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Effects of microbial transglutaminase added edible coatings based on heated or ultrasound-treated whey proteins in physical and chemical parameters of frozen Atlantic salmon (*Salmo salar*)

Laura Rodriguez-Turienzo, Angel Cobos, Olga Diaz *

Área de Tecnología de Alimentos, Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias, Universidad de Santiago de Compostela, 27002 Lugo, Spain

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ABSTRACT

The effects of the addition of transglutaminase to heated or ultrasound-treated whey protein coatings on quality parameters of frozen Atlantic salmon (*Salmo salar*) were evaluated. The influence of the type of denaturation treatment (heating or sonication) was also studied. The addition of microbial transglutaminase to the coatings did not significantly modify the yields, drip losses, colour and chemical composition of the fish fillets. The yields were higher in the samples protected with heated coatings than in those with ultrasound-treated ones. Transglutaminase addition to heated whey protein coatings delayed lipid oxidation. Ultrasound-treated coatings with or without enzyme addition were equally effective for lipid oxidation reduction after frozen storage of Atlantic salmon than heated-treated transglutaminase-added coatings.

1. Introduction

Edible films and coatings are a good alternative for the partial or total substitution of plastic packaging due to their properties: they are biodegradable, non-toxic, environmental-friendly and, in many occasions, made with by-products of the food industry.

Whey protein films and coatings are a good example of these materials. During their preparation, the application of heat treatment to the solution is necessary in order to denature the proteins and obtain insoluble edible packages with improved mechanical and oxygen barrier properties by formation of cross-linking between the protein molecules (Pérez-Gago, 2012). However, there are other treatments that can be used, such as ultrasounds, which modify the properties of the package otherwise that heating. Ultrasounds induce changes in the secondary and tertiary structures of proteins that affect the mechanical properties of milk protein-based films (Banerjee et al., 1996), and improve the appearance of gluten-based films (Marcuzzo et al., 2010).

Another option for protein modification is the use of enzymes; among them, transglutaminase has been investigated for uses in food processing, such as seafood, meat or dairy products manufacture, especially since a microbial source of the enzyme became commercially available. In proteins, transglutaminase catalyses acyl-transfer reactions, producing the formation of ε-(γ-glutamyl) lysine intra- or inter-molecular cross-links (Gerrard, 2006). Microbial transglutaminase has also been added in edible protein-based film preparation for promoting the cross-linking between molecules and improving the properties of the films. Whey, egg white and soy proteins, and mixtures of gelatin–casein or chitosan–whey protein have been used for film preparation (Chambi and Grosso, 2006; Di Pierro et al., 2006; Jiang et al., 2007; Lim et al., 1998; Mahmoud and Savello, 1992). These authors described the enhancement of mechanical resistance and water vapour and oxygen barrier properties of the films when transglutaminase was added. Nevertheless, there is no information about the application of transglutaminase-added edible coatings to a real food product.

Globular proteins are poor substrates for this enzyme due to their compact structure; they need to be previously denatured for improving the accessibility of the transglutaminase to the target amino acid residues, glutamine and lysine (Lim et al., 1998). The denaturation is often achieved by heating, but other treatments that produce structural changes in the proteins could also be useful, such as ultrasounds. However, the combination of ultrasound treatment followed by transglutaminase addition has not been proved for the formation of edible films and coatings.

In previous works (Rodriguez-Turienzo et al., 2011, 2012), heat-treated and ultrasound-treated whey protein coatings showed their capability for decreasing lipid oxidation in frozen Atlantic salmon (a fish with high lipid content) without changes in its sensory properties. The addition of transglutaminase to the whey

* Corresponding author. Tel.: +34 982 824124; fax: +34 982 285872.
E-mail address: olga.diaz.rubio@usc.es (O. Diaz).

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protein coating solutions might improve their positive effects decreasing lipid oxidation during fish preservation.

The objectives of this work were: (1) to evaluate the effects of the addition of transglutaminase to heated or ultrasound-treated whey protein coatings on quality parameters (yields, composition, colour and lipid oxidation) of frozen Atlantic salmon and (2) to investigate the influence of the type of denaturation treatment (heating or sonication) applied to the protein coatings.

2. Materials and methods

2.1. Fish fillets preparation

Atlantic salmon (*Salmo salar*) eviscerated specimens were purchased at a local market. Once in the laboratory, the fishes were hand-skinned and cut in fillets from dorsal to ventral section. The average weight of salmon fillets was 180–200 g.

2.2. Preparation of coating solutions and application to fish fillets

Whey protein concentrate (WPC), containing 80% protein, (Protarmor 800) and whey protein isolate (WPI), containing 90% protein, were purchased from Armor Proteines (Saint-Brice en Coglès, France). According to the manufacturer, the composition of the WPC was 80% protein, 4% moisture, 3.5% ash, 3.5% fat and 9% lactose, and the composition of WPI was 90% protein, 5% moisture, 2.5% ash, 0.5% fat and 2% lactose. The coating solutions were prepared by slow stirring the WPC or WPI (8% protein w/w) in distilled water for 30 min at room temperature (20 °C); then, glycerol, in a proportion protein:plasticizer 2:1, was added. The mixture was stirred for 30 min and the pH was adjusted with 2 M NaOH at a value of 7.0. Afterwards, the dispersions were divided in four portions (150 g each) and were poured into 250 ml Erlenmeyer flasks: two were heated in a water bath at 80 °C for 30 min and the other two were submitted to an ultrasound treatment (30 min) in a 35 kHz ultrasound bath Sonorex Digital 10P, power 820 W (Bandelin Electronic, Berlin, Germany). The solutions were treated at a power setting of 50%. The ultrasonic power of the generated ultrasonic wave was 20.8 W, as measured by calorimetry in 150 g of distilled water into a 250 ml Erlenmeyer flask (Kimura et al., 1996). One of the heated and one of the ultrasound-treated solution portions were added with 10 units/g protein transglutaminase Activa™ WM (Ajinomoto Co., Tokio, Japan) which contained 99% maltodextrins and 1% microbial transglutaminase with a nominal activity of 100 U/g of power, according to the information provided by the manufacturer. All the solutions, with or without enzyme, were stirred for 2 h at 20 °C.

The coatings were applied to fish before freezing. The fillet pieces were dipped in each coating solution for 1 min, drained for 15 s, packed in polyethylene freezer bags, and stored at −10 °C for 4 months. Non-coated fillets were used as controls. All experiments were made in triplicate.

2.3. Determination of yields and drip losses

Yield after coating, thawing, and drip loss after thawing were measured following the procedures described by Sathivel (2005). Drip loss after chilled storage and cooking loss determinations were carried out by the methods described by Rodriguez-Turienzo et al. (2012). All determinations were made in duplicate.

2.4. Determination of pH

The pH of the non-coated and coated salmon fillet pieces before and after 4 months frozen storage was determined introducing a penetration pH electrode in the sample and the measurement was carried out in triplicate with a pH meter model GLP 21 (Crison Instruments, Barcelona, Spain).

2.5. Dry matter, lipid and ash contents

For chemical analyses, the meat samples were finely minced in a blender (Polytron PT-20, Brinkman Instruments, Westbury, USA). The samples were analysed in triplicate for dry matter and ash contents using the AOAC standard methods 930.15 and 942.05, respectively (AOAC, 1995). Lipids were extracted and purified from the former homogenate with a chloroform–methanol mixture (1:1 v/v) according to the method of Hanson and Olley (1963). Total lipids were gravimetrically determined. The determinations were made in duplicate.

2.6. Colour parameters

Colour parameters of fresh samples, and of frozen, thawed and cooked for both coated and non-coated salmon fillets were determined using a chromameter X-Rite model SP60 (Grand Rapids, Michigan, USA). All measurements were made in the CIE $L^*a^*b^*$ colour space (CIE, 1976) using the D65 illuminant and the 10° standard observer. The instrument was standardised with the white and black tiles provided by the manufacturer before sample measurements. The colour values were expressed as L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness). From these values, whiteness was calculated according to the following formula: Whiteness = $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$.

2.7. Determination of lipid oxidation

The peroxide value was determined by the modified xylenol orange assay described by Eymard and Genot (2003). The standard curve was developed with solutions of cumene hydroperoxide in methanol. Levels of hydroperoxides were expressed as meq cumene hydroperoxide/kg of fish meat and as meq cumene hydroperoxide/kg of lipids. The thiobarbituric acid reactive substance (TBARS) value was evaluated for the salmon fillet samples using the method described by Eymard (2003). The results were expressed as mg malondialdehyde/kg of fish meat and as mg malondialdehyde/kg of lipids. Lipid oxidation parameters of raw and thawed salmon fillets were carried out in duplicate.

2.8. Statistical analysis

The statistical significance of differences among treatment means was evaluated by analysis of variance (one-way ANOVA), and the means were compared using the least significant difference test with significance at $p < 0.05$. The comparison of means of the samples coated with WPC and WPI was done using a t-test for independent samples. A significance level of $p < 0.05$ was used for all mean evaluations. Data were evaluated statistically using the SPSS version 15.0 for Windows (2006; SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Composition, pH, colour parameters and lipid oxidation of raw salmon fillets

The composition of the fresh salmon fillets used in the experiments was 35.5% dry matter, 13.2% lipids and 1.00% ash. The values of lipid oxidation parameters were: peroxide value, 0.78 meq cumene/kg fish (5.07 meq cumene/kg lipids), TBARS value, 0.24 mg malondialdehyde/kg fish (1.67 mg malondialdehyde/