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Effect of pomegranate peel extract on lipid and protein oxidation in beef meatballs during refrigerated storage

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ABSTRACT

Antioxidant effect of pomegranate peel extract (PE) to retard lipid and protein oxidation was investigated in meatballs during refrigerated storage at 4 ± 1 °C. Concentrated lyophilised water extract of pomegranate peel was incorporated into freshly minced beef meat at 0.5% and 1% concentrations and compared with 0.01% butylated hydroxytoluene (BHT) as a reference and control (without any antioxidant). PE showed high phenolic content and antioxidant activity. In PE added samples, thiobarbituric acid reactive substances (TBARS) value, peroxide formation, loss of sulfhydryl groups and formation of protein carbonyls were lower than control ($P < 0.01$) after 8 days of storage. Sensory evaluation with respect to colour and rancid odour revealed that PE incorporation in meatballs prolonged the refrigerated storage up to 8 days. Addition of both 0.5 and 1% PE in meatballs reduced lipid and protein oxidation and improved sensory scores. These results indicated that PE was effective on retarding lipid and protein oxidation.

1. Introduction

Meatball is a highly consumed meat product and has a short shelf-life due to some deterioration during storage. Oxidative changes (lipid and protein oxidation) have been implicated as one of the main factors to reduce their quality and shelf-life of muscle foods (Dave & Ghaly, 2011; Ladikos & Lougovois, 1990; Lund, Heinonen, Baron, & Estévez, 2011). These alterations occur more quickly in minced meats than in intact meat. This is because the surface area of meat to air increases substantially after grinding the meat (Naveena, Sen, Kingsly, Singh, & Kondaiah, 2008b).

A major cause of the quality loss in meat products is lipid and protein oxidation (Vuorela et al., 2005). There are many studies showing the effects of lipid oxidation on muscle foods during refrigerated storage, but the studies on protein oxidation in processed meat products are limited (Ganhão, Morcuende, & Estévez, 2010). As a result of protein oxidation, changes in amino acid structures occur and these changes lead to carbonyl formation and decreased sulfhydryl content (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013). Moreover, changes in water holding capacity and tenderness of meat could occur during storage of meat depending on protein oxidation (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004).

Oxidative rancidity results in extensive flavour changes, colour losses and structural damage to proteins (Xiong, 1996), which reduce sensory quality and consumer acceptability of meat products. Therefore, natural origin food additives have been the subject of many investigations in recent years. For example, the positive effects of several plant extracts were shown on the inhibition of protein and/or lipid oxidation, such as rosemary (Lund, Hviid, & Skibsted, 2007), *Nitraria retusa* (Mariem et al., 2014), potato peel (Kanatt, Chander, Radhakrishna, & Sharma, 2005), phenolics from pomegranate peel (Kanatt, Chander, & Sharma, 2010; Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008a; Naveena, Sen, Kingsly et al., 2008), and catechins from tea (Mitsumoto, O'Grady, Kerry, & Joe Buckley, 2005; Tang, Sheehan, Buckley, Morrissey, & Kerry, 2001).

Different parts of pomegranate fruits such as arils and rinds contain many bioactive compounds showing various activities (Wang, Ding, Liu, Xiang, & Du, 2010). Peel extracts have very high antioxidant capacity in scavenging superoxide anion, hydroxyl and peroxyl radicals as well as inhibiting low-density lipoprotein oxidation (Li, Yu, & Ho, 2006; Negi & Jayaprakasha, 2003). The antioxidant effect of pomegranate peel has been studied in cooked chicken products (Kanatt et al., 2010; Naveena Sin, Vaithiyanathan et al., 2008; Naveena Sin, Kingsly et al., 2008), in cooked goat meat patties (Devatkal, Narsaiah, & Borah, 2010), in raw pork (Shan, Cai, Brooks, & Corke, 2009), and in raw ground pork meat (Qin et al., 2013).

In Turkey, pomegranate fruits are widely processed into juice and juice concentrate, and the peels obtained from juice processing are discarded. The objective of this study was to investigate (I) the antioxidant activity of lyophilized PE and (II) the effects of addition of different levels of lyophilized PE to extend the storage shelf-life of beef meatballs during refrigerated storage.

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2. Materials and methods

2.1. Preparation of pomegranate peel extracts

Newly harvested ripe pomegranate fruits (*Punica granatum* L., cv. Hicaznar) were obtained from fruit juice pilot plant at Ankara University. After pressing, pomegranate marcs were collected and the remaining arils on the marcs were removed. The cleaned marcs were then immediately frozen at −18 °C until used in a month. The peel extraction method was adapted from the work done by Kanatt et al. (2010). Prior to experiments, the samples were thawed at 4 °C. The peels and arils left on the peels were manually separated and the resulting peels were cut into 1-cm^2 pieces using a sharp knife. A 100 g of peel was refluxed with distilled water (1000 mL) for 1 h. The extract was filtered through cheesecloth after cooling. The residue was refluxed again for an hour. All filtrates were collected and centrifuged (Hermle Z326K, Germany) at 12000 ×*g* for 20 min. The supernatant was concentrated in a rotary evaporator (Heidolph Laborata 4003, Germany) at 40 °C to 10 ± 0.5% of soluble solids. The concentrate was frozen at −40 °C, then dehydrated for 72 h in a freeze–dryer (Labconco 74200, USA) at 0.120 mbar with a chamber temperature of 18 ± 0.5 °C and a condenser at −85 ± 1 °C to obtain water soluble powder.

2.2. Determination of the phenolic content

The phenolic content of the extracts was estimated by the Folin–Ciocalteu method of Singleton and Rossi (1965). The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of powder extract.

2.3. Antioxidant activity

Antioxidant activity of PE was determined according the method described by Miller and Rice-Evans (1997) and Arts, Haenen, Voss, and Bast (2001). Triplicate determinations were carried out and the results were expressed as mmol of trolox equivalents per g extract.

2.4. Meatball manufacture

Minced meat was purchased from a commercial producer (Meat and Milk Association, Sincan, Ankara) and İnegöl-style meatballs were manufactured according to a conventional formula: 86.75% minced beef (12.5% fat content), 6.0% bread crumbs, 0.25% black pepper, 0.5% cumin, 3.5% onion, 0.5% garlic, 1.5% salt and 1.0% water. All ingredients were homogenized in a bowl mixer (Tefal, QA400, France) for 5 min and divided into 4 groups. Lyophilized powder of PE was added to sample two groups at a concentration of 0.5% (83 mg/g GAE) and 1% (165 mg GAE). The third group included BHT at 0.01% as a reference and the last one did not include any antioxidant source (control). Meatballs (20 ± 2 g) were formed by hand, placed into plastic trays, sealed with one layer of a semi-permeable film and stored in the dark at 4 ± 1 °C for 8 days. Samples were taken at 0, 2, 4, 6 and 8 days of storage.

2.5. Lipid oxidation

2.5.1. Peroxide value

Peroxide values (PV) of samples were determined according to IDF method (Shantha & Decker, 1994). Depending on the degree of peroxidation, extracted lipid of 0.01–0.30 g was transferred into test tubes. 9.8 mL of chloroform:dichloromethane (2:1, *v*/*v*), 50 μL of ammonium thiocyanate and 50 μL of Fe(II) were added and the contents of test tubes were vortexed for 2–4 s after addition of each solution. Absorbance was measured with a spectrophotometer at 500 nm after 5 min of incubation at room temperature. Results were expressed as milliequivalents of peroxide/kg fat.

2.5.2. Thiobarbituric acid reactive substances

Lipid oxidation was also evaluated by thiobarbituric acid reactive substances (TBARS) according to the method described by Ahn et al. (1998) with some modifications. Briefly, 5 g of sample was homogenized with 15 mL of distilled water and centrifuged (Hettich, Rotofix II, UK) at 2000 ×*g* for 15 min. 1 mL of the resulting slurry was transferred into a test tube containing 2 mL of trichloroacetic acid/thiobarbituric acid (TCA/TBA) solution consisting of 15% TCA (*w*/*v*) and 0.375% TBA (*w*/*v*) in 0.25 M HCl and 3 ml 2% butylated hydroxytoluene (*w*/*v*) prepared in absolute ethanol in a test tube and, the tube content was immediately vortexed. Following water bath treatment at 100 °C for 15 min, the tube content was cooled rapidly down to room temperature and centrifuged at 1000 ×*g* for 10 min. Then, absorbance was measured at 531 nm with a spectrophotometer (Labomed UVD-3200) against blind (1 mL distilled water and 2 mL TCA-TBA-HCl solution). TBARS were calculated using 1,1,3,3-tetraethoxypropane standard curve and expressed as mg MDA/kg meat.

2.6. Protein oxidation

2.6.1. Determination of carbonyls

Protein carbonyl content was determined by the method outlined by Oliver, Ahn, Moerman, Goldstein, and Stadtman (1987), with the modification of Vuorela et al. (2005). Two procedures were carried out for the determination of protein oxidation: (I) carbonyl content and (II) protein quantification. A 1 g of meatball sample was homogenized in 10 mL 0.15 M KCl buffer using an Ultra Turrax (IKA T25, Germany) for 60 s at the speed of 20980 ×*g*. A 45 μL of the resulting blend was transferred into an Eppendorf vial containing 1 mL 10% trichloroacetic acid (TCA, *w*/*v*). Samples were centrifuged (Hermle Z326K) for 5 min at 2880 ×*g* and supernatant was removed. For procedure (I), 1 ml 2 M HCl containing 0.2% 2,4-dinitrophenyl hydrazine (DNPH) and for procedure (II) 1 ml 2 M HCl was added to the Eppendorf vials. Samples were then incubated for 1 h at room temperature, with vortexing every 20 min. Following the incubation, 0.60 ml 10% TCA was added. Samples were then centrifuged (Hermle Z326K) for 5 min at 2880 ×*g* and supernatant was carefully removed by not damaging the pellets. The pellets were washed for three times with 1 mL ethanol:ethyl acetate (1:1) solution, vortexed and centrifuged for 5 min at 11500 ×*g*. After the removal of DNPH residues completely, the pellets were dried under N_2 gas and dissolved in 1.5 ml 20 mM sodium phosphate buffer containing 6 M guanidine hydrochloride buffer (pH 6.5). Samples were vortexed and centrifuged for 5 min at 2880 ×*g*. Absorbance of final solutions was measured at 370 nm (Labomed UVD-3200) against 20 mM sodium phosphate 6 M guanidine hydrochloride buffer. Carbonyl concentration was calculated using extinction coefficient of 21.0 mM^{-1} cm^{-1} and protein concentration of samples (determined at 280 nm), and was expressed as nmol carbonyl/mg protein.

2.6.2. Determination of sulfhydryl groups

Total sulfhydryl (thiol) content was determined using 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) according to the method given by Ellman (1959) with modifications of Eymard, Baron, and Jacobsen (2009). A 0.5 g of meatball sample was homogenized in a 10 ml 0.05 M phosphate buffer (pH 7.2) for 60 s with a Kinematica polytron homogenizer (10–35 GT model, Sweden). 1 mL of homogenate was mixed with 9 mL 0.05 M phosphate buffer (pH 7.2) containing 8 M urea, 0.6 M NaCl and 6 mM EDTA. The mixture was then centrifuged for 15 min at 14000 ×*g* at 5 °C. A 3 mL of supernatant was mixed with 0.01 M DTNB prepared with 0.05 M sodium acetate before incubation at 40 °C for 15 min and absorbance was measured at 420 nm with a spectrophotometer (Labomed UVD-3200). The sulfhydryl content was calculated using a molar extinction coefficient of 13,600 M^{-1} cm^{-1} and the results were expressed as mmol sulfhydryl/g sample.