Meat and Muscle Biology™

# Exploring the Effects of Incorporating Egg Powder Containing Phospholipase α2 Antibody on Ground Striploin Shelf-Life

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**Abstract:** Lipid oxidation in beef may be enhanced by the hydrolysis of phospholipids by phospholipase α2 (PLA2) during postmortem storage. Anti-phospholipase α2 (aPLA2) is an antibody that can inhibit PLA2 activity. Past research has shown that aPLA2 can be mass-produced in the form of egg powder (EP) from hens immunized against PLA2. Therefore, the present study aimed to determine the effects of incorporating different levels of EP containing aPLA2 into ground striploin (GS) to assess its ability to extend beef shelf-life. Ten striploins were collected from 10 USDA Choice beef carcasses. Each striploin was ground and equally divided into 4 batches, and each batch was mixed with 0%, 0.4%, 0.8%, or 1.6% dried EP containing aPLA2. Each treatment batch was further divided into 3 smaller batches and subjected to retail display (0, 4, or 7 d). Color descriptors, pH, proximate analysis, lipid oxidation, antioxidant capacity, and phospholipid and fatty acid (FA) profiles were measured. Percent visual discoloration and instrumental color measurements of GS were unaffected by aPLA2 EP treatments ($P > 0.05$). The 1.6% treatment GS had a higher relative percentage of phosphatidylcholine compared with those from the 0% treatment ($P < 0.05$), but the lack of lysophosphatidylcholine generation in the GS from any treatment reflects a lack of detectable level of PLA2 activity. Moreover, the addition of EP in GS increased the relative percentage of FA 11-18:1, 18:2, 20:1, and 22:6 ($P < 0.05$). As a result, there was more lipid oxidation for GS from the 1.6% treatment compared with those from the 0% treatment ($P < 0.05$), but GS from the 0.8% treatment showed higher antioxidant activity than those from the 0% treatment ($P < 0.05$). In this study, the addition of EP containing aPLA2 did not demonstrate any effect to extend shelf-life when incorporated into GS.

**Key words:** phospholipase α2 antibody, lipid oxidation, phospholipid composition, fatty acid profile, discoloration
*Meat and Muscle Biology* 7(1): 16242, 1–11 (2023) **doi:10.22175/mmb.16242**
Submitted 4 April 2023 Accepted 28 June 2023

## Introduction

Postmortem aging of meat is extensively practiced in the meat industry to improve eating quality attributes such as tenderness and flavor (Kim et al., 2018). However, prolonged aging adversely impacts the oxidative stability of lipids, resulting in substantial surface discoloration of meat and potentially rancidity under further aerobic storage (English et al., 2016). Surface discoloration of fresh meat during retail display is a major quality and food loss challenge in the meat industry. Consumers rely on meat color to determine meat freshness and reject meat cuts that are brown in color (Mancini and Hunt, 2005). Conservative estimates showed that 15% to 20% of total fresh meat sold in United States retail stores is discounted in price or discarded because of surface discoloration (Smith et al., 2000). In 2019 alone, a total of US$14.2 billion was lost globally because of discoloration (Ramanathan et al., 2021). A key driver of discoloration in meat and meat products is lipid oxidation (Domínguez et al., 2019). Conjugated dienes from unsaturated lipids interact with free radicals to produce primary and

secondary lipid oxidation products (Frankel, 2005), which further participate in subsequent reactions, resulting in various meat quality issues such as off-flavors, nutritional value reduction, and/or discoloration (Frankel, 2005; Karre et al., 2013; Domínguez et al., 2019). Therefore, it has always been a priority for the meat industry to develop technology to hinder lipid oxidation, meat discoloration, and, ultimately, meat waste for a more sustainable food system.

Phospholipids are known to contain long and highly unsaturated fatty acids (FA) at their sn-2 position (El-Bacha and Torres, 2016), and phospholipase α2 (PLA2) is a group of ubiquitous enzymes found in animals (Murakami et al., 2020) and plants (Ryu, 2004) that can hydrolyze phospholipids at the sn-2 position to form free fatty acids (FFA) and lysophospholipids. Poulsen et al. (2007) first showed PLA2 may be responsible for degrading the sarcolemma membrane and thus increased drip loss in postmortem porcine muscles. Chun et al. (2023) later showed that PLA2s are extremely efficient in cleaving off the FA chain from the sn-2 position of phospholipids, particularly the phosphatidylcholine (PC), in a beef liposome model system. Finally, Chao et al. (2020) demonstrated that phospholipids will continue to degrade through the aging process and hypothesized that PLA2 could be responsible for this phospholipid degradation in aged pork. Perhaps PLA2 acts as an FFA liberator in meat, which may instigate further implications on lipid oxidation in aged beef.

The inhibition of PLA2 has been studied for decades in the biomedical field for its relation to inflammatory diseases in humans (Nikolaou et al., 2019). In more recent years, the livestock industry has shown an increased interest in this field by investigating the effect of PLA2 inhibition by PLA2 antibody (aPLA2) on the growth performance of steers (Mercadante et al., 2015), broilers (Cook, 2004), and fish (Barry and Yang, 2008). These studies have demonstrated improved growth performance for the respective livestock species by halting the inflammation reactions stimulated by PLA2 (Cook, 2011). Furthermore, passive immunity of PLA2 can easily be achieved by immunization of avian maternal bodies, which enables the commercial production of immune-boosted eggs containing a high concentration of aPLA2 (Cook and Trott, 2010). Therefore, the development of immune-boosted egg powder (EP) presents the possibility of aPLA2 inclusion in a meat system, which could potentially diminish the degradation of phospholipids by PLA2, thus reducing lipid oxidation. The present study aimed to investigate the effects of adding EP containing aPLA2 to ground striploin (GS) to explore its possibility to extend fresh beef shelf-life.

# Materials and Methods

## *Egg powder preparation*

The EP containing aPLA2 used in this study was produced as previously described by Chun et al. (2023). Briefly, single-comb White Leghorn laying hens were injected with 0.6 mg group IB secretory phospholipase A2 (10,000 U/g; Bioseutica, Lugano, Switzerland) in 3 separate injections. Eggs were collected after the final injection for 6 wk, and the eggs were dried into powders using a spray dryer (Mini-Glatt 3 Fluid Bed Dryer; Glatt, Binzen, Germany). The titer of aPLA2 in the EP was verified by enzyme-linked immunoassay (ELISA) as described by Chun et al. (2023). The extraction from the EP yielded 435 mg antibody/kg of EP, whereas the extracted protein from the negative control yielded no reactivity to the PLA2 standard.

## *Sample collection, fabrication, and treatment application*

Boneless striploins were collected 2 d postmortem from 10 USDA low Choice beef carcasses from a processing facility in Kansas. The striploins were vacuum packaged, transported to the Kansas State University Meat Laboratory, and kept under refrigeration (2°C ± 2°C) overnight. The following day, the posterior portion of each striploin containing *gluteus medius* was removed. The remaining striploin containing only *longissimus lumborum* was trimmed to remove exterior fat and connective tissue. Each striploin was ground through a 0.32-cm grinder plate in a meat grinder (KG-12-FS, Pro-Cut, Houston, TX). The grinder was cleaned out of any residual beef between each striploin. The GS was mixed and separated into four 500-g batches for each striploin.

Each batch of GS from one striploin was assigned to 1 of 4 treatments: (1) GS containing 0% aPLA2 EP; (2) GS containing 0.4% aPLA2 EP; (3) GS containing 0.8% aPLA2 EP; and (4) GS containing 1.6% aPLA2 EP (w/w), resulting in 10 replications for each treatment ($n = 40$). The concentrations of EP from each treatment were determined based on the binding capacity of PLA2 for aPLA2 (~1 μg of aPLA2 needed for 1 mg of PLA2) as determined by ELISA in Chun et al. (2023). All EP treatments except for the 0 % EP contained enough aPLA2 to exceed the binding capacity of PLA2.

The EP was hand mixed into each batch according to the assigned treatment for 5 to 10 min until no visual

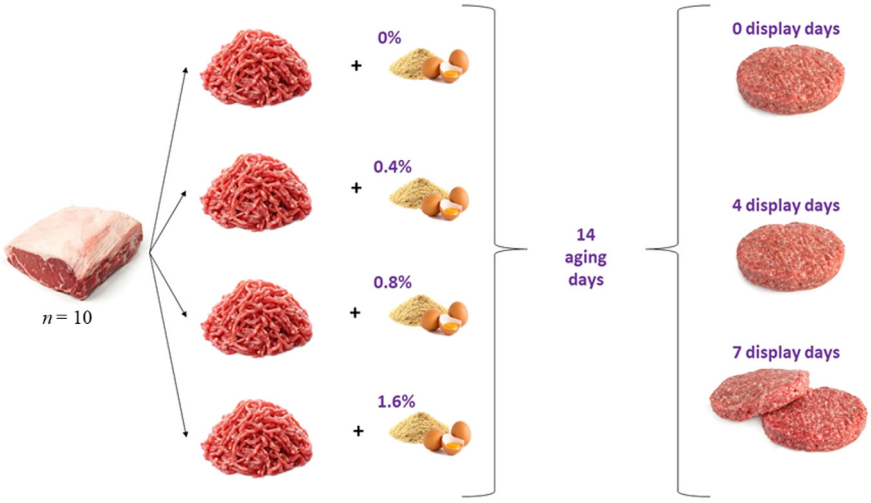


**Figure 1.** Schematic display of the sample preparation process for each striploin.

clumps of EP could be observed. The 0% EP treatment only contained GS with no addition of EP. Each completed batch was vacuumed packaged (75001840 3-Mil standard nylon/polyethylene vacuum pouches, 0.2 cc/100 $in^2$/24 h at 23°C and 0% relative humidity [RH]; Bunzl Processor Division, Riverside, MO), shielded from light and stored under refrigeration at 2°C ± 2°C for 14 d to allow time for native PLA2 activity to react with phospholipids in the samples. After the storage period, each batch of GS was hand-formed into four 114 g patties using a mold. The 4 patties from the same batch were subjected to 1 of 3 display times (0, 4, or 7 d). A schematic of the sample preparation process is illustrated in Figure 1. The patties designated for retail display were overwrapped with polyvinyl chloride (23,250 cc/$m^2$/24 h at 23°C and 0% RH; Sysco, Houston, TX) in Styrofoam trays (17S; Genpak, Charlotte, NC) lined with a tray absorbent pad (Dri-Loc AC25; Novipax, Oak Brook, IL) and displayed under continuous fluorescent lighting (32W Warm White 3,000 K; Philips Lighting Company, Somerset, NJ) averaging 2,143 ± 113 lx emission case-wide in a 2°C ± 2°C coffin-style retail case (Model DMF8; Tyler Refrigeration Corporation, Niles, MI).

## Color descriptors in simulated retail display

All color measurements followed the methods described in the AMSA Color Guidelines (King et al., 2023). Percent visual discoloration was determined using a trained panel ($N = 7$) on patties designated for 7 d of retail display. Panelists were first screened by Farnsworth–Munsell 100 Hue Color Vision Test (Munsell Color X-Rite, Grand Rapids, MI) and trained by studying a visual discoloration guide that provided 10 ground beef images ranging from 0% to 100% discoloration representing surface discoloration in increments of 10%. A percentage of 0% was used to indicate no visual discoloration, and 100% was used to indicate complete discoloration. After training, panelists visually evaluated the samples and assessed the percentage of discoloration on each day of the 7-d retail display period. Instrumental color measurements $L^*$ (lightness), $a^*$ (redness), and $b^*$ (yellowness) were measured with a HunterLab MiniScan EZ spectrophotometer (Model 2500L, Illuminant D65, 2.54 cm aperture, 10° observer; Hunter Associates Laboratory Inc., Reston, VA) on the same set of patties by averaging 6 readings from different areas of the 2 patties. Samples were randomly rearranged daily to minimize any possible lighting location effects. Upon termination of each sample's designated display period, patties were removed from the overwrapped packaging, vacuum packaged, and stored at −80°C until further processing. Finally, all GS samples were frozen by submersion in liquid nitrogen and pulverized using a commercial blender (Model 51BL32, Waring Commercial, Torring, CT), packaged in Whirl-Pak bags (Whirl-Pak, Madison, WI), and stored at −80°C for laboratory analysis.

## Proximate analysis

Proximate analyses were conducted on all day 0 samples from the 4 treatments using pulverized samples. Moisture analysis was performed by weight differentiation following a modified 950.46 AOAC oven-drying method (AOAC International, 2000a). Protein content was measured with a LECO protein analyzer (CN828; LECO Corporation, St. Joseph, MI) according to a

modified AOAC Method 992.15 (AOAC International, 2000b). Ethylenediaminetetraacetic acid (9.56% nitrogen) was used to calibrate the instrument before analysis. The percentage of nitrogen obtained was multiplied by a factor of 6.25 to calculate the percentage of crude protein. Lipids were extracted as described by Folch et al. (1957) with modifications. Briefly, 9.6 mL of deionized water, chloroform, and methanol at 2:5:5 ratio were added to 0.5 g of sample. The tubes were shaken, and 2 mL of 0.74% potassium chloride solution in ultrapure water was added. The mixture was centrifuged, and an aliquot of the bottom layer was collected into a 16 × 100 mm prelabeled, dried, and weighed glass tube. Chloroform was evaporated under nitrogen gas (Reacti-Vap Evaporator #TS-18826; Thermo Scientific, Waltham, MA), and the glass tubes containing the evaporated samples were placed in a centrifuge vacuum dryer (CentriVap DNA Vacuum Concentrator, Labconco, Kansas City, MO) for 1 h and weighed again afterward. Lipid percentage was calculated, and the lipid was resuspended in chloroform to achieve a 10 mg/mL concentration. The lipid stocks were stored in capped glass tubes sealed with parafilm in a −80°C freezer until FA analysis and phospholipid class profiling.

### Lipid oxidation analysis

For lipid oxidation evaluation, analyses were conducted on all day 0, 4, and 7 samples from the 4 treatments using pulverized samples. Procedures followed the 2-thiobarbituric acid reactive substances methodology as described by Dahmer et al. (2022). Briefly, a malondialdehyde (MDA) bis (diethyl acetal) standard curve was prepared (0 to 25 µM). Approximately 0.1 g of pulverized samples were homogenized with 700 µL of thiobarbituric acid/trichloroacetic acid solution (20 mM:15% in ultrapure water) and 50 µL of 0.3% of butylated hydroxytoluene in ethanol. The content was centrifuged, filtered, incubated at 70°C in a water bath for 30 min, and cooled in cold water. Aliquots of 200 µL of samples and standards were plated into a 96-well plate and read in a spectrophotometer at 532 nm (Eon; BioTek, Winooski, VT). The results for lipid oxidation from each sample were calculated and expressed as mg MDA/kg of muscle tissue.

### Phospholipid extraction and thin-layer chromatography

Phospholipid class profiling was conducted on all day 0 samples. Procedures for solid-phase extraction (SPE) to separate lipid classes followed the methods described by Legako et al. (2015) with modifications. Briefly, SPE columns (SPE NH2 500 mg/6 mL, JG Finneran, Vineland, NJ) were preconditioned with 2 rinses of hexane and chloroform. Following the preconditioning step, 5 mL of lipid stock prepared proximate analysis was loaded into the columns, and neutral lipids and FFA were eluted by 5 washes of 5 mL chloroform and 2% acetic acid in diethyl ether, respectively. Phospholipids were eluted with 5 washes of 5 mL methanol and evaporated using a vacuum evaporator (RapidVap, Labconco). Once evaporated, the tubes were weighed, and the dried samples were resuspended in chloroform to achieve a 2 mg/mL concentration of phospholipids for thin-layer chromatography (TLC) analysis.

The TLC was performed as described by Chao et al. (2017). Briefly, TLC plates (PE SIL G, 250 µm layer; Whatman, Little Marlow, UK) were precleaned with chloroform: methanol (1:1) and dried before use. The TLC plate was activated by spraying 2.3% boric acid in ethanol. Twenty-microgram soy reference (containing 38%, 30%, 18%, 7%, and 7% of PC, phosphatidylethanolamine [PE], phosphatidylinositol, phosphatidic acids, and lysophosphatidylcholine [LPC], respectively; soy phospholipid mixture; Avanti Polar Lipids Inc., Alabaster, AL) were used as a standard, and 60 µg of extracted phospholipid from each sample was spotted on a TLC plate. The TLC running solvent (chloroform:ethanol:water:triethylamine, 30:32:7:35 by volume) was added to the TLC tank, and the spotted plates were placed in the solvent. Once the run was complete, the plates were sprayed with a 10% cupric sulfate in 10% phosphoric acid solution, dried, and heated in the oven at 180°C until spots appeared. The area of the spots was imaged with an iBright Imaging System (FL1500; Thermo Fisher Scientific, Waltham, MA). The phospholipid classes were identified against the soy reference, and a relative percentage of each phospholipid class was calculated.

### pH analysis

The pH values were measured for all day 0, 4, and 7 samples from the 4 treatments using pulverized samples. The procedure followed the method described by Hammond et al. (2022). Briefly, 5 g of raw pulverized muscle samples were homogenized with 50 mL of ultrapure water for 20 s at 10,000 rpm using a benchtop homogenizer (Model 850; Fisher Scientific, Pittsburgh, PA). An InLab Science Pro-ISM probe (Mettler Toledo, Columbus, OH) connected to a SevenCompact pH meter (Mettler Toledo) was calibrated with pH 4 and 7 standard solutions, and the pH of the samples was

measured following the calibration. All samples were measured in duplicate.

### Fatty acid analysis

The FA analysis was conducted on day 0 samples from the 4 treatments as described by Dahmer et al. (2022). Briefly, an aliquot of the lipid stock prepared during proximate analysis was diluted with chloroform to reach a 2 mg/mL lipid concentration. For FA methyl ester preparation, 50 nmol of pentadecanoic acid (15:0) was added to the adjusted lipid stock of each sample as an internal standard, and the solvent was evaporated under nitrogen. Subsequently, 1 mL of 3 M methanolic hydrochloric acid was added to each tube and heated at 78°C for 30 min. Two milliliters of ultrapure water and 2 mL of hexane were added, vortexed, and centrifuged. The top hexane layer was removed into a glass tube and evaporated to dryness. The content was redissolved in hexane and transferred to a gas chromatography (GC) vial with an insert.

An Agilent 7683 autosampler (6890N GC system; Agilent Technologies, Santa Clara, CA) was used to inject 1 µL of the prepared sample into an Agilent 6890N GC coupled to a flame ionization detector (FID; Agilent Technologies). The GC was fitted with a DB-23 capillary column (60 m in length, 250 µm in internal diameter, and 0.25 µm of film thickness). Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The GC oven temperature ramp was operated as follows: (1) an initial temperature of 150°C with a hold of 1 min; (2) an increase of 25°C/min until a 175°C temperature was achieved; and (3) an increase in temperature rate at 4°C/min to reach 230°C and holding for 9 min. The total runtime was 24.75 min. The FID was operated at 260°C. The hydrogen flow to the detector was 30 mL/min, and the airflow was 400 mL/min. The sampling rate of the FID was 20 Hz. Individual FA peaks were processed through Agilent ChemStation software (Agilent Technologies) and expressed as relative percentages of total FA. A commercial standard (37-component FAME mix; Supelco, Bellefonte, PA) was used to identify the FA.

### Antioxidant capacity through oxygen radical absorbance capacity

The methodology used for oxygen radical absorbance capacity (ORAC) followed the methods described by Dahmer et al. (2022) on all day 0, 4, and 7 samples from the 4 treatments using pulverized samples. For lipophilic ORAC, samples were homogenized in hexane using a bead homogenizer (BeadBlaster 24; Benchmark, Sayreville, NJ) and centrifuged. The hexane layer was collected into glass tubes, and the hexane was evaporated to dryness under nitrogen (Reacti-Vap III #TS-18826; Thermo Scientific). The dried tubes were resuspended in 750 µL of 7% randomly methylated-cyclodextrin (RMCD) in 50:50 acetone:water. For hydrophilic ORAC, 80:20 water:ethanol solution was added to the bead tubes containing the meat sample previously used for the lipophilic ORAC extraction. The mixture was homogenized and centrifuged, and 750 µL of supernatant was transferred into another microcentrifuge tube. This mixture was diluted by a dilution factor of 20 with an 80% water/20% ethanol solution.

A Trolox standard curve (6.25 to 100 µM) was prepared with a 7% RMCD solution in 50:50 acetone:water or 75 mM phosphate buffer (pH of 7.4) for lipophilic and hydrophilic ORAC, respectively. The buffers were used as blanks for their corresponding treatment. A fluorescein solution (1:1,000 in 75 mM phosphate buffer) was added to all wells of a nonadhering black 96-well microplate (655906; Greiner Bio-One, Kremsmünster, Austria). Twenty-five microliters of blanks, standard dilutions, and samples were pipetted into the microplate and incubated in a Synergy HTX Multimode Reader for 30 min (BioTek Instruments, Winooski, VT). Following the incubation period, 2,2'-azobis(2-amidinopropane) dihydrochloride was added to each experimental well. Finally, the fluorescence of each well was measured in the microplate reader from the bottom of the plate at an excitation wavelength of 485 nm and an emission wavelength of 528 nm every 60 s for 120 min. ORAC results were obtained by calculating the area under the curve (AUC) and net AUC. Once the net AUC was obtained, the Trolox standard was plotted as a function of Trolox concentration to calculate the sample concentration in micromoles per liter. The results were reported as micromoles of Trolox equivalent per gram of meat.

### Statistical analysis

Proximate analysis, phospholipid profile, pH analysis, and FA profile were analyzed as a randomized complete block design, with each striploin serving as the block. Lipid oxidation and ORAC analysis were analyzed as a split-plot design with EP treatment as the whole plot factor, and the retail display day as the subplot with a treatment × display day interaction (Figure 1). Carcasses were considered as the experimental units. Meat color data were analyzed as a split-plot repeated-measures design with EP treatments as the whole plot and retail display day as the repeated

measures. The Toeplitz covariance structure was selected based on the best fit model. Multiple comparisons were obtained through Tukey method, and mean separation was conducted using least-squares mean procedures. All data points were analyzed through SAS software using the GLIMMIX procedure (v. 9.4, SAS Institute, Cary, NC). Degrees of freedom were obtained through the Kenward-Roger method. Finally, a 5% level of significance was used to detect differences among means.

## Results and Discussion

### *Color descriptors in simulated retail display*

There was no treatment × display day interaction or a main effect for treatment for discoloration ($P > 0.05$). However, there was a main effect for display day for discoloration ($P < 0.01$; Figure 2A). As display day increased, more discoloration was observed for the GS patties regardless of the treatment ($P < 0.01$). There was no treatment × display day interaction for the instrumental color measurements for $L^*$. Moreover, $L^*$ values were unaffected by treatment or display day ($P > 0.05$; Figure 2B). The values for $a^*$ showed an interaction between treatment and display day ($P < 0.05$; Figure 2C). Generally, $a^*$ values decreased as the display day progressed ($P < 0.05$). On day 3, 5, and 7 of the retail display, samples from 0% EP tended to have higher $a^*$ values than those from 1.6% EP ($P < 0.10$). There was no treatment × display day interaction for $b^*$ values, but they showed a main effect for display day. As days progressed, a lower $b^*$ was obtained ($P < 0.05$; Figure 2D).

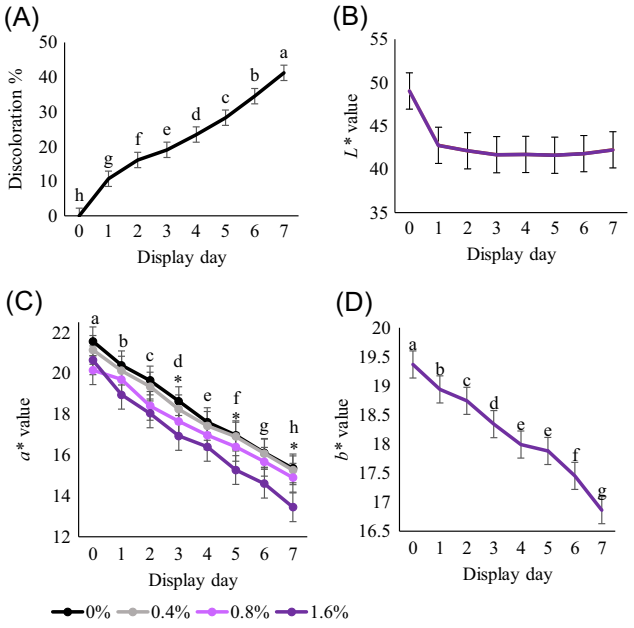


**Figure 2.** (A) Main effect of display day for discoloration %. (B) No main effect of display day for $L^*$. (C) Treatment × display day interaction for $a^*$ ($P < 0.10$); the superscripts represent the display effect only. (D) Main effect of display days for $b^*$. The treatments consisted of ground striploin patties formulated with 0%, 0.4%, 0.8%, or 1.6% egg powder containing anti-phospholipase α2. [a–h]Values with different superscripts indicate a difference at $P < 0.05$. *Asterisk indicates a tendency for differences between the 0% and 1.6% treatments ($P < 0.10$).

Meat color depends on myoglobin's oxidation state (Tewari et al., 2001), which the formation of metmyoglobin increases over time during retail display because of myoglobin oxidation (Suman and Joseph, 2013). This study is the first one to the authors' knowledge to investigate the effect of adding aPLA2 on color stability of beef. The unexpected null differences in perceived discoloration for treatment demonstrated that the incorporation of aPLA2 through EP is likely not effective in preserving GS color during retail display. In fact, the tendency found for a decrease over time in $a^*$ for the 1.6% treatment demonstrated a potential negative impact of incorporating aPLA2-containing EP into GS.

### *Lipid oxidation*

There was no treatment × display day interaction for lipid oxidation ($P > 0.05$). The GS with 1.6% treatment had higher lipid oxidation values compared with those from the other treatments ($P < 0.05$; Table 1). As

**Table 1.** Main effect of treatment for proximate analysis, phospholipid profile, lipid oxidation, and antioxidant capacity of ground striploin patties formulated with 0%, 0.4%, 0.8%, and 1.6% of egg powder containing anti-phospholipase α2

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| % Egg powder | 0% | 0.4% | 0.8% | 1.6% | SEM | *P* value |
| **Moisture (%)** | 71.19[a] | 70.93[a] | 70.82[a] | 70.17[b] | 0.19 | <0.01 |
| **Protein (%)** | 22.44 | 22.35 | 22.57 | 22.43 | 0.43 | 0.54 |
| **Fat (%)** | 6.39 | 6.47 | 6.70 | 7.04 | 0.15 | 0.45 |
| **Phospholipid profile** | | | | | | |
| **SM (relative %)** | 15.01 | 11.49 | 11.11 | 10.38 | 3.01 | 0.52 |
| **PE (relative %)** | 29.35[a] | 30.16[a] | 24.86[ab] | 18.73[b] | 2.83 | <0.01 |
| **PC (relative %)** | 54.98[b] | 58.35[b] | 63.60[ab] | 70.59[a] | 3.62 | <0.05 |
| **Lipid oxidation, mg MDA/kg of meat** | 1.10[b] | 1.11[b] | 1.11[b] | 1.41[a] | 0.10 | <0.01 |
| **pH** | 5.51[d] | 5.54[c] | 5.57[b] | 5.60[a] | 0.01 | <0.01 |
| **Hydrophilic ORAC, TE/g of meat** | 17.06[b] | 17.47[ab] | 17.97[a] | 17.06[b] | 0.34 | <0.01 |
| **Lipophilic ORAC, TE/g of meat** | 0.69 | 0.73 | 0.80 | 0.75 | 0.04 | 0.07 |

MDA = malondialdehyde, ORAC = oxygen radical absorbance capacity, PC = phosphatidylcholine, PE = phosphatidylethanolamine; SM = sphingomyelin; TE = Trolox equivalents.

[a–d]Values with different superscripts indicate a difference within each row.

**Table 2.** Main effect of display day for lipid oxidation and pH of ground striploin patties formulated with 0%, 0.4%, 0.8%, and 1.6% of egg powder containing anti-phospholipase α2

| | Display day | | | | |
|---|---|---|---|---|---|
| | 0 | 4 | 7 | SEM | *P* value |
| **Lipid oxidation, mg MDA/kg of meat** | 0.44[c] | 1.33[b] | 1.77[a] | 0.10 | <0.01 |
| **pH** | 5.51[b] | 5.54[b] | 5.57[a] | 0.01 | <0.01 |

MDA = malondialdehyde.

[a–c]Values with different superscripts indicate a difference within each row.

patties continued to stay in retail display, lipid oxidation values increased over time ($P < 0.05$; Table 2). Both myoglobin and lipid oxidation happen concurrently in beef and have been considered somewhat intertwined (Ramanathan et al., 2021), and Wang et al. (2021) showed a moderate positive correlation between the rate of lipid oxidation and visual discoloration during retail display, as found in this study.

The PLA2 is a known FFA liberator from phospholipids, and most of the FFA released from the sn-2 position is highly unsaturated (Burke and Dennis, 2009; Murakami et al., 2020). Soares et al. (2003) showed a strong relationship between discoloration and PLA2 content in heat-stressed chickens, and the same group of authors hypothesized that PLA2 may be related to meat color destabilization in pale, soft, exudative (PSE) broilers (Soares et al., 2009). Furthermore, Cheah et al. (1995) and Chen et al. (2010) also found that the PSE-prone pigs had higher levels of PLA2 activity; as the PLA2 level increased, $L^*$ values and lipid oxidation also increased in pale, soft, and exudative pork in those studies. It is possible that as heightened PLA2 activity released more highly unsaturated FFA, these FFA are very susceptible to be attacked by free radicals (Suman et al., 2014), which demonstrated PLA2 could negatively impact both lipid oxidation and discoloration.

Nevertheless, PLA2 has been reported as an antioxidant in cod and trout liposome model studies (Tatiyaborworntham et al., 2021; Tatiyaborworntham and Richards, 2018) and pork sausage (Whalin et al., 2022). Furthermore, Govindarajan et al. (1977) added PLA2 into ground beef and observed an inhibition of myoglobin oxidation that was apparent to the trained panel. In this study, we hypothesized that lipid oxidation of GS may be reduced by adding aPLA2, with the thought that aPLA2 may prevent the generation of FFA in postmortem meat. Although Chun et al. (2023) observed a reduction in lipid oxidation for treatments containing aPLA2 compared with the PLA2-only treatment in a beef liposome study, our results did not see such effect in an actual GS model.

## *Separation of phospholipids classes by thin-layer chromatography*

Sphingomyelin (SM), PC, and PE were the only 3 phospholipid classes identified in the samples by TLC, and the relative percent of the phospholipid classes are shown in Table 1. There were no differences for SM across the treatments ($P > 0.05$). The GS with 1.6% treatment showed a higher relative percent of PC and a lower relative percent of PE when compared with GS with 0% and 0.4% EP inclusion ($P < 0.05$). However, GS with the 0%, 0.4%, and 0.8% treatments did not differ in PC or PE relative percent ($P > 0.05$).

The PLA2 is known to hydrolyze primarily PCs and PEs to generate FFA and LPC or lysophosphatidylethanolamines (Bolander, 2004; Burke and Dennis, 2009; Murakami et al., 2011). Therefore, FFA and LPC levels are often used as markers for PLA2 activity. However, there was no detectable production of LPCs in this study through TLC. Arguably, this lack of LPC production and the higher content for PC in the treatments with aPLA2 EP could signify preservation of the phospholipid because of aPLA2 activity. However, Chun et al. (2023) observed no effect on PC preservation and LPC production in treatments with the addition of aPLA2 in a beef liposome study. Therefore, the total lack of LPC production in this study would suggest that there was not enough PLA2 activity in the samples to be detected by TLC in the first place. Finally, a possible explanation for the noted increase in PC for the 1.6% treatment is the inherent high PC concentration in the EP (Palacios and Wang, 2005; Blesso, 2015).

## *Moisture, protein, fat content, and pH*

Data for moisture, protein, and fat content are shown in Table 1. Moisture content was lower for the GS with the 1.6% treatment when compared with all other treatments ($P < 0.01$). There was no difference in protein and fat content among the treatments ($P > 0.05$). There was no treatment × display day interaction for pH ($P > 0.05$), but there were main effects for both treatment (Table 1) and display day (Table 2) for pH ($P < 0.01$). Day 7 samples had a higher pH value than those from samples that were only displayed for 0 and 4 d ($P < 0.01$). Additionally, the pH value increased as the aPLA2 EP percentage increased ($P < 0.01$).

The moisture result could be attributed to the low moisture level of spray-dried EP, whereas whole dried

eggs contain ~4% moisture (USDA, 2019a). The addition of 1.6% dried EP was enough to decrease the moisture level of the patties by ~1%. Nevertheless, the protein and fat contents for all treatments matched the ones expected for beef Choice loins, according to the USDA (2019b), at ~22% and ~6%, respectively.

Although there was a slight increase in pH on day 7, the values of pH obtained in this study are in the expected range for ground beef and steaks, as shown in Ramanathan et al. (2019, 2021). The difference in pH among the treatments was likely attributed to the EP's pH. In this study, the pH of the EP was 8.0. The pH of dried EP varies depending on the egg components included in the powder and manufacturing processes, usually between pH 7 and 9.5 (Vargas-del-Río et al., 2022).

The optimum pH for PLA2 activity ranges around 8.0 (Oliveira et al., 2002; Ponce-Soto et al., 2002). Nevertheless, Tatiyaborwortham et al. (2021) demonstrated strong PLA2 enzymatic activity at a pH of 6.5 in a liposome model system using washed cod muscle, and Alasnier and Gandemer (2000) showed that 50% of the maximal phospholipase A activity was retained at pH 5.5 to 6.0 in rabbit muscle. Moreover, it is not well established regarding the optimum activity for aPLA2, but aPLA2 activity delivered through EP has been reported at pH values as low as 2.0 (Cook and Trott, 2010). Enzymatic activity is known to be driven by environmental factors such as pH because their molecular structure and interaction with other molecules depend on hydrogen bonds (Campbell and Farrell, 2015). Therefore, it is possible the lack of PLA2 and aPLA2 activity in this study is partly due to the low pH of GS.

## Fatty acid profile

There were observed differences in FA profiles of GS among the treatments as listed in Table 3. The GS with 1.6% EP had higher amounts of vaccenic acid (11–18:1 trans), linoleic acid (18:2), eicosenoic acid (20:1), and docosahexaenoic acid (22:6) and a lower content of FA heptadecanoic acid (17:0) and heptadecenoic acid (17:1) when compared with those with 0% EP ($P < 0.05$). Moreover, there were no differences among the treatments for total saturated FA, monounsaturated FA, or polyunsaturated FA (PUFA) ($P > 0.05$).

Dennis (1973) stated that PLA2 follows Michaelis–Menten kinetics, in which enzymatic activity depends on substrate availability, and Murakami et al. (2020) showed that sarcoplasmic PLA2 prefers to hydrolyze phospholipids with FA 18:1 and 18:2. It is well established that nonruminants such as pigs have higher proportions of PUFAs in adipose and muscle tissue than cattle because they absorb most lipids without altering their saturation levels (Wood et al., 2008). Thus, it could be explained that because cattle have a lower content of unsaturated FA than pork or fish, PLA2 activity in cattle may not be as involved as seen in pork for postmortem phospholipid hydrolysis (Chao et al., 2020). Dried EP is known to contain high levels of 18:1, 18:2, and 22:6 FA (Javed et al., 2018; Pirkwieser et al., 2022). Accordingly, EP incorporation likely altered the FA composition of the GS patties, increasing some unsaturated FA content. Thus, it is reasonable to attribute higher lipid oxidation of the 1.6% treatment to the higher presence of those unsaturated FA, even if there was no overall difference for PUFAs.

**Table 3.** Comparison of fatty acid profiles of ground striploin patties formulated with 0%, 0.4%, 0.8%, and 1.6% of egg powder containing anti-phospholipase α2

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Fatty acid | 0% | 0.4% | 0.8% | 1.6% | SEM | *P* value |
| 14:0 | 2.60 | 2.38 | 2.31 | 2.36 | 0.15 | 0.12 |
| 14:1 | 0.62 | 0.55 | 0.54 | 0.54 | 0.05 | 0.14 |
| 16:0 | 29.24 | 29.12 | 29.26 | 29.20 | 0.39 | 0.94 |
| 16:1 | 3.44 | 3.37 | 3.07 | 3.04 | 0.22 | 0.24 |
| 17:0 | 1.01$^{a}$ | 0.98$^{b}$ | 0.96$^{bc}$ | 0.94$^{c}$ | 0.06 | <0.01 |
| 17:1 | 0.77$^{a}$ | 0.74$^{ab}$ | 0.73$^{bc}$ | 0.71$^{c}$ | 0.05 | <0.01 |
| 18:0 | 13.22 | 13.17 | 13.22 | 13.10 | 0.29 | 0.54 |
| 18:1 | 37.91 | 37.98 | 38.16 | 37.75 | 0.72 | 0.79 |
| 9-18:1 trans | 1.89 | 1.83 | 1.78 | 1.77 | 0.13 | 0.11 |
| 11-18:1 trans | 1.34$^{b}$ | 1.37$^{ab}$ | 1.39$^{a}$ | 1.38$^{a}$ | 0.03 | 0.03 |
| 18:2 | 4.93$^{b}$ | 5.38$^{ab}$ | 5.52$^{ab}$ | 6.05$^{a}$ | 0.45 | 0.02 |
| 18:3 | 0.20 | 0.20 | 0.21 | 0.21 | 0.01 | 0.18 |
| 20:1 | 0.15$^{b}$ | 0.16$^{a}$ | 0.16$^{a}$ | 0.16$^{a}$ | 0.01 | 0.03 |
| 20:3 | 0.43 | 0.43 | 0.41 | 0.40 | 0.05 | 0.72 |
| 20:4 | 1.53 | 1.59 | 1.56 | 1.62 | 0.20 | 0.93 |
| 20:5 | 0.11 | 0.11 | 0.11 | 0.11 | 0.02 | 0.93 |
| 22:4 | 0.24 | 0.25 | 0.24 | 0.23 | 0.03 | 0.92 |
| 22:5 | 0.33 | 0.32 | 0.30 | 0.30 | 0.04 | 0.60 |
| 22:6 | 0.03$^{d}$ | 0.05$^{c}$ | 0.08$^{b}$ | 0.11$^{a}$ | 0.01 | <0.01 |
| SFA | 46.07 | 45.65 | 45.75 | 45.61 | 0.53 | 0.52 |
| MUFA | 46.12 | 46.00 | 45.82 | 45.36 | 0.77 | 0.43 |
| PUFA | 7.81 | 8.35 | 8.43 | 9.03 | 0.77 | 0.23 |

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids.

$^{a–d}$Values with different superscripts indicate a difference within each row.

## Oxygen radical absorbance capacity

There was no treatment × display day interaction for lipophilic or hydrophilic ORAC ($P > 0.05$). There was a main effect for treatment for the hydrophilic ORAC ($P < 0.05$; Table 1). Interestingly, the 0.8%

treatment showed a higher antioxidant capacity than the 1.6% and 0% treatment ($P < 0.05$), but the 0%, 0.4%, and 1.6% treatment showed no differences in hydrophilic ORAC ($P < 0.05$). Similar behavior was seen for lipophilic ORAC; treatments 0.4% and 1.6% were similar to other treatments, but the 0% treatment tended to have lower lipophilic antioxidant capacity compared with the 0.8% treatment ($P = 0.07$; Table 1).

Antioxidant capacity was obtained through ORAC analysis because it has been reported to be an adequate indicator of both oxidation inhibition time and degree of inhibition by measuring peroxyl radical scavenging capacity (Huang et al., 2002; Echegaray et al., 2021). The EP addition was most likely the reason for the observed differences in antioxidant capacity among the treatments. Eggs can contain a wide variety of hydrophilic antioxidants such as peptides and vitamin C (Remanan and Wu, 2014; Zhang et al., 2019) as well as lipophilic antioxidants such as carotenoids, free amino acids, ovalbumin, vitamin E, selenium, and iodine (Nimalaratne and Wu, 2015). Finally, amphiphilic egg lecithin can also act as an antioxidant (Ribeiro et al., 2019). What is intriguing is that although the addition of these antioxidants from the EP can be used to increase the antioxidant capacity of GS samples, the addition of EP will also increase the PUFA content in the samples. The slight increase in antioxidant capacity likely offset the negative impact from the increase in PUFA in the samples and thus resulted in no differences in lipid oxidation and color changes between the 0% and 0.8% EP treatments.

However, this does not explain the reason for the decrease of antioxidant capacity for the 1.6% EP treatment compared with those from the 0.8% EP treatment. Studies have shown that the activity of some antioxidants such as PLA2 are dose-dependent, with higher levels limiting its effect (Tatiyaborworntham and Richards, 2018; Tatiyaborworntham et al., 2021). Therefore, it is possible the aPLA2 EP demonstrated a similar behavior on dose dependency. In fact, the spike in antioxidant activity of the 0.8% treatment could be the reason that the 0.8% treatment had a lower lipid oxidation level than the 1.6% treatment, given the similarities in their FA profiles.

## Conclusions and Implications

Adding EP containing aPLA2 to GS affected moisture content, FA profile, phospholipid composition, lipid oxidation, and antioxidant capacity. However, these differences could be the result from the introduction of EP and not necessarily caused by the PLA2 inhibition effect of aPLA2. The binding of aPLA2 to PLA2 was demonstrated through ELISA, but the lack of LPC production across all treatments brought up the questionable relevance of PLA2 in lipid oxidation for postmortem beef. Further research on aPLA2's ability to inhibit lipid oxidation should focus on livestock species with higher overall unsaturated FA like poultry, pork, or fish.

### *Acknowledgements*

The authors thank the Mark and Kim Young Undergraduate Research Fund for the partial support of this research. We thank Dr. Jordan Sand for providing the EP used in these studies. Part of this work was also supported by the College of Agricultural and Life Sciences at the University of Wisconsin–Madison and by the USDA National Institute of Food and Agriculture, Hatch Project 1003222.

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