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# Effects of Rosemary (*Rosmarinus Officinalis L.*) and Green Tea (*Camellia Sinensis L.*) Extracts on Sensory Properties and Shelf-Life of Fresh Pork Sausage during Long-Term Frozen Storage and Subsequent Retail Display

M. W. Schilling[1]*, A.J. Pham[1], N. Dhowlaghar[1], Y. L. Campbell[1], J.B. Williams[1], Y.L. Xiong[2], Saxon M. Perez[1], and S. Kin[1]

[1]Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS, USA
[2]Department of Animal and Food Sciences, University of Kentucky, Lexington, KY, USA
*Corresponding author. E-mail: schilling@foodscience.msstate.edu (M. W. Schiling)

**Abstract:** The quality attributes of whole hog fresh pork sausages (28% fat) formulated with combinations of rosemary extract (R: 1500, 2000, 2500 ppm), green tea extract (G: 100, 200, 300 ppm), and synthetic antioxidants (approximately 0.02% BHA, BHT, and propyl gallate based on fat %) were evaluated after 0.5, 3, and 6 mo at –20°C followed by simulated retail display (3 ± 1°C) for up to 21 d. R and G delayed lipid oxidation across all frozen storage periods. The TBARS were reduced ($P < 0.05$) in treatments with higher amounts of R compared with the control (synthetic antioxidants only) after 6 mo of frozen storage. Volatile lipid oxidation products, including 2,4-decadienal were less abundant ($P < 0.05$) in sausages with higher concentrations of R following 3 mo of frozen storage. Greater concentrations of combined R and G extracts resulted in increased ($P < 0.05$) redness and yellowness and decreased ($P < 0.05$) lightness after 6 mo of storage. Higher concentrations of R enhanced ($P < 0.05$) chroma and slowed ($P < 0.05$) discoloration throughout 14 d of retail display after 3 and 6 mo of storage. Addition of R and G resulted in greater ($P < 0.05$) consumer acceptability than the control, which displayed spoilage and detectable rancidity by d 7 following 6 mo of storage in comparison to approximately 14 d for treatments with G and R. Pork, nutmeg, ginger, spice complex, and rosemary aromas were highest ($P < 0.05$) in sausages with increased concentrations of R following 0.5 and 3 mo of storage. Increasing green tea concentration reduced the intensities of ginger, copper-herbal, rancid, off-flavor and off-odor descriptors ($P < 0.05$). In conclusion, the addition of R and G enhanced the shelf-life of fresh pork sausages throughout 6 mo of frozen storage and helped maintain their quality during retail display for up to 14 d.

**Keywords:** fresh pork sausage, frozen storage, green tea, rosemary, sensory analysis
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## Introduction

Freezing is often utilized in the processing, distribution, and storage of comminuted meat products as a reliable method for the preservation of meats and for prolonging product shelf-life. The rates of chemical and biological reactions are significantly diminished due to the reduced temperature and lessened mobility of reactants (Erickson, 1997). However, quality deterioration still occurs such as cell wall puncturing and texture modification due to the formation of ice crystals, and increased concentration and precipitation of soluble components (Leygonie et al., 2012). All of these can contribute to oxidative reactions of lipids and proteins that may affect the keeping quality of the product and hence the loss of quality and acceptability. This in turn can lead to decreased eating quality such as flavor deterioration, discoloration, reduced tenderness and juiciness, and nutritional losses (Kanner, 1994; Xiong, 2000). Another consequence of lipid oxidation is the formation of secondary aldehyde products which may alter heme chemistry and initiate pigment oxidation, which causes decreased stability of

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oxymyoglobin and loss of desirable color. These oxidative processes have also been associated with changes in protein structure and functionality of stored meats by causing cross-linking between proteins and lipid oxidation products (Howell et al., 2001; Xia et al., 2009).

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), ascorbyl palmitate, and tertiary-butyl hydroquinone (TBHQ) have been commonly used in the meat industry as effective additives to retard the development of rancidity in meat products (Hazen, 2005). However, there is growing concern among consumers regarding the potential health risks of such chemical additives, which has resulted in their strict regulation in food applications (Brewer, 2011; Shahidi and Ambigaipalan, 2015). Natural plant extracts have also been reported to possess antioxidant properties comparable with or greater than synthetic antioxidants (Schilling et al., 2018; Berdahl et al., 2010). The antioxidant properties of these plant extracts are dedicated to a combination of active components, which could act individually as well as in synergy to more effectively inhibit lipid oxidation in meats especially when extended frozen storage is needed.

Rosemary (*Rosmarinus officinalis L.*) and green tea (*Camellia sinensis L.*) extracts are 2 of the most widely accepted groups of natural antioxidants and flavoring agents in formulated meat products (Berdahl and McKeague, 2015; Karaosmanoglu and Kilmartin, 2015). However, their interactive effect has not been demonstrated in a commercial processing setting. Both plant-derived ingredients are rich in a variety of phenolic compounds that have been shown to inactivate oxidative chemical substances and break free radical chain reactions through hydrogen donation or metal chelation mechanisms (Berdahl and McKeague, 2015). These phenolic compounds could exhibit synergistic interactions among themselves and with metal chelating agents such as EDTA, citric acid, flavonoids, amino acids and peptides, Maillard reaction products, and phospholipids and thus potentially increase their antioxidant effectiveness (Tang et al., 2001). To date, limited studies have yet been conducted to explore the effectiveness of these natural plant extracts in conjunction with synthetic antioxidants for maintaining acceptable quality characteristics of pork sausages under commercial settings for long term frozen storage conditions. Therefore, the objective of this study was to evaluate the effectiveness of antioxidant combinations on the oxidative stability, microbial growth, and physicochemical and sensory properties of fresh pork sausages formulated with combinations of rosemary and green tea extracts with synthetic antioxidants during frozen storage (–20°C) for 0.5, 3, and 6 mo, followed by simulated retail display (3 ± 1°C, 800 lux).

## Materials and Methods

### *Manufacture of fresh pork sausage*

Raw well-chilled (1 ± 1°C) ground whole-hog pre-rigor meat (30 to 45 min postmortem, containing 1.5% salt to delay rigor onset) was supplied by a commercial pork processing plant, and made into sausages within 7 d of slaughter per manufacturer suggestion. The meat was stored in a walk-in cooler (1 ± 1°C) and processed within the next 24 h. Synthetic antioxidants were included in all treatments, which consisted of a proprietary combination of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) at approximately 0.02% based on fat composition, which is within the legal limits described by the USDA (9 C.F.R. §319.141, 9 C.F.R. §424.21; Code of Federal Regulations, 2015). A total of 30 (10 treatments × 3 replications) 36.3-kg batches of fresh pork sausage were manufactured in the study. Ten batches of fresh pork sausage were formulated and randomly assigned to one of the following treatment combinations (Table 1) that also contained the synthetic antioxidants described above: 1500 ppm rosemary extract (*R*) (FORTIUM Brand, Kemin Food Technologies Inc., Des Moines, IA) + 100 ppm green tea (G) (FORTIUM Brand, Kemin Food Technologies Inc.), (1500 R + 100 G), R1500 + G200, R1500 + G300, R2000 + G100, R2000 + G200, R2000 + G300, R2500 + G100, R2500 + G200, R2500 + G300, and control (synthetic antioxidants in reps 1 and 2, R1500 and synthetic antioxidants in rep 3 since it represented commercial formulations at the time that rep 3 was conducted). Each batch of pre-rigor meat with the addition of the spice blend, corn syrup solids, chilled water, synthetic and an antioxidant combination were blended for 3 min in a paddle mixer (Model 150; Butcher Boy Limited, Ayshire, Scotland, UK) and stored in a walk-in cooler (1 ± 1°C) for 48 h. This was followed by grinding with a commercial mixer-grinder (Model 80055; Hollymatic Co., Countryside, IL) equipped with a 4 mm-hole diameter plate. Aliquots of ground meat were collected prior to stuffing 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). Fat, moisture, and protein contents were 28% ± 0.70, 53% ± 0.56, and 14% ± 0.36, respectively for the 3 replications (data not shown).

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

| Treatment | Rosemary extract (ppm) | Green tea extract (ppm) | Synthetic Antioxidants (Combination of BHA, BHT, and PG) |
|---|---|---|---|
| R1500+G100 | 1,500 | 100 | Approximately 0.02% based on fat % |
| R1500+G200 | 1,500 | 200 | Approximately 0.02% based on fat % |
| R1500+G300 | 1,500 | 300 | Approximately 0.02% based on fat % |
| R2000+G100 | 2,000 | 100 | Approximately 0.02% based on fat % |
| R2000+G200 | 2,000 | 200 | Approximately 0.02% based on fat % |
| R2000+G300 | 2,000 | 300 | Approximately 0.02% based on fat % |
| R2500+G100 | 2,500 | 100 | Approximately 0.02% based on fat % |
| R2500+G200 | 2,500 | 200 | Approximately 0.02% based on fat % |
| R2500+G300 | 2,500 | 300 | Approximately 0.02% based on fat % |
| CONTROL | | | |
| Replication 1 | 0 | 0 | Approximately 0.02% based on fat % |
| Replication 2 | 0 | 0 | Approximately 0.02% based on fat % |
| Replication 3 | 1,500 | 0 | Approximately 0.02% based on fat % |

[1]BHA = Butylated hydroxyanisole; BHT = Butylated hydroxytoluene; PG = propyl gallate.

Before filling, the natural casings were rinsed to remove salt, treated for tenderization (proprietary treatment) and kept hydrated in warm water (40 ± 1°C) until stuffed with the sausage blends. Total plate counts (TPC) were determined for the untreated and treated casings using 3M Petrifilm (3M Global Headquarters, St. Paul, MN). Casings that were not treated contained 3.3 to 4.1 log cfu/g and treated casings contained between 1.4 and 2.6 log cfu/g. The ground meat was stuffed into the casing with a filling machine (Model RS1040C; Risco Vacuum Stuffer, Thiene, Italy) and hand-linked at 12.7 ± 1-cm intervals with a diameter of approximately 34 ± 1 mm (6-Inch Dial Caliper, Pittsburgh-Item 66541; Cen-Tech, Harbor Freight Tools, Camarillo, CA).

## Packaging and storage of sausage

Fresh pork sausages (5 links per tray, 300 links per treatment) were packaged in expanded polystyrene trays (20S Yellow Tray; Cascades Plastics Inc., Warrenton, MO) and overwrapped with PVC stretch film with an oxygen permeability of 121 cc/100cm$^2$ per d and water vapor permeability of 2.2 g/100cm$^2$ per d (Premium-LT 80 718; LINPAC Packaging-Filmco Inc., Aurora, OH), and stored in an air-blast freezer overnight at –30°C. The frozen pork sausages were then stored in a –20°C walk-in freezer (Model TK-3476-WF-L, K Thermo-Kool, Mid-South Industries Inc., Laurel, MS) for 0.5, 3, and 6 mos. Packages (16 to 20 per treatment) were randomly selected and arranged in a walk-in cooler (3 ± 1°C, Model TK-3476-WF-L; K Thermo-Kool, Mid-South Industries Inc., Laurel, MS) where all treatments were subjected to continuous cool white fluorescent illumination (Cool White 34 Watt; Sylvania Supersaver Ecologic, Danvers, MA) of 800 lux at the package surface for 21 d. Samples were evaluated for instrumental color, lipid oxidation, and sensory descriptive analysis after 0, 7, 14, and 21 d. Consumer acceptability was performed on d 8. Packages for psychrotrophic plate counts (PPC), pH, and proximate analysis were vacuum packaged with 3-mil standard barrier vacuum pouches (Prime Source 75001826, 0.75 gauge nylon/2.25 gauge polyethylene, OTR = 0.6 cm$^3$/100m$^2$ per d at 0°C, MVTR = 0.6g/100m$^2$ per d at 38°C, and 100% relative humidity; Rebel Butcher Supply Company, Flowood, MS) and frozen at –72°C in an ultra-cold freezer (Model, Lo-Cold Freezer, ScienTemp Corp, Adrian, MI) until subsequent analyses could be conducted.

## Fresh pork sausage characterization

***Instrumental color and pH analysis.*** Meat color measurements CIE (commission Internationale de l'EClairage) lightness ($L^*$), redness ($+a^*$), yellowness ($+b^*$), chroma (saturation index), and hue angle (discoloration) were determined for the external and internal surfaces of fresh pork sausages using a Chroma Meter (Model, CR-400; Serial No. C8202489; Minolta Camera Co. Ltd., Osaka, Japan) with a 32 mm diameter measurement area, a $D_{65}$ illuminant, and a 2° standard observer angle (American Meat Science Association, 2012). The color measurements were performed on fresh pork sausages 15 min after opening the package at room temperature (20 ± 2°C). Colorimetric values were obtained for the external surfaces of fresh pork sausages on d 0, 7, 14, and 21 of simulated retail display conditions by averaging 9 readings, 3 per sausage link per treatment for each replication. The chroma meter was standardized with a white Minolta calibration plate (CR-A43, No. 18433006; *CIE* L* = 97.81, a* = –0.11, b* = 2.00). For pH determination, 10 g of meat was homogenized with 100 mL of distilled water for 30 s using a Model 700S Waring Blender (Waring Products, Torrington, CT). The pH was measured with a pH Meter (Accumet Research Model AR25; Fisher Scientific, Pittsburgh, PA) that was equipped with a standardized combination glass electrode (Accumet Research ATC Probe; Accumet, Singapore). Two links were analyzed in duplicate for each treatment.

***Lipid oxidation analysis.*** Lipid oxidation was evaluated by determining 2-thiobartituric acid reactive substances (TBARS) based on the procedure of O'Keefe and Wang (2006). Two links were mixed in a blender (Model 700S, Waring Products, Torrington, CT) to generate triplicate samples for analysis. Results were expressed as mg of malondialdehyde equivalent per kg of fresh pork sausage (mg MDA equivalent/kg fresh pork sausage). The extent of lipid oxidation was further monitored by the gas chromatographic (GC) analysis for 2,4-decadienal and hexanal using a method described by Pham et al. (2008) for the detection, identification, and quantification of volatile flavor compounds.

***Psychrotrophic plate counts (PPC).*** The PPC were measured for fresh pork sausages that were stored for 0, 7, 14, and 21 d under simulated retail display at 0.5, 3, and 6 mos of frozen storage. Two links were aseptically removed from the tray, cut into small pieces to weigh 25-g and be placed in a sterile sample bag (52-oz. Whirl-Pak Bags; 7" × 11", Nasco, Fort Atkinson, WI) with 225 mL of 0.1% sterilized peptone solution (Difco Bacto-Peptone, Fisher Scientific, Pittsburgh, PA), followed by homogenizing for 1 min in a Stomacher Laboratory Blender (Model 400; A. J. Seward and Co. Ltd., London, England). Duplicate samples were taken from each dilution by plating on 3M petrifilm. Aerobic Count Plates (3M Global headquarters, St. Paul, MN) and incubating at 20°C for 72 h.

***Proximate analysis.*** Percentages of fat, moisture, and protein of fresh pork sausage were determined according to AOAC Official Method 2007.04 using a FOSS FoodScan Meat Analyzer Near-Infrared (NIR) Spectrophotometer (Model 78810; Foss Co., Hillerød, Denmark).

***Sensory evaluation.*** Sausage links were cooked over medium-high heat (Viking Professional 60" Custom Sealed Burner Range; Viking Range Corporation, Greenwood, MS) to an internal temperature of 75 ± 2°C in a nonstick skillet (Farberware 10.5 in Covered Fry Pan; Farberware Licensing Company LLC, Needham, MA). The cooked samples were wrapped in Reynolds extra heavy duty foil bags (Alcoa Consumer Products, Alcoa Inc., Richmond, VA), equilibrated for 15 min, sliced into 1.8-cm thick pieces and kept warm in 8-quart chafer dishes (Model ROCK-7, Rockwell Chafer Dish; Admiral Craft Equipment Corp., Hicksville, NY) until sensory analyses were conducted.

A total of 63 descriptive analysis sessions were performed for 3 replications where 9 treatments were evaluated after 0, 7, 14, and 21 d of retail display following 0.5, 3, and 6 mo of frozen storage. Freshly cooked pork sausages were evaluated by a 10-member descriptive panel, with greater than 100 h of experience pertaining to the evaluation of meat products (Civille and Carr, 2015). The panelists participated in 8 one-hour training sessions and 4 refresher sessions between replications of panel evaluations to recalibrate the panelists with the descriptive terms. Previously identified descriptors (Civille and Lyon, 1996) and terms generated during training were utilized for the sensory evaluation of pork sausage. The samples were evaluated for aroma ("aroma intensity", "pork complex", "spice complex", "ginger", "nutmeg", "rosemary", "copper-herbal", "browned", "caramelized", "liver-organy", "rancid", "fruity", and "off-odor"), basic tastes ("sweet", "sour", "bitter", "salty", and "savory"), flavor ("flavor intensity", "pork complex", "fat", "lean", "spice complex", "ginger"," nutmeg", "black pepper", "rosemary", "copper herbal", "browned", "caramelized", "liver-organy", "metallic", "rancid", "fruity", and "off-flavor"), feeling factors ("astringency" and "oily"), and texture attributes ("hardness", "chewiness", and "juiciness"). The descriptors were rated using a 15-cm line scale where 0 = not detected and 15 = extremely strong with respect to the sensory attributes. Panels were conducted in individual booths within a controlled room environments. Upon serving, sausage samples were placed in 2-oz. plastic containers with lids (Sweetheart Cup Co., Owing Mills, MD) that were coded with 3-digit random numbers. Each panelist received 2 pieces (1 piece was used to evaluate aroma and basic tastes and the other piece was used for flavor and textural attributes) of each treatment for every session (4 to 5 treatments per sensory session). A factorial rotation was applied for the order of presentation of the samples to consider the effect of rank. 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 sample evaluations.

***Consumer acceptability.*** To evaluate the acceptability of pork sausage with different natural plant extract combinations, nine consumer-based sensory panels ($n$ = 60 to 65 panelists per panel) were conducted on d 8 of storage. Only consumers who regularly eat sausage and reported no food allergies to the listed ingredients were invited to participate in the panel. Representative treatment combinations were selected as it was not feasible to evaluate all treatments at the risk of panelist fatigue. Panelists evaluated seven pork sausage samples in individual booths, under red lighting to eliminate visual difference. Each panelist received 7 samples (1 per treatment) in 2-oz. plastic containers with 3-digit random numbers. The conditions for the preparation of these samples were identical with those of the trained descriptive panel. Samples were held in the chafer dish for no more than 30 min. Panelists were provided with water, unsalted crackers, apple juice, and expectorant cups to remove residual flavors between sample evaluations. Panelists were asked to evaluate each sausage sample based on overall acceptability and the acceptability of flavor, aroma, texture, and appearance using a 9-point hedonic scale (Civille and Carr, 2015).

***Experimental design and statistical analysis.*** A randomized complete block design with a factorial arrangement was utilized to investigate the effects of rosemary and green tea extract concentration on the TBARS values, volatile decomposition products concentrations, color, psychrotrophic plate counts, and descriptive sensory attributes of fresh pork sausage over 3 different times of frozen storage (0.5, 3, and 6 mo) and four sampling days for simulated retail display (0, 7, 14, and 21 d). The data obtained for each treatment combination were submitted to a regression analysis in a second/third order polynomial equation composed of linear/main, quadratic and interaction effects for the independent variables studied. The main effects were the levels of rosemary extract ($R$), green tea extract (G), and the retail display time of products. Retail display times (0, 7, 14, and 21 d) were included in the regression model as covariate main effects to explain as much variability about the responses of interest as possible. The regression model was as follows:

$$\begin{aligned} Y = {} & \beta_0 + \beta_1(G) + \beta_2(R) + \beta_3(G*R) + \beta_4(Day) + \\ & \beta_5(G*Day) + \beta_6(R*Day) + \beta_7(Day*Day) + \\ & \beta_8(G*Day*Day) + \beta_9(R*Day*Day) + \in \end{aligned}$$

where Y is the response variable; $\beta_0$ is the constant coefficient (intercept); are the linear coefficients (main effects); is the quadratic coefficient; is the two-factor interaction coefficient, and is the random error.

The PROC GLIMMIX procedure of SAS (Statistical Analysis Software, Version 9.3, SAS Institute Inc., Cary, NC) was used to determine statistical differences among the fixed effects and their interactions ($P < 0.05$). The Student's $t$ test was used for the determination of the individual/fixed effects for each of the coefficient estimates. The nonsignificant terms ($P > 0.05$) were withdrawn from the model, and a new adjustment was made so that only significant ($P < 0.05$) term/variables were included in the fitted model/final regression model. For Fig. 1 through 7, only significant variables were included in the model. Ten treatments were included when both green tea extract and rosemary extract concentration had a significant effect ($P < 0.05$) such as with TBARS. Only 4 treatments were included for CIE a* in Fig. 2, since rosemary was significant ($P > 0.05$) and green tea was not significant ($P > 0.05$). Only 6 of the 10 treatments were evaluated for pH and PPC, so there are either 6 treatments or 4 treatments included in Fig. 4 and 5. For descriptive sensory analysis, the control treatment was not evaluated so it is not included in Fig. 5 and 6.

A randomized complete block design (replications ($n = 3$) and panelists as blocks) was used to determine the average difference ($P < 0.05$) in overall acceptability and the acceptability of aroma, appearance, flavor, and texture of natural plant extract combinations in pork sausages. When significant differences occurred among treatments, Duncan's Multiple Range Test (Statistical Analysis Software, Version 9.3, SAS Institute Inc., Cary, NC) was utilized to separate treatment means. Consumers were clustered together based on their liking for pork sausage by agglomerative hierarchical clustering (XLSTAT-Pro, Addinsoft USA, New York, NY) using the Euclidian distance and Ward's method as aggregation criterion (Pham et al., 2008)

## Results and Discussion

### *Lipid oxidation*

A quadratic relationship existed between storage time and TBARS throughout simulated retail display following 0.5 and 3 mo of frozen storage, where the TBARS values for all treatments increased from d 0 to 7 and then decreased from d 14 to 21 (Fig. 1A and B). A linear relationship was observed after 6 mo of frozen storage where maximum values for TBARS were reached on d 0, after which a decline was observed (Fig. 1C). In addition, both rosemary and green tea extracts reduced TBARS values ($P < 0.05$), particularly in treatment combinations with higher concentrations of rosemary extract for up to 6 mo of storage at –20°C (Fig. 1C). The lowest ($P < 0.05$) TBARS values of the sausages were observed when treatment combinations with 2500 ppm of rose-

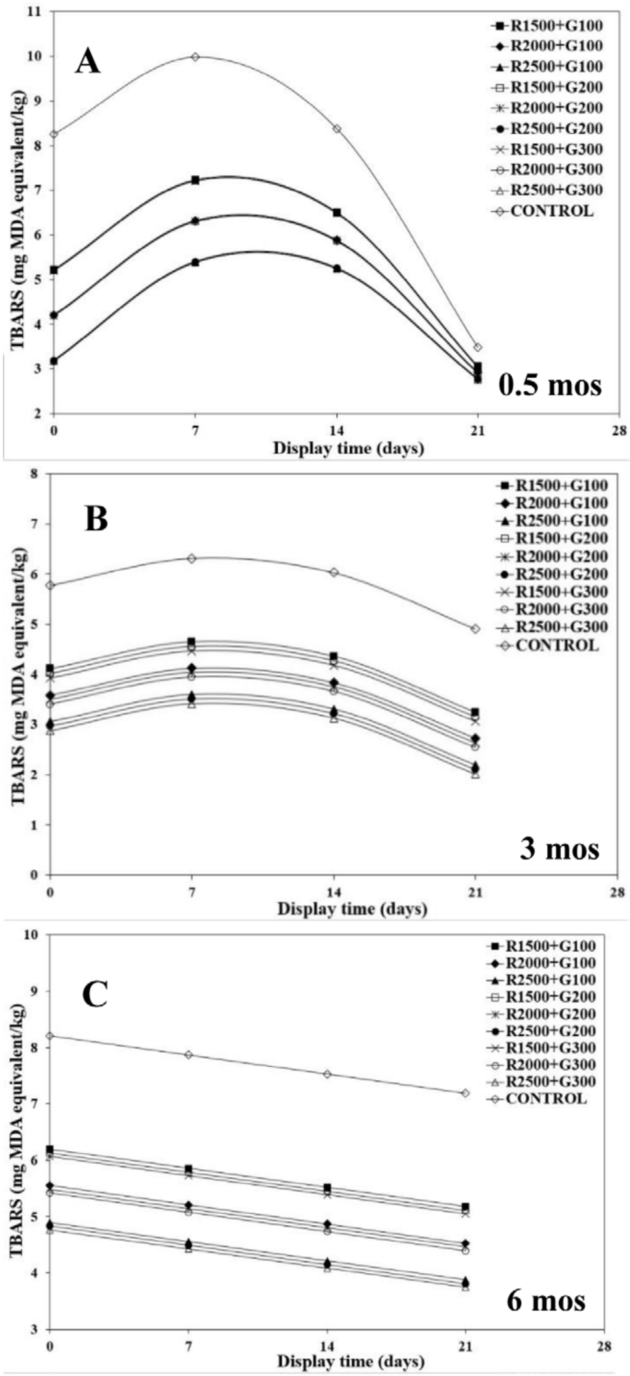


**Figure 1.** Effect of combinations of rosemary (*R*) and green tea (G) extracts on the thiobarbituric acid reactive substances (TBARS) values (mg MDA equivalent/kg fresh pork sausage) of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following frozen storage (–20°C).

mary extract were added while the highest ($P < 0.05$) TBARS values were obtained for the control. Treatment combinations with 2500 ppm of rosemary extract also provided significant reduction in lipid oxidation ($P < 0.05$) which ranged from 20 to 62%, 43 to 59%, and 40 to 48% for 0.5, 3, and 6 mo, respectively (Fig. 1).

Major volatile decomposition products are regarded as good indicators of the oxidative state of meat lipids such as 2,4-decadienal which is one of the main volatiles derived from the oxidation of oleic and linoleic acid (Wood et al., 2008). On the basis of 2,4-decadienal formation, the order of concentration was control > R1500 > R2000 > R2500 following 3 mo of frozen storage and throughout 21 d of retail display (Table 3). Hexanal concentration increased over refrigerated storage time but did not differ between antioxidant treatments (data not shown).

Lipid oxidation was negatively correlated with natural plant extract concentration, a finding in agreement with other studies that reported higher levels of rosemary or green tea extracts aid in preventing lipid oxidation. Özyurt et al. (2010) reported that the addition of rosemary extract (300 ppm) inhibited the formation of TBARS in fried sea bream fillets over a 4-mo frozen storage (–18°C) period. Similar results were obtained by Sebranek et al. (2005) who revealed that sausage patties containing 2,500 ppm rosemary extract and stored at –20°C had significantly lower TBARS values over the 4 mo sampling period when compared to the control. A recent study by Perlo et al. (2018) indicated that rosemary extract sprayed at 4,000 ppm on 2.54 cm thick pork *longissimus* chops resulted in an effective delay of lipid oxidation without affecting pH, color or water content stored at MAP conditions at 4°C for 45 d. Jongberg et al. (2018) recently reported that there was a dose-dependent concentration of green tea extract (25 to 160 ppm) that lowered TBARS values in brine-injected pork chops stored in high-oxygen atmosphere packaging over 7 d storage (5°C).

The efficacy of the phenolic diterpenes in rosemary extract such as carnosic acid and carnosol is presumably due to the greater retention of these lipophilic antioxidants in the fat globule-protein interface (Berdahl and McKeague, 2015). The incorporation of a proprietary carrier system transports it to the interface where it increases the interfacial concentrations. This results in greater association with the interfacial protein film and thus its ability to scavenge free radicals produced from the decomposition of interfacial lipid hydroperoxides, which leads to increased oxidative stability especially in sausages with higher concentrations of rosemary extract. The polar and hydrophilic green tea catechin gallates or epicatechins protect the continuous phase from lipid oxidation. The antioxidative effect of added tea catechins in meats is related to chelating free iron ions which are major lipid oxidation catalysts in muscle foods (Tang et al., 2001). These catechins are also known to trap superoxide, hydrox-

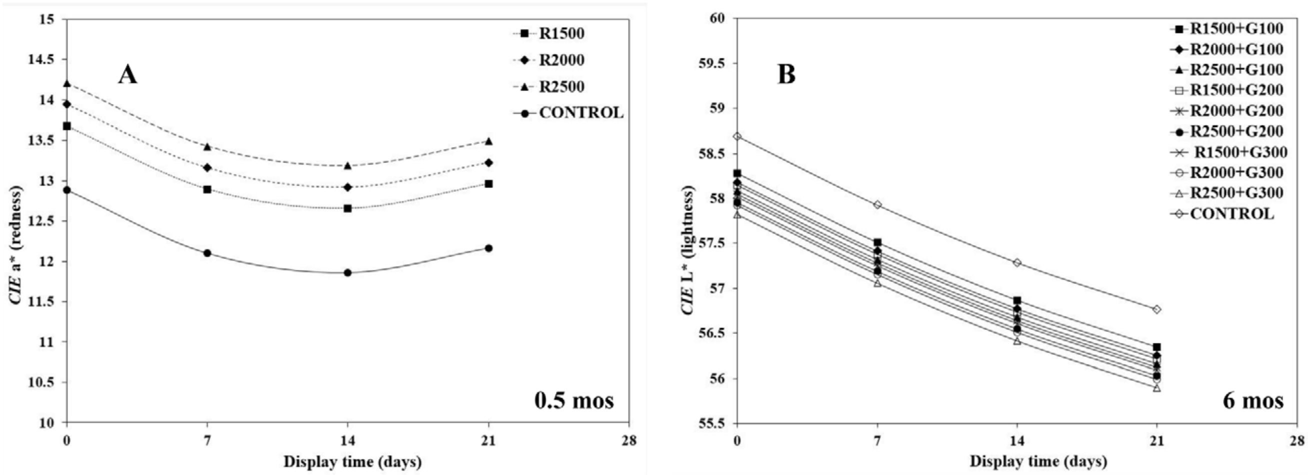


**Figure 2.** Effect of combinations of rosemary (*R*) and green tea (G) extracts on the *CIE* a* (redness) values of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following frozen storage (–20°C).

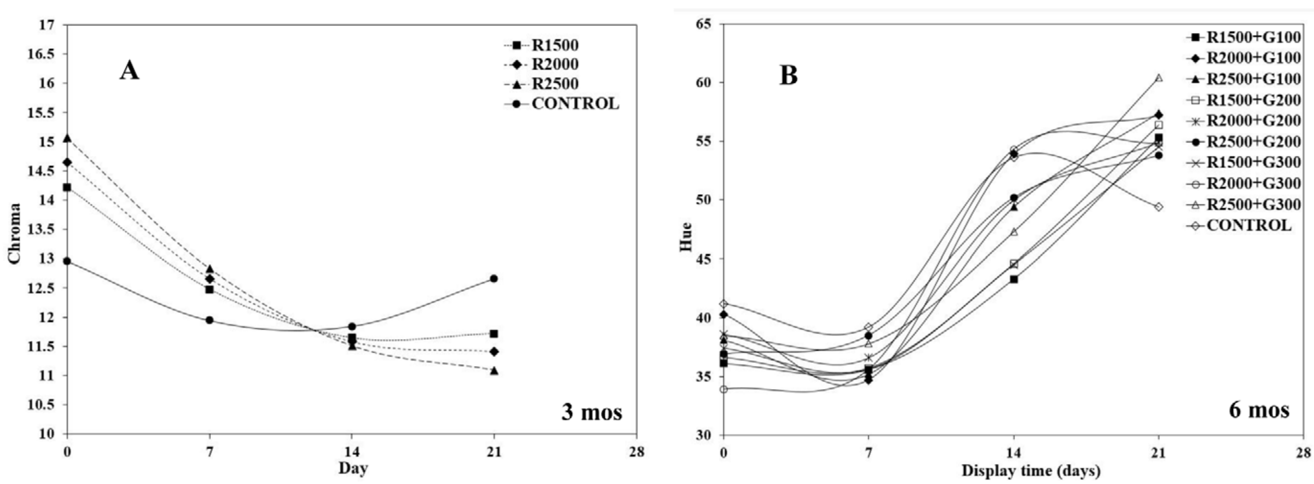


**Figure 3.** Effect of combinations of rosemary (*R*) and green tea (G) extracts on the chroma (saturation index) and hue (discoloration) values of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following frozen storage (–20°C).

yl, and peroxyl radicals and to end free-radical chain reactions in the continuous phase thereby preventing lipid oxidation in meat products (Vuong et al., 2011).

## Color

The *CIE* L* (lightness) values were higher ($P < 0.05$) for the control followed by samples containing lower concentrations of rosemary extract during 0.5 and 3 mo of frozen storage (Tables 2 and 3). The models for fresh pork sausage also demonstrate a gradual decline in the lightness values across all treatments as retail display time progressed. The *CIE* a* values (redness) were higher in fresh pork sausages with increased ($P < 0.05$) levels of rosemary extract compared with the control after 0.5 mo of frozen storage (Fig. 2A, Table 2). Green tea extract was a significant variable following 6 mo of frozen storage where redness values were higher for samples with 300 ppm of green tea extract especially on d 7 and 14 when compared to the other treatments ($P < 0.05$; Fig. 2B, Table 4). The *CIE* a* values decreased ($P < 0.05$) as frozen storage time progressed regardless of natural plant extract addition. However, it declined more rapidly in the control samples. Also noteworthy was the color reversion from brown to red across all sausage treatments during the last stages of retail display. This trend was accompanied by a gradual decline in the redness values as refrigerated storage progressed followed by an increase on d 21 as the storage period ended. This could be attributed to metmyoglobin reduction by aerobic bacterial proliferation where the release of metabolic reductants such as pyridine nucleotides [i.e., NAD(P)H] may have converted metmyoglobin to its reduced myoglobin forms in the presence of met-

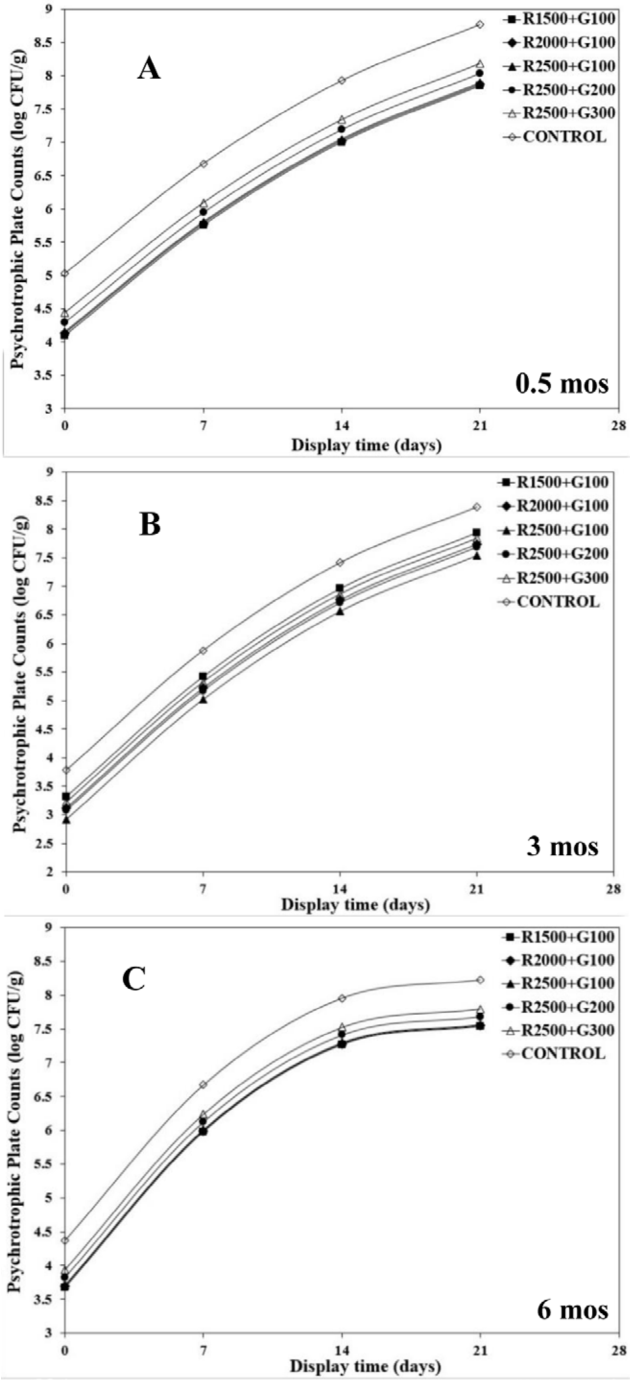


**Figure 4.** Effect of combinations of rosemary (*R*) and green tea (G) extracts on the psychrotrophic plate counts (PPC, $\log_{10}$ CFU/g) of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following frozen storage (–20°C).

myoglobin reductase (Renerre, 2000). The highest chroma (saturation index) values at 3 mo of frozen storage were found in samples containing 2,500 ppm of rosemary extract and the lowest were recorded in the control (Fig. 3A, Table 3). The trend for the chroma values was similar to that for redness and yellowness values as retail display progressed. Surface hue angles (discoloration) were lower ($P < 0.05$) for samples with higher concentrations of rosemary extract compared with the control after 6 mo of frozen storage (Fig. 3B, Table 4).

The best synergistic effect was obtained from treatment combinations with higher concentrations of rosemary extract which exhibited better color retention as well as an improvement in color relative to the control samples. Similar observations have been made by Sebranek et al. (2005) who demonstrated that rosemary extract added at a level of 2,500 ppm in pork sausage patties was more effective in maintaining color than the control and BHA/BHT treatment after 3 mo of frozen storage. Green tea extract (600 ppm) improved the red color of omega-3 fortified surimi gels at the beginning of storage; however, no significant changes during frozen storage at –18°C for 9 mo were observed (Pérez-Mateos et al., 2006). In contrast, in the current study, green tea extract addition positively affected redness values following 6 mo of frozen storage. The variation in the efficiency of green tea extract between different studies could be related to differences in the amount of polyphenols in the extract, the meat species studied, the synergistic effects brought about by the addition of other ingredients, or the oxidation pattern of oxymyoglobin under conditions of reduced enzymatic activity where storage temperature, packaging method, and light intensity are major influencing factors (Georgantelis et al., 2007).

## *Microbial growth and pH*

The models for fresh pork sausage microbial growth at each frozen storage period showed a quadratic relationship between PPC and storage time following 0.5, 3, and 6 mo of frozen storage where the PPC for all treatments increased from d 0 to 21. The lowest ($P < 0.05$) PPC of the sausages were observed when treatment combinations with 100 ppm of green tea extract were added while the highest ($P < 0.05$) PPC were obtained when the control (synthetic antioxidants only) was used. Also noteworthy were the higher counts of psychrotrophic bacteria at the beginning of the frozen storage period which ranged from 4.10 to 5.03 $\log_{10}$ CFU/g compared with 2.9 to 3.8 $\log_{10}$ CFU/g and 3.7 to 4.4 $\log_{10}$ CFU/g for 3 and 6 mo, respectively (Fig. 4ABC, Tables 3 and 4). After 14 d of storage, levels of psychrotrophic bacteria continued to increase for all treatments where the control was highest ($P < 0.05$) and ranged from 7.00 to 7.90 $\log_{10}$ CFU/g, 6.9 to 7.4 $\log_{10}$ CFU/g, and 7.3 to 8.0 $\log_{10}$ CFU/g for 0.5, 3, and 6 mo, respectively. PPC higher than 7 $\log_{10}$ CFU/g have been reported as threshold values for spoilage in

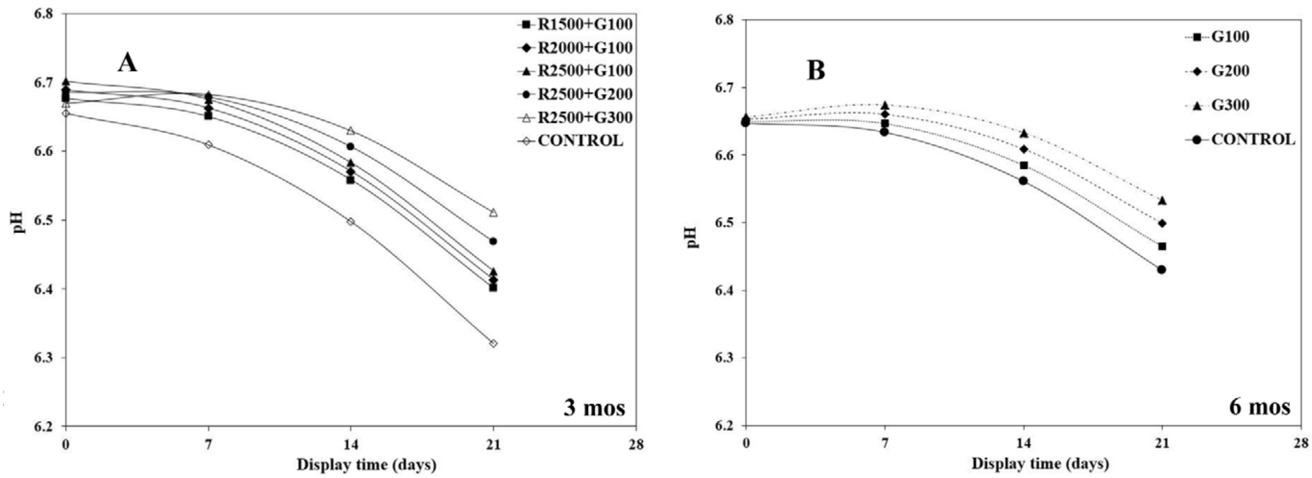


**Figure 5.** Effect of combinations of rosemary (*R*) and green tea (G) extracts on the pH values of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following frozen storage (–20°C).

meat, poultry, and seafood products (Jay et al., 2005). Perhaps the increased PPC could have been due to the presence of lactic acid bacteria (LAB) which indicates that the sausages may not be spoiled when PPC reaches 7 $\log_{10}$ CFU/g (Kin et al., 2011; Silva et al., 1995). In the present study, the co-antioxidative effect between the natural plant extracts and the synthetic antioxidants increased the microbial stability of the pork sausages allowing for frozen storage to last for up to 6 mos. Although microbial stability varied with natural plant extract combinations, it was evident that longer frozen storage was dependent on green tea extract concentration inhibiting psychrotrophic microbial growth. Our data show that natural plant extract combinations with 100 ppm of green tea extract resulted in lower PPC and were more effective in reducing microbial growth.

After 3 mos of frozen storage, the pH decreased ($P < 0.05$) as a function of storage time throughout 21 d of display (Fig. 5A, Table 3) and appear to coincide with an increase in PPC (Fig. 4B). Reduction in pH has been mostly associated with increased Gram-positive bacteria populations such as LAB or through lipolysis which liberates free fatty acids in the meat system. Fermentation is promoted by the microorganisms present, which results in the production of organic acids and a lower product pH (Papadima et al., 1999). This is also supported by the lower pH values of control samples, especially after 14 d ($P < 0.05$) of refrigerated storage (Fig. 5AB). Control samples also had the highest populations for psychrotrophic bacterial growth during the entire storage period (Fig. 4ABC). A similar effect on the pH of fresh pork sausages due to the addition of rosemary extract has been reported by Martínez et al. (2006).

## *Descriptive sensory analysis*

Following 0.5 mo of frozen storage, all treatments exhibited a decrease ($P < 0.05$) in intensity ratings for descriptors regarded as positive drivers of liking for this product such as “pork complex”, “spice complex”, “ginger”, “nutmeg”, “rosemary”, “browned”, “caramelized” aromas, “flavor intensity”, “fat”, “lean”, “spice complex”, “ginger”, “nutmeg”, “black pepper”, “caramelized”, “sweet”, “salty”, “savory” flavors and juicy texture with increased retail display time (Table 2). The data also showed that the sausages were mainly differentiated ($P < 0.05$) by “spice complex”, “nutmeg”, “rosemary” aromas, “fat, “bitter” flavors, and hardness and chewiness textural attributes following 3 mo of frozen storage (Table 3). The intensities of most of the sensory descriptive notes remained the same for all treatments within the same frozen storage period except for “pork complex”, “spice complex”, “ginger”, “nutmeg”, “rosemary”, “browned” aromas, “flavor intensity”, “fat”, “spice complex”, “ginger”, “nutmeg”, “black pepper”, “savory” flavors and “juicy” which decreased as retail display time increased (data not shown). The sausages were characterized ($P < 0.05$) by their “pork complex”, flavor intensity and oily following 6 mo of frozen storage (Table 4, data not shown for textural attributes). Also, the mean attribute intensity ratings of “pork complex”, “spice complex”, “ginger”, “nutmeg”, “rosemary”, “copper herbal”, “browned”, “caramelized” aromas, “flavor intensity”, “fat”, “lean”, “spice” “complex”, “nutmeg”, “black pepper”, “rosemary”, “caramelized”, “salty”, “savory” flavors, “astringent”, “oily” feeling factors and “juicy” texture gradually decreased ($P < 0.05$) over the 21 d display period (data not shown). In contrast, the mean attribute intensity ratings of “off-

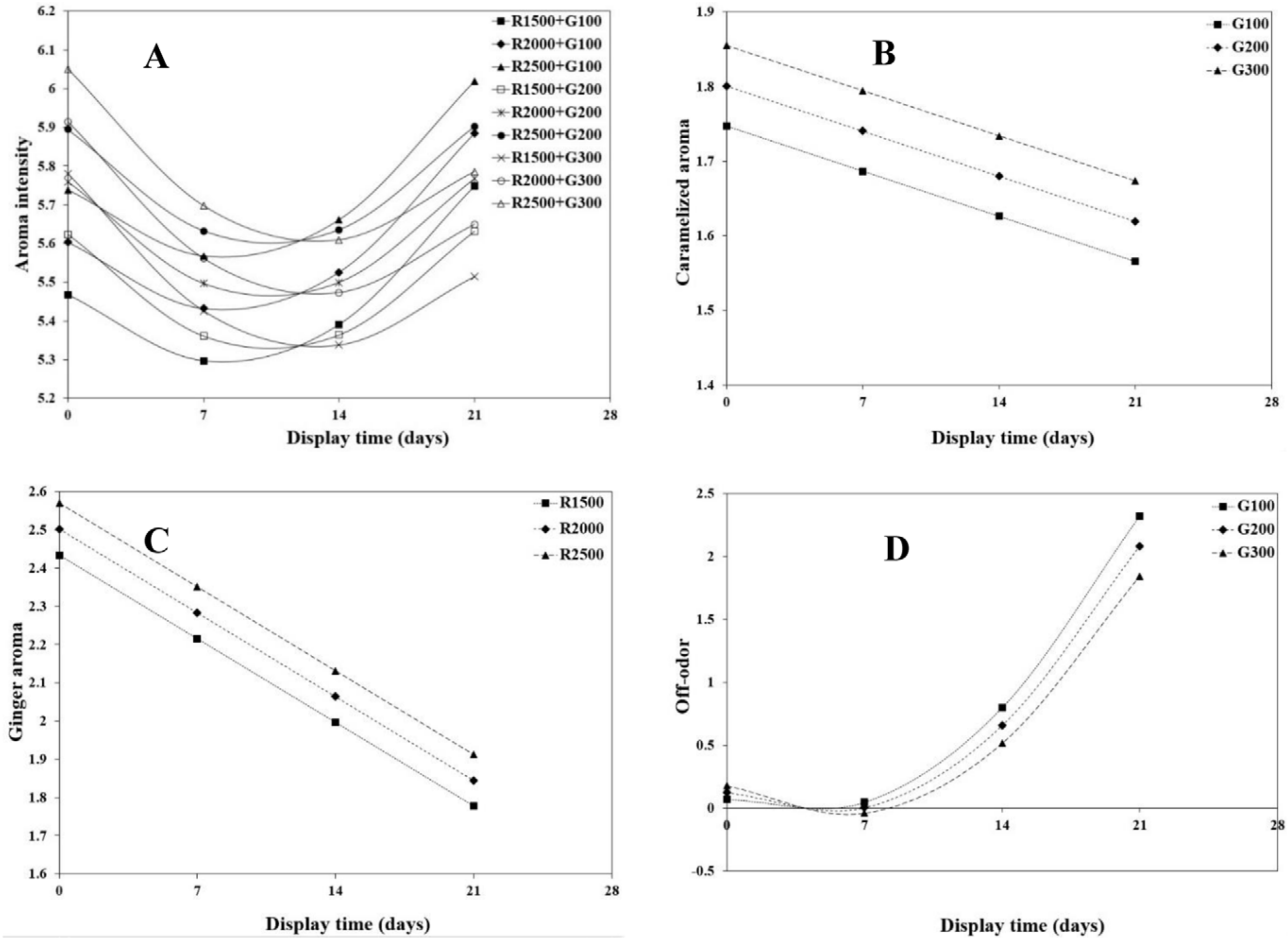


**Figure 6.** Effect of combinations of rosemary (*R*) and green tea (G) extracts on the attribute intensity ratings of Aroma intensity, Ginger, Caramelized, and Off-odor aroma attributes of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following 0.5 mo of frozen storage (–20°C).

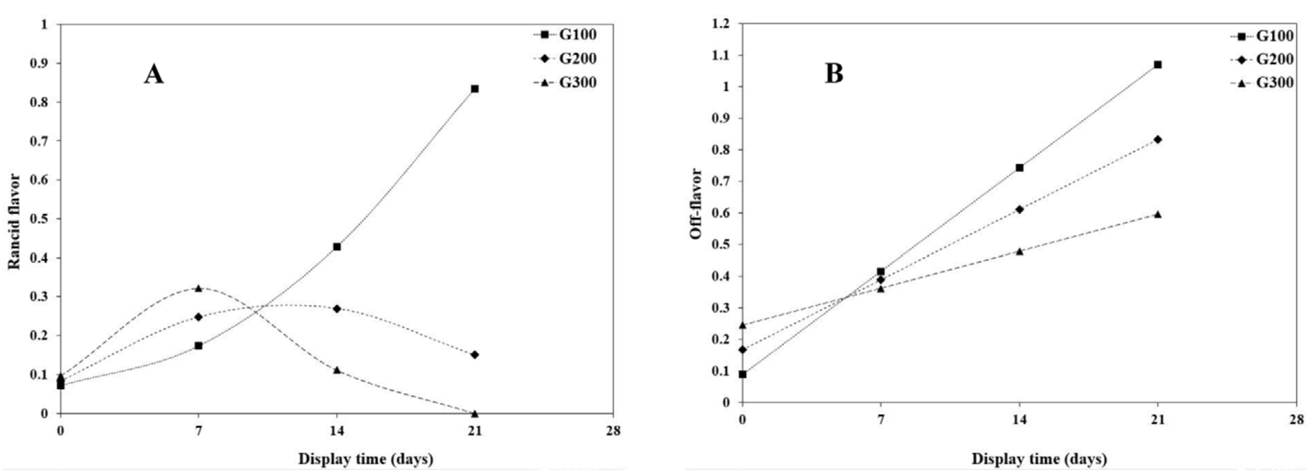


**Figure 7.** Effect of combinations of rosemary (*R*) and green tea (G) extracts on the on the attribute intensity ratings of Rancid and Off-flavor flavor attributes of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following 0.5 mo of frozen storage (–20°C).

odor", liver-organy, "rancid", "fruity" aromas, "off-flavor", "liver-organy", "metallic", "rancid", "fruity", "sour" flavors, "astringent" feeling factor and "hard" texture gradually increased ($P < 0.05$) over the 21 d retail display period and ranged between 0 and 1.5 on the 0- to 15- point scale across all frozen storage periods. The samples were considered inedible and were not evaluated for their flavor and textural attributes following 21 d of refrigerated storage due to the appearance of surface growth following all frozen storage periods.

**Table 2.** Results of the regression analysis of variables with significant effects on physicochemical and sensory attributes of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following frozen storage (-20°C, 0.5 mo)

| Attribute | Significant variables | Regression equation[1,2] |
|---|---|---|
| Color | | |
| *CIE* L* (lightness) | G, R, d | $60.2099 - 0.00216 \times \mathbf{G} - 0.00115 \times \mathbf{R} - 0.03864 \times \mathbf{d}$ |
| *CIE* a* (redness) | R, d, d × d | $12.8819 + 0.000531 \times \mathbf{R} - 0.1509 \times d + 0.005557 \times d \times d$ |
| *CIE* b* (yellowness) | R, d, d × d | $6.0953 + 0.00046 \times \mathbf{R} - 0.05808 \times \mathbf{d} + 0.005843 \times d \times d$ |
| Physicochemical | | |
| TBARS (mg MDA equivalent/kg) | G, R, d, d × d, R × d × d | $8.2613 - 0.00011 \times \mathbf{G} - 0.00202 \times \mathbf{R} + 0.4821 * \mathbf{d} - 0.0338 \times \mathbf{d} \times \mathbf{d} + 0.000003961 \times \mathbf{R} \times \mathbf{d} \times \mathbf{d}$ |
| Microbial | | |
| PPC ($\log_{10}$ CFU/g) | G, R, G × R, d, d × d | $5.0278 - 0.00995 \times \mathbf{G} - 0.00041 \times \mathbf{R} + 0.000004568 \times \mathbf{G} \times \mathbf{R} + 0.2645 \times \mathbf{d} - 0.00411 \times \mathbf{d} \times \mathbf{d}$ |
| Sensory Descriptors | | |
| Aroma-Aroma intensity | G, R, d, G × d, d × d | $4.9054 + 0.001557 \times \mathbf{G} + 0.000271 \times \mathbf{R} - 0.03035 \times \mathbf{d} - 0.00013 \times \mathbf{G} \times \mathbf{d} + 0.002702 \times \mathbf{d} \times \mathbf{d}$ |
| Aroma- Caramelized | G, d | $1.6931 + 0.000538 \times \mathbf{G} - 0.00863 \times \mathbf{d}$ |
| Aroma-Ginger | R, d | $2.2322 + 0.000135 \times \mathbf{R} - 0.03130 \times \mathbf{d}$ |
| Aroma-Off-odor | G, d, G × d, d × d | $0.01336 + 0.000544 \times \mathbf{G} - 0.0445 \times \mathbf{d} - 0.00014 \times \mathbf{G} \times \mathbf{Day} + 0.0079 \times \mathbf{d} \times \mathbf{d}$ |
| Basic Taste-Savory | G, R, G × R, d | $3.1861 + 0.005598 \times \mathbf{G} + 0.000631 \times \mathbf{R} - 0.00000267 \times \mathbf{G} \times \mathbf{R} - 0.03275 \times \mathbf{d}$ |
| Flavor- Ginger | G, d | $2.5134 - 0.00087 \times \mathbf{G} - 0.01426 \times \mathbf{d}$ |
| Flavor-Rosemary | G, R, G × R, d | $0.213 + 0.0026 \times \mathbf{G} + 0.00021 \times \mathbf{R} - 0.0000012 \times \mathbf{G} \times \mathbf{R} - 0.0055 \times \mathbf{d}$ |
| Flavor-Rancid | G, d, G × d, d × d, G × d × d | $0.0592 + 0.000126 \times \mathbf{G} - 0.02616 \times \mathbf{d} + 0.000298 \times \mathbf{G} \times \mathbf{d} + 0.004557 \times \mathbf{d} \times \mathbf{d} - 0.00003 \times \mathbf{G} \times \mathbf{d} \times \mathbf{d}$ |
| Flavor-Off-flavor | G, d, G × d | $0.01136 + 0.000777 \times \mathbf{G} + 0.06167 \times \mathbf{d} - 0.00015 \times \mathbf{G} \times \mathbf{d}$ |

[1]All bold terms are statistically significant (*P < 0.05*).

[2]Rosemary extract (R: 1,500, 2,000, and 2,500 ppm), Green tea extract (G: 100, 200, and 300 ppm), Control (synthetic antioxidants only).

The majority of the antioxidant combinations resulted in a decrease in aroma intensity from d 0 to 7 and then increased from d 14 to 21 following 0.5 mo of frozen storage (Fig. 6A, Table 2). Ginger aroma decreased as retail display progressed with intensity increasing ($P < 0.05$) as rosemary concentration increased (Fig. 6C, Table 2). Treatment combinations with higher concentrations of green tea extract resulted in sausages with more "caramelized" (Fig. 6B, Table 2) aroma and were associated with fewer negative drivers of liking such as "off-odor" (Fig. 6D, Table 2), "rancid" flavor (Fig. 7A, Table 2) and "off-flavor" (Fig. 7B, Table 2) as retail display progressed. Pork sausages with G300 had the highest intensity in "rancid" flavor at d 7 but it ultimately resulted in the lowest throughout the rest of the retail display period. These results were in agreement with the findings of Martínez et al. (2006) who reported a close relationship between the less intense rancidity notes of fresh pork sausages formulated with green tea extract (1,000 ppm) with those of lipid oxidation during the refrigerated storage of the product.

Pork sausages with higher concentrations of rosemary extract were associated with stronger "spice complex", "nutmeg", "rosemary" aromas and "fat" flavor following 3 mo of frozen storage (Table 3). The "pork complex" flavor continued to be a positive attribute for the pork sausage flavor with higher intensities found in treatment combinations with G300 for d 14 of the retail display period. Sausages formulated with G300 also displayed significantly higher ($P < 0.05$) oily feeling factor through 21 d of refrigerated storage after 3 mo of frozen storage (data not shown) and 6 mo of frozen storage (Table 4). The panelists identified the predominant off-odor at the end of shelf-life during simulated retail display (3 ± 1°C) as that of spoilage due to microbial growth and to a lesser extent deterioration related to oxidative processes. This deterioration was largely attributed to increased psychrotrophic growth for all treatments, which exceeded 7 log CFU/g after 21 d of simulated retail display. Off-flavors generated by lipid oxidation is, apart from microbial spoilage, the primary source of off-flavors in muscle foods (Gray et al., 1996). These results suggest that sausages could be successfully incorporated with rosemary and green tea extracts without significantly changing its initial positive sensory attributes. Both natural plant extracts provided protection to the product, inhibiting the formation of undesirable characteristics. From these results, it was apparent that the antioxidant effect of rosemary extract was significantly enhanced by green tea extract in preventing oxidation. This was probably due to the lipid antioxidant activity of rosemary extract and the ability of the green tea extract to regenerate rosemary extract (Grün, 2009). Also, green tea extract seemed to exert enhanced protection once the product had reached 6 mo of frozen storage and then was displayed under retail conditions.

**Table 3.** Results of the regression analysis of variables with significant effects on physicochemical and sensory attributes of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following frozen storage (–20°C, 3 mo)

| Attribute | Significant variables | Regression equation[1,2] |
|---|---|---|
| Color | | |
| *CIE* L* (lightness) | R, d, d × d | 58.3160 – 0.00054 × **R**+0.0311 × **d**-0.00413 × **d** × **d** |
| *CIE* b* (yellowness) | R, d, R × d, d × d | 5.3504 + 0.001064 × **R**-0.07092 × **d**-0.00006 × **R** × **d** + 0.01316 × **d** × **d** |
| Chroma | R, d, R*d, d*d | 12.9489 + 0.000846 × **R**-0.2087 × **d**-0.00007 × **R** × **d** + 0.009274 × **d** × **d** |
| Physicochemical | | |
| TBARS (mg MDA equivalent/kg) | G, R, d, d × d | 5.7757 – 0.00091 × **G**-0.00105 × **R** + 0.1365 × **d**-0.00846 × **d** × **d** |
| 2,4-decadienal (ppb) | R, d, R × d | -0.3395 + 0.000094 × **R** + 0.09691 × **d**-0.00003 × **R** × **d** |
| pH | G, R, d, G × d, d × d | 6.6552 – 0.00016 × **G** + 0.000025 × **R**-0.00186 × **d** + 0.000028 × **G** × **d**-0.00067 × **d** × **d** |
| Microbial | | |
| PPC ($\log_{10}$ CFU/g) | G, R, d, d × d | 3.7785 + 0.001473 × **G**-0.00040 × **R** + 0.3402 × **d**-0.00573 × **d** × **d** |
| Sensory Descriptors | | |
| Aroma-Spice complex | R, d, R × d, d × d, R × d × d | 3.9558 + 0.000039 × **R**-0.1173 × **d** + 0.000050 × **R** × **d** + 0.003370 × **d** × **d**-0.00000266 × **R** × **d** × **d** |
| Aroma-Ginger | G, d, G × d | 2.6740 – 0.00088 × **G**-0.4646 × **d** + 0.000077 × **G** × **d** |
| Aroma-Nutmeg | R, d, R × d, d × d, R × d × d | 1.9143 + 0.000170 × **R**-0.04261 × **d** + 0.000012 × **R** × **d** + 0.001590 × **d** × **d**-0.00000114 × **R** × **d** × **d** |
| Aroma-Rosemary | R, d, R × d, d × d, R × d × d | 0.6680 – 0.00000171 × **R**-0.03986 × **d** + 0.000017 × **R** × **d** + 0.002026 × **d** × **d**-0.0000011 × **R** × **d** × **d** |
| Aroma-Copper- herbal | G, d, d × d | 1.0956 – 0.00063 × **G**-0.00415 × **d** + 0.000010 × **d** × **d** |
| Basic Taste-Bitter | G, R, G × R, d | 0.3781 + 0.002443 × **G** + 0.000167 × **R**-0.00000112 × **G** × **R** + 0.005086 × **d** |
| Flavor-Fat | R, d, d × d | 3.8206 + 0.000029 × **R**-0.02965 × **d** + 0.000504 × **d** × **d** |
| Flavor- Ginger | G, d, G × d | 2.6118 – 0.00137 × **G**-0.02831 × **d** + 0.000072 × **G** × **d** |
| Flavor-Metallic | G, d, G × d, d × d, G × d × d | 1.1850 – 0.00049 × **G**-0.05427 × **d** + 0.000105 × **G** × **d** + 0.003045 × **d** × **d**-0.00000396 × **G** × **d** × **d** |
| Texture-Hardness | G, d, G × d | 4.7742 + 0.000084 × **G** + 0.01029 × **d** + 0.000018 × **G** × **d** |
| Texture-Chewiness | G, d, G × d, d × d, G × d × d | 4.7782 – 0.00039 × **G** + 0.1028 × **d**-0.00023 × **G** × **d**-0.00746 × **d** × **d**-0.000025 × **G** × **d** × **d** |

[1]All bold terms are statistically significant ($P < 0.05$).

[2]Rosemary extract (R: 1,500, 2,000, and 2,500 ppm), Green tea extract (G: 100, 200, and 300 ppm), Control (synthetic antioxidants only).

## *Consumer acceptability*

On average, consumers ($n$ = 175) preferred ($P$ < 0.05) sausages formulated with natural plant extracts after 3 mo of storage when compared to the control, but almost all sausages had average consumer scores between 6 and 7, signifying "like slightly" to "like moderately" (Table 5). Overall, the panelists did not identify any differences ($P$ > 0.05) between the sausages in terms of aroma, flavor, texture, and overall acceptability following 0.5 ($n$ = 166) and 6 ($n$ = 177) mo of frozen storage. However, this is misleading since the control treatment was already beyond shelf-life after 8 d under retail conditions when stored frozen for 6 mos. Instead, a more accurate interpretation of these results indicate that the R and G treatments were not different from the control after 0.5 mo of storage, were preferred ($P$ < 0.05) over the control treatment after 3 mo of frozen storage, and were still acceptable after 6 mo of frozen storage and 8 d of retail display, while the control was already spoiled. According to the descriptive analyses performed by the trained panel, sausages which received higher consumer acceptability scores were characterized by "spice complex", "ginger", "nutmeg", "rosemary", "copper-herbal" aromas, "ginger", "fat", "bitter", "metallic" flavors and "hard", "chewy" textures. Descriptive attributes regarded as positive drivers for the liking of the product showed a gradual decrease in their mean attribute intensity ratings with increased frozen storage time. However, the effects of such a decrease in these attributes were not as apparent in the consumer acceptability ratings of the product as sausages with combinations of rosemary and green tea extracts were rated as "liked" even after 6 mos of frozen storage followed by 8 d of retail display (Table 5). In general, rosemary and green tea extracts were able to increase the shelf-life of pork sausage links without affecting the sensory qualities that are important to regular consumers of the product. It should be noted that rosemary extract (1500 ppm) was added to the control samples for the third replication of the experiment and was still not acceptable for consumer testing after 6 mos frozen storage and 8d retail display due to spoilage.

For 0.5 mos frozen samples, cluster analysis indicated that 92% of the respondents found treatment com-

**Table 4.** Results of the regression analysis of variables with significant effects on physicochemical and sensory attributes of fresh pork sausage under simulated retail display (3 ± 1°C, 21 d) following frozen storage (–20°C, 6 mo)

| Attribute | Significant variables | Regression equation[1,2] |
|---|---|---|
| Color | | |
| *CIE a** (redness) | G, d, G × d, d × d, G × d × d | 11.9698 – 0.00480 × **G**-0.6359 × **d** + 0.001277 × **G** × **d** + 0.03114 × **d** × **d**-0.00006 × **G** × **d** × **d** |
| CIE *b** (yellowness) | R, d, d × d | 7.3687 + 0.000354 × **R**-0.00068 × **d** + 0.00315 × **d** × **d** |
| Physicochemical | | |
| TBARS (mg MDA equivalent/kg) | G, R, d | 8.215 – 0.00065 × **G**-0.0013 × **R**-0.04864 × **d** |
| pH | G, d, G × d, d × d | 6.6471 + 0.00003 × **G** + 0.00225 × **d** + 0.000015 × **G** × **d**-0.0006 × **d** × **d** |
| Microbial | | |
| PPC ($\log_{10}$ CFU/g) | G, R, G × R, d, d × d | 4.3723 – 0.00741 × **G**-0.00031 × **R** + 0.000003421 × **G** × **R** + 0.4010 × **d**-0.01035 × **d** × **d** |
| Sensory Descriptors | | |
| Flavor-Flavor intensity | G, d, G × d, d × d, G × d × d | 5.5054 – 0.000103 × **G** + 0.08666 × **d**-0.00049 × **G** × **d**-0.01161 × **d** × **d** + 0.000055 × **G** × **d** × **d** |
| Flavor-Pork complex | G, d, G × d, d × d, G × d × d | 3.6536 + 0.000395 × **G** + 0.06663 × **d**-0.00042 × **G** × **d**-0.00578 × **d** × **d** + 0.000033 × **G** × **d** × **d** |
| Flavor-Oily | G, d | 4.3435 + 0.001126 × **G**-0.01773 × **d** |

[1]All bold terms are statistically significant ($P < 0.05$).

[2]Rosemary extract (R: 1,500, 2,000, and 2,500 ppm), Green tea extract (G: 100, 200, and 300 ppm), Control (synthetic antioxidants only.

binations R1500 + G100, R1500 + G300, and R2000 + G200, to be acceptable ( ≥ 6, like slightly) after 8 d of retail storage, followed by R2000 + G100, R2500 + G200, R2500 + G300 and control which were liked by 88, 84, 81, and 77% of the respondents, respectively (Table 6). Cluster analysis revealed that a greater number of consumers liked sausages formulated with natural plant extracts when compared to the control following 3 mo of frozen storage. After 8 d of retail display, 98% of the respondents found treatment combinations R1500 + G300, R2000 + G100, R2000 + G200, and R2500 + G300 to be acceptable (≥ 6, like slightly) followed by R1500 + G100, R2500 + G200, and control which were liked by 95, 85 and 71% of the respondents, respectively (Schilling et al., 2018). After 6 mo of storage at –20°C, the organoleptic characteristics of all the sausage treatments remained acceptable both after freezing and after refrigerated storage (Table 7).

**Table 5.** Mean scores for consumer acceptability ($n = 518$) of pork sausages with varying combinations of rosemary and green tea extracts held under simulated retail display at 3 ± 1°C for 8 d

| Attribute[1] | Storage time (Months) | Treatment[2] R1500 + G100 | R1500 + G300 | R2000 + G100 | R2000 + G200 | R2500 + G200 | R2500 + G300 | CONTROL | SEM | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| Appearance | 0.5 | 6.7 | 6.8 | 6.6 | 6.8 | 6.7 | 6.7 | 6.9 | 0.1250 | 0.6988 |
| | 3 | 6.9 | 6.8 | 6.9 | 6.9 | 7.0 | 6.8 | 6.8 | 0.1084 | 0.7209 |
| | 6 | 6.9 | 6.9 | 6.8 | 6.8 | 6.9 | 7.1 | NA | 0.1177 | 0.4734 |
| Aroma | 0.5 | 6.9 | 6.9 | 6.9 | 7.0 | 6.9 | 6.8 | 6.7 | 0.1186 | 0.6114 |
| | 3 | 6.9[a] | 6.9[a] | 7.0[a] | 7.1[a] | 7.0[a] | 7.0[a] | 6.5[b] | 0.1100 | 0.0455 |
| | 6 | 7.2 | 7.3 | 7.2 | 7.1 | 7.2 | 7.3 | NA | 0.0768 | 0.3985 |
| Flavor | 0.5 | 7.0 | 7.0 | 6.6 | 7.0 | 6.8 | 6.7 | 7.0 | 0.1563 | 0.4548 |
| | 3 | 7.0[a] | 7.0[a] | 7.1[a] | 7.3[a] | 7.1[a] | 7.1[a] | 6.2[b] | 0.1922 | 0.0279 |
| | 6 | 7.1 | 7.0 | 7.1 | 6.9 | 7.0 | 7.3 | NA | 0.1521 | 0.7029 |
| Texture | 0.5 | 6.8 | 6.9 | 6.7 | 6.9 | 6.8 | 6.7 | 6.9 | 0.1623 | 0.9674 |
| | 3 | 7.0[a] | 7.0[a] | 7.1[a] | 7.1[a] | 7.0[a] | 6.9[a] | 6.5[b] | 0.1195 | 0.0287 |
| | 6 | 6.9 | 6.9 | 6.9 | 6.8 | 7.0 | 7.2 | NA | 0.1504 | 0.5041 |
| Overall Acceptability | 0.5 | 7.0 | 6.9 | 6.7 | 6.9 | 6.8 | 6.7 | 6.8 | 0.1630 | 0.7706 |
| | 3 | 7.0[a] | 7.0[a] | 7.0[a] | 7.1[a] | 7.1[a] | 6.9[a] | 6.2[b] | 0.1561 | 0.0121 |
| | 6 | 7.0 | 7.0 | 6.9 | 6.8 | 7.0 | 7.2 | NA | 0.1379 | 0.5673 |

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

[1]Scores were based on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

[2]Rosemary extract (R: 1500, 2000, and 2500 ppm), Green tea extract (G: 100, 200, and 300 ppm), Control (synthetic antioxidants only).

**Table 6.** Mean scores for overall consumer acceptability ($n$ = 166) of pork sausage with varying combinations of rosemary and green tea extracts according to different clusters of consumer segments using a hedonic scale after 0.5 mo of frozen storage

| Treatment[1,2] | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 | Cluster 6 | Cluster 7 |
|---|---|---|---|---|---|---|---|
| R1500 + G100 | 7.6[a] | 7.7[bc] | 6.0[c] | 7.2[a] | 6.1[ab] | 4.3[de] | 5.0[cd] |
| R1500 + G300 | 6.9[cde] | 7.9[ab] | 6.8[ab] | 7.2[a] | 4.4[cd] | 6.4[ab] | 6.0[bc] |
| R2000 + G100 | 6.7[de] | 7.7[bc] | 7.3[a] | 6.0[b] | 4.0[d] | 7.1[a] | 4.0[d] |
| R2000 + G200 | 7.2[bc] | 7.9[ab] | 6.0[c] | 7.1[a] | 4.6[cd] | 5.7[bc] | 7.9[a] |
| R2500 + G200 | 7.6[ab] | 7.5[c] | 6.0[c] | 5.1[c] | 6.5[a] | 5.4[bcd] | 5.1[cd] |
| R2500 + G300 | 6.5[e] | 8.0[a] | 6.2[bc] | 7.6[a] | 5.3[bc] | 4.6[cde] | 3.7[d] |
| CONTROL | 7.1[cd] | 7.9[ab] | 7.2[a] | 5.3[bc] | 5.1[c] | 3.7[e] | 7.7[ab] |
| N | 50 | 46 | 24 | 18 | 14 | 7 | 7 |
| Percentage of panelists | 30 | 28 | 15 | 11 | 8 | 4 | 4 |
| SEM | 0.16 | 0.11 | 0.23 | 0.33 | 0.37 | 0.41 | 0.62 |
| $P$-value | < 0.0001 | 0.0187 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

[a-e]Means within each column with different superscript letters are significantly different ($P$ < 0.05).

[1]Scores were based on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

[2]Rosemary extract (R: 1,500, 2,000, and 2,500 ppm), Green tea extract (G: 100, 200, and 300 ppm), Control (synthetic antioxidants only).

However, the control sausages displayed spoilage and detectable rancidity by d 8 of the retail display period following 6 mo of frozen storage and were not evaluated by consumers. The largest consumer group (50% of the consumers) rated all sausages as "like very much" and was partial ($P$ < 0.05) to R1500 + G100, R1500 + G300, and R2500 + G300 sausages over R2000 + G100 sausages (Table 7). Cluster 2 which comprised of 28% of the consumers ranged from "like slightly" to "like moderately" in their liking of the products and was partial to sausages formulated with R2000 + G100. Cluster 3 (21% of the consumers preferred ($P$ < 0.05) sausages with R2500 + G300 as "like moderately" over those from the R2000 + G100 and R2000 + G200 sausages which were rated as "neither like nor dislike". After 8 d of retail display, all of the respondents found treatment combinations R1500 + G100, R1500 + G300, R2500 + G200, and R2500 + G300 to be acceptable followed by R2000 + G100 and R2000 + G200 which were liked by 79% of the respondents.

**Table 7.** Mean scores for overall consumer acceptability ($n$ = 177) of pork sausage with varying combinations of rosemary and green tea extracts according to different clusters of consumer segments using a hedonic scale after 6 mo of frozen storage

| Treatment[1,2] | Cluster 1 | Cluster 2 | Cluster 3 |
|---|---|---|---|
| R1500 + G100 | 7.9[a] | 6.6[b] | 5.6[bc] |
| R1500 + G300 | 7.9[a] | 6.4[b] | 5.7[b] |
| R2000 + G100 | 7.6[b] | 7.2[a] | 5.1[cd] |
| R2000 + G200 | 7.7[ab] | 6.6[b] | 4.9[d] |
| R2500 + G200 | 7.7[ab] | 6.3[b] | 6.2[ab] |
| R2500 + G300 | 7.8[a] | 6.5[b] | 6.5[a] |
| N | 89 | 50 | 38 |
| Percentage of panelists | 50 | 28 | 21 |
| SEM | 0.08 | 0.20 | 0.22 |
| P-value | 0.0439 | 0.0493 | < 0.0001 |

[a-d]Least-squares means within each column with different superscript letters are significantly different ($P$ < 0.05).

[1]Scores were based on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

[2]Rosemary extract (R: 1,500, 2,000, and 2,500 ppm), Green tea extract (G: 100, 200, and 300 ppm), Control (synthetic antioxidants only).

An estimation of the samples' shelf-life in the present study was made based on the detection of off-odors or off-flavors by the descriptive panelists or when the product was deemed unacceptable when compared with the gold standard. Combinations of at least 2500 ppm of rosemary extract and green tea extract at 100 to 300 ppm as well as R2000 + G300 increased the shelf-life of fresh pork sausages to 16 to 18 d of storage after 0.5 mo of frozen storage in comparison to less than 14 d for the control treatment with synthetic antioxidants and 1500 ppm rosemary. Accordingly, for 3 mo 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). After 6 mo 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.

## *Conclusions*

The addition of combined phenolic-rich rosemary and green tea extracts with synthetic antioxidants increased effectiveness in slowing down long-term frozen storage oxidation reactions, reduced psychrotrophic bacterial growth and maintained quality during simulated retail display better than using synthetic antioxidants alone. Treatment with 2,500 ppm rosemary extract significantly reduced lipid oxidation. These results correlated positively with the protective effects against oxidative rancidity as the addition of these natural plant extracts helped preserve the intensity ratings of the positive sensory attributes and instrumental color in the product without compromising other qualities that are important to consumers. Evaluation of color showed rosemary and green tea extracts were sufficient to achieve the desired red color in the product.

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