Meat Science 95 (2013) 195–202

Contents lists available at SciVerse ScienceDirect

Meat Science

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

Relationship between the solubility, dosage and antioxidant capacity of carnosic acid in raw and cooked ground buffalo meat patties and chicken patties

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ARTICLE INFO

Article history:
Received 21 December 2012
Received in revised form 9 April 2013
Accepted 15 April 2013
Available online 25 April 2013

Keywords:
Rosemary
Carnosic acid
Antioxidant activity
Buffalo meat
Chicken
Color

ABSTRACT

Antioxidant capacity of oil soluble and water dispersible carnosic acid (CA) extracted from dried rosemary leaves using HPLC was evaluated at two different dosages (22.5 ppm vs 130 ppm) in raw and cooked ground buffalo meat patties and chicken patties. Irrespective of total phenolic content, CA extracts reduced ($p < 0.05$) the thiobarbituric acid reactive substances (TBARS) by 39%–47% and 37%–40% in cooked buffalo meat and chicken patties at lower dosage (22.5 ppm) relative to control samples. However, at higher dosage (130 ppm) the TBARS values were reduced ($p < 0.05$) by 86%–96% and 78%–87% in cooked buffalo meat and chicken patties compared to controls. The CA extracts were also effective in inhibiting ($p < 0.05$) peroxide value and free fatty acids in cooked buffalo meat and chicken patties. The CA extracts when used at higher dosage, were also effective in stabilizing raw buffalo meat color.

1. Introduction

Recent studies have put more focus on natural antioxidant compounds derived from food components, including herbs such as rosemary and oregano (McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001), spices such as cloves, cinnamon, ginger, black pepper, cumin, nutmeg, cardamom etc. (Shan, Cai, Sun, & Corke, 2005). One of the most important sources of natural antioxidants is rosemary (*Rosmarinus officinalis*) from the Labiatae family. Loliger (1983) emphasized that rosemary was one of the few spices used in the food industry primarily for its antioxidant property, whereas other spices are used for their flavoring properties. Many studies have been made to examine the antioxidative activities of crude rosemary and different rosemary extracts (Riznar et al., 2006).

Natural plant-derived antioxidants are primarily composed of polyphenolic compounds, and antioxidants containing 2 or more phenolic hydroxyl groups (Shahidi, Janitha, & Wanasundara, 1992). A number of phenolic substances were isolated from a variety of spice sources, including various phenolic compounds: monoterpenes (eteric olis), diterpene phenols (carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methyl carnosate), phenolic acids (rosmarinic and caffeic acid), flavonoids (quercitin, myrecitin, luteolin) and triterpene acids (ursolic acid, oleanolic acid, butilinic acid), volatile oils (eugenol, thymol, carvacrol) (Aruoma, Halliwell, Aeschbach, & Loligers, 1992; Shan et al., 2005). Many of these compounds have been isolated from different sources of herbs, spices, vegetables and fruits and identified by numerous authors and described in the literature. Among these phenolic compounds rosmarinic acid, carnosic acid, and carnosol are the major bioactive constituents in rosemary leaves responsible for the antioxidant, anti-inflammatory, and anticarcinogenic effects (Masuda, Inaba, & Takeda, 2001). Carnosic acid (CA) is a phenolic diterpene compound found in rosemary and sage leaves. It is the most powerful antioxidant among diterpenes and is a free radical scavenger. Antioxidative activity of CA is as high or higher as synthetic antioxidants (Cuppett, Schnepf, & Hall, 1997). Carnosic acid has a wide spectrum of actions, including antimicrobial, anticancer, and antimutagenic effects, and also has inhibitory effects on HIV-1 protease (Paris, Strukelj, Renko, & Turk, 1993). CA has increasingly become accepted as a food additive.

Many studies have been undertaken to examine the antioxidative activities of crude extracts of spices and herbs and different commercially available extracts in purified form. Today purified antioxidant products of rosemary are commercially available. It would be interesting to investigate the antioxidant activity of purified rosemary phenols without the general characteristic odor from the volatiles in raw and cooked meats. The products recommended for meat and meat products

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http://dx.doi.org/10.1016/j.meatsci.2013.04.043

are marketed either in liquid form or as powders. They may be either water or oil soluble. The effectiveness of an antioxidant in a food system is mainly dependent on concentration and the solubility. In general, studies on the antioxidant constituents of rosemary extracts have used lipid-soluble fractions (Senorans, Ibanez, Cavero, Tabera, & Reglero, 2000) as most commercially available rosemary extracts are also lipid soluble. The solubility of an antioxidant will determine its affinity for lipid or water phases, its preferential orientation at air/water/lipid interfaces (Frankel, Huang, Prior, & Aeschbach, 1996) and is likely to affect overall antioxidant activity especially in particulate systems such as comminuted meats.

It has been demonstrated that a variety of antioxidant compounds may display pro-oxidant effect especially on protein oxidation (Estevez, 2011) and that such activity is system and/or concentration dependent. Because of lower concentrations of plant extracts normally used in food formulations, the potential pro-oxidant activity of these extracts has not been widely reported. But, evidence exists for prooxidative activity of carnosol and carnosic acid (Aruoma et al., 1992), two important constituents of rosemary extract. Earlier researchers have also indicated higher antioxidant activity with higher level of CA (4% vs 20%) and increased dosage (100 ppm vs 500 ppm) in chicken frankfurters (Riznar et al., 2006) and cooked turkey (Yu, Scanlin, Wilson, & Schmidt, 2002) respectively. Susceptibility to lipid oxidation in red meat (buffalo meat) or white meat (chicken) is species dependent and it would be interesting to evaluate the antioxidant effects of rosemary extracts in these two species of meat. Considering the effect of CA content, dosage, solubility and substrate on the antioxidant efficacy, the present study was conducted to investigate the antioxidant effects of carnosic acid in raw and cooked buffalo meat patties and chicken patties.

2. Materials and methods

2.1. Materials

Fresh buffalo meat hind quarters were procured from local municipal slaughter house, Chengicherla, Hyderabad, India and chilled for 24 h at 4 °C. Hind quarter muscles were deboned and used for the study. Fresh skinless chicken drumstick, thigh and breast meat was obtained from a poultry processing plant (Sneha Poultries, Hyderabad, India), and chilled overnight at 4 °C. Chilled, boneless buffalo meat and chicken were first coarsely ground through a 13-mm plate followed by 8-mm plate in a meat grinder (SCHARFEN, Model X70, 58413 Witten, West Germany). Freshly ground meat was used for the experiment. The butylated hydroxytoluene (BHT) and 1,1-diphenyl 1-2-picrylhydrazyl (DPPH) were procured from Sigma-Aldrich Chemical Co., Germany. All chemicals were of reagent grade or greater purity.

2.2. Preparation of carnosic acid extracts and analysis using HPLC

Dried Rosemary leaves (*R. officinalis*) having a carnosic acid (CA) content of 2.5–4.5% was extracted using a mixture of organic solvent consisting of acetone and hexane by a proprietary process of KANCOR Ingredients Ltd., Ernakulam, Kerala, India, which include mainly pretreatment, extraction, de-solventization, standardization/deodorization. Different extracts includes crude oleoresin rosemary-oil soluble (OxiKan-S10); refined oleoresin rosemary-oil soluble (OxiKan-R2.5); and refined oleoresin rosemary-water dispersible (OxiKan-WD2.5). Carnosic acid content of final products was analyzed by HPLC method of Cantrell, Richeimer, Nicholas, Schmidt, and Bailey (2005) with some minor modifications. The HPLC system consisted of 1120 Compact LC by Agilent Technologies with a 100 microlitre Rhedodine injector loop, and a UV/VIS detector. The column was a LiChrospher 250-4, RP-18 (5 μm) from Merck Millipore. The analysis was run at 1.0 mL/min with a 65:35 mixture of acetonitrile and water containing 0.1% phosphoric acid with a monitoring wavelength of 230 nm. Quantification of CA was done by using a standard reference from Sigma Aldrich having 95% purity.

2.3. Antioxidant treatment and storage

Oil soluble products OxiKan-S10 and OxiKan-R2.5 and water dispersible product OxiKan-WD2.5 were prepared as per the aforesaid procedure. All the three extracts were in liquid form with a pH of 4.24, 4.22 and 4.53 respectively (Table 1). These extracts were analyzed for total phenolic content and DPPH radical scavenging activity.

Freshly ground buffalo meat and chicken were subdivided into 8 lots each and randomly assigned to eight different treatments. For each antioxidant extract, two dosages: 22.5 ppm CA phenols (low dosage, LD) and 130 ppm CA phenols (high dosage, HD) were used as recommended by the suppliers. Control (meat without any antioxidant) and BHT (100 ppm) were also compared with other treatments. Eight different treatments used in the study includes: Control; OxiKan-S10 (LD); OxiKan-S10 (HD); OxiKan-R2.5 (LD); OxiKan-R2.5 (HD); OxiKan-WD2.5 (LD); OxiKan-WD2.5 (HD); and BHT. Sodium chloride (1% w/w) dissolved in distilled water was added to all samples. The BHT was dissolved in crude, groundnut oil before addition and the same quantity of oil was added to other samples to maintain uniformity. The volume of antioxidant extracts were replaced with distilled water/oil in control and BHT samples. Immediately after adding all ingredients, samples were thoroughly mixed using a planetary mixer under medium speed for 2 min. After mixing, minced meat (100 g portions) was molded manually using glass petri-plates in the form of burger patties with uniform and smooth surface. Total of 27 patties (12-raw and 15-cooked) were made for each treatment separately for buffalo meat and chicken. The molded patties (15 × 90 mm) were loosened from the petri-plates and placed over a tray on the middle shelf of the oven in specific positions to ensure uniform heating conditions and cooked in convection type still air oven (baking oven) fitted with a fan for circulation of hot air (Cassia Siamia Technologies Ltd., Hyderabad, India). The oven temperature was fixed at 180 °C and the patties were cooked for 20 min or till the internal core temperature of the patties reaches 80 °C measured by a probe thermometer inserted into geometric centre of the patty. The oven was then switched off, the samples were extracted and left for cooling for 30 min on a open rack at room temperature. Raw patties were aerobically packaged in a low density polyethylene pouches (Famous Enterprise, Hyderabad, India) and stored in a refrigerator (SAMSUNG, Model: RT54EBTS/2010) at 4 °C for 9 days period. The patties were analyzed at 3 days interval for pH, thiobarbituric acid reactive substances (TBARS), peroxide value, free-fatty acids and sensory attributes. After cooling, cooked patties were also packaged and stored for 28 days similar to raw patties and analyzed at 7 days interval. At each designated storage period 3 patties from each treatment were used for analysis. Sensory evaluation was performed only on day 0. Buffalo and chicken studies were carried out on different occasions.

2.4. Analysis of samples

2.4.1. DPPH radical scavenging activity

The ability to scavenge 1,1-diphenyl 1-2-picrylhydrazyl (DPPH) radical by different CA extracts was estimated by the method of Singh, Murthy, and Jayaprakasha (2002). The antioxidant extracts diluted with 0.1 M Tris–HCl buffer (pH 7.4) was mixed with 1 ml of DPPH (250 μM) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and the absorbance was measured at 517 nm (SHIMADZU, Model: UV-1700 PharmaSpec, Japan). The scavenging activity was calculated by the following equation:

$$\text{Scavenging activity}\% = \left(\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Sample}} / \text{Absorbance}_{\text{Blank}}\right) \text{ x } 100$$