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Effects of Organic Acids on Mineral Contents and Composition of Fish Bone Extracts

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유기산 첨가가 생선뼈 스프에 용출되는 무기질 함량에 미치는 영향

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Abstract

본 연구는 작은 생선은 뼈째로 먹을 수 있지만 큰 생선은 생선뼈를 거의 이용하지 못하여 생선의 뼈에 포함되어 있는 무기질을 유효하게 이용할 목적으로 유기산을 첨가해서 생선뼈 스프를 만들었을 때, 유기산의 종류와 가열시간이 뼈로부터 용출되는 칼슘, 인 함량의 변화에 대하여 조사하였다. 생선뼈에 함유되어 있는 수분과 무기질 함량은 생선뼈의 종류에 따라 달랐고, 큰 생선의 뼈일수록 수분 함량은 감소하는 경향이었다. 유기산을 첨가하여 12시간 가열했을 때 스프에 용출된 칼슘은 잿방어뼈에 구연산, 사과산을 첨가하였을 때가 61.34%와 60.50%로 가장 많았고, 인의 용출도 칼슘과 같은 경향이었다. 칼슘과 인의 용출량은 참치뼈에 4%의 사과산을 첨가하여 12시간 가열했을 때에 573 mg, 78 mg으로 가장 높았다. 참치뼈 스프의 칼슘과 인의 비율은 대조군에서는 칼슘보다 인의 용출(0.21~0.35)이 많았으나, 유기산을 첨가하였을 때는 칼슘의 용출(0.98~10.86)이 많았다. 단백질과 총 유리아미노산의 용출은 대조군보다 유기산을 첨가하였을 때가 증가하였다.

Key words : Fish bone, tuna, calcium, organic acid, mineral.

Introduction

In recent years, the convenience of meals and the fastness of food preparation are developed in Japan. The intakes of process food add to phosphate has also increased. An excessive intake of phosphorus deteriorates calcium absorption from intestines. Furthermore, it is thought that calcium lack is aggravated by promoting calcium excretion of urine(Ezawa 1987).

Some young peoples go on diet for beauty purpose. The lack of nutrient intake decreases calcium density and weakens strength of bone. As a result, there was a report that the receptivity of osteoporosis increased(Doi *et al* 1993).

Calcium is main composition in bone as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). This means that bone is a huge storehouse of calcium which might be used in lack of calcium intake (Mclean and Marshall 1968).

On the other hand, the modern society greets rapidly aging society, and the neighbor problems which are osteoporosis and bone fracture prevention are occurred. Therefore, the interest of calcium intake attracts attention for people.

There was a report about addition of calcium, milk food and bone powder in cooking for the purpose of prevention and treatment of osteoporosis(Kawana *et al* 1991).

Fishes have been important protein sources for Japanese. To improve their taste nature, various cooking, processing, and preservation methods are developed(Hatae *et al* 1990, Shimomura and Suzuki 1993). Furthermore, small fishes eaten with whole bone also used as important calcium sources, but fish bones are mostly discarded as a waste. The development of ways for effective utilization of fish bones are valuable for the efficient use of natural resources. The researches for efficient utilization of fish bones were reported such as fish bones

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treated with catechin in tea extracts(Hatae *et al* 1980), softening of salmon cartilage(Hatae *et al* 1990), and horse mackerel during cooking(Shimosaka *et al* 1996), and horse mackerel cured in acetic acid solution(Shimosaka *et al* 1998). We already reported the mineral solubilization from animal bone such as beef, pig, and chicken with using various kinds of organic acids and heating times. Calcium was extracted more with citric acid solution than with vinegar from beef and pig bone(Kim 1999, Han 2000), and more with citric acid and malic acid solution from chicken bone(Lee 2002).

Large fish bones are mostly discarded as a waste. The development of ways for effective utilization of fish bones are valuable for the efficient use of calcium source.

Then, this research is focused on the change of calcium and phosphorus contents which extracted in the fish bone soup with addition of organic acids such as acetic acid, malic acid, and citric acid.

Materials and Methods

1. Materials

Fish bone was bought in a central market in Kyoto and used in the experiment. Fish bones used were sardine(*Sardinops melanostictus*), yellow tail(*Seriola quinquueradiate*), amber jack(*Seriola dumerili*), tuna (*Thunnus thynnus*), sea bream (*Pagrus major*), and sea bass(*Lateolabrax japonicus*).

The organic acids used were extra pure reagent which acetic acid, malic acid, and citric acid (anhydrous). The sizes of fish bones were shown in Table 1.

2. Preparation of Fish Bones for Measurement

The fish bones were firstly heated in distilled water at 100 ℃ for 5 min to remove flesh, blood vessels and nerves that placed to bone while washing in distilled water. Fish bone was cut to chip of which weight was 2~5g. About 10g bones were placed in glass beaker (300 mL) with concentration of 0, 0.5, 1, 2, and 4% of acetic acid, malic acid and citric acid (200 mL), and glass plate was fitted to each beaker to prevent the solution from evaporating. Amount of liquid loss by evaporation was replenished with distilled water. Heating time was 4, 6, 8 and 12 hours. Soup was filtered for filter paper, and used for the analysis. The heat source used 600W with heater (National NK-630).

Methods of Measurement

1. Moisture Contents

The fish bones were grounded to powder by cooking mixer (National, MX 915C). The fish bone powders were heated at 105℃ for 4 hours, and the weight loss was assumed as the moisture content of fish bone.

2. Calcium, Magnesium and Phosphorus Contents

After adding 5 mL of sample solution into a crucible and heating by sand bath under a draft, it dried at 105℃ for 4 hours. It was turned to dry type ashes by crucible furnace at 550℃ for 20 hours. $LaCl_3$ (2.5 mL) of 1% and 6N HCl (4.2 mL) were added to crucible, and it was made up to 25 mL with distilled water. Calcium and magnesium were measured by atomic absorption light photometer (Hitachi 508A). Phosphorus was measured by the molybdenum blue optical density method.

3. Protein and Total Free Amino Acid Contents

The protein contents of sample solution were measured by the Lowry method. Total amino acid contents were measured by the ninhydrin method.

Results and Discussion

Table 1. Length and weight of fish bones

Fish bone	Sea bream	Sardine	Sea bass
Length(cm)	24.4± 0.80*	17.0±1.06	26.6± 0.92
Weight(g)	26.2± 2.23	4.1±1.06	28.4± 1.50
Fish bone	Amberjack	Yellow tail	Black tuna
Length(cm)	44.3± 4.02	23.9±1.53	110.7± 8.44
Weight(g)	93.4±11.79	14.9±2.04	1133.2±95.38

* Mean±SD. (n=2~46).

1. Moisture and Mineral Contents of Several Fish Bones

Table 2 showed moisture and ash contents of several fish bones such as sardine, yellow tail, amber jack, tuna, sea bream and sea bass. The moisture content of sardine was the highest, and that of tuna bone was the lowest. Ash amount was the highest of tuna bone. The moisture content was different in the kind of fishes. But it was tendency decreased to fish sizes.

Fish bone composition was 10~55% of moisture contents, 30~65% of inorganic matter contents and 35~70% of organic matters contents(Ohota 1981). The main element of inorganic matter is calcium phosphate. The amount of calcium in tuna bone was the highest. However, magnesium and phosphorus were not difference among of fishes(Table 3).

2. Calcium and Phosphorus Contents of Several Fish Bones

The extraction ratio of calcium in each fish bone with various 2% organic acid at heating for 12 hours was shown Fig. 1. Although calcium was extracted hardly with water, it was extracted effectively with organic acids, especially citric acid and malic acid. Extraction ratio of amber jack bone with citric acid and malic acid was the highest such as 61.3% and 60.5%, respectively.

Fig. 2 showed the extraction ratio of phosphorus. Phosphorus was also extracted hardly with water such as 1.6~2.4%. The concentration of phosphorus in the extract was less than calcium. Although the organic acids extracted phosphorus effectively, the extraction ratio was different between 7.4~48% depend on the varieties of fish bones. The phosphorus content of fish bones did not different among fishes(Table 3).

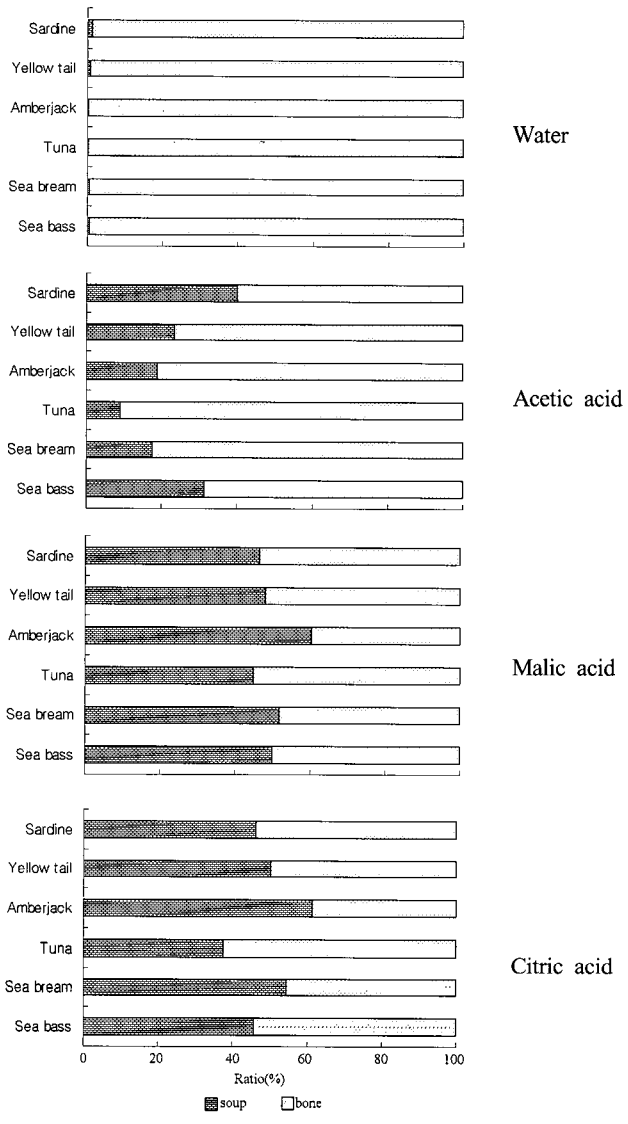

Fig. 1. Calcium soluble ratio (%) in fish bone soups after boiling for 12 hours with 2% acetic acid, malic acid or citric acid solution.

Table 2. Moisture and ash contents of several fish bones

Fish bone	Sardine	Yellow tail	Amber jack	Tuna	Sea bream	Sea bass
Moisture(%)	53	51	46	27	47	46
Ash(%)	31	30	38	45	32	32

Table 3. Mineral contents of several fish bones.

	Sardine	Yellow tail	Amber jack	Tuna	Sea bream	Sea bass
Ca	4.01±0.02*	4.03±0.05	4.26±0.02	8.94±0.04	4.73±0.02	5.27±0.05
Mg	0.11±0.00	0.11±0.00	0.12±0.01	0.18±0.00	0.12±0.01	0.14±0.01
P	1.91±0.01	1.84±0.00	1.82±0.01	1.75±0.02	1.77±0.01	1.84±0.01

* Mean(g/100g)±SD. (n=5).

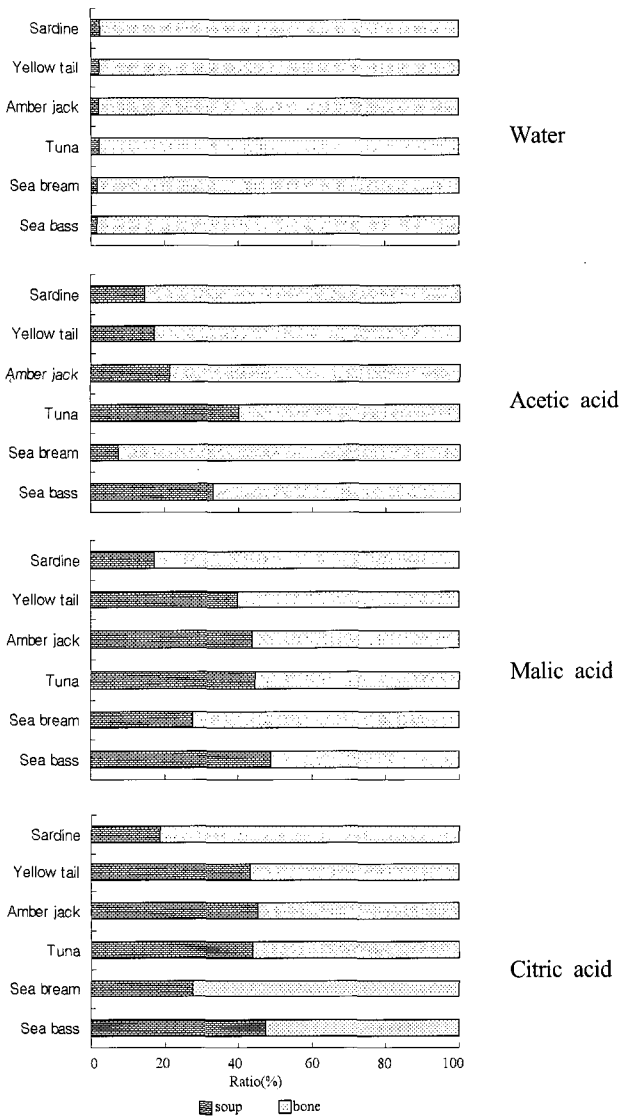

Fig. 2. Phosphorus soluble ratio (%) in fish bone soups after boiling for 12 hours with 2% acetic acid, malic acid or citric acid solution.

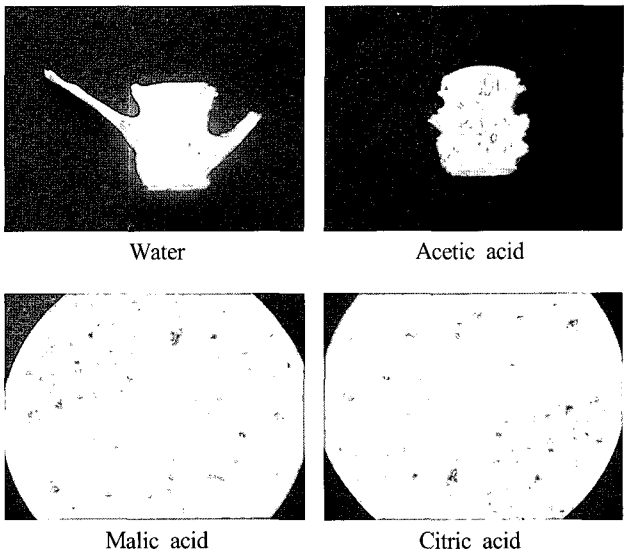

Fig. 3. Photographs of amber jack bone after boiling for 12 hours with 2% acetic acid, malic acid or citric acid solution.

However, the extract ratio was different depend on the kind of fish bone and the organic acids. It is thought that solubility of calcium phosphate was different among fish bones.

Fig. 3 was photographs of the amber jack bone after extraction for 12 hours with water and 2% of acetic acid, citric acid or malic acid. In the case of extraction with water and acetic acid, the fish bone shape was remained. However, in the case of extraction with citric acid and malic acid, their shapes were lost completely.

From the above results, it was found that calcium and phosphorus extract of fish bones were different from kinds of fish bones and organic acids. Calcium extract ratio of fish bone was the highest of amber jack bone as added 2% citric acid. However, calcium extract amount was the highest in tuna bone and it was about 401mg/ 100mL/10 g bone.

3. Changes of Calcium Content in Black Tuna Bone Soup

The acetic acid, malic acid and citric acid were added to tuna bone, and extract of calcium from the bone by heating was examined. With water it was hardly extracted as shown in Fig. 4. However, it was increased with organic acids added to concentration of acids and heating times. Extract of calcium was increased more with malic acid and citric acid solution than acetic acid solution. Especially, calcium was extracted in 573mg/100mL/10g bone with 4% malic acid for 12 hours heating. However, there was no difference with citric acid (552mg). The reason why citric acid and malic acid could extract calcium more than acetic acid was thought the chelate effect of malic acid and citric acid. Shimosaka *et al* (1998) has reported that calcium of fish bone was decreased and calcium of soaking liquid was increased when fish bone was soaked with 4% acetic acid. Fish bone had contained much minerals (Table 2). Especially, calcium was one of the main minerals of bone. The cooking method with organic acid and heating can be solubilized calcium from bone, and it can be done decrease of bone mineral. This soup is a good source for calcium supply. The prevention of osteoporosis, frac-

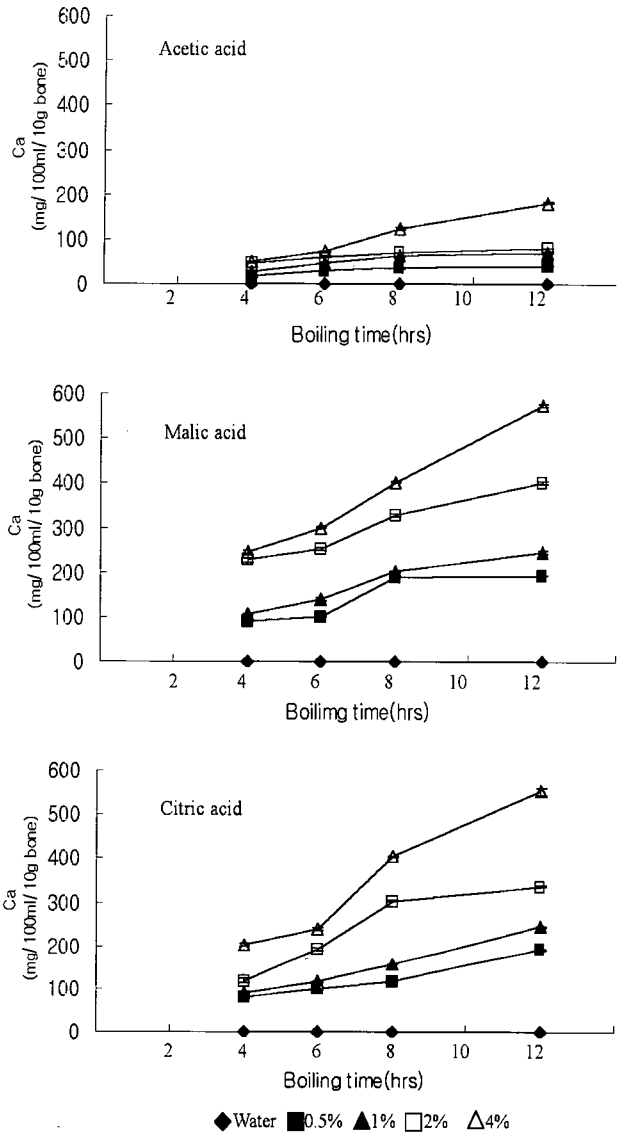

Fig. 4. Changes of calcium content in black tuna bone soup during boiling with acetic acid, malic acid or citric acid solution.

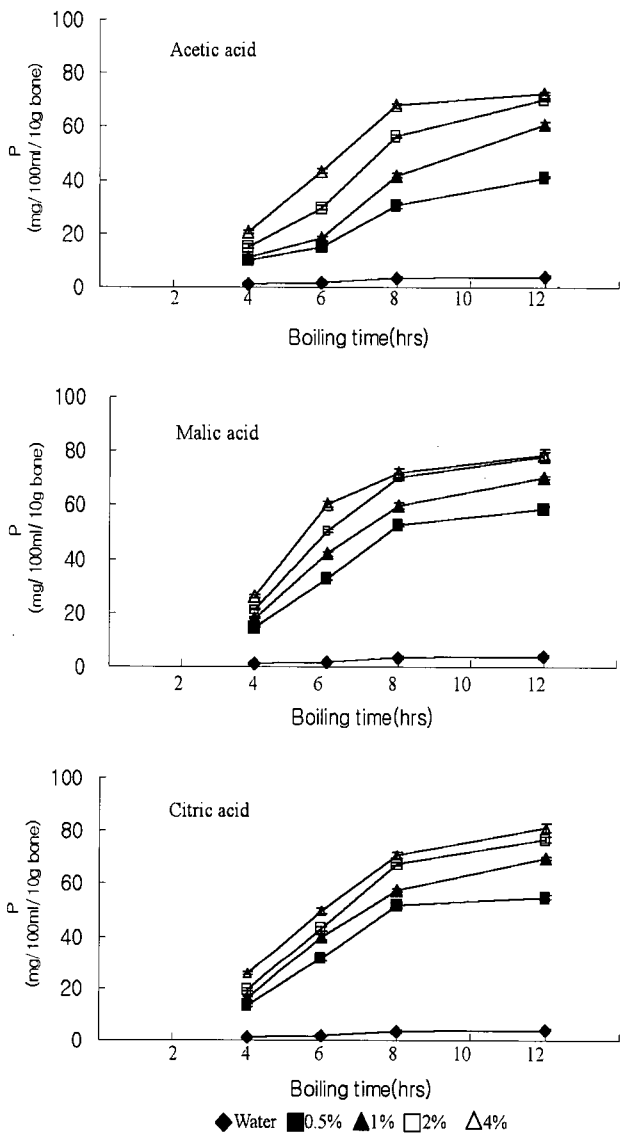

Fig. 5. Changes of phosphorus content in black tuna bone soup during boiling with acetic acid, malic acid or citric acid solution.

ture, and bedridden of the senior citizen will be done to take this soup.

4. Changes of Phosphorus Content in Black Tuna Bone Soup

Fig. 5 showed the time course of phosphorus extraction from tuna bone with acetic acid, malic acid and citric acid during heating. Their extraction were increased depend on the concentration of organic acids and heating times. In the case of added organic acids, it was increased linearly until eight hours, and then was increased small up to 12 hours. There were no significant differences among organic acids.

Studies on hardness of fish bone(Ishikawa *et al* 1989, 1990) were reported that mackerel bone was softened by heating, but mineral did not eluted from bone. It was the same result of this experiment which mineral extraction was little with water by heating.

The main element of bone is calcium phosphate. It is mostly composed with crystal of hydroxyapatite(Arnaud and Posner 1990). It was also reported that when no structural non crystalline calcium phosphate and crystalline apatite existed in beef and rabbit bone(Termine and Posner 1967 a, b). Shimo-

saka(2001) was reported that soaking in horse mackerel bone with acetic acid solution was not change crystalline of apatite, but it was extracted calcium and phosphorus. Therefore, Shimosaka was guessed that there was calcium phosphate which was not crystallized the apatite in bone. Phosphorus was extracted at early period during heating. It is thought that bone tissue was occurred change by adding organic acids and heating, and crystalline and non crystalline apatite were extracted at same time.

5. Ratio of Calcium to Phosphorus (Ca/P) in Black Tuna Bone Soups

The ratio of calcium to phosphorus is important. Absorption of calcium from intestines is the highest at less than doubles of Ca/P(Fujita 1990). Table 4 showed the Ca/P ratio of tuna bone extract for 12 hours with various concentrations of organic acid. The extract ratio of Ca/P with water was between 0.12~0.35. However, extraction ratio with organic acids was increased to 1.15~10.9.

6. Changes of Protein Content in Black Tuna Bone Soup

It was showed the changes of protein extracts which the tuna bone was heated with acetic acid, malic acid and citric acid (Fig. 6). Protein extract was increased depend on the concentration of organic acids and heating times. In the case of added to 4% citric acid and heated for 12 hours, the soup had protein of 681mg/100mL. Ishikawa *et al*(1987) was reported that extract of collagen was high with vinegar and heating. Shimosaka *et al*(1998) was reported that most of extract crud protein was collagen when horse mackerel bone was heating with acid solution. It was thought that extracted protein in this experiment was also collagen of bone tissues.

Table 4. Changes of Ca/P ratio in black tuna bone soup during boiling with acetic acid, malic acid or citric acid solution

Boiling time(hrs)		Acidity (%)				
		0	0.5	1	2	4
Acetic acid	4	0.21	1.60	2.46	3.02	2.45
	6	0.35	2.04	2.54	2.06	1.67
	8	0.30	1.17	1.48	1.26	1.82
	12	0.31	0.98	1.16	1.15	2.49
Malic acid	4		6.09	5.88	10.86	9.34
	6		3.07	3.33	5.05	4.98
	8		3.60	3.39	4.67	5.58
	12		3.28	3.48	5.14	7.33
Citric acid	4		6.08	5.64	6.01	7.93
	6		3.13	2.87	4.47	4.80
	8		2.23	2.72	4.49	5.71
	12		3.48	3.51	4.37	6.80

7. Changes of Total Free Amino Acid Contents in Black Tuna Bone Soup

Fig. 7 showed changes in the content of total free amino

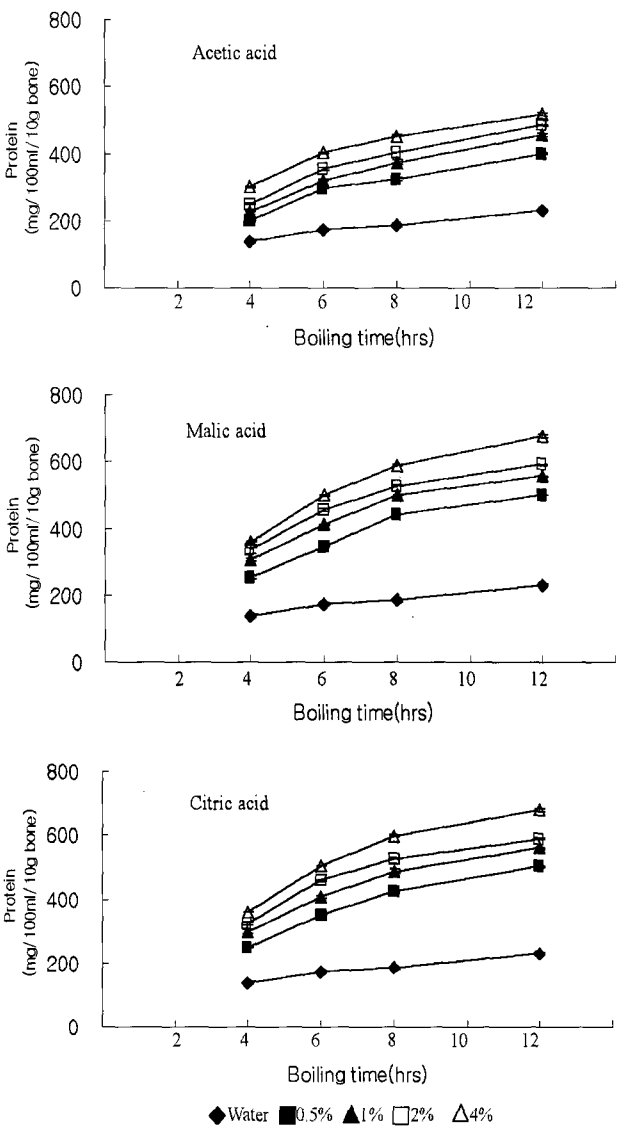

Fig. 6. Changes of protein content in black tuna bone soup during boiling with acetic acid, malic acid or citric acid solution.

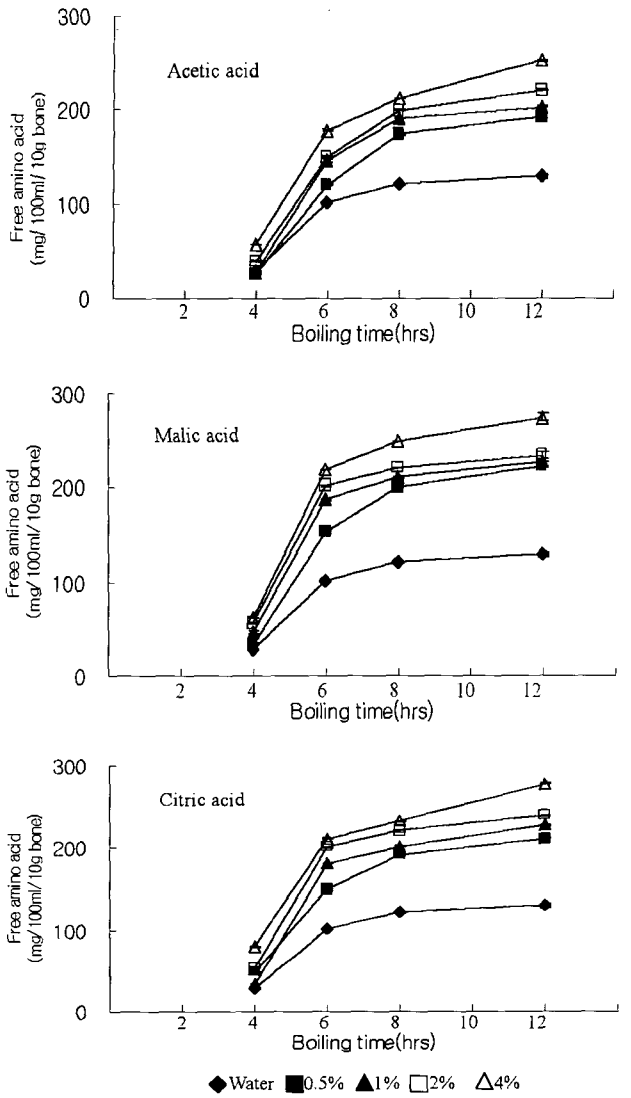

Fig. 7. Changes of total free amino acid content in black tuna bone soup during boiling with acetic acid, malic acid or citric acid solution.

acid of soup which the tuna bone was heated for 4, 6, 8 or 12 hours with acetic acid, malic acid and citric acid of 0, 0.5, 1, 2, and 4%. Extract of free amino acid were increased during heating times. Extract of free amino acid added to 4% citric acid and malic acid and heated for 12 hours was twice or more than with water.

As results, free amino acid extracts from tuna bone with malic acid and citric acid were more effective than with acetic acid.

From the above results, using with minerals of fish bone, it is a good method to solubilize it with adding organic acids and heating. Mineral contents of fish bone soup were depending on kinds of fish bone and organic acid, acid concentration, and heating time.

Kim *et al*(1999) reported that on cooking with grain vinegar and citric acid, the amount of extraction of ingredients, such as mineral, protein and free amino acid from beef bone were increased. Han *et al*(2000) reported on pork rib bone. When 2% citric acid or 2% malic acid was added, calcium, magnesium, and phosphorus of extraction from bone were increased with advance of heating times. Calcium extract of chicken thigh bone(Lee *et al* 2002) was increased with malic acid and citric acid solution than acetic acid solution.

Results of using fish bone in this experiment were the same tendency as beef, pork, and chicken thigh bone. This was shown that the effect of organic acid was different according to the amount of calcium in each bone, bone tissue and connective tissue. This respect is necessary further examination.

Calcium phosphate is known that the absorption rate from intestines is lower than calcium carbonate. Milk and dairy products are also known that the absorption rate of calcium is especially high(Yamauchi 1993). But it has problem the lactose intolerance preference. Then, there was reported to improve the absorption rate of calcium making CCM (calcium, citric acid, malic acid) that the absorption rate using carbonic acid calcium and phosphoric acid calcium is high in the solution of citric acid and malic acid in the United States(Smith 1991).

It is found that is able to solubilize minerals from fish bone with adding organic acid and heating time.

Fish bone contains high level of calcium, but it is mostly discarded as a waste. The development of ways for more effective utilization of fish bone is valuable for the efficient use of resources. It is important to examine the possibility as acid seasoning that makes the best use of abundant calcium effectively and the bioavailability of these mineral of the soup.

Summary

Fishes have been important protein sources. Large fish bone was mostly discarded as a waste. The development of ways for more effective utilization of fish bone is valuable for the efficient use of calcium source.

This research was performed to investigate the change of calcium and phosphorus contents extracted from fish bones added to organic acids such as acetic acid, malic acid and

citric acid.

The results were as follows:

1. Moisture and inorganic components were different depending on the kind of fish bones. The larger fish bones, the less their moisture content.
2. The extraction ratio of minerals increased with added organic acids. Especially, extraction ratio in amber jack bone with citric acid and malic acid were the largest as 61.34% and 60.50% of total bone minerals, respectively. Extraction pattern of phosphorus was tended to be the same as that of calcium. Calcium extraction was the highest in tuna bone. Its soup had calcium of 573 mg/100 mL/10g with 4% malic acid for 12 hours heating.
3. As for the ratio of calcium and phosphorus, extraction of calcium was less than that of phosphorus without organic acid addition. As the organic acid was added, extraction of calcium was increased.
4. The protein and free amino acid contents of bone were slightly with water. However, their extraction increased with added organic acids.

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