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Oxidative Damage to Poultry, Pork, and Beef during Frozen Storage through the Analysis of Novel Protein Oxidation Markers

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ABSTRACT: The susceptibility of meats from different animal species (beef quadriceps femoris, porcine longissimus dorsi, and chicken pectoralis major) to undergo protein oxidation during frozen storage (20 weeks/−18 °C) was studied through the analysis of novel oxidation markers. Frozen storage induced protein carbonylation (α-aminoadipic and γ-glutamic semialdehydes), carboxylation (α-aminoadipic acid), and formation of Schiff bases in meat from the three species. Major rates of protein and lipid oxidation products [thiobarbituric-acid-reactive substances (TBARS) and hexanal] were found in beef patties. Among the endogenous factors having a potential influence on the susceptibility of meat to undergo protein oxidation, heme iron seemed to play a major role. The present study illustrates the severe chemical modifications induced by oxidative stress during frozen storage of ground meat and provides original insight into the underlying mechanisms and factors.

KEYWORDS: *Protein oxidation, carbonylation, α-aminoadipic acid, Schiff bases, meat animals, frozen storage, lipid oxidation*

INTRODUCTION

Frozen storage is the most common long-term preservation technology in muscle foods because it inhibits microbiological spoilage and retards autolytic reactions.[1] However, oxidative reactions remain active during frozen storage of muscle foods.[2] The oxidation of macromolecules leads to irreversible chemical changes and impaired quality traits in meat and meat products.[1] Until recently, lipid oxidation has been acknowledged as the major form of deterioration in frozen meats. Lipid oxidation results in the formation of toxic compounds, such as malondialdehyde and cholesterol oxidation products, as well as accumulation of volatile carbonyls, alcohols, and acids responsible for off-flavors.[3] Current studies have shown that meat proteins also undergo oxidative deterioration during frozen storage induced by reactive oxygen species (ROS) or the interaction with lipid oxidation products.[4,1] Even though the influence of protein oxidation on food quality is poorly understood, recent studies have related the oxidation of muscle proteins to impaired functionality, altered sensory traits, and loss of nutritional value.[4,5]

Because protein carbonylation has been highlighted as the most remarkable change in oxidized proteins,[6,7] previous studies have focused on the impact of frozen storage on the formation of protein carbonyls using the routine dinitrophenylhydrazine (DNPH) method. Increases in protein carbonyls have been reported during frozen storage of chicken leg and breast muscles[8] and porcine muscles.[9] In contrast, lower amounts of protein carbonyls were reported when ostrich meat was subjected to freezing and thawing processes.[1] As reviewed by Estévez,[4] the inconsistency of the DNPH method urges the application of advanced methodologies to study specific protein oxidation products. The lack of chemistry background has hindered thus far the understanding of the factors and mechanisms involved in the oxidative damage to meat proteins during frozen storage and the consequences of such chemical modifications on meat quality.

Recently, specific protein carbonyls, such as the α-aminoadipic (AAS) and the γ-glutamic (GGS) semialdehydes, have been quantified in myofibrillar proteins by fluorescent high-performance liquid chromatography (HPLC−FLD),[10] and the method was further applied to burger patties,[11] cooked hams,[12] frankfurters,[13] and fish muscles.[14] Likewise, further oxidation products of lysine, such as α-aminoadipic acid (AAA) and Schiff bases (SB), had been identified in meat matrices by HPLC−FLD and spectrofluorometry (SF), respectively.[11,15] Assessing protein oxidation through the analysis of specific protein carbonyls, Estévez et al.[16] reported that the formation of protein carbonyls occurred concomitantly with the loss of water-holding capacity of pork muscles subjected to freezing. Later, Utrera and Estévez[15] established reasonable links between precise chemical changes in oxidized myofibrillar proteins (carbonylation, carboxylation, and formation of cross-links) and the loss of protein functionality. Using similar methodologies, Utrera et al.[12] recently found a connection between protein carbonylation and impaired quality traits (hardness and redness) in cooked hams elaborated from previously frozen hams. The identification of the mechanisms involved in the formation of specific protein oxidation products is required to establish the potential implication of such compounds on the quality of frozen meat. The progress in this topic requires the study of diverse protein oxidation changes using novel methodologies.

The aim of this study was to gain deeper insight into the protein oxidation mechanisms during frozen storage of beef, pork, and poultry through the application of accurate methodologies to measure novel protein oxidation markers. The susceptibility of meat proteins from different species to

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undergo particular oxidative changes was studied in relation to the chemical composition of the muscles.

MATERIALS AND METHODS

Chemicals and Materials. All chemicals were supplied from Panreac (Panreac Quimíca, S.A., Barcelona, Spain), Merck (Merck, Darmstadt, Germany), and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany). Water used was purified by passage through a Milli-Q system (Millipore Corp., Bedford, MA). Beef (muscle quadriceps femoris), pork (muscle longissimus dorsi), and chicken (pectoralis major) muscles belonged to industrial genotypes and were purchased from a local slaughterhouse in Cáceres (Spain). Meat was free from visible fat, immediately chopped into pieces (2 cm^3), kept at 4 °C for 8 h, and used as such for the manufacture of the patties.

Manufacture of Patties. Three types of patties were prepared depending upon the meat source (poultry, pork, and beef). The basic recipe was as follows (g/kg of raw batter): 732 g of meat, 244 g of distilled water, and 24 g of sodium chloride. All ingredients were minced in a cutter until a homogeneous raw batter was obtained (9 min). Patties were formed using a conventional burgermaker (~80 g/patty), to give average dimensions of 10 cm diameter and 1 cm thickness. In total, 20 patties per species were prepared in four independent manufacturing processes. Proximate and fatty acid composition of the patties was analyzed the day of the manufacture. To allow for the onset of oxidative reactions, patties were stored in individual oxygen-permeable polyethylene bags, dispensed in trays, and subsequently stored for 20 weeks at −18 °C in the dark. At each sampling time (weeks 0, 4, 8, 12, and 20), four patties per species were taken out from the refrigerator, thawed at 4 °C for 10 h, and subjected to further analysis, including oxidation markers and heme iron.

Proximate and Fatty Acid Composition of Patties. Moisture and protein contents (g/100 g of patty) were determined using official methods.[17] The method by Folch et al.[18] was used for determining the fat content (g/100 g of patty). The fatty acid profiles of patties were determined by gas chromatography of the fatty acid methyl esters (FAMEs) following the method described by Rodriguez-Carpena et al.[19] Tridecanoic acid was used as an internal standard. Results were expressed as a percentage. The heme iron content was determined by spectrophotometric quantification of myoglobin at 640 nm after extraction with acidified 80% acetone, and results were expressed as parts per million (ppm) of heme iron.[20]

HPLC−FLD Analysis of AAS and GGS. Samples (5 mg of protein) were derivatized with 50 mM aminobenzoic acid (ABA) and subsequently hydrolyzed with 6 N HCl according to the procedure described by Utrera et al.[10] Hydrosylates were dried *in vacuo*, reconstituted with 200 μL of Milli-Q water, and filtered through a polyvinylidene difluoride (PVDF) syringe filter (0.45 μm pore size, Pall Corp., Port Washington, NY). Samples were injected in HPLC using a Cosmosil (Phenomenex, Torrance, CA) C18-AR-II RP-HPLC column (5 μm, 150 × 4.6 mm,) and a guard column (10 × 4.6 mm) filled with the same material. The Shimadzu "Prominence" HPLC apparatus (Shimadzu Corp., Kyoto, Japan) was equipped with a quaternary solvent delivery system (LC-20AD), a DGU-20AS online degasser, a SIL-20A autosampler, a RF-10A XL fluorescence detector, and a CBM-20A system controller. Sodium acetate buffer (50 mM, pH 5.4, eluent A) and acetonitrile (50 mM, ACN, eluent B) were used as eluents. A low-pressure gradient program was used, varying the eluent B concentration from 0% (min 0) to 8% (min 20). The injection volume was 1 μL; the flow rate was kept at 1 mL/min; and the temperature of the column was maintained constant at 30 °C. Excitation and emission wavelengths were set at 283 and 350 nm, respectively. Identification of the derivatized semialdehydes in the FLD chromatograms was carried out by comparing their retention times (Rt) to those from a standard compound injected and analyzed under the above-mentioned conditions.[10] Peaks corresponding to AAS−ABA and GGS−ABA were manually integrated from FLD chromatograms, and the resulting areas were plotted against an ABA standard curve (ranging from 0.1 to 0.5 mM). Regression coefficients of >0.99 were obtained. The estimation of the quantities of AAS−ABA and GGS−ABA through an ABA standard curve was accomplished by assuming that the fluorescence emitted by 1 mol of ABA is equivalent to that emitted by 1 mol of the derivatized protein carbonyls. Results were expressed as nanomoles of AAS or GGS per gram of protein.

HPLC−FLD Analysis of AAA. Samples were hydrolyzed with 3 N HCl and subsequently derivatized with 0.2 mM 9-fluorenylmethyl chloroformate (FMoc) according to the procedure described by Utrera et al.[11] The derivatized samples were filtered through hydrophilic polypropylene GH Polypro (GHP) syringe filters (0.45 μm pore size, Pall Corp., Port Washington, NY) for HPLC analysis. An aliquot (1 μL) was injected and analyzed in the above-mentioned HPLC equipment (Shimadzu Corp., Kyoto, Japan) using a Zorbax Eclipse AAA column (Agilent, Santa Clara, CA) (3.5 μm, 4.6 × 150 mm) and a guard column (10 × 4.6 mm) filled with the same material. Eluent A was 20 mM ammonium acetate (pH 6.5) and 15% methanol, and eluent B was 90% acetonitrile. The flow rate was constant at 1.0 mL/min, and the column was maintained at 35 °C. The gradient profile was as follows: 0−1.5 min, 12% B; 1.5−2.0 min, 12−18% B; 2.0−9.0 min, 18% B; 9.0−9.5 min, 18−25% B; 9.5−12.5 min, 25% B; 12.5−13.0 min, 25−30% B; 13.0−16.0 min, 30% B; 16.0−17.0 min, 30−40% B; 17.0−20.0 min, 40% B; 20.0−22.0 min, 40−50% B; 22.0−23.0 min, 50% B; and 23.0−24.0 min, 50−99% B. Excitation and emission wavelengths were set at 263 and 313 nm, respectively. Identification of the derivatized AAA in the FLD chromatograms was carried out by comparing its Rt to that from a standard compound injected and analyzed in the above-mentioned conditions. The peaks corresponding to AAA−FMoc were manually integrated from FLD chromatograms, and the resulting areas were plotted against an AAA−FMoc standard curve with known amounts (ranging from 0.4 to 5 pM). Regression coefficients of >0.98 were obtained. Results were expressed as nanomoles of AAA per gram of protein.

Fluorescence Measurement of SB. The emission of fluorescence by SB was assessed using fluorescence spectroscopy.[21] Patties were ground and homogenized according to the process described by Utrera et al.[11] A 1 mL aliquot of the homogenates was diluted in 20 mL of a 20 mM sodium phosphate buffer and then dispensed in a 4 mL quartz spectrofluorometer cell. Emission spectra of SB were recorded from 400 to 500 nm with the excitation wavelength set at 350 nm (LS 55 Perkin-Elmer luminescence spectrometer, Billerica, MA). Excitation and emission slit widths were set at 10 nm, and data were collected at 500 nm/min. Results were expressed as fluorescence intensity units emitted at 460 nm. These values were corrected according to the moisture content of each sample.

Determination of Thiobarbituric-Acid-Reactive Substances (TBARS). TBARS were assessed as described by Salih et al.,[22] with some modifications. Samples (5 g) were homogenized with 15 mL of perchloric acid (3.86%) and 0.5 mL of butylated hydroxytoluene (BHT) (4.2% in ethanol). The slurry was filtered and centrifuged (1509*g* for 4 min), and 2 mL aliquots were mixed with 2 mL of thiobarbituric acid (0.02 M) in test tubes. The test tubes were placed in a boiling water bath (100 °C) for 45 min. After cooling, absorbance was measured at 532 nm. The TBARS content was calculated from a standard curve of 1,1,3,3-tetraethoxypropane (TEP) solution (ranging from 0.28×10^3 to 1.13×10^3 mg/mL) in 3.86% perchloric acid. Results were expressed as milligrams of malondialdehyde (MDA) equivalents per kilogram of sample.

Analysis of Hexanal. Hexanal was used an indicator of lipid oxidation and analyzed from the headspace of patties by solid-phase microextraction (SPME) and gas chromatography−mass spectrometry (GC−MS) following the method described by Estévez et al.[23] A total of 1 g of sample was placed in a 2.5 mL vial, and the SPME fiber (divinylbenzene−carboxen−polydimethylxilosane, 50/30 μm, Supelco, Bellefonte, PA) was exposed to the headspace, while the sample equilibrated for 30 min immersed in water at 37 °C. Analyses were performed on a HP5890GC series II gas chromatograph (Hewlett-Packard Company, Palo Alto, CA) coupled to a mass-selective detector (Agilent model 5973). Volatiles were separated using a 5% phenyl−95% dimethylpolysiloxane column (30 m, 0.25 mm inner diameter, and 1.0 μm film thickness; Restek, Bellefonte, PA). Hexanal was positively identified by comparing its mass spectra and Rt to those

Table 1. Chemical Composition and Fatty Acid Profile of Poultry, Pork, and Beef Patties[a]

	poultry	pork	beef	p
moisture (g/100 g of patty)	78.83 ± 0.30	78.39 ± 0.75	78.13 ± 0.18	ns[b]
protein (g/100 g of patty)	16.89 ± 0.11 b	18.01 ± 0.22 a	17.08 ± 0.45 b	*c*
fat (g/100 g of patty)	1.46 ± 0.19	1.38 ± 0.18	1.40 ± 0.15	ns
heme iron (mg/kg of patty)	3.44 ± 0.84 c	9.44 ± 1.13 b	33.31 ± 4.10 a	*d*
fatty acids (%)[e]				
C12	0.17 ± 0.02 c	0.28 ± 0.02 a	0.21 ± 0.02 b	*d*
C14	1.27 ± 0.15 c	2.18 ± 0.25 b	3.07 ± 0.21 a	*d*
C16	23.76 ± 1.10	24.62 ± 1.41	24.68 ± 0.95	ns
C16:1	4.94 ± 0.25 a	3.96 ± 0.21 b	3.34 ± 0.30 c	*d*
C17	0.29 ± 0.01 c	0.44 ± 0.03 b	0.92 ± 0.03 a	*d*
C17:1	0.19 ± 0.01 c	0.33 ± 0.02 b	0.51 ± 0.03 a	*d*
C18	8.92 ± 0.36 c	11.65 ± 0.53 b	18.12 ± 1.71 a	*d*
C18:1ω-9	39.92 ± 0.68	42.28 ± 1.28	40.91 ± 1.67	ns
C18:2ω-6	17.85 ± 0.47 a	11.87 ± 0.47 b	6.33 ± 0.40 c	*d*
C18:3ω-3	0.77 ± 0.03 a	0.41 ± 0.02 b	0.31 ± 0.02 c	*d*
C20	0.08 ± 0.01 b	0.16 ± 0.03 a	0.14 ± 0.02 a	*c*
C20:1	0.25 ± 0.02 b	0.37 ± 0.04 a	0.18 ± 0.01 c	*d*
C20:2	0.17 ± 0.03 a	0.18 ± 0.04 a	0.10 ± 0.01 b	*c*
C20:3ω-6	0.34 ± 0.02 a	0.26 ± 0.02 b	0.33 ± 0.05 ab	*f*
C20:4ω-6	1.12 ± 0.08 a	1.02 ± 0.07 a	0.84 ± 0.07 b	*c*
$\sum$SFA[g]	34.44 ± 1.05 c	39.33 ± 1.30 b	47.14 ± 2.38 a	*d*
$\sum$MUFA[g]	45.30 ± 0.49	46.93 ± 1.17	44.95 ± 1.94	ns
$\sum$PUFA[g]	20.26 ± 0.57 a	13.74 ± 0.56 b	7.91 ± 0.48 c	*d*

[a]Means with different letters were significantly different in ANOVA. [b]ns = not significant. [c]$p < 0.01$. [d]$p < 0.001$. [e]Percentage of total fatty acids analyzed. [f]$p < 0.05$. [g]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; and PUFA, polyunsaturated fatty acids.

displayed by the standard compound. The result from the hexanal analysis was provided in arbitrary area units (AAU).

Statistical Analysis. Four patties per species and per sampling day were prepared and used as experimental units. The effect of the species on all measurements was analyzed by one-way analysis of variance (ANOVA) and Tukey's tests (SPSS, version 15.0). To establish relationships between muscle components and the oxidation markers, Pearson correlations were calculated and a principal component analysis (PCA) was performed. Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Proximate and Fatty Acid Composition of Meat Patties. As expected, the main differences in the chemical composition of patties from different animal species were found in the fatty acid composition and heme iron content (Table 1). The total amount of saturated fatty acids (SFA) in beef patties (47.17%) was higher than those from pork and poultry (39.33 and 34.44%, respectively). Polyunsaturated fatty acids (PUFA) showed an opposite behavior (7.91, 13.74, and 20.26% in beef, pork, and poultry, respectively). Heme iron content in beef (33.31 ppm) was also the highest in comparison to pork (9.44 ppm) and poultry (3.44 ppm). The composition of the present samples is consistent with a previous study devoted to analyze the oxidative stability of lipids from beef loin, pork loin, and chicken breast in similar meat patties.[24] However, the amount of iron reported for pork and beef loins in that study was approximately 2-fold lower than the data reported in the present study. These differences can be ascribed to the fiber composition of the muscles used between studies as well as the possible difference in the age of animals at slaughter.[3]

Protein Oxidation. AAS and GGS were determined and used as early protein oxidation markers. The initial AAS values (day 0) were not significantly different between different animal species (Figure 1A). At weeks 4 and 8, the AAS content was significantly higher in beef patties and reached its maximum at week 8 (Figure 1A). A subsequent decrease was observed at

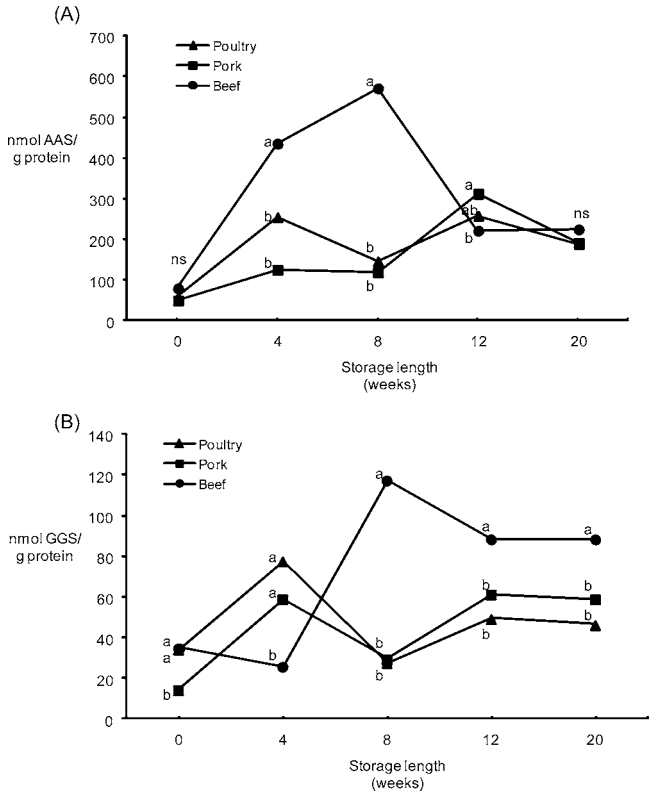

Figure 1. Evolution of the concentration of protein carbonyls, (A) AAS and (B) GGS, in poultry, pork, and beef patties during frozen storage. Different letters at a sampling point denote significant differences between means.

week 12. The AAS content in pork and poultry patties increased until week 12 and was followed by a significant decrease at week 20 (Figure 1A). At the end of the frozen storage (week 20), no significant differences were observed for the AAS content in patties from different animal species (Figure 1A). A similar pattern was found for the formation of GGS (Figure 1B). However, the highest concentration of GGS in pork and poultry patties was found at week 4 (Figure 1B). Additionally, from week 8 onward, significantly higher concentrations of GGS were found in beef samples than in the other counterparts (Figure 1B).

The formation of AAS and GGS during frozen storage of meat proves the oxidative deamination of the side chains of lysine, arginine, and/or proline in the presence of ROS and transition metals, such as iron.[4] Both semialdehydes have been highlighted as the main carbonyl products from the metal-catalyzed oxidation (MCO) of proteins.[25] The frozen storage led to a significant increase of protein carbonylation in patties from the three animal species. The formation of ice crystals during freezing causes cell disruption and concentrates pro-oxidant solutes, such as iron, H_2O_2, and myoglobin, leading to the formation of ROS.[2] The portion of unfrozen water, where the concentration of such reactive species takes place, may surround polar macromolecules, such as proteins, creating a highly oxidative environment, despite the low temperatures.[26] Among these pro-oxidants, iron, in particular, has been highlighted as the main promoter of protein carbonylation in meat systems.[4] The major source of heme iron in meat is myoglobin (up to 90%), which, in the presence of H_2O_2, can be converted to hypervalent species, which have been found to initiate protein oxidation.[7] Thus, the higher amounts of both carbonyls in beef patties may respond to the larger amounts of heme iron in these samples (Table 1). The iron-dependent carbonylation of proteins from meat subjected to frozen storage has been previously reported in porcine muscles with a diverse concentration of heme pigments.[12,21] This study confirms that the concentration of iron was plausibly more influential on the occurrence and extent of protein carbonylation than other factors, such as the fatty acid composition. Other formulation (i.e., salt content) and processing (i.e., mincing) factors could have influenced the susceptibility of muscle proteins to oxidation and, hence, contributed to the present results.

Both semialdehydes are prone to undergo further reactions because they feature highly reactive moieties.[4] Under intense oxidative conditions, AAS, in particular, undergoes a further oxidative degradation in the presence of peroxides to yield AAA. According to the results from this study, such conditions were fulfilled during the frozen storage of meat patties (Figure 2A). Besides the formation of AAA, AAS may also react with neighboring amino groups from other amino acid side chains (e.g., lysine) to form SB structures.[4] Such structures were also found to increase during the frozen storage of patties. Hence, the decrease of the AAS content at the end of the frozen storage (Figure 1A) could be reasonable, attributed to the implication of AAS in further reactions.

The AAA concentration in pork and poultry patties significantly increased at weeks 12 and 20 of frozen storage and was significantly higher than that of beef patties until week 12 (Figure 2A). After 20 weeks of frozen storage, the production of AAA increased about 2-fold times in beef patties and no significant differences were found for the content of AAA among species (Figure 2A). This trend was consistent with the loss of AAS and could be attributed to a further

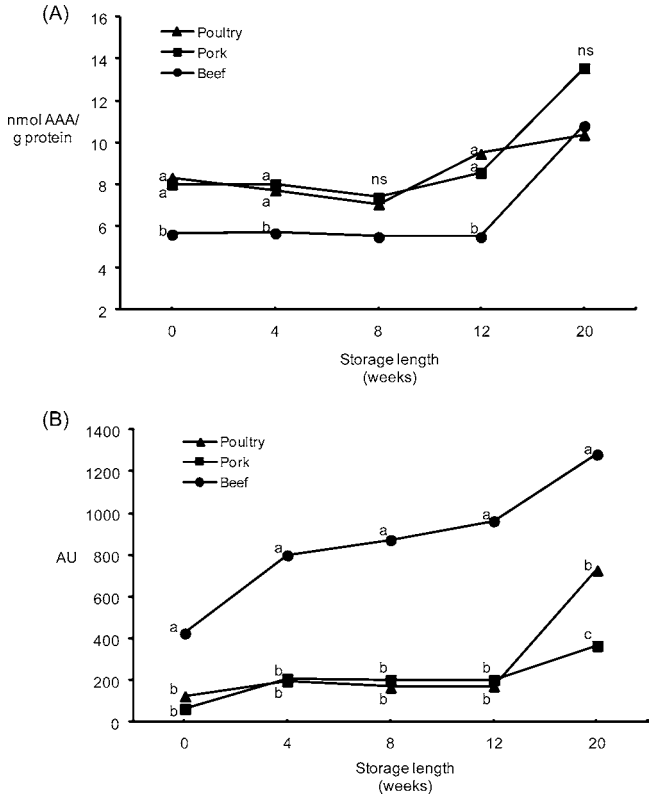

Figure 2. Evolution of the lysine oxidation end products, (A) AAA and (B) SB, in poultry, pork, and beef patties during frozen storage. Different letters at a sampling point denote significant differences between means. ns = not significant.

oxidation of the semialdehyde, as mentioned above.[4] Similar findings were reported in myofibrillar proteins oxidized in the presence of iron, copper, myoglobin, and H_2O_2[15] and cooked and chilled pork patties.[11] It is worth noting that the formation of AAA from AAS requires the presence of peroxides.[4] Thus, all of the factors having an influence on the H_2O_2 level would also affect the rate of AAA formation in meat. In this regard, catalase could play an important role because this enzyme removes H_2O_2 and has been reported to be stable for 8 weeks of storage at −20 °C.[27] In addition, the concentration of H_2O_2 may also be dependent upon the concentration of iron, because this metal is known to decompose the peroxide through the Fenton reaction to yield the hydroxyl radical. The intense production of radical species in beef patties from the iron-catalyzed decomposition of H_2O_2 would explain the intense formation of AAS (dependent upon the Fenton chemistry) and the moderate production of AAA (dependent upon the remaining H_2O_2) in beef samples. The concentration of both oxidation products (AAS and AAA) reflects that the oxidation of the carbonyl moiety explains a negligible part of the loss of AAS during the last stages of frozen storage.

The formation of SB would also contribute to explain the decrease of protein carbonyls. SB increased gradually during the frozen storage of beef patties (Figure 2B). In pork and poultry samples, however, SB did not show a significant increase during the first 12 weeks (Figure 2B). During the entire frozen storage, the fluorescence emitted by SB was significantly more intense in beef than in pork and poultry, suggesting a larger concentration of these structures in beef samples. Once again, the formation of SB was consistent with the loss of AAS,

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highlighting the fact that SB formation could be at least partially attributed to the reaction of AAS with other protein-bound AAS residues or other amino acid residues.[4] In a previous study, the formation of SB in myofibrillar proteins oxidized *in vitro* was found to be highly dependent upon the concentration of myoglobin.[15] The present study confirms that beef, with a larger concentration of heme iron than pork and poultry, is more susceptible to SB formation during frozen storage. While the oxidation of lysine residues into AAS involves the irreversible loss of an essential amino acid,[4] the formation of the end products, AAA and SB, have been shown to be implicated in the impaired functionality of oxidized meat proteins.[15] The precise oxidative changes described in the present study provide original insight into the protein oxidation pathways that occurred during frozen storage of meat and the urge for the search for antioxidant strategies to inhibit these deleterious reactions.

Lipid Oxidation. TBARS and hexanal were assessed as lipid oxidation markers. TBARS values of beef and pork patties were higher than that of poultry at day 0 (Figure 3A), suggesting that

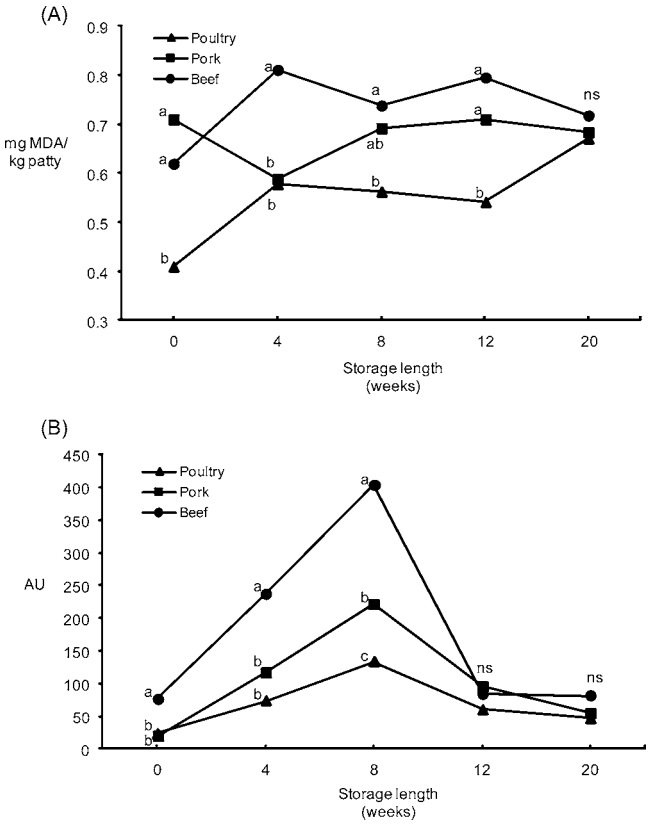

Figure 3. Evolution of lipid oxidation markers, (A) TBARS and (B) hexanal, in poultry, pork, and beef patties during frozen storage. Different letters at a sampling point denote significant differences between means. ns = not significant.

lipid oxidation was promoted more intensively during freezing in the former meats. Likewise, TBARS values were significantly higher in beef and pork patties after 12 weeks of frozen storage (Figure 3A). These results indicate that differences in the heme iron concentration could be responsible for the differences in lipid oxidation susceptibility among meats. Similar findings were found in frozen beef, pork, and chicken patties subsequently subjected to thawing and cooking.[24] However, no significant differences between animal species were found for TBARS values after 20 weeks of frozen storage (Figure 3A). Taking into consideration that PUFA are stored in adipose tissue and cell membranes, where the accessibility of pro-oxidants is reduced in frozen meats,[24] the enhanced TBARS formation in poultry patties at the end of the frozen storage could be ascribed to an extended cell damage promoted by recrystallization. This effect was also reported by Min et al.[24] when frozen patties were subjected to cooking, another technological treatment that could induce cell disruption.

Hexanal increased during the first 8 weeks of frozen storage, followed by a significant decrease at week 12 in all patties (Figure 3B). The increased susceptibility to oxidation of beef meat was confirmed by the hexanal results. Consistently, Min et al.[24] identified beef meat to be more prone to lipid oxidation when frozen beef, pork, and chicken patties were thawed and subsequently subjected to cooking.

Results reflected that hexanal was formed at early oxidation stages and underwent further reactions as the oxidation proceeded. The decrease of hexanal was consistent with the loss of AAS and the formation of SB (Figures 1A and 2B, respectively). This finding suggested strong interactions between proteins and lipid oxidation products to form SB via condensation.[28,11] In fact, lipid oxidation products (hydroperoxides, aldehydes, and ketones) can also complex with protein through hydrophobic association and hydrogen bonds and form various types of covalent bonds to also generate SB and promote polymerization.[28] Especially, free amino groups of lysine residues can cross-link with malondialdehyde and 4-hydroxy-2-nonenal.[28] Hexanal is also known to form adducts with lysine residues in meat proteins.[29] In the present study, the formation of such adducts via SB arrangement would explain the apparent loss of hexanal upon week 8 and the formation of SB after this point. In this respect, it is generally known that hexanal formation depends upon the degree of polyunsaturation in fatty acids.[30] The larger concentration of PUFA in poultry did not have a direct reflection on the hexanal counts. However, the approximately 3-fold increase of SB in poultry patties between weeks 12 and 20 could be related to the formation and consequently addition of hexanal into the proteins (Table 1). This extent, however, requires further confirmation.

Relationship between Muscle Composition and Oxidation Markers. To obtain further insight into the relationship between the muscle composition and the susceptibility of poultry, pork, and beef to undergo lipid and protein oxidation, Pearson's correlation coefficients were calculated and a PCA was performed. As expected, significant and positive correlations were found between the amount of heme iron in patties and various lipid and protein oxidation markers, namely, hexanal ($r = 0.44$; $p < 0.05$), TBARS ($r = 0.53$; $p < 0.01$), AAS ($r = 0.44$; $p < 0.05$), GGS ($r = 0.40$; $p < 0.05$), and SB ($r = 0.76$; $p < 0.001$). Iron likely promoted the oxidation of lipids and proteins under the conditions of the present work. The role of heme iron on the formation of specific protein carbonyls[31] and protein cross-links[7] in muscle foods is well-documented. In the present study, the impact of the heme iron content on the formation of SB was particularly remarkable, which is in agreement with a previous study, in which the formation of SB was induced *in vitro* by activated myoglobin.[15] This timely influence was not found for any of the other muscle components under study, including fatty acids. Significant correlations were also found between lipid and

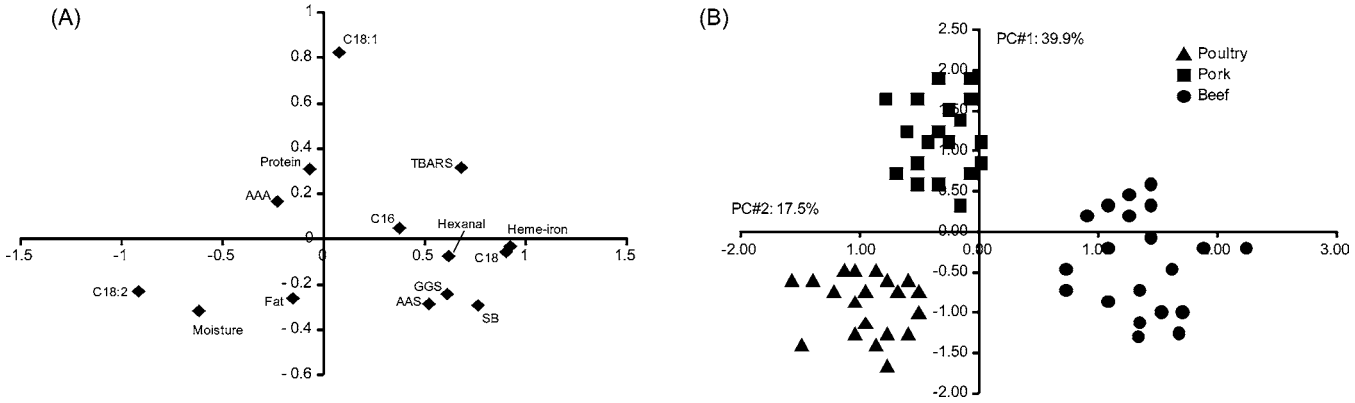

Figure 4. (A) Projection of the variables and (B) samples from the three animal species onto the space defined by the principal components (PC1/PC2).

protein oxidation markers. The particularly high and significant correlations between hexanal and AAS ($r = 0.67$; $p < 0.01$) and TBARS and SB ($r = 0.52$; $p < 0.05$) substantiate some of the hypotheses previously reported on the potential connection between both oxidation phenomena during frozen storage of meat. As aforementioned, protein carbonyls are believed to react with other protein carbonyls, lipid carbonyls, and amino groups from amino acid residues to form SB.[4]

The relative position of the variables in the PCA (Figure 4A) supports the results obtained from Pearson's correlation coefficients. These results confirmed the connection between muscle composition and susceptibility to undergo oxidative reactions during frozen storage. The projection of the samples onto the space of the two first principal components (Figure 4B) showed that the computed model clearly discriminated between patties elaborated from differences species. It is then inferred that the differences between animal species for their chemical composition has a direct impact on the formation of specific lipid and protein oxidation products during frozen storage. Beef patties were situated on the positive axis of PC1, in the plane area, corresponding to high values of heme iron, hexanal, TBARS, AAS, GGS, and SB. According to the PCA, patties from poultry and pork are mainly defined by the most abundant PUFA and MUFA, respectively, which had, in general, a negligible impact on the oxidation events.

In conclusion, the present study illustrates the severe chemical modifications undergone by proteins during frozen storage of ground meat. The formation of particular protein carbonyls leads to further reactions in which both oxidizing lipids and proteins may interact. In general, beef showed a lower oxidative stability during frozen storage, as a likely consequence of the pro-oxidant action of heme iron. Other factors, such as the fatty acid composition, may have played a minor role. The detection, in the present study, of novel protein oxidation products enables a better comprehension of the protein oxidation mechanisms and pathways that occurred during frozen storage of meat. Further studies are required to fully comprehend the effect of other meat endogenous factors on protein oxidation, the consequences of these chemical changes on particular quality traits, and the search for antioxidant strategies to control these undesirable oxidative reactions.

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Notes

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