Control of lipid oxidation with antioxidants during frozen pork shelf-life

by

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Abstract

Antioxidants are used to limit oxidation in many food products; however, there is limited published research evaluating the incorporation of antioxidants in ground pork trim. The objective of this research was to assess lipid oxidation in ground pork fat trim containing no antioxidants, nitrite at 70 ppm, citric acid-based vinegar buffered to 6.0 to 7.0 pH at 0.35% of the total formula, rosemary extract at 0.3% of the total formula, and rosemary green tea (RGT) extract at 0.28% of the total formula during 12 m of frozen storage at -17°C. The treatments (nine replications) were packaged in plastic, heat-sealed bags that were cased in a corrugate box with a weight of 22.68 kg. Treatments, quick frozen at a wind chill of -42°C, reached an equilibrated temperature of -28°C within 26 h and were stored in a commercial freezer at -17°C. Fatty acid profiles and proximate analyses were analyzed for each treatment at the beginning of the study. The control and vinegar treatments at 61.65 and 61.15% fat, respectively, had higher ($P < 0.01$) percent fat than the nitrite, RGT, and rosemary treatments and was higher than the 58 ± 2% target. This, however, was attributed to a small sample size from a larger batch that was evaluated in real time on the production line to be within 2% of 58%. There were slight differences ($P < 0.05$) in fatty acid profiles and proximate analyses among the treatments, and these differences were potentially due to sample collection in a commercial pork processing facility where hogs were sourced from multiple production facilities. The aerobic plate count and pH of the samples were measured each month for 12 m. The aerobic plate counts ranged from 2.47 to 2.98 $\log_{10}$ CFU/g throughout 12 m storage at -17°C and 2.69 to 2.80 $\log_{10}$ CFU/g among the individual treatments. Pork trim pH varied from 5.79 to 6.23 during 12 m of storage. The nitrite treatment had a higher ($P < 0.05$) pH than the pork trim containing rosemary. Lipid oxidation was measured monthly using thiobarbituric acid reactive substances (TBA), gas

chromatography, and a trained sensory panel. The malonaldehyde (MDA) of RGT and rosemary treatments remained below 0.2 mg MDA/kg during frozen storage. The control, vinegar, and nitrite treatments, however, increased resulting in higher ($P < 0.05$) MDA at 8 through 12 m than rosemary and RGT. Vinegar exceeded 1.0 mg MDA/kg at 12 m, where a consumer is likely to taste oxidation in a pork product. Hexanal, measured by gas chromatography, was similar ($P < 0.05$) for the control, rosemary, and RGT treatments during storage and stayed at or below 0.1 mg hexanal/kg throughout the 12 m of frozen storage. None of the treatments, however, exceeded 1.0 mg hexanal/kg, or reached levels of hexanal that would impact sensory. The trained sensory panel ranked rancid aroma, cardboard aroma, rancid flavor, and cardboard flavor on a 100-point scale where 0 was none and 100 was intense aroma or flavor. All treatments for all four measurements were below 7 on the 100-point scale at each month through 10 m. At 11 m, the vinegar treatment was removed from further evaluation due to due to off aroma and flavors such as meat or sour, though not defined as oxidation. The largest sensory differences among the treatments occurred at 12 m of storage where rosemary and RGT were lower ($P < 0.05$) than control for rancid and cardboard aroma and flavor. None of the treatments exceeded 30 on the sensory scale. The color was measured instrumentally and by the trained sensory panel using the National Pork Board 6-point scale. The reaction of nitrite with myoglobin impacted the color of the trim based on lower ($P < 0.05$) a* and b*; however, this color change is expected in a cured product. All the color differences were less than 3 on the color scale, and most were below two. Since most of those differences only a trained panelist will notice, the instrumental color changes do not eliminate any of the treatments. As a result, Rosemary and RGT treatments were effective at slowing oxidation of frozen pork trim during 12 m of storage and may be used to create a flexible supply chain. to maintain pork quality during frozen storage.

Table of Contents

List of Figures

List of Tables

Chapter 1 - Literature Review

Fluctuating pork prices impact the profitability of processing pork into value-added products such as sausage; therefore, purchasing pork from markets with lower pricing makes financial sense. However, working with international trade systems to identify the lowest global pork prices adds complexity to the supply chain. A longer pork trim shelf-life minimizes the time constraint resulting from trade complexities. Although freezing pork decreases enzymatic and microbial activity, extending shelf-life, freezing also facilitates dehydration, produces oxidation off flavors often characterized as “fishy,” “paint-like,” and “cardboardy” and creates gray and brown color changes in the pork (Damodaran et al., 2008). These changes indicate the limit of frozen pork shelf-life; therefore, developing methods to limit oxidation will extend the shelf-life of pork trim and add flexibility to the supply chain.

The best solution for controlling oxidation is a multiple hurdle approach, optimizing all steps of the process. Although pork oxidation cannot be prevented, it can be delayed by improving pork quality, increasing freezing rates, optimizing storage temperatures, using oxygen-excluding packaging, and adding antioxidants.

International Pork Markets

Multilateral Trade System

A global supply chain enables purchasing pork where the industry is efficient and economical; however, trade is only sustainable if protected. The global multilateral trade system is maintained by the World Trade Organization (WTO) through the work of three science-led governing bodies known as the three sisters: 1) the Codex Alimentarius Commission (Codex) focusing on food safety; 2) the World Organization for Animal Health (WOAH) focusing on animal health; and 3) the International Plant Protection Convention (IPPC) focusing on

phytosanitary concerns (World Trade Organization, 2023). Together, the WTO works through the three sisters to ensure international trade systems are free from trade barriers and to protect trade from safety concerns through regulations and equitable access to scientific information (World Trade Organization, 2023).

SPS Agreement

The heart of the multilateral trading system is the Agreement on the Application of Sanitary and Phytosanitary Measures, also known as the SPS Agreement, because it sets the framework for trade. The SPS Agreement, maintained by the three sisters, establishes the importance of setting phytosanitary and sanitary measures in trade to protect human, animal, and plant health (World Trade Organization, 2023). The SPS Agreement not only encourages members to set sanitary and phytosanitary protection measures based on international standards, it also permits countries the flexibility to set individualized measures if scientifically justifiable and equivalent to the measures set by the opposing member in the trade agreement (World Trade Organization, 2014). This allowance protects societal diversity while harmonizing global trade and reducing trade barriers (World Trade Organization, 2023).

Even with these standards in place, disagreements between countries do arise. In these cases, the protocols of the WTO Dispute Settlement Understanding are in place to solve these issues. Although many disputes are settled without it, a dispute settlement panel may be formed to resolve the concern if a problem requires it (World Trade Organization, 2014). Having an unbiased third-party mediate trade disputes removes the political and economic bias of each participating country from the settlement (World Trade Organization, 1999).

Due to the trade rules established by the SPS Agreement, the scientific based standards for sanitary and phytosanitary measures, and the mediation of trade disputes, the multilateral

trade system promotes health protection while facilitating international trade. As a result, the multilateral trade system has been successful in creating a peaceful, predictable, fair, and free-flowing trade environment, promoting increased wages, a lower cost of living, and economic growth (World Trade Organization, 1999).

Examples of Pork Trade Issues

Throughout history, the need for a multilateral trading system to keep international trade flowing has been necessary. Gignilliat (1961) summarized how the European boycott of American pork, which assumed that American pork had a higher rate of trichinae, is an example why the policies set by three sisters are important for keeping trade free flowing. Historically, America had exported mostly agricultural products until 1880 without accepting European imports. Europe had also enforced higher tariffs on U.S. goods. Rising tension led European pork industry protectionists to claim high trichinae case rates as a reason not to purchase U.S. pork, and European border inspection reinforced that the U.S. had higher rates of infected pork. Between 1870 and 1891, Spain, Portugal, Germany, France, Austria-Hungary, Italy, Greece, Denmark, and Rumania set trade barriers preventing the purchase of U.S. pork. Despite U.S. protest, the lack of scientific proof prevented the U.S. from proving that U.S. pork did not have higher rates of trichinae than any other country. Unfortunately, many Europeans genuinely no longer trusted U.S. pork. It took over a decade of political and scientific efforts to build back trade relationships. Live animal and postmortem inspection of U.S. pork became policy. This historic example demonstrates why the three sisters, manageable trade disputes, and the SPS Agreement are necessary to prevent trade barriers not based on science (Gignilliat, 1961).

The global epidemic of African Swine Fever (ASF) is a current example demonstrating the importance of regulating bodies to maintain trade. China, one of the world's largest

producers of pork, lost 40% of its supply in 2020 to ASF (Haley and Gale, 2020), a resilient virus causing severe illness and death in pigs (Rademacher et al., 2022) within 6 to 13 d of infection (Haley and Gale, 2020). The ASF virus transmits between infected and healthy pigs, by contact with infected equipment, or by contact with pork excretions or feces (WOAH, 2019). The virus also remains in uncooked or undercooked pork products for up to 6 m (WOAH, 2019).

Limiting the impact of ASF on international markets is reliant on preventing ASF transmission through science-based evidence. The WOAH published a Technical Disease Card, a reference summarizing the disease, transmission, sources, diagnosis, and prevention methods of the disease, for ASF (WOAH, 2019). This science-based information on the disease gives countries sanitary measure recommendations to implement in the instance of an outbreak, to prevent transmission to a country without ASF, and to prevent transmission from an infected country (WOAH, 2019). Disease free countries are encouraged to establish sanitary measures for ASF through import policies for both swine and pork trade (WOAH, 2019). Infected countries are asked to practice biosecurity and avoid using meat scraps as pig feed (WOAH, 2019). If an outbreak does occur, the WOAH recommends slaughtering infected pigs, cleaning of animal housing, and disposing cadavers (WOAH, 2019). Finally, these outbreaks should be investigated for sources; the area and surrounding areas should also be monitored for infection (WOAH, 2019).

Despite the work of the three sisters to limit trade impacts, trade barriers do still occur. As a result of the reduced supply, market prices of pork increased by 97% in 2020 (Haley and Gale, 2020). In efforts to protect their markets, China put tariffs on U.S. pork products in November of 2019 (Haley and Gale, 2020). In January of 2020, a trade agreement between

China and the U.S. lifted tariffs against some U.S. products, including pork and swine offal, after trade negotiations (Haley and Gale, 2020).

International trade is possible due to the support of the three sisters and the frameworks established by the SPS agreement. Due to this support, trade barriers are reduced, conflicts are peacefully resolved, and disease transmission is suppressed (World Trade Organization, 1999), enabling countries to import and export food products based on market conditions. When global trade is economical, the international supply chain must be already established for trade to be possible. Frozen storage is a logical shipping condition due to a longer shelf-life compared to that of refrigeration; however, oxidation, specifically lipid oxidation, remains a concern (Custódio et al., 2018).

Refrigerated and Frozen Pork Shelf-life

Some primary differences between refrigerated and frozen pork are the rate of microbial growth, pH shift, and enzymatic activity. Custódio et al. (2018) compared refrigerated and frozen pork loin and leg wrapped in 0.15 µm polyvinyl chloride (PVC) film. Treatments were stored at 5°C for 16 d or -18°C for 180 d. The leg and loin stored at 5°C for 16 d increased ($P < 0.05$) in pH by approximately 0.2 and 0.3, respectively, whereas the leg and loin stored at -18°C had similar ($P > 0.05$) pH throughout the 180 d. The leg and loin stored at 5°C increased ($P < 0.05$) in psychotropic bacteria by 4 $\log_{10}$ CFU/g and increased ($P < 0.05$) in mesophiles by 3 $\log_{10}$ CFU/g.

Zhang and Ertbjerg (2018) researched the impact of frozen-then-chilled storage on pork loin enzymatic activity. Vacuum packaged loins, stored at 2°C or at -20°C and thawed at 2°C overnight, were monitored for enzymatic activity over 9 d of storage at 2°C. At 1 d of chilled storage, the calpain-1 enzymatic activity of refrigerated pork loin at 80% was already higher ($P <$

0.05) than the calpain-1 enzymatic activity at 60% of frozen-thawed pork loin. By 9 d, both treatments had decreased ($P < 0.05$) in enzymatic activity, and the refrigerated loins were higher ($P < 0.05$) at approximately 90% activity than frozen-thawed pork loin at approximately 55% activity, indicating that the freezing process decreases enzymatic activity (Zhang and Ertbjerg, 2018).

Lipid Oxidation

The scientific community continuously works to understand the complex reactions of lipid oxidation. When oxygen free radicals interact with lipids, oxidation occurs, resulting in off flavors and aromas (Damodaran et al., 2008). Oxidation pathways have many natural catalysts including photosensitizers, light, heat, salt, metals, oxygen, and nitrogen reactants (Mariutti and Bragagnolo, 2017).

Lipid Oxidation Pathways

The three types of lipid oxidation are autoxidation, photo-oxidation, and enzymatic oxidation. Each pathway relies on the reaction of an unsaturated fatty acid and oxygen, so the degree of unsaturation relates to the oxidation potential of the lipid (Huang and Ahn, 2019). The most common polyunsaturated fatty acids (PUFA) in pork are linoleic acid and α-linolenic acid (Wood et al., 2008). At the site of unsaturation, the double bond will pull the shared electron toward itself, making the abstraction of a hydrogen molecule by an oxygen molecule electronegatively probable (Huang and Ahn, 2019). At this point, the reaction differs, depending on the catalysts and reactants in the environment.

Enzymatic lipid oxidation may be catalyzed by several enzymes. Lipoxygenase, the most common catalyst of enzymatic lipid oxidation, initiates the formation of a hydroperoxide (Domínguez et al., 2019). The ferrous iron site on this enzyme abstracts hydrogen from an

unsaturated fatty acid (Domínguez et al., 2019). Lipase and phospholipases hydrolyze fatty acids to mobilize oxidation reactants (Hernández et al., 2004; Wu et al., 2016). The formation of ice crystals, however, will concentrate naturally occurring enzymes, facilitating oxidation reactions (Ding et al., 2020; Medic et al., 2018). The rate of oxidation is dependent on the number of reactants in the system, so the amount of enzyme in the meat is important to the rate of enzymatic oxidation (Domínguez et al., 2019).

Photo-oxidation, reliant on energy from light as a catalyst, is common in meat products displayed under retail lights (Domínguez et al., 2019; Mariutti and Bragagnolo, 2017). Energized oxygen reacts directly with the unsaturated fatty acid in this type of reaction (Domínguez et al., 2019). Natural components of meat, myoglobin and riboflavin, are known as photosensitizers since they absorb the energy from light (Mariutti and Bragagnolo, 2017). The energy from light excites the photosensitizers becoming triplet sensitizers (Domínguez et al., 2019) at a rate faster than through autoxidation (Mariutti and Bragagnolo, 2017).

Autoxidation is often explained as a three-step process. Since triplet oxygen is in a different spin state than singlet fatty acids, a catalyst abstracting the hydrogen is necessary for oxygen to react with the unsaturated bond (Domínguez et al., 2019). This step, known as the initiation phase (Figure 1), creates free radicals to react with the triplet oxygen (Domínguez et al., 2019; Mariutti and Bragagnolo, 2017).

The formation of peroxyl radicals or hydroperoxides, primary oxidation products, is the second step of oxidation, propagation (Figure 1) (Domínguez et al., 2019; Mariutti and Bragagnolo, 2017). This step is followed by the decomposition of hydroperoxides into highly reactive peroxy, alkoxy, and hydroxyl radicals, primary products of oxidation, initiating further

oxidation reactions (Figure 2) (Domínguez et al., 2019). The decomposition of peroxy and alkoxy radicals into secondary oxidation products is shown in Figure 3.

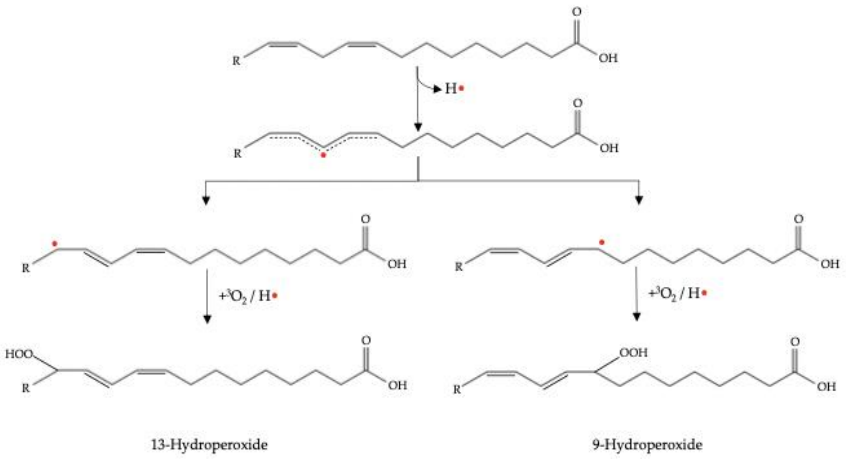

Figure 1. Oxidation initiation reaction followed by the propagation phase (Domínguez et al., 2019).

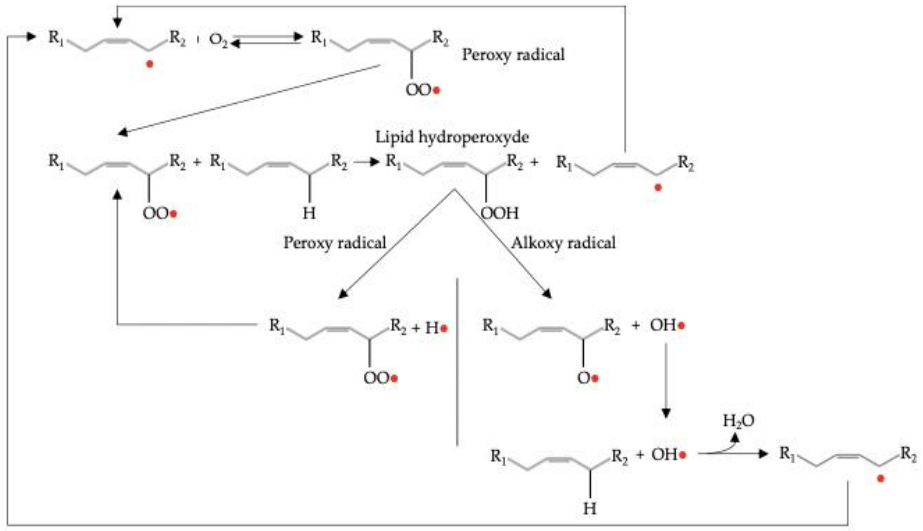

Figure 2. Formation of peroxy, alkoxy, and hydroxyl radicals to initiate further oxidation (Domínguez et al., 2019).

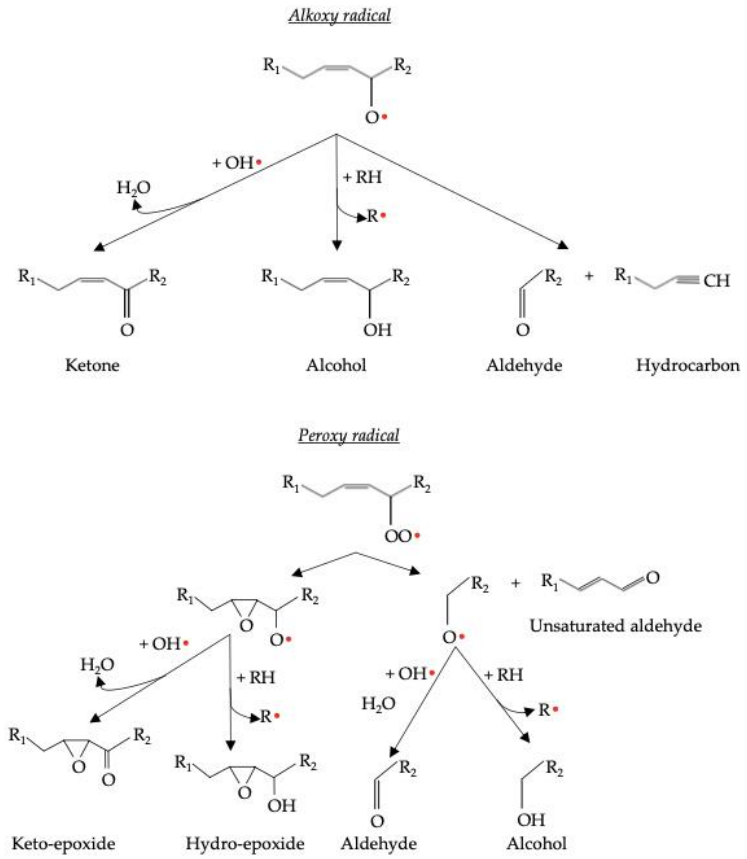

Figure 3. The decomposition of alkoxy and peroxy radicals during oxidation (Domínguez et al., 2019).

The final step known as termination occurs when there are no more fatty acid sites with which to react, radicals interact with antioxidants, or when two radicals react with each other to become non-radical compounds (Domínguez et al., 2019; Mariutti and Bragagnolo, 2017). Complete termination of oxidation in a system is uncommon since reactions often continue at some level (Domínguez et al., 2019).

Volatiles, resulting from oxidation, indicate the progression of oxidation. Primary products include hydroperoxides and conjugated dienes (Mariutti and Bragagnolo, 2017). Aldehydes, including the secondary oxidation products hexanal and malonaldehyde (MDA), are commonly measured to determine the rate of oxidation in meat products (Domínguez et al.,

2019; Fu et al., 2022; Mariutti and Bragagnolo, 2017). The products of these lipid reactions directly relate to the fatty acid composition (Meynier et al., 1998). Hexanal is the product of linoleic acid (Fu et al., 2022; Meynier et al., 1998; Pomponio and Ruiz-Carrascal, 2017). The formation of MDA is a result of the oxidation of fatty acids with three or more unsaturated bonds (Damodaran et al., 2008). Since lipid oxidation products catalyze protein oxidation (Huang and Ahn, 2019; Mariutti and Bragagnolo, 2017), it is worth noting that carbonyl is used as an indicator of protein oxidation (Blakeman et al., 1998; Ding et al., 2020). Carbonyl, however, forms as a product of both lipid and protein oxidation (Blakeman et al., 1998).

Impact of Lipid Oxidation on Meat Quality

The formation of these oxidation products results in sensory changes in meat products. Flavors and aromas developed from lipid oxidation in fresh pork also translate to oxidized flavors described as warmed over or rancid when the meat is cooked (Huang and Ahn, 2019). The products of lipid oxidation further react to instigate protein oxidation (Huang and Ahn, 2019; Mariutti and Bragagnolo, 2017), resulting in lower water holding capacity and protein solubility (Huang and Ahn, 2019). Oxidation causes protein color changes often described as browning (Lu et al., 2022). In lean protein, this is a result of myoglobin oxidation (Medic et al., 2018). These color changes are typically unappealing to consumers, reducing sale velocity (Huang and Ahn, 2019). This color preference could be because consumers associate vivid colors with fresh food since foods darken as they deteriorate (Lee et al., 2013). Research by Lee et al. (2013) compared the L*, a*, and b* of fresh and decomposed fruits and vegetables to what consumers identified as appetizing and found that, in general, consumers prefer more vividly colored foods, associating these colors with being fresh. A study by Norman et al. (2003) collected pork loins that spanned the National Pork Board 6-point color scale, sliced the loins

into chops, which were then vacuum packed, and shipped treatments to consumer households. L*, a*, and b* were measured using a Hunter Lab colorimeter. The study found 52.8% of 42 households preferred pork color that averaged within the National Pork Producers color standards 5 and 6, which was, on average, an L*, a*, and b* of 45.54, 8.75, and 13.88, respectively.

The decline in sensory qualities due to oxidation can continue, however, even during the termination of autoxidation (Mariutti and Bragagnolo, 2017). Some sensory changes due to oxidation, however, may not always be considered a decrease in quality, and some are important for the development of flavor in products such as dry cured meats (Domínguez et al., 2019).

Lipid Oxidation Methodology

Sensory evaluation, TBA, gas chromatography (GC) analysis, and instrumental color evaluation are common methods of measuring oxidation long term in food products (Damodaran et al., 2008). Comparisons among the results provide more insight into the product changes since each method measures something slightly different.

Sensory evaluation may be completed by trained or untrained panelists. The benefit of using a trained panel is correlating sensory and instrumental analyses. Trained panels must be screened for discrimination abilities and willingness to commit to the study (Meilgaard et al., 2016). Prescreening candidates should include testing attributes related to the study and may also include discriminating, ranking, matching, and identifying tests (Meilgaard et al., 2016; Wheeler et al., 2015). The American Meat Science Association (AMSA) provides guidelines for cooking and evaluating meat products (Wheeler et al., 2015). These guidelines also outline anchors for common attributes in sensory evaluation of meats, including pork, to train panelists on attributes of interest (Wheeler et al., 2015). When color is evaluated, a standard color scale should be used

(Meilgaard et al., 2016). The National Pork Board provides references for a 6-point lean color scale (National Pork Board, 2010). Sensory attributes may be measured using untrained panelists; however, larger sample sizes are necessary to identify significant differences (Meilgaard et al., 2016).

Thiobarbituric acid and GC are two measurements of oxidation products. The TBA method measures the development of MDA in meat products (Channon and Trout, 2002; Mariutti and Bragagnolo, 2017; Nielsen, 2017). Wood et al. (2008) suggested that the point where oxidation flavors surpass natural meat flavors is when oxidation levels are concerning. The point where oxidation flavors are recognized by panelists has been researched by several studies (Campo et al., 2006; Custódio et al., 2018; Gray and Pearson, 1987; Hansen et al., 2004; Miles et al., 1986; Tarladgis et al., 1960). In a study by Campo et al. (2006), 2.3 mg MDA/g was identified as the point in beef where oxidation flavors exceed natural meat flavors; however, Tarladgis et al. (1960) recommended this may be too high for pork products. Several studies have recommended 0.5 mg MDA/g as the threshold where sensory characteristics are first noticed (Custódio et al., 2018; Hansen et al., 2004; Tarladgis et al., 1960). Other studies have recommended a range depending on the sensitivity of the panelists. Gray and Pearson (1987) noted that a trained panel threshold is from 0.5 to 1.0 mg MDA/g, and an untrained panel threshold is from 0.6 to 2.0 mg MDA/g. Miles et al. (1986) indicated that 0.5 to 1.0 mg MDA/g is a common lipid oxidation threshold. In addition, MDA may further react in autoxidation resulting in a peak and decrease in MDA (Channon and Trout, 2002).

Many volatiles may be measured by GC (Nielsen, 2017); however, some studies have focused specifically on hexanal (Bak and Richards, 2021; Fu et al., 2022; Meynier et al., 1998). The volatile sample is injected into a column that separates the volatiles by characteristics such

as polarity or molecule size (Nielsen, 2017). Measuring volatiles specific to lipid oxidation, including hexanal, by GC indicates the progression of lipid oxidation (Fu et al., 2022).

Olesen et al. (2005) measured oxidation in vacuum packaged pork back fat during frozen storage at -20°C for 26 wk using TBA and found there were no differences ($P > 0.05$) in MDA values. The authors did observe an increase ($P < 0.05$) in the magnitude of all the volatiles during the study, especially hexanal that increased ($P < 0.05$) approximately 740 area units × $10^5 g^{-1}$ and 1-penten-3-ol increased ($P < 0.05$) approximately 220 area units × $10^5 g^{-1}$. A trained sensory panel found an increase ($P < 0.05$) in overall odor intensity by 1 point on a 9-point scale where 1 was no intensity and 9 was distinct intensity. Although there was no difference ($P > 0.05$), flavor intensity increased by 1.3 points between 0 and 26 wk. Paint aroma and flavor increased ($P < 0.05$) by 2.3 and 2.9 points, respectively, between 0 and 26 wk.

Meynier et al. (1998) used TBA and GC to measure lipid oxidation volatiles produced from isolated pork *longissimus dorsi* fat during iron catalyzed oxidation. In this controlled oxidation environment, total alkanals, including hexanal, 2-alkenals, 2,4-alkadienals, and alcohols were found to increase ($P < 0.05$) over 22 h. Hexanal, specifically, increased ($P < 0.05$) from 975 ng nonane eq/mg PL at 0 h to 4850 ng nonane eq/mg PL at 22 h.

In addition to sensory analyses, color may be evaluated using a colorimeter, providing three measurements: L*, measuring dark (0) to light (100); a*, measuring red (positive) to green (negative); and b*, measuring blue (negative) to yellow (positive) (Contini et al., 2014; Lu et al., 2022). The most common color change due to oxidation is a decrease in a*, becoming less red (Resconi et al., 2012; Schilling et al., 2018). Resconi et al. (2012) held modified atmospheric packaged (MAP) beef steaks containing 50, 60, or 80% oxygen in retail conditions over 8 d. At 8 d, there were no differences ($P > 0.05$) in L* or b* among any of the packaging treatments;

however, beef steaks packaged with 50% oxygen were less ($P < 0.05$) red than steaks packaged with 80% oxygen. The 60% oxygen treatment had the highest ($P < 0.05$) TBA at 3.13 mg MDA/kg which was 11.8% higher ($P < 0.05$) than 50% oxygen and 37.9% higher ($P < 0.05$) than the 80% oxygen treatment. Sensory attributes were ranked on a 10 cm line that was converted to a 10-point scale. The rancid aroma of beef steaks packaged with 50% oxygen was not different ($P > 0.05$) between 4 and 8 d. The rancid aroma of beef steaks packaged with 60 and 80% oxygen increased ($P < 0.05$) by 2.3 and 1.7 points, respectively. The rancid flavor of beef steaks packaged with 50, 60, and 80% oxygen increased ($P < 0.05$) by 1.5, 1.9, and 1.4 points, respectively.

Methods to Slow Oxidation Rates

The potential of oxidation begins at the harvest of the animal and continues throughout the shelf-life of pork (Mariutti and Bragagnolo, 2017). Although frozen environments facilitate oxidation in pork, the reaction can be minimized by improving pork quality, increasing freezing rates, optimizing frozen storage, choosing effective packaging, and adding antioxidants.

Pork Quality Factors

When deciding how to reduce oxidation in frozen pork, starting with good pork quality is the first step. Innate pork quality such as fat saturation and natural antioxidants in pork are primary factors for oxidation. Genetic factors, including gender and age, play a part in fatty acid composition. Research by Wood et al. (2008) indicated female pigs have the lowest rates of unsaturated and polyunsaturated fats followed by intact males, and that unsaturated fatty acid (UFA) content decreases with pig age. Pork fatty acid composition also tends to vary depending on the time of year the animal is harvested. Pork tends have a lower ratio of saturated fatty acids (SFA) to PUFA during the spring and summer (Wood et al., 2008). Despite these natural

variations, the average pork fatty acid profile is 38% SFA, 50% monounsaturated fatty acids (MUFA), and 12% PUFA (USDA, 2021).

A pig's diet influences the fatty acid profile of pork fat since pigs are monogastric and unable to hydrogenate fatty acids (Dinh et al., 2021). Several studies have found that the fatty acid profile of the feed is similar to the resulting pork fat (Wood et al., 2008; McClelland et al., 2012; Morel et al., 2006; and Hallenstvedt et al., 2012). Hallenstvedt et al. (2012) modified the fatty acid profile of pork diets by adjusting the amount of soybean oil and palm kernel oil in feed, otherwise comprised of barley, oat, and soybean meal. There was positive regression (R^2=0.91) between linoleic acid in the diet and the linoleic acid in the backfat layer.

Distillers dried grains with solubles (DDGS) are added to pork feed for economic reasons (McClelland et al., 2012). McClelland et al. (2012) studied the effect of DDGS on pork quality including the fatty acid profile in the resulting pork. Pigs were fed a corn-soybean meal diet containing 0, 15, 30, or 45% DDGS. The posterior end of the backfat was sampled and frozen at -22°C until the fatty acids were profiled. The DDGS in the pigs' diet had a positive linear relationship ($P < 0.05$) with PUFA content and a negative linear relationship ($P < 0.05$) with SFA and MUFA. The percentage of UFA in the inner backfat of pork fat increased ($P < 0.001$) from 60.3% when the diet contained no DDGS to 67.4% when the diet contained 45% DDGS. The percentage of MFA in the inner backfat decreased ($P < 0.05$) from 45.3% when the diet contained no DDGS to 40.3% when the diet contained 45% DDGS. The pig's diet has a great impact on the fatty acid profile, and what is economical for the farmer may not be ideal for pork processing.

In addition to the importance of the fatty acid profile, the antioxidant tocopherol, Vitamin E, added to pig feed, reduces oxidation in pork without impact on rate of gain, muscle

characteristics, or fatty acid profile (Morel et al., 2006). Morel et al. (2006) added vitamin E at 0 or 0.011% to pig diets 30 d prior to slaughter. The pork was further processed into pork products. Adding vitamin E at 0.011% to diets resulted in American-style sausages at 3.55 mg MDA/kg on average, lower ($P < 0.05$) than diets not supplemented with vitamin E at 4.37 mg MDA/kg on average.

Freezing Rates

Since oxidation reactions increase with the crystal size of frozen water, reducing water crystal size by increasing the freezing rate is another way to slow oxidation in frozen pork (Lu et al., 2022). In pork, faster freezing rates result in smaller ice crystal formation because the water has less time to move through the matrix and disrupt the muscle tissue (Lu et al., 2022; Wu et al., 2016). Slow rates allow the intercellular water to move extracellularly to form crystals whereas faster rates promote the formation of ice crystals in place (Lu et al., 2022). This has two implications for pork oxidation. First, the muscle damage caused by large ice crystal formation will increase surface area exposed to oxygen (Wu et al., 2016). Second, the nucleation of frozen water concentrates oxidation reactants, including the catalyst lipase, resulting in higher oxidation rates (Wu et al., 2016) despite enzymatic activity reduction (Teuteberg et al., 2021).

With these effects in mind, freezing pork rapidly is essential. Hou et al. (2020) compared pork muscle quality and oxidation of pork frozen by immersion solution freezing (ISF), a faster freezing rate, and air blast freezing (AF), a slower freezing rate, stored at -18°C. The ISF treatments reached an internal temperature of -17°C within 90 min whereas AF frozen pork reached -17 at 700 min. This study found less pork tissue damage in ISF pork when muscle tissue was compared under a microscope. No difference ($P > 0.05$) in a* and b* were found between the two freezing rates, but L* decreased ($P < 0.05$) from approximately 52.5 to 47.5 in

AF pork. The MDA was lower ($P < 0.05$) at 61 and 91 d in ISF pork at 0.41 and 0.55 mg MDA/kg, respectively, compared to AF pork at 0.56 and 0.61 mg MDA/kg, respectively, (Hou et al., 2020).

Frozen Storage Conditions

Consistently storing pork at an optimal temperature is the next step in oxidation reduction. In a study comparing trimmed pork loins packaged in polyethylene bags and stored at -1, -2, and -3°C, Ding et al. (2020) found TBA values increased ($P < 0.05$) from less than 0.1 mg MDA/kg for all treatments to above 0.6 mg MDA/kg for pork stored at -2°C for 30 d, above 0.3 mg MDA/kg for pork stored at -1°C for 25 d, and over 0.3 mg MDA/kg for pork stored at -3°C for 35 d.

Lee et al. (2021) researched the effects of -60, -50, and -18°C storage on pork loins frozen 48 h postmortem and packaged in air-containing packages. The L* of pork loins stored at -18°C increased ($P < 0.05$) 14.94 points between 0 and 6 m. There were no differences ($P > 0.05$) in L* at 0 and 6 m for pork loins stored at -60 and -50°C, or a* at 0 and 6 m for any of the treatments. The b* of pork loins stored at -18°C increased ($P < 0.05$) 6.51 points between 0 and 6 m, becoming more yellow. None of the treatments exceeded 0.30 mg MDA/kg, and there were no differences ($P > 0.05$) among the treatments in MDA at 6 m.

Teuteberg et al. (2021) also did not find a difference ($P > 0.05$) in MDA between pork packaged in plastic bags and held frozen at -18 and -80°C within 14 d of storage. Teuteberg et al. (2021) theorized that secondary oxidation reactions do not occur as rapidly if storage temperature is maintained since these reactions occur more rapidly during thawing. Storing at temperatures as low as -40 to -80°C may not be commercially economical, which is why focusing on maintaining frozen temperatures may be more practical for commercial storage.

Hansen et al. (2004) compared storage conditions by intentionally shifting temperature between -10 and -23°C, or -23 and -40°C as well as holding temperatures at -10, -23, and -40°C to determine the impact on frozen, minced, and salted pork formed into patties and vacuum packaged. At approximately 270 d, the patties consistently stored fluctuating between -10 and -23°C had lower ($P = 0.05$) MDA by approximately 2 µmole/kg than the patties stored at -10°C. At approximately 270 d, the patties stored fluctuating between -10 and -23°C were over 2 µmole/kg higher ($P = 0.05$) than the patties stored at -23°C, the patties stored fluctuating between -23 and -40°C were approximately 2 µmole/kg lower ($P = 0.05$) than the patties stored at -23°C, and the patties stored at -40°C were approximately 1 µmole/kg lower ($P = 0.05$) than the patties stored fluctuating between -23 and -40°C. For industry, this means that fluctuations in temperature are likely to affect oxidation, and commercial freezers should be held at the lowest temperature that is economical.

Packaging Solutions

The use of an opaque or light-blocking material limits exposure to light. A study by Reyes et al. (2022) compared color stability of ground beef when packaged with enhanced barrier film (EVOH (ethanol vinyl alcohol) and polyethylene), standard barrier film (nylon, EVOH, and polyethylene), and recycle-ready film (polyolefins and EVOH). Packaged beef was stored for the first 120 h without light at 2.2°C to simulate transportation from manufacture to retail display and then transferred to a lighted display temperature controlled at 3°C for 15 d. Although there were no interactions of packaging and film, the L* of beef packaged in enhanced barrier film was lighter ($P < 0.05$) than standard barrier and recycle-ready film. The enhanced barrier film a* also resulted in redder ($P < 0.05$) beef than standard barrier film. Beef in recycle ready film was more ($P < 0.05$) yellow than when packaged in standard barrier film and

enhanced barrier film. The authors concluded that enhanced barrier film produced the more appealing product color.

Packaging can limit exposure to oxygen by removing oxygen through vacuum or flushing out oxygen with another gas. Brewer et al. (1992) recommended oxygen-excluding packaging to reduce oxidation in pork. Their study compared pork with Saran Wrap™, Reynolds Heavy Duty Aluminum Foil™, Saran Wrap™ overwrapped with Reynolds Heavy Duty Aluminum Foil™, heat-shrunk polyvinyl chloride film on meat on Styrofoam™ trays, and nylon-polyester vacuum bags during 39 weeks of frozen storage at -17 °C. At 39 w, the vacuum packaged pork was lower ($P < 0.05$) in MDA than the Saran Wrap™, Reynolds Heavy Duty Aluminum Foil™, Saran Wrap™ overwrapped with Reynolds Heavy Duty Aluminum Foil™, and heat-shrunk polyvinyl chloride film on Styrofoam™ trays. A trained panel found no difference ($P > 0.05$) in rancid odor between fresh pork and vacuum packaged pork at week 39.

In addition to removing oxygen by vacuum, modified atmospheric packaging (MAP) flushes a package with gases, preferably ones that do not catalyze oxidation. Resconi et al. (2012) MAP packaged beef steaks in retail conditions over 8 d. MAP packages contained 50, 60, or 80% oxygen. At 8 d, there were no differences ($P > 0.05$) in L* among beef steaks packaged at 50, 60, or 80% oxygen. At 8 d, beef steaks packaged with 50% oxygen were less ($P < 0.05$) red compared to beef steaks packaged with 80% oxygen while there were no differences ($P > 0.05$) in b* among beef steaks packaged at 50, 60, or 80% oxygen. At 8 d, 60% oxygen had the highest ($P < 0.05$) TBA followed by 50% oxygen which was higher ($P < 0.05$) than 80% oxygen. Sensory attributes were ranked on a 10 cm line that was converted to a 10-point scale. The rancid aroma of beef steaks packaged with 50% oxygen was not different ($P > 0.05$) between 4 and 8 d. The rancid aroma of beef steaks packaged with 60 and 80% oxygen increased ($P < 0.05$) by 2.3

and 1.7 points, respectively. The rancid flavor of beef steaks packaged with 50, 60, and 80% oxygen increased ($P < 0.05$) by 1.5, 1.9, and 1.4 points, respectively.

Teuteberg et al. (2021) flushed packages of pork with 70% O_2 and 30% CO_2, and then froze the pork at -80°C. After freezing, the pork was stored at -18 or -80°C for 12 or 24 wk. These samples were compared to fresh pork when stored at 4°C for 14 d. Adding oxygen to packaging may catalyze oxidation (Resconi et al., 2012; Teuteberg et al., 2021); however, no difference ($P > 0.05$) in TBA between pork stored at 4 °C were found among the packaging treatments. This showed that frozen storage up to 24 wk did not have an impact on frozen, and then thawed pork oxidation levels for up to 14 d of refrigerated display in MAP packaging.

Antioxidants

In addition to packaging, frozen storage, freezing rates, and pork quality, antioxidants have been used to limit oxidation in pork products. Consumer demand for all-natural antioxidants is increasing (Domínguez, 2019; Huang and Ahn, 2019). Natural antioxidants are commonly derived from plants but can also be derived from protein peptides, although natural antioxidants often express flavors from their source (Huang and Ahn, 2019).

Antioxidant Functionality

Antioxidants slow oxidation in several ways. Enzymatic or nonenzymatic antioxidants can naturally occur in meat or be intentionally added to a meat product (Teuteberg et al., 2021). Naturally occurring antioxidants include vitamins, peptides, and carotenoids. Vitamin E competes with unsaturated fatty acids to donate hydrogen. Vitamin C, in contrast, reacts with the resulting radical to recreate α-tocopherol (Domínguez et al., 2019). Peptides, such as carnosine and anserine, in contrast, react with radicals and hydroperoxides in addition to chelating metals (Domínguez et al., 2019). Enzymes also react at differing parts of the reaction. Dismutase reacts

with O_2-. Catalase and glutathione react with H_2O_2 to block oxidation. Phenolic antioxidants scavenge free radicals reducing the reaction potential of the radicals (Huang and Ahn, 2019). Enzymes such as glutathione peroxidase and catalase scavenge peroxides (Hernández et al., 2004). Antioxidants, however, have decreased mobility in a frozen matrix (Hansen et al., 2004), and enzymatic antioxidants lose some functionality when meat is cooked (Huang and Ahn, 2019).

Antioxidants can also be added to meat products. Although there is limited published information on the application of antioxidants in pork trim, slowing lipid oxidation reactions with added antioxidants is a common method of blocking oxidation in pork and other meat products. There is opportunity to learn more about added antioxidants in pork trim, especially since food matrices impact antioxidant functionality and capability (Haak et al., 2009).

Vitamin E

A study by Channon and Trout (2002) found inconsistent results by adding vitamin E at levels of 0, 100, 200, 500, and 1000 ppm in cured, smoked pork sausage and restructured pork roast before storing the products at -18°C for 37 weeks. The pork sausage was approximately 22% fat, and the pork roast was approximately 5% fat on a total formula basis. After thermal processing, the pork sausage was packed in polyethylene bags and cased in cardboard cartons. The restructured pork roast was cooked in 90 mm fibrous cellulose casings, placed into polyethylene bags, and cased in cardboard cartons. Although there were no differences ($P >$ 0.05) in consumer acceptance, the 1000 mg/kg tocopherol treatment had the lowest consumer acceptance by 0.7 points on a 10-point scale attributed to the flavor of the tocopherol addition. Inconsistent sensory scores were explained by most treatments not reaching TBA values of 0.50 mg MDA/kg. Pork roast reached the highest TBA in the study with a value of 0.52 mg MDA/kg

at 37 weeks of frozen storage. The average mg MDA/kg in reconstructed pork roast did not exceed 0.50 mg MDA/kg except for the 500 ppm tocopherol treatment at 37 weeks. The sausage treated with 100 and 1000 ppm tocopherol exceeded 0.50 mg MDA/kg at 14 wk. The untreated sausage and 100, 200, and 500 ppm treatments exceeded 0.50 mg MDA/kg at 27 wk.

Nitrite

A study by Karwowska et al. (2020) found that nitrite was effective at postponing oxidation in ready-to-eat pork sausage when stored at 4°C for 15 d. Sausages were produced with no sodium nitrite or with 50, 100, or 150 mg/kg sodium nitrite. At 15 d, the 150 and 100 mg/kg treatments had means of 1.57 and 1.75 mg MDA/kg, respectively, which was lower ($P < 0.05$) than the mean 2.47 mg MDA/kg of the 50 mg/kg sodium nitrite treatment. All three cured treatments were lower ($P < 0.05$) on average in mg MDA/kg than the uncured sausage with a mean 3.14 mg MDA/kg at week 15. It is worth noting that the uncured sausage and sausages cured with 50 or 100 mg/kg sodium nitrite did exceed 0.50 mg MDA/kg at 0 d.

Rosemary Extract

The efficacy of adding natural antioxidants to pork has also been compared to adding synthetic antioxidants. Sebranek et al. (2005) researched the impacts of rosemary extract and synthetic butylated hydroxy anisole (BHA)/butylated hydroxy toluene (BHT) on frozen, ready-to-eat sausage with a 35% fat content. Although every treatment increased in oxidation over the 112 d, the 2500 and 1500 ppm rosemary extract treatments had less ($P < 0.001$) oxidation than the control during 16 wk. The TBA of sausage treated with rosemary extract at 1500 and 2500 ppm did not exceed 1.5 mg MDA/kg during the 16 wk whereas the control and synthetic BHA and BHT treatments exceeded 2.0 by 42 d and increased ($P < 0.05$) higher than 2.5 mg MDA/kg.

The control and BHA became less ($P < 0.05$) red. The a* of the 1500 and 2500 ppm rosemary extract treatments decreased less than 2 points.

Bak and Richards (2021) compared two brands of rosemary extract at 200, 400, and 600 mg/kg antioxidants in turkey deli meat, chicken fillets, and pulled pork produced with and without phosphates and held refrigerated for 1, 7, and 13 wk, respectively. At 13 wk, the hexanal ranged from 0.06 mg hexanal/kg in cured turkey deli meat produced with 400 mg/kg StabilEnhance OSR D 2.5 to 0.16 mg hexanal/kg ($P < 0.05$) in the control. At 7 wk, hexanal ranged from 0.23 mg hexanal/kg in chicken fillets produced with 400 mg/kg Guardian 09™ extract and 0.29% sodium tripolyphosphate to 1.52 mg hexanal/kg ($P < 0.05$) in the control. At wk 13, the hexanal ranged from 0.09 mg hexanal/kg in pulled pork produced with 400 mg/kg Guardian 09™ and 0.47% sodium tripolyphosphate to 1.26 mg hexanal/kg ($P < 0.05$) in the control. None of the rosemary treatments with phosphates added were lower ($P > 0.05$) than control at 0.27 mg hexanal/kg. In pulled pork, the 300 mg/kg Guardian 20S, 400 mg/kg Guardian 09, 800 mg/kg Guardian 09, 400 mg/kg StabilEnhance OSR 4 and 800 mg/kg StabilEnhance OSR 4 were lower ($P < 0.05$) at 0.94, 1.00, 0.77, 0.67, and 0.93 mg hexanal/kg, respectively. A trained panel evaluated the samples using a 6-point scale where 2.0 was the detection limit. There were no differences ($P > 0.05$) in oxidation flavor sensory scores for chicken fillets and cured turkey deli meat. There was a decrease ($P < 0.05$) in the oxidation sensory scores for pulled pork produced with and without phosphates at 2.1 and 3.1 at 13 wk. None of the treatments were rejected based on sensory.

Rosemary and Green Tea Extract Blends

Rosemary, green tea, or a blend of both extracts are a natural antioxidant option in the food industry. Schilling et al. (2018) added blends of rosemary extract (R) at 1500, 2000, or 2550

ppm and green tea extracts (G) at 100, 200, or 300 ppm to fresh pork sausage with 27.5% fat. Treatments were identified as the extract abbreviation and ppm. The fresh pork sausage was placed on polystyrene trays, overwrapped with PVC stretch film, and stored frozen for 3 m at -20°C. After frozen storage, treatments were displayed under fluorescent retail lighting for 21 d. Oxidation was measured by a descriptive panel, a consumer sensory panel, and TBA on 0, 7,14, and 21 d. The L* of treatments R1500 with G200 and R2000 with G100 ppm green tea extract both decreased ($P < 0.05$) by 2.2 points during the 21 d. The other treatments were not different ($P < 0.05$) between 0 and 21 d. The a* of all treatments decreased ($P < 0.05$) between 3.2 and 7.1 points during the 21 d. The control decreased ($P < 0.05$) the least by 3.2 points, however, was not different ($P > 0.05$) than any other treatment at 21 d. The b* increased ($P < 0.05$) by 1.2 to 2.6 points in all treatments except the control and R2500 with G300. The mg MDA/kg was not different ($P > 0.05$) for any of the treatments except the R1500 with G100 that increased ($P < 0.05$) by 1.5 mg MDA/kg at 7 d and decreased ($P < 0.05$) by 0.5 points at 14 d and control that decreased 2.1 mg MDA/kg between 0 and 14 d ($P < 0.05$). The authors found that although RGT did slow oxidation reactions, it did protect the meat color.

Acetic and Citric Acid

The effect of organic acids as antioxidants added to packaging and food has been studied primarily for antimicrobial efficacy and sometimes for antioxidant qualities. Lin et al. (2023) compared the quality effects of acetic acid (AA) and citric acid (CA) on ground pork stored at 0 °C for 8 d. Solutions of 16.7% AA and 33.3% CA were added at 0.5 or 1.0 g acid to 100 g of ground pork. The pork treatments were evaluated fresh and after heat treatment of 121°C for 10 or 30 min. Adding acid to pork resulted in almost a 1.0 decrease in the raw pork pH. There was not a significant interaction of time and treatment on the TBA. The TBA of 1 g CA, however,

was 0.56 mg MDA/kg, lower than the 0.66 mg MDA/kg of 0.5 g AA and the 0.76 mg MDA/kg of control. The TBA of pork heated for 10 min increased from 0.74 to 0.85 mg MDA/kg between 4 and 8 d, and the TBA of pork heated for 30 min increased from 0.67 to 0.78 mg MDA/kg between 0 and 8 d ($P < 0.05$).

Antioxidants have also been applied to packaging. A study by Contini et al. (2014) analyzed the antioxidant effect of adding a citrus based extract to packaging on cooked turkey breast and storing the meat at 4°C for 4 d. The TBA of treatments packaged in untreated packaging increased ($P < 0.05$) with time: 0 d was 1.14 mg MDA/kg, 2 d was 4.67 mg MDA/kg, and 4 d was 6.29 mg MDA/kg. The TBA of treatments stored in citric acid treated trays were lower ($P < 0.05$) at 2 and 4 d, 2.02 and 2.85 mg MDA/kg, respectively.

Conclusion

Global supply chains allow the meat industry to purchase and sell to larger markets. The protection provided by the three sisters enables global trade and facilitates science led policies. The SPS Agreement supports international trade by setting framework for trade and establishing rules of engagement during conflict. Without these regulations, trade could be barred by political barriers rather than science-based concerns. In addition, human, animal, and plant health are protected when science plays a part in trade protection.

When trade is protected, opportunities for industries to buy ingredients from around the world become possible. A longer shelf-life is necessary to allow for the transport of goods. Oxidation will eventually limit frozen pork shelf-life due to flavor, aroma, and color changes despite a longer shelf-life than refrigerated pork. Oxidation may be monitored in a variety of ways using TBA or GC analyses, measuring products of oxidation, using sensory analysis, and instrumental color evaluation.

Improving pork quality, increasing freezing rates, optimizing frozen storage, choosing effective packaging, and adding antioxidants are methods for extending pork shelf-life by limiting oxidation reactions. An opportunity to research antioxidants added directly to frozen pork trim has not been explored thoroughly in literature thus far despite in-depth research having been conducted in other meat products. Unfortunately, no one technique is completely successful by itself, and none of the treatments in the studies mentioned above were successful in stopping lipid oxidation reaction entirely. Although oxidation is an inevitable reaction during frozen pork storage, taking a multiple hurdle approach and monitoring multiple areas of interest during the processing of pork trim will limit oxidation and its unappealing sensory qualities. The objective of this research was to compare rosemary extract, rosemary green tea extract, citric acid based buffered vinegar, and nitrite as antioxidants in pork 42 trim over 12 m of frozen storage.

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Chapter 2 - Control of oxidation with antioxidants during frozen pork shelf-life

Introduction

Frozen storage extends the shelf-life of pork due to reduced enzymatic and microbial activity; however, freezing pork also results in muscle and fat tissue damage and oxidative changes of aroma, flavor, and color (Damodaran et al., 2008; Wheeler et al., 2015). The flavor and aromas characteristic of oxidation, described as warmed over, rancid, cardboardy, painty, and fishy in fresh pork (Huang and Ahn, 2019; Wheeler et al., 2015), will present themselves in a finished product if fresh pork is further processed (Huang and Ahn, 2019). Browning color is another result of oxidation (Damodaran et al., 2008; Lu et al., 2022; Medic et al., 2018). Oxidative color changes are unappealing to consumers (Huang and Ahn, 2019). Benefits of an extended frozen pork shelf-life, including supply chain and production schedule flexibility, are reasons to reduce oxidation rates.

Pork Quality and Lipid Oxidation

Lipid oxidation potential is impacted by pork quality. The total fat content of a pork product does not have as much impact on lipid oxidation as does the fatty acid profile (Damodaran et al., 2008). Lipid oxidation potential increases with the amount of unsaturated (UFA) and polyunsaturated fatty acids (PUFA), having an inverse relationship with saturated fatty acids (SFA) (Damodaran et al., 2008). The fatty acid profile is determined by factors including the pig's gender, age, diet, and season of harvest (Hallenstvedt et al., 2012; Monahan et al. 1990; Morel et al., 2006; and Wood et al., 2008). Wood et al. (2008) reviewed the research of multiple studies on fat and meat quality. They found intact males had the highest rates of UFA and PUFA, whereas female pigs had the lowest rates (Wood et al., 2008). The SFA content

increased with age (Wood et al., 2008). Research also indicates that the fatty acid profile of pig feed will translate to the fatty acid profile of the pork fat (Wood et al., 2008; Morel et al., 2006; and Hallenstvedt et al., 2012), and antioxidants in feed will result in antioxidants in the pork (Monahan et al. 1990; Morel et al., 2006; Wood et al., 2008). Finally, pork tends to be lower in PUFA when harvested during the spring and summer (Wood et al., 2008). Despite these natural variations, the average pork fatty acid profile is 38% SFA, 50% monounsaturated fatty acids (MUFA), and 12% PUFA (USDA, 2021).

Antioxidants

One method to reduce oxidation in pork products is adding antioxidants, such as organic acids, nitrite, rosemary extract, or a rosemary and green tea extract blend. Organic acids have been studied as an antioxidant in ground pork. Lin et al. (2023) added solutions of 16.7% acetic acid (AA) and 33.3% citric acid (CA) at either 0.5 or 1.0 g acid to 100 g of ground pork stored at 0°C for 8 d. Adding the acids decreased ($P < 0.05$) pH. There was no interaction ($P < 0.05$) of TBA with storage time and treatment. The 1.0 g CA treatment at 0.56 mg MDA/kg was lower ($P < 0.05$) than the 0.5 g AA treatment and control at 0.66 and 0.76 mg MDA/kg, respectively. In this study, the addition of acid resulted in a pH shift which could be solved with addition of buffered vinegar instead.

Nitrite has been successful at limiting oxidation in fully cooked pork sausage formulated with 50, 100, or 150 mg/kg sodium nitrite and stored at 4°C for 15 d (Karwowska et al., 2020). At 15 d, the sausages produced with 150 and 100 mg/kg sodium nitrite were lowest ($P < 0.05$) in MDA at 1.57 and 1.75 mg MDA/kg, respectively. The sausage with 50 mg/kg sodium nitrite was higher ($P < 0.05$) at 2.47 mg MDA/kg. The control, without cure, was the highest ($P < 0.05$) at

3.14 mg MDA/kg. Creating a cured pork trim supply could be an economical solution, even at various levels, if the trim is used in a cured product.

Since the demand for natural antioxidants is increasing (Domínguez, 2019; Huang and Ahn, 2019), many natural antioxidants are derived from plants (Huang and Ahn, 2019). Although effective at decreasing oxidation (Schilling et al., 2018; Sebranek et al., 2005), many natural antioxidants carry flavors from their source (Huang and Ahn, 2019). Natural antioxidants may be more effective than synthetic antioxidants (Sebranek et al., 2005). A study by Sebranek et al. (2005) compared 1500 or 2500 ppm of rosemary extract to a synthetic BHA and BHT blend in frozen, ready-to-eat sausage over 16 wk. The synthetic blend of BHA and BHT exceeded 2.5 mg MDA/kg whereas the two levels of rosemary extract did not exceed 1.5 mg MDA/kg. This study showed that adding rosemary as a clean label solution is effective and may be more effective than synthetic alternatives in pork products.

Schilling et al. (2018) studied blends of rosemary extract (R) at 1500, 2000, or 2550 ppm with green tea extract (GT) at 100, 200, or 300 ppm in fresh pork sausage. Treatments were stored on overwrapped polystyrene trays for 3 m at -20°C and transferred to fluorescent retail display for 21 d. Treatments were labeled with the extract abbreviation and ppm. The MDA was similar ($P < 0.05$) for all treatments except the R1500 with G100 that increased ($P < 0.05$) by 1.5 mg MDA/kg at 7 d and decreased ($P < 0.05$) by 0.5 mg MDA/kg at 14 d. The control decreased ($P < 0.05$) to 2.1 mg MDA/kg between 0 and 14 d. These results indicate that a blend of RGT is effective at reducing oxidation and could potentially work in other pork products such as pork fat trim.

Although there is limited published research on the application of antioxidants in pork trim, slowing lipid oxidation reactions with antioxidant ingredients reduces oxidation in pork

products. There is opportunity to learn more about antioxidants added to pork trim, especially since food matrices impact antioxidant functionality and capability (Haak et al., 2009). The objective of this research was to compare the effect of adding 70 ppm nitrite, citric acid-based vinegar buffered to 6.0 to 7.0 pH at 0.35% of the total formula, rosemary extract at 0.3% of the total formula, and rosemary green tea (RGT) at 0.28% of the total formula to ground pork fat trim and evaluating quality and oxidation rates during 12 m of storage at -17°C.

Materials and Methods

Experimental Design

Processing equipment and storage used within this study was proprietary. Pork fat trim, targeted at 58 ± 2% fat content, was collected from hams within 24 h of slaughter and processed at Quality Pork International, Inc. (Omaha, NE). Treatments were ground through a 12.7 mm plate and x-rayed. Forty-five 350 kg batches of trim were collected at fall. Nine batches were assigned, in order, to a proprietary antioxidant blend: control with no functional ingredients added, 70 ppm sodium nitrite, rosemary extract at 0.3% of the total formula, rosemary green tea at 0.28% of the total formula, and citric acid-based vinegar at 0.35% of the total formula. Treatments were blended for three min. The control was not blended since no additional ingredients were added. Although this could have introduced another variable, this is how an untreated pork trim would be processed in industry. The entire amount of pork trim in the blender was pumped out and packaged. Approximately 11.34 kg of pork trim was packaged into plastic, heat-sealed bags, and two bags were placed into a corrugate box with a case weight of 22.68 kg. No wash downs of equipment were completed between batches or treatments; however, the amount of pork trim that could be left in the equipment (approximately 68 kg) was pumped out, packaged, and disposed of at the beginning of each batch. Treatments were quick

frozen at -26°C with a 40 km/h wind speed (wind chill of -42°C) until an equilibrated temperature of -28°C was reached within approximately 26 h. Once frozen, the cases were shipped in temperature controlled semi-trailers set at -12.2°C. Treatments were then transferred to a commercial freezer set at -17°C and stored until the assigned sample date.

Thirteen boxes were randomly assigned a code and one of six sample dates each month. One week prior to each designated sample date, the box was pulled from the freezer to thaw in a commercial cooler to approximately 4.5°C, and one of the two bags was randomly chosen to be sampled. On the test date, a cross section of each treatment replication for all analyses were taken from the middle of the bag.

Proximate and Fatty Acid Profile Analyses

At 0, 1, 2, 3, 5, and 6 m, 500 g from the center the bag was removed for proximate analyses. A 500 g sample from the center of the sample bag was removed at 0 m for fatty acid profiling. The treatments were vacuum sealed in S-7557 20 × 30 cm vacuum bags with 3 mil film (Uline, Pleasant Prairie, WI), packaged in shipping coolers with ice packs maintaining refrigerated temperatures (< 5°C), and overnight shipped to an external lab (Merieux NutriSciences Crete, Crete, IL) for testing. Fat and moisture were analyzed using the AOCS Approved Procedure Am 5-04 (2005), protein was analyzed using the AOAC 992.23 method (1998), and the fatty acid profile was tested using the AOAC 996.06 method (2010).

APC

The AOAC official method 2015.13 was followed for aerobic plate enumeration (Bird et al., 2019). Two 25 g samples were aseptically collected in a 2,041 mL Whirl-Pak Filter Sterilized Sample Bag (Whirl-Pak Filtration Group, Madison, WI). Each sample was diluted with 225 mL of Buffered Peptone Water (Biomérieux, Marcy-l'Étoile, France) and stomached for 30 s. The

sample was serial diluted and plated (3M Petrifilm™ Rapid Aerobic Count Plate, St. Paul, MN). The two plate counts were averaged for each sample.

pH

A 10 g sample was collected in a Whirl-Pack Bag (Whirl-Pak Filtration Group, Madison, WI). Within 24 h of sampling, 100 ml of deionized water was added to the Whirl-Pak Bag, and was stomached for three min. The liquid was filtered (Whatman 4 filter paper, Cytiva, China) into a beaker until no liquid remained in the filter. The pH of the liquid filtrate was analyzed using the Orion Star A211 pH meter using an Orion 8157BNUMD ROSS Ultra pH/ATC Triode (Thermo Scientific, Waltham, MA).

Hexanal by Gas Chromatography

Hexanal was analyzed using the procedures outlined by Park et al. (2013) with the following adjustments. A 1.0 ± 0.1 g sample was collected in a 20 mL clear glass headspace vial with a 1.5 mm Silicone screw top (GC Ferrules, Santa Clara, CA). Treatments were packaged in shipping coolers with ice packs maintaining refrigerated temperatures (< 5°C), and overnight shipped to an external lab (University of Wisconsin Madison, Madison, WI) to complete the testing. GC testing began at 2 m during this study.

Thiobarbituric Acid Testing

On each sample day, a 300 g sample from the center of the sample bag was removed for TBA. These treatments were vacuum sealed, packaged in shipping coolers with ice packs, and overnight shipped at refrigerated temperatures (< 5°C) to an external lab (Merieux NutriSciences Crete, Crete, IL) for testing. The TBA methods followed those described by Witte et al. (1970).

Colorimeter

After samples for sensory, chemical, and microbiological testing were removed, half of the remaining pork 42 trim was left in the package, and the sample bag was resealed by folding and sealing with packaging tape. Color measurements by a Chroma Meter CR-400 (Konica Minolta, Inc., Tokyo, Japan) were taken through the plastic packaging. The observer was set to 2 degrees and the primary was set to C. The CR-200/300/400 white calibration plate (Konica Minolta Inc, Tokyo, Japan) was used prior to the day's measurements. Three measurements of lean tissue and three measurements of fat tissue were taken and averaged.

Trained Sensory Panel

Panelists were screened for color, aroma, and flavor discrimination abilities followed by trainings based on American Meat Science Association (AMSA) recommendations (Wheeler et al., 2015). Panelists agreed on the magnitude of anchors based on a 100-point scale where 0 was none and 100 was intense aroma or flavor at the beginning of the study using their perceptions and AMSA guidelines (Wheeler et al., 2015). The AMSA definitions for rancid and cardboard were used: "Rancid: Aromatics commonly associated with oxidized fat and oils; may include cardboard, painty, varnish, and fishy," and "Cardboardy: Aromatic associated with slightly oxidized fats and oils, reminiscent of wet cardboard packaging" (Wheeler et al., 2015). Three oil treatments (Wesson Vegetable Oil Memphis, TN) were used as references: "clean oil" was poured from a fresh, sealed bottle, "oil 1" heated to 190°C and cooled, and "oil 2" heated to 190°C, cooled, frozen for 10 h, and defrosted in a microwave (1.7 cu ft Whirlpool Over the Range Microwave, Benton Harbor, MI) for 10 min. The clean oil, oil 1, and oil 2 were assigned a rancid aroma of 0, 25, and 50, respectively, and a rancid flavor of 0, 30, and 65, respectively. Cardboard aroma was anchored using dry and wet corrugate (Kraft 44 ECT C Flute corrugate,

International Paper, Fond du Lac, WI) cut into 3.8 cm diameter circles. To prepare the wet cardboard reference, the corrugate was dipped in filtered tap water for 3 s within 30 min of the beginning of the panel. The dry cardboard anchor was assigned an aroma of 5 and a flavor of 10, and wet cardboard was assigned an aroma of 45 and a flavor of 45. Lean color was anchored using the National Pork Board 6-point lean color scale (National Pork Board, 2010).

There were six panel dates each month with eight panelists in attendance. Treatments were prepared using AMSA guidelines for pan broiling as a reference (Wheeler et al., 2015). A sample of each treatment was placed in a warmed pan (set at 107 °C to avoid browning). Samples were not formed into a patty and were served as a ground product. Samples were cooked to an internal temperature of 74°C. All treatments and anchors were served in 59.15 mL translucent plastic cups (Dart Container, Mason, MI). Cooked treatments were held at 50°C until given to the panelists. Panelists were instructed to expectorate all anchors and treatments. The panelists began by smelling and tasting anchors. Then panelists were given a sample to taste and talk about as a group. The panelists aligned on the magnitude of the sample's attributes as part of each panel's training. Each sample was evaluated first for aroma, then flavor, and finally color. Data was recorded using Red Jade sensory software (Red Jade Sensory Solutions, LLC, Martinez, California).

Several challenges impacted the sensory design of this study. First, most of the panelists were used to using a sensory scale to portray acceptability, so it took time to retrain to use the scale to represent magnitude. Also, this study began during COVID where restrictions limited the number of people allowed in one room, so the trainings were hosted in shifts. These factors did impact the length of time it took for panelists to rank similarly to each other, impacting the results of 0 to 2 m.

Statistical Design

The data analyses were generated by SAS Studio 3.8 with SAS 9.4 software (SAS Institute Inc., Cary, NC) using the PROC GLIMMIX procedure. Treatments were analyzed using a split-plot design with the whole plot factor of treatment and the subplot factor of month. One-way ANOVA was used to compare the proximate analyses and fatty acid profiles by treatment ($P < 0.05$). The effects of treatment and time on objective and sensory tests were analyzed using SAS software's least squared mean procedure ($P < 0.05$).

Results and Discussion

Interactions ($P < 0.05$) of time and treatment occurred with TBA, hexanal, fat a*, fat b*, rancid aroma, cardboard aroma, rancid flavor, cardboard flavor, and lean sensory color. Main effects ($P < 0.05$) for TPC, pH, fat L*, and lean L*, a*, and b* are shown by treatment and by month.

Proximate and Fatty Acid Profile Analyses

The least squared means of the fat, moisture, and protein analyses are listed in Table 1. The fat content of the control and vinegar treatments were higher ($P < 0.01$) than nitrite, rosemary, and RGT. The rosemary treatment had the lowest ($P < 0.01$) fat content of all treatments. The differences ($P < 0.01$) in fat, moisture, and protein among the treatments could have been due to process limitations of a commercial production facility and not randomizing collection of pork from different hog production facilities and lots. Although there were differences ($P < 0.01$) in fat content, Domínguez et al. (2019) argued that the fatty acid composition was more important for lipid oxidation potential than the fat content of the pork. These differences may also be due to small, randomized sample sizes of 300 g from to the 350 kg batch.

The fatty acid compositions of all treatments measured at 0 m are shown in Table 2. The SFA percentage of the treatments ranged between 30.75 and 34.24% ($P < 0.05$), lower than the average SFA content of 38% SFA published by the USDA (2021). Rosemary was lower ($P < 0.05$) than all other treatments. There were no differences ($P > 0.05$) in MUFA, PUFA, trans fatty acids (TFA), and conjugated fatty acids (CFA). The average MUFA content of the treatments was lower than the average MUFA content of 50% published by the USDA (2021). The PUFA content was higher than the average PUFA content of 12% published by the USDA (2021). Even though the PUFA content was higher by approximately 2%, the SFA was higher by approximately 21.50% higher than the pork industry average. Given the difference in fatty acid profile of this pork, the oxidation potential of this pork supply may be lower than average.

APC

The total aerobic plate count least squared means is shown by month in Figure 4A and by treatment in Figure 4B. There are differences ($P < 0.05$) during the 12 m of frozen storage, but the changes are inconsistent, varying by 0.51 $\log_{10}$ CFU/g. The nitrite treatment at 2.55 $\log_{10}$ CFU/g was lower ($P < 0.05$) than the rosemary, control, and RGT by 0.24 to 0.25 $\log_{10}$ CFU/g. This could be attributed to the antimicrobial effects of nitrite. None of the months or treatments exceeded 7 $\log_{10}$ CFU/g which is when microbial growth causes changes in flavor, smell, and texture in meat (Horváth et al., 2007).

Custódio et al. (2018) compared refrigerated pork loin and leg stored at 5°C for 16 d and frozen at -18°C for 180 d. The mesophilic growth of loin and leg was at 3.90 and 4.01 $\log_{10}$ CFU/g and 4.14 and 4.31 $\log_{10}$ CFU/g psychrophiles at the beginning of the study. The research did not enumerate bacterial growth during frozen storage; however, the initial microbial load and

Table 1. Least squared means of proximate analyses (% by weight) by treatment[1].

	Treatment						
Proximate Analyses	Control	Vinegar	Nitrite	RGT [2]	Rosemary	SEM[3]	*P*
Fat	61.65[a]	61.15[a]	59.65[b]	59.38[b]	57.34[c]	0.51	< 0.01
Moisture	30.21[c]	30.32[c]	31.07[cb]	31.66[b]	32.75[a]	0.34	< 0.01
Protein	8.18[c]	8.13[c]	8.49[bc]	8.71[ab]	8.97[a]	0.13	< 0.01

[1]n=9.

[2]Rosemary Green Tea.

[3]Standard error of the mean.

[a-c]Means in the same row with different superscripts are different ($P < 0.05$).

Table 2. Least squared means of fat composition (g/g of fat) by treatment[1].

	Treatment						
Fatty Acid Type	Control	Vinegar	Nitrite	RGT [2]	Rosemary	SEM[3]	*P*
Saturated Fatty Acids	34.24[a]	33.68[a]	32.81[a]	33.28[a]	30.75[b]	0.69	< 0.05
Monounsaturated Fatty Acids	44.80	43.67	42.76	43.75	40.85	0.93	= 0.06
Polyunsaturated Fatty Acids	14.28	14.09	13.52	13.21	12.72	0.44	= 0.10
Trans Fatty Acids	0.38	0.30	0.33	0.31	0.31	0.04	= 0.53
Conjugated Fatty Acids	0.12	0.11	0.11	0.11	0.11	0.00	= 0.06

[1]n=9.

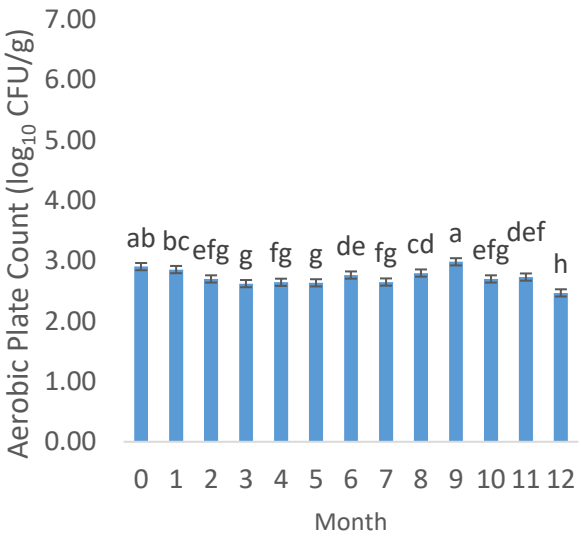
[2]Rosemary green tea.

[3]Standard error of the mean.

[a-b]Means in the same row with different superscripts are significantly different ($P < 0.05$).

the highest microbe count at 9 m of the current research was lower than the initial microbial load in this study by Custódio et al. (2018).

A)



B)

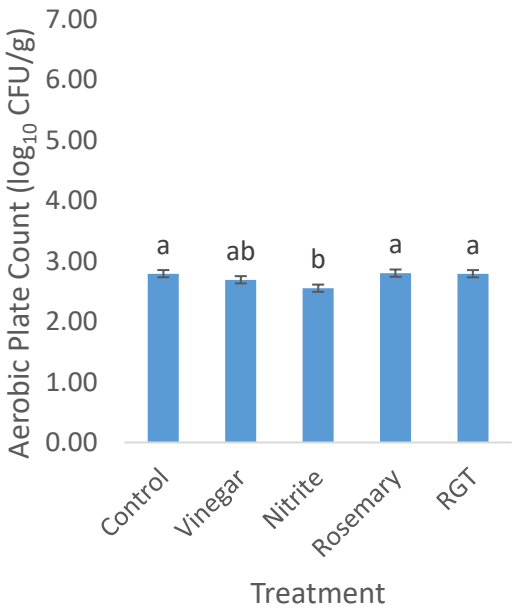


Figure 4. A) Main effects of month (n=45) on least squared mean aerobic plate count (log). Standard error = 0.040. [a-h] Least squared means with different superscripts at each month are different ($P < 0.05$). B) Main effects of treatment (n=117) on least squared mean aerobic plate count (log). Standard error = 0.06. [a-b] Least squared means of treatments with different superscripts are different ($P < 0.05$).

pH

The pH data is shown in Figure 5. There was a difference ($P < 0.05$) of 0.44 pH units during 12 m of frozen storage, and a difference ($P < 0.05$) of 0.04 pH among the treatments. The lowest ($P < 0.05$) pH was 5.79 at 1 m and the highest ($P < 0.05$) pH was 6.23 at 11 m. The pH range by month and by treatment were within the range found by Holmer et al. (2009) in fresh pork loins used to study the effect of pH on quality. A sample of 39 pork loins were selected out of 306 pork loins representing the range of 5.42 to 6.26 pH. A study by Custódio et al. (2018) comparing pork refrigerated at 5°C for 16 d or at -18°C for 180 d found no changes ($P< 0.05$) in pH of frozen pork.

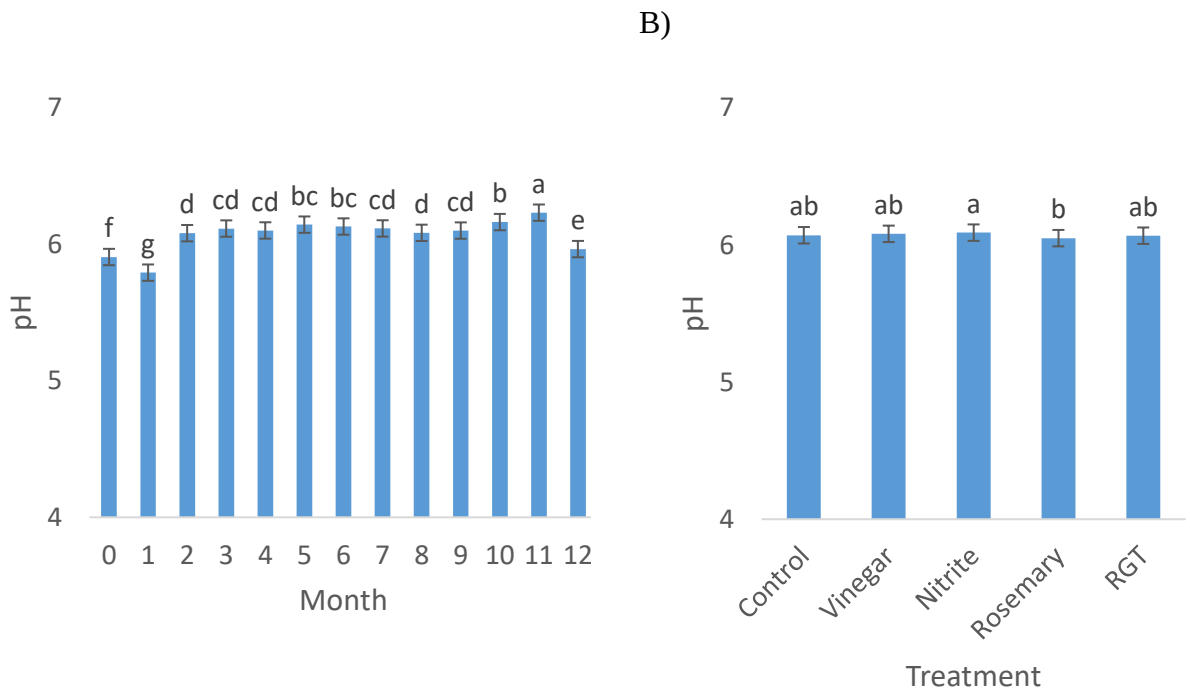


Figure 5. A) Main effects of month (n=45) on least squared mean pH. Standard error 0.04. [a-f] Least squared means with different superscripts at each month are different ($P < 0.05$). B) Main effects of treatment (n=117) on least squared mean pH. Standard error = 0.06. [a-b] Least squared means with different superscripts at each month are different ($P < 0.05$).

Hexanal by Gas Chromatography

The hexanal profile changes, beginning in month 2, are shown in Figure 6. The hexanal content of rosemary and RGT were consistently at or below 0.1 mg hexanal/kg, however, the nitrite, control, and vinegar treatments increased ($P < 0.05$) and decreased ($P < 0.05$) throughout the study. All the treatments were similar ($P > 0.05$) at 11 and 12 m. While there were differences ($P < 0.05$) in hexanal content throughout the study, these differences ($P < 0.05$) were likely not an indicator of sensory changes since the differences were less than 1.0 mg hexanal/kg, a change that did not elicit a sensory difference in a study by Bak and Richards (2021).

This study by Bak and Richards (2021) compared two brands of rosemary extract at 200, 400, and 600 mg/kg in turkey deli meat, chicken fillets, and pulled pork held refrigerated for 13, 7, and 13 wk, respectively. Chicken fillets and pulled pork were produced with and without phosphates. The treatments were evaluated for hexanal by GC and ranked by a trained panel on a 6-point scale where 2.0 was the detection limit for the panel. There were no differences ($P < 0.05$) in sensory scores ranging from 2.2 and 2.5 where the hexanal ranged ($P < 0.05$) from 0.06 to 0.16 mg hexanal/kg in control cured turkey deli meat. No practical sensory differences ($P < 0.05$) of sensory scores between 1.5 and 2.0 were found between control and antioxidant treatments in chicken fillets where the hexanal ranged ($P < 0.05$) from 0.23 to 1.52 mg hexanal/kg. The pulled pork had average sensory scores between 1.8 and 3.1 where the hexanal ranged ($P < 0.05$) from 0.09 to 1.26 mg hexanal/kg. None of the treatments were rejected based on sensory at these hexanal levels.

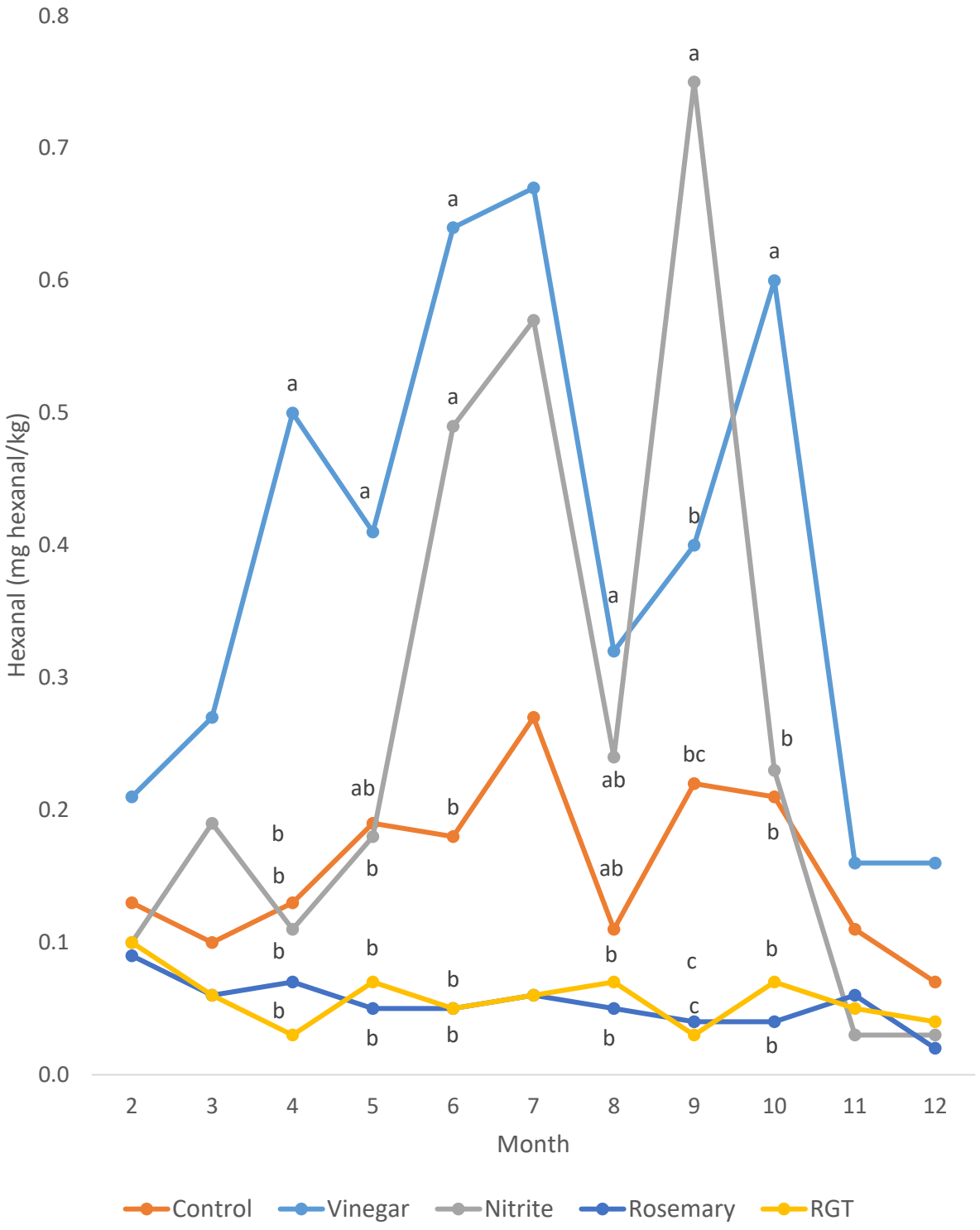


Figure 6. Main effect of month on hexanal content (mg hexanal/kg) by treatment (n=9). Standard error = 0.80. [a-c] Least squared means with different superscripts at each month are different ($P < 0.05$).

Thiobarbaturic Acid Testing

The TBA test results in Figure 7, showed rosemary and RGT treatments were effective ($P < 0.05$) at limiting oxidation reactions during the study whereas oxidation occurred more rapidly ($P < 0.05$) in control, nitrite, and vinegar treatments. The rosemary and RGT treatments remained below 0.20 mg MDA/kg, and the control, vinegar, and nitrite treatments increased ($P < 0.05$) during the 12 m of storage.

The vinegar treatment at 0.67 mg MDA/kg was higher ($P < 0.05$) by 0.51 to 0.62 mg MDA/kg than the control, nitrite, RGT, and rosemary at 0 m. The vinegar treatment, however, decreased to 0.34 in 2 m where it was not different ($P < 0.05$) than control or nitrite. At 3 m, nitrite, vinegar, and control treatments began to increase ($P < 0.05$). By 6 m, the vinegar treatment at 0.73 mg MDA/kg was again higher ($P < 0.05$) than nitrite and control at 0.39 and 0.39 mg MDA/kg. All three of these treatments were higher ($P < 0.05$) than RGT and Rosemary at 0.06 and 0.08 mg MDA/kg. The TBA results dropped unexpectedly at 7 m for the vinegar, control, and nitrite treatments, however, the reason is unclear. Vinegar and control at 0.31 and 0.29 mg MDA/kg, however, remained higher ($P < 0.05$) than the rosemary treatment 0.05 mg MDA/kg, and nitrite and RGT treatments at 0.19 and 0.10 mg MDA/kg were similar ($P < 0.05$) to control, vinegar, and rosemary treatments. These MDA levels were below the 0.50 mg MDA/kg, the sensory threshold of oxidation (Custódio et al., 2018; Gray and Pearson, 1987; Hansen et al., 2004; Miles et al., 1986; Tarladgis et al., 1960). By month 8. Vinegar, nitrite, and control treatments were higher ($P < 0.05$) than rosemary and RGT and continued to be through the remaining months. At 12 m, there was the widest range of MDA with vinegar, control, and nitrite at 1.03, 0.87, and 0.78 mg MDA/kg, respectively, higher ($P < 0.05$) than RGT and rosemary at 0.08 and 0.06 mg MDA/kg, respectively.

According to multiple studies, 0.50 mg MDA/kg is the threshold where most trained sensory panels will identify oxidation in pork (Custódio et al., 2018; Gray and Pearson, 1987; Hansen et al., 2004; Miles et al., 1986; Tarladgis et al., 1960). Neither the rosemary or RGT treatments exceeded levels of MDA that could elicit a sensory change (Custódio et al., 2018; Gray and Pearson, 1987; Hansen et al., 2004; Miles et al., 1986; Tarladgis et al., 1960). Only one treatment, vinegar at 12 m, exceeded 1.0 mg MDA/kg, the level Miles et al. (1986) identified as the consumer sensory threshold for pork (Miles et al., 1986).

The reduced rates of oxidation by rosemary and RGT blends has been observed in other studies (Schilling et al., 2018; Sebranek et al., 2005). Schilling et al. (2018) observed no differences ($P < 0.05$) in MDA in fresh pork sausage produced with rosemary and green tea extracts when stored under refrigerated retail display for 21 d although MDA did range from 2.3 to 4.6 mg MDA/kg, at a threshold most consumers would notice. Sebranek et al. (2005) compared a synthetic BHA and BHT blend to rosemary extract antioxidants in ready-to-eat sausage with 35% fat. The rosemary extract at 1500 and 2500 ppm did not exceed 1.5 mg MDA/kg whereas the control and a synthetic BHA and BHT blend were higher ($P < 0.05$), above 2.5 mg MDA/kg. Karwowska et al. (2020) found differing results when adding sodium nitrite to ready-to-eat pork sausage stored at 4°C for 15 days. Sausage with 50, 100, or 150 mg/kg at 2.47, 1.75, and 1.57 mg MDA/kg, respectively, were lower ($P < 0.05$) in MDA compared to the uncured sausage at 3.14 mg MDA/kg. Lin et al. (2023) did not record an interaction ($P < 0.05$) of time and treatment on MDA when 16.7% AA and 33.3% CA were added at 0.5 or 1.0 g to 100 g of ground pork stored raw at 0°C. The MDA of 1g CA at 0.56 mg MDA/kg was lower ($P < 0.05$) than 0.5 g AA at 0.66 mg MDA/kg and the control, which contained no organic acids, at 0.76 mg MDA/kg.

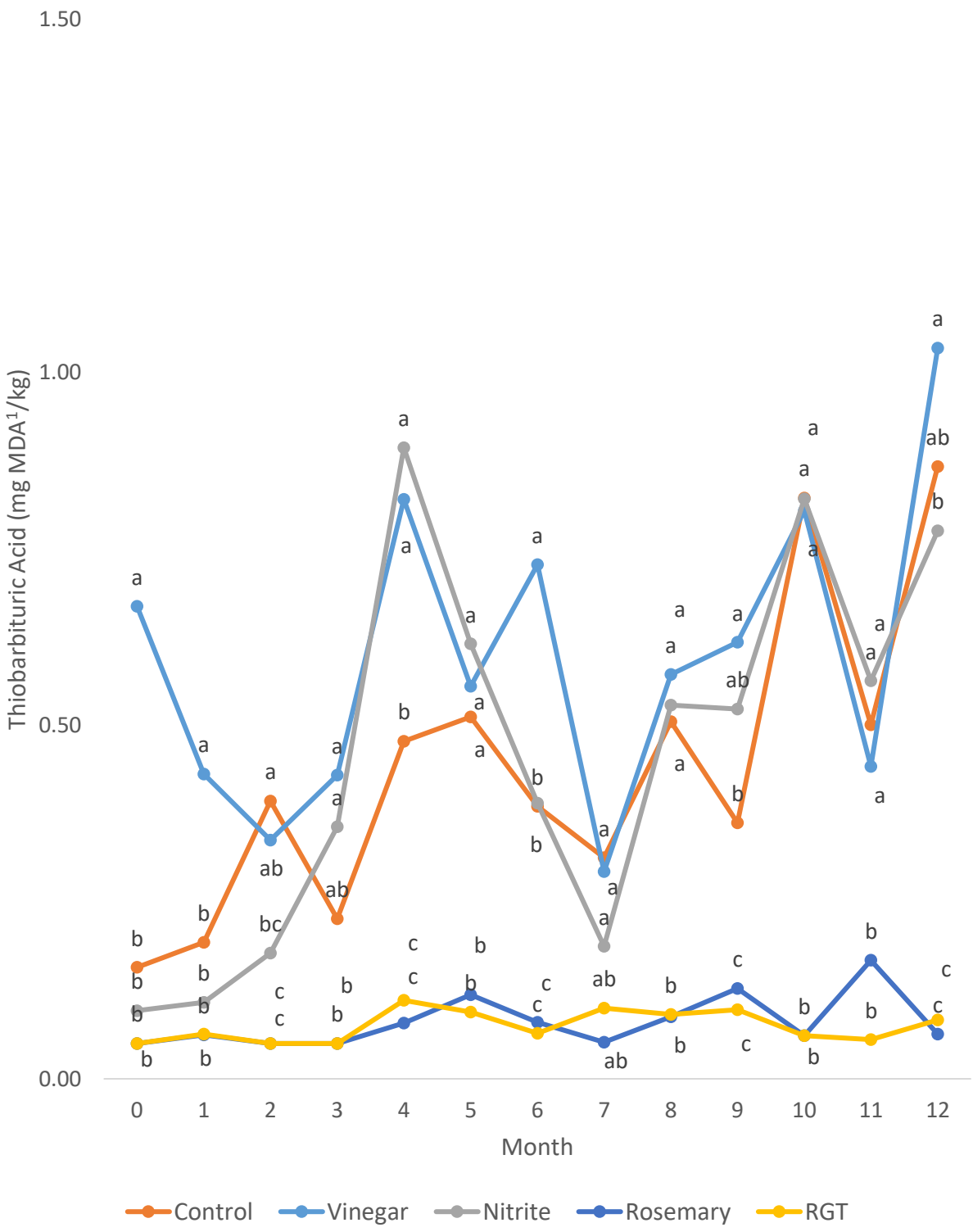


Figure 7. Main effect of month on Malonaldehyde content (g MDA/g) by treatment (n=9). [1]MDA=Malonaldehyde. Standard error = [a-c] Least squared means with different superscripts at each month are different ($P < 0.05$). Standard error = 0.075.

Colorimeter

Colorimeter has three measurements: L*, measuring black (0) to white (100); a*, measuring red (positive) to green (negative); and b*, measuring yellow (positive) to blue (negative) (Contini et al., 2014; Lu et al., 2022).

The L* of fat and protein are shown in Tables 3 and 4. The L* of fat varied by 2.26 and the L* of lean varied by 3.71. There was not a difference ($P < 0.05$) between 0 and 12 m for the fat or lean tissues. The fat L* of control and vinegar treatments were higher ($P < 0.05$), whiter, by approximately 1.00 than the rosemary and RGT treatments. Vinegar was higher ($P < 0.05$) than nitrite, rosemary, and RGT treatments by about 0.87 to 1.19, respectively. Nitrite was higher ($P < 0.05$) than the RGT treatment by 0.32.

Table 3. Least squared means of fat and lean L* by month[1].

Month	Fat[2]	Lean[3]
0	80.08^{bcd}	61.80^{cd}
1	79.93^{cde}	61.90^{cd}
2	80.31^{abc}	64.18^{a}
3	79.99^{cd}	65.04^{a}
4	79.37^{ef}	65.01^{a}
5	79.13^{fg}	63.94^{ab}
6	78.56^{g}	62.24^{cd}
7	79.54^{def}	62.81^{bc}
8	80.81^{a}	62.86^{bc}
9	80.62^{ab}	62.29^{cd}
10	80.82^{a}	61.33^{d}
11	80.72^{a}	62.18^{cd}
12	80.38^{abc}	61.46^{d}

[1]n=9.

[2]Sandard error of the mean = 0.21. $P < 0.01$.

[3]Standard error of the mean = 0.49. $P < 0.01$.

[a-c]Means in the same row with different superscripts are different ($P < 0.05$).

Table 4. Least squared means of fat and lean L* by treatment[1].

	Treatment						
	Control	Vinegar	Nitrite	Rosemary	RGT [2]	SEM[3]	*P*
Fat	80.43^{ab}	80.65^{a}	80.08^{bc}	79.60^{cd}	79.34^{d}	0.18	< 0.01
Lean	63.23^{ab}	63.56^{a}	62.69^{b}	62.39^{ab}	62.37^{ab}	0.41	< 0.01

[1]n=9.

[2]Rosemary Green Tea.

[3]Standard error of the mean.

[a-c]Means in the same row with different superscripts are different ($P < 0.05$).

The main effects of month and treatment on the fat a* are shown in Figure 8. Nitrite was lower than all other treatments by 1.47 to 1.77, respectively, at 0 m and was lower ($P < 0.05$) than all other treatments at 0, 1, 2, 3, 10, and 12 m. The a* of fat of all treatments, control, rosemary, RGT, vinegar, and nitrite decreased with time until 9 m. Rosemary and RGT were not different ($P < 0.05$) than control until 10 m when RGT and Rosemary began to increase to 3.33 and 3.71, respectively, at 11 m. Vinegar was similar ($P < 0.05$) to control, though was lower than control and all other treatments at 2 m at 2.79, 8 m at 1.35, and 11 m at 1.05. Control, vinegar, and nitrite decreased ($P < 0.05$) by 2.01, 1.95, and 1.48 during the 12 m. These changes, larger than 0.95, may be discriminated by a trained panel selected for color discrimination, although may be too small for consumer discrimination (Mancini et al., 2022).

The main effects of month as well as treatment on least squared mean a* are shown in Figures 9A and 9B. The lean a* ranged ($P < 0.05$) from 9.27 at 0 m to 7.90 at 5 m. The treatments ranged from rosemary at 7.71 which was lower ($P < 0.05$) than nitrite at 9.53. Although this difference ($P < 0.05$) of 1.82 could potentially be identified by a trained panel, the range of 1.37 is likely not identifiable by the average consumer (Mancini et al., 2022).

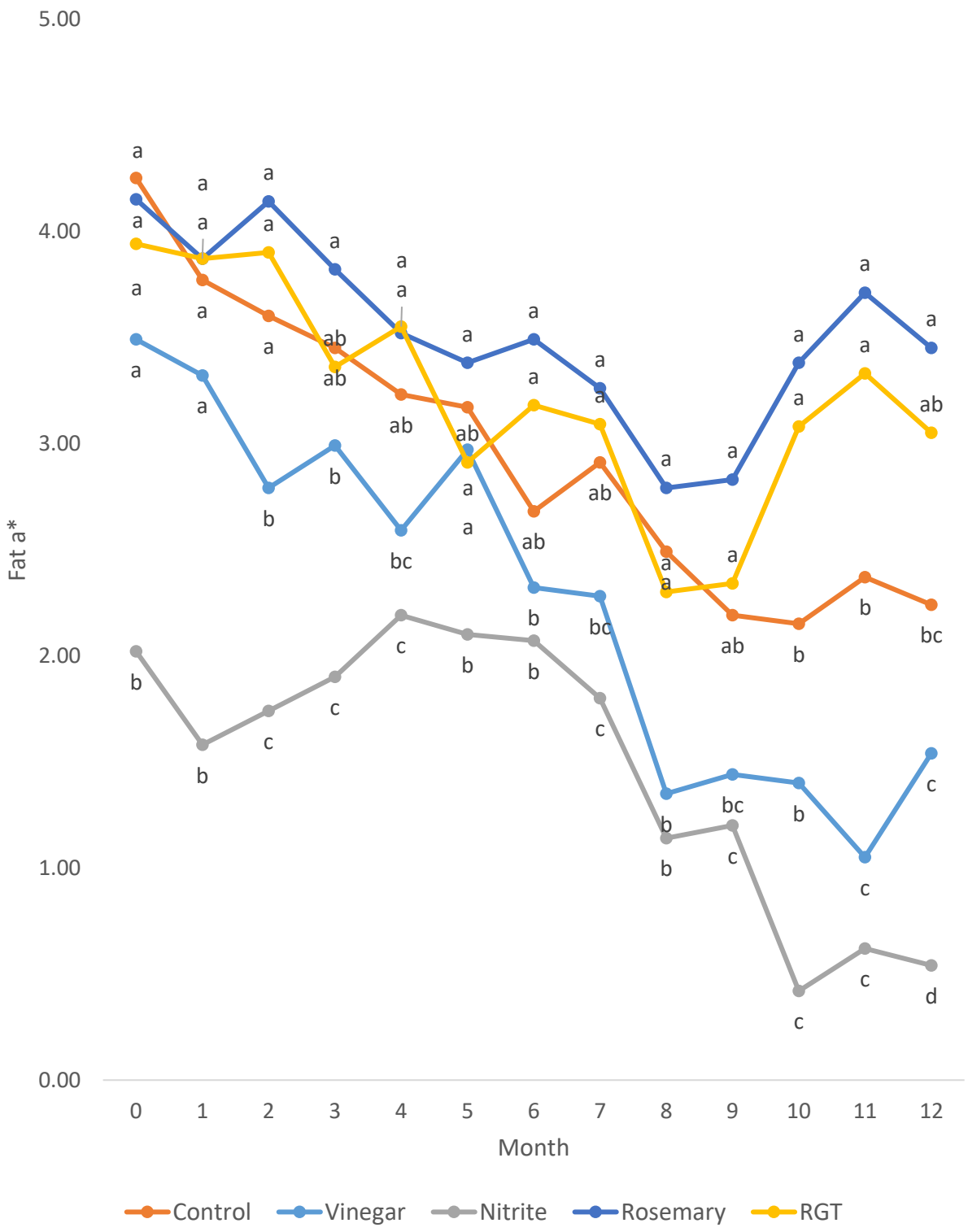


Figure 8. Main effect of month on Fat a* by treatment (n=9). [a-c] Least squared means with different superscripts at each month are different ($P < 0.05$). Standard error = 0.30.

A) B)

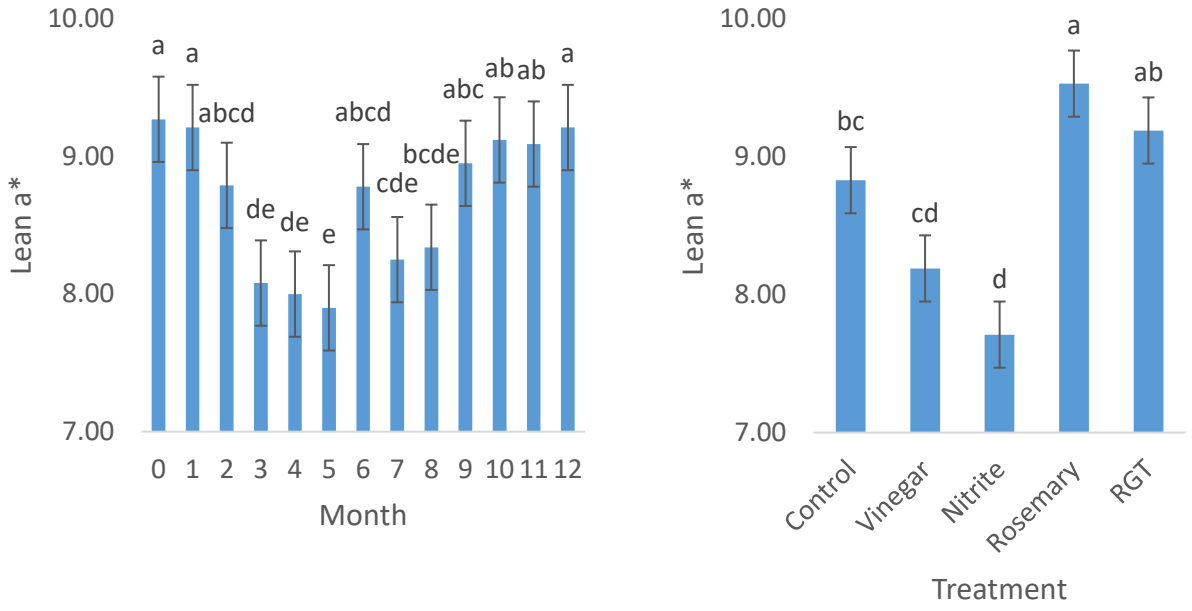


Figure 9. A) Main effects of month (n=45) on least squared mean lean a*. Standard error = 0.31. [a-e] Least squared means with different superscripts at each month are different (*P* < 0.05). B) Main effects of treatment (n=117) on least squared mean pH. Standard error = 0.24. [a-b] Least squared means with different superscripts at each month are different (*P* < 0.05).

The main effects of month and treatment on fat b* are shown in Figure 10. Although there are differences ($P < 0.05$), the least squared means of all treatments and months only varied by 1.33. This small difference, although detectable by a trained panelist, may not be perceived by the average consumer (Mancini et al., 2022).

The lean b* main effects of month and treatment are shown in Figures 11A and 11B, respectively. Although there were differences ($P < 0.05$) among the months, the range of 1.02 was again lower than most consumers will perceive (Mancini et al., 2022). Nitrite was lower ($P < 0.05$) than control, RGT, and rosemary. The difference ($P < 0.05$) between nitrite at 10.36 and control at 11.05 was only 0.69. This difference ($P < 0.05$) is lower than a trained panel will likely perceive (Mancini et al., 2022).

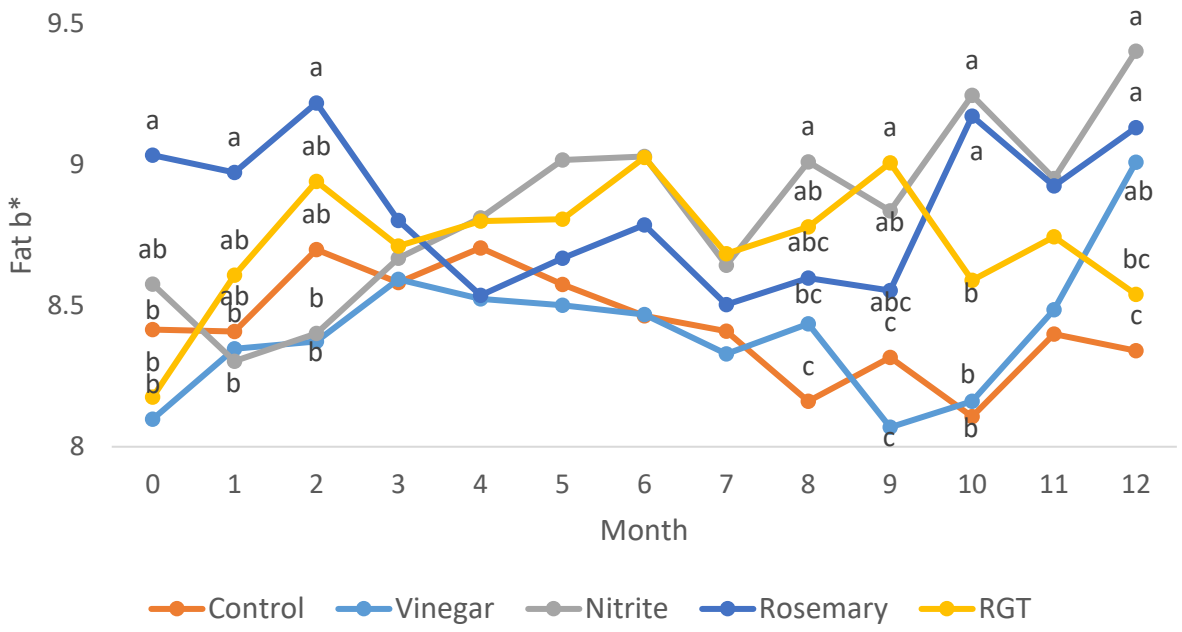
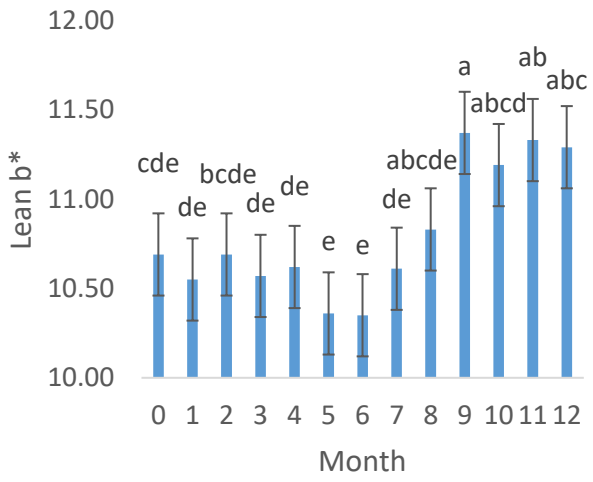


Figure 10. Main effect of month on Fat b* by treatment (n=9). [a-c] Least squared means with different superscripts at each month are different (*P* < 0.05). Standard error = 0.21.

A)



B)

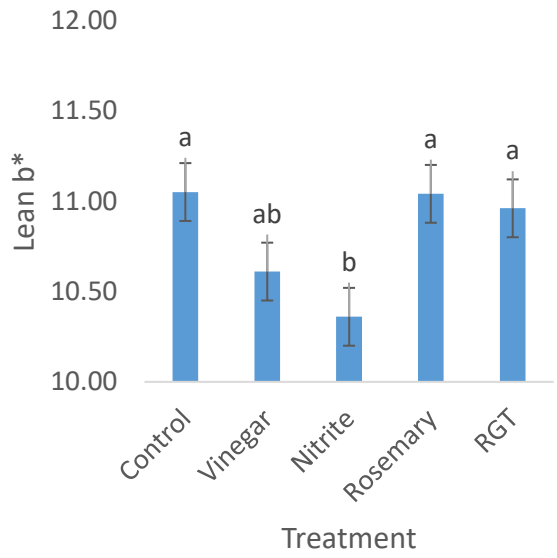


Figure 11. A) Main effects of month (n=45) on least squared mean lean b*. Standard error = 0.23. [a-e] Least squared means with different superscripts at each month are different (*P* < 0.05). B) Main effects of treatment (n=117) on least squared mean lean b*. Standard error = 0.16. [a-b] Least squared means with different superscripts at each month are different (*P* < 0.05).

Trained Sensory Panel

The sensory analyses of aroma, flavor and color were inconclusive during 0 to 2 m due to inconsistent panelist scores; however, after further training, panelists became more consistent in scoring. This was likely due to panelist prior sensory training to portray acceptability with a scale rather than magnitude, and the inability to host a training with everyone in the same room at the same time due to COVID. All treatments for all attributes evaluated were below 6 on the 100 point scale each month through 11 m. At 11 m all vinegar treatments were removed from evaluations due to off flavors that the panelists described as meaty and sour flavors, not necessarily oxidation flavors as defined by the AMSA (Wheeler et al., 2015). Treatments were ranked on a 100-point scale.

The main effects of month and treatment on rancid aroma are shown in Figure 12. All treatments were below 6 on the 100-point scale at each month through 11 m. Nitrite and control treatments were higher ($P < 0.05$) at 3.7 and 3.4 rancid aroma, respectively, than the rosemary treatment at 1.5 rancid aroma. At 12 m, control at 11.0 rancid aroma was higher ($P < 0.05$) than the nitrite treatment at 6.9 rancid aroma. Rosemary and RGT treatments at 2.8 and 2.3 rancid aroma, respectively, were similar ($P < 0.05$) to each other and lower ($P < 0.05$) than nitrite and control treatments.

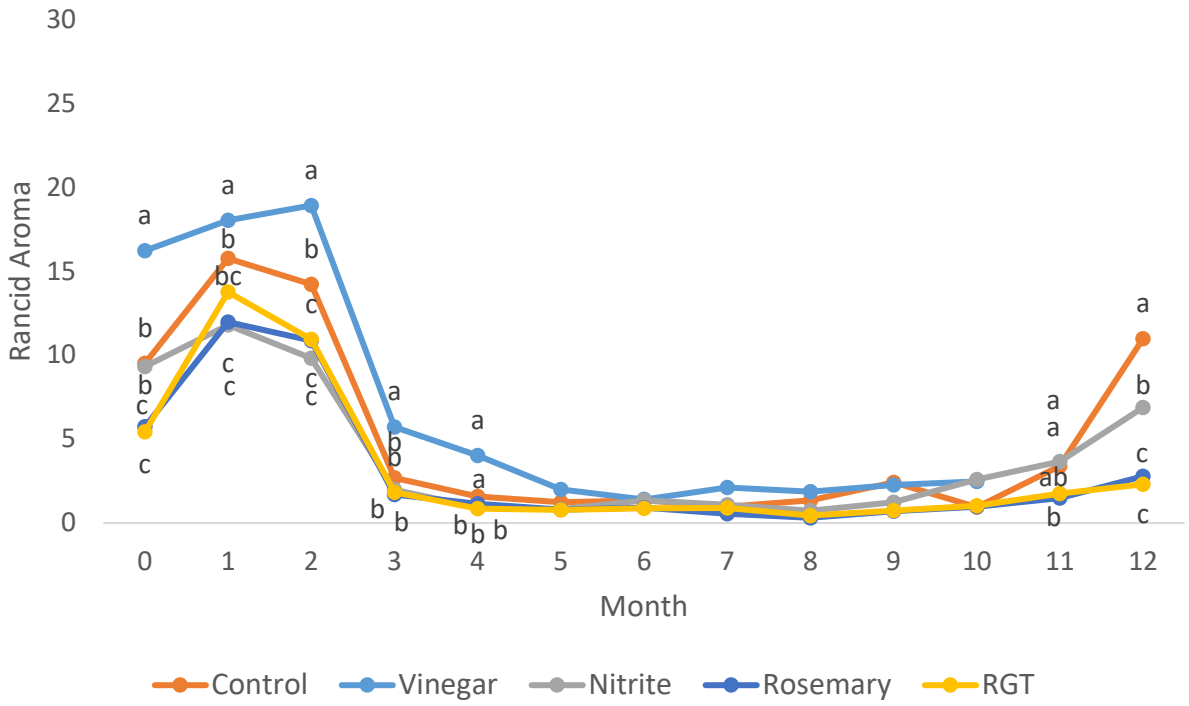


Figure 12. Main effect of month on rancid aroma where 0 is no rancid aroma and 100 is intense aroma by treatment (n=9). [a-c] Least squared means with different superscripts at each month are different ($P < 0.05$). Standard error = 0.79. *Panelists ranked aroma on a 100-point scale where 0 was no rancid aroma and 100 was intense rancid aroma.

The main effects of month and treatment on mean cardboard aroma are shown in Figure 13. At 3 m, the vinegar treatment at 5.3 cardboard aroma was higher than nitrite, rosemary, and RGT treatments at 4.5, 3.5, 3.0, and 2.6 cardboard aroma, respectively. All treatments were below 6 on the 100-point scale each month through 11 m. The treatments were different ($P < 0.05$) at 11 m. The control at 8.9 cardboard aroma was higher ($P < 0.05$) than rosemary and RGT treatments at 4.7 and 3.4 cardboard aroma, respectively. At 12 m, control at 17.9 cardboard aroma was again higher ($P < 0.05$) than nitrite, rosemary, and RGT treatments at 9.3, 6.4, and 5.7 cardboard aroma, respectively. Rosemary and RGT treatments were similar ($P < 0.05$) in cardboard aroma. The nitrite treatment, however, was higher ($P < 0.05$) than RGT in cardboard aroma.

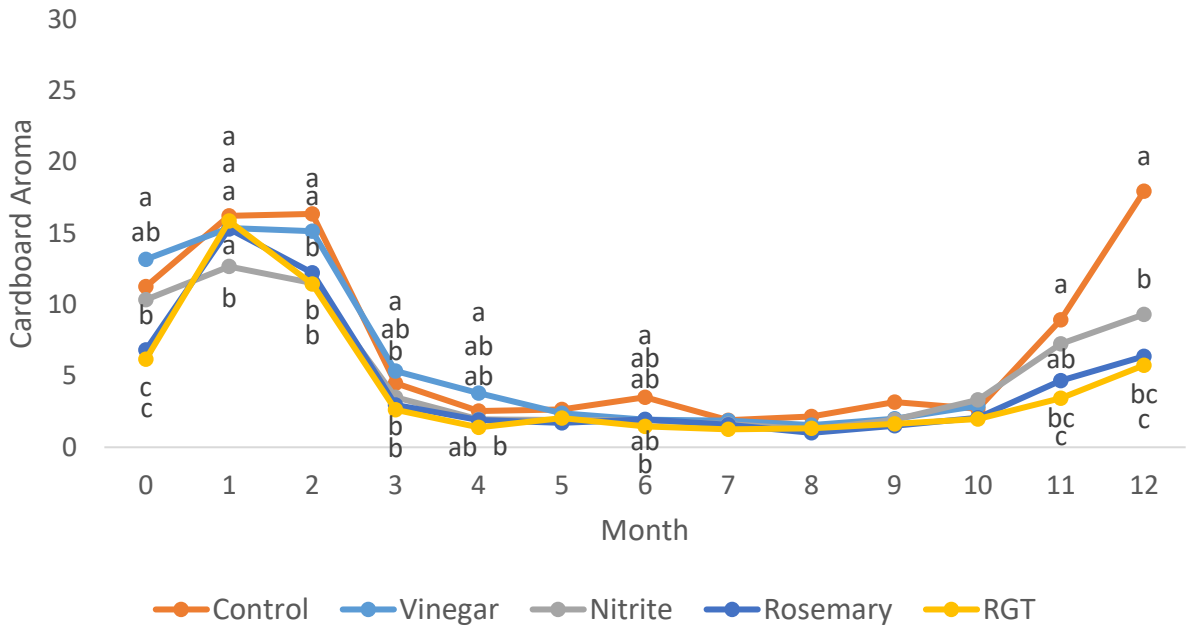


Figure 13. Main effect of month on cardboard aroma by treatment (n=9). [a-c] Least squared means with different superscripts at each month are different (*P* < 0.05). Standard error = 0.69. *Panelists ranked aroma on a 100-point scale where 0 was no cardboard aroma and 100 was intense cardboard aroma.

The main effects of month and treatment on rancid flavor are shown in Figure 14. The rancid flavor of all the treatments did not exceed 6 on the 100-point scale until 12 m. The control treatment was higher ($P < 0.05$) at 15.0 rancid flavor at 12 m than the nitrite treatment at 10.1 rancid flavor. The nitrite treatment was higher ($P < 0.05$) than rosemary and RGT treatments at 3.1 and 2.4 rancid flavor. The rosemary and RGT treatments were similar ($P < 0.05$) in rancid flavor.

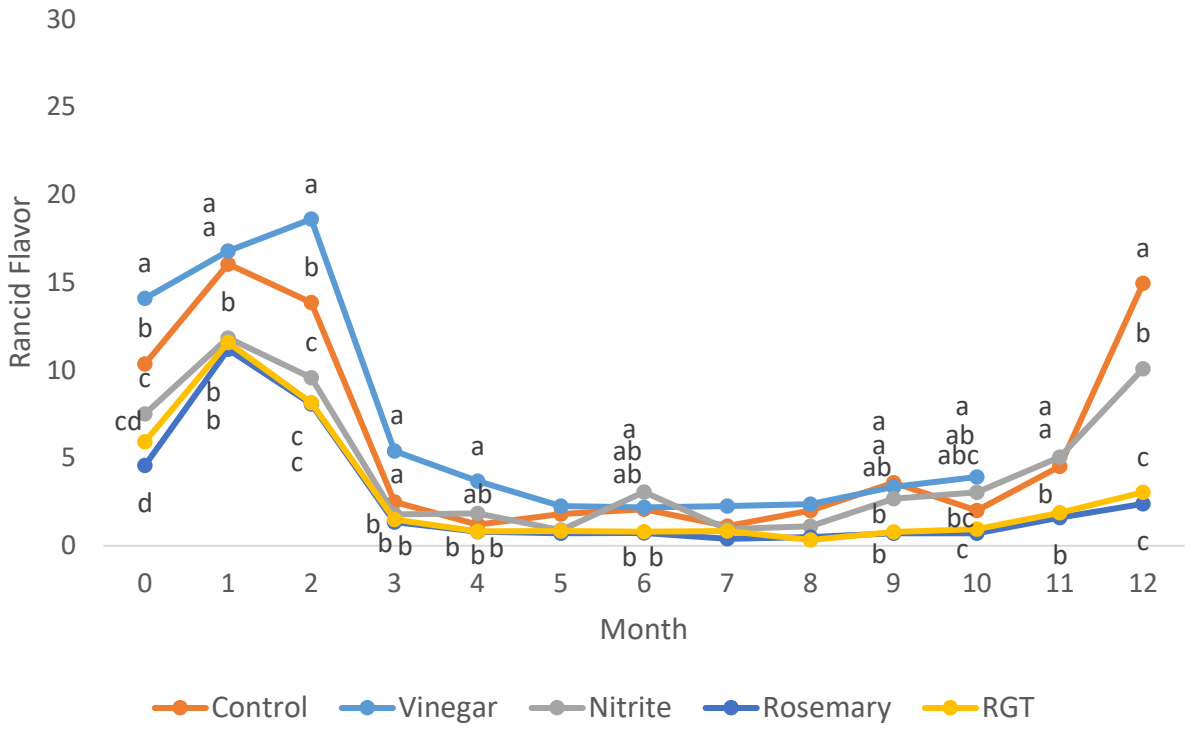


Figure 14. Main effect of month on rancid flavor by treatment (n=9). [a-c] Least squared means with different superscripts at each month are different ($P < 0.05$). Standard error = 0.79. *Panelists ranked aroma on a 100-point scale where 0 was no rancid flavor and 100 was intense rancid flavor.

The main effects of month and treatments on cardboard flavor are shown in Figure 15. All treatments were below 7 on the 100-point scale each month through 10 m. At 11 m, the control and nitrite treatments at 10.9 and 4.5 cardboard flavor, respectively, were higher ($P < 0.05$) than rosemary and RGT at 5.6 and 4.3, respectively. At 12 m, the control at 22.6 cardboard flavor was higher ($P < 0.05$) than the nitrite treatment at 14.3 cardboard flavor, and the nitrite treatment was higher ($P < 0.05$) than rosemary and RGT treatments at 7.7 and 8.2 cardboard flavor, respectively. The rosemary and RGT treatments were not different ($P < 0.05$) in cardboard flavor during the 12 m.

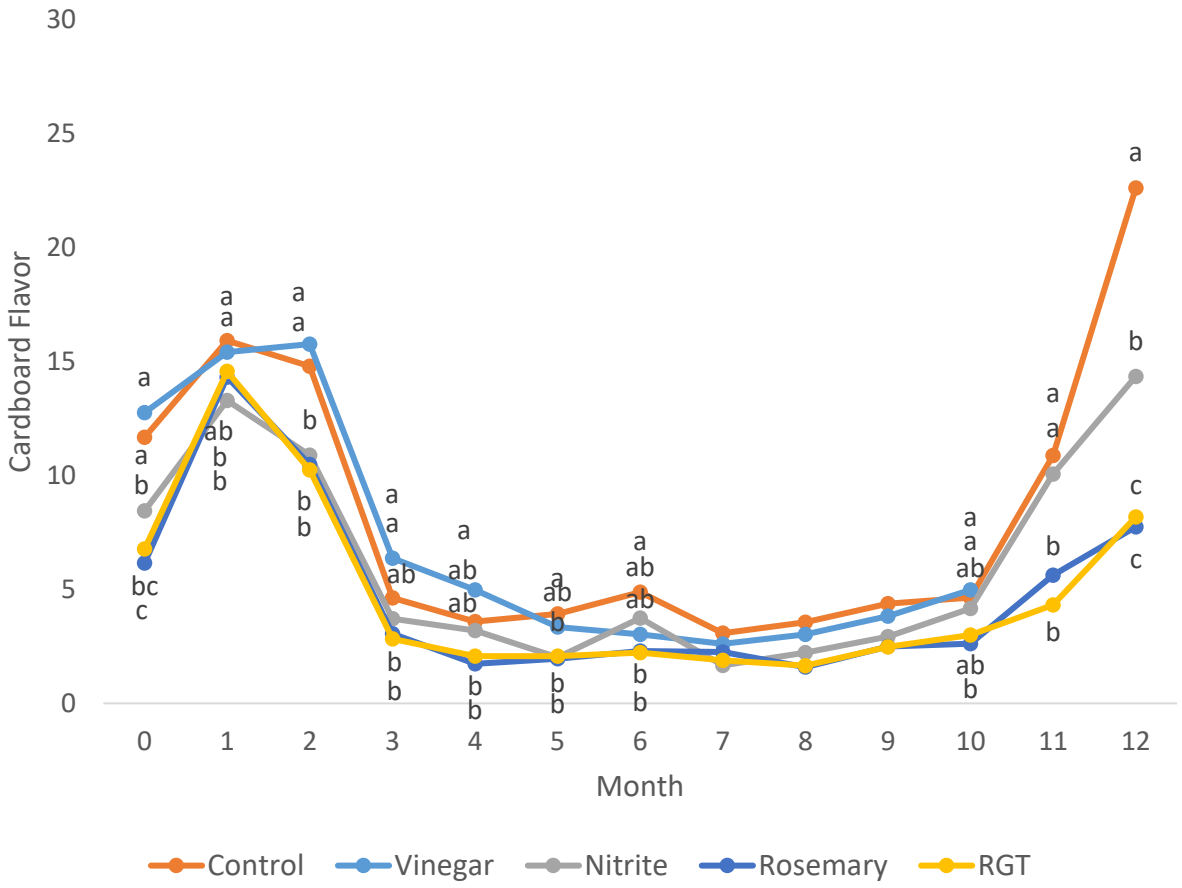


Figure 15. Main effect of month on cardboard flavor by treatment (n=9). [a-c] Least squared means with different superscripts at each month are different ($P < 0.05$). Standard error = 0.71. *Panelists ranked aroma on a 100-point scale where 0 was no cardboard flavor and 100 was intense cardboard flavor.

A trained panel, in a study by Bak and Richards (2021), ranked samples on a 6-point oxidation scale where 2.0 was the detection limit for the panel, and correlated them to hexanal content. There were no differences ($P < 0.05$) in sensory scores ranging from 2.2 and 2.5 in control cured turkey deli meat. No practical sensory differences ($P < 0.05$) of sensory scores between 1.5 and 2.0 were found between control and antioxidant treatments in chicken fillets. The pulled pork had average sensory scores between 1.8 and 3.1 (Bak and Richards, 2021). In this study on antioxidants in pork trim, the hexanal content was lower than levels that would

have rejected a meat product in the study by Bak and Richards (2021), and likely did not lead to sensory changes.

Schilling et al. (2018), however found oxidative flavors increased ($P < 0.05$) by descriptive analyses during 21 days of retail refrigerated display of fresh sausage. Acceptability was measured on a 9-point hedonic scale under red lighting by regular sausage consumers at 8 d. The aroma of all RGT treatments at 6.9-7.1 were more ($P < 0.05$) acceptable than control at 6.5. The flavor of RGT treatments at 7.0-7.3 were also more ($P < 0.05$) acceptable than control at 6.2. Control was also less ($P < 0.05$) acceptable at 6.5 for texture than all RGT treatments that were between 6.9-7. The overall acceptability of RGT treatments was higher ($P < 0.05$) at 6.9-7.1 compared to control at 6.2. The reduced oxidation impact on sensory of RGT compared to sausage not treated with an antioxidant was similar to the results of this pork trim study. RGT and rosemary were lower ($P < 0.05$) than control for aroma and flavor at 12 m.

Although there were differences ($P < 0.05$), the lean color stayed between 2 and 3, less than one point, on the National Pork Board 6-point lean color scale (National Pork Board, 2010). Norman et al. (2003) collected loins over two days of production that represented the National Pork Board 6-point lean color scale. The average L*, a* and b* of color scores 1 and 2 were 57.00, 7.44, and 15.85, and the average L*, a*, and b* of color scores 3 and 4 were 50.24, 7.68, and 14.57 (Norman et al., 2003). These differences were not large enough to elicit eliminating a treatment from the study.

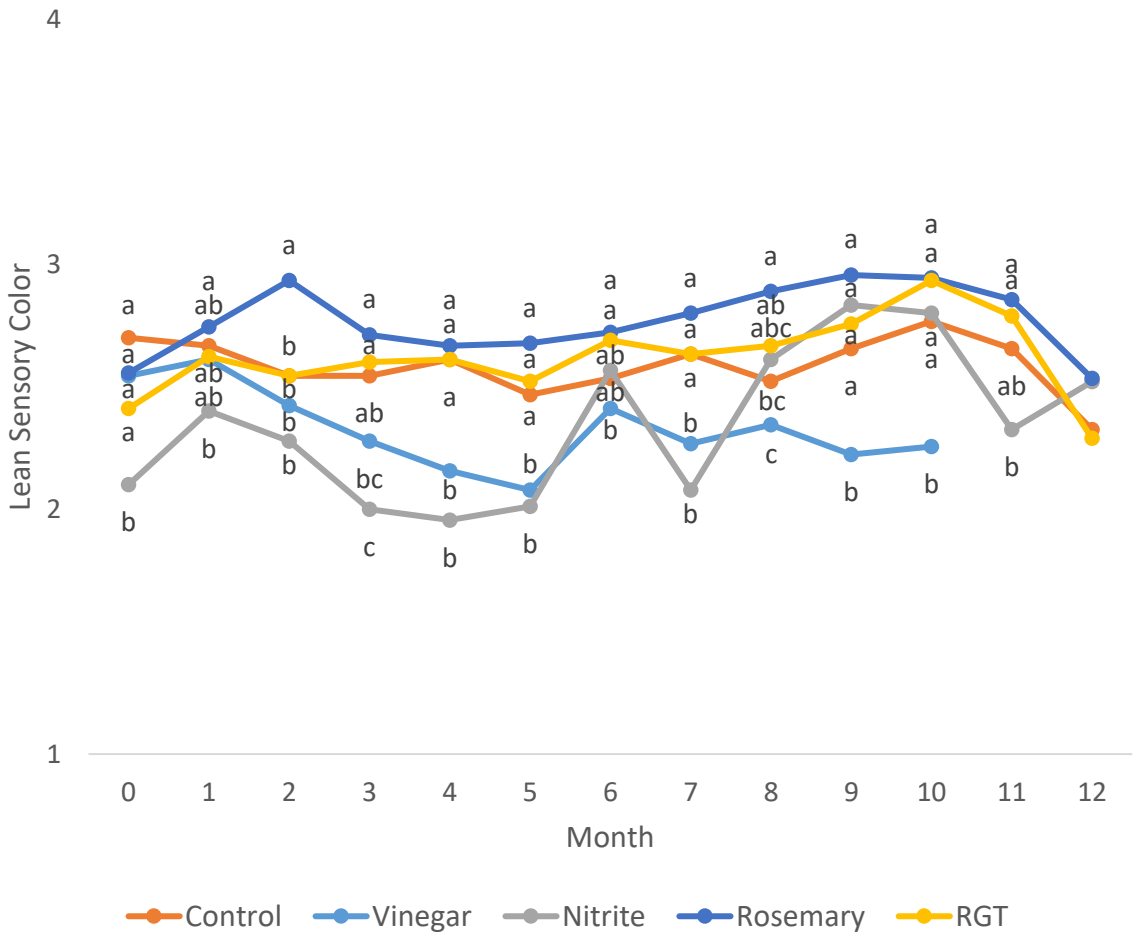


Figure 16. Main effect of month on lean color by treatment (n=9). [a-c] Least squared means with different superscripts at each month are different ($P < 0.05$). Standard error = 0.11. *Panelists ranked color on a 6-point lean color scale where 1 was light red and 6 was dark red.

Summary

The MDA and hexanal content were measured by thiobarbituric acid and gas chromatography to determine the rate of oxidation chemically. None of the treatments reached unacceptable levels of hexanal during the 12 m, and none of the samples exceeded 0.8 mg hexanal/kg. Rosemary and RGT stayed below 0.1 mg hexanal/kg throughout the 12 m; however, there were no differences ($P < 0.05$) in hexanal among the treatments at 12 m. Control, nitrite, and vinegar exceeded 0.5 mg MDA/kg, where a trained panel will likely pick up differences in sensory, before 12 m, and vinegar exceeded the average consumer's threshold of 1.0 mg

MDA/kg at 12 m. Rosemary and RGT, however, remained at or below 0.2 mg MDA/kg throughout the 12 m.

Sensory flavor and aroma evaluations of cardboard and rancid were completed on a 100-point scale using AMSA definitions and anchors as guidelines (Wheeler et al., 2015). All treatments were below 7 on the 100-point scale for all measures from 3 to 11 m. Vinegar treatments were eliminated from sensory evaluations at 11 m due to off flavor development, not necessarily oxidation flavors as defined by the AMSA (Wheeler et al., 2015). None of the treatments had average scores for aroma or flavor higher than 30 and Rosemary and RGT had lower ($P < 0.05$) rancid aroma, cardboard aroma, rancid flavor, and cardboard flavor scores than control at 12 m by approximately 8, 12, 12, and 15 points, respectively.

Color changes were measured by sensory and colorimeter. The lean sensory evaluations did result in lean color differences ($P < 0.05$) among the treatments; however, the color varied less than one point on 6-point National Pork Board lean color scale (National Pork Board, 2010). The fat and lean L* at 0 m at 80.08 and 61.80, respectively, were not different ($P < 0.05$) than the fat and lean L* at 12 m at 80.38 and 61.46, respectively. The lean a* was not different ($P < 0.05$) at 9.3 at 0 m and 9.2 at 12 m. The Lean b* also was not different ($P < 0.05$) at 10.69 at 0 m and 11.29 at 12 m. There were interactions ($P < 0.05$) of treatment and time for fat a* and fat b*. At 0 m, the nitrite treatment, was the lowest ($P < 0.05$) fat a* at 2.02 compared to control, rosemary, RGT, and BV at 4.25, 4.15, 3.94, and 3.49. At 12 m, the vinegar and nitrite treatments at 1.54 and 0.54 were lower ($P < 0.05$) than rosemary, RGT, and control treatments at 3.45, 3.05, and 2.24. At 0 m, the fat b* of the rosemary treatment at 9.03 was similar ($P < 0.05$) to the nitrite treatment at 8.58 and higher ($P < 0.05$) than the control, RGT, and vinegar treatments at 8.41, 8.18, and 8.10. At 12 m, the fat b* of nitrite, rosemary, and vinegar treatments at 9.40, 9.13, and

9.01 were similar ($P < 0.05$). The vinegar treatment was also similar ($P < 0.05$) to the RGT treatment at 8.54, and the RGT treatment was similar ($P < 0.05$) to control at 8.34.

Conclusion

The vinegar treatment was not effective at limiting oxidation rates as measured by TBA and was cut from sensory evaluations at 11 m due to off flavors not described as oxidation. The nitrite treatment did impact color; however, these color changes were expected in a cured product. Although there were differences ($P < 0.05$) in instrumental color among the months, most of the changes only a trained panel is likely to perceive. The nitrite treatment did not exceed oxidation sensory thresholds for a consumer as measured by hexanal and MDA; however, it was not as effective at postponing the oxidation reactions as effectively as the Rosemary and RGT treatments as measured by hexanal, MDA, and sensory for aroma and flavor. The Rosemary and RGT treatments remained at or below 0.2 g MDA/kg and remained at or below 0.1 mg hexanal/kg during 12 m of storage at -17°C. The rosemary and RGT treatments are ideal for use as a clean label antioxidant since they were effective at slowing the oxidation of frozen pork trim during 12 m of storage. The use of a rosemary or RGT treated pork trim will allow flexibility in production scheduling and supply chains.

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