Food Chemistry 233 (2017) 256–262

Contents lists available at ScienceDirect

Food Chemistry

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

Identification of a key *umami*-active fraction in modernized Korean soy sauce and the impact thereof on bitter-masking

Yiseul Kim [a,1], Eun-Young Kim [a,1], Hee Jin Son [a], Jai-jung Lee [b], Yong-ho Choi [b], Mee-Ra Rhyu [a,*]

[a] Division of Functional Food Research, Korea Food Research Institute, Bundang-gu, Sungnam-si, Gyeonggi-do 13539, Republic of Korea
[b] Sempio Fermentation Research Center 1, Sempio Foods Company, Osongup, Cheongwongun, Chungcheongbuk-do 28156, Republic of Korea

ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 10 April 2017
Accepted 19 April 2017
Available online 21 April 2017

Keywords:
Bitter-masking
Bitter taste receptor
Low molecular weight fraction
Modernized Korean soy sauce
Umami

ABSTRACT

Food protein hydrolysates created by natural fermentation have been used for centuries as food flavorings. The aim of this study was to define the key *umami*-active fraction of modernized Korean soy sauce (mJGN) and the impact thereof on bitter-masking of human sensory and bitter-taste receptor-expressing cells. We found strong correlations between taste profiles of mJGN and a contained fraction (F05). The latter contained compounds of less than 500 Da, and elicits a distinct *umami* taste. Both free amino acids and Glu-enriched oligopeptides are suggested to be crucial in terms of the effects of F05 on taste. F05 not only reduced human-perceived bitterness, but also effectively suppressed the intracellular Ca^{2+} response induced by caffeine in the hTAS2R43 and hTAS2R46 human bitter-taste receptor-expressing cells. This suggests that F05, a key *umami*-active fraction of mJGN, contains components that at least partially modulate human bitter-taste receptor action, improving food flavor.

1. Introduction

Fermentation is one of the oldest food-processing techniques and typically exploits the activities of a diverse array of microorganisms to induce metabolic protein breakdown. Amino acids and peptides from protein hydrolysates often make crucial contributions to the taste of fermented products. Fermentation products of soybeans contain high levels of amino acids and peptides, and are commonly used as food seasonings in Asian countries (Otsuka, 1998). Although such products are made in different ways, using raw materials and microorganisms that vary by country-by-country, biochemical breakdown via brine fermentation is a core feature when the unique *umami* taste is sought (Hajeb & Jinap, 2015; Otsuka, 1998). Fermented soy products can be categorized as sauces or pastes. *Shoyu*, a Japanese soy sauce, is probably the most common form of soy sauce worldwide. Compared to *shoyu,* which is prepared principally from soybeans mixed with wheat or rice, *Joseon ganjang*, a traditional Korean soy sauce, is made by fermenting only soybeans in brine (Chung & Sohn, 1994; Lee & Koh, 1976; Otsuka, 1998).

As most traditional soy sauces are prepared using natural bacteria and fungi, and are then ripened for a long time at room temperature, it is not easy to standardize the quality of the final product. Modernized soy sauces, prepared under controlled microorganisms and temperature have recently become commercially available. Although the microorganisms used dictate the taste of the final product (Chen, Feng, Cui, Zhan, & Zhao, 2014; Lee & Koh, 1976; Lioe et al., 2004), the taste characteristics of modernized Korean soy sauce, prepared using soybeans fermented by *Aspergillus oryzae* in brine, have not been explored. We first identified a key *umami*-active peptide fraction of modernized Korean soy sauce, and then explored the impact thereof on bitter-masking in humans. We employed sensory tests to this end; *umami* substances have been suggested to suppress the tastes of various bitter compounds (Noguchi, Yamashita, Arai, & Fujimaki, 1975; Keast, & Breslin, 2003; Kemp, & Beauchamp, 1994; Woskow, 1969). Bitter-suppression by *umami* substances was confirmed by recording single-neuron activities in C57BL/6J mice (Tokita & Boughter, 2012). These correspond to the responses of relevant receptors of taste cells. In terms of *umami*-bitter interactions with taste receptors, a recent study showed that *umami* peptides suppressed bitter tastes by binding to bitter-taste receptor(s) (Kim, Son, Kim, Misaka, & Rhyu, 2015). Thus, we defined a key *umami*-active fraction in modernized Korean soy sauce, and the bitter-masking effects thereof, using human sensory cells and cells expressing bitter-taste receptors.

* Corresponding author.
E-mail address: mrrhyu@kfri.re.kr (M.-R. Rhyu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.foodchem.2017.04.123
0308-8146/

2. Materials and methods

2.1. Materials

Caffeine, citric acid, glycyl-leucine (Gly-Leu), L-isoleucine (L-Ile), magnesium chloride, sodium chloride, and sucrose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluo 4-acetoxymetyl ester (Fluo 4-AM) was purchased from Molecular Probes (Eugene, OR, USA). Other chemicals were of cell culture grade.

2.2. Preparation of modernized Korean soy sauce and desalted concentrate thereof

Modernized Korean soy sauce was prepared according to the Korea traditional food quality certification standard methods (KS T016) enacted by National Agricultural Products Quality Management Service (Ministry of agriculture, food and rural affairs, Republic of Korea). Briefly, soybeans were washed and soaked in water at room temperature, after which they were autoclaved for 1 h at 120 °C. For fermentation, autoclaved soybeans were inoculated with *Aspergillus oryzae* and incubated at 30 °C for 40 h. The mold-treated soybeans were subsequently inoculated with other molds, *Tetragenococcus halophilus* and *Zygosaccharomyces rouxii*, in brine and maintained at 30 °C for 3 months. The resulting modernized Korean soy sauce meet the specified conditions in total nitrogen (>1.5%) and solids (>14%) by a Korean soy sauce certification of KS T016 (total nitrogen > 0.8% and solids > 8.0%), which are important factors in quality of soy sauce and proportionate to the protein content of the raw materials. After fermentation, the mixture was filtered through filter paper to remove solids. The resultant filtrate (modernized Korean soy sauce) was desalted using electrodialyzer (PS5-ED1-20, Innomeditech Inc., Gyeonggi-do, Korea). The electrode solution (2% Na_2SO_4), the concentrate solution (0.5% NaCl), and the dilute solution (sample) were circulated through the corresponding compartments of the electrodialysis stack with a flow of 1.6 L/min. A constant voltage of 25 V was used. The dialysis was terminated when the salt level of the sample fell to less than 0.5% (w/v). The desalted liquid was incubated at 60 °C for 1 h and 85 °C for 30 min and then concentrated using an evaporator (NAJ-160, EYELA, Tokyo, Japan) at 50 °C; this constituted the mJGN preparation.

2.3. Ultrafiltration and preparation of reconstituted modernized Korean soy sauce

mJGN was successively separated into six different fractions according to molecular weight (MW) distribution using YM-10 (MW cutoff; MWCO 10,000 Da), YM-5 (MWCO 5000 Da), YM-3 (MWCO 3000 Da), YM-1 (MWCO 1000 Da), and YC-05 (MWCO 500 Da) membranes (Millipore Ltd., Milford, MA, USA) at 2–4 °C under N_2 pressure. Stepwise ultrafiltration was performed; the fractions obtained were as follows: F10, the concentrate obtained after passage through a YM-10 membrane (containing materials > 10,000 Da); and F510, F35, F13, and F051; the concentrates obtained after passage of the material through YM-5, YM-3, YM-1 or YC-05 membranes, respectively. These fractions contained materials of molecular weights 5,000–10,000, 3,000–5000, 1,000–3000, and 500–1000 Da, respectively. Finally, F05 was the permeate through the YC-05 membrane (containing materials smaller than 500 Da).

To prepare complete reconstituted mJGN (re-mJGN), the six lyophilized fractions were dissolved in tap water, and the levels thereof adjusted appropriately to restore the solid content of mJGN. The six kinds of partial re-mJGN omitting F05, F051, F13, F35, F510, or F10 were named omtF05, omtF051, omtF13, omtF35, omtF510 or omtF10, respectively.

2.4. Sensory evaluation

2.4.1. Panelists and general conditions

The panelists for the sensory evaluations included male and female, ranging from 27–44 years of age were recruited from the Korea Food Research Institute. Panelists were first trained to recognize each five basic taste including sweet, bitter, sour, salty, and umami. Following training, 10 panelists participated in sensory evaluation sessions; each panelist initially tested a reference solution, and then evaluated the taste intensities of the experimental samples. Each panelist rinsed their mouth with tap water between samples. The sensory evaluation sessions were performed in an air-conditioned room at 25 ± 1 °C.

2.4.2. Taste profile analysis

Pre-trained panelists performed a quantitative descriptive analysis, based on a 15-point intensity scale ranging from 1 (no taste) to 15 (very strong taste). The 5% sucrose, 0.08% caffeine, 0.08% citric acid, 0.35% sodium chloride, and 0.04% fish soup base (Ajinomoto Co., Inc., Tokyo, Japan) were provided as reference solutions for a sweet, bitter, sour, salty and for umami taste (Rhyu & Kim, 2011), respectively. The scores for each reference solution were adapted from descriptive analysis techniques by Meilgaard, Civille, and Carr (1987).

Comparative taste profile tests for omission experiments, each of the six partial re-mJGN, omtF05, omtF051, omtF13, omtF35, omtF510, and omtF10, was presented to the panelists. And then, the intensity of the descriptors sweet, bitter, sour, salty, and umami were rated in comparison to re-mJGN (control), which assigned 7.5 points.

2.4.3. Comparative intensity analysis

Four bitter solutions, 1.35% l-Ile, 0.27% Gly-Leu, 2.7% magnesium chloride, and 0.08% caffeine, were used as reference for comparative bitter intensity study and assigned a score of 5 points. Each reference solution and 2% F05 or F510 dissolved in corresponding reference solution were presented to the sensory panelists, who asked to rate the bitter intensity on a 15-point intensity scale.

2.5. Peptide and amino acid analysis

Free amino acid content was determined using an amino acid analyzer (S-433, Sykam GmbH, Germany). The total amino acid content of mJGN and F05 was determined after processing using acidic hydrolysis for 24 h at 110 °C, and bound-type amino acid content was calculated by subtracting free amino acids from total amino acids. The lyophilized F05 subfraction (containing materials smaller than 500 Da) obtained from 10 mL of mJGN was dissolved in water to 10 mL. Thus, the total amino acid content was the same as that of mJGN.

2.6. Bitter masking effect in the cells expressing bitter taste receptor

2.6.1. Cell culture and transfection

Expression plasmids for human TAS2R43 (hTAS2R43), hTAS2R46, and G16αgust44 were kindly gift from Takumi Misaka (The University of Tokyo). The expression plasmids were subcloned into pEAK10 vector. The hTAS2R43 or hTAS2R46 expression plasmid was cotransfected with G16αgust44 expression plasmid (4:1) into HEK293T cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). At 18–24 h after transfection, the cells were used for measuring cellular responses. All cells were cultured at