Nutraceuticals & Food
Vol. 7, p.427~431 (2002)

Nutraceuticals and Food

Chemical Characteristics of Commercial Korean Soy Sauce Produced by Alkali Treatment Following Acidic Hydrolysis

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Abstract

Alkali treatment following acidic hydrolysis (ATAH) is a commonly used practice for reducing the levels of monochloropropanediol in commercial Korean soy sauce. This study investigated the chemical compounds produced in commercial Korean soy sauce made by ATAH. The levels of amino-N, total acidity, pH, salinity and Brix of the soy sauce were 6.66%, 2.52%, 19.81%, 4.57 and 35.01, respectively. The major fatty acids were C18:2n-6, C16:0, C18:1n-9 and C18:3n-6. The concentration of polyunsaturated fatty acids were especially high, with of C18:2n-6 (49.8%) being the highest followed by C18:3n-6 (3.8%) and C18:3n-3 (2.4%) in that order. Among the non-volatile organic acids, the concentration of levulinic acid (1,206.28 mg/100 g) was the highest, while the taste value of citric acid was the highest. Among the ATP related compounds, IMP concentration (31.19 mg/100 g) was highest followed by AMP, hypoxanthine and GMP in that order. The concentrations of free and total amino acids in soy sauce were 6,136.94 mg/100 g and 8,702.76 mg/100 g, respectively. On the other hand, the taste value of glutamic acid, a major amino acid flavor determinant in soy sauce, was highest of all the amino acids, which is desirable since most free amino acids such as methionine, histidine and phenylalanine have a bitter taste that detracts from the flavor of soy sauce.

Key words: alkali treatment following acidic hydrolysis (ATAH), commercial Korean soy sauce, chemical compounds

INTRODUCTION

Soy sauce has been used as a taste enhancer and seasoning by the food industry for many years in Korea. Until the mid 1990s, Korean commercial soy sauce was commonly produced by acidic hydrolysis because it produces a strong savory favor and is a less expensive production process than enzymatic hydrolysis. However, it has been reported that chloropropanols such as monochloropropanediol (MCPD) and dichloropropanol (DCP), known carcinogens, are toxic byproducts that can be formed when commercial soy sauce is made by acidic hydrolysis using hydrochloric acid. DCP is highly volatile and mostly evaporates during processing of commercial soy sauce; however, MCPD is more stable and remains in acid-hydrolyzed vegetable protein (1). The precursors of the MCPD that is formed in soy sauce has been shown to be hydrochloric acid and residual lipids from the raw materials (2). MCPD is rapidly decomposed to glycerol via the intermediate epoxide, glycidol, in aqueous alkali conditions (3). Thus, alkali treatment in the pH rage of 8.3~8.7 at temperatures of 45~60°C after acidic hydrolysis has been recently used in the production of commercial Korean soy sauce, to degrade the MCPD.

Although there have been a few studies reporting the chemical compounds in Korean soy sauce made by acidic hydrolysis without alkali treatment (4,5), to date there have been no published reports of the chemicals produced in commercial soy sauce made with alkali treatment. The objective of this study was to obtain basic data on the chemical composition of commercial Korean soy sauce made by alkali treatment following acidic hydrolysis (ATAH).

MATERIALS AND METHODS

Materials

A sample of soy sauce made by ATAH from defatted soybean protein was donated by the Pungsung Co. (Changnung, Korea) and refrigerated (4°C) until used. All chemicals used in analysis were analytical reagent-grade.

Measurement of proximate composition

The content of moisture, total sugar (Bertrand method), crude protein (semi-micro Kjeldahl method), crude lipid (Soxhlet method) and crude ash were determined by AOAC

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methods (6).

Determination of amino-N, total acidity, pH, salinity, Brix and color value

The content of amino-N was determined by the Formol method (6), and total acidity was analyzed by titration with 0.1 N NaOH in accordance with the AOAC method (6). The pH was determined with a pH meter (pH/ion meter DP-880, Dongwoo Medical System, Korea), and salinity was determined using a salinity meter (TM-30D, Takemura Electric Works, Co., Japan). Brix was determined by refractometry (Atago Hand Refractometer N-2E, Atago Co., Japan), and such color values as L, a, b, and ΔE were determined by a color difference meter (Minolta CM-3500d, Minolta Co., Japan).

Determination of fatty acid compositions

Oils were extracted by the Bligh and Dyer method (7), and fatty acid compositions were determined by gas chromatography (GC) according to the method of Suzuki et al. (8) using 200 mg of the extracted oil. A gas chromatograph (HP 6890, Hewlett-Packard Co., USA) (splitless mode; helium carrier gas at 2.5 mL/min constant flow), equipped with an HP-INNOWax™ capillary column (30 m length × 0.32 mm i.d. × 0.5 m film thickness) was used for analysis. Oven temperature was programmed at 180°C initially (10 min hold), and increased at a rate of 3°C/min until reaching 2300 C (15 min hold). Inlet and detector (FID) temperatures were set at 250°C. The concentration of each fatty acid was calculated from the ratio of the area under the curve at its respective peak of retention time to the total area of the chromatogram.

Determination of non-volatile organic acids

Non-volatile organic acids were analyzed by the modified method of Lee et al. (9) using 10 g of sample and the same GC and column as above (splitless mode; helium carrier gas at 1.2 mL/min constant flow). Oven temperature was programmed at 50°C initially (1 min hold), increased to 230°C at 10°C/min (8 min hold). Inlet and detector (FID) temperatures were set at 200 and 250°C, respectively. Duplicate analyses were performed on each extract. Identification and quantification of the organic acids were again calculated from the peaks, using standard organic acids (Sigma Chemical Co., USA) for comparison injected under identical conditions, and calculated by the procedure of Cho (10).

Determination of ATP-related compounds

ATP-related compounds were determined by the HPLC method of Lee et al. (11) using 10 g of sample. HPLC conditions were: HPLC column, HP ZOBAX™ column (XDB-C18, Hewlett Packard, USA); mobile phase; 1% triethylamine phosphoric acid buffer solution (pH 6.5); flow rate, 1 mL/min. Compounds were identified by comparing retention time against a known standard, and quantified by peak area using a standard calibration curve.

Determination of amino acids

Sample preparation and analysis of free and total amino acids were carried out according to the methods of Cha and Cadwallader (12) and Ha et al. (13), respectively. The total and free amino acids were quantitatively analyzed using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, USA). Organoleptic evaluation was used to identify taste-active compounds among the amino acids (14); the levels of taste threshold, for the determination of taste values, were calculated according to the paper of Kato et al. (15).

Statistical analysis

Statistical analysis was conducted using Students t-test, ANOVA and Pearson's correlation analysis. SPSS (Statistical Package, SPSS Inc.) system was used for the statistical analysis.

RESULTS AND DISCUSSION

Proximate composition of soy sauce produced by ATAH

The proximate composition of the commercial Korean soy sauce produced by ATAH is shown in Table 1. The contents of moisture, crude protein and crude lipid were 69.37%, 9.51% and 1.03%, respectively; whereas the content of total sugar (below 0.01%) was relatively low. It is known that soybean polysaccharides are decomposed to pentose or hexose by acidic hydrolysis, and then converted to furfural and oxymethylfurfural. Hexose can be finally converted to levulinic acid or formic acid by acidic hydrolysis of soybeans (16). On the other hand, MCPD content (below 0.08 ppm) in commercial Korean soy sauce made by ATAH was much lower than that made by acidic hydrolysis only (data not shown).

Amino-N, total acidity, salinity, Brix and color value in soy sauce produced by ATAH

The results of amino-N, total acidity, salinity, pH, Brix and color value in commercial soy sauce made by ATAH were shown in Table 2. The content of amino-N, considered a determinant of soy sauce quality, was 6.66% and

Table 1. Proximate composition of commercial Korean soy sauce produced by alkali treatment following acidic hydrolysis

(g/100 g)

Moisture	Crude protein	Crude lipid	Crude ash	Total sugar
69.37±0.05[1)]	9.51±0.05	1.03±0.01	16.26±0.06	<0.01

[1)]Mean value±SD (n=3).

Table 2. Amino-N, total acidity, salinity, pH, Brix and color value of commercial Korean soy sauce produced by alkali treatment following acidic hydrolysis

Amino-N (g/100 g)	Total acidity (g/100 g)	Salinity (g/100 g)	pH	Brix	Color value[2)]			
					L	a	b	ΔE
6.66±0.02[1)]	2.52±0.03	19.81±0.07	4.57±0.01	35.01±0.01	5.88	26.40	9.98	98.24

[1)]Mean value±SD (n=3).
[2)]L: Measures lightness and varies from 100 for perfect white to zero black (Standard plate : 99.9814).
a: Measures redness when plus, gray when zero and greenness when minus (Standard plate: -0.0081).
b: Measures yellowness when plus, when zero, and blueness when minus (Standard plate: 0.0277).
$\Delta E: \sqrt{\Delta L^2+\Delta a^2+\Delta b^2}$

accounted for nearly 70% of total crude protein, indicating that the protein was mostly decomposed by acidic hydrolysis. The total acidity, salinity, pH and Brix of the soy sauce were 2.52%, 19.81%, 4.57 and 35.01, respectively. Color values, L-, a-, b- and ΔE-value were 5.88, 26.40, 9.98 and 98.24, respectively.

Fatty acid compositions in soy sauce produced by ATAH

The fatty acid composition of the soy sauce made by ATAH is shown in Table 3. The major fatty acids were C18:2n-6, C16:0, C18:1n-9 and C18:3n-6. Polyunsaturated fatty acids (PUFA) (58.1%) were the predominant fatty acids followed by saturated fatty acids (SFA) (24.9%) and monounsaturated fatty acids (MUFA) (17.0%). C16:0 (19.89% of total fatty acids) was the predominant SFA, whereas C18:1n-9 (13.0% of total fatty acids) was the predominant MUFA, followed by C20:1n-9 (1.2%) and C18:1n-7 (1.2%) in that order. C18:2n-6 (49.8%) was highest among the PUFAs, and C18:3n-6 (3.8%) and C18:3n-3 (2.4%) followed in that order. The fatty acid composition of the soy sauce was similar to the fatty acid compositions of soybean oils (17).

Table 3. Fatty acid composition of commercial Korean soy sauce produced by alkali treatment following acidic hydrolysis[1)]

(area%)

Fatty acid	Content
12:0	0.46±0.21[2)]
13:0	0.69±0.11
14:0	0.72±0.29
16:0	19.89±1.55
18:0	2.76±0.12
19:0	0.39±0.03
Saturates	24.91
16:1n-7	0.57±0.04
16:1n-5	0.11±0.07
18:1n-9	12.99±0.40
18:1n-7	1.21±0.08
20:1n-11	0.78±0.03
20:1n-9	1.22±0.16
20:1n-7	0.10±0.04
Monoenes	16.98
18:2n-6	49.78±1.26
18:3n-6	3.79±0.15
18:3n-3	2.39±0.09
18:4n-3	0.52±0.24
20:5n-3	1.63±0.04
Polyenes	58.11

[1)]Calculated by area percent of peak of each compound except solvent peak.
[2)]Mean value±SD (n=3).

Taste compounds in soy sauce produced by ATAH

Non-volatile organic acids in the commercial soy sauce made by ATAH are shown in Table 4. A total of 7 non-volatile organic acids including: lactic acid, oxalic acid, fumaric acid, succinic acid, malic acid, citric acid and levulinic acid were detected. Among these compounds, the levulinic acid content (1,206.28 mg/100 g), which is known to be a product of the acid hydrolysis of hexose (16), was the highest, comprising 82.6% of the total non-volatile organic acids; citric acid (146.33 mg/100 g) and lactic acid (82.83 mg/100 g) concentrations followed in order. Several other investigators have reported that either lactic or succinic acid are the non-volatile organic acids in highest concentrations in traditional Korean soy sauce made by enzymatic hydrolysis (18,19). These results suggest that levulinic acid content may used to determine if soy sauce was made by enzymatic hydrolysis or acidic hydrolysis.

Table 4. Contents and taste values of non-volatile organic acid in commercial Korean soy sauce produced by alkali treatment following acidic hydrolysis

Compound	Taste threshold[1)] (mg/100 g)	Content (mg/100 g)	Taste value[2)]
Lactic acid	1.0	82.83±0.55[3)]	82.83
Oxalic acid	1.0	1.56±0.30	1.56
Malonic acid	4.5	N/D[4)]	
Fumaric acid	2.0	0.79±0.08	0.40
Succinic acid	2.0	9.75±0.66	4.88
Malic acid	1.0	13.14±2.50	13.14
Citric acid	1.0	146.33±5.56	146.33
Levulinic acid	N/A[5)]	1,206.28±29.16	
Total		1,460.68	

[1)]Jang (20).
[2)]Taste value: compound concentration divided by taste threshold.
[3)]Mean value±SD (n=3).
[4)]N/D: not detected.
[5)]N/A: not available.

On the other hand, the taste values of citric and lactic acids were higher than those of the other organic acids. Jang (20) reported that citric and lactic acids are also important flavor components in *kimchi*. Therefore, citric and lactic acids are considered to be major sour taste compounds of the non-volatile organic acids in commercial soy sauce.

ATP related compounds in the soy sauce, from high to low, were: IMP (31.19 mg/100 g), AMP, hypoxanthine and GMP (Table 5). Park and Sohn (21) reported that hypoxanthine was highest in traditional Korean soy sauce made by enzymatic hydrolysis, and that IMP, AMP, inosine, hypoxanthine and ADP followed in that order. They also reported that 5'-inosinate and disodium 5'-guanylate, which impart a savory taste, are major nucleotides and that ATP related compounds have synergistic effects with free amino acids in soy sauce. In addition, Jang (22) reported that the content of hypoxanthine was the highest, and that the average IMP concentration was 8.09 mol/100 mL in commercial soy sauce made by enzymatic hydrolysis. It is known that IMP is positively correlated with the acceptance of soy sauce (22). Therefore, it can be assumed that GMP and IMP might have a positive effect on consumer acceptance of commercial Korean soy sauce made by ATAH.

Table 5. Contents of ATP related compounds in commercial Korean soy sauce produced by alkali treatment following acidic hydrolysis (mg/100 g)

Compound	Content
ATP	N/D[1]
ADP	N/D
AMP	29.85±0.23[2]
IMP	31.19±0.28
Inosine (HxR)	5.34±0.08
Hypoxanthine (Hx)	27.50±0.22
GMP	11.96±0.13
Total	105.84

[1]N/D: not detected.
[2]Mean value±SD (n=3).

The free and total amino acids in the commercial Korean soy sauce made by ATAH are shown in Table 6. The concentrations of free and total amino acids were 6,136.94 mg/100 g and 8,702.76 mg/100 g, respectively. Free amino acids comprised 70.5% of the total amino acids. Glutamic aicd (2,707.87 mg/100 g), serine (1,159.97 mg/100 g) and aspartic acid (914.94 mg/100 g) were in highest concentrations making up 44.1%, 18.9% and 14.9% of the total

Table 6. Compositions of free and total amino acids in commercial Korean soy sauce produced by alkali treatment following acidic hydrolysis

Amino acids	Taste threshold[1] (mg/dL)	Free amino acids		Total amino acids	
		Conc[2]	T · V[3]	Conc[2]	T · V[3]
Aspartic acid	3	914.94	304.98	1,120.45	373.33
Threonine	260	153.88	0.59	153.88	0.59
Serine	150	1,159.97	7.73	1,199.06	7.99
Glutamic acid	5	2,707.87	541.57	2,816.37	563.27
Glycine	130	N/D[5]		204.47	1.57
Citrulline	N/A[4]	290.21		401.38	
α-Amino-n-butyric acid	N/A	196.38		196.38	
Valine	40	N/D		256.52	6.41
Cystine	N/A	3.60		10.49	
Methionine	30	0.28	0.01	6.49	0.22
Cystathionine	30	N/D		64.87	2.16
Isoleucine	90	N/D		234.80	2.61
Leucine	190	N/D		248.38	1.31
Tyrosine	N/A	452.49		452.49	
β-Alanine	N/A	N/D		27.89	
Phenylalanine	90	109.02	1.21	266.80	2.96
Ethanolamine	N/A	N/D		65.74	
Ammonium chloride	N/A	N/D		601.78	
Ornithine	N/A	12.34		17.64	
Lysine	50	5.67	0.11	215.35	4.31
Methylhistidine	N/A	5.64		16.88	
Histidine	20	124.65	6.23	124.65	6.23
Total		6,136.94		8,702.76	

[1]Kato et al. (15).
[2]Concentration was expressed with wet basis (mg/100 g).
[3]T · V (taste value): compound concentration divided by taste threshold.
[4]N/A: not avaliable.
[5]N/D: not detected.

mino acids, respectively with tyrosine, citrulline, α-mino-n-butyric acid, histdine and phenylalanine following ı that order. However, the sulfur amino acids, cystine 3.60 mg/100 g) and methionine (0.28 mg/100 g), were letected in very low amounts compared to other amino cids. These results were in close agreement with the mino acid profile reported in the literature for traditional Korean soy sauce made by enzymatic hydrolysis (21). The aste values of glutamic acid (541.57) and aspartic acid 304.97) in the sample were higher than for other amino cids. Most free amino acids such as methionine (0.01), ıistidine (6.23) and phenylalanine (1.21) have a bitter taste ınd therefore score lower on taste value tests. Therefore, glutamic and aspartic acids should be considered major avory taste compounds of commercial Korean soy sauce nade by ATAH.

Overall, the results obtained from this study demonstrate hat soy sauce made by ATAH is both safe, because of ts acceptable MCPD content, and flavorful because of it profile of taste compounds including: non-volatile organic ıcids, ATP related compounds, and amino acids.

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(Received September 23, 2002; Accepted November 20, 2002)