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Organic Acids Profile Comparison of Spiced Soy Sauces Prepared with Various Food Ingredients including Astragali Radix

Chang-Hwan Oh and Sung Hee Min*

Department of Oriental Medical Food and Nutrition, Semyung University, Jecheon, Chungbuk, 27136, South Korea; och35@semyung.ac.kr, shmin@semyung.ac.kr

Abstract

Objectives: Soy sauce is a famous traditional Asian sauce. Astragali Radix, the root of Astragalusmembranaceus Bunge, is an Oriental medicinal material widely used as the food ingredients in Korea. **Methods/Statistical analysis:** The spiced soy sauces with boiling meats, fish and mushrooms were mixed with Astragali Radix water extract with the different ratios. In this study, the characteristics such as pH, total acids, buffering capacity, solid contents of the spiced soy sauces (Matkanjang in Korean) were investigated with the identification and comparison of the ratio of the organic acids identified in the final products. Findings: The spiced soy sauce with Astragali Radix water extract showed higher pH value. Total organic acids contents decreased as the amount of Astragali Radix water extract increased. The spiced soy sauce of mushrooms showed the better buffering capacity than others. Twenty free organic acids were detected in the spiced soy sauces mixed with Astragali Radix water extract. Three of them were not detected in the original soy sauce. The four organic acids such as oxalic acid, 2-hydroxy caproic acid, malic acid and linoleic acid were detected in the spiced soy sauces, but they were under the detection limit in the raw soy sauce. **Improvements/Applications:** These results will be able to be used for the quality control of the spiced soy sauce that is one of the most popular traditional sauces in Korea.

Keywords: Astragali Radix, Derivatization, GC-MS, Organic Acids, Spiced Soy Sauce

1. Introduction

Sauces are not usually consumed by themselves, but served on or used in preparing other foods in cooking. The favorable dishes must be infused with delicious tastes touched by a various sauces. Soy sauce is a typical traditional condiment widely used in Korea, Japan, China and other Asian countries. It is used to be made from a fermented purée of simmered soybeans (and starch) and Aspergillus oryzae (or Aspergillus sojae)[1]. Most of the research for soy sauce were performed by Japanese researchers, such as "Studies on breeding Aspergillus, 1: mold breeding by protoplast fusion for soy sauce production" published by Furuya etc. belong to Kikkoman Corp.[2]. The domestic retail market of dressing and sauce was 348,900 million Korean Won (KW) in 2014 that is 16% higher than 300,700 million KW in 2011. Among the market, sauce took 204,000 million KW (that is 29.9% higher than the market in year 2011). In 2015, the soy sauce and the spiced sauce retail market amounts were 176,462 million KW and 74,742 million KW, respectively[3]. As the most of traditional spiced sauces are made with soy sauce, the growth potential of the spiced soy sauces based on Korean traditional recipe become a formidable contender in the near future. As one of the trend of functional food, some customers may be expecting some functionality even from sauces. To supplement the functionality to the sauce, an oriental medicinal material such as Astragali Radix (AR) could be added to soy sauce. AR, the dried root part of *Astragalusmembranaceus* Bunge, has the Korean name as "Hwang-gi" possesses that it may boost one's "wind energy (Gi in Korea)"[4]. For the matter of the AR added soy sauce, Jang *etc*. published a research paper "Quality characteristics of Korean traditional Kanjang containing*Astragalusmembranaceus*"[5]. In

*Author for correspondence

that research the soy sauce (containing AR) was tested for the physiochemical properties, such as pH, salt and sugar content, optical density, nitrogen for crude protein, total polyphenol and flavonoid content, with the sensory test except the free organic acids. The sour taste must be the important organoleptic character of soy sauce. The intensity of Sour taste was linearly correlated to the molar concentration of H^+ and the molar concentration of the organic acids that had at least 1 –COOH functional group[6]. Organic acids are organic compounds having acidic characters such as carboxyl group –COOH. Carboxylic acids contain a carbon atom functioning as a hydroxyl and a carbonyl group. Depending on their relative acidic nature, carboxylic acids will be ionized under the pH larger than pKa value of the compound, then are inadequate to be analyzed by gas chromatography (GC) due to the lack of volatility. But GC is a very efficient technique to be operated easily and cheaply. Therefore, many researches for the organic acid analysis were performed by GC after derivatization. Derivatization allows the use of GC and MS (mass spectrometry) on samples not or else amenable to these analytical methods and so extend the application of these efficient tools to new area[7]. In Silylation is the most versatile technique for extending GC performance by blocking active (protic) sites, thereby lessening intermolecular force such as dipole-dipole interactions and increasing volatility. Hydroxyl and carboxylic acids could be easily trimethylsilylated by common trimethylsilylation (TMS) reagents. But *tert.*-butyldimethylsily (TBDMS) derivatives are respectively 104 times more stable to hydrolysis than the corresponding TMS ethers. One of the most useful TBDMS reagents is *N*-*tert.*-butyldimethylsilyl-*N*-methyl trifluoroacetamide (MTBSTFA)[8]. In "Simultaneous analysis of volatile and non-volatile carboxylic acids as *tert.*-Butyldimethylsilyl derivatives", it was reported that TBDMS derivatives were stable for at least six months when stored at 4°C[9]. As the characteristic ions of TBDMS derivatives, the [M-57] ions, formed by losing of the *tert.*-butyl functional group from the molecular ions are comparatively very large, thus enabling easy identification of acids. Even though the organic acids must be the important compounds in the spiced soy sauces, the investigations for them have not been reported yet. In this study, the characteristics such as pH, total acids, buffering capacity, solid contents of the spiced soy sauces (Matkanjang in Korean) were investigated with the identification and comparison of the ratio of the organic acids identified in the final products. The spiced soy sauce was prepared by the standardized method (The composition of the product is water (45.5%), crude protein (6%), reducing sugar (11.7%) and invert sugar (32.1%))[10]. The ingredients of the spiced soy sauces, such as mushrooms, fishes and boiling meats were mixed with AR water extract (by the three different amount ratios). The result of this research will be able to be used for the quality control of the spiced soy sauce that is one of the most popular traditional sauces in Korea.

2. Experimental

2.1 Materials and Sample Preparation

The soy sauce (a thick one) used for this research was produced by the S sauce maker that is one of the major food companies (Seoul, South Korea). The granulated sugar was produced from the J Food Company (Seoul, South Korea). The food materials such as mushroom (Shiitake), fish (Pollack) and meat (Brisket) were purchased in a local market of Jecheon, Chungbuk province, South Korea. The fresh AR was purchased in Jecheon Oriental Medicine Material Store located in Jecheon. The spiced soy sauce was cooked by modified Park and Shin's method [10]. The detailed ratios of the soy sauce, food materials and AR extract are shown in Table 1. All ingredients including sugar with water were mixed until the sugar solid vanished. And they were simmered with low heat and switched to high heat for 10 minutes followed by medium heat for 40 minutes. The residual liquid of the solid ingredients was collected (by straining for 30 minutes) and mixed to the prepared solution of spiced soy sauce. The water extract of AR was prepared by the modified method of Oh. Ten time of water than the amount of AR was mixed to the

Table 1. The recipe of the spiced soy sauce

Ingredients	Amount
Soy Sauce (mL)	400
Water (mL)	400
Sugar (g)	222
Onion (g)	40
Green onion (g)	10
Garlic (g)	10
Dried red pepper (g)	2.5
Whole pepper (g)	1.5
Ginger (g)	5
Meat (or fish, mushroom) (g)	100

dried AR and boiled at 90°C for 4 hours. The ratios (v/v) of the spiced soy sauce and AR water extract were 1:1 (for Shiitake added), 1:2 (for Pollack added), and 1:3 (for Brisket). Each final spiced soy sauce with AR water extract was assigned as A, B and C in the order named.For the measuring of pH of the final solutions, Orion Star Plus pH meter (Thermo Scientific, Waltham, MA, USA) was used. To measure the total acidity, the buffering capacity and the net soluble solid contents, the methods used in "Evaluation in physicochemical properties of soy sauce fortified with soymilk residue (okarakoji)"were adapted[11]. For the analysis of the free organic acids in the final solutions, GC-Flame ionization Detector (GC-FID) and GC-MS analyses were performed after TBDMS derivatization of the extracted free organic acids[12]. The fatty acid standards were purchased from Alltech (Deerfield, IL, U.S.A.) and Sigma-Aldrich (St. Louis, MO, U.S.A.). MTBSTFA was purchased from Pierce (Rockford, IL, U.S.A.). Triethyl amine (TEA) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Sodium chloride, sodium bicarbonate and sulfuric acid (98%) were obtained from Samchun chemical (Pyungtaek, Gyoungi-do, South Korea). HPLC grade diethyl ether, methanol, dichloromethane and diethyl ether were purchased from Burdick and Jackson (Honeywell corp., Morristown, NJ, U.S.A.) All other solvents and reagents were of analytical grade. TEA was distilled over KOH. Chromos orb P (AW-DMCS, 80-100 mesh) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). For the solid phase extraction (SPE), the Chromosorb P (2.3g) packed U-shaped glass columns (6 mm I.D.) were prepared. They were washed with HPLC grade methanol, dichloromethane, and diethyl ether followed by activating in drying oven at 200°C overnight before using. Before the solid phase extraction of the free organic acids, the spiced soy sauce was centrifuged at 30,000 ×*g*. The supernatant was basified by sodium bicarbonate and washed with diethyl ether (by the principle of liquid-liquid extraction). The washed aqueous layer was acidified with concentrated sulfuric acid (0.1 mL) and salted out with 400 mg of sodium chloride. The prepared aqueous solution (1 mL) was subjected to the SPE column packed with Chromosorb P and eluted with HPLC grade diethyl ether. The aqueous layer loaded on to SPE column coated each Chromosorb P particle and diethyl ether brought out the protonated organic acids from the coated aqueous layer of the Chromosorb P particles. The first diethyl ether eluent (1.5 mL) was collected in a reaction vial containing 20 μL of TEA. The collected extract mixed with TEA was evaporated near dryness under the gentle stream of nitrogen followed by heating at 60°C for 5 hours with MTBSTFA (20μL) and iso-octane (60μL)[12]. Through the derivatization process, the carboxylic acid and alcohol functional groups were *tert*.-butyldimethylsilylated. Each organic acid was identified by [M-15] and [M-57] ions (the typical fragment ions of TBDMS derivative) and other characteristic ions. The normalized area was calculated based with the integration result of GC-FID analysis.

2.2 Instrumental

The GC×GC-TOFMS system consisted of an Agilent 6890 GC (Agilent, Palo Alto, CA, USA) and Pegasus 4D TOFMS (LECO Corporation, St. Joseph, MI, USA). The single capillary column, DB-5ms (5% phenyl-polydimethylsiloxane; 30m × 0.25mm I.D. × 0.25μm film thickness) (J&W Scientific, Folsom, CA, USA) was used. The oven was set 60 ºC for 2 min followed by ramping to 280 ºC (15 ºCmin^{-1}) and held for 30 min. The mobile phase was the ultra-high purity (99.999%) helium (1 mLmin^{-1}). The splitless injection mode at 220 ºC (the purge delay time 0.7 min) was used. The MS parameters of the electron impact ionization mode (70 eV) and 100 spectra per second of the spectral acquisition rate were adapted. The transfer line and the ion source temperatures were 260 ºC and 250ºC, respectively. The detector voltage was 1750 V, and the electron energy was −70 V. Mass spectra were collected from *m/z* 35–500. For the identification of the compounds, LECO® Software was used. For the GC-FID analysis, Agilent 6890 GC was used with the same condition of the above GC-MS. The SPSS 15.0 software was used for the statistical analyses.

3. Results

3.1 The Acidity, Total Acid, Buffering Capacity and Solid Content of the Spiced Soy Sauces

The higher AR water extract content showed higher pH value ($p < 0.001$) as well as lower total free acid levels ($p < 0.01$) as showed in Table 2. The solid content (49.44–45.28) of the three spiced soy sauces was higher than value of the spiced soy sauce made by other research (40.31–40.40). The highest solid content value of the sample A must be originated from the added Shiitake. The buffering capacity and the solid content of the three spiced soy sauces are shown in Table 3. The

Table 2. Total free acids, pH of the Astragali Radix water extract added spiced soy sauces

Sample	pH	Total free acids (mg%)
A	5.01±0.01	164.52±4.06
B	5.08±0.01	144.32±7.27
C	5.12±0.00	130.83±4.93

Table 3. Buffering capacity and solid content of the Astragali Radix water extract added spiced soy sauces

Sample	Buffering capacity	Solid content (%)
A	2.09±0.00	49.44±2.75
B	2.13±0.01	47.31±1.45
C	2.11±0.02	45.28±5.46

buffering capacity of three spiced soy sauces effectively checks large changes in pH. While the sample A (with the lowest AR water extract content) showed the lowest pH change, and the sample C (with the highest AR water extract content) exhibited the medium buffering capacity. Enfin the AR water extract content may not affect the buffering capacity of the spiced soy sauce. Meanwhile, the free amino acids extracted from Shiitake, might influence the positive effect for the buffering capacity of the sample A.

3.2 The Organic Acids Detected in the Raw and the Spiced Soy Sauces

Total twenty organic acids detected in the soy sauce material and the cooked spiced soy sauces with AR water extract Table 4. The highest normalized area (NA) portions were taken by lactic acid (in sample A and B) and fumaric acid (in sample C). The free organic acids with more than 1% of NA were lactic acid, glycolic acid, 2-hydroxy isocaproic acid, fumaric acid, phosphoric acid, phenyl lactic acid, 4-hydroxy benzoic acid, vanillic acid, azelaic acid, oxalic acid, and malic acid. Three organic acids, 2-hydroxy-3-methylvaleric acid, methyl malonic acid and 4-hydroxy phenyl acetic acid were decreased under the detection limit after cooking of the spiced soy sauce with AR water extract. The four organic acids, such as oxalic acid, 2-hydroxy caproic acid, malic acid and linoleic acid were not detected in the raw soy sauce material used in this research. Oxalic acid content was relatively high in the spiced soy sauce with sample B (Pollack added) as 3.16%, followed by 2.23% (sample C, Brisket added) and 0.84% (sample A, Shiitake added). Oxalic acid could make a salt with calcium, then could make the kidney stone in our body. It is found frequently in many brassicas such as cabbage, brocolli, brussels sprouts etc. 2-Hydroxycaproic acid (2-hydroxyhexanoic acid) is a fatty acid branched-chain alpha-keto acid substituted by a hydroxy group at position 2. 2-Hydroxycaproic acid might be the degradation product of the ingredients used for the spiced soy sauce. Malic acid (hydroxybutanedioic acid) is a dicarboxylic acid found as L form in all living organisms, contributes to the sour taste of many fruits,

Table 4. Organic acid profiles of the Astragali Radix water extract added spiced soy sauces (with Shiitake (A), Pollack (B) and Brisket (C))

No.	Organic acids	Normalized Peak Area of Organic acid TBDMS derivatives (%)			
		Raw Soy Sauce	A	B	C
1	lactic acid	58.11	30.16	33.02	18.69
2	glycolic acid	0.77	1.40	2.15	1.21
3	2-hydroxy butyric acid	0.72	30.16	0.64	0.38
4	2-hydroxy isocaproic acid	0.44	1.40	0.56	0.31
5	2-hydroxy-3-methyraleric acid	0.75	0.00	0.00	0.00
6	methyl malonic acid	21.98	0.00	0.00	0.00
7	Fumaric acid	1.44	18.08	28.60	20.29
8	phosphoric acid	0.29	0.32	1.92	2.25
9	phenyl lactic acid	0.32	0.00	1.98	1.03
10	4-hydroxy phenyl acetic acid	0.33	0.00	0.00	0.00
11	4-hydroxy benzoic acid	5.40	2.06	2.30	1.09
12	vanillic acid	6.26	3.32	3.27	1.41
13	azelaic acid	1.07	3.71	5.82	3.83
14	palmitic acid	0.78	0.39	0.41	0.23
15	oleic acid	0.58	0.56	0.69	0.33
16	stearic acid	0.77	0.29	0.13	0.08
17	oxalic acid	0.00	0.84	3.16	2.23
18	2-hydroxy caproic acid	0.00	0.72	0.32	0.18
19	malic acid	0.00	6.16	14.47	11.57
20	linoleic acid	0.00	0.43	0.56	0.17

and is frequently used as a food additive. The TBDMS derivative of malic acid showed *m/z* 419 and 461 as the [M-57] and [M-15] ion, respectively. The retention index of malic acid TBDMS derivative was 2170 that was almost similar to the value estimated by other research[12]. It causes a sour (tart) taste to wine, although the concentration decreases with increasing fruit ripeness. Malolactic conversion (also known as malolactic fermentation or MLF) is a process of malic acid (that is naturally present in grape) being converted to softer-tasting lactic acid in winemaking[13]. Even in the spiced soy sauce, the ratio between lactic acid and malic acid might be one of the important organoleptic factors. The highest NA ratio of malic acid was found in the sample B as 14.47% followed by 11.57% (sample C) and 6.16% (sample A). Therefore, the malic acid content might be affected by not the AR water extract content but the added materials (such as Shiitake, Pollack and Brisket). The lactic acid NA ratio of the spiced soy sauces was decreased from 58.11% of the original soy sauce to 33.02% (sample B), 30.16% (sample A) and 18.69% (sample C). The higher AR water extract portion brought about the lesser amount of lactic acid in the spiced soy sauce due to the dilution effect by the AR water extract. The relatively high NA ratio of the unique organic acid was found in the sample A (Shiitake added spiced soy sauce), as the 2-hydroxybutyric acid (30.16%). In the study of the formation of 2-hydroxybutyric acid in experimental animals, the use of C[14]-labelled precursors clearly showed that the amino acids methionine, threonine and homoserine could be converted to 2-hydroxybutyric acid[14]. And 2-aminobutyric acid was also converted to 2-hydroxybutyric acid, with 2-oxobutyric acid as an intermediate metabolite. The relatively high NA ratio of 2-hydroxybutyric acid in the spiced soy sauce must be caused by the added Shiitake. Other free organic acid configurations were similar with each other for the sample A, B and C.

4. Conclusion

The spiced soy sauces with each mushroom, fish and meat were mixed with AR water extract. The pH value was higher if there was more AR content. Total acid and solid content of higher AR water extract added spiced soy sauce showed lower values than the lower AR water extract added spiced soy sauce. At least 20 free organic acids were detected in the soy sauces before and after cooking with various food ingredients and AR water extract. The major organic acids, such as lactic acid, fumaric acid etc. may contribute the acidic taste of the spiced soy sauces. The organic acids detected in AR water extract added spiced soy sauces, oxalic acid, 2-hydroxy caproic acid, malic acid and linoleic acid may originated from AR water extract because they were under the detection limit in the raw soy sauce. The presence of 2-hydroxybutyric acid may be the characteristic contributing factor to the unique taste which was only found in the Shiitake and AR water extract added spiced soy sauce.

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