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Full Length Research Paper

Characterization of the gelatin of the flying gurnard (*Dactylopterus volitans*) and its interaction with starch

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Gelatin extracted from the skin of the flying gurnard (*Dactylopterus volitans L,)* was characterised and its interaction with cassava starch mixtures of different ratio concentrations was investigated by rheology and differential scanning calorimetry (DSC). Protein and moisture were the main proximate composition, whilst glycine was the main amino acid. The gelatin had high bloom (275 g) strength with high imino acids, proline and hydroxyproline, content of 217/1000 residues. Gelation temperatures increased as more starch is used in the mixtures, ranging from 19.0°C for gelatin to starch ratio 4:1 to 70.1°C for mixture 1:4. Inclusion of cassava starch also increased the elastic modulus of the gelatin phase. Therefore, gelatin from flying gurnard skin has a potential to serve as an alternative source of non-bovine or porcine gelatin for food applications.

Key words: Gelatin, amino acids, bloom strength, storage modulus, starch.

INTRODUCTION

Gelatin is widely used in the food industry at an estimated quantity of 200,000 metric tonnes (Choi and Regenstein, 2000). It is derived by hydrolytic degradation of collagen. The functional properties of gelatin are related to their chemical characteristics; molecular weight, number of each kind of amino acid residues, number of polypeptide chains and on the position of the breaks. The gel strength, viscosity, setting behaviour and melting point of gelatin depends on their molecular weight distribution and the amino acid composition. The imino acids, proline and hydroxyproline are important in the denaturation of gelatin subunits during gelling (Johnston-Banks, 1990). Gelatin with high levels of imino acids tends to have higher gel strength and melting point. The molecular weight distribution is also important in determining the gelling behaviour of gelatin. In general the quality of the gelatin depends on the composition of the raw material and factors including the species, breed, age, manner of feeding the animal, storage condition of raw materials and to some extent the manufacturing processes (Hinterwaldner, 1977).

Commercially, gelatin is obtained from mammals like bovine and porcine through acid and alkaline hydrolysis. However, periodic outbreaks of bovine spongiform encephalopathy (BSE) coupled with religious prohibitions have created a demand for alternative sources of gelatin (Choi and Regenstein, 2000). Gelatin can also be obtained from fish skins and bones, which constitute about 30% (Gòmez-Guillèn et al., 2002) of the total weight of side streams obtained after filleting of fish. The side streams constitute about 75% of the total weight of fish during normal fish processing (Shahidi, 1994).

Studies on properties of fish skin gelatins showed that fish gelatins have lower melting and gelling temperatures than those from mammals (Choi and Regenstein, 2000; Fernández-Diaz et al., 2001; Leuenberger, 1991). In addition, gelatin from low temperature fish species contains a lower amount of proline and hydroxyproline; lower level of hydrogen bonds and lower melting points than the species from a tropical environment (Gilsenan and Ross-Murphy, 2000; Arnesen and Gildberg, 2002). However these properties may be modified by the use of gel enhancers like salts, glycerol and variations of pH (Sarebia et al., 2002).

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Production of gelatin from skins and bones of fish could therefore be a very profitable value addition during general fish processing. In Ghana, the flying gurnard (*Dactylopterus volitans*) *(*Linnaeus, 1758) is abundantly harvested during the main fishing season. Unfortunately, it has very little market value as fish for home consumption. It is therefore important to find alternative uses for it. One possible good use will be the production of gelatin from the side streams.

There is a wide variety of foods that contain gelatin and starch and for that matter the properties of gelatin and starch in aqueous systems have been studied extensively, and remain the focus of substantial current research (Ross-Murphy, 1992, 1994; Keetels and van Vliet, 1994). However, it has been known that phase separation occurs in aqueous mixtures of gelatin and starch for various types of starch at concentrations above -1 wt% (Ostwald and Hertel, 1929) and this could possibly affect the functional properties of gelatin in starchy foods. Cassava starch has high amylose content, usually in the range of 13.6 to 19.1% (Defloor et al., 1998) which helps it to retrograde easily at room temperature (Thomas and Atwel, 1999) and therefore helps in stabilization/ solidification of other food components added to it. It is expected this potential of cassava starch will tremendously assist in the transforming of the gelatin from the flying gurnard into a semi-solid form which helps in food preparation and packaging

Unfortunately, there is paucity of information of the interactions of the starch and gelatin obtained from this fish skin. This study therefore concerns the characterisation of the gelatin from the skin of the flying gurnard and how it interacts with cassava starch.

MATERIALS AND METHODS

Materials

Freshly landed flying gurnard fish (*D. volitans. L)* was purchased from fishmongers at the canoe landing beach of Tema in Ghana. The fish were held in ice at –30°C. Fish skin was removed from the thawed flying gurnard.

Methods

Gelatin preparation

Gelatin was prepared from the skins of the flying gurnard according to the method of Gudmundsson and Hafsteinsson (1997).

Proximate composition and amino acid analysis

The moisture, crude fat, ash, colour and pH were determined according to the methods outlined in the Gelatin Manufacturers of Europe Monograph (GME) version 1, July 2000. Protein digestion was by methods of to ensure complete hydrolysis of collagen (Eastoe and Eastoe, 1952). The gelatin was hydrolysed with 6 N HCl (20 mL) at 110°C for 24 h to yield amino acids. The hydrolysed samples and amino acid standards (20 µL) were derivatized with phenylisothiocyanate (PITC) according to the Waters Pico-Tag method (1986). The amino acids were then were measured in triplicate by HPLC (Bildlingmeyer et al., 1987).

Bloom strength

A gelatin concentration of 6.67% (w/v) was prepared and brought up to 22°C for 3 h. Then after it was brought to 60°C (but not exceeding) on a magnetic heater stirrer for approximately 15 min, for the gelatin to dissolve completely and immediately introduced into standard bloom jars and covered after 2 min. The bloom jars were left to condition for 16 h in a water bath at 10°C. The bloom jars were centrally placed under a 0.5 mm Radius Cylinder Probe attached to a texture analyser (TA-XT2*i*) Stable Micro Systems. The bloom strength of the gelatin was determined according to the method described by Stable Micro Systems, in accordance with GME 2000.

Interaction studies with cassava starch

Samples of the flying gurnard gelatin and cassava starch were mixed in the ratio 0:10; 1:4; 1:1; 4:1 and 10:0 (w/v in distilled water) and subjected to rheological and differential scanning calorimetry (DSC) studies to evaluate the components interactions for potential development of products. These ratios were selected since phase separation occurs in aqueous mixtures of gelatin and starch at starch concentrations above -1 wt% (Ostwald and Hertel, 1929).

Rheological measurements

The blended samples of gelatin and cassava starch at the various proportions were subjected to small-deformation oscillatory measurements with a Rheumatics controlled stress rheometer and a 40 mm parallel plate geometry with a gap of 0.3 mm and frequency of 1 radian per second, to obtain sufficient data without compromising the measurements of entanglements. A dynamic temperature sweep was performed between 20 and 2°C at a rate of 1°C /min and then heated to 20°C. The applied stress was 1 Pa to keep the oscillatory strain at about 1%, sufficiently low to ensure the measurements were within the linear viscoelastic region (Hamann, 1991). The sample was surrounded by silicone oil to prevent evaporation of solvent during the temperature sweep. The viscoelastic properties of the gels measured included the storage modulus (G') and loss modulus (G") as a function of time within the linear viscoelastic range of the gels (Mitchell, 1980). The cross-over point G'/G" at which gel formation occurred and the gelation temperature were noted.

Differential scanning calorimetry (DSC) measurements

Eight hundred milligrams of gelatin samples as prepared for the rheological studies were placed in a pre-weighed DSC cell (Setaram Micro DSC VII). An equal weight of distilled water was also introduced into the reference cell to obtain a flat base line. A temperature scan of 2 to 90°C and heating rate of 0.5 °C/min were maintained through out the study. Heat absorbed or released by the transformation of the samples resulted in an endothermic or

Table 1. Proximate and amino-acid compositions of the flying gurnard gelatine.

Composition	Constituents	% Amount
Proximate	Moisture	9.7±1.4
	Crude Fat	0.2±0.05
	Ash	0.9±0.2
	Protein	89.6±1.9
Amino-Acid	Aspartic	3.57±0.43
	Glutamic	6.30±0.72
	Hydroxyproline	7.04±0.49
	Serine	2.93±0.2
	Glycine	28.43±2.21
	Histidine	0.76±0.02
	Arginne	4.28±0.37
	Threonine	2.58±0.28
	Alanine	22.57±2.13
	Proline	14.70±0.85
	Tyrosine	0.31±0.00
	Valine	1.98±0.20
	Methionine	1.45±0.06
	Cystine	0.25±0.08
	Isoleucine	0.87±0.09
	Leucine	1.97±0.15
	Phenylalanine	1.95±0.20
	Trptophan	4.09±0.37
	Lysine	3.64±0.21

Values are means of three determinations ± s.d

exothermic peak as a function of temperature. The transition temperature (T_m) was measured at the tip of the peak. Peak areas showing transition temperatures and enthalpies (ΔH) were calculated automatically by integrating the area under the peak. Each sample was scanned in triplicate.

RESULTS AND DISCUSSIONS

Table 1 show that the proximate composition of the gelatin is mainly made up of protein and water. The low ash content makes its suitable for use in food applications (Jones, 1977). Table 1 also indicates that the key amino acids in the gelatin of the skin of the flying gurnard are alanine, glycine and proline. In particular, the 28.4% of glycine in gelatin of the skin of the flying gunrand compares favourably with 31.6 and 31.13% found in the horse mackerel and the commercial gelatin, respectively (Muyonga et al., 2004).

Proline and hydroxyproline residues, referred to as the imino acid content of the gels, were expressed as the number of the residues in 1000. This was found to be 217/1000 residues. These are comparable to the residues of commercial gelatin but significantly different

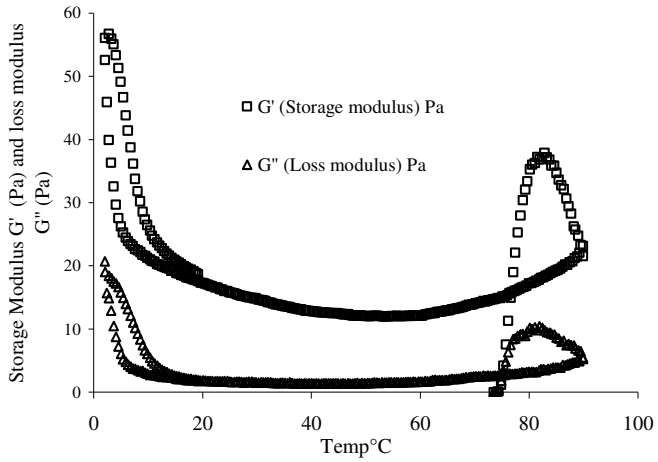

Figure 1. Gelation profile of flying gurnard skin gelatin with varying proportion of cassava starch (ratio) during a dynamic temperature ramp.

from those of the horse mackerel residues. The imino acid content is important in the re-naturation of gelatin subunits during gelling as the turns of the helix and the stability of the triple helix structure of the gelatin depend largely on these acids (Johnston-Banks, 1990). As a result, gelatin with high levels of imino acids tends to have higher gel strength and melting point. The bloom gel strength of the gelatin of the flying gurnard fish skin was estimated as 275 g. In comparison, the bloom strength of gelatin of catfish is 150 g whilst that of commercial pig gelatin is 290 g (Muyonga et al., 2004).

Interaction studies with cassava starch

Rheological studies

Figure 1 shows a typical profile obtained for the gelation of the gelatin with the cassava flour as a function of temperature. The profiles for the various mixtures followed similar trends. All the gel mixtures showed marked increase in G' and G" values respectively after 79ºC but these values dropped before reaching 90ºC during indicating a weakening of the gel structure (Tsai et al., 1997). The storage modulus and loss modulus of the mixtures (Table 2) show that at 90ºC there was a drop of viscosity and loss of rigidity of gelatin : starch (10:0) but upon cooling to 20ºC high values of G' (2530 Pa) and G" (261 Pa) were obtained. Similar observations were made for the other mixtures with the exception of the 1:1 gelatin: starch mixture which had a significantly ($P<0.05$) decrease in value of G' (22 Pa) and G" (5.4 Pa) at 90ºC to G' (16 Pa) and G" (1.7 Pa) on cooling to 20ºC respectively. These observations are in line with the fact that the viscosity of gelatin decreases with temperature.

Table 2. Storage modulus (G' Pa) and loss modulus (G" Pa) of mixtures of the Flying Gurnard gelatin and cassava starch during a dynamic temperature sweep at 90ºC and 20ºC*

Composition Gelatin: cassava starch	90 ºC		20 ºC	
	G'	G"	G'	G"
10:0	0.01±0.00	0.01±0.00	2530±191	261±29
4:1	0.05±0.02	0.17±0.04	74.9±8.25	21.7±13.97
1:1	22.2±0.94	5.4±0.18	16.1±3.71	1.7±0.21
1:4	101.8±13	30.4±8.1	156±14	28.5±8.17
0:10	246±22	84.3±6.50	729±5	99.2±15.00

* Values are means of three determinations ± s.d.

Table 3. Transition temperatures and enthalpy change mixtures of the Flying Gurnard gelatin and cassava starch.

Composition gelatin: flour	Transitions	Onset Temp (T_o) ºC	Peak Temperature (T_m) ºC	Enthalpy/ J/G (ΔH)
10:0	1	8.98	18.89	2.95
4:1	1	12.70	19.02	0.17
	2	43.31	50.66	0.03
	3	65.01	69.69	0.97
1:1	1	27.41	29.74	0.005
	2	51.35	44.44	0.0007
	3	65.35	70.07	0.313
1:4	1	48.17	53.83	0.0005
	2	65.61	69.71	0.52
0:10	1	63.82	69.47	0.67

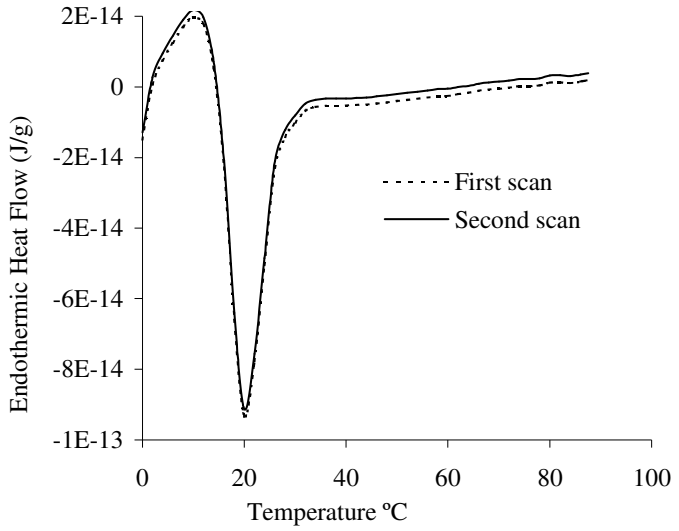

Figure 2. DSC thermograms of the gelatin of the flying gurnard skin scanned from 0 to 90ºC cooled and rescanned.

This explains why gels are easily formed at lower temperatures (Ferry, 1948). The increase in viscosity during the cooling period may be attributed to the various constituents present and in particular the swollen granules and fragments which associate or retrograde as a result of molecular interactions (Hoover, 2001). The changes in the gelation temperatures as shown in Table 2 suggest some interactions between the gelatin and the starch granule and are attributed to charges on the proteins and starch (Takeuchi, 1969). With increasing starch concentration, the cross-link density in the gel may have increased, yielding higher values of G'. A number of studies have elucidated this mechanism of interaction and the main effect of the starch component is to raise the modulus of the gelatin phase. This property could be employed in food applications (White et al., 1993).

Differential scanning calorimetry (DSC)

The thermograms for the flying gurnard gelatin gel are shown in Figure 2. The transition temperature and enthalpy change for gelatin from flying gurnard were found to be 18.89ºC and 2.95 J/g, respectively. The enthalpy reflected the amount of energy required to denature the samples and is related to the number of junction zones

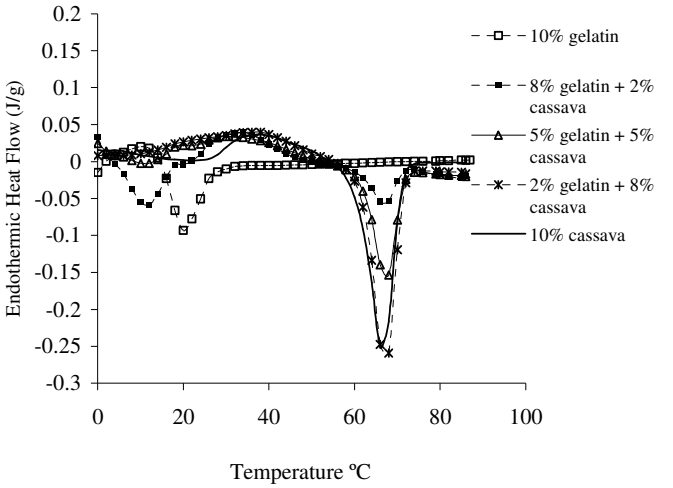

Figure 3. DSC thermograms for mixtures of gelatin of the flying gurnard gelatin and cassava starch scanned from 0 to 90ºC.

involved in the gelation of each sample (Arnesen and Gildberg, 2002). Figure 3 also shows that all the thermograms of the gelatin and cassava flour mixtures were endothermic. The DSC thermograms show that starch on its own (0: 10) indicated a single transition (T_m values 69.5 ºC). However when mixed with gelatin, three thermal transitions (T_m values of 19.0 ºC; 50.7 ºC; 69.7 ºC) and (T_m values 29.7ºC; 44.4ºC; 70.1ºC) were respectively obtained for gelatin and starch mixtures 4:1 and 1:1. However the 1:4 (gelatin: starch mixture), indicated two transitions (T_m values of 53.8°C; 69.7ºC). These results tend to offer support to the type of interactions observed in the rheological studies in this report. The mixtures which show interaction with the starch may enhance the potential of using a mixture of gelatin and cassava starch in food applications where such properties exhibited by the mixtures are required.

Conclusions

Gelation properties of the flying gurnard gelatin especially the high bloom strength and high melting point indicate that fish gelatin could be used to replace mammalian gelatins in some food applications without significantly altering the texture. The changes in gelation temperatures when fish gelatin was mixed with cassava starch due to weak electrostatic interactions confer textural changes and melting point close to that of mammalian gelatins which would be an added advantage in food applications. Developing gelatin from the flying gurnard skin would reduce the waste skin which would otherwise be discarded, to produce a value added product that may be of economic benefit to the fish industry in Ghana.

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