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# Occurrence and Synthesis Pathways of (Suspected) Genotoxic α,β-Unsaturated Carbonyls in Chocolate and Other Commercial Sweet Snacks

Alexandre Dusart,* Julie Grosjean, Manon Autuori, Séverine Goscinny, and Sonia Collin

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**ABSTRACT:** α,β-Unsaturated carbonyls are highly reactive and described as structural alerts for genotoxicity. Ten of them (either commercially available or synthesized here by combinatorial chemistry) were first investigated throughout the chocolate-making process by solvent-assisted flavor evaporation (SAFE) coupled to GC-MS/SIM. Monitored α,β-unsaturated aldehydes were formed during chocolate production, primarily through aldol condensation of Strecker aldehydes triggered by bean roasting. Notably, levels of 2-phenylbut-2-enal (up to 399 $\mu g \cdot kg^{-1}$) and 5-methyl-2-phenylhex-2-enal (up to 216 $\mu g \cdot kg^{-1}$) increased up to 40-fold. Dry conching caused evaporation of α,β-unsaturated carbonyls, while wet conching partially restored or increased their levels due to cocoa butter addition. Further analyses showed that α,β-unsaturated aldehydes also occurred in most commercial sweet snacks (up to 16 $\mu g \cdot kg^{-1}$), although often at lower concentrations than in roasted cocoa or derived chocolates. In the end, none of the monitored α,β-unsaturated aldehydes did raise a health concern compared to current maximum use levels (2−5 $mg \cdot kg^{-1}$). On the other hand, much higher levels of genotoxic furan-2(5*H*)-one were found in crepe and cake samples (up to 4.3 $mg \cdot kg^{-1}$).

**KEYWORDS:** *cocoa, chocolate, aromas, α,β-unsaturated carbonyls, genotoxicity, furan-2(5H)-one*



## 1. INTRODUCTION

Hundreds of volatiles have been identified in cocoa and chocolate.[1] These molecules can commonly be divided into three groups according to their origin: varietal aromas, fermentation aromas, and heat-generated aromas. Varietal aromas are compounds already present in the cocoa beans before harvesting. They include terpenoids, along with some esters and aldehydes. Fermentation aromas, as indicated by their name, are formed during fermentation of the beans. They mainly include alcohols, esters, and acids.[2] Finally, heat-generated aromas appear especially during roasting, but may already be partially synthesized during fermentation and drying of the beans.[3] Roasting plays a significant role in flavor development and synthesis of typical chocolate aromas such as pyrazines and Strecker aldehydes through Maillard reactions.

Among heat-generated aromas, α,β-unsaturated aldehydes have been identified as active aromatic compounds in chocolate, contributing to the typical roasted/cocoa aroma.[4] They appear as aldol condensation products between two aldehydes. One acts as an electrophile that undergoes a nucleophilic addition of the second enolized aldehyde (aldol addition). Then, if the resulting β-hydroxy aldehyde has an α-hydrogen, it can undergo dehydration (crotonization), resulting in the α,β-unsaturation of the remaining aldehyde. In heated food, typical aldehydes involved in such reactions are Strecker aldehydes (e.g., 3-methylbutanal and phenylacetaldehyde) issued from amino acids and dicarbonyl compounds. Roasting is the main step in the development of α,β-unsaturated aldehydes as optimal conditions (i.e., high temperatures) are reached for aldol condensation. Together with pyrazines, 5-methyl-2-phenylhex-2-enal (7 in Figure 1) has been shown as a good indicator for evaluating the roasting process (from 251 to 1490 $\mu g \cdot kg^{-1}$ in cocoa beans depending on the roasting temperature).[4,5] Synthesis of these aldehydes and other heat-generated compounds can continue during the conching step of chocolate-making, the concentration of 5-methyl-2-phenylhex-2-enal (7) being multiplied by two during conching.[4] New synthesis would appear mainly to occur during

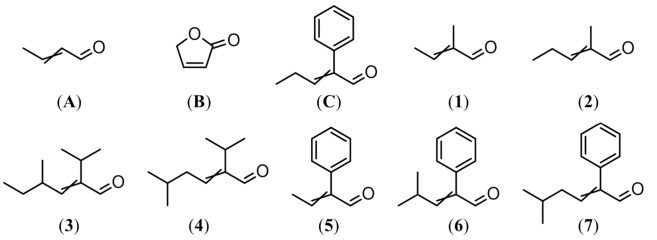


**Figure 1.** Chemical structures of different α,β-unsaturated carbonyls: but-2-enal (**A**), furan-2(5*H*)-one (**B**), 2-phenylpent-2-enal (**C**), 2-methylbut-2-enal (**1**), 2-methylpent-2-enal (**2**), 2-isopropyl-4-methylhex-2-enal (**3**), 2-isopropyl-5-methylhex-2-enal (**4**), 2-phenylbut-2-enal (**5**), 4-methyl-2-phenylpent-2-enal (**6**), and 5-methyl-2-phenylhex-2-enal (**7**). α,β-Unsaturation is shown with crossed double bonds to reflect the possibility of *Z*/*E* isomers.

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the wet conching phase, as the added cocoa butter liquifies the chocolate and allows better contact between molecules (i.e., Strecker aldehydes).

Although often organoleptically attractive, $\alpha,\beta$-unsaturated carbonyls are considered "structural alerts for genotoxicity", yet the effective (geno)toxicity is evaluated by the European Food Safety Authority (EFSA).[6] In the case of $\alpha,\beta$-unsaturated carbonyls, the carbon in the $\beta$-position, partially charged positively, is an ideal site for nucleophilic attack by electron-rich (macro)molecules such as proteins or DNA (Michael-type addition).[7,8] But-2-enal (**A** in Figure 1) and furan-2(5*H*)-one (or 2-furanone, **B** in Figure 1) are examples of confirmed genotoxic compounds.[9,10] Furan-2(5*H*)-one was only recently forbidden as a flavoring substance.[11] The absence of genotoxic compounds in food is of particular importance, as their effects can occur at very low doses (conventional threshold of toxicological concern (TTC): 0.15 $\mu$g/person per day) as compared to nongenotoxic substances (TTC: 18−1800 $\mu$g/person per day).[12] 2-Phenylbut-2-enal (**5**), 4-methyl-2-phenylpent-2-enal (**6**), and 5-methyl-2-phenylhex-2-enal (**7**) are still under safety evaluation by EFSA due to aneugenicity concern.[6] Other compounds, such as 2-methylbut-2-enal (**1**), 2-methylpent-2-enal (**2**), and 2-isopropyl-5-methylhex-2-enal (**4**), were instead evaluated as safe.[13]

In Europe, the use of flavoring substances (the legal term employed for organoleptically active substances added on purpose to food) is regulated by Regulation (EC) No. 1334/2008.[14] All "flavoring substances and source materials approved for use in and on foods" are exhaustively listed in Part A of Annex I of this Regulation, commonly called the Union list. Every new compound added to the Union list must be tested and approved.[15] The Regulation also makes Member States responsible for monitoring flavoring substances in food by a risk-based approach.[16]

In the present study, we have investigated the occurrence of 10 $\alpha,\beta$-unsaturated carbonyls (either commercially available or obtained here by combinatorial synthesis) throughout the chocolate-making process. As these molecules are known for their heat-related origin, a particular focus was on the roasting step. The impacts of different roasting temperatures on their occurrence were compared. The conching steps were also studied on an in-house-made chocolate, as heat-generated aroma synthesis is expected to continue during chocolate-making. To better understand the evolution of these molecules, they were analyzed in parallel with other heat-generated aromas, such as Strecker aldehydes and pyrazines. To expand the monitoring and identify any public health concern, the selected $\alpha,\beta$-unsaturated carbonyls were also studied in a series of 22 commercial food products such as crepes, waffles, cakes, and biscuits (all including cooking processes and/or containing chocolate).

## 2. MATERIALS AND METHODS

**2.1. Chemicals and Selection of Flavoring Substances.** 2,3,5-Trimethylpyrazine, 2,6-dimethylpyrazine, 2-acetylpyrrole, 2-acetylthiophene, 2-methylbutanal, 2-methylpyrazine, 2-phenylpent-2-enal (**C**), 3-methylbutanal, 4-methyl-2-phenylpent-2-enal (**6**), 5-methyl-2-phenylhex-2-enal (**7**), methional, *n*-decane, phenylacetaldehyde, and propanal were purchased from Merck (Overijse, Belgium). Furan-2(5*H*)-one (**B**) was purchased from Fisher Scientific (Brussels, Belgium). Acetaldehyde and but-2-enal (**A**) were issued from Fluka Chemicals (Leuven, Belgium). 2-Methylpropanal was purchased from Janssen Chimica (Geel, Belgium).

Among the 10 $\alpha,\beta$-unsaturated carbonyls selected (Figure 1), three (designated by letters) were commercially available and seven (designated by numbers) were issued from the combinatorial library synthesized here. But-2-enal (**A**) and furan-2(5*H*)-one (**B**) have been confirmed as genotoxic. The safety evaluation of 2-phenylpent-2-enal (**C**) was recently stopped due to discontinued use by industry, and therefore, its use in food is forbidden.[17] Three compounds (**5**, **6**, and **7**, designated here as suspected genotoxics) are under safety evaluation by EFSA, and they were recently subjected to maximum use levels (ranging from 20 to 5000 $\mu g \cdot kg^{-1}$ depending on the food categories).[18] The remaining compounds (**1**, **2**, **3**, and **4**) might possibly occur naturally in food, as they were found in the combinatorial library obtained here from common Strecker aldehydes and propanal (see hereunder). Compounds **1**, **2**, and **4** have been evaluated as safe by EFSA, while **3** is rarely reported in the literature and, to our knowledge, has never been evaluated by EFSA.

**2.2. Combinatorial Synthesis of $\alpha,\beta$-Unsaturated Aldehyde Standards.** NaOH at 0.1 $mol \cdot L^{-1}$ was made by diluting 100 mg of NaOH in 25 mL of distilled water. $\alpha,\beta$-Unsaturated aldehydes were synthesized under basic conditions (pH = 12) at 21 °C. First, 2.5 mL of NaOH (0.1 $mol \cdot L^{-1}$) was added into a 25 mL volumetric flask containing 12.5 mL of ethanol and 7 mL of distilled water. Common Strecker aldehyde standards (acetaldehyde, 2-methylpropanal, 3-methylbutanal, 2-methylbutanal, phenylacetaldehyde, and methional) and propanal were then added to reach 0.17 $mol \cdot L^{-1}$ of each reagent (reaction #1). After their complete solubilization, the flask was filled to 25 mL with distilled water. The reagent mix was shaken at 1000 rpm for 3 h, then extracted by liquid/liquid extraction with dichloromethane (2 × 25 mL), and later dried with anhydrous sodium sulfate. The extract was then injected into a GC-MS instrument (full scan acquisition, see Section 2.5), and the synthesized $\alpha,\beta$-unsaturated aldehydes were identified using the NIST 2014 mass spectra database (probability of identification, >70%). Afterward, a selected ion monitoring (SIM) program was created for selective detection and quantitation. The Supporting Information includes mass spectral data and retention indices of the monitored $\alpha,\beta$-unsaturated aldehydes (Table S1).

Under the above-described conditions, a second reaction was done with only acetaldehyde and 2-methylpropanal at higher concentration (1 $mol \cdot L^{-1}$). As its odor descriptor was not available in the literature, the synthesis of 2-isopropyl-4-methylhex-2-enal (**3**) was conducted with only 3-methylbutanal and 2-methylbutanal (1 $mol \cdot L^{-1}$) for further GC-O analyses.

**2.3. Selection of Samples.** *2.3.1. Cocoa Beans.* Dried fermented Haitian Criollo beans (hereafter denoted as HC beans), kindly provided by the AYITIKA Enterprise (Haiti), were used to study the impact of roasting. Homogenized beans were divided into four groups (all in duplicates, 50 g per sample): unroasted beans and beans roasted at 110, 135, or 150 °C for 30 min in a kitchen oven (maximum bean depth: 2 cm).

Dried fermented Brazilian hybrid beans (hereafter denoted as BH beans; fermentation process recently described in ref 19) were provided by Prof. H. Rogez (State University of Pará, Brazil). The beans were used here to produce an in-house dark chocolate (76.6% cocoa) for assessing the impact of the conching process. Four samples were taken during production of this chocolate: "initial dried fermented beans", "cocoa mass after roasting (145 °C for 30 min), fine grinding, and sugar addition", "chocolate after 20 h of dry conching", and "chocolate after 6 h of wet conching (=final chocolate)".

*2.3.2. In-House Chocolate Production.* BH cocoa beans (3071 g) were roasted at 145 °C for 30 min. Afterward, bean testae were discarded and the remaining cocoa nibs (2450 g) were ground for 40 min with a robot-coupe (R8VVE model from Robot Coupe SNC, France). After 35 min of grinding, 816 g of powdered sugar, equivalent to one-third of the cocoa nib mass, was added to the cocoa mass so as to obtain 25% sugar. Dry conching (Concher from Spectra Company, India) took place for 30 h, the cocoa mass being heated to 60 °C during the first hour. The conching lid was kept open during the first 2 h of conching. The cocoa mass was at 50 °C at the end of


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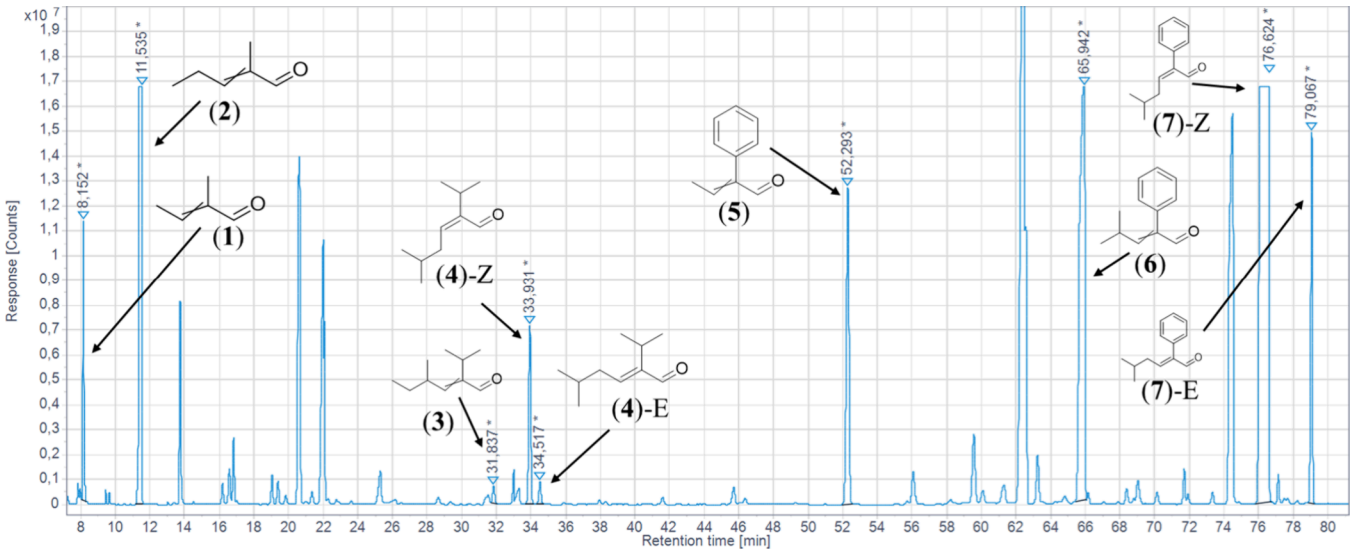


**Figure 2.** Chromatogram (SIM acquisition) of the combinatorial library synthesized by aldol condensation from 6 Strecker aldehydes (acetaldehyde, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, and methional) and propanal. A crossed doubled bond is shown if *Z*/*E* isomer separation was not observed.

dry conching. Wet conching started with adding 214 g of cocoa butter, equivalent to 7% of the cocoa mass (3066 g, taking into account the mass removed for sample analysis). Wet conching continued for 6 h until a particle size of 18 $\mu$m was obtained. The chocolate was then filtered through a metal sieve and tempered as follows: all the chocolate was heated to 50 °C, two-thirds was cooled to 28 °C, and the remaining third was then put back, bringing the chocolate to 31−32 °C.

*2.3.3. Commercial Food Products.* Twenty-two commercial food products (designated here as sweet snacks), including national and distributor brands, were purchased from Belgian supermarkets from July 2021 to March 2022. Samples with and without chocolate were selected to evaluate the contribution of chocolate to the presence of $\alpha,\beta$-unsaturated carbonyls.

**2.4. Isolation of Flavoring Substances.** The SAFE method[20] was optimized for cocoa beans, chocolate, and commercial sweet snack samples. Analyses were done in duplicate. Finely ground samples (50 g) were spiked with 150 $\mu$L of 2-acetylthiophene solution (8 mg·L$^{-1}$) as an internal standard (IST; concentration in chocolate: 24 $\mu$g·kg$^{-1}$). Samples were extracted with bidistilled dichloromethane (3 × 25 mL) for 20 min and filtered through a Büchner funnel with glass fiber filters to recover the organic phase. The combined organic extracts were extracted by SAFE[21] (water bath temperature: 40 °C; pressure below $10^{-3}$ Pa; apparatus body at 30 °C). The distillate was continuously recovered in the liquid nitrogen-cooled SAFE flask. After about 25 min of distillation, the distillate was recovered and dried with anhydrous sodium sulfate, spiked with 25 $\mu$L of *n*-decane (250 mg·L$^{-1}$) as an external standard (EST; concentration in the final extract = 12.5 mg·L$^{-1}$), and concentrated to 500 $\mu$L in a Kuderna-Danish apparatus at 45 °C. The extract was stored at −80 °C before GC-MS analysis. By using this procedure, we suspect that only orthonasal flavors are determined (additional release compounds when water is added to chocolate, as recently published[22]).

**2.5. Gas Chromatography/Mass Spectrometry and Olfactometry.** The SAFE extracts were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). One microliter of extract was injected into an Agilent 7890B gas chromatograph (Agilent, Belgium) in splitless mode (front inlet: 250 °C; splitless hold time: 0.8 min). Analytes were separated on a nonpolar column (CP-Sil 5 CB, 50 m × 0.32 mm i.d., 1.2 $\mu$m film thickness, Agilent, Belgium). Helium (99.999% purity, connected to an Agilent carrier gas filter CP17973) was used as carrier gas at 100 kPa. The oven temperature program was as follows: from 36 to 85 °C at 20 °C·min$^{-1}$, then to 145 °C at 1 °C·min$^{-1}$, and to 250 °C at 3 °C·min$^{-1}$. Finally, the oven was held at 250 °C for 30 min. The column was connected (transfer line at 250 °C) to a single quadrupole mass spectrometer (Agilent 5977B) with electron impact (70 eV), operating either in the full-scan mode ($m/z$: 40−380) or the selected-ion monitoring (SIM) mode. To assess the olfactory characteristic of 2-isopropyl-4-methylhex-2-enal (**3**), the extract from reaction #3 was analyzed by GC-olfactometry. The injection mode, column, and temperature program were identical to those used in GC-MS. The column was instead connected to a GC-O port (Gerstel ODP-4, Germany), with transfer line and exit temperature both at 250 °C. The effluent was diluted with a large volume of air (20 mL·min$^{-1}$) prehumidified with an aqueous copper(II) sulfate solution.

**2.6. Identification and Quantification of Volatiles.** Commercially available analytical standards were used for analyte identification (according to Regulation (EU) 2021/808[23]) and for quantitation as follows: [analyte X, in $\mu$g·kg$^{-1}$] = [IST, in $\mu$g·kg$^{-1}$] × (IST response coefficient/X response coefficient) × (X area/IST area). To determine the response coefficient ratio, analytical standards were externally calibrated relatively to the IST at the expected concentration ranges in the extracts. For each compound, the linearity of the relative response was verified with Mandel's fitting test ($R^2$ > 0.99). Calibration curves are presented in the Supporting Information (Figure S1). All extractions were done in duplicate. Furan-2(5*H*)-one was instead quantitated by standard addition (four points) in the commercial food products, with fortification levels adapted to the expected concentrations of the samples (Supporting Information, Figure S2). The concentration of furan-2(5*H*)-one was thus determined as follows: [furan-2(5*H*)-one, in $\mu$g·kg$^{-1}$] = [IST, in $\mu$g·kg$^{-1}$] × (1/slope of standard addition curve relative to IST) × (furan-2(5*H*)-one area/IST area). Noncommercially available compounds were identified on the basis of the combinatorial library synthesized here, and concentrations were determined in internal standard equivalents. Analyte concentrations in the chocolate samples were corrected to take into account the dilution due to sugar and cocoa butter addition. Results are thus given per initial mass of cocoa powder.

**2.7. Statistical Analysis.** Statistical analysis was performed with the JMP program (version 17), SAS Institute Inc., USA. Student tests were performed to determine significant differences between samples ($p$ < 0.05).


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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

## 3. RESULTS AND DISCUSSION

**3.1. α,β-Unsaturated Aldehyde Combinatorial Library.** Figure 2 shows the chromatogram of all detected α,β-unsaturated aldehydes formed through combinatorial synthesis from six Strecker aldehydes (acetaldehyde, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, and methional) and propanal. Only seven were detected out of the 35 theoretically expected products (Supporting Information, Table S2): 2-methylbut-2-enal (**1**), 2-methylpent-2-enal (**2**), 2-isopropyl-4-methylhex-2-enal (**3**), 2-isopropyl-5-methylhex-2-enal (**4**), 2-phenylbut-2-enal (**5**), 4-methyl-2-phenylpent-2-enal (**6**), and 5-methyl-2-phenylhex-2-enal (**7**).

Among these, **1**, **2**, and **4** have been evaluated as safe by EFSA, **5**, **6**, and **7** are under safety evaluation because of genotoxicity (aneugenicity) concerns, and **3** has rarely been reported in the literature. Addition of **3** as such to food is prohibited, as it is absent from the Union list.

Peak areas from the full-scan acquisition of the synthesized aldehydes were compared to the area of the compound with the largest peak (i.e., compound **7**) to give an order of magnitude of which compound was formed the most (Table 1). For isomers, *Z*/*E* ratios were measured when applicable.

**Table 1. α,β-Unsaturated Aldehydes Formed by the Combinatorial Synthesis from 6 Strecker Aldehydes (Acetaldehyde, 2-Methylpropanal, 2-Methylbutanal, 3-Methylbutanal, Phenylacetaldehyde, and Methional) and Propanal**

| no. | name | odor descriptors[a] | relative peak area (%) | *Z*/*E* ratio (%) |
|---|---|---|---|---|
| 1 | 2-methylbut-2-enal | fresh, fruity, almond | 1.0 | n.a. |
| 2 | 2-methylpent-2-enal | grassy, fruity | 14.7 | n.a. |
| 3 | 2-isopropyl-4-methylhex-2-enal | cocoa, roasted[GC-O] | 0.5 | n.a. |
| 4 | 2-isopropyl-5-methylhex-2-enal | herbal, woody | 2.2 | 90/10 |
| 5 | 2-phenylbut-2-enal | cocoa, roasted, honey, rum | 3.6 | 100/0 |
| 6 | 4-methyl-2-phenylpent-2-enal | cocoa, honey, nutty | 24.3 | 100/0 |
| 7 | 5-methyl-2-phenylhex-2-enal | cocoa, mocha, sweet | 100 | 91/9 |

[a]Odor descriptors according to refs 24 and 25; [GC-O]: odor description determined by GC-O analysis; n.a.: not applicable.

The abundances of **5**, **6**, and **7** suggest that phenylacetaldehyde was the best nucleophile, likely because of the higher acidity of the α-hydrogen of phenylacetaldehyde as compared to other Strecker aldehydes, due to the resonance effect. Yet, the commercially available 2-phenylpent-2-enal (**C** in Figure 1) was not found in the combinatorial library, although it is formed by aldol condensation of phenylacetaldehyde and propanal. Two reaction products (**3** and **4**) formed with 3-methylbutanal as a nucleophile were also detected but at lower levels. The small size of acetaldehyde and propanal probably facilitated their aldol condensation, leading to compounds **1** and **2**.

For each product, *Z*/*E* isomers could be expected. Molecular model calculations (MM2 in ChemDraw 3D) predicted a lower molecular energy for the *Z* than for the *E* isomers, suggesting a predominance of the *Z* form, in accordance with the literature.[26] In practice, two peaks each were indeed observed for compounds **4** and **7**, the main peak being attributed to the *Z* isomer. For **5** and **6**, the single peaks were explained by the possible presence of the *Z* form only, while insufficient chromatogram resolution at the beginning of the run probably explains the single peaks obtained for compounds **1**, **2**, and **3**.

Even with higher initial concentrations (1 mol·L$^{-1}$) of 2-methylpropanal and acetaldehyde (reaction #2), 4-methylpent-2-enal was not formed. The same applies to the other undetected products mentioned in the Supporting Information (Table S2).

The occurrence of 2-isopropyl-4-methylhex-2-enal (**3**) in the combinatorial mixture was confirmed by MS (RI = 1077 on CP-Sil5, mass spectrum in Figure 3), although it has very rarely

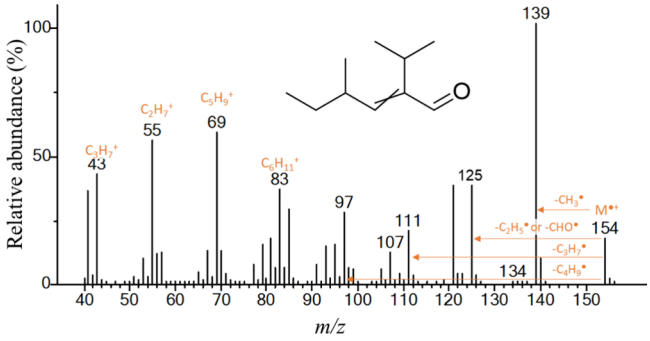


**Figure 3.** Experimental mass spectrum of 2-isopropyl-4-methylhex-2-enal (**3**) and proposed fragmentation ions.

been identified as a natural aroma in foodstuff.[27,28] Its odor perceived by GC-O was described here as "cocoa, roasted", and its mass spectrum is reported in Figure 3.

The GC-MS information (mass spectra and retention times) on the seven α,β-unsaturated aldehydes found in the combinatorial library was used to create a SIM acquisition method including the commercially available standards but-2-enal (**A**), furan-2(5*H*)-one (**B**), and 2-phenylpent-2-enal (**C**). The mass spectra data and retention indices of the monitored compounds are provided in the Supporting Information (Table S1).

**3.2. Impact of Cocoa Roasting.** *3.2.1. α,β-Unsaturated Carbonyls.* As depicted in Table 2, most of the α,β-unsaturated aldehydes investigated (2-methylbut-2-enal (**1**), 2-isopropyl-4-methylhex-2-enal (**3**), 2-isopropyl-5-methylhex-2-enal (**4**), 2-phenylbut-2-enal (**5**), 4-methyl-2-phenylpent-2-enal (**6**), and 5-methyl-2-phenylhex-2-enal (**7**)) were already slightly present in both sets of unroasted beans, the exceptions being but-2-enal (**A**), 2-phenylpent-2-enal (**C**), and 2-methylpent-2-enal (**2**). The fact that the latter (formed by the aldol condensation between two propanal molecules) was absent throughout the process could indicate very low concentrations of propanal in the samples, as compared to Strecker aldehydes. Compounds **5**, up to 20 μg·kg$^{-1}$, and **7**, up to 5.3 μg·kg$^{-1}$, are respectively also known as cocoa butenal and cocoa hexenal. They have previously been identified as compounds contributing significantly to the typical cocoa aroma.[5] Regarding the isomer ratios, the presence of compounds **4** and **7** in HC beans was mainly associated with a dominant isomer (*Z* form), accounting for 73 and 96%, respectively. In BH beans, the corresponding figures were 65% for compound **4** and 96% for compound **7**. Identifying and quantifying *Z* and *E* isomers could be relevant to toxicity, as isomers can have different reactivities.[29]


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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 2. Concentrations ($\mu g \cdot kg^{-1}$) of Heat-Generated Aromas in Haitian Criollo (HC) and Brazilian Hybrid (BH) Cocoa Bean Samples and Chocolate (from BH Cocoa Bean) Samples[f]

| | | | | | Haitian Criollo (HC) samples | | | | Brazilian hybrid (BH) samples | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | compound | RI (CPSil-5)[g] | odor descriptors[b] | threshold ($\mu g \cdot kg^{-1}$) | unroasted | roasted at 110 °C | roasted at 135 °C | roasted at 150 °C | unroasted | roasted at 145 °C | dry conching | wet conching |
| $\alpha,\beta$-Unsaturated carbonyls ($\mu g \cdot kg^{-1}$) | | | | | | | | | | | | |
| **A** | but-2-enal | 631 | pungent | 0.3−525[c] | - | 0.3 | 1.8 | 4.6 [c] | - | - | 0.2 | 0.2 |
| **1** | 2-methylbut-2-enal[a] | 722 | freshy, fruity, almond | 500[d] | 1.9 [a] | 9.5 | 11.5 | 13.1 [c] | 0.5 [b] | 1.7 [d] | 0.2 | 0.3 |
| **2** | 2-methylpent-2-enal[a] | 815 | grassy, fruity | 290[d] | - | - | - | - | - | - | - | - |
| **B** | furan-2(5*H*)-one | 866 | buttery, smoke, sweet | n/a | 21.0 [a] | 60.8 | 57.8 | 89.8 [c] | 7.7 [b] | 30.4 [d] | 15.2 | 19.5 |
| **3** | 2-isopropyl-4-methylhex-2-enal[a] | 1077 | cocoa, roasted[GC-O] | n/a | 1.6 [a] | 5.5 | 4.3 | 5.7 [c] | 4.6 [b] | 6.6 [c] | 6.8 | 6.3 |
| **4** | (Z)-2-isopropyl-5-methylhex-2-enal | 1097 | herbal, woody | n/a | 1.6 [a] | 5.9 | 10.0 | 19.8 [c] | 1.1 [a] | 34.9 [d] | 4.9 | 9.8 |
| | (E)-2-isopropyl-5-methylhex-2-enal | 1102 | | | 0.6 [a] | 1.4 | 4.4 | 9.2 [c] | 0.6 [a] | 20.0 [d] | 1.9 | 2.0 |
| **5** | 2-phenylbut-2-enal[a] | 1249 | cocoa, roasted, honey, rum | n/a | 20.0 [a] | 120.5 | 366.6 | 399.0 [c] | 2.1 [b] | 72.2 [d] | 62.4 | 88.6 |
| **C** | 2-phenylpent-2-enal | 1320 | floral, green | n/a | - | 1.3 | 3.5 | 1.9 [c] | - | 2.1 [c] | 1.4 | 1.5 |
| **6** | 4-methyl-2-phenylpent-2-enal[a] | 1360 | cocoa, honey, nutty | n/a | 4.3 [a] | 7.9 | 24.2 | 17.2 [c] | 1.1 [b] | 13.6 [c] | 3.9 | 7.8 |
| **7** | (Z)-5-methyl-2-phenylhex-2-enal | 1482 | cocoa, mocha, sweet | n/a | 5.1 [a] | 37.5 | 210.5 | 125.7 [c] | 2.6 [b] | 143.8 [c] | 24.9 | 50.3 |
| | (E)-5-methyl-2-phenylhex-2-enal | 1519 | | | 0.2 [a] | 1.3 | 5.2 | 3.4 [c] | 0.1 [a] | 2.4 [c] | 0.4 | 1.0 |
| Strecker aldehydes ($\mu g \cdot kg^{-1}$) | | | | | | | | | | | | |
| | 3-methylbutanal | 642 | acrid, cocoa, almond | 0.1−7[c] | 353.0 [a] | 3744.6 | 11,659.8 | 3583.1 [c] | 83.5 [b] | 682.9 [d] | 136.0 | 107.8 |
| | 2-methylbutanal | 651 | almond, cocoa | 1−12[c] | 139.4 [a] | 1546.9 | 5866.2 | 2520.3 [c] | 46.3 [b] | 451.0 [d] | 59.9 | 70.9 |
| | methional | 873 | cooked potato | 0.2−1[c] | 3.0 [a] | 43.8 | 84.7 | 23.7 [c] | 0.7 [b] | 16.0 [c] | 6.3 | 2.3 |
| | phenylacetaldehyde | 1019 | floral, honey | 2−30[c] | 32.2 [a] | 1637.6 | 1669.5 | 1106.1 [c] | 30.5 [a] | 159.2 [d] | 103.4 | 125.5 |
| Heat-generated heterocycles ($\mu g \cdot kg^{-1}$) | | | | | | | | | | | | |
| | 2-methylpyrazine[a] | 807 | cocoa, hazelnut | 60−100,000[d] | 2.7 [a] | 14.8 | 19.7 | 35.3 [c] | 0.7 [b] | 26.7 [c] | 5.3 | 16.0 |
| | 2,6-dimethylpyrazine | 894 | cocoa, coffee hazelnut, | 400−1500[d] | 0.6 [a] | 16.3 | 39.3 | 76.8 [c] | 1.9 [b] | 173.5 [d] | 80.4 | 110.5 |
| | 2,5-dimethylpyrazine[a] | 895 | burnt, cocoa, roasted, coffee | 80−1800[d] | 1.2 [a] | 8.8 | 22.7 | 48.3 [c] | 0.8 [a] | 117.1 [d] | 55.5 | 81.6 |
| | 2,3-dimethylpyrazine[a] | 901 | cocoa, caramel, hazelnut | 800−2500[d] | 0.1 [a] | 0.7 | 1.2 | 2.1 [c] | 0.9 [b] | 2.2 [c] | 0.2 | 2.3 |
| | 2-carboxaldehyde-1H-pyrrole[a] | 977 | musty, beefy, coffee | n/a | 4.9 [a] | 13.1 | 9.3 | 23.4 [c] | 1.4 [b] | 8.8 [d] | 4.2 | 9.1 |
| | 2-ethyl-3-methylpyrazine[a] | 984 | nutty, roasted nuts, bready | 130[d] | 16.6 [a] | 127.5 | 155.1 | 178.9 [c] | 101.9 [b] | 1125.2 [d] | 901.6 | 1005.7 |
| | 2,3,5-trimethylpyrazine | 984 | cocoa, roasted nuts | 290[e] | 23.3 [a] | 186.2 | 218.6 | 244.8 [c] | 137.2 [b] | 1543 [d] | 1 249.0 | 1263.3 |
| | 2-acetylpyrrole | 1031 | bread, cocoa, hazelnuts | 170,000[c] | 39.5 [a] | 137.5 | 233.6 | 478.6 [c] | 45.2 [a] | 546.2 [c] | 427.6 | 552.8 |
| | tetramethylpyrazine[a] | 1092 | cocoa, coffee, mocha | 1000[c] | 133.0 [a] | 565.3 | 603.0 | 707.6 [c] | 1174.4 [b] | 4475.1 [d] | 3914.9 | 4128.5 |

[a]Compounds quantified as internal standard equivalents; [GC-O]: odor described by GC-O; - = not detected. [b]Odor descriptors according to refs 24 and 25. [c]Threshold values according to ref 34. [d]Threshold values according to ref 35. [e]Threshold values according to ref 36. Significant differences ($p < 0.05$) between HC and BH beans are shown by different letters (a/b for unroasted beans and c/d for roasted beans). [f]Concentrations in Brazilian samples were corrected considering dilution factors by sugar and cocoa butter addition during the chocolate-making process, thus expressed in terms of initial cocoa mass. [g]Retention indices according to NIST database and ref 24.

Of all the $\alpha,\beta$-unsaturated carbonyls monitored in these unroasted beans, genotoxic furan-2(5*H*)-one (**C**) was found at the highest level in both bean samples (21 and 8 $\mu g \cdot kg^{-1}$ in HC and BH, respectively). The temperatures reached during fermentation (up to 50 °C) and sun-drying might allow aldol condensations and Maillard reactions to occur.[30]

As expected, an increase in $\alpha,\beta$-unsaturated aldehyde content was observed after roasting (Figure 4). In both sets of samples, this increase was the greatest for 2-phenylbut-2-enal (**5**), 5-methyl-2-phenylhex-2-enal (**7**), and 2-isopropyl-5-methylhex-2-enal (**4**), although strongly dependent on bean type. After 30 min at 135 °C, the HC samples showed an 18-fold increase in **5** and a 40-fold increase in **7**, the respective concentrations of these compounds reaching 366.6 and 215.7 $\mu g \cdot kg^{-1}$. In line with the combinatorial synthesis results, this major increase could be related to the higher aldol condensation reactivity of phenylacetaldehyde. Compound **4**, which was the second-most formed product from combinatorial synthesis (derived from two 3-methylbutanal molecules), increased 13-fold in the HC beans roasted at 150 °C, reaching


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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

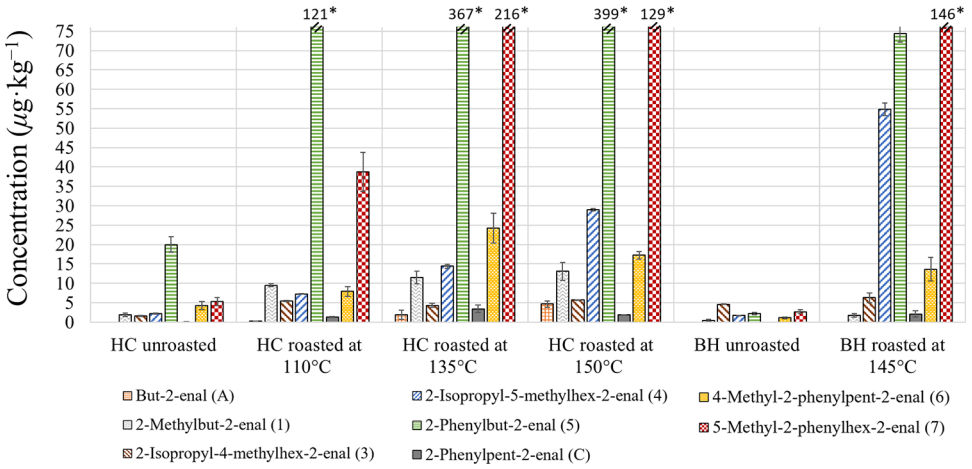


**Figure 4.** Levels ($\mu g \cdot kg^{-1}$) of $\alpha,\beta$-unsaturated aldehydes in Haitian Criollo (HC) and Brazilian hybrid (BH) cocoa bean samples before and after roasting. Asterisk (*): value above the maximum scale value.

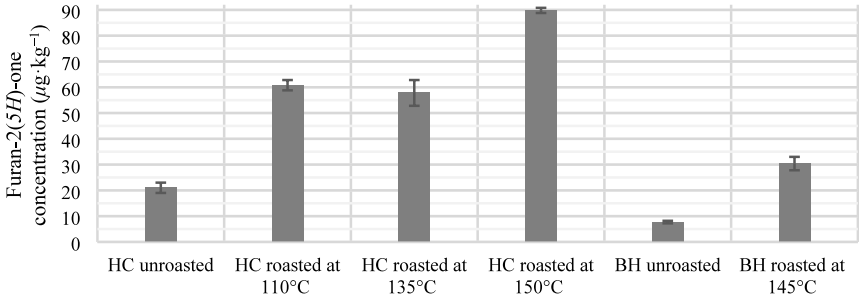


**Figure 5.** Levels ($\mu g \cdot kg^{-1}$) of furan-2(5*H*)-one in Haitian Criollo (HC) and Brazilian hybrid (BH) cocoa bean samples before and after roasting.

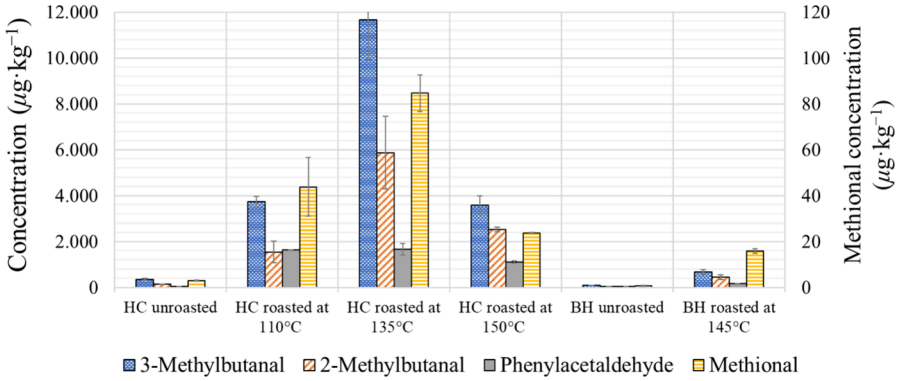


**Figure 6.** Levels ($\mu g \cdot kg^{-1}$) of Strecker aldehydes in Haitian Criollo (HC) and Brazilian hybrid (BH) cocoa bean samples before and after roasting. The methional level is represented on the right scale.

29.0 $\mu g \cdot kg^{-1}$. Levels of 4-methyl-2-phenylpent-2-enal (**6**) also increased, reaching a maximum of 24.2 $\mu g \cdot kg^{-1}$ in the HC beans roasted at 135 °C. 2-Phenylpent-2-enal (**C**) was only slightly impacted by roasting (maximum level: 3.5 $\mu g \cdot kg^{-1}$ in the HC beans roasted at 135 °C). As discussed above, this may be linked to the (probably low) propanal levels in the cocoa samples. Levels of 2-isopropyl-4-methylhex-2-enal (**3**), hardly ever reported in food matrices, remained low, with a maximum of 5.7 $\mu g \cdot kg^{-1}$ in the HC beans roasted at 150 °C.

The confirmed genotoxic furan-2(5*H*)-one (**B**) was also synthesized during roasting (Figure 5). As compared to its concentration in unroasted beans (21.0 $\mu g \cdot kg^{-1}$), it showed a 4-fold increase in both HC beans roasted at 150 °C (89.8 $\mu g \cdot kg^{-1}$) and BH beans roasted at 145 °C (30.4 $\mu g \cdot kg^{-1}$). While the exact origin of furan-2(5*H*)-one remains unclear, its level in unroasted beans is probably impacted by the bean variety, cultivation practices, and bean drying conditions.

As for genotoxic but-2-enal (**A**), it appeared after roasting at fairly low levels (0.3−4.6 $\mu g \cdot kg^{-1}$).

The impact of the roasting temperature was a general increase in $\alpha,\beta$-unsaturated carbonyls from 110 to 135 °C, whereas the effect of roasting at 150 °C depended on the compound. Only **A** and **4** showed a further increase, whereas levels of **1**, **3**, and **5** scarcely changed between 135 and 150 °C. Levels of **6**, **7**, and **C** decreased above 135 °C, although these are the least volatile compounds. Because **7** was so abundant, its 1.7-fold lower level upon roasting at 150 °C than at 135 °C compensated for the increases in the other $\alpha,\beta$-unsaturated


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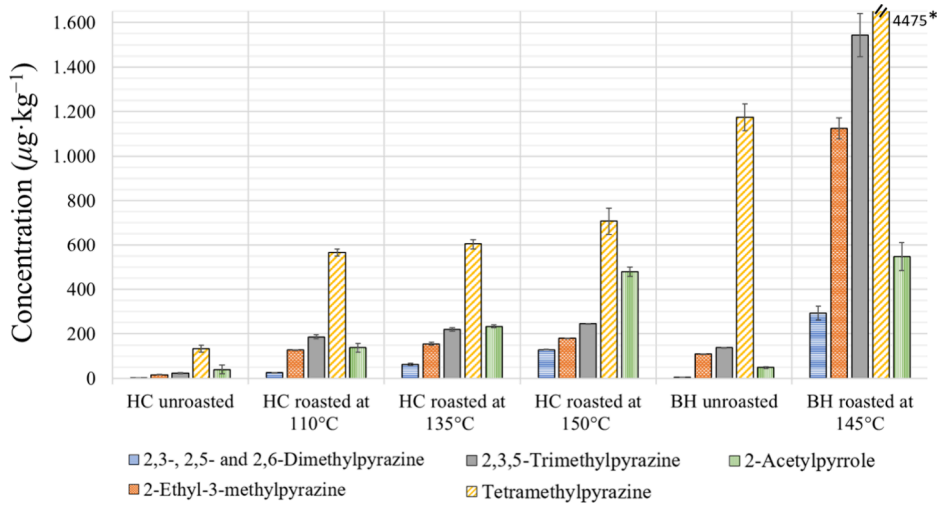


**Figure 7.** Levels ($\mu g \cdot kg^{-1}$) of nitrogen-containing heterocycles in Haitian Criollo (HC) and Brazilian hybrid (BH) cocoa bean samples before and after roasting. Asterisk (*): value above the maximum scale value.

carbonyls (combined total for all the investigated compounds: 699.8 $\mu g \cdot kg^{-1}$ at 135 °C versus 689.4 $\mu g \cdot kg^{-1}$ at 150 °C).

The isomer ratios for compounds **4** and **7** did not change significantly during the roasting step, the average contribution of the major isomer (*Z* form) in the roasted HC beans remaining at 73% for **4** and 97% for **7**.

*3.2.2. Strecker Aldehydes as Precursors.* Four Strecker aldehydes were already present above their odor thresholds in the unroasted cocoa beans (Figure 6), although at lower concentrations than previously reported for other samples.[3] 3-Methylbutanal (83 $\mu g \cdot kg^{-1}$ in BH beans, 353 $\mu g \cdot kg^{-1}$ in HC beans) and 2-methylbutanal (46 $\mu g \cdot kg^{-1}$ in BH beans, 139 $\mu g \cdot kg^{-1}$ in HC beans) were predominant in both bean sets, bringing almond/cocoa odors. Methional (potato odor) was significantly higher in HC beans (3 $\mu g \cdot kg^{-1}$), while phenylacetaldehyde (honey odor) levels were similar in samples of HC and BH beans (30−32 $\mu g \cdot kg^{-1}$).

In HC beans, all Strecker aldehydes significantly increased through roasting despite their high volatility: their combined total showed a 13-fold increase at 110 °C and a 35-fold increase at 135 °C. The increase was somewhat lesser at 150 °C because of increased evaporation.[5,31]

With a total of 1309 $\mu g \cdot kg^{-1}$ and an 8-fold increase from unroasted beans, Brazilian beans roasted at 145 °C showed much lower levels of Strecker aldehydes compared to the HC beans roasted at 150 °C. For comparison, the 3-methylbutanal concentration (cocoa aroma) was five times higher in the HC beans roasted at 150 °C. Methional was the only Strecker aldehyde to show no significant difference between HC and BH beans.

*3.2.3. Heat-Generated Heterocycles as Markers.* The occurrence of pyrazines (especially ethyl-branched ones) in chocolate is desired, as they bring roasted nut, chocolate-like, cocoa, caramel, and coffee flavors.[31,32] All the monitored pyrazines were already present in unroasted beans, although well below their thresholds except for tetramethylpyrazine, which reached 133.0 $\mu g \cdot kg^{-1}$ in HC beans and up to 1174.4 $\mu g \cdot kg^{-1}$ in BH beans (Figure 7). The main factor explaining this significant difference in pyrazine level between unroasted HC and BH beans still need to be determined, but could include variety, cultivation practices, and bean drying conditions.

As depicted in Figure 7, pyrazines also significantly increased during roasting, in both BH and HC samples. Tetramethylpyrazine was the primary compound (707.6 $\mu g \cdot kg^{-1}$ in HC beans roasted at 150 °C), followed by 2,3,5-trimethylpyrazine (244.8 $\mu g \cdot kg^{-1}$) and 2-ethyl-3-methylpyrazine (178.9 $\mu g \cdot kg^{-1}$). Although detected below their odor thresholds, the compounds 2,6-, 2,5-, and 2,3-dimethylpyrazine might synergistically participate in the roasted aroma.[33] As expected, all pyrazines continuously increased with the roasting temperature, as the optimal conditions for their formation are 150−160 °C for 30 min.[5] Positive correlations were observed between pyrazines and $\alpha,\beta$-unsaturated carbonyls levels ($R^2$ = 0.83 between 2,6-dimethylpyrazine and 2-phenylbut-2-enal; $R^2$ = 0.94 between 2-methylpyrazine and furan-2(5*H*)-one). 2-Acetylpyrrol also increased with the roasting temperature, although remaining well below its higher odor threshold as compared to pyrazines.

As in unroasted beans, pyrazines were present at higher concentrations in BH samples (roasted at 145 °C) than in HC beans (roasted at 150 °C). 2-Ethyl-3-methylpyrazine showed the greatest difference (1125.2 $\mu g \cdot kg^{-1}$ in 145 °C-roasted BH beans versus 178.9 $\mu g \cdot kg^{-1}$ in 150 °C-roasted HC beans), followed by 2,3,5-trimethylpyrazine and tetramethylpyrazine.

**3.3. Impact of Chocolate Conching.** *3.3.1. $\alpha,\beta$-Unsaturated Carbonyls.* As expected, $\alpha,\beta$-unsaturated aldehyde levels decreased during dry conching because of partial evaporation, especially for the most volatile compounds (**1**, **4**), or thermal degradation (Figure 8). Interestingly, (**3**) levels remained constant during conching. Wet conching, on the other hand, induced significant increases in compounds (**4**, **5**, **6**, **7**), issued from phenylacetaldehyde and 3-methylbutanal, yet all well below the maximum use levels (2 $mg \cdot kg^{-1}$ for (**5**), 5 $mg \cdot kg^{-1}$ for (**6**,**7**)) in chocolate by the Regulation.[18] These results showed the impact of a wet phase during chocolate conching in recreating aromas lost during the dry phase. Adding cocoa butter liquifies the chocolate, allowing better contact between Strecker aldehydes.

The isomer ratio remained the same for **7** throughout the conching process (98% *Z* form). For compound **4**, the isomer ratio increased in favor of the *Z* form (72% after dry conching and 83% after wet conching).


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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

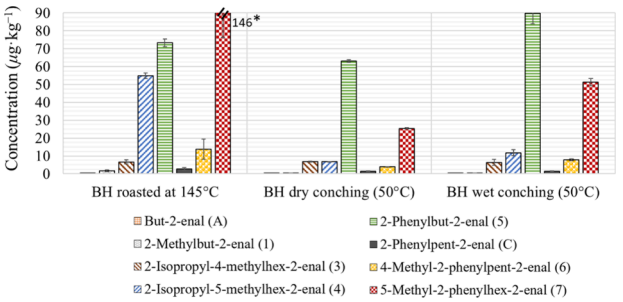


**Figure 8.** Levels ($\mu g \cdot kg^{-1}$) of $\alpha,\beta$-unsaturated aldehydes in chocolate samples from Brazilian hybrid (BH) cocoa beans before and after conching. Asterisk (*): value above the maximum scale value.

After a significant decrease during dry conching, no significant change in furan-2(5*H*)-one level was measured after wet conching (Figure 9).

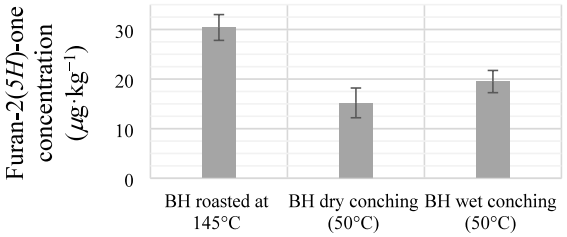


**Figure 9.** Levels ($\mu g \cdot kg^{-1}$) of furan-2(5*H*)-one in chocolate samples from Brazilian hybrid (BH) cocoa beans before and after conching.

*3.3.2. Strecker Aldehydes as Precursors.* Levels of the two most volatile Strecker aldehydes (2- and 3-methylbutanal) dropped remarkably during dry conching, as they evaporated (Figure 10). Still, no significant increase was observed during the wet phase, as opposed to $\alpha,\beta$-unsaturated aldehydes, to which they are partially converted. A similar decline in the concentrations of most aldehydes in dark chocolate, after conching at 70−80 °C, has been described previously.[4]

*3.3.3. Heat-Generated Heterocycles as Markers.* Among pyrazines, while 2,3-, 2,5- and 2,6-dimethylpyrazines partially evaporated during dry conching, the less volatile compounds 2-ethyl-3-methylpyrazine, 2,3,5-trimethylpyrazine, and tetramethylpyrazine remained stable (Figure 11), with no significant synthesis during wet conching (opposite results in the literature for higher conching temperature[4]). The two formers

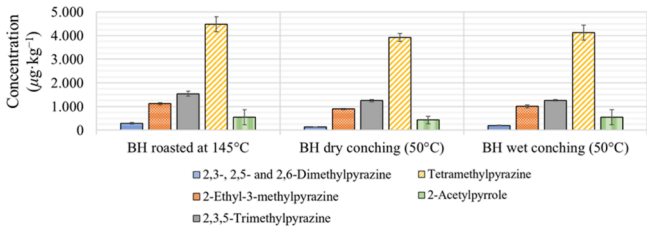


**Figure 11.** Levels ($\mu g \cdot kg^{-1}$) of nitrogen-containing heterocycles in chocolate samples from Brazilian hybrid (BH) cocoa beans before and after conching.

exceeded their odor threshold (OAV: 7.7 and 4.3, respectively), giving notes of roasted nuts, bread, and cocoa to chocolate. Despite their distinct fate through conching, correlations were still observed in the final chocolate between pyrazines and $\alpha,\beta$-unsaturated carbonyls (up to $R^2$ = 0.97 between 2,6-dimethylpyrazine and furan-2(5*H*)-one).

**3.4. Occurrence of $\alpha,\beta$-Unsaturated Carbonyls in Commercial Sweet Snacks.** Twenty-two commercial food samples (designated here as sweet snacks) were analyzed, including three crepes, four waffles, six cakes, six chocolate-containing biscuits, and three nonchocolate biscuits. Traces of genotoxic but-2-enal (**A**) were found in all three crepe samples (3−8 $\mu g \cdot kg^{-1}$) and in cake H (3 $\mu g \cdot kg^{-1}$). Six other $\alpha,\beta$-unsaturated aldehydes (**1**, **3**, **4**, **5**, **6**, and **7**) were also detected (Table 3) in several sweet snacks, albeit at levels below those reached in chocolate (maximum concentration: 16 $\mu g \cdot kg^{-1}$ for (**5**) in chocolate-containing biscuit O).

Interestingly, the *Z*/*E* isomer ratio for 2-isopropyl-5-methylhex-2-enal (**4**) was similar among food products, on average 83%/17%. 2-Methylpent-2-enal (**2**) was absent from all samples, as it was from chocolate. Overall, chocolate-containing biscuits contained more $\alpha,\beta$-unsaturated aldehydes than nonchocolate biscuits.

Concerningly, genotoxic furan-2(5*H*)-one (**B**) was found in all the sweet snacks investigated here, ranging from 17 to 4320 $\mu g \cdot kg^{-1}$ (Figure 12). Unlike the monitored $\alpha,\beta$-unsaturated aldehydes, it was found at the highest levels in the crepes, waffles, and cakes (food products not necessarily containing chocolate). Interestingly, similar furan-2(5*H*)-one levels (17−19 $\mu g \cdot kg^{-1}$) were measured in biscuits O and T of the same brand, only the former being covered with chocolate (22%). This rules out the hypothesis of a significant contribution of chocolate to the occurrence of furan-2(5*H*)-one in food. Yet,

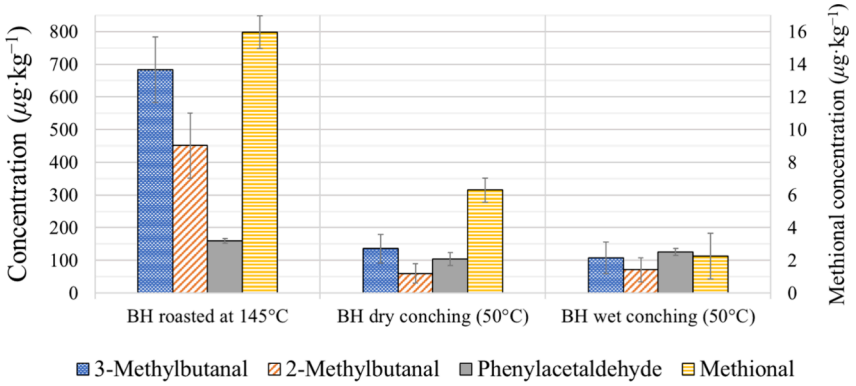


**Figure 10.** Levels ($\mu g \cdot kg^{-1}$) of Strecker aldehydes in chocolate samples from Brazilian hybrid (BH) cocoa beans before and after dry and wet conching.


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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

**Table 3. Concentrations ($\mu g \cdot kg^{-1}$) of $\alpha,\beta$-Unsaturated Carbonyls, Furfuryl Alcohol, and Furfural in Commercial Sweet Snacks**

| | | crepes | | | waffles | | | | cakes | | | | | | chocolate-containing biscuits | | | | | | nonchocolate biscuits | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | compound | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
| $\alpha,\beta$-Unsaturated aldehydes | | | | | | | | | | | | | | | | | | | | | | | |
| **A** | but-2-enal | 7.8 | 5.7 | 3.4 | - | - | - | - | 3.0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **1** | 2-methylbut-2-enal[a] | - | - | - | - | - | - | - | 0.5 | 1.3 | - | - | - | 0.9 | - | - | - | - | - | - | - | - | - |
| **2** | 2-methylpent-2-enal[a] | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **3** | 2-isopropyl-4-methylhex-2-enal[a] | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.9 | - | - | - | - |
| **4** | (*Z*)-2-isopropyl-5-methylhex-2-enal[a] | - | - | - | - | 1.9 | 0.2 | - | - | 1.9 | 0.8 | - | - | - | 0.6 | 4.5 | 4.1 | - | 14.2 | 1.3 | - | - | - |
| | (*E*)-2-isopropyl-5-methylhex-2-enal[a] | - | - | - | - | 0.3 | - | - | - | 0.3 | - | - | - | - | - | 0.9 | 0.8 | - | 1.6 | 0.2 | - | - | - |
| **5** | 2-phenylbut-2-enal[a] | - | 0.5 | - | - | 9.9 | 0.3 | 0.4 | - | 9.4 | - | - | - | 14.6 | 10.5 | 15.7 | 11.2 | 0.5 | 5.6 | 7.3 | - | - | - |
| **C** | 2-phenylpent-2-enal | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **6** | 4-methyl-2-phenylpent-2-enal[a] | - | - | - | - | - | - | - | 0.5 | 3.6 | - | - | - | - | 1.3 | 2.3 | - | - | 4.8 | - | - | - | - |
| **7** | (*Z*)-5-methyl-2-phenylhex-2-enal | - | 1.7 | - | - | 3.3 | - | - | - | 3.9 | - | 0.2 | - | - | 2.2 | 6.8 | - | - | 11.6 | - | - | - | - |
| | (*E*)-5-methyl-2-phenylhex-2-enal | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Furan-2(5*H*)-one and potential precursors | | | | | | | | | | | | | | | | | | | | | | | |
| **B** | furan-2(5*H*)-one | 997.5 | 837.4 | 363.1 | 277.4 | 459.2 | 385.2 | 130.6 | 1977.2 | 743.9 | 423.4 | 820.5 | 4320.2 | 720.3 | 47.9 | 19.3 | 17.6 | 56.5 | 46.6 | 336.4 | 17.4 | 281.9 | 8.6 |
| / | furfuryl alcohol[a] | 798.7 | 737.4 | 499.0 | 56.7 | 48.6 | 28.3 | 19.5 | 597.9 | 85.2 | 79.6 | 104.5 | 780.2 | 494.7 | 20.0 | 6.2 | 2.8 | 5.9 | 12.6 | 80.1 | 2.8 | 105.3 | 3.4 |
| / | furfural[a] | 104.4 | 744.3 | 50.8 | 12.8 | 15.9 | 20.5 | 51.1 | 8.3 | 9.3 | 7.5 | - | 38.8 | 135.8 | 10.1 | - | - | - | - | 13.3 | - | 8.2 | - |

[a]Compounds quantified as internal standard equivalents; - = not detected.



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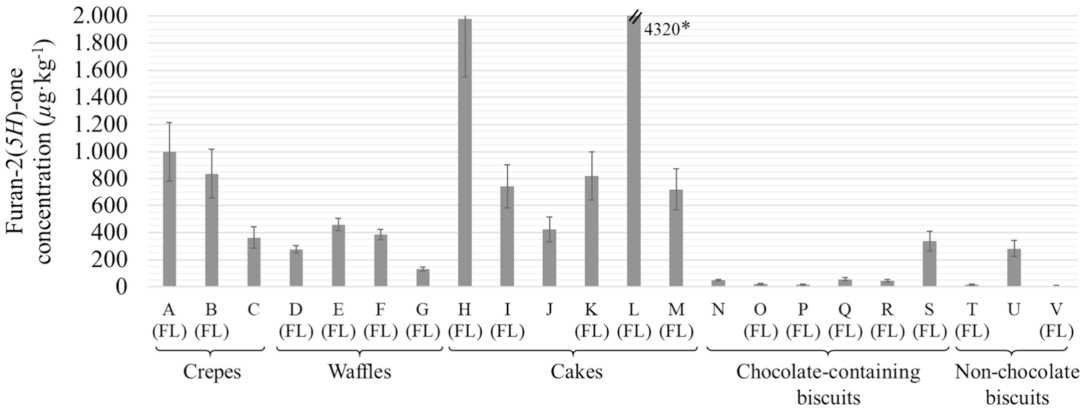


**Figure 12.** Furan-2(5*H*)-one level in commercial sweet snacks. "(FL)" stands for the mention of "flavoring(s)" (or more detailed) on the product labeling. Error bars represent confidence intervals at 95%. Asterisk (*): value above the maximum scale value.

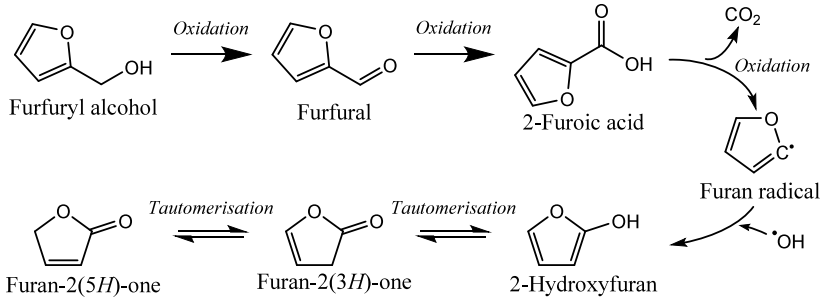


**Figure 13.** Proposed mechanism for the formation of furan-2(5*H*)-one from furfuryl alcohol or furfural.

no correlation was found between the furan-2(5*H*)-one level and the fat, protein, or carbohydrate content of the food products.

The structure of furan-2(5*H*)-one suggests a relation with Maillard products such as furfuryl alcohol or furfural. Interestingly, a positive correlation between furan-2(5*H*)-one and furfuryl alcohol levels was evidenced both within the crepe samples ($R^2$ = 0.95) and within all other food products combined ($R^2$ = 0.88), excluding two jam-filled food products (L and M). As depicted in Figure 13, the oxidation of either furfuryl alcohol or furfural (both naturally present or added as flavoring substances) may lead to furan-2(5*H*)-one through 2-furoic acid decarboxylation.[37] The exact mechanism and key factors responsible for the levels observed (especially in crepes A and B and cakes H, K, and L) are still under investigation.

In conclusion, a combinatorial synthesis allowed the investigation of 10 α,β-unsaturated carbonyls, including some of health concern, throughout the chocolate-making process and in a series of 22 commercial sweet snacks. Seven α,β-unsaturated aldehydes (**B**, **1**, **3**, **4**, **5**, **6**, and **7**) were already detected in unroasted beans. Their concentrations increased significantly during roasting, with a predominance of aldehydes with a phenyl group at the α position: 2-phenylbut-2-enal (**5**; up to 399.0 $\mu g \cdot kg^{-1}$), 5-methyl-2-phenylhex-2-enal (**7**; up to 215.7 $\mu g \cdot kg^{-1}$), and 4-methyl-2-phenylpent-2-enal (**6**; up to 24.2 $\mu g \cdot kg^{-1}$). Dry conching decreased their levels (from a total of 297 to 107 $\mu g \cdot kg^{-1}$ in chocolate from BH beans), while wet conching allowed their partial regeneration, with levels of **6** and **7** doubling to 7.8 and 51.3 $\mu g \cdot kg^{-1}$, respectively. Interestingly, pyrazines could be used as markers of their presence in chocolate. No α,β-unsaturated aldehyde levels did raise a health concern compared to current maximum use levels (2−5 $mg \cdot kg^{-1}$). Yet, working on the amount of available phenylalanine/phenylacetaldehyde in cocoa beans could probably make it possible to reduce their synthesis.[38] Lower levels were found in the 22 investigated sweet snacks, even when containing chocolate (dilution effect and/or less favorable aldol condensations through the process).

On the opposite, the highest levels of genotoxic furan-2(5*H*)-one (**B**) were found in commercial cakes (up to 4.3 $mg \cdot kg^{-1}$ vs ≤90 $\mu g \cdot kg^{-1}$ in cocoa or chocolate samples). Although correlations occured with the amount of furfuryl alcohol, complementary investigations are still required to evidence conditions critical to formation of this Maillard compound.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.4c01043.

Mass spectra data and retention time of the monitored α,β-unsaturated carbonyls (Table S1); theoretical and synthesized α,β-unsaturated aldehydes resulting from the aldol condensation of Strecker aldehydes and propanal in combinatorial synthesis (Table S2); calibration curves of the analytical standards (Figure S1); standard addition curve of furan-2(5*H*)-one (Figure S2) (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

**Alexandre Dusart** − *Unité de Brasserie et des Industries Alimentaires, Louvain Institute of Biomolecular Science and Technology (LIBST), Faculté des Bioingénieurs, Université catholique de Louvain, Louvain-la-Neuve 1348, Belgium; Department of Chemical and Physical Health Risks,*



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*Sciensano, Ixelles 1050, Belgium;* orcid.org/0000-0003-4944-5193; Email: alexandre.dusart@uclouvain.be

### Authors

**Julie Grosjean** − *Unité de Brasserie et des Industries Alimentaires, Louvain Institute of Biomolecular Science and Technology (LIBST), Faculté des Bioingénieurs, Université catholique de Louvain, Louvain-la-Neuve 1348, Belgium*

**Manon Autuori** − *Unité de Brasserie et des Industries Alimentaires, Louvain Institute of Biomolecular Science and Technology (LIBST), Faculté des Bioingénieurs, Université catholique de Louvain, Louvain-la-Neuve 1348, Belgium*

**Séverine Goscinny** − *Department of Chemical and Physical Health Risks, Sciensano, Ixelles 1050, Belgium*

**Sonia Collin** − *Unité de Brasserie et des Industries Alimentaires, Louvain Institute of Biomolecular Science and Technology (LIBST), Faculté des Bioingénieurs, Université catholique de Louvain, Louvain-la-Neuve 1348, Belgium;* orcid.org/0000-0002-1929-098X

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.4c01043

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### Notes

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*J. Agric. Food Chem.* XXXX, XXX, XXX−XXX