Thesis for the Degree of Master of Fisheries Science

Effects of Cooking Conditions on Protein Quality of Chub Mackerel (*Scomber japonicus*)

by

Frieda A. Oduro

KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

Pukyong National University

February, 2012

Effects of Cooking Conditions on Protein Quality of Chub Mackerel (*Scomber japonicus)*

고등어 단백질 품질에 미치는 조리조건의 영향

Advisor: Professor Hong-Soo Ryu

A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Fisheries Science

In KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

Pukyong National University

February, 2012

Effects of Cooking Conditions on Protein Quality of Chub Mackerel (*Scomber japonicus)*

A dissertation

by

Frieda A. Oduro

Approved by :

(Chairman) Professor Ji-Young Yang

(Member) Professor Yang-Bong Lee

(Member) Professor Hong-Soo Ryu

February 24, 2012

Table of Contents

Effects of Cooking Conditions on the Protein Quality of Chub Mackerel (*Scomber japonicus*)

Frieda A. Oduro

KOICA-PKNU International Graduate Program of Fisheries Science,

The Graduate School,

Pukyong National University

Abstract

Effects of cooking methods (grilling, frying, steaming and microwaving) on proximate composition and protein quality of chub mackerel (*Scomber japonicus*) treated with 2, 6 and 10% sodium chloride (NaCl) brine were investigated. Moisture content decreased in all cooked samples from 60.22% in the raw sample to 48.7% in the fried samples. The 10% NaCl brine treatments of all samples recorded the highest moisture loss than the 2 and 6% treatments. All cooked samples showed a decrease in their fat contents except the fried sample. Protein content increased in all cooked samples, from 47.21% in the raw sample to

63.87% in the grilled sample. The 10% NaCl brine treatment of all samples recorded the highest degree of fat oxidation (TBA value and TBARS), which was highest in the fried sample and lowest in the microwaved sample. The level of trypsin inhibitor (TI) was highest in the microwaved sample and lowest in the fried sample. In all samples, the 6% salt treatments recorded the lowest level of trypsin inhibitor and highest rates of *in vitro* protein digestibility. *In vitro* digestibility increased from 79.4% in the raw sample to 86.43% in the fried sample. The total essential amino acids of all cooked samples increased. Results from the study indicated that grilling and steaming had beneficial effects on the protein quality of chub mackerel.

1. Introduction

1.1 Importance of fish

Fish is known to be a source of protein rich in essential amino acids (lysine, methionine, cystiene, threonine and tryptophan). Fish muscle also contains micro and macro elements and fat-soluble vitamins (Larsen et al., 2007).

Approximately 14% of the animal protein consumed by humans comes from marine fisheries though there are variations between countries. Fish is an excellent source of high quality proteins, compared with those found in meat and poultry. Most raw fish is 16 – 24% protein which can give rise to as much as 35% in cooked fish (Hall, 1992). The high protein levels, with good digestibility and also low fat content are advantages of seafood (Pigott and Tucker, 1990).

Chub mackerel's popularity worldwide is due to the presence of two important fatty acids; eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These two are known to have many health benefits, particularly

with regard to heart disease, decreased risk of prostate cancer and Alzheimer disease (Huang et al., 2005).

1.2 Processing methods

Chub mackerel is rarely eaten raw and is usually cooked in different ways before consumption. Application of heat is usually the method used in the different cooking methods such as boiling, baking, roasting, frying, grilling, steaming and microwaving. All these cooking methods serve to enhance the taste, flavor as well as to increase the shelf life of the product (Garsia-Arias et al., 2003).

1.3 Effects of processing on protein quality

Protein quality of fish is affected during processing as a result of the application of heat which results in protein denaturation. The extent of protein denaturation depends on the duration of heat, the temperature as well as the processing facility (Sikorski, 2001).

It must be noted that the nutritive value of proteins is determined not only by their quantitative and qualitative composition of amino acids, but also by their availability to digestive tract proteolytic enzymes (Lee and Ryu, 1986).

Hence, the rate of digestibility of a protein is indicative of its availability to the digestive enzymes.

It is hypothesized that processing by heat increases food digestibility because it breaks proteins and carbohydrates which are less digestible. Despite this advantage however, vitamins, minerals, some essential amino acids and other beneficial nutrients are lost (Mirnezami et al., 2002).

Lipid oxidation is one factor that contributes to losses in protein quality. Fish oils are converted to ketones, aldehydes and hydroxyacids. The reactions are enhanced by iron and copper ions, so red muscle readily becomes rancid especially in tuna, swordfish, bluefish and mackerel. This appears as a thin brownish-gray layer next to the larger portion of edible flesh. Chemical reactions of oxidized lipids with amines, amino acids and proteins have received considerable attention because they are associated with changes in functional properties, nutritive value, flavor and colour of foods (Pokorny and Kolakowska, 2002; Xiong, 2000).

Oxidized lipids not only cause rancidity in food products but interact with other food components causing a change in their nutritive value (Jozef et al., 2004). Protein is one such valuable component that must be protected during food processing because fish proteins contain all the essential amino

acids (Hoffmann, 1993). Reduction of digestibility as well as limitation of the amount and degree of amino acid availability is affected by the formation of amino acid bonds with lipid oxidation products (Porkorny and Davidek, 1979; Lee and Ryu, 1986). These protein-lipid complexes contribute to the amount of indigestible substances in foods which are unavailable for proteolytic enzymes to act on.

In vitro protein digestibility is an inexpensive way of determining protein quality of seafood. This is a method for predicting the ability using a multi enzyme assay to imitate human and animal digestive systems. Digestibility depends on the amount of indigestible substrates in foods. Due to the time consuming and expensive *in vivo* method of determining protein digestibility, researchers have tried to correlate *in vivo* methods to *in vitro* methods in order to develop reliable methods for protein efficiency ratio (PER) measurement. Two such methods are computed protein efficiency ratio (C-PER) (Satterlee at al., 1982), and discriminant computed protein efficiency ratio (DC-PER) (Jewell et al., 1980). The C-PER is a PER prediction, calculated from essential amino acid information and *in vitro* protein digestibility, where as the DC-PER is solely dependent on amino acid compositional data. These two methods are known to have a high

correlation to the *in vivo* assay (Phimphilai et al., 2006). Lee and Ryu. (1986) used this model to evaluate protein quality of seafood.

Various experiments have been conducted on the protein quality of seafood. Ryu et al. (1992), observed an increase in the *in vitro* protein digestibility, a decrease in trypsin indigestible substrates (TIS), a reduction of some essential amino acids and an increase in fat oxidation of seasoned and smoked squid. Hakimeh et al. (2010) also observed an increase in the *in vitro* protein digestibility and nutritional indices in the Persian sturgeon (*Acipenser persicus*) after grilling and frying.

Cooking causes changes in proximate composition of fish as was observed by Aminullah et al. (1986). According to other reports by (Castrillon and Navarro, 1997; Piggot and Tucker, 1990), cooking practices could cause modifications in proximate composition, fatty acids and amino acids as well as changes in nutritional quality.

Chub mackerel is very popular in Ghana and is mostly consumed in the smoked form although the grilled form has also become very popular recently. The popularity of mackerel has soared because of the much publicized health benefits of the omega-3 fatty acids. Protein quality is

severely affected by the heat applied during the processing methods as equipments used do not make it possible to control the cooking conditions. It is therefore necessary to know the beneficial/optimal processing conditions that can produce superior products nutritionally rather than just satisfying the consumer's organoleptic appetite.

1.4 Objective

Although many studies have been carried out on the effects of different cooking conditions on the nutritional quality of fish, not much work has been done on chub mackerel. It is therefore the main objective of this study to investigate the effects of four different cooking methods (grilling, steaming, frying and microwaving) on the protein quality of chub mackerel and to know which cooking method(s) will be beneficial for retaining a high nutritive value ideal for human health.

2. Materials and Methods

2.1 Sample preparation

Chub mackerel samples were delivered to the laboratory as individual quick frozen (IQF) products, prepared using just landed fish from 3S Seafood Company, in Busan Korea. Average sizes of semi-dressed samples without viscera were 28.0 ± 0.1cm in length and 380.0 ± 0.2g in weight. They were immediately frozen at a temperature of -13°C after individual weights were recorded. The fish samples were then randomly divided into five units. One unit was kept raw and used as the reference or control. Each of the four units were again divided into three subunits and soaked in sodium chloride brine concentrations of 2, 6 and 10% respectively for one hour. These subunits were then cooked by frying, steaming, microwaving and grilling.

Grilling of fish was performed for twelve minutes at 250°C using an oven (Convotherm, OAS6.10 Germany). Steaming was done for twelve minutes at 200°C with the same oven. Fish were pan fried in a big frying pan with

soybean oil at a temperature of 180°C for ten minutes. Microwaving was done for six minutes using a Samsung Zipel oven (DG68-00216B-01 Korea).

2.2. Experimental procedure

2.2.1. Determination of proximate composition

The cooked mackerel meat without the skin was homogenized using a dry kitchen blender. The raw sample was also homogenized but with the skin. Moisture was determined by oven-drying at 105°C until constant weight (AOAC, 1990). Fat was determined by the method described by AOAC (1990) using the Soxhlet solvent extractor. Crude protein was determined by the semi-micro kjeldahl procedure using a conversion factor of 6.25 (AOAC, 1990). The rest of the samples were freeze dried for other tests.

2.2.2. Drip loss

This was calculated from the differences in the mass of raw mackerel samples before and after thawing in a cold room at a temperature of 6°C for 4 hours.

% Drip loss = (mass before thawing – mass after thawing)/ Mass before thawing × 100

2.2.3. Cooking loss

Cooking loss was measured according to the method of Niamnuy et al. (2008) and was calculated from the differences in mass of sample before and after each cooking method (frying, grilling, microwaving and steaming). % cooking loss = (mass before cooking – mass after cooking)/ mass before cooking × 100

2.2.4. Water activity measurements

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

2.2.5. Fat oxidation

a. Thiobarbituric acid value (TBA value)

TBA values, expressed in mg/g solid, were estimated by using the method of Tarladgis et al. (1960). Absorbance was measured at 538nm and the TBA values were obtained by multiplying the optical density by 100 and expressed in mg/g solid.

b. Thiobarbituric acid reactive substances (TBARS)

TBARS in the samples were determined by the method of Witte et al. (1970). Absorbance was measured at 530nm and the concentration of TBARS in the samples was measured by multiplying the optical density by 5.2 and expressed in mg /g solid.

2.2.6. *In vitro* protein digestibility

The *in vitro* digestibility values of raw and cooked mackerel samples were determined by the Satterlee (1979) method with modification by the AOAC procedure (AOAC, 1982). The procedure used the four enzyme method including trypsin (Sigma 17,600 BAEE units/mg solid), α-chymotrypsin (Sigma 41 units/mg solid), peptidase (Sigma 102 units/mg solid) and bacterial protease (Streptomyces griceus protease (4.5 units/mg solid, sigma). The three enzyme method (without peptidase enzyme) was also used to determine the *in vitro* digestibility rates of samples. This was to determine the correlation coefficient between the two assays. The reference protein used was ANRC casein and digestibility was calculated as follows;

% Digestibility = 234.84 – 22.56x where x is the pH of sample at 20 minutes.

2.2.7. 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 soyabean trypsin inhibitor per gram sample. The standard curve used in measuring TI content is shown in Figure 1.

The correlation coefficient between pH and TI content was 0.9914 and the equation for calculation is

y= 4.0434x – 26.281, where y = purified soyabean trypsin inhibitor (mg) and x is pH at 10 minutes incubation.

2.2.8. Amino acid profiles

This was carried out by the Feeds and Foods Nutrition Centre, PKNU, using the 6N HCl hydrolysis method with amino acid analyser (S433, Sykum, Germany).

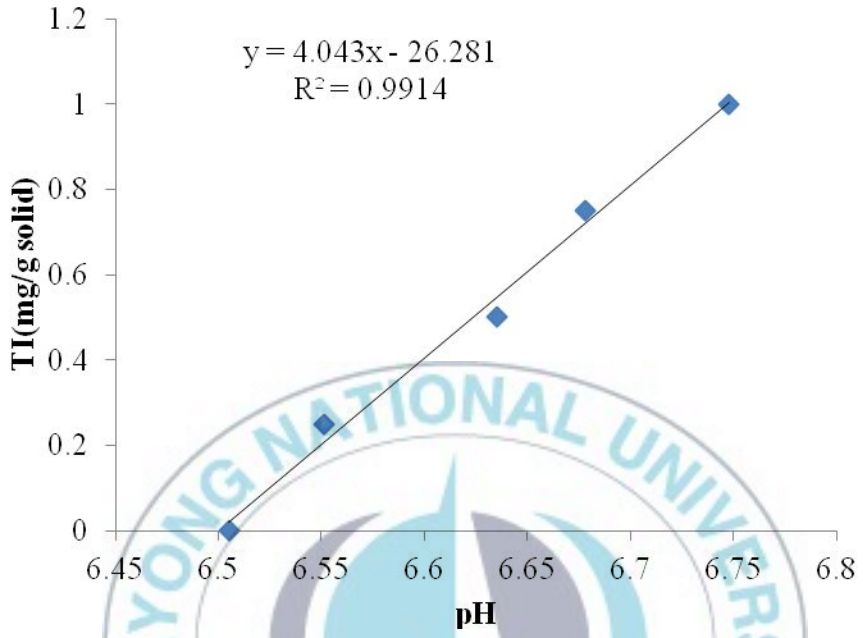


Figure 1. Relationship of pH at 10 minutes with purified soyabean trypsin inhibitor concentration.

2.2.9. Computed *in vitro* protein quality

C-PER, DC-PER and predicted digestibility were calculated by the corrected AOAC procedure (1982). Protein digestibility and amino acid profiles were used in the calculation of these *in vitro* protein quality data.

2.3. Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA), followed by Tukey's multiple range test. All data are expressed as mean ± S.D. The significance of results was at 5%. The software used was SPSS version 18.

3. Results and Discussion

3.1. Proximate composition

The proximate composition of raw and cooked fish samples are presented in Table 1. Proximate composition of raw mackerel was similar to that observed by the National Fisheries Research and Development Institute (NFRDI), in fall 2009. Moisture content decreased in all cooked samples. Moisture loss was highest in the fried sample (F-10, 48.7%) followed by grilled (G-10, 52.3%), steamed (S-10, 54.3%) and then the microwaved (M-10, 57.6%) samples. In all sample categories, the 10% NaCl brine treatments recorded the highest moisture loss and this was due to the high amount of salt that facilitated more moisture loss. Protein also increased in all cooked samples with the highest in fried, followed by grilled, steamed and microwaved samples. Based on dry basis, fat content decreased in all the cooked samples with the exception of fried which recorded an increase. The higher levels of fat in the fried sample compared to the raw and other cooked samples, was due to the absorption of fat from the vegetable oil by

the fish during frying. The grilled sample recorded the highest fat loss due to the higher temperature employed during cooking than in the steamed samples.

The decrease in moisture and an increase in protein in all cooked samples were also observed in similar experiments by Hakimeh et al. (2010) and Jucieli et al. (2008). The decrease in moisture content has been described as the most prominent change that makes the protein content increase significantly in cooked fish (Gokoglu et al. (2004). The heat and flow of gases caused drying of the cooked mackerel samples. This decreased the water content thereby causing the changes associated with dehydration such as increasing the protein concentration of the food (Morris A et al., 2004). The processing conditions employed in the steamed, grilled and microwaved samples could be said to have caused fat to be extracted from these samples hence the decrease in fat content.

Table 1. Proximate composition of raw, salted and cooked chub mackerel (g/100g sample)

Sample**	Moisture (%)*	Crude protein(%)* (N × 6.25)	Lipid (%)*
Raw	60.2±0.4^{a}	18.8±0.4^{e} (47.21)	18.9±0.8^{e} (47.54)
G-2%	56.3±0.6^{de}	28.0±0.3^{a} (61.82)	20.1±0.5^{cde} (44.48)
G-6%	54.7±0.7^{fg}	27.5±0.7^{ab} (56.08)	21.2±0.7^{bcd} (43.27)
G-10%	52.3±0.5^{h}	27.9±0.3^{a} (63.87)	20.0±0.7^{de} (45.79)
S-2%	56.6±0.6^{de}	26.4±0.6^{b} (59.92)	22.1±0.6^{bc} (44.72)
S-6%	55.9±0.2^{ef}	24.0±0.5^{d} (52.63)	22.9±0.8^{b} (44.57)
S-10%	54.3±0.9^{g}	26.3±0.6^{bc} (60.51)	22.6±0.8^{b} (46.97)
F-2%	48.9±0.2^{i}	27.2±0.3^{ab} (53.26)	25.0±0.2^{a} (49.95)
F-6%	50.2±0.2^{i}	27.6±0.3^{ab} (55.46)	25.2±0.2^{a} (50.68)
F-10%	48.7±0.2^{i}	28.6±0.6^{a} (55.74)	25.8±0.8^{a} (50.24)
M-2%	59.3±0.6^{ab}	23.9±0.6^{d} (57.88)	19.7±0.7^{de} (47.69)
M-6%	58.7±0.7^{bc}	24.9±0.3^{cd} (58.65)	18.7±0.4^{e} (44.12)
M-10%	57.6±0.4^{cd}	24.1±0.3^{d} (59.17)	20.2±0.6^{cde} (47.75)

*Mean ±SD of three determinations

[a-i] Different letters in column of each sample category show significant differences ($P<0.05$)

**Sample categories: G(grilled), S(steamed), F(fried), M(microwaved), Raw(control)
Values in brackets – g/100g solid

3.2. Drip loss

An average value of (3.72 % (g/drip/100g frozen sample) was recorded for the raw sample after thawing in a cold room at 6ºC for four hours. This low value indicated that the fish was in excellent condition before the different cooking conditions were applied. A low drip loss indicates that frozen protein denaturation has not taken place.

3.3. Water activity

The water activity values of samples showed no significant differences (Table 2). They ranged from 0.98 in the raw samples to 0.95 in the grilled samples. Foods found in this range are classified as water-rich foods and support profuse growth of microorganisms as well as other chemical reactions. This meant that all the mackerel samples had to be preserved to prevent spoilage (Pigott and Tuker, 1990).

3.4. Cooking loss

Table 3 shows cooking loss of the various cooked samples, which depended on the cooking process. The significant loss was observed in the grilled samples (G-10, 44.84%), followed by steamed (S-10, 43.33%), fried

(F-10, 42.98%) and then microwaved (M-10, 18.45%) samples. There were no significant differences between the NaCl brine treatments in each sample category. Water in the mackerel muscle is held within the myofibrils, in the space between the thick filaments (myosin) and thin filaments (actin) as well as in the connective tissue (Offer et al. (1989). As cooking proceeded, heat induced protein denaturation and aggregation leading to shrinkage of both the filament lattice and the collagen. This also led to exposing the hydrophobic areas of the myofibrillar structure, which allowed new intra and inter- protein interactions resulting in a more dense structure (Straadt et al., 2007). The subsequent aggregation and denaturation of proteins led to a loss in water holding capacity, hence the loss of water. Salts enhance denaturation by reducing the water holding capacity (de Man, 1999). The WHC values of the 10% NaCl brine treated samples in Table 2 and the cooking loss values of the 10% treatments in Table 3 clearly shows this relationship. Cooking loss leads to a significant loss of matter and is thought to have a linear relationship with the time and temperature of cooking (Garsia-Segovia et al., 2007).

Table 2. Water holding capacity and water activity of raw, salted and cooked chub mackerel

Sample**	WHC*	Aw
Raw	0.42±0.01[a]	0.98
G-2%	0.26±0.22[g]	0.95
G-6%	0.24±0.01[h]	0.95
G-10%	0.22±0.12[i]	0.95
S-2%	0.30±0.03[de]	0.96
S-6%	0.30±0.15[ef]	0.96
S-10%	0.30±0.20[f]	0.96
F-2%	0.32±0.11[d]	0.96
F-6%	0.31±0.01[de]	0.96
F-10%	0.30±0.02[de]	0.96
M-2%	0.40±0.03[b]	0.97
M-6%	0.39±0.01[b]	0.96
M-10%	0.35±0.02[c]	0.96

*Mean ±SD of three determinations
[a-i] Different letters in column of each sample category show significant differences ($P < 0.05$)
**Sample categories: G(grilled), S(steamed), F(fried), M(microwaved), Raw(control)
WHC – Water Holding Capacity; Aw – Water Activity

Table 3. Cooking loss of salted and cooked chub mackerel

Sample*	Cooking loss (%)
G-2%	44.69
G-6%	44.71
G-10%	44.84
S-2%	43.12
S-6%	43.23
S-10%	43.33
F-2%	42.80
F-6%	42.92
F-10%	42.98
M-2%	18.19
M-6%	18.12
M-10%	18.45

*Sample categories: G (grilled), S (steamed), F (fried), M (microwaved)

3.5. Fat oxidation

Fatty fish are, of course, particularly vulnerable to lipid oxidation which can create severe quality problems such as unpleasant (rancid) taste and other functional properties even on storage at subzero temperatures (Huss, 1995). This is due to the unsaturated nature of the fat in the fish. The primary products of fat oxidation are hydroperoxides which are not harmful to food quality. These hydroperoxides are however unstable and undergo scission to form volatile carbonyl compounds, such as aldehydes and ketones. Malondialdehyde, a major secondary product of fat oxidation, is shown to be the principle factor that involves protein cross-link reactions. The schiff's base formation between amino groups of lysine and other free amino groups in the protein could lead to the reduction in the availability of these amino acids (Crawford et al., 1967; Nielsen et al,. 1985). Malondialdehyde has been reported to be toxic to living cells because it can be absorbed through the digestive system (Piche et al., 1988).

TBA value and TBARS assay are widely used indicators for the assessment of degree of lipid oxidation or rancidity in foods. Table 4 presents the degree of fat oxidation as measured by the two assays. TBA values increased in all cooked samples with the highest in the fried,

Table 4. TBA value and TBARS of raw, salted and cooked chub mackerel

Sample**	TBA value (mg/g solid)*	TBARS (mg/g solid)*
Raw	415.72±0.38^{m}	10.79±0.12^{l}
G-2%	446.46±0.13^{h}	17.48±0.03^{h}
G-6%	434.62±0.21^{j}	15.65±0.06^{i}
G-10%	452.99±0.35^{g}	22.35±0.04^{g}
S-2%	537.71±0.27^{d}	29.09±0.07^{e}
S-6%	535.04±0.22^{e}	27.94±0.03^{f}
S-10%	585.22±0.60^{c}	30.54±0.03^{d}
F-2%	665.33±0.20^{b}	32.02±0.01^{c}
F-6%	533.93±0.23^{f}	33.49±0.07^{b}
F-10%	685.39±0.39^{a}	34.67±0.07^{a}
M-2%	420.28±0.51^{l}	11.65±0.03^{jk}
M-6%	424.91±0.59^{k}	11.42±0.02^{k}
M-10%	438.83.±0.37^{i}	11.89±0.23^{j}

*Mean ±SD of three determinations
$^{a-m}$ Different letters in column of each sample category show significant differences ($P< 0.05$)
**Sample categories: G(grilled), S(steamed)F(fried),M(microwaved),Raw(control)

followed by the steamed, grilled and microwaved samples. A similar trend was observed by Ryu et al. (1984a) in boiled whole anchovy. TBA values increased from 415.7mg/g solid in the raw sample to 685.39mg/g solid in 10% NaCl brine treated fried sample (Fig. 2). The lowest value recorded by the microwaved samples was probably because there was little oxygen involved in this processing method as it is a known fact that oxygen is needed for fat oxidation to occur. The high temperatures (over 150ºC) and the presence of oxygen are responsible for the high oxidation in most samples. Salt may act as a prooxidant in fish flesh with a subsequent reduction in some vitamins and increased oxidation of lipids (Daun, 1975). In all cooked samples, the degree of oxidation was highest in the 10% NaCl brine treated samples. The 6% NaCl brine treatments had the lowest degree of fat oxidation. Similar results were observed by Lee et al. (1997) when higher concentrations of NaCl resulted in higher levels of TBARS.

The TBARS results confirmed results obtained by the TBA values by exhibiting a similar trend (Fig.3). The fried samples showed the highest values followed by steamed, grilled and microwaved samples. The possible loss of amino acid availability results mainly from the interaction of proteins with oxidized lipids and their secondary products. The oxidation of protein

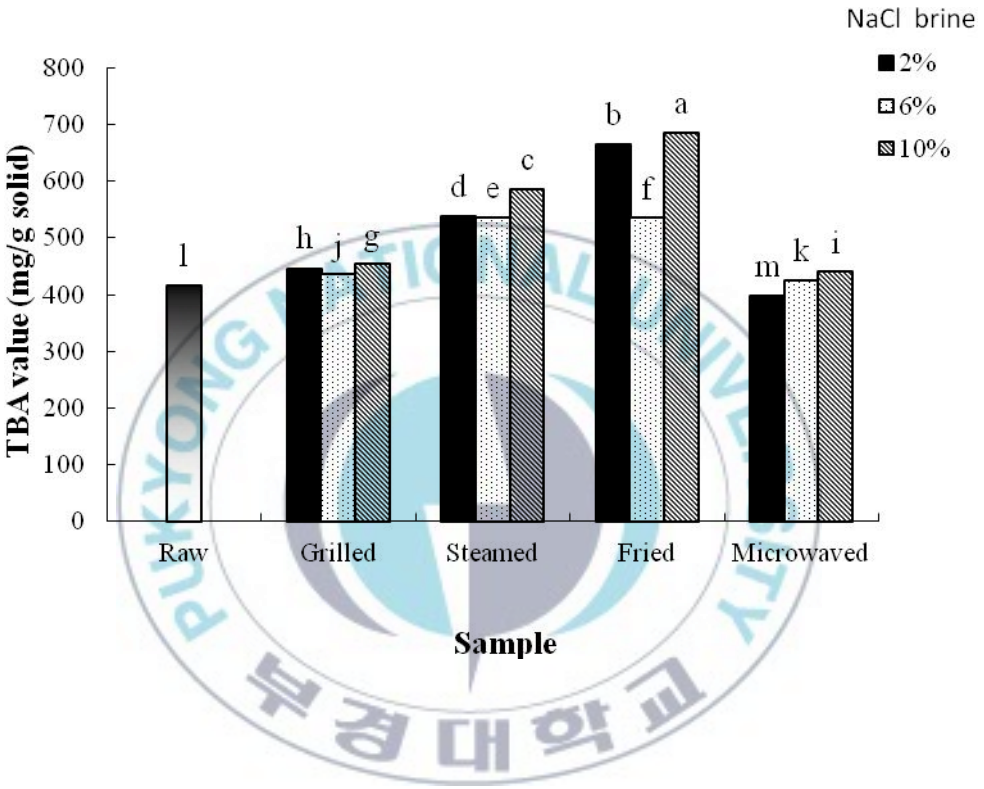


Figure 2. Comparison of TBA values of raw, salted and cooked chub mackerel.

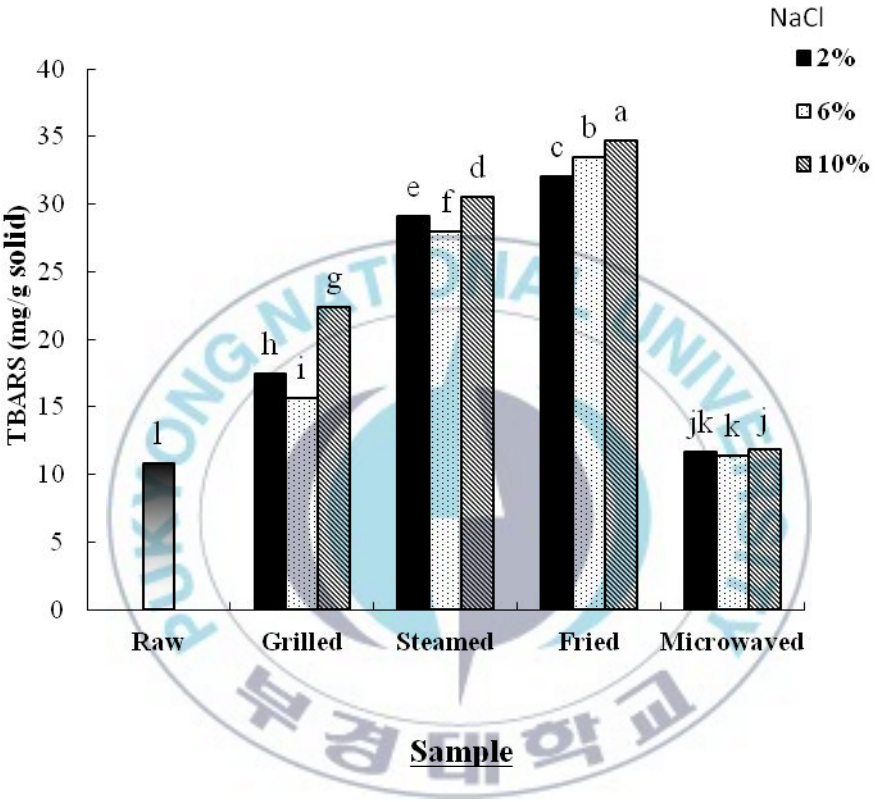


Figure 3. Different levels of TBARS in raw, salted and cooked chub mackerel.

lead to both physical and chemical changes, including amino acid destruction, decrease in protein solubility due to polymerization, formation of amino acid derivatives and reactive carbonyls (Carpenter et al., 1963), changes in protein digestibility and loss of enzyme activity (Wills, 1961). In addition, oxidative changes may give rise to altered water-binding capacity and hydration of the protein and can also lead to the formation of protein-lipid complexes.

3.6. Trypsin inhibitor

Table 5 shows the values of Trypsin inhibitor (TI) in the mackerel meat samples. Generally, TI includes typical proteinaceous inhibitory materials contained in raw sources and indigestible materials such as trypsin indigesdtible substrates (TIS) induced from the results of interaction between protein and other components such as lipid oxidation products. As shown in Fig. 4, the amount of TI decreased in all the cooked samples from an initial amount of 6.40mg/g solid in the raw sample. The microwaved sample showed the highest amount (M-2%, 4.22mg/g solid), followed by grilled (G-2%, 2.47mg/g solid), steamed (S-2%, 2.05mg/g solid) and then the least in the fried sample (F-2%, 2.02mg/g solid). Even though the microwaved sample received uniform heat enough to cook it, the duration of the process was too short to allow the inactivation of TI. This probably explains the high level of the TI contents in this sample. It was however noted that the amount of TI in each sample category showed the lowest figure in the 6% treated samples. Similar results were observed by Ryu et al., 1984b with yellow corvenia (*Pseudosciaena manchurica*) during processing and storage. The decrease in TI in the cooked samples was due to

Table 5. Level of trypsin inhibitor (TI) and *in vitro* protein digestibility of raw, salted and cooked chub mackerel

Sample**	TI(mg/g solid)	4-enzyme *in vitro* protein digestibility (%)	3-enzyme *in vitro* protein digestibility (%)
Raw	6.40±0.07^{a}	79.4±0.12^{g}	79.97±0.10^{j}
G-2%	2.47±0.02^{d}	85.79±0.06^{c}	81.59±0.11^{f}
G-6%	2.02±0.03^{ef}	85.81±0.04^{bc}	83.71±0.08^{a}
G-10%	2.13±0.02^{e}	84.84±0.04^{d}	83.09±0.05^{b}
S-2%	2.05±0.02^{ef}	84.17±0.02^{e}	82.86±0.07^{c}
S-6%	1.90±0.03^{g}	85.79±0.13^{c}	83.07±0.04^{b}
S-10%	1.93±0.02f^{g}	84.66±0.19^{d}	82.30±0.05^{e}
F-2%	2.02±0.02^{ef}	86.04±0.06^{b}	81.66±0.05^{f}
F-6%	1.74±0.04^{h}	86.43±0.04^{a}	81.10±0.09^{g}
F-10%	1.86±0.06^{g}	85.74±0.21^{c}	82.51±0.09^{d}
M-2%	4.22±0.05^{b}	81.61±0.05^{f}	80.17±0.07^{i}
M-6%	3.42±0.11^{c}	81.81±0.05^{f}	81.11±0.06^{g}
M-10%	4.10±0.09^{b}	81.77±0.07^{f}	80.54±0.07^{h}

$^{a-j}$ Different letters in column of each sample category show significant differences ($P< 0.05$)

**Sample categories: G(grilled),S(steamed)F(fried),M(microwaved),Raw(control)

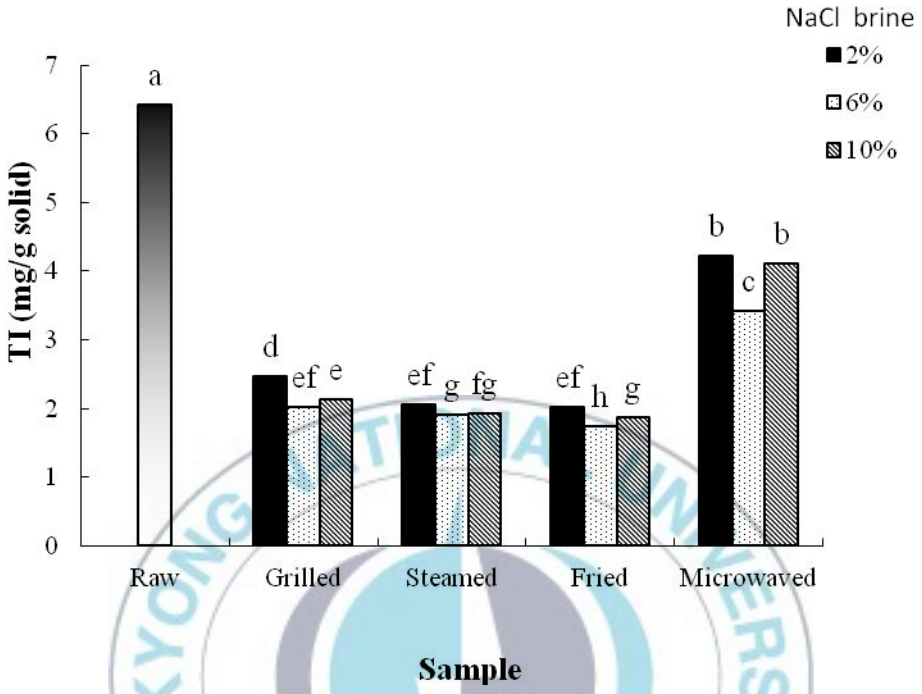


Figure 4. Comparison of TI levels in raw, salted and cooked chub mackerel.

the inactivation of these enzyme inhibitors by the heating process (Hakimeh et al., 2010). Even though fat oxidation increased in all cooked samples, decomposition of oxidized products was facilitated by the extremely high temperatures (over 150ºC). This also probably accounted for the low level of TI in the fried, grilled and steamed but not in the microwaved samples. If the TI assay was carried out using the meat samples with skin which could show severe fat oxidation and interaction between oxidized fat and denatured protein, the TI levels of the grilled, fried and steamed meat samples would be higher than the raw and microwaved meat samples and follow the same trend as the TBARS and TBA value data.

3.7. *In vitro* protein digestibility

Two methods, the three enzyme and four enzyme assays were used to determine the *in vitro* digestibility of samples (Table 5). The three enzyme assay was carried out without peptidase and the four enzyme assay was with peptidase. Fig. 5 shows the relationship between these two assays. There was an observed increase in the digestibility values of all the cooked samples from the raw sample (Fig. 6). This was also observed by Ryu et al.

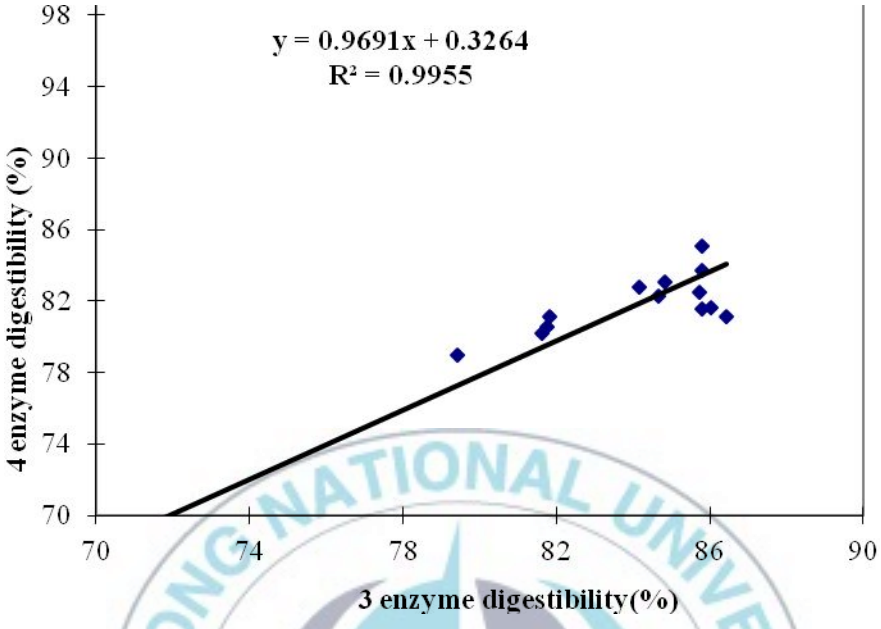


Figure 5. Relationship between the results of three and four enzyme *in vitro* protein digestibility assays according to data in Table 5.

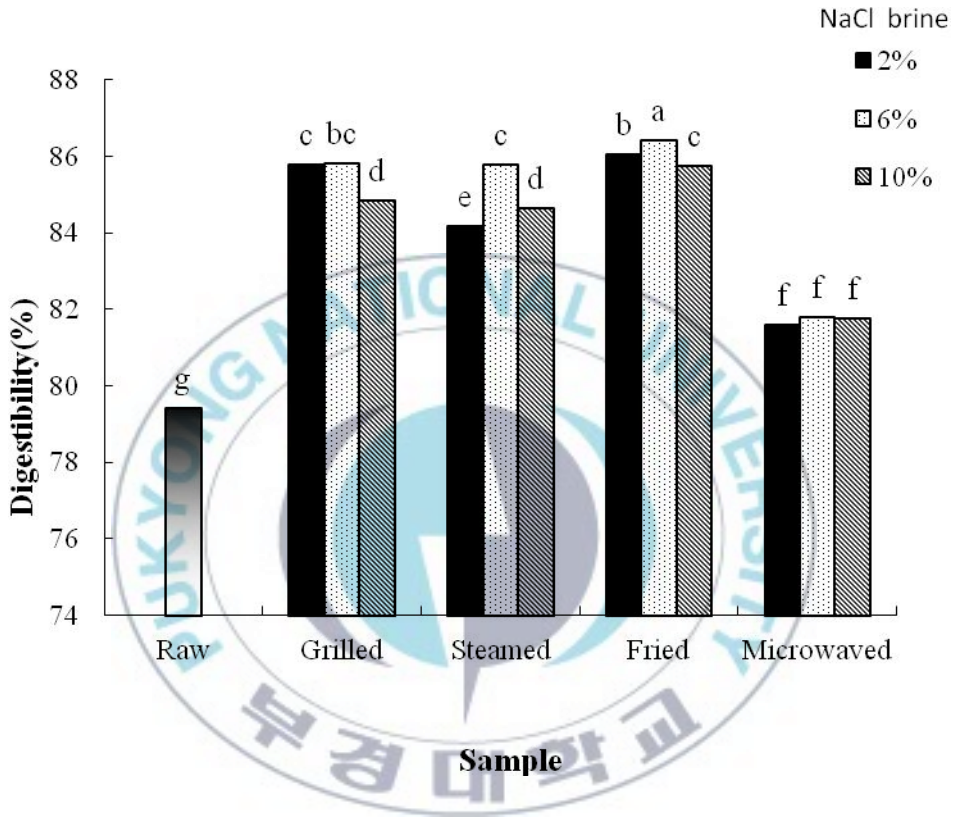


Figure 6. Comparison of the 4 enzyme *in vitro* protein digestibility of raw, salted and cooked chub mackerel.

(1984a & b), during the processing of dried anchovy and yellow corvenia and also Hakimeh et al. (2010), during the processing of the Persian sturgeon. With the four enzyme assay, the fried meat sample recorded the highest value of digestibility (86.43%), followed by grilled (85.81%), steamed (85.79%) and microwaved (81.81%) samples. Even though the 6% NaCl brine treated samples recorded the highest values, there were significant differences between the 2% and 10% NaCl brine treated samples with the exception of the fried samples. In each case, the digestibility values of the 10% NaCl brine treated samples were higher. In the three enzyme assay, all cooked samples showed an increase in their digestibility rates as compared to the raw sample. Here, however, the rates were highest in the grilled; followed by steamed, fried and the microwaved samples. There was however, a strong correlation between the two assays as seen in Fig. 5.

Protein digestibility is influenced by the presence of antinutritive factors (Liener, 1976) and different processing and cooking methods affect the levels of these antinutritive factors. In general, heating improves the digestibility of protein by inactivating enzyme inhibitors and denaturing the protein, which expose new sites to the digestive enzyme action (Sikorski,

2001). This is evidenced by the inverse relationship between *in vitro* digestibility and trypsin inhibitor as observed in Table 5.

3.8 Amino acid profiles

The amino acid profiles of chub mackerel samples (Table 6) are similar to that reported by Lee et al., 1987. Glutamic acid showed the highest concentration among all samples, which is also in agreement with Badiani et al., (1996). Tryptophan, methionine and cysteine recorded the least concentration in all samples. Total essential amino acids increased in all cooked samples (31.22 – 31.54) when compared with the raw (30.62). Aspartic acid, valine, leucine and histidine increased in all cooked samples. Serine, proline, glycine, alanine, phenylalanine, lysine and isoleucine showed a decrease in all samples. The losses of some amino acids such as lysine in all cooked samples can be due to the formation of different maillard products during heating as reported by Garcia-arias et al. (2003). Lysine is the most susceptible amino acid in intact proteins because it has a free amino group at the epsilon carbon unit that is readily available to react with reducing sugars. Free lysine is even more reactive because it has two free amino groups. Differences between serine and threonine content in raw

Table 6. Amino acid profiles of raw, salted and cooked chub mackerel

(g.a.a./16g N)

Amino acid	ANRC casein	Raw	Steamed	Fried	Grilled	Micro-waved
Aspartic acid	7.12	10.45	10.53	10.42	10.45	10.49
Threonine*	4.08	4.40	4.47	4.36	4.61	4.60
Serine	5.27	4.81	4.74	4.63	4.64	4.70
Glutamic acid	22.72	16.00	15.99	16.09	15.91	16.34
Proline	11.00	5.35	5.28	5.16	4.97	4.57
Glycine	1.83	6.93	6.19	6.57	6.14	6.28
Alanine	3.08	5.89	5.85	4.66	5.46	5.39
Valine*	6.60	5.18	5.85	5.35	5.33	5.20
Isoleucine*	5.25	4.65	4.59	4.87	4.71	4.60
Leucine	9.66	8.67	8.87	8.83	8.74	9.01
Tyrosine*	5.66	3.23	3.11	3.64	3.34	3.32
Phenylalanine*	5.21	4.73	4.56	4.63	4.58	4.70
Histidine	2.90	6.22	6.49	6.70	6.85	6.48
Lysine	8.23	7.84	7.44	7.35	7.28	7.74
Arginine	3.87	5.72	5.58	5.75	5.62	5.76
Methionine*	2.84	2.33	2.33	2.33	2.33	2.33
Tryptophan*	1.03	1.31	1.31	1.31	1.31	1.31
Cysteine*	0.58	0.85	0.85	0.85	0.85	0.85
Total	**106.93**	**104.56**	**104.53**	**104.5**	**104.12**	**104.09**

* Essential amino acid

ANRC – Animal nutrition research council

sample compared to some heat treated samples can be due to changes of these amino acids to other products, which lead to rupture of disulphide bond and liberation of a sulphide ion and free sulfur (Sikorski, 2001).

Thermal degradation of tryptophan has been reported by Rakowska et al. (1975) and also by Friedman and Cuq (1988) who found many derivatives of tryptophan in fried products. The rate of thermal decomposition of sensitive amino acid residues generally increases with temperature as well as in the presence of oxygen and reducing sacharrides (Sikorski 2001).

3.9. *In vitro* protein quality of raw and cooked chub mackerel

In vitro protein quality of raw and cooked mackerel samples are compared and presented in Table 7. Computed protein efficiency ratio (C-PER), was calculated from protein digestibility via a procedure using four enzyme and amino acid profiles. Discriminant computed protein efficiency ratio (DC-PER) and predicted digestibility were calculated solely from the amino acid profiles of sample proteins. C-PER and DC-PER are known to have a high correlation with the rat bioassay (*in vivo* method).

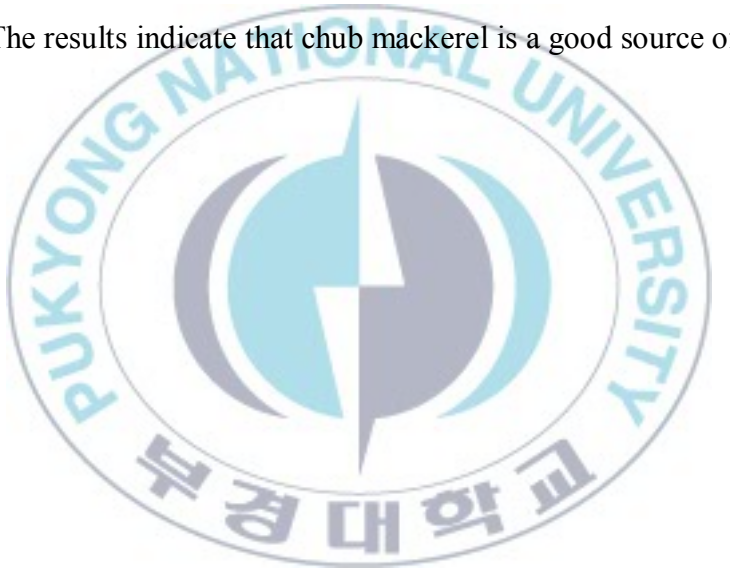
C-PER values for steamed, grilled and fried samples were higher than standard casein, raw and microwaved samples. These results are similar to

Table 7. *In vitro* protein quality of raw, salted and cooked chub mackerel

	ANRC Casein	Raw	Steamed	Fried	Grilled	Microwaved
In vitro protein digestibility (%)	90.00	79.43	85.79	86.43	85.81	81.81
Predicted digestibility (%)	90.00	89.42	88.65	89.25	89.58	88.3
C-PER*	2.5	1.97	2.60	2.60	2.60	1.97
DC-PER**	2.5	2.5	2.5	2.5	2.5	2.7

* C-PER = Computed Protein Efficiency Ratio
**DC-PER = Discriminant Computed Protein Efficiency Ratio

those reported by Azizah et al. (2002). Also, C-PER and DC-PER seem to correlate well for steamed, fried and grilled samples but not for the raw and microwaved samples. This could be due to the low digestibility values of the raw and microwaved samples. This indicates that DC-PER is not suitable for samples with digestibility values below 85%. The same trend of argument holds for the *in vitro* and predicted digestibility values for all samples. The results indicate that chub mackerel is a good source of protein.



Conclusion

As shown by the results of the study, the different cooking methods caused significant changes in the proximate composition and protein quality of all cooked samples.

Increased salt concentration facilitated moisture loss and protein denaturation. It also acted as a prooxidant, resulting in increased fat oxidation.

The duration of the cooking method and temperature employed had an impact on the inactivation of trypsin inhibitor (TI) content. This was shown in reduced levels of TI in the grilled, steamed and fried samples. The heating conditions inactivated the enzyme inhibitors and denatured the protein. This led to the exposition of greater amounts of proteins to the actions of the proteolytic enzymes. Though the fried samples showed the highest digestibility values, frying is not beneficial due to the high fat content. A high degree of fat oxidation also indicates the presence of carcinogenic compounds (malondialdehydes).

The microwaved samples showed the least digestibility values and high levels of TI. This implies much of their proteins are unavailable for digestion by proteolytic enzymes.

Grilled and steamed meat samples recorded comparatively high digestibility values and lower fat contents. It can therefore be said that these two methods retained high nutritive values and are thus recommended for processing chub mackerel. 6% NaCl brining is recommended as it gave the highest digestibility values and lower fat oxidation levels in all samples. Since the amount of moisture is essential for reactions in foods, it is believed that the 6% brine treatments gave the optimal conditions rather than the 2% treatments with their dilution effects and the 10% treatments with the extreme protein denaturation and reduced water holding capacity.

References

Aminullah Bhuiyan AKM, Ratanayake WMN and Ackman RG. 1986. Effect of Smoking on the proximate composition of Atlantic Mackerel (*Scomber scrombrus*). J Food Science 51, 327-329.

AOAC. 1982. Calculated protein efficiency ratio (C- PER and DC- PER), Official first action. J. AOAC. 65, 496-499.

AOAC. 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Washington DC.

Azizah AH, Jamilah B and Gan HB. 2002. Nutritional quality of spray dried protein hydrolysate from Black Tilapia *(Oreochromis mossambicus*). J Food Chem 78, 69-74.

Badiani A, Anfossi P, Fiorentini L, Gatta PP, Manfredini M, Nanni N, Stipa S and Tolomelli B. 1996. Nutritional composition of cultured sturgeon (*Acipenser spp*). J Food Comp Anal 9, 171-190.

Carpenter KJ, Lea CH and Parr LJ. 1963. Chemical and nutritional changes in stored herring meal. Brit. J. Nutr. 17, 151-169.

Catrillon AM, Navarro P and Alvarez-Pontes E. 1997. Changes in chemical composition and nutritional quality of fried sardine produced by frozen storage and microwave reheating. J Sci Food Agric 75, 125-32.

Crawford DL, Yu TC and Sinnhuber RO. 1967. Reaction of malonaldehyde with protein. J Food Sci 32, 332-335.

Daun, H. (1975). In: Nutritional Evaluation of Food Processing, 2nd ed. (R. Harris and E. Karmas, eds.). Avi, Westport, CT, p. 355.

deMan JM. 1999. Proteins. In: Principles of Food Chemistry. Aspen Publishers Inc., Gaithersburg, Maryland, USA, 118.

Friedman M and Cuq JL. 1988. Chemistry, analysis, nutritional value, and toxicology of tryptophan in food. A. review. J Agri Food Chem 36, 1079-1093.

Garcia Arias MT, Alvarez-Pontes E, Garcia-Linares MC, Garsia-Fernandez MC and Sanchez FJ. 2003. Cooking-freezing-reheating (CFR) of sardine (*Sardina pilchardus*) fillets. Effect of different cooking and reheating procedures on the proximate and fatty acid compositions. Food Chem 83, 349-356.

Garcia-Segovia P, Andres-Bello A, Martynez-Monzo J. 2007. Effect of cooking method on mechanical properties, color and structure of beef muscle (*M. pectoralis*). J Food Eng 80, 813-821.

Gokoglu N, Yerlikaya P and Cengiz E. 2004. Effect of cooking methods on the proximate composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). J Food Chem 84, 19-22.

Hakimeh JA, Bahareh S, Ali S and Alireza S M. 2010. Effects of cooking methods on physic-chemical and nutritional properties of Persian sturgeon (*Acipenser persicus)* fillet. Int Aquat Res 2, 15-23.

Hall GM. 1992. Fish processing Technology. USA and Canada: VCH Publishers. Inc., New York.

Hoffmann K. 1993. Nutritional value of proteins and protein requirements of people with special reference to meat proteins. Mitt Bund Fleischforsch 32, 422-429.

Huang TL, Zandi PP, Tucker KL, Fitzpatrick AL, Kuller LH, Fried LP, Burke GL and Carson MC. 2005. Benefits of fatty acid on dementia risk are stronger for those without APOE 4. Neurology 65, 1409-14.

Huss HH. 1995. Quality and quality changes in fresh fish. In: FAO Fisheries Technical Paper No. 348. Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy.

Jewell DK, Kendrick JG and Satterlee LD. 1980. The DC-PER assay: A method for predicting protein quality solely from amino acid compositional data. Nutr Rep Int, 21, 25-38.

Jozef K, Marzanna H, Gramza A and Jedrusek-Golinska A. 2004. Influence of fat oxidation on the stability of lysine and protein digestibility in frozen meat products. EJPAU 7.

Jucieli WC, Vivian C, Bochi P, Ribeiro M, Andrede and Tatiana EV. 2008. Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. Food Chem 105, 140-146.

Larsen R, Stormo SK, Dragnes BT, Elvevoli EO. 2007. Losses of taurine, creatine, glycine and alanine from cod (*Gadus Morhua L.*) fillet during processing. J Food Compos Anal 20, 396-402.

Lee KH and Ryu HS. 1986. Proceedings of the international symposium on seafood quality determination. 10-14 Nov. 1986. Pp, 473-485. Alaska. U.S.A.

Lee EH, Oh KS, Ahn CB, Jung BG, Bae YK and Ha JW. 1987. Studies on the taste compounds and manufacturing of mackerel powder soup. Bull Korean Fish Soc 20, 41-43.

Lee SK, Mei L and Decker EA. 1997. Influence of Sodium Chloride on antioxidant enzyme activity and lipid oxidation on frozen ground pork. J Meat Sci 46, 349-355.

Liener IE. 1976. Legume toxins in relation to protein digestibility-a review. J Food Sci 41: 1076-1081.

Mirnezami Ziabari SH, Hamidi Sfahani Z and Faez M. 2002. What you know about microwave? P 78-79. Tehran: Agricultural Sciences Press.

Morris A, Barnett A and Burrows OJ. 2004. Effect of processing on nutrient content of foods. Vol. 37, 3.

National Fisheries Research and Development Institute, 2009. Supplemented chemical comp. of marine products in Korea, 26-28.

Niamnuy C, Devahastin S and Saponronnarit S. 2008. Changes in protein compositions and their effects on physical changes of shrimp during boiling in salt solution. Food Chem 108, 165-175.

Nielsen HK, Finot PA, and Hurrell RF. 1986. Reactions of protein with oxidized lipids. In: Influence on protein quality and on the bioavailability of lysine, methionine, cystine and tryptophan as measures in rat assays. Br J Nutr 53, 75-86.

Offer G, Knight P, Jeacocke R, Almond R, Cousins T and Elsey J. 1989. The structural basis of the water-holding, appearance and toughness of meat and meat product. J Food Microstruct 8, 151-170.

Piche LA, Draper HH and Cole PD. 1988. Malondialdehyde excretion by subjects consuming cod liver oil versus a concentrate of n-3 fatty acids. Lipids 23, 370-371.

Pigott GM and Tucker RW. 1990. Controlling Water Activity. In: Seafood: Effects of technology on nutrition. Fennema OR and Whitaker JR, eds. Marcel Dekker, Inc., New York, U.S.A., 136-137.

Phimphilai S, Galyean RD and Wardlaw FB. 2006. Relationship of two in vitro assays in protein efficiency ratio determination on selected agricultural by-products. Songklanakarin J Sci Technol 28, 81-87.

Pokorny J and Kolakowaka A. (2002). Lipid-protein and lipid-sacharide interactions. In: Chemical and functional properties of food lipids.

Sikorski Z and Kolakowaka A, eds. FL: CRC Press, Boca Raton, 345-362.

Pokorny J and Davidek J. 1979. Influence in interactions of proteins with oxidized lipids on nutrition and sensory value of food. Acta Aliment Pol 5, 87-95.

Rakowska M, Werner J, Szkittadziowa W, Nadolna I and Zielinska Z. 1975. Comments on effect of heat treatment on the nutritive value of protein and the availability of amino acids in meat-vegetable canned foods. pp. 167-217. Zez Probl

Rhinehart D. 1975. A nutritional characterization of the distiller's grain protein concentrates, MS thesis of Univ. of Nebraska-Lincoln, 29.

Ryu HS, Lee KH and Jo JH. 1984a. Changes in TI and *In vitro* Apparent Digestibility of boiled and dried Anchovy during processing and storage. Bull Kor Fish Soc 17, 101-108.

Ryu HS, Lee KH and Jo JH. 1984b. Changes in TI and protein quality of salted and dried yellow corvenia (*Pseudosciaena manchurica*) during processing and storage. Bull Kor Fish Soc 17, 109-116.

Ryu HS and Lee KH. 1985. Effect of heat treatment on the in vitro protein digestibility and trypsin indigestible substrate content in some seafoods. J Kor Soc Food Nutr 14, 1-12.

Ryu HS, Mun SI and Lee KH, 1992. Changes in Quality of Seasoned and Smoked Squid during processing. Bull Kor Fish Soc 25, 406-412.

Satterlee LD, Kendrick JG, Marshall HF, Jewell DK, Ali RA, Heckman MM, Steinke HF, Larson P, Phillips D, Sawar G and Slump P. 1982. *In vitro* assay for predicting protein efficiency ratio as measured by rat bioassay: Collaborative study. J Assoc Off Anal Chem 65, 798-809.

Satterlee LD, Kendrick JG and Miller GA. 1979. Rapid *in vitro* assay for estimating protein quality. Food Tech 31, 78-81.

Sikorski EZ. 2001. Chemical and Functional Properties of Food Proteins. Technomic Publishing Company Inc., Pennsylvania 17604 USA, 191-215.

Straadt IK, Rasmussen M, Anderson HJ and Bertram HC. 2007. Aging-induced changes in microstructure and water distribution in fresh and cooked pork in relation to water-holding capacity and cooking loss- A Combined Confocal Laser Scanning Microscopy (CLSM)

and low-field nuclear magnetic resonance relaxation study. J Meat Sci 75, 687-695.

Tarladgis BG, Watts BM, Younathan MT and Dugan LR. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. J American Oil Chem Soc 37, 44-48.

Wills ED. 1961. Enzyme inhibition by oxidized unsaturated fatty acids. Proc Intern Conf Biochem Lipids CA. p. 74.

Witte VC, Krause GF and Bailey ME. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J Food Sci 35, 582-585.

Xiong YL. (2000). Protein oxidation and implication for muscle food quality. In: Antioxidants in Muscle Food. Decker EA, Faustman C and Lopez-bote CJ, eds. New York: John Wiley & Sons, Inc., 85-111.

Acknowledgements

I'm grateful to KOICA for funding my stay and study in Korea and also wish to thank my supervisor, Professor Hong-Soo Ryu, for his guidance and valuable contribution for the successful completion of this thesis. My appreciation to Professors Yang-Bong Lee and Ji-Young Yang for editing this script. I'm grateful to my laboratory members for their support to me during the experiments. My gratitude to Mr. Mahmoud Mohseni for the statistical analysis.

My gratitude also to my Director, Mr. Quaatey and my department for granting me the opportunity to further my education.

I am particularly grateful to my husband and children Samuel, Beatrice, Nathan and David for their patience and understanding.

Above all, I thank God for his protection and help for the successful completion of the thesis.