Int Aquat Res (2010) 2: 15-23
ISSN 2008-4935
Available online at www.intelaquares.com

International
Aquatic Research

Effects of cooking methods on physico-chemical and nutritional properties of Persian sturgeon *Acipenser persicus* fillet

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Received: 18 January 2010; Accepted: 20 March 2010

Abstract

Effects of cooking methods (frying and grilling) on proximate composition, total sulfhydryl group (SH) content, protein solubility, cooking loss, amino acid composition and nutritional parameters of Persian sturgeon (*Acipenser persicus*) fillets were investigated. Both grilling and frying decreased moisture content and increased total protein content significantly. The protein solubility was high in raw samples but decreased dramatically after cooking treatments. Heating treatments decreased total SH content from 5.33 in raw fillet to 0.43 and 0.93 (mol/10^5 g proteins) in grilled and fried samples, respectively. All computed nutritional indices, were increased after cooking. *In vitro* digestibility of raw fillet was 81.50 which increased to 95.08 and 100% in grilled and fried samples, respectively. The protein digestibility corrected amino acid scores (PDCAAS) of cooked samples for age group of 10-12 years old and adults were 100 which indicate that Persian sturgeon fillet is good source of protein for these age groups. Result indicated that both cooking methods had the beneficial effects on nutritive value of Persian sturgeon fillets.

Keywords: Persian sturgeon, *Acipenser persicus*, Cooking methods, Nutritional indices

Introduction

Fish is an excellent protein source with high nutritive value due to a favorable essential amino acid composition. Fish muscle also contains minerals, vitamins and other nutritional compounds which are necessary in a diet (Larsen et al. 2007). Fish is rarely eaten raw and usually cooked in different ways before consumption. Heating is one of the common methods in food processing. Heat is applied for food in different ways (boiling, baking, roasting, frying and grilling) to enhance their flavor and taste, and increase shelf life (Garsia-Arias et al. 2003).

Processing by heat increases food digestibility because it breaks proteins and carbohydrates which are less digestible. Despite these advantages, heating has some defects: meat processing at high temperature results in leaving water drops and soluble vitamins, and also producing one bilionium gram mutagen compounds (Mirnezami Ziabari et al. 2002).

Many of changes occurs during food processing are consequences of heat denaturation of proteins, depending on the duration of heating and temperature, as well as natural features of the protein molecules or complexes, heating brings about deconformation resulting in exposure of the reactive groups. Most proteins are compounds

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susceptible to quality and quantity changes during heat processing as loss of solubility of temperature-sensitive proteins can be utilized as an indicator of the time and temperature that had been applied in heat processing of various foods (Sikorski 2001).

The consumption of processed foods has increased due to the impositions of modern life, where food preparation time is a main factor (Saldana and Bragagnolo 2008). Processing of food products is connected with the application of high temperature treatment, high pressure, alkaline or acidic media and water elution. Changes to foods that are produced by these treatments should be known in order to limit the loss of valuable compounds of natural products, improve the process itself and achieve foods with the best nutritional value.

Persian sturgeon (*Acipenser persicus*) is one of the most important sturgeon fishes in the south coast of the Caspian Sea that has presented high amount (67.5%) of sturgeons hunting (Kose et al. 2001). This species have commercial importance not only for caviar but also for having meat with high marketability. There is no report indicating effects of heating on Persian sturgeon fillet proteins. In Iran, fish is treated by one of various cooking processes that can make changes in its composition. Since the method of preparation can affect the protein quality, therefore in the present study the effects of two different cooking methods (the most common cooking method in Iran) on the protein quality of sturgeon fillet were investigated.

Materials and methods

Preparation of samples

Female Sturgeon fish with 175 cm length and 28 kg weight (without caviar) were caught in 29th October 2008 from the south coast of the Caspian Sea (Mian Ghale region, Ashourade Island) in Golestan province, Gorgan, Iran. The fish were immediately transferred to the laboratory in ice at a ratio of 2:1 (at least 1 h) with the fish rigor-morties was not passed. Head, viscera and skin of fish were removed, then fish were washed and filleted. These fillets from middle part of fish (average weight of 220±21g) were divided in to 3 groups randomly.

One group was kept raw and used as a reference (R). The other two groups were cooked: One group was fried (F), and the other group was grilled (G). The fillets were fried in sunflower oil at 180 °C for 6 min at an automatic fryer (ADR2, Portugal).Other fillets were grilled for 30 min on a stainless steel grill (Bq100, Delongi, Germany) at 50-60 Hz frequency.

Determination of proximate composition

The fillets were first minced using a kitchen blender (Depose, moulinex, Italy) and homogenized before the analysis. The moisture was determined by drying in an oven at 70 ºC to the constant weight (AOAC 2000, Mehod No. 934.01). Total fat was extracted with petroleum ether (BP 60-40 ºC) using the Soxtec System (416 SE, Gerhardt, Germany; Garcia-Arias et al. 2003).

Ash was determined gravimetrically using a muffle furnace by heating at 500 ºC to the constant weight (AOAC 2000, Mehod No. 942.05). Protein was determined by the Kjeldahl procedure using conversion factor of 6.25 (AOAC 2000, Mehod No. 988.05). Protein loss was calculated from the difference in the protein content of sturgeon fillet before and after each cooking method (frying and grilling): %Protein loss (w.b) = % Protein before cooking (w.b) - % Protein after cooking (w.b)

Cooking loss measurement

Cooking loss was measured according to the method of Niamnuy et al. (2008) and was calculated from the difference in the mass of sturgeon fillet before and after each cooking method (frying and grilling).

% Cooking loss = (Mass before cooking – Mass after cooking) / Mass before cooking × 100

Determination of total sulphydryl content

Total sulfhydryl content was determined using 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB), using the method described by Ellman (1959), modified by Benjakul et al. (1997). Nine ml of 0.2 M Tris–HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA, were added to 1 ml of 0.4% NAM solution (natural actomyosin). 0.4 ml of 0.1% DTNB, in 0.2 M Tris–HCl (pH 8.0), was added to 4 ml of the latter mixture and was incubated at 40 °C for 25 min. The absorbance at 412 nm was measured using a UV-16001 spectrophotometer (Shimadzu, Kyoto, Japan). A blank was conducted by replacing the sample with 0.6 M KCl. Sulfhydryl content was calculated using the extinction coefficient of 13,900 M/cm.

Protein solubility and isoelecteric point

Protein solubility was determined according to the method of Lee et al. (1992), with some modifications. 40 ml of distilled water was added to 2 g sample and the mixture was stirred using a magnetic stirrer (RHB2, IKA, Germany). The pH of slurry was adjusted to pH 1-12 by the addition of 1N / 0.1N HCl or 1N / 0.1N NaOH. The volume was adjusted to 50 ml with distilled water and was shaken for 1h at room temperature (27 ºC), centrifuged at 4063g for 20 min at 4 ºC and the pH of the supernatant was recorded.

Protein content of supernatants was determined using the Kjeldahl method. Percentages of soluble protein in the supernatant, relative to the total protein, were calculated at each pH value. The *pI* was estimated as the pH value corresponding to the minimum solubility percentage.

In vitro digestibility

In vitro digestibility was determined using pepsin pancreatin enzymes based on the method described by Akeson and Stahman (1964). Pepsin and pancreatin digest were applied by incubation of 100 mg protein equivalent sample with 1.5 mg pepsin of 0.1N HCl at 37 ºC for 3h. After neutralization with 0.2N NaOH, 4 mg pancreatin in 7.5 ml of phosphate buffer of pH 8.0 was added. One ml of toluene was added to prevent microbial growth and the solution was incubated for additional 24 h at 37 ºC.

An enzyme blank was prepared in the same way without sample. After 24h, the enzyme was inactivated by the addition of 10 ml 10% TCA to precipitate undigested protein. The volume was made up to 100 ml and centrifuged at 4063 g for 20 min. the protein content of clear supernatant was determined by Kjeldahl method. The *in vitro* digestibility was calculated as percentage of the total protein solubilised after enzyme hydrolysis.

Amino acid composition

Sample preparation was conducted by hydrolysis with 6 M HCl at 110 ºC for 12 h and derivatisation using phenyl isothiocyanate prior to HPLC analysis. The total amino acids and free amino acids were analyzed by the Pico Tag method (Waters Corporation, Milford, MA), using a Pico Tag column (3.9 × 150 mm; Waters) at a flow rate of 1 ml/min with UV detection. Breez® software was applied to data analysis. Tryptophan was extracted according to the method of Concon (1975) and estimated by the method described by Swakais and Pest (1990).

Chemical score

Chemical score was calculated using the following formula (FAO/WHO 1990):
(essential amino acid in test protein / total essential amino acid in test protein) × (total essential amino acid in egg/ essential amino acid in egg) × 100

Computed protein efficiency ratio (C-PER)

C-PER was calculated by the method described by Satterlee et al. (1979) using following formula:
$C\text{-}PER = -2.1074 + 7.1312(SPC) - 2.5188(SPC)^2$

Where SPC is the essential amino acid score ratio of a sample with respect to casein.

Essential amino acid index (EAAI) and biological value (BV)

EAAI was calculated by method described by Oser (1951) and BV was calculated using the formula of Oser (1959):
$BV = 1.09(EAA\ Index) - 11.7.$

Nutritional index (NI)

NI was calculated using formula presented by Crisan and Sands (1978). NI= (EAA index × % Protein)/100

Protein digestibility corrected amino acid score (PDCAAS)

PDCAAS was calculated using the method described by Sarwar and McDonough (1990) using the essential amino acid composition of the test sample and the amino acid pattern suggested by FAO/WHO (1990) for 10-12 year old children and adults.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) and followed by the Duncan's new multiple rang test (DMRT) with 0.05 probability of Type-I error (Snedecor and Cochran 1967). Statistical analyses were performed using the SAS software version 9.1.

Results and discussion

Proximate composition

The proximate compositions of raw and cooked fish samples are presented in Table 1. Changes in dry matter and protein between raw and cooked samples were found to be significant. Cooked samples had higher content of protein compared to raw fish samples, which were in agreement with Garcia-Arias et al. (2003); Gokoglu et al. (2004); and Saldana et al. (2007). Fried fish had higher levels of fat compared to raw and grilled fish, mainly due to the absorption of frying oil by the fish.

The moisture content decreased during cooking which caused the protein and fat contents to increase significantly. Pandey et al. (2008); Musaiger and D'Souza (2008) have reported similar findings. Ash content increased after cooking but it was not statistically significant.

Table 1. Effect of different cooking methods, grilling and frying on the proximate composition of Persian sturgeon fillets (g/100 g wet basis)*

	Samples		
	Raw	Grilled	Fried
Moisture (%)	$63.2 \pm 0.41^{a**}$	52.9 ± 0.25^{b}	45.2 ± 0.44^{c}
Protein (%)	21.4 ± 0.38^{b}	31.0 ± 0.20^{a}	32.0 ± 0.55^{a}
Fat (%)	13.1 ± 1.13^{b}	14.4 ± 0.06^{b}	20.3 ± 0.33^{a}
Ash (%)	3.33 ± 0.33^{a}	3.41 ± 0.28^{a}	3.45 ± 0.01^{a}

* Mean ± SD of three determinations.

** Different superscript in each row show significant differences ($P < 0.05$).

Cooking loss

The moisture content of sturgeon fillets were 63% and reduced to around 53% and 45%, after grilling and frying, respectively. Most water in muscle is held within the myofibrils, in the space between the thick filaments (myosin) and thin filaments (actin) and some water is located 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 and also to exposition of hydrophobic areas of the myofibrillar structure, which allow new intra and inter- protein interactions that resulted in a more dense protein structure (Straadt et al. 2007). Hence, water was pressed out of the muscle cells leading to water loss.

Cooking led to a significant loss of solid matter. The cooking yield was different depending on the cooking process. The changes in cooking losses tended to have a linear relationship with the time and temperature of cooking (Garsia-Segovia et al. 2007). The amount of loss is probably related to the composition of muscle, denaturation of proteins by the ionic strength of the extra cellular fluid and oxidation of lipids, which decreases the solubility of proteins (Dyer and Dingle 1967).

Effect of cooking on sulphydryl content

Sulrhydryl (SH) content of raw, grilled and fried samples are shown in Table 2. There were significant differences in SH contents among different samples. Heat treatment decreased considerably the total SH contents in grilled and fried samples which are in agreement with Tang et al. (2009). Reduction in SH group is related to some rheological

changes which are brought about in heated meat and fish by aggregation of proteins due to the formation of new hydrogen and disulfide bonds as well as hydrophobic interactions (Takeshi et al. 1994).

Table 2. Total sulfhydryl content of raw and cooked Persian sturgeon fillets (mol/10^5g protein)*

Samples	Raw	Grilled	Fried
Total SH content	$5.33 \pm 0.33^{a*}$	0.430 ± 0.07^{b}	0.930 ± 0.06^{b}

* Mean ± SD of three determinations.
** Different superscript show significant differences ($P < 0.05$).

During cooking of foods, the thiol-disulfide exchange reactions may be mainly responsible for the aggregation of proteins. Furthermore, the SH groups in amino acid residues in proteins may also react with formaldehyde (Synowiecki and Shahidi 1991). Another researcher declared that aggregation of myofibril proteins in thermal process depends on the oxidation of sulfydryl groups, which show considerable reductions during heating (Romero et al. 2009).

Protein solubility

Figure 1 displays the protein solubility behavior of raw and cooked sturgeon filets as a function of pH. All three samples showed a typical behavior, showing a U-shaped solubility curve with a minimum solubility at isoelectric point, which is around pH 5. Solubility were increased at both side of this point.

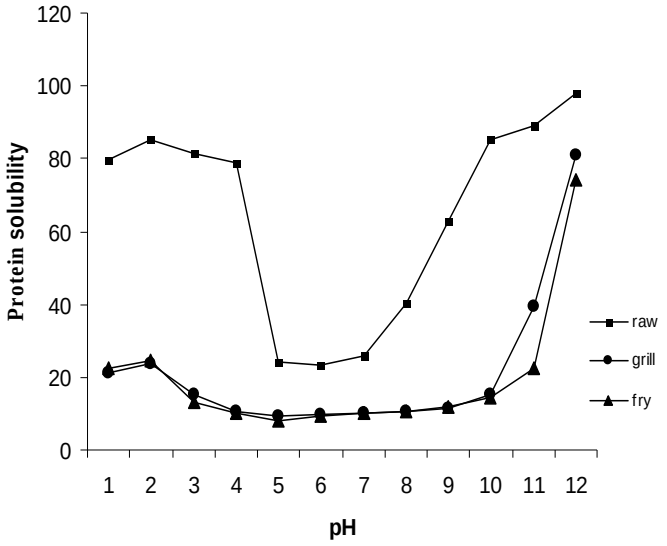

Fig. 1. Protein solubility profile of raw and cooked sturgeon fillets at different pH values

Raw samples showed high protein solubility at acidic and alkaline pH values. These data were simillar to findings of Undeland et al. (2002) on white muscle of herring (*Clupea harengus*) and Kristinsson et al.. (2005) on Atlantic croaker (*Micropogonias undulatus*) who studied protein yield using the acid and alkaline processes. Protein solubility decreased significantly in both grilled and fried samples at all pH values (Fig. 1).

In grilled samples, protein solubility at pH 2 was 19%, compared to 89% in raw samples. At pH 11, solubility in grilled sample was 35 while in raw sample was 87%. Fried samples had similar solubility behavior as grilled samples. The protein solubility in fried samples at pH 2 and 11 was 25% and 23%, respectively, which was low compared to raw samples. Reduction in protein solubility due to heat processing has been reported (Romero et al. 2009; Bourtoom et al. 2009). The data show that the heating treatments denature the proteins of sturgeon fillets and reduce their solubility in water at different pH.

Table 3. The amino acid composition (g amino acid/ 100 g protein) of raw, grilled and fried Persian sturgeon fillets*

Amino acid	Raw	Grilled	Fried
Aspartic acid	9.94±0.01[b***]	9.92±0.03[b]	10.1±0.08[a]
Threonine **	3.93±0.03[a]	3.63±0.06[c]	3.85±0.01[b]
Serine	2.74±0.01[a]	2.60±0.11[b]	2.60±0.14[b]
Glutamic acid	18.4±0.06[a]	18.2±0.08[b]	18.5±0.15[a]
Proline	4.74±0.17[a]	4.35±0.05[b]	4.12±0.01[c]
Glycine	4.45±0.11[c]	4.76±0.01[b]	4.91±0.05[a]
Alanine	5.34±0.08[c]	5.61±0.08[b]	5.78±0.12[a]
Cysteine **	0.81±0.02[c]	1.07±0.01[a]	0.900±0.07[b]
Valine **	5.41±0.05[ab]	5.39±0.01[b]	5.44±0.16[a]
Methionine **	2.29±0.06[c]	2.51±0.32[a]	2.49±0.19[b]
Isoleucine **	5.56±0.24[a]	5.25±0.13[b]	5.40±0.14[c]
Leucine **	8.45±0.19[c]	8.58±0.01[a]	8.54±0.03[b]
Tyrosine **	2.89±0.01[b]	2.96±0.04[a]	2.76±0.08[c]
Phenylalanine **	4.30±0.01[a]	4.26±0.03[b]	4.31±0.02[a]
Histidine	5.19±0.05[a]	4.85±0.01[b]	4.50±0.02[c]
Lysine	9.42±0.25[a]	9.34±0.08[b]	9.07±0.07[c]
Arginine	6.08±0.03[c]	6.55±0.09[b]	6.61±0.04[a]
Triptophan **	1.11±0.01[a]	0.0500±0.09[b]	0.0100±0.07[c]
Total essentional amino acids	42.97	42.99	42.76
Total non-essentional amino acids	57.85	56.89	57.13

*Mean ± SD of three determinations.
** Essential amino acid.
***Different superscript in each row show significant differences ($P < 0.05$).

In vitro protein digestibility (IVPD)

The *in vitro* protein digestibility of Persian sturgeon fillets are presented in Table 4. There was no significant difference in IVPD between grilled and fried samples. The highest IVPD was observed in fried samples. As protein digestibility is influenced by the presence of antinutritive factors (Liener 1976), different processing and cooking methods, that affect the levels of those antinutritive factors, will subsequently influence protein digestibility. Thermal treatments (grilling and frying) increased *in vitro* protein digestibility significantly in Persian sturgeon fillets. These results are in agreement with Wu and Mao (2008). In general, heating improves the digestibility of protein by inactivating enzyme inhibitors and denaturating the protein, which might expose new sites to the digestive enzyme action (Sikorski 2001).

Table 4. Calculated Nutritional Indices of raw and cooked samples

Parameters	Raw	Grilled	Fried
In vitro protein digestibility [#] (%)	81.5±0.35[b*]	95.1±0.49[a**]	100.±0.02[a]
Chemical score (CS)	63.0	75.0	71.0
Computed Protein Efficiency Ratio	2.32	2.74	2.85
Essentional amino acid index (EAA)	89.1	89.7	91.2
10-12 years old	100	100	100
PDCAAS			
Adults	100	100	100
Nutritional index (NI)	19.1	27.8	29.2
Predicted Biological Value(BV)	85.4	86.0	87.7

[*] Mean ± SD of three determinations.

[**]Different superscript in firs row show significant differences ($P < 0.05$).

Amino acid composition

Table 3 presents the amino acid composition of raw and cooked Persian sturgeon fillets. The amino acids composition of raw sample was similar to composition of *Huso huso* as reported by Kaya et al.. (2008). Among all Amino acids glutamic acid showed the highest concentration, that was in agreement with results by others (Badiani et al. 1996). Tryptophan and cystein were the least amino acids in all three samples.

The total essential amino acids contents of different treatments ranged from 42.7 g/100 g protein in the fried fillets to 42.9 g/100 g protein in the grilled fillets. Grilling increased total essential amino acids contents in investigated fillet whereas frying caused a slight decrease in total essential amino acids. Both cooking methods increased glycine, methionine, leucine, alanine and arginine content but decreased significantly proline, threonine, histidine, lysine, isoleucine, tyrosine and tryptophan content.

The results showed that amount of all amino acids increased after cooking except glutamic acid (in grilled samples), proline, isoleucine, histidine, lysine and triptophan. The losses of some amino acids such as lysine in grilled and fried samples can be due to the formation of different Millard product 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 serin and theronine content in raw sample compared to 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 sulphid 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 founded many derivation of tryptophn in fried products. On the other hand, oxidation in some sensitive amino acids (histidine and tryptophn) may be another reason for their reduction. The rate of thermal decomposition of sensitive amino acid residues generally increases with temperature as well as in the presence of oxygen and reducing sacharides (Sikorski 2001). The results showed no significant changes in essential amino acid content in samples after each cooking methods.

Computed nutritional indices

Different nutritional indices of raw and cooked samples are compared and presented in Table 4. The C-PER of raw fillet was 2.32 which was lower than the C-PER value of for fried and grilled samples respectively. These results are in agreement with those reported by Azizah et al. (2002).

The Essentional amino acid index (EAA) of grilled and fried samples was 89.7 and 91.2 respectively, which were higher than the value of 89.1 for raw fillet. The biological value of sturgeon fillets was 85.4, 86 and 87.7 for raw, grilled and fried samples, respectively. It can be seen that the C-PER of cooked samples was higher than that of raw fillets. However the nutritional index of cooked samples was nearly 1.5 times higher than raw fillet, indicating the positive affect of heating on nutritional quality of fillets. PDCAAS values for all age groups for both raw and cooked samples were 100.

The results clearly indicate that fish fillet is an excellent source of dietary protein. The chemical score was high in grilled and fried samples with values of 75 and 71, respectively. The lowest value was observed in raw samples with values of 63. These results agree with those reported by Ryu et al. (1994).

Conclusion

As shown in this study, grilling and frying affect the proximate composition, protein solubility, Sulfhydryl content and nutritional quality of Persian sturgeon. Both cooking treatments improved the *in vitro* protein digestibility of fillets. The protein quality, in terms of PER, CS and EAAI, was improved after the cooking treatments. The results showed that the methionine and cysteine were the limiting amino acids in all samples. Both cooking methods had a similar effect on measured parameters. Therefore, both frying and grilling methods showed beneficial effects on protein quality and can be used for fish processing.

Acknowledgements

The authors are wishing to thank chemistry laboratory staffs, Agricultural Sciences and Natural Resources University of Gorgan, Iran, for their valuable help.

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