FEEDING PEROXIDIZED SOYBEAN OIL TO FINISHING PIGS: EFFECTS ON PERFORMANCE, NUTRIENT DIGESTIBILITY, CARCASS CHARACTERISTICS, AND THE SHELF-LIFE OF LOIN CHOPS AND COMMERCIALLY MANUFACTURED BACON

BY

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DISSERTATION

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ABSTRACT

Fifty-six barrows (46.7 ± 5.1 kg initial BW) were randomly assigned to 1 of 4 diets in each of two dietary phases, containing either 10% fresh SO (22.5°C) or thermally processed SO (45°C for 288 h, 90°Cfor 72 h, or 180°C for 6 h), each infused with of 15 L/min of air. Peroxide values were 2.0, 17.4, 123.6, and 19.4 mEq/kg; 2,4-decadienal values were 2.07, 1.90, 912.15, and 915.49 mg/kg; and 4-hydroxynonenal concentrations were 0.66, 1.49, 170.48, and 82.80 mg/kg, for the 22.5, 45, 90 and 180°C processed SO, respectively. Pigs were individually housed and fed ad libitum for 81 d to measure growth performance, including a metabolism period to collect urine and feces for determination of GE, lipid, N digestibility, and N retention. Following the last day of fecal and urine collection when pigs were in the metabolism crates, lactulose and mannitol were fed and subsequently measured in the urine to evaluate gut permeability, while markers of oxidative stress were evaluated in plasma, urine, and liver. At 82 d pigs were slaughtered and hot carcass weight (**HCW**) and liver weights were recorded. Carcass characteristics and fresh loin quality were evaluated 1 d post-mortem. Loin chops from each carcass were overwrap-packaged and subjected to a 10 d simulated retail display. Daily measurements of L*, a*, b*, reflectance and visual discoloration were conducted, evaluation of cooking loss and Warner-Bratzler shear force were conducted on chops stored 0, 5, and 10 d, and thiobarbituric acid reactive substances (TBARS) were evaluated on chops stored 0 and 10 d. On d 83 carcasses were fabricated and bellies collected for recording of weight, dimensions, and flop distance. Belly adipose tissue cores were collected for analysis of iodine value (**IV**) by near-infrared spectroscopy (**NIR-IV**). Bacon was manufactured at a commercial processing facility and sliced bacon was subsequently transferred to food-service style packaging and subjected to 0, 30, 60, or 90 d storage at -20°C. Stored bacon was evaluated for thiobarbituric acid reactive

substances (**TBARS**) and trained sensory evaluation of oxidized odor and flavor. Growth performance, nutrient digestibility, carcass traits, and bacon slicing yields were analyzed as a one-way ANOVA with the fixed effect of SO. Additionally, initial body weight was used as a covariate for analyses of growth performance and carcass traits. Shelf-life traits for both loin chops and sliced bacon were conducted as repeated measures in time using the mixed model approach, with fixed effects of SO and storage time.

There were no differences observed in ADFI (P = 0.91), but ADG and GF were decreased in pigs fed 90°C SO diet (P ≤ 0.07) compared to pigs fed the other SO diets. Pigs fed the 90°C and 180°C SO had the lowest (P = 0.05) DE as a % of GE compared to pigs fed the 22.5°C SO, with pigs fed the 45°C SO being intermediate. Lipid digestibility was similarly affected (P = 0.01) as energy digestibility, but ME as a % of DE was not affected by dietary treatment (P = 0.16). There were no effects of lipid peroxidation on N digested, N retained, or the urinary lactulose:mannitol ratio (P ≥ 0.25). Pigs fed the SO processed at 90°C and 180°C had lower concentrations (P < 0.01) of plasma Trp compared to pigs fed the 22.5°C and 45°C SO treatments. Pigs fed 90°C SO had the greatest (P < 0.01) concentrations of F2-Isoprostane in plasma and urine TBARS compared to the other SO treatments.

Carcasses of 90°C pigs weighed 6.0, 8.6, and 6.9 kg less than (P < 0.03) 22.5°C, 45°C, and 180°C carcasses, respectively. Livers of 90°C and 180°C pigs were 14.3 and 11.7%, respectively, heavier (P ≤ 0.02) than those from pigs fed 22.5°C SO, with livers of 45°C being intermediate. Livers of 90°C pigs represented 0.12 percentage units less (P = 0.02) of ending live weight than livers 180°C, and 180°C liver were 0.12 percentage units less (P < 0.01) of ending live weight than those from pigs fed 22.5°C SO, with 45°C being intermediate. There was no difference (P ≥ 0.19) BF depth, LMA, or estimated carcass lean percentage among SO treatments, nor was there

an effect ($P \geq 0.13$) of SO on any early post mortem loin quality traits or loin composition. There was no effect ($P > 0.14$) of SO on cooking loss, WBSF, L*, a*, b*, hue angle, reflectance, discoloration, or TBARS; however, there was a tendency ($P = 0.09$) for chops of 45°C pigs to have greater ($P < 0.04$) chroma than either 22.5°C or 180°C, with 90°C being intermediate.

There was no effect ($P \geq 0.30$) of SO on belly weight, length, width, or thickness; but bellies of pigs fed 90°C SO had greater ($P \leq 0.04$) flop distance (more firm) than all other SO treatments. Belly fat NIR-IV of pigs fed 90°C SO were 10.22 units less ($P < 0.0001$) than pigs fed 180°C SO, which were 2.99 and 3.29 units less than belly adipose tissue of pigs fed 22.5°C and 45°C SO, respectively. There was no effect of SO on brine uptake or cooking yield of commercially manufactured bacon. There was a trend ($P = 0.09$) for bacon manufactured from bellies of pigs fed 45°C and 90°C SO to have greater slicing yields than those from pigs fed 22.5°C and 180°C SO. There were no SO × storage time interactions ($P \geq 0.27$) for any shelf life trait. There was no difference in TBARS, oxidized odor, or oxidized flavor among the four SO treatments, though all three shelf life metrics increased ($P < 0.0001$) with storage time.

Overall, the change in FA composition and/or the presence of lipid peroxidation products in thermally peroxidized SO resulted in increased markers of oxidative stress and digestibility of GE and ether extract, resulting in drastically reduced growth performance and significantly lighter carcasses. Despite the negative effect on digestibility and growth, feeding thermally peroxidized SO had no effect on the shelf life of loin chops or bacon. In conclusion, pig producers should be weary of feeding thermally peroxidized fat sources, but packers and processors have little cause for concern when it comes to the stability and quality of their pork products.

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CHAPTER 1: REVIEW OF LITERATURE

1.1 SUMMARY

The quality of the lipids fed to growing-finishing pigs has long been recognized as having profound effects on pork quality. Much effort has been put forth to understand lipid nutrition as it affects meat quality, with most research focusing on the fatty acid profile of lipids and their influence on fat deposition. Recently, there has been renewed interest in other areas of dietary lipid quality; namely, the oxidative status of lipids. Human nutrition and medicine have long recognized that consumption of oxidized lipids have deleterious effects to health. Recent research has indicated that effects of consuming peroxidized lipids may have farther reaching effects than previously believed; influencing energy metabolism, pregnancy outcomes, and prenatal development.

In regard to pigs as livestock, studies have focused on the effects of peroxidized lipids on gut health, immune response, and digestibility in the field of swine nutrition. Although research has been conducted to investigate the effects of oxidized dietary lipids on pork quality, results have been inconclusive. Still, with the continued use of ingredients such as DDGS and various plant oils in swine diets, it is of importance to understand the potential impacts the oxidative quality of these ingredients has on pork quality.

1.2 INTRODUCTION

Increased use of corn co-products such as DDGS and oils in swine diets have increased concerns over the effects of dietary lipid composition on the quality and shelf-life of fresh and processed pork products. Numerous studies have been conducted to investigate the effects of the use of DDGS in diets on the quality and shelf-life of both fresh (Leick et al., 2010) and processed (Bumsted et al., 2015) pork products. The majority of pork quality and shelf-life research on the

effects of DDGS and other ingredients that serve as a source of dietary lipids has revolved around the manipulation of the fatty acid composition of adipose tissue with the objective of reducing the proportion of oxidatively-labile polyunsaturated fatty acids (**PUFA**). This increased interest in the role of dietary lipid composition has also drawn attention to dietary lipid quality, particularly the oxidative status of the lipids. There is a growing body of evidence to indicate that consumption of oxidized lipids and their secondary reaction products have implications for health, growth, development, and metabolism that are of consequence to the pork industry. In addition to the ramifications of dietary oxidized lipids on pig performance, there are potential implications for the quality and shelf-life of pork products.

It is the objective of this presentation to review the body of literature relating to the effects of oxidized lipids in growing-finishing swine diets on the quality and shelf-life of fresh and processed pork, with an emphasis on the relationship between the oxidative state of tissues and measures of shelf-life of meat products.

1.3 EFFECTS OF FEEDING OXIDIZED LIPIDS ON PIG GROWTH PERFORMANCE AND CARCASS TRAITS

The implications of dietary oxidized lipids for pork quality and shelf-life begins with the effects that oxidized fats and oils have on nutrient digestibility, intestinal function, and growth performance; these topics have been reviewed extensively by Shurson et al. (2015).
Numerous lipid sources are available for supplementation to swine diets, and are included to increase caloric density, improve pellet quality, and provide for essential fatty acids (Azain, 2001; Lin et al., 2013). There are numerous lipid sources available, and they can vary widely in their energy content, fatty acid profile, cost, and quality. Largely, research into the quality of lipid sources have revolved around their fatty acid profiles, and what effect that may have on

growth, digestibility, and meat quality. In recent years, a growing body of evidence has indicated that the oxidative status of lipid sources should also be taken into consideration (Shurson et al., 2015). This is particular importance to the swine industry as rendered fats and unsaturated vegetable oils are routinely used in swine diets (Lin et al., 2013). Even with quality control protocols, the rendering, recycling, handling, and storage of lipid sources can further exacerbate their oxidative deterioration. Furthermore, used frying oils may be recycled and added directly to diets. Discarded frying oils are typically extremely oxidized (Sebastian et al., 2014) and are likely to represent the most extreme lipid deterioration of lipid sources used in livestock feeding. The process of lipid peroxidation changes the chemical composition of the lipid through the consumption of PUFA, yielding a lipid with a greater concentration of monounsaturated (**MUFA**) and saturated fatty acids (**SFA**), ultimately reducing the iodine value (**IV**; Johnson and Kummerow, 1957). The IV of the diet, or perhaps more precisely the fatty acid profile of the diet, can have varying effects on the growth performance (Jones et al., 1992; Benz et al., 2011) of pigs and the digestibility of lipids. Lipid sources with greater concentrations of PUFA, such as vegetable oils, are more digestible than more saturated sources, such as lard or tallow (Jones et al., 1992; Powles et al., 1994; Duran-Montgé et al., 2007). The increased saturation that occurs with oxidation and its effects on pig growth is not the only concern when feeding peroxidized lipid sources. The progression of lipid oxidation results in the formation of numerous secondary and tertiary peroxidation products that can have deleterious effects on pig health and growth performance (Shurson et al., 2015). The oxidation of linoleic acid yields at least 19 volatile compounds (Belitz Grosch, and Schieberle, 2009). Thus the number of such compounds produced by the oxidation of a complex mixture of fatty acids, such as exist in lipid sources for livestock diets, must number well into the hundreds. Primary, secondary and tertiary lipid

oxidation products have been reported to interact with other nutrients, namely amino acids and lipids, to reduce their digestibility in a rat model (Nielson, Finot, and Hurrell, 1985). This is believed to be, at least in part, due to the effect of lipid oxidation products on the integrity and morphology of the gut. Rosero et al. (2015) reported a reduction in fat and gross energy digestibility increasing peroxides in the diet, and that this was coupled with a trend towards increased gut permeability. However, such an effect on gut function has not been universal, as others have reported no effect of feeding oxidized oils on gut permeability (Liu et al., 2014a). In contrast, reductions in growth performance have been routinely reported with feeding of oxidized lipid, with most research focusing on nursery pigs. Feeding oxidized oil was reported to reduce ADG, ADFI, and G:F in nursery pigs (Liu et al., 2014b; Rosero et al., 2015; Hanson et al., 2016) and growing-finishing pigs (Boler et al., 2012; Lu et al., 2014a). Along with the poorer growth, feeding oxidized oils reduced loin muscle (**LM**) area (Lu et al., 2014b) as well as back fat thickness, hot carcass weight, and dressing percentage (Boler et al., 2012; Lu et al., 2014b). Minimal differences in fresh meat quality have been reported between carcasses of pigs fed oxidized or fresh oil, with reduced redness of the LM (lower a*) and firmer bellies reported in oxidized oil fed pigs (Lu et al., 2014b). However, Boler et al. (2012) found no difference in loin quality traits between pigs fed fresh or oxidized corn oil, but did note that boston butts (shoulders) of oxidized oil fed pigs had a greater fat content than those from pigs fed fresh oil.

1.4 ABSORPTION AND METABOLISM OF DIETARY OXIDIZED LIPIDS AND LIPID OXIDATION PRODUCTS

Although these effects on carcass composition are likely explained by the reduced growth performance, the implications of dietary oxidized lipids on pork shelf-life are more likely to lie within the realm of metabolism of those oxidized lipids and their secondary oxidation products.

Therefore, to fully understand how dietary oxidized lipids affect the functionality of pork products and their oxidative stability during storage, it is important to understand the metabolism of those products in the living animal.

Dietary lipids are absorbed from the intestine where they are packaged into chylomicrons in enterocytes, released into the lymphatic system and enter the blood stream, and are ultimately transported to muscle, adipose tissue, or the liver to be metabolized. Along with unoxidized lipids, oxidized lipids and many lipid oxidation products, such as 4-hydroxynonenal (HNE) and other α, β-unsaturated hydroxyaldehydes, are readily absorbed, with lipid oxidation products being of particular concern due to their cytotoxicity (Esterbauer et al., 1991; Grootveld et al., 1998). Furthermore, oxidized lipids have been demonstrated to be present in postprandial chylomicrons in both humans (Staprans et al., 1994) and rats (Staprans et al., 1996). It is likely that the increased concentrations of plasma thiobarbituric acid reactive substances (TBARS), a measure of lipid oxidation, observed in pigs fed diets containing oxidized oils (Fernández-Dueñas, 2009) was due to the presence of lipid oxidation products in chylomicrons. After absorption, chylomicrons containing the oxidized lipids, along with secondary oxidation products, are transported to the liver to be metabolized (Pignitter and Somoza, 2015), accounting for the increased concentration of TBARS in hepatic tissue in pigs fed diets containing oxidized oils (Fernández-Dueñas, 2009). Chylomicrons can also transport lipids to muscle or adipose tissue; however, it is yet uncertain if oxidized lipids and secondary oxidation products are also transported to, and potentially incorporated into, these tissues.

The liver serves as the primary point of detoxification in the body; thus, it is especially susceptible to the effects of lipid oxidation products. The toxicity of secondary lipid oxidation products results in an increase in the synthesis of enzymes to aid in detoxification (Huang et al.,

1988) as well as increased proliferation of hepatocytes (Dibner et al., 1996). This proliferation may underlie the increased liver weight observed in pigs (Liu et al., 2014b), as well as rats (Eder, 1999) fed oxidized oils. Additionally, feeding oxidized lipids increases expression of PPARα and other genes associated with lipid metabolism; however, the exact mechanisms, nor the regulatory role, of PPARα in lipid metabolism are fully understood (Shurson et al., 2015). Despite the livers capacity to neutralize and excrete cytotoxic lipid oxidation products, it may not be completely effective. Winter et al. (1987) injected radioactively labeled hexenal (HHE), a major secondary lipid oxidation product, which itself is a catalyst of oxidation, into the hepatic portal vein of rats, and although 77-83% of the administered radioactivity was excreted in urine, approximately 9% was recovered from skeletal muscle. However, it is unclear if this was indicative of HHE being deposited in muscle or if the labelled carbons were incorporated into other compounds, which were then transported to skeletal muscle.

Moreover, carbons from radioactively marked linoleic acid administered to rats (Strapans et al., 1996) and pigs (Suomela et al., 2005) in the form of chylomicrons were present in very low density lipoprotein (VLDL), indicating that oxidized fatty acids are metabolized and released into circulation similarly to the non-oxidized form. The same marked carbons were also detected in extrahepatic tissues, including skeletal muscle. However, it is unclear whether the marked carbons were still part of linoleic acid, or if they had been donated to other compounds. There is, in fact, evidence that oxidized lipids from the diet are not only absorbed and metabolized, but also interact with cellular structures. Buckley et al. (1989) reported the mitochondrial membranes of pigs fed oxidized corn oil (peroxide value = 9 meq/kg feed) for 10 weeks were less oxidatively stable than those of pigs not fed oxidized oil. They hypothesized that the oxidized lipids in the diet were a source of free radicals, thus destabilizing the membranes.

Therefore, feeding pigs diets containing oxidized lipids increases the presence of oxidized lipids in both circulation and liver, and these oxidized lipids and their secondary oxidation products can also be deposited in tissues. It is the deposition of oxidized lipids and secondary oxidation products in muscle and adipose tissue that are of the most concern for pork quality and shelf-life.

1.5 IMPLICATIONS FOR PORK QUALITY AND SHELF-LIFE

Before discussing the implications of feeding oxidized oil to pigs on pork quality, the mechanisms affecting shelf-life must be understood. When discussing shelf-life of meat products, consumers and the uninformed may believe that microbial growth is the primary cause of shortened shelf-life. However, if proper sanitation and hygiene is employed, other processes are more likely to reduce shelf-life before microbial spoilage takes effect. Among these processes, the chemical oxidation of the pigment protein myoglobin, as well as unsaturated fatty acids are more likely to truncate shelf-life of meat products before microbial storage.

Lipid oxidation, otherwise called lipid peroxidation, as it pertains to the development of rancidity, occurs during storage of meat products, even at refrigeration and frozen temperatures. The susceptibility of meat products to lipid oxidation is largely dependent on the fatty acid profile of the lipids. With oxidative lability increasing with increasing concentrations of PUFA (Rhee et al., 1996). This characteristic of PUFA is due to the reactive methylene bridges found adjacent to the double bound which makes the associated hydrogen atom especially reactive (Herrmann, 1982). The process can be broken down into three stage: initiation, propagation, and termination. Lipid oxidation is initiated with the removal of a hydrogen atom from a carbon adjacent to a double bond of an unsaturated fatty acid to form a free radical. This free radical then combines with an O_2 to form a peroxyradical (Damodaran and Parkin, 2008). The peroxylradical is also unstable, and in turn interacts with another fatty acid to form a

hydroperoxide and another free radical, which then leads to the propagation of the autocatalytic oxidation process (Ladikos and Lougovois, 1990). The termination phase is characterized by radicals reacting with one another to form a more stable, non-radical compound (Damodaran and Parkin, 2008). While this basic reaction has been defined for several decades (Frankel, 1984; Frankel, 1991); the exact mechanism by which the reaction is initiated remains somewhat controversial. Both spontaneous lipid radical formation and direct interaction of unsaturated fatty acids with molecular oxygen are thermodynamically favorable (Baron and Andersen, 2002). Regardless, in the case of food products, and especially in muscle foods, lipid oxidation involves transition metals. Of the transition metals, iron is considered a pivotal catalyst in the progression of lipid oxidation in meat (St. Angelo et al., 1996). This is due to the relatively high concentration of iron, both in heme and non-heme forms found in muscle. The contribution of iron as a catalyst for lipid oxidation (Younathan and Watts, 1959). Even into the modern day, there is considerable debate about which form, heme or non-heme, is the primary contributor towards lipid oxidation in muscle foods (Faustman et al., 2010). Rhee and Ziprin (1987) reported that heme pigment was a more prominent catalyst of lipid oxidation than non-heme iron. More recently, non-heme iron content was reported to exhibit a strong positive correlation with TBARS formation in chicken breast and beef loin (Min, Cordray, and Ahn, 2010). Lipid oxidation in meat ultimately results in oxidative rancidity and the development of associated undesirable odors and flavors.

Lipid oxidation is typically accompanied with deterioration of meat color from a desirable red to an undesirable brown color, due to the oxidation of oxymyoglobin to metmyoglobin (Faustman et al., 2010). Myoglobin is the major pigment protein found in muscle and is responsible for its red color. In living muscle, myoglobin serves as the oxygen binder and

transporter in muscle cells, delivering oxygen to the mitochondria (Wittenberg and Wittenberg, 2003). Myoglobin is a monomeric heme protein, consisting of a heme prosthetic group and a globin moiety. The heme portion confers myoglobin with the ability to bind oxygen; whereas the globin chain allows for myoglobin to be water soluble (Suman and Joseph, 2013). At center of its structure, the heme group holds an iron atom that can exist in either the reduced (ferrous, Fe^{2+}) or oxidized (ferric, Fe^{3+}) form. This characteristic is of utmost importance in regard to the change in color that occurs during the shelf-life of meat products. In meat products, stored in typical commercial packaging systems may exist in any four redox states (Mancini and Hunt, 2005): deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), carboxymyoglobin (COMb), and metmyoglobin (MetMb). The Deoxy-, Oxy-, and COMb forms all exist in the reduced, ferrous state. When Mb is in the Oxy- or CO- state, the meat exhibits a desirable, bright cherry red color and the difference between the two is indistinguishable to the human eye (Cornforth and Hunt, 2008). In both these form, there is an oxygen atom bound to the sixth coordinate of the heme iron. In contrast, DeoxyMb lacks any ligands attached to its iron coordinates, and exhibits a purple color. The fourth redox state of myoglobin is the oxidized MetMb, where the iron exists in its oxidized, ferric state. This form is indicative of discoloration in meat products (Faustman and Phillips, 2001; Suman and Joseph, 2013). Metmyoglobin does exist *in vivo,* with approximately 2-3% present in tissues (Halliwell and Gutteridge, 1989). But in the physiological condition, the population of metmyoglobin is constantly being reduced to the deoxy- state by enzymatic processes (Baron and Andersen, 2002). Moreover, in living tissue, there is a constant turnover of NADH, which can reduce the ferric iron of metmyoglobin back to the more stable ferrous state (Mancini and Hunt, 2005). While this mechanism is able to maintain the redox equilibrium in living tissue, its action is limited in meat. The pool of enzymes as well as NADH

are continually depleted as the time postmortem progresses, because the processes to replenish the pools have been halted (Mancini and Hunt, 2005). While the redox equilibrium cannot be maintained indefinitely in meat, it can be prolonged both by the supplementation of antioxidants to the animal while it lives, and the incorporation of antioxidants to the meat itself (Brewer, 2004). The transition from reduced oxymyglobin, to the oxidized metmyoglobin state that is the primary factor in reducing shelf-life, because meat purchasing decisions are primarily driven by color (Mancini and Hunt, 2005). In addition to endogenous factors, some of which have been previously described, the oxidation of myoglobin can be perpetuated exogenously as well. When fresh meat is exposed to oxygen it reacts with myoglobin to form oxymyglobin to produce a bright, cherry red color (Suman and Joseph, 2013). In the meat industry, this process of oxygenation is known as “bloom”. However, the maintenance of the oxygenated state is ephemeral, because eventually the oxymyoglobin further reacts with oxygen to form the brown metmyoglobin in a matter of days (McMillin, 2008). The formation of metmyoglobin in also catalyzed by lipid derived radicals produced by lipid oxidation and also accelerated by secondary lipid oxidation products, including members of the β-unsaturated hydroxyaldehydes, such as HNE and HHE (Faustman et al., 1999). The final result of lipid and myoglobin oxidation is a meat product that has an unacceptable color accompanied with objectionable odors and flavors that is unlikely to be purchased or consumed.

Shelf-life of pork products is dependent upon many factors, including the lipid content of the product, the use of preservative ingredients, packaging, storage method, and lighting. The detection threshold for rancidity in fresh pork is 0.5 to 1.0 mg-TBARS/kg of tissue (Wood et al., 2008), however it is possible that deterioration of color will occur before the threshold for rancidity is reached. Stored in typical retail display conditions in oxygen permeable packaging,

the shelf-life, as determined by the aforementioned TBARS threshold, of fresh pork loin chops may be 7 to 14 d (Sheard et al., 2000; Fernández-Dueñas, 2009;); whereas, fresh seasoned pork patties may only have a shelf-life of 5 to 7 d (Fernández-Dueñas, 2009; Qin et al., 2013). Cured meat products typically have a much longer shelf-life, due to the preservative roles of several curing ingredients, with a typical shelf-life of refrigerated vacuum-packaged sliced bacon being in excess of 90 d (Lowell et al., 2016). The premature end of shelf-life has major economic implications for the entire food industry, with the loss of meat and poultry products being the most severe. In 2010, it was estimated that 8.6 billion pounds of meat were lost at the retail and consumer points of the U.S. supply chain due to spoilage, representing an economic loss of $23.2 billion (Buzby et al., 2014).

Much of the recent improvements in prolonging shelf-life of meat products has focused on the use of novel packaging systems, such as modified atmosphere packaging (McMillin, 2008) and antioxidant-active packaging films (Gómez-Estaca et al., 2014), as well as novel display lighting sources (Steele et al., 2016). Despite the improvements in packaging and display technology, the fact that diet influences the shelf-life of meat products, remains. For example, the inclusion of DDGS in growing-finishing pig diets increased the deposition of oxidatively-labile PUFA in adipose tissue and decreased shelf-life of blade chops (Leick et al., 2010); whereas, the supplementation of antioxidants can increase or spare stores of endogenous antioxidants, thereby improving shelf-life (Boler et al., 2012). Given these results, several researchers have investigated whether absorption and deposition of dietary oxidized lipids and secondary oxidation products in muscle and adipose tissues may reduce the shelf-life and negatively affect the quality of meat products. However, the results of such experiments have been mixed (Table 1).

An early study by Buckley et al. (1989) reported that both loin chops and ground pork patties of pigs fed oxidized corn oil displayed substantially greater rates of lipid oxidation than did those from pigs fed fresh oil at all stages of either fresh or frozen storage. Furthermore, feeding rancid rice bran (15.6% FFA) increased the rate of lipid oxidation in fresh and cooked pork loin chops (Chae and Lee, 2002). Monahan et al. (1992) reported that loin chops from pigs fed oxidized corn oil tended to have greater TBARS concentrations than did chops from pigs fed fresh oil; however, the peroxide value of the oil used in their study was half that of the earlier work of Buckley et al. (1989). In contrast to this earlier research, more recent studies have reported that although feeding oxidized corn oil induced markers of oxidative stress in both liver and plasma, there was no effect of oil quality on TBARS of loin chops (Boler et al., 2012). However, chops from pigs fed oxidized oil were rated as less tender after 14 d of simulated retail display than those from pigs fed fresh oil. This may have resulted from oxidation of myofibrillar proteins. Like the oxidation of lipids, protein oxidation occurs via a free radical chain reaction. The oxidation of myosin can result in cross-linking of myosins; thereby, altering the structure of the contractile unit in such a way that it decreases tenderness, water holding capacity, and protein solubility (Lund et al., 2011). There is some evidence that protein and lipid oxidation work in tandem, with the propagation of one initiating the other, yet the exact mechanism by which this may occur is not well defined (Lund et al., 2011). Lu et al. (2014b) reported that although a* (redness) values were reduced (became less red) by feeding oxidized soybean oil, suggesting oxidation of myoglobin to brown metmyoglobin, TBARS of fresh loin muscle were not different than those from pigs fed fresh oil. In this study, however, it should be noted that the loin samples were not subjected to simulated retail storage and therefore were not truly indicative of the shelf-life of the meat. Additionally, the inclusion of 3% oxidized corn oil (150 meq/kg oil) did not

affect either lipid oxidation or color stability of loin chops compared to chops from pigs fed fresh corn oil under simulated retail display conditions (Monahan et al., 1994). The inconsistency of these results of feeding oxidized oils to pigs and subsequent effects on lipid oxidation in the product can be attributed to several factors including the particular level of oxidation in the diets, antioxidant supplementation levels, fat level in the pork products evaluated, and storage time and conditions prior to evaluation. It is also important to consider that bacon and sausage make up 37.9% of all in-home pork consumption in the U.S (Pork Check Off, 2009). The greater fat content of these products make them more susceptible to the effects of oxidation than lean cuts, such as loin chops, which have been the focus of much of the research on the effects of dietary oxidized lipids on pork quality and shelf-life. Therefore, the lack of data regarding the effects of dietary oxidized oil inclusion on the shelf-life and quality of these products is troubling.

In contrast to the mixed results reported in pork studies, studies in broilers have routinely reported that oxidized oils in diets resulted in increased rate of lipid oxidation in meat during storage (Lin et al., 1989; Jensen et al., 1997; Tavárez et al., 2011; Zhang et al., 2011). This disparity in response is likely due to differences in the endogenous antioxidant systems between poultry and pigs, which are primarily driven by the difference in muscle fiber types and their preferred metabolic pathways. Oxidative fibers make up a greater proportion of pork muscle than in chickens, which have a greater proportion of glycolytic fibers. The preference of oxidative muscle fibers to utilize fatty acid oxidation to produce energy results in greater concentrations of free radicals and secondary oxidation products. Thus, oxidative muscle fibers have more robust antioxidant systems to handle these by-products of oxidative metabolism. Therefore, pigs in contrast to chickens may be more suited to combat increased oxidative stress from the consumption of oxidized lipids.

The ability to combat oxidation in tissues lies with antioxidant enzyme systems. Many of these antioxidant enzymes are used as markers of oxidative stress. Glutathione peroxidase (GPx) is one such enzyme that is routinely measured. Glutathione peroxidases are found in several tissues, with the greatest concentrations in the liver and plasma of both pigs (Daun and Åkesson, 2004; Boler et al., 2012) and chickens (Tappel et al., 1982), but with significantly less activity in skeletal muscle of either species (Yamauchi et al., 1984). Zhou and Decker (1999) suggested that though GPx was a powerful antioxidant, concentrations were not sufficient in skeletal muscle to be the primary antioxidant in the system. In fact, the same authors demonstrated that at concentrations present in skeletal muscle, carnosine was more effective at neutralizing lipid oxidation product than GPx. Overall, chicken and pork muscle have comparable concentrations of GPx, with chickens having slightly greater levels of activity than pigs (Yamauchi et al., 1984). However, pork contains far greater concentrations of carnosine, regardless of muscle location, than chickens (Tian et al., 2007; Mora et al., 2008). In addition to carnosine, the antioxidant enzymes catalase (Rhee et al., 1996; Pradhan et al., 2000) and superoxide dismutase (Avanzo et al., 2001; Hernández et al., 2004) are present in greater concentrations in pork than in chicken. With GPx activities being similar across tissues between species, the greater concentration of carnosine, catalase, and superoxide dismutase in pork may account for the disparity in results of shelf-life studies between the two species.

1.6 CONCLUSIONS

The inclusion of fats and oils in growing-finishing pig diets can, under some scenarios, offer cost savings in diet formulation and improve some carcass characteristics. However, not all dietary lipids are created equal, as is clearly demonstrated when comparing the fatty acid composition of tallow against corn oil. Much consideration has been given to evaluating the

levels of PUFA, MUFA, and SFA in dietary lipids from various sources, but less has been given to the oxidative status of those lipids. Certainly, a growing body of data are available in the literature in regards to the effects of oxidized oils and fats on nutrient digestibility, growth performance, and immune function at all stages of pig growth and development; but, data regarding the implications of feeding oxidized oils on meat quality and shelf-life are comparatively limited. The data that are available are somewhat contradictory, and this is possibly due to the complex nature of not just the chemical composition of the dietary lipids, but also their metabolism. As researchers continue to investigate the roles and effects of lipids from various sources in pig diets, the oxidative stability and status of those ingredients should also garner consideration. Finally, future research conducted in this area should first focus on identifying whether dietary oxidized oils have any meaningful effects on the quality and shelf-life on fattier pork products (e.g. sausage, bacon) that are at a greater risk for oxidation, then determining interventions to mitigate those quality issues.

1.7 TABLE

Table 1.1 Summary of results of studies reporting the effect of dietary oxidized lipids on pork quality and shelf-life

Study	Fat source	Fat source PV[1], mEq/kg	Diet PV[1], mEq/kg	Product	Results
Lu et al. (2014b)	soybean oil	180	9	Fresh loin muscle	Oxidized oil decreased lean redness, but did not affect TBARS[2] of fresh loin muscle
Boler et al. (2012)	corn oil	150	7.5	Fresh loin chops, fresh ground pork	No effect of oxidized oil on color stability or TBARS during retail display of loin chops or ground pork. Oxidized oil reduced sensory tenderness of chops at 14 d of storage (15 d postmortem).
Chae and Lee (2002)	rice bran	NR[3]	NR[3]	Fresh ground pork, cooked ground pork	Oxidized rice bran increased TBARS during storage of fresh ground pork, but had no effect on cooked pork
Monahan et al. (1994)	corn oil	150	4.5	Fresh loin muscle	No effect of oxidized oil on discoloration or TBARS during refrigerated display
Monahan et al. (1992)	corn oil	150	4.5	Fresh loin chops, frozen/thawed loin chops, cooked ground pork	Oxidized oil tended to increase TBARS in fresh chops and cooked ground pork
Buckley et al. (1989)	corn oil	300	9	Fresh loin chops, frozen loin chops, fresh ground pork, salted ground pork	Oxidized oil rapidly increased TBARS during storage of both fresh and frozen loin chops and fresh and salted ground pork, destabilized microsomal and mitochondrial membranes

[1]Peroxide value

[2]Thiobarbituric acid reactive substances

[3]Not reported. Chae and Lee (2002) reported free fatty acid (FFA) concentrations of rice bran. Rancid rice bran = 15.6% FFA, fresh rice bran = 8.2% FFA; fed at 20% inclusion in the diet, as fed basis.

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CHAPTER 2: INFLUENCE OF FEEDING THERMALLY PEROXIDIZED SOYBEAN OIL ON GROWTH PERFORMANCE, DIGESTIBILITY, AND GUT INTEGRITY IN FINISHING PIGS

ABSTRACT: Consumption of peroxidized lipids has been shown to reduce pig performance and energy and lipid digestibility. Objectives of the current study were to evaluate the effect of feeding soybean oil (SO) with different levels of peroxidation on growth performance, lipid, N, and GE digestibility, plasma Trp, and gut integrity in finishing pigs. Fifty-six barrows (46.7 ± 5.1 kg initial BW) were randomly assigned to 1 of 4 diets in each of two dietary phases, containing either 10% fresh SO (22.5°C) or thermally processed SO (45°C for 288 h, 90°Cfor 72 h, or 180°C for 6 h), each infused with of 15 L/min of air. Peroxide values were 2.0, 17.4, 123.6, and 19.4 mEq/kg; 2,4-decadienal values were 2.07, 1.90, 912.15, and 915.49 mg/kg; and 4-hydroxynonenal concentrations were 0.66, 1.49, 170.48, and 82.80 mg/kg, for the 22.5, 45, 90 and 180°C processed SO, respectively. Pigs were individually housed and fed ad libitum for 81 d to measure growth performance, including a metabolism period to collect urine and feces for determination of GE, lipid, N digestibility, and N retention. Following the last day of fecal and urine collection when pigs were in the metabolism crates, lactulose and mannitol were fed and subsequently measured in the urine to evaluate gut permeability, while markers of oxidative stress were evaluated in plasma, urine, and liver. There were no differences observed in ADFI ($P = 0.91$), but ADG and GF were decreased in pigs fed 90°C SO diet ($P \leq 0.07$) compared to pigs fed the other SO diets. Pigs fed the 90°C and 180°C SO had the lowest ($P = 0.05$) DE as a % of GE compared to pigs fed the 22.5°C SO, with pigs fed the 45°C SO being intermediate. Lipid digestibility was similarly affected ($P = 0.01$) as energy digestibility, but ME as a % of DE was not affected by dietary treatment ($P = 0.16$). There were no effects of lipid peroxidation on N

digested, N retained, or the urinary lactulose:mannitol ratio ($P \geq 0.25$). Pigs fed the SO processed at 90°C and 180°C had lower concentrations ($P < 0.01$) of plasma Trp compared to pigs fed the 22.5°C and 45°C SO treatments. Pigs fed 90°C SO had the greatest ($P < 0.01$) concentrations of F2-Isoprostane in plasma and urine TBARS compared to the other SO treatments. These results indicate that the change in FA composition and/or the presence of lipid peroxidation products in peroxidized SO may reduce ADG, GF, and digestibility of GE and ether extract, but has little impact on N digestibility and balance or on gut permeability.

2.1 INTRODUCTION

Refined, crude, or recycling through yellow grease, soybean oil (**SO**) is commonly added to swine diets as a concentrated energy source (Pettigrew and Moser, 1991; Azain, 2001; Lin et al., 2013). Soybean oil contains a high concentration of mono- and polyunsaturated fatty acids (NRC, 2012) which unlike saturated fatty acids (**SFA**), are more susceptible to lipid peroxidation due to double bonds in their structure (Holman, 1954). Because lipids may undergo thermal stress during their processing, lipid peroxidation has the potential to affect the caloric value of a lipid when added to feed formulations. Lipid peroxidation is a free radical reaction that can be initiated by thermal processing of a lipid in the presence of oxygen, light, or metal ions (Labuza, 1971; Lundburg and Jarvi, 1971; Gray, 1978), with products generated and consumed being peroxides, lipid hydroperoxides, polar and nonpolar acids, ketones, aldehydes, and polymers (Gonzalez-Muñoz et al., 1998; Schaich, 2012). As lipid peroxidation progresses, the unsaturated:saturated fatty acid ratio (**UFA:SFA**) of a lipid will decrease and its FFA concentration may increase (Liu et al., 2014b), while antioxidants present in the lipid become depleted (Seppanen and Csallany, 2002), each of which may affect its energy value (Wiseman et al., 1998).

Consumption of peroxidized lipids have been shown to decrease lipid and energy digestibility (Liu et al., 2014c; Lindblom et al., 2018a) and growth performance parameters in swine (DeRouchey et al., 2004; Boler et al., 2012; Rosero et al., 2015), but there is a dearth of information as to which components of lipid peroxidation are most determental to lipid and energy digestibility, and ultimately animal performance. Therefore, the objective of this study was to evaluate the effect of feeding divergently thermally processed SO to finishing pigs on growth performance, plasma Trp, N, GE and lipid digestibility, and intestinal integrity.

2.2 MATERIALS AND METHODS

All animal care and use procedures for this experiment were approved by the Institutional Animal Care and Use Committee at Iowa State University.

2.2.1 Dietary Treatments

There were four thermally processed SO treatments within each phase of growth, which included either 10% fresh SO (22.5°C) or SO thermally processed at 45°C for 288 h, 90°C for 72 h, or 180°C for 6 h. Except for the 22.5°C temperature, each heating process was accompanied with constant air flow (15L/min) using an air pump and a calibrated air flow controller with air forced into the tank using a 9.5 mm copper pipe. Immersion heaters were used to heat the SO to 45°C and 90°C, while a liquid propane heater was used to heat the SO to 180°. Oil temperatures were taken at regular intervals to ensure the predetermined temperatures were maintained. The stainless steel heating pots were 53 mm in circumference and 61 cm high, and were filled 2/3 during the heating process. To generate enough SO for each treatment, multiple batches of SO were heated and subsequently pooled into a 210 L container and stored at room temperature prior to feed mixing. Multiple batches of each phase of feed was mixed with no antioxidant added before or during diet preparation, each diet was sampled at the time of mixing, and then pooled

across phase for a composite sample for subsequent analysis. Oils were also sampled at the time of mixing and pooled across all mixing times prior to analysis. Samples of the pooled oil and final mixed feed were stored at 0°C prior to analysis. Diverse analyses of each SO treatment was conducted as outlined in Tables 1 and 2 to characterize the quality of each SO treatment. During the 81 d study, diets were formulated to contain 0.90% (d 1 to 49, Phase-1) or 0.68% (d 49 to 81, Phase-2) standardized ileal digestible Lys, with AA ratios, ME, and mineral content calculated to be adequate for 44 to 90 and 90 to 135 kg pigs, respectively, according to the NRC (2012), Table 3. Diets were offered to the pigs ad libitum in meal form during the entire trial.

2.2.2 Experimental Design

Fifty-six (56) finishing barrows (Geneticporc F25 females × B6.0 sires, Hendersonville, TN) with an initial BW of 46.7 ± 5.1 kg, were randomly allotted into 56 pens equipped with partial slats, one two-holed feeder, and a single nipple waterer in a room that was mechanically ventilated with a pull-plug manure storage system located at the Swine Nutrition Farm at Iowa State University (Ames, IA). Pigs were randomly assigned to 1 of 4 dietary treatments, resulting in 14 replications per treatment. Each pig was individually penned (1.8 × 1.9 m) and had ad libitum access to feed and water.

While pigs were being fed the phase-1 diet, a metabolism experiment was conducted to evaluate N, acid hydrolyzed ether extract (**AEE**), and GE digestibility, and to collect urine to evaluate *in vivo* intestinal permeability and measures of oxidative stress. On d-21 and d-29, a group of 24 pigs each, were weighed (d 21 BW = 68.3 kg, SD = 7.1 kg) and moved to individual stainless steel metabolism crates (1.2 × 2.4 m). For the next 3 d, pigs were fed twice daily at 0700 and 1900, 1.25 kg/meal, to acclimate them to the metabolism crate and feeding schedule, with water offered ad libitum through nipple waterers. Following this 3 d adaption period, a 3 d

collection period occurred where stainless steel wire screens were placed under each metabolism crate for total fecal collection. Feces were collected twice daily and stored at 0˚C until the end of the collection period. At the end of the collection period, feces were dried in a 70˚C forced air oven, weighed, ground through a 2-mm screen, and a subsample was taken for nutrient analysis. Orts were subtracted from total feed offered to calculate net feed intake. Following this 3-d collection period, pigs were given 40 g lactulose and 4 g mannitol (Spectrum Chemical, Gardena, CA) in 100 g of feed at 2000 h and once this feed was consumed, the remainder of their feed allotment was given. Chlorohexidine (5 mL) was added to each urine container, urine was collected for the next 12 h (ending at 0800 h), quantified, and a sample obtained for subsequent analysis to assess *in vivo* intestinal permeability (Wijtten et al., 2011). After this collection, the urine buckets were cleaned and replaced under the metabolism crates with 5 mL of chlorohexidine, and an additional collection of urine occurred for 7 hr (ending at 1500 h), quantified, and a sample obtained for measures of *in vivo* oxidative stress. Pigs were returned to their individual pens for the remainder of the performance study, with feed consumption during the metabolism crated period added to the total feed intake for the performance trial. On d 78 while pigs were still on feed, each pig was bled (10 mL) at 0900 h via jugular venipuncture into heparinized tubes (158 USP units sodium heparin; BD Vacutainer, BD Diagnostics, Franklin Lakes, NJ), samples were centrifuges at 2,500 × g for 10 minutes at 4˚C, after which an aliquot of plasma was stored at -80˚C for subsequent analysis. On d 81, all pigs and feeders were individually weighted to determine ADG, ADFI, and GF.

2.2.3 Calculations and Methodologies

Diets and feces were analyzed for AEE (Thermo Scientific Application Note 361, Thermo Fisher Scientific, Salt Lake City, UT) using an accelerated solvent extraction system

(model 350, Dionex, Bannockburn, IL) and 100 mL stainless steel extraction cells to accomplish the lipid extraction. Gross energy of the SO, diets, feces, and urine was determined using an isoperibol bomb calorimeter (model 6400, Parr Instrument Co., Moline, IL) using benzoic acid as a standard. Nitrogen was analyzed by thermo-combustion (VarioMAX CNS, Elementar Analysensysteme GmbH, Hanau, Germany) where combustion gases are converted to individual gases and sorted into adsorption columns and are measured using a thermal conductivity detector. Digestibility coefficients for AEE, GE, and N were estimated using a time-based collection methodology. Metabolizable energy as a percent of DE was calculated by dividing ME intake by DE intake and N retention as a percent of N digested was calculated by dividing N retained by N digested, both reported as a percent. Detailed descriptions of metabolism experimental methods are provided elsewhere (Adeola, 2001; Kerr et al., 2013; Li et al., 2016). Urinary lactulose and mannitol concentrations were measured via HPLC as an *in vivo* indicator of small intestinal permeability and using the method as described by Kansagra et al., (2003). The ratio of lactulose:mannitol (**L:M**) was calculated back to the total amount of urine collected and reported on a recovery basis. For plasma Trp concentration, plasma was diluted 1:1 with 0.05M potassium phosphate buffer, pH 6.0, and deproteinized with 2M trichloroacetic acid. Plasma Trp levels were subsequently determined by separation on a 4 μm spherical silica gel particle column (Superspher 100 RP-18 LiChroCART, Millipore Sigma, Billerica, MA) by an automated HPLC system with a fluorescence detector (Jasco FP-1520, Jasco Analytical Instruments, Easton, MD).

Several markers of oxidative were evaluated in plasma, urine, and liver homogenates. Samples were diluted as needed in order for the measured values to fall within range of the standard curve. Assays were conducted in in triplicate with an intra-assay CV of $\leq$ 5%.

Thiobarbituric acid reactive substances (**TBARS**) and F_2-isoprostane (**ISP**) were analyzed in urine, plasma, and liver as indicators of lipid peroxidation. No preparation step was necessary prior to analyzing plasma or urine for TBARS or ISP. Liver tissue (100 mg) was homogenized in 1 ml of RIPA buffer (Cayman Chemical Co., Ann Arbor, MI; #10010263) then centrifuged at 1600 × g for 10 min at 4 ^{o}C. The TBARS assay was conducted on the resulting supernatant. Analysis of ISP were measured in undiluted plasma and 1:10 solution of urine and deionized water. Both urinary TBARS and ISP were normalized to the volume of urine excreted during the collection period by multiplying the concentration of the analytes by the volume of urine. Protein carbonyls (**PC**) were measured in plasma and liver samples only, as protein is not excreted in the urine. Plasma samples were analyzed for PC using the protocol outlined by the kit (Cayman Chemical Co., Ann Arbor, MI; #10005020); whereas, liver tissue (200 mg) was homogenized in 1 mL of 50mM phosphate buffer + 1mM EDTA then centrifuged at 10,000 × g for 15 min at 4 ^{o}C. The resulting supernatant was then used to evaluate the concentration of PC, similar to the protocol employed for plasma. Under conditions of oxidative stress, DNA may also be damaged. The most oxidatively labile nucleic acid is guanine, and therefore its' oxidation product, 8-hydroxy-2'-deoxyguanosine (**8-OH-2dG**) is commonly assayed as a measure of DNA damage due to oxidative stress (Wu et al., 2004). Thus, urine and liver samples were assayed for 8-OH-2dG. For liver samples, DNA was extracted from 25 mg of tissue (ZR Genomic DNA- Tissue MiniPrep, Zymo Research, Irvine, CA) with yields of DNA evaluated using Gen 5 software on Cytation 5 Imaging Reader (BioTek, Winooski, VT); DNA yields ranged between 20 to 40 µg. Digestion of DNA was then conducted using nuclease (P1 nuclease, Sigma-Aldrich, St. Louis, MO) which converts double stranded to DNA to single stranded DNA. Nucleotides were then converted to nucleosides by adding 1unit of alkaline phosphatase (Roche Diagnostics,

Mannheim, Germany) per 100 µg of DNA. The resulting supernatant was evaluated for 8-OH-2dG using a commercial kit (Cayman Chemical Co., Ann Arbor, MI; #589320) with no dilution being necessary. No DNA isolation step was required for urine samples prior to analysis, but urine was diluted 1: 750 in sample buffer prior to assessment.

The activity of endogenous antioxidant enzymes, such as glutathione peroxidase (**GPx**) and catalase (**CAT**), are often affected by oxidative stress (Lykkesfeldt and Svendsen, 2007; Royer et al., 2016). Therefore, GPx activity was assayed in both plasma and liver, and CAT activity was measured in liver samples. To measure GPx activity in liver and plasma, 100 mg of sample was first homogenized in 1 mL 50 mM Tris-HCl + 5 mM EDTA + 1mM DTT. The homogenate was then centrifuged at 10,000 × g for 15 min at 4 °C. The resulting supernatant was diluted 1: 20 in sample buffer prior to being assayed. Activity of CAT in liver was evaluated by first homogenizing 100 mg of tissue in 1 mL of 50 mM potassium phosphate + 1mM EDTA. The homogenate was then centrifuged at 10,000 × g for 15 min at 4 °C. The resulting supernatant was then diluted 1: 10,000 in sample buffer and then assayed for CAT activity (nmol $min^{-1}mL^{-1}$).

2.2.4 Statistical Analysis

Data were analyzed as a completely randomized design with individual pig as the experimental unit with initial BW serving as a covariate for analyses of growth performance data, using Proc MIXED procedure of SAS (version 9.4; SAS, 2009). Means were reported and separated using LSMEANS. In addition, relationships between lipid peroxidation measures with growth performance and digestibility variables were evaluated by simple linear correlation (Pearson correlation coefficients) analysis. Differences were considered significant at $P \leq 0.05$, whereas values of $0.05 \leq P \leq 0.10$ were considered statistical trends.

2.3 RESULTS AND DISCUSSION

2.3.1 Compositional changes of soybean oil due to thermal processing

Lipid peroxidation is an extremely complex process, involving free radical formation and subsequent propagation of free radicals which ultimately bind to PUFA (Holman, 1954), where the development of lipid peroxidation products is based on the duration and intensity of thermal processing and presence of oxygen (St. Angelo et al., 1996). In general, thermal processing of lipids decreases lipid quality through hydrogenation of double bonds and the formation of peroxides in the initiation phase of lipid peroxidation, which can then be degraded into polar and nonpolar acids, ketones, and aldehydes in the propagation phase, ultimately forming indigestible polymers in the termination phase (Grey, 1978; Gonzalez-Muñoz et al., 1998). Similar to the peroxidation of SO as reported by Lindblom et al. (2018a), the current experiment induced peroxidation by processing SO at different temperatures and durations prior to being mixed in the diet. To generate SO with different degrees of peroxidation, SO was heated at 45°C for 288 h, 90°C SO for 72 h, and 180°C for 12 h to reflect conditions noted in the livestock, rendering, and restaurant industries (Meeker and Hamilton, 2006) and to use temperatures which have been evaluated in previously research (Boler et al., 2012; Liu et al., 2014b; Kerr et al., 2015; Rosero et al., 2015; Hanson et al., 2016). The four SO were analyzed in detail prior to feed mixing for FA composition, lipid quality, and various lipid peroxidation products (Tables 1 and 2) which have been described in detail elsewhere (Shurson et al., 2015).

As shown in Table 2, the unprocessed SO (i.e., 22.5°C) had a higher UFA:SFA than SO processed at 90° and 180°C (5.28 versus 4.73 and 4.88, respectively), a decrease which can be explained by the slight increases in C16:0 and C18:0 with slight decreases in C18:2 and C18:3. While UFA:SFA is an important measure for energy predictability in swine (Wiseman et al.,

1998), it is fairly crude measure of the degree of unsaturation, and thus susceptibility to peroxidation. Because of this, we calculated iodine value (**IV**, Meadus et al., 2010) as an additional measure of FA unsaturation. For SO processed at 90° and 180°C there was a decrease in IV compared to SO not thermally processed (22.5°C) or processed at 45°C (126 and 127 versus 131 and 131, respectively). The observed changes in both UFA:SFA and IV were expected because as lipid peroxidation progresses, there is a general hydrogenation of FA which decreases the number of double bonds available for peroxidation (Holman, 1954; Yin et al., 2011). The changes in FA composition, UFA:SFA, and IV in the current experiment are similar to that reported by others (DeRouchey et al., 2004; Liu et al., 2014b, Hanson et al., 2015; Kerr et al., 2015; Rosero et al., 2015; Lindblom et al., 2018a).

Even though numerous quality factors were measured in an effort to expand the basis of understanding of lipid peroxidation in livestock feeds (Table 2), only a few of the more common factors will be discussed. Peroxide value, a measurement of peroxides and hydroperoxides formed in the initiation phase, was determined to be highest in the 90°C processed SO followed by the 45°C and 180°C processed SO, and lowest in the fresh SO with values of 123.6, 17.4, 19.4, and 2.0 mEq/kg SO, respectively. Oxidized fatty acids (**OFA**), a measure of lipid hydroperoxides and peroxides and saturated epoxy-, keto-, and hydroxy-acids, did not differ greatly and were low across all SO treatments, but did slightly decline in SO processed at 90°C and 180°C. Although counterintuitive,this suggests that at 90°C and 180°C of thermal processing, these lipid peroxidation products have been further degraded into aldehydes and polymers. Lastly, anisidine value (**AnV**), a measure of high molecular weight saturated and unsturated aldehydes, and total polar compounds (**TPC**), a measure of monoglycerides, diglycerides and free fatty acids, were highest in the 90°C and 180°C SO compared to the 22.5°C

and 45°C SO, indicating that our method of thermal processing generated SO to different degrees of peroxidation. The changes in PV, OFA, AnV, and total polar compounds were expected based on the overall process of lipid peroxidation and are of similar directions that reported by others (Engberg et al., 1996; Kerr et al., 2015; Lindblom et al., 2018a).

Specific aldehydes were also measured because some (i. e., hexanal) are common measures of lipid peroxidation reported in the literature (Shurson et al., 2015), and also because acrolein (Kehrer and Biswal, 2000; Abraham et al., 2011), 2,4-decadienal (**DDE**, Chang et al., 2005), and 4-hydroxynonenal (**HNE**, Esterbauer et al., 1991) are considered highly damaging aldehydes to DNA, proteins, and lipids *in vivo*. Furthermore, a ratio among two clusters of aldehydes has been shown to be closely associated with the progression of SO peroxidation (Wang et al., 2016). However, few if any of these specific aldehydes or their ratio has been evaluated in respect to lipids fed to livestock and subsequent impacts on performance or digestive functions. In the current experiment, thermal processing of SO at 45°C had little effect on acrolein, hexanal, DDE, or HNE concentrations, or a proposed aldehyde ratio, compared to the unprocessed SO (Table 2). In contrast, concentrations for each of these aldehydes and for the calculated aldehyde ratio were increased when SO was processed at 90°C or 180°C.

In addition to measuring lipid peroxidation compounds, we were interested in measuring the tocopherol concentrations status of SO. It is well known that tocopherols are natural antioxidants found in vegetable oils and function in protecting the lipid from degradation (Kamal-Eldin, 2006) so as lipid peroxidation progresses it would be expected that these compounds would be depleted. In the current study, the 90°C processed SO had the lowest total tocopherol (**TOC**) concentration with 94 mg/kg oil followed by 180°C, 45°C, and fresh oil with 798, 1.331, and 1.328 mg/kg respectively. Our data is similar to others (Miyagawa et al., 1991;

Lindblom et al., 2018a) who reported that thermally processing a blend of soybean oil and rapeseed oil or SO, respectively, would result in a degradation of tocopherols.

2.3.2 Growth performance

Performance data were collected over the 81 d trial, including the 5 d while pigs were in the metabolism crates. During the experimental period, 1 pig fed the 22.5°C SO, 1 pig fed the 45°C SO, and 1 pig fed the 180°C was removed from the trial due to health reasons unrelated to the SO treatment. As shown in Table 4, pigs fed the 90°C SO had reduced ADG (P = 0.07) in comparison to the other three SO treatment groups, with no differences noted among pigs fed the 22.5°C, 45°C, and 180°C SO treatments. Thermal processing of SO did not affect ADFI (P = 0.91) resulting in pigs fed diets containing the 90°C processed SO having a reduced GF (P = 0.04) compared to the other SO treatments, with no differences noted among pigs fed the other SO treatments. The reduction in ADG in pigs fed the 90°C SO is in agreement with others (DeRouchey et al., 2004; Boler et al., 2012; Liu et al., 2014a; Rosero et al., 2015; Hanson et al., 2016; Lindblom et al., 2018a) who reported decreased ADG in pigs fed lipids with increased concentrations of lipid peroxidation products compared to pigs fed lipids with low levels of lipid peroxidation products. The reduction in ADG by 6% due to processing SO at 90C was similar to the reduction in growth compared to pigs fed fresh lipid sources as reviewed by Hung et al. (2017). Lipid peroxidation compounds have also been shown to affect palatability and feed intake in swine (Boler et al., 2012; Liu et al., 2014a, Rosero et al., 2015), consequently we would have expected a decrease in ADFI with the different thermally processed SO. This was not, however, observed in the current experiment (Table 4). While the lack of an effect on ADFI are in agreement with Hanson et al. (2016) who fed graded levels of peroxided corn oil to nursery pigs and Lindblom et al. (2018a) who fed thermally processed SO to growing pigs, these data are

in disagreement with the review by Hung et al. (2017) who summarized that pigs fed peroxidized lipids had decreased ADFI by approximately 5% compared to pigs fed unperoxidized lipids. The reduction in GF in the current experiment by 5% for pigs fed the 90°C SO is supported by the review by Hung et al. (2017) where GF was reduced by 2% compared to pigs fed fresh lipid sources, as well as by Hanson et al. (2016) who fed peroxidized corn oil to nursery pigs and Lindblom et al. (2018a) who fed peroxidized SO to growing pigs. In contrast, no effect on GF was reported when nursery pigs were fed peroxidized choice white grease (DeRouchey et al., 2004), when finisher pigs were fed peroxidized corn oil (Boler et al., 2012), or when nursery pigs were fed peroxidized SO (Rosero et al., 2015). In the current experiment, the different thermal processing temperatures and times were selected to generate different concentrations of lipid peroxidation products from which to increase our understanding as to which lipid peroxidation products are most detrimental to pig growth. Even though thermally processing SO at 45°C or 180°C did generate different concentrations of lipid peroxidation products (Table 2), their consumption did not affect pig performance, similar to that reported by Lindblom et al. (2018a). Different lipid peroxidation product concentrations in the feed can also be accomplished by blending an unperoxidized lipid with a peroxided lipid, where it has been show that consumption of low levels of lipid peroxidation products may have little to no measurable effect on pig performance (Rosero et al., 2015; Hanson et al., 2016), indicating that continued research efforts need to be made in this area.

Plasma Trp was measured because of its involvement in brain and nervous system function as well as many behavioral and physiological processes, including feed intake (Baranyiova, 1991; Seve, 1999). Although ADFI was not affected by SO peroxidation, pigs fed the SO processed at 90°C and 180°C had lower concentrations ($P < 0.01$) of plasma Trp

compared to pigs fed the 22.5°C and 45°C SO treatments (Table 4). These data are supported by Wang et al. (2017) in mice and Lindblom et al. (2018a) in growing pigs that feeding peroxidized SO resulted in a decrease in plasma Trp.

Because measures of growth performance and plasma Trp were affected by SO processing, a correlation analysis of pig performance responses with various measures of lipid peroxidation products were conducted (Table 5). We elected to conduct correlations specifically for UFA:SFA and polymerized triacylglycerides (**PTAG**) because of its impact on energy digestibility (Marquez-Ruiz et al., 1992; Gonzalez-Munoz et al., 1998; Wiseman et al., 1998); and peroxidation indices of PV, AnV, oxygen stability index (**OSI**), and hexanal because they are common measures of lipid peroxidation in the literature (Shurson et al., 2015). In addition, OFA and TPC were included because they are measureable components in the peroxidation process and the specific aldehydes of acrolein (Kehrer and Biswal, 2000; Abraham et al., 2011), DDE (Wang et al., 2016), and HNE (Esterbauer et al., 1991; Wang et al., 2016) because these aldehydes are considered highly damaging to DNA, proteins, and lipids *in vivo*; including a ratio of aldehydes associated with SO peroxidation (Wang et al., 2016). Lastly, TOC were evaluated as they assist in providing an accurate depiction of the antioxidant status naturally occurring in SO (Seppanen and Csallany, 2002; Kamal-Eldin, 2006).

Irrespective of diet, UFA:SFA, and TOC exhibited a positive correlation to ADG, GF, and plasma Trp. In contrast, PV, TPC, hexanal, and HNE all exhibited negative correlations with ADG, GF, and plasma Trp (Table 5). Interestingly, there were no correlations between ADG and GF with AnV, OFA, PTAG, acrolein, DDE, or the aldehyde ratio. Likewise, there were no significant correlations noted between ADFI and any of the lipid quality indices, and thus not listed in Table 5. Because the difference in FFA and TBARS values among the SO treatments

were considered to be insignificant, we did not attempt to correlate these parameters to pig performance, even though it is known that FFA and PTAG can affect the energy value of a lipid (Marquez-Ruiz et al., 1992; Gonzalez-Munoz et al., 1998; Wiseman et al., 1998) and TBARS has been shown to be correlated to ADG and ADFI in swine (Hung et al., 2017). The positive correlation between ADG and GF with TOC is in contrast to Hung et al. (2017) who reported no such relationship.

2.3.3 Energy and lipid digestibility

As shown in Table 6, DE as a percentage of GE was greatest in pigs fed the fresh SO (91.13%), lowest in pigs fed the 90°C and 180°C SO (89.51 and 89.37%, respectively), and intermediate in pigs fed the 45°C SO (90.96%, $P = 0.03$). There were no differences noted among the SO treatments for ME as a percent of DE ($P = 0.16$). Similar to energy digestibility, pigs fed the fresh SO had the greatest AEE digestibility (84.60%), was lowest in pigs fed the 90°C and 180°C SO (80.99 and 80.59%, respectively), and intermediated in pigs fed the 45°C SO (82.54%, $P = 0.01$). These results are in agreement with DeRouchey et al. (2004), Rosero et al. (2015), and Lindblom et al. (2018a) who reported a reduction in energy and lipid digestibility when pigs were fed peroxidized lipids relative to unperoxidized lipids. In contrast, Liu et al. (2014c) did not report changes in DE as a percentage of GE or ME as a percentage of DE when pigs were fed various peroxidized lipids.

Multiple positive correlations were observed between DE as a percent of GE, ME as a percent of DE (even though there was not a treatment effect noted for this measure), and AEE digestibility with UFA:SFA, OFA, OSI, and TOC, Table 7. In contrast, these energy and lipid digestibility measures were negatively correlated to measures associated with lipid peroxidation (PV, TPC, AnV, acrolein, DDE, hexanal, HNE, and the aldehyde ratio). These correlations

would be expected because when SO is peroxidized there are small but consistent changes in the FA profile which are known to affect energy and lipid digestibility (Wiseman et al., 1998). Although confounded with the FA profile changes in the current experiment, the generation of lipid peroxidation products may also be independently responsible for decreased digestibility, but no known data is available to support this statement. The reduction in energy and AEE digestibility in pigs fed the 90°C SO also helps explain the reduction in ADG and GF noted in this SO treatment.

2.3.4 Nitrogen digestibility and balance

There were no differences noted among the SO treatments for N digestibility ($P = 0.94$) or N retained as a percent of N digested ($P = 0.25$; Table 6). In addition, there were no correlations observed between SO treatments with N digestibility, and N retention as a percent of N digested was only correlated to OSI (R = 0.28, $P = 0.08$; Table 7). These results are supported DeRouchey et al. (2004) who did not report any statistical differences among N digestibility in nursery pigs fed thermally processed choice white grease and Liu et al. (2014c) who did not report any differences in N digestibility or N retention due to feeding various peroxidized lipids. In contrast, Lindblom et al. (2018a) reported a decrease in N digestibility and N retention in growing pigs fed SO thermally processes at 90C for 72h; and also reported that N retention as a percent of N digested was correlated to many lipid quality indices. Differences in N digestibility and retention suggest that lipid peroxidation may affect *in vivo* metabolism, but further research is needed to confirm this statement.

2.3.5 Intestinal barrier function

Measuring the effects of lipid peroxidation products on intestinal permeability is of interest because it has been shown that intestinal permeability is increased when consuming a

diet high in saturated FA (Laugerette et al., 2012; Mani et al., 2012; Liu et al., 2014d), but little data is available on the effects of feeding peroxidized lipids on intestinal permeability. In the current study, there were no differences in the urinary L:M ratio among SO treatment groups with urinary ratios of L:M on a recovery basis averaging 0.077 (P = 0.51; Table 6). Likewise, there were no correlations observed between the L:M ratio with any lipid peroxidation product. While we might have expected an increase in intestinal permeability in pigs fed 90°C and 180°C SO because of the changes in lipid saturation, this was not the case. Our findings are, however, in agreement with Liu et al. (2014d) and Lindblom et al. (2018a) who reported no significant differences in urinary L:M ratios among lipid peroxidation levels, and as expected, no correlations to any lipid quality indices.

2.3.6 Oxidative status of plasma, liver, and urine

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (**ROS**) and the available system of antioxidant defenses (Betteridge, 2000). If the pool of antioxidant defenses is insufficient, ROS are left unchecked and may cause damage to tissues. One of the most routinely used markers of oxidative stress are TBARS, which is a measure of compounds produced from lipid oxidation (Dalle-Donne et al., 2006). Previous studies involving feeding peroxidized fat sources have reported increased TBARS in blood and tissues (Ringseis et al., 2007; Boler et al., 2012; Varady et al., 2012). However, in the present study, there was no effect ($P \geq 0.39$) of SO peroxidation status noted on plasma TBARS, PC, or GPx, Table 8. This is supported by Hanson et al. (2015) who reported a similar result in comparing serum TBARS of pigs fed either peroxidized- or fresh-dried distillers grains with solubles. In the current pexeriment, plasma ISP of pigs fed 90°C SO was 1.4- to 1.6× greater ($P < 0.01$) than pigs fed the other SO treatments, with no difference ($P \geq 0.32$) noted in plasma ISP

among pigs fed the 22.5°C, 45°C, or 180°C SO. Because TBARS are a non-specific metric of lipid peroxidation that detects numerous compounds and ISP is specifically a product of the oxidation of arachidonic acid (Morrow et al., 1995), ISP is a preferred measure of lipid damage (Dalle-Donne et al., 2006). The discernable difference in plasma ISP among SO treatments in the current experiment, where other markers were not indicative of oxidative stress, may be due to the stability and specificity of the ISP molecule as an indicator of lipid damage.

It was hypothesized that the most pronounced oxidative stress would be observed in the livers of pigs fed oxidized oils. Although there was liver hypertrophy observed with oxidized oil feeding of these pigs (Overholt et al., 2018), there was no effect ($P \geq 0.12$) of SO noted on TBARS, PC, 8-OH-2dG, GPx activity, or CAT activity (Table 8). In light of the enlarged livers in pigs fed peroxidized SO, we had expected there to be accompanying evidence of cytoxic damage, especially in the form of DNA damage. DNA is particularly susceptible to oxidative damage (Wu et al., 2004) and 8-OH-2dG concentrations are known to increase in livers damaged by toxicity or oxidative stress, such as with hepatitis and cirrhosis (Shimoda et al., 1994), as well as in non-alcoholic fatty liver disease (Seki et al., 2002).

Amounts of TBARS detected in urine of pigs fed 90°C were 1.8- to 3.3× greater ($P < 0.03$) than pigs fed the other SO treatments, with no difference ($P > 0.22$) in urinary TBARS among pigs fed 22.5°C, 45°C, or 180°C SO (Table 8). Excretion of TBARS, in particular malondialdehyde, are reported to increase with the onset of oxidative stress (Draper et al., 2000). Because the excretion of TBARS in urine is dependent on the quantity of fat consumed, fatty acid profile and other factors, it is therefore not considered a reliable marker of oxidative stress in clinical settings (Draper et al., 2000; Dalle-Donna et al., 2006). In this study, however, the pigs were fed similar diets (save for treatment imposed on the SO) and were raised under

controlled conditions. Consequently, the increased urinary TBARS observed in pigs fed 90˚C SO should be considered an indication of lipid damage caused by oxidative stress. However, due to the number of factors that can influence urinary TBARS, they should not be relied upon as the sole marker of oxidative stress. The increased urinary TBARS is supported by the 2.5× greater ($P < 0.01$) amounts of urinary ISP in pigs fed 90°C SO compared with pigs fed either 22.5°C or 45°C SO, with 180°C being intermediate.

It is likely that the suppression of pig growth and nutrient digestibility could be attributed, at least in part, to specific or combinations of physicochemical characteristics of the oils. Because of this, we conducted correlation analyses to examine the relationships among the physicochemical traits of the oil with markers of oxidative stress (Table 9). There were no correlations observed between SO treatment and plasma TBARS, plasma PC, plasma GPx, liver TBARS, liver 8-OH-2dG, liver CAT, or urinary 8-OH-2dG so they were omitted from the table. Among the oil traits, TPC, heptanal, and octenal, total tocopherols were the most consistently correlated with measures of oxidative stress. Urinary ISP was moderately ($P < 0.01$) correlated with all of the oil traits presented; whereas, urinary TBARS and Plasma ISP were weakly to moderately correlated ($P \leq 0.05$) with all oil traits presented except for free glycerin, OFA, acrolein, undecenal, and the ratio of aldehydes. Inconsistent, but largely weak correlations existed among liver PC and GPx activity and the oil traits. The consistent correlation among ISP in both urine and plasma may be reflective of the compounds stability, and may provide preliminary support for its potential use as a viable marker of oxidative stress in finishing pigs. Based on these results, it remains uncertain exactly what measure of oil quality may be most indicative of the potential for a SO to induce oxidative stress. It is of not that these results are dissimilar from the analyses conducted by Lindblom et al. (2018b), which reported that OFA was

the oil trait most consistently and strongly correlated with markers of oxidative stress in growing pigs. Furthermore, Lindblom et al. (2018b) reported at least 8 different markers of oxidative stress were correlated with specific physicochemical traits of the oils; whereas, only 5 such oxidative stress markers were found to correlate with any oil traits. Although it is unclear why such disparity occurred between these two studies, which were conducted under similar conditions, the more mature finishing pigs used in the present study may have had more developed antioxidant defense systems than the growing pigs, which may have ameliorated the effect of the diets.

2.3.7 Conclusion

In conclusion, thermal processing of SO reduced UFA:SFA and IV, but increased numerous lipid peroxidation compounds including PV, AnV, TPC, and several aldehydes. The combination of changes in FA profile and the formation of lipid peroxidation products were found to be greatest in the SO thermally processed at 90°C for 72 h in comparison to other SO treatment groups, which resulted in reduced ADG, GF, and GE and AEE digestibility. In contrast, peroxidation of SO did not affect N digestion, N retention, or intestinal permeability as measured by urinary L:M. There were minimal effects of feeding peroxidized SO on measures of oxidative stress, although plasma ISP and urinary TBARS and ISP were found to be greatest in pigs fed the SO thermally processed at 90°C for 72 h in comparison to other SO treatment groups. Using simple correlation coefficients, there were some common indices of lipid peroxidation products correlated with growth and digestibility which may provide insight on which lipid peroxidation products should be further assessed in determining lipid quality impacts on animal productivity.

2.4 TABLES

Table 2.1. Method of analysis for thermally processed soybean oils

Analyte	Method
Aldehydes[1]	Wang et al., 2016
p-Anisidine value[2]	AOCS Cd 18-90
Fatty acids[2]	AOCS Ce 1a-13
Free fatty acids[2]	AOCS Ca 5A-40
Free glycerin[2]	AOCS Ca 14-56
Insoluble impurities[2]	AOCS Ca 3-46
Moisture[2]	AOCS Ca 2c-25
Oil stability index[2]	AOCS Cd 12b-92
Oxidized fatty acids[2]	AOCS G 3-53
Peroxide value[2]	AOCS Cd 8b-90
Polymerized triacylglycerides[3]	AOAC 993.25
Thiobarbituric acid value[2]	AOCS Cd 19-90
Tocopherols[2]	AOCS Ce 8-89
Total polar compounds[2]	AOCS Cd 20-91
Unsaponifiable matter[2]	AOCS Ca 6a-40

[1]Analyzed by University of Minnesota, St. Paul, MN.
[2]Analyzed by Barrow-Agee, Memphis, TN.
[3]Analyzed by the USDA-ARS, Peoria, IL.

Table 2.2. Composition and peroxidation analysis of thermally processed soybean oils

Heating temperature, °C	22.5	45	90	180
Time heated, h[1]	0	288	72	6
Fatty acids, % of total fat[2,3]				
C8:0, Caprylic	ND[4]	ND	ND	0.07
C16:0, Palmitic	10.69	10.74	11.70	11.21
C16:1, Palmitoleic	0.09	0.08	0.09	0.08
C17:0, Margaric	0.10	0.10	0.11	0.10
C18:0, Stearic	4.19	4.13	4.52	4.42
C18:1, Oleic	23.46	23.49	25.15	24.26
C18:2, Linoleic	53.07	52.86	50.79	51.65
C18:3, Linolenic	7.14	7.15	6.14	6.43
C19:0, Nonadecanoic	0.26	0.25	0.23	0.35
C20:0, Arachidic	0.32	0.32	0.35	0.34
C20:1, Gadoleic	0.18	0.19	0.20	0.30
C22:0, Behenic	0.35	0.35	0.49	0.41
C24:0, Lignoceric	ND	0.12	ND	0.13
Other FA	0.15	0.22	0.23	0.27
UFA:SFA[4]	5.28	5.23	4.73	4.88
IV[5]	131	131	126	127
Free fatty acids, %[2]	0.10	0.10	0.46	0.14
Free glycerin, %[2]	1.04	3.72	0.82	0.82
Moisture, %	0.02	0.02	0.04	0.04
Insoluble impurities, %	0.02	0.04	0.04	0.04
Unsaponifiable matter, %	0.51	0.39	0.41	0.47
Oxidized FA, %[2]	3.0	2.8	2.5	1.9
OSI @ 110 C, h[2,4]	6.65	4.65	2.70	3.65
p-Anisidine value[2,6]	1.11	1.33	121	165
Peroxide value, meq/kg[2]	2.0	17.4	123.6	19.4
Polar compounds, %[2]	3.61	3.28	20.25	11.58
PTAG[4,7], %	ND	ND	3.16	3.10
TBA value[2,6]	0.14	1.14	0.14	0.14
Aldehydes, mg/kg[8]				
2,4-decadienal	2.07	1.90	912.15	915.49
4-hydroxynonenal	0.66	1.49	170.48	82.80
Acrolein	6.15	6.06	27.12	44.60
2-Decenal	0.16	0.19	55.21	81.60
2,4-Heptadienal	0.47	1.19	268.62	151.65
2-Heptenal	2.33	1.82	254.48	90.68
Hexanal	2.01	2.02	33.69	6.28
2-Octenal	0.40	0.67	212.60	51.96
Pentanal	5.36	1.05	10.76	2.84
2,4-Undecadienal	0.06	0.07	43.73	53.34
2-Undecenal	0.19	0.19	50.29	110.38

Table 2.2 (Continued)

Ratio[9]	0.12	0.21	0.58	1.14
Total tocopherols, mg/kg[2]	1,328	1,331	94	798
Alpha	98	97	< 10	< 10
Beta	< 10	< 10	< 10	< 10
Delta	196	209	15	169
Gamma	1,034	1,025	79	629

[1]Thermally processed oils had constant air flow rate at 15L/min.
[2] Analyzed by Barrow-Agee, Memphis, TN.
[3] No other FA were detected besides those listed.
[4]Abbreviations: ND, not detected; FA, fatty acid; UFA:SFA, unsaturated:saturated fatty acid ratio; TBA, thiobarbituric acid; OSI, oil stability index; PTAG, polymerized tryacylglycerides; IV, iodine value.
[5] Iodine values were calculated using the FA profile data following the equation: VI = (16:1 × 0.95) + (18:1 × 0.86) + (18:2 × 1.732) + (18:3 × 2.616) + (20:1 × 0.795) + (20:2 × 1.57) + (20:3 × 2.38) + (20:4 × 3.19) + (20:5 × 4.01) + 22:4 × 2.93) + (22:5 × 3.68) + (22:6 × 4.64); Meadus et al., 2010.
[6] There are no units for p-anisidine value or TBA value.
[7]Analyzed by the USDA-ARS, Peoria, IL.
[8]Analyzed by University of Minnesota, St. Paul, MN.
[9]Ratio of 2-decenal, 2,4-hydroxynonenal, 2,4-undecadienal, and 2-undecenal as a percent of total aldehydes to acrolein, 2,4-heptadienal, and 2-heptenal as a percent of total aldehydes; Wang et al., 2016.

Table 2.3. Ingredient and calculated composition of treatment diets, as-is basis

Ingredient, %	Phase-1[1]	Phase-2[1]
Corn	66.45	73.23
Soybean meal, 46% CP	20.45	14.20
Soybean oil heat treatment[2]	10.00	10.00
Limestone	1.05	0.90
Monocalcium phosphate	0.85	0.64
Sodium chloride	0.35	0.35
Vitamin mix[3]	0.25	0.25
Trace mineral mix[4]	0.15	0.15
L-lysine•HCl	0.30	0.21
L-threonine	0.08	0.06
DL-methionine	0.07	0.01
TOTAL	100.00	100.00
Calculated composition		
ME, kcal/kg	3,800	3,800
CP, %	15.6	12.8
Lys, %	1.01	0.77
Ca, %	0.63	0.52
P, %	0.50	0.43
Analyzed composition[5]		
CP, %	15.1	12.6
Crude fat, %	12.1	11.9

[1] The Phase-1 diet was fed for 49 d (pig BW 46.7 to 98.2 kg) and the Phase-2 diet was fed for 32 d (pig BW 98.2 to 130.0 kg).

[2] Dietary treatments consisted of 10% refined soybean oil that was either unheated (22.5°C), or heated at 45°C for 288 h, 90°C for 72 h, or 180°C for 6 h, with 12 L/min of air supplied by a bubbled into the heating vesicle, excluding the 22.5°C temperature which was not aeriated.

[3]Provided the following per kilogram of diet: vitamin A, 7,656 IU; vitamin D_3, 875 IU; vitamin E, 62.5 IU; vitamin K, 3.75 mg; vitamin B_{12}, 0.06 mg; riboflavin, 13.75 mg; niacin, 70 mg; and pantothenic acid, 33.75 mg.

[4]Provided the following per kilogram of diet: Cu (as $CuSO_4$), 16.5 mg; Fe (as $FeSO_4$), 165 mg; I (as $Ca(IO_3)_2$), 0.3 mg; Mn (as $MnSO_4$), 39 mg; Zn (as $ZnSO_4$), 165 mg; and Se (Na_2SeO_3), 0.3 mg.

[5]Average analysis across all four soybean oil treatments.

Table 2.4. Growth performance and plasma Trp of pigs fed soybean oil with differing peroxidation levels.

	Processed soybean oil[1]				Statistics	
Parameter	22.5	45	90	180	SEM	P value
ADG, kg	1.04[ab]	1.09[a]	0.98[b]	1.07[a]	0.03	0.07
ADFI, kg	2.77	2.77	2.72	2.80	0.07	0.91
GF	0.38[ab]	0.39[a]	0.36[b]	0.38[ab]	0.01	0.04
Plasma Trp, μM/mL	62.6[a]	63.0[a]	50.4[b]	50.3[b]	1.65	0.01

[1]Data are least square mean of 13 observations for 22.5, 45, and 180; and 14 observations for 90. 22.5 = fresh oil; 45 = oil heated for12 d at 45°C with constant compressed air flow rate at 15L/min; 90 = oil heated for 72 h at 90°C with constant compressed air flow rate at 15L/min; 180 = oil heated for 6 h at 180°C with constant compressed air flow rate at 15L/min. Performance data was collected over 81 d with initial average BW of 46.8 ± 5.2 kg (P = 0.99) and final average BW of 131.2 ± 11.5 kg (P = 0.06).

[2]Superscripts reflect peroxidized soybean oil treatment differences (abc, $P \leq 0.07$).

Table 2.5. Pearson correlation coefficients among lipid composition and peroxidation measures with performance and plasma Trp[1]

Lipid quality indices[2]	ADG		GF		TRP	
	R value[1]	P value[1]	R value	P value	R value	P value
UFA:SFA	0.23	(0.10)	0.26	(0.06)	0.72	(0.01)
PV	-0.32	(0.02)	-0.33	(0.01)	-0.46	(0.01)
AnV	-	-	-	-	-0.73	(0.01)
OFA	-	-	-	-	0.63	(0.01)
TPC	-0.29	(0.04)	-0.32	(0.02)	-0.66	(0.01)
PTAG	-	-	-	-	-0.74	(0.01)
OSI	-	-	-	-	0.63	(0.01)
Hexanal	-0.33	(0.01)	-0.35	(0.01)	-0.49	(0.01)
Acrolein	-	-	-	-	-0.69	(0.01)
DDE	-	-	-	-	-0.74	(0.01)
HNE	-0.29	(0.04)	-0.32	(0.02)	-0.66	(0.01)
Ratio	-	-	-	-	-0.64	(0.01)
TOC	0.30	(0.03)	0.33	(0.02)	0.64	(0.01)

[1]Correlation (*r* value) and correlation significance (*P*-value). If no value is given, it was not found to be significant (-) at $P \leq 0.10$. There were no correlations between ADFI and any lipid quality indices.

[2]UFA:SFA, unsaturated:saturated fatty acid ratio; FFA, free fatty acids; PV, peroxide value; AnV, p-anisidine value; TBARS, thiobarbituric acid reactive substances; OFA, oxidized fatty acids; TPC, total polar compounds; PTAG, polymerized triacylglerides; OSI, oxygen stability index; DDE, 2,4-decadienal; HNE, 4-hydroxynonenal; Ratio, ratio of aldehydes as described by Wang et al., 2016; TOC, total tocopherols.

Table 2.6. Energy and lipid digestibility, nitrogen balance, and intestinal permeability in finishing pigs fed various levels of peroxidized soybean oil.

	Processed soybean oil[1]				Statistics	
Parameter	22.5	45	90	180	SEM	P value[2]
DE, % of GE	91.13[a]	90.96[ab]	89.51[b]	89.37[b]	0.52	0.03
ME, % of DE	98.35	98.19	97.95	97.96	0.15	0.16
AEE digestibility, %	84.60[a]	82.54[ab]	80.99[b]	80.59[b]	0.86	0.01
Nitrogen digested, %	90.53	90.17	90.24	90.09	0.53	0.94
Nitrogen retained, %[3]	80.45	75.98	76.12	75.37	1.96	0.25
Urinary L:M ratio[4]	0.086	0.065	0.086	0.069	0.012	0.51

[1]Data are least square mean of 14 observations for 22.5 and 90; and 13 observations for 45 and 180. 22.5 = fresh oil; 45 = oil heated for12 d at 45°C; 90 = oil heated for 72 h at 90°C; 180 = oil heated for 6 h at 180°C. All oil groups had a constant compressed air flow rate at 15L/min.

[2]Superscripts reflect peroxidized soybean oil treatment differences (abc, $P \leq 0.05$).

[3]Nitrogen retained as a percent of N digested.

[4]Urinary lactulose:mannitol ratio.

Table 2.7. Pearson correlation coefficients among soybean oil composition and peroxidation measures with digestibility responses[1]

Lipid quality indices[2]	DE:GE		ME:DE		EE digestibility		N retention	
	R value[1]	P value[1]	R value	P value	R value	P value	R value	P value
UFA:SFA	0.43	(0.01)	0.32	(0.03)	0.45	(0.01)	-	-
PV	-0.25	(0.10)	-	-	-0.29	(0.07)	-	-
AnV	-0.44	(0.01)	-0.31	(0.04)	-0.45	(0.01)	-	-
OFA	0.40	(0.01)	0.28	(0.06)	0.44	(0.01)	-	-
TPC	-0.38	(0.01)	-0.29	(0.05)	-0.38	(0.01)	-	-
PTAG	-0.44	(0.01)	-0.32	(0.03)	-0.45	(0.01)	-	-
OSI	0.38	(0.01)	0.33	(0.03)	0.49	(0.01)	0.26	(0.08)
Hexanal	-0.27	(0.08)	-	-	-0.28	(0.08)	-	-
Acrolein	-0.42	(0.01)	-0.29	(0.05)	-0.43	(0.01)	-	-
DDE	-0.44	(0.01)	-0.32	(0.03)	-0.45	(0.01)	-	-
HNE	-0.38	(0.01)	-0.29	(0.05)	-0.39	(0.01)	-	-
Ratio	-0.41	(0.01)	-0.28	0.28	-0.43	(0.01)	-	-
TOC	0.37	(0.01)	0.28	(0.06)	0.37	(0.02)	-	-

[1]Correlation (*r* value) and correlation significance (*P*-value). If no value is given, it was not found to be significant (-) at $P \leq 0.10$. There were no correlations observed between N digested or lactulose:mannitol and any lipid indices.

[2]UFA:SFA, unsaturated:saturated fatty acid ratio; FFA, free fatty acids; PV, peroxide value; AnV, p-anisidine value; TBARS, thiobarbituric acid reactive substances; OFA, oxidized fatty acids; TPC, total polar compounds; PTAG, polymerized triacylglerides; OSI, oxygen stability index; DDE, 2,4-decadienal; HNE, 4-hydroxynonenal; Ratio, ratio of aldehydes as described by Wang et al., 2016; TOC, total tocopherols.

Table 2.8. Oxidative stress biomarkers in urine, plasma, and liver of pigs fed soybean oil with differing peroxidation levels.

	Oil treatment				Statistics	
Item[1]	22.5°C	45°C	90°C	180°C	SEM	*P*-value
Plasma, number of observations	14	14	14	14	--	--
TBARS, mM/mL	11.4	11.1	11.4	12.0	0.9	0.91
ISP, pg	44. 9[b]	46.4[b]	72.0[a]	51.2[b]	4.7	< 0.01
PC, nmol/mL	38.1	36.0	47.1	35.9	5.2	0.39
GPx, $nmolmin^{-1}mL^{-1}$	2,991	2,870	3,238	2,999	378	0.92
Liver, number of observations	13	14	14	13	--	--
TBARS, mM/mg	94.3	94.9	83.1	109.4	11.9	0.48
PC, nmol/mg	292.8	318.3	268.5	302.3	15.1	0.12
8-OH-2dG, pg	1,459	1,435	1,637	1,547	134	0.68
GPx, $nmolmin^{-1}mg^{-1}$	35.1	33.9	43.7	39.4	3.9	0.24
CAT, $nmolmin^{-1}mg^{-1}$	721.7	812.4	908.0	879.8	105.4	0.59
Urine, number of observations	12	12	12	12	--	--
TBARS, mM/mL	6.3[b]	7.7[b]	20.6[a]	11.3[b]	2.8	< 0.01
ISP, pg	1,956[b]	1,969[b]	4,839[a]	3,534[ab]	628	< 0.01
8-OH-2dG, µg	96.3	78.0	91.6	118.7	162	0.36

[1]TBARS, thiobarbituric acid reactive substances; ISP, F2-isoprostanes; 8-OH-2dG, 8-hydroxy-2'-deoxyguanosine; PC, protein carbonyls; GPx, glutathione peroxidase activity; CAT, catalase activity.

[a-b]Least square means with a row with different superscripts are different at $P \leq 0.05$.

Table 2.9. Pearson correlation coefficients among soybean oil composition and lipid peroxidation products with measures of oxidative status[1]

	Criterion[2]				
Soybean oil composition and peroxidation measures[2]	Plasma ISP	Liver PC	Liver GPx	Urine TBARS	Urine ISP
FFA	0.56 (< 0.0001)	-0.29 (0.03)	-	0.50 (< 0.01)	0.45 (< 0.01)
Free Glycerin	-	-	-	-	-0.29 (0.04)
OFA	-	-	-	-	-0.29 (0.04)
OSI	-0.45 (< 0.01)	-	-	-0.44 (< 0.01)	-0.44 (< 0.01)
AnV	0.32 (0.02)	-	-	0.34 (0.02)	0.41 (< 0.01)
PV	0.54 (< 0.0001)	-0.27 (0.05)	-	0.50 (< 0.01)	0.44 (< 0.01)
TPC	0.54 (< 0.0001)	-0.27 (0.05)	0.28 (0.04)	0.50 (< 0.0001)	0.50 (< 0.0001)
PTAG	0.42 (< 0.01)	-	-	0.41 (< 0.01)	0.47 (< 0.01)
DDE	0.41 (< 0.01)	-	-	0.41 (< 0.01)	0.46 (< 0.01)
HNE	0.54 (< 0.0001)	-	0.28 (0.04)	0.50 (< 0.01)	0.50 (< 0.01)
Acrolein	-	-	-	-	0.35 (0.01)
Decenal	0.52 (< 0.0001)	-	0.28 (0.04)	0.49 (< 0.01)	0.50 (< 0.01)
Heptadienal	0.52 (< 0.0001)	-	0.28 (0.04)	0.49 (< 0.01)	0.50 (< 0.01)
Heptenal	0.55 (< 0.0001)	-0.28 (0.04)	0.28 (0.04)	0.51 (< 0.01)	0.49 (< 0.01)
Hexanal	0.56 (< 0.0001)	-0.29 (0.03)	-	0.50 (< 0.01)	0.46 (< 0.01)
Octenal	0.56 (< 0.0001)	-0.28 (0.04)	0.27 (0.05)	0.51 (< 0.01)	0.48 (< 0.01)
Pentanal	0.48 (< 0.01)	-0.32 (0.02)	-	0.41 (< 0.01)	0.37 (< 0.01)

Table 2.9 (Continued)

Undecadienal	0.35 (< 0.01	-	-	0.36 (0.01)	0.43 (0.03)
Undecenal	-	-	-	-	0.32 (0.03)
Ratio	-	-	-	-	0.31 (0.03)
Total tocopherols	-0.54 (< 0.0001)	0.27 (0.05)	-0.28 (0.04)	-0.50 (< 0.01)	-0.50 (< 0.01)

[1]Top value represents correlation (*r* value) and bottom value in parenthesis represents significance (*P*-value). If no value is given, it was not found to be significant (-) at $P < 0.05$. There were no correlations observed between SO treatment and plasma TBARS, plasma PC, plasma GPx, liver TBARS, liver 8-OH-2dG, liver CAT, or urinary 8-OH-2dG so they were removed from the table.

[2]FFA, free fatty acids; PV, peroxide value; AnV, p-anisidine value; TBARS, thiobarbituric acid reactive substances; OFA, oxidized fatty acids; TPC, total polar compounds; PTAG, polymerized triacylglerides; OSI, oxygen stability index; HEX, hexanal; DDE, 2,4-decadienal; HNE, 4-hydroxynonenal; Ratio, ratio of aldehydes as described by Wang et al., 2016; TOC, total tocopherols; PC, protein carbonyls; GPx, glutathione peroxidase activity; ISP, F_2-isoprostanes; 8-OH-2dG, 8-hydroxy-2'deoxyguanosine; CAT, catalase activity.

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CHAPTER 3: INFLUENCE OF FEEDING THERMALLY PEROXIDIZED SOYBEAN OIL TO FINISHING PIGS ON CARCASS CHARACTERISTICS, LOIN QUALITY, AND SHELF LIFE OF LOIN CHOPS

ABSTRACT: The objective of this study was to evaluate the effect of feeding soybean oil (SO) with varying levels of peroxidation on carcass traits and shelf life of loins. Fifty-six barrows were randomly assigned to 1 of 4 diets containing 10% fresh SO (22.5°C) or thermally processed SO (45°C for 288 h, 90°C for 72 h, or 180°C for 6 h), each infused with air at a rate of 15L/min. Individually housed pigs were provided ad libitum access to feed for 81 d. At 82 d pigs were slaughtered and hot carcass weight (HCW) and liver weights were recorded. Carcass characteristics and fresh loin quality were evaluated 1 d post-mortem. Loin chops from each carcass were overwrap-packaged and subjected to a 10 d simulated retail display. Daily measurements of L*, a*, b*, reflectance and visual discoloration were conducted, evaluation of cooking loss and Warner-Bratzler shear force were conducted on chops stored 0, 5, and 10 d, and thiobarbituric acid reactive substances (TBARS) were evaluated on chops stored 0 and 10 d. Shelf life related data were analyzed as a completely randomized design with repeated measures in time, with storage location (shelf) as a random effect. Carcasses of 90°C pigs weighed 6.0, 8.6, and 6.9 kg less than ($P < 0.03$) than 22.5°C, 45°C, and 180°C carcasses, respectively. Livers of 90°C and 180°C pigs were 14.3 and 11.7%, respectively, heavier ($P \leq 0.02$) than those from pigs fed 22.5°C SO, with livers of 45°C being intermediate. Livers of 90°C pigs represented 0.12 percentage units less ($P = 0.02$) of ending live weight than livers 180°C, and 180°C liver were 0.12 percentage units less ($P < 0.01$) of ending live weight than those from pigs fed 22.5°C SO, with 45°C being intermediate. There was no difference ($P \geq 0.19$) BF depth, LMA, or estimated carcass lean percentage among SO treatments, nor was there an effect ($P \geq 0.13$) of SO on any early post

mortem loin quality traits or loin composition. There was no effect ($P > 0.14$) of SO on cooking loss, WBSF, L*, a*, b*, hue angle, reflectance, discoloration, or TBARS; however, there was a tendency ($P = 0.09$) for chops of 45°C pigs to have greater ($P < 0.04$) chroma than either 22.5°C or 180°C, with 90°C being intermediate. Overall, feeding SO cooked at 90°C for 72 h resulted in reduced carcass weight and dressing percentage; however, there was no evidence that feeding peroxidized SO was detrimental to shelf life of loin chops.

3.1 INTRODUCTION

Interest in dietary lipid quality has focused on the influence of fatty acid profile on carcass and pork product quality. However, it has been increasingly recognized that the oxidative status of the diet is also important, and may be of special interest considering rendered fats like white or yellow grease, and soy bean oil (**SO**) are routinely included in swine diets (Lin et al., 2013). Feeding peroxidized lipids has been shown to reduce lipid digestibility (Liu et al., 2014a; Lindblom et al., 2017), growth performance (Rosero et al., 2015), as well as HCW and BF depth (Boler et al., 2012) in growing-finishing pigs.

Though the negative effects of peroxidized lipids on growth and carcass traits have been regularly reported, the influence of feeding peroxidized lipid on the quality and shelf life of pork loin chops has been inconsistent. Buckley et al. (1989) reported feeding corn oil with a peroxide value (**PV**) of 9 mEq/kg resulted in greater concentrations of thiobarbituric acid reactive substances (**TBARS**) during display of loin chops compared with chops from pigs fed fresh oil. This is supported in part by Monahan et al. (1992), where there was a tendency for loin chops of pigs fed corn oil (PV = 4.5 mEq/kg) to have greater TBARS than pigs fed fresh oil. Recent studies, however, have reported that feeding peroxidized corn oil do not affect lipid peroxidation or color stability of loin chops during display (Monahan et al., 1994; Boler et al., 2012). The

inconsistencies in these results may be due to inconsistencies in oil processing or dosage, which ranged from 4.5 to 9 mEq/kg, with the most significant response in product stability observed at the greater dietary PV.

Therefore, the objective of this study was to determine the effect of feeding divergently thermally processed SO on carcass characteristics and early post-mortem loin quality and to determine what role, if any, peroxidized SO in the diet had on the shelf life of pork loin chops, by feeding diets with PV of 0.2 to 12.4 mEq/kg.

3.2 MATERIALS AND METHODS

All animal care and use procedures for this experiment were approved by the Institutional Animal Care and Use Committee at Iowa State University.

3.2.1 Dietary Treatments and Experimental design

Fifty-six individually housed barrows (Geneticporc F25 females × B6.0 sires, Hendersonville, TN) were randomly allotted to 1 of 4 diets containing 10% fresh soybean oil (SO; 22.5°C) or SO that was thermally processed at 45°C for 288 h, 90°C for 72 h, or 180°C for 6 h throughout a 2 phase, 81 d finishing trial. Diets were formulated to meet or exceed the nutrient requirements for finishing pigs according to the NRC (2012), and no antioxidants were added to diets before or during their preparation. Oil and diet preparation, as well as design of the feeding trial were described previously (Overholt et al., 2018).

3.2.2 Slaughter and carcass evaluation

At the conclusion of the growth trial, pigs were transported approximately 613 km from Ames, IA to the University of Illinois Meat Science Laboratory in Urbana, IL. One barrow fed the 22.5°C SO diet was deemed unfit for transport and therefore was removed from further experiments. Once arriving at the University of Illinois Meat Science Laboratory, barrows were

held overnight (~16 h) with no access to feed but ad libitum access to water. Barrows were weighed to determine ending live weight (**ELW**) before being immobilized using the head-to-heart electrical stunning technique immediately followed by exsanguination. Upon evisceration and inspection of internal organs, livers from each carcass were weighed and a section was removed from the right lateral lobe and snap-frozen in liquid nitrogen for subsequent analysis of oxidative status. Hot carcass weight (**HCW**) was recorded after each carcass had been inspected and deemed wholesome by an USDA-FSIS inspector. Carcasses were allowed to chill at 4°C and 24 h after slaughter, the left side of each carcass was ribbed between the 10^{th} and 11^{th} ribs, and carcasses were evaluated fat depth at the 10^{th} rib (**BF** depth) at a point perpendicular to the skin, three-fourths the length of the LM. Loin muscle area (**LMA**) was traced onto double matted acetate paper and the LMA outlines were later traced in duplicate with a digitizer pad (Intuos Pro Digitizer Tablet and Stylus; Wacom Technology Corp., Vancouver, WA), and the mean area of the 2 tracings was reported as LMA. Estimated carcass lean percentage (**ECL**) was calculated using the equation of Burson and Berg (2001): estimated carcass lean, % = [8.588 + (0.465 × HCW, lbs) − (21.896 × BF, in) + (3.005 × LMA, in^2)/HCW, lbs × 100]/HCW, lbs. Adipose tissue cores, measuring 2.54 cm in diameter and consisting of all 3 adipose layers were collected from the clear plate between the scapula and cervical vertebra near the dorsal midline of the left side of each carcass. Iodine values (**IV**) of both adipose depots were determined using a Bruker MPA Multi-Purpose FT-NIR Analyzer (Bruker Optics Inc., Billerica, MA).

3.2.3 Early post-mortem loin quality evaluation

Ultimate pH, loin color, marbling, and firmness were evaluated 30 min after carcasses had been ribbed. Ultimate pH was measured by inserting a pH meter probe fitted with a glass electrode (MPI pH-Meter, Topeka, KS; calibrated to pH 4 and 7) into the ventral surface of each

loin at the posterior termination of the spinalis dorsi. Instrumental color evaluation (L*, a*, b*; CIE, 1978) was conducted with a Minolta CR-400 Chroma-meter (Minolta Camera Co., Ltd., Osaka, Japan) using a D65 light source and a 2° observer with an 8 mm aperture. Calibration of the instrument was conducted using a white tile. Subjective color, marbling (NPPC, 1999) and firmness (NPPC, 1991) were determined by a single trained evaluator. After early post-mortem loin quality evaluation, carcasses were fabricated to yield Canadian back loins [North American Meat Processors Association (NAMP) #414]. The anterior portion of Canadian back loins were removed with a cut immediately posterior to the spinalis dorsi and, working from an anterior to posterior direction, three 2.54 cm and two 1.27 cm chops were cut for use in simulated retail display. An additional 2.54 cm and 1.27 cm chop were removed for analysis of moisture and lipid content using the drying and extraction by chloroform: methanol method described by Novakofski et al. (1989) and evaluation of drip loss by the suspension method (Boler et al., 2014), respectively.

3.2.4 Loin chop display

There were three 2.54 cm and two 1.27 cm chops saved for the simulated retail display experiment. One chop of each thickness was designated for 0 and 10 d of simulated retail display; with the 2.54 cm chop used for Warner-Bratzler shear force (**WBSF**) and the 1.27 cm chop to be used for analysis of lipid oxidation. A single 2.54 cm chop was designated for 5 d of display and to also be used for WBSF. Pairs of chops designated for 5 and 10 d of storage were placed on polystyrene trays and overwrapped with polyvinylchloride film (**PVC**; oxygen transmission rate = 1,627.9 cc/m^2/d; moisture vapor transmission rate = 170.5 g/m^2/d). Packages were arranged on wire mesh shelves lined with white butcher paper in two single-layer rows. Lighting was provided by two 122 cm long 32 W fluorescent bulbs (Ecolux with Starcoat,

3000K, General Electric, Boston, MA) that were suspended 38 cm above each row of packages. Packages were stored at 4 °C. Color evaluations were conducted through the PVC film on the 1.27 cm chop designated for 10 d of display following the procedure described by Holmer et al. (2009). Daily evaluations of instrumental color (L*, a*, b*, 650/580 ratio) were conducted using a Hunter Lab Miniscan XE Plus (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA) using an A illuminant, 10° observer and a 25 mm aperture. Calibration of the device was conducted prior to each daily evaluation using a black glass and a white ceramic tile covered with the same PVC film used to package the loin chops. Daily visual discoloration scores were determined by a single trained observer using a 10 cm unstructured line scale anchored at 0 and 100% surface discoloration, with each 1 cm increment equal to 10% discoloration of the chop surface. On the designated day of display (0, 5, or 10 d) the chops were removed from display once they had been evaluated (with the exception of the 0 d chops, which were not displayed), chops were then packaged in individual bags and vacuum sealed. The 1.27 cm chops, which were designated to for analysis of lipid oxidation, were stored at -80°C until later analysis. The 2.54 cm chops, which were designated for analysis of WBSF were stored at -20°C until later analysis.

3.2.5 Warner-Bratzler shear force

Vacuum-packaged chops were allowed to thaw at 4°C for 18 h before analysis, trimmed of excess fat, and weighed before being cooked on a Farberware Open Hearth grill (model 455N; Walter Kidde, Bronx, NY). Chops were flipped once at an internal temperature of 35°C and then cooked until they reached an internal temperature of 70°C. Internal temperatures were monitored using copper-constantan thermocouples (Type T; Omega Engineering, Stamford, CT) connected to a digital scanning thermometer (model 92000-00; Barnant Co., Barrington, IL). Cooked chops

were cooled to 25°C and weighed before four 1.25-cm-diameter cores were removed parallel to the orientation of the muscle fibers. Cores were then sheared once through the center using a Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems Ltd., Godalming, UK) with a blade speed of 3.3 mm/s and a 100-kg load cell. Shear force was reported as the average peak force of the 4 cores. Cooking loss was calculated as the difference between the pre- and post-cooked chop weights divided by the precooked chop weight (Boler et al., 2011).

3.2.6 Thiobarbituric acid reactive substances

One 1.27 cm chop representing 0 d of display, and another that had been displayed for 10 d, were trimmed of external fat, connective tissue, and any accessory muscles. Chops were cut into cubes and pulverized in a blender (Waring Products, Torrington, CT). Duplicate 5 g samples of pulverized loin muscle were combined with 1 mL of 0.2 mg/ml butylated hydroxytoluene and 45.5 ml of 10% trichloroacetic acid in 0.2 *M* phosphoric acid, and blended for 30 s (Waring Products, Torrington, CT). The homogenate was filtered through Whatman No. 1 filter paper and collected in flasks. Two 5 ml aliquots of filtrate were transferred from flasks into 15 ml conical tubes. To one tube, 5 ml of 0.02 *M* thiobarbituric acid was added, and to the other was added 5 ml of deionized water to serve as a blank. An additional 2 pulverized loin muscles were randomly selected, representing 0 and 10 d of display, to serve as spiked samples in order to estimate percent recovery. The spiked samples were prepared in the same way as previously described, except that 12 ml of 10μ*M* 1, 1, 3, 3-tetramethoxypropane was added in substitution for an equal volume for 10% trichloroacetic acid in 0.2 *M* phosphoric acid. A standard curve was made to represent 0, 1.25, 2.5, 5, and 7.5 mg malondialdehyde/ml using 25 μ*M* 1, 1, 3, 3-tetramethoxypropane. An aliquot of the 25 μ*M* 1, 1, 3, 3-tetramethoxypropane was combined

with 5 ml of 0.02 *M* thiobarbituric acid and volumized to 10 ml with 10% trichloroacetic acid in 0.2 *M* phosphoric acid solution. All tubes were capped, inverted to mix, and then stored in the dark at 23°C for approximately 16 h to allow pigment development. After 16 h, 150 µl from each tube was transferred onto 96 well round bottom plates and read at 530 nm with a Beckman Du-640 Spectrophotometer (Beckman Coulter Inc., Fullerton, CA). Thiobarbituric acid reactive substances (TBARS) were reported as mg-MDA/kg tissue and as mg-MDA/kg-extractable lipid.

3.2.7 Statistical analyses

All analyses were conducted with the MIXED procedure of SAS (SAS v9.4, SAS Inst. Inc., Cary, NC). Because pigs were fed individually, pig carcass served as experimental unit. Carcass and liver data were analyzed as a completely randomized design with the fixed effect of SO treatment, with initial bodyweight (Overholt et al., 2018) serving as a covariate. There were 2 carcasses determined to be outliers for carcass traits, one each from the 45°C and 180°C, therefore they were omitted from related analyses. Early post-mortem loin quality data were also analyzed as a completely randomized design, but no covariate was used nor were there any outliers in the data.

Simulated retail display data; including color stability, TBARS, cooking loss, and WBSF were analyzed as a completely randomized design with repeated measures in time. Therefore, fixed effects were SO treatment, storage time, and the interaction of SO and storage time. Storage location (shelf) served as a random effect. Analysis of repeated measures was conducted using the REPEATED statement of PROC MIXED. Multiple covariance structures were compared using Akaike's Information Corrected Criterion for each dependent variable. Based on these comparisons, a heterogeneous Toeplitz (TYPE = TOEPH) covariance structure was used for analyses of L*, a*, b*, chroma, hue, reflectance, and discoloration; whereas, a heterogeneous

compound symmetric [TYPE = CSH] covariance structure was used for analyses of TBARS, cooking loss, and WBSF. Least square means were separated with the PDIFF option of the MIXED procedure. Main effects and interactions were considered different at $P \leq 0.05$ and main effects were considered trending at $0.05 < P \leq 0.10$.

3.3 RESULTS AND DISCUSSION

3.3.1 Characteristics of soybean oils

A full characterization of the oxidative status and composition of the thermally processed SO are presented in Table 1 and detailed elsewhere (Overholt et al., 2018). However, certain characteristics of the oils merit discussion in the context of the current study. The four thermal processing treatments were chosen because they reflected conditions that fat sources and diets routinely experience in the livestock, rendering, and restaurant industries (Meeker and Hamilton, 2006), and because they also represented temperatures previously evaluated (Boler et al., 2012; Liu et al., 2014b; Rosero et al., 2015; Hanson et al., 2016).

Although statistical comparisons were not made, there are several numerical differences among the SO profiles of interest. Peroxide value (**PV**) has been a common metric by which the oxidative status of lipids used in swine diets have been characterized, and therefore are an important measurement available to draw comparisons between the current and previous studies. In these previous experiments, PV ranged from 5.7 to 300 mEq/kg in the oil and 0.2 to 9 mEq/kg in the diets (Liu et al., 2014b,c; Rosero et al., 2015; Dilger et al., 2016). Among the thermally processed oils evaluated in the current study, SO heated at 90°C had the greatest PV at 123.6 mEq/kg compared to 2, 17.4, and 19.4 mEq/kg of the 22.5°C, 45°C, and 180°C treatments, respectively. The 10% SO inclusion rate used in the experimental diets represents a range of dietary PV of 0.2 to 12.4 mEq/kg. This range fully covers the dietary PV observed in previous

studies investigating the effects of peroxidized lipids on swine growth, digestibility and carcass traits, as well as studies investigating the influence on peroxidized lipid feeding on the shelf life of pork products. Further evidence that the thermal treatments resulted in a varying degrees of peroxidative damage was the range in p-anisidine values present the different oils; ranging from 1.11 to 165. Previous experiments have evaluated oils subjected to varying degrees of thermal abuse, resulting in various oxidative profiles; however, no study to date has investigated such an array of thermal processing treatments under the same experimental conditions, nor have they evaluated the effect such treatments may have on the shelf life of pork products. These previous studies have yielded inconsistent results; therefore, it was an underlying objective of the current study to test a wide battery of thermal processing conditions with the aim of drawing definitive conclusions to the question of the effect of dietary peroxidized lipids on pork carcass traits and loin shelf life.

3.3.2 Carcass characteristics and early postmortem loin quality

Carcasses of pigs evaluated in the present study were previously part of a feeding trial in which pigs fed 90°C SO had reduced ADG and G:F as well as reduced DE and AEE digestibility compared with the other SO diets and G:F as well as reduced DE and AEE digestibility compared with the other SO diets (Overholt et al., 2018). The poorer performance of the pigs fed the 90°C was reflected in the relationships among treatments in regards to carcass characteristics. Ending live weight of pigs fed 90°C diets were 8.9 and 7.6 kg less than ($P \leq 0.03$) than pigs fed 45°C and 180°C diets, respectively, with ELW of pigs fed 22.5°C being intermediate (Table 2). This difference in ELW persisted with HCW, as carcasses of pigs fed 90°C weighed 6.0 to 8.6 kg less than ($P \leq 0.03$) carcasses of pigs fed any of the other SO diets, which did not differ ($P \geq 0.36$) in HCW. Although the reduced HCW of pigs fed 90°C diets was largely a function of

having reduced ELW, it was exacerbated by a 1.25, 1.26, and 0.76 percentage unit reduction ($P \leq 0.05$) in dressing percentage compared with pigs fed 22.5°C, 45°C, and 180°C SO diets, respectively. This observation was in agreement with results of previous studies, in which the reduction in dressing percentage observed in pigs fed peroxidized DDGS (Song et al., 2014) and corn oil (Boler et al., 2012) was due to reduction in performance and carcass fatness. Although the pigs fed 90°C in the current study grew less efficiently and at a slower rate than those fed the other SO diets, there was no difference in BF depth ($P = 0.44$), LMA ($P = 0.19$), or estimated carcass lean percentage ($P = 0.80$) among SO treatments. Therefore, it is unlikely that the reduced dressing percentage of pigs fed 90°C was due to differences in carcass composition. The reduction in dressing percentage may, in part, be attributable to liver enlargement, as livers of pigs fed 90°C and 180°C SO were 14.3 and 11.7%, respectively, larger ($P \leq 0.02$) than livers of pigs fed 22.5°C SO, with livers of pigs fed 45°C being intermediate. This relationship was even more pronounced when liver weight was expressed as a percentage of ELW, as livers of pigs fed 90°C accounted for 0.11 percentage units more ($P = 0.02$) of ELW than did livers of pigs fed 180°C. Likewise, livers of pigs fed 180°C accounted for 0.13 percentage units more ($P < 0.01$) of ELW than livers of pigs fed 22.5°C SO, with the proportional weight of livers of pigs fed 45°C being intermediate. Liver enlargement has been previously reported in both pigs (Liu et al., 2014c) and rats (Eder, 1999) fed peroxidized lipids, and is an indication of oxidative stress. Lipid peroxidation products, such as 4-hydroxynonenal (**HNE**), of which the 90°C and 180°C SO had the greatest numerical concentrations in the oil, are known cytotoxins (Esterbauer et al., 1991; Grootveld et al., 1998). The liver is the primary site of detoxification in the body, and upon prolonged intake of toxins, the liver responds by increasing synthesis of microsomal enzymes (Huang et al. 1988) and increasing hepatocyte proliferation (Dibner et al., 1996), resulting in

liver hypertrophy. Compounded with the previously reported increase in F_2-isoprostane and TBARS in plasma and urine of pigs fed 90°C SO (Overholt et al., 2018), the observed liver hypertrophy is a clear sign that feeding 90°C SO induced oxidative stress to a greater degree than did feeding the other SO treatments.

Iodine value has become the most accepted metric of determining the technological quality of pork fat (Seman et al., 2013), and is strongly influenced by the qualities of dietary lipids. Thermal processing conditions resulted in divergent IV among SO treatments (22.5°C = 45°C > 180°C ≈ 90°C), and these differences were reflected in the IV of clear plate adipose tissue. Clear plate IV of carcasses from pigs fed 45°C SO were 3.4 units greater ($P < 0.01$) than IV of from carcasses of pigs fed 180°C SO, with adipose tissue of pigs fed 22.5°C SO being intermediate. Clear plate IV of pigs fed 90°C SO drastically reduced compared to the other 3 SO treatments; being 13.9, 15.1, and 11.2 units less than ($P < 0.0001$) the 22.5°C, 45°C, and 180°C SO treatments, respectively. It is unlikely the stark reduction in IV observed in the 90°C was due to entirely to the relative reduction in SO IV. This is in spite of the fact that lipid digestibility is reduced when peroxidized lipids are included in the diet (DeRouchey et al., 2004; Rosero et al., 2015; Lindblom et al., 2017) and *de novo* fatty acid synthesis increases as the amount or digestibility of dietary fat is decreased (Kloareg et al., 2007). Furthermore, the chain length and unsaturation of dietary fatty acids are determinants of the rate of *de novo* fatty acid synthesis, as palmitic and palmitoleic acids have a greater effect of suppressing *de novo* fatty acid synthesis than longer chain fatty acids, such as linoleic or oleic acid (Smith et al., 1996). We previously reported that pigs fed 90°C and 180°C had the lowest digestibility of acid hydrolyzed ether extract (**AEE**) of the SO treatments (Overholt et al., 2018). Moreover, the 90°C SO had, numerically, the greatest concentration of palmitic acid, which may have also contributed to the

disproportionate reduction in IV of the 90°C SO fed pigs compared with the other 3 SO treatments, and especially the IV of pigs fed 180°C SO.

It is interesting to note that oil composition, carcass weights, or liver weights did not necessarily display a commensurate change with the increasing SO cooking temperatures. When considering this, it is important to note that the SO treatments were specific combinations of time and temperature (selected to represent processing and storage conditions of feeds and feedstuffs). In addition to being thermally processed at different temperatures, the 90°C and 180°C treatments were also heated for different lengths of time (72 h and 6 h, respectively). The progression of lipid peroxidation, and the production of oxidation products, is dependent on both time and temperature (Labuza and Dugan, 1971).

There was no effect ($P \geq 0.13$) of SO on any early postmortem loin quality traits or loin composition, in agreement with the results reported by Boler et al. (2012), but in contrast to the study by Lu et al. (2014). The latter experiment compared loin quality traits of pigs fed either a diet containing 5% oxidized SO and 10% PUFA supplement (diet PV = 9 mEq/kg) or a standard corn-soy diet, and reported that despite there being no difference in NPPC color scores among the 2 treatments, loin chops of pigs fed oxidized SO were less red (reduced a*) than chops from pigs fed a corn-soy diet.

3.3.3 Shelf life of loin chops

Previous studies investigating the effects of diet on pork product shelf life have focused on either the influence of the fatty acid composition of the diet or the supplementation of dietary antioxidants. Few studies have investigated the impact of feeding peroxidized ingredients, and most have done so as a secondary objective to testing the efficacy of dietary antioxidant interventions. The present study was designed with the intent to determine whether there was any

effect of feeding peroxidized SO on the shelf life of loin chops, without antioxidant interventions as has been investigated in previous research. Generally, the loins would have been subjected to a 'wet-aging" period prior to the initiation of display in order to best simulate industry-typical conditions. However, it was decided that to best determine if peroxidized oil feeding affected the shelf life of loin chops, it would be necessary to observe any changes in color beginning on the same day the chops were excised from the carcass (1 d postmortem). Therefore, no "wet-aging" period was employed and chops were packaged, displayed, and baseline color evaluations were recorded 1 d postmortem (approximately 4 hours after early postmortem evaluations were conducted).

In the current study, there were no SO × Day interactions ($P \geq 0.09$) for any of the shelf life traits. During the course of the simulated retail display period, there was no effect ($P \geq 0.14$) of SO on either cooking loss, WBSF (Figure 1), lightness, redness, yellowness, hue angle (Figure 2), reflectance, discoloration score (Figure 3), or on TBARS calculated on a tissue or extractable lipid-basis (Figure 4). There was a trend ($P = 0.09$) for loin chops from pigs fed 45°C SO to have greater ($P \leq 0.09$) chroma than chops from pigs the other 3 SO treatments. Early studies reported feeding peroxidized corn oil to pigs increased the rate of lipid oxidation, compared with feeding fresh corn oil, in loin chops (Buckley et al., 1989; Monahan et al., 1992) and ground pork patties (Buckley et al., 1989). A similar effect was reported both fresh and cooked loin chops of pigs fed rancid rice bran (Chae and Lee, 2002) and peroxidized SO (Murphy et al., 1991). In agreement with the results of the current study, both Monahan et al. (1994) and Boler et al. (2012) reported that despite having reduced tissue vitamin E concentrations, the color stability and lipid oxidation of loin chops of pigs fed peroxidized corn oil did not differ from chops of pigs fed fresh oil. The penetration of O_2 into whole muscle meat is limited to the outermost 1 to 2 mm

(O'Keeffe and Hood, 1982), and it is possible that if these outer-most portion of the chops had been evaluated for lipid oxidation, differences would have been detected. However, no differences were detected among SO treatments for any of the color traits, all of which were measured on the surface of the chops. Feeding peroxidized lipids to broilers has routinely elicited a decrease in muscle vitamin E levels and increased susceptibility to lipid oxidation of both breast and thigh meat (Jensen et al., 1997; Tavárez et al., 2011; Zhang et al., 2011). Only one study (Murphy et al., 1991) reported an analogous result in pork. It is not entirely clear why peroxidized lipid feeding results in reduced shelf life in poultry meat, but not pork, despite the intake of peroxidized lipids causing measurable indications of oxidative stress in plasma, liver, and even adipose tissue of both species (Tavárez et al., 2011; Boler et al., 2012). The differential response to diet induced oxidative stress in muscle may be due to the physiological differences that exist between the species. In general, porcine muscle has a greater proportion of oxidative fibers than chicken breast muscle, which is almost entirely composed of glycolytic fibers. Oxidative muscle fibers have a preference for aerobic metabolism, relying more heavily on β-oxidation of fatty acids and the electron transport chain to produce ATP, than glycolytic fibers. Reactive oxygen species (**ROS**), such as superoxide, are produced as a by-product of aerobic metabolism and because ROS can damage cellular structures, antioxidant enzymes such as superoxide dismutase (**SOD**) and glutathione peroxidases (**GPx**), are synthesized by cells to neutralize ROS (Schieber and Chandel, 2014). Activity of catalase (**CAT**), SOD, and GPx were correlated with the relative number of oxidative fibers in 6 different muscles in rats (Laughlin et al., 1990). This relationship was later confirmed in a comparison of antioxidant enzyme activity in pork psoas major and longissimus dorsi (Lauridsen et al., 1999). The difference in muscle fiber type composition being a driving force to cause the disparity in the oxidative stress/shelf

life relationship between species is further evidenced by the fact that pork has greater concentrations of CAT (Rhee et al., 1996; Pradhan et al., 2000) and SOD (Avanao et al., 2001; Hernández et al., 2004), as well as carnosine (Tian et al., 2007; Mora et al., 2008) than do chickens, regardless of which muscles are evaluated. Though muscle fiber type differences seem a likely contributor to the differential susceptibility of peroxidized lipid intake to cause reduced oxidative stability of muscle and meat products, further research must be conducted to establish whether a causal link exists.

Lightness, yellowness, hue angle, and discoloration score over storage time increased ($P < 0.0001$) with storage time; whereas, redness, chroma, reflectance, and WBSF all decreased ($P < 0.0001$) with storage time. These results were consistent with previous studies documenting color stability and deterioration in pork loin (Lindahl et al., 2006; Tikk et al., 2008).

3.3.4 Conclusions

The extent to which peroxidized soybean oil affected carcass characteristics was dependent on the severity of the thermal processing treatment, with pigs fed the 90°C SO having reduced HCW and dressing percentage, and feeding SO processed at 45°C and 180°C having little effect on carcass characteristics compared to pigs fed the 22.5°C SO diets. Despite the peroxidized SO feeding inducing signs of oxidative stress, feeding peroxidized SO was not detrimental to the color stability or lipid oxidation of loin chops. Therefore, peroxidized SO feeding is deleterious to carcass value, but appears to be of little concern as it applies to the quality and shelf life of pork loins.

3.4 FIGURES

Figure 3.1.

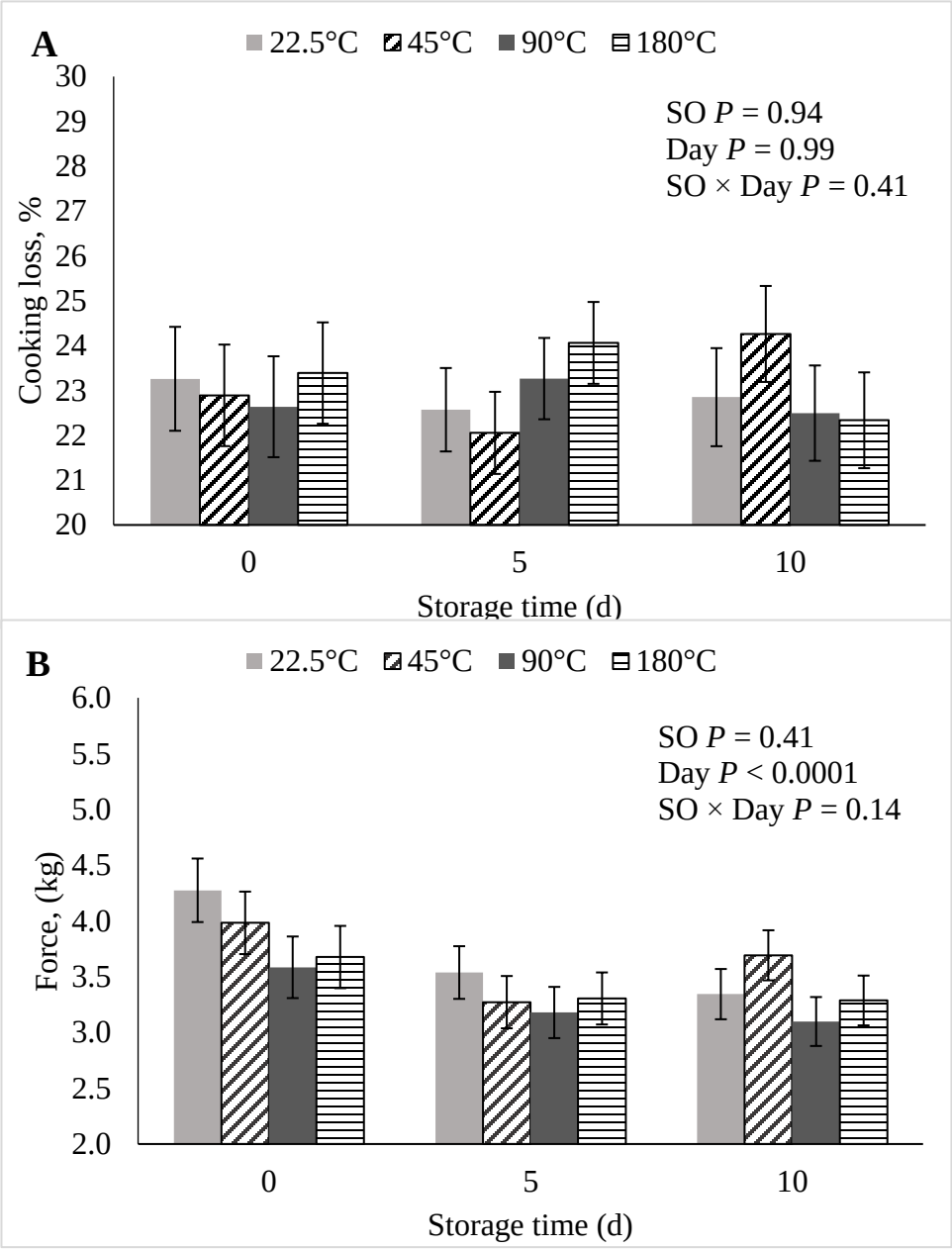

Figure 3.1. Effects of feeding peroxidized SO to finishing pigs on A) Cooking loss and B) Warner-Bratzler shear force of loin chops subjected to 0, 5, or 10 d of simulated retail display.

Figure 3.2.

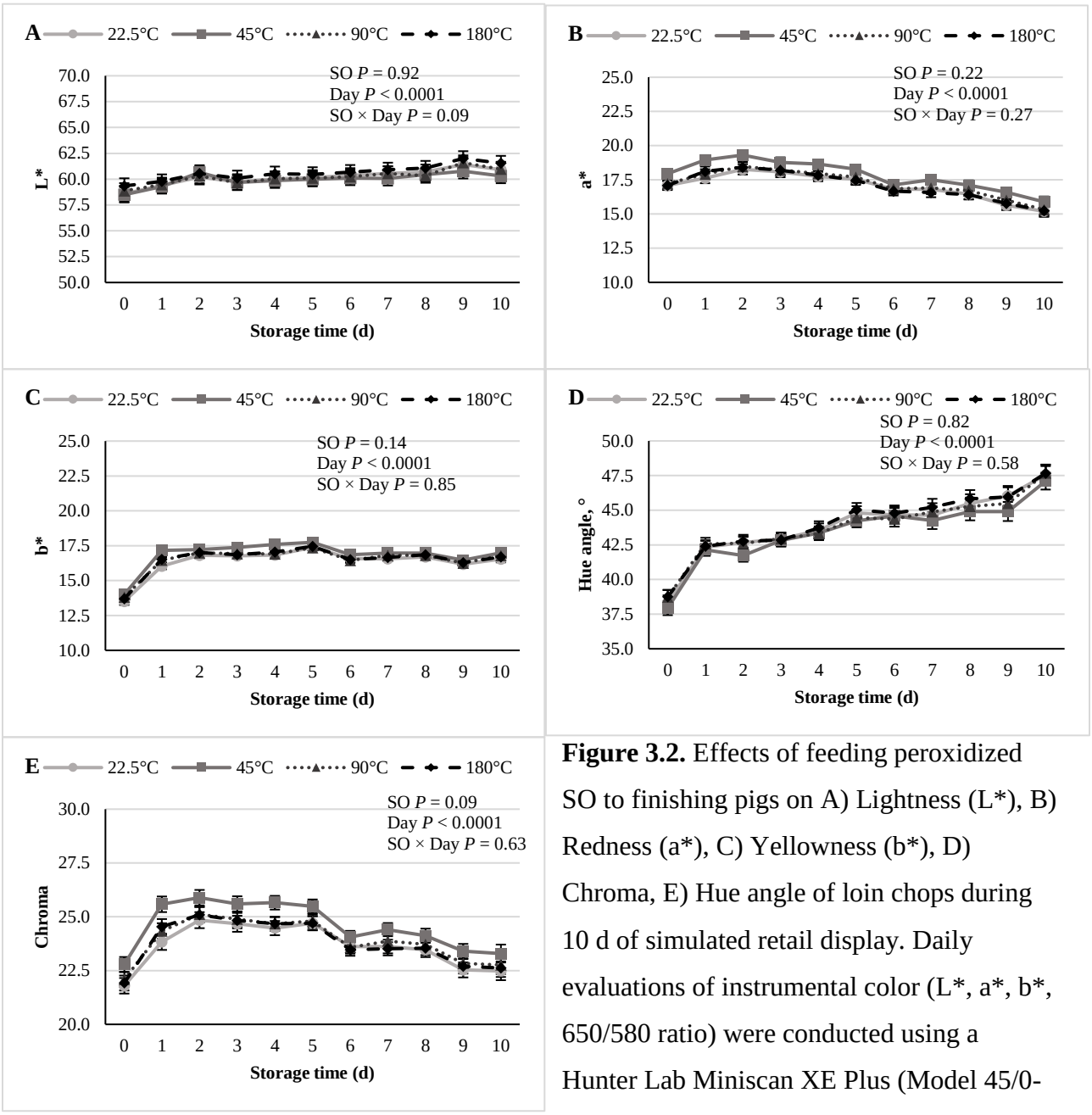

Figure 3.2. Effects of feeding peroxidized SO to finishing pigs on A) Lightness (L*), B) Redness (a*), C) Yellowness (b*), D) Chroma, E) Hue angle of loin chops during 10 d of simulated retail display. Daily evaluations of instrumental color (L*, a*, b*, 650/580 ratio) were conducted using a Hunter Lab Miniscan XE Plus (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA) using an A illuminant, 10° observer and a 25 mm aperture. Calibration of the device was conducted prior to each daily evaluation using a black glass and a white ceramic tile covered with the same PVC film used to package the loin chops. Daily visual discoloration scores were determined by a single trained observer using a 10 cm unstructured line scale anchored at 0 and 100% surface discoloration, with each 1 cm increment equal to 10% discoloration of the chop surface.

Figure 3.3.

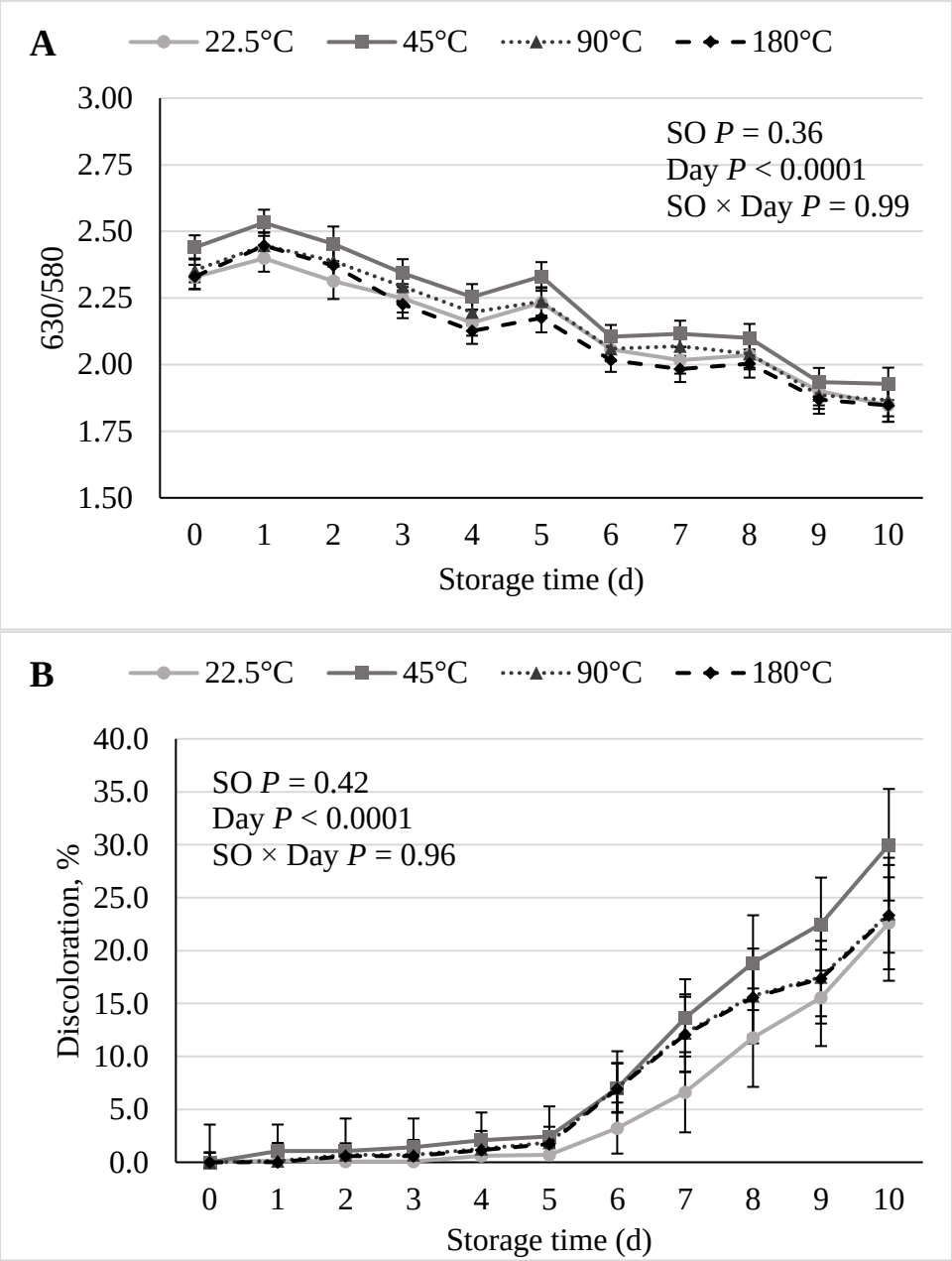

Figure 3.3. Effects of feeding peroxidized SO to finishing pigs on A) Reflectance and B) Discoloration of loin chops during 10 d of simulated retail display.

Figure 3.4.

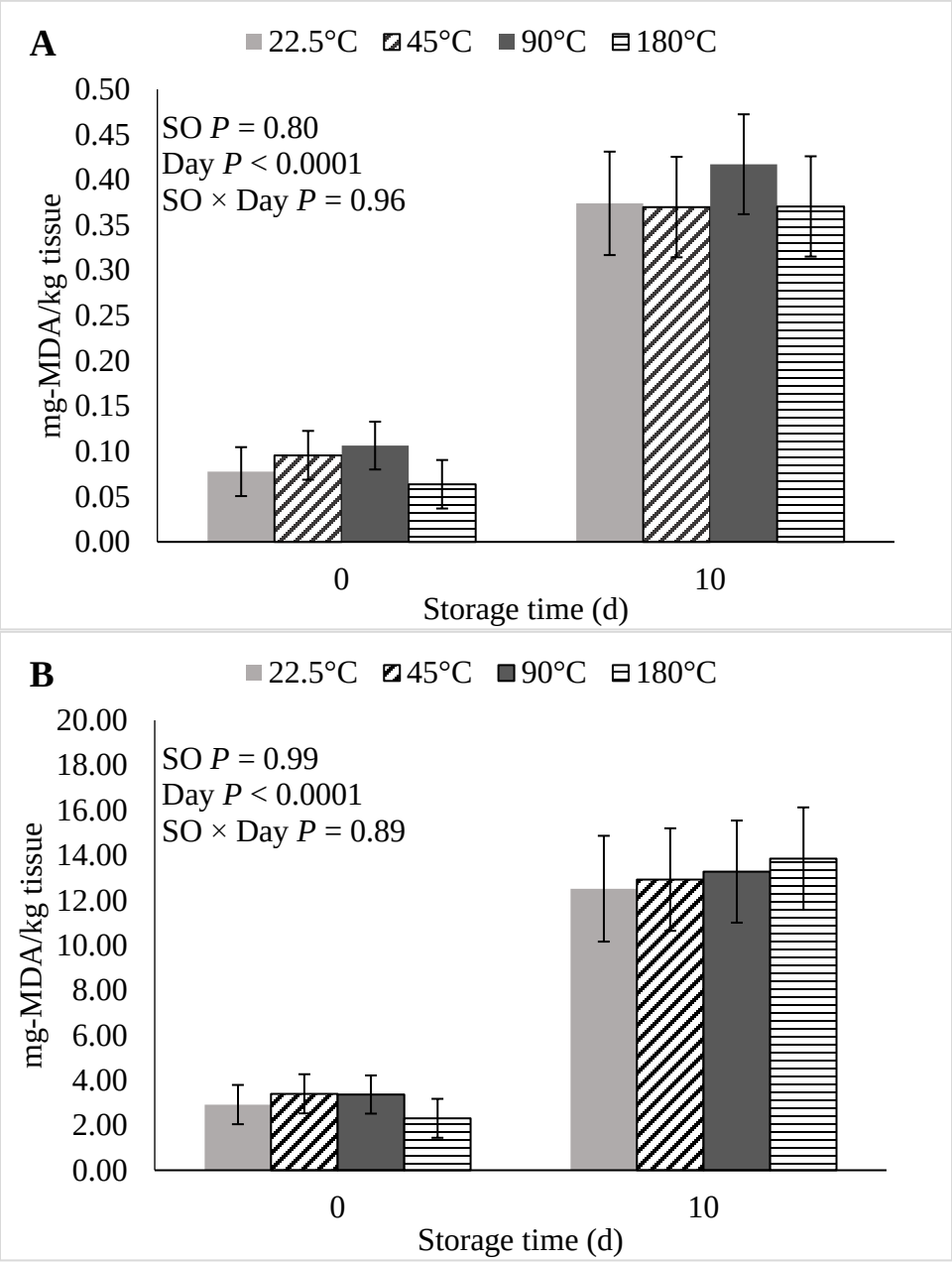

Figure 3.4. Effects of feeding peroxidized SO to finishing pigs on thiobarbituric reactive substances (TBARS) of loin chops subjected to 0 or 10 d of simulated retail display calculated on A) Tissue-basis or B) Extractable lipid content-basis.

3.5. TABLES

Table 3.1. Composition and peroxidation analysis of thermally processed soybean oils

Heating temperature, °C	22.5	45	90	180
Time heated, h[1]	0	288	72	6
Fatty acids, % of total fat[2,3]				
C8:0, Caprylic	ND[4]	ND	ND	0.07
C16:0, Palmitic	10.69	10.74	11.70	11.21
C16:1, Palmitoleic	0.09	0.08	0.09	0.08
C17:0, Margaric	0.10	0.10	0.11	0.10
C18:0, Stearic	4.19	4.13	4.52	4.42
C18:1, Oleic	23.46	23.49	25.15	24.26
C18:2, Linoleic	53.07	52.86	50.79	51.65
C18:3, Linolenic	7.14	7.15	6.14	6.43
C19:0, Nonadecanoic	0.26	0.25	0.23	0.35
C20:0, Arachidic	0.32	0.32	0.35	0.34
C20:1, Gadoleic	0.18	0.19	0.20	0.30
C22:0, Behenic	0.35	0.35	0.49	0.41
C24:0, Lignoceric	ND	0.12	ND	0.13
Other FA	0.15	0.22	0.23	0.27
UFA:SFA[4]	5.28	5.23	4.73	4.88
IV[5]	131	131	126	127
Free fatty acids, %[2]	0.10	0.10	0.46	0.14
Free glycerin, %[2]	1.04	3.72	0.82	0.82
Moisture, %	0.02	0.02	0.04	0.04
Insoluble impurities, %	0.02	0.04	0.04	0.04
Unsaponifiable matter, %	0.51	0.39	0.41	0.47
Oxidized FA, %[2]	3.0	2.8	2.5	1.9
OSI @ 110 C, h[2,4]	6.65	4.65	2.70	3.65
p-Anisidine value[2,6]	1.11	1.33	121	165
Peroxide value, meq/kg[2]	2.0	17.4	123.6	19.4
Polar compounds, %[2]	3.61	3.28	20.25	11.58
PTAG[4], %				
TBA value[2,6]	0.14	1.14	0.14	0.14
Aldehydes, mg/kg[7]				
2,4-decadienal	2.07	1.90	912.15	915.49
4-hydroxynonenal	0.66	1.49	170.48	82.80
Acrolein	6.15	6.06	27.12	44.60
2-Decenal	0.16	0.19	55.21	81.60
2,4-Heptadienal	0.47	1.19	268.62	151.65
2-Heptenal	2.33	1.82	254.48	90.68
Hexanal	2.01	2.02	33.69	6.28
2-Octenal	0.40	0.67	212.60	51.96
Pentanal	5.36	1.05	10.76	2.84
2,4-Undecadienal	0.06	0.07	43.73	53.34

Table 3.1 *(Continued)*

2-Undecenal	0.19	0.19	50.29	110.38
Ratio[8]	0.12	0.21	0.58	1.14
Total tocopherols, mg/kg[2]	1,328	1,331	94	798
Alpha	98	97	< 10	< 10
Beta	< 10	< 10	< 10	< 10
Delta	196	209	15	169
Gamma	1,034	1,025	79	629

[1]Thermally processed oils had constant air flow rate at 15L/min.
[2] Analyzed by Barrow-Agee, Memphis, TN.
[3] No other FA were detected besides those listed.
[4]Abbreviations: ND, not detected; FA, fatty acid; UFA:SFA, unsaturated:saturated fatty acid ratio; TBA, thiobarbituric acid; OSI, oil stability index; PTAG, polymerized tryacylglycerides; IV, iodine value.
[5] Iodine values were calculated using the FA profile data following the equation: IV = (16:1 × 0.95) + (18:1 × 0.86) + (18:2 × 1.732) + (18:3 × 2.616) + (20:1 × 0.795) + (20:2 × 1.57) + (20:3 × 2.38) + (20:4 × 3.19) + (20:5 × 4.01) + 22:4 × 2.93) + (22:5 × 3.68) + (22:6 × 4.64); Meadus et al., 2010.
[6] There are no units for p-anisidine value or TBA value.
[7]Analyzed by University of Minnesota, St. Paul, MN.
[8]Ratio of 2-decenal, 2,4-hydroxynonenal, 2,4-undecadienal, and 2-undecenal as a percent of total aldehydes to acrolein, 2,4-heptadienal, and 2-heptenal as a percent of total aldehydes; Wang et al., 2016.

Table 3.2. Carcass characteristics of finishing pigs fed peroxidized soybean oil[1]

Item	Oil treatment				SEM	*P*-value
	22.5°C	45°C	90°C	180°C		
No.	13	13	14	13		
ELW, kg	127.21[ab]	130.48[a]	121.59[b]	129.15[a]	2.37	0.05
Lairage loss, %	3.06	3.20	3.27	3.32	0.25	0.90
HCW, kg	101.83[a]	104.40[a]	95.79[b]	102.70[a]	1.94	0.01
Dressing percentage, %	79.99[a]	80.00[a]	78.74[b]	79.50[a]	0.28	< 0.01
Liver weight, kg	1.54[b]	1.66[ab]	1.76[a]	1.72[a]	0.05	0.03
Liver % ELW	1.21[c]	1.27[bc]	1.45[a]	1.33[b]	0.04	< 0.0001
10th rib BF depth, cm	2.65	2.55	2.29	2.54	0.16	0.44
LMA, cm^2	52.46	53.01	48.51	51.38	1.59	0.19
Estimated carcass lean, %	51.06	51.48	52.12	51.29	0.80	0.80
Clear plate IV	95.68[ab]	96.88[a]	81.75[c]	92.99[b]	1.06	< 0.0001

[a-b]LS means within a row having different superscripts are statistically different ($P \leq 0.05$)

[1]ELW, ending live weight; HCW, hot carcass weight; BF, back fat; LMA, loin muscle area; clear plate IV, Iodine value of clear plate fat measured using a Bruker MPA Multi-Purpose FT-NIR Analyzer (Bruker Optics Inc., Billerica, MA).

Table 3.3. Early post-mortem, fresh loin quality characteristics of finishing pigs fed peroxidized soy bean oil

	Oil Treatment					
Item	22.5°C	45°C	90°C	180°C	SEM	*P*-value
No.	13	14	14	14		
24 h pH	5.46	5.44	5.48	5.43	0.02	0.36
[1]L*	49.61	49.76	50.60	49.91	0.72	0.78
[1]a*	7.60	8.48	7.96	7.82	0.42	0.49
[1]b*	0.35	0.48	0.85	0.80	0.29	0.55
[2]Chroma	7.65	8.55	8.08	7.92	0.43	0.48
[3]Hue angle, °	2.23	3.33	5.47	5.19	2.04	0.62
[4]NPPC color	2.23	2.39	2.11	2.04	0.11	0.13
[4]NPPC marbling	1.00	1.21	1.14	1.07	0.09	0.33
[5]NPPC firmness	2.00	2.14	1.79	1.57	0.20	0.20
Drip loss, %	2.70	3.18	2.92	3.55	0.30	0.20
Moisture, %	74.11	74.22	74.11	74.44	0.31	0.84
Extractable lipid, %	3.36	3.20	3.28	2.93	0.26	0.65

[1]Measurements conducted with a Minolta CR-400 Chroma-meter (Minolta Camera Co., Ltd., Osaka, Japan) using a D65 light source and a 2° observer with an 8 mm aperture.

[2]Chroma = $\sqrt{a^{*2} \times b^{*2}}$

[3]Hue angle, ° = $\tan^{-1}$(b*/a*) × 57.296.

[4]NPPC (1999).

[5]NPPC (1991).

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CHAPTER 4: INFLUENCE OF FEEDING THERMALLY PEROXIDIZED SOYBEAN OIL TO FINISHING BARROWS ON PROCESSING CHARACTERISTICS AND SHELF LIFE OF COMMERCIALLY MANUFACTURED BACON

ABSTRACT: Objectives were to evaluate effects of feeding soybean oil (SO) with varying levels of peroxidation on fresh belly characteristics, processing yields, and shelf life of commercially manufactured bacon stored under food-service style conditions. Fifty-six barrows were randomly assigned to 1 of 4 diets containing 10% fresh SO (22.5°C) or thermally processed SO (45°C for 288 h, 90°C for 72 h, or 180°C for 6 h), each infused with air at a rate of 15L/min. Individually housed pigs were provided ad libitum access to feed for 81 d. On d 82 pigs were slaughtered and on d 83 carcasses were fabricated and bellies collected for recording of weight, dimensions, and flop distance. Belly adipose tissue cores were collected for analysis of iodine value (**IV**) by near-infrared spectroscopy (**NIR-IV**). Bacon was manufactured at a commercial processing facility and sliced bacon was subsequently transferred to food-service style packaging and subjected to 0, 30, 60, or 90 d storage at -20°C. Stored bacon was evaluated for thiobarbituric acid reactive substances (**TBARS**) and trained sensory evaluation of oxidized odor and flavor. Fresh belly and bacon processing traits were analyzed as a one-way ANOVA with the fixed effect of SO; whereas, shelf life traits were analyzed as a one-way ANOVA repeated in time. There was no effect ($P \geq 0.30$) of SO on belly weight, length, width, or thickness; but bellies of pigs fed 90°C SO had greater ($P \leq 0.04$) flop distance (more firm) than all other SO treatments. Belly fat NIR-IV of pigs fed 90°C SO were 10.22 units less ($P < 0.0001$) than pigs fed 180°C SO, which were 2.99 and 3.29 units less than belly adipose tissue of pigs fed 22.5°C and 45°C SO, respectively. There was no effect of SO on brine uptake or cooking yield of commercially manufactured bacon. There was a trend ($P = 0.09$) for bacon manufactured from

bellies of pigs fed 45°C and 90°C SO to have greater slicing yields than those from pigs fed 22.5°C and 180°C SO. There were no SO × storage time interactions ($P \geq 0.27$) for any shelf life trait. There was no difference in TBARS, oxidized odor, or oxidized flavor among the four SO treatments, though all three shelf life metrics increased ($P < 0.0001$) with storage time. Overall, feeding SO thermally processed at 90°C and 180°C reduced belly adipose tissue IV, but feeding peroxidized SO did not affect processing yields or shelf life characteristics of commercially manufactured bacon.

4.1. INTRODUCTION

Research on lipid quality has focused on the role of dietary fatty acid profile and its effects on the characteristics of adipose tissue. There has been a growing realization that when characterizing dietary lipid quality, the oxidative status of the lipid source should also be considered (Shurson et al., 2015). This is of importance to the swine industry because rendered fats and vegetable oils are commonly included in swine diets (Lin et al., 2013). It is well documented that feeding peroxidized lipids reduces nutrient digestibility and growth performance (Rosero et al., 2015; Lindblom et al., 2017) leading to reduced HCW (Boler et al., 2012). However, the effect of peroxidized lipid feeding on the quality and shelf life of pork products has been inconclusive. Buckley et al. (1989) reported that feeding peroxidized corn oil increased lipid oxidation of loin chops and ground pork during storage while others reported no effect of peroxidized lipid feeding on shelf life of loin chops or ground pork (Monahan et al., 1994; Boler et al., 2012). Peroxidation of lipids results in the hydrogenation of UFA (Yin et al., 2001), thereby decreasing the iodine value (**IV**) of the lipid (Lin et al., 2006). Reducing the IV of swine diets results in firmer fat, which is implicated in improving belly quality and the slicing yield of bacon (Shackelford et al., 1990; Kyle et al., 2014). Because bacon is the most valuable

pork product in the U.S market (U.S. Bureau of Labor Statistics, 2017) and its quality is dependent on fat quality, the effect of feeding peroxidized feedstuffs on the quality of bellies and the shelf life of bacon warrant investigation.

Therefore, the objectives of the study were to evaluate effects of feeding thermally abused soybean oil (SO) the iodine value of pork bellies and how that may affect fresh belly characteristics, processing yields, and shelf life of commercially manufactured bacon stored under food-service style conditions.

4.2. MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Iowa State University approved all animal care and use procedures for the live phase of the experiment.

4.2.1. Dietary Treatments and Experimental design

Fifty-six finishing barrows (Geneticporc F25 females × B6.0 sires, Hendersonville, TN) with an initial BW of 46.7 ± 5.1 kg, were randomly allotted into 56 pens equipped with partial slats, one two-holed feeder, and a single nipple waterer in a room that was mechanically ventilated with a pull-plug manure storage system located at the Swine Nutrition Farm at Iowa State University (Ames, IA). Pigs were randomly assigned to one of four dietary treatments, resulting in 14 replications per treatment. Each pig was individually penned (1.8 × 1.9 m) and had ad libitum access to feed and water (Overholt et al., 2018).

The four thermal processing treatments applied to the SO were selected to induce different degrees of peroxidation that would reflect conditions found in the feed processing, rendering, and restaurant industries (Meeker and Hamilton, 2006), and further, to reflect temperatures previously evaluated (Boler et al. 2012, Liu et al., 2014a, Kerr et al., 2015, Rosero et al., 2015, Hanson et al., 2016). The four SO treatments were included each phase of growth, which included either 10% fresh SO (22.5°C) or SO thermally processed at 45°C for 288 h, 90°C

for 72 h, or 180°C for 6 h. Except for the 22.5°C temperature, each heating process was accompanied with constant air flow (15L/min) using an air pump and a calibrated air flow controller with air forced into the tank using a 9.5 mm copper pipe. Immersion heaters were used to heat the SO to 45°C and 90°C, while a liquid propane heater was used to heat the SO to 180°. Oil temperatures were taken at regular intervals to ensure the predetermined temperatures were maintained. The stainless steel heating pots were 53 mm in circumference and 61 cm high, and were filled 2/3 during the heating process. To generate enough SO for each treatment, multiple batches of SO were heated and subsequently pooled into a 210 L container and stored at room temperature prior to feed mixing (Overholt et al., 2018). Multiple batches of each phase of feed was mixed with no antioxidant added before or during diet preparation, each diet was sampled at the time of mixing, and then pooled across phase for a composite sample for subsequent analysis (Table 1). Diets were formulated to meet or exceed the nutrient requirements for finishing pigs according to the NRC (2012), and no antioxidants were added to diets before or during their preparation.

4.2.2. Bacon processing

At the end of the 81 d feeding trial, pigs were transported approximately 613 km from Ames, IA to the University of Illinois Meat Science Laboratory in Urbana, IL. One barrow fed the 22.5°C SO diet was not fit for transport and therefore was euthanized on the farm prior to transportation. Upon arriving at the University of Illinois Meat Science Laboratory, barrows were held overnight (~16 h) with no access to feed, but ad libitum access to water. All slaughter procedures were conducted under the inspection the USDA Food Safety and Inspection Service (**FSIS**). Barrows were immobilized using the head-to-heart electrical stunning technique immediately followed by exsanguination. Following evisceration, carcasses were chilled at 4°C

for approximately 24 h following slaughter. After chilling, carcasses were fabricated according to North American Meat Processors Association specifications for (**NAMP**, 2010) #408 bellies. Fresh bellies were weighed and with length, width, depth, and flop distance measures obtained using the methods described by Overholt et al. (2016). Adipose tissue cores, measuring 2.54 cm in diameter and consisting of all 3 adipose layers, were collected from the anterior end along the dorsal edge of each belly. Lean tissue and skin were excised from the adipose cores before IV of the whole sample was determined using a Bruker MPA Multi-Purpose FT-NIR Analyzer (Bruker Optics Inc., Billerica, MA). Adipose tissue cores were then dissected by making a cut following the natural seam between the two most proximal adipose tissue layers (inner and medium layers) and the most distal layers (outer layer), which were then analyzed for IV in the same manner as the whole core.

Fresh (never frozen) bellies were then skinned to yield NAMP #409 skinless belly, packaged with individual identification tags, and transported by refrigerated truck to an USDA-FSIS inspected facility to be manufactured into bacon. Bellies were grouped by SO treatment and injected using a multi-needle injector with an industry-typical commercial curing solution formulated to deliver 1.5% sodium chloride at a targeted curing solution uptake of 13% of belly weight. Bellies were weighed immediately after injection to calculate brine uptake:

Brine uptake = [(Injected weight, kg – Initial weight, kg)/initial weight, kg] × 100.

Injected bellies were hung on smoke house racks and thermally processed to an internal temperature of 53.3°C. Neither natural or liquid smoke was used during processing. Bellies were chilled for approximately 24 h until they reached an internal temperature between -5.6°C and -4.4°C. Once cooked and chilled, bacon slabs were weighed to calculate cooked yield:

Cooked yield = [(cooked weight, kg – initial weight, kg)/ initial weight, kg] × 100.

Bacon slabs were then pressed and sliced, anterior end first, to obtain a target of 31 to 35 slices per kg (14 to 16 slices per pound). Slices were sorted by trained plant-personnel based on grading procedures of the manufacturer to remove incomplete slices, end pieces, and slices of unacceptable quality. Sliced bacon slabs were placed on U-boards and boxed individually such that anatomical orientation was maintained and only 1 sliced bacon slab was packaged per box. Sliced bacon slabs were subsequently transported to the University of Illinois Meat Science Laboratory for further analysis.

After arriving at the University of Illinois, bellies were removed from boxes and sliced bacon slabs were weighed in order to calculate sliced yield from initial weight and cooked weight:

$$\textit{Sliced yield (initial weight)} = (\textit{sliced weight, kg} / \textit{initial weight, kg}) \times 100;$$

$$\textit{Sliced yield (cooked weight)} = (\textit{sliced weight, kg} / \textit{cooked weight, kg}) \times 100.$$

Sliced bacon slabs were then divided into 5 zones (A, B, C, D, and E) with approximately equal number of slices per zone, in an anterior to posterior order, and a single slice from the center of each zone was collected (Overholt et al., 2016). These 5 slices were subsequently homogenized analyzed for moisture and extractable lipid content using the oven-drying and chloroform:methanol solvent extraction method described by Novakofski et al. (1989). An additional 20 slices were collected from the center of zone C and were randomly assigned in groups of 5, to be stored for 0, 30, 60, or 90 d. Five slices, per each of the 4 storage times, were placed flat on a piece of parchment paper. Slices designated for 0 d of storage were then placed in plastic bags, vacuum-packaged, and stored at -80°C to prevent degradation until further analyses were conducted. Slices allotted to the other 3 storage times, arranged on the parchment paper, were stacked in boxes (1 box per storage time), and subjected to 30, 60, or 90 d of

simulated storage at -20°C. At the conclusion of each storage time, bacon slices were removed from their packaging and transferred to plastic bags, vacuum-sealed, and stored at -80°C until they were needed for sensory evaluation and analysis of lipid oxidation.

4.2.3. Sensory evaluation

Sensory evaluation procedures were approved by University of Illinois Institutional Review Board (IRB #17753) and training of panelists was conducted in accordance with American Meat Science Association Guidelines for sensory evaluation (AMSA, 2015). A pool of 16 voluntary panelists were selected and trained, before each set of sensory sessions, to evaluate cooked bacon for oxidized odor and flavor using the protocol described by Lowe et al. (2014). From this pool of panelists, 6 panelists were chosen to participate in sensory sessions based on their availability such that every sensory panel was not made up of the exact same combination of panelists.

Frozen bacon slices were removed from -80°C storage and removed from their packaging 1 h prior to cooking. Three of the 5 slices were placed on raised wire grates oriented on cooking trays and allowed to thaw. The remaining 2 frozen slices were transferred into plastic sample bags and stored at -80°C until they were analyzed for lipid oxidation at the conclusion of sensory panels for that storage time. Bacon samples were prepared and presented to panelists according to the protocol described by Leick et al. (2010). Briefly, bacon slices were cooked in a convection oven (Southbend Model V-15, Fuquay-varina, NC) at 204.5°C for 7 min, trays were rotated 180°, and cooking resumed for an additional 7 min. Cooked slices were cut into 2.54 cm portions, placed in plastic serving cups with lids, and presented to panelists. Panelists then smelled and tasted the bacon samples, marked their score for oxidized odor and oxidized flavor on a 15 cm unstructured line scale, took a bite of unsalted cracker, a drink of apple juice, and

smelled ground coffee before repeating this process on the next sample. There were a total of 9 sensory sessions per storage time and 2 sessions were conducted per day with at least 1 h between sessions. Panelists' scores were averaged for each sample at each storage time for analyses.

4.2.4. Thiobarbituric acid reactive substances

Upon completion of sensory evaluations after each storage time, bacon samples were removed from the -80°C freezer and allowed to temper at approximately 23°C for 20 min; such that they were softened but still frozen. Slices were then cut into cubes, pulverized, and homogenized in a blender (Waring Products, Torrington, CT). Duplicate 5 g aliquots of each bacon sample were combined with 1 mL of 0.2 mg/ml butylated hydroxltoluene and 45.5 mL of 10% trichloroacetic acid in 0.2 *M* phosphoric acid then blended for 30 s. One mL of sulfanilamide was added to the homogenate before it was filtered through Whatman No. 1 filter paper (GE Healthcare, Chicago, IL) and collected in flasks. Two 5 mL aliquots of the filtrate were then transferred into 15 mL conical tubes. To one tube, 5 ml of 0.02 *M* thiobarbituric acid was added and to the other tube 5 ml of deionized water was added to serve as a blank. An additional 2 pulverized samples were randomly selected at each storage time to serve as spiked samples in order to estimate percent recovery of TBARS. The spiked samples were prepared in the same way as described above, except that 12 ml of 10μ*M* 1, 1, 3, 3-tetramethoxypropane was added in substitution for an equal volume for 10% trichloroacetic acid in 0.2 *M* phosphoric acid solution. A standard curve was made to represent 0, 1.25, 2.5, 5, and 7.5 mg malondialdehyde/ml using 25 μ*M* 1, 1, 3, 3-tetramethoxypropane. An aliquot of the 25 μ*M* 1, 1, 3, 3-tetramethoxypropane was combined with 5 ml of 0.02 *M* thiobarbituric acid and volumized to 10 ml with 10% trichloroacetic acid in 0.2 *M* phosphoric acid solution. All tubes were capped,

inverted to mix, and then stored in the dark at 23°C for approximately 16 h to allow pigment development. After 16 h, 150 µl from each tube was transferred onto 96 well round bottom plates and read for absorbance at 530 nm (Beckman Du-640 Spectrophotometer, Beckman Coulter Inc., Fullerton, CA). Thiobarbituric acid reactive substances (TBARS) were reported as mg-MDA/kg tissue and as mg-MDA/kg-extractable lipid.

4.2.5. Statistical analyses

All analyses were conducted with the MIXED procedure of SAS (SAS v9.4, SAS Inst. Inc., Cary, NC). Pigs were fed individually during the growth trial such that belly served as the experimental unit for all analyses. Fresh belly and bacon processing characteristics were analyzed as a completely randomized design with the fixed effect of SO treatment. Analyses of lipid oxidation (TBARS) was analyzed as a completely randomized design repeated in time with fixed effects of SO treatment and storage time. Sensory data were analyzed as a partially balanced incomplete block design repeated in time. In both TBARS and sensory analyses, storage time was the REPEATED variable. Analyses of repeated measures for each variable were conducted with multiple covariance structures and their appropriateness was determined by comparing Akaike's Information Corrected Criterion. Based on these comparisons, a 1st order Autoregressive Heterogeneous [TYPE = ARH(1)] covariance structure was selected. Least square means were separated with the PDIFF option of the mixed procedure. Main effects and interactions were considered different at $P \leq 0.05$. Statistically trends were defined when $0.05 > P \leq 0.10$.

4.3. RESULTS AND DISCUSSION

4.3.1. Fresh belly characteristics

There was no SO treatment effect ($P \geq 0.30$) on fresh belly weight, length, width, or thickness (Table 2). Because the pigs used in this study were fed 10% SO throughout the finishing period, adipose tissue IV of bellies yielded from these pigs were unusually high, regardless of treatment. Seman et al. (2013) reported mean IV of bellies sourced from a commercial processor ranged from 65.7 to 71.4, and the 2005 U.S. pork supply chain audit reported a mean belly IV of 67.51 (Wright et al., 2005). This is in contrast to the across-treatment mean IV of 88.82 observed in the present study, with maximum IV of 98.56. The remarkably poor quality of these bellies lended the opportunity to gain insight on the processing characteristics of bellies at one extreme of what is physiologically possible in the pork industry. The four thermal processing regiments resulted in divergent qualities of the oils (Table 1.), most apparent of which were fatty acid profile and oil IV. Though not statistically compared, there were notable numeric differences among the SO treatments with the 22.5°C, 45°C, 90°C, and 180°C SO having IV of 131, 131, 126, and 127; respectively. The disparity in IV of the oils were echoed in the firmness and IV of the bellies themselves. Flop distance of bellies of pigs fed 90°C/72h SO were 1.56 to 1.86 greater (more firm; $P \leq 0.04$) than the other three SO treatments. This relationship in flop distance was reflective of the differences in NIR-IV. Iodine value of whole core belly adipose tissue samples, consisting of all three adipose tissue layers, of pigs fed 90°C were the least saturated among all treatments ($P < 0.0001$), but would still would be considered high (IV = 79.66) under typical industry thresholds of an IV less than or equal to 74 (Seman et al., 2013). Despite a lack of statistical difference in flop distance among bellies of pigs fed the other three SO treatments, IV of whole core belly adipose tissue of pigs fed 180°C SO were 2.99 and 4.09 units less than ($P \leq 0.02$) than that of pigs fed 22.5°C and 45°C SO, respectively. This same pattern was observed after belly adipose tissue samples had been

bisected into combined inner-middle layers and an outer layer for analysis. While the relative differences were the same among treatments, IV of the inner-middle and outer layer were, on average, 2.65 and 0.65% greater, respectively, compared with the IV measured on the whole core sample, with the combined inner-middle adipose layers having marginally greater IV than the outer layer. Though differences in belly adipose tissue IV were a reflection of the IV of the SO, the magnitudes of difference observed between treatments, specifically when comparing IV of adipose tissue from pigs fed 90°C and 180°C were unexpected based on the relative similarities between the IV of the respective SO (IV = 126 vs. 127). Feeding peroxidized oil is known to reduce lipid digestibility (Liu et al., 2014b; Lindblom et al., 2017), which would cause a shift towards more *de novo* fatty acid synthesis (Kloareg et al., 2007) and deposition of medium chain, more saturated fatty acids. However, there was no difference in lipid digestibility between pigs fed 90°C and 180°C SO in the present study (Overholt et al., 2018). Perhaps the disproportionate difference in IV between belly adipose tissue of these two treatments is due to specific differences in the fatty acid profiles or other characteristics of the SO treatments; however, no known data is available to support this hypothesis at this time.

The level of unsaturation, as indicated by IV, observed in the present study were considerably greater than what would typically be observed in the U.S. pork industry (Seman et al., 2013). For perspective, the mean belly adipose tissue IV of pigs raised and slaughtered under industry-typical conditions is reported to range between 65 and 72 (Wright et al., 2005; Seman et al., 2013); whereas, the mean across all four treatments in the present study was 88.82. Although this level of unsaturation exceeds the typical range observed in the current industry environment, where the current use of high-fat grain co-products are limited, the bellies in the current study are

comparable to those yielded from pigs fed distillers dried grains with solubles (**DDGS**) in excess of 30% of the diet, which can have iodine values in excess of 88 (Leick et al., 2010).

4.3.2. Bacon processing characteristics

There was no difference ($P \geq 0.76$) among SO treatments for initial or injected weight (Table 3). In contrast, brine uptake of bellies from pigs fed 90°C was 1.37, 1.31, and 1.23 percentage units less ($P < 0.01$) than bellies from pigs fed 22.5°C, 45°C, and 180°C SO, respectively. Despite this, there was no effect ($P \geq 0.14$) of SO treatment on cooked weight, cooked yield, or sliced weight; and there was no SO effect ($P \geq 0.37$) on percent moisture or extractable lipid of finished bacon among any of the SO treatments. Iodine value is among the most reliable metrics of fat quality used to predict commercial bacon slicing yields (Seman et al., 2013; Kyle et al., 2014), and slicing yield is reduced as the level of unsaturation increases (Shackelford et al., 1990). Because there were stark differences in IV among the SO treatments, it was expected there would be equally stark differences in commercial bacon slicing yields. However, there was only a trend for bellies from pigs fed 45°C and 90°C to have greater ($P \leq 0.09$) bacon slicing yield, whether calculated from initial weight or cooked weight, than bellies from pigs fed 22.5°C/0h; with slicing yield of bacon manufactured from pigs fed 180°C being intermediate. Though there were meaningful numerical differences in slicing yield among treatments, with a difference of approximately 12 percentage units between the lowest and greatest SO treatments, the variability in yield was greater than anticipated, thus preventing the detection of any statistical differences. A second experimental population of bellies with a similar number of observations were processed on the same day, using the same equipment, reported substantially less variability in bacon slicing yields (SEM = 0.12 to 0.86; Lowell et al., 2017) compared with our study (SEM = 3.65 to 3.77). The primary difference between these two

populations was the IV of the belly adipose tissue. The mean-across-treatment IV reported by Lowell et al. (2017) was 69.05 compared with the 88.82 across-treatment mean observed among the current study. This appears to indicate that, although increased IV reduces slicing yield, it may also result in more inconsistent slicing. This hypothesis was previously suggested by the study by Overholt et al. (2016) who reported that the number of slices per kg tended to deviate from targeted slice counts dictated by slicing machine settings when slicing bacon with greater IV.

4.3.3. Bacon shelf life

Most previous research investigating the influence of diet on the shelf life of pork products has focused on the effects of the dietary fatty acid profile and the efficacy of antioxidant supplementation. Fewer studies have had the primary objective of investigating what role the oxidative status of the diet may have on product quality and shelf life. Although there have been a handful of studies that have investigated the relationship between feeding peroxidized lipids and product quality and shelf life, they have exclusively focused on loin chops and ground pork. These studies have reported inconsistent results in these products, particularly in respect to the shelf life of loin chops, with peroxidized lipid feeding resulting in a more consistent reduction in the shelf stability of the fattier, ground pork products. If the consumption of peroxidized lipids were to have any effect on the oxidative stability of pork products, it would likely be most readily observable in products with greater lipid contents, such as bacon and sausages. For this study, bacon packaged and stored under food-service-style conditions was selected as the model product. This was done because food-service represents the largest market for sale and consumption of bacon (NPD Group, 2014), representing 1.4 billion pounds of bacon sold in 2016 (NPD Group, 2017). Moreover, food service-style packaging typically lacks an atmospheric

barrier (Lowe et al., 2014), thus being more conducive towards lipid oxidation than sealed retail packaging. Furthermore, no study to date has investigated the effect of feeding peroxidized lipids on the oxidative stability of bacon.

There were no interactions ($P \geq 0.27$) between SO treatment and storage time for any bacon shelf life trait. Despite stark differences in the IV of belly adipose tissue among SO treatments and the oxidative profile of the oils themselves, there was no difference ($P \geq 0.46$) in lipid oxidation as measured by TBARS, whether calculated on a tissue- or extractable lipid content-basis (Figure 1). Similarly, there was no effect of SO on either oxidized odor ($P = 0.69$; Figure 2) or oxidized flavor ($P = 0.79$). Because no previous experiments have reported the effect of peroxidized oil feeding on bacon shelf life and oxidative stability the discussion of the results of the present study must rely upon comparisons with other pork products that have been investigated. Previous studies reported feeding peroxidized corn oil to finishing pigs increased the rate of TBARS development in loin chops during retail display compared with chops from pigs fed fresh oil (Buckley et al., 1989; Monahan et al., 1992). However, due to intrinsic differences in composition, minimal comparisons can be made between pork loin chops and bacon. A more apt comparison is that between bacon and ground pork products due to being more similar in their fat contents than with loin chops. Buckley et al. (1989) reported that both salted and unsalted ground pork manufactured from pigs fed peroxidized corn oil oxidized more rapidly than those made from pigs fed fresh oil. A similar finding was reported by Chae and Lee (2002) investigating the effects of feeding rancid rice bran on the oxidative stability of fresh, ground pork. In contrast to the results of these earlier experiments, and in congruence with the results of the current study, Boler et al. (2012) reported no difference in the rate of lipid oxidation when comparing ground pork from pigs fed either fresh or peroxidized corn oil,

despite the fact that feeding the peroxidized oil had resulted in increased plasma TBARS and reduced vitamin E concentrations in tissues.

4.3.4. Conclusions

Feeding finishing pigs peroxidized SO did not reduce the quality of fresh bellies or the shelf life of sliced bacon compared with feeding fresh SO. Moreover, the differences in IV resulting from feeding the different SO diets did not result in significant differences in commercial bacon slicing yield. However, it should be noted that this population of bellies were of considerably poorer quality compared with what is typically observed in the US pork industry. It is highly likely that the bellies in the present study would have drastically lower slicing yields compared with industry-typical bellies with iodine values within the normal range. With this in mind, it remains apparent that bacon quality and shelf life is not affected by the oxidative status of dietary lipids in finishing pig diets.

4.4. FIGURES

Figure 4.1.

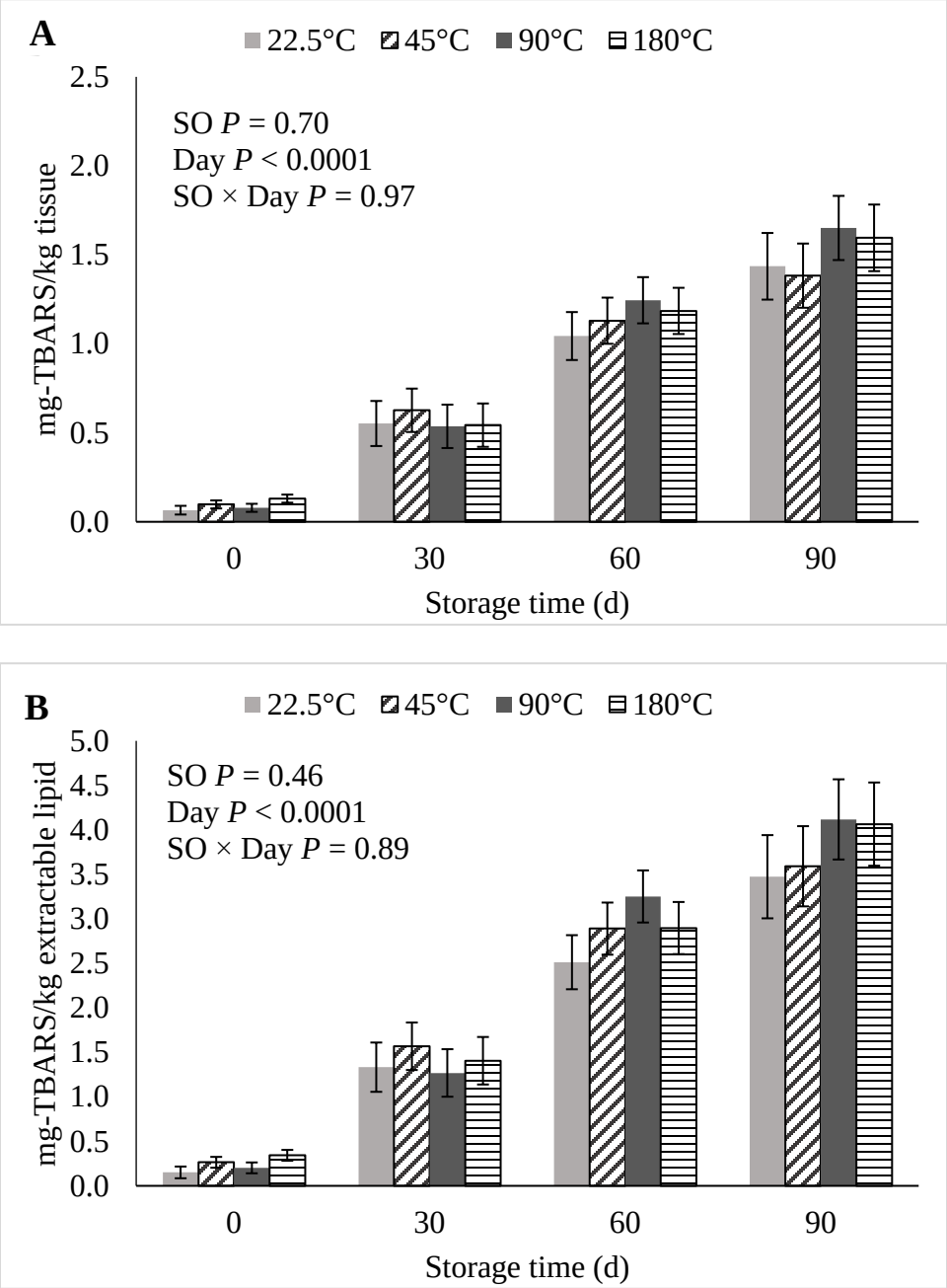

Figure 4. 1. Effects of feeding peroxidized soybean oil to finishing barrows on thiobarbituric acid reactive substances (TBARS) of bacon stored for 0, 30, 60, and 90 d calculated on a A) Tissue-basis or B) Extractable lipid-basis.

Figure 4.2.

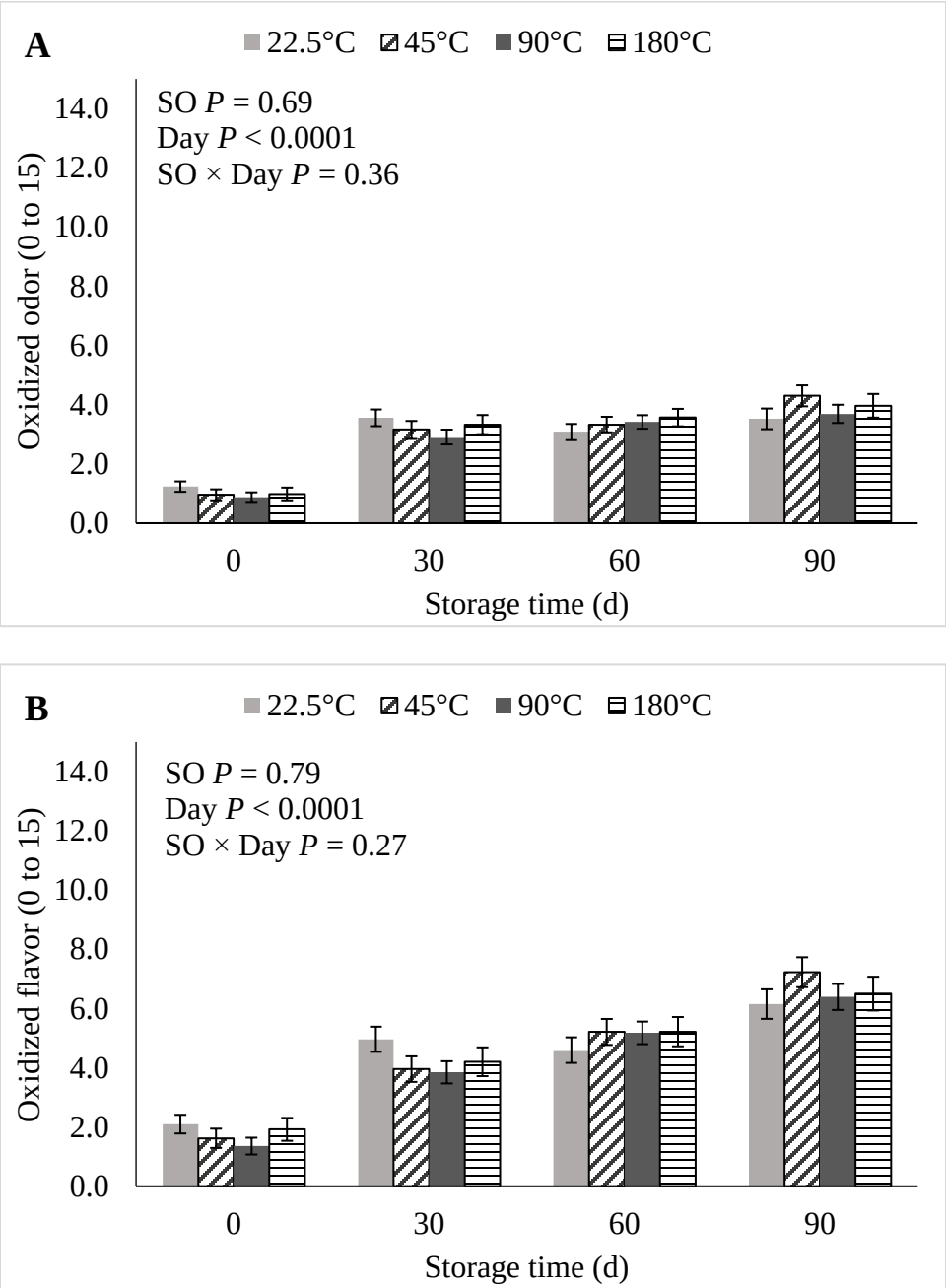

Figure 4.2. Effects of feeding peroxidized SO to finishing barrows on trained sensory panelists' evaluation of A) Oxidized odor and B) Oxidized flavor, of bacon stored for 0, 30, 60, and 90 d. Panelist evaluations were scored on a 15 cm unstructured line scale with a score of 0 representing no oxidized odor or flavor, and a score of 15 representing an extreme oxidized odor or flavor.

4.5. TABLES

Table 4.1. Composition and peroxidation analysis of thermally processed soybean oils

Heating temperature, °C	22.5	45	90	180
Time heated, h[1]	0	288	72	6
Fatty acids, % of total fat[2,3]				
C8:0, Caprylic	ND[4]	ND	ND	0.07
C16:0, Palmitic	10.69	10.74	11.70	11.21
C16:1, Palmitoleic	0.09	0.08	0.09	0.08
C17:0, Margaric	0.10	0.10	0.11	0.10
C18:0, Stearic	4.19	4.13	4.52	4.42
C18:1, Oleic	23.46	23.49	25.15	24.26
C18:2, Linoleic	53.07	52.86	50.79	51.65
C18:3, Linolenic	7.14	7.15	6.14	6.43
C19:0, Nonadecanoic	0.26	0.25	0.23	0.35
C20:0, Arachidic	0.32	0.32	0.35	0.34
C20:1, Gadoleic	0.18	0.19	0.20	0.30
C22:0, Behenic	0.35	0.35	0.49	0.41
C24:0, Lignoceric	ND	0.12	ND	0.13
Other FA	0.15	0.22	0.23	0.27
UFA:SFA[4]	5.28	5.23	4.73	4.88
Iodine value[5]	131	131	126	127
Free fatty acids, %[2]	0.10	0.10	0.46	0.14
Free glycerin, %[2]	1.04	3.72	0.82	0.82
Moisture, %	0.02	0.02	0.04	0.04
Insoluble impurities, %	0.02	0.04	0.04	0.04
Unsaponifiable matter, %	0.51	0.39	0.41	0.47
Oxidized FA, %[2]	3.0	2.8	2.5	1.9
OSI @ 110 C, h[2,4]	6.65	4.65	2.70	3.65
p-Anisidine value[2,6]	1.11	1.33	121	165
Peroxide value, mEq/kg[2]	2.0	17.4	123.6	19.4
Polar compounds, %[2]	3.61	3.28	20.25	11.58
PTAG[4], %				
TBARS[2,6]	0.14	1.14	0.14	0.14
Aldehydes, mg/kg				
2,4-decadienal	2.07	1.90	912.15	915.49
4-hydroxynonenal	0.66	1.49	170.48	82.80
Acrolein	6.15	6.06	27.12	44.60
2-Decenal	0.16	0.19	55.21	81.60
2,4-Heptadienal	0.47	1.19	268.62	151.65
2-Heptenal	2.33	1.82	254.48	90.68
Hexanal	2.01	2.02	33.69	6.28
2-Octenal	0.40	0.67	212.60	51.96
Pentanal	5.36	1.05	10.76	2.84
2,4-Undecadienal	0.06	0.07	43.73	53.34
2-Undecenal	0.19	0.19	50.29	110.38

Table 4.1 (Continued)

Ratio[7]	0.12	0.21	0.58	1.14
Total tocopherols, mg/kg[2]	1,328	1,331	94	798
Alpha	98	97	< 10	< 10
Beta	< 10	< 10	< 10	< 10
Delta	196	209	15	169
Gamma	1,034	1,025	79	629

[1]Thermally processed oils had constant air flow rate at 15L/min.

[2] Analyzed by Barrow-Agee, Memphis, TN.

[3] No other FA were detected besides those listed.

[4]Abbreviations: ND, not detected; FA, fatty acid; UFA:SFA, unsaturated:saturated fatty acid ratio; TBARS, thiobarbituric acid reactive substances; OSI, oil stability index; PTAG, polymerized tryacylglycerides; IV, iodine value.

[5]Calculated using the FA profile data following the equation: VI = (16:1 × 0.95) + (18:1 × 0.86) + (18:2 × 1.732) + (18:3 × 2.616) + (20:1 × 0.795) + (20:2 × 1.57) + (20:3 × 2.38) + (20:4 × 3.19) + (20:5 × 4.01) + 22:4 × 2.93) + (22:5 × 3.68) + (22:6 × 4.64); Meadus et al., 2010.

[6] There are no units for p-anisidine value or TBA value.

[7]Ratio of 2-decenal, 2,4-hydroxynonenal, 2,4-undecadienal, and 2-undecenal as a percent of total aldehydes to acrolein, 2,4-heptadienal, and 2-heptenal as a percent of total aldehydes; Wang et al., 2016 (doi: 10.1021/acs.jafc.6b01127).

Table 4.2. Fresh belly characteristics of finishing barrows fed peroxidized soybean oil

Item	Oil Treatment				SEM	*P*-value
	22.5°C	45°C	90°C	180°C		
No.	13	14	14	14		
Belly weight, kg	7.41	7.46	7.24	7.61	0.28	0.82
Belly, % of side weight	14.82	14.95	15.35	15.34	0.29	0.45
Flop distance, cm	6.37b	6.51b	8.07a	6.21b	0.54	0.05
Length, cm	69.61	70.62	70.67	70.58	1.12	0.89
Width, cm	28.38	28.53	27.35	28.67	0.56	0.30
Thickness, cm	3.67	3.87	3.82	3.64	0.17	0.72
[1]NIR-IV						
Whole core	92.87[a]	93.17[a]	79.66[c]	89.88[b]	0.89	< 0.0001
Middle and inner layer	95.57[a]	94.88[a]	82.86[c]	91.70[b]	1.08	< 0.0001
Outer layer	93.22[a]	93.99[a]	80.11[c]	90.57[b]	0.95	< 0.0001

[a-b]Least square means within a row having different superscripts are statistically different ($P \leq 0.05$).

[1]Near-infrared spectroscopy iodine value. Combined middle and inner layer includes the 2 most proximal layers of belly adipose tissue. Outer layer includes only the most distal layer of belly adipose tissue.

Table 4.3. Processing characteristics of commercially manufactured bacon from finishing barrows fed peroxidized soybean oil

	Oil Treatment					
Item	22.5°C	45°C	90°C	180°C	SEM	*P*-value
No.	13	14	14	14		
Initial weight, kg	5.80	5.78	5.59	5.88	0.25	0.85
Injected weight, kg	6.88	6.87	6.57	6.99	0.30	0.76
[1]Injection uptake, %	18.76[a]	18.70[a]	17.39[b]	18.62[a]	0.33	< 0.01
Cooked weight, kg	6.22	6.23	5.98	6.28	0.28	0.87
[2]Cooked yield, %	107.19	107.63	106.72	106.42	0.47	0.27
Sliced weight, kg	3.38	4.11	4.00	3.88	0.24	0.14
[3]Sliced yield (green weight), %	59.10	71.35	71.45	66.95	3.77	0.08
[4]Sliced yield (cooked weight), %	55.20	66.37	66.99	63.12	3.65	0.09
Moisture, %	41.26	45.95	44.77	45.51	2.07	0.37
Extractable lipid, %	42.64	39.63	39.16	39.11	2.05	0.56

[a-b]Least square means within a row having different superscripts are statistically different ($P \leq 0.05$).

[1]Injection uptake = [(Injected weight, kg – Initial weight, kg)/initial weight, kg] × 100.

[2]Cooked yield = [(cooked weight, kg – initial weight, kg)/ initial weight, kg] × 100.

[3]Sliced yield (initial weight) = (sliced weight, kg / initial weight, kg) × 100.

[4]Sliced yield (cooked weight) = (sliced weight, kg / cooked weight, kg) × 100.

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