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Characteristics of Salt-Fermented Sauces from Shrimp Processing Byproducts

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A salt-fermented sauce from shrimp processing byproducts (heads, shells, and tails) was prepared and characterized. Three types of sauces were prepared; sauce C, with 30 g of salt/100 g of byproduct (high salt); sauce E, with 30 g of salt and 0.2 g of sodium erythorbate (high salt); and sauce L, with 20 g of salt, 0.2 g of sodium erythorbate, 6 g of sorbitol, 0.5 mL of lactic acid, and 5 mL of ethanol (low salt). Sauces C and E showed higher exopeptidase activities than sauce L, whereas sauce L showed the highest endopeptidase activity. After 3 months of fermentation, the amino N content of sauce increased from 150−200 to 500−600 mg/100 g and the nonprotein nitrogen content increased from 300 to 950−1050 mg/100 g. Volatile basic nitrogen content increased significantly from 18 to 60 mg/100 g. The total carotenoids retained in sauces C, E, and L were 26.3, 76.2, and 73%, respectively, thus indicating that the addition of sodium erythorbate to sauces E and L retarded oxidation. Water activities of sauces C, E, and L were 0.753, 0.751, and 0.773, respectively. According to the omission test, the taste of sauces was influenced by the content of free amino acids, mainly glutamic acid and aspartic acid. All three sauces examined showed a 35% higher total amino acid content than commercial salt-fermented shrimp sauces. Therefore, shrimp processing byproducts may lend themselves to the preparation of high-quality salt-fermented sauces.

KEYWORDS: Shrimp processing byproduct; salt-fermented sauce; free amino acids; sensory quality

INTRODUCTION

Fermented seafoods are very popular and important in the Korean diet. Typical fermented fish foods in Korea are divided into three groups, namely, salt-fermented fish (jeotkal or jeot), fermented fish with cereals (sikhae), and fish sauce (the liquid part of salt-fermented fish—aekjeot) (*1*). Salt-fermented fish is produced by fermentation of the meat, viscera, or whole body of fish or shellfish by the action of endogenous hydrolyzing enzymes and/or microorganisms (*2*). The hydrolysis of proteins is an essential step in the fermentation process and depends primarily on the activity of proteolytic enzymes present in the fish (*3*). During proteolysis, flavor and aroma substances are formed and a characteristic consistency develops. Proteolytic products formed during fermentation are composed mainly of soluble nitrogenous compounds such as amino acids, peptides, nucleotides, and their decomposition products (*3*). Salt-fermented fish is nutritionally valuable as it contains high amounts of amino acids and minerals. In Korea, salt-fermented fish products, such as salt-fermented anchovy and its sauce, salt-fermented sand eel sauce, and salt-fermented shrimp, have been used as a side dish for steamed rice and added to other foods such as kimchi as a seasoning agent.

The byproducts from shrimp processing, including heads, shells, and tails, could provide a good source of useful enzymes. Proteases in shrimp are involved in the development of black spot or melanosis phenomenon during storage (*4*, *5*). Thus, shrimp processing byproducts (heads, shells, and tails) are usually removed to prevent quality deterioration due to autolysis by endogenous enzymes (*6*). It is difficult to synthesize seafood flavorants because they are more complex than plant-based flavorants. Therefore, shrimp byproducts may serve as a potential source of flavorants such as shrimp sauce. Studies on shrimp fermented foods include investigation on taste compounds of salt-fermented shrimp (*7*, *8*), fatty acid composition (*9*), flavor composition (*10*, *11*), enzymatic properties (*12*, *13*), processing conditions (*14*, *15*), improvement of flavor quality (*16*), and quality evaluation (*17*). In the present study, shrimp processing byproducts were used for the preparation of salt-fermented shrimp sauce. The physicochemical properties, taste active compounds, and nutritional components of fermented sauces so produced were subsequently studied.

MATERIALS AND METHODS

Preparation of Fermented Shrimp Byproduct Sauce. Composite southern rough shrimp (*Trachypena curvirostris*), caught near Tongyeong, Korea, were hand-peeled and transported to the laboratory from a

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commercial fish market in ice. Before sauce preparation, byproducts were divided into two parts; one was presoaked in water and the other in a 0.2% solution of sodium erythrobate for 30 min. Presoaked samples were drained, frozen at −20 °C for 5 h, and then pulverized (pulverizer M-12S, Korea Fujee Co., Seoul, Korea). Three types of sauce were prepared by fermentation at 20 ± 2 °C for 6 months. Sauce C was prepared with 30 g of salt/100 g of sample, and sauce E was the same as sauce C but also contained 0.2 g of sodium erythorbate. Sauce L contained only 20 g of salt/100 g of byproduct along with 0.2 g of sodium erythorbate, 6 g of sorbitol, 0.5 g of lactic acid, and 5 mL of ethanol. Additives were selected for antioxidant activity (sodium erythorbate), lowering of water activity (sorbitol), drop in pH (lactic acid), and antimicrobial (ethanol) effects. The byproduct sauces were then centrifuged at 12000*g* for 15 min. The supernatant was considered to be the fermented shrimp byproduct sauce and used for subsequent analyses. Yield of sauce was represented by the amount of supernatant per 100 g of byproduct.

Determination of Nitrogen Compounds. The supernatant was used to determine nitrogen compounds. Amino nitrogen (NH_2-N) was measured according to the formol titration method of Kobara et al. (*18*). Nonprotein nitrogen (Ex-N) was determined by using the Kjeldahl method (*19*). Volatile basic nitrogen (VBN) was determined according to the Conway (*20*) method.

Gel Permeation HPLC Analysis. Twenty microliters of sauce were injected onto a Shodex protein KW-804 column (i.d. 8 mm × 300 mm, Showa Denko, Tokyo, Japan) equilibrated with 50 mM sodium phosphate buffer, pH 6.0, containing 100 mM NaCl and analyzed using a Shimadzu HPLC (LC-10AT vp, Shimadzu, Kyoto, Japan) at a flow rate of 1 mL/min. The elution profile of protein was monitored using a Shimadzu UV−vis detector (SPD-10AV vp, Shimadzu) at 280 nm. Molecular weights of standard proteins (Sigma-Aldlrich Chemical Co., St. Louis, MO) used were as follows: aprotinin (6500 Da), cytochrome *c* (12400 Da), carbonic anhydrase (29000 Da), bovine serum albumin (66000 Da), and alcohol dehydrogenase (150000 Da).

Determination of Enzyme Activity. The protein concentration was measured according to the method of Lowry et al. (*21*) using bovine serum albumin as the standard protein. Protease activity on azocasein was assayed by Starky's method (*22*) with minor modifications. A 40 μL aliquot of sauce was mixed with 2 mL of 0.2% azocasein in 50 mM sodium phosphate, pH 6.0, and Tris-HCl, pH 8.0, buffer incubated at 40 °C for 30 min. The reaction was stopped by the addition of 2 mL of 5% trichloroacetic acid (TCA) solution, and the mixture was then centrifuged at 5000*g* for 15 min. The absorbance of the supernatant was read at 410 nm for the released azo dye. Amidase activity on benzoyl-arg-β-naphthylamide (BANA), arg-β-naphthylamide (ArgNA), and leu-β-naphthylamide (LeuNA) was determined according to the procedure described by Barrett (*23*, *24*) with slight modifications. The assay mixture was composed of 1.9 mL of 0.2 mM solution of amino acid derivatives in 50 mM sodium phosphate buffer, pH 7.0, and 0.1 mL of diluted sauce. The incubation was carried out at 40 °C for 30 min, and mersalyl reagent was used to terminate the reaction and to couple the liberated β-naphthylamide with Fast Garnet GBC. The intensity of the red color was measured at 520 nm.

One unit of activity is defined as the amount of enzyme that causes an increase of absorbance of 0.1 unit per minute. Specific activity (units per mg) is defined as the activity units per milligram of protein.

Total Carotenoids. The content of carotenoids was determined according to the method of McBeth (*25*). Twenty milliliters of sauce sample was extracted with 4 volumes of 95% ethanol using a separatory funnel for 1 h in a dark room and then suction filtered. The filtrate was transferred to *n*-hexane by the addition of a saturated sodium chloride solution in a separatory funnel, and the absorbance at 473 nm was read immediately. The content of total carotenoids present in 100 mL of sauce was calculated from the following equation: (g of carotenoid/100 mL of sauce) = (A_{473} × total volume × 10^3)/(2400 × mL of sauce).

Sensory Evaluation. Sensory evaluation of shrimp by product sauce was carried out by a 10-member trained panel using the structured scaling test. A 5-point hedonic scale (5, excellent; 4, good; 3, fair; 2, poor; 1, unacceptable) was used to grade the overall quality in terms of flavor, taste, and color.

Proximate Composition, pH, Salinity, and Total Viable Cells. The proximate composition of byproducts was determined according to the AOAC (*19*) methods. Moisture content was quantified by oven-drying at 105 °C, total lipid by Soxhlet extraction with diethyl ether, crude protein by Kjeldahl method, and crude ash by incineration at 550 °C. The amount of carbohydrate was calculated by difference. A calibrated pH meter (Metrohm model 744) was used for pH determination. Salinity was determined using a salt meter (model 460CP, Istek Co., Seoul, Korea). Samples for pH and salinity measurements were prepared by homogenizing 5 g of each sample with 10 volumes of deionized water. The total viable cells were determined by the standard plate count method (*26*).

Water Activity. Water activity was determined directly using a Thermoconstanter (Novasina MS1).

Omission Test. An omission test was performed as described by Lee et al. (*27*). For preparation of amino acid free sample (A), 20 mL of sauce was loaded onto an Amberlite IR-120 (H^+ form, 100−200 mesh) column (i.d. 10 × 250 mm) and eluted with deionized water. The eluate was concentrated to 20 mL using a vacuum rotary evaporator (R114, Eyela Co., Tokyo, Japan). A Dowex 1X8 (formic form, 100−200 mesh) column (i.d. 10 × 250 mm) was used for the preparation of ATP-related compounds free sample (N) and an Amberlite IRA-400 (H^+ form, 100−200 mesh) column (i.d. 10 × 250 mm) for the organic acid free sample (O) were used, respectively. Samples of AN (the broth from which amino acids and ATP-related compounds were eliminated), NO (the broth from which ATP-related compounds and organic acids were eliminated), OA (the broth from which organic acids and amino acids were eliminated), and ANO (the broth from which amino acids, ATP-related compounds, and organic acids were eliminated) were prepared by using a series of columns mentioned above. Eluate from each column was collected and subjected to sensory evaluation against the control (C, the original broth).

Composition of Total and Free Amino Acids. The total amino acid composition of samples was determined using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, Uppsala, Sweden). Samples were hydrolyzed in 6 M HCl in evacuated sealed tubes at 110 °C for 24 h. For determination of sulfur-containing amino acids, samples were added to dimethyl sulfoxide (DMSO) to obtain a final concentration of 0.2 M and then hydrolyzed in 6 M HCl (*28*). Free amino acids were extracted into 80% ethanol and deproteinized with 5-sulfosalicylic acid and determined as before.

Minerals and Phosphorus Determination. Minerals and phosphorus were determined by using the wet nitric acid ashing method of Tsutagawa et al. (*29*). The sample was then analyzed by an inductively coupled plasma (ICP) spectrophotometer (Atomscan 25, Thermo Electron Co., Waltham, MA).

ATP and Its Related Compounds. ATP and its related compounds were determined according to the method of Ryder (*30*) after their extraction with cold 10% perchloric acid and adjusted to neutral pH with 1 M potassium hydroxide solution. The samples were injected into a μ-Bondapak C_{18} column (i.d. 3.9 × 300 mm, particle size = 10 μm, Waters) equilibrated with 50 mM potassium phosphate buffer, pH 6.4. Analyses were performed using a Shimadzu HPLC (LC-10AT vp, Shimadzu) at a flow rate of 1 mL/min. The elution profile was monitored with a Shimadzu UV−vis detector (SPD-10AV vp, Shimadzu) set at 254 nm.

Statistical Analysis. Statistical treatment of data was performed by analysis of variance using the ANOVA test. Significant differences between means were determined using Duncan's multiple-range test at $P < 0.05$ (*31*).

RESULTS AND DISCUSSION

Yield of Salt-Fermented Sauce Prepared from Processing Byproducts of Shrimp. The changes in the yields of shrimp sauces prepared from shrimp processing byproducts are shown in **Figure 1**. Yields of shrimp sauces C (44−56%), E (44−55%), and L (47−61%) increased from initial values by 11−14% after 3 months of fermentation but leveled off afterward. Sauce L experienced the highest increase of 14%, presumably

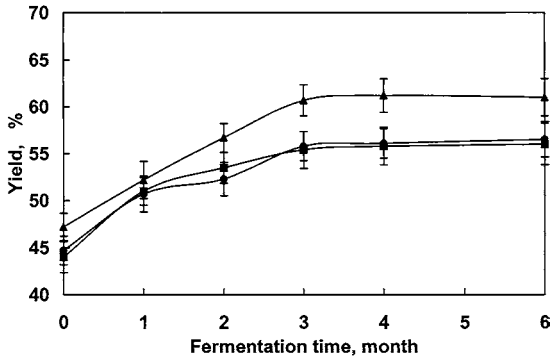

Figure 1. Changes in yields of sauce prepared from processing byproducts of spotted shrimp during fermentation. Product codes (C, ●; E, ■; L, ▲) are as explained in the text.

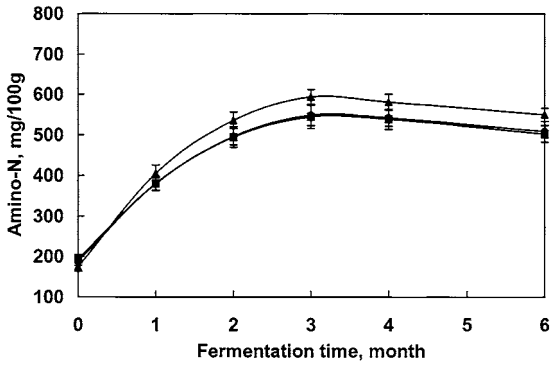

Figure 2. Changes in amino nitrogen contents of sauce prepared from processing byproducts of spotted shrimp during fermentation. Product codes (C, ●; E, ■; L, ▲) are as explained in the text.

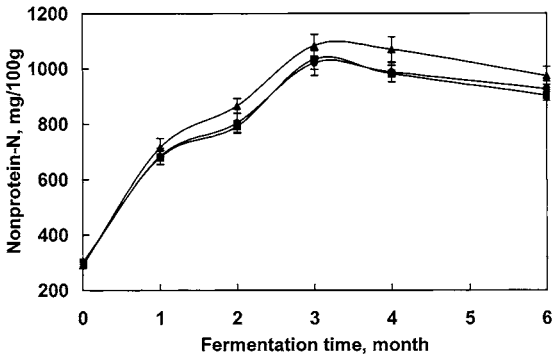

Figure 3. Changes in nonprotein nitrogen contents of sauce prepared from processing byproducts of spotted shrimp during fermentation. Product codes (C, ●; E, ■; L, ▲) are as explained in the text.

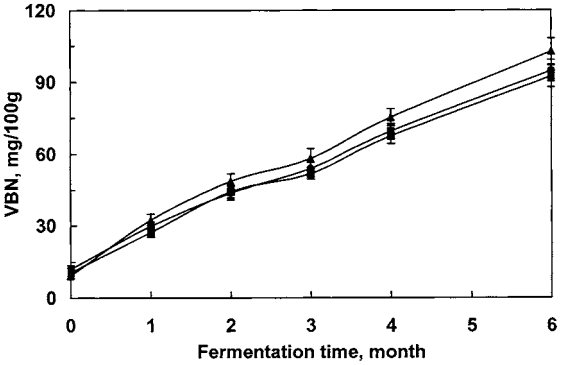

Figure 4. Changes in volatile basic nitrogen (VBN) of sauce prepared from processing byproducts of spotted shrimp during fermentation. Product codes (C, ●; E, ■; L, ▲) are as explained in the text.

arising from the hydrolysis of proteins in shrimp byproducts by endogenous proteases and their liquifaction.

Contents of Amino, Nonprotein, and Volatile Basic Nitrogen. Amino nitrogen contents (**Figure 2**) of shrimp sauces during fermentation increased rapidly (C, 188.3−548.1 mg/100 g; E, 193.7−544.6 mg/100 g; L, 173.8−593.6 mg/100 g) through 3 months. The increase in amino nitrogen contents was higher for sauce L than for sauces C and E by ∼45 mg/100 g. Lee et al. (*17*) reported that the amino nitrogen content of commercial salt-fermented shrimp prepared with whole body and 30% salt was 30−265 mg/100 g, which is considerably lower than that of 3 months fermented shrimp sauce in this study. Thus, shrimp sauces made from heads and tails possessed strong protease activities, leading to large amounts of extractive compounds.

The content of nonprotein nitrogen compounds consisted mostly of taste active components. The amounts increased rapidly and reached a maximum at month 3 with 1021 mg/100 g for C, 1033 mg/100 g for E, and 1084 mg/100 g for L (**Figure 3**).

The VBN of sauces C, E, and L increased in proportion to the length of fermentation period. Upon 6 months of fermentation, the values reached 102 mg/100 g for L, 95 mg/100 g for C, and 92 mg/100 g for E (**Figure 4**). Mok et al. (*15*) reported a rapid increase in VBN contents during the early stages of fermentation; this was ascribed to microbial deterioration rather than fermentation, and high contents in the latter period of fermentation were due to possible spoilage. Therefore, the increase in VBN beyond month 3 in our study was believed to be due to microbial deterioration rather than fermentation.

Patterns of Protein Hydrolysis and Enzyme Activity. In **Figure 5**, the protein molecular weight distributions of sauces C, E, and L indicated six groups of peaks: peak 1 (MW 150000 Da); peak 2 (MW 60000 Da); peak 3 (MW 20000 Da); peak 4 (MW 10000 Da); peak 5 (MW 6000 Da); and peak 6 (MW <3000 Da). As the fermentation proceeded, peaks 1−4 tended to decrease for C, E, and L. However, peak 5 increased rapidly from 1 to 3 months; the increase for C and E was ∼3-fold and ∼2.5 times for L from 0 months. Peak 6 increased throughout the fermentation period, but L revealed the highest concentration. Therefore, sauce L showed a faster decrease in molecular weight due to more extensive protein hydrolysis than that found for sauces C and E. Sauce L (salinity of ∼18%) had a higher concentration and distribution of peaks 5 and 6, with molecular weights lower than those of C and E (salinity of ∼24%; see **Table 3**). Thus, hydrolyzing activities of proteases and peptidases in shrimp byproducts were more pronounced under low salt conditions than in the presence of high salt.

Changes in protein concentration and proteolytic activity toward azocasein and changes in amidolytic activity toward synthetic substrates of shrimp processing byproduct sauces during fermentation are shown in **Figures 6** and **7**, respectively. In the case of sauces C and E, hydrolyzing activity toward azocasein (pH 6.0 and 8.0) at 1 month of fermentation decreased by 35−50% in comparison with 0 months of fermentation, and it tended to decrease as the fermentation period was extended. In the case of sauce L, enzyme activities upon 1 month of fermentation were 0.078 unit/mg (pH 6.0) and 0.121 unit/mg (pH 8.0), compared to 0 months of fermentation (0.045 unit/mg, pH 6.0; 0.06 unit/mg, pH 8.0), after which hydrolyzing activity toward azocasein nearly leveled off. The reason for the low proteolytic activities of C and E may be related to changes in the structure of enzymes under high salt conditions with a concurrent decrease in their hydrolyzing activities (*9*). In C, E,

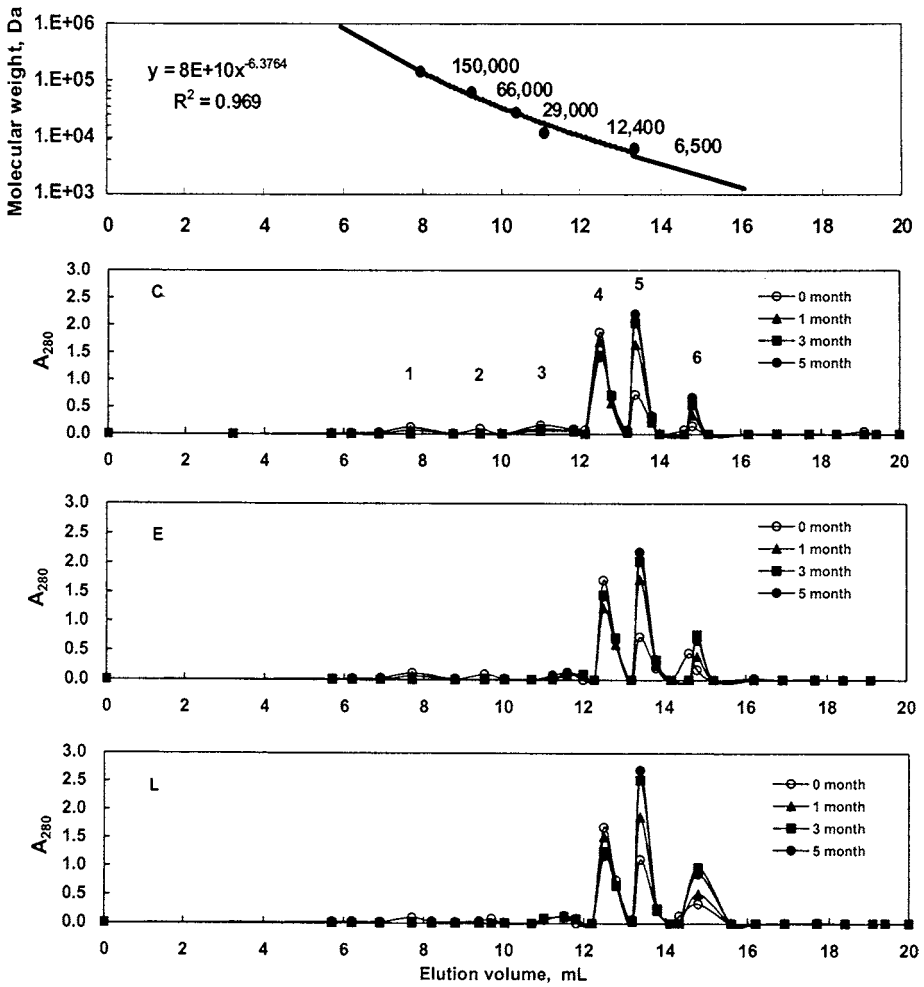

Figure 5. Changes in HPLC gel permeation chromatograms using a Shodex protein KW-804 column of sauce prepared from processing byproducts of spotted shrimp during fermentation. Product codes (C, E, and L) are as explained in the text.

and L, activities at pH 8.0 were 1.5-fold higher than those at pH 6.0, possibly due to the presence of more alkaline proteases with stronger activities at pH 8.0.

The hydrolyzing activities of benzoyl-arg-β-naphthylamide (BANA, pH 7.0; **Figure 7**) during fermentation of shrimp sauce revealed rapid hydrolysis of sauce L over the first month of fermentation (0.076 unit/mg) and a subsequent leveling off. For sauces C and E, however, the hydrolysis remained constant throughout the fermentation period, reaching only 30% of that of L. BANA shows a peculiar hydrolyzing activity for trypsin-like enzymes, especially for hydrolyzing proteins for low-salt L formulation. Ezquerra et al. (*32*) reported the presence of both trypsin and chymotrypsin in hepatopancreas extract of white shrimp (*Penaeus vannamei*). Therefore, alkaline proteases such as trypsin and chymotrypsin may be implicated in the fermentation of shrimp during sauce production. In the case of hydrolyzing activities toward ArgNA, the activity of sauce C increased 4 times from 0.03 unit/mg (0 month) to 0.12 unit/mg (2 months) but decreased to 0.04 unit/mg after 6 months of fermentation. The activity of E reached a peak of 0.11 unit/mg in the 3 months and then decreased to 0.04 unit/mg in 6 months. Meanwhile, the activity of L increased from 0.03 unit/mg (0 month) to 0.07 unit/mg (1 month) and then leveled off. Hydrolyzing activity toward LeuNA (**Figure 7**) was almost the same as that toward ArgNA, but the latter was stronger than the former.

Therefore, exopeptidases such as leucine aminopeptidase and arginine aminopeptidase may represent the main proteolytic force in the case of high-salt sauces C and E. However, trypsin-like endopeptidase and leucine and arginine aminopeptidase exopeptidase may be active under the low salt conditions experienced in sauce L.

Carotenoid Content. The carotenoid contents in all sauces decreased during fermentation, as shown in **Table 1**. The remaining carotenoid content in sauce C after 3 months of fermentation was 26.3% of the original amount, whereas those in sauces E and L, both containing sodium erythorbate, were 76.2 and 73.0%, respectively. Antioxidant-treated sauces (E and L) had distinctly less degradation of carotenoids than the untreated sauce C, indicating that sodium erythorbate was effective in controlling the oxidation of carotenoids. Cho et al. (*33*) reported that the browning in the salt-fermented ascidians caused by carotenoid oxidation could be retarded by antioxidants.

Sensory Evaluation. For sensory evaluation, commercially salt-fermented shrimp was used as a reference with a grade designation of 3 (fair). The color of sauce C was rated 2.14 $\pm$ 0.38, which is acknowledged as a meaningful grade in 2 months fermented samples and lower than that of the commercial products, whereas the antioxidant-treated sauces (E and L) received scores of 3.29 $\pm$ 0.49 and 3.71 $\pm$ 0.49, respectively, after month 3, which were higher than that of their commercial counterpart (**Table 2**). However, after 6 months (2.43 $\pm$ 0.53 for E; 2.57 $\pm$ 0.53 for L), the color scores of all three sauces were lower than that of the reference salt-fermented shrimp. In the taste sensory evaluation, all three sauces (C, E, and L) attained values which were lower than that of commercial

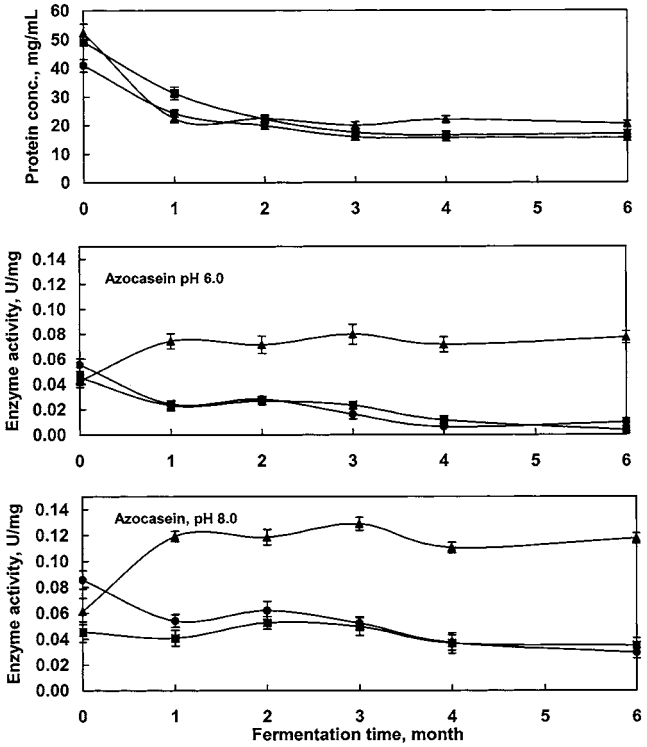

Figure 6. Changes in protein concentration and proteolytic activity of sauces prepared from wastes of spotted shrimp during fermentation. Product codes (C, ●; E, ■; L, ▲) are as explained in the text.

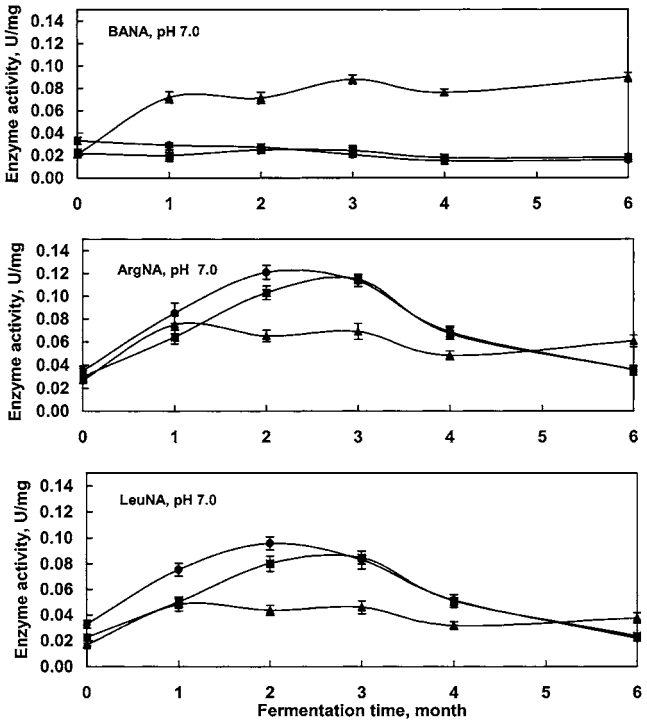

Figure 7. Changes in amidolytic activity of sauces prepared from wastes of spotted shrimp during fermentation. Product codes (C, ●; E, ■; L, ▲) are as explained in the text.

salt-fermented shrimp during the early stages of fermentation, probably because of the low contents of extractive compounds. However, at month 3, they received higher taste scores (4.29 ± 0.49 for C, 4.14 ± 0.38 for E, and 4.29 ± 0.49 for L) than the commercial sauce (**Table 2**). As high molecular weight compounds were hydrolyzed, low molecular weight extractive compounds were released, hence intensifying the taste of the sauce. After 3 months, there was little change in taste, which began to weaken.

Table 1. Changes in Total Carotenoid Contents of Sauce Prepared from Processing Byproducts of Spotted Shrimp during Fermentation (Grams/100 mL of Sauce)

fermentation time (months)	product code[a] C	E	L
0	0.129 ± 0.005	0.130 ± 0.002	0.126 ± 0.004
1	0.087 ± 0.006	0.119 ± 0.004	0.115 ± 0.007
2	0.051 ± 0.004	0.108 ± 0.007	0.100 ± 0.005
3	0.034 ± 0.003	0.99 ± 0.005	0.092 ± 0.005
4	0.021 ± 0.004	0.87 ± 0.004	0.081 ± 0.004
6	0.017 ± 0.003	0.64 ± 0.005	0.079 ± 0.002

[a] Product codes: C, 30 g of salt/100 g of byproduct; E, same as C, but also with 0.2 g of sodium erythorbate; L, 20 g of salt/100 g of byproduct along with 0.2 g of sodium erythorbate, 6 g of sorbitol, 0.5 g of lactic acid, and 5 mL of ethanol. Values are means of three determinations ± standard deviation.

Table 2. Results of Sensory Evaluation of Sauce Prepared from Processing Byproducts of Spotted Shrimp during Fermentation

product code[a]	fermentation time(months)	color[b]	taste[b]	flavor[b]
commercial sauce[c]	3	3.00 ± 0.00ef	3.00 ± 0.00d	3.00 ± 0.00d
C	0	3.71 ± 0.49g	2.29 ± 0.49b	3.14 ± 0.38de
	1	3.14 ± 0.69f	2.86 ± 0.38c	3.43 ± 0.53defg
	2	2.14 ± 0.38bc	3.29 ± 0.49de	3.86 ± 0.38gh
	3	2.00 ± 0.00ab	4.29 ± 0.49j	4.29 ± 0.49i
	4	1.86 ± 0.38a	4.00 ± 0.00hij	4.00 ± 0.00hi
	6	1.57 ± 0.53a	3.57 ± 0.53fg	3.43 ± 0.53defg
E	0	3.86 ± 0.38h	2.14 ± 0.38b	3.00 ± 0.00de
	1	3.71 ± 0.49gh	3.00 ± 0.00de	3.29 ± 0.49def
	2	3.71 ± 0.49gh	3.43 ± 0.53ef	3.71 ± 0.49fg
	3	3.29 ± 0.49f	4.14 ± 0.38ij	4.29 ± 0.49i
	4	3.00 ± 0.00ef	4.00 ± 0.00hij	3.71 ± 0.49fgh
	6	2.43 ± 0.53cd	3.71 ± 0.49fgh	3.29 ± 0.49defg
L	0	4.00 ± 0.58h	1.29 ± 0.49a	1.43 ± 0.53a
	1	4.00 ± 0.00h	2.14 ± 0.38b	2.29 ± 0.49b
	2	3.86 ± 0.38h	2.43 ± 0.53bc	2.86 ± 0.38c
	3	3.71 ± 0.49gh	4.29 ± 0.49j	3.43 ± 0.79defg
	4	3.14 ± 0.38f	4.14 ± 0.38ij	3.43 ± 0.79defg
	6	2.57 ± 0.53d	3.86 ± 0.38ghi	3.57 ± 0.53efg

[a] Product codes are the same as in **Table 1**. [b] Values are means of 10 determinations ± standard deviation. Means with different letters within the same column are significantly different ($P < 0.05$). [c] Salt-fermented shrimp on the market was prepared at 20 °C and was 3-months fermented at the time of purchase.

Table 3. Physicochemical and Microbiological Properties of Salt-Fermented Sauce from Spotted Shrimp Byproduct for 3 Months

measurement	product code[a] C	E	L	commercial sauce[b]
moisture (%)	68.0 ± 0.4	68.0 ± 0.2	71.6 ± 0.0	65.7 ± 0.5
crude protein (%)	7.1 ± 0.0	7.3 ± 0.0	7.0 ± 0.1	5.0 ± 0.1
total lipid (%)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
ash (%)	24.0 ± 0.0	23.9 ± 0.0	18.1 ± 0.1	28.0 ± 0.4
pH	7.46 ± 0.06	7.35 ± 0.05	7.37 ± 0.03	8.49 ± 0.06
salinity (%)	24.0 ± 0.5	23.8 ± 0.4	18.0 ± 0.2	24.6 ± 0.3
alcohol (%)	0.21 ± 0.07	0.20 ± 0.06	3.23 ± 0.14	ND
water activity	0.751	0.753	0.774	ND
viable cells (CFU/g)	2.9×10^4	3.2×10^4	3.4×10^4	9.8×10^4

[a] Product codes are the same as in **Table 1**. Values are means of three determinations ± standard deviation. [b] Salt-fermented shrimp on the market. ND, not determined.

Table 4. Results of Omission Test of Salt-Fermented Sauce from Spotted Shrimp Byproduct for 3 Months

	sample code[a]							
product code[b]	C	A	N	O	AN	NO	OA	ANO
E	5.00 ± 0.00f	1.44 ± 0.53b	4.11 ± 0.33e	4.44 ± 0.53e	1.11 ± 0.33a	3.44 ± 0.53d	1.78 ± 0.44c	1.78 ± 0.44c
L	5.00 ± 0.00f	1.67 ± 0.50b	4.22 ± 0.44e	4.56 ± 0.53e	1.00 ± 0.00a	3.33 ± 0.50d	1.78 ± 0.44c	1.67 ± 0.50c

[a] C, original broth; A, broth from which amino acids were eliminated; N, broth from which ATP-related compounds were eliminated; O, broth from which organic acids were eliminated; AN, broth from which amino acids and ATP-related compounds were eliminated; NO, broth from which nucleotide-related compounds and organic acids were eliminated; OA, broth from which organic acids and amino acids were eliminated; ANO, broth from which amino acids, ATP-related compounds, and organic acids were eliminated. Means with different letters within the same row are significantly different ($P < 0.05$). Values are means of 10 determinations ± standard deviation. [b] Same as in **Table 1**.

Table 5. Free Amino Acid Contents of Salt-Fermented Sauce Prepared from Shrimp Processing Byproducts during Fermentation (Milligrams/100 g)

	taste		0 months		3 months		commercial
amino acid	threshold[a] (g/dL)	byproducts	E[b]	L[b]	E	L	sauce[c]
phosphoserine	NA[d]	11.5	7.9	6.6	6.8	5.4	18.8
taurine	NA	130.8	84.8	87.1	3.6	4.0	–[e]
aspartic acid	0.003	39.9 (13.30)[f]	25.5 (8.50)	23.0 (7.67)	170.8 (56.94)	176.3 (58.78)	59.3 (19.77)
threonine	0.26	104.9 (0.52)	67.8 (0.26)	70.7 (0.27)	189.5 (0.73)	214.2 (0.82)	65.8 (0.25)
serine	0.15	58.9 (0.39)	40.7 (0.27)	35.6 (0.24)	186.6 (1.24)	203.2 (1.35)	55.4 (0.37)
asparagine	0.10	85.0 (0.85)	58.1 (0.58)	55.0 (0.55)	171.2 (1.71)	226.6 (2.27)	28.4 (0.28)
glutamic acid	0.005	92.5 (18.50)	62.5 (12.50)	70.3 (14.06)	514.8 (102.9)	420.0 (84.00)	62.4 (12.48)
glutamine	NA	78.5	55.2	46.8	35.8	38.5	26.8
sarcosine	NA	0.7	0.4	–	8.0	8.7	0.9
α-AA[g]	NA	1.1	0.7	–	1.7	13.4	0.6
proline	0.30	108.6 (0.36)	76.1 (0.25)	82.8 (0.28)	280.9 (0.94)	176.4 (0.59)	37.0 (0.12)
glycine	0.13	52.5 (0.40)	38.4 (0.30)	37.0 (0.28)	312.9 (2.41)	307.2 (2.36)	56.6 (0.44)
alanine	0.06	75.8 (1.26)	66.4 (1.11)	64.5 (1.08)	381.8 (6.36)	408.3 (6.80)	61.9 (1.03)
citrulline	NA	35.1	21.3	23.9	–	6.3	54.5
valine	0.14	93.0 (0.66)	67.3 (0.48)	64.0 (0.46)	306.4 (2.19)	348.6 (2.49)	69.2 (0.49)
cysteine	NA	16.4	8.5	5.5	37.0	69.1	–
methionine	0.03	39.2 (1.31)	26.7 (0.89)	30.4 (1.01)	143.7 (4.79)	121.2 (4.04)	20.0 (0.67)
isoleucine	0.09	82.8 (0.92)	60.1 (0.67)	64.7 (0.72)	257.7 (2.86)	284.9 (3.17)	65.7 (0.73)
leucine	0.19	114.2 (0.60)	81.0 (0.43)	88.8 (0.47)	411.1 (2.16)	438.4 (2.31)	97.4 (0.51)
tyrosine	NA	101.1	60.1	52.4	50.2	62.2	66.4
β-alanine	NA	4.1	2.0	2.4	16.6	14.7	–
phenylalanine	0.09	118.7 (1.32)	79.2 (0.88)	90.0 (1.00)	218.0 (2.42)	285.8 (3.18)	62.9 (0.70)
ethanolamine	NA	–	–	–	14.5	19.3	–
DL-AL[g]	NA	4.1	1.4	3.4	5.5	20.6	1.1
ornithine	NA	77.9	53.8	49.3	290.0	286.4	65.7
lysine	0.05	108.8 (2.18)	81.4 (1.63)	110.2 (2.20)	486.6 (9.73)	442.1 (8.84)	100.9 (2.02)
histidine	0.02	62.0 (3.10)	44.1 (2.21)	39.8 (1.99)	72.1 (3.61)	102.9 (5.15)	23.0 (0.46)
arginine	0.05	213.5 (4.27)	155.7 (3.11)	177.2 (3.54)	142.7 (2.85)	137.6 (2.75)	100.4 (2.01)
total		1911.6	1327.2	1381.4	4716.5	4840.3	1201.5

[a] These data were quoted by Kato et al. (*35*). [b] Same as in **Table 1**. [c] Salt-fermented shrimp on the market. [d] NA, not available. [e] –, not detected. [f] Values in parentheses represent taste values of free amino acid concentration divided by taste threshold. [g] Abbreviations: α-AA, β-aminodipic acid; DL-AL, DL+-allohydroxylysine.

As already mentioned, there was a difference in taste or flavor between the high-salt (C and E) and low-salt (L) sauces. Sauces C and E had a deeper and more desirable flavor than sauce L, and the presence of ethanol positively influenced the flavor of the sauce.

On the basis of the sensory evaluation data, low-salt sauce L was found to fare favorably with high-salt sauces (C and E) in color, taste, and flavor. The yield of extractive compounds (amino nitrogen, nonprotein nitrogen, and VBN) and sensory results further indicated that a 3 month fermentation period was optimum for production of the three types of shrimp sauces examined.

Physicochemical and Microbiological Properties. According to the proximate composition shown in **Table 3**, the moisture contents of sauces C and E (68%) were the same, but the low-salt sauce (L, 71.6%) had a higher moisture and a lower (by 6%) ash content (**Table 3**). The amount of common salt in L was relatively lower than that in high-salt sauces (C and E) as reflected in these results. The contents of crude protein and lipid were nearly the same for all types of sauces produced. Thus, all sauce samples prepared had higher moisture and crude protein contents and a lower crude ash content than commercial salt-fermented shrimp, but their total lipid contents were about the same.

The pH values of the three sauces were similar (~7.4), which is ~1 unit lower than the pH of 8.49 observed for commercial salt-fermented shrimp. Salinities of high-salt sauce C and antioxidant-treated sauce E were 24% compared to that of low-salt sauce L (18%). All sauces examined here, however, had a lower salinity than commercial salt-fermented shrimp. According to the standards of Fisheries Products Inspection of the Republic of Korea (*34*), it is prescribed that the moisture content of shrimp sauce should be <68% and its total nitrogen content be >1% with a salinity of <27%. Thus, high-salt sauces C and E meet this requirement. For the low-salt sauce (L) its total nitrogen content and salinity meet the above requirement, but its moisture content is higher than that recommended by ~3%. A viable cells count of shrimp sauce made under optimum fermentation time was, regardless of the type, $(2.9-3.4) \times 10^4$ colony-forming units (CFU)/g, which is far below that of commercial

Table 6. Changes in Contents of ATP and Its Related Compounds of Salt-Fermented Sauce Prepared from Shrimp Processing Byproducts during Fermentation (Milligrams/100 g)

		0 months		3 months		commercial
nucleotide	byproducts	E[a]	L[a]	E	L	sauce[b]
ATP	1.47	1.11	1.23	–[c]	–	–
ADP	8.55	6.22	6.48	–	–	4.00
AMP	0.90	0.72	0.70	1.59	1.72	1.20
IMP	60.63	43.33	43.19	56.15	53.47	47.63
inosine	18.02	12.81	13.79	8.64	10.65	11.34
hypoxanthine	44.85	35.88	37.45	36.55	40.84	16.38
total	134.42	100.07	102.84	102.93	106.68	80.55

[a] Same as in **Table 1**. [b] Salt-fermented shrimp on market. [c] –, not detected.

salt-fermented shrimp (9.8×10^4 CFU/g). It appears that a portion of viable cells was removed in the process of washing of raw material and subsequent centrifugal separation for obtaining shrimp sauce. Water activities in 3 months fermented C and E were 0.751 and 0.753, respectively, and that of L was 0.774. Kim (*6*) reported that the water activities, equivalent to growth limiting of common bacteria, yeast, and fungi, were 0.90, 0.88, and 0.80, respectively, and that nonenzymatic browning occurred in the water activity range of 0.7−0.8. Therefore, it appears that three sauces (C, E, and L) in this study may retain their integrity against microbial deterioration due to the growth of common bacteria, yeast, and fungi. However, it is likely that nonenzymatic browning occurs, and countermeasures are necessary to control its development.

Omission Test. In both E and L samples devoid of free amino acids (A: E, 1.44; L, 1.67), those devoid of amino acids and nucleotide-related compounds (AN: E, 1.11; L, 1.00), those devoid of amino acids and organic acids free samples (OA: E and L, 1.78), and those devoid of amino acids, nucleotide-related compounds, and organic acid free samples (ANO: E, 1.78; L, 1.67) distinct differences existed in their taste with a strong sour taste (**Table 4**). The removal of nucleotide-related compounds (N: E, 4.11; L, 4.22), organic acids (O: E, 4.44; L, 4.56), and nucleotide-related compounds and organic acids (NO: E, 3.44; L, 3.33) did not lower the taste score as markedly as removal of A, OA, and ANO (**Table 4**). The results demonstrated that free amino acids were most important to the taste of shrimp sauce, whereas nucleotide-related compounds and organic acids were only marginally important.

Free Amino Acids. The composition of free amino acids in sauces examined was determined because free amino acids are most important to the taste of shrimp sauce as shown by the omission test. **Table 5** summarizes the contents of free amino acids in shrimp sauce during fermentation and its calculated taste values. Some 30 free amino acids were identified in shrimp byproducts and sauces. The total free amino acids content of shrimp processing byproducts was 2018.1 mg/100 g, and contents of arginine (213.5 mg/100 g), phenylalanine (118.7 mg/100 g), leucine (114.2 mg/100 g), lysine (108.8 mg/100 g), proline (108.6 mg/100 g), and threonine (104.9 mg/100 g) were high. Right after the production of shrimp sauce (E and L, 0 months), the total contents of free amino acids were approximately 1398.6 mg/100 g for E and 1443.1 mg/100 g for L. The total free amino acid contents of E and L were reduced by 30.7 and 28.5%, respectively, from that of shrimp processing byproducts (2018.1 mg/100 g) because of the addition of ingredients, mainly salt. The major free amino acids of shrimp sauce (E and L), immediately after production, were arginine, phenylalanine, leucine, lysine, and proline, similar to those in the byproducts. The total free amino acids content of high- (E) and low-salt (L) sauces after 3 months of fermentation were 4726.7 and 4855.0 mg/100 g, respectively, increases of 3- and 4-fold, respectively. During fermentation, there were almost no changes in the content of phosphoserine, taurine, glutamine, citrulline, α-aminoadipic acid, tyrosine, and arginine; however, increases were observed for others, especially glutamic acid (E, 823.7%; L, 597.4%), glycine (E, 814.8%; L, 830.3%), alanine (E, 575.0%; L, 633.0%), valine (E, 455.3%; L, 544.7%), leucine (E, 507.5%; L, 493.7%), and lysine (E, 597.8%; L, 401.2%). The total free amino acid content of commercial salt-fermented shrimp was 1222.5 mg/100 g, equivalent to 85% of those in sauces E and L at the beginning and 25% of those after 3 months of fermentation.

Among amino acids, glutamic acid (18.50) and aspartic acid (13.30) had the highest taste values calculated from the ratio of amino acid content to taste threshold (*35*) throughout the fermentation period. The taste values increased considerably

Table 7. Protein Amino Acid Contents of Salt-Fermented Sauce Prepared from Shrimp Processing Byproducts during Fermentation (Milligrams/100 g)

		0 months		3 months		
amino acid	byproducts	E[a]	L[a]	E	L	commercial sauce[b]
aspartic acid	982.2 (9.5)[c]	452.3 (10.0)	415.4 (9.2)	741.5 (10.9)	776.4 (11.2)	669.6 (13.4)
threonine	538.4 (5.2)	210.1 (4.7)	218.1 (4.8)	369.0 (5.4)	367.9 (5.3)	265.6 (5.3)
serine	511.9 (5.0)	187.3 (4.1)	214.6 (4.7)	340.6 (5.0)	339.1 (4.9)	256.6 (5.2)
glutamic acid	1342.7 (13.0)	709.1 (15.7)	671.9 (14.9)	1049.9 (15.4)	1058.5 (15.3)	798.9 (16.0)
proline	478.5 (4.6)	286.2 (6.3)	233.0 (5.2)	393.4 (5.8)	415.8 (6.0)	212.5 (4.3)
glycine	657.8 (6.4)	331.9 (7.4)	339.5 (7.5)	432.6 (6.4)	432.0 (6.2)	255.6 (5.1)
alanine	582.8 (5.7)	281.9 (6.2)	266.1 (5.9)	351.5 (5.2)	396.5 (5.7)	253.9 (5.1)
cystine	202.1 (2.0)	25.8 (0.6)	15.1 (0.3)	59.0 (0.9)	63.0 (0.9)	21.0 (0.4)
valine	650.0 (6.3)	288.1 (6.4)	347.7 (7.7)	484.5 (7.1)	472.4 (6.8)	338.7 (6.8)
methionine	86.8 (0.8)	47.2 (1.0)	31.9 (0.7)	56.0 (0.8)	63.2 (0.9)	12.3 (0.2)
isoleucine	443.8 (4.3)	176.4 (3.9)	219.6 (4.9)	296.3 (4.4)	291.4 (4.2)	269.8 (5.4)
leucine	672.7 (6.5)	299.7 (6.6)	340.2 (7.5)	448.8 (6.6)	445.7 (6.4)	385.3 (7.7)
tyrosine	195.0 (1.9)	83.6 (1.9)	47.5 (1.1)	167.9 (2.5)	187.6 (2.7)	114.8 (2.3)
phenylalanine	858.6 (8.3)	180.2 (4.0)	180.0 (4.0)	391.5 (5.8)	387.6 (5.6)	204.2 (4.1)
histidine	268.6 (2.6)	128.2 (2.8)	121.3 (2.7)	200.5 (2.9)	201.1 (2.9)	107.7 (2.2)
lysine	922.9 (9.0)	498.2 (11.0)	591.0 (13.1)	614.8 (9.0)	602.5 (8.7)	552.3 (11.1)
arginine	902.1 (8.8)	328.0 (7.3)	267.4 (5.9)	410.2 (6.0)	429.2 (6.2)	263.0 (5.3)
total	10296.9 (99.9)	4514.2 (99.9)	4520.3 (100.1)	6808.0 (100.1)	6929.9 (99.9)	4972.8 (99.9)

[a] Same as in **Table 1**. [b] Salt-fermented shrimp on market. [c] Values in parentheses are the percentage of total amino acid content.

Table 8. Changes in Minerals and Phosphorus Contents of Salt-Fermented Sauce Prepared from Shrimp Processing Byproducts during Fermentation (Milligrams/100 g)

	0 months		3 months	
mineral	E[a]	L[a]	E	L
calcium	20.5	20.5	100.2	124.2
iron	0.3	0.2	2.1	2.8
magnesium	2.7	2.8	4.1	4.3
phosphorus	4.1	5.5	35.3	39.7

[a] Same as in **Table 1**.

after fermentation. In commercial salt-fermented shrimp, on the other hand, aspartic acid showed the highest taste value, followed by glutamic acid. From these results, one may conclude that taste was influenced by glutamic acid for umami and by aspartic acid for sweetness.

ATP and Its Related Compounds. The total content of nucleotide-related compounds decreased by ~23% in 3 months (E, 102.93 mg/100 g; L, 106.68 mg/100 g) for both sauces regardless of the amount of common salt added. Nucleotide-related compounds of E and L (3 months) were higher by 27.8 and 32.6% than those in the commercial salt-fermented shrimp sauces (**Table 6**). Nucleotide-related compounds at the beginning included ATP, ADP, AMP, IMP, inosine, and hypoxanthine, but after 3 months both ATP and ADP were no longer present. Among the four identified nucleotides, IMP, which was assumed to have a strong influence on the taste of shrimp sauce, increased and inosine decreased, whereas others remained unchanged during the fermentation process (**Table 6**).

Total Amino Acids. Changes in the content of total amino acids during fermentation of shrimp processing byproducts are shown in **Table 7**. Total amino acids content of byproducts was 10296.9 mg/100 g, as compared to that of E (0 month, 4514.2 mg/100 g) and L (4520.3 mg/100 g), which were ~44% of that of the byproducts. After 3 months of fermentation, the values were 6808.0 mg/100 g for E and 6929.9 mg/100 g for L, equivalent to ~67% of that of the byproducts.

The increase in the total amino acids content during fermentation appears to be due to liquification by autohydrolytic and microbial proteases. The total amino acids contents of sauces E and L after 3 months of fermentation were higher by 37−39% than that of the commercial salt-fermented shrimp tested (4972.8 mg/100 g). Major constituent amino acids of byproducts E and L were aspartic acid, glutamic acid, and lysine, similar to those in commercial salt-fermented shrimp. Thus, the high amounts of lysine, which is a limiting amino acid in grains such as rice, a staple food for Koreans, in the shrimp salt-fermented sauce may act as a nutritional supplement.

Mineral Composition. Changes in the contents of minerals and phosphorus during the fermentation of shrimp byproducts are shown in **Table 8**. In shrimp sauce, regardless of the fermentation period, calcium (20−124 mg/100 g) was present in the highest amount, followed by phosphorus (4.1−39.4 mg/100 g), magnesium (2.7−4.3 mg/100 g), and iron (0.3−2.8 mg/100 g); manganese was not detected. Obviously, calcium contained in the shells was partially liquified and included in the solution. The Korean Institute for Health and Social Affairs (*36*) recommends a daily intake of 600 mg of calcium, 10 mg of iron, and 300−350 mg of magnesium for adults. Taking into consideration the minerals contained in shrimp sauc, with regard to recommended daily intake it may be concluded that shrimp sauce provides an excellent source of calcium. Thus, shrimp sauces E and L, made from shrimp byproducts, provide a valuable source of high-quality amino acids such as lysine and functional minerals, including calcium.

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