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Optimizing color and lipid stability of beef patties with a mixture design incorporating with tea catechins, carnosine, and α-tocopherol

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ABSTRACT

The interactions of three natural antioxidants, tea catechins, carnosine, and α-tocopherol and their effect on color, lipid stability, Metmyoglobin percentage and Metmyoglobin-reducing activity of raw beef patties were evaluated. Increase in the content of tea catechins in the mixture lead to higher lipid stability and lower Metmyoglobin percentage. Carnosine had a notable effect on improving Metmyoglobin-reducing activity and producing better color stability at a low concentration. α-Tocopherol and tea catechins enhanced lipid stability. Interaction between tea catechins, carnosine, and α-tocopherol improved lipid stability and color stability of raw beef patties. The optimal values of tea catechins, carnosine, and α-tocopherol in the mixture were 3–19%, 78–94%, and 0–12%, respectively.

1. Introduction

Oxidative processes lead to the degradation of lipids and proteins (including pigments) and are one of the primary mechanisms of quality deterioration in meat and meat products. They cause loss of flavour, color and nutritive value and limit the shelf-life of meat and meat products (Kanner, 1994). Many studies have indicated that lipid oxidation in meat can be effectively controlled or, at least, minimized by adding antioxidants (Djenane et al., 2004). Due to concerns about toxicological safety of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), naturally derived antioxidants are perceived by consumers as better and safer than synthetics. Then meat products containing natural antioxidants, as opposed to synthetic derivatives, are more desirable from a consumer viewpoint (Mitsumoto et al., 2005; Yen et al., 2002). In the past few years, various plant materials containing phenolic compounds have been demonstrated to be effective antioxidants in model systems.

α-Tocopherol (VE) is generally regarded as an acceptable 'consumer friendly' supplement and incorporated into animal diets as a highly effective lipid-soluble chain-breaking antioxidant (Faustman et al., 1989) and can prolong color stability of beef (Chan et al., 1995; Eikelenboom et al., 2000) and lipid stability of fresh pork (Lanari et al., 1995; Monahan et al., 1994).

Carnosine (b-alanyl-L-histidine), an endogenous dipeptide found in the skeletal muscle of most vertebrates, is synthesized from b-alanine and L-histidine by carnosine synthetase (Kozan et al., 2008; Lee and Hendricks, 1997). Its antioxidant properties may result from its ability to chelate transition metals such as copper, its enzyme-like activity, and its free-radical scavenging (Lee et al., 1998). It also can interfere in the initiation step of oxidation, decrease the amount of preformed peroxides and react with some secondary products (Kansci et al., 1997). In recent years, there have been some interests in the antioxidant potential of carnosine in meats (Calvert and Decker, 1992; Lee et al., 1999; O'Neill et al., 1999). Research results indicated that carnosine (0.5–1.5%) inhibited lipid oxidation in pork (Decker and Faraji, 1990; Decker and Crum, 1991), turkey (Calvert and Decker, 1992), and beef (Shantha et al., 1995). Carnosine exerted significant efficacy in maintaining an acceptable visual red color for irradiated ground beef and raw patties during storage and has been suggested as a useful natural food antioxidant (Badr, 2007).

Tea catechins are polyphenolic antioxidants which possess a range of health promoting properties, such as anti-carcinogenic, anti-mutagenic properties in numerous human, animal and *in vitro* studies (Dreosti, 1996; Mandel et al., 1999). Tea catechins, have been reported to be effective natural antioxidants in fish muscle model systems (He and Shahidi, 1997), chicken meat (Tang et al., 2000) and red meat systems (Tang et al., 2001a,b). The strong free-radical-scavenging ability plus the iron-chelating effects of tea catechins provide a plausible mechanism for the antioxidant effects of added tea catechins in meat system *in vitro* (Tang et al., 2002).

Many studies have shown that synergetic effect was exist between different antioxidants (Salah et al., 1995; Decker and Faraji,

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1990). The chain-breaking effect of α-tocopherol, the copper chelating effect and enzyme-like activity of carnosine, the iron-chelating effect and free-radical-scavenging ability of tea catechins suggesting that they could act synergistically to inhibit beef patty lipid oxidation and discoloration.

The objective of present work was to apply the simplex–centroid mixture design methodology, to determine the effect of α-tocopherol, tea catechins and carnosine and their interaction on the color attributes of raw beef patties and their optimal combination to preserve meat color during chilled storage.

2. Materials and methods

2.1. Reagents

L-carnosine ($C_9H_{14}N_4O_3$), tea catechins (polyphenols P 98%, catechins P 90%,) and α-tocopherol were all food grade and purchased from Qinghua Kechuang Food Additives Co. Ltd. (Beijing, China). NADH and equine Myoglobin were obtained from Sigma (St. Louis, USA). All other chemicals and solvents used were of analytical grade and purchased from Lanyi Chemical Articles Co. Ltd. (Beijing, China).

2.2. Experimental design

A three component, simplex–centroid mixture design was used to investigate the effect of carnosine (X_1), α-tocopherol (X_2) and tea catechins (X_3) on the color, lipid stability, percentage of Metmyoglobin and Metmyoglobin-reducing activity of raw beef patties, and to determine the optimum combinations of the antioxidants to preserve the meat color during chilled storage. In a mixture experiment design, the total amount is held constant and a measured property of the mixture changes when the proportions of the components of the mixture are changed. Therefore, the main purpose of using this methodology is to verify how the properties of interest are affected by the variation of the proportions of the mixture components (Nardi et al., 2004). Component proportions were expressed as fractions of the mixture with a total content ($X_1 + X_2 + X_3$) of 0.03% (Table 1).

According to the mixture design (Fig. 1), the vertex of the triangle indicated single antioxidant treatment, the point on the border indicated two antioxidants mixture treatment and the inner point of the triangle indicated three antioxidants mixture treatment, thus there were seven treatments generated: three single antioxidant treatments, three two antioxidants mixtures treatments and one three antioxidants mixture treatment, respectively. Seven combinations of the three antioxidants were tested.

2.3. Sample preparation

Six beef strip lions (*Longissimus dorsi* muscle, LD) of 24 months old Simmental breeds with live weight of 450–470 kg were obtained at 48 h post mortem. The animals were stunned by captive bolt and slaughtered in a commercial plant following the industry practices. After dressing, the carcasses were held at 4 °C for 48 h. The average ultimate pH was 5.70 ± 0.05. The loins were cut into small pieces after removal of visible fat and connective tissues and then grounded using a food processor (CombinMax600, China) along with different combinations of antioxidants stipulated in the experiment design and total antioxidants content was 0.03 mg/kg meat. Treated meat samples (180 g) were formed into patties using a conventional burgermaker. The patties were placed on round glass plates with diameter of 10 cm and height of 1.5 cm and stored at 2 °C in the dark in a cabinet for 7 days. Two sub-samples were taken from each of the meat patties at 7th day and analyzed.

Table 1
Mixtures composition in beef patties formulated with tea catechins, carnosine, and/or α-tocopherol in a three-component constrained simplex–centroid mixture design

Formulation[a]	Antioxidants proportion[b]		
	X_1 (CR)	X_2 (VE)	X_3 (TC)
1	1	0	0
2	0	1	0
3	0	0	1
4	0	0.5	0.5
5	0.5	0	0.5
6	0.5	0.5	0
7	0.333333	0.333333	0.333333

[a] Formulation numbers correspond to Fig. 1.
[b] CR, carnosine; VE, α-tocopherol; TC, tea catechins.

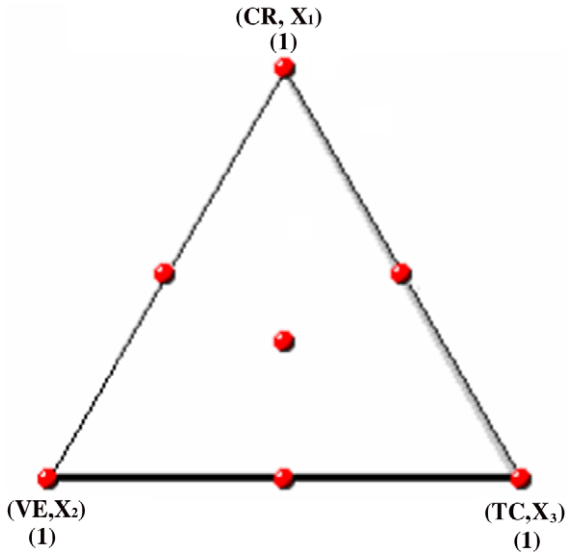

Fig. 1. Seven point simplex–centroid design for the interaction of carnosine (CR, X_1), α-tocopherol (VE, X_2) and tea catechins (TC, X_3) in beef patties. The term (1) at each angle means that at this point, there is only one kind of antioxidant in the mixture and its concentration is 0.03%.

The experiments were replicated three times and mean values were used for calculation.

2.4. TBARS values

Lipid oxidation of treated beef patties were measured at 7th day. Thiobarbituric acid-reactive substances (TBARS) assay was performed as described by Faustman et al. (1992) with little modification. Pattie samples (10 g) were mixed with 45 ml of stock solution (25 ml 20% TCA–20 ml water) homogenized for 30 s with a superfine homogenizer (F6-10, Fluko, Shanghai, China) and centrifuged at 1000g for 20 min at 4 °C. The supernatant was filtered through Whatman No. 1 filter paper. The solution (3 ml supernatant–3 ml 0.02 M TBA) heated in a boiling water bath (95–100 °C) for 30 min to develop the pink color. Samples were cooled under running tap water, centrifuged at 4500g for 25 min and the absorbance of the supernatant measured at 532 nm using an Unicam UV4 spectrometer (Unicam Ltd., China). TBARS were expressed as mg malonaldehyde/kg sample.

2.5. Percentage of Metmyoglobin, Oxymyoglobin and Myoglobin

Pattie samples (5 g) were homogenized in 25 ml ice-cold 40 mM phosphate buffer (pH 6.8) for 10 s using a superfine