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Effects of active gelatin coated with henna (*L. inermis*) extract on beef meat quality during chilled storage

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ABSTRACT

The effect of a beef meat coating prepared using cuttlefish skin gelatin with henna aqueous extract (HAE) as natural preservative was studied during chilled storage of meat at 1, 3, 6 and 8 days. The combined coating increased the shelf-life of meat by decreasing the total and psychrophilic vial bacteria counts at the end of storage. In addition, the lipid oxidation of meat decreased during the period of storage in gelatin-HAE coated samples measured using the thiobarbituric acid reactive substances test. Furthermore, coating significantly preserved color properties in comparison with uncoated samples. According to free amino acid contents, coating decreased the rate of proteolysis process, whereas it was faster in uncoated samples. Thus, the gelatin coatings offer an excellent protection to avoid meat deterioration, which was significantly improved by the addition of HAE to the gelatin gel.

1. Introduction

The stability of fresh meat during chill storage use to be compromised after a period of time causing a decrease in quality and even safety. Main reasons for this deterioration are food spoilage induced by psychotropic bacteria, color change caused by metmyoglobin formation and heme iron conversion, and flavor deterioration due to the lipid oxidation.

Edible coatings have been widely employed to preserve meat quality although their use in meat and poultry industry is still limited (Gennadios, Hanna, & Kurfh, 1997). Gelatin is one of the main ingredients used as edible coating mainly due to its film forming ability, and its use in meat have been described to prevent moisture loss and lipid oxidation at chilling storage (Herring, Jonnalongadda, Narayanan, & Coleman, 2010). Furthermore, active coating is one of the most important tools used to preserve food quality via the release of active agents. This release can be used to extend the quality and shelf-life of the final product, avoiding the incorporation of bioactive substances directly on the foodstuff (Lee, 2010; Zhou, Xu, & Liu, 2010).

In this context, henna (*Lawsonia inermis*) is one of the most studied plants in herbal medicine. It belongs to Lythraceae's family and it is well-known for the natural orange-red color of its leaves. Leave extracts have been reported to show several biological activities such as antidiabetic (Arayne, Sultana, Mirza, Zuberi, & Siddiqui, 2007), antifungal (Subbaiah & Savithramma, 2012) anti-inflammatory (Imam, Uddin Mahbub, Khan, Kabir Hana, & Rahman, 2013), and antioxidant (Adetutu, Owoade, & Oyekunle, 2013; Dasgupta, Rao, & Yadava, 2003). In fact, the antioxidant activity described in extracts could be attributed to several phenolic compounds (Jridi et al., 2016). Additionally, some reports have indicated that extract from rosemary can retard lipid oxidation and prolong the shelf life of processed meat products.

The purpose of this study was to evaluate the effect of gelatin coating enriched with aqueous extracts of henna (*L. inermis*) in preserving the quality of beef meat during 8 days of chill storage. Particularly, weight loss, color changes, metmyoglobin concentration, lipid oxidation, heme iron amount and free amino acids concentration of coated and uncoated samples were determined at 1, 3, 6 and 8 days of storage.

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2. Materials and methods

2.1. Preparation of gelatin extracts

By-products derived from cuttlefish were washed several times with water to eliminate residues and dark ink. Gelatin was extracted from the cuttlefish outer skin. Firstly, non-collagenous proteins were removed using a solution of NaOH 0.05 M in a ratio 1/10 (w/v) during 2 h at 4 °C that was changed every 30 min. Then, skins were washed several times with 4 °C distilled water to reach again their characteristic neutral pH. In order to extract the gelatin, skins were immersed in 0.1 M acetic acid in a ratio 1:10 (w/v) and subjected to hydrolysis with pepsin at 5 U/g of skin and incubating for 48 h at 4 °C (Jridi et al., 2016). To inactivate enzymes, the pH of the mixture was then raised to 7.5 using 10 M NaOH and agitated for 1 h at 4 °C. Then, skin was incubated at 40 °C for 18 h in agitation to extract the gelatin. Finally, samples were centrifuged at 10,000 g for 30 min at 4 °C to remove insoluble material. Gelatin was in the supernatant (cuttlefish-skin gelatin) which was freeze dried (Bioblock Scientific Christ ALPHA 1–2, IllKrich-Cedex, France) and stored in cold (4 °C) until use. In order to prepare the active gelatin solution, HAE was dissolved in the gelatin extract at a concentration of 50 μg/mL.

2.2. Preparation of henna aqueous extract

Lawsonia inermis fresh leaves were obtained from Gabes city-Tunisia and stored at −20 °C. Henna aqueous extract (HAE) was prepared according to Mittal and Aguwa (1983), with minor modifications. Briefly, after washing the leaves of henna were sun dried and grounded into powder. A total of fifty grams of the power was mixed with 500 mL of distilled water and agitated during 24 h at room temperature (25 °C). Finally, the mixture was centrifuged (10,000 × g, 15 min, 4 °C) to remove insoluble material and the supernatant was collected and freeze dried.

2.3. Meat sample preparation

Beef muscle (*Longissimus dorsi*) was obtained from a local market in Valencia City, Spain, the day of slaughtering. Muscle was cut in eighteen squares, sizing 5 × 5 × 2 cm, and divided in three groups according to the treatment: control samples with no treatment; samples dipped into the gelatin solution during 30 s at 40 °C and samples dipped into gelatin solution enriched with HAE during 30 s at 40 °C. After treatment, all samples were weighed and stored at 4 °C without packaging to simulate fresh retail display setting. Eighteen samples (six of each batch) were removed from the chamber at 1, 3, 6 and 8 of storage for microbial, sensorial, free amino acids and physico-chemical analysis.

2.4. Weight loss

The weight loss of the meat samples on days 3, 6 and 8 days was calculated using the equation: WHC (%) = $(W_0 - W_i)/W_0 \times 100$, where W_0 is the initial weight of sample and W_i is the weight of the same sample after 3, 6 and 8 days of chilled storage.

2.5. Color, pH and water activity measurements

The internal color of meat samples was determined using a CR-410 colorimeter (Minolta Chroma Meter Measuring Head, Osaka, Japan) with D65 illuminant and 10° observer. Thus, the gelatin was removed from meat sample and three different measures from three different surfaces of the cube were done. The instrument was calibrated using a standard white plate. An average value was determined by taking observations from six different meat samples on days 1, 3, 6 and 8 of storage. Only the Control sample (n = 6) was evaluated at day 1 as no changes due to the gelatin and gelatin + HAE occurred. CIE lightness (L*), redness (a*) and yellowness (b*) were recorded. Hue was calculated using the standard formula [Arc tan (b*/a*)], whereas chroma (C*) was calculated with the equation: $C^* = (a^{*2} + b^{*2})^{1/2}$.

In order to measure meat pH from each treatment and storage day, 5 g of sample was homogenized in 20 mL of bidistilled water for 2 min using a Stomacher (IUL Instrument, Barcelona, Spain).

Water activity (a_w) was determined using a Fast-lab water activity meter (Gbx, Romans sur Isère Cédex, France). Briefly, after calibration with sodium chloride and potassium sulphate, an internal section from each sample was placed in the instrument and a_w measured.

2.6. Lipid oxidation

The lipid oxidation was evaluated according to Witte, Krause, and Bailey (1970). Briefly, 5 g of meat sample and 20 mL of trichloroacetic acid (TCA) diluted to 5% were homogenized in a Polytron homogenizer (PT 2100, Kinematica AG, Switzerland) for 5 min. The homogenate was centrifuged at 12,000 g for 10 min at 4 °C. 4 mL of the supernatant was mixed with 4 mL of thiobarbituric acid (TBA) 0.02 M and incubated in a water bath at 100 °C for 60 min. Then, the mixture was centrifuged at 2500 g for 10 min at 4 °C. The absorbance of the supernatant was measured at 532 nm. The value of TBA reactive substances (TBARS) was calculated using the standard curve of the organic compound malonaldehyde (MDA) and results were showed in μmoles MDA/g sample.

2.7. Metmyoglobin content

Metmyoglobin content (Mmb) was determined according to the previous study of Fernández-López et al. (2003). Briefly, 5 g of meat was homogenized with 50 mL of phosphate buffer 0.04 M and pH 6.8 for 15 s at 4 °C using a Polytron homogenizer. Then, sample was centrifuged for 30 min and 15,000 rpm at 4 °C. The supernatant was filtered through glass wool and the obtained solution was analyzed using a spectrophotometer to measure the absorbance at 525, 572 and 730 nm. The percentage of metmyoglobin was determined according to the formula: Mmb (%) = $1.395 - ((A_{572} - A_{730})/(A_{525} - A_{730})) \times 100$.

2.8. Heme iron content

Total heme iron content was determined as previously described by Clark, Mahoney, and Carpenter (1997). Meat sample (2 g) was homogenized with 9 mL of acidified acetone (90% acetone, 8% deionized water, 2% HCl). The homogenate was placed for 1 h at 25 °C in dark and then filtered through glass wool. The absorbance of filtrate was determinate at 640 nm. The amount of heme iron was calculated as: Heme iron (μg/g of meat) = $A_{640} \times 680 \times 0.0882$.

2.9. Microbiological analysis

Microbiological activity assay was performed according to the method described by Berghe and Vlieinck (1991). 10 g of sample was homogenized with 90 mL of 0.85% NaCl in order to determine bacteriological counts in different dilutions. Total viable aerobic bacterial counts were determined using plate count agar by the pour plate method. The inoculated plates were incubated at 37 °C during 2 days for total viable counts, and at 10 °C during 7 days for psychrotrophilic bacteria counts. All microbial counts were converted to logarithms of colony-forming units per gram of meat (log