Published June 27, 2019

Meat and Muscle Biology™

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

M. W. Schilling[1]*, A. J. Pham-Mondala[1], N. Dhowlaghar[1], Y. L. Campbell[1], T. T. Dinh[2], A.C. Tolentino[1], J. B. Williams[1], Y. L. Xiong[3]

[1]Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS 39762, USA
[2]Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS 39762, USA
[3]Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40506, USA
*Corresponding author. Email: schilling@foodscience.msstate.edu (M. W. Schilling)

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
Meat and Muscle Biology 3(1):194–209 (2019) **doi:10.22175/mmb2019.03.0007**
Submitted 1 Mar. 2019. Accepted 2 May 2019.

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 binding 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 (Marsili, 2007). The GCO-Osme technique has been applied to various products such as liver pâtés (Estévez et al.,2004) and Iberian dry-cured ham (Carrapiso 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-

Table 1. Treatment combinations of rosemary and green tea extracts used in the manufacture of fresh pork sausage based on the salted pork weight

Treatment	Rosemary extract, mg/kg	Green tea extract, mg/kg	Synthetic antioxidants (combination of BHA, BHT, and PG[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 %

[1]Abbreviations: BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; PG = propyl gallate.

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-butanone, diacetyl, ethyl acetate, acetic acid, 3-methylbutanal, 1-butanol, acetoin, methyl butanoate, 3-methyl-butanol, 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_5–C_8 (Aldrich; Sigma-Aldrich Chemical Co., St. Louis, MO) and C_8–C_{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).

Manufacture 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

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 violatile 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 (Reactitherm 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 carboxen/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, Folsom, CA), coupled to a Saturn 2100T Ion-Trap Mass Selective Detector (MSD; Varian Inc., Walnut Creek, CA). Ultra-high purity helium (Airgas, Columbus, MS) was used as the carrier gas with a flow rate of 1.2 mL/min. The GC oven temperature program was isothermal for 1 min at 35°C, ramped to 250°C at a rate of 10.75°C/min, and held at 250°C for 4 min. The GC–MS transfer line and the manifold were maintained at 250°C and 180°C, respectively. The MSD operated in the electronic ionization mode (70 eV), in a scan mode from m/z 35 to m/z 350 at a rate of 0.5 s per scan.

For identification of the aroma-impact compounds, Osme-GCO analyses were conducted using a modified Varian CP-3800 gas chromatographic system (Varian Inc.) equipped with a sniffing port (ODO-I, SGE, Kramer Lane, Austin, TX), a flame ionization detector (FID), 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 C_5–C_{18} 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 response 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:

$$\mathrm{Y} = \beta_0 + \beta_1(G) + \beta_2(R) + \beta_3(G*R) + \beta_4(\mathrm{day}) + \beta_5(G*\mathrm{day}) + \beta_6(R*\mathrm{day}) + \beta_7(\mathrm{day}*\mathrm{day}) + \beta_8(G*\mathrm{day}*\mathrm{day}) + \beta_9(R*\mathrm{day}*\mathrm{day}) + \in$$

where Y is the response variable; β_0 is the constant coefficient (intercept); β_1, β_2, and β_4 are the linear coefficients (main effects); β_7 is the quadratic coefficient; β_3 is the two-factor interaction coefficient, and $\in$ 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)

			Concentration, mg/kg[2]				
Peak no.	Compound	LRI[1]	d 0	d 7	d 14	d 21	SEM
	Alcohols						
2	Ethanol	545	0^{b}	0^{b}	5.5^{ab}	19.1^{a}	5.2
4	1-propanol	567	27^{b}	101^{ab}	147^{a}	115^{a}	27.6
9	1-butanol	663	7.7	10.9	15	10.2	4.7
12, 20	3-methyl-1-butanol	741, 878	6.1^{c}	13^{c}	243^{b}	384^{a}	43.4
19	1-hexanol	871	18^{ab}	19.9^{ab}	47.5^{a}	9^{b}	11.0
29	1-octen-3-ol	979	16.1^{b}	36.3^{a}	56.4^{a}	40.5^{a}	7.0
44	Benzeneethanol	1,115	4.3^{b}	3.5^{b}	12^{b}	62.7^{a}	5.6
53	2,3-dimethyl-2,3-butanediol	1,244–1,250	6.3	6.8	4.1	4.7	0.93
	Aldehydes						
8	3-methylbutanal	648	1.4^{b}	10.1^{b}	159^{a}	112^{ab}	41.3
15	Hexanal	799	89.1	138.3	111	108	21.9
22	Heptanal	901	6.1^{bc}	4.9^{c}	16.9^{ab}	25.6^{a}	3.4
28	2-heptenal	955	7^{c}	112^{b}	194^{a}	221^{a}	23.2
45	Benzeneacetaldehyde	1,043	14.3	10.5	36.6	292	95.2
56	2,4-decadienal	1,320	0^{b}	0^{b}	< 1^{b}	1^{a}	0.13
	Carboxylic acids						
7	Acetic acid	637	31.5^{c}	110^{c}	323^{b}	743^{a}	52.1
16	3-methylbutanoic acid	850, 860	0^{b}	0^{b}	3.1^{ab}	4.6^{a}	1.3
51	Octanoic acid	1,176	27.2^{b}	26.3^{b}	30.3^{b}	53.5^{a}	7.7
	Esters						
6	Ethyl acetate	618	7.5^{c}	5.8^{c}	62.4^{b}	183^{a}	16.0
11	Methyl butanoate	725	1.9	< 1	3.8	4.1	2.8
13	Ethyl isobutyrate	764	1.7^{b}	0^{b}	< 1^{b}	10.4^{a}	2.2
14, 17	Methyl isovalerate	779–786, 852–857	<1^{b}	<1^{b}	1.5^{b}	11.6^{a}	2.4
24	Methyl hexanoate	925	11.7^{a}	9.2^{ab}	8.4^{ab}	6.8^{b}	1.4
35	Ethyl hexanoate	999	0	1.4	2.9	3.6	1.7
52	Ethyl octanoate	1,197	0^{b}	0^{b}	7.2^{b}	38.2^{a}	3.8
57	Ethyl decanoate	1,395	0^{b}	0^{b}	10.4^{b}	158^{a}	10.8
	Ketones						
5	2-butanone	613	8.9^{b}	7^{b}	19.4^{a}	26.4^{a}	2.8
10	3-hydroxy-2-butanone	722	41.7^{c}	57.1^{bc}	123^{b}	225^{a}	24.3
21	2-heptanone	890	10.8^{b}	10.1^{b}	18^{ab}	22.1^{a}	2.9
30	2,3-octanedione	982	4.6^{b}	6.2^{ab}	9.1^{a}	8.3^{ab}	1.3
31	6-methyl-5-hepten-2-one	986	133	153	133	146	21.3
	Monoterpene hydrocarbons						
25	α-thujene	928	591^{a}	398^{ab}	236^{b}	430^{ab}	85.4
26	α-pinene	934	1,704^{a}	1453^{ab}	864^{b}	1207^{ab}	249
27	Camphene	948	90.8	88.3	62.5	92.5	12.1
32	α-phellandrene	1,005	415^{a}	270^{b}	249^{b}	166^{b}	44.1
33	3-carene	1,011	1,566^{a}	1352^{ab}	1047^{b}	1034^{b}	135
34	α-terpinene	1,018	1,233^{a}	753^{b}	519^{b}	529^{b}	99.6
36	*p*-cymene	1,026	1,806^{a}	1407^{a}	965^{b}	777^{b}	152
37	Limonene	1,031	441	527	532	420	66.8
39	2-methyl-cis-3a,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						
54	δ-elemene	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

Table 2. (cont.)

Peak no.	Compound	LRI[1]	Concentration, mg/kg[2] d 0	d 7	d 14	d 21	SEM
	Terpenes with oxygen						
38	Isopulegol	1,031	1,434[a]	1135[ab]	1305[a]	924[b]	153
40	1,8-cineole	1,033	11.6	2.7	18.8	7.7	7.2
41, 50	α-terpineol	1,071, 1,194	407[b]	384[b]	339[b]	545[a]	42.7
47	Sabinene hydrate	1,099	808[a]	642[ab]	548[b]	470[b]	67.3
48	cis-*p*-menth-2-en-1-ol	1,125–1,144	162	152	139	122	15.4
49	Terpinen-4-ol	1,188	1,982[a]	1807[ab]	1453[b]	1442[b]	145
60	Caryophyllene oxide	1,609	79.9	90.6	60.7	53.9	17.7
	Terpenoid phenols						
58	Myristicin	1,531	865	912	828	1079	98.6
	Sulfur-containing compounds						
1	Methanethiol	500–510	0[b]	0[b]	49.9[b]	345[a]	73.6
3	Carbon disulfide	545–567	61.7[a]	49.1[ab]	24.2[b]	13.5[b]	12.5
	Furans						
18	2-furanmethanol	862	2.2	2.4	3	2.6	0.71
	Pyrroles						
23	2-acetyl-1-pyrroline	920	1.9[c]	3.7[c]	72.7[b]	111.5[a]	13.8

[a–c] Means within each row with different superscript letters are significantly different ($P < 0.05$).

[1] Linear Retention Indices (LRI) calculated for DB-5 capillary column (J & W Scientific: 30 m × 0.25 mm inner diamter × 0.25 mm film thickness) on a gas chromatograph equipped with a mass selective detector.

[2] Results expressed as means of nine samples in ng/g sausage.

Results and Discussion

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 sabinene (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, *p*-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

		LRI[3]			Aroma intensity[5]				
Peak no.	Compound[1,2]	GC-MS	GCO	Aroma[4] note/descriptor	d 0	d 7	d 14	d 21	SEM
1	Methanethiol	500–510	< 500	Alcoholic, sweet	0^{b}	0^{b}	3^{a}	4^{a}	0.45
2	Ethanol	545							
3	Carbon disulfide	545–567	< 500, 601	Sulfury, meat-like-thiol-like	0^{b}	1^{b}	3^{a}	4^{a}	0.53
4	1-propanol	567							
5	2-butanone	613	568–626	Buttery, milky	1.5^{b}	2^{b}	4.5^{a}	5^{a}	0.38
6	Ethyl acetate	618							
7	Acetic acid	637	568–626	Sour, cheesy	$<1^{b}$	1^{ab}	1^{ab}	2^{a}	0.37
8	3-methylbutanal	648							
9	1-butanol	663	670	Green, dirty socks	0^{b}	0^{b}	2^{a}	3^{a}	0.47
10	3-hydroxy-2-butanone	722	741	Fruity, dirty socks, spoiled fruit, berry-like	0^{c}	0^{c}	1^{b}	5^{a}	0.37
11	Methyl butanoate	725	748						
12	3-methyl-1-butanol	741							
13	Ethyl isobutyrate	764	760–765		0^{b}	0^{b}	$<1^{b}$	6^{a}	0.30
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	0^{c}	0^{c}	4^{b}	9^{a}	0.40
17	Methyl isovalerate	852–857							
18	2-furanmethanol	862	868–870	Vitamin	6^{c}	6.5^{bc}	7^{b}	8.5^{a}	0.31
19	1-hexanol	871							
20	3-methyl-1-butanol	878							
21	2-heptanone	890	905–913	Baked potato	6^{a}	2^{b}	0^{c}	0^{c}	0.39
22	Heptanal	901	905–913	Musty, yeasty, baked potato	0^{c}	4^{b}	9^{a}	9^{a}	0.40
23	2-acetyl-1-pyrroline	920	883, 920	Popcorn, cooked rice	3.5^{a}	4^{a}	3^{a}	0^{b}	0.67
	2-acetyl-1-pyrroline	920	883, 920	Stale	0^{d}	3^{c}	6^{b}	10^{a}	0.56
24	Methyl hexanoate	925	933	Fruity	1	1	1	0	0.38
	Methyl hexanoate	925	933	Spoiled fruit	1	2	1.5	2	0.50
25	α-thujene	928	935–938	Sausage, spicy, ginger, nutmeg	8^{a}	5^{b}	0^{c}	1^{c}	0.43
26	α-pinene	934							
27	Camphene	948							
28	2-heptenal	955	935–938	Oxidized sausage	0^{c}	2^{b}	6^{a}	6^{a}	0.44
	2-heptenal	955	969	Fecal	0^{b}	0^{b}	0^{b}	2^{a}	0.31
29	1-octen-3-ol	979	980–987	Metallic,	5^{b}	8.5^{a}	8^{a}	8.5^{a}	0.34
30	2,3-octanedione	982		mushroom	6^{b}	8^{a}	8^{a}	8^{a}	0.35
31	6-methyl-5-hepten-2-one	986							
32	α-phellandrene	1005	996–1029	Citrusy, herbal, grainy, meaty	$<1^{b}$	3.5^{a}	4^{a}	2^{b}	0.48
33	3-carene	1011							
34	α-terpinene	1018							
35	Ethyl hexanoate	999	996–1029	Oxidized citrus	0^{b}	0^{b}	$<1^{b}$	3.5^{a}	0.43
36	*p*-cymene	1026	1025, 1031	Sweet cola, minty	2^{a}	$<1^{b}$	$<1^{b}$	$<1^{b}$	0.41
37	Limonene	1031							
38	Isopulegol	1031							
39	2-methyl-cis-3a,4,7,7a-tetrahydroindan	1031							
40	1,8-cineole	1033	1044–1099	Minty, eucalyptol	8^{a}	6^{b}	2.5^{c}	0^{d}	0.39
41	α-terpineol	1071							
42	α-terpinolene	1091							
43	Unknown		1044–1099	Oxidized mint	0^{c}	0^{c}	3^{b}	7^{a}	0.39
44	Benzeneethanol	1115	1055	Rose	0^{b}	0^{b}	$<1^{b}$	2^{a}	0.40
45	Benzeneacetaldehyde	1043							
46	α-terpinolene	1091	1087–1093	Grainy, herbal	6	5	6	6	0.41
47	Sabinene hydrate	1099							

Continued

Table 3. (cont.)

Peak no.	Compound[1,2]	LRI[3] GC-MS	GCO	Aroma[4] note/descriptor	Aroma intensity[5] d 0	d 7	d 14	d 21	SEM
48	cis-*p*-menth-2-en-1-ol	1125–1144	1113–1136	Floral, spicy, cocoa, grainy	6.5^{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	α-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	δ-elemene	1349	1342–1396	Ginger-nutmeg, spicy, minty	4^{a}	4^{a}	<1^{b}	0^{b}	0.42
55	α-cubebene	1364							
56	2,4-decadienal	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

[a–d] Means within each row with different superscript letters are significantly different ($P < 0.05$).

[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.

and 2,4-decadienal (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 hexanal (4.5 mg/kg), heptanal (3 mg/kg), 2-heptenal (13 mg/kg), and 2,4-decadienal (0.07 mg/kg; Leffingwell and Leffingwell, 1991). Presence of these aldehydes at similar concentrations in sausage indicates that the product is near the end of retail shelf life. Methyl-branched aldehydes such as 3-methylbutanal and benzeneacetaldehyde have been established as major flavor contributors in various meat products due to their low odor thresholds of 0.2 mg/kg and 4 mg/kg, respectively, and distinctive aroma characteristics (Buttery et al., 1997; Xie et al., 2008). Formation of these compounds has been attributed to the oxidative deamination-decarboxylation of amino acids through Strecker degradation and could be related to proteolysis (Lund et al., 2011).

Five aroma impact ketones, 2-butanone, 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 β-keto acid decarboxylation (Ramirez and Cava, 2007) or β-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-butanone, 3-hydroxy-2-butanone, and 2,3-octanedione 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

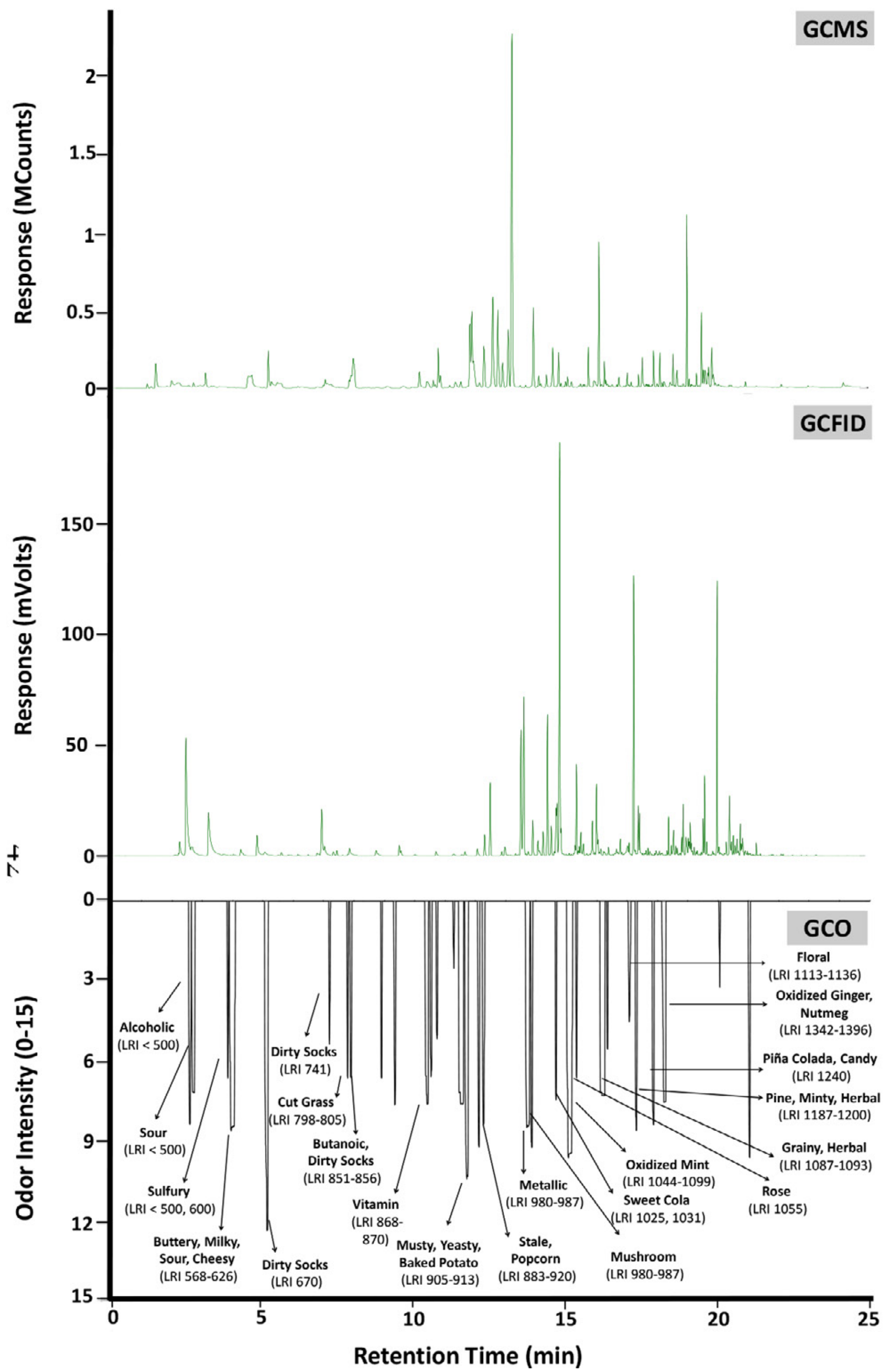

Figure 1. Comparison of the GC–MS (top) and GC-FID (middle) chromatograms with identification of some aroma impact compounds against GCO Osme time-intensity osmegram (bottom) for fresh pork sausage with rosemary extract (2500 mg/kg green tea extract (300 mg/kg) and synthetic antioxidants under simulated retail display (3 ± 1°C, 14 d) following 90 d of frozen storage (–20°C). 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 sniffing port and flame ionization detector

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[1]	Compound	Approximated R^2	Regression equation
15		Alcohols		
	545	Ethanol	0.913	–18.8201+0.002779*R+10.9342*Day–0.00349*R*Day
	979	1-octen-3-ol	0.973	–25.477+0.07486*G+0.01497*R+5.0862*Day
	1244–1250	2,3-dimethyl-2,3-butanediol	0.911	9.3348–0.02216*G + 0.2484*Day + 0.01464*G*Day +0.02166*Day*Day–0.00066*G*Day*Day
		Aldehydes		
	648	3-methylbutanal	0.889	–9.994–0.04986*R+11.1759*Day+0.01111*R*Day
	799	Hexanal	0.941	0.6871+ 0.2871*G+4.6331*Day
	901	Heptanal	0.929	2.4631–0.03882*G+0.5144*Day+0.007857*G*Day
	955	2-heptenal	0.952	–49.4434+0.2598*G+21.4443*Day–0.726*Day*Day
	1043	Benzeneacetaldehyde	0.923	12.9532–0.00576*R–5.1099*Day + 0.001519*R*Day + 0.3528*Day*Day
		Carboxylic acid		
	637	Acetic acid	0.979	–9.7975+0.1621*G+11.6123*Day–0.1097*G*Day–0.1391*Day*Day+0.00634*G*Day*Day
		Esters		
	779–786, 852–857	Methyl isovalerate	0.929	16.6433–0.00697*R–9.0765*Day + 0.001505*R*Day + 0.5211*Day*Day
	925	Methyl hexanoate	0.684	1.8423+0.004272*R+0.249*Day
		Ketones		
	613	2-butanone	0.959	–4.9951+0.006592*R+7.3548*Day–0.00458*R*Day–0.2731*Day*Day+0.000239*R*Day*Day
	982	2,3-octanedione	0.833	5.976–0.01311*G–0.081*Day+0.002425*G*Day
		Monoterpene hydrocarbons		
	928	α-thujene	0.764	153.09+0.1789*R+15.163*Day
	934	α-pinene	0.888	217.91+7.9777*G+0.7548*R–0.00422*G*R–51.6538*Day+6.5939*Day*Day
	948	Camphene	0.938	18.9012+0.3588*G+0.03454*R–0.0002*G*R–3.589*Day+0.3908*Day*Day
	1011	3-carene	0.973	623.11+0.3455*R–34.2726*Day+4.9796*Day*Day
	1026	*p*-cymene	0.914	362.13+0.4041*R+45.1136*Day
	1031	Limonene	0.921	114.42+0.7861*G+0.1042*R–0.00048*G*R–3.8661*Day +0.9121*Day*Day
	1031	2-methyl-cis-3a,4,7,7a-tetrahydroindan	0.963	210.11+2.1763*G+0.2859*R–0.00133*G*R+36.436*Day
	1091	α-terpinolene	0.935	266.63+3.5135*G+0.2525*R–0.00181*G*R–9.274*Day + 2.2611*Day*Day
		Sesquiterpene hydrocarbons		
	1349	δ-elemene	0.882	359.83+0.97*G+22.8469*Day
	1536	Cedrene	0.894	314.23–0.3646*G+3.7581*Day+0.09537*G*Day
		Terpenes with oxygen		
	1031	Isopulegol	0.961	175.98+3.7379*G+0.4458*R–0.00227*G*R–6.0387*Day + 2.7044*Day*Day
	1125–1144	cis-*p*-menth-2-en-1-ol	0.937	143.02+0.5007*G+17.2326*Day
		Terpenoid phenols		
	1531	myristicin	0.873	386.89+3.1553*G+0.1015*R–0.00142*G*R+33.5741*Day
90		Alcohols		
	1115	Benzeneethanol	0.972	–5.4271+0.006585*R–0.8309*Day–0.0015*R*Day + 0.3141*Day*Day
		Aldehydes		
	901	Heptanal	0.909	9.2393–0.08839*G–0.00609*R +0.000061*G*R + 0.9413*Day
	955	2-heptenal	0.959	9.1915+0.2103*G+9.3795*Day
	1320	2,4-decadienal	0.945	–0.3395+0.000094*R+0.09691*Day–0.00003*R*Day
		Carboxylic acid		
	637	Acetic acid	0.972	181.78–0.1087*R+31.5202*Day

Continued

Table 4. (cont.)

Frozen storage time, d	LRI[1]	Compound	Approximated R^2	Regression equation
		Esters		
	618	Ethyl acetate	0.979	69.8307–0.02989*R–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	–6.1013+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–49.5658*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–27.4358*Day+0.02167*R*Day+1.8035*Day*Day–0.00134*R*Day*Day
	1091	α-terpinolene	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	Sabinene hydrate	0.856	535.4+0.1206*R+5.551*Day–0.01119*R*Day
	1125–1144	cis-*p*-menth-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–0.02387*R–6.2257*Day+0.01841*G*Day
180		Alcohols		
	545	Ethanol	0.821	–4.0193–0.6291*G–0.02954*R+0.000449*G*R+3.5536*Day
	741, 878	3-methyl-1-butanol	0.973	234.02–1.7306*G–0.134*R+0.000933*G*R–17.7311*Day+1.9931*Day*Day
		Esters		
	764	Ethyl isobutyrate	0.955	0.1996–0.02413*G–0.00118*R+0.000019*G*R–0.5037*Day+0.04376*Day*Day
	779–786, 852–857	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.

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 been associated with desirable meat-type sulfur notes in dry-cured ham (Carrapiso and García, 2004). Carbon disulfide decreased as retail display time progressed; however, its associated "sulfury" 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 degradations 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 (Mottram, 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 odorants 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-tetrahydroindan, 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)

Treatment[1]	15 d Shelf life, d	90 d Shelf life, 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).

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 antioxidant 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 (+) gallocatechin (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 Fe^{2+}/Fe^{3+}. 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, 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 panelists 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). There 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.

Acknowledgments

Approved for publication by the Mississippi Agricultural and Forestry Experiment Station. This research did not receive any specific grant funding from agencies in the public, commercial, or not-for-profit sectors.

Literature Cited

Acree, T. E., and H. Arn. 2015. Flavornet gas chromatography-olfactometry (GC-O) of natural products. http://flavornet.org/ (accessed 7 June).

Ahn, D. U., K. C. Nam, M. Du, and C. Jo. 2001. Volatile production in irradiated normal, pale soft exudative (PSE) and dark firm dry (DFD) pork under different packaging and storage conditions. Meat Sci. 57:419–426. doi:10.1016/S0309-1740(00)00120-0

AOAC. 2007. AOAC Official Method 2007-04. In Official Methods of Analysis. 18th ed. Rev. 3. AOAC Int., Washington, DC.

Aruoma, O. I., B. Halliwell, R. Aeschbach, and J. Loligers. 1992. Antioxidant and pro-oxidant properties of active rosemary constituents: Carnosol and carnosic acid. Xenobiotica 22:257–268. doi:10.3109/00498259209046624

Belitz, H. D., and H. Grosch. 1987. Food chemistry. Berlin: Springer-Verlag.

Berdahl, D. R., and J. McKeague. 2015. Rosemary and sage extracts as antioxidants for food preservation. In: F. Shahidi, editor, Handbook of antioxidants for food preservation. Woodhead Publishing, Waltham, MA. p. 177–217. doi:10.1016/B978-1-78242-089-7.00008-7

Berdahl, D.R., R.I. Nahas, and J.P. Barren. 2010. Synthetic and natural antioxidant additives in food stabilization: Current applications and future research. In: E.A. Decker, R.J. Elias, and D.J. McClements, editors, Oxidation in foods and beverages and antioxidant applications. Woodhead Publishing, Oxford, UK. p. 272–320. doi:10.1533/9780857090447.2.272

Brewer, M.S. 2011. Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. Compr. Rev. Food Sci. Food Saf. 10:221–247. doi:10.1111/j.1541-4337.2011.00156.x

Buttery, R.G., L.C. Ling, and D.J. Stern. 1997. Studies on popcorn aroma and flavor volatiles. J. Agric. Food Chem. 45:837–843. doi:10.1021/jf9604807

Carrapiso, A. I. 2007. Effect of fat content on flavour release from sausages. Food Chem. 103:396–403. doi:10.1016/j.foodchem.2006.07.037

Carrapiso, A. I., and C. García. 2004. Iberian ham headspace: Odorants of intermuscular fat and differences with lean. J. Sci. Food Agric. 84:2047–2051. doi:10.1002/jsfa.1911

Carrapiso, A. I., Á. Jurado, M. L. Timón, and C. García. 2002. Odor-active compounds of iberian hams with different aroma characteristics. Journal of Agricultural and Food Chemistry of Agricultural 50:6453–6458. doi:10.1021/jf025526c

Chen, J., and C. T. Ho. 1998. The flavour of pork. In: F. Shahidi, editor, Flavor of meat, meat products and seafood. 2nd ed. Blackie

Academic and Professional, London. p. 61–83.

Chevance, F. F. V., and L. J. Farmer. 1999. Identification of major volatile odor compounds in frankfurters. J. Agric. Food Chem. 47:5151–5160. doi:10.1021/jf990515d

Dirinck, P., F. Van Opstaele, and F. Vandendriessche. 1997. Flavour differences between northern and southern European cured hams. Food Chem. 59:511–521. doi:10.1016/S0308-8146(97)00012-5

El-Sayed, A. M. 2015. The pherobase: Database of insect pheromones and semiochemicals. http://www.pherobase.com/ (accessed 7 June).

Estévez, M., S. Ventanas, R. Ramírez, and R. Cava. 2004. Analysis of volatiles in porcine liver pâtés with added sage and rosemary essential oils by using SPME-GC-MS. J. Agric. Food Chem. 52:5168–5174. doi:10.1021/jf0496705

Estévez, M., S. Ventanas, R. Ramírez, and R. Cava. 2005. Influence of the addition of rosemary essential oil on the volatiles pattern of porcine frankfurters. J. Agric. Food Chem. 53:8317–8324. doi:10.1021/jf051025q

James, S. S., and C. James. 2012. Quality and safety of frozen meat and meat products. In: D.-W. Sun, editor, Handbook of frozen food processing and packaging. 2nd ed. CRC Press, Taylor & Francis Group, Boca Raton, FL. p. 303–323.

Jo, C., and D. U. Ahn. 2000. Volatiles and oxidative changes in irradiated pork sausage with different fatty acid composition and tocopherol content. J. Food Sci. 65:270–275. doi:10.1111/j.1365-2621.2000.tb15992.x

Jongberg, S., M. A. Tørngren, and L. H. Skibsted. 2018. Dose-dependent effects of greentea or maté extracts on lipid and protein oxidation in brine-injected retail-packed pork chops. Medicine 5:1–12.

Karaosmanoglu, H., and P. A. Kilmartin. 2015. Tea extracts as antioxidants for food preservation. In: F. Shahidi, editor, Handbook of antioxidants for food preservation. Woodhead Publishing, Waltham, MA. p. 219–233.

Leffingwell, J. C., and D. Leffingwell. 1991. GRAS flavor chemicals-detection thresholds. Perfum. Flavor. 16:1–19.

Leygonie, C., T. J. Britz, and L. C. Hoffman. 2012. Review: Impact of freezing and thawing on the quality of meat. Meat Sci. 91:93–98. doi:10.1016/j.meatsci.2012.01.013

Lund, M. N., M. Heinonen, C. P. Baron, and M. Estévez. 2011. Protein oxidation in muscle foods: A review. Mol. Nutr. Food Res. 55:83–95. doi:10.1002/mnfr.201000453

Lynch, M. P., and C. Faustman. 2000. Effect of aldehyde lipid oxidation products on myoglobin. J. Agric. Food Chem. 48:600–604. doi:10.1021/jf990732e

Marsili, R. T. 2007. Comparing sensory and analytical chemistry flavor analysis. In: R.T. Marsili, editor, Sensory-directed flavor analysis. CRC Press, Boca Raton, FL. p. 1–22.

Martínez, L., I. Cilla, J. A. Beltrán, and P. Roncalés. 2006. Antioxidant effect of rosemary, borage, green tea, pu-erh tea and ascorbic acid on fresh pork sausages packaged in a modified atmosphere: Influence of the presence of sodium chloride. J. Sci. Food Agric. 86:1298–1307. doi:10.1002/jsfa.2492

Masuda, T., Y. Inaba, T. Maekawa, Y. Takeda, H. Tamura, and H. Yamaguchi. 2002. Recovery mechanism of the antioxidant activity from carnosic acid quinone, an oxidized sage and rosemary antioxidant. J. Agric. Food Chem. 50:5863–5869. doi:10.1021/jf025605o

McDaniel, M. R., R. Miranda-Lopez, B. T. Watson, N. J. Michaels, and L. M. Libbey. 1990. Pinot noir aroma: A sensory/gas chromatographic approach. Flavor and offflavors. In: G. Charalambous, editor, Proceedings of the 6th International Flavor Conference. Elsevier, Rethymnon, Crete, Greece Amsterdam, The Netherlands. p. 23.

Mottram, D. S. 1991. Meat. In: H. Maarse, editor, Volatile compounds in foods and beverages. Marcel Dekker, Inc., New York, NY. p. 107–177.

Qian, M. C., H. M. Burbank, and Y. Wang. 2007. Preseparation techniques in aroma analysis. In: R. Marsili, editor, Sensory-directed flavor analysis. CRC Press, Boca Raton, FL. p. 111–154.

Ramirez, R., and R. Cava. 2007. Volatile profiles of dry-cured meat products from three different Iberian × Duroc genotypes. J. Agric. Food Chem. 55:1923–1931. doi:10.1021/jf062810l

Rouseff, R., R. Bazemore, K. Goodner, and M. Naim. 2001. GC-olfactometry with solid phase microextraction of aroma volatiles from heated and unheated orange juice. In: R. Rouseff and K. Cadwallader, editors, Headspace analysis of food and flavors: Theory and practice. Kluwer Academic/Plenum Publishers, New York, NY. p. 101–112. doi:10.1007/978-1-4615-1247-9_8

Schilling, M. W., A. J. Pham, J. B. Williams, Y. L. Xiong, N. Dhowlaghar, A. C. Tolentino, and S. Kin. 2018. Changes in the physiochemical, microbial, and sensory characteristics of fresh pork sausage containing rosemary and green tea extracts during retail display. Meat Sci. 143:199–209. doi:10.1016/j.meatsci.2018.05.009

Sebranek, J. G., V. J. Sewalt, K. L. Robbins, and T. A. Houser. 2005. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat Sci. 69:289–296. doi:10.1016/j.meatsci.2004.07.010

Srinivasan, S., Y. L. Xiong, P. Blanchard, and J. H. Tidwell. 1997. Physicochemical changes in prawns (*Machrobrachium rosenbergii*) subjected to multiple freeze-thaw cycles. J. Food Sci. 62:123–127. doi:10.1111/j.1365-2621.1997.tb04381.x

van den Dool, H., and P. D. Kratz. 1963. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. J. Chromatogr. A 11:463–471. doi:10.1016/S0021-9673(01)80947-X

Varlet, V., C. Prost, and T. Serot. 2007. Volatile aldehydes in smoked fish: Analysis methods, occurence and mechanisms of formation. Food Chem. 105:1536–1556. doi:10.1016/j.foodchem.2007.03.041

Xia, X., B. Kong, Q. Liu, and J. Liu. 2009. Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze-thaw cycles. Meat Sci. 83:239–245. doi:10.1016/j.meatsci.2009.05.003

Xie, J., B. Sun, F. Zheng, and S. Wang. 2008. Volatile flavor constituents in roasted pork of mini-pig. Food Chem. 109:506–514. doi:10.1016/j.foodchem.2007.12.074

Xiong, Y. L. 2017. The storage and preservation of meat: I – Thermal technologies. Chapter 7. In: F. Toldra, editor, Lawrie's meat science. Elsevier/Woodhead Publishing, Duxford, UK.

United States Code of Federal Regulations. 2015. Code of federal regulations. Title 9, Part 319, Section 319.142, Part 424, Section 424.21. https://www.gpo.gov/fdsys/granule/CFR-2012-title9-vol2/CFR-2012-title9-vol2-sec319-141 (accessed 4 Mar. 2019).

Yoo, S. S., S. H. Kook, K. B. Chin, and J. H. Shim. 2005. The comparison of physico-chemical and textural properties, as well as volatile compounds, from low-fat and regular-fat sausages. Int. J. Food Sci. Technol. 40:83–90. doi:10.1111/j.1365-2621.2004.00911.x