

The investigation of proximate composition and protein solubility in processed mullet fillets

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Abstract: The influence of different cooking methods (grilling, frying and steaming) on proximate composition and protein solubility of golden grey mullet (*Liza aurata*) fillets were evaluated. The protein and ash contents increased in all cooked fish. Decrease in moisture and increase in fat contents was the most prominent changes in proximate composition. Steaming had no significant influence on fat content of fillets whereas after frying and grilling fat content was increased significantly ($P < 0.05$). After cooking, protein solubility of fillets decreased by declining pH with the minimum solubility being observed at a pH range 5-6 confirming the isoelectric point of fillets. Grilled sample showed lower solubility compared to steamed samples.

Keywords: Golden grey mullet, cooking methods, proximate composition, protein solubility

Introduction

Fish has long been recognized as a valuable source of high-quality protein in the human diet (Weber, 2008). The high protein levels, with good digestibility and also low fat content are advantages of Seafood (Pigott and Tucker, 1990). Among the large groups of fish species which have been consumed in Iran, golden grey mullet is one of the most important fish in southern Caspian Sea that are consuming extensively Iranian people (Abdoli *et al.*, 2009). Grey mullet are mainly a catadromous family, excluding a few member species (Katselis, 2006). The golden grey, like other species of mullet, is euryhaline (from freshwater to 38‰) and eurythermic (from 3 to 35°C) (Fazli *et al.*, 2008). The juveniles of Black Sea grey mullet (*Mugil cephalus*, *Liza aurata* and *L. saliens*) were successfully introduced from Black Sea into the Caspian Sea as brackish water in 1930 to 1934 but only the two last species have successfully acclimated, adapted and propagated in the Caspian Sea (Oren, 1981; Kosarev and Yablonskata, 1994). Nowadays they provided one of the principal fishing resources, especially in the southern Caspian Sea and they are important food fishes.

Fish is usually treated by various processes before consumption. Heating (grilling, frying and steaming) is applied to food to improve its flavour and taste (Bognar, 1998). The basic scientific reason for heating a food product is to make it safe to eat or to prevent or minimize spoilage during storage and these processes before can give rise to major changes in composition. The effects of different cooking methods on nutritive values such as proximate composition of different fish have been previously studied (Weber *et al.*, 2008; Ersoy and Ozeren,

2009). Solubility of protein is considered as the most important factor and an excellent index for their functionality of dehydrated products. In addition this is an important factor because of its relevance to other properties such as viscosity, gelation, foaming and emulsification (Hall, 1992). Protein solubility refers to the amount of total muscle protein that goes in to solution under specified conditions (Zayas, 1997) and depends on protein structure, pH, concentration of salt, temperature, duration of extraction and many other intrinsic factors. The conformation of proteins, which is related to the environment to which the protein is exposed, plays an important role in defining the functional properties of the protein. The method of processing affects the solubility of protein specially if they are exposed to heat (Kilara and Harwalkar, 1996). Denaturation and aggregation of muscle proteins are associated with the formation of disulfide bond formation (Jiang *et al.*, 1989). Although the pH-solubility relationship for proteins has been studied (Geirsdottir *et al.*, 2007; Bourtoom *et al.*, 2009) and specially has been reported for *Mugil cephalus* in mugilidae (Mohan *et al.*, 2007), there is lack of information on the chemical composition and nutritional profile of cooked *Liza aurata* in southern Caspian Sea. This study was therefore, conducted to determine the influence of three cooking methods (grilling, frying and steaming) on physicochemical properties of brackish water fish, *Liza aurata* fillets.

Materials and Methods

Samples preparation

The fish, golden grey mullet (*Liza aurata*) were purchased from Bandar-Torkman fish market in Golestan, Iran, during the autumn 2009. They were

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about 500 g in weight. The fish were transported to the laboratory for sample preparation and analysis. On arrival at the laboratory the fish were washed with tap water several times to remove adhering blood and slime, they were then prepared such as eviscerated, beheaded, removing backbone, skin, tail and fin yielding two fillets. At least they were randomly divided in to 4 lots, which were assigned to the three repetitions of each one of the 3 cooking methods and to the raw group that was used as a reference.

Cooking methods

Common ways of cooking were used. The samples were cooked by frying, grilling and steaming. The fish fillets were fried in frying vegetable oil (Bahar frying oil, Iran). The temperature of oil during the frying process was 150°C for 10 min in an automatic fryer (ADR2, Moulinex, Portugal). The grilling process was carried out with an electrically operated stainless steel grill (Bq100, Delongi, Germany) at 50-60 Hz frequency and maximum temperature level. After the set temperature was attained the fillets were grilled for 20 min (10 min on each side). Steaming was performed in a domestic steamer at approximately 98°C for 20 min (Tefal Steam Cuisine, Berkshire, UK). Samples of raw or cooked fish fillets were immediately homogenized using a kitchen blender and analyzed to determine proximate composition and protein solubility.

Proximate composition

Proximate composition analyses of cooked and uncooked fish fillets were done in triplicate for moisture, protein, lipid and ash contents. The moisture was determined by oven-drying at 100-105°C until a constant weight was obtained (AOAC, 1993). The crude protein content was calculated by the kjeldahl procedure using conversion factor of 6.25 (AOAC, 1993). Fat was determined by the method described by AOAC (1990) using the Soxhlet System (416 SE, Gerhardt, Germany) with petroleum ether as the solvent. Ash was gravimetrically determined using a muffle furnace by heating at 500°C to constant weight (AOAC, 1993).

Cooking loss

Cooking loss was measured according to the method of Niamnuy *et al.* (2008) and was calculated from the differences in the mass of golden grey mullet fillets before and after each cooking methods (grilling, frying and steaming). Calculation of cooking loss is below:

$$\text{Cooking loss\%} = \frac{\text{Mass before cooking} - \text{Mass after cooking}}{\text{Mass before cooking}} \times 100$$

Protein solubility

Protein solubility was determined according to the method of Lee *et al.* (1992), with some modification. 2 g homogenized fillet sample was added to 40 ml of distilled water and the mixture was stirred using a magnetic stirrer at speed 2 at room temperature (RHB2, IKA, Germany). The pH of slurry was adjusted to desired pH (1-12) by the addition of 1 or 0.1 N HCl and 1 or 0.1 N NaOH to desired acidic and alkaline pH values, respectively. The volume was adjusted to 50 ml with distilled water. It was shaken for 1h at room temperature (about 27°C), centrifuged at 5000 rpm for 20 min at 4°C and the pH of the supernatant noted. Protein content of supernatants was determined using the kjeldahl method. Percentages of soluble protein in the supernatant compared to the total protein were calculated at each pH value as follows:

$$\text{Protein solubility\%} = \frac{\text{Protein concentration in supernatant}}{\text{Protein concentration in homogenate}} \times 100$$

The isoelectric point (PI) was estimated as the pH value corresponding to the minimum solubility percentage. All treatments were conducted on triplicate.

Statistical analysis

The effects of cooking and pH and their interactions were evaluated. The effect of cooking proximate composition and color of the fillet was analyzed by one-way (ANOVA). Analyses were performed using SAS learning edition 2.0 software. Differences were considered to be significant when $p < 0.05$. Data were presented as mean \pm STD.

Results and Discussion

Proximate composition

The changes in proximate composition such as moisture, ash, protein and fat content of samples after cooking processes are shown in Table 1. The composition of raw fillets is similar to that observed are similar to findings of other researchers on mullet (Kalay, 2008). The proximate composition of golden grey mullet was significantly affected by all the cooking methods ($p < 0.05$). The moisture content of the fish fillets ranged from 79.5% to 51.5%, decreasing after cooking (Table 1). The decrease in the moisture content has been described as the most prominent change that makes the protein, fat and ash contents increase significantly in cooked fish fillets (Garcia-Arias *et al.*, 2003). Accordingly, the increase in ash, protein and fat content found in cooked golden grey mullet fillets is explained by the reduction in

Table 1. Proximate composition of raw and cooked golden grey mullet fillets

	Moisture%	Protein%	Ash%	Fat%
Raw	79.50±0.050 ^c	17.02±0.84 ^a	1.13±0.11 ^a	1.34±0.12 ^a
Grilled	64.010±0.74 ^c	28.66±0.83 ^c	1.90±0.025 ^c	4.42±0.10 ^c
Fried	51.58±2.20 ^a	31.96±0.84 ^d	2.50±0.10 ^d	11.53±0.125 ^d
Steamed	71.43±1.17 ^d	25.27±1.02 ^b	1.20±0.070 ^b	1.17±0.28 ^a

Values are shown as mean ± standard deviation.
Within the column values with different letters are significantly different (P<0.05).

moisture. The protein content increased after cooking in all evaluated methods. Fat also increased by frying and steaming; however the increase in fat content was most obvious in fried fillets (Table 1), mainly due to the absorption of fat by the fish, the absorption of fat through frying also caused an increase of ash. Fat increase can be due to the oil penetration in to the food after water is partially lost by evaporation (Saguy and Dana, 2003). Similar results have been reported for fried African catfish in sunflower oil (Rosa *et al.*, 2007) and fried rainbow trout in sunflower oil (Gokoglu *et al.*, 2004). The least and the most value of crude protein was seen in raw (17%) and fried (31.9%) fish fillets respectively. The ash content also increased after cooking. Steaming had no significant influence on fat and ash content of fillets (P>0.05).

Cooking loss

Cooking loss in golden grey mullet muscle was measured after each cooking treatment. The cooking loss was different depending on the cooking process. Fried sample and steamed fillet showed significantly the highest (47.32%) and lowest (32.61%) cooking loss respectively. Aggregation and denaturation of protein in golden grey mullet muscles were induced by heating, leading to the loss in water holding capacity of proteins. As a result, drastic cooking loss was observed. Niamnuy *et al.* (2008) reported occurrence of drip loss in shrimp muscle throughout the boiling in salt solution.

Protein solubility profile

Fish muscle homogenate is considered to be a heterogeneous system of proteins with fat particles dispersed in an aqueous phase (Thawornchinsombut, 2006). Figure 1 shows the protein solubility profile of cooked and uncooked fish fillets at different pH levels between 1 and 12. When the protein solubility of the homogenate at different pH was compared (Table 1), it showed a specific behavior as the most solubility observed at acidic and alkaline and the least at isoelectric points (PI). Heat processing affected the solubility of proteins. In general, raw samples possess higher protein solubility than that of cooked ones.

As it can be seen in Figure 1, the steamed samples showed maximum level of protein solubility between cooked preparations.

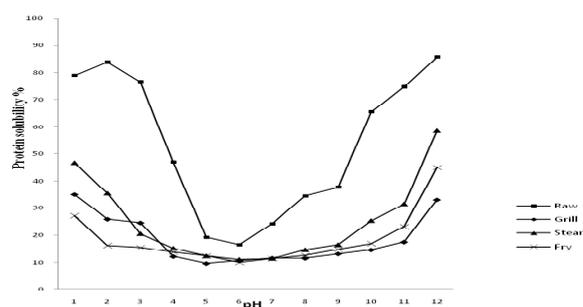


Figure 1. Protein solubility profile of raw and cooked golden grey mullet fillets at different pH values

In this study the minimum protein solubility that called isoelectric point, in raw and cooked samples exhibit at pH 5-6. The protein of raw and cooked fillets showed decreasing solubility with increasing pH to isoelectric point then increased again to high pH. The protein were least soluble in raw, grilled, fried and steamed fillets in value of 16.5%, 9.72%, 10.09% and 11.09% respectively at pH 5-6.

Signs of denaturation of protein are reflected in changes in solubility. Method of processing affects the solubility of protein especially if they are exposed to heat (Kilara and Harwalkar, 1996). As it can be seen in Figure 1, steamed samples as received minimum heat during steaming, showed higher solubility compare to two other processing methods. It is conducted that this method has less negative effect on the nutritional parameters. Solubilities of grilled and fried samples were whatever near to each other; fried samples had higher solubility at alkalic values. Reduction in protein solubility due to heat processing has been reported (Romero *et al.*, 2009; Bourtoom *et al.*, 2009). The degree of protein solubility in an aqueous medium is the result of electrostatic and hydrophobic interactions between proteins is greater than hydrophobic interactions (Zayas, 1997). In isoelectric point and in neutral condition, electrostatic absorbance takes place between two neighbor ions which lead to orientation and also aggregation of molecules. On the other hand, hydrogen-bonding result in more adjacency of molecules to each other.

Nevertheless, because of numerous hydrogen-bonding and electrostatic linkages in isoelectric point, there could be the most stability and minimum solubility. Also, Damodaran (1997) states that the minimum solubility occurs at about the isoelectric point of protein, and that the majority of food proteins are acidic proteins. Data of this study on isoelectric point were in agreement with Fatemi (2000). At pH above or below isoelectric pH, the proteins become negatively or positively charged depending on the pH, resulting in electrostatic repulsions between molecules and hydration charged residues, contributing to the solubility of proteins. Strong electrostatic repulsions among proteins molecules and the increased protein-solvent interaction at this pH could have contributed to this increased solubility (Mohan *et al.*, 2007).

Conclusion

The results obtained from this study indicated that conformational changes taking place in fish muscle proteins when exposed to different pH and Cooking methods vary and also showed the proximate composition of golden grey mullet fillets in different cooking methods. Changes in proximate composition were more prominent in fried fillets. Protein solubility increased five fold by altering the pH in raw fish to extremes. Proteins, which are not normally extracted at neutral pH, became soluble at this pH. Steaming of fillets had minimum effect on protein solubility of samples and because in food systems, existence of protein in soluble form is necessary, thus indicates on a positive effect of using this method. In general it can be said that although steamed samples didn't have a good acceptability compared to other methods, they showed the least undesirable effects of heating such as protein denaturation and resulted in maintaining nutritional value of fish, can be selected as the best. Different conformational changes imparted to proteins can greatly contribute to alterations and enhancement in functional properties of proteins and hence their use in the development of value added products.

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