EFFECT OF BONE PARTICLE SIZE OF CARP (*CYPRINUS CARPIO*) HEADS IN GELATIN EXTRACTION

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SUMMARY

Gelatin properties of fish skin are different from mammals gelatins, however literature on gelatin from fish bones is limited. The aim of this study was to evaluate the effect of the bone particle size of common carp (*Cyprinus carpio*) heads in the yield, melting point and gel strength of gelatin extracted from the bone fraction of carp heads. Four gelatin extractions were carried out for bones particle sizes of 1 and 2 mm, with pH and temperature of each extraction (5.3 – 60°C), (4.4 – 70°C), (3.8 – 80°C) and (3.6 – 85°C), respectively. Higher gelatin yield (4.86%) was found with particle size 1 mm. The higher gel strengths (128 and 131 g) were not significantly different, and they were found in the first extraction with bones particle sizes 1 and 2 mm, respectively. Using particle size of 2 mm, it was possible to obtain higher melting point values, of 28.5°C to the first extraction. All values of yield, melting point and gel strength of gelatin bones were lower than those found by the carp heads gelatin skins.

KEYWORDS: Carp heads. Collagen. Fish bones. Gelatin.

1. INTRODUCTION

The common carp (*Cyprinus carpio*) is a major fish species in world aquaculture production [17]. The waste products generated during carp processing such as skins, heads, bones and viscera are around 60% of the raw material [11], common carp heads being approximately 22% of the raw material [8]. The majority of these waste products are frequently used to produce fish oil, animal food and fertilizers [7]. However, these new products have low aggregate value [10].

Gelatin is an important functional biopolymer which is widely used in many industrial fields, such as photography, pharmacy and food due to its specific chemical and physical properties [12]. In food it has been used to improve elasticity, consistency and stability [20]. Gelatin is a soluble polypeptide derived from insoluble collagen. The procedure to convert the collagen into soluble gelatin consists of reducing the cross-links between collagen components in acid or alkaline solution. When the tissues containing collagen are exposed to

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the processes of light degradation, the fibrillar structure of the collagen is irreversibly divided, then, the gelatin is formed [18]. The main raw materials used in the gelatin production are bovine bones and hides, pork skin and fish skin and bones [10].

The industrial process to produce gelatin consists of three steps: raw-material pre-treatment, gelatin extraction and purification/drying. The industrial preparation consists of controlled hydrolysis of the organized structure of the collagen to obtain soluble gelatin, through an acid or alkaline treatment of the raw material. This process defines the type of extracted gelatin. Gelatins obtained from acid treatment are named type A, whereas type B are obtained from alkaline treatment [9].

Some studies found that the gelatin properties (such as gel strength and melting point) of fish skin are different from mammal gelatins, and vary between the species. However, literature on gelatin from fish bones are limited [12,14], it is known that the bones particles size affects the extraction yield [16]. The gelatin functional properties depend on the physical, chemical, and structural properties which will define its applicability. The gel strength is the main rheological property of the gelatin whose commercial value is mainly based on its bloom degree, the gelling force [5]. Another important characteristic is the temperature where the gelatin goes from solid to liquid and vice-verse (solidification/melting point). The high bloom gelatins have high solidification temperature [6]. The amino acid content (proline and hydroxyproline) are particularly important to both properties and the gelatins with high levels of these amino acids have greater gel strength and melting point [3,15].

This study aims to evaluate the effect of the bones particle size in the process of gelatin extraction from the common carp (*Cyprinus carpio*) head bones, which are obtained after the gelatin extraction from fish head skin. Results as gelatin yield, gel strength, melting point of the gelatins extracted from skin and bone were analyzed.

2. MATERIAL AND METHODS

2.1. Raw material

The raw materials used as collagen sources in the gelatin production were common carp (*Cyprinus carpio*) heads. These are waste products discharged during fish industrialization. The material was kept in freezers (Consul CHB53C, Brazil) inside plastic containers at -18°C until its use. The chemical reagents used in the analyses were all of analytical grade.

The sample preparation was done according to the applied methodology of Arnesen and Gildberg [3]. The common carp heads stored were defrosted at 4ºC for 20 h, and then

ground to size 5 mm in a knife mill (Wiley Mill Standard, model nº3, USA). In each experiment, approximately 1 kg of carp head was used. The samples were washed with water (1:6) at 4ºC for 10 min, and centrifuged (Centrifuge Sigma 94317, Germany) at 7000xg for 5 min to eliminate excess water.

2.2. Sample treatment and gelatin extraction

The carp heads (skin and bone) treatments, and the gelatin extraction conditions followed the applied methodology according to Arnesen and Gildberg [3] with some changes.

The carp head was treated using alkaline solution 3 $molL^{-1}$, time period of treatment of 105 min and with one exchange of NaOH solution. The gelatin extraction conditions were using distilled water (1:1) at 52ºC, in thermostatic bath (Quimis 214 D2, Brazil), for 2 h in pH 4. Later, the material was centrifuged (Sigma 94317, Germany) at 4000×g for 15 min for the removal of the skins gelatin solution.

After gelatin was extracted from the head skins, the carp bones were dried at ambient temperature (25ºC) for 10 h. Later, the bones were ground according to the studied particle size (1 and 2 mm), before demineralization step (acid treatment). The material was suspended in HCl 0.6 mol L^{-1} for 20 h in thermostatic bath (Quimis 214 D2, Brazil) at the temperature of 10°C, and later centrifuged (Sigma 94317, Germany) at 4000×g for 15 min to remove the excess acid, and washed with distilled water (1:4). Four extractions were carried out in thermostatic bath (Quimis 214 D2, Brazil) with distilled water (1:1), at pH (Marte MB10/MB-10P, Brazil) and extraction temperatures of 5.3 at 60ºC , 4.4 at 70ºC, 3.8 at 80ºC, and 3.6 at 85ºC , respectively, each extraction for 30 min.

The gelatin solutions extracted from skin and bone fractions were filtered in Büchner funnel with Whatman nº 4 paper filter, before using for analysis.

2.3. Centesimal composition

The moisture (stove method: nº 950.46), protein (Kjeldahl method: nº 928.08), ash (oven method: nº 920.153) and lipids (Soxhlet method: nº 960.39) contents of ground heads and bones followed the AOAC [2] methods.

2.4. Characterization of the gelatin solutions

To determine the gelatin yield, the method described by Yang, Wang, Zhou and Regenstein [19] was used. The gelatin solution concentration was determined by refractometer (Atago n° 1, Japan), and the yield calculated according to Equation 1.

$$RG = \frac{C_{gelatin} - V_{solution}}{m_{heads}} \quad (1)$$

where RG is the gelatin yield ($g_{gelatin}\ 100g^{-1}_{heads}$), $C_{gelatin}$ the protein concentration in the gelatin solution (g mL^{-1}), $V_{solution}$ the solution volume of the extracted gelatin (mL) and m_{heads} the grinded head mass (g).

The gel strength was determined based on the applied methodology of Zhou and Regenstein [20]. The extracted gelatin solutions were diluted to a concentration 3.3% with distilled water and heated to 40°C in thermostatic bath (Quimis 214 D2, Brazil) for 30 min. Each dilution had 10 mL added to bottles prepared for analysis (25 × 54 mm, smooth bottom). The bottles with the diluted solutions were put inside the refrigerator (Eletrolux R 250, Brazil) to mature at 7°C for 17±1 h. A thermometer was used to check the temperatures. The gelatin gel strength (g) was measured by a texture analyzer (TA.Xtplus, Stable Micro Systems, England), using teflon probe of diameter 12.5 mm, compressing 4 mm of gelatin at speed 1 mm s^{-1}. The determinations were carried out in triplicate.

The melting point was determined according to Choi and Regenstein [5]. Gelatin solutions at 3.3% were prepared and 5 mL aliquots were transferred to small tubes. The tubes were heated in thermostatic bath (Quimis 214 D2, Brazil) at 60°C for 15 min, chilled in ice bath, and matured at 10°C in the refrigerator (Eletrolux R 250, Brazil) for 17±1 h. The following step included the addition of five drops of a mixture of 75% chloroform and 25% methylene blue dye to the gel. The tubes with gel were put in thermostatic bath at 15°C. The temperature was raised 0.5°C each 5 min. The melting point was the average value between the temperature in which the dye mixture drops visibly penetrated the solutions, and the temperature in which the solutions were totally dyed.

2.5. Statistical analysis

The statistical data analysis was carried out by the Tukey test which was used to determine the significant differences in the averages of the analyses completed in the gelatins, with confidence interval of level 95% ($p \leq 0.05$) [4]. The program Statistica® 6.0 (Statsoft, USA) was used for the calculations.

3. RESULTS AND DISCUSSIONS

3.1. Centesimal composition

The centesimal compositions of the common carp ground heads and bones after the step of gelatin extraction from the heads skin are shown in TABLE 1.

In TABLE 1, the protein levels in the head bones were high although myofibril and sarcoplasmic proteins (non-collagen proteins) were eliminated during the raw material treatment of ground heads in the gelatin extraction from skins. The total protein reduction was not shown as percentage because the ossein material moisture was greatly reduced.

Table 1 – Centesimal compositions of the common carp ground heads and bones after the step of gelatin extraction from the heads skin.

	Ground heads* (%), wet basis	Bones after extraction* (%), wet basis
moisture content	75.9±1.4	15.1±0.9
ash content	9.2±0.7	51.2±1.7
protein content	9.7±0.6	28.3±1.3
lipid content	3.6±0.6	3.3±0.5

* mean values ± standard error (for three experiments).

TABLE 1 shows that the moisture content found in the common carp head bones was lower than the one found by Alfaro, Costa, Fonseca and Prentice [1] in the fish bones, which was around 44%. All the other values, except for the lipids, were higher than the ones found by these authors (proteins, ashes and lipids contents of 14.6%, 34.8% and 3.9%, respectively).

The higher difference in the values in the centesimal composition of the remaining bones in relation to the values of Alfaro, Costa, Fonseca and Prentice [1] took place during the drying step at 25°C, not used by these authors before the removal of the aliquot to determine the bones composition. The reduction in the material moisture is responsible for the high content of the other components. The high ashes content is a result of the high amount of minerals present in the bone fraction, which indicates, at the end of the process of gelatin production, presence of high content of calcium and other minerals. The low lipids content may be related to the alkaline treatment, which might have been enough for their removal.

3.2. Gelatin yield, gel strength and melting point

The values of the gelatin yield, gel strength and melting point for gelatin extracted from common carp head skins were 2.01±0.08 $g_{gelatin}$ $100g^{-1}_{heads}$, 243±8 g and 28.6±0.2°C, for two repetitions.

In TABLES 2–4 are shown the gelatin yield, gel strength and melting point values for the four gelatin extractions from common carp head bones, with particle size 1 and 2 mm, respectively.

Gelatin yield, gel strength, and melting point values for gelatin of carp head skins were higher than all gelatins extracted from fish head bones (TABLES 2–4). Arnesen and Gildberg [3] found that the use of more severe conditions in gelatin extraction from bones was needed due to the presence of stable cross-links in the ossein collagen chains and the presence of protective mineral structure. Therefore, the individual yield per extraction and the physical characteristics of the gelatin from carp head skins were higher for the bones.

Table 2: Gelatin yield values for the four gelatin extractions from common carp head bones, with particle sizes 1 and 2 mm.

			yield ($g_{gelatin}$ $100g^{-1}_{head}$)*	
	T (°C)	pH	1 mm	2 mm
GO1**	60	5.3	$1.54±0.6^{a, A}$	$1.40±0.05^{b, A}$
GO2**	70	4.4	$1.23±0.05^{a, B}$	$0.93±0.04^{b, B}$
GO3**	80	3.8	$1.43±0.08^{a, C}$	$1.07±0.07^{b, C}$
GO4**	85	3.6	$0.66±0,08^{a, D}$	$0.82±0,06^{b, D}$
Total			$4.86±0,08^{a}$	$4.23±0,13^{b}$

* mean values ± standard error (for two experiments). Different minuscule superscript letters in same line, and different capital superscript letters in same column are difference significance at level 95% ($p \leq 0.05$).
**GO1: first extraction gelatin of bones; GO2: second extraction gelatin of bones; GO3: third extraction gelatin of bones; GO4: fourth extraction gelatin of bones.

TABLE 2 shows that all gelatin extractions from carp head bones presented a significant difference to the level of 95% in the yields. Liu, Han and Guo [14] showed gelatin

extracted from catfish head bones with particle size 0.5 mm and maximum yield 8.4%. Nicolas-Simonnot et al. [16] showed higher gelatin yield when extracting from very small pieces (0.125-0.20 mm).

Table 3: Gel strength values for the four gelatin extractions from common carp head bones, with particle sizes 1 and 2 mm.

			Gel strength (g)*	
	T (°C)	pH	1 mm	2 mm
GO1**	60	5.3	$128.2\pm1.8^{a, A}$	$131.5\pm3.2^{a, A}$
GO2**	70	4.4	$108.2\pm2.0^{b, B}$	$123.6\pm3.1^{a, B}$
GO3**	80	3.8	$96.7\pm4.1^{a, C}$	$93.6\pm3.9^{a, C}$
GO4**	85	3.6	$74.4\pm4.3^{a, D}$	$54.7\pm3.5^{b, D}$

* mean values ± standard error (for two experiments). Different minuscule superscript letters in same line, and different capital superscript letters in same column are difference significance at level 95% ($p \leq 0.05$).
**GO1: first extraction gelatin of bones; GO2: second extraction gelatin of bones; GO3: third extraction gelatin of bones; GO4: fourth extraction gelatin of bones.

TABLE 3 shows that there was no significant difference ($p \leq 0.05$) between both gel strength values in the first gelatin extraction from common carp head bone fractions with two particle sizes. However, there was a difference to the level 95% in each extraction for the same particle size, the values being lower than the ones found by Muyonga, Cole, Duodu [14] in the nile perch head bones at 50°C (179 g). The results show that the gel strength was reduced as the extraction temperature increased with both particles sizes. According to Arnesen and Gildberg [3] the extraction temperature increase is responsible for the gel strength values decrease.

TABLE 4 shows that, similar to the gel strength behavior, the higher melting points were found at lower extraction temperatures, and there was significance difference ($p \leq 0.05$) between values for each extraction with same particle size. Melting point values of gelatin extracted from bones with particle size 2 mm were higher than the ones with size 1 mm. Arnesen and Gildberg [3] used 2 mm diameter to evaluate the viscoelastic properties of the gelatin solutions from codfish head bones, and stated that the melting point temperature of the first and second extractions were 25.1 and 22.9°C, respectively, these values being lower

than the ones found in the first and second gelatin extractions from common carp head bones. Studies found by Liu, Han and Guo [14], with catfish (*Ictalurus punctatus*) head bones, showed the same behavior with maximum and minimum values of 25.1 and 20.7°C, respectively, with particle size 0.5 mm.

Table 4: Melting point values for the four gelatin extractions from common carp head bones, with particle sizes 1 and 2 mm.

			Melting point (°C)*	
	T (°C)	pH	1 mm	2 mm
GO1**	60	5.3	$26.2\pm0.3^{a, A}$	$27.8\pm0.3^{b, A}$
GO2**	70	4.4	$25.0\pm0.4^{a, B}$	$27.3\pm0.3^{b, A}$
GO3**	80	3.8	$23.2\pm0.2^{a, C}$	$26.5\pm0.3^{b, B}$
GO4**	85	3.6	$22.1\pm0.2^{a, D}$	$24.6\pm0.2^{b, C}$

* mean values ± standard error (for two experiments). Different minuscule superscript letters in same line, and different capital superscript letters in same column are difference significance at level 95% ($p\leq0.05$).
**GO1: first extraction gelatin of bones; GO2: second extraction gelatin of bones; GO3: third extraction gelatin of bones; GO4: fourth extraction gelatin of bones.

According to Liu, Han and Guo [14], there is a relationship between the gelatin melting point and the molecular weight, gelatins with higher molecular weight will present higher melting point. Arnesen and Gildberg [3] showed, by the molecular weight distribution, that the peptide chain size decreases with the increase in extractions. The decrease in the gel strength and melting point values were due to a higher protein chemical hydrolysis rate caused by a bigger surface in the material with small particle size [14].

4. CONCLUSIONS

The best condition to obtain gelatin from carp head bones took place while using bones with particle size 1 mm, where the yield was higher than the gelatin yield from carp bones with particle size 2 mm and significantly difference ($p\leq0.05$). In addition, the gel strength values were almost the same in both particle sizes and there was no significant difference between first and third extractions.

The extracted gelatin of the carp heads skins showed higher gelatin yield, gel strength and melting point when compared individually to the extracted gelatin of the bones. The total

yield in obtained gelatin of the carp heads (skins and bones) was of 6.87% in relation to the ground heads samples.

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