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Edible film with antioxidant capacity based on salmon gelatin and boldine

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ABSTRACT

Salmon gelatin and boldine as a natural antioxidant were used to prepare edible films by a cold casting method. The concentration of each component was optimised by applying a Box-Behnken experimental design (BBD) with the goal of maximising radical scavenging capacity of film forming suspensions (FFS) measured by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical assay. The results showed synergistic effect between gelatin and boldine for the antioxidant capacity (radical scavenging of over 80%) and antimicrobial activity of gelatin against *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ISP 6508. The release of boldine into the food simulant was faster for films containing 2 % gelatin than for those containing 4 %. Kinetic data for boldine release from films fitted to the Weibull model ($r = 0{,}99$). Possible molecular interactions between gelatin and boldine were observed in the FTIR spectrum of the composite films. Within the range of 1638 to 1628 cm-1 a strong interference caused by boldine in the hydrogen bonding between water and imide residues was observed. Owing to the simultaneous antioxidant and antimicrobial activities displayed for the gelatinboldine films, there is potential for application in the preservation of some perishable fresh food such as fish, meat and cheese.

1. Introduction

One of the focuses of the research on edible films and coatings for food applications is the use of biodegradable polymers obtained from natural sources. They can be used as substitutes for the current synthetic polymers in a wide spectrum of different products. Edible films and coatings can provide a protective layer for fresh food and thereby preventing major losses in quality and quantity. Some successful active edible coatings can give a desirable internal gas composition, appropriate for extending the shelf life of specific food products (Park, Byun, Kim, Whiteside, & Bae, 2014, pp. 257–275).

Edible biopolymer films have been generally classified according to the source of the original polymer utilized and the main raw materials are lipids, polysaccharides, and proteins. Among these, the polysaccharide-based or protein-based materials are the most widely used for the formation of edible biopolymer films. Other edible films components such as plasticizers and active agents with different properties (antioxidant, antimicrobial, pigments, etc.) are also included (Park et al., 2014, pp. 257–275).

Gelatin is an animal protein obtained by controlled hydrolysis of the fibrous insoluble collagen present in the bones and skins generated as waste materials during animal slaughtering and processing (Patil, Mark, Apostolov, Vassileva, & Fakirov, 2000). Gelatin has been one of the most studied biopolymers due to its film-forming ability and its use as an outer film to protect food from drying and exposure to light and oxygen (Arvanitoyannis, 2002). The unique property of proteins to form networks and induce plasticity and elasticity are considered beneficial in the preparation of biopolymer-based packaging materials (Voon, Bhat, Easa, Liong, & Karim, 2010). Fish gelatin has gained importance in recent years due to three main reasons: the outbreak of bovine spongiform encephalopathy during the second half of the 90's, the banning on consumption of collagen from pig skin and bone in some religions (e.g. Halal and Kosher) and some technological advantages over mammalian gelatins (Boran & Regenstein, 2010, chap. 5; López,

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Enrione, & Matiacevich, 2014, pp. 257–268). Several studies on fish gelatin films have used gelatin from big eye snapper (Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006), brownstripe red snapper (Jongjareonrak et al., 2006), Baltic cod, tilapia or tuna (Kolodziejska & Piotrowska, 2007; Kolodziejska, Piotrowska, Bulge, & Tylingo, 2006) and salmon (Díaz, López, Matiacevich, Osorio, & Enrione, 2011; Matiacevich, Celis, Schebor, & Enrione, 2013). However, physical properties of fish gelatin films influence its quality and potential application, since they are related to gelatin structure and biochemical characteristics (Díaz et al., 2011; López et al., 2014, pp. 257–268). Besides, natural active compounds such as antioxidants and antimicrobial agents could be added to these films in order to improve the shelf-life of fresh foods (Gómez-Estaca, Montero, Fernández-Martín, Alemán, & Gómez-Guillén, 2009; Li, Miao, Wu, Chen, & Zhang, 2014).

Oxygen is responsible for many degradation processes in foods, such as lipid oxidation, microbial growth, enzymatic browning and vitamin loss (Ayranci & Tunc, 2003). The oxidation of lipids results in the generation of off-flavours, colour changes and nutrients loss (Hong & Krochta, 2006). Oxidative processes cause the degradation of meat proteins, pigments and lipids which reduce the shelf life (Liu, Dai, Zhu, & Li, 2010). Therefore, in order to produce active edible films that avoid the negative effects of oxygen, the addition of antioxidants has been investigated. Various synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ter-butylhydroquinone have been successfully used to prevent oxidative deterioration of foods (Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010). However, synthetic antioxidants and additives have, in general, a negative impact on some groups of consumer more conscious of possible effects on human health. On the other hand herbs and spices contain many phytochemicals which are potential sources of natural antioxidants including cathequins, phenolic ditherpenes, flavonoids, tannins and phenolic acids some of which also have anti-inflammatory and anticancer activities (Dawidowicz, Wianowska, & Baraniak, 2006; Li et al., 2014), When applied in food systems, they can enhance flavour, retard lipid oxidation, inhibit growth of microorganisms, and also play roles in decreasing the risk of some diseases (Achinewhu, Ogbonna, & Hart, 1995; Tanabe, Yoshida, & Tomita, 2002).

The Boldo tree (*Peumus boldus Molina,* Monimiaceae) grows abundantly from the more humid ecosystems of the Mediterranean climatic region of central Chile to the rainiest zone of the country in the X Region of the territory. As stated by O'Brien et al. (O'Brien, Carrasco-Pozo, & Speisky, 2006) Boldo leaves contain at least 17 different alkaloids belonging to the large benzylisoquinoline-derived family (Ruegger, 1959). Boldine is the major alkaloid obtained from Boldo tree as it accounts for 12–19% of the total alkaloid content (Van Hulle, Braeckman, & Van Severen, 1983). The structure of boldine is analogue to natural and recognized antioxidant substances, in particular structures featuring phenolic hydroxyl groups showing antioxidant activity in solution (O'Brien et al., 2006). The objective of this work was to develop a new edible film with high antioxidant capacity based on salmon gelatin incorporating boldine. In order to evaluate the antioxidant properties of these films, the 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of the films was measured. Antimicrobial and physical properties of these films as well as possible interaction between film components were also investigated.

2. Material and methods

2.1. Material

Salmon gelatin (SG) was obtained from the skins of Atlantic salmon (*Salmo salar*), which was kindly donated by Fiordo Austral Company located in Puerto Montt, Chile. The skin was treated by acidic/alkaline extraction method following the methodology proposed by Zhou and Regenstein (2005) with some minor modification according to Díaz et al. (2011).

(6aS)-boldine (Indena, Chile) was a generous donation of SAPhyChem (South American Phytochemical), Santiago, Chile.

2.2. Preparation of films forming suspensions (FFS)

Films were prepared containing salmon gelatin as the matrix polymer, sorbitol (Blumos, Santiago, Chile) as plasticizer, and boldine (SAPhyChem, Santiago, Chile) as antioxidant agent. Salmon gelatin was used at concentrations of 2, 3 and 4% w/v, sorbitol was used at concentrations between 45 and 75% w/w with respect to gelatin (0.3 and 3% w/v of the filmogenic solution), and boldine at 0.00075, 0.0045 and 0.00825% w/v. The mixture was made up to a final volume of 10 mL with distilled water and all components were dissolved using a hot plate stirrer at 50 °C (Arex hot plate stirrer, VELP Scientifica, Italy) connected to a temperature controller (VTF digital thermoregulator, VELP Scientifica, Italy). The pH of all FFS was adjusted at 4.5 ± 0.3 (pH Meter model 3505, Jenway, UK). The concentrations of the different components of the FFS were obtained from the optimum results of the experimental design described below (Table 1).

2.3. Antioxidant capacity of FFS by DPPH

DPPH assay is one of the most widely employed and preferred method for measuring the radical scavenging activity of plant extracts (Umamaheswari & Chatterjee, 2008). The antioxidant assay was performed according to Li et al. (2014) with some modifications. A solution of 0.1 mM DPPH (Calbiochem, USA) in 95.5% ethanol was prepared. The different FFS samples (1.5 mL) were mixed with 1.5 mL of ethanolic DPPH solution, stirred in a vortex for 10 s and left in the dark for 30 min at room temperature. A control of the ethanolic DPPH solution was prepared by mixing 1.5 mL of 95.5% ethanol with 1.5 mL of ethanolic DPPH solution. A second control of each 1.5 mL experimental samples was prepared adding 1.5 mL of distilled water. The absorbance at 517 nm was measured in a spectrophotometer (UVmini-1240, Shimadzu, Japan). The percentage of DPPH radical scavenging was calculated using Equation (1) (Li et al., 2014):

$$\textit{Scavenging activity}\ (\%) = \left(1 - \frac{(A_m - A_b)}{A_c}\right) \times 100 \qquad (1)$$

where: A_m: Absorbance of the samples with DPPH.A_b: Absorbance of the samples with distilled water.A_c: Absorbance of the ethanolic solution with DPPH.

Table 1
Levels of the experimental factors, in coded and uncoded units, used for the Box-Behnken design for the optimization of the radical scavenging activity of film forming suspensions.

Experimental Factor	Low −1	Medium 0	High +1
X_1: Gelatin (% w/v)	2.0	3.0	4.0
X_2: Boldine (mg/mL)	0.0075	0.045	0.0825
X_3: Sorbitol (%w/w of gelatin)	15.0	45.0	75.0
Response variable			Goal
Y: Radical scavenging activity (%)			Maximize