LWT - Food Science and Technology 61 (2015) 352–358

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Stabilization of cooked cured beef color by radical-scavenging pea protein and its hydrolysate

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ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form
9 December 2014
Accepted 21 December 2014
Available online 30 December 2014

Keywords:
Cured meat
Color
Pea protein
Protein hydrolysate
Antioxidant

ABSTRACT

The study was to investigate the role of pea protein for color stability of cooked cured beef and to understand potential mechanisms of color stabilization. Ground beef was either untreated or treated with 2 g/100 g pea flour (PF), pea protein isolate (PPI), or pea protein hydrolysate (PPH), nitrite-cured, then cooked to 72 °C. During storage at 2 °C up to 9 days in PVC package, color parameters (L^*, a^*, b^*, nitrosylhemochrome, and pigment UV spectrum) were analyzed. Radical-scavenging activity [2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid)] and ferric-reducing power of PF, PPI, and PPH and their inhibition of protein and lipid oxidation in the sarcoplasmic protein fraction were measured. All three pea protein products, most notably PPH, retarded color fading (7.0–16.9% less a^* reductions, $P < 0.05$) and decomposition of nitrosylhemochrome (reduction rates of 41.4%, 37.7% and 28.1% for PF, PPI, and PPH, respectively, compared to 69.5% for control, $P < 0.05$) of cured beef after 9 days. These color protections corresponded with improved oxidative stability of sarcoplasmic proteins and residual lipids, corroborating the remarkable radical-scavenging and reducing power of these pea products. Meanwhile, the affinity of certain nitrogen-containing constituents for porphyrin iron was implicated in the cured meat color protection.

1. Introduction

Nitrite-cured meat represents a main category of processed muscle foods, which encompasses a variety of ready-to-eat meats that are widely consumed in the modern society. Although the color of cured meats is notably more stable than that of untreated meat, discoloration during storage is a common problem that limits the shelf-life of this type of product (Møller et al., 2003).

The internal color of cooked beef is known to be influenced by intrinsic (e.g., myoglobin redox status and muscle source) as well as extrinsic (e.g., lighting, microorganisms, and non-meat ingredients) conditions (King & Whyte, 2007). Among various contributing factors, heme pigment oxidation is recognized to be the primary cause promoting color fading, resulting in a reduced consumer acceptability of both raw and cooked meats. The predominant pigment in cooked cured meat is nitrosylhemochrome (NO-Heme). Myoglobin in meat products treated with nitrate or nitrite is converted into bright-red NO-Heme in which the ferrous ion is coordinated with nitric oxide and it changes into pink-reddish NO-Heme after the meat has been cooked (Cassens, Greaser, Ito, & Lee, 1979).

Based on a plethora of spectroscopic investigations of nitrosylheme complexes, it is believed that during thermal processing of nitrite-cured meat, the globin portion of nitrosylmyoglobin denatures and subsequently detaches itself from the heme moiety to form NO-Heme (Sun, Zhou, Xu, & Peng, 2009). NO-Heme is relatively stable due to the protection of the NO group combined with porphyrin iron, but the sensory color is still readily subjected to change. Based on our previous investigation, one causative factor for the color instability is the modification of the conjugated structure of NO-Heme due to auto-oxidation, and another factor is the disruption of the porphyrin ring which occurs under strong free radical attack (Sun et al., 2009). Many attempts have been made to protect and stabilize the pigments in cured meat products either through strategies that promote the endogenous redox potential or through the addition of exogenous antioxidants (Sun, Zhang, Zhou, Xu, & Peng, 2010).

The ligand of the heme porphyrin iron is important and worth consideration when describing cured beef color stability. The color

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http://dx.doi.org/10.1016/j.lwt.2014.12.048
0023-6438/

of myoglobin depends on the kind of molecule coordinating to iron (Sakata, 2000). Dymicky, Fox, and Wasserman (1975) surveyed a large number of compounds as substitutes for nitrite in producing the characteristic cured color in meats. According to their findings, nitrogen-containing compounds generally had a high affinity for the heme porphyrin iron forming bright pigments with different color intensities. The best color that resembled the NO-Heme color was produced by pyridine compounds. In agreement with this observation, we have noted a significant effect of amino acids on the color of NO-Heme and of heated cured beef slurries, indicating the potential role of proteins and peptides in modifying cured meat colors.

Pea is a major crop in the world and the protein fraction has emerged as a novel functional ingredient in food processing. Considering that pea protein is a rich source of nitrogen, and based on our previous investigation that pea protein hydrolysate has antioxidant activity (Zhang, Xiong, Chen, & Zhou, 2013), the present study was conducted to test the hypothesis that components in pea protein and pea protein hydrolysate could interact with myoglobin pigments in nitrite-curing reactions so as to modify the color and influence the color stability of cured beef during storage.

2. Materials and methods

2.1. Materials

Biceps femoris (NAMP 184D) muscle samples were obtained from 21-day dry-aged carcasses of a pool of ten steers slaughtered at the University of Kentucky Meat Lab, a USDA-inspected facility. After the removal of external fat, individual muscle samples were sliced into ~200 g pieces, placed in plastic bags (0.06 mm, 3–6 cm^3/m^2/24 h oxygen transmission rate at 23 °C; Cryovac Division, Sealed Air Corp., Duncan, SC, U.S.A.) and vacuum-sealed before being frozen and subsequently stored at −30 °C. Before use, the beef was thawed in a 2 °C cooler for 24 h. Dry yellow peas (*Pisum sativum*) were obtained from a local store. All chemicals used in various analytical and extraction procedures, from either Fisher Scientific (Fair Lawn, NJ, U.S.A.) or Sigma–Aldrich (St. Louis, MO, U.S.A.), were at least reagent grade.

2.2. Preparation of pea flour (PF) and pea protein isolate (PPI)

Three batches ($n = 3$) of PF and PPI were prepared from yellow peas. Peas were dried in a 50 °C oven (Premium Oven 725 F, Thermo Fisher Scientific, Waltham, MA, U.S.A.) overnight to a moisture content of about 12 g/100 g then crushed with a blender. After the removal of the hull, the broken beans were milled to a fine powder using a grain mill (L'EQUIP Nutrimill, St. George, UT, U.S.A.). The fine powder, that is PF, was treated with *n*-hexane:ethanol (10:1, mL:mL) to extract fat, and the extraction procedure was repeated twice. The defatted flour was subjected to the process of protein extraction according to the method of Jiang, Xiong, and Chen (2010) using a 2 mol/L NaOH solution (pH 8.0) followed by the titration with 2 mol/L HCl to pH 4.5 to precipitate pea protein at its isoelectric point. The pellet was washed twice with distilled water followed by centrifugation at 6000× *g* for 10 min at 4 °C. Thereafter, the protein pellet was resuspended in distilled water and neutralized to pH 7.0 with 2 mol/L NaOH. The PPI suspensions were freeze-dried and stored at 4 °C before use. Because all three batches were found to have strong radical-scavenging and reducing activities and the assay results were extremely consistent (described later), they were pooled then applied to the testing for cured meat color protection as well as the inhibition of lipid and protein oxidation which were tested in three independent trials ($n = 3$).

2.3. Preparation of pea protein hydrolysate (PPH)

A 5 g/100 mL protein solution of PPI was hydrolyzed for 0.5 h with Flavourzyme (Novo Nordisk, Bagsvard, Denmark) at 50 °C to produce PPH. Flavourzyme was used because it was identified in our previous study to be the most effective of seven proteases tested to produce antioxidative peptides from pea protein (Zhang et al., 2013). The enzyme-protein ratio was 1/100 and the hydrolysis pH was 6.0. The termination of hydrolysis was achieved by heating the hydrolysate at 80 °C for 15 min. After cooling to room temperature, the hydrolysate was titrated to pH 7.0 with 1 mol/L NaOH, centrifuged at 9000× *g* for 10 min to remove insoluble particulates then freeze-dried. The PPH powder was kept at 4 °C before use.

2.4. Preparation of cooked, cured beef

The experiments described below were repeated three times ($n = 3$) in independent trials (replicates) on different days. Before use, randomly selected frozen *B. femoris* muscle samples were thawed at 2 °C overnight, pooled, then ground through a 4.5-mm orifice plate. In each replication, four aliquots (ca. 250 g each) of ground meat were assigned to the following four treatments: control group, PF (2 g/100 g), PPI (2 g/100 g), and PPH (2 g/100 g). The 2 g/100 g protein addition represents a level equivalent to the maximum level allowed for soy protein isolate used in ground meat products by the regulation of the USDA [MPI Regulations section 318.7(c) (4)]. All samples were blended with 30 mL of a mixed curing solution containing appropriate amounts of sodium chloride (NaCl), sodium nitrite ($NaNO_2$), and sodium tripolyphosphate to obtain the final concentrations of 2 g/100 g, 150 mg/kg, and 0.3 g/100 g in the beef sample, respectively. Prior to use, PF, PPI or PPH (at a final 2 g/100 g concentration in cured meat) was dissolved in the curing brine. Ground muscle samples were then mixed with different brine treatments by hand mixing and then incubated at 4 °C for 48 h to allow the curing reaction to complete. Cured samples were placed and shaped into 8.5 (diameter) × 1.0 cm (thickness) patties (60 ± 0.5 g each) in Petri dishes and heated in an enclosed water bath chamber (80 °C) to a center temperature of 72 °C, which was measured using a digital thermometer probe. Cooked patties (four from each formulation treatment) were sliced into two equal halves (0.5 cm thick), individually wrapped in an oxygen permeable polyethylene film (ca. 18,500 cm^3/m^2/24 h/atm of oxygen permeability), and placed in a 2 °C cooler in the dark up to 9 days to monitor the surface color change as described below.

2.5. Surface color measurements

Objective measurement of color (L^*, a^* and b^*) was performed over a 9 day period on the surface of the same cooked meat samples (3 samples per treatment) using a CR-400 Chroma Meter (Observer 2°, illuminant D65, Minolta Co., Osaka, Japan). The chromameter was standardized with a white tile ($L^* = 97.63$, $a^* = -0.04$, and $b^* = 1.69$). Chroma (C^*) and hue-angle (h) were calculated by the formulas: $C^* = (a^{*2} + b^{*2})^{1/2}$, $h = \tan^{-1}(b^*/a^*)$. Retarding color fading is indicated by the less a^* reduction on 9th day of storage, namely, a^* reduction $= [(a^*_{\text{treatment}} - a^*_{\text{control}})/a^*_{\text{control}}] \times 100\%$.

2.6. Measurement of pigment NO-Heme

The concentration of cooked meat pigment NO-Heme was measured according to the method described by Hornsey (1956). All the procedures were carried out under anaerobic condition within a fume hood in a dimmed room illuminated by a weak red light (ca. 1.04 lx) at room temperature (ca. 20 °C). All solvents were deoxygenated by flushing with a stream of nitrogen gas. Minced samples (10 g each)