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Comparison of chitosan–gelatin composite and bilayer coating and film effect on the quality of refrigerated rainbow trout

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ABSTRACT

The effect of chitosan–gelatin coating and film on the rancidity development in rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage (4 ± 1 °C) was examined over a period of 16 days. Composite and bilayer coated and film wrapped fish samples were analysed periodically for microbiological (total viable count, psychrotrophic count) and chemical (TVB-N, POV, TBARS, FFA) characteristics. The results indicated that chitosan–gelatin coating and film retained their good quality characteristics and extend the shelf life of fish samples during refrigerated storage .The coating was better than the film in reducing lipid oxidation of fillets, but there was no significant difference between them in control of bacterial contamination.

1. Introduction

Interest in edible coatings and films on highly perishable unmodified and/or fresh foods has intensified in recent years (Antoniewski, Barringer, Knipe, & Zerby, 2007; López-Caballero, Gómez-Guillén, Pérez-Mateos, & Montero, 2005). Edible coating is a thin layer of material formed as a coating on a food product, while an edible film is a preformed thin layer, made of edible material, which once formed can be placed on or between food components (Falguera, Quintero, Jiménez, Munoz, & Ibarz, 2011). Some of their functions are to protect the product from mechanical damage, physical, chemical and microbiological activities, edibility, biocompatibility, barrier properties, being non-toxic and polluting and serving as carriers of food additives (i.e. antioxidants, antimicrobials). Therefore, preventing bacterial growth and delaying lipid oxidation can promote the shelf-life extension of foods (Falguera et al., 2011; Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009).

The most commonly used materials for edible film production are biopolymers such as carbohydrates and proteins (Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011). Among these biopolymers, gelatin and chitosan are hydrophilic ones with good affinity and compatibility, to form composite and bilayer films with good properties (Arvanitoyannis, Nakayama, & Aiba, 1998; Pereda et al., 2011; Rivero, García, & Pinotti, 2009). Gelatin is an animal protein resulting from partial hydrolysis of collagen (Pereda et al., 2011; Rivero et al., 2009). It can be visualised as a copolymer build up from triads of α-amino acids with glycine at every third position (soft blocks) and triads of hydroxyproline, proline and glycine (rigid blocks), with a narrow molar mass distribution (Pereda et al., 2011). Gelatin has been extensively studied for its film-forming capacity and applicability as an outer covering to protect food against drying, light, and oxygen (Gómez-Guillén et al., 2009).Gelatin film itself, as most protein films, does not have ideal water vapour barrier properties. Thus, some chemical treatments can be applied to modify the polymer network through cross-linking of the polymer chains to improve the hydrocolloid film functionality (Rivero et al., 2009).

Chitosan, a linear β-1,4-D-glucosamine, biopolymer is a natural polysaccharide obtained from deacetylation of chitin, the second most abundant natural polymer in nature after cellulose (Rivero et al., 2009; Sathivel, Liu, Huang, & Prinyawiwatkul, 2007; Vásconez et al., 2009). Due to its intrinsic antimicrobial properties and good film-forming ability, chitosan can be used as active antimicrobial coatings and films (Pereda et al., 2011; Rivero et al., 2009; Sathivel et al., 2007). Composite and bilayer films of chitosan and gelatin have been reported to have improved mechanical, transport and physical properties compared with those of single component based films. This was attributed to the formation of polyelectrolyte complexes through electrostatic interactions operating between the protonated amino groups of chitosan and the negatively charged side-chain groups in gelatin or collagen (i.e. carboxylate groups) at the operating pH (Pereda et al., 2011). It is reported that bilayer chitosan–gelatin films have higher perfor-

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mance in terms of water vapour permeability and mechanical properties than composite counterpart (Pereda et al., 2011; Rivero et al., 2009).

Fish is an extremely perishable food compared with other fresh commodities (Sathivel, 2005; Vásconez et al., 2009). During storage, fish quality is reduced quickly because of chemical and enzymatic reactions and microbial spoilage. The application of an edible film or coating is a new method to protect its quality (Vásconez et al., 2009). A mixture of fish gelatin and chitosan, both derived from marine sources, would seem to be suitable to protect seafood products (López-Caballero et al., 2005). The aim of the present study was to investigate and establish a comparative basis antimicrobial and antioxidant effect of chitosan–gelatin composite and bilayer edible film and coating on quality of rainbow trout, *O. mykiss*, fillets under refrigerated conditions (4 ± 1 °C).

2. Materials and methods

2.1. Preparation and treatment of fish samples

2.1.1. Fish preparation

Fresh water rainbow trouts with an average weight of 550–650 g were purchased at a public market and were transferred to the Seafood Processing Laboratory in Fishery Department at Gorgan University, Iran, filleted by hand. The fish were harvested during the period of January–February 2011. Two fillets were obtained from each fish after removing the head and bone.

2.1.2. Preparation of coating solutions, films and treated fillets

Chitosan and gelatin solutions were prepared separately. Chitosan solution was prepared with 1% (w/v) chitosan (Sigma Chemical Co., medium molecular weight, viscosity 200–800 cP) in 1% (v/v) acetic acid (Ojagh, Rezaei, Razavi, & Hosseini, 2010). To achieve complete dispersion of chitosan, the solution was stirred at room temperature to dissolve completely. The dry cold water fish skin gelatin (Sigma Chemical Co., solid form, bioreagent) was dissolved in water (3%, w/v), first being allowed to swell at 7 °C for 15 min and then warmed to 55 °C for 30 min (López-Caballero et al., 2005). Glycerol was added at 0.75 ml/g concentration as a plasticizer and stirred for 10 min (Ojagh et al., 2010). All films were obtained by casting 100 ml film forming solution on a nonstick surface (16 ∗ 27 cm). Bilayer films (BF) were prepared by a two-step casting technique; 60 ml gelatin solution were poured onto the surface, dried at ambient temperature (20 °C) until a firm surface but still with adhesive properties was obtained. The second layer of 40 ml chitosan solution was added onto these preformed gelatin based films and finally the system was dried again. To prepare composite film (CF) 60 ml of the gelatin solution and 40 ml of chitosan solution were blended and poured on the surface. After evapouration the films were peeled off from the plates.

Fillet samples were randomly assigned into five treatment lots consisting of: one control lot (uncoated), two lots wrapped with composite and bilayer films and two lots treated with the following coating methods: composite coating (CC) (fillets were immersed for 30 s in composite chitosan–gelatin solution and then allowed to stand for a 2 min period followed by a second immersion in the solution for 30 s) and bilayer coating (BC) (fillets were immersed for 30 s in chitosan solution and then allowed to stand for a 2 min period followed by immersion in gelatin solution for 30 s). Then the fish fillets were removed and allowed to drain for 2 h at ambient temperature (20 °C) in order to form the edible coating. All samples were placed in polyethylene bags, stored at 4 ± 1 °C for 16 days. Chemical and microbiological analyses were performed at 4-day intervals to determine the overall quality of fish.

2.2. Proximate composition analyses

The moisture content and crude ash were determined in an oven at 103 and 550 °C, respectively, until the weight became constant. The total crude protein was determined by the Kjeldahl method (AOAC, 1984) and lipid content was analysed according to Bligh and Dyer (1959).

2.3. Bacteriological analysis

Bacteriological counts were determined by homogenising 10 g sample in 90 ml of 0.85% NaCl solution. Other decimal dilutions were prepared from this dilution and plated in the appropriate media. Total viable aerobic bacterial counts were determined by the pour plate method, using plate count agar (PCA, Merk, Darmstadt, Germany). The inoculated plates were incubated at 37 °C for 2 days for total viable counts, and at 10 °C for 7 days for psychrotrophilic counts. All counts were expressed as $\log_{10}$ cfu/g (Sallam, 2007).

2.4. Chemical analyses

2.4.1. Determination of total volatile base nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) value was estimated by the micro-diffusion method (Goulas & Kontominas, 2005). The micro diffusion method was determined by distillation after the addition of MgO to homogenised fish samples. The distillate was collected in a flask containing aqueous solution of boric acid and methyl red as an indicator. Afterward, the boric acid solution was titrated with sulphuric acid solution. The TVB-N value (mg N/100 g of fish) was determined according to the consumption of sulphuric acid.

2.4.2. Determination of peroxide value (POV)

The peroxide content was determined in the lipid extract following the method of Woyewoda, Shaw, Ke, and Burns (1986) according to Eq. 1. Results were expressed in meq peroxide/1000 g lipid.

$$POV = \frac{V \times N \times 1000}{W} \quad (1)$$

V = ml of thiosulphate for titration$N$ = normality of thiosulphate$W$ = weight (g) of lipid

2.4.3. Determination of thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid (TBA) measurement was determined following Tarladgis, Watts, and Younathan (1960) with some modification. 10 g of homogenised sample were added with 97.5 millilitres of distillated water and 2.5 millilitres of 4 M HCl. The mixture was heated with steam distillation. Five millilitres of distillate was added to 5 millilitres of thiobarbituric reactive reagent containing 0.02 M TBA in 90% glacial acetic acid and incubated in boiling water for 35 min. After cooling, the absorbance of the pink solution was measured at 532 nm using a spectrophotometer. The constant 7.8 was used to calculate the TBA number using Eq.2 (Tarladgis et al., 1960). The TBA value is expressed as mg malonaldehyde equivalents/kg sample.

$$\text{TBARS value} = 7.8\text{Abs}_{532} \quad (2)$$

2.4.4. Free Fatty Acid (FFA)

The Free fatty acid content was determined in the lipid extract according to the Eq. 2. Results were expressed in % of oleic acid (Woyewoda et al., 1986).