Removal of Mercury from Shark Using Sodium Borohydride and Product Characterization

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Abstracts: Predatory fish, such as shark, can accumulate mercury (Hg) at levels that are incompatible in relation to consumption. Fish containing Hg can be decontaminated and potentially used as food. It was studied a procedure for the removal of Hg from shark using sodium borohydride ($NaBH_4$) and characterized the product obtained from shark (POS). The Hg was reduced to Hg^o and eliminated by shaking. The efficiency of Hg removal was high (> 86 %) and the residual levels of Hg in POS were compatible with human consumption. Physical appearance, color, no fish odour, texturing capacity shown by POS were potentially compatible with its use as food. The process used did not significantly affect the POS protein, except for the partial reduction (24.5 %) of bioavailable lysine. Although significant, the reduction of lysine still kept its quantitative nutritional attractiveness. The $NaBH_4$ performed strong potential decontaminant in relation to Hg, but his use requires more studies.

Keywords: Aminoacids, Bioavailable lysine reduction, Fish decontamination, Fish odour removing, Texturizing under freezing.

1. INTRODUCTION

It is recognized that fish and shellfish are vulnerable to contamination by mercury (Hg). For this reason, they are the largest source of Hg in the human diet. Hg is very toxic mainly as methylmercury (CH_3Hg), which is the chemical form of highest occurrence in fish and shellfish. Almost completely absorbed from the diet, CH_3Hg has high biological half-life (50 days, on average), which determines its toxicity. CH_3Hg primarily affects the central nervous system (CNS), and in particular the developing CNS [1].

Predatory fish located at the top of the trophic chain, such as shark, swordfish and tuna, can accumulate Hg at levels that are incompatible in relation to consumption. Legal limits are established to avoid the consumption of fish contaminated with Hg. In Brazil, 0.5 µg/g for non-predatory species and 1.0 µg/g for predatory species are tolerated [2]. Human exposure to Hg can also be controlled through the Provisional Tolerable Weekly Intake (PTWI). PTWI of 5 µg/kg body weight of total Hg was established by the Joint FAO/WHO Expert Committee on Food Additives, of which, no more than 3.3 µg/kg body weight (66 %) should be in the form of CH_3Hg [3].

Fish containing Hg in excess can be decontaminated and potentially used as food. Cysteine has been the substance most widely used for this purpose due to the chemical affinity of its sulfhydryl group to Hg [4,5]. On the other hand, the potential demonstrated by sodium borohydride ($NaBH_4$) has not received adequate attention. $NaBH_4$ has been successfully used for Hg removal during the production of fish protein concentrate (FPC). During this process, Hg is reduced to its volatile form (Hg^o) and immediately eliminated by vigorous shaking, and these procedures do not affected the FPC nutritional value [6].

The aim of this work was to study a procedure designed for the removal of Hg from shark muscle using $NaBH_4$ and to characterize the product obtained in relation to its possible use as food.

2. MATERIAL AND METHODS

2.1. Glassware, Reagents and Samples

Glassware was washed with water and detergent, immersed in nitric acid solution (20 %), during 24 h, and rinsed with distilled and deionized water before use. The reagents used were compatible analytically. Shark samples (commercially identified as *mangona*) were obtained from the Municipal Market of Pinheiros, Sao Paulo, Brazil. Samples (skin-free white muscles) were triturated in a domestic food processor (Black & Decker), sieved in nylon screen (1 mm^2) for the removal of connective tissue, subdivided into aliquots of 50 g each, packed in plastic bags and kept frozen (-18 ± 1 °C).

2.2. Mercury Removal

A procedure for the removal of Hg from shark muscle was designed, based on Cohen and Schrier [6].

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By the action of $NaHB_4$, Hg was reduced to its volatile form (Hg^o) and eliminated under shaking. The procedure was developed in a common laboratory chapel and the gases generated (Hg^o, mainly) were eliminated by exhaustion.

The frozen samples (50 g) were added to distilled water (450 mL) and sufficient amounts of $NaHB_4$ to obtain concentrations of 1, 3 or 5 % in relation to the sample, followed by immediate and vigorous shaking. Stirring was conducted in domestic blender (Wallita, 1774), using the medium speed through 5 cycles of 1 minute each stirring for 1 minute rest and also through 20 stirring cycles of 1 minute each, for 2 minutes of rest. While stirring, temperature was maintained between 23 and 28 °C. Excess $NaBH_4$ was eliminated as metaborate and for this, distilled water (400 mL), antifoaming (80 ppm; Antifoaming C, Dow Corning), and hydrochloric acid (4 M) were added until pH 4.5 was reached, followed by shaking in mechanical platform (Heidolph, Unimax 2010, at 100-120 cycles/minute) during 20 minutes, followed by centrifugation (12,000xg/10 minutes). Distilled water (800 mL) was added to the resulting solid product, and the suspension obtained was centrifuged at 12,000xg for 10 minutes two times consecutively, adjusting the pH (6.8-7.0) by adding solid sodium bicarbonate before the last centrifugation.

2.3. *Mercury Determination*

The lyophilized samples were digested in sulfuric acid (Baker 9673-33) and hydrogen peroxide at 50 % (*Peróxidos do Brasil*), 1:1 (v/v), reduction of Hg^{2+} to Hg° was made with stannous chloride (Sigma, S-2752) (10 %) in hydrochloric acid solution (Merck, 100318) (5 %), and quantification was performed by using atomic absorption spectrometer Perkin Elmer model 3110 equipped with cold steam generator coupled to an automatic flow injection system (FIAS, model 200) [7].

2.4. Boron Determination

The samples were digested in sulfuric acid (Baker, 9673-33) and hydrogen peroxide 30 % (*Peróxidos do Brasil*), 1:1 (v/v), and quantification was based on the formation of a complex of B with curcumin [8].

2.5. Color Measurement

The samples placed in a Petri dish (5.5 cm diameter) had its color measured by reflectance in Hitachi spectrophotometer, model U-3410. The following parameters were used: luminosity (L*), from the *Committee Internationale de L'Eclairage* (CIE), with anhydrous magnesium sulfate (Mallinckrodt, 6070) as a reference, and operational conditions involved scanning from 380 to 780 nm, scan rate of 120 nm/min and illuminant C [9].

2.6. Texturing under Freezing and Hardness Measurement

The product obtained from shark (POS) was maintained on a Petri dish (8.8 cm diameter), covered with plastic wrap, frozen at -18 ° C for 15 hours, and characterized as for its texture. Hardness was measured in the previously frozen POS (-18 °C/15 hours) in a cylindrical plastic container (3 cm in diameter x 5 cm in height) using a texturometer TA-XT2 (Stable Micro System) and non-frozen product as control [10].

2.7. Texturing and Dehydratation

The product obtained from shark (POS) was frozen at -18 ºC for 15 hours and thawed at ambient temperature. For elimination of the water released the product was manually pressed and centrifuged (660xg/5 minutes). Obtained product (POST) was crushed into particles of convenient size and dehydrated at 50±1 ºC for 15 hours and pulverized, resulting in the dehydrated product (POSTD).

2.8. Basic Chemical Composition and Non-Protein Nitrogen Determination

Moisture, ash and protein (semi-micro Kjeldahl method) were determined by routine methods [11] and total lipids were gravimetrically quantified after extracted with chloroform (Synth, C 1062.01.BJ) and methanol (QM 12350.1000), 2:1 (v/v) [12].

Total volatile bases nitrogen (TVB-N) and trimethylamine oxide nitrogen (TMAO-N) were quantified by distillation and subsequent titration [13]. Distillation was performed by steam distillation in alkaline medium (pH ≥ 12) in a semi-micro Kjeldahl distiller [11]. An extract was obtained with the addition of trichloroacetic acid (Synth A1066.01.LB) at 7.5 %, mechanical shaking for 1 hour, filtration on filter paper and adjustment of the final volume (100 mL). In the case of TMAO-N, there was a prior reduction of trim ethylamine oxide (TMAO) to trimethylamine (TMA) with titanium trichloride (Merck, 110789) at 15 %. Urea was quantified by reaction with p-dimethylamin-obenzaldehyde (Sigma D 2004), after extracted from the samples in medium containing active charcoal (Vetec V000159), zinc acetate (Sigma-Aldrich 383058)

and potassium ferrocyanide (Sigma-Aldrich P3289) [11].

2.9. Solubility of Protein Determination

Samples (0.5 g) were solubilized in NaCl (Synth 36300) (0.1 M) under pH values of 5, 6, 7 and 8 and the protein quantified by biuret reaction [14].

2.10. Hydratation Ability Determination

Two grams of pulverized samples and 15 mL of buffers were added into tubes of centrifuge (40 mL). Phosphate buffer [$Na_2HPO_4.7H_2O$ (Sigma S 9390)/NaH_2PO_4 (Sigma S 0751)], at pH 7, and at pH 8, and bicarbonate/carbonate buffer [$NaHCO_3$ (Sigma S 5761)/Na_2CO_3 (Sigma S 6139)], at pH 9, and at pH 10, were used. The obtained mixture was homogenized, re-homogenized at each 30 minutes for until 3 hours, and centrifuged at 660xg/5 minutes. The sediment was added to distilled water (10 mL), homogenized and centrifuged (660xg/5 minutes), for three times. At final, the tube was weighted and the hydratation was calculated as sample hydrated/ sample dehydrated, g/g.

2.11. Protein Electrophoresis Determination

Samples were solubilized in solution containing 5 % of 2-mercaptoethanol (Sigma M 5174), pH 7.5, and protein was determined at 280 nm. Electrophoresis was developed in sodium dodecyl sulfate gel (SDS, Sigma, G 5750) and polyacrylamide (PAGE) at concentration of 10 % acrylamide (Sigma, A 8887), 200 Volts, in Mini Protean II System (Bio Rad). Staining was performed with Coomassie brilliant blue R250 (Serva, 17525) for 2.5 hours, and bleaching with glacial acetic acid (Synth, 31310), methanol (QM 12350.1000) and distilled water (1:4:5) for 2.5 hours. Raw material (RM) and RM treated under the same conditions as the POS, replacing $NaBH_4$ by sodium hydroxide (Synth 36,420), pH 9.5-9.7 were compared. A mixture of proteins with known molecular weight (kDalton, kD) (High Molecular Weigth Standard Mixture, Sigma, SDS-6H) was included as reference: myosin (rabbit), 205 kD; β-galactosidase (*Escherichia coli*), 116 kD, phosphorylase b (rabbit), 97.4 kD, bovine albumin, 66 kD; egg albumin, 45 kD; and carbonic anhydrase (bovine erythrocyte), 29 kD [15].

2.12. Aminoacids Determination

The lyophilized samples were hydrolyzed with hydrochloric acid (Merck, 100318) 6 M at 110 °C for 24 hours in glass ampoule fire sealed under vacuum. The acid was removed, exposing and keeping the open ampoule in a desiccator containing sodium hydroxide pellets (Sinth, 36420) under vacuum. The residue obtained was resuspended in citrate buffer, pH 2.2, filtered through a Millipore membrane (0.22 mm) with appropriate further dilution [16]. Quantification was performed by high-performance liquid chromatography (HPLC) with post-column derivatization using orthophthalic aldehyde (OPA, Merck, 821,027) and fluorescence detector [17].

2.13. Hydroxyproline Determination

Collagen was isolated from whole shark muscle. The ground samples were added to 10 parts of distilled water, homogenized and sieved through nylon mesh (1 mm^2) for 10 consecutive times. The samples were extracted with phosphate buffer containing dithiothreitol for two consecutive times, with final wash with distilled water [18]. A portion of the isolated collagen was treated with $NaBH_4$ (in the same proportions and conditions used for POS) and the other portion was not (control), and both were subsequently lyophilized. The collagen was hydrolyzed in hydrochloric acid (Synth 30,650) 6 M, oxidized with chloramine T (Merck, 102424) and hydroxyproline was quantified with p-dimethylaminobenzaldehyde (Sigma D 2004) [19].

2.14. Chemically Viable Cysteine and Cysteine +1/2 Cystine Determination

Chemically viable cysteine was determined with the dissolution of the samples (0.1 g) in sodium phosphate monobasic buffer (NaH_2P0_4, Sigma S 0751) and hydrochloric acid (Merck, 100,318), 0.05 M, pH 8.2, and reaction with the Ellman's reagent [5,5 '-dithiobis (2-nitrobenzoic acid), Sigma, D 8130] [20]. For the quantification of chemically viable cysteine + 1/2 cystine, prior to reaction with Ellman's reagent, cystine contained in the samples (0.05 g) was reduced to cysteine with $NaBH_4$ (Nuclear, 3057) [20,21].

2.15. Bioavailable Lysine Determination

The samples were solubilized in sodium dodecyl sulfate (SDS, Sigma, L 5750) at 15 %, pH 9; protein was quantified by semi-micro Kjeldahl method [11] and available lysine was determined with orthophthalic aldehyde (OPA Merck , 821,027) [22].

2.16. Statistical Analysys

Data were submitted to analysis of variance and comparison of means by Tukey test at 5 % probability level [23].

3. RESULTS AND DISCUSSION

3.1. Mercury Removal

Using 3 and 5 % $NaBH_4$ and shaking during 20 minutes, the efficiency of Hg removal as high as 86.5 and 91.3 %, respectively, were obtained (Table **1**). Residual Hg levels found in the product obtained from shark (POS) were respectively 4 and 6.25 times lower than the limit of 1 µg/g allowed by the Brazilian legislation for predatory fish species [2], suggesting the use of this product as food. The results shown in Table **1** are consistent with those reported by Cohen and Schrier [6]. It is necessary, however, optimize the procedure used. In this context, the reduction of the $NaBH_4$ concentration, the adjust of the stirring (form and time), and the care with the Hg volatilized are important.

Hg removal that occurred most efficiently in this study (86.5 % and 91.3 %, Table **1**) using $NaBH_4$, mostly exceeded removal obtained using cysteine (40-90 %). Several fish species were used and with rare exceptions, these studies used specimens highly contaminated with Hg, as frequently observed in several shark species [4].

Derived almost exclusively from $NaBH_4$, the boron concentration (B) in POS of 31.00 ± 11.77 µg / g (wet matter with moisture of 80 g/100 g) (n = 5), offers no risk of toxicity. The tolerable intake of B, 0.4 mg / kg body weight / day or 28 mg daily, for an individual weighing 70 kg [24], would only be achieved with the unlike intake of 903 g/day of POS.

Cohen and Schrier [6] speculated about the possibility of $NaBH_4$ to be also useful for the removal of selenium, cadmium, arsenic and lead in addition to mercury. However, such removal would be quantitative only in relation to lead and mercury [25]. Tenuta-Filho, Macedo and Favaro [26] showed that 60.3 % of the selenium present in shark was retained after mercury removal using $NaBH_4$. Selenium retention is important because it is a nutrient and has the ability to reduce the Hg toxicity.

3.2. Evaluation of the Product Obtained From Shark (Pos) As Food

POS was prepared using 3 % $NaBH_4$ under shaking during 20 minutes (Table **1**). In this case, it was considered that at 5 %, $NaBH_4$ showed almost the same efficiency than at 3 %. The residual presence of the antifoaming agent used was not considered due to the small amount used (80 ppm) and the washes with water suffered by POS.

3.2.1. Yield, Physical Appearance, Color And Odour

The POS yield was 11.00±1.55 g/100 g (n = 5) expressed in wet matter (moisture of 80 g/100 g) and its physical appearance was a homogeneous mass, hydrated and with a favorable aspect. POS showed lighter color (L * = 74.17 ±1.06) in relation to the raw material (L * = 64.56±0.92) (n = 7, $p < 0.05$), suggesting a bleaching action of $NaBH_4$ [25, 27]. With lighter color, POS could possibly be used as food. On the other hand, when the raw material was composed exclusively of dark muscle, $NaBH_4$ was not fully efficient as bleacher.

Table 1: Mercury Removal Efficiency from Shark using Sodium Borohydride ($NaBH_4$)

	Treatments - $NaBH_4$ (%): Shaking Time (in minutes)						
	Control	**1:5**	**3:5**	**5:5**	**1:20**	**3:20**	**5:20**
Hg µg/g dm	9.37 ±0.83	7.57 ±0.31	3.54 ±0.16	2.06 ±0.02	2.25 ±0.06	1.27 ±0.05	0.82 ±0.01
Hg µg/g wm	1.87	1.51	0.71	0.41	0.45	0.25	0.16
% Hg removal[(1)]	-	19.2	62.2	78.0	76.0	86.5	91.3

Mean (n=3) ± standard deviation. Control= Raw material. dm = Dry matter (freeze-dried product corrected for residual moisture). wm = Wet matter (calculated based on moisture content of 80 g/100g, from dm). [(1)] In relation to control in dm.

Surprisingly, POS was free from odour of fish and virtually odorless. Urea, trimethylamine oxide and volatile bases, typically involved in fish odour, were eliminated during the process of Hg removal. Thus, POS shows a great potential of use in association with marine and non-marine foods.

3.2.2. Texturing by Freezing

Freezing POS to -18 °C/15 h resulted in product with clear texture. After the elimination of water formed during thawing, by pressing or centrifuging, the product exhibited characteristic porosity. The moisture level (g/100 g) of the raw material, 81.58±0.03, was lower than that of textured POS, 94.45±0.03, (n= 3, $p < 0.05$), as opposed to hardness (gram-force), respectively 144.74±7.37 and 858.43 ±47.88 (n=3, $p < 0.05$), featuring the texture formed.

As seen before, POS freezing led to inevitable product texturing. However, this phenomenon may be desirable, including the possibility of manipulating the texturizing conditions to yield products with appropriate texture. In addition, texturing can serve for the partial POS dehydration, achieved by pressing and/or centrifugation.

Prior to texturing by freezing involves protein-protein interaction. In the freezing of aqueous protein dispersion, similar to that which occurred in the case of POS, ice crystals align themselves predominantly in one direction, and the protein concentrates in crystals form by physical compression. When the product is thawed, the protein material appears aligned, simulating muscle fibers [28].

3.2.3. Chemical Composition

High hydration was found in POS. Minerals (ash) and lipids showed reduced contents probably by dissolution and drag through the water. The hydration level found in POST was similar to that of the raw material (RM) as a result of POS texturing by freezing and elimination of water formed during thawing by centrifugation (Table **2**).

3.2.4. Hydratation Ability

The protein of the POS showed low solubility in NaCl (0.1 M) under pH values between 5 and 8 (0.4 to 1.2 g/100g protein). Under the same conditions the protein of the POST was practically insoluble. On the other hand, the POST previously dehydrated and pulverized resulted in a product (POSTD) with appreciable hydratation (measured by relation between hydrated product and dehydrated product, in g/g). The hydratation of the POSTD was 7.2 and 9.2 times its weight original when hydrated with phosphate buffer at 7.2 and 8.0, respectively. With bicarbonate/carbonate buffer at pH 8.0 and 10.0 the hydratation was of 11.8 and 14.7 times, respectively. Significative correlations between the hydratation and pH (r^2=0.9890) and between the hydratation an moisture (r^2=0.9904) were also observed.

3.2.5. Protein Electrophoresis

With molecular weight of ~45 and 205 kDa each, two more pronounced protein bands composed the electrophoresis profiles of POS (A) and raw material (B) (Figure **1**). Protein bands with 45 and 205 kDa, corresponding to actin and myosin, respectively, were reported in fish as Alaskan pollock (*Theragra chalcogramma*), Atlantic croaker (*Micropogan undulatus*) and silver carp (*Hypophthalmichthys molitrix*) [29,10]. POS differed from RM in relation to other protein bands. Little pronounced, however, such differences suggest that protein from POS has not been seriously affected, mainly because in the process of Hg removal, alkaline medium (pH 9.5-9.7) used was not so high and temperature (25-28 °C) and time (20 minutes) were compatible. When $NaBH_4$ was replaced by NaOH (C), at pH 9.7, in the process of Hg removal, apparently, there was no evidence that the POS protein had been seriously affected.

Table 2: Chemical Composition of the Raw Material (RM) and Product Obtained from Shark Before (POS) and after Texturing (POST)

		Moisture	Ash	Protein	Lipids
RM		80.35^{a}±1,08	1.26^{a}±0,07	16.53^{a}±1.06	0.61^{a}±0.06
POS		96.20^{b}±1,50	0.05^{b}±0.01	3.39^{b}±0.02	0.14^{b}±0.02
POST		81.13^{a}±2.77	0.23^{c}±0.04	17.44^{a}±3.08	0.71^{a}±0.10

Mean (n=5)±standard deviation (g/100g, in wet matter). POST= textured, thawed and centrifuged POS, crushed into particles of convenient size. Different superscripted letters in columns indicate statistically significant differences ($p < 0.05$).

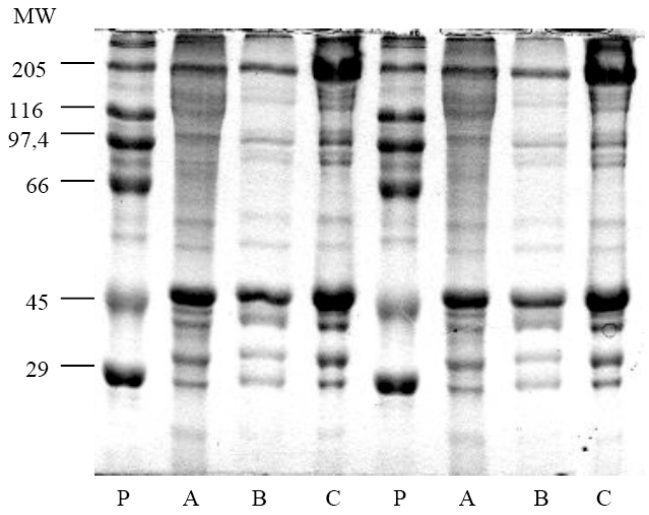

Figure 1: Electrophoresis profile of protein from product obtained from shark (POS), raw material (RM) and RM treated with NaOH (pH 9.7), developed in sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE, 10 %).

P = Molecular weight pattern (MW), in kDa (kilodaltons). A = Product obtained from shark (POS). B = Raw material (RM). C = RM treated with NaOH (pH 9.7). Analysis carried out in duplicate.

3.2.6. Aminoacids

No difference ($p > 0.05$) was found in the proline levels between MP and POS. Regarding the hydroxyproline levels found in collagen (isolated from shark muscle) submitted or not submitted (control) to the same treatment as POS, no difference was found (5.58±0.22 g and 5.51±0.43 g/100g, n=3, $p>0.05$, respectively). A possible effect of the process of obtaining POS on imino acids proline and hydroxyproline was not confirmed, considering the fact that $NaBH_4$ could have a reduction action on the imine group [25].

The results shown in Table **3** suggest that the treatment used for Hg removal did not affect the protein content of POS in relation to the concentration of sulfhydryl (-SH) and disulfide (S-S) groups. Cysteine corresponded to about 50 % of the sum of cysteine and ½ cystine, found in the raw material and POS. $NaBH_4$ was used to reduce S-S to –SH, whose efficiency depends on the S-S accessibility. Thus, a non-homogeneous accessibility of $NaBH_4$ in relation to the S-S groups among POS samples could justify the high variation coefficient (40.89 %) observed in the case of cysteine.

The results shown in Table **4** suggest that there was no influence of the process on the protein composition of the product obtained ($p> 0.05$), except for bioavailable lysine, whose concentration was compromised in 24.5 %. Nevertheless, POS maintained its high nutritional attractiveness in relation to lysine. Due to its high lysine concentration, fish has been regarded as ideal supplementary source to correct or improve the nutritional value of vegetable protein. Compared with standard protein of FAO/WHO/UNU [30], the protein value of POS given by the essential amino acids analyzed, meets human nutritional needs in all age groups except for children ≤ 1 year of age. Literature lacks of nutritional and / or toxicological studies involving the use of $NaBH_4$ in foods.

As already mentioned, Cohen and Schrier [6] used $NaBH_4$ in Hg decontamination during the preparation of fish protein concentrate, and no changes in the biological value of protein and no apparent toxic problems were found (study on birds). Johnson and Wen [27] obtained protein isolates from sunflower meal with the use of $NaBH_4$, with improvement in color, palatability and nutritional value (assessed in rats), being pointed the possibility of its use in foods. The treatment involved alkaline pH (9.0 to 9.5), 75-200 ppm of $NaBH_4$ and heating (45-55 °C/30 minutes).

Using $NaBH_4$ Lee *et al.* [31] chemically modified casein. There was impairment of lysine and therefore

Table 3: Cysteine and Cysteine +½ Cystine Chemically Viable (g/100 g of Protein) in the Raw Material (RM) and Product Obtained from Shark (POS).

	RM				POS		
	Cysteine	Cysteine + ½ Cystine	Cysteine % ([1])		Cysteine	Cysteine + ½ Cystine	Cysteine % ([1])
Mean (n=4)	0.64 [b]	1.28 [a]	50.30		0.80 [b]	1.57 [a]	50.13
±SD	0.04	0.14	4.25		0.33	0.21	15.71
VC (%)	5.55	11.17	8.45		40.89	13.17	31.34

(1) = In relation to cysteine + ½ cystine. Equal superscripted letters in the line indicate lack of statistically significant differences ($p > 0.05$). SD = standard deviation. VC = Variation coefficient.

reduction of the product's biological value. The conditions used in the casein treatment were very different and also very different from that used in the preparation of POS. Thus, they did not allow obtaining subsidies able to clarify the reduction of bioavailable lysine found in this study.

Table 4: Aminoacids (g/100 g protein) Present in Raw Material, Product Obtained from Shark (POS) and FAO/WHO/UNU [30] Standard Protein

Aminoacids	Raw material	POS	Standard protein
Not essentials			
Alanine	6.7[a] ± 0.1	6.3[a] ± 0.6	-
Aspartic acid	11.6[a] ± 0.2	10.9[a]±1.3	-
Glutamic acid	19.3[a] ± 0.4	18.1[a] ± 1.8	-
Glycine	4.0[a] ± 0.1	3.9[a] ± 0.3	-
Proline	3.7[a] ± 0.1	3.6[a] ± 0.3	-
Serine	5.0[a] ± 0.3	4.6[a] ± 0.3	-
Tyrosine	3.3[a] ± 0.1	3.1[a] ± 0.1	-
Essentials			
Histidine	2.8[a] ± 0.3	3.9[a] ± 0.4	1.9
Isoleucine	4.0[a] ± 0.1	3.9[a] ± 0.3	2.8
Leucine	9.0[a] ± 0.3	8.7[a] ± 0.8	6.6
Lysine bioavailable	9.4[a] ± 0.2	7.1[b]± 0.1	5.8
Phenylalanine	4.2[a] ± 0.2	4.7[a] ± 0.4	-
Phenylalanine+tyrosine(*)	7.5	7.8	6.3
Threonine	5.5[a] ± 0.2	5.3[a] ± 0.4	3.4
Valine	3.8[a] ± 0.1	3.7[a] ± 0.2	3.5

Mean (n=3) ±standard deviation. Equal superscripted letters, or not, in the line indicate lack of statistically significant differences ($p > 0.05$) and statistically significant difference ($p < 0.05$), respectively. (*) Sum of the mean values of tyrosine and phenylalanine (aromatic aminoacids)

In the event of the optimizing the process proposed in this paper to obtain POS, bioavailable lysine should also be included as variable, like $NaBH_4$ concentration, shaking, etc. Monitoring through bioavailable lysine would be aimed at obtaining a final product with low or no protein damage.

CONCLUSIONS

Hg removal was high and the residual levels of Hg and B in POS are compatible with human consumption. Physical appearance, color, no fish odour, texturing capacity under freezing (restructuring) shown by POS are potentially compatible with its use as food. The process used did not significantly affect the POS protein, except for the partial reduction of bioavailable lysine. Although significant, the reduction of lysine still kept its quantitative nutritional attractiveness. In the event of process optimization, bioavailable lysine should be included as a monitoring variable in order to obtain a final product with less or no protein damage.

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