Food Chemistry 215 (2017) 200–208

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of sonication on the extraction of free-amino acids from moromi and application to the laboratory scale rapid fermentation of soy sauce

Kok Ming Goh [a], Oi Ming Lai [b], Faridah Abas [c], Chin Ping Tan [a,*]

[a] Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[c] Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 12 April 2016
Received in revised form 16 June 2016
Accepted 27 July 2016
Available online 28 July 2016

Keywords:
Ultrasound-assisted extraction
Sonication
Free-amino acid
Fermentation
Soy sauce

ABSTRACT

Soy sauce fermentation was simulated in a laboratory and subjected to 10 min of sonication. A full factorial design, including different cycles, probe size, and amplitude was used. The composition of 17 free-amino acids (FAAs) was determined by the AccQ-Tag method with fluorescent detection. Main effect plots showed total FAAs extraction was favoured under continuous sonication at 100% amplitude using a 14 mm diameter transducer probe, reaching 1214.2 ± 64.3 mg/100 ml of total FAAs. Moreover, after 7 days of fermentation, sonication treatment caused significantly higher levels ($p < 0.05$) of glutamic acids (343.0 ± 22.09 mg/100 g), total FAAs (1720.0 ± 70.6 mg/100 g), and essential FAAs (776.3 ± 7.0 mg/100 g) 3 days sooner than the control. Meanwhile, enzymatic and microbial behaviours remained undisturbed. Collectively, the sonication to moromi resulted in maturation 57% faster than the untreated control.

1. Introduction

Soy sauce is one of the most commonly used liquid condiments in most Asian countries. It is typically used to deliver intense umami, a salty sensation and unique aroma to most Asian cuisine (Lioe, Wada, Aoki, & Yasuda, 2007). Although the recipe of fermenting soy sauce can differ due to regional and ethnic practices, there are typically Chinese and Japanese soy sauces available in the market. The main ingredients are always soy beans, wheat grains and salt. One of the main types of Japanese soy sauce, Koikuchi-shoyu (common soy sauce) uses soybeans and wheat in equal amounts, which has also been adapted as a standard recipe (Kataoka, 2005). During a long period of soybean fermentation with certain ingredients, soybean proteins are degraded by proteolytic enzymes, leading to the formation of free-amino acids (FAAs) (Han, Rombouts, & Nout, 2004). Studies have shown a substantial increase in FAAs in the fermented state compared to the original state (Dajanta, Apichartsrangkoon, Chukeatirote, & Frazier, 2011). An amino acid is an organic compound composed of an amino (NH_2) group with basic properties and a carboxyl (COOH) group with acidic properties. Both groups are attached to the same carbon atom. There is a side chain, commonly known as the R group that is also attached to the carbon atom and differs between amino acids. Fundamentally, amino acids can be grouped based on their chemical structure. The categories are basic (Lys, His, Arg), acidic (Asp, Glu, Arg), charged (basic, acidic), hydrophilic (charged, Thr, Ser), hydrophobic (Val, Leu. Ile, Phe, Tyr, Met), and apolar (hydrophobic except Tyr) (Sarkar, Jones, Craven, Somerset, & Palme, 1997). However, in food applications, taste perception is a high priority because it is one of the important quality indicators. In that regard, FAAs can be categorized according to their sensory attributes, which are the monosodium glutamate-like (MSG-like) FAAs (Asp, Glu), the sweet FAAs (Ala, Gly, Ser, Thr), the bitter FAAs (Arg, His, Ile, Leu, Met, Phe,Trp, Tyr, Val) and the tasteless FAAs (Cys, Lys, Pro) (Tseng, Lee, Li, & Mau, 2005). Therefore, one can imagine the importance of the FAAs present in soy sauce for their sensory qualities. To achieve a high amount of FAAs in soy sauce products, fermentation becomes a crucial process. However, fermentation is time consuming because the liberation of amino acids is dependent on proteolytic enzyme reactions.

Alternatively, ultrasound is a promising technology for the preparation of foods that delivers generally positive results rapidly, efficiently and reliably compared to conventional food processing methods, such as heat processing (Chemat, Zill, & Khan, 2011).

To the best of our knowledge, fermentation can be accelerated by ultrasound-assisted technology (UAT). Ultrasound indirectly accelerates the cell growth in a fermentation system, resulting in the formation of substances that indicate a faster maturation rate

* Corresponding author.
E-mail address: tancp@upm.edu.my (C.P. Tan).

http://dx.doi.org/10.1016/j.foodchem.2016.07.146
0308-8146/

or a higher quantity (Sulaiman, Ajit, Yunus, & Chisti, 2011). However, the most discussed aspects always relate sonication to cell growth, which indirectly results in a higher fermentation speed. Therefore, in our study, we wanted to investigate the effects of sonication on FAA liberation in a soybean and wheat grain fermentation system (moromi) that simulates the fermentation of soy sauce. In this case, 3 factors of the sonication conditions, namely the sonicator probe size, number of cycles, and sonication amplitude were chosen and applied in a general $2 \times 2 \times 3$ full factorial design to identify the best combination to liberate the most FAAs when applied after a continuous 7-day fermentation period.

The aim of the current study was to achieve rapid soy sauce fermentation with a high level of FAA liberation using UAT and to compare the maturation rate by means of the composition of free amino acids between the control and the sonicated moromi.

2. Materials and methods

2.1. Materials

Soybeans, wheat grains, and salt were purchased from the local Giant Malaysia hypermarket. The starter culture (*Candida* spp and *Aspergillus oryzae*) was purchased from Angel Yeast Co., Ltd. in China.

2.2. Soy sauce fermentation mash (moromi) preparation

Whole soybeans and wheat grains were measured at a ratio of 1:1 dry weight. The soybeans were washed and autoclaved at 121 °C for 15 min to achieve partial protein denaturation, while the wheat grain was lightly roasted at 200 °C for 5 min and ground into coarse particles using a kitchen blender. Cooked soybeans and roasted wheat grains were mixed and inoculated with an *Aspergillus oryzae* starter culture at 0.1% of the total dry ingredients' weight. The solid fermentation was incubated in a compressor-cooled incubator (ICP450, Memmert) at 30 °C for 28 h. Then, the fermented koji was blended with a 5% brine solution in a Waring blender at high speed for 1 min to yield moromi (koji:brine = 1:9). Lastly, a 1% (v/v) solution of activated industrial soy sauce yeast (*Candida* spp.) was injected into the moromi. All samples, in a final volume of 100 ml, were then subjected to different conditions of sonication. Moromi sampling was performed as needed, placed into amber bottles, and stored in a freezer at −20 °C until analysis.

2.3. Sonication treatment of the moromi samples

Sonication was performed using a Sartorius Stedim Biotech ultrasonic homogenizer (Labsonic P., Germany). A generator was used to convert the supplied voltage of 230 V/50 Hz into a longitudinal mechanical vibration at 24 kHz through a titanium alloy standing probe. The ultrasonic probe was inserted to a depth of 1-inch into the sample contained in a 100 ml volume laboratory bottle using the different probe diameter, cycle, and amplitude conditions.

2.4. Free amino acid sample preparation and pre-column derivatization

Moromi samples (0.2500 ± 0.0050 g) were measured, added to 1 ml of 2.5 μmol/ml HPLC internal standard (alpha-Aminobutyric acid, AABA), and diluted using a 50 ml volumetric flask. The diluted samples were filtered through Whatman No.4 filter paper, and the filtrates were then passed through a Pall 0.45 μm GHP filter (Waters).

Pre-column derivatization was performed using the AccQ-Tag Reagent Kit purchased from Waters (Research Instrument, Malaysia). Briefly, 10 μl of filtered and diluted sample was mixed with 70 μl of AccQ-Tag Fluor Borate Buffer. Then, 20 μl of AccQ-Tag Fluor Reagent (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; AQC) was added and the content was heated at 55 °C for 10 min on a heating block. The samples were kept in the auto sampler vial for a maximum of one week prior to HPLC injection.

2.5. Separation and analysis of free amino acids by HPLC

The free amino acids contained in the samples were separated using an AccQ-Tag amino acid Nova-Pak C18 column, 4 μm (150 × 3.9 mm) on a HPLC system (Shimadzu, Prominence LC-20AD) and detected using a fluorescence detector with E_λ = 250 - nm, E_m = 395 nm, Gain = 4, and Filter = 1.5 s. The injection volume was set at 10 μl and the column was kept at 36 °C.

The gradient mobile phase, consisting of eluent A (prepared by mixing the AccQ-Tag Eluent A concentrate with Milli-Q water at a ratio of 1:10) and eluent B (60% acetonitrile, 40% Milli-Q water), was injected at a flow rate of 1 ml/min throughout the experiment. The gradient program was defined as follows: 100% A at start, 98% A and 2% B at 0.5 min, 90% A and 10% B at 15 min, 87% A and 13% B at 19 min, 65% A and 35% B at 32 min for 1 min, 100% B at 34 min for 3 min, and 100% A at 38 min, allowing the column to equilibrate for 22 min until the 60th min. Lastly, the free amino acids were identified and quantified based on 5 points calibration curves of the respective amino acids prepared from an Amino Acid Hydrolysate Standard (Thermo) solution which contained a 2.5 mM mixture of 17 amino acids except Cys, which was present at 1.25 mM. AABA was used as the internal standard at a constant concentration (50 pmol in a 10 μl injection volume).

2.6. Proteolytic enzyme activity of the moromi

Proteolytic enzyme activity was determined using the method published by Kim et al., with slight modifications. Briefly, 5 g of moromi was diluted in 95 ml of distilled water and then mixed for 20 min in a cold water bath maintained at 4 °C. The solution was centrifuged at 20,000*g* for 15 min and the supernatant was collected as the enzyme extract. Then, 0.35 ml of a 0.6% casein solution was mixed with an equal volume of enzyme extract and incubated at 37 °C for 10 min. The reaction was stopped immediately by adding 0.7 ml of 0.44 M trichloroacetic acid (TCA), followed by incubation at 37 °C for 30 min. Then, the reagent mixture was centrifuged at 13,000*g* for 15 min, and 1 ml of clear supernatant was collected. The supernatant was mixed with 2.5 ml of 0.55 M Na_2CO_3, immediately followed by the addition of 0.5 ml of Folin-phenol reagent and incubated at 37 °C for 30 min. The absorbance was measured at 660 nm. One unit of proteolytic enzyme activity was expressed as μmol of tyrosine produced per gram of sample (Kim, Hwang, & Lee, 2010).

2.7. Total antioxidant capacity

Samples were diluted 20-fold in distilled water, and 2 ml of a 0.1 mM DPPH solution in methanol was added to 1 ml of diluted sample, followed by incubation at 25 °C for 30 min. The absorbance of the mixture was measured at 517 nm. An aliquot of 100% methanol was used as the blank, while 1 ml of distilled water instead of sample was used for control. The DDPH scavenging activities were calculated based on the following equation (Yang, Yang, Li, Li, & Jiang, 2011):

$$\text{DPPH radical scavenging activity } (\%) = [1 - (A_{\text{sample}} - A_{\text{blank}})/A_{\text{control}}] \times 100,$$