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The effect of drying temperature on the properties of gelatin from carps (*Cyprinus carpio*) skin

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Abstract: The influence of drying temperature on the characteristics and gel properties of gelatine from *Cyprinus carpio* L. skin was studied. Gelatine was extracted from the carp skin using NaOH and ethanol pre-treatment method, extracted in water in 45°C and then dried in 4 different temperatures: 50, 70, 80°C and freeze-dried. The electrophoresis and functional properties of gelatines were investigated. Freeze drying allowed to obtain a high gelling force, and all other methods did not give satisfactory results. The proteins in gelatines dried at higher temperatures separated by electrophoresis gave severely blurred bands. It may be explained by thermal hydrolysis of collagen fibrils. Freeze drying is the only effective method for drying this product, which can be used in industry.

Keywords: carp; gelatin; skin; temperature of drying

Gelatine is a polypeptide commonly used in the food industry for obtaining the right texture, stability and chewiness, as a fat substitute in low fat products and to improve water binding in meat products. The main sources of gelatine are skins and bones of pigs and cattle (Karim & Bhat 2009; Boran & Regenstein 2010).

Fish farming accounts for a significant percentage of all food production, and fish consumption is constantly growing due to their health benefits.

Popular skinless fish products leads to higher generation of waste which go to the landfill and is heavily burdened by the environment and the producer has to pay for its storage. Losses in the fishing industry are estimated at ~7.3 million tonnes per year at half yearly average of 91 million tonnes (Kelleher 2005). Due to this, researchers are still looking for methods to utilise fish industry wastes and one of them is the production of food gelatine.

Carp (*C. carpio* L.) is one of the most commonly cultivated fish species on the earth, mainly due to its high growth rate and feed utilisation efficiency (Tokur *et al.* 2006). The development of an efficient method for obtaining gelatine from carp skins will enable producers to reduce waste costs while being beneficent for the environment at the same time.

There is more and more research conducted to develop a technology of gelatine production, with various methods of collagen extraction presented (Grossman *et al.* 1992; Kołodziejska *et al.* 2008; Duan *et al.* 2011). However still little is known about the effect of drying temperature and method on the properties of produced gelatine. The aim of this study was to determine the effect of temperature of drying on the quality and functional properties of the gelatine from carp's skin.

MATERIAL AND METHODS

Raw material. Fish skins from common carp (*C. carpio*) were obtained from Sona sp. z o.o, (Poland). The

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skins were a by-product from carp filleting process. Firstly the skins for gelatine production were washed and cleaned by removing residual flesh and scales. Afterwards, the skins were washed one more time, blended and stored frozen at –22°C until used for gelatine extraction. Frozen skins were thawed at 4°C and pre-treated.

Skin pre-treatment and gelatine extraction. The pre-treatment method used prior to gelatine extraction was based on Duan *et al*. (2011) with slight modifications. The skins were mixed with 0.1 M NaOH (Sigma Aldrich) for 6 h with continuous stirring to remove non-collagenous proteins. The alkali solution was changed twice. Next, the samples were washed with cold distilled water, until neutral pH of the washing water was obtained. The skins were then soaked using food grade ethanol (95.6%), left overnight and washed with cold distilled water repeatedly. Gelatine was extracted from the pre-treated skins using a solid/distilled water ratio of 1:3 (g/l) for 4 h at 45 ± 1.5°C. Drying of gelatine solution was performed through lyophilization (Gelatine L) using LYO-QUEST-55ECO lyophilizator (Telstar, Spain) and drying in 50°C (Gelatine A), 70°C (Gelatine B) and 80°C (Gelatine C) in a SML 48/250 dryer (Zalmed, Poland). Drying time was adjusted to achieve the moisture less than 5%. All chemicals were standard reagent grade laboratory chemicals.

Colour measurement. To measure the colour parameters L^* (lightness/brightness), a^* (redness/greenness) and b^* (yellowness/blueness) of gelatine gel (6.67% g/l) CR 200 Minolta Chromameter (Japan) was used. The values were calculated by using the CIE system. The colorimeter has been calibrated to a white standard before analysis.

Determination of bloom strength of gelatine gels. To measure the gel strength, 7.5 g of gelatine and 105 ml of distilled water were weighed into the Bloom jar yielding a 6.67% solution. The sample was mixed using a glass rod and allowed to rest under cover for 3 hours. After this time, gelatine was heated for about 20 min in a water bath at 65°C using a magnetic stirrer to completely dissolve the gelatine. The covered samples were allowed to cool down at room temperature (22°C) for 15 minutes. Until the analysis was performed, the samples were stored overnight (17 h) in a water bath at 10°C. To test the gel strength, TA-XT2 texturometer (Stable Micro Systems, UK) was used to penetrate with a standard 0.5 mm radius cylinder (P/O.5R) probe, to a depth of 4 mm at 0.5 mm/s. The maximum force reading (the resistance to penetration) is the Bloom strength (g) of the gel. The analysis was performed in triplicate.

Electrophoretic analysis. Sample (1 g) was homogenised with 20 ml distilled water. Homogenates were diluted (1:1) with a Leammli Sample Buffer (Bio Rad, cat. number #161-0737) and heated for 90 s in a boiling water bath. The extracts were centrifuged for 3000 g for 3 min (Centrifuge MPW-352R) and the clear supernatant was collected. SDS-PAGE was carried out according to the method by Laemmli (1970) using a 12% g/l gel concentration. Chemicals for electrophoresis were obtained from Bio-Rad (Electrophoresis Purity Reagents).

Functional properties. Method Cho *et al*. (2004) was used to study gelatine properties. To determine the foam formation ability, 0.5 g of the test sample was placed in 50 ml of distilled water and waited for 15 min and then heated to dissolve at 60°C. To obtain the foam, a homogenizer (Unidrive X 1000; CAT Scientific, USA) was used to homogenise the sample at 10 000 rpm for 5 minutes. The obtained homogenate was poured into a 250 ml measuring cylinder and the foam capacity was read by comparing the ratio of foam to liquid. The foam stability was calculated as the ratio of the initial volume of foam to the volume of foam after 30 minutes.

To test the water-holding capacity and fat-binding capacity, up to 1 g of gelatine was added 50 ml of distilled water or 10 ml of sunflower oil. Samples were left for 1 hour at room temperature and mixed for 5 s with a vortex mixer every 15 minutes. After this time, the samples were centrifuged at 450 g for 20 min, then the top phase was removed and the remaining contents of the centrifuge tube drained by tilting the tube at 45°C on a paper filter.

Tested values were calculated from the mass difference of filtered sample and dried gelatine.

Statistical analysis. All analyses were conducted in triplicate and data was subjected to a statistical analysis using Statistica 12 software (Dell Software, USA). The normality of the results was established using the Shapiro–Wilk test and to compare the results between the groups, one-way analyses of variances (ANOVA) were used. The significance of differences between means (P < 0.05), was established using the Tukey post-hoc test. The results are presented as average ± standard deviations.

RESULTS AND DISCUSSION

Colour measurement and determination of bloom strength of gelatine gels. There was a statistically significant relationship between the drying method and the colour of gelatines (Table 1). The colour

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parameters of the samples dried in the presence of oxygen (Gelatine A, B and C) did not differ from each other. On the other hand Gelatine L had significantly higher lightness than Gelatine A and B and significantly higher yellowness than all other gelatines.

The darker colour of gelatine is due to the contamination with inorganic compounds, proteins and mucosubstance (Avena-Bustillos *et al.* 2006), which are further darkened by oxidation processes in the presence of oxygen and elevated temperatures. No differences were found between the methods with respect to the *a** axis. It is desirable to obtain the highest brightness and the ratio of green to blue, and gelatine *L* best meets these requirements (GMIA 2012). Ninan *et al.* (2011) points out that the colour of gelatine is dependent on the fish species of raw material, while Boran and Regenstein (2009, 2010) and Gudmundsson and Hafsteinsson (1997) claim that it depends on the extraction method. Because of the use of one fish species and the same gelatine extraction method, it can be assumed that the drying process also affects the colour of the gelatine.

Each drying method showed statistically significant differences in gel strength (Table 2). The higher gel strength of lyophilized gelatine may result from lower thermal damage to the collagen structure that can occur during air drying (Montero & Gomez-Guillen 2000). High temperature of drying have the most negative impact on bloom value 50°C, less when 80°C is applied, and most preferable is 70°C. Low gel strength after drying at 50°C may result from the longest exposure to elevated temperatures, despite its lower value. Drying at 70°C will be more advantageous than drying at 80°C and 50°C, because drying time and temperature are both optimal in one time. Furthermore according to Muyonga *et al.* (2004) high content of β fraction of collagen is associated with high Bloom value of gelatines. It can be assumed that gelatine A has the highest content of β fraction. However, further research should be carried out to verify the content of β chain in analyzed gelatines.

Nevertheless the gel strength in commercial gelatine is 100 to 300 Bloom value, and most desirable is 250–300 Bloom value (Jellouli *et al.* 2011). Therefore air drying at high temperature of carp skin gelatine results in the loss of its gelling properties and does not provide the desired gelling force which would be required for commercial application. The higher gelling strength of the freeze-dried gelatine might have occurred due to lower degradation of gelatine at the low drying temperature. Moreover, freeze drying produces more cross-linking in the proteins which usually causes freeze-dried proteins to have higher proportions of β- and γ-fractions (Kwak *et al.* 2009). Freeze-drying is the best way of gelatine drying in terms of gel strength, which is confirmed by numerous studies. Normand *et al.* (2000) reported that high extraction temperature caused protein degradation, producing protein fragments with lower molecular weight and lowering gelling ability. By lyophilizing gelatine of different species, the following gelling strengths were obtained (in Bloom value): Red tilapia 128.11 and Black tilapia 180.76; clown featherback ~280; seabass 369; African catfish 234; amur sturgeon 141 and gray triggerfish 168.3 (Jamilah & Harvinder 2002; Duan *et al.* 2011). The results obtained for the lyophilized gelatine from common carp are reproducible with the literature data. Duan *et al.* (2011) compared the gel strength of gelatines from skins of carps (*C. carpio*) caught in winter to those obtained for the summer equivalents. The gel strength gelatine from summer season carp was 76 g, winter season carp 61 g. This gelatines does not meet industry requirements. In Duan *et al.* (2011) study, the gel strengths of carp skin gelatines were lower than those of gelatines presented in this study, which may be due to high extraction temperatures of the samples.

The shorter drying time at high temperatures is an economical benefit; however, it is useless for food applications where gelling is required. There-

Table 1. Colour and gel strength of gelatines

Gelatine	*L**	*a**	*b**	Bloom value (g)
A	$8.83^{a} \pm 3.27$	$0.65^{a} \pm 0.1$	$1.10^{a} \pm 0.24$	$2.79^{a} \pm 0.84$
B	$7.83^{a} \pm 5.20$	$0.62^{a} \pm 0.16$	$1.14^{a} \pm 0.31$	$21.70^{c} \pm 0.08$
C	$10.83^{ab} \pm 2.18$	$0.62^{a} \pm 0.16$	$1.14^{a} \pm 0.33$	$16.30^{b} \pm 1.39$
L	$16.62^{b} \pm 2.93$	$0.47^{a} \pm 0.10$	$2.42^{b} \pm 0.28$	$158.70^{d} \pm 3.71$

Different letters indicate significant differences at $P < 0.05$; values are presented as a mean ± s.d.

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fore, lower drying temperatures with less protein denaturation are desirable for making gelatines with acceptable gel strength.

Electrophoretic analysis. The approximate molecular weight distribution of gelatines from carp dried in four different methods was compared and presented in Figure 1.

The maximum molecular weights of gelatine A, B, C and L were in the order of 200, 200, 200 and 250 kDa. The minimum molecular weight was in the > 25, > 25, > 25, and > 40 kDa. In each gelatine, typical locations of bands derived from α_1 chains (150 kDa), α_2 (~120 kDa), β (200 kDa) of collagen were observed. In the case of gelatine dried at higher temperatures, the bands are severely blurred with visible smudges which means that the thermal hydrolysis of collagen proteins occured, which explains their no gelling strength. The lyophilized gelatine gives clear bands in characteristic spots, blurring is much smaller and smudges are shorter. However, lower molecular weight proteins can be observed in this case too, but in lesser numbers than in other samples. This indicates a slight hydrolysis of gelatine proteins and explains the high gel strength of the gelatine after freeze-drying. Jellouli *et al.* (2011) lyophilized gray triggerfish gelatine and obtained similar results by observing distinct bands of α_1, α_2 and β collagen chains. Duan *et al.* (2011) lyophilized carp skin gelatine and reported clearly marked all collagen chains, although, with low visibility of the α_2 band in some samples, which could be due to thermal decomposition during extraction. The thermostability of α chains depends on the species of origin of the gelatine. Muyonga *et al.* (2004), dried gelatine from Nile perch skins at 42°C and observed many low molecular weight smudges and bands and a clear band at the spot of the α chain. Boran *et al.* (2010) dried gelatine from silver carp at 60°C and obtained clear bands at the heights of α_1, α_2 and β chains of collagen. This suggests that the method of gelatine drying should be chosen for each fish species individually.

Functional properties. Foam formation ability is one of the most important properties of gelatine for commonly used food products. Foam formation ability and foam stability of carp gelatines are shown in Figure 2.

The foam formation ability of gelatine from carp was 1.03 (gelatine L), 1.02 (gelatine A), 1.01 (gelatine B) and 1.01 (gelatine C), which means that the gelatines were almost not foaming at all. The foam

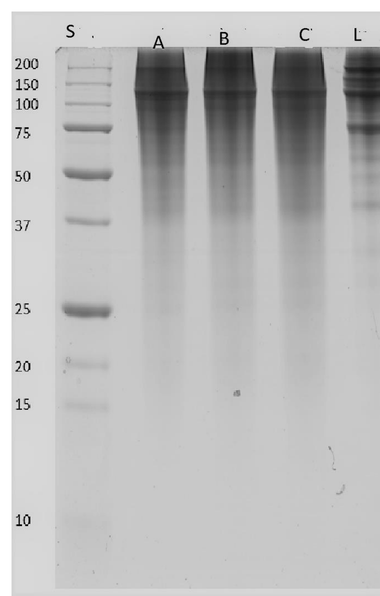

Figure 1. Electrophoretic analysis of the gelatines
S – standard; A – gelatine A; B – gelatine B; C – gelatine C; L – gelatine L

formation ability did not differ significantly among all the studied gelatines. The foam stability of gelatines was also low (1.00–0.92). Cho *et al.* (2004) suggested that reduced foam formation and stability may be due to aggregation of proteins which interfere with interactions between the protein and water needed for foam formation. Foam formation ability might be influenced by the source of the protein, intrinsic properties of protein, protein composition, and conformations of protein in solution at the air/water interface (Jellouli *et al.* 2011). The tested gelatines was differed only by the method of drying. The results showed that the drying conditions did not affect the foam formation ability of the carp gelatine.

Gelatine L has a significantly higher water binding capacity (WHC) than gelatine A and B and significantly higher fat-binding capacity (FBC) than all other gelatines. Based on the results, it can be assumed that both WHC and FBC are affected by the drying method, with lyophilisation being the best method to obtain gelatine from carp skin. High WHC and FBC allows the gelatine to be used as food stabilizer in terms of preventing the moisture loss of the frozen products during heat treatment or thawing and to protect against separation of the fat fraction. According to Cho *et al.* (2004) FBC might depend on the amount of hydrophobic residues in the molecule, and that the drying process with hot air reveals

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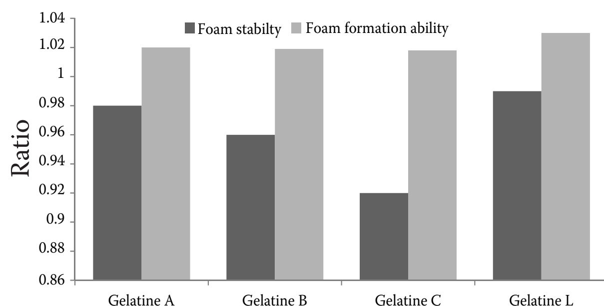

Figure 2. Foam formation ability and foam stability of carp's gelatine

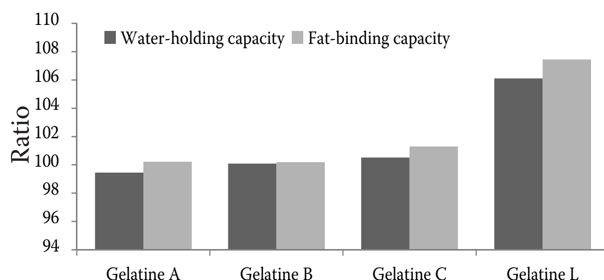

Figure 3. Water-holding capacity and fat-binding capacity of carp's gelatine

these groups. However, the results obtained in this study do not confirm this.

CONCLUSIONS

The best quality of carp skin gelatine was obtained by lyophilization. This drying method allowed to obtain, more desirable colour, significantly higher WHC and FBC and stable structure of natural collagen chains. Moreover freeze drying allowed to obtain gelatine with high gelling force, while drying with all the other methods did not result in satisfactory gel strength of the acquired gelatine. Given the gelation strength of gelatine from carp skins, freeze drying is the only effective method for drying this product, which can be used in industry to produce an alternative to mammalian gelatine.

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