Impact of storage conditions on protein oxidation of rendered by-product meals[1]

Carl A. Frame, Elisabeth J. Huff-Lonergan, and Mariana C. Rossoni Serao[2]

Department of Animal Science, Iowa State University, Ames, IA 50010

ABSTRACT: Rendered products used in animal feed and pet food undergo extreme temperatures during manufacturing and may be stored up to 2 yr. No information is available on protein oxidation in these products. The objective of this study was to determine the extent to which typical antioxidant inclusion at different storage conditions may limit protein oxidation in typical rendered protein meals. Two experiments were conducted on 14 rendered products stored at either 45 °C for 7 or 14 d, or at 20 °C for 3 or 6 mo to determine the extent to which time, temperature, and antioxidants affect protein oxidation. Results from this study show that fish meal and chicken blood meal are susceptible to protein oxidation during storage at 45 °C ($P = 0.05$; 0.03) as well as during storage at 20 °C ($P = 0.01$; 0.04). Natural antioxidants were effective at limiting carbonyl formation in fish meal during short-term storage at 45 °C, whereas ethoxyquin was effective at limiting the extent of protein oxidation in fish meal stored long term at 20 °C.

Key words: antioxidant, pet food, protein carbonyl, protein oxidation, rendering

INTRODUCTION

It is essential to understand how protein oxidation can impact the quality of rendered protein meals used for livestock and companion animal feeds. Consumption of oxidized proteins may lead to oxidative stress of various tissues throughout the body (Estévez and Xiong, 2019). From a performance standpoint in livestock species, consumption of oxidized lipids can negatively impact the production efficiency of livestock species (Dibner et al., 1996; Takahashi and Akiba, 1999; DeRouchey et al., 2004; Tavárez et al., 2011). Consumption of oxidized protein resulted in decreases in daily weight gain and feed intake (Wu et al., 2014; Chen et al., 2015). In other species, where longevity is the overall goal (such as companion animals or humans), oxidative stress may lead to specific health issues. For example, in humans, it is hypothesized that protein oxidation plays a part in certain age-related diseases such as Alzheimer's, Parkinson's, inflammatory bowel disease, muscular dystrophy, and even diabetes (Estévez and Luna, 2017).

The rendering process is a vital step in nearly all meat processing facilities to ensure feed safety, sustainability, and economic reasons. Rendering transforms unusable tissues into valuable feed ingredients (Meeker and Meisinger, 2015). This process separates lipids, protein, and moisture, as well as increases the shelf life and safety by destroying spoilage

organisms and pathogens through the use of high heat. Additionally, this step eliminates potential spoilage and putrefaction bacteria that could decrease product quality. Without this step, these products would be unusable and would result in waste that would fill landfills within the United States in 4 yr (Hamilton et al., 2006). It is rendering that allows the meat and animal feed industries to be sustainable. In the production of rendered protein meals, raw materials are cooked, pressed, ground, and finally dried. Typically during the cooking process, products are exposed to temperatures of 115–145 °C for 40–90 minutes (Anderson, 2006).

Within the rendering industry, there can be a wide variation in the quality of products produced. Renderers and their customers measure many quality traits. One of these traits is lipid oxidation. Lipid oxidation can cause quality deterioration, including nutritional quality loss and consumer acceptance decline. Consumer acceptance is especially important in the pet food industry because of its association with distinct objectionable odors and flavors. The industry recognizes that the lipid content of these protein meals is susceptible to oxidation. Thus antioxidants (synthetic and natural) are used to control lipid oxidation.

Because of greater perceived stability, oxidative deterioration of the protein fraction of the meals receives less attention. In most protein meals, tests are only performed to ensure the product matches the legal definition for composition. These tests generally include a minimum or maximum values for protein, moisture, or fat. Whereas studies examining dietary oxidized protein in broilers and rodents have shown decreased organ and immune function, the risks to livestock or companion animals that consume oxidized lipids and proteins are not well defined (Wu et al., 2014; Li et al., 2016; Ding et al., 2017; Estévez and Luna, 2017; Yang et al., 2017).

Currently, there is no information on the extent of protein oxidation present in rendered products. Just as importantly, the rate and extent of further oxidation in proteins in these products during storage is unknown. Heat induces protein oxidation in soy protein isolate (Tang et al., 2012; Zhang et al., 2017). Cooking lamb loins will increase the amount of protein oxidation (Roldan et al., 2014). It is also recognized that when lipids become oxidized, free radicals that are generated can initiate protein oxidation (Nielsen et al., 1985). In beef patties, greater lipid content was associated with more protein oxidation (Utrera et al., 2014). Rendered products from the meat industry are highly susceptible to lipid oxidation and thus have the capacity to be susceptible to protein oxidation. There are steps during the rendering process that can lead to oxidation of both lipids and proteins. However, it is unclear how stable the product is, especially the protein component, during storage post-production. While antioxidants are added to control lipid oxidation, it is not known how well these antioxidants protect from the oxidation of proteins.

A variety of rendered meals are used in animal diets. These products are used for various nutritional purposes due to differences in composition (Table 1). For example, blood meals are used as they contain the highest levels of lysine among natural feed ingredients. The high calcium in meat and bone meal is appealing to egg-laying operations (Meeker and Hamilton, 2006). A variety of methods are employed to make these products. Greater amounts of heat and time are needed to produce blood meal to drive off the added moisture. Specific processes allow manufacturers to reduce the amount of inorganic material as in the case for chicken by-product compared to low ash chicken by-product meal. As a result, this increases the concentration of other nutrients. These differences in composition and production practices may all impact the extent and rate of oxidation in both lipids and proteins and make it difficult to compare between products. It could be expected that different catalysts of oxidation could have different impacts depending on the product.

Rendered protein sources are widely used in both the livestock and companion animal feeds due to their high protein content and relatively low cost. Through the rendering process as well as post-production transportation and storage, many

Table 1. Composition of rendered meals as % dry matter

Product[*]	DM, %	Ash, %	Protein, %	Lipid, %
Beef				
Spray dried plasma	99.4	8.1	86.7	0.5
Blood meal	97.2	1.2	98.1	0.7
Meat and bone meal	94.2	33.2	52.7	11.0
Poultry				
Blood meal	96.5	2.0	99.5	0.8
Chicken by-product	97.3	13.2	70.3	10.3
Low ash chicken by-product meal	98.9	8.2	74.0	13.4
Fish				
Fish meal	96.9	19.9	71.4	8.3

DM, dry matter.

[*]Proteins were sourced from commercial renderers and did not contain antioxidants.

factors may induce protein oxidation. Considering these products are not thermally controlled during storage, these products could reach 45 °C for shorter periods. The expected shelf life of rendered products is 1–2 yr. The average storage temperature for extended periods would be around 20 °C. These conditions generate an environment that promotes protein oxidation. The use of antioxidants should limit protein oxidation. Understanding the extent to which protein meals may be oxidized is critical as it may impact health (Estévez and Xiong, 2019) and performance (Wu et al., 2014; Chen et al., 2015) in animal species. This study aimed to determine the extent to which typical antioxidant inclusion at different storage conditions may limit protein oxidation in common rendered protein meals. This information is vital to understand if protein oxidation should be considered in quality measures of rendered meals.

MATERIALS AND METHODS

Materials Used

Beef, poultry, and fish protein meal sources were used to give a general overview of common rendered products. All products were obtained from commercial renderers. Products containing antioxidants were treated at the manufacturer using currently employed application methods. Fish meal containing synthetic antioxidant was stabilized with ethoxyquin. The synthetic antioxidants used in bovine meat and bone meal, chicken by-product, and low ash chicken by-product meal was a commercial mixture containing butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Fish meal, chicken by-product meal, and low ash chicken by-product meal that contained natural antioxidants utilized mixed tocopherols in a total commercial product. The manufacturer added antioxidants under industry protocols, and the target levels were as appropriate for the antioxidant used. In addition to these products, blood meals from poultry and beef, as well as bovine spray-dried plasma were analyzed. These ingredients do not typically contain antioxidants as the lipid content is 0.5%, 0.7%, and 0.8% for spray-dried plasma, beef blood meal, and poultry blood meal, respectively (Table 1).

Experimental Design

Samples of spray-dried bovine plasma, bovine blood meal, bovine meat and bone meal with and without synthetic antioxidants, chicken by-product with synthetic, natural, and no antioxidants, low ash chicken by-product meal with synthetic, natural, and no antioxidants, and fish meal with synthetic, natural, and no antioxidants were utilized. Each product was equally split into 25 samples that were randomly assigned to 1 of 5 storage conditions (control, 45 °C for 7 or 14 d, or 20 °C for 3 or 6 mo), resulting in 5 replicates per storage condition ($n = 5$). To imitate different storage conditions, this study was split into two experiments. In experiment 1, products were held at 45 °C for 7 or 14 d to simulate maximum feasible storage temperatures that would last for shorter periods. Experiment 2 was designed to keep products at 20 °C for 3 or 6 mo to mimic normal storage temperatures for extended periods.

Material Handling

Products were obtained from each respective facility by collecting samples during loadout at 10-min intervals during processing and pooling samples together. For each product type containing antioxidants, the antioxidants were added during the production process immediately prior to storage or loadout. Upon delivery to the Iowa State University Companion Animal Nutrition Lab, products were vacuum sealed and stored at −20 °C until the start of each experiment (beef products–9 mo, poultry–2 mo, fish–2 d). Each product was divided into 25 samples containing 200 g and randomly assigned to 1 of 5 treatments. Control samples were immediately vacuum packaged and stored at −20 °C. Samples in the remaining treatments were placed in rectangular aluminum pans (14 × 12 × 5 cm) and placed in an incubator for assigned time and temperature. In experiment 1, samples were held at 45 °C for either 7 or 14 d and were rotated within the incubator daily. In experiment 2, samples were held at 20 °C for 3 or 6 mo and were rotated within the incubator each week. Each sample was removed from the incubator after the assigned time and immediately vacuum packaged and stored at −20 °C until completion of all incubation periods (6 mo).

Chemical Analysis

Control products were analyzed in duplicate for dry matter (AOAC 930.01), ash (AOAC 942.05), crude protein by using a LECO Nitrogen Analyzer (AOAC 992.15), and lipid by ether extract (AOAC

2003.06). All samples were analyzed for carbonyls following a modified protocol (Levine et al., 1990). Protein meal (2 g) was dissolved in 50 mL of 5% w/v sodium dodecyl sulfate (SDS), vortexed, and placed in a water bath for 1 h at 100 °C (Soglia et al., 2016). Samples were removed and immediately put on ice and vortexed, followed by centrifugation for 10 min at 3,000 × *g* at 4 °C to remove any remaining insoluble particles. This solution was then corrected to 15 mg of protein/mL and was used for carbonyl analysis as an indication of protein oxidation (Levine et al., 1990). Protein concentration was determined after the completion of the assay using biuret method (Robson et al., 1968), and values of carbonyls were calculated as nmol carbonyl per mg protein using the following equation.

$$\text{Corrected carbonyl (nmol/mg)} = \frac{\text{Carbonyl concentration (nmol/mL)}}{\text{Protein concentration (mg/mL)}}.$$

Statistical Analysis

In samples that did not have antioxidants (bovine spray-dried plasma, bovine blood meal, chicken blood meal), products were analyzed for the effect of storage time only within each experiment. Products that contained only synthetic antioxidants (beef meat and bone meal) were analyzed with a 3 × 2 factorial design with the effects of time and antioxidants within each experiment. Products that contained natural, synthetic, and no antioxidants (chicken by-product, low ash chicken by-product meal, Alaska Pollock fish meal) were analyzed with a 3 × 3 factorial design with the effects of time and antioxidant within each experiment. Five samples were analyzed in triplicate for protein oxidation at each time point for each level of antioxidant resulting in *n* = 5. Due to the substantial difference in composition of products, results were analyzed by each product for each experiment using the following model.

$$\gamma_{ijk} = \mu + T_i + A_j + (A \times T)_{ij} + \varepsilon_{ijk}.$$

In the above model, γ is the observed value; μ represents the overall mean; T is the ith level for the fixed effect of time (if a product contained variation in antioxidant), A is the jth level of the fixed effect of antioxidant, and ε_{ijk} represents the random error associated with γ_{ijk} assuming that $\sim N(0, I\sigma_\varepsilon^2)$. P-values ≤ 0.05 were considered significantly different, with P-value of $0.05 < P \leq 0.10$ considered trends. Data were analyzed as a completely random design using Proc MIXED procedure of SAS (version 9.4; SAS) with means reported using LSMEANS. Additionally, the day of carbonyl analysis was used as a random effect, and the Tukey post hoc test was utilized.

RESULTS AND DISCUSSION

Impact of Storage Time on Protein Meals

Rendered products are commonly expected to have up to a 2-yr shelf-life. Throughout that time, it will likely be shipped in either rail car, truck, or be held in some enclosed facility while still being exposed to oxygen. These products will probably be held in storage units that are not temperature controlled, leaving them vulnerable to climate temperature variations. As a result, it is feasible the most extreme storage conditions a product may experience would be during the seasonal summer heat, which would last for a relatively short period.

Protein carbonyl content did not increase in many products. However, in experiment 1, chicken blood meal (P-value = 0.03) had significantly greater carbonyl when stored at 45 °C for 7 d compared with control (Table 2). Beef meat and bone meal (P-value = 0.04) and fish meal (P-value < 0.01) also had greater carbonyl content after storage at 45 °C for 7 d compared with control (Table 3). When stored at 20 °C (experiment 2), chicken blood meal (P-value = 0.04; Table 4) and fish meal (P-value < 0.01; Table 5) exhibited increased carbonyl content at 6 mo compared with control. Additionally, low ash chicken by-product meal

Table 2. Protein oxidation measured by carbonyls in rendered by products without antioxidants for experiment 1 stored at 45 °C over short-term storage

| Protein source[†] | Carbonyl[*], nmol/mg protein | | | Time | |
	Control	7 d	14 d	SEM	*P*-value
Beef					
Spray dried plasma	7.1	6.2	6.9	0.7	0.64
Blood meal	17.4	23.5	21.7	4.0	0.56
Poultry					
Blood meal	55.8[b]	110.4[a]	127.2[a]	16.9	0.03

SEM, standard error of the mean. Analysis was conducted with five replicates at each time and protein source combination (*n* = 5). Differences in superscripts [a–b] within a row indicate a significant difference between time.

[*]Carbonyls were analyzed as a measure of protein oxidation. Values are reported as nmol of carbonyl/mg of protein and are the average of five replicates.

[†]Proteins were sourced from commercial renderers and did not contain antioxidants.

Table 3. Protein oxidation measured by carbonyls in rendered by products for experiment 1 stored at 45 °C over short-term storage

Protein source	Carbonyl*, nmol/mg protein			SEM	P-value		
	Control	7 d	14 d		Time	AOX	Time*AOX
Beef meat and bone meal							
No antioxidant	14.7	22.4	17.2	3.1	0.04	0.56	0.24
BHA/BHT	12.8	21.4	15.7				
Chicken by-product							
No antioxidant	15.0[ab]	21.0[a]	20.7[a]	5.6	0.15	0.07	0.05
BHA/BHT	15.3[ab]	18.6[a]	10.1[b]				
Natural antioxidant	16.1[ab]	15.1[ab]	11.0[b]				
Low ash chicken by-product meal							
No antioxidant	13.6	19.5	16.8	2.5	0.09	0.87	0.40
BHA/BHT	16.5	16.1	14.6				
Natural antioxidant	12.8	20.4	13.7				
Fish meal							
No antioxidant	11.5[c]	24.3[a]	15.3[bc]	3.0	<0.01	0.02	0.01
Ethoxyquin	14.8[bc]	21.3[ab]	13.8[bc]				
Natural antioxidant	9.4[c]	14.4[bc]	8.9[c]				

AOX, antioxidant; SEM, standard error of the mean for the interaction. The analysis was conducted with five replicates at each time and antioxidant combination ($n = 5$). The difference in superscripts [a–b] indicates significant interaction between time and antioxidant across antioxidant inclusion and time within a product source.

*Carbonyls were analyzed as a measure of protein oxidation. Values are reported as nmol of carbonyl/mg of protein and are the average of five replicates.

Table 4. Protein oxidation measured by carbonyls in rendered by products without antioxidants for experiment 2 stored at 20 °C over extended storage

Product[†]	Carbonyl*, nmol/mg protein			Time	
	Control	3 mo	6 mo	SEM	P-value
Beef					
Spray dried plasma	7.1	8.5	9.4	1.2	0.41
Blood meal	17.4	29.8	22.0	4.3	0.16
Poultry					
Blood meal	55.7[b]	87.2[ab]	123.1[a]	16.2	0.04

SEM, standard error of the mean. The analysis was conducted with five replicates at each time and protein source combination ($n = 5$). Differences in superscripts [a–b] within a row indicate a significant difference between time.

*Carbonyls were analyzed as a measure of protein oxidation. Values are reported as nmol of carbonyl/mg of protein and are the average of five replicates.

[†]Proteins were sourced from commercial renderers and did not contain antioxidants.

had increased carbonyl content after 6 mo at 20 °C compared with control (P-value = 0.01). These results suggest that poultry blood meal and fish meal are not only susceptible to high-temperature storage but also extended storage time at a moderate temperature. Additionally, beef meat and bone meal is susceptible to protein oxidation during storage at 45 °C, yet low ash chicken by-product meal is susceptible during storage at 20 °C.

The manufacturing process of poultry blood meal has the potential to increase the amount of free iron released from hemoglobin. This increase in free heme iron can increase metal-induced lipid oxidation (Grunwald and Richards, 2006). This free iron can similarly induce protein oxidation by way of Fenton reactions (Amici et al., 1989). Lipid oxidation products also initiate protein oxidation. However, this is not likely the case for poultry blood meal due to its low lipid content (0.8%). The increase in protein oxidation of beef meat and bone meal may be due to the high ash content (33.0%) of meat and bone meal. Metal ions in beef meat and bone meal, such as iron or copper, could induce protein oxidation (Amici et al., 1989; Rahal et al., 2014).

Fish meal contains 8.3% lipid (Table 1) and contains the least saturated as well as most polyunsaturated fatty acids (PUFA) compared with other products in this study. Although not analyzed in this study, literature values for unsaturation:saturated ratio for Alaskan pollock are 3.27 compared with 2.19 for chicken and 1.17 for beef (U.S. Department of Agriculture, 2007). Additionally, of the fat that is unsaturated, fish meal contains the highest percentage of PUFA at 77% compared with 34% for chicken and 8% for beef (U.S. Department of Agriculture, 2007). An increase in the content of unsaturated fatty acids—specifically PUFAs,

Table 5. Protein oxidation measured by carbonyls in rendered by products for experiment 2 stored at 20 °C over extended storage

| | Carbonyl*, nmol/mg protein | | | | P-value | | |
Protein source	Control	3 mo	6 mo	SEM	Time	AOX	Time*AOX
Beef meat and bone meal							
No antioxidant	14.7	20.8	14.9	4.3	0.14	0.52	0.42
BHA/BHT	12.8	23.9	20.5				
Chicken by-product							
No antioxidant	15.0	20.7	23.8	3.4	0.70	0.19	0.45
BHA/BHT	15.3	12.1	16.0				
Natural antioxidant	16.1	18.3	14.2				
Low ash chicken by-product meal							
No antioxidant	13.6	23.7	26.1	4.2	0.01	0.58	0.08
BHA/BHT	16.5	17.8	18.8				
Natural antioxidant	12.8	18.0	30.2				
Fish meal							
No antioxidant	11.5[d]	31.9[b]	53.9[a]	4.8	<0.01	0.29	<0.01
Ethoxyquin	14.8[cd]	27.9[bc]	38.1[b]				
Natural antioxidant	9.4[d]	30.1[b]	59.1[a]				

    AOX, antioxidant; SEM, standard error of the mean for the interaction. The analysis was conducted with five replicates at each time and antioxidant combination ($n = 5$). The difference in superscripts [a–d] indicates significant interaction between time and antioxidant across antioxidant inclusion and time within a product source.

    *Carbonyls were analyzed as a measure of protein oxidation. Values are reported as nmol of carbonyl/mg of protein and are the average of five replicates.

increases the susceptibility of lipid to oxidation (Frankel, 1998).

    Though short-term storage at high heat resulted in a tendency for increased protein oxidation at 7 d in low ash chicken by-product meal (P-value = 0.09), this product is vulnerable to extended storage time at 20 °C (P-value = 0.01). This could also be a result of the lipid in this meal being readily oxidized, thus leading to free radical formation and protein oxidation.

Impact of Antioxidants on Limiting Protein Oxidation During Storage of Protein Meals

    Processors must include antioxidants, especially in products susceptible to lipid oxidation to maintain acceptable quality. Peroxide value is a contemporary method to verify the effectiveness of antioxidants. However, when lipids are oxidized, the resulting products can induce protein oxidation. Typical antioxidants work to limit the impact of catalysts (reactive oxygen species, metal catalyst, heat) on lipid oxidation. These same catalyst can also impact protein oxidation. Therefore, it is possible that typical antioxidants could have some efficacy toward limiting protein oxidation.

    Fish meal treated with natural antioxidants contained fewer carbonyls than fish meal without or with ethoxyquin (P-value = 0.02). A time × antioxidant interaction was observed (P-value = 0.01) for carbonyl values from fish meal stored at 45 °C (Table 3). Fish meal treated with a natural antioxidant had less carbonyl development after 7 d of storage than fish meal with no antioxidant (Table 3). This would suggest that the natural antioxidant was effective in controlling for protein oxidation in fish meal stored at 45 °C for 7 d. However, these findings were not consistent for a longer storage at a more moderate temperature. When stored at 20 °C for 6 mo, fish meal with ethoxyquin had less carbonyl content compared with fish meal without or with natural antioxidant (P-value = 0.01). A time × antioxidant interaction was observed (P-value < 0.01) for carbonyl values from fish meal stored at 20 °C (Table 5). Fish meal treated with ethoxyquin had less carbonyl development after 6 mo of storage than fish meal with no antioxidant or with natural antioxidants (Table 5). However, it is essential to point out that while the use of synthetic antioxidants did limit the extent of protein oxidation after 6 mo, fish meal with ethoxyquin was still greater than the control suggesting carbonyl formation still occurred (P-value < 0.01). Low ash chicken by-product meal containing BHA/BHT stored for 6 mo at 20 °C tended to contain fewer carbonyls than low ash chicken by-product meal without antioxidants or with natural antioxidants (Table 5;

P-value = 0.08). The inclusion of antioxidants in other products did not measurably decrease protein oxidation in those products.

In saturated lipids, free radicals favor reacting with tocopherols over fatty acids. Once the free radical reacts with the tocopherol, it is stabilized in the phenolic ring, thus terminating free radical propagation (Verleyen et al., 2002). As saturation decreases, as in the case of fish meal, free radicals favor fatty acid over tocopherols, resulting in reduced effectiveness of the antioxidant (Verleyen et al., 2002). As a result, tocopherols are more effective in controlling lipid oxidation in more saturated fats. This has been shown in studies that as the unsaturation of a lipid increases, tocopherols are consumed at a slower rate (Verleyen et al., 2002; Barrera-Arellano et al., 2005). This slower consumption rate could be an explanation of why tocopherols were not effective at preventing carbonyl development in products with a higher proportion of unsaturated lipids stored at 20 °C. Granted, the study by Marinova and Yanishlieva (1992) showed that α-tocopherol effectiveness in limiting lipid oxidation in lard increased with increasing temperature at 25, 50, 75, and 100 °C. This increase in activity with increased temperature may explain why mixed tocopherols were effective in the fish meal when stored at 45 °C but not at 20 °C.

The thermal stability of both natural and synthetic antioxidants is not fully understood in rendered meals. Comparing BHA/BHT with ethoxyquin, it was shown that BHA has greater thermal stability than ethoxyquin (Sanhueza et al., 2000). Santos et al. (2012) showed that BHA vaporized in 155 min, while BHT vaporized in 90 min when heated to 110 °C. The same study showed that α-tocopherol withstood the greatest temperature before decomposition, followed by BHA and BHT. A similar study examined the effectiveness of antioxidants in sunflower oil after heated to 180 °C for 1 h. Results from this study showed BHA and BHT were more susceptible to thermal inactivation compared wih mixed tocopherols (Allam and Mohamed, 2002). The results of these studies provide evidence that tocopherols are more stable at elevated temperatures compared with BHA/BHT. Although temperatures that proteins experienced during this study were less than these studies, this could explain why when products were stored at 45 °C, no synthetic antioxidants were effective in limiting protein oxidation as evaluated by measures of carbonyl formation.

The evidence presented supports the conclusion that commonly used antioxidants did not directly protect proteins. Protection was only provided when antioxidants were included in products that are most susceptible to lipid oxidation. This secondary effect is likely a result of antioxidants controlling lipid oxidation products that may would have promoted protein oxidation.

It should be noted that in chicken by-product stored at 45 °C, an unexpected decrease in carbonyls was observed between 7 and 14 d of storage at 45 °C in product containing antioxidants (P-value = 0.05). This was also seen in fish meal stored at 45 °C without antioxidants and with natural antioxidants (P-value = 0.01). Estévez et al. (2009) made a similar observation in which carbonyls measured in bovine serum albumin at 37 °C increased from 0 to 9 d and then decreased at day 14 of storage (Estévez et al., 2009). They proposed that carbonyls may not be the end product of oxidation and could be further oxidized. This degradation of the carbonyl may result in the observed decrease in total carbonyls. The decrease in carbonyls at 14 d of storage could be a result of carbonyls degrading and further oxidizing into unknown secondary products.

From this study, it was determined that poultry blood meal and fish meal when not treated with antioxidants are susceptible to protein oxidation (as measured by carbonylation) during a wide range of storage conditions (45 °C for 7 d, or 20 °C for 6 mo). In the case of fish meal, the use of natural antioxidants did prevent protein carbonylation when stored at 45 °C for up to 7 d. However, when fish meal stored at 20 °C, protein oxidation was limited after 6 mo with the inclusion of ethoxyquin.

Many factors could play a part in why the products studied here are susceptible. It is hypothesized that free iron, ash content, and lipid profile could influence protein and lipid oxidation. While these could be possible impacts, the substantial difference in composition between products makes it difficult to determine the exact cause for increased protein oxidation. Factors such as aggregation that would impact the availability for protein to participate in oxidation (Promeyrat et al., 2010), to the inherent amino acid profile of each protein source (Davies, 2016) and its mineral content all play a potential role.

This study was successful in characterizing protein oxidation measured by carbonyl formation during storage of rendered meals and in identifying products that are susceptible to protein oxidation. While others have determined that protein oxidation may impact health (Estévez and Xiong, 2019) and

performance (Wu et al., 2014; Chen et al., 2015) in animal species, further work should focus on levels observed in these feed ingredients. It is important to note that carbonyls may be degraded as a result of further oxidation, leading to an underestimation of protein oxidation. Thus, it is important to consider time course studies and the inclusion of more measures of protein oxidation end products when evaluating antioxidants for their efficacy in preventing/limiting protein oxidation. A comprehensive understanding of the specific modes of action and intrinsic features that influence protein oxidation will inform the development of technologies to preserve product nutrient and chemical quality.

Conflict of interest statement. None declared.

LITERATURE CITED

Allam, S. S. M., and H. M. A. Mohamed. 2002. Thermal stability of some commercial natural and synthetic antioxidants and their mixtures. J. Food Lipids. 9:277–293. doi:10.1111/j.1745-4522.2002.tb00226.x

Amici, A., R. L. Levine, L. Tsai, and E. R. Stadtman. 1989. Conversion of amino acid residues in proteins and amino acid homopolymers to carbonyl derivatives by metal-catalyzed oxidation reactions. J. Biol. Chem. 264:3341–3346.

Anderson, D. 2006. Rendering operations. In: Meeker, D. L., editor. Essential rendering all about the animal by-products industry. Alexandria (VA): National Renderers Association; p. 31–52.

Barrera-Arellano, D., M. C. Dobarganes, and C. J. Steel. 2005. The influence of natural tocopherols during thermal oxidation of refined and partially hydrogenated soybean oils. Grasas y Aceites. 56:46–52. doi:10.3989/gya.2005.v56.i1.133

Chen, X., Y. P. Chen, D. W. Wu, C. Wen, and Y. M. Zhou. 2015. Effects of heat-oxidized soy protein isolate on growth performance and digestive function of broiler chickens at early age. Asian-Australas. J. Anim. Sci. 28:544–550. doi:10.5713/ajas.14.0609

Davies, M. J. 2016. Protein oxidation and peroxidation. Biochem. J. 473:805–825. doi:10.1042/BJ20151227

DeRouchey, J. M., J. D. Hancock, R. H. Hines, C. A. Maloney, D. J. Lee, H. Cao, D. W. Dean, and J. S. Park. 2004. Effects of rancidity and free fatty acids in choice white grease on growth performance and nutrient digestibility in weanling pigs. J. Anim. Sci. 82:2937–2944. doi:10.2527/2004.82102937x

Dibner, J. J., C. A. Atwell, M. L. Kitchell, W. D. Shermer, and F. J. Ivey. 1996. Feeding of oxidized fats to broilers and swine: effects on enterocyte turnover, hepatocyte proliferation and the gut associated lymphoid tissue. Anim. Feed Sci. Technol. 62:1–13. doi:10.1016/S0377-8401(96)01000-0

Ding, Y.-Y., X.-R. Cheng, Z.-Q. Li, S.-J. Wu, Y. Yang, Y.-H. Shi, and G.-W. Le. 2017. Effect of dietary oxidized tyrosine products on insulin secretion via the oxidative stress-induced mitochondria damage in mice pancreas. RSC Adv. 7:26809–26826. doi:10.1039/C7RA02945D

Estévez, M., and C. Luna. 2017. Dietary protein oxidation: a silent threat to human health? Crit. Rev. Food Sci. Nutr. 57:3781–3793. doi:10.1080/10408398.2016.1165182

Estévez, M., V. Ollilainen, and M. Heinonen. 2009. Analysis of protein oxidation markers α-aminoadipic and γ-glutamic semialdehydes in food proteins using Liquid Chromatography (LC)−Electrospray Ionization (ESI)−Multistage Tandem Mass Spectrometry (MS). J. Agric. Food Chem. 57:3901–3910. doi:10.1021/jf804017p

Estévez, M., and Y. Xiong. 2019. Intake of oxidized proteins and amino acids and causative oxidative stress and disease: recent scientific evidences and hypotheses. J. Food Sci. 84:387–396. doi:10.1111/1750-3841.14460

Frankel, E. N. 1998. Lipid Oxidation. Dundee (Scotland): Oily Press.

Grunwald, E. W., and M. P. Richards. 2006. Mechanisms of heme protein-mediated lipid oxidation using hemoglobin and myoglobin variants in raw and heated washed muscle. J. Agric. Food Chem. 54:8271–8280. doi:10.1021/jf061231d

Hamilton, C. R., D. Kirstein, and R. E. Breitmeyer. 2006. The rendering industry's biosecurity contribution to public and animal health. In: Meeker, D. L. editor. Essential rendering all about the animal by-products industry. Alexandria (VA): National Renderers Association. p. 71–94.

Levine, R. L., D. Garland, C. N. Oliver, A. Amici, I. Climent, A. G. Lenz, B. W. Ahn, S. Shaltiel, and E. R. Stadtman. 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 186:464–478. doi:10.1016/0076-6879(90)86141-h

Li, Z. L., Y. Shi, G. Le, Y. Ding, and Q. Zhao. 2016. 24-Week exposure to oxidized tyrosine induces hepatic fibrosis involving activation of the MAPK/TGF-β1 signaling pathway in Sprague-Dawley rats model. Oxid. Med. Cell. Longev. 2016:1–12. doi:10.1155/2016/3123294

Marinova, E. M., and N. V. Yanishlieva. 1992. Effect of temperature on the antioxidative action of inhibitors in lipid autoxidation. J. Sci. Food Agric. 60:313–318. doi:10.1002/jsfa.2740600307

Meeker, D. L., and C. R. Hamilton. 2006. An overview of the rendering industry. In: Meeker, D. L. editor. Essential rendering all about the animal by-products industry. National Renderers Association, Alexandria (VA). p. 1–16.

Meeker, D. L., and J. L. Meisinger. 2015. Rendered ingredients significantly influence sustainability, quality, and safety of pet food. J. Anim. Sci. 93:835–847. doi:10.2527/jas.2014-8524

Nielsen, H. K., P. A. Finot, and R. F. Jurrell. 1985. Reactions of proteins with oxidizing lipids 2. Influence on protein quality and on the bioavailability of Lysine, Methionine, Cyst(e)Ine and Tryptophan as measured in rat assays. Br. J. Nutr. 53:75–86. doi:10.1079/BJN19850012

Promeyrat, A., M. L. Bax, S. Traoré, L. Aubry, V. Santé-Lhoutellier, and P. Gatellier. 2010. Changed dynamics in myofibrillar protein aggregation as a consequence of heating time and temperature. Meat Sci. 85:625–631. doi:10.1016/j.meatsci.2010.03.015

Rahal, A., A. Kumar, V. Singh, B. Yadav, R. Tiwari, S. Chakraborty, and K. Dhama. 2014. Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed Res. Int. 2014:761264. doi:10.1155/2014/761264

Robson, R. M., D. E. Goll, and M. J. Temple. 1968. Determination of proteins in "Tris" buffer by the biuret reaction. Anal. Biochem. 24:339–341. doi:10.1016/0003-2697(68)90188-7

Roldan, M., T. Antequera, M. Armenteros, and J. Ruiz. 2014. Effect of different temperature-time combinations on lipid

and protein oxidation of sous-vide cooked lamb loins. Food Chem. 149:129–136. doi:10.1016/j.foodchem.2013.10.079

Sanhueza, J., S. Nieto, and A. Valenzuela. 2000. Thermal stability of some commercial synthetic antioxidants. J. Am. Oil Chem. Soc. 77:933–936. doi:10.1007/s11746-000-0147-9

Santos, N. A., A. M. T. M. Cordeiro, S. S. Damasceno, R. T. Aguiar, R. Rosenhaim, J. R. Carvalho Filho, I. M. G. Santos, A. S. Maia, and A. G. Souza. 2012. Commercial antioxidants and thermal stability evaluations. Fuel. 97:638–643. doi:10.1016/j.fuel.2012.01.074

Soglia, F., M. Petracci, and P. Ertbjerg. 2016. Novel DNPH-based method for determination of protein carbonylation in muscle and meat. Food Chem. 197(Pt A):670–675. doi:10.1016/j.foodchem.2015.11.038

Takahashi, K., and Y. Akiba. 1999. Effect of oxidized fat on performance and some physiological responses in broiler chickens. Japanese Poult. Sci. 36:304–310. doi:10.2141/jpsa.36.304

Tang, X., Q. Wu, G. Le, and Y. Shi. 2012. Effects of heat treatment on structural modification and in vivo antioxidant capacity of soy protein. Nutrition 28:1180–1185. doi:10.1016/j.nut.2012.03.011

Tavárez, M. A., D. D. Boler, K. N. Bess, J. Zhao, F. Yan, A. C. Dilger, F. K. McKeith, and J. Killefer. 2011. Effect of antioxidant inclusion and oil quality on broiler performance, meat quality, and lipid oxidation. Poult. Sci. 90:922–930. doi:10.3382/ps.2010-01180

U.S. Department of Agriculture, Agricultural Research Service. 2007. USDA National Nutrient Database for Standard Reference, Release 20. Methods and Application of Food Composition Laboratory Home Page. http://www.ars.usda.gov/nea/bhnrc/mafcl

Utrera, M., D. Morcuende, and M. Estévez. 2014. Fat content has a significant impact on protein oxidation occurred during frozen storage of beef patties. Food Sci. Technol. 56:62–68. doi:10.1016/J.LWT.2013.10.040

Verleyen, T., A. Kamal-Eldin, R. Mozuraityte, R. Verhé, K. Dewettinck, A. Huyghebaert, and W. De Greyt. 2002. Oxidation at elevated temperatures: competition between α-tocopherol and unsaturated triacylglycerols. Eur. J. Lipid Sci. Technol. 104:228–233. doi:10.1002/1438-9312(200204)104:4<228::AID-EJLT228>3.0.CO;2-5

Wu, D. W., X. Chen, X. Yang, Z. X. Leng, P. S. Yan, and Y. M. Zhou. 2014. Effects of heat treatment of soy protein isolate on the growth performance and immune function of broiler chickens. Poult. Sci. 93:326–334. doi:10.3382/ps.2013-03507

Yang, Y., H. Zhang, B. Yan, T. Zhang, Y. Gao, Y. Shi, and G. Le. 2017. Health effects of dietary oxidized tyrosine and dityrosine administration in mice with nutrimetabolomic strategies. J. Agric. Food Chem. 65:6957–6971. doi:10.1021/acs.jafc.7b02003

Zhang, X., P. Lu, W. Xue, D. Wu, C. Wen, and Y. Zhou. 2017. An evaluation of heat on protein oxidation of soy protein isolate or soy protein isolate mixed with soybean oil in vitro and its consequences on redox status of broilers at early age. Asian-Australas. J. Anim. Sci. 30:1135–1142. doi:10.5713/ajas.16.0683