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Changes in fatty acid composition and lipid profile during *koji* fermentation and their relationships with soy sauce flavour

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ABSTRACT

Evolution of lipids during *koji* fermentation and the effect of lipase supplementation on the sensory properties of soy sauce were investigated. Results showed that total lipids of the *koji* samples were in the range of 16–21%. The extracted lipid of initial *koji* consisted mainly of triacylglycerols (TAGs, >98%), followed by phospholipids (PLs), diglycerides (DAGs), monoacylglycerols (MAGs) and free fatty acids (FFAs). As the fermentation proceeded, peroxide value of the lipids decreased while carbonyl value increased ($p < 0.05$). Linoleic acid was utilised fastest according to the fatty acid composition of total lipids, and preferential degradation of PLs to liberate FFAs was also observed. Moreover, phospholipase supplementation had significant influence on the sensory characteristics of soy sauce, especially enhanced ($p < 0.05$) scores for the umami and kokumi taste attributes. All these results indicated that the control of PLs utilisation during fermentation was a potential method to improve soy sauce's characteristic taste.

1. Introduction

Soy sauce is used as a condiment or seasoning sauce around the world due to its characteristic aroma accompanied by the intense umami taste. Chinese traditional soy sauce involves two important fermentation stages, *koji* fermentation and *moromi* fermentation (Gao et al., 2009). Previous studies showed that the evolution of volatile components largely depended on lipid oxidation in the *koji* fermentation stage (Feng et al., 2013). In addition, soy sauce aroma was significantly influenced by the content of lipids in the raw materials (Gao et al., 2009). Thus, influence of 20% fat in soybean, the main material, on the flavour of traditional Chinese-type soy sauce is worthy of investigation.

Lipolysis, release of fatty acids, and secondary reactions of fatty acids resulted in the development of some volatile substances, such as aldehydes, ketones and alcohols, which are responsible for the characteristic aroma of fermented foods (Gambacorta et al., 2009; Visessanguan, Benjakul, Riebroy, Yarchai, & Tapingkae, 2004). It has been reported that lipolysis and lipid oxidation make significant contributions to the thickness, mouthfulness and continuity taste in *migaki-nishin*, a dried fish product (Azad Shah, Tokunaga, Kurihara, & Takahashi, 2009). Moreover, evolution of the lipid in some fermented soya products has been proved to be the main factor in forming aroma, such as natto, Japanese miso and Indonesian tempe (De Reu, Ramdaras, Rombouts, & Nout, 1994; Kiuchi, Ohta, Itoh, Takahayahsi, & Ebine, 1976; Sarkar, Jones, Gore, Craven, & Somerset, 1996; Shieh, Beuchat, Worthington, & Phillips, 1982). Much literature has concentrated on degradation of proteins (Chou & Ling, 1998; Gao et al., 2011; Lertsiri, Maungma, Assavanig, & Bhumiratana, 2001), and umami peptides (Lioe et al., 2004) of soy sauce. However, little attention has been paid to the changes of lipid and its relationship with volatile compounds during the *koji* fermentation of soy sauce.

Therefore, the aim of this study was to investigate the changes in lipid compositions through the *koji* fermentation, and to evaluate their potential effects on the characteristic flavour of soy sauce. Furthermore, the effects of adding exogenous lipase on the sensory quality of soy sauce were described, in order to explore the relationship between lipolytic and sensory parameters of soy sauce. Results from this study would make us better understand the relationships between lipid compositions in soybeans and the flavour of soy sauce.

2. Materials and methods

2.1. Materials and chemicals

Soybean (Guanghui Agricultural Products Co., Ltd., Heilongjiang, China), wheat flour (Runfon Flour Co., Ltd., Guangdong, China) and

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edible salt (Zhongshan Salt Industrial Co., Ltd., Zhongshan, Guangdong, China) were purchased from a local market. *Aspergillus oryzae* HN3.042 spores were obtained from Jiaming Fermentation Food Co., Ltd. (Shanghai, China). Phospholipase (Lecitase® ultra) and Lipozyme IM 4350 were purchased from Novozymes (Copenhagen, Denmark). Heptadecanoic acid, methyl ester was purchased from Sigma Co., Ltd. (St. Louis, MO). Chloroform, *n*-hexane and isopropanol were of the highest commercial grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of samples

Two kilograms raw soybeans were soaked in 4000 mL water for 8 h at room temperature, and steamed at 125 °C for 15 min. Wheat flour and steamed soybean were mixed at a ratio of 1:4 (w/w) and cooled to 40 °C. The mixture was then inoculated with 0.05% (w/w) of *A. oryzae* HN 3.042 spores, and incubated at 30 °C for 16 h and 28 °C for another 32 h. The relative humidity was about 95% during *koji* fermentation. The preparation of *koji* was completed when the colour of the culture began turning a greenish yellow. Five soy sauce *koji* samples were periodically taken at 0, 12, 24, 36 and 44 h during the *koji* fermentation. All the samples were ground and held at −18 °C until analysis.

A primary supplementation test was conducted to further clarify the effect of metabolism of lipids on the flavour of soy sauce. Lipase (IM4350, Novozymes, 0.05% koji weight) or phospholipase (Lecitase® ultra, 0.03% koji weight, maintaining the same enzymatic activity) was mixed with *koji* to yield *moromi*, and the main processes of soy sauce were made according to the method of Gao et al. (2011). The fermented soy sauce samples were taken on the 6th month. All samples were filtered through filter papers (18-cm diameter, Shuangquan Co. Ltd., Hangzhou, China), then stored in a refrigerator at −18 °C until analysis.

2.3. Chemical analysis

Moisture and lipid contents of the *koji* were determined according to AOAC (2000). To determine pH value, 5 g of each sample were homogenised with 10-fold volume of distilled water and the pH was measured directly by using PHS-3E pH meter (TecFront Electronics Co. Ltd., Shanghai, China).

2.4. Lipid extraction

Total lipids were extracted from the *koji* samples with a solvent mixture of chloroform:methanol (2:1, v/v) according to the method of Azad Shah et al. (2009). The extracts were dried under vacuum on a rotary evaporator and finished with a nitrogen flow. The lipid samples were stored under nitrogen gas in the dark at −18 °C until further analysis.

2.5. Measurement of lipid oxidation

Acid value and peroxide value of the extracted lipid were determined according to AOAC (2000). Acid value was analysed by titration of approximately 0.5 g of lipid, dissolved in a mixture of 100 mL of ethanol and diethyl ether (1:1, v/v), with 0.01 N potassium hydroxide. Phenolphthalein was used as the indicator. The results of acid value and peroxide value were expressed as mg KOH/g lipid and meq/kg of lipid, respectively. The peroxide value was defined as the oxidised potassium iodide content, expressed as meq of hyperoxide per kg of lipid. According to the method of Gambotti and Gandemer (1999), carbonyl compounds were evaluated by the ratio of the absorbance at 275 nm to the absorbance at 215 nm from the lipid (125 μg/mL in cyclohexane solution). An increase of the ratio A_{275}/A_{215} is related to an increase in carbonyl compounds.

2.6. Fractionation of total lipids

Neutral lipids (NLs), free fatty acids (FFAs) and phospholipids (PLs), were separated from the total lipids by using Strata® NH_2 cartridges containing 500 mg of amine-propylic resin (Phenomenex, Torrance, CA) as described by Regueiro, Gibert, and Diaz (1994). The cartridge was activated with 6 mL of chloroform before use. The extracted lipids (10–20 mg of total lipid) were redissolved in chloroform and then loaded on the top of the cartridges. The NLs, FFAs and PLs were eluted with 2.5 mL chloroform:isopropanol (2:1, w/w), 3 mL 2% (w/w) of acetic acid/ether, and 3 mL methanol in sequential order.

2.7. Fatty acid composition analysis

Fatty acid methyl esters (FAME) were prepared from total lipids and the isolated fractions (NLs, FFAs and PLs) of soy sauce koji lipids according to the method of Morrison and Smith (1964). The contents of the fatty acid methyl ester in each fraction were quantified using heptadecanoic acid, methyl ester as internal standard. The GC–MS system consisted of a Trace Ultra GC, a Trisplus autosampler and a quadropole DSQ II MS (Thermo Finnigan, San Jose, CA). Separation was performed with a TR-5MS capillary column (30 m × 0.2 mm, 0.25 μm, J&W Scientific, Folsom, CA). Helium was used as carrier gas with a flow rate of 1.0 mL/min. Sample volume of 1.0 μL was injected with a split ratio of 100:1. The analytical conditions were as follows: the temperature of the column was maintained at 40 °C for 2 min, ramped to 150 °C at 10 °C/min, holding for 2 min, and then rose to 280 °C at a rate of 10 °C/min and held at 280 °C for 5 min. The mass spectrometer was operated in electron-impact (EI) mode. The ionisation energy, detector voltage, scan range and scan rate applied for the analysis were 70 eV, 350 V, *m*/*z* 35–350 and 3.00 scans/s, respectively. Both injector and ion source temperature were 250 °C. FAME were identified by matching the retention times and mass spectra with those of reference standards in the standard NIST 08 library and standard FAME analysed under the same experimental conditions.

2.8. Analysis of lipids composition

The compositions of triacylglycerols (TAGs) and lipids were determined by a reversed-phase high-performance liquid chromatography. The chromatographic apparatus consisted of a Waters P600 pump with a quaternary gradient system (Waters, Milford, MA), and a 3300 evaporative light-scattering detector (Alltech Associates Inc., Deerfield, IL) with an atmosphere compression pump (Tianjin, China). A Purospher® STAR RP-18e column (250 mm × 4.6 mm i.d., particle size 5 μm, Merck, Darmstadt, Germany) was used. The temperature was 40 °C, and flow rate of gas and liquid phases were 1.5 L/min and 0.6 mL/min, respectively. The injection volume was 10 μL. The gradient elution was achieved by mobile phases **A** (acetonitrile:acetic acid = 99.95:0.05, v/v) and **B** (dichloromethane). The gradient was operated as follows: 0–4 min 100% **A**; 4–12 min 90% **A**; 12–15 min 70% **A**; 15–19 min 20% **A**; 19–31 min 80% **A**; 31–36 min 90% **A**; 36–39 min 100% **A**; 39–42 min 100% **A**. The compounds in the samples were identified by HPLC/MS (Bruker Daltonics Inc., Billerica, MA), and the conditions were the same as described by Liu et al. (2012).

2.9. Identification of volatile compounds in the soy sauce

The sample preparation and SPME technique were used according to the methods of Feng et al. (2013). The SPME Trisplus auto-