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Effect of extracting time and temperature on yield of gelatin from different fish offal

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Abstract

The aim of the study was to determine the optimal conditions for preparing gelatin from different kinds of fish offal: heads and backbones of Baltic cod, skins of fresh and cold-smoked salmon, and skins of salted and marinated herrings. The yield of gelatin extraction at 45 °C was 71–75% for fresh salmon skins or cod backbones, and 86%, for smoked salmon skins. When heating marinated herring skins for 15 min or salted herring skins for 45 min, about 100% of collagen was converted to gelatin. For fish skins, 45 °C and 15–60 min extraction time, depending on the kind of skins, were established as optimal conditions for preparing gelatin. The yield of gelatin extraction from the cod heads did not exceed 70%, even when a three stages process was used. In the case of backbones, 100% of collagen in the form of gelatin was isolated using this procedure. SDS-PAGE analysis showed that gelatin from fish skins was much less degraded than gelatin from pigskins.

Keywords: Fish skins; Fish heads and backbones; Fish gelatins

1. Introduction

The offal separated during processing of fish can amount to 60% of the raw material mass. The parts that constitute this offal are mostly skins, heads and backbones. They can serve as an additional source of proteins, especially collagen or gelatin. Collagenous material is widely used in the food, pharmaceutical, cosmetic and packaging industries, as well as in some medical and biotechnological applications.

Heating collagen in water leads to its conversion into soluble gelatine, forming colloidal solutions and gelling at appropriate concentration and temperature. Thermal solubilisation of collagen is due to cleavage of a number of intra- and intermolecular cross-linking bonds in collagen (Veis, 1964). Moreover, some amide bonds in the elementary chains of collagen molecules undergo hydrolysis (Bailey, 1985). As a result the obtained gelatin has molecular weight lower than native collagen and constitutes a mixture of fragments with a molecular weight in the range of 16–150 kDa (Asghar & Henrickson, 1982). The conversion rate of collagen into gelatin depends on processing parameters (temperature, time, and pH), the properties of the raw material and its pretreatment.

The collagen of warm- and cold-blooded animals differs in some physical and chemical properties, such as the amino acid composition, solubility, thermal stability and chemical reactivity (Bailey & Light, 1989; Sikorski, Scott, & Buisson, 1984; Yamaguchi, Lavéty, & Love, 1976; Yata, Yoshida, Fujisawa, Mizuta, & Yoshinaka, 2001). Therefore, the methods used for isolation of collagen and preparation of gelatin from cattle and pig connective tissue cannot be used in the case of fish offal.

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The conditions for obtaining gelatin have been determined mainly by using fresh or frozen fish skins as the raw material (Cho, Gu, & Kim, 2005; Fernández-Díaz, Montero, & Gómez-Guillén, 2003; Giménez, Gómez-Guillén, & Montero, 2005a; Giménez, Turnay, Lizarbe, Montero, & Gómez-Guillén, 2005b; Gómez-Guillén, Giménez, & Montero, 2005; Gómez-Guillén et al., 2002; Gudmundsson & Hafsteinsson, 1997; Kołodziejska, Kaczorowski, Piotrowska, & Sadowska, 2004). So far, the offal from further processing of semi-processed fish products such as skins of salted, marinated or smoked fish has not been used as a source of collagen and gelatin. In such materials, the applied processing conditions can affect the properties of collagen and obtained gelatin. For example, collagen from skins of smoked fish can be cross-linked with components of smoke and then not soluble in acids or resistant to thermal hydrolysis. On the other hand, collagen from skins of marinated herring could display opposite properties.

The objective of the investigations was to estimate the usefulness of different kinds of fish offal as a source of gelatin, as well as to determine the optimal conditions for its extraction.

2. Materials and methods

2.1. Raw material

The skins, heads and backbones of Baltic cod (*Gadus morhua*), skins of fresh and cold-smoked salmon (*Salmo salar*), and skins of salted and marinated herrings (*Clupea harengus*), kindly provided by Polish industrial plants, were used as the source of gelatin. Tissue residue was removed manually. The raw material, in the partially frozen state, was minced in a meat grinder with 3-mm diameter mesh, mixed thoroughly, and stored at −20 °C until use. The dry weight, total protein, hydroxyproline, lipids and ash in raw material were determined.

2.2. Preparation of gelatin

In order to determine the optimal conditions for gelatin extraction, the minced skins, previously washed with NaCl solution (with the exception of salted and marinated herring skins) and water (Fig. 1), were gently stirred with water (1:6, w/v) for 15–120 min at 45, 70 or 100 °C. The samples were centrifuged at 10 000*g* for 30 min at 15 °C and the hydroxyproline content was determined in the supernatants. In the case of backbones and heads of cod, gelatin was also obtained by extracting of the raw material three times with water. In the first stage, samples were heated for 45 min at 45 °C, in a ratio of raw material to water of 1:6 (w/v). After centrifuging, the resulting sediments were mixed with water (1:4 or 1:6, w/v, in relation to the raw material) and heated for 45 min at 60 °C. The samples were centrifuged and water was again added to the sediment (1:2 or 1:6, w/v, in relation to the raw material). The samples were heated for 45 min at 70 °C and centrifuged. Again, the content of hydroxyproline was determined in all supernatants.

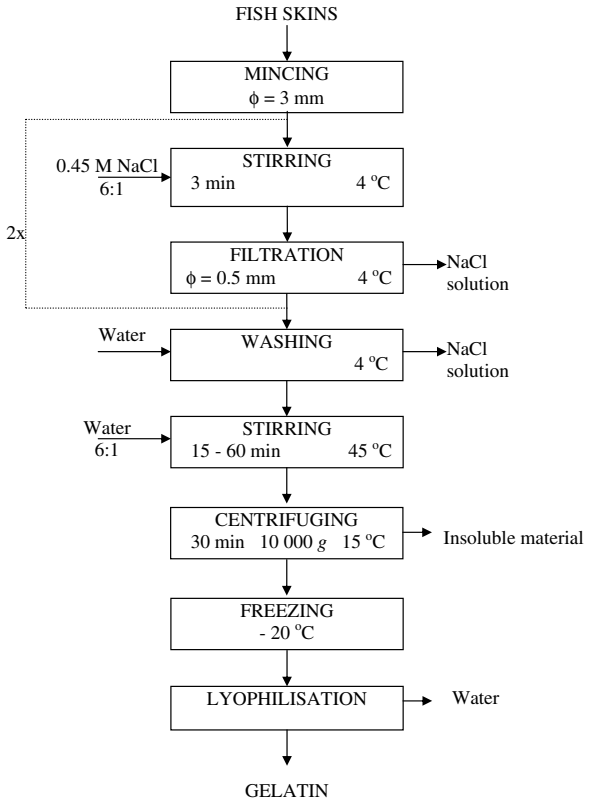

Fig. 1. Flowchart of the procedure used for extraction of gelatin from fish skins.

The preparation of gelatin extracts was carried out using the three separated samples of raw material treated in identical conditions. For each extract the determination of hydroxyproline was made in triplicate. Based on the obtained results the optimal parameters for gelatin extraction were established.

Yield of gelatin was calculated from

Yield (%) = hydroxyproline content of supernatant (g/ml) × volume of supernatant (ml) × 100/hydroxyproline content of raw material (g/g) × weight of raw material used (g)

The data shown in Tables represent the mean values ± standard deviations from three parallel experiments.

The portions of gelatin in the laboratory scale experiment were prepared to characterise some of their properties and to analyse the composition of the thermal degradation products of collagen. Extraction of gelatin from cod and herring skins (salted or marinated) was conducted for 45 min and from skins of smoked salmon for 60 min at 45 °C. The solutions of gelatin were freeze-dried (Fig. 1).

2.3. Chemical and physical analyses

The dry weight, total nitrogen, ash and lipids were determined according to AOAC methods (1990).

Hydroxyproline was determined after hydrolysis of the material in 6 M HCl for 6 h at 105 °C, using the colorimetric method recommended by ISO (Anonymous, 1978).

The gelling temperature was determined based on the viscosity value. The viscosity was continuously measured during cooling of gelatin solution from 40 °C to 5 °C at (0.2 °C/min). The temperature at which a sharp increase in viscosity appeared was assumed to be the gelling temperature (Arnesen & Gildberg, 2007). The viscosity of gelatin solutions was measured using a Brookfield DV-III viscometer with small sample adapter and SC4-27 spindle at 25 rpm (Brookfield Engineering Laboratories Ltd., Middleboro, MA).

The electrophoresis of products of thermal hydrolysis of collagen was performed, in principle, according to the methods of Bollag and Edelstein (1991) using 5% stacking gel and 8% separating gel. After electrophoresis, the gel was stained with Coomassie Blue R-250 dye in methanol:acetic acid:water solution (5:1:4, by volume) and destained in methanol:acetic acid:water solution without dye (1:1:8, by volume).

3. Results and discussion

3.1. Chemical composition of raw material used as the source of gelatin

As shown in Table 1, the raw materials used in the experiments are a rich source of proteins. The skins of smoked salmon contained the most protein, as a result of moisture loss during cold smoking. The content of protein in the heads and backbones of cod and in the skins of marinated and salted herrings was similar but about 50% lower than that in fresh cod and salmon skins. Collagen in cod and salmon skins constituted about 80% of the total protein. The skins of marinated or salted herring were low in collagen. This was not a result of collagen solublisation during processing of fish because in the fresh skins the content of collagen was similar. More collagen, about 35% of total protein, was found in the heads and backbones of cod. The muscle proteins adhering to the bone elements of heads and backbones constitute the rest of the total protein. Thus, from one tonne of heads and backbones 115 kg of valuable muscle proteins and about 50 kg of collagen or gelatin can be recovered.

3.2. Effect of time and temperature on the yield of gelatin

The yield and properties of gelatin depend on the kind of raw material, its pre-treatment and parameters of the process. Fish skins are especially suitable as a source of gelatin because it is easily extracted with high yield at relatively moderate temperature, usually at or below 50 °C (Giménez et al., 2005a; Gómez-Guillén et al., 2002; Gudmundsson & Hafsteinsson, 1997; Kołodziejska et al., 2004). Moreover, using minced skins instead of wholeskins, significantly shortens the time of extraction of gelatins (Kołodziejska et al., 2004).

These experiments showed that depending on the raw material, 30–100% of collagen was solubilised during heating for 15 min at 45 °C (Tables 2–4). The increase in the thermal solubility of collagen was very small or was not observed when extraction time at 45 °C was longer than 45–60 min. Increasing the extraction temperature to 70 °C also did not affect collagen solubility, with the exception of fresh salmon skins. Based on these results, for fish skins, a temperature of 45 °C and time of extraction 15–60 min, depending on the kind of skins, were established as optimal conditions for preparing gelatin.

In the process conducted at 45 °C, the yield of gelatin extraction was 74% from fresh salmon skins and 86%, when smoked skins were used as the source of gelatin (Table 3). These results show that smoked salmon skins are a suitable raw material for efficient gelatin extraction. They also suggest that cross-linking of collagen with components of smoke is not sufficient to affect thermal solubility of collagen. Under optimal conditions of extraction of fresh and smoked salmon skins, the yield of gelatin

Table 1
Chemical composition of fish offals'[a]

Raw material	Content (%)				
	Dry weight	Total protein [$N \times 6.25$]	Collagen[b]	Lipids	Ash
Cod:					
skins	30 ± 1.3	27 ± 0.6	20.4 ± 0.7	0.3 ± 0.05	2.2 ± 0.10
heads	20 ± 0.5	15 ± 0.4	5.3 ± 0.3	0.2 ± 0.10	5.0 ± 0.18
backbones	22 ± 0.4	17 ± 0.8	5.1 ± 0.3	0.2 ± 0.08	6.2 ± 0.28
Salmon:					
skins of fresh	35 ± 0.2	30 ± 0.1	24.8 ± 0.9	1.9 ± 0.08	3.1 ± 0.19
skins of smoked	69 ± 0.2	42 ± 0.3	31.8 ± 0.7	22.3 ± 0.30	7.1 ± 0.22
Herring:					
skins of fresh	44 ± 1.5	ND[c]	3.4 ± 0.3	ND[c]	ND[c]
skins of salted	31 ± 0.6	15 ± 0.4	3.1 ± 0.3	15.5 ± 0.17	1.6 ± 0.39
skins of marinated	40 ± 0.2	16 ± 0.4	3.5 ± 0.4	22.1 ± 1.41	1.8 ± 0.33

[a] Mean value ± standard deviation of six separated samples.
[b] The conversion factor for calculating the content of skin collagen from hydroxyproline was 14.7.
[c] Not determined.

Table 2
Yields of gelatins extracted from backbones (a) and heads (b) of cod at various temperatures and times[a]

Heating time (min)	Yield of gelatin (%)		
	45 °C	70 °C	100 °C
(a)			
15	43 ± 2.9	46 ± 6.5	62 ± 2.8
30	53 ± 4.4	58 ± 2.6	64 ± 2.1
45	64 ± 6.2	61 ± 4.0	70 ± 1.1
60	64 ± 4.1	65 ± 3.2	73 ± 0.2
90	67 ± 6.4	64 ± 4.7	83 ± 5.8
120	71 ± 4.3	69 ± 5.1	94 ± 4.6
(b)			
15	31 ± 2.9	40 ± 4.7	50 ± 2.4
30	40 ± 1.6	40 ± 6.7	56 ± 3.2
45	48 ± 2.3	42 ± 3.6	60 ± 2.7
60	48 ± 7.0	47 ± 0.8	61 ± 2.0
90	53 ± 3.9	52 ± 3.7	64 ± 4.8
120	57 ± 2.4	56 ± 5.4	69 ± 7.0

[a] Mean value ± standard deviation of three replications.

Table 3
Yields of gelatins extracted from skins of fresh (a) and smoked (b) salmon at various temperatures and times[a]

Heating time (min)	Yield of gelatin (%)		
	45 °C	70 °C	100 °C
(a)			
15	41 ± 4.8	55 ± 7.1	90 ± 8.4
30	60 ± 4.4	72 ± 2.4	99 ± 6.4
45	60 ± 4.4	74 ± 3.9	100 ± 9.3
60	75 ± 5.4	81 ± 5.6	101 ± 7.2
90	71 ± 7.5	78 ± 4.7	101 ± 4.1
120	74 ± 10.2	88 ± 7.1	98 ± 8.5
(b)			
15	64 ± 1.2	80 ± 1.4	85 ± 1.7
30	72 ± 1.8	80 ± 2.0	90 ± 1.2
45	76 ± 3.3	82 ± 2.6	94 ± 1.3
60	80 ± 0.9	85 ± 0.4	96 ± 1.6
90	78 ± 1.7	84 ± 1.7	96 ± 1.1
120	86 ± 2.8	84 ± 1.5	95 ± 1.4

[a] Mean value ± standard deviation of three replications.

Table 4
Yields of gelatins extracted from skins of salted (A) and marinated (B) herrings at various temperatures and times[a]

Heating time (min)	Yield of gelatin (%)		
	45 °C		70 °C
	A	B	A
15	79 ± 8.2	104 ± 7.5	101 ± 10.0
30	90 ± 2.1	104 ± 2.5	103 ± 5.5
45	96 ± 2.4	102 ± 3.4	100 ± 1.1
60	102 ± 5.6	108 ± 5.6	98 ± 3.5
90	94 ± 3.5	106 ± 11.2	101 ± 4.8
120	104 ± 4.1	103 ± 6.8	104 ± 6.5

[a] Mean value ± standard deviation of three replications.

amounted to 19% and 25% of the weight of raw material, respectively (the content of collagen in raw material was about 25% and 32%, respectively). In our previous work (Kołodziejska et al., 2004), 85% of collagen from minced cod skins was converted to soluble gelatin after 30 min at 45 °C. The yield of gelatin on a large laboratory scale was lower; it amounted to 64% of collagen content in skins (12.3% of the weight of raw material). In comparison, Gudmundsson and Hafsteinsson (1997), using prolonged extraction of whole cod skins, obtained a yield of gelatin between 11% and 14%, depending on the conditions used in the preliminary treatment of raw material. A lower yield of gelatin from cod skin, about 7%, was obtained by Gómez-Guillén et al. (2002). In the case of other species of fish, the extraction yield of gelatin from skins ranged from about 5.5–21% of the weight of raw material (Giménez et al., 2005a, 2005b; Grossman & Bergman, 1992; Jamilah & Harvinder, 2002; Muyonga, Cole, & Duodu, 2004; Osborne, Voight, & Hall, 1990). The reason for such large differences in the yield of gelatin can result, amongst other things, from different contents of collagen in the raw material. However, very often this information is not available among published data.

The highest yield of extraction was achieved from herring skins. During heating of marinated herring skins for 15 min, or salted herring skins for 45 min, about 100% of collagen was converted to gelatin. However, it constituted only about 3.5% of the weight of raw material because of the low content of collagen in herring skins (Table 1).

In the case of backbones 100% of collagen in the form of gelatin was isolated (about 5% of the weight of raw material) when the three-time extraction procedure was used. As reported by Muyonga et al. (2004) the yield of gelatin extraction in a four stage process from young and adult Nile perch (*Lates niloticus*) backbones was 1.3% and 2.4%, respectively. It was lower than that from cod backbones. However, a long leaching process was used in preparing gelatin from Nile perch backbones. This process could lead to loss of collagen. In our experiment gelatin was extracted directly from the raw material without preliminary treatment.

The lowest yield of gelatin was obtained from whole cod heads. It did not exceed 57%, even after 120 min extraction at 45 °C (Table 2). The yield of gelatin from the cod heads increased to about 70% in the three stage procedure (Table 5). As reported by Arnesen and Gildberg (2006), in a five stage process at elevated temperature and pH 3.5–5.3, about 55% of gelatin was prepared from cod head bones, after separation of soft head connective tissue.

3.3. Electrophoretic characteristic of gelatins

The SDS-PAGE patterns of gelatins obtained from different sources are presented in Figs. 2 and 3. In the case of commercial bovine and pigskin gelatin of 75–100 Bloom value (BL), the distinct bands corresponding to the main components of collagen were not observed, even at higher protein concentrations. Over the whole length of the gel only a smudged band was visible. These results indicate

Table 5
Yields of gelatins obtained from heads and backbones of cod using a three-stage procedure[a]

Temperature (°C)	Raw material: water (w/v)	Time (min)	Yield of gelatin (%)	
			Heads	Backbones
(a) Varying ratio of raw material to water				
45	1: 6	45	46 ± 2.8	59 ± 3.8
60	1: 4		18 ± 0.6	33 ± 1.9
70	1: 2		4.5 ± 0.3	13 ± 2.3
(b) Constant ratio of raw material to water				
45	1: 6	45	44 ± 4.3	59 ± 3.6
60			15 ± 2.1	29 ± 1.9
70			8 ± 1.2	13 ± 0.8

[a] Mean value ± standard deviation of three replications.

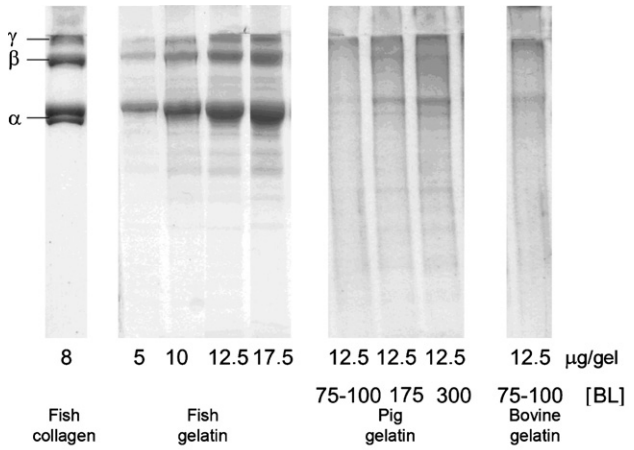

Fig. 2. SDS-PAGE patterns of cod skin collagen and gelatins of different origins; α, β, and γ – the main components of denatured collagen.

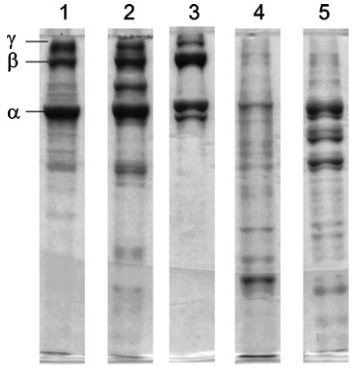

Fig. 3. SDS-PAGE patterns of gelatins from:(1) cod skins heated for 45 min at 45 °C; (2) fresh salmon skins heated for 45 min at 45 °C and (3) for 45 min at 90 °C; (4) skins of salted and (5) marinated herrings heated for 45 min at 45 °C; α, β, and γ – the main components of denatured collagen.

advanced hydrolysis of collagen. Probably some small molecular weight products of hydrolysis were also not stopped in the gel. Only in the case of pigskin gelatin of 175 and 300 BL were low intensity bands corresponding to the main components of denatured collagen seen on electrophoregrams. Gelatin from cod skins was much less degraded (Fig. 2). The distinct bands corresponding to β and γ components of collagen and to the one α chain in the electrophoretic pattern were visible. However, the second α chain was not observed. According to Gómez-Guillén et al. (2002), damage or partial loss of $\alpha_1$chains can occur during the extraction procedure. In analysed samples, at a higher concentration of gelatine, additional bands with molecular weight lower than 100 kDa appeared below the α chain. They could be the products of hydrolysis of elementary chains of collagen or a residue of non-collagenous proteins. The gelatin extracted from salmon skins at 45 °C showed a similar electrophoretic pattern (Fig. 3). It was also seen that the main components of collagen were still present in gelatin when the process was conducted at 90 °C (Fig. 3). A different electrophoretic pattern was obtained in the case of gelatin from skins of salted and marinated herrings. The β and γ components of collagen did not appear on the electrophoregrams. Only the bands corresponding to α chains of collagen and components with smaller molecular weight were present. However, these changes of collagen probably took place in the salting and marinating process, not during extraction of gelatin. Moreover, differences in the qualitative and quantitative composition between these two gelatins were also observed.

3.4. Gelling temperature of gelatin solutions

The gelling properties of gelatin are greatly influenced by the origin of raw material used in the process. This is the effect of differences in the content of proline and hydroxyproline in collagens of different species and is connected with the temperature of the habitat of the animals. The thermal shrinkage, denaturation temperature of collagens and melting temperature of gelatins derived from the skins of cold-water fish are significantly lower than those of collagens and gelatins from skins of warm-blooded animals and fish living in warm waters, due to their lower imino acid content and decreased proline hydroxylation degree (Gilsenan & Ross-Murphy, 2000; Gómez-Guillén et al., 2002; Norland, 1990; Piez & Gross, 1960; Yamaguchi et al., 1976). The differences in ratio of glycine to imino acids and glycine to glutamic acids also exist in gelatins of different origin (Gudmundsson & Hafsteinsson, 1997). The properties of gelatin depend as well on the molecular weight distribution of colagenous components and on the α_1/α_2 ratio (Gómez-Guillén et al., 2002). According to Normand, Muller, Ravey, and Parker (2000) only α, β, and γ components contribute to the elastic properties of gelatin.

As was expected, gelatin solutions from tested skins of fish living in moderate-temperature water gelled at lower temperature than the pigskin gelatin (Fig. 4). Gelatins from skins of salted and marinated herrings formed a gel at 5 °C and from cod skins at about 5.5 °C. According to Gómez-Guillén et al. (2002) gelling of cod gelatin occurred at 12–13 °C. The discrepancy in these data may be caused

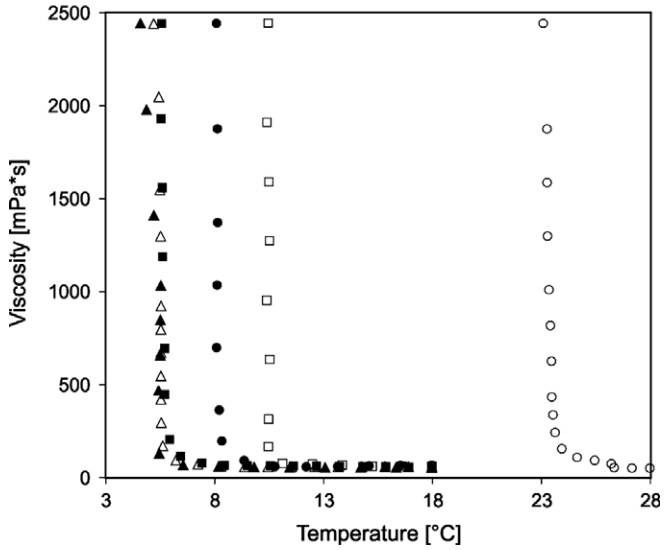

Fig. 4. Gelling temperature of gelatins extracted from different sources: cod skins heated for 45 min at 45 °C (■); fresh salmon skins heated for 45 min at 45 °C (□) and for 45 min at 90 °C (●); skins of salted (Δ) and marinated (▲) herrings heated for 45 min at 45 °C; pigskins (○).

by different conditions used during preparation of gelatin and different methods for determination of gelling temperature of gelatin. As reported by Norland (1990), the gelatin produced from skins of fish living in cold waters formed a gel below 8–10 °C. Gelatin extracted from salmon skins heated for 60 min at 45 °C showed the highest temperature of gelling. The increase in temperature to 90 °C during extraction decreased the gelling temperature of the obtained gelatin by about 2 °C.

4. Conclusions

The skins of semi-processed fish can be a valuable source of gelatin. The most suitable for this purpose are the skins of smoked salmon. This raw material is rich in collagen. Moreover, the collagen in skins after smoking of fish is still susceptible to thermal denaturation. Similarly to fresh salmon skins, about 80% of collagen contained in smoked salmon skins is converted to soluble gelatin after heating of raw material for 60 min at 45 °C. The obtained gelatin is less degraded than gelatins from skins of marinated and salted herrings and its gelling temperature is also higher. On the other hand, collagen from herring skins is completely solubilised in these conditions. The yield of extraction of gelatin from smoked salmon skins can be increased to almost 100% at 100 °C. However, as a result of higher degradation of the main components of collagen, the gelling temperature of the obtained gelatin was lower than that from the process conducted at lower temperature. Heads and backbones can also be used as sources of gelatin. About 70% and 100% of gelatin were obtained, respectively, from cod heads and backbones in a three stage extraction procedure.

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