Thesis for the Degree of Master of Fisheries Science

Effects of Processing Conditions on Protein Quality of Fried Anchovy Kamaboko

by

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멸치 어육을 이용한 어묵의 단백질 품질에 미치는 가공조건의 영향

Advisor: Prof. Hong-Soo Ryu

by

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Effects of Processing Conditions on Protein Quality of Fried Anchovy Kamaboko

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Abstract

The effects of processing conditions and frozen storage on the quality of anchovy (*Engraulis japonica*) fried kamaboko products (raw anchovy, minced, surimi and kamaboko) were investigated. The results revealed significant variation in moisture content from 73.8 (raw meat) to 83.2% (surimi) and those decreased nearly of 64.8% in kamaboko products. Protein content decreased after kamaboko processing from 19.6 (raw meat) to 12.1% (kamaboko) due to the added ingredients. Lipid content decreased from 2.8 (raw meat) to 1.3 in minced and 0.5 % in surimi, but fried kamaboko showed 6.9 % levels in lipid. TBA values and TBARS levels were highest in kamaboko samples 89.5 and 1.9 mg/g solid and increased gradually with the storage time until 101.8 and 4.6 mg/g solid respectively. *In vitro* protein digestibility increased from 79.2% in raw anchovy to 88.5% in kamaboko samples, while levels of TI were gradually decreased with the processing

conditions and during the storage time from 2.43 in raw anchovy to 0.31 mg/g solid in the kamaboko sample for 60 days of frozen storage.

Noticeable changes were not observed in total essential amino acid during processing conditions. Computed protein efficiency ratio (C-PER) for kamaboko was highest (2.59) compared with whole anchovy (1.96), minced (1.94) and surimi (2.50). Anchovy kamaboko showed a higher digestibility (88.5%), and C-PER value (2.59), compared with the other surimi products.

Fresh anchovy kamaboko showed almost similar values of hardness, springiness, gumminess and chewiness than commercial kamaboko, highest value of fracturability and adhesiveness, lowest value of cohesiveness and resilience, while frozen and thawed anchovy kamaboko showed the highest values for all of these rheological parameters.

Anchovy kamaboko showed the lowest Lightness (62.9) and redness (0.16) and similar yellowness (11.9) compared with commercial kamaboko.

Frozen storage and vacuum packaging were effective to maintain the shelf life of anchovy kamaboko within 30 days, but not effective after 45 days due to fat oxidation.

Introduction

Anchovy (*Engraulis ringens*) is a small pelagic fish and the most important resource in the Peruvian's fishing grounds, approximately 97% of the annual landing of anchovy stocks is mainly used in the manufacture of indirect human consumption products. Only 3% is used for direct human consumption, and recently on a smaller scale is designed to process cured and frozen, highlighting a greater use for the canning industry, in different presentations, due to the scarcity of resources such as sardine, an increase that is noticeable from 2006 (PRODUCE, 2010). According to this data, there is a possibility to develop new products with this species. One possible alternative is the production in main scale of anchovy surimi and supply of these fisheries products to Asia, United States and many other countries. Surimi is an excellent high-protein base for a new generation of foods (Pigott and Tucker, 1990).

Since the discovery of frozen surimi, the level of quality has been based on the use of surimi in the preparation of kamaboko, and many kinds of value added seafood products. Its production used primarily low value marine species like Pacific whiting and Alaska pollock. In recent

years, the demand for fish protein ingredients is growing worldwide. The quality and characteristics of these products are highly dependent on the source of muscle protein and the processing procedures applied. Species with white flesh and low fat content are considered most suitable for manufacturing surimi; however, a big drop in production due to the scarcity and high cost of marine species used to produce it has prompted the use of other fishery resources, including pelagic resources such as anchovy. The future will see expanded use of underutilized foods from the sea in the form of this highly refined fish protein product (Pigott and Tucker, 1990).

Dark muscle fish species currently make up 40-50% of the total fish catch in the world. There is great interest in using the large quantities of these low values, fatty, pelagic fish for human food. Moreover, anchovy can be used as a potential raw material for surimi manufacture.

By cooking fish meat ground with salt and seasonings, a food product called “Neriseihin” is obtained. A representative product of neriseihin is kamaboko. Making kamaboko, which is mainly the utilization of muscle

protein, has many interesting facets from the point of view of protein chemistry (Suzuki, 1981).

The variation of levels of nutrients in raw material is due to more than just processing. Most nutrients loss occurs during preparation, but some losses occur during harvest, processing, storage and distribution. Stability of nutrients varies with pH, oxygen, heat and light. When food is processed, the tissues are damaged and nutrients interact with other components. The choice must be made between the risk of nutrients loss and the benefit of food availability. Some benefits of processing are destruction of anti-nutritional factors, such as amylase and trypsin inhibitors and increased digestibility of starch and protein (Pigott and Tucker, 1990).

While cooking improves both digestibility and flavor, care must be taken to retain nutritional benefits. Protein represents one of the most valuable compounds of our diet and it should be protected during processing. The maintenance of protein nutritive value is particularly important during production of meat products, because meat proteins are balanced, containing all essential amino acids. The nutritive value of meat

proteins is determined not only by quantitative and qualitative composition of amino acids, but also by their availability to digestive tract proteolytic enzymes (Korczak et al., 2004). Important chemical reactions that decrease the nutritional value of seafood are lipid oxidation and nonenzymatic browning (Pigott and Tucker, 1990). Reduction of digestibility as well as limitation of the amount and the degree of amino acid availability is affected by the formation of amino acid bonds with lipid oxidation products. Fat oxidation products react first of all with functional groups of proteins and amino acids, oxidized lipids effects are the main cause of rancidity formation in food products but they also interact with other food components and change nutritional value (Korczak et al., 2004).

An important and frequently observed effect of food processing is the reduction of protein nutritive quality. These changes may depend on the denaturation of the protein and reduction in amino acid availability by cross-linking, racemization, degradation and formation of complexes with sugar and may result in loss of digestibility. Therefore when attempting to estimate protein quality, one of the first factors that must be evaluated is

its digestibility. Because the nutritional quality of a protein is related both to its amino acid content and the capacity of digestive enzymes to liberate them, method using digestion enzymes have been tried (Gauthier et al., 1982).

This investigation aimed to know the possibility of utilizing anchovy catches for kamaboko products and study the food qualities of those products including the evaluation of protein quality. To compare the food qualities of anchovy kamaboko with commercial kamaboko from white muscle fishes, protein nutritional value, degree of lipid oxidation, textural properties and color value were experimented using fried kamaboko products from anchovy (*Engraulis japonica*) surimi.

Materials and Methods

Sample Preparation

Eighty kilograms of fresh anchovy samples were obtained as quick frozen block in Gijang Market, Busan South Korea, in January 19th, 2012. Average sizes of whole anchovy were 14.3 ± 0.48 cm, total length, 12.5 ± 0.44 cm standard lengths and 18.1 ± 2.28g in weight.

Each frozen anchovy block was divided into IQF (individual quick frozen), and those samples were lightly thawed at room temperature for 5 min and then headed and gutted. The skin and backbone samples were removed in order to obtain the ordinary muscle. The minced meat was obtained from the ordinary muscle by using a meat chopper M-125 FUJEE. Then the samples were weighed and washed with cool water (below 5˚C), in a 3:1 water/minced fish ratio. The minced fish was washed for three times, the first washed with 0.5% $NaHCO_3$ solutions, and the last wash water was added with 0.3% of NaCl, after each washing excess water was removed by pressing.

Surimi was prepared by mixing 0.4% sucrose, 0.4% sorbitol and 0.3% polyphosphate and stored in freezing conditions, (Maza and Llave, 2006).

Kamaboko base was prepared by mixing some ingredients like surimi (48.4%), salt (2.9%), sugar (1.7%), soybean oil (2.5%), monosodium glutamate (1.0%), white egg (3.5%), mirin (2.0%), wheat flour (8.0%), and cool water (30.0%). Mixed kamaboko base was formed into thin slices of 5.50±0.66 mm in thickness and fried in 220 ˚C boiling soybean oil for 3 min. Fried kamaboko was stored at freezing conditions (-24˚C) in vacuum package, for 0, 15, 30 45 and 60 days. Whole anchovy (raw material), ordinary muscle (minced), and surimi samples were also used to study the effects of frozen storage on food quality.

Experimental Procedure

Proximate Composition

The proximate compositions of the all samples were determined using the following AOAC (1990) procedure. Moisture (%) samples were dried in a drying oven Model J-DS4 JISICO at 105˚C until constant weight. Crude fat (%) was determined using the Soxhlet solvent extractor (Model VELP SCIENTIFICA SER 148). Crude protein (%) was determined by the semi-micro Kjeldahl method using 6.25 as a conversion factor after acid digestion (Digestor Model Gerhardt Turbotherm, distillator model Gerhardt Vapodest). Crude ash (%) was determined by incineration in an electric muffle furnace model KUKJE SCIEN HY-8000S series (250˚C for 2 h and 570˚C for 1 h).

The percentage contents of protein, fat and ash were determined in relation to the dry basis of the samples analyzed, dry basis being expressed in g x 100. The carbohydrates content were calculated by the difference of proximate composition. All determinations were made in triplicate for each run.

Water Activity Measurements

Water activity measurements were taken for all the samples, using the water activity-measuring equipment (BT – RSI – 7557 012, Switzerland).

Fat Oxidation

To measure fat oxidation, two tests were used in all the samples to estimate the extent of oxidation.

a) Thiobarbituric Acid Value (TBA Value)

TBA values were determined by the method of Tarladgis et al. (1960). Absorbance was measured using UV spectrophotometer Model UV mini 1240 SHIMADZU at 538 nm and the TBA values were calculated by multiplying the sample absorbance by 100 and expressed in mg/g solid.

b) Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were determined by the method of Witte et al. (1970). Absorbance was measured using UV spectrophotometer Model UV mini 1240 SHIMADZU at 530 nm and the resulting colors in all the samples were calculated by multiplying the absorbance by 5.2 and expressed in mg/g solid.

Protein Quality Determination

a) *In vitro* Protein Digestibility

The *in vitro* protein digestibility values of all the samples were determined by the method of Oduro et al. (2011) with modification by the AOAC method (1982), the procedure used four enzymes method. Oduro et al. (2011) tried the different method for protein digestibility using three proteolytic enzymes. They determined the correlation coefficient between two assays and it showed high correlation (R^2= 0.9955).

The α-chymotrypsin (Sigma 38 units/mg solid), trypsin (Sigma 13,390 BAEE units/mg solid) and protease (*Streptomyces griceus*, Sigma 46 units/mg solid) were used in three enzymes method. The reference protein used was ANRC casein. Digestibility was calculated as follows:

% Digestibility (three enzymes) = 234.84 – 22.56x. Where x is the pH of sample at 20 minutes.

% Digestibility (four enzymes) = 1.03x (three enzymes digestibility) - 0.34

b) Amino Acid Profiles

Amino acid profiles were determined using the 6N HCl hydrolysis method with amino acid analyser (S433, Sykum, Germany).

c) Trypsin Inhibitor Assay

Trypsin inhibitor (TI) concentration in all samples was determined using the procedure of Ryu and Lee (1985), which is a modification of the

Rhinehart method (1975). Results of TI are expressed in trypsin inhibitor equivalents which equals the mg of purified soybean trypsin inhibitor per gram sample. The standard curve used in measuring TI content is shown in Fig. 1.

The correlation coefficient between pH and TI content was 1.000 and the equation for calculation is:

Y=5.1335x - 35.339, where y = purified soybean trypsin inhibitor (mg) and x is pH at 10 minutes incubation.

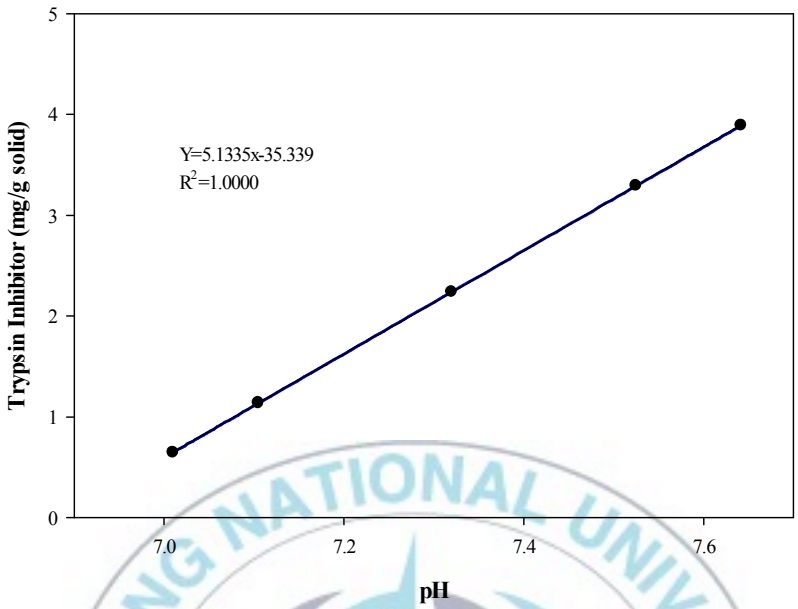


Fig. 1. Relationship for pH at 10 minutes with purified soybean trypsin inhibitor concentration

d) Computed Protein Quality

C-PER was calculated in a software, using the data obtained from the *in vitro* protein digestibility (four enzymes) and amino acid profiles. The calculation was based on the procedure of AOAC (1982), to obtain the protein quality data compared to casein as a standard protein.

Starch Digestibility

The *in vitro* starch digestibility (IVSD) was determined using the freeze dried kamaboko samples (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml of pancreatic amylase (500,000 U/mg) suspension (0.44 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 37°C for 2 h according to the method of Singh, et al., (1982). At the end of the incubation period, 4 ml of 3,5-dinitrosalicylic acid reagent were added and the mixture was boiled for 5 min. After cooling, the absorbance of the filtered solution was measured using UV spectrophotometer Model UV mini 1240 SHIMADZU at 550 nm with maltose used as standard. IVSD was expressed as percentage.

Texture Analysis

Texture Profile Analysis (TPA) was performed using TA-XT2 Texture Analyser (model MHK Trading CO.) according to the method of Schubring (2002). Prior to the testing, kamaboko samples were equilibrated to room temperature for 30 min and cut into 1.5 x 1.5cm sections. The samples were compressed twice at 50% of initial height, using a 2.0 cm diameter cylindrical probe, at a test speed 1.5 mm/s. The mechanical properties of hardness, fracturability, adhesiveness, springiness, cohesiviness, gumminess, resilience and chewiness were evaluated.

Color Measurement

Spectral reflectance was determined using a portable colorimeter (Color Techno System Co. LTD Model JC801-Japan). The kamaboko samples without fried surface were placed in a plastic petri dish. The color measurement was repeated 3 times by using the method of Schubring (2003). L denotes lightness on a 0 to 100 scale from black to white; a* denotes (+) red or (-) green; and b* denotes (+) yellow or (-) blue.

Statistical Analysis

The results were presented as average and standard deviations for each sample, done in triplicate. Statistical analysis of data was performed using SAS V.9.1.3. Tukey's multiple range tests was carried by one-way analysis of variance (ANOVA), to determine significance of mean SD differences among treatments. The significance level was set at P=0.05 for all analysis.

Results and Discussion

Proximate Composition

Among the samples analyzed in this study, proximate composition (Table 1) of raw anchovy, moisture and carbohydrate are relatively similar (73.8 ± 0.39 and 0.1 ± 0.37 % respectively) to those observed by the National Fisheries Research and Development Institute (NFRDI, 2009), 73.4 and 0.3% and food composition table of Rural Nutrition Institute (R.D.A., 1991) (74.8 and 0.2%). The protein and ash contents in the muscle tissue of anchovy (19.6 ± 0.00 and 3.6 ± 0.06%) were slightly higher than the references cited above, (NFRDI, RDA: 17.7% and 3.2% respectively), but the content of lipids (2.8 ± 0.00%) (NFRDI, 5.4%, RDA 4.1%), was low probably due to small size of anchovy and caught in winter season. Huss (1995) reported that the chemical composition of the different fish species will show variation depending on seasonal variation, migratory behavior, sexual maturation and feeding cycles, etc. The variation in the percentage of fat is reflected in the percentage of water, since fat and water normally constitute around 80 % of the fillet.

Table 1. Proximate composition of raw anchovy, minced, surimi and kamaboko

g/100g sample (% dry basis)

Processing steps	Moisture %	Protein %	Lipid %	Ash %	[1]Carbohydrate %
Whole anchovy	$73.8^{c} \pm 0.39$	$19.6^{b} \pm 0.00$ (75.02)	$2.8^{d} \pm 0.00$ (10.80)	$3.6^{a} \pm 0.06$ (13.87)	$0.1^{c} \pm 0.37$
Minced	$75.6^{b} \pm 0.17$	$20.5^{a} \pm 0.10$ (84.35)	$1.3^{e} \pm 0.06$ (5.33)	$1.8^{e} \pm 0.10$ (7.20)	$0.8^{c} \pm 0.12$
Surimi	$83.2^{a} \pm 0.53$	$14.8^{c} \pm 0.16$ (88.08)	$0.5^{f} \pm 0.04$ (2.67)	$0.6^{f} \pm 0.00$ (3.60)	$0.9^{c} \pm 0.63$
Kamaboko (0 days)	$70.8^{d} \pm 0.24$	$12.1^{f} \pm 0.00$ (41.53)	$5.5^{c} \pm 0.12$ (18.80)	$2.9^{d} \pm 0.00$ (10.00)	$8.6^{b} \pm 0.34$
Kamaboko (15 days)	$65.4^{ef} \pm 0.78$	$13.2^{e} \pm 0.07$ (38.14)	$6.5^{b} \pm 0.29$ (18.76)	$3.2^{bc} \pm 0.08$ (9.07)	$11.7^{a} \pm 0.92$
Kamaboko (30 days)	$66.3^{e} \pm 0.54$	$12.9^{e} \pm 0.12$ (38.50)	$6.9^{a} \pm 0.08$ (20.67)	$3.2^{c} \pm 0.08$ (9.47)	$10.6^{a} \pm 0.65$
Kamaboko (45 days)	$66.5^{e} \pm 0.12$	$13.0^{e} \pm 0.06$ (38.73)	$6.7^{ab} \pm 0.14$ (20.00)	$3.2^{c} \pm 0.08$ (9.47)	$10.6^{a} \pm 0.17$
Kamaboko (60 days)	$64.8^{f} \pm 0.21$	$14.1^{d} \pm 0.07$ (42.23)	$6.7^{ab} \pm 0.08$ (18.13)	$3.4^{ab} \pm 0.16$ (9.47)	$11.0^{a} \pm 0.43$

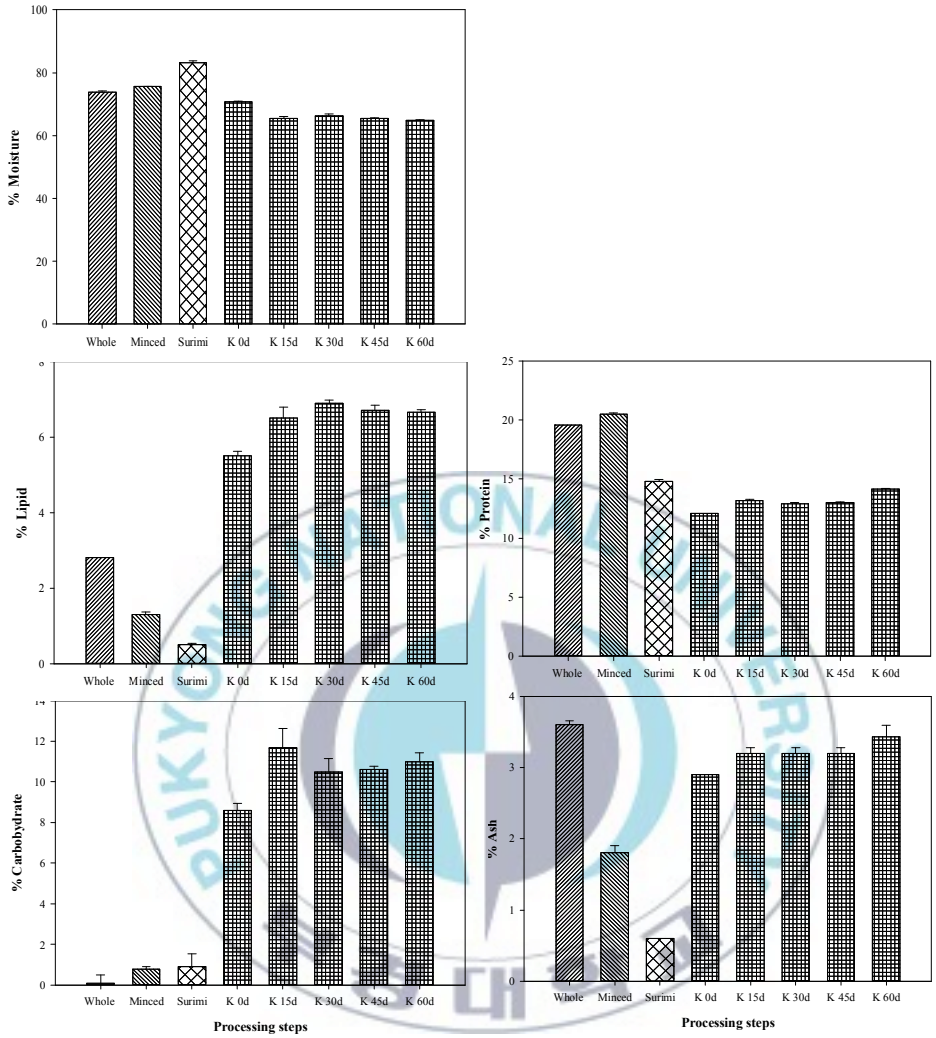
1) Obtained by calculation

Mean ±SD of three determinations

$^{a-f}$Different letters show significant differences ($P<0.05$)

The percentage of protein and moisture (Fig 2) increased slightly in minced (20.5 ± 0.10 and 75.6 ± 0.17% respectively), while lipid (1.3 ± 0.06%) and ash (1.8 ± 0.10%) decreased significantly after removing the skin and bones. Less fat and altered mineral composition was reported by Pigott and Tucker (1990). Akman (1982), cited by Huss (1995) reported that normally the skin bears about 40% of the total fat in the fish. The fat cells making up the lipid depots in fatty species are typically located in the subcutaneous tissue, in the belly flap muscle and in the muscles moving the fins and tail.

Surimi showed lower protein (14.8±0.16%), lipid (0.5±0.04%) and ash (0.6±0.06%) content compared with minced due to alkaline solution used for washing the minced fish. Washing changed the mineral composition, but the wash water quality and equipment type affected the range and extent of these changes (Pigott and Tucker, 1990). Similar result (protein 14.1, lipid 0.5, and ash 0.7%) was observed by Maza and Solari (2006) in anchovy refined surimi. Reduction in total ash increased protein quality and more favorable mineral composition, so generally, the nutritional quality is improved.



K: Kamaboko; 0, 15, 30, 45 and 60 (storage days)

Fig. 2. Proximate composition of raw anchovy, minced, surimi and kamaboko.

The key to nutrition retention appears to be proper deboning to get a mince nutritionally equal to fillet (Pigott and Tucker, 1990). Moisture content was highest (83.2±0.53%), probably due to traditional method of dewatering. Minced fish was wrapped in a tofu bag and squeezed manually. Similar value (83.13%) was observed in Alaska pollack surimi, control sample by Ryu et al. (1994b) during the study of the effects of cryoprotectants on the protein qualities of Pollock surimi. The moisture content from the final screened product is reduced to 80-85% by pressing in a conventional screw press (Pigott and Tucker, 1990). Mixed ingredients and frying in hot oil resulted to lower moisture content from 64.8 to 70.8±0.24% in the kamaboko samples and those decreased the protein content slightly (12.1 to 14.1±0.07%). Commercial kamaboko showed moisture content ranged from 54.27±1.51 to 66.4±0.35%, protein content showed similar values to commercial kamaboko which ranges from 11.2±0.0 to 13.48±0.0%, (Ryu and Choi, 2012). Pigott and Tucker (1990) reported that the lower protein content is not so much due to washing as to dilution by adding carbohydrates. The lipids showed a higher level (ranges

from 5.5 to 6.9±0.08%) than commercial kamaboko (2.8±0.15 to 5.2±0.26%) (Ryu and Choi, 2012). Oil absorption by fried foods may range from 10 to 40%, depending on the conditions of frying and the nature and size of the food (deMan, 1999). The percentage of ash was higher (2.9 to 3.4±0.16%) through the addition of some ingredients like salt compared with commercial kamaboko (2.0±0.0 to 2.93±0.23%) (Ryu and Choi, 2012). Carbohydrates (8.6 to 11.7±0.92%), were increased in the kamaboko samples due to the added wheat flour, compared with commercial kamaboko ranged from 13.88±0.0 to 28.26±0.0% (Ryu and Choi, 2012).

Water Activity

The results presented in the Table 2, showed significant differences between the raw samples and fried samples. Water activity values (Fig. 3), were higher in the raw samples (0.96) and highest in the surimi (0.97) rather than in the kamaboko samples (0.91 to 0.92) during frozen storage time. The a_w in commercial kamaboko samples ranged from 0.92 to 0.96 (Ryu and Choi, 2012). Usually the range a_w 0.90 ~1.0 is classified as water-rich foods (Pigott and Tucker, 1990) and considered to be a susceptible range to

Table 2. Changes in water activity of raw anchovy, minced, surimi and kamaboko during frozen storage (-20 ˚C)

Processing steps	Aw
Whole anchovy	$0.96^{a} \pm 0.00$
Minced	$0.96^{a} \pm 0.00$
Surimi	$0.97^{a} \pm 0.00$
Kamaboko (0 days)	$0.92^{b} \pm 0.00$
Kamaboko (15 days)	$0.91^{b} \pm 0.01$
Kamaboko (30 days)	$0.92^{b} \pm 0.01$
Kamaboko (45 days)	$0.92^{b} \pm 0.00$
Kamaboko (60 days)	$0.92^{b} \pm 0.00$

Mean ±SD of three determinations

[a-b]Different letters show significant differences ($P<0.05$)

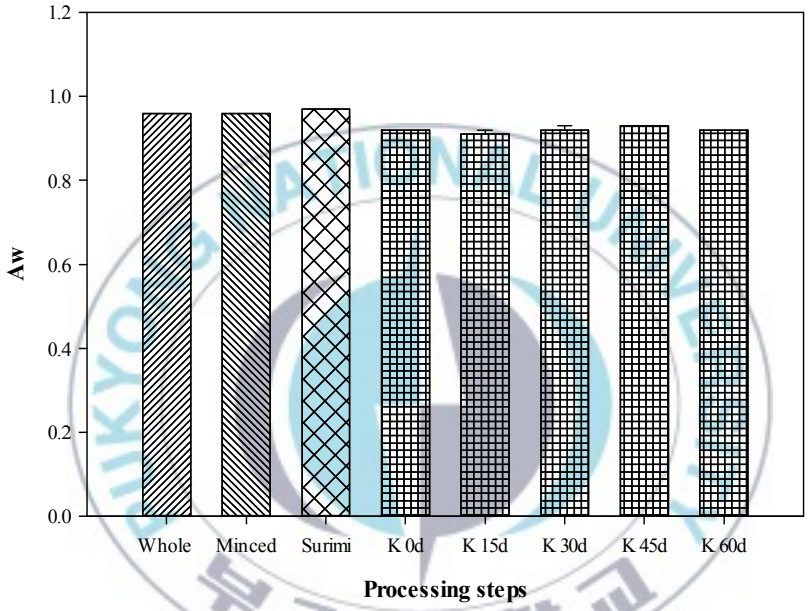


Fig. 3. Changes in water activity of raw anchovy, minced, surimi and kamaboko during frozen storage.

bacterial growth. Therefore, it was thought that anchovy kamaboko used in this study had a weakness in storage stability even in frozen products.

The control of the moisture content in foods is one of the oldest exploited preservation strategies, but the a_w of a food describes the degree to which water is “bound” in the food, its availability to participate in chemical/biochemical reactions, and its availability to facilitate growth of microorganisms (IFT/FDA, 2003). The most important component of foods, water, is the controlling ingredient for most chemical and physical reactions and interactions in foods, the freedom of water to move or interact with other ingredients, not just the water content, is the key to product stability, chemical reactions during processing and storage and the growth of microorganisms (Pigott and Tucker, 1990). The water activity (a_w) played an important factor in fish spoilage. Food designers use water activity to formulate products that are shelf stable. Water activity values can also help limit moisture migration within a food product made with different ingredients. In addition, a_w helps limit or slow certain undesirable reactions, such as non-enzymatic browning, fat oxidation, vitamin degradation,

enzymatic reactions, protein denaturation, starch gelatinization and starch retrogradation (Aberoumand, 2010).

Fat Oxidation

TBA value and TBARS assay are indicators of lipid oxidation or rancidity, the results in the Table 3 shows the degree of fat oxidation for these two assays. TBA value was relatively high in the raw anchovy (136.6±0.06 mg/g solid), as anchovy is a fatty species. A lower value showed in minced fish (41.1±1.51 mg/g solid) without skin, head, viscera and bones, and surimi showed the lowest value (40.0±0.45 mg/g solid), probably because washing effectively removed oil from the minced fish. Washing removed about 37% of the solids which included blood, pigments, fats and soluble protein (Pigott and Tucker, 1990). Chen et al. (1997) observed in horse mackerel, the oil content of unwashed mince was 2.24% and decreased with washing time. The oil elimination efficiency reached 95%.

Table 3. TBA value and TBARS of raw anchovy, minced, surimi and kamaboko

Processing steps	TBA (mg/g solid)	TBARS (mg/g solid)
Whole anchovy	$136.6^{a} \pm 0.06$	$1.7^{b} \pm 0.29$
Minced	$41.1^{d} \pm 1.51$	$0.3^{c} \pm 0.08$
Surimi	$40.0^{d} \pm 0.45$	$0.2^{c} \pm 0.03$
Kamaboko (0 days)	$89.5^{c} \pm 2.07$	$1.9^{b} \pm 0.14$
Kamaboko (15 days)	$96.3^{bc} \pm 1.16$	$2.0^{b} \pm 0.20$
Kamaboko (30 days)	$97.8^{b} \pm 3.84$	$2.0^{b} \pm 0.16$
Kamaboko (45 days)	$100.5^{b} \pm 6.14$	$2.3^{b} \pm 0.15$
Kamaboko (60 days)	$101.8^{b} \pm 2.10$	$4.6^{a} \pm 0.52$

Mean ±SD of three determinations

[a-d/ a-c]Different letters show significant differences ($P<0.05$)

TBA values increased significantly after frying the kamaboko and increased progressively with the storage time (from 89.5±2.07 to 101.8±2.10 mg/g solid), compared to commercial kamaboko samples ranges from 24.8±0.34 to 45.6±0.22 mg/g solid (Ryu and Choi, 2012), due to different deep frying condition. When the food is heated by immersion in hot oil (160 ˚C to 195 ˚C), practiced in commercial frying for a short time (2 min), the oil is not in contact with air, which eliminates the possibility of oxidation and thermal alteration. In continuous industrial frying, oil is constantly being removed from the fryer with the fried food and replenished with fresh oil so that the quality of the oil can remain satisfactory. This is more difficult in intermittent frying operations (deMan, 1999). In the kamaboko samples the water activity values were 0.92. Smith et al. (1990) reported that lipid oxidation increased most rapidly in proteins stored at a_w of 0.85.

The TBARS confirmed the results obtained by TBA values, because they have a similar trend (Fig. 4). The fried samples showed a little higher level (1.9 to 2.3 mg/g solid) than the raw samples (1.7 mg/g solid). Similar trend was observed by Oduro et al. (2011) in the fried mackerel samples.

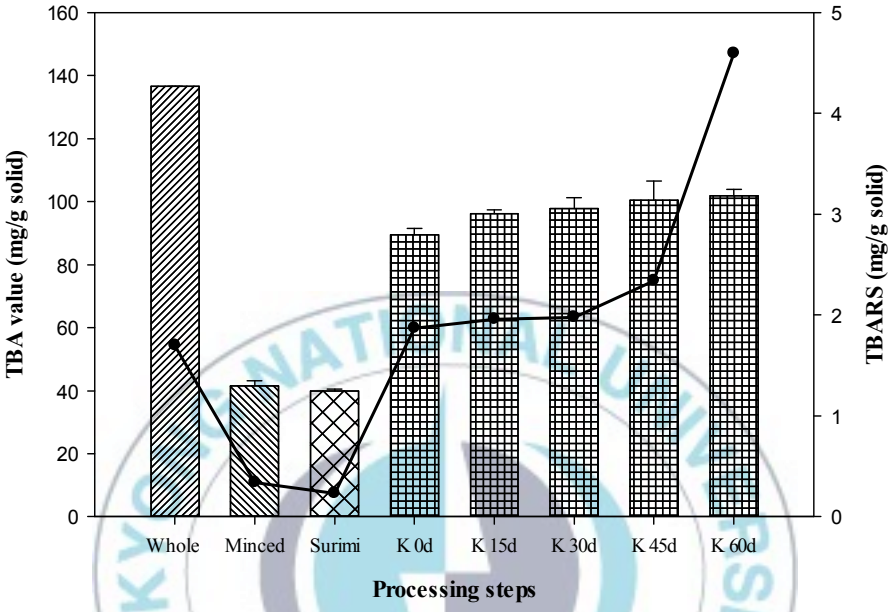


Fig. 4. Comparison of TBA value vs TBARS levels in raw anchovy, minced, surimi and kamaboko.

A significant increase of TBARS values was observed at 60 days of storage (4.6±0.52 mg/g solid). Studies for commercial kamaboko storage during 50 days showed the same trend (0, 10, 20, 30, 40 and 50 days: 1.93±0.03, 2.06±0.0, 2.08±0.2, 2.41± 0.41. 3.97±0.36 and 4.38±0.47 mg/g solid respectively) (Ryu and Choi, 2012). The increased rancidity denoted by the TBA index is due chiefly to formation of malonaldehyde by hydroperoxides from fatty acids containing three or more double bonds (Sikorsky et al., 1984) cited by Marti de Castro et al. (1997).

Changes on fat oxidation determinants, TBA value and TBARS indicate considerable increase of fat oxidation degree in the fried kamaboko products during storage in frozen conditions. Freezing slows enzyme activity and inhibits microorganism growth but cannot retard lipid oxidation. Long-term storage will release free fatty acids from lipid/protein reactions, which form oxidized products and cause denatured protein, which toughens. The nutritional value of proteins is lowered by reactions with peroxides, which also destroy pigments, bleach foods, produce toxins and or carcinogens, and cause off-flavors and odors. Vacuum packaging significantly minimizes autooxidation (Pigott and Tucker, 1990).

In vitro Protein Quality

Amino Acid Profile

Raw anchovy amino acid profile is nearly similar to that reported by NFRDI (2009). Glutamic acid had the highest concentration in all kamaboko samples (Table 4). The high concentration may also be attributed to added MSG in kamaboko products. Histidine level was gradually decreased through dissolving and removing during washing out stage in surimi processing. Cysteine and tryptophan showed the lowest value in all the samples (Fig. 5). Decrease of amino acid availability is the major and most serious nutritive loss associated with nonenzymatic browning. Lysine is the most easily damaged essential amino acid and becomes biologically unavailable. The loss of sulfur-containing amino acids in meat and fish can be significant. Effects of feeding heat-damaged protein include lowered protein efficiency ratios as essential amino acids become biounavailable. Many compounds formed during frying and barbecuing meats and fish are products of the Maillard reaction (Pigott and Tucker, 1990).

Table 4. Amino acid profile of raw anchovy, minced, surimi and kamaboko

Amino acid	ANRC Casein	Whole Anchovy	Minced	Surimi	Kamaboko
Aspartic acid	7.12	6.80	6.93	7.26	6.40
Threonine*	4.08	4.89	5.35	5.19	4.61
Serine	5.27	3.63	3.65	3.82	3.77
Glutamic acid	22.72	13.73	14.44	17.01	21.36
Proline	11.00	4.40	3.40	3.61	3.97
Glycine	1.83	6.10	4.73	4.46	4.18
Alanine	3.08	6.52	6.16	6.08	5.48
Valine*	6.60	5.09	5.14	5.03	4.73
Isoleucine*	5.25	4.47	4.58	4.73	4.35
Leucine	9.66	7.82	7.99	8.58	7.87
Tyrosine*	5.66	2.52	2.81	3.55	2.96
Phenylalanine*	5.21	4.20	4.18	4.07	3.94
Histidine	2.90	5.07	5.39	2.85	2.99
Lysine	8.23	8.51	9.06	9.51	8.17
Arginine	3.87	5.88	5.74	6.50	5.71
Methionine*	2.84	3.12	3.16	2.97	2.45
Tryptophan*	1.03	1.15	1.16	1.09	0.97
Cysteine*	0.58	1.11	1.13	1.06	1.08
Total	**106.93**	**95.00**	**95.00**	**97.38**	**95.00**

*Essential amino acid

ANRC – Animal nutrition research council

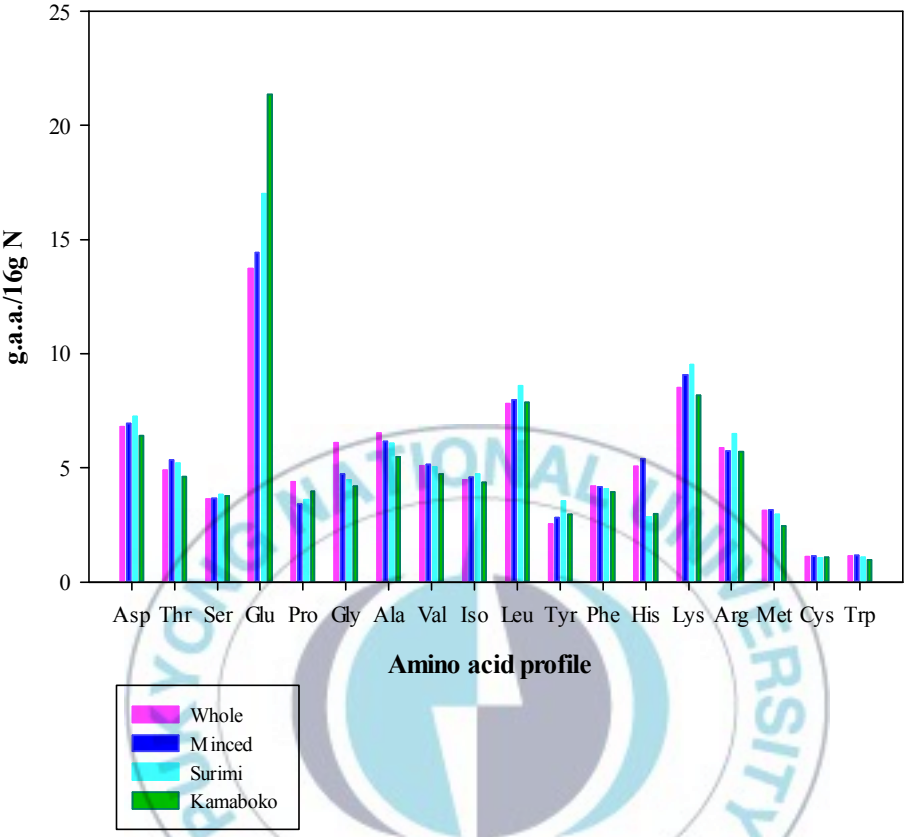


Fig. 5. Amino acid profile of whole anchovy, minced, surimi and kamaboko

Protein Digestibility

Three enzymes were used to determine the *in vitro* digestibility of samples and reference protein casein. The *in vitro* methods for assaying digestibility all rely on the use of proteolytic enzymes to correlate with the digestion of protein *in vivo*. In general, *in vivo* (rat) protein digestibility for raw fish range from 90.6 to 96.6% (McDoonough et al., 1990; FAO/WHO, 1990), cited by El and Kavas (1996).

Table 5 shown the *in vitro* protein digestibility and level of trypsin inhibitor in raw anchovy, minced, surimi and kamaboko and Fig. 6 shows the relationship between these two assays. Whole anchovy and minced have almost the same value (79.2±1.01 and 79.4±0.53% respectively). A similar result (78.50%) was observed by Lee et al. (1984a) and Lee and Ryu (1986), during the processing and storage of boiled and dried anchovy, and in the evaluation of seafoods protein quality as predicted by C-PER assays, respectively for the same species in raw sample.

Table 5. *In vitro* protein digestibility and level of trypsin inhibitor in raw anchovy, minced, surimi and kamaboko

Processing steps	Digestibility (%)	TI (mg/g solid)
Whole anchovy	$79.22^{d} \pm 1.01$	$2.43^{a} \pm 0.01$
Minced	$79.39^{d} \pm 0.53$	$1.83^{b} \pm 0.07$
Surimi	$84.81^{c} \pm 0.16$	$0.88^{c} \pm 0.06$
Kamaboko (0 days)	$88.47^{a} \pm 0.51$	$0.77^{d} \pm 0.02$
Kamaboko (15 days)	$87.18^{ab} \pm 0.94$	$0.60^{e} \pm 0.02$
Kamaboko (30 days)	$87.37^{ab} \pm 1.13$	$0.43^{f} \pm 0.01$
Kamaboko (45 days)	$86.56^{abc} \pm 0.90$	$0.39^{fg} \pm 0.02$
Kamaboko (60 days)	$86.24^{bc} \pm 0.40$	$0.31^{g} \pm 0.02$

Mean ±SD of three determinations

$^{a-d/\ a-g}$Different letters show significant differences ($P<0.05$)

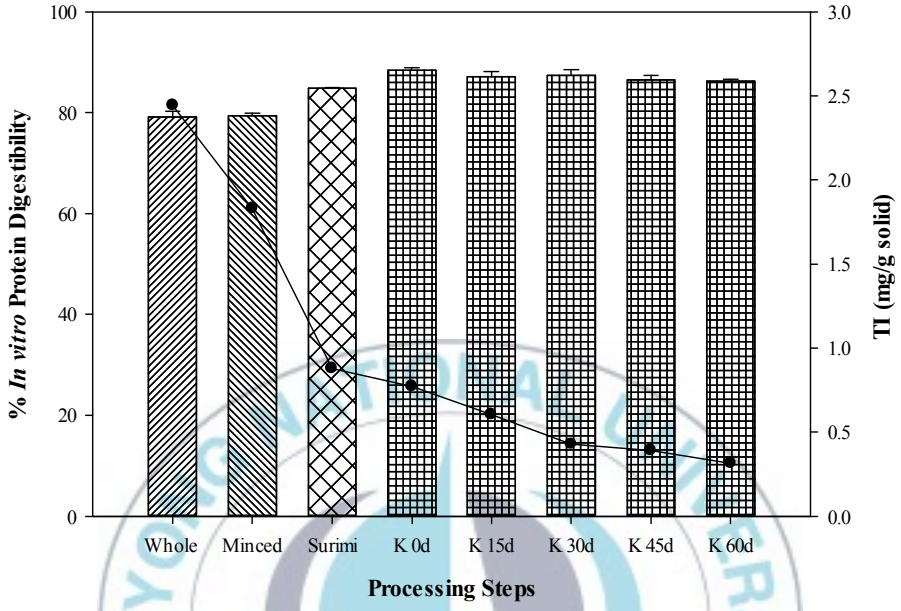


Fig. 6. **Comparison of *in vitro* protein digestibility vs TI levels in raw anchovy, minced, surimi and kamaboko.**

There was an increase in the degree of digestibility of the surimi (84.8±0.16) and kamaboko samples compared with the raw samples, but the protein digestibility of kamaboko was decreased slightly during the frozen storage time. The value of digestibility for 0 days (88.5±0.51%) is similar to the reported result of marketed steamed kamaboko and crab meat analog (86.4 to 88.2%) by Ryu et al. (1994a). Protein digestibility in fried commercial kamaboko ranged from 80.9 to 91.9% (Ryu and Choi, 2012). Mauron (1984) cited by El and Kavas (1996) reported that protein digestibility was reduced as a result of complex chemical (crosslinking) reactions such as protein interactions or protein-fat interactions when food was broiled at high temperatures.

***In vitro* Protein Quality**

The percentage of protein digestibility and computed protein efficiency ratio (C-PER) of the kamaboko samples are shown in the Table 6.

Table 6. *In vitro* protein qualities of whole raw anchovy, minced, surimi and kamaboko

	ANRC Casein	Whole Anchovy	Minced	Surimi	Kamaboko
In vitro protein digestibility (%)	90	79.2	79.4	84.8	88.5
C-PER*	2.50	1.96	1.94	2.50	2.59

*C-PER = Computed Protein Efficiency Ratio

The C-PER assay is an *in vitro* assay that has been developed to predict or estimate the PER of food proteins. By use of a computer, a model was developed that matched *the in vitro* digestibility and essential amino acid (EAA) profile of the samples respective rat-based PERs. This model is termed the C-PER (Pellet and Young, 1980). Computed protein efficiency ratio (C-PER) for kamaboko was highest (2.59) compared with whole anchovy (1.96), minced (1.94) and surimi (2.50). Values of C-PER ranged from 1.97 to 2.63 were reported for commercial kamaboko (Ryu and Choi, 2012), values ranged from 2.32 to 2.56 for surimi and from 2.13 to 3.13 for steamed kamaboko was documented by (Ryu et al., 1994b; Ryu et al., 1994a) respectively. Protein efficiency ratio of minced flesh is almost equal to that of whole fillet, indicating retention of the high quality found in the protein of fish flesh. Surimi is a highly functional pure fish protein-water-cryoprotectant combination prepared from washed fish flesh. Surimi PER can be higher than in mince and higher than in a raw fillet due to loss of the less functional proteins. Surimi product can upgrade the nutrition image of fast foods (Pigott and Tucker, 1990).

Trypsin Inhibitor

Table 5 shows the changes of trypsin inhibitor (TI) in anchovy kamaboko during frozen storage (-20 ˚C) for 60 days. The amount of TI is highest in the raw anchovy (2.43±0.01 mg/g solid). In minced and surimi, the TI value decrease probably due to lipid extraction (1.83±0.07 and 0.88±0.06 mg/g solid respectively). Lee and Ryu (1986) reported that in several instances it was thought that the boiling and salting processes resulted in lipid extraction, which diminished the formation of the enzyme-indigestible substrate. In the kamaboko samples, TI gradually decreased during the storage time (from 0.77 to 0.31 mg/g solid) probably due to oxidation confirmed by the high TBA value and TBARS (Fig.6). Similar trend was observed by Oduro et al. (2011) in the fried, grilled and steamed mackerel. TI includes typical proteinaceous inhibitory materials contained in raw sources and indigestible materials such as trypsin indigestible substrates (TIS) induced from the result of interaction between protein and other components such as lipid oxidation products (Oduro et al., 2011) .

Starch Digestibility

Table 7 and Fig. 7 shows the values and changes *in vitro* starch digestibility of kamaboko samples during storage (-20˚C). The amount of starch digestibility decreased lightly with the storage time from 14.2 to 12.5%, probably due to lipid oxidation, commercial kamaboko ranged from 15.4 to 17.5 % (Ryu and Choi, 2012). Measurement of different starch fractions provides a means for predicting the rate and extent of digestion in the human small intestine. It is of importance that the *in vitro* procedure should simulate starch digestion at the best possible rate. Specifically a greater increase of temperature (170-180°C) is known to occur while steaming which might have increased the total starch content causing higher gelatinization of the starch molecules thereby increasing the available starch content. Cooking increases susceptibility of starches to hydrolysis, a wide variation in the level of starch modification exists, depending on the processing intensity and storage conditions. The most important parameters are the water content and the maximum temperature reached. The heat used during cooking can be dry as in baking or wet as in boiling and steaming.

Table 7. *In vitro* starch digestibility in kamaboko samples stored at -20˚C

Processing steps	Starch Digestibility (%)
Kamaboko (0 days)	$14.2^{a} \pm 0.36$
Kamaboko (15 days)	$14.0^{a} \pm 0.12$
Kamaboko (30 days)	$12.5^{b} \pm 0.14$
Kamaboko (45 days)	$12.5^{b} \pm 0.27$
Kamaboko (60 days)	$12.7^{b} \pm 0.30$

Mean ±SD of three determinations

[a-b]Different letters show significant differences ($P<0.05$)

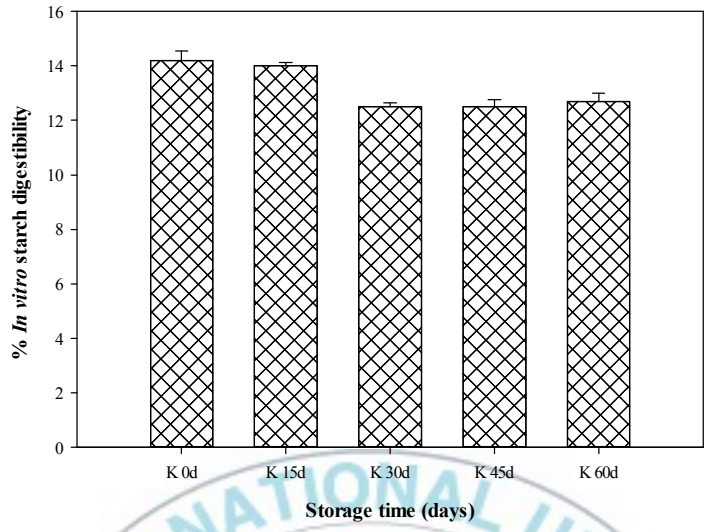


Fig. 7. Changes *in vitro* starch digestibility of kamaboko samples during storage (-20˚C).

Heat increases the nutrient availability. Processing may also reduce the nutritional value as a result of losses and changes in major nutrients (Faiyaz and Urooj, 2008).

Texture Properties

Table 8 shows that the hardness, springiness, gumminess and chewiness of fresh anchovy kamaboko (FAK) had non-significant difference with commercial kamaboko (CK) but showed very significant difference with frozen and thawed kamaboko (FTAK), which showed some higher values in all of these textural properties, probably because freezing storage caused structural damage to the texture. Marti de Castro et al. (1997), observed that the compression parameters (hardness, elasticity and cohesiveness) of the frozen product were mainly influenced by freezing temperature.

FAK and FTAK showed similar fracturability and adhesiveness, and difference with CK, which showed low value in fracturability.

Table 8. Texture properties for fresh, frozen and thawed anchovy kamaboko and commercial kamaboko

	Hardness g	Fracturability g	Adhesiveness gs	Springiness %	Cohesiveness %	Gumminess g	Chewiness	Resilience
FAK	1483.70^{b}±78.60	20.18^{a}±2.19	1.86^{a}±0.74	84.0^{b}±0.02	42.7^{b}±0.00	634.37^{b}±38.74	533.10^{b}±43.01	0.155^{b}±0.02
FTAK	1994.29^{a}±163.66	18.76^{a}±1.14	-1.82^{a}±1.80	100.7^{a}±0.05	68.3^{a}±0.03	1364.23^{a}±176.25	1375.3^{a}±202.08	0.318^{a}±0.02
CK	1303.67^{b}±72.85	10.45^{b}±1.31	-25.19^{a}±19.28	85.8^{b}±0.07	69.0^{a}±0.05	888.47^{b}±47.48	764.00^{b}±97.49	0.305^{a}±0.04

Mean ±SD of three determinations

$^{a-b}$Different letters show significant differences (P<0.05)

FAK : Fresh anchovy kamaboko

FTAK : Frozen and thawed anchovy kamaboko

CK : Commercial kamaboko

FTAK and CK showed non-significant difference in cohesiveness and resilience and very significant difference with FAK, which showed the lowest values for both rheological parameters. According to Lanier (1986) cited by Marti de Castro et al. (1997), and Gomez-Guillen and Montero (1996) cohesiveness is the most sensitive parameter for evaluating the state of surimi proteins. Moreover, cohesiveness and hardness can vary independently (Hamann & Mac Donald, 1992), cited by Gomez-Guillen and Montero (1996). According to Uresti et al. (2004), cohesiveness and chewiness showed a similar behavior to hardness.

Color Value

Lightness (L) value for anchovy kamaboko was lower compared with commercial kamaboko samples (Table 9). There was non-significant differences with CK1 in lightness, probably because this type of kamaboko contained vegetables, but showed very significant differences compared with the samples CK2 and CK3, (samples without vegetables).

Table 9. Color value for frozen anchovy kamaboko and different types of commercial kamaboko

Type of kamaboko	L	a	b
FTAK	$62.99^{c} \pm 0.78$	$0.16^{c} \pm 0.30$	$11.96^{b} \pm 0.49$
CK 1	$63.67^{c} \pm 0.84$	$1.68^{ab} \pm 0.20$	$18.01^{a} \pm 0.67$
CK 2	$80.29^{a} \pm 1.34$	$0.85^{c} \pm 0.39$	$11.55^{b} \pm 0.04$
CK 3	$72.11^{b} \pm 1.57$	$2.33^{a} \pm 0.91$	$12.65^{b} \pm 1.24$

Mean ±SD of three determinations

$^{a-c}$Different letters show significant differences ($P<0.05$)

FTAK : Frozen and thawed anchovy kamaboko

CK 1: Commercial kamaboko with vegetables

CK 2: Commercial kamaboko without vegetables

CK 3: Commercial kamaboko without vegetables

The lower redness (a) value for anchovy kamaboko showed significant difference compared with all commercial kamaboko samples, probably due the natural dark color of surimi, because anchovy is a dark-fleshed fish. A major problem is the washing process, because of the high lipid content, water soluble proteins as well as the pigments and trimethylamine oxide (TMAO), in dark-fleshed minced fish (Shimizu et al., 1992) cited by Chen et al. (1997). The heme pigments are much less easily extracted from dark fleshed species because the dark muscle structure is much more tough and rigid than white muscle (Shimizu et al., 1992) cited by (Hsu and Chiang, 2002), since myoglobin contributes to the color of muscle (Pearson and Young, 1989) cited by Chen et al. (1997). The increase in lightness (L) and decrease in redness (a) of washed mince are related to increased transparency as well as loss of myoglobin. The color remaining after washing was the result of insoluble pigments. Structures such as mitochondria, which contain cytochromes, would not be removed from intact muscle fragments by washing with water (Park et al., 1996) cited by Chen et al. (1997). Also the amount of fat level in the kamaboko product and unsaturated fatty acids might influence lipid oxidation, which decreases in redness.

Non-significant differences of yellowness (b) value was observed between anchovy kamaboko and some commercial kamaboko (CK2 CK3) samples, but significant difference was observed with CK1, which observed the highest value of the samples.

Decrease in lightness and increase in redness and yellowness were observed by Koo et al. (2001), Ha et al. (2001), Son et al. (2003) and Kim et al. (2003), due to the addition of increasing percentages of mushroom in fish paste. Bae and Lee (2007), observed that increasing the amount of small size and large size anchovy powder resulted to decrease in redness and increase in yellowness. Hsu and Chiang (2002) observed that the yellowness decreased with the increase of moisture content. It means that the higher the moisture content, the whiter and the yellow was the color. They reported that the increase in the lightness was not species dependent, while the decrease in the yellowness tended to be species dependent. The addition of protein additives to improve the textural properties of dark-fleshed fish species could further worsen their color properties, for example the use of whey protein, beef plasma protein and soybean protein could increase the yellowness of the final products (Park, 1994). Color and texture

properties are important sensory attributes affecting the market acceptance of surimi products. Generally, gels with high lightness, low yellowness and high whiteness are highly demanded by consumers (Hsu and Chiang, 2002).

Conclusions

Processing conditions caused significant changes in the proximate composition and protein quality of all the samples especially in the fried kamaboko product.

Storage time in frozen conditions and the use of vacuum package were effective in maintaining the shelf life of anchovy kamaboko within 30 days, but not effective after 45 days due to fat oxidation.

Anchovy kamaboko showed the highest digestibility (88.5%), and C-PER value (2.59), among surimi, minced and raw anchovy.

Fresh anchovy kamaboko showed almost similar values of hardness, springiness, gumminess and chewiness to commercial kamaboko, highest value of fracturability and adhesiveness, lowest value of cohesiveness and resilience, while frozen and thawed anchovy kamaboko showed the highest values for all of these rheological parameters.

Anchovy kamaboko showed the lowest lightness and redness and similar yellowness compared with commercial kamaboko.

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