quality of odour mixtures (2). Sensory testing of volatile flavour compounds and their perception when detected retronasally via the olfactory epithelium of the nose during maceration has also been investigated and documented (3).

Possibly due to the challenging nature of accurately delivering tastants and measuring actual concentrations during *in vivo* testing, the perception of tastants in the mouth has been investigated to a far lesser degree. For many years orthosteric and allosteric binding modes of umami tastants to human taste receptors have been known (4), though the link between the *in vivo* release and persistence of umami compounds on the tongue and the perception of umami flavour appears not to have been investigated. Hort and co-workers have reported the use of a Dynataste apparatus to deliver constant flow of tastants into the mouths of panellists allowing the synergistic effect of tastants to be investigated (5) and Davidson and co-workers (6) have reported the use of a swabbing technique to investigate the release of flavour compounds *in vivo*. The approach adopted during the course of this work seeks to combine elements of previous work in the area of tastant perception to investigate the synergistic effects of orthosteric and allosteric binding umami compounds.

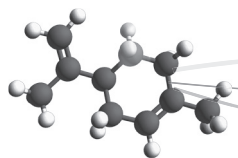
Umami perception was monitored in trained panellists who consumed a gel matrix containing known concentrations of umami-inducing compounds for a measured period of time. Umami taste perception in the panellists was monitored using time intensity techniques with FIZZ® sensory analysis software. Simultaneously, the actual levels of umami inducing compounds on the tongue were monitored as a function of time by regular swabbing, combined with LC-MS analysis. The relationship between the persistence of umami inducing compounds on the tongue was compared to the perceived intensity and persistence of the umami taste sensation.

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3.02

SYNERGISTIC/SUPPRESSIVE EFFECTS OF BINARY AND TERNARY MIXTURES OF SWEETENERS IN SEMI-SKIMMED MILK

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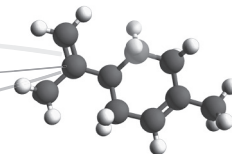
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Keywords: Sweeteners, Sweetness, Synergy, Suppression, Binary mixtures, Ternary mixtures, Semi-skimmed milk

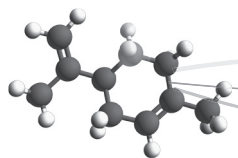
The use of sweetener blends in low-cal products has several advantages. These include an increase in low-cal product choices for consumers; reduction of the product costs; improvement of the product taste and stability (1); and reduction of the decline in sweetness intensity experienced over repeated exposure (2). While there is plenty of information on the properties of sweetener blends in aqueous solutions (3, 4, 5), there is very little about them in product matrices (1). For dairy companies, there is a need to understand the sweetness properties of different blends in dairy products. Consequently, we designed a study to assess the sweetness of binary and ternary mixtures of 4 bulk sweeteners (sucrose, glucose, fructose, and lactose) and 7 intense sweeteners (aspartame, acesulfame-K, cyclamate, erythritol, neotame, saccharin, and sucralose) in semi-skimmed milk. The data were interpreted statistically by a linear regression through backward elimination. The number of synergistic mixtures turned out to be lower than expected (15% of binary mixtures and 3% of ternary mixtures). The sweetness increase due to synergy reached up to 20% for binary mixtures (aspartame/saccharin) and up to 44% for ternary mixtures (aspartame/neotame/sucrose). Suppressive effects, leading up to 40% reduction of sweetness, were observed in 13% of the binary mixtures and in 5% of the ternary mixtures. Surprisingly, some binary mixtures did not significantly contribute to enhancing or decreasing sweetness in a ternary mixture. In other cases, positive binary synergistic effects resulted in negative ternary synergistic effects. Observed results could not all been clearly explained. Common bulk sweeteners sucrose, fructose, glucose and lactose did not (or only marginally) result in significant synergy effects in binary mixtures. Neotame and saccharine had, in all cases, a negative impact in binary mixtures while cyclamate and erythritol were always suppressive in ternary mixtures. Aspartame was definitively more successful in ternary mixtures than in binary mixtures.

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3.03

**CORRELATIONS BETWEEN AROMA-ACTIVE COMPOUNDS AND THE
FOODBORNE TOXICANT STYRENE DURING WHEAT BEER PROCESSING:
FORMATION PATHWAYS FROM SELECTED PRECURSORS**

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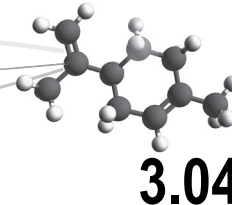
Keywords: wheat beer, aroma-active compounds, AEDA, SIDA, styrene

During beer brewing, different processing steps occur: on the one hand enzymatic processes and on the other hand thermal processes, which are responsible for the generation of desired compounds like aroma-active substances formed from precursors. But, also undesired compounds with toxicological relevance, the so-called “food-borne toxicants”, might be formed. In the past, styrene has become a substance of interest in wheat beer due to its classification as “possibly carcinogenic to humans” (2B; International Agency for Research on Cancer).

Thus, the aims of the present study are i) to identify and quantify the key aroma compounds in wheat beer on the basis of the molecular sensory science concept, ii) to develop a stable isotope dilution analysis for the quantitation of styrene in wheat beer and iii) to elucidate the formation of both, selected aroma-active compounds and styrene from different precursors, namely phenylcarbonic acids, during the processing steps to ensure wheat beer with a typical aroma but a low content of styrene.

For this purpose, the most important aroma-active compounds in wheat beer were identified using aroma extract dilution analysis in combination with GC-MS and then quantified by stable isotope dilution assays. Next, quantitation methods for various phenylcarbonic acids (e.g., cinnamic acid, vanillic acid, ferulic acid) as well as for the corresponding decarboxylated products (e.g., styrene, guaiacol, 4-vinylguaiacol) were developed. With these methods at hand, different wheat beers were analyzed revealing huge differences in the respective amounts. To get deeper insights into the critical processing steps, the precursors as well as the decarboxylated products were quantified, beginning with malts and ending with the bottled beers including important intermediate products.

The lecture will summarize all these data and will show the possibility to produce a healthy wheat beer with its characteristic aroma on the one hand, but with a reduced level of styrene on the other hand.



IMPORTANT AROMA COMPOUNDS IN SALAMI AND CORRELATIONS WITH BIOGENIC AMINES

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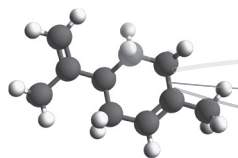
Keywords: Fermented sausage, aroma, biogenic amines, salami

Salamis are cured, fermented, and air-dried sausages made of meat of one or diverse animals. They are suitable for consumption in an uncooked and matured form after a period of storage, which is associated with the loss of water. Beside muscle tissue, salami contains fat tissue, microbiological cultures, herbs, sugar, nitrate or nitrite, and pepper. During storage important aroma compounds and undesirable biogenic amines are generated. Not only exogenous but also endogenous microorganisms play a major role, in this process.

The first aim of this study was to get an insight in the substances responsible for the characteristic aroma profile of salami. Therefore, a commercial sausage was investigated using the concept of molecular sensory science, including gentle distillation of volatile compounds in high vacuum, aroma extract dilution analysis, quantification by stable isotope dilution analysis (SIDA), calculation of odor activity values (OAV), and imitation of the aroma profile by combination of key aroma compounds in their natural concentrations.

Fifty five aroma active compounds were identified in commercial Hungarian winter salami. 2-methoxyphenol, 2-methoxy-4-(2-propenyl)-phenol, 2-methoxy-4-(E)-(1-propenyl)-phenol, 5-methyl-2-methoxyphenol, 3-(methylthio)propanal, phenylacetaldehyde, 4-ethyl-2-methoxyphenol, 4-propyl-2-methoxyphenol, and 3-ethylphenol were found with high flavor dilution factors. After quantification and calculation of OAVs twenty nine compounds were received with an OAV greater or equal one. Hence, these compounds are suggested to the overall aroma profile of Hungarian salami. The most important ones (OAV > 100) were acetic acid (8800), acetaldehyde (1600), 3-(methylthio)propanal (740), phenylacetaldehyde (330), 2-methoxyphenol (210), and 2-acetyl-1-pyrrolin (140).

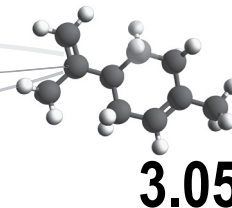
In order to investigate the correlation between aroma compounds, in particular strecker aldehydes, and biogenic amines, a sausage was produced using a defined procedure. By application of the concept of molecular sensory thirty nine aroma active compounds were finally identified. The highest concentrations were found for acetic acid, 3-methylbutyric acid, butyric acid, 2-methylbutyric acid, and phenylacetic acid. Concentrations of further aldehydes, like phenylacetaldehyde, 3-methylbutanal, and 3-(methylthio)propanal were determined as well. In order to provide a correlation of these aldehydes; with the corresponding biogenic amine a SIDA method was developed (1). Based on a derivatisation with benzoyl chloride followed by an RP-LC separation and tandem-mass-spectrometric detection, a method was developed with superior selectivity and precision. Using this method, it was possible to quantify fourteen biogenic amines, with a detection limit of 0.05 µg/kg. Highest concentrations were found for tyramine and β-alanine. The presentation will show data concerning the aroma of commercial salami as well as the concentrations of selected



aldehydes and their corresponding biogenic amines of a sausage which was produced using a defined procedure. Additionally, an overview concerning the developed SIDA method will be given.

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ELUCIDATION OF ASHTRAY ODOR

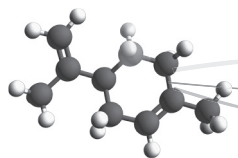
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Ashtray Odor, Tobacco, Gas Chromatography-Olfactometry

The aroma characteristics and the chemical composition of aged smoke and its residue have not been characterized. In this study, ‘ashtray aroma’ was analyzed using Gas Chromatography Olfactometry (GC-O) combined with Aroma Extract Dilution Analysis (AEDA) of ashtray solvent extracts and headspace of ashtrays. The headspace over an ashtray was sampled, cryo- trapped in a Programmable Temperature Vaporizing (PTV) injector, and transferred to the capillary column. A total of 31 odor active regions were detected in the headspace, including highly volatile compounds such as methanethiol (sulfury, foul) and 2,3-butanedione (buttery), and less volatile compounds such as 2-ethyl-3,5-dimethylpyrazine (earthy) and dimethyl trisulfide (sulfury, cabbage). In addition to the headspace analysis, the content of an ashtray was extracted with dichloromethane, followed by Solvent-Assisted Flavor Evaporation (SAFE) to separate volatile aroma from non-volatile compounds. The distillate was concentrated and aroma compounds were identified by Gas Chromatography-Mass Spectrometry-Olfactometry (GC-MS- O) in combination with AEDA. The AEDA of the ashtray extract yielded a total of 55 odor active regions with a Flavor Dilution (FD) factor ≥ 32 . With the highest FD factors found for 3,5- dimethyl-2(E-1-)propenyl pyrazine (earthy, FD 8192), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like, FD 4096) and β -damascenone (cooked apple, FD 4096) followed by 2-ethenyl-3,5- dimethylpyrazine (earthy, FD 2048), 2,6-dimethylphenol (phenolic, medical, FD 2048) and 3- methylindol (faecal, FD 2048). Furthermore, the AEDA revealed the important contribution of pyrazines, in particular alkylpyrazines such as 2-isobutyl-3-methylpyrazine (bell pepper), 2,3- diethyl-5-methylpyrazine (earthy), and 2-isopropyl-3-methylpyrazine (earthy), but also of phenolic compounds such as 2,6-dimethylphenol (phenolic, medical) and 2-methoxyphenol (smoky) to the ashtray aroma. In addition, the ashtray aroma was assessed descriptively by a trained panel. Panelists were asked to evaluate six predefined aroma qualities and one trigeminal sensation. This resulted in highest scores for ‘phenolic’, followed by ‘smoky’ and ‘earthy’. Based on headspace analysis and solvent extraction investigations, it was possible to identify the key aroma compounds contributing to ashtray aroma.



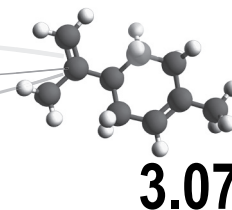
3.06

USE OF THE MICRO SCALE PLATFORM FOR HIGH THROUGHPUT SCREENING OF
FLAVOUR CHARACTERISTICS IN STRAINS (YEAST/LAB) FOR ALCOHOLIC
BEVERAGES

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The characterization, development or improvement of (new) beer and wine varieties with respect to yeast performance during alcoholic fermentation requires lab/pilot scale screenings of relatively large amounts of yeast strains in real product settings. This laborious and time consuming process could be enhanced by using a micro scale product screening: MicroBeer and MicroVinification. Alcoholic fermentation of beer and wine by using 24-wells micro plates are compared with lab scale fermentation. In the miniaturised microvinification system, fast measurement of growth, viable cell count and volatile flavor formation are combined, which significantly increases the predictive power of the outcome of pilot/full scale productions.



VOLATILE FLAVOUR COMPOUNDS OF PROVENANCE-SPECIFIC *VALENCIA* LATE ORANGE JUICES

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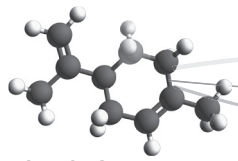
Keywords: Orange juice, volatile flavour compounds, aroma profile, thermal desorption, sensory evaluation

In order to study the influence of geographic origin, fresh orange juice samples of provenance-specific, single-cultivar oranges from various growing areas were analyzed. Juices of Valencia late oranges from different European countries were compared to juices from Valencia late oranges originating from Northern Africa as well as Central and South America. All fresh juices were processed under identical conditions at the same plant (1) with 'flash-pasteurization-technique', assuring gentle pasteurization conditions. Samples were provided to our laboratory within 3 days after production, all analytical investigations were carried out without delay. Special emphasis was placed on the evaluation of the aroma profile. The headspace flavour patterns of all Valencia orange juices were established. An individually designed technique of purge and trap, consisting of a thermal desorption unit coupled with gas chromatography on OV 1701, was employed for quantification purposes. Tenax® TA polymer resin (0.5 ml in glass sample tubes) was used as adsorbent.

When comparing the aroma levels of the obtained profiles, special emphasis was put on the important flavour compounds, such as ethyl butyrate, monoterpene aldehydes, linalool and monoterpene hydrocarbons. Additionally, the physicochemical properties of the juices were also established. Sensory evaluation with a test panel was performed, clearly documenting the significant influence of Brix value and ratio; however, an unambiguous correlation to aroma compound values, quality and sensory evaluation could not be determined. Questions of standardisation of aroma values in juices, often considered a necessity, will be discussed with respect to consumer needs. While aroma patterns of orange juices from various geographic origins often differ considerably, sensory investigation did not automatically lead to pronounced differences in the evaluation.

Aknoeweledgements:

1. The authors would like to thank Eckes-Granini Group GmbH for providing especially produced juices from geographically non-blended Valencia oranges.



3.08

**VOLATILE FLAVOUR COMPOUNDS AND SENSORY EVALUATION OF
COMMERCIALY AVAILABLE APPLE JUICES AND FRESHLY SQUEEZED, NON-
BLENDED APPLE JUICES**

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Keywords: Apple juice, volatile flavour compounds, aroma profile, aroma index, thermal desorption, sensory evaluation

The correlation between the composition of the volatile flavour compounds of apple juices and their sensory evaluation was investigated. Commercially available juices (cloudy direct juices and clear juices from concentrate) as well as freshly squeezed, non-blended, cloudy juices from different varieties of apple were analytically studied.

Special emphasis was placed on establishing the respective aroma profiles. The headspace flavour patterns of all apple juices were analyzed and quantified. Using an individually designed technique of purge and trap, the aroma compounds were transferred onto a Tenax® TA polymer resin as adsorbent (0.5 ml in glass sample tubes). A thermal desorption unit coupled with gas chromatography was employed for establishing the flavour profiles.

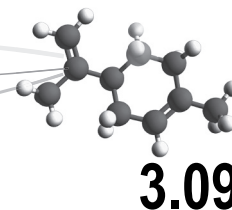
These patterns were then compared quantitatively, especially focussing on esters, aldehydes and alcohols as important flavour compounds of apple juices. While the industrially produced, concentrate-derived blends exhibited aroma values situated within a narrow range, the values of freshly squeezed, single-cultivar juices often showed significant variance, reflecting the variety of nature.

Calculations of the Aroma Index and assessments of the aroma values, according to the GfL-system (1) for the evaluation of apple juices, were also carried out. However, these theoretical, calculated classifications automatically resulted in a negative rating for a majority of the freshly squeezed, single-cultivar juices. On the other hand, these juices predominantly received positive sensory evaluations.

In this context, questions of standardization of aroma values in juices, considered a helpful tool for valuation purposes, will be discussed in the light of consumer needs and natural diversity.

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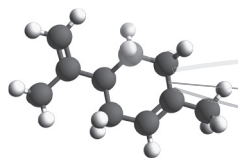


REACTIONS OF PROPYLENE GLYCOL WITH THE CONSTITUENTS OF FOOD FLAVOURINGS

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Propylene glycol (1,2-propanediol) is a solvent commonly used for the preparation of commercial food flavourings. Under acidic or basic conditions, the hydroxyl groups in propylene glycol can react with components of food flavourings to give new compounds. The reaction of propylene glycol with aldehydes and ketones to give acetals and ketals, respectively, has been widely reported. However, propylene glycol can also react with organic acids commonly used in flavourings, such as acetic, butyric and lactic acids, to give both mono- and di-esters. In addition, propylene glycol can transesterify; in particular, it can react with lactones to give dihydroxy esters. These three reactions will be described and the spectral information of reaction products of some compounds commonly found in food flavourings will be provided. In addition, gas chromatography/olfactometry data from these flavour compounds and their reaction products will be presented, and the effect of propylene glycol on the sensory quality of stored food flavourings will be discussed.



3.10

IDENTIFICATION OF A SWEET TASTE ENHANCING VANILLIN ISOMER FROM *MONDIA WHITEI* VIA SENSORY GUIDED ANALYSIS

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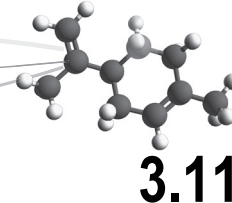
Keywords: sweetness, taste modulation, sensory guided analysis, vanillin isomers, sweet taste receptor

Mondia whitei (Hook.f.), also called umondi by the Zulu people is a woody creeper from Eastern, Southern and Central Africa (1). Apart from medicinal uses, the roots are drunk as a tea-like beverage or added to porridge due to its vanilla-like flavour and sweet aftertaste (1). They are reported to contain the vanillin isomers isovanillin, as well 2-hydroxy-4-methoxybenzaldehyde (2,3). 2-Hydroxy-4-methoxybenzaldehyde, responsible for the characteristic coumarinic flavour of *Mondia*, was described to act as a sweetness inhibitor as a neat compound (3). Extracts of the roots, however, tasted on 5 % sucrose solution by a trained panel were described to be sweeter than the reference sample: an aqueous-ethanolic extract showed a sweet enhancement of more than 25%.

In order to elucidate these sweet enhancing characteristics of an aqueous-ethanolic *Mondia* extract and to identify the relevant fractions, sensory-guided fractionation based on a LC Taste® protocol (5) in combination with a novel large capacity sorptive extraction (LCSE) tool, so called SymStixx®, was employed. Interestingly, one fraction containing 2-hydroxy-4-methoxybenzaldehyde as a main compound was perceived sweeter than the reference sucrose solution. Results were confirmed using the neat compound in a duo-comparison test (6). At low and medium concentrations (0.1- 0.5 ppm), a clear sweetness enhancing effect could be detected (up to 30%), while increasing concentrations (1.0 ppm) did not result in stronger sweetness enhancement, probably due to the strong coumarinic aroma value. This strong intrinsic flavor might also explain, why the neat compound was described as sweetness reducing as neat compound. Consequently, the sensory evaluation was repeated using nose clip and compared to vanillin and ethylvanillin. In these experiments, the test compounds up to 150 ppm neither showed intrinsic sweetness nor did they lead to sweetness enhancing effects. These results obviously support the hypothesis to consider vanilla like molecules to act as congruent flavors to increase sweetness perception.

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LC-MS/MS STUDIES ON THE INFLUENCE OF THE PH VALUE ON THE FORMATION OF ISO- α -ACID DEGRADATION PRODUCTS IN BEER

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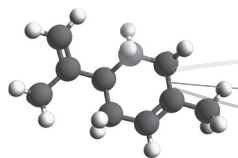
Keywords: pH studies, degradation of iso- α -acids, LC-MS/MS analysis

The flavour of beer is heavily dependent on time of storage. The typical bitterness of fresh beer is well-known to slightly decrease in intensity and to change in quality with increasing age of the beverage. Non-volatile bitter compounds of beer have been investigated in the last decades, and it is agreed that the typical beer bitterness is caused by adding hop products during wort boiling. A number of isomerization processes during the wort boiling process have been reported to be of major importance for bitter taste development in the final beer product. Moreover, the iso- α -acids have been identified as the major bitter contributors in beer and were demonstrated to be generated upon a re-arrangement reaction of their hopderived

precursors, namely the α -acids. Already *De Cooman et al.* (2000) pointed out that particularly the *trans*-iso- α -acids are prone to degradation (1). In contradiction to previous findings, *Intelmann et al.* (2009) revealed an acid-catalytic decomposition pathway for *trans*-iso- α -acids to tri- and tetra-cyclic degradation products (2). Evaluation of beer flavor stability is usually based on the determination of one or a few analytical parameters (3). The main quality problem of beer is the change of its chemical composition during storage, which alters the sensory properties. In the present study, we investigated the influence of the pH value of beer on the formation of these degradation products with focus on tricyclohumol and tricyclocohumulol by means of quantitative HPLC-MS/MS experiments.

Commercial pilsener-type beer samples were analyzed fresh and after storage for 6 months at three different pH values. Lowering the pH value about 0.1 and 0.2 effected an increase of tricyclic degradation products around 10 % and 15 % compared to the beer sample with pH value of 4.5. In previous studies it was possible to determine the bitter recognition threshold value of tricyclocohumulol in unhopped beer (4). In contrast to the bitter taste recognition threshold concentration of tricyclocohumulol in water (30 $\mu\text{mol/L}$, 2), tricyclocohumulol showed with 11 $\mu\text{mol/L}$ (4) a three times lower threshold concentration in unhopped beer. The fact that after six months of storing the concentration of tricyclocohumulol in beer is just around the threshold of bitter taste detection, clearly demonstrates that, besides aroma-active volatiles, also the generation of nonvolatile degradation products originating from iso- α -acids contribute to the flavour instability of beer and underlines the complex nature of the deterioration of beer products.

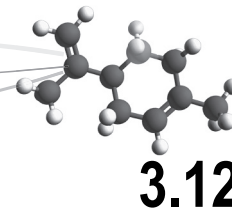
The results exhibited a direct correlation between the pH value and the decomposition of *trans*-iso- α -acids to tri-cyclic degradation products. In this connection tricyclohumol presents a further analytical marker of beer ageing, beside tricyclocohumulol. Due to the findings, the



brewing process is to reconsider with respect to pH modification since already a slight raise of pH shows an effect of flavour stability of aged beer.

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USE OF THE ALPHA-MOS HERACLES ELECTRONIC NOSE FOR MAILLARD REACTION FLAVOURS BLENDING AND EVALUATION

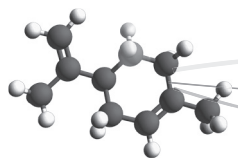
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Keywords : reaction flavours, volatiles, electronic nose, GC, PCA, blending

A commercial flavour compound is a mixture of a yeast extract and a core note (roasted chicken in this case) produced by the Maillard reaction of a sugar and a sulfur amino acid. This core note has to display a powerful enough roasted chicken note without falling on the toasted or burnt side. Therefore the proportions of the core note and the yeast extract in the blend will depend on the strength of the former. Traditionally, this blending is done after flavour assessment of the core note by the flavourist and the feasibility of using the electronic nose for doing such has been investigated. The electronic nose measurement is done directly on the powder or liquid sample through a headspace, purge and trap double column (non polar and polar) ultra fast GC whose chromatograms are submitted to a Principal Component Analysis (PCA) in order to get a “fingerprint” of the volatiles. A set of core notes samples is first evaluated by the flavourist and ranked according to their percentage of incorporation in the recipe. This percentage is correlated to the result of the PCA as reduced to one point for each product and a regression line is established with a good coefficient. This correlation is then used for predicting the percentage of incorporation from the result of the electronic nose. In a further attempt, the PCA fingerprint of the final flavour compound is correlated to the intensity as perceived and rated by a human panel (n =30) for some flavours (chicken, toasted, sulphur...) in order to predict its profile from the measurement by the equipment. The last step relies on the exploitation of the chromatograms in order to retrieve and identify the volatile molecules which have the more impact on the flavour profile.

These experiments demonstrate the usefulness of the electronic nose for the purpose of assembling reaction flavours whereas for the evaluation of the final product, it needs to be completed by an evaluation of the non volatile taste active compounds.



3.13

FLAVOUR RELEASE FROM HOT CHEESE IN RELATION TO AROMA COMPOUND PROPERTIES

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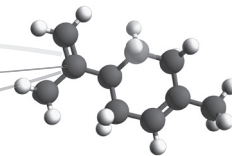
Keywords: Real-time flavour release; Proton transfer reaction mass spectrometry (PTR-MS); Melted cheese; Physicochemical properties; Partition coefficient

While cheese has been of considerable interest in flavour release studies (1-4), there is very little literature regarding the equilibrium or dynamic flavour release from cheese in cooked applications, for example cheese eaten hot as part of a mixed food. Further, the texture of cheese differs greatly when it is melted as opposed to eaten cold, and it is well known that the texture of a food can influence the release and/or perception of flavour as the food is eaten (5-7). It is also possible that factors such as oiling off could alter the relative partitioning and temporal release of flavour compounds, which again may alter flavour release and perception. The objective of this poster is to show how the release of a range of aroma compounds from hot cheese during eating is related to their physicochemical properties, using theoretical calculations to predict equilibrium release and comparing this with real-time release to determine if texture plays a role.

We have tested four variations (two fat levels, and two levels of a processing variable) of a flavoured mozzarella-type cheese base. In a study carried out at CSIRO in Australia, proton transfer reaction mass spectrometry was used to measure the release of six aroma compounds as the cheese was eaten while hot (approximately 50–55 °C) and while cold (12 °C). The aroma compounds were chosen to cover a wide range of physicochemical properties, and were included at concentrations typical of natural cheese. The equilibrium air/product partition coefficient was calculated from the physicochemical properties of each aroma compound, and in an earlier study (8) this was confirmed to be similar to the measured air/product partition coefficients.

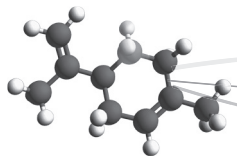
The breath concentration of each aroma compound increased over time to a maximum intensity (I_{max}) at a time defined as T_{max} , and then decreased. In general, compounds with a higher air/product partition coefficient (i.e., those compounds for which equilibrium release was greater) had a greater I_{max} and an earlier T_{max} .

During the period of increasing breath concentration, the average rate of aroma release (as a proportion of the total level of that aroma compound in the cheese) was related to the theoretical air/product partition coefficient via a power law function. This function was very similar for all four cheese bases, and applied whether the cheese was eaten hot or cold. Our future modelling studies can therefore use this relationship as a starting point to model flavour release during eating.



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3.14

QUANTITATIVE MAPPING OF TASTE-ACTIVE COMPOUNDS IN DASHI INGREDIENTS

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Keywords: Dashi, Seaweed, Fushi, Niboshi, Umami Taste, Recombinant, Sensometabolome

Umami is an important taste attribute in savoury food which is associated to a palatable and pleasurable flavour. This flavour impression is characteristically imparted by glutamate and 5'-ribonucleotides such as inosinate and guanylate. Especially traditional Japanese cuisine is very renowned for excellent umami dishes and the typical Japanese stock "dashi" is used as a convenient flavor base for many soups and sauces since centuries in Asia. Several studies have been performed in the past to identify the non-volatile key players evoking the typical taste profile of a dashi ingredients (typically seaweeds and dried fishes) (1-4) but little information are available which combine quantitative data and taste attributes.

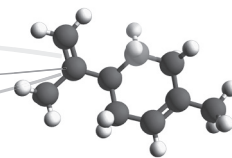
Extracts of four different edible seaweeds (konbu) and eight variations of dried fishes (fushi and niboshi) were investigated using a set of analytical methodologies (e.g. RP-HPLC-UV, HPLC-MS/MS, ion chromatography, enzymatic tests). Applying a sensometabolomic approach, a total of 41 alleged taste-active metabolites and minerals were quantitatively determined in the methanol/water extracts prepared from the seaweeds and dried fishes, respectively, and then ranked in their sensory impact after assessing the individual dose-overthreshold (DoT) factors.

For the seaweeds highest DoT values were received for aspartic and glutamic acid, potassium and sodium chloride, respectively. In the five fushi samples glutamic acid, γ -aminobutyric acid, lactic acid, histidine, 5'-inosine monophosphate, sodium and potassium chloride were predominant, whereas the three niboshi samples revealed high DoT values for glutamic acid, γ -aminobutyric acid, lactic acid, creatinine, 5'-inosine monophosphate, sodium and potassium chloride.

Based on the set of analytical data, recombination experiments were conducted for selected targets to investigate the taste contribution of individual tastants and to demonstrate that the compounds identified can create the typical savoury taste of the dashi ingredients. The reconstitutes, containing the quantified tastants in natural concentrations, were evaluated in a comparative taste profile analysis ranking the basic taste qualities (salty, sour, bitter, umami, sweet).

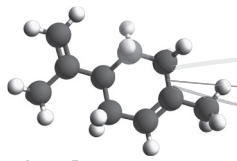
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3.15

THE ROLE OF ETHYL- β -D-GLUCOSIDE IN PLEASANTNESS OF SEA BUCKTHORN JUICE

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Keywords: bitterness, ethyl- β -D-glucoside, chromatography, pleasantness, sea buckthorn

Sea buckthorn (*Hippophaë rhamnoides*) berries are known to be very acidic and sour and astringent (1, 2). Berries contain also various sugar alcohols and alkylated sugars and the contents vary during ripening (3). Our study examined the effect of ethyl- β -D-glucoside on sensory properties (taste and astringency) and pleasantness of sea buckthorn juice. Three samples from two varieties were chosen to the study: variety with naturally high contents of ethyl- β -D-glucoside (picked optimally ripe and slightly over-ripe, a month difference) and another with a lower content of ethyl- β -D-glucoside (optimally ripe = control) growing in the same place and conditions.

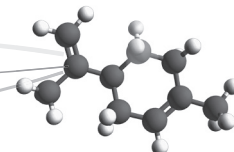
Three cold-pressed juice samples were prepared. The contents of ethyl- β -D-glucoside and various sugars and acids in samples were determined by GC (1, 3). 39 voluntary subjects were asked to rate the taste pleasantness of the juices on a 9-point scale (1 = extremely unpleasant, 9 = extremely pleasant). The intensities of sensory attributes (sourness, bitterness, soft and rough astringencies) were evaluated from the juices by a trained panel (n = 26). The panel evaluated intensities from the samples on a gLMS a total of three times in separate sessions. Reference samples were given to panellists to anchor the scales in every session. The pleasantness and sensory analyses were performed in a sensory laboratory in accordance with ISO 8589-1988 standard.

Two optimally ripe berry juices did not differ significantly in pleasantness of flavor. A significant decrease of pleasantness from optimal to over-ripe juice was detected ($p < 0.05$). The three juices differed from each other only in bitterness, the over-ripe being the most bitter. Based on the GC-analyses, the content of ethyl- β -D-glucoside was clearly highest in the over-ripe juice and the content of glucose decreased simultaneously with the increasing content of ethyl- β -D-glucoside. Sugar contents were the highest in the control juice and higher in optimally ripe samples than the over-ripe berry juice. Sugar/acid ratio was the same in optimal and in over-ripe samples. Ethyl- β -D-glucoside may be a negative factor for pleasantness of sea buckthorn juices and therefore may influence the utilization of berries. Higher sugar content and sugar/acid ratio combined with the lower content of ethyl- β -D-glucoside may all have contributed to the higher pleasantness of the control variety.

Also the higher content of glucose and various fruit acids in optimally ripe juice sample may mask the bitterness. The content of the ethyl- β -D-glucoside varies significantly between subspecies and varieties (3). This study provides important information concerning the flavor contributing compounds of sea buckthorn and their role in pleasantness of berry.

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MONITORING OF BACTERIA CAUSING OFF-FLAVOURS IN BOLOGNA TYPE SAUSAGES BY SPME-GC-MS

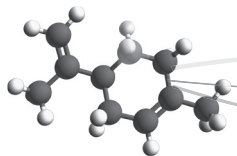
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Keywords: Meat, meat products, spoilage, lactic acid bacteria, GC-O

Bologna type sausages are among the most consumed meat products in Europe. Originally it is made out of pork or beef, water, lard, starch and spices. The occurring flora of microorganisms in the fresh product is strongly dependent on the hygienic circumstances present in the producing company. The common spoilage process of fine precooked meat products is predominated by different homo- and heterofermentative Lactobacteriaceae, which are able to suppress other pathogenic spoilage bacteria.

General quality assessments regarding the freshness are mainly based on microbiological techniques by means of total plate counts without strictly regulated limits, but by indicative bench marks. If the microbiological contamination reaches critical values the decision on the tradability of the product is handed over to human sensory. Human sensory is a very useful tool in quality assessment, but needs a considerable, continuously and well trained panel -requirements which often cannot be fulfilled by small enterprises. The aim of this work is to render the decision of tradability more objective by the implementation of GC techniques. Further on, a multivariate model based on microbiological, various GC (GC-MS, GCO) and sensory data sets will be developed, allowing the prediction of the quality of a certain meat product with a single GC run. Numerous strains of *Lactobacilli spp.*, *Streptococcus spp.*, *Enterococcus spp.* and yeasts have been isolated from Bologna type sausage samples during a storage period of 5 days at 10°C. The species were easily distinguished by their production of different patterns of alcohols, aldehydes, ketones and sulfides. One fast growing strain of *Lactobacillus* was chosen for further experiments on fresh sliced Bologna sausage (cfu <103) applied at different concentrations. The pure culture was used to monitor the process of spoilage of sliced Bologna sausage under microaerophilic conditions at 12°C, performing contemporaneously microbiological and analytical methods every 24 hours, the latter being SPME-GC-MS, SPME-GCO and human sensory analysis. After 24 hours of incubation no other strain than the added *Lactobacillus sp.* was detected on the sausage slice and no significant difference neither in odour nor in the spectral data (GC-MS) between the reference and the inoculated samples was detected as long the number of cfu was beyond 105. Above this concentration the sensory panel described the samples as sour and realized a strong loss of a typical odour of meat. After 72h of incubation the samples reached values around 107 cfu and the panel's descriptions changed to "sweet" and less "sour". Samples with a cfu between 108 and 109 were even not recognized as Bologna type sausages anymore. These results were confirmed by GC-MS and GCO, which evidenced amongst others, an increase of acetic acid and ethanol, followed by a significant increase of acetoin, typical bacterial fermentation products. Possible interactions between microorganisms involved in spoilage have to be investigated. The impressing correlation between microbiological, GC and sensory data will lead for sure to an extension regarding matrices and relevant strains.



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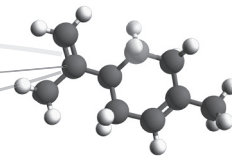
**QUANTITATIVE STUDY OF ESTER RECOVERY DURING THE
CONCENTRATION OF CASHEW APPLE (ANACARDIUM
OCCIDENTALE L) JUICE IN A THERMAL-SIPHON TYPE
EVAPORATOR OPERATING IN A CLOSED SYSTEM**

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Keywords: cashew apple, volatile compounds, Osme, gas chromatography-mass spectrometry, water phase

During the concentration of cashew apple juice, the esters, one of the most important chemical classes contributing to beverage aroma and flavour, are lost by evaporation, reducing the sensory quality of the concentrate (1). These volatiles can be recovered by condensation and subsequently added back to the processed juice, improving its sensory quality. Hence a qualitative and quantitative knowledge of the evaporation and recovery of the esters during juice concentration, as yet little studied, is fundamental. The objectives of the present research were to identify and quantify the loss and recovery of esters during the different steps of cashew apple juice concentration. Three and a half litres of fresh juice were concentrated in a thermal-siphon type evaporator at 35°C, operating in a closed system under vacuum (700mmHg), condensation occurring at 2°C. Two condensates were produced: one containing volatiles recovered during concentration from 10.6 °Brix (fresh juice) to 12 °Brix, and the other during concentration from 10.6 °Brix (fresh juice) to 40 °Brix. The volatiles in the headspaces of the condensates were stripped by vacuum (70mmHg) to a Porapak Q trap during 2h, eluted with 300 µl of acetone, identified by GC-MS and quantified by external standardization using 12 standards representing the following chemical classes: esters, aldehydes, alcohols, ketones, terpenes, acids, lactones and sulphur compounds. The odoriferous importance of the isolated volatiles was assessed by GC-olfatometry using the Osme technique (2). A total of 581.28 mL condensate was recovered during concentration from 10.6 to 12.1 °Brix, and 2307.14 mL from 10.6 to 40 °Brix. The first condensate contained 580.31µg/L of esters, representing 90% of the total mass of volatiles present in the sample. The esters ethyl hexanoate (158.45µg/L), ethyl isovalerate (137.47µg/L), ethyl crotonate (89.34µg/L), ethyl butanoate (52.49µg/L), ethyl 2-methylbutanoate (31.57µg/L), ethyl propanoate (30.59µg/L) and isoamyl acetate (14.03µg/L), together represented 80% of the total mass of volatiles recovered in the first condensate. Twenty-nine odiferous volatiles were identified in this condensate by GC-olfactometry (Osme), of which 21 were esters, the following showing the greatest odour impact: ethyl crotonate (cashew), ethyl butanoate (cashew), ethyl isovalerate (fruit/sweet), ethyl hexanoate (fruit/floral), ethyl 2-methylbutanoate (cashew/strawberry), propyl acetate (sweet/fruit), methyl 3-methylpentanoate (sweet/grassy), 2-methylpropyl acetate (fruit/grassy), ethyl octanoate (grainy/mould) and methyl 2-methylbutanoate (fruit/cashew). The condensate obtained

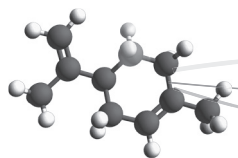


during concentration from 10.6 °Brix to 40 °Brix contained 46µg/L esters, also representing about 90% of the total mass of volatiles present in the sample. All the esters were present at much lower concentrations than in the first condensate, including those of greater odoriferous importance, such as ethyl crotonate (5.64µg/L), ethyl butanoate (4.62 µg/L) and ethyl isovalerate (14.28µg/L). The results indicated that the esters represented the major class of volatiles recovered in the condensate during cashew apple juice concentration. It appears that the majority of the esters present in the cashew apple juice evaporated at the beginning of concentration, suggesting that the recovery of volatiles during the rest of the process only dilutes the material, without significantly altering the quantitative and qualitative profiles of the esters present in the final condensate.

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Acknowledgements: to CAPES (AUX-PE-PNPD-1470/2008) and FAPESP (2008/55986-0) for the financing support.



THE POTENTIAL USE OF RAW AND DEODORISED NON-CONVENTIONAL PROTEIN POWDER IN HUMAN FOOD

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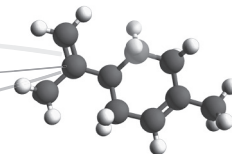
Keywords: food fortification, non-conventional proteins, deodorised proteins, sensory evaluation.

Eisenia foetida (Ef) protein powder is a non-conventional source of protein which contains about 62% of protein and has a good composition of essential amino acids (1). Medina *et al.* (2) demonstrated that the Ef proteins were not toxic to human cell lines at low concentrations. Cayot *et al.* (3) characterized the organoleptic and functional quality of this protein powder and found it had a low solubility and a strong off-flavour. The present work aims to evaluate the potential use of an Ef protein powder in human food. Because of its low solubility, it could not be used for food fortification in gels, foams or emulsions. Cookies were chosen as fortified food. Sensory analyses were done in order to determine the acceptable threshold of fortification of cookies with Ef protein powder.

A first part of this work aims to verify the acceptability of the fortified food using Ef protein powder and masking agents. Two kinds of fortified cookies were prepared: cocoa cookie and cocoa/cinnamon cookie. They were evaluated by a panel of 70 students and staff recruited from the University of "Los Andes" Merida, Venezuela. The participants were volunteers and they were informed that the aim of the study was to taste cookies that may contain earthworm protein powder. The results of this study have shown that cocoa cookie can be fortified with a substitution level of 3.87 % (w/w) of Ef proteins and that cocoa/cinnamon cookie can be fortified with a substitution level of 5.16 % (w/w) of Ef proteins.

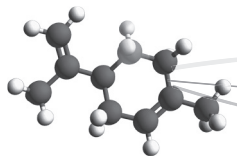
A second part of this work aims to verify if the acceptability of the fortified food could be improved by reducing the off-flavour of the Ef protein powder. Auto oxidation of lipids is the main origin of the odour active compounds of the Ef powder (4). A delipidation method already

optimised (5) was used to prepare deodorised protein powder. Cookies fortified with raw and delipidated powders were prepared. Triangle olfactory sensory tests have shown a significant difference between these two types of cookies. In order to determine the acceptable level of fortification, cookies fortified with delipidated powder were evaluated by olfaction by a panel of 12 students recruited from the engineer school AgroSup Dijon. Each panel classified 7 samples from the worst to the best preferred. The best substitution level was evaluated at 5.60 % (w/w) of protein powder. This result is acceptable since, in this second experiment, the prepared cookies do not contain any masking agent like cocoa or cinnamon.



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3.19

DIRECT FLAVORING OF FOOD SUBSTRATES BY USING IN-SITU STEAM DISTILLATION AND PRESSURIZED INFUSION

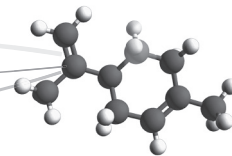
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Keywords: Aromatic plants, simultaneous steam distillation-cooking, pressure infusion-profusion

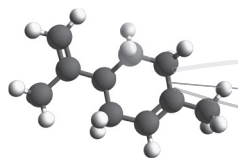
Molecular Cuisine is known as a new style of cooking embracing science, research, technological advances in equipment and various natural gums and hydrocolloids produced by the commercial food processing industry. It allowed to create numerous deconstructed foods and surprising textures (alginate pearls, espumas, solid food after treatment by liquid nitrogen, ...) which were presently criticized. Modern Cuisine was based on the use of cooking techniques allowing healthy homemade recipes preparation, especially steaming. But unfortunately according to consumers, prepared dishes were flavorless. Recently, Progressive Cuisine introduced the direct use of essential oils as a creative way to add flavor to soup, stew or salad dressing while Environmental Cuisine recommended the use of underutilized aromatic plants and vegetables for flavoring dishes. But due to the high concentration in aromatic compounds of essential oils, their use in cuisine could be problematic and even deceptive while as forgotten plants being not cultivated, their use depended exclusively of their harvesting. In the framework of the GASTRONOMIC program, we first focused on the development of various headspace concentrators devices for better characterizing native flavor compounds from Midi-Pyrénées regional medieval (aka forgotten) aromatic plants, especially *Achillea millefolium*, *Agastache foeniculum*, *Meum athamanticum*, *Chrysanthemum balsamita*, *Calamintha grandiflora*. With the objective to study plant aroma “from the field to the fork”, these devices were: i) HeadSpace Bell Jar (HSBJ) for collecting volatiles emitted by uncrushed living plants in field, ii) Artificial Crushing Finger device (ACF) and Flash Aroma Dispenser (FAD) based respectively on a mechanical and a gas flash pressure crushing of leaves in order to capture native volatile compounds emitted after exposure of essential oils storage vesicles; iii) HeadSpaceDome (HSD) for studying aromatic impact of such plants or their respective essential oils when they were added to dishes by collecting emitted volatiles directly in the plate (1). In the present paper, we reported the second part of the program, i.e. a combination of the four cuisine concepts previously described, in order to produce in « one pot » flavored food either solid nor liquid. To reach these goals, two different approaches were developed : i) a simultaneous steam distillation&cooking process, ii) an infusion&profusion processing using gas flash-pressure. The first one was based on the use of an electrical steamer in which the generated essential oil from aromatic plants by steam distillation was directly adsorbed on a steamed solid food substrate (rice). The second ones



used a cream whipper filed with edible liquid and fresh or dried aromatic plants pressurized with gas cartridges in order to express essential oils under cold and pressure conditions. Different kind of rices (easy cook, round, glutinous or basmati), various liquids (oil, vinegar or sugar sirup) and gases (nitrous oxide or carbon dioxide) were studied . Major volatiles and odor active compounds emitted by flavored products were analyzed by HSD-SPME-GC-MS and SPME-GC-O. Various experimental processing (cooking or infusion time, minerality of waters, ...) and sampling (trapping temperature and duration, SPME fibers types, ...) parameters were optimized.

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3.20

AGROREFINERY CONCEPT APPLIED TO MYRHIS ODORATA L: SEASON-DEPENDENT VARIATION IN ESSENTIAL OIL COMPOSITION AND ANTI-OXIDANT ACTIVITY OF EXTRACTION BY-PRODUCTS

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Keywords: Aromatic plants, essential oil, season dependent, antioxidant activity, by-products valorization

An increasing demand for essential oils as natural food additives (flavorings, conservatives,...) generates a great interest for both sustainable culture of aromatic plants taking in account bioaccumulation of secondary metabolites vs season and global valorization of vegetal matter including extraction by-products use. The agrorefinery concept, i.e. valorization of the entire plant by sequential extractions of molecules of interest, fits with these objectives. So, in the present paper, Sweet Cicely (*Myrrhis odorata*) belonging to the Labiatae family, a perennial herb having a strong and penetrating anise odour which is presently used in fresh state as a condiment in French “Nouvelle Cuisine”, was selected as model plant for application of agrorefinery concept to aromatic plants (1).

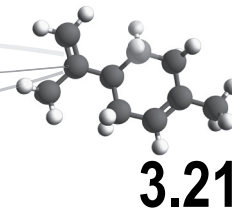
First, essential oils of Lithuanian sweet cicely are extracted by hydrodistillation from leaves collected at different growth times and compared to French ripe sweet cicely. The constituents of essential oil have been analyzed by GC-FID and GC-MS allowing identification of four volatile compounds. Essential oils were characterized by high content of E-nerolidol, which varied from 10.1% to 43.6% of the total volatiles content.

Then, in order to study the chemical potential of extraction by-products, the residues obtained after hydrodistillation were separated into liquid and solid fractions. The solid fraction was dried and then extracted in cascade with acetone, methanol and ethanol, while the liquid fraction (aromatic waters) was freeze-dried and spray-dried. The antioxidant potential of different plant extracts and pure compounds can be measured using numerous *in vitro* assays. Two main types of antioxidant activity tests were employed: i) assays to evaluate oxidation of fats, oils and other fat-containing foods (Oxipress); ii) assays to evaluate radical scavenging activity in model systems (DPPH, ABTS, FRAP). The antioxidant activities were expressed as gallic acid equivalents (GAE) to standardize these methods and to allow data comparisons. Results clearly stated that methanol and ethanol extracts presented highest antioxidant activities.

If chemical compositions varied throughout the plant vegetation, highest values for both essential oil extraction yields and by-products phenol contents, and the correlated free radical-scavenging activity, are reported for sweet cicely leaves collected at flowering stage.

References:

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RETENTION OF CAVACROL AND ETHYL ACETATE IN AQUEOUS GELS OF POTATO STARCH AND KONJAC GLUCOMANNAN

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Keywords : konjac glucomannan, potato starch, partition coefficient, PRV, flavour control

When formulating a food product, great attention must be devoted to flavour control because it is affected by the interactions taking place between the food components. In this context, the present study aimed to measure the retention of flavour compounds in gels based on potato starch and konjac glucomannan (KGM).

The KGM is a neutral polysaccharide which has been introduced into Europe (E425) as a food additive due to its gelling and emulsifying properties (1-2).

The addition of any hydrocolloid to starch is known to increase the viscosity of starch and influence its swelling and retrogradation. Various authors (1- 3) found that the high water holding capacity of KGM prevented syneresis occurring in starch gels and slowed down the retrogradation rate of starch during longer storage.

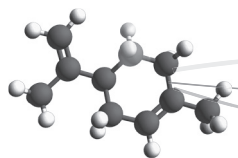
Additionally, starch forms interactions with small molecules such as aroma compounds. More precisely, amylose, one of the starch polymer, is able to form semicrystalline complexes which result in a high aroma retention (4). Each parameter affecting swelling and retrogradation is potentially affecting these interactions and, as a consequence, is affecting flavour retention and release.

In the present study, two aroma compounds were selected : ethyl acetate (non forming complexes with amylose) and carvacrol (forming complexes with amylose). These aroma compounds were added to different matrices : water, KGM, potato starch, and starch – KGM mixtures. The addition was done either before starch swelling or at the very beginning of retrogradation.

Polysaccharides mixtures were prepared in a RVA® device allowing a reproducible preparation process through controlled parameters such as time, temperature, stirring, and viscosity.

The retention of aroma compounds in these mixtures was estimated at thermodynamic equilibrium by the PRV (phase ratio variation) method which is based on the relationship between the partition coefficient K and the phase volume ratio in the vial (gas phase volume/matrix volume).

After preparation, the mixtures were sampled and stored at 25°C for 24 h for equilibration. During sampling, different quantities of each mixture were poured into headspace vials. Four repetitions were done per volume which represented 28 vials for one measurement of the K



coefficient. The reported values were obtained by averaging the results of four series and analysis of variance was used to determine significant differences ($p < 0.05$). As foreseen, retentions of both aroma compounds were increased in the presence of starch as compared to water.

When adding KGM, the results obtained for the two aroma compounds were differing:

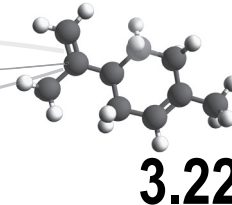
- the retention of carvacrol, which was forming complexes with amylose, was not modified by the presence of KGM
- the retention of ethyl acetate, which was not forming complexes with amylose, was lowered by the presence of KGM.

In starch suspension, we also found that the retention of carvacrol was better when added at the very beginning of retrogradation. The retention of ethyl acetate by starch was not depending on the moment of its addition into the mixture.

The retention of the aroma compounds added in the starch-KGM mixtures was clearly driven by the interactions with starch.

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PRODUCTION OF 6-PENTYL- α -PYRONE BY *TRICHODERMA HARZIANUM* USING BRAZILIAN ESPRESSO COFFEE GROUNDS

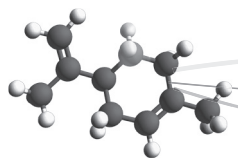
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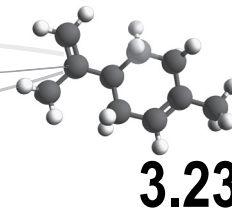
Keywords: 6-pentyl- α -pyrone, aroma, solid state fermentation, espresso coffee grounds

Demand for flavor compounds has increased substantially in the last few years. The 6-pentyl- α -pyrone (6-PP) compound is a lactone with a characteristic coconut-like aroma and approved by the Food and Drug Administration (FDA) for food usage. The increasing demand for flavors production and the consumers preference for natural flavors, rather than synthetic, increased the interest for aromas production by biotechnological route. Recently, solid state fermentation (SSF) had been used as a model for the study of metabolism and physiology of microorganisms. It is well known that the filamentous fungus *Trichoderma harzianum* produces the 6-PP as a volatile secondary metabolite in both liquid and solid state fermentation (SSF) (1,2,3). The aim of this work was to quantify a 6-PP production by *T. harzianum* using Brazilian espresso coffee grounds as support for solid state fermentation. The coffee grounds were dried in a tray dryer at 60 °C until the initial moisture being reduced to 3%. *T. harzianum* 4040 was cultured in a medium PDA enriched by MgSO₄ and CaCO₃ at 28°C for 7 days when spores were suspended in physiological saline solution (0.9% NaCl). SSF was carried out by taking the solid support placed in 250mL Erlenmeyer flasks and impregnated with nutrient solution containing (g l⁻¹): dextrose (30), (NH₄)₂SO₄ (0.94), MgSO₄ (5.0), KH₂PO₄ (1.0), KCl (5.0), CaCl₂·2H₂O (0.008), FeSO₄·7H₂O (0.01) and ZnSO₄·7H₂O (0.001). The flasks were inoculated with 1mL of the spores suspension and incubated at 28°C without stirring. Headspace solid-phase microextraction coupled with gas-chromatography mass-spectrometry (HS-SPME-GC/MS) was used to quantify the 6-PP produced in SSF by calibration curve using standart 6-PP. The compound was extracted on days 3, 5, 7 and 9 of SSF using polydimethylsiloxane (PDMS) fiber with addition of 25% (w/v) NaCl solution at 80°C with stirring. Hence, the fiber was thermally desorbed during 5 minutes at 240°C. The quantitative analysis was carried out using a gas chromatograph (Agilent 6850) coupled to a quadrupole mass spectrometer (Agilent 5975C) with ionization by electronic impact (70eV) and a DB5-MS capillary column (30m×0.25mm×1μm). The kinetic production results showed that the production reached a maximum of 3.40 mg 6-PP/g dry mass in the fifth day of solid state fermentation. The central composite design is being used to optimize the SSF conditions.



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FLAVOR STUDIES ON THE ELABORATION OF ARTISAN TYPE MEXICAN BEER: EFFECT OF DIFFERENT CONDITIONS OF HOPS ADDITION

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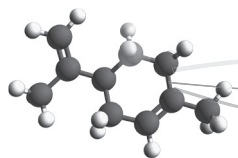
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Beer's physical and chemical properties have been described through analytical laboratory methods, the same as the assessing of expert tasters. Meilgard's flavour wheel (1) has become a reference descriptive system for beer sensory evaluation. Nowadays there is plenty of information that has been generated by beer international institutes (2,3), which could support a reference system for establishing the relation method-compound-sensory descriptor. During the last five years, Mexican artisan beer industry has experienced an accelerated growing, where producers have joint in a society (ACerMex) whose objective is to impulse their consolidation through improving quality standards in collaboration with research institutions. Since 2009, the Universidad Autonoma Metropolitana has been working on setting up a prototype facility to carry out research directed to support artisan Mexican beer producers. The first study consisted on the evaluation of the effect of three different alternative conditions for hops addition (*Humulus lupulus*, var. Saaz) during must cooking, on selected physicochemical properties, volatile composition, sensory perception and the iso-humulones profile as bitterness responsible compounds. Volatiles were analyzed by GC-MS with a previous collection using a SPME device, while iso-humulones were analyzed by HPLC. Sensory evaluation was carried out through Quantitative Descriptive Analysis (QDA, 1) complemented with standard references of international beer organizations (2,3). Color, alcohol contents, bitter index and pH were also analyzed.

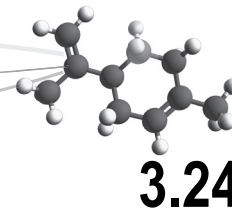
Results showed the presence of volatile compounds related to beer aging (4,5) the same as those important for flavor impact such as isoamyl acetate, isomayl alcohols, ethyl esters among others. Regarding iso-humulones, the concentration of isomerized compounds was related to the time that hops were exposed during must cooking. Relationships between physicochemical parameters, volatile and sensory descriptions were explored in order to characterize the profile of the resulting beers, and to explain these effects on the behavior of different hops related compounds. A better understanding of the relationship between the physicochemical composition and sensory evaluation, all of them using methods adapted and validated for artisan Mexican beer, will enable a reference system to characterize beer sensory quality as a powerful tool for industry to carry out product diversification and technological innovations, but preserving the distinctive sensory character expected in artisan beer.

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CLEAN LABEL FATTY CHICKEN FLAVOUR

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The postulated primary reactions for meat flavour development on heating include pyrolysis of amino acids and peptides, degradation of carbohydrates, ribonucleotides, thiamine and lipids, and interaction of sugars with amino acids or peptides. It has also been reported that heating of the lean portion of beef, pork, chicken and lamb resulted in non-species-specific meaty flavour, while heating of the fat in meats led to species-specific flavours (1, 2). Hornstein and Crowe (3) were among the first to report that the fat, and more specifically the carbonyl compounds resulting from lipid oxidation, contributed to differences in flavour among species. Ramarathnam et al. demonstrated that the presence and absence of certain carbonyls, or the differences in their concentrations in the volatiles among cooked beef, chicken and pork, can be a major contributory factor to the differences in the aroma nuances observed in them (4). Finally, carbonyl compounds formed by peroxidation of unsaturated acyl lipids have been discussed by Minor et al. as important contributors to the “chicken-like” aroma, since their removal from the volatile fraction produced during the heating of chicken meat resulted in a loss of the “chicken odour” and in an intensification of the “meaty odour” (5). These results were later confirmed by Kerler and Grosch (6).

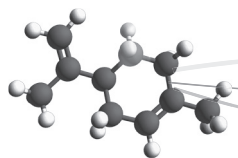
Although heating of animal fat produces species-specific flavours, the fact that these flavours are based on animal derived raw materials imposes severe issues. In a number of cases these flavours will not be kosher or halal, making them unsuitable for a large group of potential consumers. Because of their animalic origin, these flavours will also not be vegetarian or vegan. Finally, a number of food crises in recent years (e.g. BSE and birds flu) have urged food manufacturers to look for flavours that are based on raw materials from non-animal origin.

In an attempt to generate clean label species-specific fatty-type flavours we have turned to vegetable oils and fats. Adequate blending of vegetable oils and fats readily available in bulk quantities, produces a mixture that closely mimics the fatty acid composition of chicken fat. Upon oxidation of this blend, chicken-specific fatty-type flavours can be generated.

Next to generating the desired flavour profile, keeping the flavour in a chemically stable form that is physically easy to handle is another challenge. It has been observed that reacting the oxidised oil blend with a yeast autolysate through DSM’s proprietary DYSC technology produces a powder that still possesses the typical fatty chicken note and is stable for extensive periods of time. The powder efficiently protects the oxidation sensitive materials and thus prevents the flavour from further oxidation.

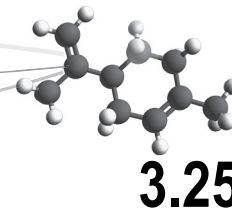
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DETERMINATION OF VOLATILE COMPOUNDS FOR THE ASSURANCE OF QUALITY, SECURITY AND HEALTH IN THE USE OF ALIMENTARY OILS AND ITS APPLICATION TO HOME APPLIANCES INDUSTRY

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Keywords: home appliances, SPE, frying odour

Competition in home appliance industry is very tough; therefore, companies must make efforts to develop innovative products, which satisfy consumer needs. However, it is important to take into account that the careless use of some home appliances could cause some troubles such as kitchen fires or unpleasant odours and it would be quite interesting to control or avoid these kinds of consequences.

Most common home appliances are used for cooking. There are several cooking methods, but frying is probably the most ordinary. In this method oils and fats are used at high temperatures suffering different reactions generating volatile compounds from several chemical families. Using repeatedly the same oil fraction or using oil at too high temperature causes unpleasant organoleptic properties in food and it can also have repercussion in the environment. In addition, some compounds generated in this process could be toxic (1,2).

Understanding conditions where responsible compounds of unpleasant odours of frying process are originated could be useful with the aim of developing a device for kitchens which doesn't allow reach such conditions, and therefore this kind of compounds will not be generated.

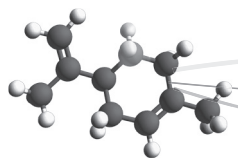
Thus a procedure based in solid phase extraction (SPE) has been developed. With this technique the fume generated while oil is heated is retained in a cartridge of LiChrolut EN sorbent. The trapped volatiles are eluted with a mixture of dichloromethane and 5% of methanol.

A total number of 29 different compounds have been identified. Their identity has been confirmed by comparison of their mass spectrum with the spectrum library, by identification with a chemical standard and/or Kovats index. Compounds belonging to different chemical families have been found, for example: carbonyl compounds, alkanes or acids.

Once compounds have been identified, a study has been made to evaluate the contribution of each compound to the characteristic flavour generated in the frying process.

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3.26

NITROGEN SUPPLEMENTATION OF SUGAR CANE MUST: EFFECTS ON FLAVOUR AND GENE EXPRESSION PROFILES IN CACHAÇA FERMENTATION

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Keywords: flavour, yeast, nitrogen, cachaça

Cachaça is a traditional Brazilian alcoholic beverage obtained by distillation of fermented sugar cane juice. Apart from ethanol and carbon dioxide, organoleptic substances are produced during fermentation that impart subtle and complex flavours¹. Among these, higher alcohols and esters are the most important aroma compounds because they confer floral and fruity notes, highly desired in fermented beverages².

It is widely accepted by the Brazilian cachaça producers that nitrogen complementation might be critical to maintain the fermentative activity of yeast during the fermentation process. However, there is no information about the effect of this factor on the volatile composition and sensory properties of the fermentation product.

The objective of the present work was to investigate the influence of nitrogen supplementation on the accumulation of higher alcohols and esters and on the expression levels of genes in the corresponding biosynthetic pathways. Studies were performed in small-scale fermentations using sterilized natural sugar cane juice and the commercial Brazilian yeast strain JP1. Results of the nitrogen supplemented conditions LAS (low ammonium sulphate), HAS (high ammonium sulphate) and ILV (isoleucine, leucine and valine) were compared to those obtained with non-supplemented sugar cane must.

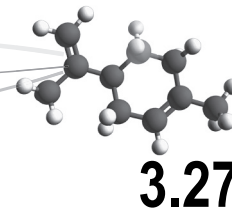
This work shows that LAS complementation significantly increased the accumulation of 3-methylbutyl and ethyl acetate esters. The effect was different in the HAS condition where the accumulation of 3-methylbutanol and its corresponding acetate ester was reduced while that of ethyl octanoate was increased. In the ILV condition, 2-methylbutyl and 3-methylbutyl alcohols as well as ethyl hexanoate and octanoate esters accumulation was significantly increased while that of ethyl and particularly 3-methylbutyl acetate esters was reduced.

The expression of genes coding for decarboxylation activities was globally reduced, particularly that of *PDC5* under HAS and ILV supplementations. *ARO10* transcript was considerably induced in these conditions. Among alcohol dehydrogenase genes, only the ILV condition significantly induced *ADH4* and repressed *ADH3*. With respect to esters biosynthesis/ hydrolysis, *EEB1* expression was induced in HAS and that of *IAH1* in both HAS and ILV conditions.

Our results show that the type and level of nitrogen supplementation affect the flavour and gene expression profiles in cachaça fermentation and reveal correlations between them.

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EVALUATION OF GAMMA AND ELECTRON-BEAM IRRADIATION ON THE AROMATIC PROFILE OF BLACK TRUFFLE (*TUBER MELANOSPORUM*) AND SUMMER TRUFFLE (*TUBER AESTIVUM*)

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Key words: aroma compounds; *Tuber melanosporum*; *Tuber aestivum*, electron-beam irradiation and gamma irradiation

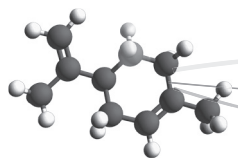
Changes in aroma composition of truffles (*Tuber aestivum* and *Tuber melanosporum*) after electron-beam and gamma irradiation at doses of 1.5 and 2.5 kGy were investigated by solid phase microextraction methodology (HS-SPME). In particular, the effect was evaluated on specific compounds chosen for their aromatic importance according to previously collected olfactometric data. Under this criterion, there are eleven relevant odorants in the aroma of the *Tuber melanosporum* specie and only four in the case of *Tuber aestivum*. The presence of dimethyl sulfide and dimethyl disulfide as important odorants in both species is worth noting. However, it should be remarked the exclusive presence of methional and p-cresol in summer truffles (*Tuber aestivum*).

The main aim of this study is to evaluate the effect of electron-beam and γ -irradiation on the aroma of different truffles. Some changes were produced by these treatments, although none were enough to be seen in a sensory test. Data obtained from these analyses provided relevant conclusions. Some of these findings are presented below.

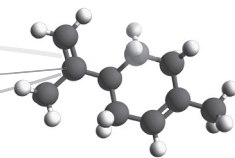
In the case of *Tuber melanosporum* there has been a great variability between all the samples chosen for this study. In spite of this lack of homogeneity, it has been appreciated as the electron-beam treatment induces important changes in the aromatic profile of this type of truffles, while γ -irradiation didn't make any significative change. Most different samples had been irradiated with doses at 1.5 kGy of electron-beam.

On the other hand, in the case of *Tuber aestivum*, γ -irradiation was the treatment that has induced the greatest aromatic differences in comparison with the non irradiated samples (that have been considered as control). Again, the dose of 1.5 kGy has had the greater impact on truffles aroma.

It has been known that irradiation process implies an oxidative degradation of fatty acids (lipidic oxidation). According to this fact, it has been evaluated the presence or no of

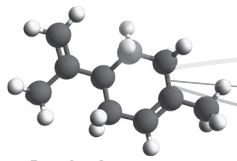


some aldehydes which come from this degradation. As a result of this complementary research, some of these carbonyls, (as is the case of hexanal, E,E-2,4-nonadienal), showed higher levels in the irradiated samples mentioned previously: In the cases of *Tuber melanosporum* treated with electron-beam (1.5 kGy) and in *Tuber aestivum* treated with γ -irradiation (1.5 kGy).



Topic:
Flavour systems

**Poster
Presentations**



**BEER VOLATILE COMPOUNDS FORMATION AT DIFFERENT
FERMENTATION TEMPERATURE USING IMMOBILISED YEASTS**

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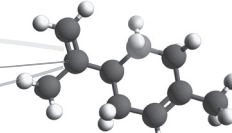
Keywords: beer, yeast, *Saccharomyces*, flavour, volatile by-product, calcium pectate, κ -carrageenan;
DEAE-cellulose

Beer flavour is the result of a complex combination of components that give each brew its distinctive personality. Yeast metabolism makes an important contribution to flavour. Higher temperatures increase the rate of yeast metabolism but the quantitative influence of a temperature change will be different for each biochemical reaction, changing the balance of flavour compounds. The production of bottom fermented beer at lower temperatures is in recognition of the fact that fermentation at temperatures above 14°C results in a product with significantly poorer aroma and taste. Immobilised cell systems increase productivity and improve the economy of bioprocesses, but also influence yeast metabolism and consequently, beer flavour.

The aim of this study was to determine the influence of fermentation temperature and immobilisation on fermentation parameters and beer quality in the first step of wort fermentation using the bottom fermenting yeast *Saccharomyces cerevisiae*. A suitable fermentation temperature of yeast immobilised was sought while maintaining desirable analytical and flavour characteristics of the beer produced.

Beers produced by yeast entrapped in calcium pectate and κ -carrageenan contained lower amounts of diacetyl and higher alcohols at all temperatures studied (from 5 to 20°C). Ester formation was lower at temperatures from 5 to 15°C and acetaldehyde formation at temperatures from 5 to 12°C. The contents of total nitrogen and free amino nitrogen were higher at all temperatures studied due to lower amino acid uptake by entrapped cells. The character of beers produced by yeast adsorbed on DEAE-cellulose at different temperatures was similar to beers produced by free yeast. The concentration of diacetyl in beers fermented by entrapped yeasts decreased as the temperature was increased in all beers. The aroma and flavour of beers produced by yeast entrapped in calcium pectate or κ -carrageenan at temperatures around 15°C were similar to beers produced at lower temperatures using free yeast. Seven of ten tasters considered beer produced using calcium-pectate-entrapped yeast cells at 15°C in our continuous system to be comparable to a beer produced by classical fermentation technology. This fact has high practical significance, because fermentation at higher temperatures is much cheaper and the process using immobilised cells in continuous fermentation can be carried out with significantly reduced residence times.

This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Academy of Sciences, registration number 1/0096/11.



EXPLOITATION OF ORANGE PEEL FOR THE PRODUCTION OF FLAVOUR-ACTIVE COMPOUNDS WITH THE USE OF A COMMERCIAL WINE STRAIN

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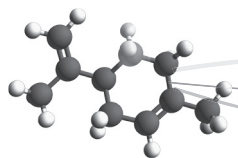
Keywords: orange peel, volatile esters, commercial wine strain, semianaerobic conditions

Flavour is one of the most important factors determining the product's quality and acceptance. Flavour compounds derived from microbial sources is an alternative "bio" product considering that US and European legislations have recognized these products as "natural" (1). Biotechnological production of flavour compounds occurs at mild conditions and does not generate toxic wastes. Because of the growing consumers' demand for "natural" food additives, the commercial importance of biotechnologically produced flavours will certainly grow further in the near future. The main limitations of the process of microbial flavour compounds include the low yield of the final product and the considerably higher market price of natural aromas compared to synthetic ones (2).

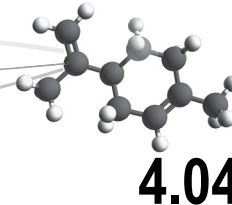
Citrus pulp and peel, the main fruit residues, are currently generated at appreciable quantities in citrus production. Their disposal is correlated with a number of economic and environmental problems. On the basis of the upgrading concept, orange peel offers excellent possibilities to be used as low-cost substrate for the production of value-added products by biotechnological processes, such as single cell protein production (3). Moreover, orange peel contains high amounts of important compounds for microbial nutrition such as sugars, organic acids, proteins, flavonoids, polysaccharides and vitamins.

In the present work, the dynamics of orange peel, a cheap raw material, was examined for the production of flavour compounds with the use of a commercial wine strain (*Saccharomyces cerevisiae*). Experimentation took place to evaluate the effect of orange peel as a supplementary substrate to glucose for bioflavour production in oxygen limited and non limited cultures (semianaerobic vs aerobic culture). Results were compared with those obtained from conventional processes on glucose in the absence of orange peel. The overall flavour formation pattern in distinct phases of the bioprocesses under different fermentation conditions was monitored by GC-MS. Orange peel was found to stimulate the *de novo* synthesis of isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate by *S. cerevisiae*. This was strongly evident in the case of limited oxygen supply under semianaerobic conditions, indicating that oxygen negatively regulates ester production (4). Depending on the volatile ester, a 4- to more than 100- fold increase in yield was observed in the culture containing orange peel with regard to the respective values determined in the presence of glucose as a sole carbon source, with the synthesis of ethyl octanoate, ethyl decanoate and ethyl dodecanoate to be mainly enhanced. The above findings indicate the potential of orange peel exploitation towards the microbial production of value-added flavour-active volatile esters.

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**BIOSYNTHESIS OF VANILLIN: ENZYME INVOLVING THE CONVERSION OF FERULIC ACID TO VANILLIN IN *VANILLA PLANIFOLIA***OSAMU NEGISHI¹ and Yukiko Negishi²¹Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan and²Institute of Nutrition Sciences, Kagawa Nutrition University, Sakado, Saitama 350-0288, Japan

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Keywords: *Vanilla planifolia*, biosynthesis, vanillin, ferulic acid, lyase

Previously we carried out pulse-chase experiments with ¹⁴C-labeled compounds in disks of green vanilla pods. The results suggest the biosynthetic pathway for vanillin is 4-coumaric acid → ferulic acid → vanillin → glucovanillin in *Vanilla planifolia* (1,2). Furthermore, we detected the activity of the enzyme forming vanillin from ferulic acid, supporting this suggested pathway. In order to confirm the conversion of ferulic acid to vanillin in *Vanilla planifolia*, we purified the key enzymes.

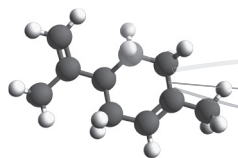
The enzymes for shortening of the phenylpropanoid side chain were extracted with ascorbic acid-K₂HPO₄ solution (pH 7.0) containing 2-mercaptoethanol and Triton X-100 from the acetone powder prepared from the green vanilla pods. The enzymes were partially purified by a combination of DEAE-cellulose, SP-Toyopearl 650M and Toyopearl HW-55F column chromatographies after (NH₄)₂SO₄ precipitation. Activities were measured by the reaction with (¹⁴C-labeled) ferulic acid (or 4-coumaric acid) in a solution containing ATP, dithiothreitol (DTT), MgSO₄ and MES-KOH buffer (pH 6.5), and determination of the resulting vanillin by HPLC. The molecular weights of the enzymes were estimated by HPLC with TSKgel G3000SWXL column.

The enzyme activities were separated into three fractions by cation exchange column chromatography with SP-Toyopearl 650M. Activities were dependent on the presence of DTT.

The optimum pH and the molecular weight of the enzyme in one fraction were approximately 7.0 and 17,000, respectively. The enzyme activity of ferulic acid toward 4-coumaric acid in the same fraction was about 16%, which is higher than that in tissue cultures of vanilla (3). This relatively high enzyme activity suggests the enzyme is able to catalyze the reaction from ferulic acid to vanillin in vanilla pods. The reaction seems to proceed more effectively by the glucosylation of vanillin in vivo (1,2) than in vitro, where the enzyme activities are low. To confirm whether the lyase for ferulic acid is directly involved in vanillin biosynthesis, further investigations activating the enzyme in vitro are in progress.

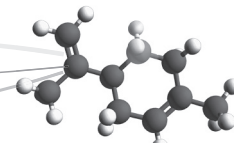
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INFLUENCE OF COMPOSITION (CO₂ AND SUGAR) ON AROMA RELEASE AND PERCEPTION OF MINT-FLAVORED CARBONATED BEVERAGES

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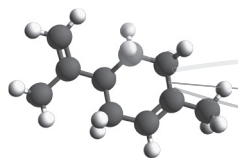
Keywords: soft drink; flavor compounds; sensory analysis; release; nose space

The main sensory properties of soft drinks induced by carbonation are sparkle and effervescence and are responsible for flavour enhancement and refreshing sensation. These properties largely contribute to consumer choices and preferences. A better understanding of the phenomena involved represents thus a real challenge for the food industry. The oral sensations produced by carbonated beverages could either be of chemogenic origin (formation of carbonic acid) or mechanical origin (bursting of CO₂ bubbles that stimulates tongue mechanoreceptors) [1], [2]. However, the composition of the aqueous phase (natural or artificial sugars, hydrocolloids, etc.) may also contribute to enhancing the effects of carbonation due, in particular, to an increase in the surface active charges of the liquid. The presence of carbon dioxide in beverages is known to modify their taste and flavour perception, even if results described in literature can be controversial [3]. And to our knowledge, it has not yet been investigated how carbonation of a beverage influences aroma perception (biological, physicochemical, or sensory origins).

Despite short residence time in the mouth, food beverages undergo changes during consumption (mixing with saliva, temperature increase in the mouth, biochemical reactions) [4]. To better understand retronasal aroma perception, the dynamic aspects of drinking processes have thus to be taken into account.

The present study aims at identifying and quantifying the mechanisms of aroma release that are responsible for their perceptions in the case of the consumption of flavoured carbonated beverages. The original aspect of this study is the use of an integrated approach combining physicochemical and sensory methodologies to investigate the effects of sucrose and CO₂ (the major constituents of carbonated soft drinks). Four panellists were recruited for the study. The influence of beverage composition on the dynamic release of aroma compounds was studied using a proton transfer reaction mass spectrometer (in *vitro* and *in vivo* measurements). During the nose-space measurement of aroma release, subjects scored the perceived overall aroma intensity at three main consumption times: (i) upon introduction of the beverage into the mouth; (ii) when swallowing (6 s after introduction of sample into the mouth); and (iii) 60 s after introduction of the beverage into the mouth (persistence).

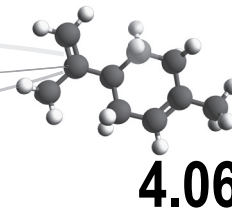
Sensory results revealed that the presence of CO₂ increased aroma perception regardless of the sugar content. In agreement with volatility parameters, *in vivo* measurements showed that carbonated drinks released a greater quantity of aroma compounds in the nose space than non-carbonated ones. CO₂ seemed thus to induce large modifications of the physicochemical mechanisms responsible for the aroma release and flavour perception of soft drinks. Moreover, sugar content seemed to have an impact (increase) on aroma perception only in the



case of non-carbonated beverages. Sensory interactions were thus observed, in particular, between sweet and aroma perceptions. For carbonated beverages, sugar content had an impact only on aroma release, but not on their perception [5, 6].

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EFFECT OF LIPID OXIDATION ON THE FORMATION OF PYRAZINES AND ON COLOUR IN COMPLEX MAILLARD SYSTEMS WITH MEATY-LIKE FLAVOUR

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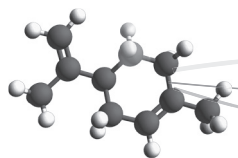
Keywords: Maillard reaction, pyrazine, lipid oxidation, fat, meat flavor

Flavour in food depends upon constituents obtained during its production through biochemical reactions, but also upon changes occurring during thermal processing. Aroma generation in meat, occurring during cooking, results from a complex interaction between volatiles formed within the Maillard reaction and lipid degradation compounds derived from fat oxidation (1). As a matter of fact the Maillard reaction, resulting from the condensation between an amino source and a reducing sugar, and lipid oxidation pathways are interrelated and important flavour compounds formed during Maillard reaction are heavily influenced by the oxidation of lipids (2).

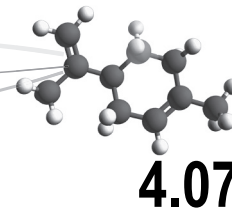
Pyrazines, an important class of aroma compounds formed in the Maillard reaction, are Strecker degradation products with a significant impact on food flavour. Their formation depends on the level and type of dicarbonyls formed in the Maillard reaction, and therefore are sensitive to temperature, time and pH but also the type of amino acid (3). Several studies also reported the influence of the type and level of fat on pyrazines formation in Maillard model systems. Until now, scientists have tried to understand the release of pyrazines in fat systems, the effect of saturation of the fat and the effect of temperature in presence of fat on the formation of pyrazines, but also the interaction between lipid oxidation and Maillard reaction by studying simple model systems containing several degradation products of these reactions (4; 5). However, despite all the research that has been done in this field, no studies have reported the formation of pyrazines in complex model Maillard systems with fat to compare the effect of lipid oxidation on their formation.

In the present study, Maillard complex systems giving a meat-like aroma and containing at least two amino acids and two sugars were used to investigate the effect of pH, temperature and addition of sunflower oil on pyrazines formation and colour. Sunflower oil addition clearly impacted colour formation and increased the level of the ten pyrazines that were identified, although it also changed their distribution. Moreover, it was found that the way fat is inserted has an influence on the interaction occurring between lipid oxidation and Maillard reaction, for instance on colour formation that was found to decrease when adding oil in an emulsion when literature reports an increase with simply adding fat without further mixing. Lipid oxidation and Maillard reaction are two very complex set of reactions and this study constitutes a step forward in understanding how both reaction interact and influence each others.

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INFLUENCE OF THE MANUFACTURING PROCESS ON CHANGES IN THE CONCENTRATIONS OF SELECTED KEY AROMA COMPOUNDS OF DORNFELDER RED WINE

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Keywords: red wine, aroma, molecular sensory science concept, recombine, winemaking process

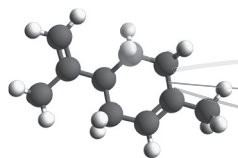
The aroma is an important quality attribute of wine, and thus, the identification of volatile compounds has been a research topic of numerous investigations in the past, and so far about 400 volatiles have been identified (1). It is well-accepted in the literature that, besides the grape variety, the manufacturing process and aging in barrels has a clear influence on the overall aroma of the final product.

In close collaboration with a small winemaker in Germany, red wines were produced from the Dornfelder grape variety by a traditional process. Using the concept of molecular sensory science as a “retro” version, first the key odorants in a finished wine stored in oak barrels were characterized by means of aroma extract dilution analyses and quantitation of the key aroma compounds by stable isotope dilution assays. The non-volatile fraction of the same batch of wine was analysed in parallel by our partners at the chair of food chemistry and molecular sensory science. On the basis of the quantitative data obtained for a total of thirtyfive key tastants and twenty-eight key odorants, a complete flavour recombine of red wine was successfully established (2).

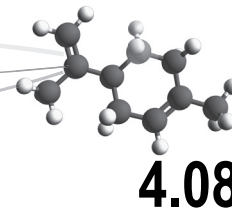
In further experiments, the key odorants in Dornfelder grapes and must as well as in young red wine from the same vintage and grape variety were characterized. By application of the aroma extract dilution analysis, the highest FD-factor among the twenty-three odour-active compounds in the grape distillate was found for (Z)-3-hexenal. 2-Phenylethanol was established as the most potent odorant among the twenty-one aroma compounds in must, and in young red wine the highest FD-factors were assigned to 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol. Selected compounds, identified as key odorants in the final red wine, were then quantified to indicate changes in the most important odorants on the way “from grapes to wine”. As to be expected, for example 2-phenylethanol known as an amino acid metabolite formed during must fermentation, increased by a factor of 300. Altogether the data obtained for thirteen wine aroma compounds allowed a clear conclusion on the formation of single key odorants during the wine making process. Formation pathways from odourless precursors during the manufacturing process will be discussed.

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OXIDIZED GUAIENES AND THEIR RELATIONSHIP TO THE AROMA COMPOUND ROTUNDONE

STACEY BURRETT, Dennis K. Taylor

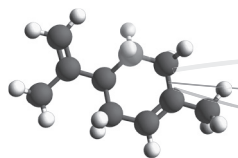
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Keywords: Oxidation, Wine, Guaiene, Rotundone.

Rotundone and guaiene are two of the many known important aroma compounds found in grapes & wine (1, 2). They have a similar base structure and both give a desirable (but differing) peppery aroma (1, 3). Rotundone gives a desirable black pepper aroma to wine and has a very low aroma threshold of 8ng/L in water and 16ng/L in wine, making it easily detected (1). Rotundone was the first compound found in black or white peppercorns to have a distinctive peppery aroma, and with an OAV in the order of 50000-250000, is the most powerful aroma compound yet found in that most important spice (1). Rotundone appears to be an oxidised form of guaiene, so the question arises as to whether other oxidised products of guaiene possess interesting aroma properties? Wine itself is such a complex mixture containing a vast number of compounds, that multiple interactions and reactions occur amongst the individual components (4). This makes it very difficult to determine the origins of compounds and their mechanism of formation. By taking a known grape/wine compound, oxidising it as a single compound and then comparing the products to wine/must/juice removes this difficulty. This research looks at the oxidation of guaiene, in order to identify any new aroma compounds formed and thus determine if such oxidation can account for the formation of naturally occurring rotundone. A variety of different chemical oxidation methods were performed, to simulate what might occur in nature. The process then required identification / characterization of products, and rationalization of the mechanisms by which they form. From this we hope to establish what oxidation processes occur in grapes, must or wine. We also will aim to identify new compounds from grapes, must or wine and to assess the aroma properties these compounds might impart to wine. Results will be presented at the symposium.

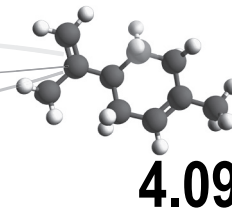
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PRECURSORS TO THE POTENT ODORANT; WINE LACTONE

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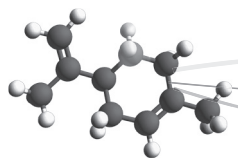
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Keywords: wine lactone, enantiomer, precursors, acid hydrolysis.

Wine lactone (3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one) was originally discovered by Southwell in Koala urine (1). Guth later discovered this compound in white wines, and named it “wine lactone”(2). This monoterpene imparts desirable characters such as *coconut*, *woody* and *sweet* aromas to wine (2). It is an important and potent odorant that is seldom found in wines at more than trace levels (2). Of the eight possible isomers of this compound, only the (3*S*, 3a*S*, 7a*R*) enantiomer (1) has been identified in wine (3). This isomer has been shown to be the most potent of the eight, and is also one of the most potent wine aroma compounds known, with thresholds of 0.02 ng/L in air and 10 ng/L in model wine (3). Studies by Winterhalter *et al.* have shown that there are two possible precursors to wine lactone in wine, these being (*E*) 2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid and its corresponding glucose ester (4). Further hydrolytic studies of the former compound showed that it is, in fact, converted into wine lactone under normal wine conditions. However, upon chiral analysis, the wine lactone obtained was found to contain both the natural isomer plus its opposite (3*S*,3a*S*,7a*R*)-enantiomer in small amounts (5). It has been postulated that this isomer is actually present in wine but went undetected due to its low concentration. Studies of the acid hydrolysis of the glucose ester under normal wine conditions showed that it is not a realistic precursor to wine lactone under these conditions (5). However, it is possible that the glucose moiety could be cleaved by the esterase activity of the yeast used in winemaking producing the free acid which might then be converted to wine lactone *via* acid hydrolysis. Our study concerns the quantification of wine lactone in wine and its chiral analysis. Wine lactone was found in wines of differing variety and age and these samples were analysed for their chirality. The precursor acid was also quantified in grapes and wines and the fermentation of the glucose ester was conducted.

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4.10

CHARACTERISATION OF DRY RIESLING WINES AROMATIC TYPICALITY USING SENSORY AND INSTRUMENTAL ANALYTICAL METHODS - A COMPARATIVE APPROACH

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Keywords: typicality, sensory analysis, varietal thiols, Riesling

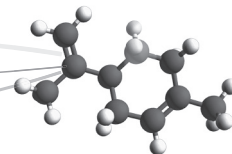
Wines from *Vitis Vinifera* var. Riesling grapes are known for their complex aromatic expression and their distinctive aroma which leads to high recognition among wines from other varietal grapes. It is known that a lot of different aromatic compounds intervene in this sensorial impression. Numerous works have been carried out to reveal marking molecules like terpenic compounds (1,2,3,4), C13-norisoprenoid compounds (5), sulfur volatile compounds (6) and varietal thiols (7).

To our knowledge, no study has investigated the correlation between the sensorial attribute of 'aromatic typicality' and instrumental analytical data obtained from gas chromatographic analysis including varietal thiols so far.

Therefore, in this study a comparative approach has been chosen using the methodology of identification of sensory space described by Ballester and Le Fur (8) as well as using the A-Not-A-Method (9) as an indirect statistical method. In addition the tasters have been asked to name olfactory descriptors.

A series of orthonasal sensory analysis with two expert panels (one German, one French) from 12 to 20 persons was conducted in two locations (Geisenheim, Bordeaux) using the two methods to assess in total 41 wines (Riesling wines from different origins and wines of other varieties) for Riesling wine typicality.

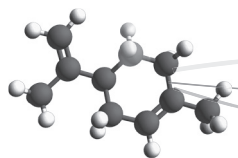
The same wines have been analyzed for different aromatic active compounds like thiols, terpenes and C13-norisoprenoides to correlate their concentrations to Riesling wine typicality. Sensorial results have shown that Riesling wines are significantly distinguishable from other grape varietal wines due to their aromatic expression and they belong to a specific sensorial space. There is also a good consistence of chosen descriptors of both panels after grouping them into major groups so that it could be stated that there is a universal qualitative aromatic profile which correlates with typicality.



The qualitative aromatic profile shows some variability in quantitative intensity of the descriptors, which was measured as relative frequency. This leads to a variable aromatic expression at similar typicality ratings. Therefore, depending on the compounds, the quantitative analysis of the chosen volatile marker molecules presented various levels of correlation with the typicality determined by sensory analysis.

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4.11

DIFFERENCES IN CHEMICAL COMPOSITION OF AROMA AMONG RED WINES OF DIFFERENT PRICE CATEGORY

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Keywords: red wine; aroma; price category

The price of a wine is not decisive for its characteristics and quality, however, a more expensive wine is usually related to a more exhaustive and careful making process. Differences in making can lead to a different chemical composition and sensory notes, therefore wines of the same price category are expected to have some common sensory and chemical characteristics which make them different from wines of other price category.

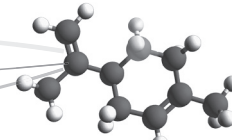
The aim of this work was to compare the chemical composition of aroma of three different price categories of Spanish red wines. To achieve this, 116 odorants were quantified in 96 wines (32 of each price category) by means of 9 different analytical methods.

Significant differences in concentration were found between price categories for 76 compounds by means of ANOVA tests. The group of wines with the highest price (>15€/bottle) presented the highest levels of compounds related to oak aging like eugenol, whiskylactones or volatile phenols extracted from wood. Brett character related odorants, volatile sulphur compounds and oxidation related aldehydes were also found more concentrated in these wines. These observations are in accordance to the long aging process that these extra-premium wines normally undergo (1). On the other hand, low priced wines (<6€/bottle) showed the highest amounts of fermentative compounds like fusel alcohols, fatty acids, ethyl esters or acetates derived from alcohols. It must be taken into account that this category was mainly composed of young wines and compounds formed during fermentation have hardly suffered chemical transformations. Medium priced wines (6-15€/bottle) were in an intermediate situation and only a few compounds like diacetyl were more concentrated in this category.

Moreover a Principal Components Analysis (PCA) allows separating low priced wines from medium and high price categories according to chemical composition of aroma. However, no separation was found between medium and high priced wines.

Finally, differentiation ability of the odorants was measured by comparing median concentrations in each category. Aldehydes like methional and phenylacetaldehyde, aging related compounds like eugenol, Z-whiskylactone and 4-ethylphenol, and some fermentative molecules like diacetyl, 3-methylbutyric acid and methionol presented the greatest differences in concentration between categories.

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LARGE-SCALE ANALYSIS OF GROWTH INHIBITION MECHANISMS INVOLVED IN YEAST TOLERANCE TO 2-PHENYLETHANOL

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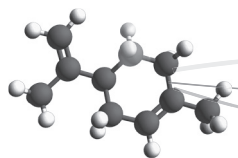
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Keywords: flavour, yeast, tolerance, phenylethanol, large-scale analysis

2-Phenylethanol (2PE) is an important flavour and fragrance compound with a rose-like odour. It occurs naturally in the essential oils of many flowers and plants, especially in rose oil. Most of the world's production is obtained by chemical synthesis with benzene and styrene oxide as starting compounds. Still consumer's increasing preference for natural products drives the expansion of the market of flavours produced with microbial systems. In yeast, 2-Phenylethanol is produced from Phenylalanine by the Ehrlich pathway (1). However, as many flavour molecules, 2PE inhibits the growth of microorganisms limiting hence its production. The purpose of this study were: (i) to compare the inhibitory effect of 2PE with those of its precursors Phenylpyruvate and Phenylacetaldehyde and (ii) to search for cellular targets of 2PE in yeast by a systematic screening of the Yeast Knock-Out library from Open Biosystems (BY4741 genetic background).

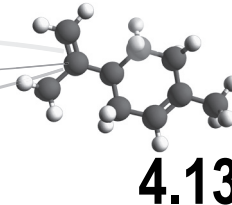
For the first purpose, the experiments performed in liquid and in solid YPD media showed that it is 2PE, the final product of the pathway, which is mainly responsible of the growth inhibition observed during Phenylalanine bioconversion (total growth inhibition at 3 g.L⁻¹). The high-throughput screen was carried out searching for 2PE sensitive and resistant mutants. The mutant candidates identified from the initial screen were arranged into new subsets and submitted to a second screening. After organization of the results into functional classes and categories, a series of mutants were submitted to detailed growth inhibition analysis in liquid and solid YPD media.



As a result of this work, the single-deletion mutants *stb5*, *zwf1* and *gnd1*, among others, were identified as sensitive, whereas the resistant trait found in a few set of candidates turned out to be too weak. *Zwf1p* and *Gnd1p* produce NADPH in the pentose phosphate pathway and are involved in adaptation to oxidative stress. *Stb5p* is an activator of these genes expression (2). Hence the availability of NADPH seems to be directly or indirectly implicated in 2PE tolerance. We performed a backcross analysis of the *stb5* mutant with BY4741 WT strain, which showed a 2:2 segregation for 2PE sensitivity together with the Geneticin resistance marker. This result confirmed that the sensitivity of the *stb5* mutant towards 2PE is due to a single mutation, the deletion of *STB5* gene. This phenotype was reverted by overexpression of *IDP2* gene coding for isocitrate dehydrogenase, an enzyme which catalyzes the oxidation of isocitrate to α -ketoglutarate with the concomitant reduction of NADP^+ to NADPH. Furthermore, the 2PE sensitivity phenotype of the *stb5* mutant was also observed in the CEN.PK genetic background. Moreover, the *stb5* mutant showed no sensitivity to Congo Red, weak sensitivity to Calcofluor White (CFW) and Sodium Dodecyl Sulfate (SDS) and revealed high sensitivity to Caffeine, indicating possible cell integrity defects. These defects might be explained by lower NADPH availability due to impairment of the pentose phosphate pathway and to other possible implications of the transcriptional regulator *Stb5p* in resistance to Caffeine, CFW and SDS or in cellular integrity.

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ENHANCEMENT OF CHERRY JUICE AND WINE AROMA BY YEAST GLYCOSIDASE – THE MODEL STUDIES

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Aroma of fruit juice and wine products represented by several classes of compounds is the most important quality criteria. These compounds are formed by primal flavours originated from fruits, synthesized during fermentation and derived during maturation. In a great number of grapes and fruits a significant part of flavor components is accumulated in the form of non-volatile flavourless glycoconjugates known as glycoside aroma precursors. Due to limited effect of glycosidase from fruits and *Saccharomyces cerevisiae* yeast in wine making many of aroma precursors are still presented in young wine. The effect of yeast glycosidase on flavour recovery from aroma precursors in cherry fruit juice production and in winemaking was studying in the model solutions of wine.

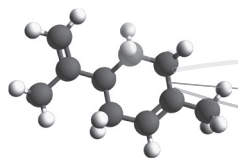
Beta-galactosidase activity on agar plates with arbutin was screened for 33 wine yeasts from LOCK culture Collection at the Institute of Fermentation Technology and Microbiology at the Technical University of Lodz, Poland and for *S.cerevisiae* D4 wine yeast strain from Begerow, Germany. Glycoside fractions were extracted from the samples of cherry juice using Bakerbond spe- Octadecyl columns and the model juice and wine solution was constructed to the one described by Ugliano et al (1). Samples were analyzed by SPME-GC/MS method for aroma compounds.

Almost all tested yeast strains showed beta- glucosidase activity and selected yeast strains of Porzecznica, Deidesheim, Brusznica, Madeira, Pisport and Zeltinger were displayed this activity towards synthetic glycosides, like arbutin, MUG, p-NPG and ability of liberation flavour compounds from natural bounded forms in the winemaking conditions, enhancing wine aroma. Among the wine yeast studied Pisport showed the highest glucosidase activity in releasing aroma substances, like terpenes and benzene derivatives. Also model cherry wines supplemented with extracts of cherry aroma precursors and inoculated with different strains were enriched after fermentation in aroma substances, mainly terpenes and benzene derivatives. Some yeast strains, like D4 didn't displayed beta-glucosidase activity and was used as the control.

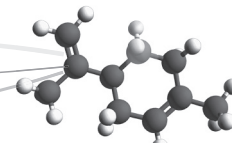
By using selected yeast strains with high beta-glucosidase activity increasing of the total level of volatiles in wines during fermentation compared to control can be achieved enhancing wine aroma and total bouquet.

The research was founded by a PhD grant from the Ministry of Sciences and High education in Poland

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THE EFFECT OF METHYLOBACTERIA APPLICATION ON STRAWBERRY FLAVOUR INVESTIGATED BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY MASS SPECTROMETRY

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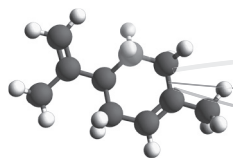
Keywords: strawberry, flavour, methylobacteria, comprehensive GC x GC-qMS

Fragaria ananassa, the cultivated strawberry, represents a very important fruit crop. Strawberries are popular worldwide. The annual worldwide production of this fruit with the very pleasant flavour is estimated to about 3.2 million tons. Strawberry cultivation is very difficult, as - due to their structure - most strawberry varieties show a high risk of deterioration. Varieties with a firm pulp often lack the intense and pleasant strawberry aroma.

The flavour of fresh strawberries is very complex with over 350 identified volatile compounds mainly consisting of esters, furanones, aldehydes, ketones and alcohols. Moreover, the composition of the flavour is highly dependent on the variety, ripeness, climatic conditions, or time of the harvest. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF) with its pronounced

‘caramel’ or ‘cotton-candy’ odour is considered to be one of the most important flavour compounds in the fruits (1, 2). In addition to its odour properties, DMHF is a known flavour enhancer (3). We showed recently that a DMHF increase in strawberries does not primarily lead to an increase in the typical DMHF odour in the fruits, but that it boosts the perceived sweetness and the perceived ripeness attributes of the berries (4).

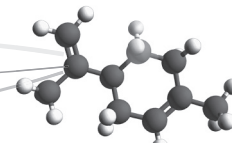
Plant-associated bacteria may fulfil a number of important functions on their host like for example health and growth of the plant, but they might also influence the fruit quality and flavour (5). Methylobacteria have been described to interact with the strawberry callus cultures. In this interaction, the methylobacteria are said to oxidise 1,2-propanediol, which is emitted from the plant itself, to lactaldehyde which is proposed to react with dihydroxyacetone phosphate in a kind of ‘in vivo aldol condensation’ to DMHF as reaction product (6). In our investigations with strawberry plants in the greenhouse as well on the field, we observed an increase in DMHF concentrations in the fruits when methylobacteria were applied on the plant (5). In addition to DMHF analysis, multivariate analysis of aroma profiles (headspace SPME with subsequent GC-MS) of fruits of treated and untreated plants showed a clear differentiation which was not based on DMHF concentrations, as DMHF was not extracted by headspace SPME. Due to the high number of compounds, co-elution was observed in many cases in the one-dimensional GC-chromatogram. As a consequence, comprehensive two-dimensional gas chromatography was applied to strawberries from treated and untreated plants. GC x GC-qMS of the strawberry volatiles will give a more detailed insight into the volatile composition of the



strawberries and will help to understand the reactions that are promoted by the mutualistic association between the strawberry plant and the methylobacteria.

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CHANGES IN THE AROMA COMPOSITION OF SAVELOY INOCULATED WITH THREE POTENTIAL SPOILAGE BACTERIA

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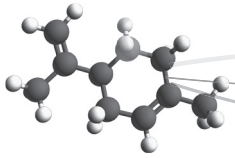
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Keywords: Saveloy, shelf-life, microbial spoilage, aroma composition, chemical markers.

The shelf-life of cooked and sliced meat products is closely related to the development in the aroma composition. During manufacturing the products are subjected to the in-house microbial flora. This microbial flora produces volatile organic compounds (VOC's) that can cause spoilage of the product during storage (1). The most characteristic VOC's could potentially serve as chemical markers for shelf-life. *Chryseomonas luteola*, *Leuconostoc carnosum* and *Carnobacterium piscicola* are potential spoilage organisms (2;3). In this study saveloy (seasoned sausage of minced pork meat) samples were sliced and inoculated with these three bacteria individually and studied in relation to a series of control samples. The inoculated samples were packed in modified atmosphere and stored for three weeks at 5 °C followed by package opening and one additional week of storage with fluctuating temperatures. At seven points throughout the experiment the aroma composition of the saveloy samples was measured using dynamic headspace sampling followed by GC-MS analysis. This enabled a study of the production of VOC's by the three potential spoilage organisms through the shelf-life period.

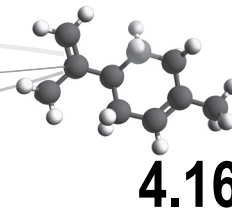
C. luteola and *C. piscicola* both produced elevated amounts of acetoin and diacetyl after package opening compared to the control samples. These compounds have butter-like odours and have previously been described as important contributors to spoilage of meat products (4;5). 2- and 3-methylbutanal and their corresponding alcohols 2- and 3- methylbutanol are also well known microbial metabolites that has also been associated with spoilage. In this study *C. piscicola* produced both 2- and 3- methylbutanal and 2- and 3- methylbutanol whereas *C. luteola* only produced 2- and 3- methylbutanol. The production of VOC's from *Leuc. Carnosum* followed a different pattern as neither diacetyl nor acetoin were produced. The most remarkable change in the aroma composition of saveloy inoculated with *Leuc. Carnosum* was an increase in 1-Hexanol during the first three weeks of storage. 1-hexanol appears to be formed by a reduction of hexanal since the amount of this compound is lowered compared to the level in the control samples. A similar pattern was observed for 2-heptanone and 2-heptanol in samples inoculated with *Leuc. Carnosum*.



This study showed that sliced saveloy inoculated with three different bacteria developed different aroma profiles during storage. The production of diacetyl and acetoin by *C. luteola* and *C. piscicola* is likely to cause spoilage of sliced meat products, whereas the impact of the production of 1-hexanol by *Leuc. Carnosum* is considered more uncertain.

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**MICROBIAL CONVERSION OF (±)-LINALOOL TO LINALOOL OXIDES BY
*CORYNESPORA CASSIICOLA***

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Keywords: terpene biotransformation, linalool, linalool oxides, *Corynespora cassiicola*

Linalool oxides are interesting for the flavor and fragrance industry because of their lavender notes. The production of pure (2R) or (2S) configured linalool oxides is preferable, because this stereocenter determines their olfactorial properties (earthy, leafy or floral, creamy).

The recently described biotransformation of (±)-linalool to linalool oxides by *Corynespora cassiicola* DSM 62475 [1] was further investigated.

While the use of racemic substrate (±)-linalool resulted in (2R) and (2S) configured linalool oxides, it was possible to produce nearly pure (2R) configured products ($ee \geq 90\%$) from (R)- (–)-linalool, which is available from natural sources with high enantiomeric purity. In fed- batch shaking flask cultures, product concentrations above 1 g l^{-1} were obtained. Substrate limitation was shown to be the key factor limiting the productivity, which was about $80\text{ mg l}^{-1}\text{d}^{-1}$. For both linalool and linalool oxides, severe growth inhibition occurred for concentrations above 450 and 800 mg l^{-1} , respectively.

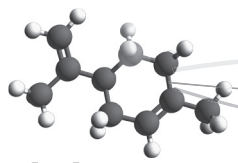
This led to the development of a combined *in situ* substrate feeding and product removal (SFPR) approach. The polystyrene adsorber Lewatit VP OC 1163 showed the highest loading capacity ($0.55\text{ g linalool per g resin}$) while maintaining a subinhibitory substrate concentration (150 mg l^{-1}).

The bioprocess was transferred into a bioreactor and optimized by circumventing substrate limitation via a linalool-saturated air stream. The SFPR process resulted in a more than 4-fold increase in productivity ($920\text{ mg l}^{-1}\text{d}^{-1}$) compared to the fed-batch biotransformation in a bioreactor ($216\text{ mg l}^{-1}\text{d}^{-1}$).

This way, it was possible to load the bioreactor with 10 g l^{-1} substrate of which 42 % were converted to 4.8 g l^{-1} product after 5 days. More than 80 % of the product was found adsorbed onto the resin as well as 35% of the substrate, which can be recycled after elution and separation from the product.

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4.17

**BIOTECHNOLOGICAL PRODUCTION OF NATURAL FLAVORS BY
*SAPROCHAETE SUAVEOLENS (GEOTRICHUM FRAGRANS)***

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Keywords: Biotechnology; Yeast; Flavors; *Saprochaete suaveolens*; *Geotrichum fragrans*; Ethyl Tiglate; 2-phenylethanol

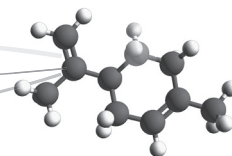
Flavors and fragrans play nowadays an important role in food, cosmetic, pharmaceutical and chemical industries (1). In 2010, the worldwide market of this industry was estimated to US\$ 22 billion (2).

Consumer's preference toward "natural flavors" (3) and the inconvenient of conventional routes for production, such as chemical synthesis and extraction from plants (4), have motivated industrials to study and develop new resource for natural flavor. Since 1923, the microbial production of natural flavors has been extensively studied and many reviews on this fields have been published (5,6,7).

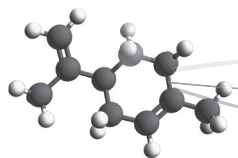
As part of a program aiming at the selection of strains that might be of interest as source of natural flavors, many yeast strains isolated in Reunion island were investigated. One of these strains, namely one isolated from Dragon fruit or Pitaya (*Hylocereus polyrhizus*) and identified as *Saprochaete suaveolens* (formerly *Geotrichum fragrans*), was further investigated for its aroma generation ability's. Bio-flavor production and physiological properties of this indigenous strain were studied in bioreactor and were compared with reference strains such as *Saccharomyces cerevisiae* and *Geotrichum fragrans*. The influence of amino acids in the production of fruity aroma by these microorganisms was also investigated.

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4.18

2-ACETYL-1-PYRROLINE SYNTHESIS DURING RICE PLANT (*ORYZA SATIVA* L.) GROWTH UNDER CONTROLLED SALINITY CONDITIONS

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Keywords: salinity stress, fragrant cultivar, Aychade, 2-acetyl-1-pyrroline, biosynthesis

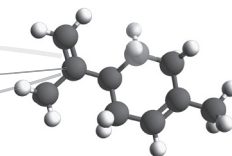
Abstract

Soil salinity in the field during rice plant development (*Oryza sativa* L.) can impact on growth, yield and 2-acetyl-1-pyrroline (2AP) level, a characteristic flavor compound of fragrant rice cultivars (1). The aim of this study was to better understand the synthesis of 2AP in rice plant under controlled salinity conditions. The experiments were conducted in a greenhouse. The soil was treated with a NaCl solution yielding 3500 S/cm electrical conductivity at different growth stages of a fragrant rice cultivar (Aychade). This conductivity was chosen to have sufficiently salt stress without hindering rice grain production. Rice leaves were sampled at 4 growth stages: beginning tillering (DT), middle tillering (MT), panicle initiation (IP) and flowering (FLO) stages. Rice grains were sampled at harvest stage. The quantification of 2AP was performed through a stable isotope dilution assay involving SPME / GC-MS-MS analysis (2). L-proline and gamma amino butyric acid (GABA) taking part in the biosynthesis of 2AP were also quantified in rice samples. Results of growing and yield of rice plant and that of grain indicated that there were no significant changes under salt stress condition. In rice leaves a significant increase in 2AP level was observed mainly for salinity stress applied during DT to IP stage. In contrast, L-proline and GABA accumulation in rice leaves did not change significantly under salt stress. Interestingly, 2AP level increased significantly in rice grains issued from the plants subjected to salt stress at different periods of growth.

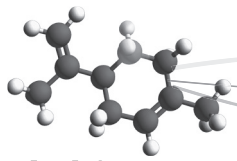
The highest increase was observed for salinity stress during DT to IP and IP to FLO stage, respectively. The data here indicates that salinity as well as the period of salinity stress application on rice plant can have an impact on the 2AP biosynthesis.

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4.19

BIOTECHNOLOGICAL PRODUCTION OF FATTY ALDEHYDES

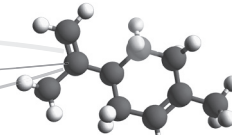
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Keywords: fatty aldehydes, α -dioxygenase,

Fatty aldehydes are an important group of fragrance and flavor compounds with an odor described as fresh, citrus and waxy. Although they can be found in plants, their concentration in oils is rather low.

Here we present a biotechnological synthesis route using an α -dioxygenase most probably responsible for fatty aldehyde biosynthesis via hydroperoxidation of fatty acids in plants. This heme containing enzyme is highly attractive for production of these valuable substances as it is cofactor independent and amenable to overexpression in *Escherichia coli*. Whole-cell biotransformations were performed with resting cells and different even-numbered fatty acids as substrates. In a highly selective manner they are converted to the respective n-1 fatty aldehydes. Our system allows an economically attractive production of many different commercially important fatty aldehydes in a natural way.



SHORT-TERM AND MODERATE UV-B IRRADIATION CHANGES AROMA VOLATILES IN TOMATO FRUITS

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Keywords: aroma volatiles, ultraviolet irradiation, tomato

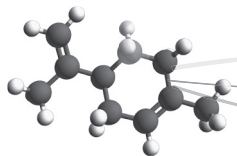
At present, UV radiation is mainly applied to vegetables and food products for the elimination of foodborne pathogens or to delay product ripening. Plants subjected to UV radiation respond with an up-regulation of the plant's protective stress genes. Therefore, short-term and moderate UV-B irradiation could be used as postharvest treatment to increase phytochemicals (1). However, no information is available on the effect of short-term and moderate UV-B irradiation on aroma volatiles in tomato fruits, which strongly contribute to the flavour and thus consumer acceptability. Therefore, the objective of the present study was to determine whether a short-term and moderate UV-B exposure could be used as targeted pre-harvest stress treatment to trigger the biosynthesis of aroma volatiles in tomato.

Tomato plants were grown in a controlled greenhouse environment and a short-term moderate UV-B radiation level of 0.075 W h m⁻² was applied within 10 h using a UV-B fluorescence light source. Thereafter, red fruits remained for 22 h without UV-B irradiation on the plant and were then harvested. Aroma volatiles were determined with stir bar sorptive extraction and GC-MS. Furthermore, carotenoids - known as precursors of few volatiles - were analyzed by HPLC.

Short term UV-B exposure at moderate level increases the biosynthesis of two amino acid related volatiles (3-methylbutanal and the corresponding alcohol 3-methylbutanol) and of two fatty acid derived volatiles (hexanal and 1-penten-3-on) up to 84% in comparison to the control fruits. In contrast, two carotenoid related volatiles (β -ionone and β -damascenone) as well as the lignin related guaiacol decreased. Interestingly, the carotenoid analysis showed that the concentration of lycopene was not affected by moderate UV-B exposure while the concentration of β -carotene slightly increased up to 11%. Generally, the volatiles discussed can contribute to the flavour of tomatoes with green, grassy, unpleasant, sweet, and violet-like odour notes.

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4.21

THE OFF-FLAVOUR OF PEA FLOUR: SENSORY REPRESENTATIVITY OF HEADSPACE EXTRACTS

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Keywords : *Pisum sativum*, Off-flavour, SPME, Purge and Trap, D-GC-O

Pisum sativum represents an economical and nutritional interest due to its protein content. Nevertheless, pea products are underused as protein source in human food because of their organoleptic characteristics. Indeed, the off-flavour, described as “beany”, “green”, “vegetal”, “haylike”...[1], is often evocated as a limiting factor for the use of this protein source, as milk replacer for example [2].

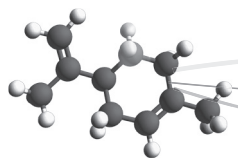
In order to identify the volatile compounds responsible of this off-flavour, it is necessary to analyse the headspace composition of a pea flour suspension. The objective of the present study is to select an extraction method being the most representative for the sensory perception of the off-flavour.

In a first step, two headspace analysis procedures were chosen and optimised: a static method, the Solid Phase Micro Extraction (SPME) and a dynamic method, the Purge and Trap (using Tenax® trap). Various parameters were optimised: temperature and duration for both methods plus gas flow rate for Purge and Trap. The extracts, obtained with optimised parameters, were analysed by Gas Chromatography coupled with Mass Spectrometry (GC-MS). The performance of each method was checked through the number of identified compounds and the sum of their surface area.

In a second step, the sensory representativity of the extracts obtained thanks to the optimised extraction methods was assessed. As proposed by Rega *et al.* [3], Direct Gas Chromatography-Olfactometry (D-GC-O) was used to compare SPME and Purge and Trap extracts to a 10% pea flour suspension in water (used as reference). SPME fibres and Tenax® trap were injected in D-GC-O and 9 panellists ranked the representativity of the perceived global odour on a 10 cm scale (from 0: “very representative of the reference” to 10: not representative of the reference). Results have been statistically analysed by an ANOVA test.

It appeared that the SPME method was more suitable because of its good representativity of the odour of the pea suspension despite the fact that the extracted compounds were less numerous than with Purge and Trap. Future experiments will allow us to identify key flavour- active compounds using SPME-GCO.

References:



4.22

BIOCHEMICAL CHARACTERIZATION OF AN ARYL-ALCOHOL DEHYDROGENASE FROM THE WHITE-ROT FUNGUS PHANEROHAETE CHYRSOSPORIUM

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Keywords: *AAD*, aryl-alcohol dehydrogenase, *P. chrysosporium*, natural flavours and fragrances production

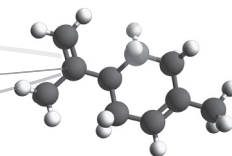
Phanerochaete chrysosporium is a white-rot fungus recognized for its ability to degrade the abundant aromatic polymer lignin¹. We have cloned an aryl-alcohol dehydrogenase (*AAD*) from *P. chrysosporium* using the Rapid Amplification of cDNA Ends (RACE) method. The cloned *AAD* ORF (1158bp) shares 96.8% nucleotide sequence identity and encodes 100% identical amino acid sequence with a previously cloned *AAD* from the same fungus².

Reverse transcription quantitative PCR (RT-qPCR) analysis of *AAD* expression revealed a continuous accumulation of the transcript during nitrogen-limited culture of the fungus. The RACE cloning method was applied on total RNA purified from cells harvested after 6 days of cultivation.

The *P. chrysosporium* *AAD* ORF obtained was sub-cloned into two *Escherichia coli* expression vectors (pGEX-6p-1, pGS-21a) and expressed in three different *E. coli* strains. The highest concentration of soluble heterologous recombinant Gst fusion protein was obtained with *E. coli* BL21 STAR strain and the pGS-21a vector after Glutathione–Sephadex affinity chromatography.

The optimum catalytic temperature of the recombinant enzyme was determined to be 37°C with an optimum pH of 6.1. In these conditions, the purified recombinant protein was able to catalyze the reduction of veratraldehyde with NADPH as cofactor. NADH can also be the electron donor, while having a higher K_m (215 μ M) compared to that of NADPH (39 μ M). With a 6xHis-Gst tag at the amino terminus and a 6xHis tag at the carboxyl terminus, the specific activity of the recombinant protein was about one-third of that previously measured on the fungus-derived purified extract, indicating that the tags may have affected the enzyme.

The substrate specificity of the recombinant protein for the reduction and oxidation reactions was assayed with a broad range of aldehydes and alcohols. The enzyme was active with all aryl-aldehydes tested as well as with several aliphatic aldehydes (particularly with C6, C7 and C8-aldehydes). For the oxidation reaction, the enzyme showed activity with only some

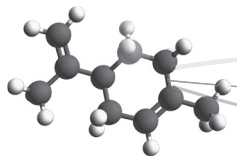


aryl-alcohols. The kinetic parameters K_m and k_{cat} were determined for a subset of molecules. The highest catalytic efficiencies were obtained with 3,4 and 3,5-dimethoxybenzaldehyde. The specificity constants were respectively 20 and 100-fold higher for the reduction of vanillin and of 3,4-dimethoxybenzaldehyde than for the oxidation of the corresponding alcohols.

Applications of this aryl-alcohol dehydrogenase from *Phanerochaete chrysosporium* are hence possible in biotechnological processes for natural flavours and fragrances production.

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4.23

ODORANT POLYFUNCTIONAL THIOLS ISSUED FROM BOTTLE BEER REFERMENTATION

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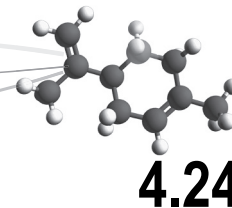
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Keywords : bottle refermentation, polyfunctional thiols, beer aroma, yeast vitality

Bottle refermentation which imparts beer effervescence and resistance against infection and oxidation is also known to improve flavor profile and stability. By this process, some stale off-flavors exhaled by aldehydes (*trans*-2-nonenal, 3-methylthiopropionaldehyde, 3-methylbutanal ..) are reduced into alcohols (1, 2). Unfortunately, yeast esterases can also strongly affect the beer fruity character by hydrolyzing isoamyl acetate, ethyl hexanoate and ethyl octanoate (1, 2). Thiols are known to have a strong impact on the overall aroma of fermented beverages (3). More than ten polyfunctional thiols were detected in fresh lager beers (4). The thiols profile of refermented beer has been little studied. In this work, the polyfunctional thiol contents of commercial and pilot beers submitted or not to bottle refermentation were compared. A strong organoleptic impact of the bottle refermentation process was evidenced in all samples by a training panel (70%). A specific *p*HMB thiols extraction (5) was applied and the extracts analyzed by GC-MS, GC-PFPD and GColfactometry (AEDA). A large number of sulfanylalcohols, sulfanylacetates and sulfanilylcarbonyls revealed produced during the refermentation process, especially after three weeks. Among them, 3-sulfanylpropanol reached a FD olfactometric values up to 32768. According to their beta-sulfanyl structures, hop cystein adducts are suspected to be hydrolyzed by refermentation yeast-derived lyases. Therefore, a better control of the refermentation process requires both an excellent control of yeast vitality and a strict selection of the hop varieties.

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**ORIGINS OF *S*-CYSTEINYLATED AND *S*-GLUTATHIONYLATED THIOL
PRECURSORS IN *VITIS VINIFERA*
GRAPES: STIMULATION OF THEIR PRODUCTION BY *BOTRYTIS*
CINEREA IN GRAPEVINE CELLS**

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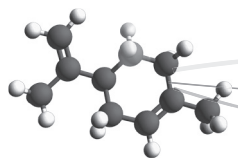
Keywords: cysteine *S*-conjugates; glutathione *S*-conjugates; thiol precursor; (*E*)-2-hexenal

Volatile thiols are responsible for the sensory characteristics and quality of various foods and beverages. They are highly volatile compounds with powerful aromas and are known to contribute to the varietal aroma of many white wines. Recently, the major role of volatile thiols in the citrus aromas of Sauternes wines was demonstrated. 3-sulfanylhhexanol (3SH), 3-sulfanylpentan-1-ol (3SP), and 3-sulfanylheptan-1-ol (3SHp) concentrations in botrytized wines are particularly affected by the development of *Botrytis cinerea* on grapes (more than 30-fold for 3SH) (1). This positive effect, due to the presence of *B. cinerea* on the berries, in fact results from a strong enrichment of cysteine *S*-conjugate precursors in botrytized berries. For example, the *S*-3-(hexan-1-ol)-L-cysteine (P-3SH) levels increased approximately 100-fold between the healthy and first botrytized stages, within a one week period (2, 3).

In the present study, a convenient model was investigated to reproduce and therefore study this phenomenon. A *Vitis vinifera* cell culture was used as a simple model and we focused on *S*-3-(hexan-1-ol)-L-cysteine (P-3SH), the cysteinylated precursor of 3-sulfanylhhexanol. We demonstrated that grapevine cells were able to produce P-3SH and the presence of *B. cinerea* considerably increased the precursor level (up to 1000-fold). This positive result was determined to be due to metabolites secreted by the fungus.

These metabolites were temperature sensitive, unstable over time and their production was activated in the presence of grapevine cells.

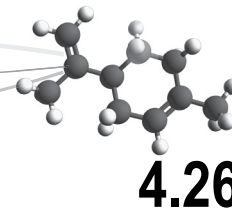
Moreover, we confirm that like some cysteine *S*-conjugates, P-3SH was produced by the breakdown of the corresponding glutathione *S*-conjugate (P-GSH), which itself was generated after a conjugation of glutathione on (*E*)-2-hexenal. It appears that P-3SH was produced in grapes by the intermediate of the detoxification pathways. The conversion rates of (*E*)-2-hexenal and P-GSH in P-3SH by grapevine cells were estimated at 20 and 12%, respectively. Therefore, (*E*)-2-hexenal is a more valuable and useful compound, compared to P-GSH, in terms of helping the plant in P-3SH production.



This result may be explained by the fact that reactive aldehydes including (*E*)-2-hexenal induce glutathione *S*-transferase (4). This mechanism was amplified by *B. cinerea* infection.

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SULPHUR-CONTAINING COMPOUNDS IN BUTTER AND THEIR INFLUENCE ON BUTTER AROMA

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Keywords: Butter, sulphur compounds, aroma, SPME, PFPD

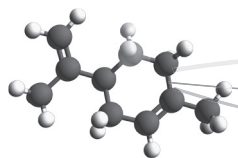
Volatile sulphur compounds, with their often occurring low odour thresholds, are known to influence the flavour of a variety of foods and beverages and their presence in a matrix, even in sub-ppb quantities, might cause a significant effect on the sensory properties of that matrix [1 2]. Although butter aroma has been widely investigated [3], little is known about the influence of sulphur compounds on its flavour [4 5]. The formation of these compounds in butter might include oxidative, thermal, enzymatic and microbial reactions [5].

The odour-active compounds of four sour cream butters (produced in France, Germany, Ireland and Switzerland) with different flavour characteristics were analysed by gas chromatography mass spectrometry combined with olfactometry (GC-MS-O), using head-space solid phase microextraction (HS-SPME). Additionally, the butter samples were analysed by sulphur specific detection (GC/pulsed flame photometric detection GC/PFPD) using static headspace extraction, to focus on the most volatile sulphur compounds.

The French butter was characterised by nutty notes (4-ethyl-benzaldehyde, 2-acetyl-pyrroline), whereas the German butter was high in green (hexanal) and caramel odour notes (ethyl furaneol). In the Irish butter mainly green (*E*-2-hexenol) and fatty notes (nonanal) were perceived. The Swiss butter was characterised by fruity odours (2-undecanone, δ -octalactone). Results of GC-MS-O analyses showed that, 2-methyl-3-furanthiol, a sulphur compound with a meaty/broth-like odour, was perceived in all butter samples. Methional (boiled potato) was found in the German and Irish butter, whereas dipropyl disulphide (garlic-like) was detected only in the Swiss and German samples. Dimethyl trisulphide (cheesy/sulphury) was found in French and German butter.

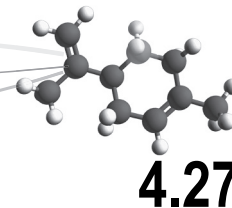
Furthermore, additional sulphur compounds such as methanethiol, carbon disulphide, dimethyl sulphide and dimethyl disulphide were identified in all butter samples when using sulphur specific detection by GC/PFPD. In a recombination study the influence of the sulphur compounds on butter aroma was investigated. The aroma of the four butter samples was reconstituted, using sweet cream butter as matrix and spiking it with the most important odour compounds found by GC-O

analyses. Odour-active sulphur compounds such as 2-methyl-3-furanthiol, methional, dipropyl disulphide and dimethyl trisulphide were stepwise added on top. The effects on the overall butter odour were evaluated by a sensory panel.



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STRAWBERRY SULFUR VOLATILE CHANGES DUE TO FRUIT MATURITY AND CULTIVAR DIFFERENCES

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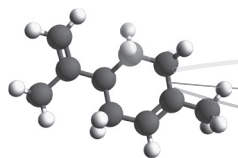
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Keywords: Sulfur volatiles, Fruit maturity, Strawberry cultivar, Strawberry volatiles

Sulfur volatiles have been identified as aroma impact compounds in some fruit such as melon, durian, strawberry, pineapple, kiwi fruit, yellow passion fruit, grapefruit, and pear. In this study, eight alkyl sulfur and nine thioesters strawberry sulfur volatiles were examined using SPME-GC-PFPD (pulsed flame photometric detector) and sensory evaluation. The assayed sulfur volatiles include hydrogen sulfide (H₂S), sulfur dioxide (SO₂), methanethiol (MeSH), carbon disulfide (CS₂), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), methional, methyl thioacetate, methyl thiopropionate, methyl thiobutyrate, ethyl thiobutanoate, methyl thiohexanoate, methyl (methylthio)acetate, ethyl (methylthio)acetate, methyl 3-(methylthio)propionate, and ethyl 3-(methylthio)propionate. Seven of these thioesters are the newly identified sulfur volatiles (*1*). Their olfactory qualities include: rotten egg, cabbage, vegetative, garlic, onion, potato, tropical fruit, and fruity aroma notes. Aroma quality was concentration dependent. Headspace volatiles of intact strawberries produced higher levels of DMS, DMDS, and DMTS, while fresh strawberry puree had relatively more CS₂ and thioesters. The change of sulfur volatiles in strawberry at five fruit developmental stages (white, 1/2 red, 3/4 red, full ripe and overripe) with multiple harvest dates was investigated. Most sulfur volatiles, especially thioesters, increased with fruit maturation with the most rapid increases between full ripe and overripe stages. Only a few alkyl sulfides presented at the white and half red stages. The sulfur volatile profiles of 11 Florida cultivars were examined in multiple harvest seasons. Thioesters varied in quality and quantity and thioester content varied as much as 70 fold among the cultivars. However, alkyl sulfides varied only in quantity and there was no big difference of alkyl sulfides among those cultivars. Industry standard ‘Strawberry Festival’ had the highest amount of thioesters, while older cultivars such as ‘Dover’ and ‘Sweet Charlie’ had the lowest thioester content. Principal Component Analysis, PCA, of sulfur volatile composition was used to examine breeding classifications.

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4.28

FORMATION OF KEY FLAVOUR PRECURSORS IN BISON RIBEYE POST-MORTEM: EFFECT OF CHILLED STORAGE CONDITIONING

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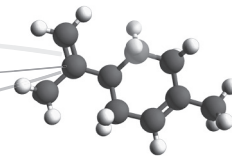
Keywords: Flavour precursors, bison, ribeye, chilled storage, post-mortem conditioning, water-soluble, lipids, sensory evaluation.

The volatile compounds responsible for contributing to meat flavour and aroma develop during cooking by complex reactions between natural components present in raw meat. These components are divided into two categories, **(a)** water-soluble precursors including nucleotides, reducing sugars, free amino acids and **(b)** lipids (1). Reducing and phosphorylated sugars are limiting flavour precursors since even small changes in their natural concentration can have a significant impact on the eating quality of cooked meat (2). Chilled storage conditioning on the formation of different flavour precursors has been shown to be crucial to the eating quality of different types of meat (3, 4). Identification of these precursors is important due to their potential impact on flavour formation in standard food production. Bison is vastly becoming a popular red meat with Canada currently having approximately 2,000 bison producers. Bison meat is a lean meat alternative to beef with higher levels of unsaturated fatty acids (5). The health benefits and identification of limiting precursors in local bison meat will improve the marketability of this product nationally, while benefiting both producers and consumers.

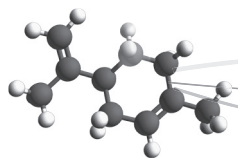
The effect of chilled storage conditioning on the formation of different flavour precursors and fatty acids on sensory attributes of post-mortem bison ribeye has been studied. In order to evaluate how post-mortem conditioning may affect flavour formation in meat, ribeye steaks from six individual freshly slaughtered grain-fed two year old bison heifers were vacuum packed and monitored while stored at 4 °C. Extractions were done for water-soluble and lipid components at 2, 4, 8, 15 and 21 days. Research on the importance of lipid-derived components in meat during variable post-mortem conditioning periods has been limited in previous flavour studies and therefore total lipids including cholesterol, triglycerides, free fatty acids, phospholipids and neutral lipids were tested in bison ribeye. Sensory evaluation with trained panelists was used to measure on a 15 cm line scale the flavor, aroma and texture attributes in the same six bison ribeyes during baked conditions at 250 °F for 10 minutes. Correlation studies between chemical and sensory evaluation results were used to compare any flavour trends optimized during cooking.

The effects of amino acids, reducing sugars and nucleotides as well as lipid changes in post-mortem bison ribeye during chilled storage conditioning at 4°C are discussed.

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4.29

**THE EFFECT OF pH ON THE FLAVOR FORMATION AND ANTIOXIDANT
ACTIVITY OF AMINO ACID AND SUGARS INTERACTION**

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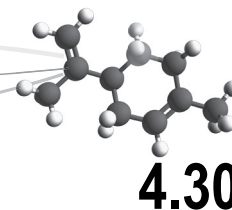
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Keywords: Meat, flavor, beef fat, phenolic contents, non-enzymatic browning, caramalizin, Maillard reaction, meat model system, pH, antioxidant activity, natural antioxidants

Flavor is the crucial part of eating quality. In the preparation of natural identical flavor in different model systems like cysteine-ribose (rib-cys), cysteine-glucose (glu-cys) and cysteine-beef fat affected by different pHs such as 4.5, 7 and 10 pH a wide range of flavor was obtained. In sugar and amino acid model system roasted and burnt meat flavor was obvious while in beef fat model system boiled meat flavour was dominated. Which was strongly supported by sensory evaluation. In rib-cys and glu-cys model system total phenolic contents were highest at pH 7 and pH 4.5 respectively along with browning, leading to strong antioxidant activity. In beef fat –cys model system it was found that as pH increases TPC and browning increases and antioxidant activity become maximum at basic pH. All result indicated that there is a positive relationship between the TPC, browning and antioxidant activity of all model systems



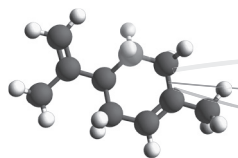
COMPARISON OF MAILLARD-DERIVED FLAVOUR VOLATILES OF COOKED MILLED AND COOKED BROWN RICE

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Keywords: milled rice, brown rice, fragrant rice, non-fragrant rice, Maillard reaction, flavour volatiles

Flavour is the key driver of quality and the main discriminator between rice varieties and it is an important determinant of global rice prices. The major volatiles reported to contribute to the flavour of boiled rice include n-hexanal, (*E*)-2-heptenal, 1-octen-3-ol, n-nonanal, (*E*)-2-octenal, (*E*)-2, (*E*)-4-decadienal, 2-pentylfuran, 4-vinylguaiacol and 4-vinylphenol. A very important character-impact flavour compound in fragrant varieties of rice is 2-acetyl-1-pyrroline (2AP), whereas cooked non-fragrant rice contains little or no 2AP. Brown rice contains more outer bran layer than milled rice. Furthermore, outer portion of a rice kernel contains more nitrogen and minerals (P, Mg, K and Mn) than the inner portion. During cooking, non-enzymic browning, such as the Maillard reaction, may occur, so Maillard reaction products are expected in the flavour volatiles profile of cooked rice. This paper presents a comparison of the Maillard reaction flavour volatiles of cooked milled rice of both fragrant (Basmati and Jasmine) and non fragrant varieties (long grain rice) and the cooked brown rice (non-fragrant). Rice was washed, and then cooked using an automatic electric rice cooker. Flavour volatiles were extracted using dynamic headspace collection, and identified using GC MS. In agreement with previous reports, 2AP was identified in all fragrant rice, but was not found in the non-fragrant rice. In contrast, 1-penten-3-ol, 2-pentanone, pyrrole, 6-methyl-5-hepten-2-one and E-2-nonenal were identified in the non-fragrant rice, but were not found in the fragrant rice. In addition, brown rice contained more Maillard reaction products than milled rice. The products of Maillard reaction were Strecker aldehydes (2-methylbutanal, 3-methylbutanal and phenylacetaldehyde), derivatives of methionine (methanethiol), pyrazines, and furans (furfural, 2-acetylfuran, and 5-methylfurfural). The levels of Maillard precursors in the rice varieties were quantified. The potential to optimise the Maillard reaction in cooked rice is discussed.



4.31

COMPARISON OF RIBOSE AND ASCORBIC ACID IN MODEL PROCESS REACTIONS

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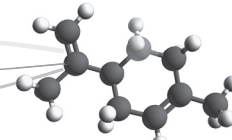
Keywords: Meat flavour, process reactions, ribose, cysteine, ascorbic acid, volatiles

It is well established that cysteine and ribose are important precursors essential for the thermal generation of characteristic and highly potent meat flavour aroma compounds in process reactions. The odour impact compounds are a series of sulfides derived from 2-methyl-3-furanthiol (MFT) and the formation pathways are well known (1). In addition, these compounds can also be formed from thiamine degradation (2).

In this paper, the use of ascorbic acid as an alternative source of reactive carbonyls is investigated. Thermally treated model reactions of cysteine/ascorbic acid and cysteine/ribose (either buffered or unbuffered) were prepared. The volatile profiles of the resulting products were extracted using dynamic headspace extraction and the semi-polar compounds were extracted using solid phase extraction. Both extracts were analysed by GC-MS. This paper discusses the similarities and differences between the ribose and ascorbic acid systems and relevant mechanisms will be discussed. One major revelation is the formation of MFT derivatives from the buffered system containing ascorbic acid and cysteine. This provides a third reaction pathway for the generation of these important meat aroma compounds.

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INFLUENCE OF BENZEALDEHYDE, ETHYL DECANOATE, ETHYL OCTANOATE AND PHENETHYL ALCOHOL ON THE AROMA OF NEW ZEALAND PINOT NOIR

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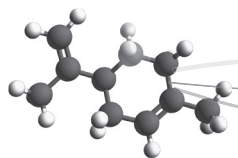
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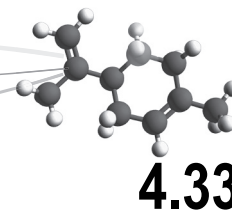
Pinot noir wine has a much more complex aroma chemical profile than many other wines, such as Sauvignon blanc. This complexity is because there is no one main group (or groups) of aroma chemicals that can explain the specific aromas associated with Pinot noir. A further challenge occurs as the majority of the main aroma compounds are found at levels below their perception threshold. Determining correlations between sensory data and volatile chemical data is currently the best way to uncover the role of specific aroma compounds. SPME-GC-MS was used to determine the concentration range of 34 aroma chemicals in 32 New Zealand Pinot noir wines. The intensity of 15 aroma attributes was investigated using visual analog line scales. Correlations between the aroma intensity and aroma chemicals were calculated using canonical correlation analysis, by correlating the canonical results with the X and Y variables (1). Results show that ethyl decanoate, ethyl octanoate and phenethyl alcohol positively correlate with red fruit aromas and negatively correlate with dark fruit aromas. The second most significant correlation involves benzealdehyde, which positively correlates with jam aromas and negatively correlates with spice and oak aromas. While the statistics suggest that these aroma chemicals are responsible for specific aromas, this may not be the case in reality. Synergistic or antagonistic interactions may occur that cause aroma perception to differ from what is specified by the canonical correlation results. Additional sensory testing is required to verify the statistical results. The correlations were tested by spiking different concentrations and combinations of these four chemicals into a wine matrix. In this case the wine matrix was a Pinot noir wine diluted by 10% with a dearomatized sample of the same Pinot noir. The resulting wine matrix still smelled like a commercial Pinot noir but contained low levels of the four compounds of interest. All other measured compounds in the matrix were found to lie within the compound ranges found in the 32 Pinot noir wines. Using a matrix that is an actual wine is important. The additional compounds in the wine that were not measured, or compounds that are currently unknown, play an important part in sensory perception. This is the first time that an actual wine has been used as a wine matrix in this way, as wines used in previous studies were dearomatized or chosen specifically due to the neutral characteristic of the wine aroma (2). A high concentration of each of the 10 possible chemical spikes were tested against the average



concentration matrix using triangle tests. Panelists were able to differentiate all of the spiked wines from the wine matrix ($\alpha=0.05$). The intensity of the aromas of each spiked wines were also evaluated using visual analog wine scales. The resulting sensory correlations do agree with the original correlation and provide further information on the influence of each aroma chemical.

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KEY ODORANTS OF JURA FLOR-SHERRY WINES

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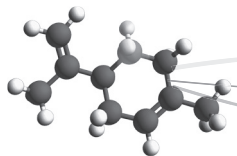
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Keywords: theaspirane, sotolon, abhexon, Jura wines, aroma, grenadine

The aromatic profile of Jura flor-sherry wines has been little studied, yet. Only acetaldehyde, diethoxy-1,1-ethane and sotolon have been described as key-odorants (1-4). In the present work, two “Yellow wines” from vintage 2002 were investigated. Two extractions were applied. The XAD extraction was used to determine the complete aroma profile. As XAD 2 resin does not allow very efficient recovery of hydrophilic molecules like sotolon, an adapted procedure, including pH variation, was optimized (5). After extraction, both samples were analyzed by GC-FID, GC-MS and GC-O AEDA. The specific extraction dedicated to hydrophilic compounds allowed us to evidence for the first time in Jura wines abhexon, the ethyl analog of sotolon. This lactone was described recently in aged Sauternes wines (6). Its concentration (23-31 µg/kg) revealed to be under the range reported for sotolon (78-140 µg/kg). Because of the very long barrel aging (6 years and 3 months), many oak-related odorants were found in the XAD 2 flavor extracts, mainly homofuraneol (cotton candy, FD = 256-512), cis-β-methyloctalactone and isovaleric acid. Two candy/fruity esters issued from yeast also exhibited high FD values : ethylisobutyrate and ethylisovalerate, as well as β-phenylethanol and 4-ethylguaiacol. As expected, very few varietal aroma survived to this long aging. However, theaspirane and two derived compounds tentatively identified as dihydrodehydro-β-ionone and 4-hydroxy-7,8-dihydro-β-ionone were found. The latter ($IR_{CPsil5CB} = 1373$), never reported before in wine research, exhaled an exceptional grenadine/blackcurrant odor. Theaspirane or its glycosilated precursors are suspected to be oxidized and hydrolyzed during the long oak-aging, allowing the occurrence of these unreduced carbonyles in wine.

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4.34

DETERMINATION OF AROMA COMPOSITION IN TROPICAL WINES FROM NORTHEAST OF BRAZIL

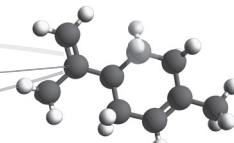
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Keywords: *Vitis vinifera* L.; climate variability; grapes; tropical wines; aroma profile.

The production of traditional wines in the world is located in temperate climates in Europe, in North and South America, South Africa and Oceania. In these places, grapes can be harvested only once a year. The tropical wines are being produced in Thailand, India, Venezuela and northeastern Brazil. In these regions, it's possible to harvest two or three times a year. The Sub-middle São Francisco river Valley is the second largest region producing fine wines in Brazil since 25 years ago, and presents a tropical semiarid climate, with edaphoclimatic characteristics that allow to get harvests throughout the year. These characteristics favor to obtain different wine types, presenting quality and regional identity. The aim of this study was to determine the aroma composition of white and red tropical wines elaborated in two seasons of 2009. Grape harvest was carried out in June (harvest I) and November (harvest II) for white cultivars Vedejo (VE), Viognier (VI) and Sauvignon Blanc (SB) and reds Petit Verdot (PV), Tempranillo (TE) and Syrah (SY). The winemaking process was carried out by applying the traditional method, in stainless steel tanks of 200 L, with alcoholic and malolactic fermentations conducted at 25°C and 18°C, respectively. After stabilization of the wines by cold (0°C for 30 days) they were bottled and then analyzed. Esters, acids and 2-phenylethanol were extracted by liquid-liquid extraction (LLE) with ether and hexane and it was added 3-octanol and heptanoic acid as internal standards. While, alcohols and ethyl acetate were quantified by direct injection of the wine distillates with addition of 4-methyl-2-pentanol as internal standard. All compounds were analyzed in triplicate by gas chromatography with flame ionization detector (GC-FID) and quantified using internal standardization. Multivariate statistical analysis was applied (principal components analysis – PCA) to discriminate groups and also Tukey means test (p=5%). Twenty four aroma compounds were quantified: 6 carboxylic acids, 8 alcohols and 10 esters. PCA allowed to discriminate between samples according to the two crops, as well as to the wine type. Differences found between the concentrations of aroma compounds in red and white wines highlight the importance of genetic and environmental factors influencing quality and typicality. The potential key odour- active compounds of the Brazilian tropical wines were ethyl acetate, 3-methyl-1-butanol, 2- phenyletanol and octanoic acid. This work represents the first step of researches to characterize the identity and typicality of tropical wines produced in Brazil and worldwide.



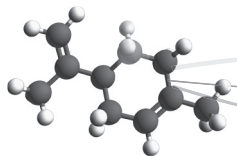
THE EFFECT OF NITROGEN SOURCE ON AROMA COMPOUNDS FORMATION BY YEAST DURING ALCOHOLIC FERMENTATION

Catarina Barbosa, Ana Mendes-Ferreira, Arlete Mendes-Faia

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Yeasts have the ability to use a wide range of nitrogen-containing compounds as the sole nitrogen source. Aroma compounds found in wine are originated directly in the grapes but the majority result from the activity of yeasts during the alcoholic fermentation, in particular from sugar and nitrogen compounds. In this study experiments were carried out to examine the effects of nitrogen source, diammonium phosphate (DAP) or a mixture of amino acids (AAA) with or without methionine, on aroma compound formation, in *Saccharomyces cerevisiae* grown in synthetic grapejuice media. A decrease in butyric, hexanoic, and octanoic acids as well as on 1-octanol, linalool and isoamyl alcohol and the esters, ethyl butyrate, diethyl succinate, ethyl decanoate, ethyl octanoate and isoamyl acetate, was detected in media in which DAP was replaced by AAA. Others such as ethyl 2-methylbutyrate, ethyl isobutyrate and 2-phenylethyl acetate increased after that substitution. Absence of methionine led to an increase in sulphide, acids, alcohols, except linalool, and their respective acetate esters. From an oenological point of view, several aspects could be highlighted from this study: selection of specific yeast strains to conduct fermentation emerges as a useful tool for producing wines with specific flavour profiles and adjustments of grape-juice' yeast assimilable nitrogen could lead to the production of interesting flavour compounds.

This work was supported by FCT projects PTDC/AGR-ALI/71460/2006, PTDC/AGR372-ALI/68284/2006 and FCOMP-01- 0124-FEDER-014043 (PTDC/AGR-ALI/111224/2009). C.B. is recipient of a PhD grant from FCT (SFRH/BD/61881/2009)



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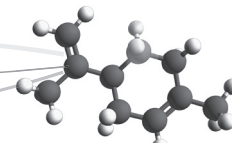
IDENTIFICATION OF A POTENT VOLATILE ODORIFEROUS THIOL, ETHYL-2-SULFANYLACETATE, CONTRIBUTING TO WINE'S OFF FLAVOURS

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Among odoriferous compounds, sulfur-containing molecules and especially thiols or sulfanyls are probably some of the most recognized key flavor compounds in many foods and beverages. They are often characterized by detection threshold as low as the ppt level. In enology, sulfur derivatives have been considered for many years as solely responsible for sensory defects, the nauseous qualifier being associated with the word sulfur. But studies concerning the characterization of impact wine aroma compounds carried from the 1990's made it possible to demonstrate the role of some powerful volatile thiols as 3-sulfanylhexas-1-ol, 3-mercaptohexyl acetate and 4-methyl-4-sulfanylpentan-2-ol in the typical fruity nuances of wines varietal flavour. Other thiols as benzenemethanethiol, 2-furanmethanethiol and ethyl 3-mercaptopropionate participate to the empyreumatic ageing nuances as contributors of wine aroma "*bouquet*".

White wines finesse is one very important aspect of their organoleptic appreciation. In order to progress in the knowledge of the aromatic composition of dry white wines, direct high-pressure liquid chromatography analysis of a wine organic extract was performed. Thus, various Sauvignon blanc wines were submitted to liquid-liquid extraction then the extracts were injected by semi-preparative HPLC analysis. The sensory descriptive analysis was done on the HPLC fractions and the most differentiated fractions were re-extracted for analysis by gas chromatography-olfactometry technique. Doing so, it was possible to put in evidence an odoriferous zone reminiscent of an unpleasant odor. Use gas chromatography analysis coupled with mass spectrometry allowed identifying a new powerful sulfanyl ester in wines, ethyl-2-sulfanyl acetate (E2SA), recalling mustard and *Fritillaria meleagris* root. E2SA perception threshold in water and model solution was determined at 70 ng/L and 200 ng/L, respectively, and in a range from 267 to 400 ng/L in different dry white wines. Moreover, using a paired comparison procedure, the concentration at which E2SA was identified as an off-odor in wines was situated between 300 and 500 ng/L depending on the dry white wine origin. These results meant, as expected, that the evaluation of E2SA contribution to wine off odor was more likely based on the wine style. The perception and rejection thresholds of this compound in dry white wines made it possible to demonstrate its contribution to the wine off-flavors. The levels of E2SA of different white and rosé wines was also reported.



OXIDATION MECHANISMS OCCURRING IN WINES

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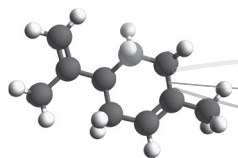
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Keywords: Wine; Oxidation; Phenolic Compounds; Antioxidant agents; Copper(I) / Copper(II); Iron(II) / Iron(III).

The present review aims to show the state of the art on the oxidation mechanisms occurring in wines. Enzymatic browning almost entirely occurs in grape must, and is largely correlated with the content of hydroxycinnamates such as caffeoyltartaric acid and *para-coumaroyltartaric* acid (1). Non-enzymatic browning, also called chemical oxidation of wine, prevails in fermented wine.

During the process of non-enzymatic oxidation the oxidative processes begin by the oxidation of polyphenols containing an catechol or a galloyl group such as (+)-catechin/(-)-epicatechin, galocatechin, gallic acid and its esters, and caffeic acid, which are the most readily oxidized wine constituents (2). However, oxygen thus not reacts directly with phenolic compounds. The limitation on the reactivity of triplet oxygen is overcome by the stepwise addition of a single electron, which can be providing by reduced transition metal ions, essentially iron(II) and copper(I) (3). The initial transfer of an electron leads to the formation of superoxide radical anion ($O_2^{\bullet-}$) which, at pH wine, exists in the protonated hydroperoxide radical form ($HOO^{\bullet}$). The transfer of a second electron will produce peroxide anion (O_2^{2-}) which, at pH wine, exists in the protonated hydrogen peroxide form. The next reduction step creates the hydroxyl radical ($OH^{\bullet}$), via the Fenton reaction. The hydroxyl radical will oxidize almost any organic molecule found in wine and will react with the first species it encounters, 2 depending on their concentration. Oxidation via hydroxyl radical and radical species was confirmed (4), evidencing the Fenton reaction in wine.

Sulfur dioxide (SO_2) is an important antioxidant in wine. Nevertheless, it does not react directly with oxygen but with the oxygen reduced form hydrogen peroxide. In this way, SO_2 can inhibit aldehydes formation by competing for hydrogen peroxide (4). SO_2 also play an important role in reducing quinones, formed during oxidation process, back to their phenol form, where the presence of transition metal ions is crucial (5). Ascorbic acid is added to white wine due to its ability to efficiently scavenge molecular oxygen, but in the process it is initially converted to dehydroascorbic acid and hydrogen peroxide. Alternative options have been assessed for the prevention of oxidation during wine storage, including caffeic acid, gallic acid and glutathione, where their mixture protects several aromatic volatiles of white wines with reduced SO_2 (6).

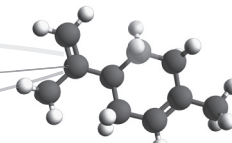


Many aldehydes that occur in wines resulting from the oxidation of alcohols, sugars and amino acids degradations are described to have an important contribution to the sub-qualities of wine aroma. Besides, these aldehydes may react with flavonoids during ageing yielding new colored and taste compounds (7, 8). Acetaldehyde formed in ageing wine has been shown to have important function in the chemical transformations occurring in wine. Apart from the methylnmethine-linked flavonoid adducts, other compounds are also derived from acetaldehyde-induced reactions, for instance, decomposition of the methylnmethine-linked adducts may yield reacting vinylflavonoids directly involved in the formation of some pyranoanthocyanins.

The complexity of the overall mechanisms implicated in wine oxidation is not fully understood and the identification of all mediators' reactions and its characterization needs to be done.

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**PARCIAL REPLACEMENT OF SULFUR DIOXIDE BY ANTIMICROBIAL
PHENOLIC EXTRACTS AS A PRESERVATIVE OF WINE: EFFECTS ON
WINE ORGANOLEPTIC CHARACTERISTICS**

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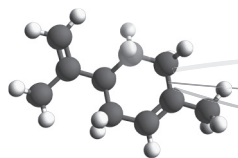
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Keywords: Antimicrobial phenolic extracts, SO₂ replacement, Wine organoleptic properties

During winemaking sulfur dioxide (SO₂) is added to wine in order to avoid undesirable microbiological effects that could affect negatively to the sensorial characteristic of wines and also generate toxic compounds such as biogenic amines. However, the doses of SO₂ should be controlled since high doses could affect the organoleptic characteristics of wine and produce risks to humans. For that reason, some natural phenolic extracts has been investigated as natural preservative agents to replace SO₂ either totally or partially. Therefore, the aim of this work was to evaluate the effect of the partial replace of SO₂ by two antimicrobial phenolic extracts on the organoleptic characteristics and volatile and phenolic composition of a white wine during its ageing in wood.

The assays were carried out with a white wine from Verdejo variety that had just finished the alcoholic fermentation. Phenolic extracts (A and B) were added to wines at 0.1 g/L together with 50% of the dose of SO₂ normally added to this type of wines. In addition, a wine treated with the usual doses of SO₂ was aged in the same way and a control wine was stored in a stainless steel tank. Samples were taken at initial time and after one and two months of ageing. The analysis of volatile compounds and phenolic compounds were carried out by SPME-GC-MS and HPLC-ESI-MS, respectively. All assays were carried out in duplicate. The aroma profile was evaluated by 12 trained judges in three formal sessions in different days. The correlation between sensory and instrumental data (global composition, volatile and phenolic compounds) was performed using different statistical methods.

The results have shown that the addition of phenolic extracts avoid the development of lactic acid bacteria and therefore the malolactic fermentation. Chemical and sensorial analysis of wines at two months in barrels has shown no relevant differences between wines treated with phenolic extracts + SO₂ and only with SO₂. These results suggest that these phenolic extracts could be an alternative to the use of SO₂, although the total replacement of SO₂ has not been evaluated so far.



4.39

**ENZYMATIC ACTIVITIES OF WINE LACTIC ACID BACTERIA:
PRODUCTION OF VOLATILE SULPHUR COMPOUNDS AND VARIETAL
AROMAS**

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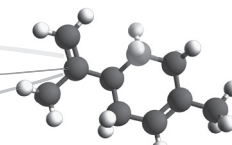
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Keywords: Lactic Acid Bacteria, Enzymatic activity, Volatile Sulphur Compounds, Wine aroma

Lactic acid bacteria (LAB) are responsible for malolactic fermentation that is often encouraged, since it improves wine stability and quality. Furthermore, LAB exhibit different enzymatic activities that could have an impact on the sensory quality of wine due to either the production of aroma compounds or the transformation of both grape- and yeast-derived volatile compounds and aroma precursors. The role of esterase and C-S lyase activities from LAB on cheese aroma development has been extensively reported. Nevertheless, information on these enzyme activities including their potential use in winemaking is limited. In wine, esterase activities could modify the profile of esters that are largely responsible for the fruity aroma of wine while C-S lyase activities could confer characteristic sulphur aromas and pleasant or unpleasant flavours. Moreover, recent studies have shown that glycosidase activities of LAB are involved in the release of grape derived aroma compounds from their glycosylated precursors.

Here, cell-free extracts from selected LAB strains, mainly responsible for the wine malolactic fermentation, were screened for esterase, C-S lyase and glycosidase activities. The VSC-producing ability of these strains towards L-methionine was also characterized by measuring the formation of methanethiol (MTL), dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) by solid phase microextraction coupled to gas-chromatography-mass spectrometry (GC-MS). Moreover, the impact of these LAB strains on the release of volatiles from aroma precursors was evaluated using extracts from skin Verdejo grapes.

Preliminary assays shows a large natural biodiversity in enzyme capabilities and high inter- and intra-species variability was found among the LAB strains investigated. From these data, knowledge of the enzymatic potential of wine-associated LAB will be of great importance for the selection of starter cultures with desired organoleptic characteristics, and therefore may provide an efficient approach to enhance wine aroma.



COMPARISON OF VOLATILE PROFILES OF MUSCADINE WINES PRODUCED USING PRE-TREATMENT WITH PECTINASE AND TRADITIONAL METHODS

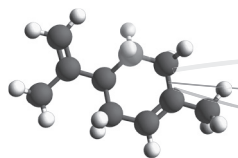
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Keywords: Wine, Muscadine, Enzyme, Aroma, GC-PFPD, GC-MS

The present study was designed to determine the effect of enzyme treatments on Muscadine wine flavor. It focused on comparing ester and sulfur compound development in the wines and the aroma activity of the components that contribute to the wine flavor while utilizing the different applications on the musts. Muscadine (Noble) red native cultivar was purchased from the Gainesville Region vineyard of Florida University. Pectolytic enzyme treatment and skin contact maceration were applied on musts of the Muscadine grape (*Vitis Rotundifolia*). The resulting wine was made with enzymatic and classical treatments then racked and filtrated according to traditional wine processes. Samples included the initial year of the wine and three years later. The samples' volatile composition and aroma characteristics were investigated by gas chromatography-sulfur pulsed flame photometric, and mass spectrometry. Those treatments which included longer time frames (10 hours-enzyme and 48 hours-skin contact) resulted in a 67-74% reduction in sulfur volatiles over the three year period of time. While the shorter time frame of the skin contact samples also had a similar reduction, the shorter time frame of the enzyme treatment caused a 10 % increase in sulfur volatiles over the three years. Ester volatile levels of enzyme-treated wines decreased 42-55% during the three years studied. Samples with 48 hours skin contact also exhibited a similar decrease. However, those samples with 24 hours skin contact displayed a 10% increase in ester volatiles.



4.41

EFFECT OF TEMPERATURE DURING BOTTLE AGING ON THE FLAVOR PROFILE AND ANTIOXIDANT CAPACITY OF RUBY CABERNET RED WINE

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Keywords: volatiles, aroma, aging conditions, maturation, DPPH

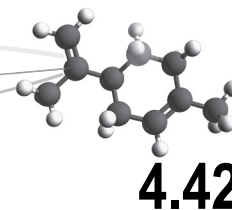
It is a fact that red wine provides health benefits. Investigations have shown in vitro that some compounds prevent free radical oxidation reducing the risk of cancer, cardiopathies and arteriosclerosis (1). Wine is a complex system with a great amount of chemical compounds that contribute to the sensory profile. Anthocyanins and phenolic compounds are the main responsible for the antioxidant capacity. Regarding flavor compounds, these can be increased or modified during maturation; therefore, the objective of this study was to explore the effect of red wine bottle aging at 4 and 18 °C both in antioxidant capacity and the flavor profile of ruby cabernet Mexican wine from Queretaro State.

Wine from the same batch was bottled. Half of them were stored at 4 °C and the other half at 18 °C. Acidity, sulfurous and total phenols were analyzed at the beginning and the end of the aging period. Color and DPPH test for antioxidant capacity were measured every 15 days during 6 months. At the end of maturation period, flavor volatiles were quantified by CG-FID and GC-MS after a liquid-liquid extraction using dichloromethane with a subsequent concentration with nitro gen. Concurrently, sensory evaluation was carried out by Quantitative Descriptive Analysis using 20 trained assessors.

Results showed that there were no important differences in color between the wines matured at both temperatures while antioxidant capacity was higher in wine aged at 18 °C. Volatile compounds with sensory relevance (2) were explored including alcohols, aldehydes, esters, lactones and a pyrazine characteristic of cabernet grapes. The complete sensory profile was obtained after developing a descriptive vocabulary relevant for this study. Differences of the volatile composition of wines from both 4 and 18 °C, and their correlation with sensory profile and physicochemical parameters have provided information for the potential application of cold temperatures during bottle aging of ruby cabernet Mexican wine.

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MONITORING OF FLAVOR COMPONENTS DURING FERMENTATIONS OF CABERNET SAUVIGNON GRAPES

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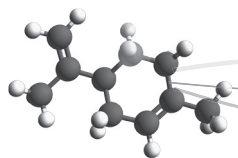
Keywords: Volatile compounds; fermentation; wine; GC-MS, β -damascenone

Aroma is one of the most important indicators of wine quality, which is formed by a huge number of volatile compounds from different origins (1). In order to improve wine aroma in a controlled and systematic manner, the processes and mechanism that are involved in the formation of wine aroma from grapes need to be understood. Flavor compounds formed from grape precursors have a different set of processing parameters that control their release into wines. Many of these compounds exist as glycosidically bound precursors in grapes and are hydrolyzed either enzymatically or chemically to the free compounds during fermentation (2). C-13 norisoprenoids such as β -ionone and β -damascenone fall into this category. Although the viticultural parameters that affect norisoprenoids concentration have been studied, the understanding the processes that can modulate the release of these aroma compounds could give winemakers an important tool to improve or control wine quality. According to this, the aim of this work was to determine how grape processing decisions affect the partitioning and release of the flavor compounds present in Cabernet Sauvignon grapes and how these decisions affect the final flavor concentration in wine.

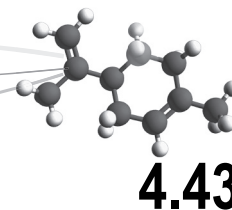
A total of 12 fermentations were carried out considering two treatments, “OUT” and “IN”. For the “OUT” treatment, skins were present from the beginning of fermentation and removed at various points during fermentation. For the “IN” treatments, the fermentation began with no skins in the juice and skins were added back in to the fermentation at various points. Volatile compounds were monitored by solid-phase microextraction- gas chromatography –mass spectrometry (SPME-GC-MS) (3) starting at inoculation and continuing every 12 or 24 hours until the end of fermentation. The duration and timing of the skin contact during fermentation showed a measurable effect on volatile composition. Hence, some compounds reached higher concentration in those fermentations performed with skins while, in other cases, skins acted to trap or delay release of volatiles, particularly the β -damascenone release. Surprisingly, this compound reached lower concentrations in presence of skins.

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RELATION BETWEEN DIFFERENT AROMA EXTRACTION TECHNIQUES AND SENSORY PERCEIVED QUALITY – WITH CARROTS AS AN EXAMPLE

VIBE BACH, Sidsel Jensen and Merete Edelenbos

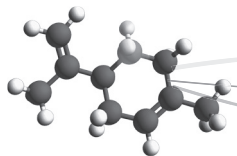
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Keywords: Dynamic headspace, SPME, solvent extraction, carrot aroma, sensory evaluation

Sensory evaluation is considered the ideal way to characterize the aroma profile of a food product, but it is time-consuming and expensive. Often chemical analyses are carried out instead, and it is important that the chemical analysis reflects the sensory quality experienced by panelists and consumers. The question then arises on which chemical techniques to choose and how to interpret the results? The use of different methods to extract aroma compounds from food results in different aroma profiles of the same sample material, and it can be unclear which of the extraction methods represents the sensory profile best. Comparison of aroma profiles obtained by different extraction techniques with the sensory quality, can give insight into which of the tested sampling techniques, that is the most suitable in expressing the sensory perceived aroma and flavor composition of a food product. In this study carrots were used as a model to study the aroma profile obtained by different extraction techniques, and the results were related to data from sensory evaluation.

The chemical composition of aroma compounds in three varieties of carrot was investigated using solvent extraction with hexane or ethyl acetate, dynamic headspace sampling with collection on Tenax TA and SPME using CAR/PDMS fiber. The aroma profile was characterized and quantified using GC and GC-MS. The sensory quality was determined by quantitative descriptive analysis by a trained panel of eight assessors. Results from the chemical and sensory analyses were related using multivariate data analysis.

SPME, dynamic headspace and solvent extraction resulted in chromatograms showing three very distinct aroma profiles. The identified aroma compounds mainly belonged to the groups of mono- and sesquiterpenes. The dynamic headspace sampling extracted more of the very volatile compounds from the carrots than the solvent extractions and the SPME method did, with the most prominent peaks in the chromatogram being terpinolene and β -caryophyllene. The solvent extraction methods extracted quantitatively less volatile compounds than the headspace methods, with the ethyl acetate extract showing more volatile compounds than the hexane extract. The CAR/PDMS fiber extracted mostly compounds with a medium volatility and the most dominant peak was *p*-cymene. The results on aroma volatiles extracted from carrots by solvent extraction, dynamic headspace and SPME are discussed in relation to the sensory perceived quality. These results can be used in the development of chemical methods to supplement sensory descriptive analysis.



4.44

INFLUENCE OF THE FERMENTATION TIME AND TEMPERATURE DRYING ON PROFILE VOLATILE COMPOUNDS IN COCOA BEANS

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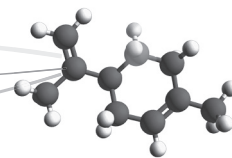
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Keywords: cocoa bean quality; fermentation-drying process; GC-MS; profile volatile

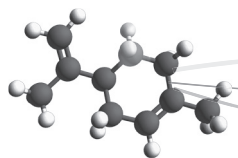
Two principal steps of the processing of cocoa beans are essential in the change of the flavour precursor and formation of flavour compounds, which are crucial for quality of cocoa products, such as fermentation and drying processes (1). In Mexico the most common method employed is the Sun drying. This is considered the best method to obtain the flavour development maximum (2). However, this method has disadvantages due to its long times and the labors required, producing cocoa with heterogeneous quality during rainy weather (3). In this research, the effect of fermentation time and drying temperature on profile of volatile compounds were evaluated with 2, 4, 6, and 8 fermentation days followed of drying at 60, 70 and 80 °C. Samples of fermented and drying cocoa were compared with dried cocoa in Samoa drier and Sun. The principal component analysis (PCA) was used to identify the most important volatile compounds and discriminate the effect of the fermentation time and drying temperature in the treatments. We found 58 volatile compounds identified by SPME-HS/GC-MS, which esters 20, alcohols 12, acids 11, aldehydes & ketones 8, pyrazines 4 and other 3 compounds were grouped. The fermentation process had higher effect on total concentration of alcohols, aldehydes & ketones, ester and acids, while the drying process had higher effect on total concentration of pyrazines and phenols. The concentrations of some undesirable compounds at 8 fermentation days suggests over-fermentation, therefore that is not necessary to extend the fermentation at long times. Furthermore, 6 days of fermentation were enough to produce volatile compounds with flavor notes desirable from cocoa bean quality and also avoid production of compounds with off-flavor notes. Using the PCA we observed that the volatiles profile in treatments at 70 and 80 °C of drying temperature with 6 days of fermentation were associated with Sun drying however, the drying at 70 °C could represent a low cost. Therefore, in this research the optimal conditions by fermentation and drying process were 6 days of fermentation followed of a drying process at 70 °C.

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4.45

ANALYSIS AND SENSORY EVALUATION OF GOOSEBERRY (*RIBES UVA CRISPA* L.) VOLATILES

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Keywords: *Ribes uva crisper* L., gooseberry, volatiles, aroma, GC-O

Gooseberries (*Ribes uva crisper* L.) are cultivated in Europe since the beginning of the 17th century. Although their popularity has dropped over the last years, they are still being widely used as fresh fruits as well as for the preparation of desserts, juices or jams. The worldwide annual production of gooseberries amounts to approximately 121000 t.

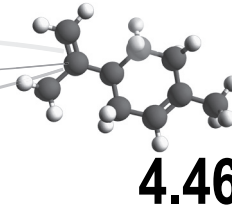
Previous analyses focused on non-volatile organic acids [1], selected volatile constituents, such as theaspiranes [2], or phenolic ingredients [3,4]. So far, knowledge on the aroma profile of gooseberries was lacking. Therefore, the objectives of the present study were to identify and to quantify volatile gooseberry constituents and to assess their contributions to the aroma by gas chromatography-olfactometry (GC-O).

Volatile constituents were isolated from gooseberries by liquid-liquid-extraction and vacuum-headspace-extraction, respectively, and the obtained concentrates were analyzed by capillary gas chromatography-mass spectrometry. C6-components (e.g. (Z)-hex-3-enal, (E)-hex-2-enal) and short-chain esters (e.g. methyl butanoate, ethyl butanoate) turned out to be the major compound classes. The variability in their distribution was demonstrated by analysis of several batches. Changes in the spectrum of C6-compounds formed enzymatically by lipidoxidation after disruption of the plant matrix were followed by inhibition-trials using calcium chloride.

The contributions of volatiles to the gooseberry aroma were assessed by (i) GC-O in combination with aroma extract dilution analysis, (ii) determination of odour thresholds of the most relevant compounds in water as well as in a berry-like matrix, and (iii) calculation of odour activity values. C6-components and esters were shown to be responsible for the green and fruity character of gooseberries.

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SENSORY AND MOLECULAR CHARACTERIZATION OF THE AROMA PROFILES OF FISH OIL SUPPLEMENTS

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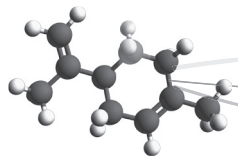
Keywords: two-dimensional high resolution gas chromatography, mass spectrometry, olfactometry, quantification, flavour, fish oil

A series of studies demonstrated the protective effects of n-3 polyunsaturated fatty acids (n-3-PUFAs) with regard to coronary heart disease in adults, as well as the fact that a diet supplemented with n-3-PUFAs may decrease mortality from myocardial reinfarction and sudden death. These effects have been attributed to eicosapentaenoic acid and docosahexaenoic acid, which are present in fish oil, as well as to alpha-linolenic acid (1, 2). Apart from that, n-3-PUFAs have been shown to be beneficial for diverse aspects of foetal and neonatal development such as vision, cognition, and several others (3-6). Accordingly, supplementation with fish oil products (commonly encapsulated) has become increasingly popular, especially during pregnancy and the breastfeeding period to provide long-chain n-3 PUFAs for improved development of brain and cardiovascular health function (7, 8), but also as a common supplement to the everyday diet. Nevertheless, supplementation with such prescriptions repeatedly elicits complaints from consumers/patients about fishy impressions that appear after ingestion of the encapsulated material. These seem to be, to some extent, related to the respective product (unpublished data).

Accordingly, the aim of our study was to characterize three commercially available encapsulated fish oil supplements with regard to their odor qualities and their odor constituents utilizing sensory profiling as well as high-resolution gas chromatography/olfactometry (HRGC-O) and two-dimensional HRGC-O/mass spectrometry with qualitative and quantitative characterization of the target compounds.

Our data show that all preparations exhibited a considerable odour load that was dominated by characteristic fishy, fatty, rancid-oil-like, tang-like and metallic odour qualities. Nevertheless, significant differences between prescriptions were observed both with regard to overall aroma intensity and rating of single odour attributes. GC-O evaluation revealed that the molecular odorant composition was dominated by substances that are common (poly)unsaturated fatty acid oxidation products (9-12).

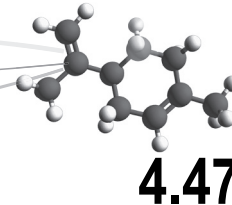
Accordingly, a series of oxidation markers such as (Z)-octa-1,5-dien-3-one, hexanal, (Z)-hex-3-enal and (E)-non-2-enal was quantified in comparative assays in all three supplements using stable isotope dilution assays. These experiments showed that the respective prescriptions indeed differed with regard to the quantitative composition of the respective odour constituents, in single cases even up to a factor of more than 10. Based on these results,



the sensory relevance of fish oil oxidation status as well as implications with regard to nutritional quality of the respective fish oil supplements will be discussed.

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THE FLAVOUR OF ELDERFLOWER – SPECIES DIFFERENTIATION VIA FLAVOUR COMPOUNDS

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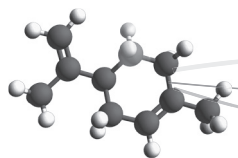
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Keywords: elder flower, flavour, species differentiation, aroma profile, multivariate data analysis

In southern Austrian regions, elderberries (*Sambucus nigra* L.) have developed to be a very important crop. With respect to export data alongside apples, elderberries represent Austria's second important fruit species. The ripe elderberry contains a number of bio-active compounds and, as a consequence, shows a number of health-promoting effects. Due to its very intense colour, the berry is a very interesting raw material for the production of natural colourings for the food industry.

In Austria, the selection of elder species was primarily based on the quality of the berries and not on the flavour of the flowers. The most frequently cultivated elder varieties are *Haschberg* and *Rubin*. In addition, there is a large population of wild forms. Other elder varieties are mainly cultivated in experimental stations only. Within the last years, raising request for the flowers can be noticed due to the very pleasant flavour which of interest for the food but also for the cosmetics industry. In contrast to the properties of the berries, the very fragile flavour of the elder flowers has not been studied very intensely up to now (1-3). Over 100 volatile compounds have been identified and reported in literature. Nevertheless, the results concerning the composition of the elderflower flavour are not consistent. The fragile flavour of the flowers in combination with different extraction techniques may be the reason for this fact.

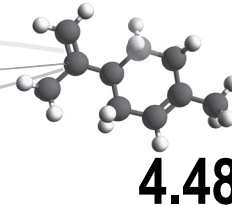
In the present study, we investigated the volatile compounds of the flowers several elder varieties and wild forms with the aim to investigate (i) differences and/or similarities in the composition of the aroma compounds, (ii) the effect of growing site and vegetation period on the flavour composition within a three-years vegetation time and (iii) if the wild forms can be related to any elder variety via the aroma profile. To minimize the influence of any extraction steps on the flavour composition, we used headspace solid phase microextraction as extraction technique. The whole umbels of elder flowers were harvested when they were in full bloom. The small white flowers were cut from the umbel immediately before the measurements and transferred directly into the headspace vial for the extraction of the volatiles. The subsequent gas chromatographic separation was performed on a column of medium polarity followed by mass spectrometry. Comparison of the aroma profiles was performed by semi-quantitative interpretation and multivariate statistics of the GC-MS data. First results show that the composition of the elder flower volatiles is highly dependent on the variety. Large differences of the aroma profiles were observed. The allocation of the wild



forms via the flavour compounds to any varieties is hardly possible. The habitat does not significantly influence the flavour compounds – flowers of the same species from different locations show a comparable flavour composition. The influence of the vegetation period will be evaluated by comparing the data from 2009 and 2010 with the results from the vegetation period 2011. As further steps, GC-olfactometry as well as sensory evaluation will be performed to investigate the sensory differences of the flowers.

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THE AROMA ANALYSIS OF SWEETENED CONDENSED MILK BY SOLVENT ASSISTED FLAVOUR EVAPORATION (SAFE) AND GC-O/MS

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Key words: sweetened condensed milk, solvent assisted flavour evaporation (SAFE), GC- O/MS

Sweetened condensed milk (SCM) is a key industrial ingredient in the manufacture of a number of confectionery products. The rheology and texture of SCM has been well characterised¹⁻⁴, however little appears to be published on SCM aroma. Full fat SCM is typically concentrated to 28% total milk solids in the presence of 45% sucrose. Thus it can be expected that the aroma composition of the SCM will be a combination by the intrinsic and extrinsic (process-related) aroma compounds.

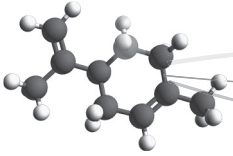
The objective was to develop methodology that would allow the aroma profile of industrial grade SCM to be characterised with the long term objective to control flavour development during processing.

SCM was collected from the factory-site within six hours of manufacture and stored at -18°C until analysed. A mixture of SCM (110 g), water (110 mL), calcium chloride (11 g) and ethyl acetate (50 mL) was prepared with eugenol (1ppm) as an internal standard and distilled by solvent assisted flavour evaporation (SAFE)⁵. The ethyl acetate was recovered from the distillate after the addition of 30 g sodium chloride and dried. Four distillation runs were combined and concentrated to a final volume of 1mL. The concentrated extract was analysed by GC-O on a non-polar column (HP5) by six panellists in triplicate measuring direct intensity using a hand-span device. The SCM extract was analysed by GC-MS using two column polarities (HP5 and HPwax).

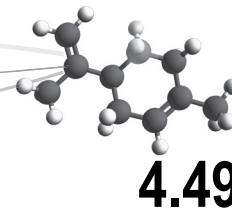
The GC-O panel qualitatively determined that the SCM extract was characteristic of SCM. The odours detected by GC-O panel could be classified as cheesy; lolly/flowery/fruity; citrus/mint; leather/spicy; musty/mushroom; and green/grassy. The cheesy odour corresponding to Butanoic acid was the most intense odour detected. Free fatty acids were identified in the SCM extract by GC-MS from acetic acid to tetradecanoic acid. In addition both 2-methyl and 3-methyl butanoic acid were detected. Terpene compounds were detected including alpha-pinene, beta-pinene and limonene. A series of lactones were present including delta-decalactone and delta- dodecalactone. Nonanal was also identified.

The odour profile of SCM does appear to be mostly due to extrinsic factors like heat exposure for formation of lactones and storage and process-related factors for the free fatty acids, though diet and lipid biosynthesis may also influence free fatty acid concentrations.

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EFFECTS OF DISTRIBUTION CHAIN ON FLAVOUR FORMATION IN RIPENING FRESH TOMATOES

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KEYWORDS: distribution chain length, tomato, aroma compounds, SBSE

In order to evaluate the effects of short, compared to medium or long distribution chains, on flavour formation in ripening fresh tomatoes, a post-harvest experiment was carried out by reproducing in the lab the most common temperature-relative humidity conditions and storage-transport times occurring in the real distribution chain of horticultural fresh products.

Cv. Nerina tomatoes picked at three different ripening stages (mature green, turning, red) were subjected to conditions corresponding to a short (1 day at room temperature), a medium (cold storage at 6÷9°C and 60÷80% R.H. for 2 days, followed by ripening at room temperature) and a long distribution chain (cold storage at 6÷9°C and 60÷80% R.H. for 6 or 9 days, followed by ripening at room temperature). In addition fruits harvested at the three different ripening stages were also subjected to optimal storage conditions (8°C, turning and red tomatoes; 13°C green tomatoes, all at 95% R.H.) for 16 days.

At the end of the post-harvest experiment fruits were analyzed for volatile aroma compounds, organic acids and soluble sugars. About 40 tomato aroma compounds were determined by a SBSE-GC-MS method (1) developed in our lab.

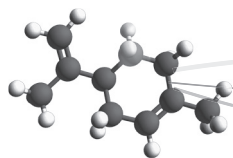
Conditions of medium and long distribution chain (harvest at the green stage followed by cold storage-transport) markedly affected the formation of some aminoacid-derived key odorants: the formation of 1-nitro-2-phenylethane was strongly inhibited when compared to vine ripened fruit or to fruit subjected to short chain conditions. On the contrary, other phenylalanine derivatives, phenylacetaldehyde and 2-phenylethanol, showed increased levels in fruits subjected to medium chain conditions, similarly to 2- and 3-methylbutanal, which in turn derive from isoleucine and leucine, respectively. A significant inhibitory effect associated to cold storage conditions was also observed on another aminoacid-derived key odorants, isobutylthiazole.

Formation of lipid-derived key odorants, such as (Z)-3-hexenal, hexanal, (E)-2-hexenal, 1-penten-3-one was scarcely influenced by the considered post-harvest conditions. Differential effects were observed on carotenoid-derived odorants. A somewhat reduced level of β -ionone and 6-methyl-5-hepten-2-one was found in fruits subjected to medium chain conditions when compared to vine ripened or short chain tomatoes, whereas beta-damascenone content tended to be only slightly affected by post-harvest conditions.

Minor effects were observed on organic acids and sugar formation. Among all the flavour related compounds analysed, the group of aminoacid-derived aroma compounds was that more strongly affected by the distribution chain conditions considered.

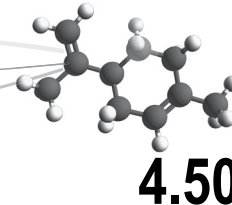
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Acknowledgement: The present study was performed within the project BIOVITA (W.P.3.1- Supply chain of horticultural products) funded by the Italian Ministry of Agriculture, Food and Forestry.



CONTRIBUTION OF VOLATILE COMPOUNDS TO THE AROMA OF FERMENTED SAUSAGES DURING PROCESSING

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Keywords: fermented sausages, multiple SPME, odour activity value, aroma, volatile compounds.

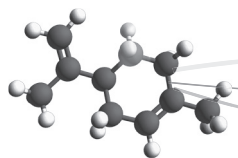
Abstract

Meat products are complex matrix where many volatile compounds are generated and interact with the food components. The composition of the odorants in the air above the food can be obtained by headspace analysis however, it is necessary to estimate the proportion of volatile compounds in the whole product to predict their impact on the aroma. In this sense, the quantitative analysis of volatile compounds in a complex matrix was described by Kolb et al. (1) with a procedure called multiple headspace extraction (MHE). The method is based on a stepwise gas extraction at equal time intervals allowing the total area for the compound to be calculated and eliminating the influence of the matrix. Multiple headspace solid-phase microextraction (multiple HS-SPME) has the same aim than MHE (2).

In the present work, the most important aroma compounds in fermented sausages were selected by calculating their odour activity values (OAVs). The compound quantification in the headspace (HS) was carried out by SPME and the total concentration in the sausage by multiple HS-SPME using gas chromatography and mass spectrometry analyses. Then, the odour-activity values (OAVs) in air or oil of each compound present in the HS or in the matrix were calculated (3). For this purpose, the concentration of the compound in the HS or in the sausage (total concentration) was divided by the detection threshold in air or oil respectively (4, 5).

The fermented sausages analyzed were processed under a slow fermented process (6). The OAV in oil obtained during the processing stages revealed that several compounds showed positive OAV since the beginning of the fermented process (2-methyl butanal, 3-methyl butanal, octanal, 2,3-butanedione and ethyl 2-methyl butanoate). In addition, during the fermented process other compounds increased in concentrations showing positive OAV (propanal, pentanal, hexanal, ethyl 3-methyl butanoate, 1-octen-3-ol, 3-methyl butanoic, 2-methyl propanoic, ethyl hexanoate and nonanal).

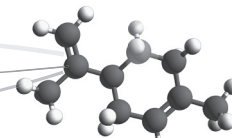
The concentration of the aroma compounds was also determined in the headspace of fermented sausages cured for 42 d by SPME-GC-MS. The concentration of all the compounds analyzed was lower than 20 % of the total concentration determined in the matrix by multiple SPME analysis except for the compounds; 2-heptenal (24%), nonanal (35%), 2-nonanone (24%), ethyl acetate (93%), ethyl pentanoate (79%), and ethyl hexanoate (47%). In addition, the OAV calculated in the matrix and in the air



above the fermented sausages cured during 42 days were compared. However, when the OAV were calculated from the headspace concentrations and using the detection threshold in air only few compounds showed high OAV in air (3-methyl butanoic, ethyl 2-methyl butanoate, ethyl hexanoate, hexanal, octanal and nonanal). In many cases, the OAV in the matrix were higher than those calculated in air except for the OAV of octanal and nonanal. These results indicate the high retention of aroma compounds produce by the meat matrix. In summary, the generation of aroma compounds during the fermented process depends on many processing factors but at the same time, matrix composition affects their contribution to the final aroma.

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**AROMA VOLATILES OF CHINESE-STYLE FRIED PRAWN OIL**

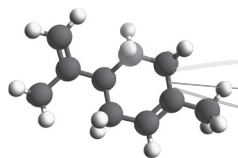
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Key words: prawn, shrimp, seafood, flavour volatiles, GC-olfactometry

Fried prawn oil is traditionally used by Asian chefs as a flavour enhancer for both broths and cooked dishes. Frying the prawn heads and shells in relatively hot oil (up to 140 °C) generates a strong desirable aroma that is quite different from that of steamed or boiled prawns due to the higher temperature applied. Care is taken to ensure that the temperature does not exceed

140 °C as this would result in an undesirable burnt aroma. Surprisingly limited information is available in the literature on fried prawn aroma. Hence, this study aims to elucidate the key character impact volatile compounds of fried prawn (*Penaeus monodon* and *Melicertus plebejus*) oil through headspace, SAFE and SDE (Likens-Nickerson) extraction followed by GC-MS analysis and GC-Olfactometry. Sensory evaluation of the flavour isolates from Headspace, SAFE and SDE demonstrated that each extraction method emphasized a different aspect of the flavour profile. Headspace trapping was able to isolate low boiling reactive volatiles such as dimethyl sulfide, pyrrolidine, 2-methyl piperidine, 1-butyl pyrrolidine, 1-methyl pyrrolidone and trimethylamine. The application of SAFE allowed the identification of higher boiling components important for fried prawn aroma like longer chain ketones, aldehydes, lactones and pyrazines, as well as ionone derivatives. These ionone-derivatives, possibly breakdown products from carotenoids, were identified in prawns for the first time and found to be important for the characteristic fried prawn aroma. These included alpha-ionene, alpha-ionone, beta-ionone and dihydro gamma-ionone. SDE emphasized not only the higher boiling components but also exhibited a higher proportion of Maillard volatiles and sulphur components compared to the other methods. The latter are generated during the cooking process from the breakdown of sulfur-containing amino acids, and include dimethyl trisulfide, dimethyl disulfide and methional. Information from all 3 extraction methods is necessary to enable a more complete, well-rounded re-constitution of the aroma of fried prawn oil.



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EATING QUALITY OF NEW DEVELOP PINEAPPLE ACCESSIONS CORRELATIONS BETWEEN FLAVOR PROFILES AND SENSORY LIKING TEST OF TWO PINEAPPLE CULTIVARS AND THEIR NEW GENOTYPE

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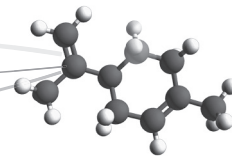
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Keywords: flavor, pineapple, sensory, genotype, cultivar, correlation

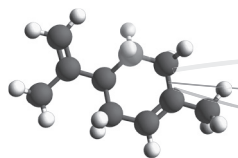
Pineapple has an important role in Indonesia's national economy as well as one of the main export commodities. In 2003, Indonesia has been ranked as 10th in the world as a country-exporting pineapple (4). Efforts to increase the competitiveness of pineapple fruit has been done by the Centre of Tropical Fruits Study (PKBT) at Bogor Agricultural University (IPB), Indonesia, through a long term breeding program in order to obtain superior varieties of pineapple with high consumer preferences. Flavor is one of the main attributes of foods and is given by a combination of volatile molecules. These compounds especially odor-active compounds are often considered to play dominant role in the flavor of fruit, while flavor is one determinant of consumer acceptance. (1, 2, 3, 7). There are two varieties of pineapples and one of their genotype those have been developed, namely 'Delika Subang', 'Mahkota Bogor', and 'Pasir Kuda' (5, 6). The hedonic test has been conducted by 81 untrained panelists to evaluate the acceptability of color, aroma, taste, texture and overall. A rank test has also been conducted to evaluate the overall acceptability of the samples. The flavor profiles have been determined by Quantitative Descriptive Analysis (QDA). The volatiles were extracted using different sample preparation methods (liquid-liquid-extraction and solid-phase micro extraction/SPME). For identification and semi-quantification GC-MS was carried out. Character impact compounds which contribute to the aroma impression were characterized by Gas chromatography-olfactometry (GC-O). The so called nasal impact frequency method (NIF) was used (8). Based on the sensory liking test, 'Mahkota Bogor' was characterized by the highest acceptability in terms of overall attributes, whereas 'Delika Subang' was the least accepted. This result has also been supported by the results of the rank test which showed 'Mahkota Bogor' has the highest rank, followed by 'Pasir Kuda' (new genotype) and 'Delika Subang', respectively. Significant positive correlations between the preference flavor and olfactory detectable aroma impact compounds like methyl 2-methyl butanoate and ethyl 2-methyl-butanoate were indicated thorough the analysis results. The



correlations between the flavor compounds in pineapples, flavor profiles and their sensory acceptance will be discussed further on the presentation.

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AROMA ANALYSIS AND DATA HANDLING IN THE EVALUATION OF NIECHE JUICE PRODUCTS FROM 160 LOCAL DANISH APPLE CULTIVARS

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Keywords: aroma, apple juice, PCA, PARAFAC2, multi way analysis

Apple juice has traditionally been a low cost product in Denmark. It is made from fruit not meeting the quality demands for fresh consumption due to factors like appearance, firmness or sensitivity to bruising. These criteria are however not critical in juice processing and some of the old local cultivars may have unique flavour qualities that can be attractive in juices.

Nowadays, most of the apple juice available on the Danish market is imported as concentrates, produced from a mix of different apple cultivars. However, there is a growing interest in local speciality products with unique flavour attributes.

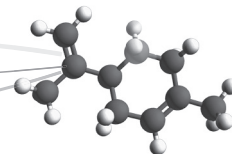
The present study is part of a project aiming to promote the utilization of Danish apple cultivars and increase the competitiveness of growers in the market, by identifying the suitability of local varieties for niche markets for fruit juices with tailored sensory and nutritional labels. Results will be integrated into the online "Apple PC-Key" database which is being developed to promote interest in the old Nordic apple cultivars and provide public access to the most current knowledge.

Aroma analysis was performed on juices from 160 local apple cultivars from the collection of European fruit genotypes (Pometum, University of Copenhagen). Preliminary data evaluation showed that the cultivars possessed a high degree of variability being characterised by either acetate esters, butyrate esters or a general low level of most aroma compounds.

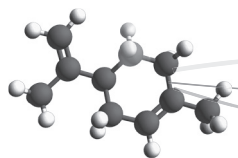
However, analysing such large number of samples, with a great genetic variation, introduce some challenges like baseline drifts, peak shift, co-elution and a tedious job manually desicating all the chromatograms. Hence, advance methods for baseline correction (e.g. b-splines) and peak alignment (icoshift) (1,2) will be used to obtain a comparable set of chromatograms. MS fingerprint will be used to elucidate patterns in the chromatograms. Moreover, multi-way analysis (Parallel Factor Analysis 2, PARAFAC2) (3,4) will be applied in selected chromatogram areas to resolve areas with overlapped peaks and to identify those analytes of interest.

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4.54

CHARACTERIZATION OF ODOUR-ACTIVE COMPOUNDS IN AROMATIC CARAMEL USING GC-OLFACTOMETRY AND GC-MASS SPECTROMETRY

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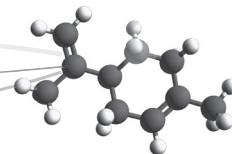
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Keywords: caramel; aroma-active compounds; SAFE; GC/Olfactometry

Aromatic caramel is a product widely used in food industry. Considered as food ingredient, it is commonly used to aromatize dairy products, ice cream or ready to eat meals. Sugar and water are the basic ingredients for caramel production. Heat treatment of this mixture induces a non- enzymatic browning reaction. Due to its importance in food science, this type of reaction has been studied since the beginning of the 20th century, especially the Maillard reaction which involves amino acids. However, only a few studies dealt with caramel itself. The most recent studies provided an identification of more than 57 compounds in aromatic caramel and vapour during cooking (1) and 15 of them were associated to olfactory properties (2).

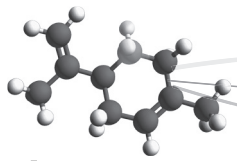
The main purpose of the present study was to characterize aroma-active compounds in 4 aromatic caramels, differing in carbohydrate composition and cooking process (temperature/time). The volatile fraction was isolated by Solvent Assisted Flavour Evaporation (SAFE) technique and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Aroma-active compounds were evaluated with GC-Olfactometry (GC-O) performed by 9 judges in duplicate and results were processed with detection frequency method (3). Identification of compounds was achieved by comparing mass spectra, linear retention indices (LRI) and odour of standards or from data available in the literature. In parallel, caramel sensory profiles were performed with a panel of ten trained assessors.

A total of 75 odorant areas (detection frequency $\geq 28\%$) were detected and the related aroma compounds have been identified. Among them, heterocyclic oxygen compounds and cyclopentenone derivatives appeared as main contributors to the aroma and were responsible for the empyreumatic character. These compounds are the characteristic products of caramelization reaction formed by thermal degradation of carbohydrates. Carboxylic acids (pungent, sour), aldehydes (vegetal), esters (fruity) and ketones (fatty, buttery) were also highlighted. Results from partial least-squares regression (PLSR) between odour-active compounds detection frequencies and sensory attribute intensities showed a relationship between chemical composition and sensory properties. Cyclopentenones and furanic compounds, for example 2-(hydroxyacetyl)-furan and 5- methyl-2(3H)-furanone, were mostly detected in caramels that are described as burnt, roasted and sour. Whereas esters and aldehydes were mostly detected in other caramels which were described as sweet, honey-like and fruity. These differences could be linked to caramelization level.



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4.55

IDENTIFICATION OF KEY ODORANTS IN CEREAL COFFEE

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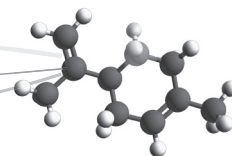
Keywords: cereal coffee, key odorants, AEDA, roasting

Analysis of coffee flavor aroma has gained a lot of attention and great number of research papers have been already presented (1). On the other hand beverages prepared from cereal roasted grains have been very popular in middle and eastern European countries for many years. At the beginning they were treated as a cheap coffee substitutes but right now they are becoming more and more popular due to its health attributes as compare to regular coffee such as lower acidity and lack of caffeine but also they are becoming appreciated for its specific roasted flavor.

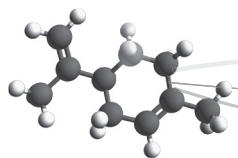
In presented studies typical Polish cereal coffee made from roasted barley, rye, beetroot and chicory has been presented to key odorants analysis with the application of gas chromatography – olfactometry (GC-O) and aroma extract dilution analysis (AEDA). In the analyzed cereal coffee extract obtained with the use of solvent assisted flavor evaporation method (SAFE) 30 aroma-active volatiles have been identified with the FD factors ranging from 4 to 4096. The highest FD value (4096) was obtained for furfuryl mercaptan with the roasted, coffee-like aroma and very low odor threshold of 0.01 µg/kg in water (2). Flavor compounds identification and quantification has been performed with application of comprehensive multidimensional gas chromatography GCxGC and time of flight mass spectrometry (TOF) which have been already reported as a sensitive tool for analysis of low odor threshold flavor compounds. Quantification of most potent odorants has been carried out with the labeled internal standard addition and odor activity values has been calculated. Analytical results have been correlated with sensory analysis as well. Finally results of studies on key odorants of individual grain ingredients such as barley, rye, beetroot and chicory will be presented and their effectiveness in generating the overall flavor of analyzed cereal coffee has been verified as all analysis were performed on cereal coffee as well as on individual ingredients.

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4.56

MUSK STRAWBERRIES – FORMERLY FAMOUS FRUIT REASSESED

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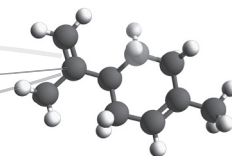
Keywords: flavor, strawberry, characterization

Abstract

The demand for new, exotic food and beverage flavors initiated in recent years many “treks” into the tropic areas of the Earth. Nonetheless, there still exist not-fully characterized fruits growing in the “normal” world, whose flavor could surprise the interested consumer. Strawberries belong to one of the most sought fruits all around the globe. Even if for consumers is the aroma of strawberries the most important quality indicator, factors such as color and size of the fruits, texture, shelf life and yield of the crops has been until recently in the center of breeding aims. Not surprisingly, the constant criticism of consumers on sensory quality of commercial garden strawberries is pervasive.

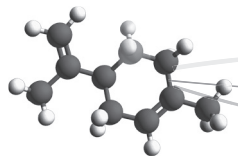
Musk strawberry (*Fragaria moschata*) is the only hexaploid ($2n = 6x = 42$) from all strawberry species. Until the outbreak of garden strawberry in the 19th century, it was the musk strawberry, together with wood strawberry, which was mostly planted [1]. Musk strawberry is believed to be the first strawberry with a given cultivar name, which was “*Gallobelgis des Chapirons*” [1]. It was also the preferred strawberry variety of Jane Austen and F.D. Roosevelt, who was encouraging the strawberry breeders to experiment with musk varieties [2].

Fruits of wildly growing ripe musk strawberries from two highland sites were studied during two seasons in this work. The fruits were in situ characterized by flavorists and the full profile of volatile compounds was extracted immediately after collection using the Solid Phase Extraction (SPE) technique. The aroma of whole fruits was later characterized with the help of dynamic headspace, and the SPE extract was analyzed by Gas Chromatography – Mass Spectrometry and Gas Chromatography - Olfactometry. Single fruits are characterized by green, spicy, seedy and sweet exotic notes, which turn in bunchy fruits into a complex mango- like smell. More than one hundred distinctive volatile compounds were observed by Gas Chromatography – Mass Spectrometry. Many of them are reported for the first time in *Fragaria* species, most notably the abundant Coniferyl alcohol and Chavicol. GC-Olfactometry revealed Mesifurane, Eugenol, Methyl butyrate, Furaneol and 3-Mercaptohexyl acetate as the key components of highlands musk strawberry flavor.



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4.57

COMPARISON OF DIFFERENT EXTRACTION METHODS TO ISOLATE AROMA VOLATILES FROM CASHEW APPLE JUICE

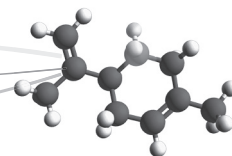
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Keywords: cashew apple, volatile compounds, Liquid-liquid extraction (LLE), Solid-phase microextraction (HS-SPME), Dynamic headspace.

Of the methodologies available for extracting the volatiles of greater importance for the aroma and flavour of beverages, liquid-liquid extraction (LLE) is one of the most traditional, but consumes large amounts of expensive, generally toxic, solvents. Dynamic headspace methodology (DHS) is also widely used, but recently solid phase micro-extraction (SPME) has gained popularity, being easy to use, quick, requiring no solvents and using a small sample volume. Thus the objective of the present research was to compare the efficiency of LLE, HS-SPME and DHS on the profile of the odour volatiles isolated from fresh cashew-apple juice. Initially, DHS was used to strip the volatiles from the headspace of 300mL fresh cashew apple juice (cv CCP76) for 2 h to a PorapakQ[®] trap using vacuum at 70mmHg and room temperature and then elute with 300µL acetone. SPME extraction was carried out for 45min in the headspace of 5ml of fresh cashew juice at 22±2°C using a DVB/Carboxen/PDMS fibre. After extraction, the SPME device was introduced into the splitless injector of a Gas Chromatograph (GC), and maintained at 200°C for 5min. Finally, the volatiles present in 40mL of fresh cashew juice were extracted by LLE with 15mL of methylene dichloride using 3 consecutive extractions. A second extraction was carried out in the same way, and the extract concentrated to 0.5mL under a nitrogen flow, generating a concentrated LLE extract. The four isolates were evaluated by gas chromatography coupled to mass spectrometry (GC-MS), and GC-olfactometry (1) was carried out on both the DHS and the two LLE isolates. Less volatiles were isolated by HS-SPME ($n=72$ volatiles) as compared to DHS ($n=100$ volatiles), and LLE with ($n=116$ volatiles) and without concentration ($n=100$ volatiles). Slightly more odoriferous volatiles were detected in the DHS isolate ($n=44$) as compared to isolates generated by LLE with ($n=38$) and without concentration ($n=39$). Ten volatiles, all esters obtained from the DHS isolate showed average odour intensity equal or above 4.0 on a 10-point scale, indicating that the panel members perceived their intensity as between moderate and high. Six volatiles from the concentrated LLE isolate were perceived with an average odour intensity ≥ 4 , including 4 esters, 1 alcohol and 1 acid. Finally, 7 volatiles from the non-concentrated LLE isolate showed average odour intensity ≥ 4 , including 3 esters, 3 terpenes and 1 aldehyde. The GC-MS technique confirmed that the esters were best isolated using the DHS technique, representing 33% of the total chromatogram area of the DHS isolate, 16% of that of the concentrated LLE isolate and 11% of that of the non-concentrated one. Esters represented 39.53% of the total chromatogram area of the

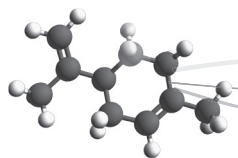


HS-SPME isolate but ethyl hexanoate alone corresponded to 31% of the total area. GC-MS also indicated that terpenes were best isolated by the non-concentrated LLE extract where they represented 41% of the total chromatogram area, whereas for the DHS isolate they represented 11% of the total area, less than 2% for the concentrated LLE extract, and less than 1% for the HS-SPME isolate.

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Acknowledgements: to CAPES (AUX-PE-PNPD-1470/2008) and FAPESP (2008/55986-0) for their financial support.



4.58

EFFECT OF REFRIGERATION ON SELECTED ALDEHYDE AND ALCOHOL AROMA VOLATILES OF TOMATO AND THE IMPACT ON ITS FLAVOUR PERCEPTION

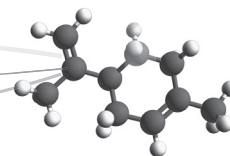
Ponce-Valadez M., Sanchez-Lecuona M., Chong-Sanchez F., Cordero-Garcia H.G., Diaz de Leon-Sanchez F., ESCALONA-BUENDIA H.*, Rivera-Cabrera F. and Perez-Flores L.J.

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Keywords: aroma, alcohol dehydrogenase, storage, quantitative descriptive analysis

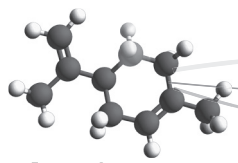
Refrigeration is the main postharvest technology to increase shelf life in horticultural products. Even though, optimal storage temperatures have been established for different commodities, there are few studies on the effect of low temperature on flavour. Previous research (1) showed that storage of saladette tomato at 10 °C resulted in changes both in volatile profile and sensory perception. Fresh tomato characteristic flavour is the result of complex interactions between organic acids, soluble sugars and over 400 volatile compounds, derived from different biochemical pathways such as the catabolism of lipids, aminoacids, lignins and carotenoids (2). Some of the more abundant aroma volatiles are derived by the oxidative degradation of fatty acids. Lipoxygenase (LOX) is a key enzyme in the regulation of this pathway. Alcohol dehydrogenase (ADH) is an important enzyme which contributes to flavour development by interconverting aldehyde and alcohol forms of volatiles originated from lipids and aminoacids. In this study, we analyzed the effect of the recommended storage temperature of tomato (12.5°C) both on the physical quality and on the volatile profile determined by GC-FID and its correlation with LOX and ADH activity and gene expression and sensory perception. Refrigeration delayed loss of weight, firmness and colour changes. Regarding the effect of refrigeration on the aroma volatile profile, the most dramatic changes were observed at longer storage times when compared to fruit stored at 20°C. LOX activity and gene expression decreased through storage independently of the temperature suggesting that this enzyme participates in the aroma development at earlier ripening stages than the one commonly harvested and refrigerated. Low temperature inhibited ADH activity and gene expression and as a consequence the behavior of some alcohols and aldehydes volatiles was altered. Descriptive sensory analysis and a consumer study showed that changes of tomato stored at 12.5 °C had a lower impact on the aroma than those observed at 10 °C when comparing both with tomato stored at 20 °C. This research suggests an optimal storage temperature for maintaining the integral quality of saladette tomato and contributes to understand some of the biochemical changes that affect the biosynthesis of aroma volatiles under refrigeration.



This research was funded by UAM, CONACYT (No. 35126), CA PROMEP (No. 34775), Apoyo Exbecario PROMEP and Programa de Reincorporación de Profesores UAM. We thank UAM for Monica Ponce postdoctoral fellowship.

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4.59

EFFECT OF SOIL NUTRITION ON AROMA COMPOUND FORMATION IN ORGANICALLY GROWN APPLES (CV. GOLDEN DELICIOUS).

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Keywords: apple, Stir Bar Sorptive Extraction, aroma compounds, organic, fertilizer

In organic apple production only organic fertilizers can be used to supply the soil with nitrogen. In organic fertilizers the mineralization process, needed to provide the plants with the required amount of available nitrogen, tends to be slower than in the synthetic counterparts. This in turn may adversely affect aroma compounds formation in the fruit, even though little is known about the effect of nitrogen soil nutrition on biogenesis of fruit volatile compounds (1).

The aim of the present study was to assess the effect of some commercial fertilizers used for organic apple production on formation of volatile flavour compounds in cv. Golden Delicious apples. Fruits obtained in experimental fields by using three distinct organic fertilizers were considered: Azocor 105 (both animal and vegetable origin), Agrobiosol (fungal biomass) and compost + biogas slurry. In addition fruits from a plot without the use of fertilizer ("control") and a plot where a mineral fertilizer ("ammonium sulphate") was used, were examined for comparison. The rate of N application was normalized for all fertilizers. In addition, only for Azocor 105, two different N rates were tested.

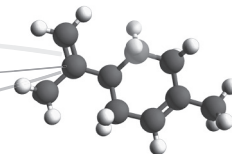
Fruit volatile aroma compounds were determined by a SBSE-GC-MS (2) method developed in our laboratory.

Preliminary results from a first harvesting year showed significant differences in the total amount of volatiles: higher levels were found in the "compost + biogas slurry" sample, whereas a lower amount was observed in the "ammonium sulphate" sample. In "compost + biogas slurry" sample and in the "control" butyl acetate level was significantly higher than in the "ammonium sulphate" and in all Azocor 105 samples; in the "control" hexyl acetate concentration was higher than in the "ammonium sulphate" sample and the high N rate Azocor 105 sample. On the contrary, no significant differences were found in the levels of the branched-chain (aminoacid derived) 2- methylbutyl acetate.

Preliminary results suggest that both rate of N application and, for the same rate, also the nature of the used organic fertilized have the potential to significantly affect flavour formation in apple fruit.

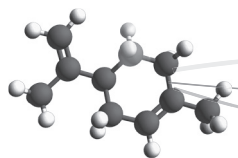
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Acknowledgement: The present study was carried out within the project "Qualita' nutrizionale ed organolettica e impatto ambienta/e di produzioni biologiche. Un caso studio: il meZo" funded by the Italian Ministry of Agriculture, Food and Forestry.



4.60

PROFILE OF NEW IMPROVED BRAZILIAN *CAPSICUM CHINENSE* PEPPERS.

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DEBORAH S. GARRUTI

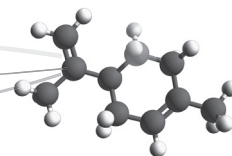
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Keywords: aroma, flavor, dynamic headspace, solid phase micro-extraction, GC-MS

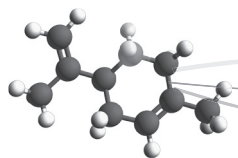
Capsicum peppers have a significant international market (1). Their attributes such as pungency, color, aroma and flavor, desirable in a variety of culinary dishes around the world, make them widely appreciated. In order to expand the Brazilian *Capsicum* agribusiness, the Brazilian Agricultural Research Corporation (EMBRAPA) is carrying out a breeding program in order to develop new genotypes with characteristics of agronomical and industrial interest. Besides the capsaicin content, which is responsible for the burning sensation, the program is looking after strains able to add aroma and flavor to foods (2). In the present work the volatile profiles of two accesses of the Pepper's Active Germoplasm Bank with strong pungency, CNPH 3931 (yellow *Murupi*) and CNPH 4080 (*Cumari-do-Pará*), were compared to the profile of a very scent Brazilian variety, called "orange *Biquinho*", all *Capsicum chinense* Jacquin cultivars (3). The headspace volatile components were isolated by Solid Phase Micro-Extraction (SPME), separated by Gas Chromatography (GC) and identified by GC-MS and retention indices. In total, 57 compounds were detected. *Biquinho* showed a richer profile (46 compounds versus 35 in CNPH peppers), but CNPH 4080 presented more total volatile quantity. In all samples esters were the predominant chemical class, but were also detected alcohols, aldehydes and terpenes. Sousa *et al.* (4) also found similar results for several red, yellow and purple Brazilian *Capsicum chinense* peppers. Odoractive compounds of *Biquinho* pepper, determined in a previous work (5), were used as target compounds to compare profiles. Compounds with medium to high intensity of pepper-like aroma were more abundant in the novel cultivars (hexyl isobutanoate, hexyl isovalerate, dimethyl-2-nonyl-ciclopropane and a non-identified terpene, amongst others). Two contributor compounds with green notes (citronellyl isovalerate and hexyl valerate) were also found in larger quantities in CNPH samples. However many compounds present in *Biquinho* pepper with strong sweet, floral and fruity notes were found in the novel material only in small quantities or were even not detected. i.e. pentyl isopentanoate, pentanal, squalene, α -cardinol. *Biquinho* showed 13 peaks (28% of peaks) described with sweet, floral and fruity notes, while the CNPH 3931 and CNPH 4080 showed 7 (19%) and 5 (14%), respectively. Also the *Biquinho*'s chromatogram total area referring to these notes was bigger than the other peppers. Results show that the volatile profiles of novel varieties present high quantity of compounds with pepper-like and herbal odor, and lacks desirable esters which can add a variety of different pleasant notes. These genetic materials could undergo future cross breeding to enhance their flavor quality.

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4.61

VARIABILITY OF ALLYL HEXANOATE CONCENTRATION IN PINEAPPLE-FLAVOURED BEVERAGES AND YOGURTS.

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Keywords: allyl caproate, pineapple, SBSE, beverage, yogurt.

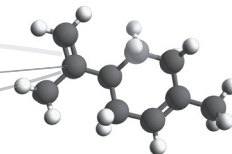
One of the main sources of uncertainty in the estimation of dietary exposure to flavouring substances is the uncertainty in presence/absence and in concentration level of flavouring substances, naturally present or added to foodstuffs.

Allyl hexanoate is a character impact compound, commonly added to give a “pineapple” flavour, which does not occur naturally in pineapple itself. In the present work two simple and rapid methods for the quantification of allyl hexanoate in pineapple-flavoured fruit based beverages and yogurts were developed in order to assess the variability of its concentration levels in these products and to provide data to be used for a refined estimate of dietary exposure to this substance.

The analytical methods were based on the SBSE (Stir Bar Sorptive Extraction) technique for the isolation step and the GC-MS analysis for the final determination. An SBSE extraction time of 90 minutes on a 1:200 diluted filtered juice was set for the beverages, 60 minutes and 1:400 dilution for yogurts. A suitable internal standard was identified in hexyl propanoate. Performance characteristics of the two methods were: calibration on a linear range from 0.09 to 26.61 mg kg⁻¹, with $r^2 = 0.9997$ (for beverages) and from 0.04 to 61.20 mg kg⁻¹, with $r^2 = 0.9979$ (for yogurts); R.S.D. of 0.72 %, and 1.43%, respectively, for repeatability; recoveries of 99÷104% and 97÷99%, respectively, for accuracy.

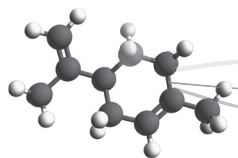
Thirty-five beverages (24 with pineapple juice as main ingredient and 11 “tropical” or multi-fruit juice beverages) and 30 yogurts (18 from whole-fat-milk, 12 from low-fat milk) purchased from the local market (Rome) were analysed.

In the fruit -based beverages, allyl hexanoate concentration ranged from trace levels to 16.7 mg kg⁻¹. In beverages with pineapple juice as main ingredient, in 14 out of 24 products, allyl hexanoate level was within a relatively narrow range (0.5÷3.7 mg kg⁻¹), in 9 products it was low (<0.1 mg kg⁻¹), whereas in a single product it showed a markedly higher concentration (16.7 mg kg⁻¹). In the group of “tropical” or multi-fruit juice beverages the level was in most cases lower than 0.1 mg kg⁻¹. Regarding yogurts, the most populated group (18 products) was that with allyl hexanoate level within 1 and 10 mg kg⁻¹; 6 samples showed low levels (<1 mg kg⁻¹), 4 products were in the range from 10 to 20 mg kg⁻¹, whereas in 2 samples (from the same brand) markedly higher concentrations (74 and 89 mg kg⁻¹) were found.



In most of the products allyl hexanoate levels were close to the use levels reported by recent industry surveys, even though in single cases concentrations more than 10-fold higher were found.

Acknowledgement: This work was co-funded by the European Union under Grant Agreement 211686 (Project FACET -Flavours, Additives and Food Contact Material Exposure Task).



4.62

INVESTIGATION OF FOUR TROPICAL UNIFLORAL HONEY AROMAS USING SENSORY AND SPME GC-OLFACTOMETER AND GC-MS ANALYSES

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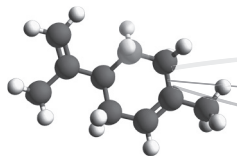
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Keywords: aroma-active compounds, QDA, Sabsua honey

Honey is one of the most ancient foods and has long been used as a source of sweetness and for its perceived health benefits (1). Honey can be produced from many flower types as multifloral or single flower types for unifloral honeys. Most unifloral honeys have unique aromas and generally greater commercial value. Some of the most popular unifloral tropical honeys are produced from longan, lychee, common floss and coffee flowers. Instrumental based marker compounds and sensory analysis can be used to differentiate these honeys and depending on the extent, also indicate possible adulteration. Headspace SPME has been used to identify volatiles in unifloral honeys from flowers grown in temperate regions (2,3). However, few studies have examined the uncommon aroma profiles honey produced from tropical or subtropical flowers. The purpose of this study was to identify the key volatiles that are responsible for the unique aroma profiles from four different unifloral honeys using both instrumental and sensory techniques. Solid phase microextraction (SPME) combined with GC-O and GC-MS was used to analyze the volatiles in four Thai unifloral honeys. These honey types included: longan honey (*Dimocarpus longan*), lychee honey (*Litchi chinensis*), coffee honey (*Coffea arabica*) and common floss flower honey (*Eupatorium odoratum* L.). Aroma profiles were established with a trained sensory panel using quantitative descriptive analysis. The aroma profiles of the four honeys were decidedly different. Longan honey was described as a floral (citrus blossom) honey with balsamic (liquorice and menthol) figs candy and chemical notes. Lychee honey was described as possessing an intense balsamic and woody odor with also a floral odor character. Coffee honey had odor notes of stone/seed fruit (peaches) and toasted (malt). The common floss flower honey was described as having dried fruits (figs) odor with an animal note in nose and by having toasted (caramel) and floral (withered flowers) notes in mouth. GC-O data indicated there were 26, 28, 22 and 19 aroma active volatile compounds in longan, lychee, coffee and common floss flower honey respectively. There were seven aroma active compounds common to all four honeys: (E)- β -damascenone, phenylacetaldehyde, acetic acid, 2,3-butanedione, guaiacol and 1-

References:

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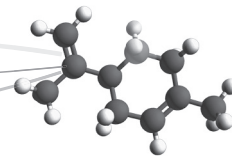
VARIETAL DIFFERENCES IN THE VOLATILE PROFILE OF BANANAS WITH RESISTANCE TO BLACK SIGATOKA

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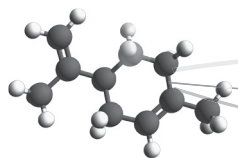
Keywords: Musa sp.

Very appreciated all over the world, banana is the fourth largest agricultural crop in the world and Brazil is the fourth largest world producer, with a production of more than 7 million ton/year (1). This production is almost entirely absorbed in the domestic market, given the importance of this fruit in the population's diet. However, the main cultivars sold in Brazil are susceptible to the fungus *Mycosphaerella fijiensis* Morelet, causes the Black Sigatoka disease which, depending on conditions, may cause losses up to 100% in production (2). As an alternative to counter the spread of this disease the Brazilian Agricultural Research Corporation (Embrapa) is developing new resistant varieties (3). Before these materials are made available to producers, it is necessary that its flavor and the acceptance are evaluated. The objective of this study was to compare the volatile profile of two most popular banana cultivars in Brazil (Prata and Pacovan), susceptible to Black Sigatoka, with some resistant hybrids of them: Vitoria, Maravilha, Galil 18, Preciosa, Fhia 18, Pacovan Ken, Garantida, Japira and PA 4268. Fruits at full ripening stage were homogenized with the addition of NaCl (40%) and then frozen in small portions. After dilution (1:1 in water), the headspace volatiles were trapped by solid- phase microextraction (SPME) (DVB/CAR/PDMS, 60 min, 25 °C, under agitation), separated and identified by GC-mass spectrometry (DB-Wax, 1 mL/min, 55 min, splitless). Multivariate analysis of data was performed by Principal Component Analysis (PCA) using the chromatogram peak areas. The cultivars were well discriminated by the first two dimensions. The non-resistant varieties Prata and Pacovan and their hybrids Galil 18 (Prata) and Pacovan Ken (Pacovan) showed similar profiles, mainly characterized by compounds 2-heptyl butanoate, 2-pentanol and 2-heptanol, located in the lower left region of the graph. These resistant varieties could replace the susceptible cultivars without impairing their characteristic flavor. PA 4268, Vitoria and Garantida, plotted very close to each other in upper left region, showed greater amounts of isopentyl isopentanoate, 2-methyl-1-propanol and 1-butanol. Compounds arranged in upper right region such as butyl butanoate, isobutyl acetate, isopentyl acetate and especially 2-methylbutyl 2-methylbutanoate were characteristic of Fhia 18 and Preciosa, which showed similar volatile profile. Cultivar Maravilha showed to be poor in volatile compounds while Japira presented the richer profile, composed mainly by esters with characteristic aroma of banana such as isopentyl acetate, isopentyl butanoate, isobutyl butanoate, isopentyl isobutanoate and isopentyl isopentanoate. This cultivar seems to be promising in terms of flavor. We are finishing the sensory profile of these varieties and will correlate sensory with analytical data in a future work.



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4.64

VOLATILE COMPOUNDS CHARACTERIZATION IN DARK CHOCOLATES BY HS-SPME AND GC- MS. INFLUENCE OF COCOA ORIGIN AND ROASTING CONDITIONS.

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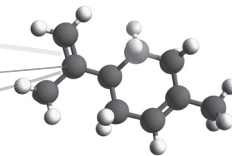
Key words: dark chocolate, cocoa origin, roasting, volatile compounds, HS-SPME, CG-MS.

Chocolate is one of the most popular foods around the world because of its sensory properties, which traditionally has been consumed mainly for its taste and flavour. The secret of chocolate flavour so highly appreciated worldwide, resides mainly in its volatile aromatic fraction. Parameters such as processing conditions and cocoa bean composition can be key factors in generating characteristic volatile compounds in chocolate, affecting the final taste and flavour (1, 2). The objectives of this study were to characterize volatile compounds in dark chocolates and to evaluate the effects of cocoa origin and roasting conditions on chocolate flavour quality.

Six dark chocolate samples with different cocoa geographical origin (Ecuador or Ghana) varying in roasting time (30.5, 34.5 and 38.5 min) were used in this study following a traditional chocolate manufacturing process (3). Chocolates containing 51% (w/w) of cocoa were prepared in a local chocolate factory.

An extraction method based on static headspace solid-phase microextraction (HS-SPME) with polydimethylsiloxane-divinylbenzene coating (PDMS-DVB) and carboxen-polydimethylsiloxane coating (CAR-PDMS) fibers was applied. GC-MS analyses were performed in a Voyager M800 detector coupled to a GC8000 Thermo Quest Chromatograph, equipped with a Teknochroma TRB-WAX capillary column (60m x 0.25mm x 0.25µm). The SPME-extracted volatiles were desorbed (5 min) into the split-splitless injector at 250°C, with Helium. The oven programmed temperature was: 60°C (5 min); 3°C/min to 200°C (6 min); finally, 4°C/min to 250°C (7 min). The mass spectrometer operated in EI+ mode, filament emission current 150µA, source temperature 200°C, interface temperature 250 °C and detector voltage 550V. Masslab and Xcalibur softwares were used for acquisition data. Identification of volatile compounds was carried out comparing retention times (area abundance > 10⁴) and their mass spectra with those of the US National Institute of Standards and Technology, NIST'08 mass spectra database library. Two identical samples were prepared for each analysis.

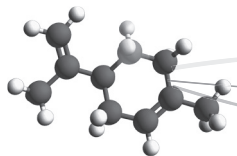
A large number of compounds were identified in dark-chocolate samples (more than 75), comprising alcohols, aldehydes, acetals, esters, ketones, furans, hydrocarbons, pyrans, pyrazines, pyridines, pyrroles, phenols, thiazoles and acids. Composition of the volatile



fraction varied among chocolates made with cocoa beans from different origin. Differences in the volatile compounds generated during roasting were also detected and quantified.

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4.65

IDENTIFICATION AND QUANTIFICATION OF ODOUR-ACTIVE COMPOUNDS IN STEAMED AND PAN-FIRED GREEN TEAS

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Keywords: odour-active compounds, green tea, quantification, GCxGC-TOFMS.

Green tea is one of the most consumed beverages in China and Japan but its popularity has not dethroned black tea much beyond these regions. Behind a common green tea name, a variety of cultivation, harvesting and manufacturing practices are in use and result in brewed green teas with very distinct sensorial characteristics. In a simplified view, typical green tea process involves an enzyme deactivation step on fresh leaves before cutting and drying the leaves. Such deactivation step is traditionally performed via steaming the leaf in Japan and pan-firing (dry heat treatment) in China.

To date, about 400 volatile compounds have been reported in green tea composition (1). Lists of odour-active compounds identified in different green teas based on GC-O dilution methodologies have been published (1-5) but the relation between these odour-active compounds and main processes practices has not been well established.

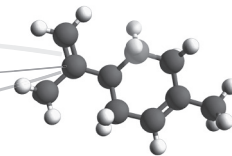
In this paper, the odour-active compounds from a commercial steamed and a pan-fired green tea have been identified based on a frequency of detection GC-O method with 7 trained assessors (6). Respectively 24 and 31 odour-active peaks were detected by at least 5 assessors in steam and pan- fired green tea extracts. Pan-Fired was seen to generate higher aroma concentration than steamed tea.

To comprehend better compositional differences between steamed and pan-fired green teas, our data were compared with GC-O results reported in green teas. A list of 35 odour-active compounds relevant for most green teas was established and was used to compare the aroma composition in 6 Chinese (pan-fired deactivation) and 6 Japanese (steamed deactivation) green teas.

Quantification and determination of the Odour Activity Values for the 35 odour-active compounds in these 12 green teas is reported. A SPME-GCxGC-TOFMS method was used for the quantification, as the improved sensitivity and separation power compared with traditional GC-MS was much needed for some aroma compounds.

Reference:

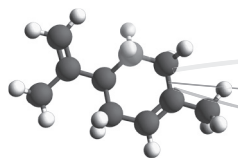
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4.66

CHARACTERIZATION OF GINS BY SENSORY AND CHEMICAL ANALYSIS

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Keywords: gin; descriptive analysis; napping, SPME-GC-MS

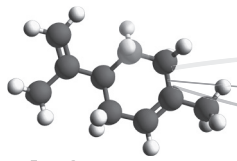
Gin is a distilled beverage developed in northern Europe in the 17th century. It has several classes and formulations. According to European Union (EU) regulation, it belongs to the “Distilled gin” class, which is produced by redistillation of alcohol 96% (v/v) in the presence of juniper berries (*Juniperus communis*), and other natural, botanic ingredients rich in essential oils, which contribute to the aroma of most gins (1). Although gin is well-known and widely consumed, there are few documented studies on its composition and sensory characteristics available. Currently, scientific literature on the sensory attributes of gins is sparse (2).

In this context, the objective of this work was to study if gins from four countries (England, Germany, France and the United States of America) differed sensorially and/or based on their volatile profiles. Twenty five gins were evaluated by 15 panelists in triplicate by descriptive analysis using 12 sensory attributes. These gins were also evaluated in duplicate by 12 panelists using a relatively new sensory technique called napping. Additionally the gins were analysed by headspace solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-HS-GC-MS) to determine their volatile profiles. The descriptive and napping data sets were compared and essentially described the same sensory space. It may be possible to substitute napping for descriptive analysis in cases where there are clear-cut differences in the sample sensory space. However, the volatile profiles were not very similar to the sensory space indicating that individual chemical compounds do not describe sensory responses but that matrix effects are more important. The gins from France and Germany were similar to one another (they were high in alcohol and caramel intensities relative to the other gins) and very different from the English and American gins. Some of English (for example Bombay and Beefeater) and American gins (Bellringer and Lords) were very similar to each other, while others were quite different (New Amsterdam and Booths – USA; Juniper Organic and Plymouth – England).

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4.67

EVALUATION OF TEQUILAS BY INSTRUMENTAL AND SENSORY ANALYSIS

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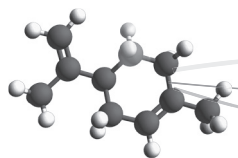
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Keywords: tequila; descriptive analysis; napping, SPME-GC-MS

Tequila is an internationally known distilled spirit produced in Mexico. This beverage is obtained by distillation of fermented juice from cultivated *Agave tequilana weber azul*, by processes that date back to more than three centuries (1). There are four categories of tequila 100% agave depending on the maturation process. 1) Tequila Blanco, which is un-aged, 2) Tequila Reposado, aged for a period between 2 to 12 months, 3) Tequila Añejo, matured for at least one year. 4) Tequila Extra Añejo, aged for at least 3 years. For the purpose of this analysis only Tequilas Reposado were used. Although tequila is a very popular drink that is well-known internationally, there are few studies on its volatile composition (2) and there is no scientific literature on the sensory attributes of Tequilas Reposado from Mexico.

On the other hand, in the market there is great variability in the price of this beverage. Hence, the aim of this work was to determine whether the price of Tequila Reposado affected its sensory attributes and its position on the sensory space. For that, twenty Tequilas Reposado, ranging in price from US\$10 to US\$50 for a 750 ml bottle were evaluated by 11 panelists in triplicate by descriptive analysis and using 11 sensory attributes. These Tequilas were also subjected to a napping exercise where 16 panelists who evaluated the Tequilas in duplicate. Additionally, the tequilas were analysed by solid phase microextraction- gas chromatography- mass spectrometry (SPME-GC-MS) to determine their volatile profiles. The napping data indicated that twelve descriptors (fruity, citrus, floral, soapy, agave, lime, smoke, wet hay, chemical, Play Doh[®], woody and dried fruit) completely describe the Reposado Tequila napping sensory space. The descriptive analysis sensory space was described by the following attributes: Play Doh[®], lemon, smoky oak, tea-tree oil, herbal, fruity, floral, orange, soapy, honey and alcohol. The two sensory spaces were quite similar and it seems as if one could substitute napping for descriptive analysis. The tequilas did not only separate by price in the sensory space (although some of the less expensive Tequilas were quite different from the more expensive ones) but more interesting and quite unexpectedly, Tequilas made by the same house were more similar to one another regardless of price. Two examples of this similarity would be Azul Tenampa (US\$26) and Cuervo Tradicional (US\$38) and Cazadores (US\$33) and Corzo (US\$46).

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IMPACT ODORANT IN STRAWBERRY VINEGARS

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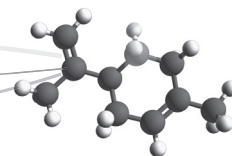
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Keywords: strawberry; vinegar; GC-O; GC-MS.

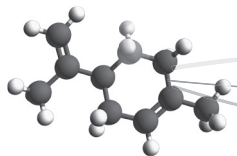
Vinegar is one of the most widespread and common products in the world because it is available in every country in several different varieties (1). In Spain, traditionally is produced from grapes by means of double fermentation (alcoholic and acetous). Today, innovation in the production of vinegar advances in two ways, improving elaboration processes and employing different raw materials. Strawberry is a fruit prized for its aroma and flavour. Spain is the second strawberry producer in the world and consequently there are surpluses of second quality fruit. These facts make strawberry a good candidate to be used as raw material in elaboration of new kind of vinegar. Aroma is certainly one of the most important determinants of food quality and acceptance. Aroma is determined by large number of compounds that are involved in different ways in it. Among them, odor-active compounds play an important role in perceived aroma.

In this work we have studied odor-active compounds in strawberry vinegars obtained by means of double fermentation and in their raw materials by gas chromatography coupled with olfactometry (GC-O). The panellists were asked to provide a descriptor to characterize the eluted odor and to rate its intensity using a 3-point category scale (1=low, 2=medium and 3= high). The quantification of results for each odor region was performed by “modified frequency” (MF), in which frequency and intensity average of the odor region are used. This is calculated with the formula proposed by Dravnieks(2). The odorants were identified by comparison of their odors and chromatographic retention index with literature data and identification using the NIST 98 library after analysis by gas chromatography coupled with mass spectrometer detector (GC-MS). The results showed 4 odor zones with a MF of 100, the odor description of them were boiled potato, cheese (tentatively identified as butyric acid), sweet (tentatively identified as pantolactone), rose/honey (tentatively identified as phenylacetic acid). Moreover, we found 10 odor zones with MF higher than 80, the corresponding odor descriptions of them were butter (diacetyl), pungent (acetic acid), river water/vapour, cheese (tentatively identified as isovaleric acid), plastic/licorice, licorice, fruit/blackberry/sweet, mint/licorice/curry, coconut/flowers and vanilla. Thus, these odorant with high frequency of detection and medium/high intensity could be determinant in the aroma of these strawberry vinegars.



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4.69

AROMA-ACTIVE COMPOUNDS OF *CAPSICUM CHINENSE* PEPPER VAR. *BIQUINHO*

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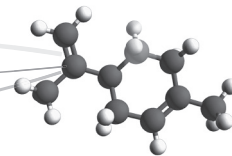
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Keywords: dynamic headspace, solid phase micro-extraction, volatile compounds, gas chromatography, olfactometry.

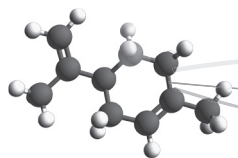
The genus *Capsicum* comprises five big species and the *Capsicum chinense* is one that usually has an extremely strong pungency and aroma (1, 2). The variety called orange *Biquinho*, however, presents the peculiarity of presenting a strong aroma of pepper without a strong burning sensation. Its characteristic aroma combined with a sweet flavor and mild pungency makes *Biquinho* pepper well appreciated in culinary as a flavoring agent and even as an appetizer. The objective of this work was to evaluate its volatile profile and determine the odor importance of these compounds, looking for markers that could help geneticists in obtaining other strains able to add desirable characteristics of aroma and flavor to food preparations. The headspace volatile components were isolated by Solid Phase Micro-Extraction (SPME), separated by Gas Chromatography (GC-FID) and identified by GC-MS and retention indices. The odoractive compounds were assessed by Osme time-intensity GC-O technique (3) where three judges previously trained evaluated the aroma of GC effluents, in three replicates, describing its aroma quality and registering the perceived intensity using the Data Collection Time-Intensity System (SCDTI), in an unstructured scale of 10 cm. Major components in the chromatogram were 3,3-dimethylcyclohexanol, four esters (hexyl pentanoate, heptyl butanoate, decyl pentanoate and heptyl isopentanoate) and three terpenes (α -copaene, α -humulene and β -cubebene), however only heptyl isopentanoate, described with a green note, showed high aroma intensity. The major compounds profile showed similarity with the profile of spicier peppers (1, 2), but many compounds in small quantities (area < 0.1 to 1%) presented high importance to the sweet and pepper-like *Biquinho* characteristic flavor. The aromagram showed 72 compounds with some odor activity, which were divided into four groups: "pepper-like, spicy" (n=25), "sweet, floral" (n=24), "herbal, green, citric" (n=13) and "miscellaneous" (n=10). Among compounds with higher aroma intensity (6.5 to 10) were found hexyl isobutanoate (described as bell pepper), a non identified terpene (sweet pepper), α -cardinol and squalene (sweet), heptyl isopentanoate and isohexanol (herbal, green), compounds that may be potential markers for the sweet aroma and mild flavor of this pepper.

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4.70

IMPACT OF FLAVOUR CARRIER SOLVENT ON VANILLIN STABILITY AND THE AROMA OF SHORTCAKE BISCUITS

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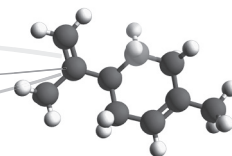
Keywords: Flavour solvent, propylene glycol, triacetin, vanillin stability, biscuit, aroma, texture, flavour, storage

Commercial liquid flavourings are created by a mixture of aroma compounds dissolved in a carrier solvent. The carrier solvent could affect the stability of aroma compounds in different ways: it may interact chemically or physically with aroma compounds or change the physical properties of the food product. For example, propylene glycol (PG), the most commonly used flavour carrier, can form acetals and ketals readily with carbonyl flavour compounds, such as vanillin (1). While, triacetin (TA) is preferentially used in chewing gum instead of PG as TA contributes to the desired gum texture by acting as a plasticiser whereas PG hardens the gum (2).

The impact of carrier solvent on the chemical and physical stability of volatile aroma compounds was evaluated in a biscuit model system. PG and TA was applied into the biscuit dough separately at 0.02% and compared directly with biscuits containing no solvent (Blank system). 40 replicates of each system were produced within one tray spatially located in a random design to minimise oven effects. The impact of spatial location, flavour addition and the presence of carrier solvent on biscuit colour and texture were evaluated. The natural biscuit aroma compounds generated as intermediate in Maillard Reaction (MR) were also assessed.

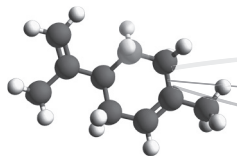
Across the oven tray there was a distribution of adsorbed heat which resulted in inhomogeneous baking kinetics. When peripheral samples are excluded and remaining samples were evaluated, a statistically significant impact of choice of flavour solvent on colour and texture was identified (ANOVA, Tukeys, $P < 0.05$). With TA samples being more brittle, less yellow and red in colour. Moreover, the biscuit aroma compounds classically generated from Maillard reaction chemistries (Hydroxyl Methyl Furfural) were presented at a significant higher level in biscuits with TA than with PG.

Additionally, vanillin stability was evaluated in the stored biscuits, 10% vanillin was mixed into either PG or TA to make a simple vanilla flavouring and applied at 0.2% respectively within sample biscuits, the produced were then stored at 20 °C, 32.5 °C and 45 °C. Samples were evaluated for changes in texture and loss of volatile aroma compounds over the storage period (8 weeks). Sensory evaluation was also carried out after 3, 6 and 8 weeks of storage using 30 consumer-type panellists to evaluate sensory perceived fracturability, vanilla flavour strength and fat rancidity note, the results of which are detailed within the poster.



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4.71

**THE CYSTEINE OR GLUTATHIONE REACTION WITH DIACETYL UNDER
WINE-LIKE CONDITIONS: PROPOSED MECHANISMS FOR MIXED ORIGINS
OF 2-METHYL HETEROCYCLES**

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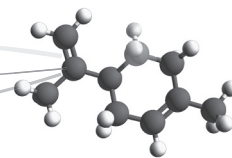
Keywords: Cysteine, glutathione, carbonyls, heterocycles, wine aroma.

Since the beginning of the 2000's, we know that a part of the "bouquet of wines" can be produced during chemical reactions between S-amino acids (mainly cysteine) and dicarbonyl compounds. Products are sulphurated and nitrogenous heterocyclic compounds comparable to those formed by Maillard reaction (1). In particular, products of the diacetyl reaction with cysteine, under wine ageing physico-chemical conditions (20±2°C, ethanol/water 12% v/v, pH 3.5), include a number of 1,3-N,S and 1,3-N,O five member heterocycles having methyl groups attached at carbon number two (CH₃-C(2)). The origin of this CH₃-C(2) fragment was not clear; it could be supplied from diacetyl or from cysteine. To explore this question, a parallel reaction was run in which diacetyl was replaced by 3,4-hexanedione. The reaction mix, were wine like model solutions (1). The heterocyclic were extracted by dichloromethane, separated by GC with a BP-21 column and detected by electronic impact Mass Spectrometry. The 2-ethyl substituted heterocycles levels were compared to 2-methyl substituted heterocycles ones. With the C(1) and C(4) carbons of diacetyl thus marked with methyl groups, the product distribution demonstrated that in the diacetyl and cysteine reaction, both diacetyl and cysteine provided the CH₃-C(2) to varying degrees in the formation of 2-methylthiazole, 2-methyl-3-thiazoline and 2,4,5-trimethyloxazole but only cysteine supplied this fragment for 2-methylthiazolidine. In the series 2-methylthiazole, 2-methyl-3-thiazoline, 2-methylthiazolidine; cysteine supplies increasing proportions of this CH₃-C(2) unit. The results are interpreted in terms of reaction paths appropriate for the mild conditions (2). These pathways permit to understand the mechanisms leading from dicarbonyls to heterocyclic compounds. These results accords with the fact that a mild version of Maillard reaction exists (3, 4) and can occurs during wine ageing. Like all the chemical pathways it permits to anticipate the impact of other compounds and physicochemical parameters on heterocyclic generations, it suggests the presence of unexplored odorous compounds in wines and, finally, proposes strategies to quantify them in the complex wine matrix.

In addition, the possible impact of peptides containing cysteine such as glutathione was examined and first results, obtained using mainly by sensorial analysis and GC/MS, shows the generation of a new set of heterocyclic compounds from these precursors under wine like conditions.

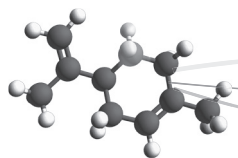
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4.72

IMPORTANCE OF 3-ALKYL-2-METHOXYPIRAZINES IN RED WINES FROM SPAIN

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Keywords: 3-alkyl-2-methoxypyrazines, wine, flavour, SPE, GC-MS, selective isolation

Some 3-alkyl-2-methoxypyrazines (MPs) are particularly relevant in the aroma of wine due to their low olfactory thresholds (1-10 ng/L) and to their characteristic green or vegetative aroma. Depending on their concentration these compounds are considered as an off-flavour of wine.

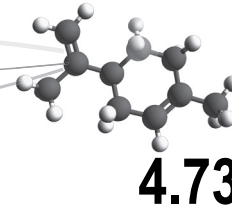
The low concentrations of MPs in wine (ng/L) difficult their analysis and make necessary the use of highly selective methods. A method recently developed in our lab has been used in this study (1). This method consists in the selective isolation by solid phase extraction using cation exchange mixed-mode sorbents and further gas chromatography mass spectrometry (SPE-GC-MS).

Spanish wines are not characterized by their content of MPs. However, a thoroughly study which could show what the real role of these compounds is in Spanish wines has not yet been made. The objective of this work was to study the importance of MPs and its sensory role in red wines from Spain: more than 70 red wines made with different grape varieties from several regions of Spain have been analysed. In the election of these wines was also taken into account that they covered a high range of quality. Furthermore, a panel of 20 experts made the sensorial analysis of the wines which consisted in a descriptive analysis and a classification depending on the quality perceived by the panellists.

In this work we present the quantitative results obtained in these analyses and a statistical study which tries to explain the different variables related with the presence and concentration of MPs.

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CONSUMER REJECTION THRESHOLD OF ETHYL PHENYLACETATE AND PHENYLACETIC ACID, COMPOUNDS RESPONSIBLE FOR SWEET-LIKE OFF ODORS IN SOUR ROTTEN WINES

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Keywords: Sour rot, wine, consumer rejection threshold, ethylphenylacetate, phenylacetic acid

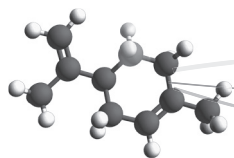
Sour rot is a disease which damage the grape skin and cause mechanical and physiological injuries to the berry. A recent work (1) studied the effect of sour rot on the sensory profile and volatile composition of wines affected by different levels of infection. This study highlighted that wines produced from damaged berries exhibited clear sweet-like off-odors not evoked by healthy samples and that ethyl phenylacetate (EPhA) and phenylacetic acid (PhAA), both presenting sweet, honey aromas, emerged as key aroma compounds of sour rotten wines. The levels of these compounds in infected wines were one order of magnitude above those found in healthy samples.

There is evidence that wine experts (producers, retailers...) prefer wines without the sweet-like off-odor but it is difficult to predict if regular wine consumers consider this character acceptable at low levels before becoming objectionable at higher concentrations. The main aim of the present work was to determine a consumer rejection threshold (CRT) for ethylphenyl acetate (EPhA) alone, and for both EPhA and PhAA in wine, since both compounds exhibit similar odors and therefore an additive or synergic effect could be expected.

Regular wine consumers received pairs of samples confronting a control wine against a spiked wine with ascending concentrations of the target compounds, and were asked to indicate which sample they preferred (2). Results estimated a conjoint CRT for EPhA and PhAA of 128 µg L⁻¹ and 522 µg L⁻¹, respectively. Data revealed that a minority of consumers interviewed (14 %) did not find EPhA and PhAA objectionable even at the highest levels assayed. Consumers were also asked to rate hedonic preference and to describe the aroma profile of samples in order to know the attributes driving consumer preference.

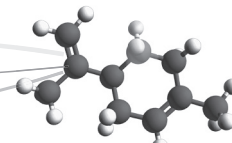
Results suggest that at concentrations of EPhA and PhAA around the CRT, much before that a clear “honey” off-odor appears, consumers are able to perceive undesirable “dried fruit” odors that lead to a clear decrease of the general aroma quality of spiked samples, making this wines being significantly rejected by consumers.

The measured CRT provides a first estimation of risk concentrations for EPhA and PhAA in red wine as they represent a “taint” for regular wine consumers. This data can be taken by wine producers in order to establish maximum allowed percentages of sour rotten grapes employed as raw material in wines released to the market.



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ESTIMATION OF THE AROMA POTENTIAL OF GRAPES

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Keywords : potential aroma, precursors.

The stabilization, maturation and fining of the organoleptic character of wine occurs since the end of the fermentation process till the moment of consumption, due to the permanent modifications of the aromatic composition[1]. At present, the existing knowledge does not provide neither a rational criterion to predict the evolution of a wine, nor the aging parameters most appropriate for achieving the desired final sensory characteristics.

Within this context, the goal of the present work is to report the changes taking place during the wine ageing process. In particular, we aim to study the potential aromatic composition of wine as a function of its content in aroma precursors along with the generation and degradation kinetics under the ageing conditions occurring in wine.

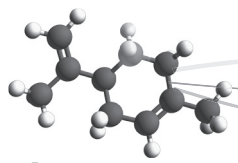
For this purpose volatile compounds released from the target precursors were extracted by SPE and determined by GC-MS after 0, 12 hours, 1, 2, 3,6, 8, 10, 14, 21, 28, 35, 42, 56, 70, 84 days of accelerated ageing (45°C for 3 months) simulating wine maturation in bottle[2]. A series of model solutions supplemented with, either flavor precursors extracted from different grape varieties, or with pure aromatic compounds, were used to study the hydrolytically release and stability of the aromatic compounds in the model media.

Concerning the solution spiked with flavor precursors, major differences in concentration were observed during the first week of accelerated ageing. Most compounds exhibited an initial significant increase and further steady decrease in their concentrations. From this data, a degradation/formation mathematical function could be obtained. On the other hand, the model wine spiked with the pure aromatic compounds was employed to monitor the kinetics and compounds stability.

Results from this experiment were employed to establish a mathematical model allowing predicting the aromatic potential of grapes.

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4.75

GAS CHROMATOGRAPHY-OLFACTOMETRIC PROFILES OF EIGHT DIFFERENT VARIETIES OF PERUVIAN PISCO SPIRITS

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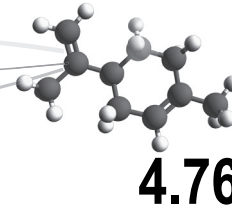
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Keywords: Piscos, gas chromatography-olfactometry, aroma compounds

The aromatic composition of nearly 190 Peruvian Pisco spirits was evaluated by gas chromatography-olfactometry (GC-O). Eight different varieties of Pisco have been studied. Four of them belonged to non aromatic Piscos, and the others were included into the group of aromatic Piscos. Among the non aromatic varieties it can be found Quebranta, Negra Criolla, Mollar and Uvina, while aromatic varieties studied were Italia, Torontel, Moscatel and Albilla. Furthermore, these spirits come from different precedence as Lima, Ica, Arequipa, Tacna and Moquegua.

The volatiles of these spirits samples were collected using a purge and trap system. The LiChrolut EN cartridge (400 mg) was placed on the top of a bubbler flask containing 80 mL of Pisco sample. These samples (without previous dilution) were purged by a stream of nitrogen at 25°C during only 20 minutes. Volatile constituents released in the headspace were trapped in the cartridge and were further eluted with 3.2 mL of dichloromethane containing 5% methanol.

For the sake of simplicity, those odorants not reaching a maximum GC-O score (MF) of 30% in any of the eight studied Piscos were eliminated and considered as noise. After this operation, the number of odorants present was more than 20, mostly of them were identified. Heart-cutting GC techniques made easier us the identification of some unknown compounds, however some of them could not be successfully identified even if the same heart cutting strategy was employed.



FLAVOUR-PACKAGING INTERACTIONS OF A CONCENTRATED MODEL FLAVOUR SYSTEM DURING STORAGE

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Keywords: flavour concentrate, packaging, interactions, sensory, HDPE

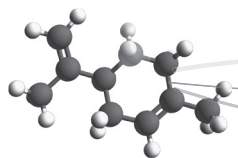
Flavourings are concentrated aroma chemicals systems used in foods and beverages formulation. Such ingredients are very important as they provide the main aroma of foodstuff. Due to its very high aroma chemicals level, flavour concentrate could undergo various interactions, especially with packaging materials during shelf-life. One of the most broadly used packaging materials is high density polystyrene (HDPE). The latter, had been largely used in foodstuff packaging due to its low cost, low safety risk (unbreakable) and relatively good storage protection over time. Despite HDPE advantages and various uses in foodstuff, little is known on its interaction with highly concentrated aroma chemicals systems, such as flavour concentrate, especially during shelf-life, and on its sensory impact on the packed foodstuff. This work deals with the measurement of aroma compounds in a highly concentrated model system stored in HDPE packaging during shelf-life and glass (as control), as well as the sensory evaluation of carbonated beverages made from the stored model concentrate.

Model concentrate: several aroma compounds found in citrus flavours (limonene, alpha-terpineol, neral, geranial) were added into a water-ethanol system (70:30) at a concentration of 8500, 1600 and 2500 ppm, respectively. The later system was stored in 1 gallon HDPE containers and stored at 21 and 32 °C over 12 months. GC-MS analysis was used to quantify the aroma compounds.

Carbonated beverages: At 12 months carbonated beverages were prepared from the model concentrate in a pilot plant, and then evaluated by consumers using r-index/multi-match methodology. Correlation between aroma compounds and sensory acceptability was done by principal components analysis (PCA).

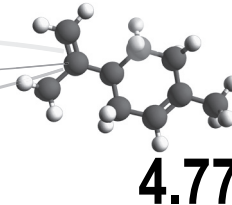
After 12 months, limonene in concentrate stored in glass decreased 23% (respect to time zero) at both 21 & 32 C. In HDPE containers, limonene decreased 67% at 21C and 75% at 32 C. Neral in concentrate stored in glass decreased 16% (respect to time zero) in both 21 & 32 °C. In HDPE containers, Neral decreased 12% at 21 °C and 16% at 32 °C. Geranial in concentrate stored in glass decreased 16% (respect to time zero) at 21 °C, and 21% at 32 °C. In HDPE containers, geranial decreased 18% at 21 °C and 21% at 32 °C. Higher losses of limonene in HDPE could be explained by sorption into HDPE, as limonene is a more hydrophobic molecule (Log P=4.83), whereas Neral and geranial are less (Log P=3.45).

Consumers did not notice a significant difference between control and variants in beverages made from the stored concentrate even though analytical differences were present in the samples assessed. Interestingly samples stored at 32 C were found to be more acceptable than those stored at 21 C.



When PCA analysis was conducted on the aroma compounds in concentrates and sensory acceptability results, compounds such as α -terpineol and p-cymene, were highly correlated to the acceptable samples, while samples presenting high levels of limonene and citral correlated with non-acceptable samples.

A possible explanation for this could be that consumers are more familiar with degraded lemon-lime flavor (higher in α -terpineol and p-cymene) and less familiar with a fresher lemon-lime flavor (higher in limonene, Neral and geranial). This study contributes to performance of citrus flavors in highly concentrated systems, as concentrates, in HDPE containers in conjunction with consumer acceptability.



GAS CHROMATOGRAPHIC-OLFACTOMETRIC CHARACTERIZATION OF KEY AROMA COMPOUNDS IN FRESH AND FROZEN LAMB MEAT USING NEW EXTRACTION METHODS

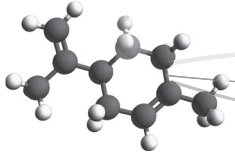
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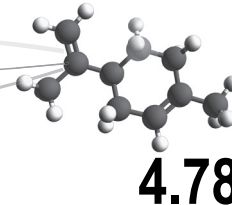
Keywords: Lamb, Meat, Aroma, Flavour, GC-Olfactometry, Odorants, Sample Preparation, Freezing

The seasonal supply of lambs is a fact in the ovine market, with a high offer during spring and a high demand at Christmas time. Moreover, meat producers try to freeze meat attempting to stabilize its price. Freezing procedures should ensure not only the nutritional quality, which has already been demonstrated, but also the sensory quality of frozen meat. The latter is closely linked to meat aroma and this can only be controlled if the chemical compounds responsible for the most relevant sensory properties of fresh and frozen (1-2) lamb meat are known. Two different techniques for obtaining extracts representing the most relevant aroma chemicals from cooked lamb have been developed and applied to the elucidation of the Gas Chromatographic-Olfactometric (GC-O) profiles of cooked lamb from fresh or frozen samples. The first one attempted to collect the aroma released during cooking; and the second one, the aroma released during eating. GC-O data made it possible to rank the potentially most important aroma compounds in the different samples. The GC-O work was complemented with a thorough isolation and identification study comprising liquid chromatography and dual Gas Chromatography with simultaneous MS and Olfactometric detection. Overall, the GC-O profiles were composed of 44 odor zones, comprising at least 54 aroma compounds of which 45 could be identified. It is remarkable that no compounds smelling 'lamb' or 'wool' were detected, what suggests that their characteristic lamb aroma is caused by a combination of chemicals. Eight different aroma compounds with meaty odors were detected of which Z-2-heptenal, 2,5-dimethylpyrazine, Z-2-decenal, 2-butyl-2-octenal, 2-acetyl-2-tiazoline and E,E-2,4-decadienal could be identified. Moreover, 2-isopropyl-3-methoxypyrazine, 2-methylbenzaldehyde, 2,6-dichlorophenol and 4-hydroxy-3-methoxybenzaldehyde (vanillin) were described for the first time in lamb. Seven out of 44 odour zones detected (corresponding to 11 aroma compounds) were found to vary significantly among the different extracts. Frozen samples contained higher levels of ethyl hexanoate and butyric and 3-methylbutyric acids, while samples obtained directly from the grill were richer in pyrazines. Furaneol was only important in samples directly collected from the mouth.



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FERMENTATION PROFILES OF FREE AND IMMOBILIZED INDUSTRIAL YEAST STRAINS

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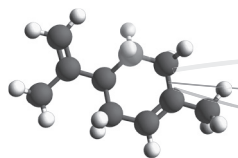
Keywords: yeasts, immobilization, GC profiles

GC or HPLC chromatographic profile of metabolites formed during fermentation is an important factor in evaluation of biotechnological properties of yeast strain species. In the case of wine and beer making, contribution of specific aroma substances to the flavor and final bouquet of alcoholic beverages is an important factor in its quality judgment.

GC-chromatographic profiles of potential aroma substances synthesized during alcoholic fermentation conducted with four different industrial strains of yeasts. The profiles of brewery bottom fermenting *Saccharomyces pastorianus*, brewery top fermenting *Saccharomyces cerevisiae*, Crabtree-positive distillery strain *Saccharomyces cerevisiae* and Crabtree-negative unconventional amylolytic strain *Debaryomyces occidentalis* were compared for free and immobilized cells. Yeast cells were immobilized (encapsulation) in multichamber alginate cores formed by droplet method but with foamed basic solutions. For increasing mechanical stability and preventing cell leakage from alginate beads different outer membranes first from poly-L-lysine and then from alginate or silica were formed. Additionally yeast cells were immobilized (adhesion) on different solid ceramic carriers - hydroxyapatite or chamotte.

The GC-assay profile included the quantitative determination of acetaldehyde, ethyl acetate, methanol, ethanol, 1-propanol, ethyl butyrate, 2-methyl-1-propanol, isopentyl acetate, ethyl caproate, ethyl caprylate from small-scale fermentation trials.

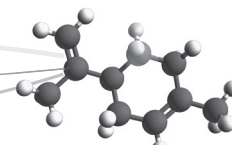
Each strain has unique characteristics closely related to its practical application in fermentation industry. Distillery yeasts exhibited the Crabtree effect which was seen in yield of ethanol formation in batch culture at high concentrations of 120 g glucose/L. In other strains, e.g. in brewery strains or amylolytic *Debaryomyces occidentalis*, clear Crabtree effect wasn't observed. Similar fermentation profiles were seen for free and immobilized yeasts, especially for green beer but aroma compounds were produced at higher level for both types of brewery strains to comparison to distillery or amylolytic yeasts. The immobilized cells showed higher ethanol production. Yeast cell activity and behavior during incubation were individual characteristic of given specific strain and type of carrier.



Topic:

**Advances
in sensory
science/
psychophysics**

**Poster
Presentations**



QUANTIFICATION OF RELEVANT FLAVOR COMPOUNDS IN BEEF STOCKS AND CORRELATION TO SENSORY RESULTS BY "REVERSE METABOLOMICS"

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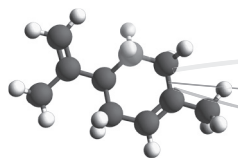
Keywords: sensory, analysis, correlation, metabolomics, beef

The meaningful correlation of sensory data with analytical data is one of the most challenging tasks in flavor research (1). Especially in beef stocks, due to the presence of low levels of aroma-active compounds and the taste contribution of non-volatile molecules to the typical 'juiciness' character, one encounters a complex matrix situation. The goal of our study was to carry out a comprehensive analysis of all relevant flavor molecules and the correlation to human sensory data.

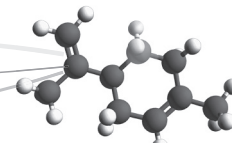
A technique recently developed at the IPB and termed 'reverse metabolomics' has been used to link biological activity (i.e. sensory data) with variations in the metabolic profile (i.e. analytical data). We have used this methodology for the first time to correlate sensorial attributes and GC-MS, LC-MS and NMR data in culinary beef stocks.

The basic principle of reverse metabolomics is as follows: For a number of related, but not identical samples a metabolic profile is generated using chromatographic techniques. For each sample, the taste properties are also determined in a quantitative way. Then certain algorithms are applied that rank correlations between biological and analytical patterns resulting in a list headed by those chromatogram peaks showing the best correlation with the bioactivity. In more detail, 'reverse metabolomics' has been applied to study the link between sensory and chemical composition with a series of freshly prepared culinary beef stocks. A set of 10 different beef stocks has been prepared. 20 sensorial attributes have been recorded by a trained panel (n = 15). In addition, the liking of the samples was also recorded on a hedonic 1-9 scale. The analysis of the stocks has been performed by LC-MS, GC-MS and NMR. ¹H NMR data directly obtained from the meat stock was very complex. Analysis of this data set by reverse metabolomics revealed some basic structural elements of the key taste compounds such as carnosine or anserine. The reverse metabolomics correlation with LC-MS data revealed the relevant taste contribution of carnosine, anserine and creatinine to the taste of meat stocks (2). This has been confirmed by additional sensory experiments. In our study, reverse metabolomics proved as an efficient tool to correlate sensorial attributes with single aroma-, and taste-active molecules.

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THE IMPACT OF VISION ON FLAVOR PERCEPTION

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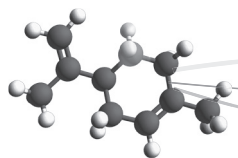
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Keywords: multimodal, perception, flavor, olfaction, vision, congruency

Flavor is most commonly experienced through multimodal input. We examine food with our eyes far before we taste it. The colors and shapes of food inform our perceptions. However, the mechanisms behind the influence of vision on flavor perception and flavor perception on vision have not been thoroughly investigated. Prior research has shown the indisputable influence of color on our perceptions of food (1, 2). The influence of a visual signal on flavor is most powerful when the two are congruent, such as the color red and strawberry (3, 4). These associations are learned; however, the elements of vision necessary to produce an enhanced perceptual effect have never been investigated. This study examines the cross-modal interactions between vision and olfaction by presenting individuals with black and white outlines of familiar fruit shapes along with their fruit odors. In order to study these two modalities, classical psychophysical methods are applied in a novel cross-modal experiment, demonstrating the impact of the combined processing of two senses (5, 6.) Of utmost importance, many prior visual olfactory crossmodal experiments, improperly assessed one of the sensory functions, using methods that compromised the sensitivity of one of the senses (7-10). Rarely, is the congruency of the visual and olfactory stimuli examined, this is often assumed.

The stimuli chosen for this experiment were four familiar fruits: banana, cherry, grape, and orange. They were selected for their distinctive shape as well as differentiable odors. The four matching olfactory stimuli were isoamyl acetate (banana), benzaldehyde (cherry), methyl anthranilate (grape), and octanal (orange). The first experiment consisted of testing the congruency of odors and visual images. Based on the best fitting visual and olfactory stimuli, two images and two matching fruit odors were chosen for the rest of the experiment. In the second part of the experiment, both the visual and olfactory thresholds were determined for each individual by the method of ascending limits. In order to assess the visual thresholds for individuals, all visual stimuli were delivered on a computer through a Python based computer program called PsychoPy (11, 12). The first two experiments described are not novel, but are necessary for part three. The third part of the experiment tested the cross-modal influence of both vision on olfaction and vision on olfaction. Part three also assessed the impact of congruency. One condition evaluated the influence of olfaction on vision, and the other tests the influence of vision on olfaction.

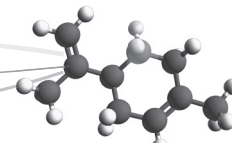
This is controlled through the timing of the stimulus delivery. Subjects evaluated both congruent stimuli (when the odor and visual stimuli match) and incongruent pairings (when the odor and the visual stimuli do not match). This experiment examined shifts in sensitivity due to the influence of two modalities. The results from these experiments inform our current



understanding of how an image can influence our flavor perceptions and will be discussed further.

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MULTIPLE TIME-INTENSITY PROFILING (MTIP) AS AN ADVANCED EVALUATION TOOL FOR COMPLEX TASTANTS

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Keywords: time-intensity profiling, epigallocatechin gallate, green tea, rebaudioside A, *Stevia rebaudiana*

Many tastants do not show only a single taste quality but meet a whole dimension of descriptors. For an adequate description of various taste qualities and their aftertaste properties a timeintensity profile is fundamental. This is especially important for compounds showing lingering taste effects, such as many high impact sweeteners or astringent tastants. The standard time-intensity (TI) method is used to monitor the intensity of one attribute only over a period of time (1). To minimize the time effort for evaluation of a complex matrix with more than one descriptor dual-attribute time-intensity (DATI) ratings are commonly used. Thereby the mouse is employed to move a cursor on a two-dimensional plane consisting of a horizontal and vertical intensity scale (2). Other methods described in the literature combine time-intensity measurements and traditional sensory profiling (3). In the time-intensity profiling (TIP) method, for example, time-intensity ratings for a set of descriptors are collected consecutively (3).

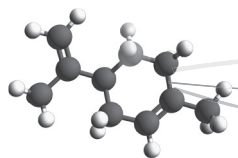
To combine the advantages of DATI, reducing the number of tests, and of TIP, which allows evaluation of more than two attributes per session, we developed an advanced protocol, referred to as multiple time-intensity profiling (mTIP). Multiple attributes can be rated in parallel over a certain period of time by using horizontal unstructured line scales for each descriptor.

As prototypical taste problems we investigated the taste of rebaudioside A, a steviol glycoside of the plant *Stevia rebaudiana*, for the descriptors sweet, as well as bitter and astringent off-tastes (4). Furthermore we evaluated the bitter and astringent taste of epigallocatechin gallate, an antioxidative catechin occurring in green and black tea. For the evaluation of a more complex food system, various descriptors of a green tea application were described.

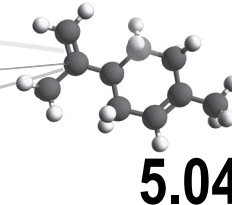
As shown for rebaudioside A and epigallocatechin gallate, the mTIP protocol allows a more dimensional evaluation of the time course of intensity of several taste descriptors in parallel. The use of half-time parameters, as applied in these experiments, may be very helpful to quantify these effects. The tool seems to be especially valuable for the proper characterization of selective taste modulators.

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PERCEPTUAL INTERACTIONS IN ODOUR MIXTURES: THE BLENDING EFFECT

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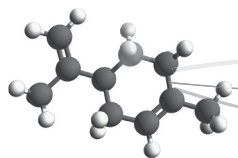
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Keywords : perception, odour mixture, blending effect, odour quality space, human

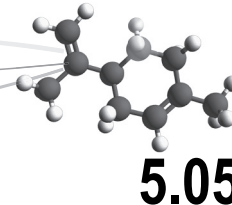
The odour perceived from a mixture of odorants varies depending on several factors as the context, individual physiological abilities, experience... but also as the perceptual interactions occurring over the processing of odorant mixtures. Sometimes, these interactions which appear during odour information coding and processing, lead to synergy or masking of odour notes (1). In other occasions, they lead to a blending effect. Odour blending appears when a mixture is perceived as a unique odour different from the odour of its components (1, 2). This type of mixture is well known by perfumers and flavourists who daily construct and use it in an empirical fashion. Here, we conducted a series of experiments to evaluate the contribution of two factors suspected to contribute to such a blending phenomenon, the number and the proportions of the odorants included in the mixture. We took advantage of previous results that showed, through a typicality rating test, that mixtures of 2 and 6 components were rated as more typical of a target odour than each of their components (3). In our experiments, we performed sorting and similarity tasks of several mixtures and their components in order to evaluate the perceptual qualitative distance existing between components and mixtures. We depicted the results as odour quality spaces. As a result, the binary and senary mixtures were perceived as significantly different from their components, in contrast to mixtures with same components but in different proportions and supposed to not induce a blending effect. Regarding the 6 components mixture, it was discriminated from all its components and it was interestingly not located at the barycenter of the odour quality space formed by the components. Concerning the binary mixture it was not either located in between the position of the two components in the odour quality space. This suggests that the quality of the mixture is not a mix of the qualities of the components but rather a distinct (new) one and that it is a blending effect. Complementary results on the 6 components mixture showed that the proportion of each component in the mixture is also a key factor for blending occurrence but that each component did not have the same impact on the blending effect. To conclude, some mixtures appear as inducing perceptual blending, a processing depending on specific odorants mixed in specific proportions.

Supported by grants from the Burgundy Regional council, EU-ERDF and IFR 92 to GC and TTD and a fellowship from the French MESR to CS.

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THE DYNAMICS OF AROMA RELEASE DURING THE CONSUMPTION OF CANDIES WITH DIFFERENT STRUCTURES. RELATIONSHIP WITH TEMPORAL PERCEPTION

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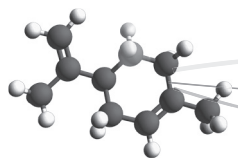
Keywords: aroma compounds, gels, structure, dynamic of release, PTR-MS, perception over time, eating behaviour

The effects of product structure on aroma release result from the combination of physicochemical (entrapment of aroma compound in product structure and/or obstruction to their mass transport) and physiological phenomena (modification of oral behaviour) (1). Most of the time, increasing product viscosity or firmness results in decreasing aroma release and perception, even if some contradictory results exist. A better understanding of relationships that can exist between sensory perceptions and physicochemical properties of foods has always been a tempting objective to better control food sensory properties. In literature, only a few studies focused on perception over time by applying the Time-Intensity sensory method and proposed some relations with the dynamics of *in vivo* aroma release.

The objective of the present study was to evaluate the impact of candy structure on the dynamics of *in vivo* aroma release and to propose some relationship with temporal sensory perception (method of Temporal Dominance of Sensation, TDS). The use of instrumental and sensory methods in parallel constitutes an original approach to better understand aroma release and perception and to identify some relationships between these two phenomena.

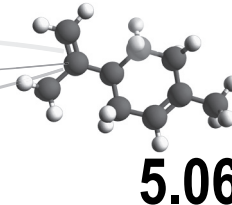
Four candy textures were established by varying gelatine content between 0 and 15% w/w. They were flavoured with 3 aroma compounds having different physicochemical properties and different sensory attributes. *In vivo* aroma release kinetics were measured using a High-Sensitivity Proton Transfer Reaction-Mass Spectrometer (PTR-MS) (Ionicon Analytik, Innsbruck, Austria) (2). Sensory analysis was performed simultaneously with nose-space measurements using the method of Temporal Dominance of Sensations (3). Six aroma and taste attributes were selected: sweet, sour, strawberry, peach, green grass and butter.

The highest *in vivo* release, monitored using Proton Transfer Reaction Mass Spectrometry with a trained panel, was obtained for the 2%-gelatine sample for all aroma compounds. The dynamics of aroma and taste perception were characterized using the Temporal Dominance of Sensations (TDS). The dominant sensation for the liquid product was the “strawberry” note. For other products, the temporal characteristics of perceptions were more complex. The global duration of the dominance period (all sensory attributes taken together) increased linearly with gelatine content. Data highlighted that aroma release resulted from interaction between product properties and oral behaviour. Some relations with the dynamics of perception have been established, essentially between temporal parameters (2).



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FLAVOR DESCRIPTION PROJECT - CAN INTERNET HELP FLAVOR SCIENCE?

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Keywords: interactivity, flavor descriptors, web

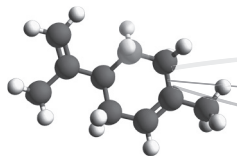
Over twenty years we are facing the ever increasing influence of internet on our lives. In this communication we will discuss the development of a comprehensive set of descriptors with the help of dedicated web page [www. flavor.isgreat.org](http://www.flavor.isgreat.org) and a community forum “Flavor and fragrance industry” running on the social network for professionals LinkedIn. The project deals with an extension of the well known flavor wheel (1) of odor classes - Green, Grassy; Fruity, Ester-like; Citrus, Terpenic; Minty, Camphoraceous; Floral; Spicy, Herbaceous; Woody, Smoky; Roasty, Burnt; Caramel, Nutty; Bouillon, HVP; Meaty, Animalic; Fatty, Rancid; Sulphurous, Alliaceous; Mushroom, Earthy; Celery, Soupy; Dairy, Buttery - with a list of descriptors, completed with additional sections for Taste and Mouthfeel modalities as well as with a class of the Miscellaneous, mostly chemistry-related descriptors. We all know how difficult is to describe olfactory sensation with only one descriptor. Likewise, assignment of one descriptor to single sensory class is many times not possible. In this project we, as first, allowed descriptors to be included simultaneously in various classes. Second, the primary character of a descriptor is expressed in the color of its most characterizing sensory class.

Within five months we published three successive versions of the table, which meant increasing number of descriptors as well as various rearrangements of primary assignments. When looking at number of descriptors, the most abundant classes are Floral and Carmel, Nutty. The table of descriptors visually reveals also some expected relationships such as between the classes Green, Grassy vs. Spicy, Herbaceous or the classes Roasty, Burnt vs. Caramel, Nutty.

This project proves that with the help of internet-based communities it is possible to get rapidly valuable ideas and corrections from the professionals of all around the world.

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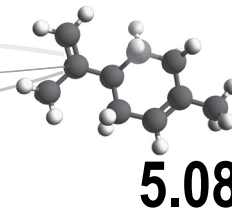


5.07

EVALUATION OF THE COMPOUND METALLIC IN MEAT FLAVOURM.L. Mitterer-Daltoé¹, R.O. Treptow², E. Martins³, V.M.V. Martins³, M.I. QUEIROZ¹¹ Biotechnology Laboratory, Federal University of Rio Grande, 475, 96201-900 Rio Grande, RS, Brasil.² Federal University of Pelotas, Pelotas – RS, Brasil.³ State University of Santa Catarina, Lages – SC, Brasil.*mariaisabel.queiroz@mailcity.com*

Keywords: beef, ferrous sulfate, threshold, metallic flavour.

The term “quality” when referring to meat, consists of many factors, such as appearance, color, juiciness, odor, flavour and texture. However, other factors, such as metallic taste, also affect the quality of the meat. Various researches report that a metallic flavour may be multimodal, including taste and olfactory sensations. This is particular to ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), which has been proposed as a reference standard in the training and monitoring of selected assessors during food sensory evaluation. The objective of this paper was to propose the procedure to select and train assessors to evaluate metallic flavour in beef and evaluate the compound metallic in meat flavour. The detection threshold of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was determined using the method of limits. Twenty-six undergraduates participated in the test, and a single detection threshold was determined. Candidates with a threshold lower than or equal to the general value threshold were accepted. Next, the performance of the accepted candidates was evaluated using meat from the semimembranosus muscle of bovines (Crioula Lageana) at an internal cooking temperature of 65, 72 or 75°C. The metallic flavour in the meat was evaluated using a ranking test and a continuous line scale from zero (not perceptible) to nine (highly perceptible). The assessors were selected to help the team analyze the discriminative power, reproducibility and overall use of scale. The equivalence of metallic sensation was evaluated by analyzing ferrous sulfate concentrations using a continuous line scale from zero (not perceptible) to nine (highly perceptible). A linear regression was developed with ferrous sulfate concentration (dependent variable) and sensory response (independent variable). The regression model described presents a correlation of $r = 0.9689$, and this value indicates that viability was obtained with the model. The metallic sensation equivalent in ferrous sulfate should be calculated using the sensory response values obtained from the subjects who tasted the meat. The procedure proposed for selecting and training the panel to evaluate metallic sensations was successful. The results demonstrated that an internal cooking temperature at 65°C can be an adequate temperature to use as a standard reference for evaluating metallic sensations in meat. The semimembranosus muscle of the bovine Crioulo Lageano is characterized as having low metallic flavour.



DYNAMICS OF AROMA RELEASE DURING CHEESE CONSUMPTION: INFLUENCE OF THE PHYSIOLOGICAL STATE

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Keywords: Aroma release, satiation, cheese, APCI-MS

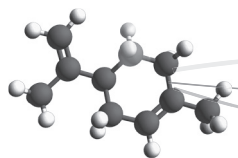
Studies dealing with food intake usually focus either on *in vivo* aroma release or on subject physiology and behaviour. However, no approach combining all the parameters is known and the effect of changes in the physiological state (for instance satiation state) has been slightly studied until now. Yet, the evolution of aroma perception during food intake is important because it can modify the consumption behaviour, in particular when the subject is satiated. The objective of this project is to study the dynamics of the evolution of aroma release from model cheese, according to physiological modifications such as satiation attainment.

The present study is a preliminary test led on a small number of healthy subjects selected without known pathology and with a normal weight ($19 < \text{BMI} < 25$). The main criterion of evaluation was the intensity of aroma release in function of the nutritional state. This criterion was calculated for the seven aroma compounds present in the cheeses. The aroma release was followed during cheese consumption both before and after a satiating meal (made up of pasta eaten *ad libitum*). The technique used to evaluate the intensity of aroma release was the nose-space Atmospheric Pressure Chemical Ionization- Mass Spectrometry (APCI-MS).

The secondary criteria of evaluation were: -the activity of the muscles involved in chewing, followed by surface electromyography, -the jaw movements during mastication, followed by motion capture measurement (Optotrak®), -the respiratory flow rate, measured with a flowmeter, -the subjective evaluation of satiation attainment before and after a meal, determined with a questionnaire.

The influence of the nutritional state (satiation), before and after the pasta meal has been studied through the comparison of all these criteria. Results showed that the aroma compounds were on average more released during model cheese consumption after satiation than before. This difference can be explained by an effect of satiation on masticatory behaviour. Indeed, it has been shown that the total chewing work was higher after satiation than before. However, there were no significant differences observed before and after satiation for the respiratory flow rate.

Further studies will be conducted on a larger number of subjects and with other flavour compounds in order to complete these results and to go into details about the potential interactions.



5.09

Olfactory mental imagery: an example of learning

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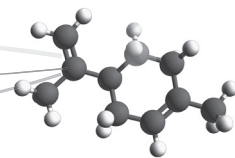
Keywords: mental imagery, experts, olfactory learning, sensory training.

The wine experts' olfactory performances play an important role in wine tasting. The differences in judgment among the professionals may be explained by their individual variation. This justifies the observed sensory variables during the wine tasting. It is therefore important to examine sensory training procedures.

Olfactory mental imagery, ability to mentally evoke the odour perceptual qualities in the absence of real stimulus, could alternate a common training. The effectiveness of a mental imagery in the context of learning has been confirmed in several areas such as sport or even music.

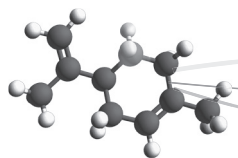
Olfactory mental imagery may play an important role in the perceptual process when neurophysiological mechanisms are sharing with perception. We have tested the training influence of a mental image on both sensibility and olfactory identification. Our results demonstrated that olfactory mental image can affect subjects' olfactory capacity. For example, it has a strong impact on experts' detection performance. Their specific detection thresholds of certain molecules are increased after mental imagery training. This effect can be extended as well as generalized to non-trained molecules.

The magnitude of the gain depends on the tested compounds.



Topic:
**Modeling
sensory
perception**

**Poster
Presentations**



6.01

A STANDARDISED APPROACH TO *IN VIVO* MEASUREMENT OF VOLATILE RELEASE: THE EFFECT OF FOOD MATRIX ON RELEASE AND INTRA- AND INTERINDIVIDUAL DIFFERENCES.

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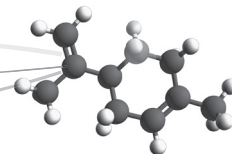
Keywords: *in vivo*, flavour release, food structure, proton transfer reaction mass spectrometry (PTRMS), consumption protocol

The timing and amount of aroma released during consumption is an important sensory aspect of food (1, 2), largely dictated by structure and composition, but also dependent on individual oral processing. Accurate and reproducible measurement of *in vivo* release phenomena remains a technical challenge, especially as individuals vary in their eating and release behaviour (3, 4, 5). The amount and manner in which individual's release aroma may also play a role in regulation of eating behaviour (6, 7); accurate methods are required to measure this. The objectives of this study were to: (i) apply techniques to measure the impact of food structure and composition on *in vivo* volatile release, and (ii) obtain reliable data on inter- and intra-individual variation in release across a range of food products and develop some reference values of characteristic release behaviour.

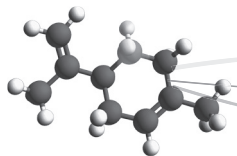
A rigorous breathing and consumption protocol with either a "fixed" or "free" swallow time was applied using human subjects. The fixed swallow protocol was expected to be more suited to understanding the influence of structure on release whereas the free protocol would better suit investigations where understanding idiosyncratic differences in oral processing and release is the focus. In both approaches breath by breath release could be measured accurately and easily aligned across subjects and products. Eight subjects were presented with twelve common foods representing liquid, semi-solid and solid states, encompassing a variety of textures and compositions. Subjects were interfaced with a proton transfer mass spectrometer (PTR-MS) for measurement of exhaled breath volatiles. Pre- and post-swallow release parameters such as maximum concentration (*I_{max}*), time to *I_{max}* (*T_{max}*), and area under the concentration curve (AUC) were measured as well as more detailed breath by breath information. Multivariate statistical analysis (MANOVA) was applied to understand the effect of structure on release and individual differences. Differences in pre- and post-swallow and breath by breath volatile release parameters (AUC, *I_{max}*, *T_{max}*) were found across food types - liquid, semi-solid and solid, as well as the relative proportion of aroma released in pre- and post-swallow segments. The variability of release behaviour between and within subjects across the range of food types using the fixed and free swallow time methods was characterised.

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PERCEPTIVE INTERACTIONS ON WINE TYPICAL FRUITY AROMA

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Keywords: Wine, perceptive interactions, global aroma, reconstitution.

In wine, most of volatiles are present at lower or similar levels to their individual perception thresholds. Considering possible perceptive interactions among them, it is difficult to determine accurately their contribution to the global aroma (1).

Considering the difficulties in wine aroma reconstitution from only “key compounds”, we developed aromatic reconstitution from wine fractions instead of pure compounds, mainly in order to prioritize the role of these different fractions in relation to the global wine aroma. For this, we developed a methodology to prepare aromatic reconstitutions from fractions of an organic wine extract and we assessed these reconstitutions both wine model solution and in desaturated wine.

This study was carried out with fruity merlot red wines (2008 vintage). Reverse High-Performance Liquid Chromatography method was developed for wine extracts fractionation (2). From each aromatic extract, 25 fractions with various flavors were obtained and some fractions were selected after a 3 members expert panel sensory evaluation and preliminary tests (3).

Sensory analyses were performed by a 27 trained judges panel. Thanks to triangular tests, we showed that it was not possible to reproduce global wine aroma using this reconstitution technique. We also observed that the absence of some fruity fractions had no influence on the global fruity aroma expression indicating that these fractions volatiles did not play a significant role.

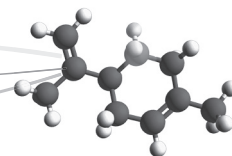
Sensory profiles concerning red, black, fresh and jammy fruits aromas were evaluated with an intensity scale test. Using fractions with fruity notes it was possible to obtain aromatic reconstitutions having red and fresh fruity intensity notes statistically similar to the corresponding wine but black and jammy fruits intensities were significantly lower when compared to wine.

We also explored particular interactions. Thus, we observed an additive effect on the fresh fruity aroma due to fractions with the least intense fruity note and a masking effect on the same aroma due to some not fruity fractions.

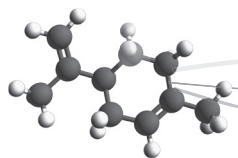
Further analysis showed that these fractions composition was linked to yeast and lactic acid bacteria metabolism. Impact of the involved compounds on perceptive interactions was evaluated by aromatic reconstitutions, including omission tests, of these fractions.

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6.03

MEASUREMENT OF TIME-INTENSITY SENSORY PERCEPTION AND COMPARISON TO IN-VIVO AROMA RELEASE

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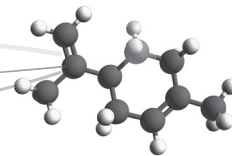
Keywords: Time-intensity sensory perception, flavour release, proton transfer reaction mass spectrometry (PTR-MS), oral breakdown, mastication behaviour

Perception of flavour is a complex integrated response incorporating taste, aroma, texture and chemesthesis. During consumption of food, perception of flavour also has a temporal dimension, related to the breakdown of food during mastication and flavour release (1, 2). Despite considerable research, relating flavour release to dynamic sensory perception remains a complex challenge (3, 4). The objective of this study was to determine the temporal perception of flavour intensity of a range of foods and relate it to aroma release measured by *in vivo* proton transfer reaction mass spectrometry (PTR-MS).

To achieve this, time-intensity (TI) sensory analysis was used to determine the temporal perception of flavour intensity relative to the oral breakdown of the sample during consumption. Two attributes were evaluated: *overall flavour intensity* and a texture attribute (*resistance to chewing* for solids; *viscosity* for semi-solids) as a holistic measure of the oral breakdown. Two different methods of time-intensity sensory analysis were used and critically compared: (i) a “conventional” method, where a single attribute was rated continuously for 90 seconds; and (ii) a discrete-interval method, where the two attributes were rated simultaneously on 100mm line scales at six time points during consumption relative to the swallow time for each individual subject in the panel (n=8). Twelve food samples were selected for investigation, varying in the amount of oral processing required before swallow, including solid (n=6), semi-solid (n=3) and liquid (n=3) samples.

The two time-intensity methods were critically compared to evaluate advantages and disadvantages with respect to which method provides the greatest insight into understanding the relationship between temporal sensory perception and dynamic aroma release. Timeintensity results were correlated to parameters of *in vivo* aroma release measured using PTRMS and to time-intensity perception of flavour recorded simultaneously during release experiments. A controlled breathing and consumption protocol during PTR-MS release experiments allowed comparisons of aroma release and perception at specific points during oral breakdown (e.g. initial, during chewing, just before swallow, just after swallow).

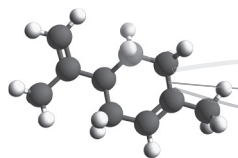
Reproducibility and discrimination between samples were better for the discrete-interval TI method compared to the continuous TI method. However, resolution was lower than the continuous TI method due to the fewer number of data points, but this was found not to impact upon the precision of results. Duration information was also lacking in the discreteinterval TI method, but this may be incorporated into the method for the future. A key advantage of the discrete-interval TI method is that the two attributes (flavour, texture) were evaluated simultaneously, so that they could be directly related, as opposed to the continuous method where they were evaluated separately. The discrete-interval method also allowed



comparison between *in vivo* aroma release and sensory perception at key points during consumption relative to the swallow of each subject. This provided greater understanding of the relationships between oral breakdown, aroma release and sensory perception.

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RELATIONSHIPS BETWEEN ORAL CHARACTERISTICS, BOLUS FORMATION AND AROMA COMPOUND RELEASES DURING THE CONSUMPTION OF FAT SPREAD IN HUMAN

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Keywords: aroma release, food bolus properties, tongue coating, fat spread, oral physiology

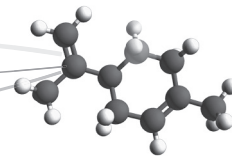
The release and perception of flavour compounds is an important factor for the acceptance of a food product. It is a complex mechanism that depends not only on the food's chemical composition and structure, but also on in-mouth mechanisms involved in its breakdown. To date, most of the studies have been focused on hard or semi-hard product such as cheese for instance (1, 2).

Among the works published on dairy product, fat spreads were not considered while they represent an important market. However these products are particularly interesting by their structure and composition (water-in-oil emulsions, solid fat content and different sources of fat (vegetal and animal)) supposing specific in-mouth mechanisms. In this context, there is a necessity to conduct of a novel scientific approach to assess these mechanisms.

For this purpose, our objective was to better understand the impact of some bolus properties i.e. viscosity, incorporated saliva rate and tongue coating on the release of flavour from fat spreads. To complete this approach, inter individual variability related to oral physiological parameters i.e. salivary flow (at rest and stimulated) and composition (protein, lipase, amylase, lysozyme, and proteolysis) as well as oral characteristics have been considered.

In the current study, 15 subjects highly contrasted regarding their salivary characteristics were selected. In parallel, 4 experimental fat spreads were designed with two levels (53% and 20%) and 2 qualities (mix of animal and vegetable, and only vegetable) of fat. The matrices were aromatized with a specific dairy flavouring ingredient to improve inmouth aroma release. Curcumin was added to the matrices and was used as a marker of the lipid mouth coating. Aroma release (3), food bolus (4) and tongue coating measurements were determined according to both free and imposed protocols. The assessment of the thickness of the lipid on the tongue was obtained by a fluorescent probe methodology (5). The release of aroma was measured by APCI-MS on 4 molecules differing by their polarity.

Results show that for HFP (High Fat Product), no influence of the fat quality (mix or vegetal) was observed on tongue coating and bolus properties i.e. viscosity and rate of incorporated saliva. However there is an important subject's effect, particularly on salivary flow (resting and stimulated), saliva viscosity and oral volume. For LFP (Low Fat Product), an effect of the fat quality was observed. Pure vegetal product coated less the tongue than mix fat product.



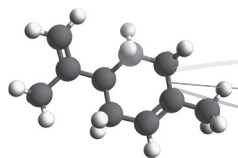
Moreover, the rate of saliva incorporated in the bolus is higher for LFP than for HFP but inter individual variability on this factor is less important than for HFP. These results suggest that a decrease of the fat content leads to attenuate subject effect.

It is likely that these differences in food bolus formation, tongue coating and oral movement will lead to differences in flavour release. Data analyses concerning this aspect are in progress and the results will be presented and discussed.

This work shows the importance to consider subject's oral physiology and inter individual variability to explain and predict flavour release from fat products.

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6.05

PTR-TOF-MS MONITORING OF IN-VITRO AND IN-VIVO FLAVOUR RELEASE IN CEREAL BARS WITH VARYING SUGAR COMPOSITION

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Keywords: PTR-TOF-MS; in-vivo, in-vitro, cereal bars, sweeteners; polydextrose

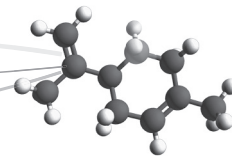
Proton transfer reaction mass spectrometry (PTR-MS) is a well-established technique used to assess volatile compounds release from real or model food matrices and, in particular, it has been applied to the in-vivo monitoring of flavour release during food consumption (nose space analysis) to demonstrate the influence of parameters related with the food matrix (composition, microstructure, textural and rheological properties) or the assessor's oral physiology e.g. saliva flow, swallowing, mouth volume and breathing pattern (1,2). The recently developed PTR-ToF-MS combines the advantages of the quadrupole based PTR-MS (sensitivity, dynamic on-line capability) with improved mass and time resolution providing unambiguous determination of chemical formulas and the possibility to monitor complex matrixes without the necessity to select few compounds at a time (3).

Type and amount of sugars can modify the physical properties of food products influencing texture, appearance and possibly flavour release. Therefore changing sugar content, in view e.g. of sugar reduction, can influence the partitioning of aroma compounds, posing a critical issue for food manufacturers (4).

The present study has a twofold goal: firstly, to describe the application of the novel PTRToF-MS in nose-space analysis and, secondly, to investigate by this technique the impact of changes in the level of glucose syrup substitution by polydextrose (from 0% to 70% on glucose syrup weight) both on the “in-vitro” and “in-vivo” flavour release from a complex system as flavoured cereal bars.

After describing the performances of PTR-ToF-MS we show how in the case of in-vitro analysis, the concentration of the flavor compounds was significantly affected by the sugar composition. The substitution of glucose syrup solids by polydextrose facilitated the release of acetaldehyde, esters, menthol and menthone whereas no significant effects were observed for other flavor compounds such as methyl cinnamate or vanillin. Flavor release is possibly driven by a “salting-out” effect due to the ability of polydextrose to enhance the rubbery – glass transition state of the systems and consequently to reduce the water free volume.

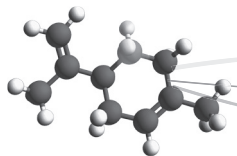
In the case of in-vivo measurements, three parameters were calculated from the curves describing the nose-space concentration of each assessor: the total area (A_{total}), the maximum concentration (I_{max}) and the time needed to reach it (t_{max}). Moreover the time of the swallowing events has also been recorded. Similarly to the in-vitro analysis, esters, carboxylic



acids, acetaldehyde, menthone and menthol exhibited significantly ($p < 0.05$) higher values for A_{total} , I_{max} , and t_{max} at a 50 or 75% glucose syrup substitution level. A similar mechanism related with salting out effects can be also used for explaining the observed behavior. For methyl cinnamate and vanillin A_{total} , and I_{max} were maximized at 75% glucose syrup substitution level but t_{max} seems to be related only to the swallowing time. This, together with the observed dependence of the swallowing behavior on sugar concentration, suggests that factors related with individual chewing behavior (free chewing protocol) and oral physiology may have led to the increased variability of the calculated parameters, particularly in the case of the time between the first swallowing event and t_{max} .

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6.06

BEYOND ODOUR THRESHOLD VALUES - NEW INSIGHTS DERIVED FROM A CUMULATIVE EXHALED ODORANT MEASUREMENT (EXOM) APPROACH

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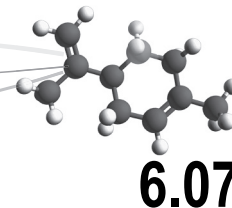
Keywords: cumulative exhaled odorant measurement (EXOM), breath analysis, odour threshold values

Historically, the idea of linking the relevance of an aroma compound to its odour threshold value in the respective food, known as the odour activity value concept, presented a milestone in aroma analysis by introducing a measure of relevance rather than just concentration of an aroma compound (1-3). However, odour threshold values in a particular food matrix tell us little about the breath concentration of an odorant as it is transported towards the olfactory receptors during the process of eating. Vanillin for example has been reported to exhibit an odour threshold value around 1 ng/L air in GC/Olfactometry (4) while its odour thresholds were reported to be 25 µg/kg in water (5), 180 µg/kg in sunflower oil (6) and up to 3 mg/kg in a matrix as complex as yogurt. It may be assumed that partitioning between the respective matrix during the process of eating leads to similarly low breath concentrations as reported in GC-Olfactometry. In order to have a deeper look into these aspects, the present study focussed on determining absolute breath concentrations of selected odorants during consumption.

Buettner and Schieberle (7) first introduced Exhaled Odorant Measurement (EXOM) as a method for absolute quantitation of breath odorants. In the present study, absolute quantitation of aroma compounds such as ethyl butanoate, ethyl hexanoate, (Z)-3-hexenyl acetate, limonene, diacetyl, ethyl-2-methyl butanoate, (Z)-3-hexenol, δ-octalactone, δ-decalactone, γ-decalactone, 4-Methoxy-2,5-dimethyl-3(2H)-furanone (mesifurane), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furanol), methyl cinnamate and vanillin as they appear in the breath of a panellist during the process of eating, was achieved by a cumulative EXOM approach for the first time. For example, recovery rates for vanillin from a yogurt matrix into the breath of a panellist were found to be 0,017 % resulting in substantially lower breath concentrations than would be expected from the above mentioned threshold values. Correlations between the determined breath concentrations and their odour threshold values as well as implications for the process of perception of the respective compounds will be discussed.

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RELEASE OF FRUIT AROMA COMPOUNDS FROM CHEWING GUM

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Keywords: aroma release, delivery systems, chewing gum, PTR-MS

One important segment in the chewing gum market is fruit flavoured chewing gum. Due to the more hydrophilic nature of most “fruity” aroma compounds it is more difficult to achieve a longlasting effect with these compounds as it is e.g. in the case of mint flavoured gums. As consumers look for a product that tastes good and continuously releases a pleasant flavour sensation over the total time of chewing, the flavour industry needs to understand how “fruity” aroma compounds are set free during chewing gum consumption and develop flavour solutions - like different kinds of delivery systems - accordingly.

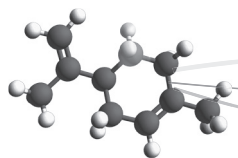
Thanks to the development of soft-ionisation mass-spectrometry techniques it is possible to measure concentrations of volatile organic compounds in air in very short time intervals. With these techniques aroma concentration in the breath of panellists can be followed directly when they chew gum. Several of these so-called in-vivo measurements were performed in the past (1-3). It is well known that aroma release kinetic is not the only driving force of aroma perception (4, 5). Nevertheless we could demonstrate earlier that shifting the menthol / menthone release curves by application of delivery systems also shifts the time intensity curves of mint perception (6).

In this study a Proton Transfer Reaction Mass Spectrometer (PTR-MS) was used to investigate the impact of the immediate aroma compound environment (either chewing gum matrix or delivery system) on its release during eating. All aroma compounds were selected from fruit flavours. Their physico-chemical properties covered a broad range which allowed investigating the mutual influence of delivery system's and aroma compound's properties on aroma release. Delivery systems were chosen from different technologies: spray dries, coacervates, and coated matrices. All results were compared to the release curves of the non-encapsulated (liquid) flavours in the chewing gum.

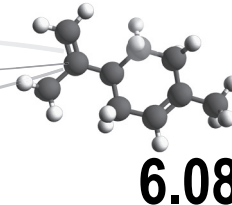
Depending on the nature of the aroma compound and the nature of the delivery systems different amounts of aroma were released (expressed as Area Under the Curve, AUC) and the maximum intensity of release was reached at different times (expressed as Tmax). For each given flavouring system these parameters could be successfully correlated to physico-chemical parameters of the different aroma compounds.

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AROMA PROFILE OF A RED BERRIES YOGHURT DRINK BY HS-SPME-GC-MS-O AND INFLUENCE OF MATRIX TEXTURE ON VOLATILE AROMA COMPOUND RELEASE OF FLAVOURED DAIRY PRODUCTS

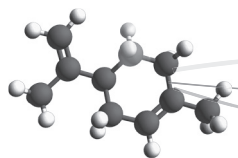
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Keywords: GC-Olfactometry, matrix effect, aroma release, texture, solid-phase microextraction

Headspace-solid-phase microextraction (HS-SPME) sampling followed by gas chromatography (GC) separation is widely employed for the analysis of volatile odorant compounds in dairy products (1, 2). In a previous study, the odour representativeness of HS-SPME extracts of a red berries yoghurt drink was investigated by direct-GC-Olfactometry, i.e. without chromatographical separation. The odour of the product was best represented by the DVB/CAR/PDMS 50/30 µm fibre (3). In the present work, we report on the volatile odorant compound profile of the yoghurt drink by HS-SPME-GC-MS/FID-O with the described fibre to determine the chemical and sensorially perceived aroma composition. As aroma release in dairy products is known to be influenced by multiple interactions with the matrix (4, 5), the effect of the dairy product texture on volatile compound release was investigated.

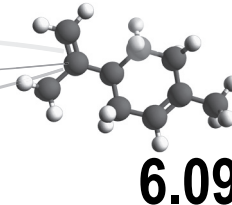
The two predominant constituents are methyl hexanoate (~54 % of the total FID peak area) and ethyl 2-methylbutanoate (~24 %), but various other esters, alcohols, and ketones are present. Further compounds detected at ≥ 1 % are ethyl 2-methylpropanoate (~1 %), ethyl butanoate (~3 %), (Z)-hex-3-enol (~2 %), (Z)-hex-3-enyl acetate (~4 %), and cyclohexyl acetate (~1 %). Methyl octanoate was added as an internal standard (IS). During GC-O analyses, seven odour-active zones were perceived by the panellists at a detection frequency of ≥ 50 %, whereby fruity notes were dominant. Main odour compounds were ethyl 2-methylpropanoate (fruity, red berries, pineapple), ethyl 2-methylbutanoate (fruity, strawberry), ethyl butanoate (fruity, banana, pineapple), and (Z)-hex-3-enol (grassy, fruity). Based on these results, a model red berries aroma was composed and added to several dairy matrices differing in texture (milk, neutral yoghurt drink, yoghurt, curd). GC-MS/FID-profiles were compared in order to study the matrix effect on aroma release for the main components ethyl 2-methylbutanoate, methyl hexanoate, and methyl octanoate. In all four matrices, the sum of added compounds constituted ≥ 95 % of the total GC/FID-profile. A slight shift in percentage among the matrices is most likely due to the presence of other volatile compounds such as e.g. butanone, diacetyl, acetoin or limonene. Deviations between peak areas over sample repetitions were observed, and the highest deviations were found in curd. This might be due to a less homogeneous distribution and release of the compounds compared to a liquid matrix such as milk. Compound volatility might also influence deviations as methyl octanoate area deviations were found to be among the highest in most cases.



Furthermore, ethyl 2- methylbutanoate showed similar FID-areas in all four matrices, methyl hexanoate areas were slightly higher in the yoghurt drink, and methyl octanoate areas varied strongly between the matrices. These results indicate once more that matrix effects should not be neglected when analysing food aroma. Therefore, and especially for quantitative analyses, it is highly important to employ suitable internal standards.

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THE FLAVOUR OF MARGARINES: ROLE OF AROMA-MATRIX INTERACTIONS ON PERCEPTION

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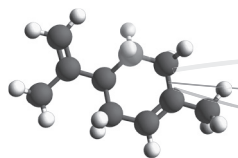
Keywords: flavour, structured emulsion, in-vivo aroma release, PTR-MS, sensory perception, composition

In literature, few information is available on the behaviour of aroma compounds during oral processing of complex food products such as margarines. Margarines are water-in-oil emulsions stabilised by fat crystals. Aroma release during consumption is mainly governed by the melting of solid fats, causing a phase inversion of emulsion in the mouth (1). In order to better understand mechanisms leading to flavour perception in margarines, several studies were performed using sensory and in-vivo aroma release methodologies.

The experimental set-up was defined by investigating whether the way of consumption of margarines, pure or spread on bread, would impact aroma release in the mouth and perceived aroma intensity. Both tasting conditions were examined in margarines flavoured with 10 aroma molecules from different chemical classes typically used in spreads. Trained panelists evaluated the perceived aroma intensity using a Rank and Score method. If margarines could be discriminated on aroma intensity when they were evaluated spread on bread, they were not when tasted pure in a spoon. In parallel, in-vivo aroma release during consumption was monitored with 5 panellists using Proton Transfer Reaction Mass Spectrometer (PTR-MS). Results showed that when margarine was spread on bread, the signal intensity of aroma release was 30% (2-nonanone) to 70% (trans-2-hexenal) higher than under spoon tasting condition. The spreading protocol was thus selected for the follow-up experiments.

The influence of the composition and structure of the model emulsions on in-vivo aroma release was investigated by PTR-MS. Compositional parameters included the nature of fat (vegetable vs. dairy), the fat level (60-39%), the amount of solid fat in the fat fraction (6-15%) and the protein level (0-0.5%) in the water phase. Within the range of parameters studied, aroma release in mouth was found to be more affected by the nature of aroma compounds than by changes in margarine composition. For example, the effect of the type of fat seemed to be directly depending on the hydrophobicity of the aroma compounds (LogP).

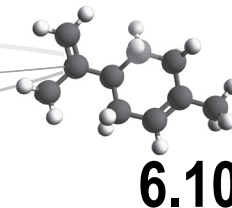
A Time-Intensity sensory test was then run with trained panellists on 4 margarines varying in fat level (60-39%) and flavoured with 2 commercial flavours of different hydrophobicity compositions. Margarines composition was found to affect Time-Intensity results, in a flavour-dependent manner. Margarines flavoured with a more hydrophilic flavor were perceived with a higher maximum intensity in the 39% fat margarine as compared to the 60% fat margarine when no significant differences were observed between high and low fat products for the most hydrophobic flavour composition.



As a conclusion, these studies showed that changing margarine composition may affect flavour perception via specific interactions between aroma compounds and emulsion components and different melting behaviours. A full characterisation of the mechanisms of the in-mouth emulsion breakdown (phase inversion) and interactions with salt release would be necessary to further understand the links between aroma release and flavour perception in margarines and design tailor-made margarine flavours.

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FLAVOUR RELEASE MEASUREMENT USING MODEL MOUTH EQUIPPED WITH FORCE LOAD CELL DEVICE FOR MONITORING MASTICATORY FORCE DURING MIMIC MASTICATION

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Keywords: flavour release, model mouth, masticatory force

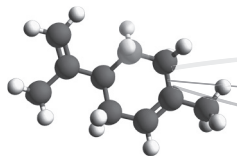
Flavour release during mastication is very important because it is one of the factors to determine the final palatability of food. Model mouth systems were used to study release behavior of volatile compounds during mastication to avoid inter- and intra- variances among panelists (1-4). It was reported masticatory force had an effect on the release of volatile compounds using the model mouth (4).

Masticatory force decreased as food was broken down into a bolus in panelist-experiments. To simulate the real mouth conditions, model mouth system needs to monitor the wide range of masticatory force. Force load cells are generally equipped to model mouth system to monitor masticatory force during mimic mastication. Each cell, however, has its own available range of force, such as 0-0.98 N, 0-98 N, and 0-980 N, and the optimal force range of measurement is also fixed, for example the value from 200 N to 800 N is guaranteed in a 0-980 N cell. To monitor the change of masticatory force of hard food sample from the first bite to the end of the mastication process, it is not enough to use only one force load cell.

Combining multiple force load cells made possible to monitor masticatory force from the first bite, showing higher value, to the end of the mastication, showing very lower one. The model mouth equipped with the multiple load cells was also useful to simulate different mastication behaviors, such as elderly, younger and childhood generations using different masticatory forces.

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6.11

FLAVOUR DELIVERY THROUGH NATURAL EMULSIONS

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Keywords: Flavour, Aroma, Delivery, Oil Bodies, Natural Emulsion

Oil bodies are small sub-cellular organelles that are found to exist in a number of different plant structures (although principally they are found in storage structures such as seeds) that store neutral lipid as energy reserves. Structurally oil bodies are oil droplets stabilised by surface proteins (oleosins) and a phospholipid monolayer (1). Oil bodies can be extracted intact using a solvent free protocol forming a natural emulsion isolate which shows high levels of stability both physically (2) and chemically (3). The extraction protocol is based on wet milling of the seed structures and sucrose gradient centrifugation to separate sub-cellular structures on a density basis, oil body isolates can then be processed as normal protein stabilised emulsions.

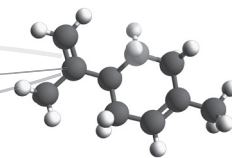
The use of fresh oil body isolates from sunflower seed (*Helianthus annuus*) as a carrier agent for volatile aroma compounds will be discussed in light of existing literature on cloud emulsions.

Cloud emulsions are stable oil in water emulsions that are widely used in beverage systems to impact colour to the beverage and deliver lipophilic flavours in aqueous products (4). Oil bodies offer interesting functional properties in regard their delivery of volatile aroma compounds. In the work to be detailed the static partitioning of a range of volatile compounds to oil bodies will be reviewed and the respective partitioning of aroma compounds when exposed to dynamic headspace dilution will be detailed. The impact of the findings on their inclusion in food will also be highlighted.

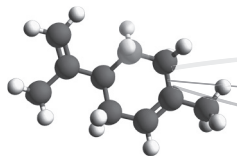
Oil bodies are shown to act comparably to artificial emulsions when in a static equilibrium state, the partitioning of the volatile compound is driven principally by the lipid content of the emulsion, as is found in typical cloud emulsions (5). When exposed to dynamic headspace dilution then natural emulsion system has significantly greater volatile headspace persistence when compared to processed emulsions.

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ENCAPSULATION AND CONTROLLED RELEASE OF FOOD FLAVOURS

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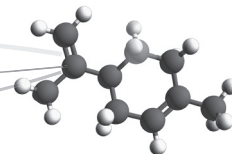
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Keywords: Flavour, Encapsulation, Flavour Retention

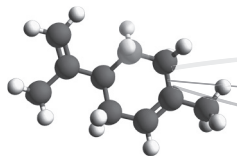
Flavour characteristic as well as appearances and texture/rheological properties of foods is the most important factor for preference of food and evaluation of food quality (1). Flavour compounds are volatile molecules. They are formed during processing or storage of food products by several chemical and biochemical reactions. Many factors including storage condition, packaging material, heating temperature, mastication, affected flavours in food (2). For example, citrus flavour compounds in orange juice was decomposed and lost during heat treatment of orange juice. Knowledge of the stability, retention and release of flavour compounds in food are crucial in terms of food and flavour manufacturers. Encapsulation is one way for stabilizing and protecting the flavours from thermal degradation, loss by evaporation and oxidation. Encapsulation is also used for controlled release of flavours in food systems (3). In this technology, flavours is coated or entrapped by another material or system in micron size. Coated material is called core or active material and coating material is called wall material. Carbohydrates (starches, maltodextrins, cellulose, alginates, carrageenans etc.), waxes and lipids (beeswax, candelilla, carnauba waxes etc.), proteins (whey proteins, zein, soy proteins, etc) and food grade polymers (polypropylene, polyvinylacetate, polybutadiene etc) are used as a wall-forming material (encapsulant) in the encapsulation process of flavours. Several encapsulation techniques have been applied for flavour encapsulation (2, 4). However, spray drying, extrusion, coacervation, molecular inclusion (Cyclodextrins) and spray chilling/cooling are the most common commercial techniques (3, 4, 5). Selection of encapsulation technique and coating material are depend on flavour structure, wall material properties and interaction between food matrix and flavour compounds (3). In this review, encapsulation processing for food flavours, encapsulation materials used for encapsulation of flavour, recent developments in the encapsulation of food flavour and mechanisms of controlled release of encapsulated flavour in food will be discussed

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6.13

EXPLAINING THE PLEASANTNESS OF BILBERRY AND CROWBERRY JUICES BY COMBINING SENSORY AND CHEMICAL DATA

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Keywords: astringency, bilberry, crowberry, flavonols, fruit acids and sugars, liking, taste

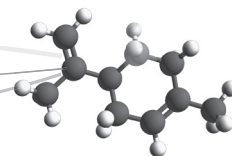
Bilberries (*Vaccinium myrtillus*) and crowberries (*Empetrum nigrum*) are growing wild and widely in the northern hemisphere. The former is very well known and recognised, but the latter is poorly known and utilized in food industry. Our study examined the roles of sensory (taste and astringency) and chemical properties (various phenolic compounds, sugars and fruit acids) in pleasantness of cold-pressed bilberry and crowberry juices.

Four juice samples were prepared according to Laaksonen et al. (1). The first ones were cold-pressed berry juices and to the other juice samples the two first extracts of the press residue were added. The ratio of juice and extract was according to yields of these fractions (1, 2). 39 voluntary subjects were asked to rate the taste pleasantness of the juices on a 9-point scale (1 = extremely unpleasant, 9 = extremely pleasant). The intensities of sensory attributes (sourness, bitterness, soft and rough astringencies) were analysed from the juices by a trained panel (n=39) using generic descriptive analysis.

The panel evaluated intensities from the samples in triplicate sessions on a labeled magnitude scale. Additionally, they were asked to mark and comment the sweetness and any other significant taste properties, if found. Reference samples were given to panelists to anchor the scales. The pleasantness and sensory analyses were performed in sensory laboratory in accordance with ISO 8589-1988 standard. Contents of various phenolic compounds and sugars in the juices and acids were determined with HPLC and GC (1, 2). All data was used to explain the pleasantness of the juices by using PLS-regression model.

Bilberry juices were significantly more pleasant than crowberry juices ($p < 0.05$, $n = 39$). Addition of phenolic extracts to juices did not decrease the pleasantness of the juices. Both bilberry juices were more sour and less bitter than crowberry juices ($n = 117$). All samples had more soft, velvety astringent properties than rough, puckering astringency. However, the crowberry juices had more rough astringent properties and crowberry juice with added phenolic extract was more intense in this attribute. Crowberry juices were more frequently marked as sweet than bilberry juices and in both berries the frequency declined with addition of the extracts. Addition of extracts doubled the content of anthocyanins in the juices and significantly increased the content of flavonol glycosides and hydroxycinnamic acids. Crowberry juices had more sugars, less acids and higher sugar/acid ratio.

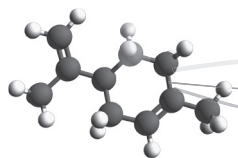
PLS-regression model showed strong correlation between sourness, acids and the pleasantness of taste as they were positive drivers of liking. Many phenolic compounds correlated either with rough or soft astringency. Rough astringency was negative driver of liking with bitterness. Results show that certain sourness in addition to sweetness is crucial



for berry liking and masking astringency and bitterness of phenolic extracts (1, 2). Also the unfamiliar profile of crowberry in comparison to bilberry may affect as negative driver for liking. Comprehensive understanding of the roles chemical and sensory properties of berries is needed to enhance the exploitation in food industry.

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6.14

HIGHBUSH BLUEBERRY VARIETAL FLAVOUR CHARACTERS

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Keywords: SPE, SPME, Descriptive Sensory Analysis, Free Choice Profiling, Flavour Modeling

Blueberries are widespread fruiting plants from the genus *Vaccinium* categorized as: wild lowbush (*V. angustifolium* Ait.), highbush (*V. corymbosum* L.) and rabbiteye (*V. ashei*). Rabbiteye blueberries have a different aroma volatile profile from highbush and wild lowbush the last having the highest content (1). Highbush blueberries contain - 1-hexanol, cis-3-Hexen-1-ol, trans-2-hexen-1-ol, linalool, citronellol, α -terpineol, nerol, geraniol, hydroxycitronellol farnesol, farnesyl acetate, 2-hexenal, benzaldehyde, benzylalcohol, 2-phenylethanol, phenol, eugenol, trans-cinnamyl alcohol, vanillin (2). Lowbush blueberries contain - methyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, methyl butanoate, linalool (3). Rabbiteye blueberries are reported to contain - 1-penten-2-one, γ -terpinene, carveol, acetone, cis-caran-3-ol, ecineralone, α -cedrene, sabinol, geranyl framate, linalyl acetate, undecan-2-one, tridecan-2-one, ethyl acetate, ethyl tetradecanoate, dimethyl octanedioate, toluene, p-cymene (4).

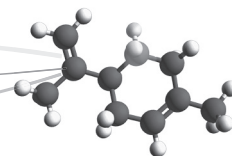
The aim was understanding sensory differentiation of flavour in fruit from ten highbush cultivars grown in the UK. A two-phase descriptive sensory analysis was used: initial free-choice profiling to understand the range of descriptors used by consumers to differentiate the fruit; subsequent conventional profiling by trained assessors using a consensus vocabulary to generate a multivariate product space describing relationships between the cultivars.

To clarify the basis of varietal flavours, fruit aroma volatiles were subjected to qualitative and semi-quantitative analyses using solid phase microextraction (SPME: Combipal TM autosampler) and solid phase extraction (SPE) and gas chromatography with mass spectrometric (electron impact) detection. Of these SPE stabilized fruit flavour, enhanced sensitivity and allowed analyses using a conventional autosampler and by GC-olfactometry.

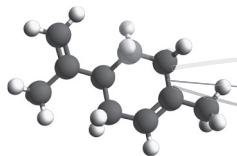
Relationships between varietal fruits in multivariate spaces derived from the two extraction strategies suggest caution should be used in interpreting aroma volatiles data. Modeling relationships between sensory characters and compositional data also suggests differences.

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6.15

CLASSIFICATION OF THE AROMA QUALITY OF PYRAZINE DERIVATIVES USING RANDOM FOREST TREE TECHNIQUE

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Keywords: Molecular odor, Molecular descriptors, Random Forest Tree, pyrazine molecules, QSAR.

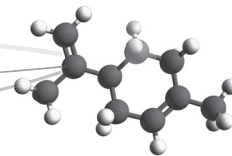
Aroma quality classification can be performed with molecular Structure – Odor Relationships (mSOR) despite mathematical modeling remains very difficult due to the subjectivity of odor quality (1-2). In the open literatures, several models were developed and proposed with a wide variety of molecular descriptors and statistical equations to classify odor molecules (3). However, none of them is reported as universal ones. Ivanciuc (4) has classified odor of the pyrazine molecules based on Support Virtual Machine approach and molecular descriptors while Klocker et al. presented methodology to classify pyrazine derivatives using Self-Organizing Molecular Field Analysis and Artificial Neural Network (5).

In this paper, we present an alternative classification of the odor molecules based on molecular descriptors (⁰D, ¹D, ²D and ³D) and Random Forest Tree (RFT) (6). RFT is considered as a powerful technique in the field classification and so far not used in molecular odor classification. This technique is applied to classify 98 molecules of pyrazine derivatives representing three classes of aromatic notes: Green (32), Nutty (23) and Peppery (43). The molecular database is divided between training and test sets (OOB validation) with 70% and 30% respectively. The number of descriptors chosen after a selection and reduction step is 8 for ⁰D, 10 for ¹D, 11 for ²D and 24 for ³D.

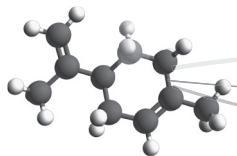
The results obtained with descriptors ⁰D, ¹D, ²D and ³D give a correct classification rate of molecules for the learning phase: 72.1%, 70.6%, 82.4% and 85.3% respectively. For the test phase, the classification rate is 80%, 86.7%, 93.3% and 90%. The classification model has numbers of trees in the forest (model dimension) of 180, 40, 45, 50 trees each for ⁰D, ¹D, ²D and ³D. The results show that RFT is most effective in developing the mSOR models, based on selected molecular descriptors and a forest of large size, which mean the dimensionality of the model. RFT could be an efficient predictive approach useful in the synthesis of new aromatic molecules.

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6.16

TANNIN-PROTEIN INTERACTIONS IN RELATION TO WINE MOUTHFEEL: IS THERE ANY SPECIFICITY?

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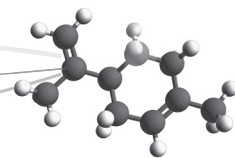
Earlier considered as a taste, astringency is now clearly accepted as a mouthfeel, defined by the American Society for Testing and Materials as “the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins”(1) . Very typical for wine (2), it also appears with tea (3), cacao (4) or fruits (5). This mouthfeel has been the focus of numerous studies which have underlined the importance of its specificity (6), the proline content being of the salivary proteins often being cited (7). In this work, this specificity of the interaction was investigated for the first time in wine tasting conditions, by mean of several in vitro and ex vivo experiments.

A first experiment involved various proteins (bovine serum albumin, α -amylase, ovalbumin, fetuin, β -glucosidase, thyroglobulin, gelatin) and polyphenol extracts (grape seed extract and wine). Thanks to an high pressure liquid chromatography method enabling to globally and simultaneously measure the tannin and protein content of the medium, very different stoichiometries were seen depending on the various concentration used. The non-stoichiometry of the interaction could then be proven in all cases. Moreover, apart from the influence of protein molecular weight, no specificity factor could be drawn out.

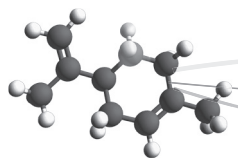
To go further, an ex vivo tasting was developed: saliva collected from volunteers was added to wine in wine tasting conditions. The reaction between saliva's proteins and wine's tannin was then followed by means of 2D gels electrophoresis and HPLC. The supernatant proteins (i.e. proteins that have not reacted with wine tannin) were analysed. It appeared that, in wine tasting condition, all salivary protein react with wine (including α -amylase, acidic, basic and glycosylated proline rich proteins, histatins) and no specificity factor (proline content, glycosylation) could be drawn out. One can then asks about the specificity of the interaction between salivary protein and condensed tannin in the frame of astringency.

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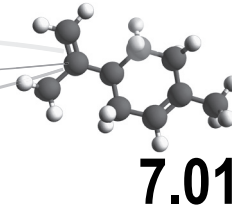
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Topic:

**Instrumental
aspects and
other tools
of the trade**

**Poster
Presentations**



NIGHTMARE PROBLEMS IN THE ANALYSIS OF VSCS AND IN THE WORK WITH “OXYGEN-FREE” ATMOSPHERES

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Keywords: stability, VSCS, PFPD, airtight container, indigo carmine.

Providing of stable standard solutions along the time is highly important in order to calibrate and thus it can be possible to quantify correctly. There are lots of compounds that submit problems in their quantification due to their instability, so it is essential to achieve tools that allow us to control their concentration. In the case of volatile sulfur compounds, VSCS: MeSH (methanethiol), EtSH (ethanethiol), DMS (dimethylsulfide), DES (diethylsulfide), DMDS (dimethyldisulfide) and DEDS (diethyldisulfide) there are two issues: one of them is their lability in presence of oxygen and the other is their low boiling points. We use solid phase microextraction (SPME) to quantify this VSCS in wine so the concentration of the solutions is very low and this fact has a great influence in the stability (1). The study of the stability of the VSCS is based on previous experiments with polyfunctional mercaptans (2, 3). To prepare stable solutions, a glove box under purified inert gas P[BOX] from Jacomex was used, which allows oxygen levels lower than 1 ppm. Vials with Mininert® valves, which ensure a minimum entrance of oxygen, were also used. The control of solutions was carried out by liquid injection in split mode and SPME depending on the concentration. All analyses were carried out by using a Varian CP-3800 gas chromatograph fitted with a PFPD system. The column was a DB-WAXetr.

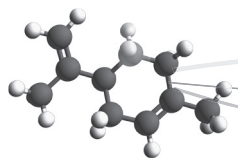
To control these solutions, the percentage of area of each compound as regards the sum of all the areas of the compounds was taken into account. Solutions were discarded when the percentage of the compounds changed along the time. A suitable stability of the solutions for the analysis was achieved.

To obtain a “perfect” airtight container that could be manipulated and after that it continues being airtight is not so easy and obvious. In order to study how a wine changes when is put with different well-known quantities of oxygen an airtight container is essential.

A qualitative study with different containers has been done. A colour indicator (a solution of indigo carmine) was used in order to know if air was passing through the seal (4). Commercial sealings have showed not to be appropriate to get a “perfect” airtight container. Therefore some modifications have been done in these commercial sealings with the purpose of reducing the quantity of oxygen passing through the seal. Moreover a system with different barriers has been tested ensuring a minimum entrance of air.

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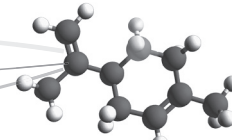


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INTEREST OF ONLINE HIGHER ALCOHOL AND ESTER DETERMINATIONS DURING WINEMAKING FERMENTATIONS

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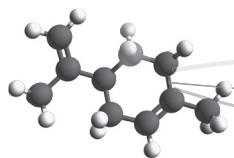
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Keywords: *Saccharomyces cerevisiae*, winemaking, online monitoring, aromatic compounds, gas-liquid transfer, dynamic modelling

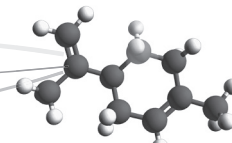
In winemaking, the synthesis of higher alcohols and esters during the alcoholic fermentation makes a major contribution to wine quality, especially to young wines. Therefore, accessing the kinetics of production of these compounds is a key issue.

We developed an experimental device to on-line measure, by gas chromatography, the production of volatile compounds in the off-gas of fermentations run in 10 and 100 L tanks. 16 compounds considered as markers of organoleptic and/or metabolic interest and corresponding to the main higher alcohols, esters and aldehydes were measured every hour. The high frequency measurement (once hourly) made it possible a very precise description of their synthesis with, for the first time, the calculation of rates and specific rates of production. The dynamics of higher alcohol synthesis, with maximal specific rates at the start of fermentation, was entirely consistent with the hypothetical metabolic pathways. These alcohols were mostly synthesised from their precursor amino acids, but were also formed, albeit with a lower metabolic flux, from other nitrogen sources and, for isobutanol and isoamyl alcohol, from carbon metabolites. Propanol seemed to be a highly pertinent marker of nitrogen metabolism. Its synthesis was strongly correlated with the presence of assimilable nitrogen, in both the growth and stationary phases. Acetate ester concentrations were linearly correlated with the concentrations of the corresponding higher alcohols, consistent with the maintenance of a constant yield throughout synthesis.

In parallel, the relationship between the aroma compound concentrations in the gas and liquid phases was studied and modelled. It enabled us to calculate gas-liquid balances. Losses highly depended on the studied compound. They were negligible for the studied higher alcohols. By contrast, 56 % of the ethyl hexanoate and 34 % of the isoamyl acetate were stripped by the CO₂ when the temperature profile simulated red winemaking conditions. Even at a moderate temperature of 20°C, typical of white wine fermentations, 40 % of the ethyl hexanoate and 21% of the isoamyl acetate were lost. Effect of temperature on the production of the volatile compounds studied, was assessed by running fermentations at different temperatures, with the same medium and strain. Changes in volatile compound production were found to be smaller than those usually calculated from the concentrations in the wine. These findings highlight the



potential importance of knowledge concerning aroma gas-liquid balances, for both an understanding of yeast metabolism and the identification of innovative control strategies minimizing aroma losses.



OAK AROMA PRECURSORS

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Keywords: oak, precursor, aroma, HPLC

Malolactic fermentation (MLF) usually occurs spontaneously at the end of alcoholic fermentation. The MLF favorable effects on wine quality (microbial stability, decrease wine acidity and improve taste, flavor) are desirable in red wines and in some white wines as well. The presence of lactic acid bacteria (LAB) in oak barrels induces an increase in wood volatile compound concentration (1). This phenomenon could be due to the glycosidic activity of LAB (2). The glucosidic precursor of a major woody aroma compound (oak lactone) has already been identified and quantified in oak wood (3, 4) but no data are available concerning the presence of other flavor precursors in oak wood

A multi-step protocol was developed with the aim to obtain an analytical profile of the flavor content of different oak woods.

Precursors was first extracted from 200 g of oak chips by stirring in H₂O / MeOH : 50/50 during 48 hours. After concentration, wood extract was fractioning by semipreparative HPLC. Each fraction was submitted to acidic hydrolysis to cleavage aglycone moiety and finally analyzed by GC-MS. We have screened different types of oak wood for their content in flavor precursors.

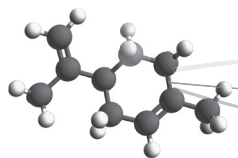
Synthesized standards have been used to optimize HPLC separation. Previous works (2) have shown that xylosidase, glucosidase and arabinosidase enzymes released vanillin and coniferaldehyde from hydroalcoholic oak extracts, so vanillin- β -D-glucopyranose, vanillin- β -D-xylopyranose, coniferaldehyde- β -D-glucopyranose were prepared via a diastereoselective glycosylation of vanillin or coniferaldehyde by 1-bromo-per-Oacetylated sugar under phase transfer conditions. The synthesized molecules were characterized by high resolution LC-NMR spectrometer and ESI-HPLC-MS.

With these molecules the HPLC separation conditions have been optimized.

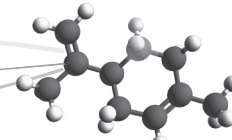
A multistep SPE purification method has been settled down and optimized. Oak wood extracts have been analyzed by HPLC-DAD-MS, and preliminary results have shown the presence of precursors in oak wood used by cooperage.

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THE EFFECT OF GAS-CHROMATOGRAPHY INJECTION PARAMETERS ON ANALYSIS OF THIOLS AND POLYSULFIDES

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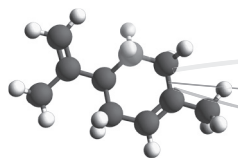
Keywords: artefacts, redox, dimerisation, meaty odour, GC

Many sulfur-containing compounds have been identified in the aroma of savory flavours where they are often key contributors to the odour quality (1). Thiols and disulfides have been identified as particularly important in allium species (2) in beer (3) and meat flavours (4). Many of these compounds have very low aroma thresholds (5), and are also found at low concentrations in food products. Thiol compounds are known to react easily, especially through sulfide transfer in the presence of other thiols (6, 7) and the possibility that this effect can occur during analysis e.g. when a mixture of thiols is exposed to heat in a GC injector. This means that interpretation of GC analysis of thiol containing mixtures is not clear cut. For example, 2-methylfuran-3-thiol (MFT) is a particularly important compound in cooked meat flavour and both the monomer and the disulfide form (bis(2-methylfuryl)disulfide) are found after GC analysis of meat flavour.

Pure MFT has been synthesised and analysed by GC using conventional heated injection as well as cold on-column injection to ascertain whether changes occur at this stage or elsewhere in the analytical system e.g. in the heated transfer line leading to the mass spectrometer. Since the odour thresholds of MFT and the disulfide form are very different (7 ng/L and 0.02 ng/L (5)), the inter-conversion has potential significant effects on the sensory quality of meat odour.

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7.05

MULTIPLE HEADSPACE EXTRACTION – A SIMPLE WAY TO QUANTIFY AROMA COMPOUNDS FROM BREAD

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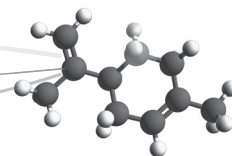
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Keywords: Multiple headspace extraction, dynamic headspace extraction, gas chromatography mass spectrometry, aroma, wheat bread

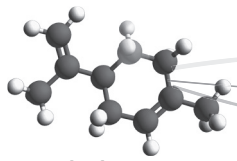
Bread is a complex food matrix and quantification of the wide range of aroma compounds identified in bread can be difficult. A simple quantification method “Multiple Headspace Extraction” (MHE) was developed in 1977 (1, 2), but the method has only rarely been applied within food science (3). MHE is a method developed by Kolb and Pospisil to quantify aroma compounds present in a sample. The method is particularly interesting when extracting aroma compounds from a complex sample matrix, since the method is independent of the sample matrix (2). MHE was originally developed for static headspace extraction. The purpose of this study has been to investigate the possibility of using MHE combined with dynamic headspace extraction in order to quantify the aroma compounds in bread crumb. The principle was that stepwise headspace extractions of aroma compounds were performed and analysed by gas chromatography mass spectrometry (GC-MS), each successive extraction resulting in a lower area of the aroma compound. After an infinite number of headspace extractions all aroma compounds would be extracted from the sample. The total of all peak areas would then correspond to the total amount of aroma compounds (A_i). The decline in peak area does, however, follow first order kinetics, therefore an infinite number of extractions are not needed, but enough extractions to get a correlation coefficient (R^2) close to 1 were performed in order to calculate A_i . To quantify the amount of each aroma compound in the sample, standard solutions of authentic standards were dissolved and injected directly to a Tenax-TA trap and analysed exactly as the samples. MHE was found to be a successful method in quantification of 39 aroma compounds identified in wheat bread crumb. 3-Methyl-1-butanol, 3-methylbutanal, 2-nonenal and 2,3-butanedione were found to be the sensory most important aroma compounds identified in bread crumb based on their odour activity values (OAV's). MHE was found to be a relatively simple method for quantification of aroma compounds in bread crumb and the method is well suited for quantification of aroma compounds from other food products, since food in general consists of complex matrices.

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EXPERIENCES WITH OFF-FLAVOUR RESEARCH OVER THE LAST DECADE

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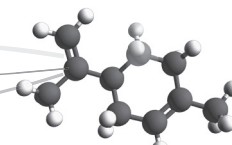
Keywords: off-flavour, GC analyses, chlorophenols, fire incidences

TNO triskelion BV, a fully owned daughter of TNO, has a very long history in flavor research; off-flavour investigations form the most prominent activities in bilateral research. When an off-flavour at a food product is noticed at the stage that the product has already reached the consumer, the finding of the source of the complaint as soon as possible is of outmost importance. Recognition of the character of the off-flavour is possible in many cases at TNO on bases of the long experience.

An overview of off-flavour cases will be presented. Contaminations with chlorophenols and/or chloroanisoles are still the most common sources of off-flavours. Three decades ago chloroanisoles formed the most occurring sources while the last decade the contaminations with chlorophenol were most prominent. Finding the precise isomer causing the off-flavour is of outmost importance for the indication of the right source. This is only possible by using the GC-sniff technique.

The authors are of the opinion that informal sensory evaluations of the contaminated and reference products in combination with GC-sniff are indispensable tools in off-flavour research. However sometimes ethical objections are made to this approach. The health of the researchers might be questioned. Certain food producers are performing research to investigate on forehand all possible sources of contamination with off-flavour compounds in their production and storage facilities. Once consumer complaints are received the product can be evaluated for these compounds. This approach requires that all possible variations in the normal volatile fingerprint of their products are known.

The investigators of TNO are asked in an increasing number of cases to give their opinion about the safety for consumers or the risk of off-flavour complaints after a fire incident for food products, packaging materials, machineries, producing facilities and a large range of consumer products. Informal sensory research by a small number of investigators is the only possible way to give a fast opinion over a large range of products and equipments. Instrumental research of the off-flavour compounds, often present in minute quantities, would in these cases be too expensive and long lasting. However also in these cases the health implications for the investigators might be questioned; certainly in a recent incident where a large range of toxicological products/compounds caught fire. Our approach in these cases is outlined.



EVALUATION OF MONOLITHIC MATERIAL SORPTIVE EXTRACTION (MMSE) AS AN ALTERNATIVE AROMA EXTRACTION TECHNIQUE

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Keywords: MMSE, LVSH, SBSE, representative aroma extract, strawberry flavored milk

One of the preconditions in finding relations between results from aroma analysis and sensory analysis is that the aroma extract prepared resembles the aroma of the product of investigation.

In this study we compared the extraction capability of the well known sample preparation techniques of Large Volume Static Headspace (LVSH) and Stir Bar Sorptive Extraction (SBSE) with a relative new extraction technology called Monolithic Material Sorptive Extraction (MMSE).

MMSE makes use of a new generation media for adsorption and extraction developed by using silica monolith technology. Based on this silica monolith technology an innovative hybrid adsorbent of silica and activated carbon (or graphite carbon) having a large surface area bonded with octadecyl silane (C18, ODS) was manufactured. For the ODS bonded hybrid medium application notes showed quite effective adsorption capability to a wide variety of compounds. It was also reported that an advantage of MMSE is a large surface area, a high recovery and a short extraction and conditioning time (1, 2).

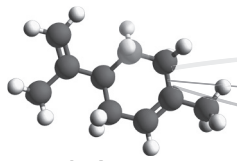
In our study strawberry flavored milk was used as a model solution and was extracted with the MMSE technique under comparable conditions (time, temperature) followed by GC/MS analysis. Analytical data showed that GC profiles of LVSH and MMSE were very similar. For the SBSE technique it was observed that the balance of the aroma components was not comparable to LVSH and MMSE.

After eluting from the GC column volatiles were trapped in cooled milk and evaluated by trained sensory panelists. Sensory results showed that the recombined made by MMSE was most comparable to the strawberry flavored milk. The reproducibility, linearity and detection limit of the MMSE technique was also investigated and showed good results.

The obtained data showed clearly that an extract made by the relative new MMSE technique could be very powerful in finding the relation between analytical and sensory data.

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7.08

SUCRALOSE ANALYSIS IN MILK WITHOUT PROTEIN PRECIPITATION

CARLOS IBÁÑEZ, Josep Solà and Pere Peiró

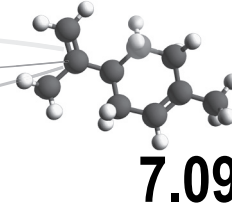
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Keywords: Sucralose, HPLC, milk, SPE, filter

Sucralose is a chlorinated high intensity sweetener, around 600 times sweeter than saccharose, common now in many applications in the food industry: beverages, dairy products, ice creams, chewing gums, etc. One of these applications is the sweetening of milk products. The direct sucralose analysis in milk samples is not possible due to presence of multiple interfering compounds like proteins, fats and sugars, but rapid analytical methods are needed to control the presence of this sweetener in milk samples. Usually some previous cleaning or derivatization steps (1) were needed to allow good analytical results but some difficulties were found in the sweetener extraction process: clogging of filters and or emulsion formation, and also were found in the final analytical process due to the presence of multiple UV absorbing substances coeluting in this complex matrix. To simplify and accelerate the analysis of sucralose in milk samples a new extraction protocol was proposed, avoiding protein precipitation and using a combination of a special filter and two solid phase extraction cartridges. That permits us to clean the sample enough to realize the posterior analysis by HPLC without special coelution problems and at the same time to speed up the previous cleaning steps. The method consist first in a simultaneous sample filtration with a commercially available special filter designed for difficult samples made from hydrophilic PVDF and including a glass fiber prefilter, and a cleaning step made with a C-18 SPE cartridge. Afterwards a second cleaning purification step was made by attaching a NH₂ SPE at the end of a C-18 SPE cartridge and applying a simultaneous optimized elution step to both cartridges. Finally the resulting extract was analyzed using a classical reverse phase isocratic HPLC separation method (2) with methanol-water as mobile phase and detection with a Diode Array Detector. Some applications with real milk samples were also presented and advantages, drawbacks and future improvements of this method were finally exposed.

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GAS CHROMATOGRAPHY OLFACTOMETRIC ANALYSIS OF SOME COOKED HAM SAMPLES

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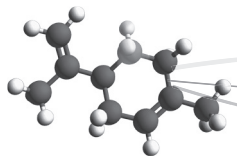
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Keywords: GCO, Surface Nasal Impact Frequency, SNIF, cooked ham, flavor, pig breeds

Iberian pig (IB) is a breed found in some areas of the Iberian Peninsula (Spain and Portugal). The IB has special meat features such as a specific composition of both extra and intramuscular fat deposits that result in pork sensory properties of high culinary interest. The aim of this work was to compare the volatile compounds of cooked ham coming from two different pig breeds, commercial Large White (LW) and Iberian (IB), by gas chromatography-olfactometry (GC-O) following distillation of the samples, to look for qualitative and quantitative differences in the respective flavour profiles of the extracts. Distillation of the samples were made using Simultaneous Distillation Extraction SDE glass equipment, the so called Likens-Nickerson apparatus, often used as the preferred extraction system for many flavour producing samples like cooked or cured meat products (1) Dichlorometane was the extraction solvent chosen for this case. Samples were analysed by gas chromatography using a FID detector and a sniffing port. Effluents from the column were smelled, recorded and studied with the Surface Nasal Impact Frequency SNIF method (2) This method had some advantages for our work comparing with classical methods like AEDA or OSME. Components of both flavor extracts were identified by GC/MS. A panel of eight different panellist, four men and four women, none trained for this experiment but used to work with organoleptic tests, smelled the effluents of both samples IB and LW at the exit of a polar chromatographic column. Samples were tested in duplicate along different randomized trials and finally the response of all trials were summed up for each sample and compared, to obtain the so-called aromagrams where the highest peaks correspond to the most olfactive intense components of the flavour samples. In this way is possible to detect differences in the overall flavour profile between samples. Results indicated that qualitatively both chromatograms and aromagrams were quite similar but not quantitatively were some differences in the intensities and the FID areas of the strongest odorant peaks were found. Those differences can explain the variation found in the initial flavour of both cooked ham distillate samples.

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7.10

IMPROVEMENT OF PARTITION COEFFICIENTS DETERMINATION OF AROMA COMPOUNDS IN FOOD MATRICES BY THE PHASE RATIO VARIATION METHOD

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Keywords: PRV; partition coefficient; liquid vapour equilibrium; aroma retention-release

Before its perception, aroma compounds must move from the liquid phase towards the vapour phase, and aroma release from food matrices constitutes a determinant step for its perception. In previous works, we used Quantitative Structure-Property Relationships (QSPR) approach to identify the nature of interactions involved between constituents of food matrix and aroma compounds (1). In this way, study of $K=C_{\text{vap}}/C_{\text{liq}}$ at equilibrium is an usual method to account for the balance between liquid phase and vapour phase. Because QSPR methodology is based on statistical regression tools, a limit of QSPR approach is the size of data set. Indeed, good applicability conditions require sufficient large training set constituted by at least 10 compounds belonging to the same chemical space (2). However, partition coefficient determinations are based on headspace methods and are time consuming because quantification of aroma compounds in vapour phase by chromatographic experiment requires substance-response factor detector calibration.

The Phase Ratio Variation (PRV) method (3) allows to overcome the calibration step. Nevertheless, some limits are linked to this method, particularly when compounds are strongly retained by food matrices (4), but also in case of very volatile molecules. However, as the PRV approach is a very valuable method for partition coefficients data acquisition, we have carefully examined its principle in order to improve the reliability of results.

According to Ettre *et al.* (3), the applicability of PRV equation $1/A=1/f_i C_{\text{liq}} x(C_{\text{liq}}/C_{\text{vap}}+\beta)$, with $\beta=V_{\text{vap}}/V_{\text{liq}}$, is based on the assumption that concentration of solute in liquid phase remains almost constant, thus close to the initial concentration C_0 ($C_{\text{liq}}\approx C_0$). Indeed, considering a vapour phase sampling of 1 mL, the PRV equation becomes $1/C_{\text{vap}}=1/C_{\text{liq}}*(V_{\text{vap}}/V_{\text{liq}}+C_{\text{liq}}/C_{\text{vap}})$, so that $1/C_{\text{vap}}=V_{\text{vap}}/(C_{\text{liq}}*V_{\text{liq}})+1/C_{\text{vap}}$. The equality between the two equation terms is ensured only if $V_{\text{vap}}/(C_{\text{liq}}*V_{\text{liq}})\ll 1$.

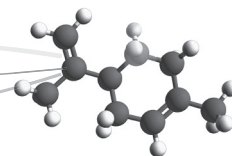
On the other hand, $1/C_{\text{vap}}$ vs $V_{\text{vap}}/V_{\text{liq}}$ linear regression calculation leads to obtain an ideal regression line, which slope and intercept are equal respectively to $1/C_0$ and $1/C_0*C_{\text{liq}}/C_{\text{vap}}$.

Taken together, the two equations lead to highlight a “correction term” equal to n_0/n_{liq} . This term must be as closest as possible to 1 to correctly apply the PRV method.

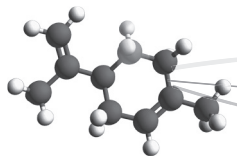
We performed an examination of several cases values allowing identification of the ranges of K values for which a carefully choice of β values is crucial for reliability of the regression calculation $1/A$ vs β . Some experimental examples illustrate this statement.

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7.11

A COMPARISON OF HEADSPACE SAMPLING TECHNIQUES FOR THE ANALYSIS OF AROMA IN A MODEL GEL SYSTEM

MATTHEW D. TALBOT and Lewis L. Jones

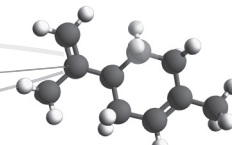
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Keywords:

Headspace sampling and analysis is used as a representation of the odour signal that is experienced before eating. Various methods for the analysis of headspace have been reported previously by authors such as Marsili (1). Headspace methodologies can be categorized into two variants, those that involve the direct injection of a volume of gas and those that incorporate various forms of absorption. Both have their experimental merits and downsides. Direct injection is an equivalent snapshot of what is actually being smelt, but suffers from interfering partition coefficients, whereas absorption gives the ability to concentrate the odours, but compounds are absorbed selectively and thus quantification of data requires external standardisation. A study has been undertaken to compare three widely used headspace sampling techniques using a model gel system with a defined odour mixture. The odour mixture contained compounds that spanned the range of hydrophobicity and volatility values and the sample size used for analysis was standardized across all three methods. A static equilibrium method was investigated as an example of direct injection headspace methodology, whereas dynamic headspace–thermal desorption (2) and solid phase micro extraction (SPME) (3, 4) were used as examples of absorption. Identification and quantification of the compounds of interest was performed using Gas Chromatography– Mass Spectrometry. The analytical data produced during this study, e.g. level of detection and variability will be reported.

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AUTOMATIC HS-SPME-GC-IT/MS FOR QUANTITATIVE ANALYSIS OF WHITE WINE VOLATILES: METHOD DEVELOPMENT AND OPTIMIZATION

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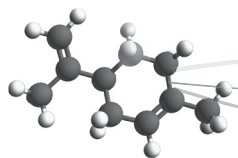
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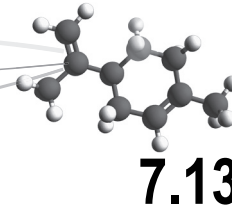
Keywords: HS-SPME-GC-IT/MS (Headspace Solid-phase MicroExtraction-Gas Chromatographic-Ion Trap/ Mass Spectrometry), wine, aroma, volatile compounds

Solid-Phase Microextraction (SPME) is a fast, simple, and solventless alternative sampling technique (1, 2) and has been applied to flavor and taint analyses of beverages (3,4). In this work an automated headspace solid-phase microextraction (HS-SPME) combined with gas chromatography/ion trap-mass spectrometry was developed in order to quantify a large number of volatile compounds in wines such as alcohols, ester, carbonyl compounds, norisoprenoids and terpenes. The first step of this study was to compare the efficacy of five commercial SPME fibers, namely PDMS, PDMS/DVB, PA, DVB/CAR/PDMS and CAR/PDMS, for the extraction of volatile compounds in Alvarinho white wine. The capillary column used was VF-5ms (30m×0.25mm×0.25mm) from VARIAN (5). The same extraction condition was used: extraction for all fibres temperature – 45°C; pré-incubation time – 5 minutes; extraction time – 20 minutes and 0,5g of NaCl was added). The second step was to optimize the HS-SPME conditions. A central composite experimental design, which was based on a 24 factorial plus eight axial points plus five replicates in the center point of the design was applied. The variables chosen were the extraction temperature (40°C from to 50°C), pre-incubation time (from 5 to 15 minutes), extraction time (from 10 to 30 minutes) and the presence of salt (NaCl from 0.5g to 1.5g). According to ANOVA, the results of the first step of this study showed that the most appropriate fiber to extract and detect the largest number of volatile compounds – such as alcohols, esters, carbonyl compounds and norisoprenoids and terpenes – was the DVB/CAR/PDMS fiber. This fiber also showed the highest reproducibility and good chromatographic resolution. According to the optimization, the significance variables ($p < 0.05$) were : the salt and the extraction time. The best response of optimization was obtained after 30 minutes of the extraction time, with 2.0g of salt. The extraction temperature and the pre-incubation time were not significant variables ($p > 0.05$) – the center point values were used to the extraction of volatile compounds in white wines from different varieties and origins.

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AN APPROACH FOR IDENTIFICATION OF POTENT ODORANTS IN WINE AND COFFEE BREW USING GAS CHROMATOGRAPHY-OLFACTOMETRY AND COMPREHENSIVE GAS CHROMATOGRAPHY

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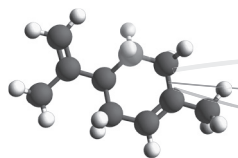
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Keywords: comprehensive two-dimensional gas chromatography, gas chromatography-olfactometry, wine, coffee, volatile analysis, sulfur speciation

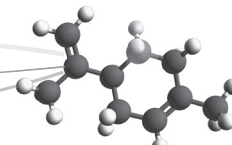
In the volatile analysis of food products that exhibit complex matrix such as wine and coffee (1, 2), verification of the responsible odour compounds is difficult to achieve due to coincidence of co-eluting peaks within the odour region in gas chromatography-olfactometry (GC-O). Sometimes no peak signal is observed in the correspond mass spectrometric detection (3). Multiple hyphenated chromatographic systems have to be used for the identifying responsible odorants but an efficient data matching approach among various GC instruments continues to be an emerging challenge (4). In this work, volatile constituents in wine and coffee beverages were analyzed using a combined system incorporating GC-O and comprehensive two-dimensional gas chromatography-flame ionization detection (GC×GCFID) techniques. A column set consisting of a first dimension (¹D) DB-FFAP phase, and a short second dimension column (²D) DB-5 phase was applied to achieve desired GC×GC separation of the volatile extract isolated using solid phase extraction (SPE). Whilst single dimensional GC resulted in many overlapping peaks, GC×GC allowed resolution of coeluting compound clusters which coincided with the odourous zone located in the GC-O aromagram. Character-impact odorants were adequately identified through data correlation of GC×GC contour plot maps across the results obtained using different systems, coupled with either time-of-flight mass spectrometry (TOFMS), or with flame photometric detection (FPD) for sulfur speciation. Odorants of 2-methyl-2-butenal, 2-(methoxymethyl)-furan, dimethyl trisulfide, 2-ethyl-5-methyl-pyrazine, 2-octenal, 2-furancarboxaldehyde, 3-mercapto-3-methyl-1-butanol, 2-methoxy-3-(2-methylpropyl)-pyrazine, 2-furanmethanol and isovaleric acid were suspected to be particularly responsible for coffee aroma using this approach. Aroma composition in wine varieties can be compared while attribution of responsible odorants for wine varieties can be proposed. The volatile sulfur compound 2-mercapto-ethyl acetate was discovered to contribute a fruity, brothy, meaty, sulfur odour to some Australian wines. By coupling of such approaches with existing flavor data libraries, rapid and informative analytical identification of character-impact odorants from complicated matrices can be accomplished.

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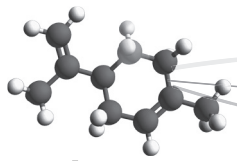
**STUDY OF THE VOLATILE COMPOUNDS USEFUL FOR THE
CHARACTERISATION OF FROZEN ANCHOITA (*Engraulis anchoita*) BY SPME-
GC-MS**

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Fish and fish products fulfil an important role in human nutrition as a source of biologically valuable protein, fat and fat-soluble vitamins. Freezing is an efficient way of storing fish but, nowadays, consumer interest in fresh products is increasing. In such products, aroma is a very important attribute, which can easily be altered by storing time. Thus, the aim of the present work was to study the formation of volatile compounds derived from frozen anchoita. In order to evaluate these modifications in anchoita (*Engraulis anchoita*), it was submitted to 120 days of frozen storage at -20 °C. Anchoita was captured during cruises performed by FURG ship, close to the limit of Brazil and Uruguay. After capture, the fishes were stored on board in thermal recipients containing the same proportion (1:1) of fish and ice/seawater mixture. In the laboratory, the anchoita was kept frozen at -20 °C until the analysis moment. The Peroxide value and the thiobarbituric acid-reactive substances (TBARS) index of fish muscle were determined. The volatile compounds were isolated by headspace-solid phase micro-extraction (SPME) with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber 50/30 µm. The SPME fiber was inserted into the headspace of the vial containing the cashew apple juice system for 45 min at 35 °C. After this period, the fiber was removed from the vial and immediately desorbed into the injector of the GC equipment. The volatile compounds were separated and tentatively identified by mass spectrometry (HSSPME-GC-MS). Variations in the chromatographic responses of a number of volatile compounds during storage were detected and the observed differences. Of these, several alcohols (1-butanol, 2-cis-penten-1-ol, 1-penten-3-ol, 1-hexanol, 2-ethyl-hexanol, 1-octen-3-ol) and aldehydes (butanal, hexanal, heptanal, octanal, nonanal) were identified as potential markers for anchoita until 60 days storage. Several other volatiles (acetic acid, ethyl benzene, styrene, propionic acid, vinyl ester) were identified as potential markers for anchoita spoilage.



7.15

**METHOD DEVELOPMENT AND OPTIMIZATION OF LIQUID-LIQUID
EXTRACTION FOR THE QUANTITATIVE ANALYSIS OF VOLATILE
COMPOUNDS FROM BRAZILIAN GRAPE JUICES**

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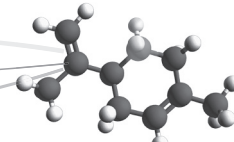
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Keywords: grape juice, volatile compounds, comparative analysis

The study of volatile compounds from grape juice is quite complex, because these compounds have different chemical properties, are in extremely small quantities, and are generally thermolabile (1). In recent years, one of the analytical techniques used to prepare the complex samples is the liquid-liquid extraction (LLE). This is based on the solubility of analytes in the sample in two solvents, ideally immiscible. (2) The aim of this study is to compare the volatile compounds of two brands of grape juice (in order to protect the companies, the grape juices were coded by CB and AL) by LLE, using different solvents, namely dichloromethane and hexane. The identification of volatile compounds by gas chromatograph coupled to a quadrupole mass spectrometry (GCMS), using a DB5 capillary column (polar) and another capillary column with DBWax (polar) (both with 30m x 0.25mm I.D.). In each LLE, it was used 50 mL of juice in a 250 ml conical flask, with 5 mL of solvent and stirring for 5 minutes without heating. After stirring, the solution was placed in a separation funnel and the organic phase was collected. This organic phase was dried with sodium sulfate and the volume was concentrated to 1 mL under nitrogen flow. In the grape juices tested, CB and AL, compounds such as alcohols, terpenes and esters were the most exploited during the LLE using dichloromethane as solvent. During the LLE with hexane, it was observed a higher number of aldehydes, although the other compounds were less observed. Chromatographic peaks showed better resolution in DBWax column than in DB5 column, because essential compounds of greater polarity, used to identify the juice aroma, as esters and ketones, were not identified in DB5 column. Hence, the best results were obtained when LLE using dichloromethane solvent extracted more volatile compounds from both grape juices. The most efficient identification of these compounds occurred using the DBWax capillary column. New alternatives are being tested in order to extract more components from grape juice, one of which is to replace the agitation in the ultrasonic bath for LLE. The conditions for solvent extraction are also being tested in an experimental design.

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COMPARISON OF FAST GAS CHROMATOGRAPHY- SURFACE ACOUSTIC WAVE SENSOR AND CAPILLARY GC-MS FOR DETERMINING STRAWBERRY, BLUEBERRY AND TOMATO VOLATILES ASSOCIATED WITH MATURITY AND QUALITY

X. Du ¹, A. Plotto ², E. Baldwin ², and R. ROUSEFF ¹

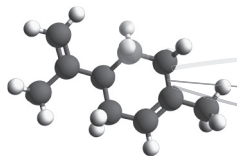
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Keywords: z-Nose TM, principle component analysis, rapid analysis

Development of rapid, simple and sensitive analytical techniques for volatile analysis has been an active area of research during the last decade. Much of this development has been spurred by the security/military industry for the detection of explosives. However these instruments have also been employed for the analysis of food and flavor volatiles. A typical GC-MS run will take 30-60 min whereas a typical fast gas chromatography- surface acoustic wave sensor (FGC/SAW) analysis will take 15 to 20 s. As expected there was less chromatographic resolution with the faster analyses. For strawberries at the same maturity stage, 19 peaks were observed with the FGC/SAW and 81 peaks for the GC-MS. In similar fashion: tomatoes, 18 versus 52 peaks and for blueberry 18 peaks versus 40 peaks were observed. It must therefore be inferred that each FGC/SAW probably contained more than a single compound. Although the qualitative and quantitative analyses of fruit volatiles using FGC/SAW was inferior to GC-MS, the usefulness of the data to distinguish strawberries, blueberries and tomatoes of different maturity stages was equivalent. Five strawberry maturity stages were evaluated at three harvest dates with two cultivars. Total volatiles and principle component analysis (PCA) results from the FGC/SAW data were very similar to those from GC-MS. Total volatiles increased exponentially from white (most immature) to over ripe (dark red) using both systems. PCA analysis showed that shipping ripe, full ripe and overripe fruit samples were tightly grouped and clearly separated with both systems. Four blue berry cultivars from five different harvest dates were separated into separate PCA quadrants using FGC/SAW data. The clustering and separation were similar using 40 variables (peaks) from the GC-MS data. In the case of tomato, two cultivars were sampled at three maturities, namely: green, turning and pink. The GC-MS PCA employed all 52 identified volatiles and completely separated the two cultivars, but the maturity levels were only roughly separated. The FGC/SAW data using only 19 peaks produced similar separation between the two cultivars but superior separation and clustering of the three maturity stages.



7.17

A ROBUST SPME METHOD FOR THE ANALYSIS OF WINE VOLATILES BASED ON MULTIPLE INTERNAL STANDARDS AND MULTIVARIATE REGRESSION

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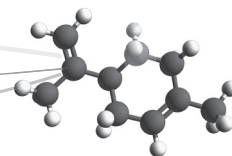
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Keywords: HS-SPME, wine, aroma compound, PLSR, matrix effects

Head space solid phase microextraction (HS-SPME) has been lately chosen for the analysis of volatile compounds in beverages. It is a solvent-free technique that allows an almost complete automatization, reducing the total time of analysis and getting amazing sensitivities. In addition, getting beautiful chromatograms (symmetric peaks) with HS-SPME is very easy, what explains its widespread use. In some sense it can be said that automated HS-SPME has made apparently easy otherwise quite complicated analytical procedures. The hidden risk of SPME lies in the fact that as the amount of analyte really extracted is very low; it is extremely sensitive to any experimental parameter that may affect the liquid-gas and gas-solid distribution coefficients. In the case of wine, ethanol levels (that affect both coefficients), the retentive power of the non-volatile wine matrix (that affects l-g coefficients) and the volatile composition (that affect g-s distribution), can be identified as major potential sources for matrix effects. Our aims are to measure the relative weight of these factors on the lack of accuracy, and to design a robust calibration system able to avoid or limit their effects.

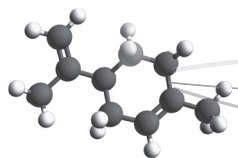
For the first goal, synthetic but real-like wines containing a fixed amount of selected analytes (70) and variable amounts of ethanol (3 levels), non-volatile constituents (3 levels) and major volatile constituents (3 levels) were prepared following a 3-Factor complete Factorial design. The non-volatile matrix was obtained from three different real wines after complete dealcoholization and de aromatization by vacuum distillation and solvent extraction. The analytes in those synthetic samples were analyzed by automated HS-SPME-GC-MS using a CAR-PDMS-DVB fiber, 0.2 mL of sample in 4.80 mL of brine in a 20 mL standard vial, with extraction carried out at 60°C for 30 min. The study of the relevance of the Factors was carried out by analysis of variance (ANOVA) and by Principal Component Analysis and revealed that the levels of major volatile constituents affected the extraction of most analytes, while ethanol and matrix affected particularly low volatile compounds. Lipophilic esters are most influenced by major volatile compounds, while acids, phenols and lactones are affected by the non-volatile matrix.

13 different internal standard compounds belonging to different chemical classes and representing different properties were used in the calibration experiment. This was similar to



the aforementioned experiment (including again different alcohol levels, non-volatile matrixes and major volatile levels), but including as well 5 different concentration levels following an incomplete Factorial Design. In 29 out of 65 cases, a single internal standard provided a robust calibration guaranteeing an accuracy better than 10%, while in others a Partial Least Square Regression analysis was run in order to find a model using the 13 Internal Standards able to provide maxima accuracy. Satisfactory models fulfilling all of them reasonable quality criteria in terms of precision, linearity and recovery could be built for 30 other compounds, so that the method can quantify up to 59 relevant wine volatile compounds.

Acknowledgements: This work has been funded by the Spanish MICYT (Ministerio de Industria, Comercio y Turismo) project AGL2010-22355-C02-01



7.18

IDENTIFICATION OF KEY AROMA COMPOUNDS IN GIN: A MULTIDIMENSIONAL APPROACH BASED ON AN ORIGINAL SELECTION PROCEDURE

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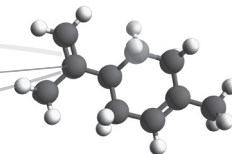
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Keywords: GC-Olfactometry, Heart-cut GC-GC-O/MS, key aroma compounds, detection frequency, gin, chemometrics

Food companies are constantly looking for efficient strategies to select their raw material based on a limited number of parameters. The identification of key aroma compounds is therefore an important step in food product analysis. This task is challenging because it requires the drastic selection of a few key compounds from a very large number of compounds separately evaluated by Gas Chromatography-Olfactometry (GC-O) (1). To achieve this goal, we established a procedure for the identification of key aroma compounds. First we pre-selected the potential impact compounds through an original GC-O and heart-cut GC-GC-O procedure based on detection frequency. Then we refined the selection of compounds based on different physical, chemical and sensory characteristics. For this purpose, we decided to work on a product that was not widely studied: the gin.

We performed on two different gins a liquid-liquid extraction, which was previously described as representative and efficient on alcoholic products (2). The main drawback of the detection frequency procedure is the lack of discrimination between major and minor compounds (3), which is why we decided to use two concentrations of the extracts. These 4 concentrated extracts were sniffed by 10 untrained assessors with 2 replicates. The generated odour events were regrouped in 209 Odorant Areas (O.A.) by a software developed for this analysis. Based on the literature (4) we decided to discard the O.A. perceived by less than 25% of the assessors which left us with a final number of 60 O.A.. The O.A were described by the assessors, and their descriptors were regrouped using words classification (5). In parallel, the product chemical profiles were obtained using GC-MS. The coelutions have been resolved using heart-cut GC-GC-O/MS. Secondly, we gathered different physical and chemical data on the pre-selected compounds corresponding to the 60 O.A., i.e. their concentrations in the products, their partition coefficient or their ebullition temperature and sensory information, i.e. their GC-O indices for the two gins at each concentration, the descriptors obtained in GC-O or their perception thresholds. We used a multivariate chemometric analysis on this heterogeneous dataset to obtain a final selection of 20 keyaroma compounds.

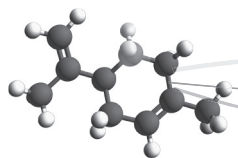
This method allowed us to isolate potential key aroma compounds by GC-O and GC-GC-O detection frequency. Our original procedure gave us information on minor and major compounds which were implemented in a database regrouping information on the pre-selected



compounds. The chemometric approach allowed us to take various physical, chemical and sensory parameters into account to perform our final selection. In a future work, we will validate our selection by recombination experiments in a neutral alcoholic media and compare the recombinates with the original products.

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7.19

PROTON TRANSFER REACTION TIME OF FLIGHT MASS SPECTROMETRY TO DETERMINE CHANGES IN FLAVOR COMPOUNDS DURING LAGREIN RED WINE MATURATION IN WOODEN AND STAINLESS STEEL VESSELS

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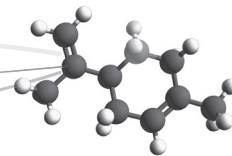
Proton Transfer Reaction-Mass Spectrometry allows the rapid, non-invasive on-line monitoring of volatile organic compounds released from complex matrices such as foodstuffs or from dynamic processes such as manufacturing processes and food consumption. The recently developed PTRToF-MS is characterized by high sensitivity, dynamic on-line capability, rapid response time, and remarkably improved mass resolution that provides in many cases the unambiguous discrimination of chemical formula and detection of the VOCs. PTR-MS has been successfully implemented for the discrimination of red and white Italian wines and the temporal flavour release during wine swallowing (1, 2).

Lagrein wine is a typical PDO Italian red wine produced in the Trentino-Alto Adige area hosted in the northern part of Italy. Along with Marzemino, it is a descendant of Teroldego, and related to Syrah, Pinot Noir and Dureza wines (3).

The flavour of wine can be attributed to the grape variety, oenological, vinification and maturation processes and in addition the wine barrel blending ratios. The vinification process gives rise to principal flavour components under the influence of grape variety and oenological processes resulting in formation of esters, carboxylic acids, alcohols, ketones, aldehydes, terpenes, lactones and phenolics (4). The use of wooden barriques or oak chips is among the well-established methods for wine maturation. The maturation process contributes to the flavour of wine by elimination of undesirable compounds, extraction of desirable compounds from the barrel, maintenance of desirable varietal compounds and increasing complexity of compounds present (5). These reactions when successfully conducted enable a characteristic flavour bouquet and superior quality traits.

In the present study we apply, for the first time, PTR-ToF-MS to wine samples to investigate the effects of oak (three French brands, one American brand), acacia and stainless steel vessels on the volatile compound profile of Lagrein wine during maturation. Wine from the same vinification process was transferred to the six vessels and wine sub-samples removed after 6 and 11 months, diluted (wine:water 1:40 w/w) and headspace gas was measured using a PTR-ToF-MS instrument (Ionicon Analytik GmbH, Innsbruck, Austria).

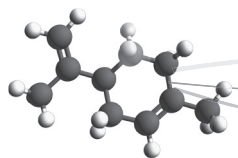
Two-way ANOVA demonstrated that major flavour compounds and their fragments significantly ($p < 0.001$) differentiated samples on the basis of maturation time. In particular, methanol, acetaldehyde, ethyl acetate, hexanoic acid/hexanoates and fragment at m/z 41.038 (related to alcohols) exhibited a significant increase during ageing. In contrast, peaks related to 2-ketones, alpha-diketones, hexen-1-ol, terpineol, and most of the higher esters/carboxylic



acids decreased. From the mass peaks related to phenolic compounds such as furfural, hydroxymethylfurfural (HMF), guaiacol, ethylphenol, benzaldehyde, and whiskey-lactone showed a significant increase ($p < 0.05$). Principal components analysis of the entire spectral data set acquired for wine samples analysed at 6 and 11 months maturation, discriminated wine matured in stainless steel tank from those aged in oak and acacia. These results show that PTR-ToFMS can be used to understand process- and storage-induced volatile compound changes and due to the high throughput capability can play a valuable role in elucidating maturation mechanisms in wine.

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7.20

**AN INSTRUMENTAL APPROACH OF THE ROBINSON TEST (EN 1230:2-2009) BY
MEANS OF MASS SPECTROMETRY-BASED ELECTRONIC NOSE
TECHNOLOGY**

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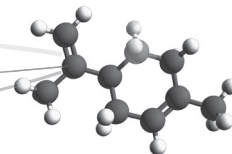
Keywords: SPME, MS-nose, Robinson, packaging, chocolate

The possibility of chemical transfer from cellulose-based packaging materials to food is a major concern to the packaging industry and food producers. Migration of volatiles from the packaging material to food products may lead to consumer dissatisfaction, claims and expensive recalls. In order to avoid off-flavour problems, a normalised sensory test, EN 1230:2-2009 (1), also known as Robinson test (2), is usually applied as a sensory procedure for testing the influence of packaging materials on the organoleptic quality of food products. As sensory analysis always contains an inevitable level of subjectivity, there is an important need for a fast and objective complementary instrumental analysis method. In this work a chemical-analytical approach for the Robinson test was carried out by means of a mass spectrometry based electronic nose system, indicated as MS-nose technology.

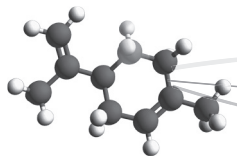
Four commercial unprinted paper and paperboard samples (virgin fibre and recycled board) were subjected to the standardised sampling procedure of the Robinson test (EN 1230:2-2009), with milk chocolate as a simulant. The four sampled chocolates were organoleptically compared to untreated chocolate by means of the standardised sensory procedure. Supplementary, all chocolates were analysed by means of MS-nose-technology. The MS-nose configuration consisted of an autosampler for on-line and automated isolation of the chocolate volatiles and migrated packaging volatiles using headspace-solid phase microextraction (HS-SPME). The HS-SPME sampling method was optimised for isolation of packaging volatiles migrated to cocoa products (3). Mass fingerprinting was performed on the volatile composition without prior chromatographic separation and the mass fingerprints were imported in the chemometric modeling software. Principal components analysis (PCA) was used as an exploratory algorithm to visualise the differences in volatile composition of the sampled chocolates in comparison to the reference chocolate. Furthermore, HS-SPME isolation combined with conventional gas chromatography-mass spectrometry (GC-MS) was applied on all samples to validate the HS-SPME-MS-nose classification.

This work showed that the results of fast HS-SPME-MS-nose technology were in good relation with the time-consuming Robinson test and can be used for prediction of the influence of cellulose-based packaging materials on the sensory quality of food products. In order to avoid undesirable off-flavours caused by cellulose-based packaging materials, the technique can be very useful for paperboard manufacturers, printing companies as well as food producers.

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7.21

EVALUATION OF TWO ISOLATION TECHNIQUES RELATED WITH SENSORY ANALYSIS FOR THE DETERMINATION OF THE VOLATILE COMPONENTS IN SOURDOUGH BREADS MADE FROM DIFFERENT FLOURS

ANN DE WINNE and Patrick Dirinck

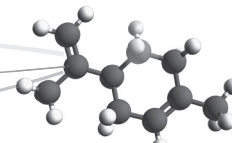
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Keywords: sourdough bread, SDE, HS-SPME, GC-MS, sensory analysis, PCA-analysis

Because of their excellent nutritional properties, consumers have a growing interest for sourdough breads. Sourdough fermentation is based upon an earlier spontaneous process of the flour with lactic acid bacteria. The type of flour has a significant impact on the volatiles formed in the bread and its flavour characteristics. In this study, the aroma components of four sourdough breads made from different flours (white, rye, wheat and spelt) were investigated by two different isolation techniques and sensory analysis.

The volatile compositions of the four sourdough breads were studied by means of steam distillation-extraction (SDE) and headspace-solid phase microextraction (HS-SPME), followed by gas chromatography-mass spectrometry (GC-MS). SDE was performed twice on each type of bread; for the HS-SPME isolation, five replicates for each type of bread were carried out. The identified aroma compounds included several alcohols, saturated and unsaturated aldehydes, acids, furans, esters, lactones, pyrazines, pyrroles, phenols and ketones. SDE-GC-MS profiles provided more chemical information of the volatile composition compared to HS-SPME-GC-MS profiles. Semi-quantitative results of both techniques were statistically interpreted by multivariate analysis. Principal component analysis (PCA) showed clear differences between white and rye sourdough bread for both aroma isolation techniques. SDE-GC-MS-PCA was more discriminative between rye bread and wheat/spelt bread compared to HS-SPME-GC-MS-PCA.

These analytical results were related to descriptive sensory analysis of the same breads. A ranking test for seven descriptors by a laboratory panel ($n = 12$) was used to evaluate the odour and taste differences. PCA on the instrumental and sensory results clearly demonstrated analytical differences between the four breads in good relation with sensory analysis. Besides odour intensity differences, also taste differences (flour taste and acid taste) were observed between the four breads.



**CORRELATION BETWEEN ODOR AND TASTE SENSORY RATINGS AND
ELECTRONIC NOSE ANALYSIS: MAPPING THE ORGANOLEPTIC PROFILE
OF DIFFERENT POLYETHYLENE RESINS USED IN DRINKINGWATER
TRANSPORTATION AND PACKAGING**

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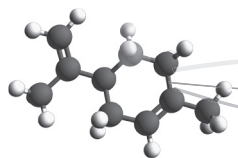
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Keywords: polyethylene; e-Nose; human sensory panel; taste; odor; chemometrics

Polyethylene (PE) materials used in food packaging have been intensively investigated for their sensory impact on packaged food (1, 2). Odors are usually a mixture of hundreds or even thousands of chemical compounds. Consequently, it is difficult to describe a set of reference odors that describe the olfactory input. A similar situation happens in the case of taste. In recent years, new technology has been developed for organoleptic evaluation. This is the case of electronic nose instruments (e-Nose). There are numerous investigations where e-Nose has been applied to the food area like beer, wines and alcoholic beverages (3, 4), fruit juice, olive oil, vinegar, milk and fatty liquids (5, 6). However, little has been published on PE material used for drinking water transportation or packaging, two market segments where Dow PE resins are used and low taste and odor (T&O) are key performance properties. To ensure good organoleptics to our customers, Dow performs qualitative and quantitative organoleptic evaluations by an experienced human sensory panel (HSP). However, this testing is time consuming and manpower intensive making it critical that we try to avoid T&O panelists' fatigue.

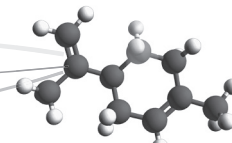
In this study, we have performed an organoleptic evaluation by HSP and e-Nose of three PE resins (low density PE (LDPE), high density PE (HDPE) and linear low density PE (LLDPE)) which are used in packaging and potable water transportation pipe systems. e-Nose technology was evaluated in order to speed up T&O evaluation since this instrument offers several advantages compared to the HSP: it is an automated system and also has an autosampler which allows higher sample throughput. The e-Nose instrument used has 18 metal oxide sensors where the electrical resistance of the sensors changes as a function of time when they are exposed to vapor. In the present study, e-Nose signal has been correlated with the input from the HSP for both qualitative and quantitative purposes. In the case of HSP, the input provided on T&O does not clearly differentiate resin type since each resin type has overlapping odor and taste rating intensity ranges. However, in the case of e-Nose, when Principal Component Analysis (PCA) is applied, the instrument is able to discriminate between resin families, enabling the mapping of the organoleptic space of each resin type. For quantitative analysis, e-Nose signals have been correlated with HSP by applying multivariate calibration (Partial Least Squares, PLS). The models obtained show good correlation between



both methodologies for T&O, indicating that e-Nose provides comparable results to HSP. The implementation of these models allow quick detection if samples pass the internal requirement on T&O. This opens an opportunity to consider e-Nose analysis as a routine quality tool in PE resin manufacturing.

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SENSORY BENCHMARKING OF FOOD PRODUCTS USING FAST MASS FINGERPRINTING

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Keywords: volatiles, aroma compounds, sensory, benchmarking

The feasibility of mass spectrometry-based electronic nose technology (MS-nose) for fast sensory benchmarking of commercial food products (e.g. cola-like soft drinks, coffee blends, energy drinks, pilsner beers) was evaluated. The performance of the MS-nose technology was measured in terms of the capability to discriminate between different commercial samples in a benchmarking study and to obtain good correlations with the sensory properties of commercial products.

The hyphenated configuration of a sample preparation autosampler for aroma isolation using headspace-solid phase microextraction (HS-SPME) with quadrupole mass spectrometry as a sensing system in combination with online pattern recognition software was used (1). The isolated aroma compounds were directly sent to the MS system without chromatographic separation. Total mass spectra of the isolated volatiles were obtained and converted to so-called mass fingerprints by the instrument software. The obtained mass fingerprints of different commercial products were submitted to chemometrical algorithms, such as principal component analysis (PCA) and soft independent modelling of class analogy (SIMCA).

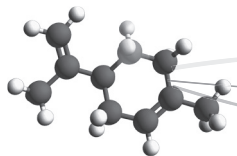
The HS-SPME-MS-nose technology resulted in fast classification of commercial food products:

- 8 cola-type soft drinks
- 16 coffee blends
- 5 energy drinks
- 8 pilsner beers

The feasibility of mass fingerprinting for fast sensory benchmarking of foods and beverages was clearly demonstrated. In this study good correlations with the sensory reputation of the commercial products were obtained. Further validation showing good relationships with time consuming gas chromatography-mass spectrometry (GC-MS) profiling and sensory analyses by trained expert panels is necessary. MS-nose technology delivers only limited chemical data. For detailed insight into the relation between flavour characters and specific volatiles GC-MS is still an irreplaceable technique.

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7.24

SCREENING OF WINE OFF-FLAVOURS BY MEANS OF GAS CHROMATOGRAPHY OLFACTOMETRY

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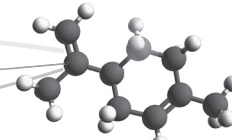
Keywords: olfactometry; screening; aroma analysis

Human nose has been recurrently used as a detector in the instrumental technique known as Gas Chromatography-Olfactometry (GC-O). In most of the cases, it has been applied as a qualitative technique, however several attempts have been made to use GC-O as a quantitative technique. In 1999, Pollien et al published the first attempt to quantify an odorant by means of GC-O¹. In that work, they carried out an external calibration of 1-octen-3-one with GC-O to determine this compound in coffee. The registered answers of judges were binary, being the odorant concentration correlated with the nasal impact frequency. The method produced results of quality comparable with those obtained by GC-MS for the same odorant. The other attempt to use GC-O as a quantitative technique was published by Ferreira et al in 2003². In that work, the judges used a simple intensity scale to evaluate the odorants. The calibration was carried out with 15 odorants with dramatic variations in the slopes of the calibration curves depending on the compound studied. Both experiments proved that GC-O could be used as a quantitative technique, although some limitations are implicit in the methods. The work presented in this communication searches to simplify these limitations by using GC-O as a screening technique.

In the present work, four known off-flavours of wine 2-isobutyl-3-methoxypyrazine (IBMP), 2,4,6-trichloroanisole (TCA), 4-ethylguaiacol (EG) and 4-ethylphenol (EF) were studied by quantitative GC-O. A panel of 12 judges was trained to recognize the off-flavours. Sniffings were performed only in selected periods of the chromatographic run for a total time of 4 min. Sample preparation for GC-O analysis was carried out with solid phase extraction following a previous method³. Repeatability of the method was observed in 3 complete analysis of the same sample. Calibration of the response was carried out through complete GC-O analysis at 6 different concentration levels and registering the number of positive responses.

Phenol off-flavours presented several problems of coelution, which affected their correct identification by the panel, however the results showed that in all cases the method was able to detect concentrations above the levels causing depreciation of wine aroma. The method was able to give a reliable positive response when the concentration was above 1 ng/l for IBMP, 0.25 ng/l for TCA, 2 µg/l for EG and 8 µg/l for EF with a relatively short analysis time for the concentration levels studied.

- (1) Pollien, P.; Fay, L. B.; Baumgartner, M.; Chaintreau, A. *Anal. Chem.* **1999**, *71*, 5391–5397.
- (2) Ferreira, V.; Petka, J.; Aznar, M.; Cacho, J. *J. Chromatogr. A.* **2003**, *1002*, 169–178.
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AUTOMATIC AND TOTAL HEADSPACE IN-TUBE EXTRACTION FOR THE ACCURATE DETERMINATION OF POLAR VOLATILE COMPOUNDS FROM WINES

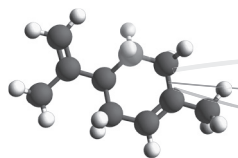
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Keywords: Wine, polar volatile compounds, ITEX, GC-MS.

Some volatile compounds, such as diacetyl, acetaldehyde, ethyl acetate or butyric acid have remarkable sensory and biochemical properties and are very good markers of wine microbiological state. The extraction (liquid-liquid or liquid-solid) of these compounds from wine is extremely difficult due to its high solubility. On the other hand, although these compounds can be easily detected by headspace solid phase microextraction (SPME) analysis, the low recoveries caused by the high level of ethanol and other major volatiles compounds make that those strategies are extremely matrix-dependent and highly inaccurate (1). We have developed an automatic and fast alternative based on In-tube extraction (ITEX) for the complete extraction of these compounds present in the headspaces of small volumes of wine. In-Tube Extraction (ITEX) is a completely automatic solventless extraction technique for headspace sampling in which a headspace syringe with a needle body filled with a sorbent is used. The analytes are extracted from the sample headspace by repeated pumping this through the sorbent. The needle body is surrounded by a heater which is used for the thermal desorption of analytes into the injection port of a GC system. In tube extraction offers the advantage that a variety of commercially available sorbent materials and higher amounts of these materials can be used to obtain higher extraction yields than in other related microextraction methods such as solid-phase microextraction (SPME) or liquid-phase microextraction (LPME) (2).

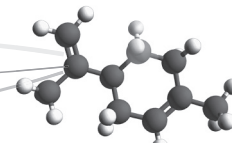
In this work, a method for the analysis of acetaldehyde, ethyl acetate, diacetyl, ethyl butyrate, methyl butyrate, isoamyl alcohol and isobutanol from only 2 μ l of wine was developed and validated in different matrixes. Two sorbent materials were evaluated: TENAX and BOND ELUT-ENV. All extraction and desorption parameters were optimized. Furthermore, different matrixes (reconstructed and synthetic wine, red wine, white wine and beer), alcohol degree (between 10-15%) and concentrations of major compounds were evaluated in order to test the effect of these parameters in the method. Finally, the method was validated in wine. A 2 ml vial containing the sample was incubated at 60 $^{\circ}$ C, and 100 extraction cycles (strokes with the HS-syringe containing Bond Elut ENV sorbent) were chosen, after that, desorption was carried out at 240 $^{\circ}$ C. Linearity of the method was satisfactory with coefficients $R^2 > 0.999$ in all cases. Reproducibility was between 2-10 %. Detection limits were below of the detection thresholds for these target compounds in wine. Recovery of the method (evaluated in samples spiked with the analytes) was 95-106% and independent of the alcohol degree, kind of wine and concentration of major compounds. The extraction efficiency calculated by multiple extractions from the same sample ranged from 85-95%.



In conclusion, the proposed strategy based on ITEX makes it possible to get nearly quantitative recoveries of the most volatile compounds present in to the headspace of complex solution, which constitutes ITEX a clear advantage over microextraction techniques.

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**THE NEXT LEVEL IN FOOD AND FLAVOUR ANALYSIS - RECENT
IMPROVEMENTS IN PROTON TRANSFER REACTION MASS SPECTROMETRY
(PTR-MS)**

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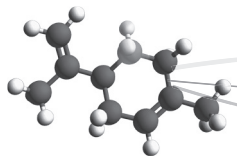
Keywords: PTR-MS, real-time analysis, TOF, PTR-TOFMS, low detection limit

The innovative technology called "Proton Transfer Reaction – Mass Spectrometry" (PTR-MS) was invented and developed by scientists of the "Institut für Ionenphysik" at the Leopold-Franzens University in Innsbruck and commercialized by the spin-off company IONICON Analytik GmbH. (for details see (1) and very recently (2)). Since the early years PTR-MS was used in food and flavor science: in online measurements of fruit flavor in nose space air, in determining the freshness of food or in sensory profiling of espresso coffee (3), just to mention a few examples.

Here we report on the latest instrumental developments that are especially beneficial for food studies, namely (i) the improvement of the detection limit that now allows for measuring trace gas compounds in a concentration range from several ppmv down to the ppqv (parts-per-quadrillion) region with a typical response time well below 100 ms, (ii) the coupling of our sophisticated PTR source to two different types of time-of-flight (TOF) mass analyzers (one with an outstanding mass resolution called PTR-TOF 8000 (4) and one with an increased sensitivity called PTR-TOF 2000 (5)) and (iii) the possibility to switch between H_3O^+ , NO^+ and O_2^+ as reagent ions (6).

Whereas development (i) allows us to enter new fields of application where extremely high sensitivities are needed, the use of TOF detectors in (ii) is especially important for applications where not only high sensitivity but also unambiguous identification is needed (e.g. identification of flavor compounds). The high mass resolution of up to 8.000 m/ Δ m and high mass accuracy of the PTR-TOF 8000 allow for separation of most isobaric compounds and for substance identification via the exact mass. As there might be applications where an enormous mass resolution is not necessarily needed, but the sensitivity has to be as high as possible, the PTR-TOF 2000 performs with an enhanced sensitivity at the expense of a somewhat lower mass resolution. We present comparison data which demonstrate that the resolution of this PTR-TOF 2000 is still around 2.000 m/ Δ m while showing an increased sensitivity by a factor of five compared to the PTR-TOF 8000.

For (iii) we show, that the sensitivities obtained with NO^+ and O_2^+ are comparable or even better than the outstanding sensitivity of the established PTR-MS instruments and therefore well above those from e.g. SIFT-MS instruments. To demonstrate the advantages of this so called "SRI" (switchable reagent ions) setup we e.g. measured acetone and propanal (isomeric molecules at nominal mass 58 amu) utilizing NO^+ as the precursor ion. According to Spanel

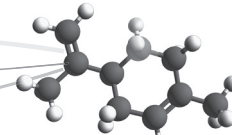


et al. (7) NO^+ interactions with aldehydes follow the reaction: $\text{NO}^+ + \text{XH} \rightarrow \text{X}^+ + \text{NOH}$ whereas ketones follow: $\text{NO}^+ + \text{XH} \rightarrow \text{XH}^+ + \text{NO}$ (and clustering). This means that we see isomeric compounds on different nominal masses and can identify them unambiguously. Furthermore, by using O_2^+ precursor ions we are able to ionize molecules via charge transfer reactions that cannot be measured via hydronium proton transfer reaction (e.g. ethylene and acetylene which are of great importance in food research).

Acknowledgements: Work was supported by the EC, Brussels, the Land Tirol, Innsbruck and the FFG, Vienna. SJ acknowledges the support of the Community under a Marie Curie Industry-Academia Partnership and Pathways grant (Grant Agreement Number 218065).

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DETECTING DANGEROUS SUBSTANCES IN FOOD - DIRECT LIQUID AND HEADSPACE ANALYSIS WITH PTR-MS

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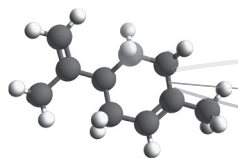
Keywords: PTR-MS, real-time analysis, direct injection, illicit drugs, knockout drugs

Researchers working in food and flavor science already recognized the high potential of the PTR-MS technology shortly after it had been invented at the University of Innsbruck in the 1990s and commercialized by the spin-off company Ionicon in 1998. Nowadays about 30% of all customers work in this field; see (1) for a comprehensive review and (2) and (3) for two very recent examples of food and aroma studies utilizing PTR-MS.

In a PTR-MS instrument water vapor originating from a reservoir filled with distilled water enters a hollow cathode ion source, where the H₂O is transformed into H₃O⁺ (primary ions). Alternatively, since the recent introduction of the so-called SRI (switchable reagent ions) functionality also O₂⁺ (generated from an external oxygen supply) and NO⁺ (generated from normal air) can be used as primary ions. Due to the sophisticated design of the ion source, the ionization process is highly efficient, i.e. the purity of the primary ions is greater than 99%, which means that no signal-diminishing mass filter (as is used by techniques similar to PTR-MS like e.g. SIFT) is needed between the ion source and the adjacent drift tube. The actual ionization of the trace compounds present in the sample air takes place in the drift tube either via proton transfer from H₃O⁺ to all molecules that possess a higher proton affinity than water or via charge transfer reactions in case O₂⁺ or NO⁺ is utilized. Depending on the kind of instrument the protonated molecules are subsequently introduced into either a quadrupole mass filter or a time-of-flight (TOF) mass analyzer, where especially the latter one (PTR-TOFMS) proved to be a great step towards unambiguous substance identification (4).

Here we present very recent studies on potentially dangerous substances that are predominantly (and often unknowingly) consumed highly diluted in liquids, e.g. γ -butyrolactone and 1,4-butanediol traces mixed in different concentrations into plain water, tea, and red and white wine. Both substances are metabolized in the human body to γ -hydroxybutyric acid ("liquid ecstasy") and are therefore frequently abused as recreational drugs (in lower doses) or so-called "date rape or knockout drugs" (in higher doses). Utilizing complementary simple headspace analysis above the liquids' surface and our recently introduced Direct Aqueous Injection (DAI) system coupled to a PTR-MS (5), which already proved to work well on the detection of explosives in liquid and gas phase (6), we were able to detect both substances in all above-mentioned liquids with great linearity down to concentration levels far below the activation threshold for effects in human beings.

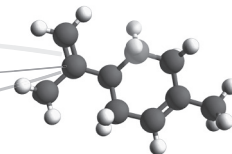
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Work was supported by the EC, Brussels and the FFG, Vienna. SJ acknowledges the support of the Community under a Marie Curie Industry-Academia Partnership and Pathways grant (Grant Agreement Number 218065).

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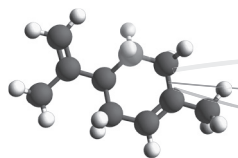
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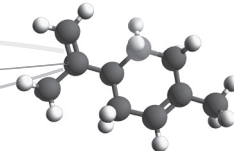
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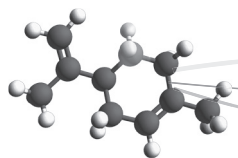


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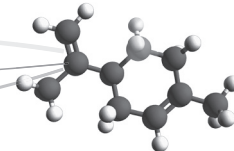


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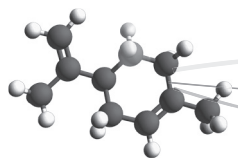


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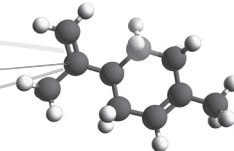


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