Changes in the Volatile Composition of Fresh Pork Sausage with Natural Antioxidants During Long-Term Frozen Storage

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Abstract: Pre-rigor meat was formulated into fresh pork sausages with a combination of synthetic antioxidants (butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate) or the same synthetic antioxidants in combination with rosemary (R, 1500, 2000, 2500 mg/kg) and green tea (G, 100, 200, 300 mg/kg). Sausages were stored frozen (-20°C) for 15, 90, or 180 d followed by refrigerated storage (3 ± 1°C). The volatile compounds from these sausages were identified using solid phase microextraction (SPME), gas chromatography coupled with a mass selective detector (GC-MSD), and OSME-gas chromatography-olfactometry (GCO-OSME). Fifty-five aroma compounds were identified from the headspace of pork sausage where spice-derived volatiles such as terpenes (α-pinene, α-thujene) and terpenoids (isopulegol, 1,8-cineole) were the most abundant compounds in the headspace of the fresh product (0 d). Aldehydes (heptanal, 2-heptenal, (E,E)-2,4-decadienal) and alcohols (1-octen-3-ol, 1-penten-3-ol) characteristic of lipid degradation and microbial metabolites (methanethiol, 3-methylbutanoic acid, acetoin) were associated with more intense odorants as the product neared the end of shelf life at 14 d of refrigerated storage. Incorporation of R resulted in lower levels of hexanal (cut grass) and 1-octen-3-ol (mushroom) across all frozen storage periods. After 180 d of frozen storage, higher levels of G contained lower concentrations of ethanol (alcoholic), 3-methylbutanoic acid (sweaty), and 2-acetyl-1-pyrroline (popcorn). As R and G concentration increased in the sausage, there were greater (P < 0.05) concentrations of terpenes and less (P < 0.05) acetic acid throughout refrigerated storage. Incorporation of R resulted in less (P < 0.05) 2,4-decadienal (oxidized ginger-nutmeg), and methanethiol (sulfur) following 90 d of freezing. After 180 d frozen storage, higher levels of G led to less (P < 0.05) 3-methyl-1-butanol and methyl isovalerate (spoiled fruit). Enhanced protection by natural plant extract combinations was observed, especially beyond 90 d of frozen storage where oxidation associated aroma-impact volatiles were reduced in sausages with higher rosemary and/or green tea extract concentrations.

Keywords: aroma-impact compounds, fresh pork sausage, gas chromatography, green tea, rosemary

Introduction

Freezing is utilized to preserve fresh sausage during transportation and storage until they are eventually thawed for retail display (James and James, 2012). Although microbial spoilage is effectively terminated, quality deterioration still takes place since meat that is frozen and stored at temperatures greater than –20°C is prone to oxidation (Leygonie et al., 2012), osmosis of water, myosin denaturation, mechanical damage of proteins, and cross-linking and aggregation of myofibrillar proteins that induces quality losses (Xia et al., 2009; Xiong, 2017). After thawing, tissue damages may initiate free radical reactions due to destruction of the protective cellular membrane that separates unsaturated lipids and pro-oxidant metals, the inactivation of cellular antioxidants, and the liberation of damaging metal ions from metal bind-
ing proteins. This facilitates interactions between pro-oxidants and unsaturated fatty acids, which results in free radicals and the propagation of oxidative reactions (Srinivasan et al., 1997). Oxidation leads to the formation of secondary oxidation products that contributes to undesirable odors, including aldehydes such as n-alkanals, trans-2-alkenals, 4-hydroxy-trans-2-alkenals, and malondialdehyde (Lynch and Faustman, 2000). When these aldehydes covalently bind to myoglobin, they cause accelerated heme oxidation and metmyoglobin formation, and thus meat discoloration.

Because of the extensive tissue disruption caused by grinding, mincing and blending, sausages are particularly susceptible to lipid and protein oxidation. To minimize oxidative damage, a variety of antioxidants with different modes of action and partitioning ability can be incorporated into fresh sausage to inhibit lipid oxidation. Rosemary (Rosmarinus officinalis L.) extracts contain several phenolic diterpenes such as carnosic acid, carnosol, and rosmarinic acid, while green tea (Camellia sinensis L.) extracts are rich in catechins (flavan-3-ols) such as (–) epicatechin, (–) epicatechin gallate, (–) epigallocatechin, (–) epigallocatechin gallate, (+) catechin, and (+) gallocatechin (Berdahl and McKeague, 2015; Karaosmanoglu and Kilmartin, 2015). These natural plant extracts exhibit synergistic interactions via regenerative and metal chelation mechanisms, which enhances their combined antioxidant efficacy (Karaosmanoglu and Kilmartin, 2015). Sebranek et al. (2005) reported that 2500 mg/kg of commercial rosemary extract had greater antioxidant capacity than 200 mg/kg of butylated hydroxyanisole (BHA)/ butylated hydroxytoluene (BHT) when used in refrigerated fresh pork sausage. In addition, green tea extract slows oxidation in fresh pork sausages and pork chops (Martínez et al., 2006; Jongberg et al., 2018). Schilling et al. (2018) reported that combinations of green tea at 100–300 mg/kg, rosemary extract at 1500–2500 mg/kg, and synthetic antioxidants extended color and flavor acceptability of fresh pork sausage from approximately 10 d to greater than 14 d of refrigerated storage under lights.

Analysis of the volatile profiles of sausages supplemented with different combinations of natural and synthetic antioxidants may provide insights into the possible mechanisms of their interactions for enhancing antioxidant capability, which results in extended color and flavor shelf life. Headspace volatiles can be identified by solid phase micro-extraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS) and gas chromatography–olfactometry–Osme (GCO-Osme). These methods have been used to determine the volatile components in complex mixtures that contribute most to the overall aroma in terms of their odor intensities. Additionally, some volatiles may be present at very low concentrations (mg/kg levels), which are too low for their identification using GC–MS alone (Qian et al., 2007). The GCO is a sensitive instrumental technique that is utilized for the identification of aromatic compounds in foods (Marsi, 2007). The GCO-Osme technique has been applied to various products such as liver pâtés (Estévez et al., 2004) and Iberian dry-cured ham (Carrapipo et al., 2002). Because of the complexity of the volatile composition of sausages due to the presence of different spices, potential oxidative reactions, as well as the interaction of spice volatiles with oxidized lipids, GCO-Osme is a valuable means to analyze the flavor profile in sausages that were previously frozen.

The hypothesis of this study is that the concentration of volatile compounds that are indicators of off-flavor would be less during refrigerated storage at each frozen storage time as concentrations of rosemary and green tea extracts increased. Specific objectives include: (1) evaluating the change in volatile composition of fresh pork sausage over refrigerated storage time that was formulated with concentrations of rosemary and green tea extracts that are commonly used in the food industry after 15, 90, and 180 d of frozen storage; (2) to use regression analyses to estimate changes in volatile composition over refrigerated storage time based on rosemary and green tea concentration for each of 15, 90, and 180 d frozen storage.

Materials and Methods

Raw materials and chemicals

Fresh pork sausages were processed using whole-hog, pre-rigor meat (30–45 min postmortem, containing 1.5% salt to delay rigor onset) obtained from a commercial pork processing plant and stored at 1 ± 1°C until the manufacture of the product (<24 h). Food grade green tea (G, GTFORT) and rosemary (R, FORTIUM Brand R10) extracts, propyl gallate (PG), BHA, and BHT were obtained from Kemin Food Technologies Inc. (Des Moines, IA). A three-phase solid phase microextraction (SPME) fiber (2 cm-50/30 mm StableFlex divinylbenzene [DVB]/carboxen [Car]/polydimethylsiloxane [PDMS]; Supelco, Bellefonte, PA) was used to extract the volatile compounds from the samples. Deodorized-distilled water was prepared by boiling distilled water in a flask until the water volume was decreased by one-third. Ultra-high purity helium, compressed air, nitro-
gen, and hydrogen were supplied by Airgas USA, LLC (Columbus, MS). Gas chromatographic results were verified by authentic standards, including methanethiol, ethanol, carbon disulfide, 1-propanol, 2-butanol, diacetyl, ethyl acetate, acetic acid, 3-methylbutanal, 1-butanol, acetoin, methyl butanoate, 3-methylbutanol, 1-pentanol, ethyl butyrate, hexanal, 3-methylbutanoic acid, 2-furanmethanol, 1-hexanol, 2-heptanone, heptanal, methyl hexanoate, α-pinene, camphene, 2-heptenal, 1-octen-3-ol, 2,3-octanedione, sulcatone, 3-carene, α-terpinene, ethyl hexanoate, p-cymene, eucalyptol, limonene, 2-octenal, benzeneacetaldehyde, 2-phenylethanol, octanoic acid, ethyl caprylate, 2,4-decadienal, ethyl caprate and caryophyllene oxide. Sodium chloride (25% non-iodized; Morton Salt Inc., Chicago, IL) was added to improve the extraction of volatile compounds. An internal standard, 1,3-dichlorobenzene (80 mg/kg), and n-paraffin mixtures C\textsubscript{5}–C\textsubscript{8} (Aldrich; Sigma-Aldrich Chemical Co., St. Louis, MO) and C\textsubscript{8}–C\textsubscript{20} (Fluka; Sigma-Aldrich Chemical Co.) were used to standardize the results and calculate Linear Retention Indices (LRI), respectively, (van den Dool and Kratz, 1963).

Manuf acture of fresh pork sausage

The addition of synthetic antioxidants in all treatments included a proprietary combination of BHA, BHT, and PG at approximately 0.02% based on fat composition, which is within the legal limits described by USDA (9 C.F.R. § 319.141, 9 C.F.R. § 424.21; United States Code of Federal Regulations, 2015). Treatments (Table 1) were based on the salted pork weight with either (1) R1500 + G100 (1500 mg/kg rosemary extract + 100 mg/kg green tea extract + synthetic antioxidants), (2) R1500 + G200, (3) R1500 + G300, (4) R2000 + G100, (5) R2000 + G200, (6) R2000 + G300, (7) R2500 + G100, (8) R2500 + G200, (9) R2500 + G300 and (10) Control (synthetic antioxidants only). The control consisted of synthetic antioxidants in reps 1 and 2. A control was also manufactured for replication 3, but its data was not included in the statistical analyses since it included R1500 and synthetic antioxidants, which was representative of commercial formulations at the time that replication 3 was conducted. The usage rate of rosemary and green tea concentration was recommended by the supplier and falls within the range that is commonly used in the United States (Kemin Food Technologies Inc. Des Moines, IA).

A standard fresh pork sausage mixture (9 C.F.R. § 319.141, 9 C.F.R. § 424.21) was formulated to contain 36.3 kg of pre-rigor meat (fat, 27.7%; moisture, 52.9%; protein, 13.6%). A proprietary combination of a spice blend, corn syrup solids, chilled water, and synthetic antioxidants were added with each treatment combination to an experimental unit of pre-rigor meat and blended for 3 min in a commercial paddle mixer (Model 150, Butcher Boy Limited, Ayshire, Scotland, UK). Treatments were replicated on 3 separate production days. The natural plant extracts were added dry and dispersed in the spice blend prior to addition to the meat block. The blended meat was stored in a walk-in cooler (1 ± 1°C) for 48 h prior to grinding (Model 80055 Mixer-Grinder, Hollymatic Co., Countryside, IL) through a 4-mm grinder plate. Aliquots of ground meat were collected and analyzed for fat, moisture, and protein contents (Method 2007.04; AOAC, 2007) using a FOSS FoodScan Meat Analyzer Near-Infrared (NIR) Spectrophotometer (Model 78810; Foss Co., Hillerød, Denmark) prior to stuffing. Fat, protein, and moisture content ranged from 26.9 to 28.1%, 13.3 to 13.9%, and 52.3 to 53.4%, respectively. After grinding, the meat was vacuum stuffed (Model RS1040C; Risco Vacuum

| Treatment | Rosemary extract, mg/kg | Green tea extract, mg/kg | Synthetic antioxidants (combination of BHA, BHT, and PG\textsuperscript{1}) |
|-----------|-------------------------|--------------------------|-----------------------------|
| R1500+G100 | 1,500                   | 100                      | ~0.02% based on fat %       |
| R1500+G200 | 1,500                   | 200                      | ~0.02% based on fat %       |
| R1500+G300 | 1,500                   | 300                      | ~0.02% based on fat %       |
| R2000+G100 | 2,000                   | 100                      | ~0.02% based on fat %       |
| R2000+G200 | 2,000                   | 200                      | ~0.02% based on fat %       |
| R2000+G300 | 2,000                   | 300                      | ~0.02% based on fat %       |
| R2500+G100 | 2,500                   | 100                      | ~0.02% based on fat %       |
| R2500+G200 | 2,500                   | 200                      | ~0.02% based on fat %       |
| R2500+G300 | 2,500                   | 300                      | ~0.02% based on fat %       |
| CONTROL  | 0                       | 0                        | ~0.02% based on fat %       |

\textsuperscript{1}Abbreviations: BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; PG = propyl gallate.
Stuffer, Thiene, Italy) into natural hog casings (Model 10003, 32/35 mm; Wolfson Casing Corporation, Mount Vernon, NY). Natural casings were tenderized (proprietary procedure), washed with water to eliminate salt, acid-treated and kept in warm water (40 ± 1°C) prior to use. After packaging, trays were stored at −20°C for 15, 90, and 180 d followed by simulated retail display for 0, 7, 14, and 21 d at 3 ± 1°C after each frozen storage period. Packages were randomly arranged under refrigerated (3 ± 1°C) display conditions (800 lux; Cool White 34 Watt; Sylvania Supersaver Ecologic, Danvers, MA). Volatile flavor composition was evaluated on d 0, 7, 14, and 21 of retail display.

**Volatile flavor analysis**

**Isolation and analysis of volatile compounds.** Fresh pork sausage homogenates (1:1 dilution, 50% w/w) were prepared with a saturated salt solution (25%) and a commercial blender (Model HC306, Waring Corporation, Towson, MD). A 10-g aliquot of the homogenized sample was transferred into a 40-mL amber glass vial (28-mm outer diameter × 98-mm H, Supelco, Bellefonte, PA) with an open-center propylene screw cap and Teflon faced silicone septum (22 mm outer diameter; Supelco, Bellefonte, PA). An internal standard (1,3-dichlorobenzene; 80 mg/kg; Sigma-Aldrich Chemical Co., Milwaukee, WI) was added for the quantification of the volatile compounds in the sausage. Samples were equilibrated at 20°C for 30 min, followed by equilibration for 30 min at 50°C in a thermostatic heating block (Reacti-Therm Heating/Stirring Module; Pierce Biotechnology Inc., Rockford, IL) with constant stirring using a magnetic octagonal stirring bar (8-mm outer diameter × 13-mm L; Fisher, Pittsburgh, PA). A 3-phase SPME fiber (2 cm-50/30 um carbobox/polydimethylsiloxane/divenylbenzene [Car/PDMS/DVB]) was inserted into the vial through the septum and was exposed to the generated sample headspace for 1 h at 50°C. This was followed by thermal desorption of the volatiles from the SPME fiber into an injector port of a gas chromatographic system in a splitless mode at 250°C for 5 min.

The analysis of the volatile compounds was performed on a Varian 3900 gas chromatographic system equipped with a CP-1177 split/splitless injector and a DB-5 capillary column (30 m × 0.25 mm inner diameter × 0.25 mm film thickness (df), J &W Scientific). The column extended from the oven and was split by a column flow splitter to the FID and the olfactometer that was connected to a stainless steel sniff port with a custom-made glass nose cone (SGE, Kramer Lane). In addition, the glass nose cone was purged with humidified air at a flow rate of 30 mL/min. The operating conditions were identical to those used for the GC–MS. Two panelists trained in sensory and GCO analyses described the aroma properties of the volatile compounds present in the samples that were separated by the GC (Rouseff et al., 2001). The assessors were trained in GCO analyses for 10 h by sniffing original samples and volatile flavor compounds extracted by SPME from pork sausage homogenates. The intensity of the perceived aroma was rated by each panelist using a 0–15 potentiometric sliding scale, 0: none; 15: maximum intensity (McDaniel et al., 1990) interfaced to a computer (Osme Software, Starkville, MS). Authentic standards and n-alkane series C5–C18 were separated and quantified by the GC–FID system under the same operating conditions. Headspace volumes were drawn for the authentic and alkane standards using a gastight digital syringe (1700 Series GASTIGHT Digital Syringe, Hamilton Company, Reno, Nevada) and immediately introduced into the injector.

Identification of the aroma-impact compounds was based on comparing sample mass spectra with those in the NIST02 Mass Spectral Database (NIST, Maryland; purchased from Varian Inc.), the linear retention index and aroma quality perceived at the sniffing port with those of an authentic standards, the linear retention index (n-alkanes C5–C18, Sigma-Aldrich Chemical Co.) and the aroma quality perceived at the sniffing port with those in literature and retention index databases (Acree and Arn, 2015; El-Sayed, 2015). Approximate quantities for the aroma compounds were calculated from the multiplication of the area ratio (area of compound/area of internal standard) with the concentration of the internal standard using a re-
Sensation factor of 1. The content of each aroma compound in the fresh pork sausage sample was expressed as ng/g fresh pork sausage (relative abundance mg/kg).

**Determination of sausage flavor shelf life**

Pork sausages were evaluated daily following 14 d of retail display by 3 trained panel members with greater than 200 h of experience pertaining to the evaluation of meat products and greater than 100 h determining the sensory shelf life of fresh pork sausage. Packaged trays containing fresh pork sausages were equilibrated to room temperature (20 ± 2°C) for 15 min prior to cooking. The sausages were cooked in a lidded nonstick pan (Farberware 10.5-in. covered fry pan; Farberware Licensing Company LLC, Needham, MA) over medium-high heat (Viking Professional 60” Custom Sealed Burner Range; Viking Range Corporation, Greenwood, MS) to an internal temperature of 75 ± 2°C. The sausages were placed with 227 mL of water in the lidded pan for 5 min. The sausages were then turned over and cooked for another 5 min in the lidded pan. The cover was removed after 10 min and the sausages continued cooking and were turned every 2 min and cooked until the desired internal temperature (Model 00645W2; Acu-Rite Digital Thermometer, Schaumburg, IL) was achieved. The cooked samples were then wrapped in Reynolds extra heavy-duty foil bags (Alcoa Consumer Products, Alcoa Inc., Richmond, VA) to rest at room temperature (20 ± 2°C) for 15 min before slicing. The samples were sliced into 1.8-cm thick pieces and kept warm (60–70°C) until sensory analyses were conducted. Upon serving, sausage slices were placed in 56.7 mL plastic containers with lids (Sweetheart Cup Co., Owing Mills, MD) and each panelist received two pieces of each treatment during every session. Panelists were provided with water (Natural Spring Water, Crystal Springs, Atlanta, GA), unsalted crackers (Premium Nabisco, East Hanover, NJ), apple juice (Lucky Leaf Apple Juice, Knouse Foods Co-op Inc., Peach Glen, PA), and expectorant cups to remove residual flavors between samples. Shelf life was ended when the product was deemed unacceptable in comparison to fresh pork sausage (d 0 storage) or when off-flavors and off-odors were detected by the panelists. The day before the product was deemed unacceptable was considered the end of shelf life for that specific treatment combination.

**Statistical analysis**

A split plot design with the whole plot as sausage batch and whole-plot factor as antioxidant treatment (Table 1), and split plot as sausage package and split-plot factor as refrigerated storage time was utilized to investigate the effects of treatment and simulated retail display time on the aroma impact compound concentrations and aroma intensities of fresh pork sausage separately at each frozen storage time of 15, 90, and 180 d. When significant differences occurred among treatments, Duncan’s Multiple Range Test (Statistical Analysis Software, Version 9.3, SAS Inst. Inc., Cary, NC) was utilized to separate treatment means. The data obtained for each treatment combination were submitted to a regression analysis according to a second-order polynomial equation composed of linear/main, quadratic and interaction effects for the independent variables studied. The main effects are the levels of rosemary extract (R), green tea extract (G), and the retail display time (D) of products. Replication was included in the model as a random effect.

The regression model takes the following form:

\[
Y = \beta_0 + \beta_1G + \beta_2R + \beta_3(G \times R) + \beta_4(d) + \beta_5(G \times d) + \beta_6(R \times d) + \beta_7(d \times R \times d) + \epsilon
\]

where Y is the response variable; \(\beta_0\) is the constant coefficient (intercept); \(\beta_1, \beta_2, \text{ and } \beta_3\) are the linear coefficients (main effects); \(\beta_4\) is the quadratic coefficient; \(\beta_3\) is the two-factor interaction coefficient, and \(\epsilon\) is the random error. The PROC GLIMMIX procedure of SAS (Vers. 9.3, SAS Inst. Inc.) was used to determine statistical differences among the fixed effects and their interactions at the 5% probability level \((P < 0.05)\). The significance of the model was tested by an analysis of variance (F-test, \(P < 0.05\)). The nonsignificant terms \((P > 0.05)\) were withdrawn from the model in a stepwise fashion, and a new adjustment was made so that only significant \((P < 0.05)\) terms were included in the final regression model. Since a general linear mixed model was used, an accurate \(R^2\) by definition cannot be calculated since that assumes a lack of interaction between the random and fixed effects. \(R^2\) was approximated by using the F statistics and covariance estimates from the SAS output and assuming that there was a lack of interaction between random and fixed effects.

Analyses included quantification and determination of aroma active volatile flavor compounds and sensory shelf life. Tables 2, 3, and Fig. 1 were selected as a representation of the data to demonstrate the impact of retail display time on volatile composition, aroma intensity of volatile compounds, and an example chromatogram generated from the GC–MS, GCO, and GCFID data.
Table 2. Aroma impact compounds identified and quantified in the headspace of fresh pork sausage with rosemary extract (2500 mg/kg), green tea extract (300 mg/kg) and synthetic antioxidants under simulated retail display (3 ± 1°C, 21 d) following 90 d of frozen storage (−20°C)

| Peak no. | Compound | LRI ¹ | d 0 | d 7 | d 14 | d 21 | SEM |
|----------|----------|-------|-----|-----|------|------|-----|
| 2        | Ethanol  | 545   | 0b  | 0b  | 5.5ab| 19.1a| 5.2 |
| 4        | 1-propanol| 567   | 27b | 101ab| 147a | 115a | 27.6|
| 9        | 1-butanol | 663   | 7.7 | 10.9 | 15   | 10.2 | 4.7 |
| 12, 20   | 3-methyl-1-butanol | 741, 878 | 6.1c | 13c  | 243b  | 384a | 43.4 |
| 19       | 1-hexanol | 871   | 18ab| 19.9ab| 47.5a | 9b   | 11.0|
| 29       | 1-octen-3-ol| 979   | 16.1b| 36.3a| 56.4a| 40.5a| 7.0 |
| 44       | Benzenecethanol | 1,115 | 4.3b| 3.5b | 12b  | 62.7a| 5.6 |
| 53       | 2,3-dimethyl-2,3-butanediol | 1,244–1,250 | 6.3 | 6.8 | 4.1 | 4.7 | 0.93 |

**Alcohols**

| Peak no. | Compound | LRI ¹ | d 0 | d 7 | d 14 | d 21 | SEM |
|----------|----------|-------|-----|-----|------|------|-----|
| 8        | 3-methylbutanal | 648   | 1.4b| 10.1b| 159a | 112a | 41.3|
| 15       | Hexanal   | 799   | 89.1| 138.3| 111  | 108  | 21.9|
| 22       | Heptanal  | 901   | 6.1bc| 4.9c | 16.9ab| 25.6a| 3.4 |
| 28       | 2-heptenal | 955   | 5c  | 112b | 194a | 221a | 23.2|
| 45       | Benzenecetaldehyde | 1,043 | 14.3| 10.5| 36.6 | 292  | 95.2|
| 56       | 2,4-decadienal | 1,320 | 0b  | 0b  | < 1b | 1a   | 0.13|

**Carboxylic acids**

| Peak no. | Compound | LRI ¹ | d 0 | d 7 | d 14 | d 21 | SEM |
|----------|----------|-------|-----|-----|------|------|-----|
| 7        | Acetic acid | 637   | 31.5c| 110c | 323b | 743a | 52.1|
| 16       | 3-methylbutanoic acid | 850, 860 | 0b  | 0b  | 3.1ab| 4.6a | 1.3 |
| 51       | Octanoic acid | 1,176 | 27.2b| 26.3b| 30.3b| 53.5a| 7.7 |

**Esters**

| Peak no. | Compound | LRI ¹ | d 0 | d 7 | d 14 | d 21 | SEM |
|----------|----------|-------|-----|-----|------|------|-----|
| 6        | Ethyl acetate | 618   | 7.5c| 5.8c | 62.4b| 183a | 16.0|
| 11       | Methyl butanoate | 725   | 1.9 | < 1  | 3.8  | 4.1  | 2.8 |
| 13       | Ethyl isobutyrate | 764   | 1.7b| 0b  | < 1b | 10.4a| 2.2 |
| 14, 17   | Methyl isovalerate | 779–876, 852–857 | < 1b | < 1b | 1.5b | 11.6a| 2.4 |
| 24       | Methyl hexanoate | 925   | 11.7a| 9.2ab| 8.4ab| 6.8ab| 3.8 |
| 35       | Ethyl hexanoate | 999   | 0   | 1.4 | 2.9  | 3.6  | 1.7 |
| 55       | Ethyl decanoate | 1,197 | 0b  | 0b  | 7.2b | 38.2a| 3.8 |

**Ketones**

| Peak no. | Compound | LRI ¹ | d 0 | d 7 | d 14 | d 21 | SEM |
|----------|----------|-------|-----|-----|------|------|-----|
| 5        | 2-butanone | 613   | 8.9b| 7b  | 19.4a| 26.4a| 2.8 |
| 10       | 3-hydroxy-2-butanone | 722   | 41.7c| 57.1bc| 123b | 225a | 24.3|
| 21       | 2-heptanone | 890   | 10.8b| 10.1b| 18ab | 22.1a| 2.9 |
| 30       | 2,3-octanediol | 982   | 4.6b| 6.2ab| 9.1a | 8.3ab| 1.3 |
| 31       | 6-methyl-5-hepten-2-one | 986   | 133 | 153 | 133  | 146  | 21.3|

**Monoterpene hydrocarbons**

| Peak no. | Compound | LRI ¹ | d 0 | d 7 | d 14 | d 21 | SEM |
|----------|----------|-------|-----|-----|------|------|-----|
| 25       | α-thujene | 928   | 591a | 398ab| 236b | 430ab| 85.4|
| 26       | α-pinene  | 934   | 1,704a| 1453ab| 864b | 1207ab| 249|
| 27       | Camphene  | 948   | 90.8 | 88.3 | 62.5 | 92.5 | 12.1|
| 32       | α-phellandrene | 1,005 | 41.5a| 270b | 249b | 166b | 44.1|
| 33       | 3-carene  | 1,011 | 1,566a| 1352ab| 1047b | 1034b| 135 |
| 34       | α-terpinene | 1,018 | 1,233a| 753b | 519b | 529b | 99.6|
| 36       | p-cymene  | 1,026 | 1,806a| 1407a | 965b | 777b | 152 |
| 37       | Limonene  | 1,031 | 441  | 527  | 532  | 420  | 66.8|
| 39       | 2-methyl-cis-3,4,7,7a-tetrahydroindan | 1,031 | 1,197 | 1495 | 1605 | 1364 | 188 |
| 42, 46   | α-terpinolene | 1,091 | 893  | 751  | 667  | 681  | 92.7|

**Sesquiterpene hydrocarbons**

| Peak no. | Compound | LRI ¹ | d 0 | d 7 | d 14 | d 21 | SEM |
|----------|----------|-------|-----|-----|------|------|-----|
| 54       | δ-cembrene | 1,349 | 422  | 401  | 387  | 473  | 163 |
| 55       | α-cubebene | 1,364 | 200  | 186  | 164  | 203  | 23.2|
| 59       | Cedrene   | 1,536 | 331  | 330  | 222  | 295  | 47.8|

Continued
Identification of the aroma impact compounds of fresh pork sausage

Fifty-five aroma impact compounds were identified using GC–MS, GC-FID, and GCO (Table 2) and aroma descriptions of the compounds, repeatedly sniffed by both trained panelists, were summarized in Table 3. Tables 2 and 3 contain data for the sausage treatment with 2,500 mg/kg rosemary extract and 300 mg/kg green tea extract. This data was reported because the specific compounds were representative of all other treatments as refrigerated storage time increased, just in different concentrations. Nearly all of the aroma compounds in this study have been previously identified in the headspace volatiles of cooked pork as well as contributors to the distinct aromas and flavors of spices, herbs, and seasonings (Carrapiso, 2007; Estévez et al., 2005; Jo and Ahn, 2000; Yoo et al., 2005; Chen and Ho, 1998; Mottram, 1991). The flavor characteristics of natural plant extract-based compounds, which possess minty, herbal, ginger-nutmeg, and spicy notes, include a family of structures that contain a terpene group. Twenty-two terpenes included monoterpene hydrocarbons, sesquiterpene hydrocarbons, and terpenoid phenol, the concentrations of which ranged from 3 mg/kg to 1,982 mg/kg (Table 2). Results from the present study regarding the abundance of terpenes in the volatile composition of the product agree with those obtained in previous research pertaining to the study of headspace volatiles from frankfurters and liver pâté (Chevance and Farmer, 1999; Estévez et al., 2004, 2005). The volatiles in black pepper comprised mostly of monoterpene hydrocarbons, including sabine (19% of the total volatile composition), limonene (17%), and β-pinene (10%), and also a large proportion (14%) of β-caryophyllene (sesquiterpene hydrocarbon) and 4% of 1,8-cineole (terpene alcohol). As retail display progressed, a concomitant decrease ($P < 0.05$) in the relative concentrations of α-phellandrene, 3-carene, $\alpha$-cymene, sabinene hydrate, terpinen-4-ol, and isopulegol was observed (Table 2). The aromas related to these compounds such as “sausage/spicy/ginger/nutmeg”, “sweet cola/minty”, “minty/eucalyptol”, “floral/linalool/spicy/cocoa/grainy, pine/minty/rosemary/green tea”, “ginger-nutmeg/spicy/minty”, and “spice mix/sweet” also decreased ($P < 0.05$) in their intensities, denoting them as possible markers for product freshness (Table 3). These odorants were among the most potent, as indicated by aroma intensities as high as 8 on a 15-point scale from d 0 to 7.

Six aldehydes were identified as aroma impact compounds in the headspace of pork sausage (Tables 2 and 3), including 3-methylbutanal (sour, cheesy), hexanal (cut grass), heptanal (musty, yeasty, baked potato), 2-heptenal (oxidized sausage, fecal), benzeneacetaldehyde (rose)
Table 3. Retention indices for aroma impact compounds detected in the headspace of sausage under simulated retail display (3 ± 1°C, 21 d) following 90 d of frozen storage (−20°C) during SPME-GC–MS, SPME-GCO-Osme, and SPME-GC-FID

| Peak no. | Compound1,2 | LRI1 | Aroma4 note/descriptor | Aroma intensity5 |
|----------|-------------|------|------------------------|-----------------|
|          |             | GC-MS | GCO |                      | d 0 | d 7 | d 14 | d 21 | SEM |
| 1        | Methanethiol | 500–510 | < 500 | Alcoholic, sweet | 0b | 0b | 3b | 4a | 0.45 |
| 2        | Ethanol     | 545   |     |                        |     |     |     |     |     |
| 3        | Carbon disulfide | 545–567 | < 500, 601 | Sulphury, meat-like-thiol-like | 0b | 1b | 3a | 4a | 0.53 |
| 4        | 1-propanol  | 567   |     |                        |     |     |     |     |     |
| 5        | 2-butanoate | 613   | 568–626 | Buttery, milky | 1.5b | 2b | 4.5a | 5a | 0.38 |
| 6        | Ethyl acetate | 618  |     |                        |     |     |     |     |     |
| 7        | Acetic acid | 637   | 568–626 | Sour, cheesy | <1b | 1ab | 1ab | 2a | 0.37 |
| 8        | 3-methylbutanal | 648 |     |                        |     |     |     |     |     |
| 9        | 1-butanol   | 663   | 670 | Green, dirty socks | 0b | 0b | 2a | 3a | 0.47 |
| 10       | 3-hydroxy-2-butane | 722 | 741 | Fruity, dirty socks, spoiled fruit, berry-like | 0c | 1b | 5a | 0.37 |
| 11       | Methyl butanoate | 725 | 748 |                        |     |     |     |     |     |
| 12       | 3-methyl-1-butanol | 741 |     |                        |     |     |     |     |     |
| 13       | Ethyl isobutyrate | 764 | 760–765 |                        |     |     |     |     |     |
| 14       | Methyl isovalerate | 779–786 |     |                        |     |     |     |     |     |
| 15       | Hexanal     | 799   | 798–803 | Cut grass | 3 | 4 | 3 | 4 | 0.43 |
| 16       | 3-methylbutanoic acid | 850, 860 | 851–856 | Butanoic, dirty socks | 0c | 0c | 4b | 9a | 0.40 |
| 17       | Methyl isovalerate | 852–857 |     |                        |     |     |     |     |     |
| 18       | 2-furanmethanol | 862 | 868–870 | Vitamin | 6c | 6.5bc | 7b | 8.5a | 0.31 |
| 19       | 1-hexanol   | 871   |     |                        |     |     |     |     |     |
| 20       | 3-methyl-1-butanol | 878 |     |                        |     |     |     |     |     |
| 21       | 2-heptanone | 890   | 905–913 | Baked potato | 6a | 2b | 0c | 0c | 0.39 |
| 22       | Heptanal    | 901   | 905–913 | Musty, yeasty, baked potato | 0c | 4b | 9a | 0.40 |
| 23       | 2-acetyl-1-pyrrole | 920 | 883, 920 | Popcorn, cooked rice | 3.5a | 4a | 3a | 0b | 0.67 |
| 24       | 2-acetyl-1-pyrrole | 920 | 883, 920 | Stale | 0d | 3c | 6b | 10a | 0.56 |
| 25       | Methyl hexanoate | 925 | 933 | Fruity | 1 | 1 | 1 | 0 | 0.38 |
| 26       | Methyl hexanoate | 925 | 933 | Spoiled fruit | 1 | 2 | 1.5 | 2 | 0.50 |
| 27       | α-thujene   | 928   | 935–938 | Sausage, spicy, ginger, nutmeg | 8a | 5b | 0c | 1c | 0.43 |
| 28       | α-pinene    | 934   |     |                        |     |     |     |     |     |
| 29       | Camphene    | 948   |     |                        |     |     |     |     |     |
| 30       | 2-heptenal  | 955   | 935–938 | Oxidized sausage | 6a | 2b | 0c | 6a | 0.44 |
| 31       | 2-heptenal  | 955   | 969 | Fecal | 0b | 0b | 0b | 2a | 0.31 |
| 32       | 1-octen-3-ol | 979 | 980–987 | Metallic | 5b | 8.5a | 8a | 8.5a | 0.34 |
| 33       | 2,3-octanedione | 982 |     | mushroom | 6b | 8a | 8a | 8a | 0.35 |
| 34       | 6-methyl-5-hepten-2-one | 986 |     |                        |     |     |     |     |     |
| 35       | α-phellandrene | 1005 | 996–1029 | Citrusy, herbal, grainy, meaty | <1b | 3.5e | 4a | 2b | 0.48 |
| 36       | 3-carene    | 1011  |     |                        |     |     |     |     |     |
| 37       | α-terpine | 1018  |     |                        |     |     |     |     |     |
| 38       | Ethyl hexanoate | 999 | 996–1029 | Oxidized citrus | 0b | 0b | <1b | 3.5a | 0.43 |
| 39       | p-cymene    | 1026  | 1025, 1031 | Sweet cola, minty | 2a | <1b | <1b | <1b | 0.41 |
| 40       | Limonene    | 1031  |     |                        |     |     |     |     |     |
| 41       | Isopulegol  | 1031  |     |                        |     |     |     |     |     |
| 42       | 2-methyl-cis-3a,4,7a-tetrahydroindan | 1031 |     |                        |     |     |     |     |     |
| 43       | 1,8-cineole | 1033  | 1044–1099 | Minty, eucalyptol | 8a | 6b | 2.5c | 0d | 0.39 |
| 44       | α-terpineol | 1071  |     |                        |     |     |     |     |     |
| 45       | α-terpinolene | 1091 |     |                        |     |     |     |     |     |
| 46       | Unknown     | 1044–1099 |     | Oxidized mint | 0c | 0c | 3b | 7a | 0.39 |
| 47       | Benzenecethanol | 1115 | 1055 | Rose | 0b | 0b | <1b | 2a | 0.40 |
| 48       | Benzeneacetaldehyde | 1043 |     |                        |     |     |     |     |     |
| 49       | α-terpinolene | 1091 | 1087–1093 | Gravy, herba | 6 | 5 | 6 | 6 | 0.41 |

Continued
and 2,4-decadial (oxidized ginger-nutmeg). With the exception of hexanal and benzeneacetaldehyde, the remaining aldehydes and their corresponding aroma intensities increased \( (P < 0.05) \) as retail display progressed (Tables 2 and 3). Linear saturated, unsaturated, and polyunsaturated aldehydes are typical fat degradation compounds formed by lipolysis autoxidation mechanisms (Belitz and Grosch, 1987) and have been associated with rancidity notes that develop during the storage of fatty foods (Mottram, 1991). It has been suggested that these aldehydes contribute to the loss of desirable flavors in meats due to their high rate of formation during lipid oxidation and low odor thresholds. The aldehydes and their corresponding odor thresholds in literature include 3-hydroxy-2-butanone, 2-heptanone, 2,3-octanedione, and 6-methyl-5-hepten-2-one were described as having “buttery, milky”, “fruity, dirty socks”, “baked potato” and “mushroom” aromas, respectively (Tables 1 and 2). Methyl ketones arise from \( \beta \)-keto acid decarboxylation (Ramirez and Cava, 2007) or \( \beta \)-oxidation products (Dirinck et al., 1997) of fatty acids and are considered to be among the meat flavor precursors that contribute to the fatty aromas associated with cooked meat (Chen and Ho, 1998). In addition, ketones are often produced by microbial oxidation of fatty acids or by decarboxylation pathways (Mottram, 1991). For example, 2-heptanone is an oxidation product of linoleic acid. Based on Osme results, this compound was the most intense odorant in the product at the beginning of the retail display (Table 3). Although the pleasant baked potato aroma of 2-heptanone was strongly detected by olfactometry, it was not perceived on d 14 and 21, even with the continued increase \( (P < 0.05) \) in its estimated concentration (Tables 2 and 3). This may be because greater concentrations of 2-heptanone can lead to musty and fruity flavors. It may also be attributed to the “musty” and “yeasty” notes afforded to the “baked potato” aroma from heptanal, 2-butanol, 3-hydroxy-2-butanol, and 2,3-octanone and their corresponding aroma descriptors, which were more predominant toward the end of refrigerated storage.

Ethanol eluted early and had a strong and sweet alcoholic aroma. The alcoholic note of ethanol was a

Five aroma impact ketones, 2-butanone, 3-hydroxy-2-butanol, 2-heptanone, 2,3-octanone, and 6-methyl-5-hepten-2-one were described as having “buttery, milky”, “fruity, dirty socks”, “baked potato” and “mushroom” aromas, respectively (Tables 1 and 2). Methyl ketones arise from \( \beta \)-keto acid decarboxylation (Ramirez and Cava, 2007) or \( \beta \)-oxidation products (Dirinck et al., 1997) of fatty acids and are considered to be among the meat flavor precursors that contribute to the fatty aromas associated with cooked meat (Chen and Ho, 1998). In addition, ketones are often produced by microbial oxidation of fatty acids or by decarboxylation pathways (Mottram, 1991). For example, 2-heptanone is an oxidation product of linoleic acid. Based on Osme results, this compound was the most intense odorant in the product at the beginning of the retail display (Table 3). Although the pleasant baked potato aroma of 2-heptanone was strongly detected by olfactometry, it was not perceived on d 14 and 21, even with the continued increase \( (P < 0.05) \) in its estimated concentration (Tables 2 and 3). This may be because greater concentrations of 2-heptanone can lead to musty and fruity flavors. It may also be attributed to the “musty” and “yeasty” notes afforded to the “baked potato” aroma from heptanal, 2-butanol, 3-hydroxy-2-butanol, and 2,3-octanone and their corresponding aroma descriptors, which were more predominant toward the end of refrigerated storage.

Ethanol eluted early and had a strong and sweet alcoholic aroma. The alcoholic note of ethanol was a

Table 3 (cont.)

| Peak no. | Compound\(^{1,2}\) | LRI\(^3\) | Aroma\(^4\) note/descriptor | Aroma intensity\(^5\) |
|---------|----------------|----------|----------------------------|---------------------|
|         |                | GC-MS    | GCO                        | d 0 | d 7 | d 14 | d 21 | SEM  |
| 48      | cis-\(\beta\)-menth-2-en-1-ol | 1125–1144 | 1113–1136 | Floral, spicy, cocoa, grainy | 6\(^{a}\) | 6\(^{ab}\) | 5.5\(^{ab}\) | 5\(^{b}\) | 0.41 |
| 49      | Terpinen-4-ol  | 1188     | 1187–1200 | Pine, minty, herbal          | 5\(^{a}\) | 5\(^{a}\) | 5\(^{a}\) | 2\(^{b}\) | 0.60 |
| 50      | \(\alpha\)-terpineol | 1194     |                   |                     |                |                |                |                |    |
| 51      | Octanoic acid  | 1176     | 1187–1200 | Oxidized pine                | 0\(^{b}\) | 0\(^{b}\) | 1\(^{b}\) | 5\(^{a}\) | 0.53 |
| 52      | Ethyl octanoate| 1197     |                   |                     |                |                |                |                |    |
| 53      | Pinacol        | 1244–1250| 1240           | Pina colada candy, waxy     | 0\(^{c}\) | 0\(^{c}\) | 3\(^{b}\) | 9\(^{a}\) | 0.47 |
| 54      | \(\delta\)-elemene | 1349     | 1342–1396 | Ginger-nutmeg, spicy, minty | 4\(^{a}\) | 4\(^{a}\) | <1\(^{b}\) | 0\(^{b}\) | 0.42 |
| 55      | \(\alpha\)-cubebene | 1364     |           |                     |                |                |                |                |    |
| 56      | 2,4-decadial   | 1320     | 1342–1396 | Oxidized ginger-nutmeg     | <1\(^{b}\) | 0\(^{b}\) | 2\(^{a}\) | 2\(^{a}\) | 0.35 |
| 57      | Ethyl decanoate| 1395     | 1335–1399 |                     |                |                |                |                |    |
| 58      | Myristicin     | 1531     | 1522–1536 | Spice mix, sweet           | 4\(^{a}\) | 3\(^{ab}\) | 3\(^{ab}\) | 2\(^{b}\) | 0.46 |
| 59      | Cedrene        | 1536     |                   |                     |                |                |                |                |    |
| 60      | Caryophyllene oxide | 1609     | 1522–1536 | Oxidized spice             | 0          | 0          | 0          | 1          | 0.30 |

\(^{1}\) Compounds correspond to those in Fig. 1 and Table 3.

\(^{2}\) Aroma impact compounds are presented in order of their elution on the DB-5 capillary column.

\(^{3}\) Linear Retention Indices (LRI) calculated for DB-5 capillary column (J & W Scientific: 30 m × 0.25 mm inner diameter × 0.25 mm film thickness) on a gas chromatograph equipped with a mass selective detector and with a sniffing port and flame ionization detector.

\(^{4}\) Aroma quality perceived by at least two panelists during SPME-Osme-GCO.

\(^{5}\) Average aroma intensity perceived at the sniffing port during SPME-Osme-GCO where 0: none; 15: maximum intensity.
possible marker for the end of shelf life. Ethanol may be derived from the reduction of aldehydes formed via Strecker degradation from the amino acid valine or from bacterial spoilage. In pork sausage, 1-propanol, another highly volatile aroma compound, contributed to sulfury notes in the product. At low concentrations, 3-methyl-
Table 4. Results of the regression analysis of variables with significant effects on the concentrations of the aroma impact compounds of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following frozen storage (−20°C, 15, 90, and 180 d)

| Frozen storage time, d | LRI | Compound | Approximated $R^2$ | Regression equation |
|------------------------|-----|----------|---------------------|---------------------|
| 15                     |     | Alcohols | 0.913               | $-18.8201 + 0.002779 \times R + 10.9342 \times Day - 0.00349 \times R \times Day$ |
| 979                    |     | 1-octen-3-ol | 0.973         | $-25.477 + 0.07486 \times G + 0.01497 \times R + 5.0862 \times Day$ |
| 1244–1250              | 0.911| 2,3-dimethyl-2,3-butanediol | | $9.3348 - 0.02216 \times G + 0.2484 \times Day + 0.01464 \times G \times Day + 0.02166 \times Day \times Day - 0.00066 \times G \times Day \times Day$ |
| 648                    | 0.889| 3-methylbutanal | | $-9.994 - 0.04986 \times R + 11.1759 \times Day + 0.01111 \times R \times Day$ |
| 799                    | 0.941| Hexanal | | $0.6871 + 0.2871 \times G + 4.6331 \times Day$ |
| 901                    | 0.929| Heptanal | | $2.4631 - 0.03882 \times G + 0.5144 \times Day + 0.007857 \times G \times Day$ |
| 955                    | 0.952| 2-heptenal | | $-49.4434 + 0.2598 \times G + 21.4443 \times Day - 0.726 \times Day \times Day$ |
| 1043                   | 0.923| Benzenecetaldehyde | | $12.9532 - 0.00576 \times G - 5.1099 \times Day + 0.001519 \times R \times Day + 0.3528 \times Day \times Day$ |
| 637                    | 0.979| Acetic acid | | $-9.9775 + 0.1621 \times G + 11.6123 \times Day - 0.1097 \times G \times Day - 0.1391 \times Day \times Day + 0.00634 \times R \times Day \times Day$ |
| 779–786, 852–857       | 0.929| Methyl 4-isovalerate | | $16.6433 - 0.00697 \times R - 9.0765 \times Day + 0.001505 \times R \times Day + 0.5211 \times Day \times Day$ |
| 925                    | 0.684| Methyl hexanoate | | $1.8423 + 0.004272 \times R + 0.249 \times Day$ |
| 613                    | 0.959| 2-butanone | | $-4.9951 - 0.006592 \times R + 7.3548 \times Day - 0.00458 \times R \times Day - 0.2731 \times Day \times Day + 0.000239 \times R \times Day \times Day$ |
| 982                    | 0.833| 2,3-octanedione | | $5.976 - 0.01311 \times G - 0.081 \times Day + 0.002425 \times G \times Day$ |
| 928                    | 0.764| α-thujene | | $217.91 + 7.9777 \times G + 0.7548 \times R - 0.00422 \times G \times R - 51.6538 \times Day + 6.5939 \times Day \times Day$ |
| 934                    | 0.888| α-pinene | | $18.9012 + 0.3588 \times G + 0.03454 \times R - 0.00022 \times G \times R - 3.589 \times Day + 0.3908 \times Day \times Day$ |
| 948                    | 0.938| Camphene | | $623.11 + 0.3455 \times R - 3.4276 \times Day + 4.9796 \times Day \times Day$ |
| 1011                   | 0.973| 3-carene | | $362.13 + 0.4041 \times R + 45.1136 \times Day$ |
| 1026                   | 0.914| p-cymene | | $-0.3395 + 0.000094 \times R + 0.09691 \times Day - 0.0003 \times R \times Day$ |
| 1031                   | 0.921| Limonene | | $210.11 + 2.1763 \times G + 0.2859 \times R - 0.00133 \times G \times R + 36.436 \times Day$ |
| 1091                   | 0.935| α-terpinolene | | $26.63 + 3.5135 \times G + 0.2525 \times R - 0.00181 \times G \times R - 9.274 \times Day + 2.2611 \times Day \times Day$ |
| 1349                   | 0.882| ß-elemene | | $359.83 + 0.97 \times G + 22.8469 \times Day$ |
| 1536                   | 0.894| Cedrene | | $314.23 - 0.3646 \times G + 3.7581 \times Day + 0.09537 \times G \times Day$ |
| 1031                   | 0.961| Isopulegol | | $175.98 + 3.7379 \times G + 0.4458 \times R - 0.00227 \times G \times R - 6.3837 \times Day + 2.7044 \times Day \times Day$ |
| 1125–1144              | 0.937| cis-p-methyl-2-en-1-ol | | $143.02 + 0.5007 \times G + 17.2326 \times Day$ |
| 1531                   | 0.873| myristicin | | $386.89 + 3.1553 \times G + 0.1015 \times R - 0.00142 \times G \times R + 33.5741 \times Day$ |
| 1115                   | 0.972| Benzenecetaldehyde | | $-5.4271 + 0.006585 \times R - 0.8309 \times Day - 0.0015 \times R \times Day + 0.3141 \times Day \times Day$ |
| 901                    | 0.909| Heptanal | | $9.2393 - 0.08839 \times G - 0.00609 \times R + 0.00061 \times G \times R + 0.9413 \times Day$ |
| 955                    | 0.959| 2-heptenal | | $9.1915 + 0.2103 \times G + 9.3795 \times Day$ |
| 1320                   | 0.945| 2,4-decadienal | | $-0.3395 + 0.00094 \times R - 0.09691 \times Day - 0.0003 \times R \times Day$ |
| 637                    | 0.972| Acetic acid | | $181.78 - 0.1087 \times R + 31.5202 \times Day$ |

Continued
1-butanol provided “fruity” and “vitamin” notes (Table 2 and 3), and contributed unpleasant spoiled fruit and vitamin notes as storage time increased. The mushroom/metallic-like aroma of 1-octen-3-ol was identified as a significant contributor to the distinct flavor of this product due to its frequent detection and high Osme intensity value. This compound has been described as an important component of meat volatiles particularly with the fatty characteristics of meat flavors and is a product of the autoxidation of linoleic acid or other polyunsaturated fatty acids such as the 12-hydroperoxide of arachidonic acid (Chen and Ho, 1998; Mottram, 1991).

Other potentially important alcohols included 1-butanol, 1-hexanol (vitamin notes), benzeneethanol (rose-like aroma), and 2,3-dimethyl-2,3-butanediol.

Methanethiol (alcoholic, sweet) and carbon disulfide (sulfury, meaty, thiol-like) were the two predominant sulfur-containing flavor compounds in the sausage (Table 2). The low thresholds of these sulfur compounds (0.02 to 0.2 mg/kg; Carrapiso et al., 2002) make them important contributors to flavor development, even in minute concentrations. Sulfur-containing volatiles are known as primary contributors to the meaty note in cooked meats and carbon disulfide has

### Table 4. (cont.)

| Frozen storage time, d | LRI | Compound | Approximated R² | Regression equation |
|------------------------|-----|----------|-----------------|---------------------|
|                        |     | Esters   |                 |                     |
|                        | 618 | Ethyl acetate | 0.979 | 69.8307–0.02989*R–0.29.009*Day + 0.01236*R*Day + 2.1319*Day*Day – 0.00078*R*Day*Day |
|                        | 764 | Ethyl isobutyrate | 0.909 | 5.268–0.01998*G–1.3774*Day + 0.003102*G*Day + 0.05729*Day*Day |
|                        | 1197 | Ethyl octanoate | 0.971 | –6.103+0.003754*R–0.3367*Day–0.00085*R*Day + 0.1821*Day*Day |
|                        | 1395 | Ethyl decanoate | 0.987 | 20.2778–0.07687*G+0.002783*R–10.1604*Day +0.02016*G*Day– 0.00201*R*Day+0.8161*Day*Day |
|                        |     | Ketones   |                 |                     |
|                        | 613 | 2-butanone | 0.941 | –3.0125+0.01726*G+0.002306*R+2.9261*Day–0.00105*G*Day–0.00087*R*Day |
|                        | 986 | 6-methyl-5-hepten-2-one | 0.709 | 244.72–0.463*G–28.5018*Day+0.1371*G*Day+1.1697*Day*Day– 0.00571*G*Day*Day |
|                        |     | Monoterpene hydrocarbons |       |                     |
|                        | 1011 | 3-carene | 0.769 | 928.09+0.3018*R+8.5829*Day–0.0195*R*Day |
|                        | 1018 | α-terpinene | 0.940 | 688.21+0.261*R–4.9568*Day–0.01718*R*Day+2.3876*Day*Day |
|                        | 1026 | p-cymene | 0.921 | 960.27+0.4142*R–0.09678*Day–0.02644*R*Day |
|                        | 1031 | Limonene | 0.768 | 401.77+0.01338*R–27.4538*Day+0.02167*R*Day+1.8035*Day*Day–0.00134*R*Day*Day |
|                        | 1091 | α-terpinolone | 0.716 | 510.86+0.168*R+13.4293*Day–0.01208*R*Day |
|                        |     | Sesquiterpene hydrocarbons |       |                     |
|                        | 1536 | cedrene | 0.727 | 138.6+0.0975*R+11.102*Day–0.00751*R*Day |
|                        |     | Terpenes with oxygen |       |                     |
|                        | 1031 | Isopulegol | 0.817 | 1108.14+1.3492*G–17.9632*Day |
|                        | 1099 | Sabine hydrate | 0.856 | 535.4+0.1206*R+5.551*R–0.0119*R*Day |
|                        | 1125–1144 | cis-p-ment-2-en-1-ol | 0.735 | 119.89+0.02158*R+3.1879*Day–0.00264*R*Day |
|                        | 1188 | Terpinen-4-ol | 0.840 | 1394.23+0.2846*R+24.1694*Day–0.02748*R*Day |
|                        |     | Terpenoid phenols |       |                     |
|                        | 1531 | Myristicin | 0.729 | 609.73+0.1101*R+32.251*Day–0.01267*R*Day |
|                        |     | Sulfur-containing compounds |       |                     |
|                        | 500–510 | Methanethiol | 0.929 | –45.1015+0.03149*R–3.4201*Day–0.00777*R*Day+1.631*Day*Day |
|                        | 545–567 | Carbon disulfide | 0.918 | 143.37–0.1749*G–0.02837*R–6.2257*Day+0.01841*G*Day |
|                        | 741, 878 | 3-methyl-1-butanol | 0.973 | 234.02–1.7306*G–0.134*R+0.00093*G–17.7311*Day+1.9931*Day*Day |
|                        | 764 | Ethyl isobutyrate | 0.955 | 0.1996–0.02413*G–0.000118*R+0.000019*G*R–0.5037*Day+0.04376*Day*Day |
|                        | 779–786 | Methyl isovalerate | 0.951 | 2.8097 – 0.1345 * G – 0.00381 * R + 0.000061 * G * R + 1.4152 * Day |

1 Linear Retention Indices (LRI) calculated for DB-5 capillary column (J & W Scientific: 30 m × 0.25 mm inner diameter × 0.25 mm film thickness) on a gas chromatograph.
been associated with desirable meat-type sulfur notes in dry-cured ham (Carrapizo and García, 2004). Carbon disulfide decreased as retail display time progressed; however, its associated “sulphury” note continued to increase, which may have been due to microbial growth and carbon disulfides reactivity with functional groups that donate electrons such as amino groups. Other compounds with high aromatic impact due to the low flavor thresholds, such as disulfides, are mainly formed through the conversion of sulfur-containing amino acids (methionine, cysteine, and cystine) to thiols via Strecker degradation or through complex enzymatic reactions. The majority of these sulfur-containing volatiles have been reported to dissipate from aerobically packaged samples or react with fatty acids while levels of oxidative volatiles increase (Ahn et al., 2001).

The “sour”, “dirty socks”, and “oxidized” aromas that formed as retail display progressed can be attributed to the formation of volatile carboxylic acids (Chen and Ho, 1998). These decomposition products can arise from fermentation and via the action of bacterial enzymes during refrigerated storage where free fatty acids, peptides, and amino acids can be made available for further oxidative reactions. The highest Osme time-intensity values for the acids such as acetic acid, 3-methylbutanoic acid, and octanoic acid were found on d 21 of refrigerated storage and ranged from 2 to 9 on a 15-point scale (Fig. 1; Table 3). Oxidation of unsaturated fatty acid residues leads to aldehydes and eventually to short chain fatty acids.

Ester compounds were described by panelists as having fruity and berry-like aromas (Fig. 1; Table 3). Ethyl acetate, ethyl isobutyrate, methyl isovalerate, and methyl hexanoate contribute to the “fruity” notes in pork sausage at the beginning of the retail display period. Ethyl octanoate and ethyl decanoate together with the aforementioned esters are responsible for undesirable notes in pork sausage such as “spoiled fruit”, “oxidized citrus”, and “oxidized ginger-nutmeg” as the product nears the end of shelf life. Furans have been associated with the overall odor of broiled and roasted meats but are insignificant contributors to the basic meaty taste (Varlet et al., 2007). For example, 2-furanmethanol was detected and associated with positive vitamin-like aromas when perceived at the sniff port. Because of its fairly high Osme intensity value, it might contribute significantly to the meaty aroma of this product. There are several suggested pathways for the formation of 2-furanmethanol, including the Maillard reaction or as a result of the deamination and dehydration of Amadori products during cooking (Motttram, 1991). Like furans, pyrroles identified in pork arise from Maillard-type reaction between amino acids and reducing sugars or other carbonyl compounds. One of these pyrroles, 2-acetyl-1-pyrroline, is the primary characteristic aroma compound in freshly prepared popcorn and has been reported as a Maillard reaction product in the headspace of grilled pork (Buttery et al., 1997). At higher concentrations, 2-acetyl-1-pyrroline exhibited a “stale” note. The stale aroma provided by this compound was among the most potent odors in the headspace of pork sausage at the end of its shelf life.

**Aroma impact compounds**

The extracts did not impart any deleterious flavors or aromas characteristic of rosemary or green tea such as astringent or bitter tastes, especially when incorporated at high concentrations in food products and are typically one of the reasons for consumer rejection. Possible spoilage markers formed through microbial growth or oxidative deterioration in the product such as ethanol and acetic acid were significantly inhibited ($P < 0.05$) by natural plant extract addition (R2500 and G300, respectively) following 15 d of frozen storage and particularly on d 7 and 14 of simulated retail display (Table 4). On the other hand, 2,3-dimethyl-2,3-butanediol and 2-heptenal were greater ($P < 0.05$) in samples with increased amounts of green tea extract (Table 4). However, the corresponding fecal aroma of 2-heptenal had lower aroma intensities ($P < 0.05$) with increased amounts of green tea extract as retail display progressed.

The relationship of lipid-derived volatiles formation to flavor deterioration was substantiated with sensory evaluation of the actual samples (Table 5). The sausages with the higher concentrations of the natural plant extracts, particularly R2500 as opposed to sausages with synthetic antioxidants alone had the best flavor (Schilling et al., 2018). Monoterpenes α-thujene, 3-carene, and p-cymene were higher in sausages with increased rosemary extract compared with the control (Table 4). Sausages with increased amounts of green tea extract had greater concentrations of δ-elemene (ginger-nutmeg aroma intensity), cedrene, and cis-p-menth-2-en-1-ol (Table 4). In the current study, higher concentrations of antioxidative terpenes were in the treatments with greater concentrations of R and G, which enhanced antioxidative protection. For example, combinations of both plant derived extracts had higher concentrations of terpenic compounds such as α-pinene and camphene, limonene, isopulegol, 2-methyl-cis-3a,4,7,7a-tetraydroindan, and myristicin in comparison with the control (Table 4).

Green tea extract concentration was a significant variable following 90 d of frozen storage where 2-heptenal and 6-methyl-5-hepten-2-one were greater...
Table 5. Effect of combinations of rosemary and green tea extracts on the sensory shelf life of pork sausage held under simulated retail display at 3 ± 1°C for 21 d following 15 and 90 d of frozen storage (–20°C)

| Treatment¹ | 15 d | 90 d | Sensory defect |
|------------|------|------|---------------|
| R1500 + G100 | 14 | 16 | Oxidized, slightly fruity |
| R1500 + G200 | 16 | 15 | Strong oxidized |
| R1500 + G300 | 15 | 15 | Oxidized, spoiled |
| R2000 + G100 | 14 | 15 | Sour, oxidized |
| R2000 + G200 | 15 | 16 | Oxidized, spoiled |
| R2000 + G300 | 18 | 16 | Oxidized, slightly fruity |
| R2500 + G100 | 16 | 16 | Very strong oxidized, slightly fruity |
| R2500 + G200 | 16 | 15 | Oxidized, spoiled |
| R2500 + G300 | 18 | 16 | Oxidized, slightly fruity |
| CONTROL | <14 | <14 | Oxidized, spoiled |

¹ Rosemary extract (R: 1500, 2000, and 2500 mg/kg), Green tea extract (G: 100, 200, and 300 mg/kg), Control (synthetic antioxidants only).

Table 5 shows the effect of combinations of rosemary and green tea extracts on the sensory shelf life of pork sausage held under simulated retail display at 3 ± 1°C for 21 d following 15 and 90 d of frozen storage (–20°C). The table lists the sensory defects observed for different treatments.

In sausages with G300, especially on d 7 and 14 than any of the other treatments (P < 0.05; Table 4). In contrast, 2-butanone was most concentrated in control samples and lowest in sausages with R2500, which is similar to the 15 d frozen storage period (Table 4). Similarly, benzeneethanol was greatest in the control sausages when compared with those having R2500 (Table 4). Methanethiol concentration increased (P < 0.05) as rosemary concentration decreased (Table 4). Carbon disulfide which was associated with the sulfury and meaty notes of the product decreased (P < 0.05) as retail display progressed regardless of natural plant extract addition; however, its concentration fluctuated the least in sausages with G300 (Table 4). Acetic acid and ester ethyl acetate and ethyl octanoate were greater in the control sausages and least in samples with R2500 (Table 4). However, ethyl isobutyrate and ethyl decanoate were highest in sausages with G300 when compared to the control after 90 d of frozen storage (Table 4).

Spice-derived terpenes such as monoterpenes (3-carene, α-terpinene, p-cymene, limonene, α-terpinolene), sesquiterpenes (cedrene), terpenes with oxygen (sabinene hydrate, cis-p-menth-2-en-1-ol, terpinen-4-ol), and terpenoid phenol (myristicin) were higher in sausages with higher rosemary extract mostly after d 0 and 14 of refrigerated storage (Table 4). Green tea extract concentration led to an increase in terpenes with oxygen such as isopulegol (Table 4). Similar to the 15 d frozen storage time, the phenolic compounds present in both rosemary and green tea extracts enhanced product protection against oxidative reactions after 90 d frozen storage. The majority of the oxidation decomposition products were higher in treatments with lower concentrations of the natural plant extracts (Table 4).

The antioxidant quality of rosemary extracts has been mainly attributed to the presence of the phenolic diterpene compounds carnosic acid and carnosol. These active ingredients have been reported to function as chain breaking radical scavengers by donating hydrogen to free radical intermediates of the oxidation reactions (Berdahl et al., 2010). Pure carnosic acid and carnosol are good peroxyl radical scavengers while other constituents of rosemary extract such as rosmarinic acid is a superior superoxide scavenger (Aruoma et al., 1992; Berdahl et al., 2010). Additionally, the vicinal–OH groups chelate pro-oxidant metals, thereby providing an additional protective mechanism toward oxidation (Brewer, 2011). Moreover, carnosic acid has been reported to initiate the so-called ‘carnosic acid cascade” or oxidation cascade of carnosic acid. In this reaction, carnosic acid and carnosol are readily oxidized, reduced, and isomerized into quinone and lactone products accompanied by the quenching of free radicals via hydrogen atom donation from the phenolic groups (Masuda et al., 2002). The proposed oxidation cascade from carnosic acid and the observation of new antioxidant products imply that carnosic acid may still contribute to the antioxidative activity after its oxidation. The catechins in green tea extract have been shown to exhibit antioxidant activities such as (–) epicatechin (EC), (–) epicatechin gallate (ECG), (–) epigallocatechin (EGC), (–) epigallocatechin gallate (EGCG), (+) catechin (C), and (+) gallo-locatechin (GC; Berdahl et al., 2010). The presence of multiple hydroxyl groups in the molecules of these compounds makes them effective radical scavengers via hydrogen donation mechanisms. Some functional groups such as the galloyl group have been shown to chelate metals for the protection against multivalent metal catalysts such as Fe²⁺/Fe³⁺. Furthermore, the donation of hydrogen atoms from the catechol or galloyl moieties results in the formation of relatively stable phenoxyl radicals. When these species accumulate, polymerization reactions between adjacent phenoxyl radicals may occur via substitution of carbon atoms in their aromatic ring. This reaction leads to the regeneration of hydroxyl groups and, consequently rejuvenate their ability to donate hydrogen atoms and their antioxidant capacity.

Both rosemary and green tea extracts had a significant effect on the volatile profile of fresh pork sausages for up to 180 d of storage at −20°C. Although the panellists reported no objectionable odors in the sausages at the beginning of the retail display period, the lack of freshness or flavor dissipation in the samples was noticeable after 180 d of storage (data not shown).
was a significant decrease in the levels of volatile compounds of pork sausage with R2500 + G300 after 15, 90, and 180 d of frozen storage (data not shown). Both rosemary and green tea extracts showed a significant effect on the concentrations of 3-methyl-1-butanol and methyl isovalerate in the sausages where these aroma impact compounds were higher ($P < 0.05$) in the control and lower in treatment combinations with G300 especially R1500+G300 (Table 3).

**Flavor shelf life of pork sausage**

Combinations of at least 2500 mg/kg of rosemary extract and green tea extract as well as R2000+G300 increased the shelf life of fresh pork sausages to 16–18 d of storage after 15 d of frozen storage (Table 5). Accordingly, for 90 d of frozen storage, all treatment combinations retained the shelf life of fresh pork sausages to 15 d of storage compared with the control whose shelf-life was limited to less than 14 d (between 8 and 13 d; Table 5). After 180 d of frozen storage, all sausage samples reached the end of their flavor shelf life following 14 d of retail display, compared with the control, which displayed spoilage and detectable rancidity by d 7 under similar storage conditions. The panelists identified the predominant off-odor as that of spoilage due to microbial growth and to a lesser extent deterioration related to oxidative processes. Addition of rosemary and green tea extracts resulted in product shelf lives comparable to those of commercial pork sausages which would normally contain an antimicrobial agent, color protector, and are processed under more controlled conditions. These results indicate that the addition of natural plant extracts could be helpful for improving the sensory attributes of the sausages.

**Conclusions**

Fifty-five aroma impact compounds that contribute to pork sausage flavor were identified using SPME, GC–MS, and GCO-Osme. Similar compounds were present in all sausages but were differentiated through the relative concentrations of their aroma impact compounds. The typical aroma of the sausage product was mainly associated with the presence of a high number of volatile terpenes as well as lipid-derived volatiles such as aldehydes and alcohols. An enhanced protection by natural plant extract combinations was observed across all storage periods, especially beyond 90 d of frozen storage where oxidation associated aroma-impact volatiles were reduced in sausages with higher rosemary and/or green tea extract concentrations. This enhanced protection was attributed to a multi-antioxidant approach allowing the active ingredients to partition in all of the phases of the product to provide antioxidant protection where it is needed the most.

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