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Effects of cross-linking in nanostructure and physicochemical properties of fish gelatins for bio-applications

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ABSTRACT

The development of advanced materials from proteins requires their modification to improve functional properties, mainly barrier properties. In order to obtain the improvement of properties desirable for some bio-related uses, such as food, pharmaceutical and biomedical applications, Maillard reaction, a natural and non-enzymatic reaction between proteins and sugars, can be promoted and controlled by sugar contents and heating treatments. In this paper, Maillard reaction improves the barrier properties of fish gelatin films, providing an excellent protection against UV light and decreasing solubility due to the cross-linking induced by heating and lactose addition. Furthermore, a fluorescence compound is formed during this reaction and can be used as an indicator of the reaction progress. The knowledge of reaction kinetics as well as the changes observed in the nanostructure as a consequence of the cross-linking between fish gelatin and lactose provides the scientific information needed to spread the field of application of the products that can be manufactured using these natural reactive polymers.

1. Introduction

Growing environmental concerns have led to increase the emphasis on research and development of biodegradable materials based on renewable resources for biomedical and industrial applications. Biopolymers such as collagen or gelatin, chitin or chitosan, soy protein, starch, or cellulose are used to prepare composite films and coatings. Among them, gelatin is a natural hydrocolloid derived from pig, bovine and fish connective tissues, skin and bones and has many applications in pharmaceutical, biomedical, food, photographic and cosmetic industries. Gelatin is a protein derived from collagen trough an acid (type A-gelatin) or alkali (type B-gelatin) hydrolysis. The conversion of collagen into soluble gelatin has been described as a denaturation process where the triple-helix conformation is lost due to the destruction of intra- and inter-molecular cross-links [1–4].

The microstructure and physical performance of gelatin can be modified by cross-linking and thus, properties can be adjusted to specific uses, such as food, medical, and industrial applications. Chemical cross-linking methods typically use chemicals like aldehydes, which interact with the functional groups of proteins, such as the amino function in lysine and hydroxylysine or the carboxyl group in aspartic and glutamic acids. Additionally, physical cross-linking methods such as heating, drying, and irradiation are also commonly applied to proteins. Specifically, heating in the presence of sugars can alter the conformation and interactions within proteins, leading to a natural and complex cross-linking process, known as Maillard reaction or non-enzymatic glycation [5]. The initial step of the Maillard reaction is well-characterized. A non-enzymatic condensation reaction takes place between the carbonyl group of the reducing sugars, which may exist in open-chain aldehyde form, and the amino group of proteins, forming an unstable Schiff base with release of water. The Schiff base is subsequently transformed via the Amadori rearrangement into the protein-bound Amadori product [6,7]. The initial form of the Amadori product is subjected to breakdown and antioxidant brown pigments, known as Maillard reaction products (MRPs) or melanoidins, are generated [8–10]. However, the Amadori compound follows diverse ways toward the formation of advanced glycation end-products (AGEs), which are still not well-known. Since some products can be fluorescent and used as indicators or markers of the Maillard reaction extension, this reaction has been investigated in medical and food sciences in relation to aging and illnesses [11]. The main variables affecting the extension of the Maillard reaction are temperature, time, initial pH, carbonyl/sugar ratio, and water activity [12–14], so analyzing these factors allows controlling the extension of the reaction in order to obtain the properties required for a specific application, preventing the formation of non-desirable compounds and expanding the use of proteins for many applications.

Although fish gelatins are being increasingly researched as alternative materials to mammalian gelatins, the progress of Maillard reaction by UV–visible (UV–vis) spectroscopy, the formation of fluorescent compounds as AGEs, and the analysis of the protein secondary structure during the reaction by Fourier transform infrared (FTIR) spectroscopy have not been previously reported in the literature. In this context, the aim of this study was focused on the effect of lactose content and heating time on the morphology and physical performance of cross-

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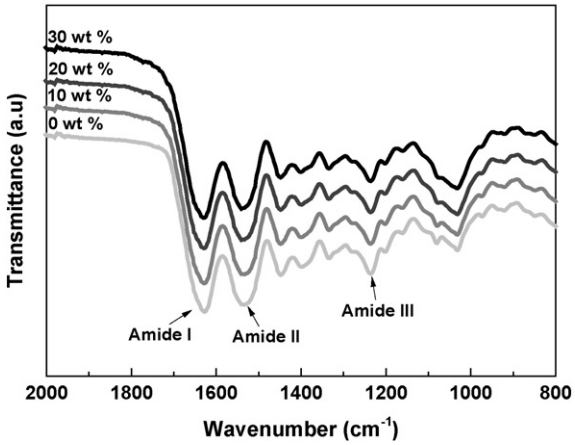

Fig. 1. FTIR spectra of fish gelatin films as a function of lactose content.

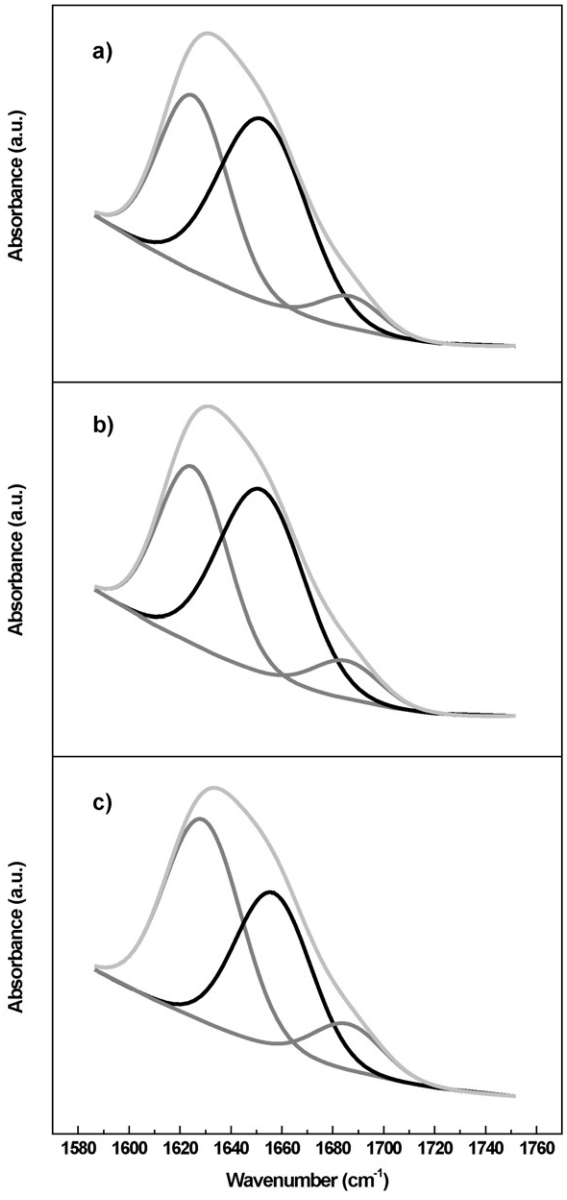

Fig. 2. Curve fitting spectra of amide I for fish gelatin films with 30 wt.% lactose content as a function of heating time: a) 0 min, b) 150 min, and c) 1440 min.

Table 1
Resulting percentage of the curve fitting of amide I as a function of lactose content and heating time.

Lactose (wt.%)	Time (min)	Area amide I		
		1625 cm^{-1}	1651 cm^{-1}	1688 cm^{-1}
10	0	35.34	59.09	5.57
	90	38.54	52.23	9.23
	150	39.87	50.21	9.92
	210	42.21	47.74	10.05
	270	50.43	39.26	10.31
	1440	50.24	40.15	9.62
20	0	35.12	59.73	5.15
	90	37.12	57.11	5.77
	150	41.32	50.14	8.54
	210	51.23	39.38	9.39
	270	52.12	38.12	9.76
	1440	53.87	36.77	9.36
30	0	34.89	60.09	5.02
	90	40.23	52.89	6.88
	150	49.54	41.23	9.23
	210	50.12	40.23	9.65
	270	50.97	39.14	9.89
	1440	52.18	38.49	9.33

linked fish gelatin films in order to select the most appropriate composition to develop technically valuable products.

2. Experimental

2.1. Materials

A commercial cod fish gelatin type A was employed in this study. It has bloom 200, 11.06% moisture and 0.147% ash. Fish gelatin was kindly supplied by Weishardt International (Liptovsky Mikulas, Slovakia) and meets the quality standard for edible gelatin (1999/724/CE). Glycerol and lactose were food grade and were used as a plasticizer and a cross-linking agent, respectively; both additives were obtained from Panreac (Barcelona, Spain).

2.2. Film preparation

Fish gelatin films with different lactose contents (10, 20 and 30 wt.% on gelatin dry basis) were prepared by casting. Firstly, 5 g gelatin and the amount of lactose required for each composition were dissolved in 100 mL distilled water for 30 min at 80 °C under continuous stirring to obtain a good blend. After that, 10 wt.% glycerol (on gelatin dry basis) was added to the solution, pH was adjusted to 10 with NaOH (1.0 N) and solution was maintained at 80 °C for other 30 min under stirring. Then, the solution was poured into Petri dishes and kept at room temperature during 48 h to evaporate water and form the film. Finally, all films were conditioned in a controlled bio-chamber (ACS Sunrise 700 V) at 25 °C and 50% relative humidity for 48 h before testing.

Table 2
Total soluble matter (TSM) values of fish gelatin films as a function of lactose content. Mean values followed by the same letter are not significantly ($P > 0.05$) different thought the Tukey's multiple range test.

Lactose (wt.%)	Time (min)	TSM (%)
10	0	100^{a}
	150	61.9 ± 0.2^{b}
	270	31.0 ± 0.7^{c}
	1440	16.0 ± 2.4^{d}
20	0	100^{a}
	150	52.2 ± 0.8^{f}
	210	33.1 ± 1.1^{c}
	1440	13.0 ± 4.0^{e}
30	0	100^{a}
	150	33.1 ± 0.9^{c}
	1440	12.8 ± 3.3^{e}