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Characterization of taste and aroma compounds in *Tianyou*, a traditional fermented wheat flour condiment

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ABSTRACT

Taste and aroma compounds in *Tianyou* were determined using HPLC and GC–MS/GC-olfactometry. By comparison with light soy sauce (control), the contents of salt, sugar, total nitrogen and total acid in *Tianyou* were higher, while the contents of umami, sweet and bitter free amino acids, and the percentage of 1–5 kDa peptides in *Tianyou* were lower. Thirty-one aroma-active compounds were identified in both *Tianyou* and the control (30 compounds in common). Aroma extraction dilution analysis indicated that most flavor dilution factors of aroma-active compounds were lower in *Tianyou* than the control. Quantitative descriptive analysis showed that *Tianyou* had significantly stronger salty and sweet tastes, weaker umami taste and weaker malty, caramel-like and smoky notes when compared to the control ($p < 0.05$), which were in agreement with the analyses of taste and aroma compounds. This confirms that *Tianyou* has a distinctively different flavor from light soy sauce.

1. Introduction

Tianyou, which originated from Jiangsu province in the Ming dynasty, is a fermented wheat flour condiment and is widely used as a dipping sauce and in Chinese-type salads, soups and varieties of other dishes in Jiangsu province, China (Xie, 2012). Foods with light flavor have become a growing trend, thus, *Tianyou* is becoming more popular due to its light and soy sauce-like flavor. In fact, *Tianyou* had been listed as part of Xuzhou city's (Jiangsu, China) intangible cultural heritage owing to its high popularity and potential commercial and cultural values (Xie, 2012).

Tianyou is often mistakenly regarded as a type of light soy sauce. Indeed, both are made from the same kinds of raw materials (wheat flour, soybean, edible salt and water) and fermentation strains (i.e., *Aspergillus oryzae* and yeast). However, the manufacturing processes and raw materials ratio (wheat flour/soybean) are different (Feng et al., 2013; Xie, 2012). As shown in Fig. 1, the manufacturing process for *Tianyou* is mainly composed of preparations of soybean *koji* and wheat flour *koji* (solid state fermentation), brine preparation, moromi fermentation (liquid state fermentation) and raw *Tianyou* extraction, sterilization and bottling. However, no wheat flour *koji* is used during soy sauce preparation. Furthermore, a high ratio of wheat flour *koji* to soybean *koji* (> 3:1, w/w) is used to prepare *Tianyou*, while only a small quantity of wheat flour is used to supply the energy for the growth of fermentation strains during soy sauce *koji* preparation (Feng et al., 2013; Xie, 2012).

Soybean comprises approximately 40% protein, 17% lipid and 18% carbohydrate, while wheat flour contains approximately 11% protein, 1.9% lipid and 72% carbohydrate. Furthermore, the compositions of amino acids and carbohydrate are also different (Eldridge, Black, & Wolf, 1979; Grieshop & Fahey, 2001; Nandini & Salimath, 2001; Prabhasankar & Rao, 2001). The degraded products of protein, lipid and carbohydrate, i.e. free amino acids (FAA), unsaturated fatty acids and monosaccharide, are flavor compounds and/or the source of aroma-active compounds (Feng et al., 2013; Lee, Seo, & Kim, 2006). For example, 2-methylbutanal and 3-methylbutanal originate from leucine and isoleucine, respectively (Hall, Andersson, Lingnert, & Olofsson, 1985; Hartman, Scheide, & Ho, 1984). Thus, *Tianyou* should be considered a distinctive fermented condiment.

Although *Tianyou* has gained in popularity in East China, elsewhere in China *Tianyou* is still mistaken for light soy sauce which inhibits its sales. Thus, further elucidation of the taste and aroma characteristics of *Tianyou* may help consumers understand that *Tianyou* is different. A large number of investigations concerning the flavor characteristics and chemical compounds of soy sauce have been reported (Feng et al., 2014; Gao et al., 2011; Kaneko, Kumazawa, & Nishimura, 2013; Lee et al., 2006; Lioe, Wada, Aoki, & Yasuda, 2007; Steinhaus & Schieberle, 2007; Wanakhachornkrai & Lertsiri, 2003). More than 300 chemical

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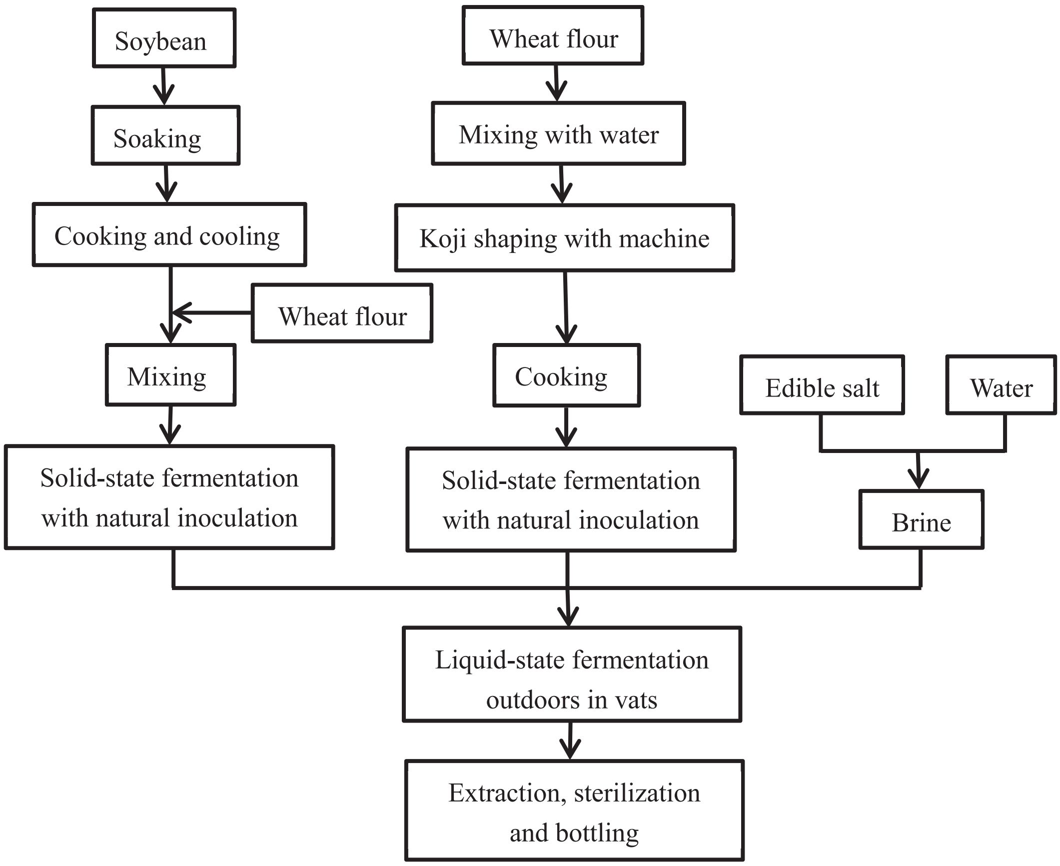

Fig. 1. Schematic diagram of the *Tianyou* manufacturing process.

compounds in soy sauce have been identified including dozens of aroma-active compounds and key taste compounds (FAA and 1–5 kDa peptides). In this study the characteristic taste and aroma compounds in *Tianyou* were evaluated using light soy sauce as the logical control to show the differences between the two. This work should also help *Tianyou* producers improve and control the flavor quality.

Thus, the objectives of this work were to (i) determine and identify the key taste and aroma compounds in *Tianyou*, and (ii) characterize the taste and aroma of *Tianyou*.

2. Materials and methods

2.1. Materials and chemicals

Tianyou (Xinyi Longjiao Food Co., Ltd., Xinyi, China) and light soy sauce (Foshan Haitian Flavoring & Food Co., Ltd., Foshan, China) were purchased from a local supermarket (Zhenjiang, China).

3-Methylbutanal, 2-methylbutanal, 3-methylbutanoic acid, 3-methylthiopropanal, benzeneacetaldehyde, ethyl propanoate, ethyl 3-methylbutanoate, 2-phenylethyl acetate, 2-phenylethanol, 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone, (*E*)-β-damascenone, acetic acid, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 4-ethylphenol, 2-acetyl-1H-pyrroline, phenylacetic acid, 2-ethylbutyric acid (internal standard) and 2-methyl-3-heptanone (internal standard) were purchased from Sigma–Aldrich (Shanghai, China). 2,3-Butanediol (*meso*), 3-methylbutanol, 2-methylbutanol, 1-octen-3-ol, 2-methylpropanoic acid, butanoic acid, ethyl acetate, methyl benzoate, 2-furanmethanol, 4-ethyl-2-methoxyphenol, ethanol, benzoic acid, 2-methoxyphenol, 2-methoxy-4-vinylphenol, 3-methylthiopropanol, 2-ethyl-3-methylpyrazine and C_6–C_{33} n-alkanes were obtained from Aladdin Holdings Group (Shanghai, China). Other chemicals used in this work were of the highest commercial grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Proximate analysis

Total solids content was determined using AOAC method 990.2 (AOAC, 2000). Salt content (expressed as NaCl, g/100 mL) was determined using a volumetric titration with $AgNO_3$ using Mohr's method (Hamilton & Simpson, 1964). Non-salt soluble solids content (g/100 mL) was calculated as the total solids content (105 °C until constant weight) minus salt content. Contents of total nitrogen (using 6.25 as the conversion factor), total sugar and reducing sugar in samples were measured according to AOAC methods 992.23, 925.35 and 923.09, respectively (AOAC, 1995). Formaldehyde nitrogen and total titratable acidity were measured using titration methods (Jiang, Zeng, Zhu, & Zhang, 2007). Twenty mL of diluted sample (20-fold dilution with distilled water, w/v) was mixed with 60 mL of distilled water and titrated to pH 9.6 with 0.05 M NaOH, which was used to determine the total titratable acid. Then, 10 mL of formalin solution (37%) was added and the amount of NaOH needed to return the solution to pH 9.2 was used to determine the formaldehyde nitrogen.

2.3. Determination of molecular weight distribution of water soluble peptides

Prior to analysis, each sample was diluted 10-fold with distilled water, then filtered through a micropore filter with 0.45 μm pore size (Sangon Biotech, Shanghai, China). The molecular weight distribution of the water soluble peptides in the samples was determined using gel permeation chromatography on a Superdex peptide 10/300 GL column (Amersham Biosciences Corp., Piscataway, NJ, USA) with UV detection at 214 nm. Elution was done using an isocratic procedure with 0.02 M sodium phosphate buffer (pH 7.2) containing 0.25 M NaCl at a flow rate