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Effect of addition of commercial rosemary extracts on potent odorants in cooked beef

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ABSTRACT

Solid-phase microextraction-gas chromatography-olfactometry (SPME-GCO) and aroma extract dilution analysis (AEDA) were applied to measure the effects of the addition of two commercial rosemary extracts (RE) on the potent odorants in cooked beef extracts (BE). On the basis of the results of SPME-GCO and AEDA, the addition of RE imparted sweet and floral notes to BE as a result of the addition of esters and terpenes of RE. In addition, RE suppressed the formation of odorants derived via lipid oxidation and Maillard reactions. The most potent lipid oxidation volatiles consisted of 1-octen-3-one (mushroom-like), (*E*)-2,4-epoxy-(*E*)-2-decenal (metallic), and eight different aldehydes (fatty). The Maillard reaction volatiles, necessary for typical cooked beef flavor, included compounds with meaty [2-methyl-3-furanthiol, 2-methyl-3-(methylthio)furan, 2-methyl-3-(methyldithio)furan], roasty (2-furanmethanethiol), caramel-like [4-hydroxy-2,5-dimethyl-3(2*H*)-furanone], baked potato-like [3-(methylthio)propanal], and spicy [3-hydroxy-4,5-dimethyl-2(5*H*)-furanone] attributes. The suppressive effects of RE may be caused by the action of antioxidative substances in RE alone or in combination with the pH increase in BE induced by the matrix components of RE.

1. Introduction

Lipid oxidation causes the deterioration of critical nutritional and sensory attributes in foods during processing and storage (Kubow, 1992). For this reason it is often necessary to add antioxidants to food, especially for products containing high contents of lipids, such as meat products and edible oils. Synthetic antioxidants, such as *tert*-butyl-4-hydroxyanisole (BHA) and di-*tert*-butyl-4-hydroxytoluene (BHT), are effective inhibitors of lipid oxidation and are widely used in the industry; however, in recent years concerns have grown over the possible toxicity of synthetic antioxidants. This has in turn led to an increased demand for alternative antioxidants originating from natural sources such as plants (McBride, Hogan, & Kerry, 2007; Pan et al., 2007; Paradiso, Summo, Pasqualone, & Caponio, 2009). Rosemary (*Rosmarinus officianalis* L.) is a popular source of natural antioxidants, composed mainly of phenolic diterpenes including carnosol, epirosmanol, rosmanol, carnosic acid, and rosmadial (Löliger, 1983; Zhen & Wang, 2001). A number of studies have reported rosemary extracts have high potency as antioxidants in meat products; for example, in beef (Ahn, Grun, & Mustapha, 2007; Pennisi Forell, Ranalli, Zaritzky, Andres, & Califano, 2010), pork (Haak, Raes, & De Smet, 2009; Nissen, Byrne, Bertelsen, & Skibsted, 2004), and chicken (Keokamnerd, Acton, Han, & Dawson, 2008; Nissen, Mansson, Bertelsen, Huynn-Ba, & Skibsted, 2000). Sensory results of some previous studies indicated that the odor characteristics related to lipid oxidation, such as rancid, was significantly decreased in meat products treated with rosemary extracts (Nassu, Gonçalves, da Silva, & Beserra, 2003; Nissen et al., 2004). This observation is supported by analytical studies where it was found that selected lipid-derived volatiles, such as aldehydes and alcohols, were in lower abundance in meat products treated with rosemary extracts (Barbut, Josephson, & Maurer, 1985; Estévez, Ventanas, Ramírez, & Cava, 2004). However, no study has systematically examined the effect of natural antioxidants such as rosemary on the potent odorants in cooked meat using gas chromatography-olfactometry (GC-O) methods.

It is hypothesized that rosemary constituents will affect reactions responsible for the formation of essential meat-like odorants, such as sulfur-containing furanes and thiophenes and related disulfides which are known to have strong meat-like aroma characteristics (Mottram, Madruga, & Whitfield, 1995) and will also suppress formation of odorants derived via lipid-oxidation. It is also possible that rosemary extracts may affect the formation of volatiles derived from other pathways.

The aim of the present study was to apply solid phase microextraction-GC-O and aroma extract dilution analysis to evaluate the effect of the addition of commercial rosemary extracts on potent odorants in cooked beef extracts.

2. Materials and methods

2.1. Materials

Commercial rosemary extract powders, RM-BD™ (oil-soluble type; composed of oil-soluble diterpenes, including carnosic acid (34%) and

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carnosol (15%), as main active ingredients), RM-MP™ (water-soluble type; formulated with 1% RM-BD™ and other buffering agents composed of sodium bicarbonate, trisodium citrate dehydrate and sodium chloride in order to improve its antioxidative activity in aqueous solution), and MAT (buffering agents of RM-MP ™ without RM-BD™) were provided by Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Beef Loin, Top Sirloin Butt, Boneless (*m. gluteus medius*, IMPS item No. 184, USDA Choice) was purchased from Agri Star Meat and Poultry, LLC. (Postville, IA, USA).

Authentic reference standards for the compounds listed in Table 2 and Table 3 were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) or Lancaster (Windham, NH, USA). 2-Acetyl-1-pyrroline was synthesized using the method described by Fuganti, Gatti, and Serra (2007). (*E*)-4,5-Epoxy-(*E*)-2-decenal was synthesized by closely following the method described by Schieberle and Grosch (1991). (*Z*)-2-Nonenal was synthesized from (Z)-2-nonen-1-ol (Bedoukian Research Inc., Danbury, CT, USA) by oxidation with Dess-Martin periodinane (0.3 M in dichloromethane; Sigma-Aldrich Co.) following the procedure described by Meyer and Schreiber (1994).

2.2. Preparation of beef extract

Beef was processed at the Meat Science Laboratory at the University of Illinois (Urbana, IL, USA). It was ground for 10 min (Hobart grinder model 4152, Hobart Mfg. Co., Troy, OH, USA), and was comminuted through a 3-mm plate. Temperature of the processing lab was kept under 7 °C throughout the processes. 450 g portions of the comminuted meat was vacuum-packaged individually and was stored frozen until needed (−22 °C). Beef extract (BE) was prepared by following the method of Balagiannis et al. (2009) with slight modification. The comminuted meat (400 g) was mixed with an equal quantity of distilled-deodorized water, and homogenized using an Ultra Turrax® (T18 basic, IKA, Wilmington, NC, USA) at 18000 rpm for 5 min. The slurry was separated into 250-mL Teflon bottles and centrifuged for 10 min at 3000 rpm (1500 ×*g*). The supernatants were collected in a 1-L glass beaker. The raw BE (100 mL) was placed into a 200-mL glass bottle (52 mm O.D.×110 mm high) with rosemary extract powders (RE). All REs were dissolved in ethanol:distillated water (1:1 by volume) at the designated dosage levels as follows; control, no RE added (treatment code, CON); 500 ppm of RM-BD™ (RMD); 3000 ppm of RM-MP™ (RMP); 3000 ppm of RM-MP matrix (MAT). These dosage levels were suggested by the manufacturer. Each treatment was heated in an oil bath (130 °C) for 15 min after the temperature in BE reached to 95 °C. The cooked BE was cooled for 30 min at room temperature, and was filtered through a stainless sieve (size no. 45; Fisher Scientific, Pittsburgh, PA, USA) prior to further analysis. The pH value of cooked BE was obtained with a pH meter (Accumet AB15, Fischer Scientific). All treatments were prepared in triplicate.

2.3. Headspace solid phase microextraction-gas chromatography–mass spectrometry (SPME-GC-MS)

Volatile compounds were isolated by solid phase microextraction (SPME) method. The SPME fiber, coated with 50/30 μm divinylbenzene/carboxen/polydimethyl siloxane (Supelco, Bellefonte, PA, USA) was preconditioned prior to analysis at 270 °C for 1 h. Five grams of sample was introduced into a 20-mL headspace glass vial along with a magnetic stir bar (PTFE, Ø 3 mm×12 mm length) and 2 μL of internal standard (IS; 2-methyl-3-heptanone; 7.73 μg/mL of methanol). The vial was sealed with a magnetic open-top screw cap (PTFE/silicon septa, thickness 1.3 mm; Supelco). All samples were stored in a freezer (−70 °C) until SPME-GC-MS analysis. A 6890 gas chromatograph/5973 N mass selective detector (MSD) (Agilent Technologies Inc., Palo Alto, CA, USA), equipped with a Combi PAL auto sampler (CTC Analytics AG, Zwingen, Switzerland) was used for the analysis. The operating conditions of the autosampler was as follows: pre-incubation time, 10 min; pre incubation and absorption temp., 40 °C; absorption time, 20 min; agitating speed during pre-incubation and absorption, 250 rpm; desorption time, 14 min (purged after 4 min); desorption temp., 260 °C. Separations were performed using a Stabilwax column (30 m length×0.25 mm i.d.; 0.25 μm film thickness; Restek, Bellefonte, PA, USA). The GC oven was maintained at 35 °C for 5 min, raised to 225 °C at a ramp rate of 4 °C/min, and then held for 20 min. Helium was used as carrier gas at constant flow mode of 1.0 mL/min. The MSD conditions were as follows: capillary direct interface temp., 250 °C; ionization energy, 70 eV, mass range, 35–300 a.m.u; EM voltage, autotune + 200 V; scan rate, 5.27 sans/s. Analyses were performed in duplicate.

2.4. Aroma extract dilution analysis (AEDA)

AEDA was conducted to determine the relative potency of individual odorants according to the method employing successive dilution technique (Martí, Mestres, Sala, Busto, & Gausch, 2003). Each sample was stepwise diluted (1:3 ratio; 1 part BE to 2 parts buffer) with 0.2 M citric acid buffer (pH 5.6 for CON and RMD; pH 6.6 for RMP and MAT; prepared by the method of Perrlin & Dempsey, 1974) in order to keep the diluted samples in the same environment as the initial BE. Each dilution (5 g) was kept in the SPME vial at −70 °C until analysis. The gas chromatography-olfactometry (GCO) system consisted of a 6890 GC equipped with a flame ionization detector (FID) (Agilent Technologies Inc.) and an olfactory detection port (ODP2, Gerstel, Mülheim an der Ruhr, Germany). Two columns of different polarity, including a polar column (Rtx-Wax, 15 m×0.53 mm i.d.; 1.0 μm film thickness, Restek) and a nonpolar column (Rtx-5, 15 m length×0.53 mm i.d.; 1.0 μm film thickness, Restek), were used for the separations. Column effluent was split 1:1 between the FID and olfactory detection port using deactivated fused silica tubing. A manual type of SPME holder (Supelco) was used for the analysis using the conditions previously described above for SPME-GC-MS. The GC oven was maintained at 40°C for 5 min and then raised to 225°C (hold for 15 min) at a rate of 10°C/min. Helium was used as carrier gas at constant flow mode of 2.2 mL/min. The FID and olfactory detection port temperatures were held at 250°C. GCO analysis was conducted in duplicate for each dilution.

2.5. Relative concentration

Relative concentration of each peak in SPME-GC-MS data was estimated by dividing total ion peak area with that of IS, and multiplied by the concentration of IS spiked into the sample. Relative concentrations were expressed as ng/g of sample.

2.6. Identification of odor-active compounds

Compounds were positively identified by matching retention indices [on two different GC column phases and calculated using homologous series of *n*-alkanes (van den Dool & Kratz, 1963)], mass spectra, and odor properties of unknowns with those of authentic standard compounds analyzed under identical experimental conditions. Compounds were considered to be tentatively identified when only two of the above criteria were met.

2.7. Triangle difference test

Triangle difference tests (Meilgaard, Civille, & Carr, 1991) were employed to determine if perceivable differences in odor properties existed between CON and the other three treatments. All treatments were prepared as described above and were cooled to room temperature for 1 h before testing. Treatments (15 mL) were placed into 125-mL squeeze PTFE bottle (Nalgene, Rochester, NY, USA) with siphon tube removed from the cap. The bottles were covered with aluminum foil to prevent any visual bias, and were labeled with random three-digit codes. Samples were presented in random order including all six possible