



Brewing and volatiles analysis of three tea beers indicate a potential interaction between tea components and lager yeast



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ARTICLE INFO

Article history:

Received 26 May 2015

Received in revised form 3 October 2015

Accepted 19 October 2015

Available online 19 October 2015

Keywords:

Tea beers

Comparison model

Triangular test

HS-SPME GC–MS

PCA

Interaction

Tea components

Lager yeast

ABSTRACT

Green tea, oolong tea and black tea were separately introduced to brew three kinds of tea beers. A model was designed to investigate the tea beer flavour character. Comparison of the volatiles between the sample of tea beer plus water mixture (TBW) and the sample of combination of tea infusion and normal beer (CTB) was accomplished by triangular sensory test and HS-SPME GC–MS analysis. The PCA of GC–MS data not only showed a significant difference between volatile features of each TBW and CTB group, but also suggested some key compounds to distinguish TBW from CTB. The results of GC–MS showed that the relative concentrations of many typical tea volatiles were significantly changed after the brewing process. More interestingly, the behaviour of yeast fermentation was influenced by tea components. A potential interaction between tea components and lager yeast could be suggested.

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1. Introduction

Beer is one of the most widely consumed alcoholic beverages in the West. Normal beer is made of barley malt, grains, water, yeast, hop (*Humulus lupulus* L.), and other raw materials. The volatile compounds of beer are extremely complex and are primarily derived from these raw materials by yeast fermentation (Riu-Aumatell, Miró, Serra-Cayuela, Buxaderas, & López-Tamames, 2014).

Volatile compounds are especially important to beer as they contribute to the quality of final product and have been extensively studied (Callemien, Dasnoy, & Collin, 2006; de Silva et al., 2012; Fritsch & Schieberle, 2005; Langos, Granvogl, & Schieberle, 2013; Saison, De Schutter, Delvaux, & Delvaux, 2009). The methods of extracting volatiles from beer include liquid–liquid extraction, stir bar sorptive extraction, headspace and headspace–solid phase microextraction (HS-SPME) (de Silva, Augusto, & Poppi, 2008;

Rossi, Sileoni, Perretti, & Marconi, 2014). HS-SPME is a simple and solvent-free sample preparation method. It can avoid contamination of nonvolatile compounds from samples by extracting and concentrating volatile compounds in headspace (Lv et al., 2012). In recent years, SPME has been widely used for the analyses of volatiles of beer (de Silva et al., 2008; Rossi et al., 2014), ranging from identification of specific groups of compounds, such as monophenols (Pizarro, Pérez-del-Notario, & González-Sáiz, 2010), terpenes (Takoi et al., 2010; Van Opstaele, De Rouck, De Clippeleer, Aerts, & De Cooman, 2010) and carbonyl compounds (Saison et al., 2009; Vesely, Lusk, Basarova, Seabrooks, & Ryder, 2003) to comprehensive characterisation of total volatiles (Langos et al., 2013; Rossi et al., 2014).

As an essential plant material, hop provides bitter taste and noble hop aroma for beer and therefore plays a key role in modern beer brewing (Aberl & Coelhan, 2012). Over 1000 compounds have been characterised in hop essential oil (Roberts, Dufour, & Lewis, 2004). Terpene alcohols are an important factor in hoppy aroma (Kishimoto, Wanikawa, Kono, & Shibata, 2006; Peacock, Deinzer, Likens, Nickerson, & McGill, 1981; Takoi et al., 2010).

On the other hand, tea (*Camellia sinensis*) is a popular aromatic beverage, and particularly in China. According to different

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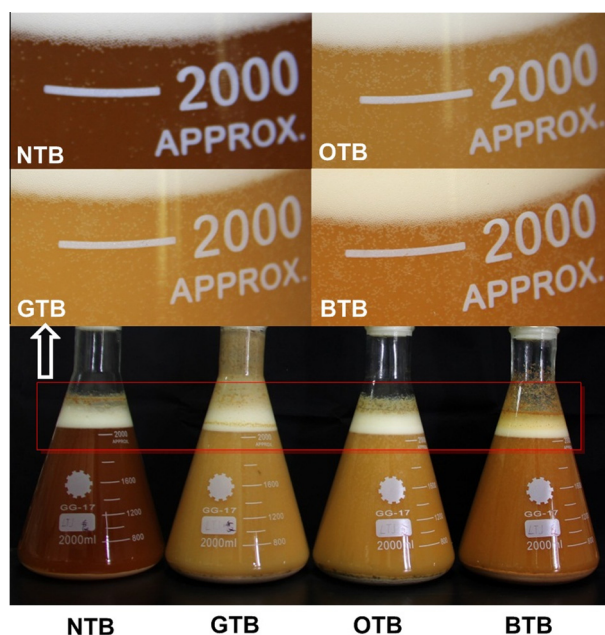


Fig. 1. The brewing of NTB and the three TBs (2.5 d).

manufacturing processes, Chinese commercial teas are classified into 6 categories, namely green tea, oolong tea, black tea, white tea, yellow tea and dark tea. Among them, the most popular teas are green tea (non-fermented), oolong tea (semi-fermented) and black tea (full-fermented). The volatile compounds, which are critical for flavour of tea, were reported to be significantly different among the three teas (Wang et al., 2008). In addition to the unique flavour, teas provide health benefits. The health functions are attributed to nonvolatile compounds in tea, such as polyphenols, caffeine and amino acids which possess antioxidant, antimicrobial, anti-carcinogenic, and hypolipidemic effects. In addition, these constituents contribute to some taste characteristics; e.g. hop contributes to bitterness of beer.

As tea is harmonious with hop in features of both taste and aroma, an integration beverage of tea and beer should be welcomed for its enhanced flavour and bio-functions.

In the present work, typical green, oolong, and black teas were separately introduced into wort to brew three kinds of tea beers (TBs, Fig. 1). A preliminary sensory evaluation showed a very special good flavour of TBs, which led to a supposition that the volatiles of tea in TB were influenced by brewing, i.e., the volatiles of TB were not the simple combination of volatiles in corresponding tea infusion and normal beer. Therefore, a simple but effective comparison model was designed to evaluate the supposition. The comparison was accomplished by triangular sensory evaluation test and HS-SPME gas chromatography–mass spectrometry (GC–MS) analysis methods.

2. Materials and methods

2.1. Raw materials and samples

Saaz hop was obtained from the Czech Republic. Three typical Chinese commercial tea samples (all made from leaves of *C. sinensis* var. *sinensis*) were chosen carefully from the market in China as follows: green tea (Huangshan Mao Feng), black tea (Keemun Black Tea), and oolong tea (Tie Guan Yin). The teas were stored at -20°C prior to beer brewing.

2.2. Brewing process

TBs were produced by a modified lager-type beer brewing method, with addition of selected teas mentioned above (Supplementary material). The wort was prepared, using commercially available two-rowed malts [mashed by manual grinder (Corona, China) for just stripping bran from barleycorn] according to the following mashing programme: 60 min at 52°C , 60 min at 62°C , and a further 30 min at 68°C . The mash was then heated to 76°C and filtered to yield wort, which was kept boiling for 90 min. Hops were added at the beginning of boiling (two thirds of total dosages) and at 10 min before the end of boiling (one third of total dosages). The teas were added together with the last dosage of hops, for independent test brewing of green tea beer (GTB), oolong tea beer (OTB), and black tea beer (BTB), respectively (Table 1). After cooling, the fermentation was started by adding 15.0×10^6 cells/ml of the lager yeast to each type of wort (12°P). The primary fermentation was carried out at 10°C for 5 days. Maturation was held at 12°C for 3 days and then at 0°C for 4 weeks. On the other hand, the brewing of non-tea beer (NTB, normal beer without tea) was carried out by a method similar to that mentioned above (Table 1).

2.3. Preparation of tea infusion (TI)

The three tea samples (green, oolong, and black teas, each 5 g) were added to 1000 ml of boiling water, respectively, and boiled for 10 min. After cooling on ice water, the supernatants were obtained as the green tea infusion (GTI), oolong tea infusion (OTI), or black tea infusion (BTI).

2.4. Design of the comparison model

A mixture of NTB and TI (1:1, v/v) was named as CTB (combination of TI and NTB). For comparison, an equivalent volume mixture of TB and water (TBW, 1:1, v/v) was prepared.

As the volatiles in TB were supposed to be not a simple combination of volatiles in corresponding TI and NTB, there should be significant differences between the volatiles of TBW and CTB, with regard to quality or quantity features. Therefore, in the designed model, each CTB versus TBW group was arranged to be compared by properties of both triangular sensory evaluation test and GC–MS analysis (Fig. 2).

Table 1
Conditions of brewing.

	Hop dosage 1 ^a (g/l)	Hop dosage 2 ^b (g/l)	Tea dosage ^b (g/l)
NTB	1	0.5	0
GTB	1	0.5	5
OTB	1	0.5	5
BTB	1	0.5	5

^a Added at the beginning of wort boiling.

^b Added at 10 min before the end of wort boiling.

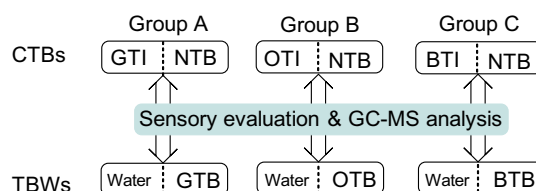


Fig. 2. The comparison model for sensory evaluation and GC–MS analysis.

2.5. Sensory evaluation

The triangular test (Lermusieau, Bulens, & Collin, 2001) was applied to assess aroma difference between CTB and TBW. In the test, 12 trained panellists were asked to smell the headspace of each sample. 50 ml aliquots of each sample solution were prepared in dark-glass cups. The significance of the difference of results was calculated.

2.6. Sample pretreatment and HS-SPME procedure

The exaction of the volatile compounds was carried out by the HS-SPME method, using a PDMS/DVB fibre, with 65 µm film thickness (Supelco, Bellefonte, PA, USA). The fibre was conditioned before initial use for 30 min in the GC injection port and preconditioned for 10 min before volatiles extraction.

Before the extraction, NTB and TBs were cooled on ice water and degassed in an ultrasonic bath for 8 min. First, 4 ml each TB sample and 4 ml distilled water were placed in a 20 ml glass vial with 3 g of sodium chloride, separately, as the TBW sample. 20 µl of 3-nonanone (98%, Heowns, Tianjin, China) were put into each glass vial as internal standard solution (21.1 µg/ml in 5% ethanol solution) (Goodner & Rouseff, 2010). Second, 4 ml of NTB and 4 ml of each TI were placed into a 20 ml glass vial (as CTB), respectively, together with 3 g of sodium chloride and 20 µl of 3-nonanone (Fig. 2).

The above glass vials were sealed and preincubated by stirring, using a magnetic rotor at controlled temperature. The SPME fibre was exposed to the sample headspace in a 30 °C oven while the sample was continuously stirred for 60 min (Supplementary material). After the absorption, the extracted analytes were immediately desorbed in the injection port of a GC at 230 °C. The fibre was kept in the GC injector for 15 min to guarantee total desorption and avoid inter-run carryover (de Silva et al., 2008). All analyses were triplicated.

2.7. GC–MS analysis

GC–MS analysis was used for identification and semi-quantification analysis, and was done with an Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometry (Agilent Technologies, Wilmington, DE, USA). The GC instrument was equipped with an HP-5MS capillary column or DB-FFAP (both are 30 m × 0.25 mm i.d., 0.25 µm film thickness) (Agilent, Santa Clara, CA, USA). Ultra high purity grade helium was used as the carrier gas, with flow of 1.0 ml/min. The injector was equipped with a 0.75 mm internal diameter liner (Supelco, Bellefonte, PA, USA). Injection mode was splitless and injection port temperature was 230 °C. For all the analyses on the HP-5MS column, the oven temperature was programmed to 35 °C for 5 min, then at 4 °C/min to 130 °C, held for 3 min, and raised at 5 °C/min to 230 °C and held for a further 3 min. For the analysis on a DB-FFAP column, the oven temperature was programmed to 40 °C for 2 min, then at 4 °C/min to 230 °C and held for 10 min.

The MS detector was at 70 eV in electron impact mode, and scan range was from 35 to 350 amu. Identification of the detected peaks was carried out, using the automated mass spectral deconvolution and identification system (AMDIS) software coupled to the NIST 11.L mass spectrometry database (NIST, Washington, DC, USA) and confirmed by retention indices (RIs). RIs of the volatiles were calculated, using the C₈–C₂₀ n-alkane (Sigma–Aldrich, St. Louis, MO, USA) (HP-5MS column), or C₈–C₂₅ n-alkane (o2si, Charleston, SC, USA) (DB-FFAP column) standards. The relative content of each volatile compound was calculated by means of the ratio of the volatile compound peak area to the internal standard peak area

in select ion monitoring (SIM) mode. The semi-quantification was based on the results of GC–MS analysis on HP-5MS.

2.8. Date analysis

The experiments were performed in triplicate. A T-test was used for analysis of the differences between TBW and CTB. A difference was considered significant when $P < 0.05$. Principal component analysis (PCA) was performed, using SIMCA-P12.0 software (Umetrics, Umeå, Sweden).

3. Results and discussion

3.1. General

A NTB, and three kinds of TBs, namely GTB, OTB and BTB, were brewed independently in the present work. A comparison model (TBW versus CTB) was designed for both the triangular sensory evaluation test and GC–MS analysis.

3.2. Sensory evaluation

Significant differences were clearly demonstrated between CTBs and TBWs in each group (A, B, C) (Table 2). The results showed that the volatiles of TB were not a simple combination of volatiles from tea and beer.

3.3. Identification and quantification of volatile compounds in the TBWs and CTBs

3.3.1. General

The volatile compounds of TBWs and CTBs were analysed by HS-SPME GC–MS (Figure S1). The qualitative and quantitative differences among all samples were analysed. As shown in Table S1, in total, 84 volatile components, including 16 terpenes, 27 esters, 15 volatile higher alcohols, 2 phenolic compounds, 11 aldehydes, 6 ketones, 3 aromatic hydrocarbons, 1 acid and 3 miscellaneous compounds, were identified in TBWs and CTBs. Among them, 67 and 81 volatile compounds were found from three TBWs and three CTBs, respectively. In addition, the relative contents of most of the volatile compounds were significantly different between TBWs and CTBs.

3.3.2. Terpenes

Terpenes, especially linalool, geraniol and citronellol, are important aroma compounds contributing to flowery flavour for normal beer and tea (Kishimoto et al., 2006; Lam, Foster, & Deinzer, 1986). Fifteen terpenes, including 6 terpene hydrocarbons, 5 terpene alcohols, 2 terpene alcohol oxides and 2 other terpenes were identified in all these samples.

Compared to the three CTBs, the contents of some monoterpene alkenes, namely β -myrcene, limonene and β -ocimene were lower in the corresponding TBWs (Table S1).

The contents of most of the oxygenated terpenoids were not significantly different ($P > 0.05$) between TBWs and CTBs, except

Table 2
Rate of correct answer in triangular tests.

Group	Comparison ^a		Rate of corrections	
	CTBs	TBW		
A	NTB + GTI	vs	GTB + Water	12/12 ^b
B	NTB + OTI	vs	OTB + Water	12/12 ^b
C	NTB + BTI	vs	BTB + Water	11/12 ^b

^a The sample was made of 50% NTB and 50% TI (CTB), or 50% TB and 50% water (TBW).

^b $P < 0.05$.

for citronellyl acetate. The content of citronellyl acetate in TBWs was much higher ($P < 0.05$) than that in the corresponding CTBs, especially in the BTB group (Group C). A potential biotransformation route of some monoterpene alcohols by yeast fermentation was proposed previously (Fig. 3) (King & Dickinson, 2000, 2003). In those studies, geraniol was mainly metabolized to citronellol. Both citronellol and geraniol could be further transformed to their acetate esters by lager yeast. Geranyl acetate can also be transformed to citronellyl acetate.

Surprisingly, the content of geraniol of TBW was significantly higher than that of CTB in Group A (green tea group, $P < 0.05$). Green tea is a non-fermented tea that contains abundant important aroma precursors (Wang, Kurasawa, Yamaguchi, Kubota, & Kobayashi, 2001; Wang, Yoshimura, Kubota, & Kobayashi, 2000). The increase of geraniol was considered to be caused by glycosidic hydrolysis in GTB (Daenen, Sterckx, Delvaux, Verachtert, & Derdelinckx, 2008). On the other hand, many flavour precursors of oolong tea and black tea were hydrolysed during tea processing, as they are in semi- and full-fermented tea, respectively (Wang, Kurasawa et al., 2001). Therefore, many volatile compounds may be released into wort during boiling, and decreased during yeast fermentation. As shown in Group C (Table S1), the content of geraniol was significantly lower, while both citronellol and its acetate were significantly higher in TBW, compared with those in CTB. This might be caused by fermentation of yeasts, as shown in Fig. 3. In BTB, geraniol was mainly converted to a relatively high quantity of citronellol, which was further transformed to citronellyl acetate by esterification during yeast fermentation. Hence, citronellol and citronellyl acetate could be highly accumulated in BTB.

Nerolidol is one of the principal aroma compounds in oolong tea (Lin et al., 2013). In group B, the content of nerolidol was high in both TBW and CTB samples. But the statistical analysis still showed a significant difference between the two samples (Table S1).

As an important volatile compound, jasmine lactone provides jasmine-like floral and fruity odours for oolong tea (Wang, Kubota, Kobayashi, & Juan, 2001; Wang et al., 2000). In group B, the content of jasmine lactone in TBW was significantly lower than that in CTB.

3.3.3. Esters

Esters are the most important compounds in beer aroma (Pires, Teixeira, Brányik, & Vicente, 2014; Riu-Aumatell et al., 2014). Among them, ethyl acetate (solvent-like aroma), isoamyl acetate (banana aroma), isobutyl acetate (fruity aroma), phenylethyl acetate (roses and honey aroma), ethyl hexanoate (sweet apple aroma), and ethyl octanoate (sour apple aroma) are the most important aroma compounds in lager beer (Verstrepen et al., 2003). In this

research, a total of 27 esters was found in all samples (Table S1). The contents of these esters in TBWs were significantly different from those in CTBs.

In group A (Table S1), ethyl octanoate showed the highest relative concentration in TBW, followed by isoamyl acetate, ethyl hexanoate, ethyl 9-decenoate, ethyl acetate and β -phenylethyl acetate. Compared with CTB, the amounts of isoamyl acetate, ethyl acetate, ethyl hexanoate and ethyl decanoate were significantly lower ($P < 0.05$) in TBW of group A. By contrast, the content of ethyl octanoate in TBW was significantly higher ($P < 0.05$) than that of CTB in this group. Similarly, as shown in groups B and C, ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, β -phenylethyl acetate, ethyl 9-decenoate and ethyl decanoate were major ester compounds in OTB and BTB. In both groups B and C, the contents of ethyl octanoate, ethyl 9-decenoate and ethyl decanoate were significantly higher, while ethyl acetate, isoamyl acetate and ethyl hexanoate were significantly lower in TBWs compared to CTBs. These esters are important contributors to the flavour of the final beer (Verstrepen et al., 2003).

3.3.4. Volatile higher alcohols (VHAs)

Fifteen VHAs were identified in three groups. VHAs are important organoleptic components present in beer. Though VHAs are few in tea (Lin et al., 2013; Schuh & Schieberle, 2006), they could be formed by yeast metabolism via the Ehrlich pathway in beer (Pires et al., 2014). In lager beer, the most important VHAs were propanol, isobutanol, isoamyl alcohol, amyl alcohol and phenylethyl alcohol (Pires et al., 2014; Riu-Aumatell et al., 2014). In our results, isoamyl alcohol, 2-methylbutanol and phenylethyl alcohol, which have the associated flavour notes of malty, solvent and flowery, respectively (Langos et al., 2013), were found in high quantities in all TBWs and CTBs (Table S1). In addition, the contents of the above three VHAs in TBWs were all significantly lower than those in the corresponding CTBs ($P < 0.05$).

3.3.5. Phenolic compounds

Methyl salicylate is an important aroma compound in tea, especially in black tea. The compound imparts sweet, spicy and minty notes for tea (Wang et al., 2008). However, as far as we know, there is no report of methyl salicylate from beer yet. Hence, methyl salicylate in TBs was considered to be derived from teas (Table S1). In groups A and B, the content of methyl salicylate in TBWs was significantly higher ($P < 0.05$) than that in CTBs. This could be explained by glycosidic hydrolysis by yeast fermentation in brewing (Daenen et al., 2008), as methyl salicylate occurs in non- or semi-fermented tea in glycoside form (Wang, Kubota et al., 2001; Wang et al., 2000). But, in black tea, most of the methyl salicylate glycoside is hydrolysed during tea processing (Wang, Kurasawa et al., 2001). Therefore, the contents of the methyl salicylate in group C, though was far more than those in groups A and B, were not significantly different between TBW and CTB.

Another phenolic compound, 2-methoxy-4-vinylphenol, was identified in the samples. The compound plays an important role in the flavour profile of wheat beer, and contributes to wheat beer flavour with clove-like, phenolic odour notes (Langos et al., 2013). The compound was suggested to be a degradation product of ferulic acid by a thermal effect and yeast fermentation in brewing (Coghe, Benoot, Delvaux, Vanderhaegen, & Delvaux, 2004). In groups A and B, the content of 2-methoxy-4-vinylphenol in TBWs was significantly higher than that in CTBs. The results suggested a potential enhancement of green or oolong tea contribution to decarboxylase activity of lager yeast.

3.3.6. Aldehydes/ketones

Aldehydes and ketones are important aroma compounds in tea. Benzaldehyde, *trans*-2-hexenal and methyl-5-hepten-2-one, are

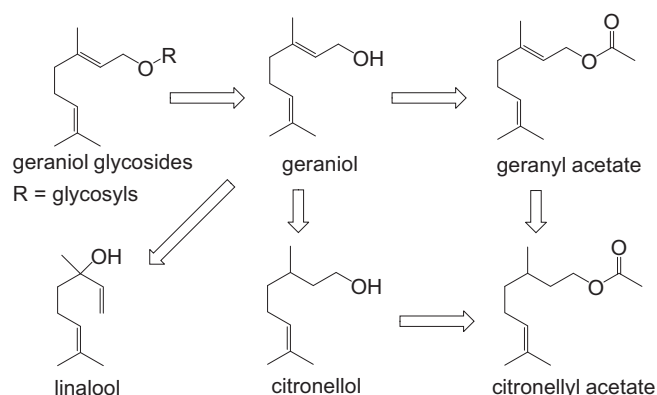


Fig. 3. Biotransformation of some monoterpene alcohols and their derivatives by yeast fermentation.

able to discriminate unfermented and fermented teas (Wang et al., 2008). Benzaldehyde and benzeneacetaldehyde have odour attributes of almond and sweet. β -Ionone is one of the most important aroma-active compounds in tea, contributing significantly to the woody flavour (Schuh & Schieberle, 2006).

In this work, 11 aldehydes and 6 ketones were detected. The aldehydes/ketones in TBWs were significantly lower than those in CTBs in terms of both variety and quantity (Table S1).

3.3.7. Acids/aromatic hydrocarbons

One acid, namely octanoic acid, was detected in all the six samples. The content of octanoic acid was significantly lower in TBWs than in CTBs (Table S1). Since octanoic acid (sweaty, goat-like) corrupts beer flavour with rancid notes (Langos et al., 2013), the introduction of tea into fermentation might promote the quality of beer.

In total, three aromatic hydrocarbons were detected (with very low contents) in these samples. Isopropenylbenzene and styrene were not found in any TBs.

3.3.8. Miscellaneous compounds

In the present study, three miscellaneous compounds, namely benzyl nitrile, N-ethyl pyrrole and indole, were identified only in a few samples (Table S1).

Among them, indole is an important aroma compound in oolong tea, generated in the processing of the tea (Wang, Kubota et al., 2001; Wang et al., 2008). The CTB of oolong tea had a relatively abundant amount of the compound (Table S1). It is interesting that indole was not detected in TBW of oolong tea (Group B). Hence, the compound was suggested to be metabolized by yeast during brewing.

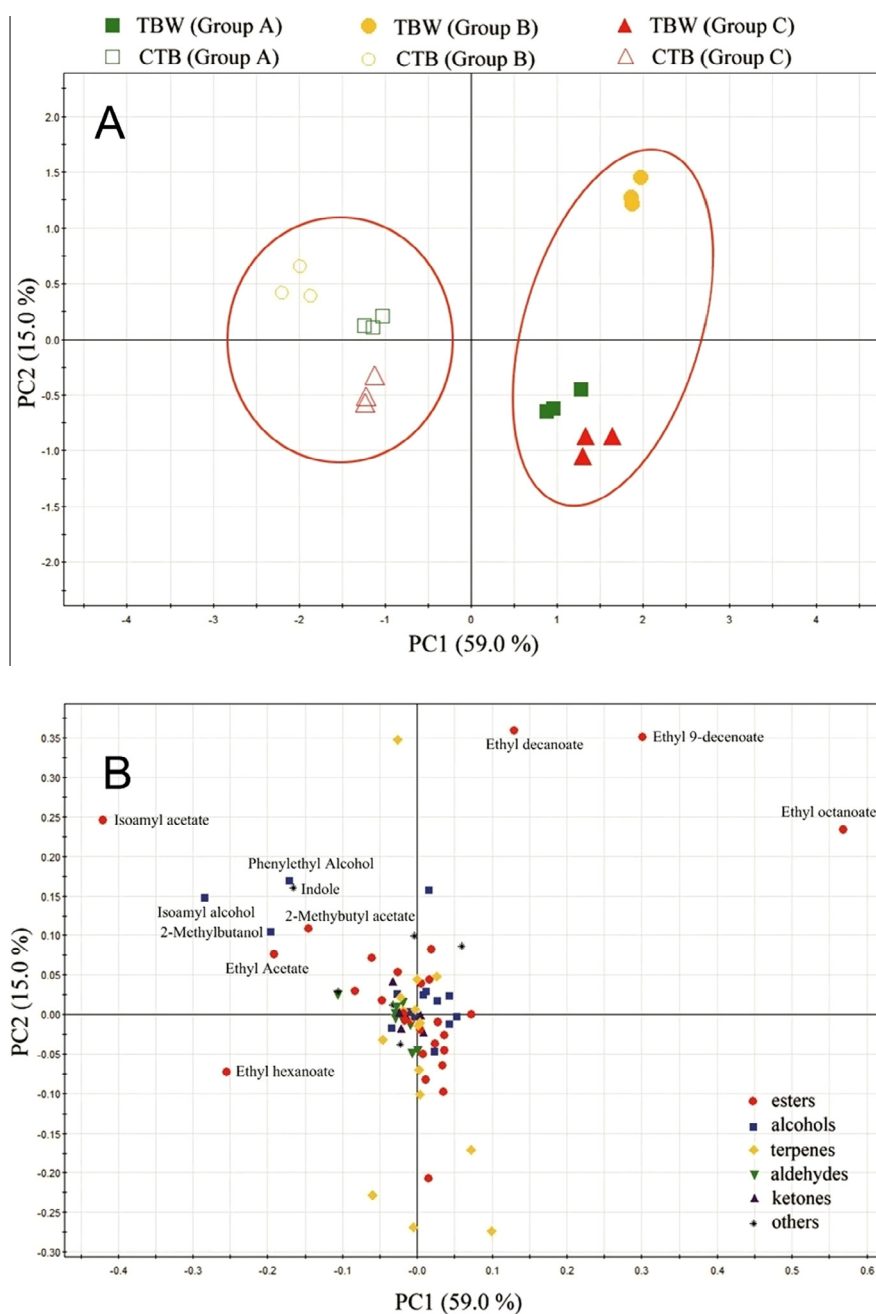


Fig. 4. PCA score plots (A) and loading plots (B) of TBWs and CTBs.

3.4. PCA

As an unsupervised statistical model, PCA is a data reconstruction and dimensional reduction method by means of principal components (PCs) (Chen, Zhao, Chen, Lin, & Zhao, 2011). Each PC is from a linear transformation of original characteristic variables. Hence, PCA could not only obtain useful information and eliminate the redundant information, but also acquire visual cluster trends of these samples and establish relationships between samples and variables by utilising score and loading plots (Lv et al., 2014).

In this work, PCA was performed by analysis of GC–MS data (HP-5MS column) by SIMCA software. In the score scatter plots (Fig. 4A), 74.0% of the raw data were explained by the first two PCs (PC1 and PC2). The score plots clearly demonstrated the profiles of TBW and CTB samples. PC1 showed the difference between the volatiles of CTBs and TBWs, which displayed negative and positive values on PC1, respectively. Therefore, introduction of tea into beer brewing led to a significant variation of the original aroma in beer or tea. The volatiles of TB were not formed by simple combination of TI and NTB volatiles. In addition, PCA loading plots were applied in order to identify key compounds that contributed to the difference between TBWs and CTBs (Fig. 4B). Though SPME–GC–MS is not a perfect method to accomplish absolute quantitative analysis without standards of each compound, the loading plots nevertheless provided some interesting volatile profiles of TBWs and CTBs (Fedrizzi et al., 2011; Inui, Tsuchiya, Ishimaru, Oka, & Komura, 2013; Lv et al., 2014). The key volatile compounds, which have higher positive/negative values in loading plots, made significant contributions to distinguish samples. As shown in Fig. 4B, the key volatile compounds seemed to be 7 esters (ethyl octanoate, ethyl 9-decanoate, ethyl decanoate, isoamyl acetate, ethyl acetate, ethyl hexanoate, and 2-methylbutyl acetate), 3 VHAs (isoamyl alcohol, phenylethyl alcohol, and 2-methylbutanol) and indole. Among them, ethyl octanoate, ethyl 9-decanoate, and ethyl decanoate were identified as the characteristic volatiles of TBWs. While other key compounds, namely isoamyl acetate, ethyl acetate, ethyl hexanoate, 2-methylbutyl acetate, isoamyl alcohol, phenylethyl alcohol, 2-methylbutanol and indole, were identified as the characteristic volatiles of CTBs.

4. Conclusion

The present work designed a simple but effective comparison model (Fig. 2), which provided an equal matrix for samples of TBW and CTB, and was crucial for the following sensory test and GC–MS analysis.

The results showed that both relative contents and varieties of most of the typical volatile compounds of teas were significantly different between TBWs and CTBs. Among these compounds, the concentration differences of monoterpene alkenes, jasmine lactone and aldehydes/ketones, between TBW and CTB, might be caused by absorption of hydrophobic yeast biomass (King & Dickinson, 2003), or even by evaporation during the long brewing process due to their structural properties of low polarity and low boiling point. The other typical tea volatile compounds, including methyl salicylate, indole, especially geraniol and its derivatives (Fig. 3), were modified by yeast fermentation during brewing.

The contents of the typical metabolites of lager yeast, namely, esters and VHAs (Table S1, Pires et al., 2014), were also significantly different between TBWs and CTBs. These results generated a speculation that the fermentation behaviour of yeast might be affected by tea compositions, which was echoed by an interesting phenomenon that, the foam appearances (both gas generation speed and foam level) were significantly different between NTB and TBs (Fig. 1, Supplementary material). Therefore, the present

work may indicate an interesting interaction between tea components and lager yeast, which should be responsible for the characteristic flavour of TBs.

TB is a good juncture of western and oriental culture. Some parameters, such as quality guarantee period should be investigated before the launch of the TB industry. Furthermore, there are many other kinds of commercial teas, namely, white tea, yellow tea and dark tea, which are also of potential for invention of new TBs products. TBs will be welcomed in future, not only for their special good flavour, but also because of the combined functional compounds from tea, hops and malt/wheat. In addition, the present work also suggests some fundamental work for TBs. In the next stage of TBs R&D, the interesting potential effects of tea compounds on yeast fermentation will be studied, and the generation mechanism of some crucial volatiles for the unique flavour of TBs. These aroma compounds should be very important for the special flavour of TBs, and deserve a more extensive investigation. Furthermore, the present work not only suggests a good solution for aroma investigation of other new complex combined beverages, but also implies a potential interesting future for research of the nonvolatile compounds in TBs, which may include, not only the derivatives of tea polyphenols or theanine, but also the non-volatile compounds derived from tea volatiles in the brewing process. As a new branch of brewing chemistry, the investigation will help to understand the generation mechanism of the special TB flavour.

Acknowledgments

We thank Mr. Yi-Min Wu, Technical Department of China Resources Snow Breweries (Anhui) Co. Ltd., Hefei, PR China, for advice and organization of sensory evaluation, and Dr. Jing-Tian Ni, Life School of Anhui Agricultural University, for technical advice in beer brewing. This work was supported by the National Modern Agriculture Technology System (CARS-23), Anhui Major Demonstration Project for Leading Talent Team on Tea Chemistry and Health, and National Natural Science Foundations of China (Grant No. 31101335, No. 31471720), as well as Anhui Scientific and Technological Project (1406C085017).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2015.10.088>.

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