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Taste and chemical characteristics of low molecular weight fractions from tofuyo – Japanese fermented soybean curd

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ABSTRACT

Tofuyo, a Japanese traditional food, is a fermented soybean curd manufactured in Okinawa region. Due to its original cheese-like flavor, the current study was designed to evaluate the sensory and chemical characteristics of three stepwise ultrafiltration fractions, using 10,000, 3000 and 500 Da membranes and further chromatographic fractions from tofuyo. The results showed that umami, sweet and salty were the characteristic tastes of all fractions, with umami intensity evaluated for the fraction with MW less than 500 Da (F-500) as the most prominent among the three fractions. Subsequent Sephadex G-25 SF fractions and RP-HPLC fractions were subjected to sensory and chemical analyses. The tastiest fraction contained sodium chloride, sugars, organic acids, umami and sweet free amino acids, at concentrations above their thresholds. The abundant presence of umami and sweet free amino acids with certain concentrations of sodium chloride and glucose might provide the typical savory taste of tofuyo.

1. Introduction

Tofuyo is a fermented soybean curd which has been traditionally manufactured in Okinawa, Japan (Yasuda, 2010). This fermented product has specific cheese-like characteristics at low salt content, resulting from the action of proteases, carbohydrases and other catabolic enzymes existing in red koji (*Monascus* fungus) and/or yellow koji (*Aspergillus oryzae*) during ripening or maturation process of tofuyo (Tachibana & Yasuda, 2007; Yasuda, 2010; Yasuda, Tachibana, & Kuba-Miyara, 2012). Red koji or a mixture of red and yellow koji (made from inoculated cooked non-glutinous rice) is used for the production of tofuyo (Yasuda, 2010). During the ripening period, the soybean curd immersed in koji paste, a mix of koji and awamori, a traditional Okinawan liquor, with sodium salt concentration at less than 4%, forms smooth cheese-like texture (Yasuda, 2010). This texture is due to the unique degradation of indigenous soybean proteins, such as β-conglycinin and glycinin, into short peptides and to some extent of free amino acids by proteinases and peptidases in koji, in the presence of ethanol (15–20%) from the use of awamori, for 3–5 months of fermentation (Liu & Yasuda, 2005; Yasuda, 2010; Yasuda & Sakaguchi, 1998).

The presence of peptides in tofuyo has been investigated. Some of them were revealed to have functional properties (Kuba, Tanaka, Tawata, Takeda, & Yasuda, 2003). However, the chemical compounds responsible for the unique savory taste of tofuyo remain unclear. In comparison, umami amino acids, L-glutamic acid and L-aspartic acid, followed by bitter amino acid, L-leucine, were the most abundant amino acids in a similar fermented tofu product in China called red sufu, with a high salt concentration of 8−14% *w/v* (Han, Rombouts, & Nout, 2004).

The umami and bitter taste amino acids are often found together as important compounds for the typical umami taste of fermented soy products, such as over-fermented tempe, *shoyu* (a Japanese soy sauce), and *kecap* (an Indonesian soy sauce) (Lioe, Selamat, & Yasuda, 2010; Lioe, Takara, & Yasuda, 2006; Lioe, Wada, Aoki, & Yasuda, 2007; Lioe et al., 2004; Utami, Wijaya, & Lioe, 2016). Amino acids themselves are known to impart several basic tastes, such as umami, sweet and bitter, to food (Nishimura & Kato, 1988). In addition, several glutamyl, aspartyl, and/or glycyl peptides formed in some fermented foods are also known to impart umami taste, either by their own or by their

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interaction with other food components (Kim et al., 2017; Rhyu & Kim, 2011; Zhao, Schieber, & Gänzle, 2016; Zhuang et al., 2016). The umami amino acids and peptides might be present in tofuyo and contribute to the mild cheese-like taste of tofuyo. We therefore were interested in examining the chemical characteristics and sensory evaluation of tofuyo by analyzing its water-soluble extract and separated fractions, in order to know the umami fraction of tofuyo and its chemical composition.

2. Materials and Methods

2.1. Materials

Tofuyo was obtained from Benihama Co. Ltd. (Okinawa, Japan) as the only tofuyo manufacturer established in Okinawa. Tofuyo was homogenized and stored at 4 °C prior to use. Milli-Q water was obtained from a Milli-Q SP reagent water system (Millipore Corp., Bedford, MA). Ultrafiltration membranes, Sephadex G-25 SF, and all analytical grade chemicals as well as HPLC-grade chemicals used in the analyses are described in detail below.

2.2. Methods

2.2.1. Stepwise ultrafiltration

Original tofuyo sample, 100 g, was homogenized three times for 1 min (at intervals of 2 min) in the presence of three-fold volume of Milli-Q water, using an Ultra-Disperser Model LK-21 (Yamato Scientific Co. Ltd., Tokyo, Japan), to obtain water-soluble extract (WSE) of tofuyo. The supernatant for the subsequent ultrafiltration was obtained after a brief centrifugation (using refrigerated centrifuge CR20G; Hitachi Co., Tokyo, Japan) at 40,000*g* for 5 min at 4 °C and filtration through 0.2-μm cellulose acetate membrane filter unit (ADVANTEC, Toyo Roshi Kaisha Ltd. Tokyo, Japan). The tofuyo sample was then ultrafiltered using an ultrafiltration cell and molecular membranes with ultrahigh purity nitrogen gas for providing the needed pressure. The ultrafiltration cell was an Amicon Model 202 ultrafiltration unit (Amicon Inc., Beverly, MA). Ultrafiltration was done at 4 °C under 1.5–2.0 bar N_2 pressure. Stepwise ultrafiltration for tofuyo was carried out to obtain fractions with molecular weights (MW) below 500 Da (namely, F-500), 500 − 3000 Da (F-3000), and 3000 − 10,000 Da (F-10,000) by a combination of Q0100 (MW-cutoff at 10,000 Da, ADVANTEC), YM3 (MW-cutoff at 3000 Da, Millipore), and YC05 (MW-cutoff at 500 Da, Millipore) membranes. All ultrafiltration fractions, were collected, freeze dried using a TAITEC model VD-80 freeze drier (TAITEC Corp., Saitama, Japan) and then reconstituted to the same final volume, 50 mL, using Milli-Q water.

2.2.2. Capillary zone electrophoresis (CZE) profiles

CZE profiles of tofuyo ultrafiltration fractions were obtained in order to recognize the characteristic patterns. The CZE analyses were performed on a Photal CAPI-3300 capillary electrophoresis instrument (Otsuka Electronics Co., Osaka, Japan) using an uncoated fused silica column (50 cm length, 37.8 cm effective length, 50 μm inner diameter). The conditions for CZE analysis were as follows: 15 kV voltage; 30 sec hydrodynamic injection time; 45 min running time; room temperature; 50 mM phosphate (pH 2.5) as a running buffer; and UV 214 nm detection (using photodiode array detector with a scan range of 190 − 400 nm). Filtration through a 0.45-μm cellulose acetate membrane (ADVANTEC) was applied to sample solutions prior to CZE analysis.

2.2.3. Fractionation by gel filtration chromatography of F-500 fraction

Samples (5 mL) of F-500 fraction of tofuyo WSE were loaded on a Sephadex G-25 superfine (SF) column (2.6 × 90 cm; Pharmacia, Uppsala, Sweden). The separation was performed at 4 °C with Milli-Q water as the eluent at 0.45 mL/min. Aliquots of the eluate (5 mL each) were collected using an Iwaki FRC-2120 fraction collector (Iwaki Glass, Co. Ltd., Tokyo, Japan), and their UV absorbances were measured at 214 and 280 nm, using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). The sodium chloride concentration of each tube was also analyzed by titrimetry following the Mohr method (Fischer & Peters, 1968) to discover the fractions containing NaCl. Both UV absorbance and salt concentration were plotted against the tube number to obtain a chromatogram. According to this chromatogram, eluates were divided into several fractions. All resulting fractions were thereafter freeze dried. The fractions were combined from three chromatographic runs and reconstituted to the same total volume of 15 mL with Milli-Q water. This volume is the same as the initial volume of F-500 sample solution applied to the gel filtration in the three separation runs.

2.2.4. Chemical and sensory analyses

Analyses of dry matter, total peptides, NaCl concentration, total sugars, total acids, pH and free L-glutamic acid concentration. Analyses of these chemical parameters were performed for ultrafiltration fractions and their separated fractions, as well as RP-HPLC subfractions in duplicate. Dry matter of samples was obtained by gravimetry after using a freeze dryer. Total peptide concentrations were determined by spectrophotometry following Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951), using a DC protein assay reagent kit (Bio-Rad Laboratories, Hercules, CA) and a Benchmark Plus microplate spectrophotometer (Bio-Rad). The sodium chloride concentration was quantified by titrimetry as described above. Total sugars were analyzed by spectrophotometry (Clegg, 1956) using anthrone reagent (Nacalai Tesque, Kyoto, Japan), D-glucose standard (Cica, Kanto Chemical Co., Tokyo, Japan) and a Shimadzu UV-160 spectrophotometer. Due to the presence of a large amount of D-glucose in tofuyo, total sugars were measured as total D-glucose equivalent. Total acids were determined by titrimetry with 0.1 N NaOH as a titrant and phenolphthalein as an indicator, according to the method described by the Association of Official Analytical Chemists (AOAC, 1990). The total acids were calculated as lactic acid equivalents, since lactic acid was used as a major indicator for organic acids in fermented soybean products (Lioe et al., 2006). The pH was measured by a pH meter (Horiba model F.8L; Horiba Corp., Kyoto, Japan) and ADVANTEC pH test paper. The free L-glutamic acid concentration was quantified by spectrophotometry using a glutamic acid reagent kit (Boehringer Mannheim, Mannheim, Germany), following the manufacturer's protocol.

Analysis of amino acid composition. Ultrafiltration fractions and their resulting fractions were analyzed by HPLC-fluorescence with *o*-phthalaldehyde (OPA) precolumn derivatization (Harris, 1988) before and after acid hydrolysis, respectively, for their free and total amino acid compositions. The acid hydrolysis was conducting with 6 N HCl at 110 °C for 20 h under vacuum. In these analyses, L-tryptophan and L-cysteine could not be detected, because they were destroyed during acid hydrolysis. Determination by HPLC was performed on a Shimadzu LC-10A using a Shim-pack Amino-Na column (i.d. 6.0 mm × 10 cm). The OPA fluorescence detector used was set at 350 and 450 nm as excitation and emission wavelengths, respectively. HPLC was run at 60 °C, with Na-form buffer solvents of amino acid analytical grade (Shimadzu, Kyoto, Japan) (from pH 3.2 to 10.0) with mobile phase flow rate of 0.60 mL/min, using an injection volume of 10 μL for sample or standard. Amino acid standard solutions of 2.5 μmol/mL from Wako (Japan), diluted 25 times in sodium citrate buffer (pH 3.2), then injected into HPLC under the same conditions as for the sample, for amino acids identification and quantification. The peptides content was calculated by the difference between total and free L-amino acids concentrations.

Taste dilution analysis. Training of the panelists. Six subjects from the Department of Bioscience and Biotechnology of University of the Ryukyus, Okinawa, Japan, were selected and trained using a triangle test (Carpenter, Lyon, & Hasdell, 2000). The panelists were selected and trained to respond to the five basic tastes using five taste solutions