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Effect of pH and lactose on cross-linking extension and structure of fish gelatin films

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ABSTRACT

Lactose-modified gelatin films were prepared at two different pHs and then, films were heated to promote the cross-linking reaction between gelatin and lactose. All films were transparent and homogeneous with hardly any pores (8–12%).The extension of the cross-linking reaction was assessed by means of film colour, solubility, and the formation of a fluorescence compound (pentosidine). It was observed that both pH and lactose affected the extension of the cross-linking reaction between the carbonyl group in lactose and the amino groups in gelatin. Films processed at native pH (5.4) showed higher decolouration and lower solubility, highlighting a further extension of cross-linking at this pH than at pH 2.0. Furthermore, the curve fitting of amide I and II profiles indicated that the secondary structure of gelatin films was also affected by lactose and pH.

1. Introduction

Environmental concerns have led to reinforce the emphasis on research and development of biodegradable materials based on renewable resources for food industries. Proteins (gelatin, soy protein), polysaccharides (chitosan, cellulose, starch) and mixtures of them are good alternatives in order to prepare composite films and coatings for a wide variety of applications. Among them, gelatin global use has notably increased in the last years [1–3].

Gelatin is a natural hydrocolloid derived from pig, bovine and fish connective tissues, skin and bones [4–5]. Its demand has considerably increased for applications such as food packaging [6], membranes [7], and controlled release of active compounds [8,9]. In fact, gelatin shows functional properties such as film forming ability and biodegradability, features that promote its application in food and pharmaceutical industries. However, materials based on gelatins show some drawbacks due to their hydrophilic nature which limit their application. The improvement of properties can be achieved by means of protein cross-linking [10,11]. In fact, heating in presence of sugars can alter conformations and interactions within proteins, leading to a complex cross-linking reaction, known as Maillard reaction or non-enzymatic glycation [12]. Although much has been achieved in the knowledge of this reaction, many challenges still remain in order to understand how it affects materials' properties and, thus, to control its extension in order to obtain the required properties for specific applications.

Maillard reaction is a condensation reaction that takes place between the carbonyl group of sugars, which may exist in the open-chain aldehyde form, and the amino group of lysine residues in proteins, forming an unstable Schiff base with release of water. Subsequently, this Schiff base is transformed into the protein-bound Amadori product. At this stage of the reaction, no colour is produced [13,14]. However, in the advance stage, the Amadori product breakdowns and gives advanced glycation end products (AGEs), brown compounds with high molecular weight, high reducing potential, and strong antioxidant capacity [15,16]. The chemical decomposition route depends on the medium pH since it affects protein charge as well as the degree of protein denaturation and aggregation [14,17]. Each route leads to different reactive intermediates, whose transformation gives products which present some colouration or even fluorescence and, thus, they can be used as indicators of the Maillard reaction extension.

The extension of Maillard reaction and the type of Maillard reaction products (MRPs) formed depend on temperature, time, carbonyl/sugar ratio and pH [18,19]. The effect of those factors at pH 10 was analyzed in a previous work [20]. Also, a preliminary research was carried out at different pHs to determine the relevance of controlling the extent of cross-linking and design films with tailored properties [21]. In this work, the aim is focused on the effect of lactose and pH on the extension of Maillard reaction (assessed by means of some reaction markers, such as colour formation, protein solubility, and fluorescence compounds) and, consequently, on the morphology and structure of gelatin films.

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2. Materials and methods

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 plasticizer and cross-linking agent, respectively; both additives were obtained from Panreac (Barcelona, Spain).

2.2. Film preparation

Gelatin films with different lactose contents (10, 20 and 30 wt% on gelatin dry basis) were prepared by casting. Firstly, 5 g of gelatin and the amount of lactose required for each formulation were dissolved in 100 mL of distilled water for 30 min at 80 °C under continuous stirring. After that, 10 wt% glycerol (on gelatin dry basis) was added to the solution, the pH was adjusted to 2.0 with 1 N HCl, and the blend was maintained at 80 °C for other 30 min under stirring. Films were also prepared without modifying the solution pH (5.4), named native pH. Afterwards, film forming solutions were poured onto Petri dishes and left drying for 48 h at room temperature. These films were used as reference films to measure colour difference. Finally, films were heated at 105 °C for 24 h to promote glycation and carry out experiments, except fluorescent measurements, in which the heating process was carried out during the assay. All films were conditioned in a controlled bio-chamber (ACS Sunrise 700 V, Madrid, Spain) at 25 °C and 50% relative humidity for 48 h before testing.

2.3. Film thickness

Film thickness was measured to the nearest 0.001 mm with a hand-held QuantuMike digimatic micrometer (Mitutoyo Spain, Elgoibar, Spain). Five measurements at different positions were taken on each sample. Thickness values were in the range of 48–54 μm in all cases.

2.4. Colour measurement

Colour was determined with the CR-400 Minolta Croma Meter colorimeter (Konika Minolta, Valencia, Spain). Films specimens were placed on the surface of a white standard plate (calibration plate values $L^* = 97.39$, $a^* = 0.03$ and $b^* = 1.77$) and colour parameters L^*, a^*, b^* were measured using the CIELAB colour scale: $L^* = 0$ (black) to $L^* = 100$ (white), $-a^*$ (greenness) to $+a^*$ (redness), and $-b^*$ (blueness) to $+b^*$ (yellowness). Colour difference (ΔE^*) of films as a function of lactose content was calculated as follows:

$$\Delta E^* = \sqrt{(L^*_{sample} - L^*_{reference})^2 + (a^*_{sample} - a^*_{reference})^2 + (b^*_{sample} - b^*_{reference})^2}$$

where sample refers to the films heated to promote reaction and reference is related to the films before heating.

2.5. Fluorescence spectroscopy

Fluorescence emission measurements were performed with the spectrophotometer of Photon Technology International (PTI) with the controlled cuvette Holder TLC50 (Quantum North West) to heat the films at 105 °C. Gelatin films were excited at 335 nm and emission spectra were recorded from 360 to 650 nm, using FeliX32 software.

2.6. Total soluble matter (TSM)

The films were weighed (m_0) and immersed into 30 mL of distilled water in the presence of sodium azide (0.02 g/100 mL) in order to prevent microbial growth. Flasks were stored in an environmental chamber at 25 °C for 24 h with occasional gentle stirring. After that, specimens were dried in an air-circulating oven at 105 °C for 24 h and weighed (m_f). TSM was expressed as the percentage of film dry matter

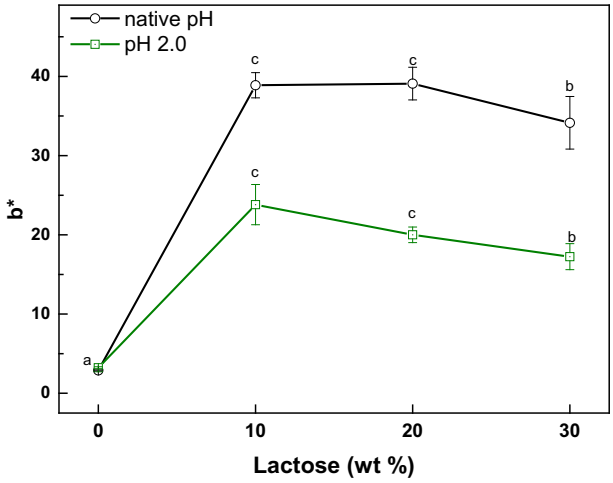

Fig. 1. b^* values of gelatin films as a function of pH and lactose content. Two mean values with the same letter in the same curve are not significantly ($P > 0.05$) different.

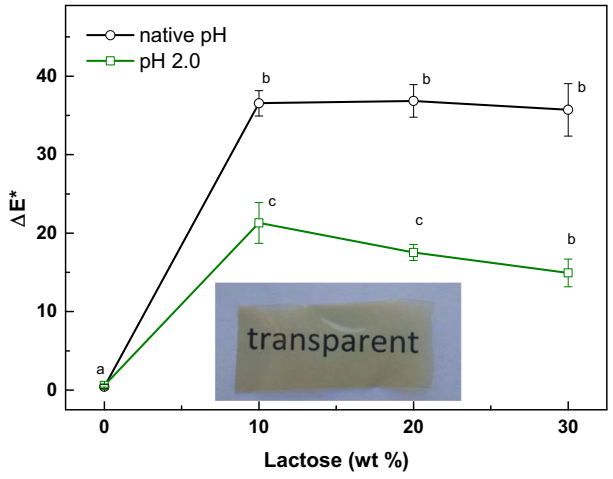

Fig. 2. ΔE^* values of gelatin films as a function of pH and lactose content. Two mean values with the same letter in the same curve are not significantly ($P > 0.05$) different.

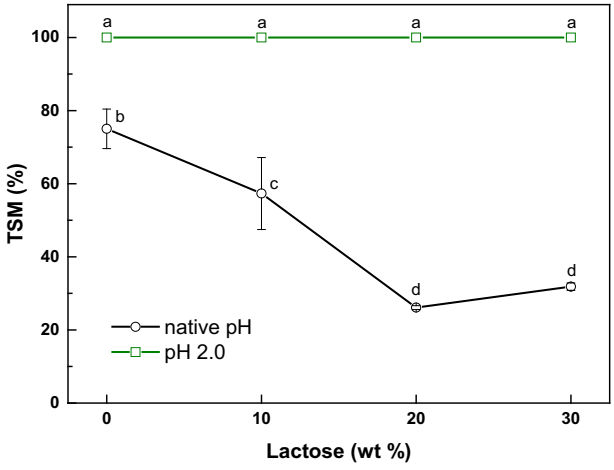

Fig. 3. Total soluble matter (TSM) of films as a function of lactose content and pH. Two mean values with the same letter in the same curve are not significantly ($P > 0.05$) different.