Food Sci. Biotechnol. Vol. 17, No. 1, pp. 114 ~ 117 (2008)

Food Science and Biotechnology

Prevention of Precipitation in Sand Lance Fish Sauce by Chelating Agents

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Abstract Chelating agents to sand lance fish sauce for the prevention of precipitate formation were applied. The precipitates consisted of crude protein (74.4%), ash (18.7%), and other components (6.9%). Sand lance sauce was mainly composed of glutamic acid (3.69 mg/g), alanine (2.96 mg/g), and lysine (2.64 mg/g). However, there was an increase in the amount of hydrophobic amino acids, phenylalanine, isoleucine, and leucine, in the precipitates. Sodium ions were not detected in the precipitate; rather, the main elements were Mg (1.98×10^4 μg/g), K (1.36×10^4 μg/g), and Ca (6.66×10^2 μg/g). In HPLC analysis, fish sauce was composed of 2 main peaks with molecular weights of 85.5 and 528.4 Da, respectively. However, the precipitate contained one peak with a molecular weight of 1,513.5 Da. The addition of 0.2% malic acid and citric acid caused 55 and 70% prevention of the precipitate, respectively. Citric acid was the most effective chelating agent and efficiently prevented precipitation in the fish sauce.

Keywords: fish sauce, sand lance sauce, precipitate prevention

Introduction

As a traditional seasoning, fish sauce is widely used in large quantities in southeast Asian countries. In east Asian countries such as China, Korea, and Japan, however, fish sauce has been overtaken by soy sauce, which has a much milder taste and smell. As a result, only a few fish sauces have survived in Korea as characteristic products in specific regions. Recently, however, fish sauces have been rediscovered, even in Korea, because of renewed consumer interest in authentic tastes and traditional foods (1). Fish sauce is now used in a variety of processed products in Korea, and the amount of imported fish sauce increases year after year. Anchovy (*Engrulis japonicus*) and sand lance (*Ammodytes personatus*) sauces are popular ingredients in preparing *kimchi* in Korea (2-4). Because it has better taste and flavor than anchovy sauce, there is increasing demand for sand lance sauce. Sand lance is common in many areas of the West Sea in Korea and are a principal prey for many marine birds, commercial fish, and marine mammals (5,6). Although sand lance is important in the food web, no countries have attempted to use these fish for processed foods except Korea.

To prepare sand lance sauce, salt is added to a level of 20-30% to raw fish, and the mixture is then fermented for a long period of time to allow the taste to develop. The fermentation period varies depending on the salt concentration and fermentation temperature: 6 months for most sand lance sauces with low salt levels and 1 year for a sauce with high salt (7,8).

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Received July 4, 2007; accepted August 27, 2007

The favored taste and flavor develop gradually during fermentation through several enzymatic reactions and microbial degradation processes (9,10). Thus, sand land sauces contain relatively high amounts of amino acids, the degradation products of fish protein. Although sand lance sauce has a good flavor and taste, a precipitate forms during storage and preservation. To solve this problem, the fish sauce can be heated before packing. However, the characteristics of the taste and flavor of the sand lance sauce are modified or weakened by heat treatment. Although the precipitate is not harmful, the precipitate worsens the appearance and customers become hesitant to use the sauce.

Thus, it is very important to prevent or remove the precipitate that forms in sand lance sauce during storage without changing the taste and flavor. Therefore, in this study, we tested the effects of chelating agents on the prevention of the formation of precipitate in fish sauce.

Materials and Methods

Materials Sand lance sauce was purchased within 2 weeks of its distribution to a local retail store and stored at room temperature. Total nitrogen and amino nitrogen contents of sand lance sauce were 119.2 and 6.3 mg/g, respectively. Malic acid, citric acid, lactic acid, and tartaric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Prevention of the precipitate in sand lance sauce Ten mg of each chelating agent, including malic acid, citric acid, tartaric acid, and lactic acid, were added to commercial sand lance sauce (10 mL), and the mixtures were heated at 100°C for 30 min. After centrifugation at 8,000×g for 10 min, the weight of the precipitate was measured. The precipitate-forming ratio was calculated as follows:

$$\text{Precipitate-forming ratio (\%)} = \frac{W_{c\text{-}ppt} - W_{ppt}}{W_{c\text{-}ppt}} \times 100$$

where, $W_{c\text{-}ppt}$ is the weight of precipitate in the control sauce and W_{ppt} is the weight of the precipitate in sauce with added chelating agents.

Assay of the components of the precipitate The amino acid content was analyzed by HPLC (Waters Pico-Tag column, Waters Associates, Medford, MA, USA) after hydrolysis with 6 N HCl at 115°C for 24 hr under vacuum. For the trace element analysis, 0.1 g of the precipitate was dissolved in a solution of 0.2 N HNO_3 and the final volume was adjusted to 100 mL. The solution was analyzed for Mg, Ca, P, Fe, Sr, Al, Zn, Se, Mo, and Cu using an ARL (model 3410) inductively coupled plasma (ICP) Emission Spectrometer (Ecublens, Switzerland).

Molecular weight determination The fish sauce and precipitate were applied to an Agilent 1100 HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with the Shodex Asahipak GS-520HQ/320/220 (Tokyo, Japan) gel-filtration chromatographic column, maintained at a temperature of 60°C, and eluted with 0.02 M phosphate buffer (pH 7.2) containing 0.25 M NaCl. The flow rate was set at 0.5 mL/min.

The calibration curve was obtained by running bovine carbonic anhydrase (29,000 Da, Sigma-Aldrich Co.), horse heart cytochrome C (12,400 Da, Sigma-Aldrich Co.), bovine insulin (5,800 Da, Sigma-Aldrich Co.), bacitracin (1,450 Da, Sigma-Aldrich Co.), gly-gly-tyr-arg (451 Da, Sigma-Aldrich Co.) and gly-gly-gly (189 Da, Sigma-Aldrich Co.).

Results and Discussion

Component and molecular weight distribution of the precipitate in sand lance sauce After centrifugation at 2,800×g for 15 min, the precipitate was obtained during cold storage. The precipitates consisted of crude protein (74.4%), ash (18.7%), and other components (6.9%). The amino acids in the crude protein of the fish sauce and the precipitate are listed in Table 1. Sand lance sauce consisted mainly of glutamic acid (3.68 mg/g), alanine (2.96 mg/g), and lysine (2.64 mg/g). A high amount of glutamic acid may give a palatable taste. However, the amount of hydrophobic amino acids (phenylalanine, isoleucine, and leucine) was greater in the precipitate. In particular, the amount of phenylalanine increased greatly from 0.18 to 2.53 mg/g.

The trace elements in the fish sauce and the precipitate are given in Table 2. The fish sauce was mainly composed of Na (1.07×10^5 μg/L). Mg, K, and Ca amounts in the fish sauce were 0.8, 2.7, and 1.2 μg/L, respectively. However, sodium was not detected in the precipitate; its main elements were Mg (1.98×10^4 μg/g), K (1.36×10^4 μg/g) and Ca (6.66×10^2 μg/g). The precipitate in fish sauce may be caused by the divalent magnesium ion.

Table 1. Amino acid composition of the fish sauce and the precipitate

Amino acid	Fish sauce (mg/g)	Precipitate (mg/g)	Amino acid	Fish sauce (mg/g)	Precipitate (mg/g)
Asp	1.56	0.09	Met	0.49	0.87
Thr	1.46	0.21	Ile	1.92	1.94
Ser	1.36	0.28	Leu	2.04	1.97
Glu	3.68	0.84	Tyr	0.04	0.03
Pro	0.71	1.02	Phe	0.18	2.53
Gly	0.74	0.12	His	0.91	0.57
Ala	2.96	2.88	Lys	2.64	0.71
Cys	0.82	0.34	Arg	0.75	0.08
Val	1.85	1.74			

Table 2. Trace elements of precipitate in sand lance sauce

Trace element	Na	Mg	K	Ca
Fish sauce (μg/L)	1.07×10^5	0.8	2.7	1.2
Precipitate (μg/g)	-	1.98×10^4	1.36×10^4	6.66×10^2

Figure 1 shows the molecular weight distribution of the fish sauce and its precipitate. Fish sauce was composed of 2 main peaks with molecular weights below 10 kDa. The molecular weights of the first and second peaks in the fish sauce were 85.5 and 528.4 Da, respectively. Contents of the sauce that were less than 150 Da were assumed to correspond to free amino acid.

However, the precipitate contained only one peak with a molecular weight of 1,513.5 Da. Thus, the precipitate contained a larger peptide than the fish sauce. Magnesium ion might be contributing to the formation of this insoluble peptide.

Sand lance sauce contains all of the essential amino acids and is especially high in glutamic acid (Table 1). When comparing all fish sauces, aspartate, glutamate, alanine, and lysine contents were found to be the most variable (1). This sauce also contains many vitamins, as well as minerals such as sodium, calcium, magnesium, iron, manganese, and phosphorus (11). According to our analyses, sand lance sauce contained sodium, magnesium, potassium, and calcium (Table 2). The precipitate mainly contained magnesium and potassium, as well as a peptide with a molecular weight of 1,513.5 Da (Fig. 1).

Even though fish sauce contains a wide range of nutrients, part of its nutritional value is due to the high salt concentration, which is also the cause of the precipitation. Gelation and aggregation of myosin molecules (fish muscle structural protein) involves partial denaturation, followed by irreversible aggregation of myosin heads through the formation of disulphide bonds and helix-coil transitions in the tail part of the molecules, resulting in a 3-dimensional network (12,13).

The physico-chemical properties of individual protein molecules, as well as environmental conditions (pH, temperature, ionic strength and species, solvent polarity and type) will influence the type of primary particle formed (morphology, size, and surface properties), the interactions between the primary particles, and ultimately the resulting 3-dimensional network structure. Protein molecules are

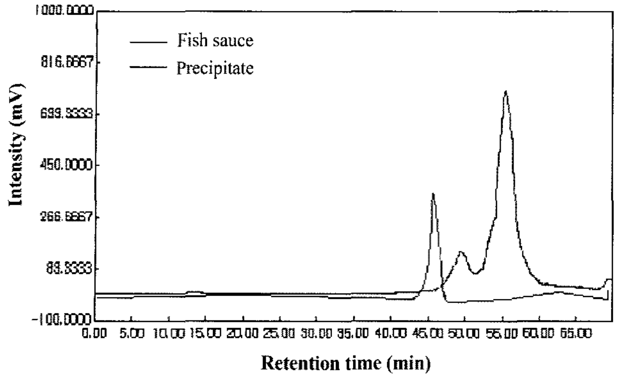

Fig. 1. Molecular distribution of sand lance sauce and its precipitate.

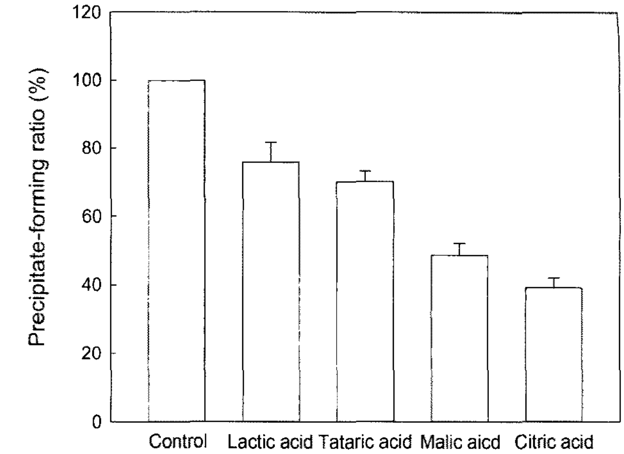

Fig. 2. Precipitate-forming ratio in sand lance sauce with the addition of chelating agent.

destabilized and form primary particles. These primary particles aggregate into large structures, which further associate to form larger structures, until a continuous 3-dimensional network is formed. Salt addition probably induces the aggregation of these particles into larger clusters, or flocks. These flocks, then, interact with each other until a 3-dimensional network, i.e., precipitate, is formed. Marangoni *et al.* (14) reported that the mechanisms by which calcium chloride, as a divalent cation, and sodium chloride, as a monovalent cation, induced the aggregation process were, however, different. The addition of NaCl leads to the slow and gradual decrease in the concentration of both the large aggregate and the smaller aggregates. NaCl-induced aggregation is a one-step process involving the slow and simultaneous aggregation of very large aggregates and smaller aggregated species of denatured whey proteins. The addition of $CaCl_2$, however, results in a different method of aggregation, that is, a very rapid and drastic decrease in the concentration of the large aggregates, followed by a slower disappearance of the smaller aggregates. $CaCl_2$-induced aggregation is a 2-step process with a rapid aggregation of large protein aggregates, followed by a slower aggregation of smaller protein aggregates. For example, soy protein isolate-based gels contain a variety of food ingredients such as sodium chloride to enhance the flavor (15), as well as minerals used as coagulant agents such as calcium chloride (16). At high ionic strength more protein matrix was formed (17). As shown in Table 2, Mg^{+2} and Ca^{+2} ions are the major trace elements in the precipitate of sand lance sauce. The precipitation in sand lance sauce during early and late stages of storage might be caused by the presence of these 2 divalent cations as well as the monovalent cation (K^{+}).

Prevention of the precipitate in the sand lance sauce
The precipitate of sand lance sauce worsens the appearance, making consumers hesitant to use the product. Therefore, the precipitate must be removed or prevented from forming in the fish sauce. Chelating agents (malic acid, tartaric acid, lactic acid, and citric acid) were used as food additives to prevent the precipitate; the results are shown in Fig. 2. The precipitate-forming ratios of lactic acid and tartaric acid were 75.9 and 70.3%, respectively. The addition of 0.1% malic acid and citric acid resulted in precipitate-forming ratios of 47.6 and 39.2%, respectively, which were significantly different from control. In other words, the addition of citric acid to the fish sauce prevented approximately 60% of the precipitate formation. Figure 3 shows the prevention effect by various concen-trations of malic acid and citric acid on the formation of the precipitate. As the concentration of the chelating agent increased, the precipitate-forming ratio decreased. The precipitate-forming ratio sharply decreased until 0.1% of malic acid and citric acid were added and then decreased gradually with increasing concentrations; citric acid was more effective than malic acid. The addition of 0.2% malic acid and citric acid resulted in 55 and 70% prevention of the precipitate formation, respectively. Based on these results, the addition of 0.2% citric acid was appropriate for the prevention of precipitate formation and for keeping the palatable taste of fish sauce.

The addition of a chelating agent increased the solubility of these elements in sand lance sauce. Therefore, the precipitate formed in the fish sauce decreased with the addition of chelating agent (Fig. 3). Natural chelating agents may play a key role in the solubilization of food

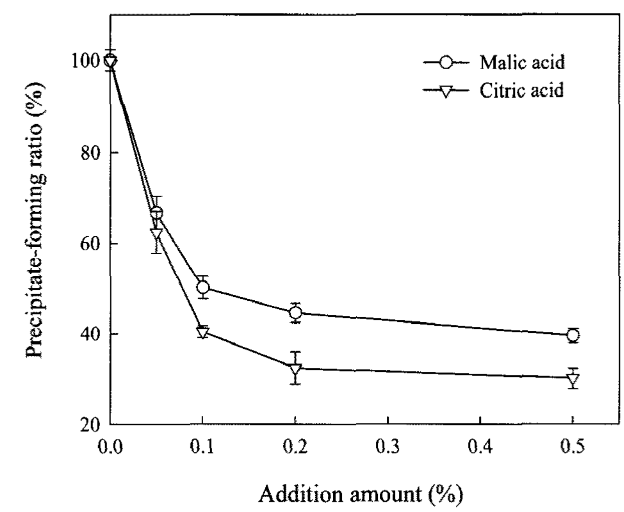

Fig. 3. Precipitate-forming ratio in sand lance sauce with different amounts of citric and malic acid.

materials and trace elements (18,19). The solubility of minerals also increased with increasing concentrations of the citric acid and malic acid. Citric acid was the most effective chelating agent and efficiently prevented the precipitation in the fish sauce. Ekholm *et al.* (20) also reported that citric acid was the most efficient chelating agent in oat bran and oat flakes. The solubilities of Ca^{+2}, Zn^{+2}, Mg^{+2}, and Mn^{+2} ions increased significantly when citric acid was added. Therefore, since Mg^{+2} and Ca^{+2} ions were the main components of the precipitate of the fish sauce, the addition of citric acid prevented the precipitate formation. Malic and lactic acid also increased the solubility of these minerals significantly, although to a lesser extent than citric acid (20). Malic acid significantly increased the solubility of Mg^{+2} ions and also affected the solubility of Mn^{+2} and Ca^{+2} ions in oat bran samples. The same trend was seen with the solubilities of Mn^{+2} and Ca^{+2} ions in the oat flake samples. Our results also followed the same trend as those of Ekholm *et al.* (20). The carboxylic group in citric and malic acids seems to be the most important structural element of the molecule and may affect the solubility of the minerals. The carboxylic group is able to form relatively stable complexes with most of the metal ions and thus could prevent the precipitation in the sand lance sauce.

The precipitate of sand lance sauce was composed of metal ions, especially magnesium ion, and of hydrophobic amino acids (Phe, Ile, and Leu). However, the solubility of the minerals increased with the addition of citric acid and malic acid. Citric acid was the most effective chelating agent and efficiently prevented precipitation in the fish sauce.

Acknowledgments

This research was supported by the 'GRRC' Project of the Gyeonggi Provincial Government, Korea.

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