Food Chemistry 175 (2015) 478–484

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of physical state of gelatin-plasticizer based films on to the occurrence of Maillard reactions

N. Riquelme [a], P. Díaz-Calderón [b], J. Enrione [b], S. Matiacevich [a,*]

[a] Food Properties Research Group, Departamento de Ciencia y Tecnología de los Alimentos, Facultad Tecnológica, Universidad de Santiago de Chile, Obispo Umaña 050, Estación Central, Santiago, Chile

[b] Biopolymer Research and Engineering Lab (BiopREL), Escuela de Nutrición y Dietética, Facultad de Medicina y Escuela de Administración de Servicios, Universidad de los Andes, Monseñor Álvaro del Portillo 12455, Las Condes, Santiago, Chile

ARTICLE INFO

Article history:
Received 11 June 2014
Received in revised form 1 December 2014
Accepted 3 December 2014
Available online 9 December 2014

Keywords:
Bovine gelatin
Salmon gelatin
Plasticizers
Glassy state
Maillard reaction

ABSTRACT

The aim of this study was to evaluate the occurrence of the Maillard reaction on gelatin-based films (bovine and salmon) in the glassy state, in mixtures with low molecular weight plasticizers (e.g. glycerol, glucose and trehalose) at different storage times. For testing, the gelatin-plasticizer films were stored under glassy conditions (Tg-10 °C), previously determined by calorimetric tests. Studies under accelerated conditions ($T \gg \mathrm{Tg}$) were also developed. Color, opacity and browning index (Br) were evaluated by computer vision at all storage times. Results showed in samples stored under glassy conditions that the Maillard reaction did not occur, independent of gelatin origin and type of plasticizer. Changes in color stated by opacity and Br were only significant ($p < 0.05$) in gelatin–glucose systems under accelerated storage conditions. The inhibition of reaction in gelatin films in the glassy state was related to the well-known conditions of low molecular mobility of glassy matrices, but also with the non-Maillard reactive characteristics of glycerol and trehalose.

1. Introduction

Gelatin is a hydrocolloid widely used in food, pharmaceutical and biomedical industries due to its well-known properties of viscoelasticity, thermo-reversibility and biocompatibility that make it a very versatile material (Karim & Bhat, 2009). Gelatin is obtained through the partial hydrolysis of collagen under acidic-alkaline conditions (Badii & Howell, 2006). Normally gelatin is obtained from mammal sources (e.g. pig or bovine), however recently some studies have been oriented to obtain gelatin from alternative sources, such as fish or other marine specimens. The latter is due to cultural and religious reason and also due to the bovine spongiform outbreak in the 90s (Rahman, Al-Saidi, & Guizani, 2008; Karim & Bhat, 2009). However, gelatin from marine source has significant differences in rheological, mechanical and thermal properties compared with those from mammal origin (Gómez-Guillén et al., 2009; Gómez-Estaca, Montero, Fernández-Martín, & Gómez-Guillén, 2009; López, Enrione, & Matiacevich, 2014), which have been directly correlated with differences in molecular weight and amino acid profile (lower content of proline and hydroxiproline than mammal gelatin) (Díaz-Calderón, López, Matiacevich, Osorio, & Enrione, 2011).

Nowadays, technological alternatives to add value to gelatin are in the field of functional compounds encapsulation such as vitamins, fatty-acids, flavors, drugs or probiotics in order to protect them from the contact with oxygen and water and in the development of controlled release systems (Karim & Bhat, 2008; Elzoghby, 2013). These modern applications require the use of biomaterials in the glassy state, however below the glass transition temperature (Tg) biomaterials have the disadvantage to be highly brittle, so during processing, handling and/or storage, the matrix can be affected by failures (breaks or cracks) that can produce a non-controlled release or the migration of surrounding molecules (e.g. oxygen, water) allowing the loss of functionality of the capsule (Vanin, Sobral, Menegalli, Carvalho, & Habitante, 2005). In order to solve this problem, gelatin matrix can be modified by the addition of low molecular weight compounds or plasticizers, such as glucose, glycerol, sorbitol among others. The use of these plasticizers has been related with changes in mechanical, thermal, sorption and transport properties of the polymer system (Vanin et al., 2005; Cao, Yang, & Fu, 2009; Al-Hassan & Norziah, 2012). However, recent studies carried out using the technique of Positron Annihilation Lifetime Spectroscopy (PALS) have also shown that presence of plasticizers modifies the structure of the polymer matrix at the nanoscale (Roussenova, Murith, Alam, & Ubbink, 2010; Roussenova et al., 2012). This behavior has been related with the ability of low molecular weight compounds to act as an enhancer of molecular packing of matrix in the glassy state

---

* Corresponding author. Tel.: +56 2 2718 4517.
E-mail address: silvia.matiacevich@usach.cl (S. Matiacevich).

http://dx.doi.org/10.1016/j.foodchem.2014.12.008
0308-8146/

or anti-plasticizer, and has been reported in maltose-maltopolymer (Townrow, Roussenova, Giardiello, Alam, & Ubbink, 2010), glycerol-maltopolymer (Roussenova et al., 2010), glycerol–gelatin and sorbitol–gelatin systems (Roussenova et al., 2012). The enhancement of molecular packing is observed through the reduction of hole volume size (V_h, Å^3), defined as those local free spaces localized between polymer chains due to irregular packing and the existence of static and dynamic structural disorder characteristic of amorphous systems (Roussenova et al., 2012).

The ability of plasticizers to act as enhancers of molecular packing suggest an explanation to changes in sorption and transport properties observed in polymer-plasticizer systems. Thus, for the more densely packed matrices (containing plasticizer, which acts as anti-plasticizers), there is less local free volume available to accommodate water molecules leading to a lower water content, and more difficult should be for oxygen cross through a polymer based barrier (Roussenova et al., 2010). Furthermore, changes observed at the nanoscale open interesting discussions about the role of the structure and physical state of plasticized polymer matrices and its effect onto the occurrence of some reactions related with chemical stability during the storage. One of the most common approaches in terms of the assessment of chemical stability is study the occurrence of Maillard reaction.

The Maillard reaction is part of reactions called non-enzymatic browning because the end products are brown colored melanoidins and can be considered as one of the most important chemical reaction in food science and technology. Briefly, the reaction starts with the addition of a non-protonized amine group to the electrophilic carbonyl group of a reducing sugar (aldosyl condensation), which is followed by a series of complex reactions (rearrangement, enolyzation, dehydration, degradation, among others) resulting in a mixture of compounds responsible for the typical flavor and color of brown products (Buera, Petriella, & Lozano, 1986). Moreover nutritional value and safety of foods may decrease due to loss of valuable compounds or formation of some mutagenic and carcinogenic compounds (Matiacevich, Santagapita, & Buera, 2005).

Although some strategies have been addressed to control, avoid or promote the occurrence of Maillard reaction (pH, temperature, water activity, sulfites) in certain foods, so far the effect of physical state of matrix is not completely elucidated, specifically in amorphous systems in the glassy state.

In glassy conditions the molecular motion is mainly local and restricted to rotational and vibrational mobility due to the high viscosity of the system (10^{14} Pa s) (Slade & Levine, 1995). Since the high viscosity reduces the encounter between reactants the Maillard reaction kinetic is significantly reduced (Rahman et al., 2008). Indeed Karmas, Buera, and Karel (1992) reported a lower rate reaction at $T < \mathrm{Tg}$ in model systems (amino acid-carbohydrate) and dehydrates vegetables. However, the occurrence of Maillard reaction in glassy food matrices has been also clearly demonstrated. Schebor, Buera, Karel, and Chirife (1999) reported the browning development in polymeric matrix (gelatinized starch, maltodextrins and polyvinylpyrrolidone) and skim-milk powder samples, stored below the glass transition temperature. Results suggest that Tg cannot be considered as an absolute parameter of food stability. Although the effect observed was not very significant, the densification of the matrix upon relaxation seems to be the responsible of slower rate reaction. The rotational mobility at molecular level, the aging of glassy materials and diffusion through pores or defects of the glasses may explain the occurrence of chemical reactions in the glassy state (Schebor et al., 1999). Additionally, the structural characteristic of amorphous materials should also be considered, due to micro and nano-heterogeneity with low-density regions localized between high-density regions (Liu, Bhandari, & Zhou, 2006) allows micro-environments, where the reactants can encounter each other without physical restrictions.

As part of a comprehensive and fundamental research focused on the study of addition of low molecular weight compounds (polyols and carbohydrates) as modifiers of the structure of gelatin films (bovine and salmon origin) at macro and nanoscale, and its effect on the structural relaxation kinetic of material, the objective of this work was to analyze the role of the physical state of polymer matrix on the occurrence of Maillard reaction in gelatin-plasticizer films stored below and above the glass transition temperature during a define time of isothermal storage.

2. Materials and methods

2.1. Materials

Commercial bovine gelatin was used in this study (type B, Bloom 220, Rousselot, Brazil) while salmon gelatin was obtained from skins of Atlantic salmon (*Salmo salar*) specimens by an acidic-alkaline extraction following the method proposed by Zhou and Regenstein (2005). Further details about extraction protocol can be found in the study described by Díaz-Calderón et al. (2011). Previous studies performed by Díaz-Calderón et al. (2011) allowed determining the molecular weight (MW) of gelatins by electrophoresis (SDS–PAGE), which resulted in salmon gelatin presenting α-chains ~95 kDa and β-chains ~195 kDa, where as bovine gelatin presented α-chains ~135 kDa and β-chains ~210 kDa.

2.2. Preparation of films

Gelatin films were prepared by hot casting (50 °C) from gelatin suspensions prepared at 7% w/v using distilled water as solvent. Sugars and polyol of different molecular weight (MW) were used as plasticizers: glycerol (Merck, Germany, MW: 92.10 g/mol), glucose (Arquimed, Chile, MW: 180.16 g/mol) and trehalose (Blumos, Chile, MW: 342.29 g/mol). Plasticizers were added at 0%, 2% and 10% w/w (dry basis). Suspensions were continuously stirred (Barnstead, SP18420-26) at 50 °C for 30 min, and pH was adjusted to 5.0 with HCl or NaOH 0.05 M (Merck, Darmstadt, Germany) using a pHmeter (Jenway 3505, UK). This pH level was chosen to be consistent with previous studies performed using this both gelatin (Díaz-Calderón et al., 2011) and also due to the well-know pH effect on reaction rate (Matiacevich et al., 2005; Matiacevich & Buera, 2006; Matiacevich, Santagapita, & Buera, 2010). Suspensions were after deposited on Petri dishes (40 mL in volume) and held at 50 °C in a horizontal flow oven (WiseVen WOF-105, Daihan Scientific, Seoul, Korea) for at least 24 h, in order to promote the formation of an amorphous structure in the film. Thus, gelatin-plasticizers films with ~0.25 mm in thickness were obtained. Films were stored for 7 days in desiccators conditioned at ~0% of relative humidity (RH) using silica (Merck, Darmstadt, Germany), and then they were held in environments hermetically closed at 44% RH (20 °C) using saturated salt solutions of K_2CO_3 (Merck, Darmstadt, Germany), until the sample reached constant weight followed by gravimetric measurements. A sample was considered to be at the equilibrium with RH when the difference between two consecutive weight measurements was lower than 0.5% (Yakimets et al., 2005). The moisture content of equilibrated samples was determined by gravimetric method in oven at 105 °C during 24 h. The moisture content of films after equilibrium varied between 3.5% and 14.7% in dry basis (Table I). Samples were sealed in hermetically closed high moisture barrier packages and stored at −18 °C until use.

2.3. Thermal analysis

In order to define the experimental conditions of storage in the glassy state, the glass transition temperature (Tg) was measured by