FAS Fisheries and Aquatic Sciences

Original Article

Fish Aquat Sci 14(4), 257-265, 2011

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

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Abstract

The effects of cooking method (grilling, frying, steaming, and microwaving) on the 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. Brine (10% NaCl) treatment recorded the highest moisture loss. All cooked samples showed a decrease in fat content, except fried samples. Protein content increased in all cooked samples, from 47.21% in the raw sample to 63.87% in the grilled sample. Brine treatment resulted in the highest degree of fat oxidation (thiobarbituric acid-reactive substances), which was highest in the fried samples and lowest in the microwaved samples. The trypsin inhibitor (TI) concentration was highest in the microwaved samples and lowest in the fried samples. In all samples, 6% salt treatment caused the lowest TI level and the highest *in vitro* protein digestibility. *In vitro* digestibility increased from 79.4% in the raw sample to 86.43% in the fried samples. The total essential amino acids of all cooked samples increased. Results suggested that grilling and steaming had beneficial effects on the protein quality of chub mackerel.

Key words: Chub mackerel, *Scomber japonicus*, Cooking methods, Protein quality

Introduction

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

Chub mackerel's popularity worldwide is due to the presence of two important fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These are known to have many health benefits, particularly with regard to heart disease and a decreased risk of prostate cancer and Alzheimer's disease (Huang et al., 2005).

Chub mackerel is rarely eaten raw and is usually cooked in different ways before consumption. Application of heat is achieved by boiling, baking, roasting, frying, grilling, steaming, or microwaving. Each of these serves to enhance the taste and flavor as well as increase the shelf life of the product (García-Arias et al., 2003).

The protein quality of fish is affected during processing as a result of the application of heat, which causes protein denaturation. The extent of protein denaturation depends on the duration and temperature, as well as the processing facility (Sikorski, 2001). Note that the nutritive value of proteins is determined not only by their quantitative and qualitative amino acid composition, but also by their availability to digestive tract proteolytic enzymes (Lee and Ryu, 1987).

Processing by heat is hypothesized to increase food digestibility due to breakdown of complex proteins and carbohydrates. Despite this, 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 loss of protein quality. Fish oils are converted to ketones, aldehydes, and hydroxyacids. These reactions are enhanced by iron and cop-

Open Access http://dx.doi.org/10.5657/FAS.2011.0257

pISSN: 2234-1749 eISSN: 2234-1757

Received 15 September 2011; **Revised** 25 October 2011; **Accepted** 26 October 2011

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per 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 color of foods (Xiong, 2000).

Reduced digestibility, as well as limiting the amount and degree of amino acid availability, is mediated partially by the formation of amino acid bonds with lipid oxidation products (Lee and Ryu, 1987). These protein-lipid complexes contribute to the quantity of indigestible substances in foods that are not available to proteolytic enzymes.

In vitro protein digestibility is an inexpensive way of determining the protein quality of seafood. This method uses a multi-enzyme assay to imitate human and animal digestive systems. Due to the time-consuming and expensive *in vivo* method of determining protein digestibility, researchers have tried to correlate *in vivo* and *in vitro* methods to develop reliable methods for determining the protein efficiency ratio (PER) measurement. Two such methods are the computed protein efficiency ratio (C-PER) (Satterlee et al., 1982) and the 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, whereas the DC-PER is solely dependent on amino acid compositional data. These two methods are known to have a high correlation with *in vivo* assays (Phimphilai et al., 2006). Lee and Ryu (1987) used this model to evaluate the protein quality of seafood.

Various studies have determined the protein quality of seafood. Ryu et al. (1992), observed an increase in *in vitro* protein digestibility, a decrease in trypsin indigestible substrates (TIS), a reduction in some essential amino acids, and an increase in fat oxidation of seasoned and smoked squid. Jannat Alipour et al. (2010) also observed an increase in *in vitro* protein digestibility and nutritional indices in the Persian sturgeon (*Acipenser persicus*) after grilling and frying. According to Pigott and Tucker (1990), cooking practices could cause modified 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 popular. The popularity of mackerel has soared because of the much publicized health benefits of omega-3 fatty acids. Protein quality is severely affected by the heat applied during processing as the equipment used does not allow control of cooking conditions. Therefore, one must know the beneficial/optimal processing conditions that result in the production of nutritionally superior products, rather than just satisfying the consumer's organoleptic appetite.

Although many studies have examined the effect of different cooking conditions on the nutritional quality of fish, little has been done on chub mackerel. Therefore, the main objective of this study was to investigate the effect of four cooking methods (grilling, steaming, frying, and microwaving) on the protein quality of chub mackerel and to determine which results in the retention of a high nutritional value.

Materials and Methods

Sample preparation

Fresh chub mackerel samples, caught in the Korean Southern Sea during February 2011, were delivered to the laboratory as individual quick frozen products. Products were prepared using just-landed fish from 3S Seafood Company, Busan, Korea. The average sizes of semi-dressed samples without viscera were 28.0 ± 2 cm in length and 380.0 ± 12 g in weight. They were immediately frozen at -13°C after weighing. Samples were then randomly divided into five units. One unit was kept raw and used as the control. Each of the other four units were again divided into three subunits and soaked in 2, 6, and 10% sodium chloride for 1 h. These subunits were then cooked by frying, steaming, microwaving, or grilling.

Grilling of fish was performed for 12 min at 250°C using an oven (OAS6.10, Convotherm, Eglfing, Germany). Steaming was done for 12 min at 200°C in the same oven. Fish were pan-fried in a large pan with soybean oil at 180°C for 10 min. Microwaving was performed for 6 min in an oven (Zipel DG68-00216B-01, Samsung, Gwangju, Korea).

Determination of the proximate composition

Cooked mackerel meat without the skin was homogenized using a kitchen blender (Super Power Mixer HMF 985, Han-Il Electric, Busan, Korea). A raw sample was also homogenized with the skin. Moisture was determined by drying at 105°C to constant weight (Association of Official Analytical Chemists, 1990). Fat was determined using the method described by the Association of Official Analytical Chemists (1990) using a Soxhlet solvent extractor. Crude protein was determined by the semi-micro Kjeldahl procedure using a conversion factor of 6.25 (Association of Official Analytical Chemists, 1990). The remaining samples were freeze-dried and stored.

Drip loss and water-holding capacity (WHC)

Drip loss was calculated from the difference in the mass of raw mackerel samples before and after thawing in a cold room at 6°C for 4 h.

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

Weight of drip from the all thawed samples ranged from 3.5 to 3.9 g/100 g sample with a mean of 3.7 g/100 g sample.

The WHC was determined by the press method introduced in the Handbook of Food and Nutrition, published by The Korean Society of Food Science and Nutrition (2000) using a 35-50 kg/cm^2 compressor.

Cooking loss

Cooking loss was measured according to the method of Niamnuy et al. (2008), and was calculated from the difference in sample mass before and after cooking (frying, grilling, microwaving, and steaming).

% Cooking loss = (mass before cooking - mass after cooking)/ mass before cooking × 100

Water activity measurements

Water activity measurements were taken for all raw and processed samples using water activity-measuring equipment (BT-RSI-7557 012; Rotronic, Grindelwald, Switzerland).

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Fat oxidation

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

In vitro protein digestibility

The *in vitro* digestibility values of raw and cooked mackerel samples were determined by the Satterlee method (1977), as modified with the Association of Official Analytical Chemists procedure (Association of Official Analytical Chemists, 1990). The procedure used four enzymes: trypsin (17,600 BAEE units/mg solid; Sigma, St. Louis USA), α-chymotrypsin (41 units/mg solid; Sigma), peptidase (102 units/mg solid; Sigma), and bacterial protease (*Streptomyces griceus* protease, 4.5 units/mg solid; Sigma). The three-enzyme method (without peptidase) was also used to calculate the *in vitro* digestibility of samples to determine the correlation 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 the sample at 20 min.

Trypsin inhibitor (T1) assay

The amount of TI in samples was determined using the procedure of Ryu and Lee (1985), which is a modification of the Rhinehart (1975) method. TI levels are expressed in TI equivalents, which equal the milligrams of purified soybean TI per gram sample.

The correlation coefficient between pH and TI content was 0.9914, calculated by the equation:

y = 4.0434x - 26.281, where y = purified soybean TI (mg) and x = pH at 10 min incubation.

Amino acid profiles

Amino acid compositions were determined using an amino acid analyzer (Sykam S433, Eresing, Germany). Samples were hydrolyzed with 6 N HCl *in vacuo* at 110°C for 25 h. Cysteine was determined by the method of Felker and Waines (1978) using a reduced glutathione standard. Tryptophan was determined using an alkaline hydrolysis (5 N NaOH) by the method of Hugli and Moore (1972).

Computed *in vitro* protein quality

C-PER, DC-PER, and predicted digestibility were calculated using the corrected Association of Official Analytical Chemists (1982) procedure. Protein digestibility and amino acid profiles were used to calculate these *in vitro* protein quality data.

Statistical analysis

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

Results and Discussion

Proximate composition

The proximate composition of raw and cooked mackerel samples are presented in Table 1. The proximate composition of raw mackerel was similar to that reported by the National Fisheries Research and Development Institute (NFRDI) in fall 2009. Moisture content decreased in all cooked samples. Moisture loss was highest in fried samples (F-10, 48.7%), followed by grilled (G-10, 52.3%), steamed (S-10, 54.3%), and then microwaved (M-10, 57.6%) samples. In all sample categories, 10% NaCl brine treatment recorded the highest moisture loss, which was due to the high level of salt. Protein also increased in all cooked samples, with the highest in fried, followed by grilled, steamed, and microwaved samples. On a dry weight basis, fat content decreased in all cooked samples, with the exception of those that were fried, which increased, which was likely due to absorption of fat from the vegetable oil by the fish during frying. Grilled samples recorded the highest fat

Table 1. Proximate composition of raw, salted and cooked chub mackerel.

Sample**	Moisture (%)*	Crude protein(%) (N × 6.25)*	Lipid (%)*
Raw	60.2 ± 0.4[a]	18.8 ± 0.4[e] (47.2)	18.9 ± 0.8[e] (47.5)
G-2%	56.3 ± 0.6[de]	28.0 ± 0.3[a] (61.8)	20.1 ± 0.5[cde] (44.5)
G-6%	54.7 ± 0.7[fg]	27.5 ± 0.7[ab] (56.1)	21.2 ± 0.7[bcd] (43.3)
G-10%	52.3 ± 0.5[h]	27.9 ± 0.3[a] (63.9)	20.0 ± 0.7[de] (45.8)
S-2%	56.6 ± 0.6[de]	26.4 ± 0.6[b] (59.9)	22.1 ± 0.6[bc] (44.7)
S-6%	55.9 ± 0.2[ef]	24.0 ± 0.5[d] (52.6)	22.9 ± 0.8[b] (44.6)
S-10%	54.3 ± 0.9[g]	26.3 ± 0.6[bc] (60.5)	22.6 ± 0.8[b] (46.9)
F-2%	48.9 ± 0.2[i]	27.2 ± 0.3[ab] (53.3)	25.0 ± 0.2[a] (49.9)
F-6%	50.2 ± 0.2[i]	27.6 ± 0.3[ab] (55.5)	25.2 ± 0.2[a] (50.7)
F-10%	48.7 ± 0.2[i]	28.6 ± 0.6[a] (55.7)	25.8 ± 0.8[a] (50.2)
M-2%	59.3 ± 0.6[ab]	23.9 ± 0.6[d] (57.9)	19.7 ± 0.7[de] (47.7)
M-6%	58.7 ± 0.7[bc]	24.9 ± 0.3[cd] (58.7)	18.7 ± 0.4[e] (44.1)
M-10%	57.6 ± 0.4[cd]	24.1 ± 0.3[d] (59.2)	20.2 ± 0.6[cde] (47.8)

Values in brackets are presented as g/100g solid.
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.
*Mean ±SD of three determinations.

Table 2. Water activity (Aw), cooking loss and water holding capacity (WHC) of salted and cooked chub mackerel.

Sample**	Aw	Cooking loss	WHC*
Raw	0.98		0.42 ± 0.01[a]
G-2%	0.95	44.69	0.26 ± 0.22[g]
G-6%	0.95	44.71	0.24 ± 0.01[h]
G-10%	0.95	44.84	0.22 ± 0.12[i]
S-2%	0.96	43.12	0.30 ± 0.03[de]
S-6%	0.96	43.23	0.30 ± 0.15[ef]
S-10%	0.96	43.33	0.30 ± 0.20[f]
F-2%	0.96	42.80	0.32 ± 0.11[d]
F-6%	0.96	42.92	0.31 ± 0.01[de]
F-10%	0.96	42.98	0.30 ± 0.02[de]
M-2%	0.97	18.19	0.40 ± 0.03[b]
M-6%	0.96	18.12	0.39 ± 0.01[b]
M-10%	0.96	18.45	0.35 ± 0.02[c]

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.
*Mean ±SD of three determinations.

loss due to the higher temperature employed during cooking.

The decrease in moisture and increase in protein in all cooked samples was also reported by Jannat Alipour et al. (2010). 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 dehydration-associated changes, such as an increased protein concentration. The cooking conditions employed in the steamed, grilled, and microwaved samples may have caused fat extraction, hence the decrease in fat content.

Drip loss

An average of 3.72% (g drip/100 g frozen sample) (data not shown) was recorded for the raw sample after thawing in a cold room at 6°C for 4 h. This suggests that the fish was in excellent condition before cooking. A low drip loss indicates that frozen protein denaturation has not taken place.

Water activity

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

Cooking loss

Table 2 also shows cooking loss of the various cooked samples, which depended on the cooking process. Significant losses occurred 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. No significant differences were observed among 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 lead to shrinkage of both the filament lattice and the collagen. This also led to exposure of the hydrophobic areas of the myofibrillar structure, which allowed new intra- and inter-protein interactions, resulting in a denser structure (Straadt et al., 2007). The subsequent aggregation and denaturation of proteins led to a loss in WHC, and hence the loss of water. Salts enhance denaturation by reducing the WHC (de Man, 1999). The WHC values of the 10% NaCl brine-treated samples and the cooking loss values of the 10% treatment in Table 2 clearly show this relationship. Cooking loss leads to a significant loss of matter and is thought to

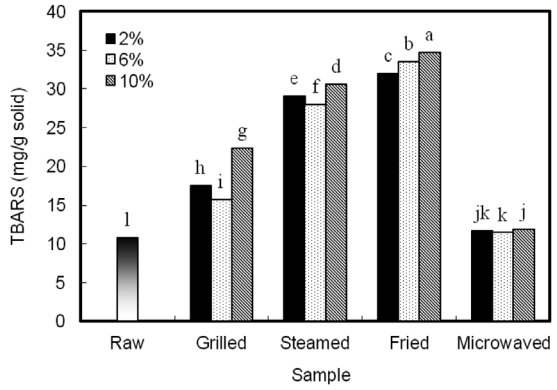

Fig. 1. Different levels of thiobarbituric acid-reactive substances (TBARS) in raw, salted and cooked chub mackerel.

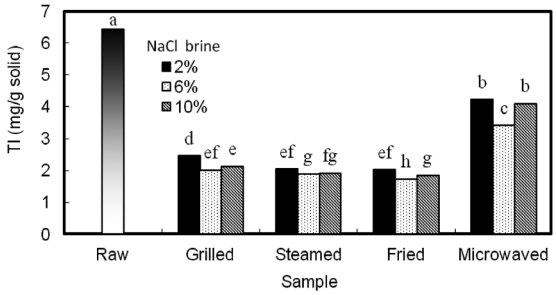

Fig. 2. Comparison of trypsin inhibitor (TI) levels in raw, salted and cooked chub mackerel.

have a linear relationship with cooking time and temperature (GarcÍa-Segovia et al., 2007).

Fat oxidation

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 the principal factor involved in protein cross-linked reactions. Schiff base formation between the amino group of lysine and other free amino groups could lead to a reduction in the availability of these amino acids (Crawford et al., 1967). Malondialdehyde has been reported to be toxic to living cells because it can be absorbed through the digestive system (Piché et al., 1988).

TBARS increased in all cooked samples, with the highest in the fried, followed by the steamed, grilled, and microwaved samples (Fig. 1). A similar trend was observed by Ryu et al. (1984a) in boiled whole anchovy. The lowest value being in microwaved samples was likely because little oxygen was involved in this processing method; oxygen is needed for fat oxidation to occur. The high temperatures (over 150°C) 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 lipid oxidation (Daun, 1975). The degree of oxidation was highest in the 10% NaCl brine-treated samples. Treatment with 6% NaCl brine caused the lowest degree of fat oxidation. Similar results were reported by Lee et al. (1997) when higher NaCl concentrations resulted in higher TBARS levels.

The fried samples showed the highest values, followed by steamed, grilled, and microwaved samples. Loss of amino acid availability results mainly from the interaction of proteins with oxidized lipids and their secondary products. The oxidation of protein leads 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, changes in protein digestibility, and loss of enzyme activity (Carpenter et al., 1963). In addition, oxidative changes may give rise to altered water-binding capacity, and protein hydration and can also lead to formation of protein-lipid complexes.

Trypsin inhibitor

Table 3 shows the TI values of the mackerel samples. TI includes typical proteinaceous inhibitory materials contained in raw sources and indigestible materials such as TIS induced by interactions between protein and other components, such as lipid oxidation products. As shown in Fig. 2, the amount of TI decreased in all samples from 6.40 mg/g solid in the raw sample. Microwaved samples showed the highest amount (M-2%, 4.22 mg/g solid), followed by grilled (G-2%, 2.47 mg/g solid),

Table 3. 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%	$1.93 \pm 0.02f^{g}$	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}

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.

steamed (S-2%, 2.05 mg/g solid), and then the fried sample (F-2%, 2.02 mg/g solid). Even though the microwaved sample received uniform heat, the duration of the process was too short to inactivate TI, which likely explains the high TI levels in those samples. However, the amount of TI in each sample category was noted to be lowest in the 6% treated samples. Similar results were observed by Lee et al. (1984b) with yellow corvenia (*Pseudosciaena manchurica*) during processing and storage. The decrease in TI in the cooked samples was due to inactivation of these enzyme inhibitors by the heating process (Jannat Alipour et al., 2010). Even though fat oxidation increased in all cooked samples, decomposition of oxidized products was facilitated by the extremely high temperatures (over 150°C). This also probably accounted for the low TI level in the fried, grilled, and steamed, but not the microwaved, samples. If the TI assay were carried out using meat samples with skin, which could show severe fat oxidation and interactions between oxidized fat and denatured protein, the TI levels of the grilled, fried, and steamed meat samples would be higher than those of the raw and microwaved samples.

In vitro protein digestibility

The three- and four-enzyme assays were used to determine the *in vitro* digestibility of samples (Table 3). The three-enzyme assay was carried out without, and the four-enzyme assay with, peptidase. Fig 3 shows the relationship between these two assays. An increase in the digestibility values of all samples was observed (Fig. 4).

Such a phenomenon was also observed by Lee et al. (1984a, 1984b) during the processing of dried anchovy and yellow corvenia, and also by Jannat Alipour et al. (2010) during processing of the Persian sturgeon. With the four-enzyme assay, the fried meat sample recorded the highest 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, significant differences were observed between the 2% and 10% NaCl brine-treated samples, with the exception of those that were fried. 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 as compared to the raw samples. Here, however, the rates were highest in the grilled, followed by the steamed, fried, and microwaved samples. A strong correlation, however, was observed between the two assays, as seen in Table 3 and Fig. 3.

Protein digestibility is influenced by the presence of antinutritive factors (Liener, 1976), the levels of which are affected by different processing and cooking methods. In general, heating improves digestibility by inactivating enzyme inhibitors and denaturing the protein, which exposes new sites to digestive enzyme action (Sikorski, 2001). This is evidenced by the inverse relationship between *in vitro* digestibility and TI (Table 3).

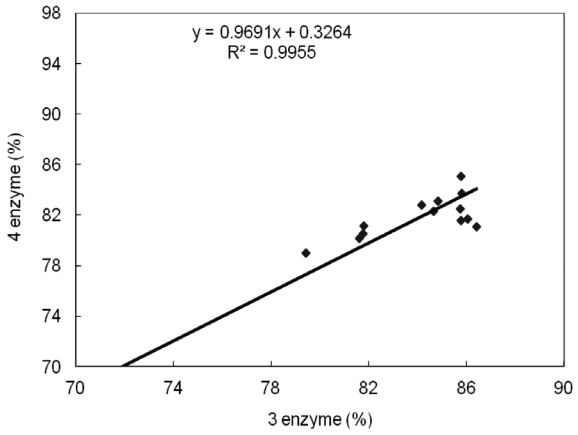

Fig. 3. Relationship between the results of three and four enzyme *in vitro* protein digestibility assays according to data in Table 3.

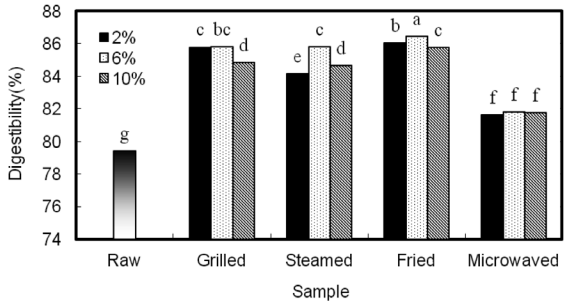

Fig. 4. Comparison of the four-enzyme *in vitro* protein digestibility of raw, salted and cooked chub mackerel.

Amino acid profiles

The amino acid profiles of chub mackerel samples (Table 4) were similar to those reported by Lee et al. (1987). Glutamic acid showed the highest concentration in all samples, which is also in agreement with Badiani et al. (1996). Tryptophan, methionine, and cysteine recorded the lowest concentrations in all samples. The total essential amino acid level increased in all cooked samples (31.22-31.54) when compared with the raw sample (30.62). Aspartic acid, valine, leucine, and histidine levels also increased in all cooked samples. Serine, proline, glycine, alanine, phenylalanine, lysine, and isoleucine levels decreased in all samples. The losses of some amino acids, such as lysine, may have been due to the formation of different Maillard products during heating, as reported by GarcÍa-Arias et al. (2003). Lysine is the most susceptible amino acid in intact proteins because it has a free amino group at the ε-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 samples compared to some heat-treated samples may be due to their conversion of these amino acids to other compounds, leading to the rupture of disulfide bonds

and liberation of a sulfide ion and free sulfur (Sikorski, 2001). Thermal degradation of tryptophan was reported by Rakowska et al. (1975), 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 saccharides (Sikorski, 2001). The results showed a significant change in the essential amino acid content of samples after cooking.

In vitro protein quality of raw and cooked chub mackerel

The *in vitro* protein quality of raw and cooked mackerel samples was compared and presented as the C-PER, calculated from protein digestibility using the four-enzyme method and amino acid profiles (Table 5). The DC-PER and predicted digestibility were calculated solely from the amino acid profiles of sample proteins. C-PER and DC-PER are known to be highly correlated 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 those of Abdul-Hamid et al. (2002). Also, C-PER and DC-PER seemed 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, indicating that DC-PER is not suitable for samples with digestibility values below 85%. The same argument holds for the *in vitro* and predicted digestibility values for all samples. The data suggest that chub mackerel is a good source of protein. The different cooking methods caused significant changes in the proximate composition and protein quality of all cooked

Table 4. Amino acid profiles of raw, salted and cooked chub mackerel.

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	4.4	4.47	4.36	4.61	4.6
Serine	5.27	4.81	4.74	4.63	4.64	4.7
Glutamic acid	22.72	16	15.99	16.09	15.91	16.34
Proline	11	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*	6.6	5.18	5.85	5.35	5.33	5.2
Isoleucine*	5.25	4.65	4.59	4.87	4.71	4.6
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	4.7
Histidine	2.9	6.22	6.49	6.7	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
Metionine*	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	**106.9**	**104.6**	**104.5**	**104.5**	**104.1**	**104.1**

Values are presented as g.a.a./16g N.
*Essential amino acid, Animal Nutrition Research Council (ANRC).

Table 5. *In vitro* protein quality of raw, salted and cooked chub mackerel.

	ANRC casein	Raw	Steamed	Fried	Grilled	Microwaved
In vitro protein igestibility (%)	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.

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 the temperature employed had an impact on TI content, as indicated by the reduced TI levels in the grilled, steamed, and fried samples. Heating inactivated the enzyme inhibitors and denatured the protein, which led to the exposure of greater amounts of protein to proteolytic enzymes. Although 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 lowest digestibility values and high TI levels. This suggests that much of their protein is unavailable for digestion by proteolytic enzymes.

Grilled and steamed meat samples exhibited comparatively high digestibility values and low fat content. Therefore, these methods retained the highest nutritional values and are thus recommended for processing of chub mackerel. Brining using 6% NaCl is recommended, since it gave the highest digestibility value and lowest fat oxidation level. Since the amount of moisture is essential for reactions in foods, 6% brine treatment is believed to have been optimal; the 2% treatment exhibited dilution effects and the 10% treatment, extreme protein denaturation, and reduced WHC.

Acknowledgments

We are grateful to the Korean International Cooperation Agency (KOICA) for funding this study.

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