

EVALUATING EFFECTS OF AN OMEGA-3 ENRICHED FINISHING DIET ON GROUND  
BEEF COMPOSITION, PALATABILITY, AND COLOR STABILITY

by

CAMERON CAYE CATRETT

(Under the Direction of Alexander M. Stelzleni)

ABSTRACT

Beef products are often scrutinized in the scientific community and media for containing elevated omega-6 (**n-6**) fatty acids and negligible amounts of omega-3 (**n-3**) fatty acids due to ruminal biohydrogenation of polyunsaturated fatty acids (**PUFA**). Increasing dietary n-3 in relation to n-6 fatty acids determines dietary healthfulness; thus, red meat producers are interested in increasing their products' n-3 PUFA content. Unfortunately, literature documents increasing meat n-3 content caused detrimental effects on fresh quality and palatability attributes, but when animal diets were managed correctly, these effects were eliminated. In the present study, a microalgae/flaxseed supplement was fed to steers to evaluate effects on ground beef composition, color stability, and palatability. Overall, supplementation did not negatively affect product color stability nor palatability as other feeding regimens have in previous studies.

INDEX WORDS: color stability, fatty acid, finishing diet, flaxseed, ground beef, microalgae

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## DEDICATION

I dedicate this degree to Fred and Virginia Oglesby for valuing higher education.

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## CHAPTER 1

### INTRODUCTION

Red meat in the diet played a key evolutionary role in human development and is regarded as a rich, protein source which is more bioavailable than alternative protein sources (Zanovec et al., 2010). Meat consumption for each species has increased and with a forecasted 34% world population growth by 2030, demand for red meat will continue to rise. Additionally, consumers are becoming increasingly more aware of where their food originates and the nutritional value associated with it. Thus, producing large volumes of high-quality red meat is and will remain a large goal for both the livestock and meat industry.

Specifically, beef is the third most consumed species in the world and the second largest in the United States. In 2020, ground beef consumption was estimated to be around 12.3 kg per capita in the United States (Schulz, 2021). Ground beef remains popular among consumers due to its preparation flexibility and attractive price but is often scrutinized in the scientific community and media due to its elevated saturated fat content and omega-6 to omega-3 fatty acid ratio. While beef is a nutritionally dense source of protein and essential nutrients, it is practically devoid of essential omega-3 fatty acids primarily due to ruminal polyunsaturated fatty acid (PUFA) biohydrogenation (Harfoot, 1978). Omega-3 fatty acid consumption has been acknowledged to have health benefits including prevention of cardiovascular and neural diseases. Major dietary sources rich in omega-3 fatty acids include seafood, namely trout and salmon; however, these fatty acids are limited in human diets due to underconsumption of naturally rich sources. The United

States Department of Agriculture and Department of Health and Human Services (2020) report 90% of Americans do not meet daily recommendations for seafood consumption. The global impact of beef coupled with the demand for healthful food products has shifted research efforts to enhancing beef's fatty acid profile.

In the lifecycle of a beef product, color is one of the most impressionable fresh quality attributes visually appraised at retail with eating satisfaction completing the consumer beef experience. Consumers associate a bright, cherry-red beef color with product wholesomeness and freshness. Hood and Riordan (1973) reported bright, cherry-red colored beef outsells discolored beef in a 2:1 ratio. Meat discoloration impacts purchasing decisions and accounts for an annual \$3.73 billion industry loss in the United States (Ramanathan et al., 2022). Fatty acids containing two or more double bonds in their chains are more susceptible to oxidation, thus PUFA enhanced beef products have a greater likelihood to oxidize and discolor more rapidly. Furthermore, literature reports PUFA enhanced meat products may have decreased palatability satisfaction due to decreased beef-flavor identity and juiciness ratings, coupled with increased off-flavor presence. In beef, tenderness, juiciness, flavor, off-flavors, and their interaction influence product palatability (Smith and Carpenter, 1974; Platter et al., 2003; Drey and O'Quinn, 2017). Industry and research emphasis on tenderness has caused the modern beef supply to become more reliably tender. Thus, consumers have begun to place greater emphasis on product flavor and juiciness (Guelker et al., 2013).

Literature demonstrates PUFA content can be improved and omega-6 to omega-3 ratios can be decreased in beef products originating from cattle supplemented with flaxseed, fish oil, linseed, and other marine sources; however, many studies report detrimental effects on product appearance and palatability. Today, beef fatty acid profile enhancement continues to be further

researched with various supplements; however, there is a lack of extensive research understanding the effects of blended omega-3 feed supplement sources and their influence specifically within the ground beef sector.

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## CHAPTER 2

### LITERATURE REVIEW

#### **2.1 Red Meat in Society**

##### *Meat consumption*

Dietary red meat inclusion played a key evolutionary role in human development. Milton (2003) claimed meat was integrated into human ancestral diets over 2 million years ago. Without dietary integration of red meat, it is highly likely humans would not have developed a complex brain and neural system (Wyness, 2016). When included in a balanced diet, red meat is a rich, protein source which is more bioavailable than alternative protein sources (Zanovec et al., 2010). Jones et al. (2018) reported meat consumption for each species has increased since 2012 representing an elevated demand for animal protein.

Meat consumption trends vary among differing cultures and nations; however, with an expected 34% world population increase by 2030, it is predicted dietary demand for meat will reach 255,877 metric tons by 2027 (Fiala, 2008; OECD-FAO, 2018). The United States Department of Agriculture (**USDA**) predicts United States consumers will have access to 101.9 kg per capita of red meat and poultry in 2024 on a retail weight basis (Jones et al., 2018). The projected increased retail accessibility to animal protein sources also indicates an increase in meat product waste. Meat waste results from a variety of factors including excessive portions, poor cookery methods, expiration, product over purchase, and namely, discoloration. Globally, in 2020, meat loss due to discoloration was estimated at 5.8 metric tons (Maia Research Analysis, 2020).

Although meat consumption trends differ regionally, red meat continues to be a vital protein source in human diets and contributes to feeding the world.

### ***Ground beef market***

Ground beef product preparation versatility and affordability allows the beef industry to compete favorably with other protein sources. Collectively, ground beef/hamburger is the largest fundamental beef product category. Ground beef sales represent 63% and 49% of total food service and retail sales volume annually, respectively (Speer et al., 2015). In 2020, ground beef consumption was estimated to be around 12.3 kg per capita in the United States (Schulz, 2021). As beef prices increase, retailers have begun to prominently highlight ground beef in advertisements due to its competitive price point and establish it as a valuable product to consumers. Since 1998, consumers have increased their beef spending habits by approximately \$113 per person (Speer et al., 2015). The observed shift in purchasing trends translates to the beef industry capturing a greater share of red meat sales. Nonetheless, ground beef remains popular among consumers due to its preparation flexibility and attractive price while substantially contributing to beef sales.

### ***Beef health perception***

Consumers are becoming increasingly more aware of where their food originates and the nutritional value associated with it. Beef is a protein source containing many essential vitamins and minerals. A single, 85 g (3 oz) beef serving contains 25 g of protein as well as 101%, 53%, and 14% of daily recommended intake values for Vitamin B<sub>12</sub>, Zinc, and Iron, respectively (Zanovec et al., 2010). Despite being a nutritionally dense protein source, beef has been placed

under public scrutiny due to exhibiting elevated saturated fatty acid (**SFA**) contents and an increased Omega-6 (**n-6**) to Omega-3 (**n-3**) ratio. The *Dietary Guidelines for Americans* recommends an individual to obtain 20 to 35% of daily calories from fat, with less than 10% derived from saturated fat sources (USDA and U.S. Department of Health and Human Services, 2020). The American Heart Association recommends reduction of dietary cholesterol, saturated fat, and total fat intake to prevent cardiovascular disease (Eckel et al., 2013). Papier et al. (2023) conducted a meta-analysis of beef consumption trends and cardiovascular disease incidence and found consumers who ate 50 g of unprocessed beef or 50 g of processed beef products daily were respectively 9% and 18% more likely to develop cardiovascular disease than consumers who did not consume beef products. While study limitations and bias exist, the previous scientific claims decrease consumer confidence and increase concern regarding the health profile of beef. Nevertheless, the intake of fat via meat consumption has been and will likely remain a major public health topic.

Moreover, beef contains negligible amounts of essential omega-3 fatty acids primarily due to rumen biohydrogenation of polyunsaturated fats (**PUFA**; Harfoot, 1978). In the last decade, consumers have grown concerned regarding saturated fat content and the n-6 to n-3 fatty acid ratio of their ground beef. Lusk and Parker (2009) reported consumers would prefer compositionally improved ground beef originating from cattle with decreased saturated fats and smaller n-6 to n-3 ratios. It is critical to understand consumer preferences since their concerns, demands, and purchasing decisions direct the entire industry. Modern day consumers place greater emphasis on what type of fat they are consuming while remaining cognizant of how much fat they are consuming. For these reasons, beef research has shifted focus to increasing product health perception specifically via n-3 fatty acid profile enhancement.

## 2.2 Omega-3 fatty acids

### *n-3 attributes and sources*

Globally, developed societies are increasing dietary saturated fat and n-6 fatty acid while decreasing n-3 fatty acid intake (Simopoulos, 2010). The term “omega” is used to describe the first double bond related to the methyl end of the acyl chain. Interest in PUFA enhancement has increased due to n-3 underconsumption and their potential role in health promotion. Health benefits from  $\alpha$ -linolenic acid (**ALA**), eicosapentaenoic acid (**EPA**), and docosahexaenoic acid (**DHA**) were first chronicled in Greenland eskimos who consumed primarily seafood diets and presented decreased incidence of coronary heart disease, asthma, diabetes, and multiple sclerosis (Ruxton et al., 2004; Calder, 2016). Alpha linolenic acid is an important precursor for long-chain n-3 PUFA synthesis of longer chain n-3 PUFA, including EPA and DHA (Juárez et al., 2011). These fatty acids often are limited in human diets due to inadequate intake of naturally rich ALA, DHA, or EPA sources such as fish. Since the initial observation, beneficial n-3 health effects have expanded to include neural development, psoriasis, mental health, and neurodegenerative diseases (Simopoulos, 2010). A recent report from the American Heart Association suggested that n-3 PUFA supplementation did not prove beneficial in preventing cardiovascular disease among patients at risk for diabetes, but reported supplementation may reduce death from coronary heart disease among patients with prior coronary heart disease. The American Heart Association recommends n-3 supplementation of 1 g/day for patients with cardiovascular disease. Shahidi and Ambigaipalan (2018) recommended consuming two fish servings per week to obtain 0.30 to 0.45 g of EPA and DHA. The United Nations Food and Agricultural Organization (FAO, 2010) recommends a total n-3 PUFA daily intake of 0.5–2% for adults to prevent deficiency symptoms. While great emphasis is placed on increasing n-3 intake, decreasing the n-6 to n-3 ratio also plays

a critical role in balancing and promoting a healthy diet as elevated n-6 to n-3 ratios promote disease pathogenesis. A ratio of 1:1 to 2:1 n-6 to n-3 ratio should be the target ratio for optimizing health (Simopoulos, 2010). While focus is often set on enhancing a singular food item, positive improvements in one item can benefit the entire diet. Nonetheless, research continues to explore and characterize the impact of n-3 PUFA consumption and the n-6 to n-3 ratio on human health.

Primary sources rich in ALA, DHA, and EPA are certain natural or modified plants, fatty fish, and algal biomasses (Shahidi and Ambigaipalan, 2018). Challenges exist with n-3 content due to the variability among source quantity and quality. Common n-3 rich foods include fatty fish; however, 90% of Americans do not meet daily seafood dietary recommendations (USDA and HHS, 2020). Rahman and Islam (2020) reported fish underconsumption leads to inadequate omega-3 fatty acid concentrations in the diet. Givens and Gibbs (2006) reviewed meat's role as a dietary n-3 PUFA source and concluded human intake of these beneficial fatty acids could be enhanced via animal diet supplementation. Medeiros et al. (2007) utilized a rat model to demonstrate that ALA-rich meat obtained from cattle dietarily supplemented with flaxseed during finishing increased the DHA concentration in the heart and liver when consumed by rats. Thus, research focus shifted towards product fatty acid profile manipulation as an alternative n-3 fatty acid source via cattle diet supplementation.

### ***Ruminant fatty acid profile and biohydrogenation***

According to the Great Britain National Diet and Nutrition Survey, meat products and milk contribute half of dietary saturated fat intake, with meat products being named the largest SFA source (Henderson et al., 2003). While containing numerous essential vitamins and minerals, beef specifically contains negligible amounts of essential n-3 fatty acids due to rumenal PUFA

biohydrogenation (Harfoot, 1978). Meat fatty acid content is influenced by several factors including diet composition as well as the animal's digestive system and the biosynthetic processes within it. Comparatively, beef contains greater proportions of SFA and MUFA than pork or poultry. Ruminant derived meat products contain many minor fatty acids (branched, odd-numbered) and intermediate ruminal biohydrogenation products (Woods and Fearon, 2009). Bhattacharya et al. (2006) reported that despite being found in negligible amounts, fatty acids present in beef have elevated biological activity levels. Microbial enzymes are responsible for dietary lipid isomerization, hydrolysis, and MUFA conversion to various partially and fully saturated derivatives (Woods and Fearon, 2009). Rumen microorganisms have a strong influence on the composition of fatty acids departing the rumen for absorption in the small intestine (Jenkins, 1993). The intestinal absorption coefficient of individual fatty acids is elevated in ruminants than non-ruminants, ranging from 80% to 92% for PUFA in conventional diets (Woods and Fearon, 2009). The increased ruminal absorption efficiency coefficient has been attributed to the greater capacity of bile salts and the lysophospholipid micellar system to solubilize fatty acids, coupled with the acidic conditions within the duodenum and jejunum (pH 3.0–6.0).

Some unsaturated fatty acids may flow to the small intestine where they are available for absorption; however, most are saturated to different degrees by ruminal microorganisms via biohydrogenation (Jenkins et al., 2008). Polyunsaturated fatty acids are toxic to ruminal bacteria and biohydrogenation is a protective mechanism used to decrease their toxicity. Maia et al. (2007) claimed cellulolytic bacteria growth was totally inhibited at PUFA concentrations of 50 µg/mL. Approximately 90% of PUFA can be hydrogenated in the rumen and 75 to 80% of free fatty acids which flow to the small intestine can be saturated (Harfoot, 1978). Dietary fats which flow to the small intestine are absorbed, re-esterified, and transported via the lymphatic system and plasma

and deposited within adipose and intramuscular tissue using lipoproteins as carriers (Bauchart, 1993). Geay et al. (2021) found that SFA and MUFA can account for up to 50% and 40 %, respectively, of total fat in ruminant adipose tissue; however, this composition could be influenced by the animal's diet.

### ***n-3 supplementation and meat fatty acid composition***

The basal diet may contribute to the resulting animal product fatty acid composition because of its dietary replacement effect on the targeted fatty acid source; however, the basal diet impact on product fatty acid composition can be difficult to quantify due to source variability. Novel oil sources such as marine algae, chia seed, lupin, hemp, and camelina have recently been investigated as lipid sources in animal feeds but considerable variations in both the oil content and fatty acid profile persist due to country of origin and botanical species.

Literature demonstrates beef n-3 fatty acid content was enhanced via oilseed/lipid, forage, and fish-derived product supplementation when added directly to finishing diets (Woods and Fearon, 2009; Vahmani et al., 2015). In a study evaluating muscle fatty acid composition originating from bison and beef cattle fed either a range diet or feedlot diet, Rule et al. (2002) reported a greater PUFA and n-3 proportions in range-fed animals but found feedlot animals had decreased total fat content. Vatansver et al. (2000) evaluated the impact of supplementing beef steers with linseed, fish oil, and linseed/fish oil on ground beef fatty acid composition. Linseed, fish oil, and linseed/fish oil treatments increased total PUFA content by 0.5, 10.6, 0.4 mg/g muscle respectively, with fish oil presenting the greatest increase ( $P < 0.05$ ). Fish oil and linseed/fish oil treatments reduced n-6 linoleic acid content by 0.25 and 0.15 g/100 g muscle respectively, while linseed and linseed/fish oil treatments increased n-3  $\alpha$ -linolenic acid content by 0.23 and 0.4 g/100

g muscle ( $P < 0.05$ ). Wistuba et al. (2007) evaluated the impact of supplementing beef steers with fish oil on *Longissimus dorsi* fatty acid composition. Dietary fish oil supplementation increased n-3 content by 0.37 g/100 g muscle and decreased the n-6 to n-3 ratio by 5.89 g/100 g muscle ( $P = 0.01$ ).

Maddock et al. (2004) evaluated the impact of supplementing beef steers with whole, rolled, and ground flaxseed on *Longissimus dorsi* fatty acid composition. Researchers found whole, rolled and ground flaxseed supplementation increased total n-3 content by 2.36, 4.05, and 4.13 nmol/100 nmol of total fatty acid respectively ( $P < 0.001$ ). Moreover, n-6 content was reduced by 1.98, 2.57, and 3.13 nmol/100 nmol and the n-6 to n-3 ratio was decreased by 1.72, 2.32, and 2.35 nmol/100 nmol of total fatty acid respectively for whole, rolled, and ground flaxseed treatments ( $P < 0.04$ ).

Phelps et al. (2016a) evaluated the impact of supplementing beef heifers with increasing microalgae meal concentrations (0, 50, 100, 150) on *Longissimus lumborum* fatty acid composition. As dietary microalgae meal increased, the amount of 18:1 *trans*-11 increased (quadratic,  $P < 0.01$ ) and 18:2n-6 *cis* and 20:3n-6 decreased (linear;  $P < 0.01$ ). Feeding increasing microalgae meal levels did not impact total SFA or MUFA ( $P > 0.25$ ) but tended ( $P = 0.10$ ) to increase total PUFA (quadratic;  $P = 0.03$ ). Total n-6 PUFA decreased (linear;  $P = 0.01$ ) and total n-3 content increased (quadratic;  $P < 0.01$ ) as microalgae meal increased dietarily which caused a decrease (quadratic;  $P < 0.01$ ) in the n-6 to n-3 ratio. Phelps et al. (2016b) evaluated the impact of supplementing beef heifers with increasing microalgae meal levels (0, 50, 100, 150) on 85/15 ground beef fatty acid composition. As microalgae supplementation increased, n-6 content decreased (linear;  $P = 0.01$ ), n-3 content increased (quadratic;  $P < 0.01$ ), and the n-6 to n-3 ratio decreased (quadratic;  $P < 0.01$ ).

Alberti et al. (2014) evaluated the impact of supplementing finishing bulls with linseed and linseed + vitamin E on *Longissimus dorsi* fatty acid composition. Supplementation increased n-3 fatty acids, namely ALA by 0.6 and 1.21 g/100 g and decreased the n-6 to n-3 ratio by 3.99 and 4.41 g/100 g respectively for the linseed and linseed + vitamin E groups, thus yielding perceivably “healthier” beef (Alberti et al., 2014).

While literature strongly illustrates positive fatty acid profile enhancement to meat products via animal dietary supplementation, there are documented adverse fresh quality and sensory effects which will be further chronicled throughout the review.

## **2.3 Palatability**

### ***Beef palatability defined***

Palatability is the term characterizing the overall eating experience of a food product. In beef, tenderness, juiciness, flavor, and their interaction influence product palatability (Smith and Carpenter, 1974; Platter et al., 2003; Drey and O’Quinn, 2017). Drey and O’Quinn (2017) conducted a meta-analysis on studies exploring beef consumer preferences and developed a model of consumer overall liking where tenderness, juiciness, and flavor were, respectively, responsible for 42%, 7%, and 48% of overall consumer satisfaction ( $r^2 > 0.99$ ). Moreover, samples deemed unacceptable for a single attribute increased the likelihood of overall product failure: tenderness: 69% likely to fail; juiciness: 66% likely to fail; flavor: 76% likely to fail (Drey and O’Quinn, 2017). Previously, tenderness was considered the most important palatability trait and attracted the greatest research attention (Savell et al., 1987; Miller et al., 1995; Platter et al., 2003). Huffman et al. (1993) reported tenderness was ranked the most important palatability trait 51% of the time while flavor was only ranked the most important 39% of the time. Industry and research emphasis

on tenderness has caused the modern beef supply to become more reliably tender via genetic selection and technological advancement. Thus, consumers have begun to place greater emphasis on product flavor and juiciness (Guelker et al., 2013). Lucherker et al. (2016) determined approximately 50% of consumers identify flavor as the most important trait with tenderness ranked the most important 39.3% of the time. Flavor has been reported to be correlated to overall palatability success, once tenderness is deemed acceptable (Legako et al., 2015; Lucherker et al., 2016). In addition to the attributes above, off-flavor presence also influences palatability perception and consumer satisfaction. While certain off-flavors may be deemed desirable, most non-characteristic flavors identified by beef consumers have a negative impact on eating satisfaction. Off-flavors are produced by a complex volatile compound mixture and can present undesirable flavors namely, oxidized flavors [American Meat Science Association (AMSA, 2016). Thiobarbituric-acid reactive substances (**TBARS**) quantification is an assay commonly used to evaluate oxidation in meat. Research has established a strong correlation between TBARS values and sensory panel flavor scores (Mielche & Bertelsen, 1993; Spanier et al., 1993). Younathan and Watts (1959) reported consumers may be able to detect product rancidity and oxidized off-flavors when a TBARS value greater than 1 mg malonaldehyde per kg of muscle tissue is recorded. Thus, TBARS is an accepted assay to characterize oxidation and its influence on product flavor.

### ***Sensory perception of n-3 enriched meat***

Vatansever et al. (2000) evaluated the impact of supplementing beef steers with linseed, fish oil, and linseed/fish oil on ground beef color stability and palatability. Product oxidation (TBARS) increased for each treatment throughout the display period consistent with ground beef discoloration ( $P < 0.05$ ), with no differences between control, linseed, and linseed/fish oil groups

( $P > 0.05$ ); however, fish oil differed and presented the greatest TBARS at each time point ( $P < 0.01$ ). Fish oil TBARS values were approximately 2 and 4 mg/kg muscle at d 3 and 10, which is past the threshold TBARS value of 1 for consumer detection (Younathan and Watts, 1959). Trained panelists evaluated samples for multiple palatability attributes and significant treatment effects were reported for rancidity, fishy flavor, and overall liking ratings ( $P < 0.05$ ). Panelist reported no differences between control, linseed, and linseed/fish oil groups ( $P > 0.05$ ) but reported greater rancid and fishy flavor scores and decreased overall liking ratings for the fish oil treatment translating to increases of 0.6 and 3.9 and decreases of 0.5, respectively ( $P < 0.05$ ). While statistically significant, without consumer panel evaluation, it is challenging to determine if the observed differences are biologically significant.

Maddock et al. (2004) evaluated the impact of supplementing beef steers with whole, rolled, and ground flaxseed on *Longissimus dorsi* palatability. Sensory panelists did not discern treatment effects for tenderness nor beef flavor intensity ( $P > 0.35$ ) but observed effects for juiciness where control steaks received the greatest ratings while whole and ground flaxseed treatments had decreased juiciness ratings which were 0.31 less than control steaks ( $P = 0.04$ ).

Phelps et al. (2016a) evaluated the impact of supplementing beef heifers with increasing microalgae meal concentrations (0, 50, 100, 150) on *Longissimus lumborum* palatability. Trained panelists indicated no difference in steak juiciness, beef flavor intensity, connective tissue, nor overall tenderness ( $P > 0.18$ ); however, as microalgae meal supplementation increased off-flavor prevalence increased by 0.07, 0.36, 0.89 for each respective treatment (quadratic;  $P < 0.01$ ). Phelps et al. (2016b) evaluated the impact of supplementing beef heifers with increasing microalgae meal levels (0, 50, 100, 150) on 85/15 ground beef palatability. Trained panelist indicated there were no discernable differences in firmness, overall tenderness, and juiciness ( $P > 0.20$ ); however, as

microalgae supplementation increased, off-flavor presence increased (quadratic;  $P < 0.05$ ) and beef flavor and cohesion ratings tended to decrease (linear;  $P = 0.09$ ).

## **2.4 Meat color**

### ***Myoglobin redox forms***

Pigments influence meat color by reflecting and absorbing light at different wavelengths. Myoglobin (**Mb**) is the sarcoplasmic heme protein primarily responsible for the red color of meat and is mostly found in three redox states: deoxymyoglobin (**DMb**), oxymyoglobin (**OMb**), and Metmyoglobin (**MMb**; Suman and Joseph, 2013). These redox forms differ in the iron valence state and the ligand bound to the sixth site. When no ligand is bound to the central heme iron, DMb, a purple-red color is presented (Mancini and Hunt, 2005). Oxygenation (bloom) occurs when Mb is exposed to oxygen and consequently binds to the heme iron sixth site (Mancini and Hunt, 2005), producing a bright, cherry-red color (Suman and Joseph, 2013). Both DMb and OMb contain a central iron in the ferrous state (Mancini and Hunt, 2005). Extended oxygen exposure forms MMb where the central iron changes to a ferric state with water bound to the sixth site, resulting in a brown meat surface color. Metmyoglobin has no oxygen binding capabilities (Suman and Joseph, 2013). The formation of MMb is the primary contributor to meat discoloration featuring an undesirable brown surface color; however, there are exogenous factors such as muscle type, retail case parameters, and oxygen partial pressure which can influence the amount and Mb redox form present (Mancini and Hunt, 2005).

### ***Consumer and economic impact***

Color is one of the most impressionable fresh quality attributes visually appraised in retail display. Consumers associate a bright, cherry-red beef color with product wholesomeness and freshness (Suman and Joseph, 2013). Killinger et al. (2004) evaluated the importance of beef product attributes including color, fat, marbling, appearance, and palatability on purchasing trends and found color to account for approximately 66% of consumer purchasing decisions regardless of personal color preference. Hood and Riordan (1973) reported bright, cherry-red colored beef outsells discolored beef in a 2:1 ratio. Thus, color, excluding price, is the leading factor influencing consumer meat purchasing decisions.

Meat discoloration not only impacts consumer purchasing decisions, but also product discounts and discardment. When MMb reaches 20% surface coverage, meat is discounted and after reaching 40% coverage, meat is rejected by consumers (McMillin, 2008). Ramanathan et al. (2022) reported \$1.18 billion is lost due to discounted, discolored meat and \$2.55 billion is lost due to discarded, discolored meat in the United States annually. Combined, these translate to a \$3.73 billion loss for the beef industry from meat discoloration (Ramanathan et al., 2022). Globally, meat discoloration product and revenue loss are estimated to be 5.8 metric tons and \$14.2 billion annually (Maia Research Analysis, 2020).

### ***Color methodology***

Evaluating and characterizing meat color can be achieved via objective color scans taken with a colorimeter and subjective visual panels. Modern colorimeters use Commission Internationale de l'Eclairage (CIE)  $L^*a^*b^*$  values for color interpretation where  $L^*$  measures black to white,  $a^*$  measures green to red, and  $b^*$  measures blue to yellow. Additionally, spectral

reflectance is measured from 400 to 700 nm. These values can be used to calculate hue angle (color), chroma (color saturation), delta E, and surface Mb redox forms to further describe meat surface color objectively (AMSA, 2012). Visual panels are used to simulate retail setting consumer evaluations but present research challenges by introducing human error. Inconsistencies may occur due to panelist fatigue, personal preferences, lighting, visual deficiencies, and product presentation. A common method to mitigate the previously mentioned challenges is through panelist orientation (AMSA, 2012), allowing subjective visual panels to serve as a benchmark for objective color measurements. Objective color differences can be detected by trained color panelists at unit changes of 0.95, 0.99 and 0.50 for  $a^*$ ,  $b^*$ , and chroma values respectively (Mancini et al., 2022). Chroma values 18 and below are indicative of an unacceptable surface color appearance when evaluated by consumers (Mancini et al., 2022). The interrelationship between objective color measurements and subjective color perception allows researchers to draw strong conclusions regarding color stability study results.

### ***Color stability of n-3 enriched meat***

Fatty acids containing double or triple covalent bonds in their chains are more susceptible to oxidation. Beef products containing elevated PUFA content are more susceptible to oxidation during retail display (Yang et al., 2002). Oxidation of EPA and DHA can cause myoglobin pigment oxidation leading to increased surface metmyoglobin formation on beef products (Jacobsen, 2008). When measured objectively, Jacobsen (2008) found meat products present reduced  $a^*$  values, increased calculated surface myoglobin percentage, increased hue angle, and decreased chroma values.

Vatansever et al. (2000) evaluated the impact of supplementing beef steers with linseed, fish oil, and linseed/fish oil on ground beef color stability. Product oxidation (TBARS) increased for each treatment throughout the display period consistent with ground beef discoloration ( $P < 0.05$ ), with no differences between control, linseed, and linseed/fish oil groups ( $P > 0.05$ ); however, fish oil differed and presented the greatest TBARS at each time point ( $P < 0.01$ ). Time and treatment effects were observed for chroma saturation where values declined throughout display for each group consistent with discoloration ( $P < 0.05$ ), with the fish oil treatment presenting the smallest values at each time point ( $P < 0.05$ ). Consumer rejection occurs at chroma values below 18 and at display d 6 fish oil samples chroma values were below the threshold, while all other treatments did not fall below it until display d 10. The advanced product discoloration and oxidation could be attributed to the increased presence of EPA and DHA within the fish oil.

Phelps et al. (2016a) evaluated the impact of supplementing beef heifers with increasing microalgae meal concentrations (0, 50, 100, 150) on *Longissimus lumborum* color stability. Phelps et al. (2016a) observed treatment  $\times$  time interactions for all color attributes and TBARS values, as well as time effects consistent with beef discoloration ( $P < 0.01$ ). As microalgae meal supplementation increased in the diet,  $a^*$  and surface metmyoglobin percentage decreased and TBARS values increased, with Algae 150 presenting the smallest  $a^*$  values and greatest TBARS values (quadratic;  $P < 0.01$ ). Thiobarbituric acid reactive substances values greater than 1 mg/kg muscle tissue may be detected during consumption as oxidized off-flavors and it is important to note on display d 0 each of the microalgae supplemented groups were near or beyond this threshold value, thus presenting an undesirable product.

Phelps et al. (2016b) evaluated the impact of supplementing beef heifers with increasing microalgae meal levels (0, 50, 100, 150) on 85/15 ground beef color stability. While PUFA content

was increased, negative effects on color stability were reported. Phelps et al. (2016b) observed treatment  $\times$  time interactions for all color attributes and time effects consistent with beef discoloration ( $P < 0.01$ ). Although feeding microalgae meal affected ground beef color early in the display period, it took nearly half of the display period for a linear treatment effect on discoloration to be detected. Unlike the Phelps et al. (2016a) data, during the final objective color measurement period,  $a^*$  and surface metmyoglobin were not impacted by treatment due to patties reaching maximum metmyoglobin formation prior to the end of display.

Alberti et al. (2014) evaluated the impact of supplementing finishing bulls with linseed and linseed + vitamin E on *Longissimus dorsi* color stability. Contrary to previous literature, Alberti et al. (2014) reported no detrimental color stability effects for linseed and linseed + vitamin E diets ( $P > 0.46$ ). Steaks discolored following trends consistent with beef discoloration. Experimental factors including decreased PUFA rancidity levels, dark storage conditions, and antioxidant use could influence observed color results. Elevated MUFA/PUFA proportions makes meat more susceptible to oxidation (Jacobsen, 2008) but these effects were not observed by Alberti et al. (2014). Moreover, light is a potent oxidant known to trigger oxidation, thus experimental use of dark storage limited light's influence on product discoloration. Lastly, the introduction of vitamin E could be responsible for the color stability observed in the linseed + vitamin E group but does not explain the added stability observed in the linseed group. Vitamin E concentration within the muscle differed ( $P < 0.05$ ) with the linseed + vitamin E group containing 88% greater content (1.32 mg/kg muscle tissue) than the control group. Liu et al. (1995) reported a minimum  $\alpha$ -tocopherol concentration of 1.2 mg/kg muscle tissue is needed to increase product color stability. Thus, the observed vitamin E concentrations could enhance product color. Nonetheless, linseed

supplementation did not negatively impact color stability nor did the inclusion of vitamin E enhance the color observed.

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## CHAPTER 3

### FEEDING MICROALGAE AND FLAXSEED (*Nannochloropsis oculata*) TO BEEF STEERS I: EFFECTS ON 90/10 ROUND AND 80/20 CHUCK GROUND BEEF PATTY COMPOSITION AND PALATABILITY<sup>1</sup>

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<sup>1</sup>Catrett, C.C., A.M. Stelzleni, J.S. Drouillard, S.A. Devane, G.A. McKinney, X. Li, K.A. Turner,

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## ABSTRACT

The study objective was to determine effects of feeding greatOplus on ground beef composition and palatability. Steers ( $N = 700$ ) were weighed, stratified into 14 weight blocks, allocated to 28 pens ( $N = 25$  head/pen), with pens randomly assigned to one of two treatments consisting of being fed either a conventional finishing diet (**CON**;  $n = 14$ ) or a conventional feedlot diet containing a 10% extruded proprietary flaxseed, wheat middling, and *Nannochloropsis oculata* algae blend (**OMG**;  $n = 14$ ). After 173 d on feed, one head per pen was randomly removed, transported, harvested, and fabricated at a commercial abattoir. Round and Chuck subprimals were vacuum packaged, boxed, and shipped to the University of Georgia Meat Science Technology Center 17 d postmortem. On d 21 and 23 postmortem, respectively, Round and Chuck muscles were coarse (13-mm) and fine (6-mm) ground, with ninety ( $113 \pm 2$  g, 13-mm thick) patties produced per carcass and primal. Patties were blast frozen ( $-40^{\circ}\text{C}$ ), randomly allocated to proximate analysis, Allo-Kramer shear force, consumer sensory, and trained sensory evaluation, vacuum packaged, and placed in frozen storage ( $-25^{\circ}\text{C}$ ) until later analyses. There were no Treatment main effects for both Round and Chuck patty proximate composition measures, shear force values, or cooked patty measures ( $P > 0.15$ ). There were no Treatment main effects for Round trained sensory cohesion, juiciness, and off-flavor scores, nor for Chuck trained sensory cohesion and off-flavor scores ( $P > 0.23$ ); however, there were observed Treatment effects for Round beef flavor and Chuck juiciness scores ( $P < 0.02$ ) where CON patties had decreased sensory scores for each attribute compared to OMG patties. Chuck CON beef flavor scores tended ( $P = 0.09$ ) to be less than OMG patty scores. There were no Treatment main effects for Round consumer ratings for cohesion, juiciness, beef flavor, or overall palatability, nor for Chuck consumer off-flavor scores ( $P > 0.15$ ); however, there were observed Treatment effects for Chuck consumer cohesion, beef

flavor, and overall palatability scores ( $P < 0.05$ ), where CON patties had decreased scores for each aforementioned attribute than OMG patties. Round OMG consumer off-flavor scores tended to be less than CON patty scores and Chuck OMG juiciness scores tended to be greater than CON patty scores ( $P < 0.07$ ). Overall, greatOplus enhanced beef patty sensory perception contrary to other supplements used in previous studies.

**KEY WORDS:** consumer, finishing diet, ground beef, omega-3 fatty acids, patty

## INTRODUCTION

Meat consumption trends vary among differing cultures; however, with an expected 34% world population increase by 2030, it is predicted global demand for meat will reach 255,877 metric tons by 2027 (Fiala, 2008; OECD-FAO, 2018). Beef is a nutritionally dense protein source containing many essential nutrients (Zanovec et al., 2010), but due to ruminal polyunsaturated fatty acid (PUFA) biohydrogenation, beef contains negligible amounts of essential omega-3 fatty acids (Harfoot, 1978). Lusk and Parker (2009) reported consumers would prefer compositionally improved ground beef originating from cattle with decreased saturated fats and decreased Omega-6 (n-6) to Omega-3 (n-3) ratios. Thus, beef research has shifted focus to increasing product health perception by enhancing meat PUFA content via dietary supplementation.

Literature demonstrates beef PUFA content was elevated through supplementation of oilseed, forage, and marine-derived products (Woods and Fearon, 2009; Vahmani et al., 2015), but negative palatability effects were observed. Phelps et al. (2016a) observed feeding microalgae meal (*Schizochytrium limacinum* CCAP 4087/2) elevated *Longissimus lumborum* eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content; however, increased off-

flavor presence was reported as supplementation increased. Vatansever et al. (2000) noted increased lipid oxidation and greater off-flavor development in ground beef patties derived from cattle fed flaxseed and fish oil. Maddock et al. (2004) sensory panel results indicated flax-fed cattle produced steaks with reduced product juiciness and decreased flavor acceptability. Palatability studies report flavor as the leading attribute influencing beef consumption satisfaction (Killinger et al., 2004; Lucherik et al., 2016; O'Quinn et al., 2018). Despite literature reporting successful beef fatty acid profile enhancement via novel feed additives, the negative palatability effects observed from these products create a less desirable product for consumers to purchase.

Thus, the study objective was to examine effects of feeding a microalgae and flaxseed blend (greatOplus; *Nannochloropsis oculata*; NBO3 Technologies, Manhattan, KS) to finishing steers on 90/10 Round and 80/20 Chuck ground beef composition and palatability.

## **MATERIALS AND METHODS**

All experimental procedures involving animals were approved by the Kansas State University Institutional Animal Care and Use Committee and the University of Georgia (UGA) Institutional Review Board approved all procedures for human subject use in sensory panel evaluations.

### ***Live Cattle Management***

Black-hided yearling steers ( $N = 773$ ) were purchased from sale barns in Kansas and Nebraska and transported to the Kansas State University Beef Cattle Research Center (Manhattan, KS) between March 30th and April 8th, 2022. Steers were weighed, vaccinated against common viruses (Titanium 5; Elanco Animal Health, Indianapolis, IN) and clostridial pathogens (Ultrabac

7; Zoetis Animal Health, Parsippany, NJ), treated for internal and external parasites (Safe-guard; Ultra Saber; Merck Animal Health, Rahway, NJ), identified with visual and radio frequency identification tags, placed into feeding pens, and fed a common diet containing approximately 50% concentrate and 50% roughage. Following a two-week acclimation period, cattle were individually weighed and stratified from heaviest to lightest body weight. From the original 773 steers, a group representing the smallest standard deviation was selected for experimental use ( $N = 700$ ).

Cattle were separated into 14 weight blocks, implanted (Component TE-200; Elanco Animal Health), and allocated to 28 feedlot pens ( $n = 25$  head/pen). Pens were randomly assigned to one of two treatments consisting of a control diet without supplemental n-3 fatty acids (**CON**; Table 1) or a diet containing 10% (dry basis) of an extruded proprietary blend of flaxseed, wheat middlings, and *Nannochloropsis oculata* algal biomass (**OMG**; greatOplus; NBO3 Technologies, Manhattan, KS). A three-week acclimation period was used to transition cattle to their final finishing diets. Cattle were fed twice daily, *ad libitum*, and on study d 173, one animal was randomly selected from each pen ( $n = 14$  / treatment), weighed, and transported to a commercial abattoir (Hertzog Meat Company, Butler, MO) for harvest and fabrication. Cattle were harvested 24 h apart as two groups (7 heavy blocks and 7 light blocks). Carcasses were fabricated 3 d after the final harvest into round and chuck subprimals, vacuum packaged, boxed, refrigerated ( $2 \pm 2^\circ\text{C}$ ), shipped 13 d postmortem, and delivered 17 d postmortem to the UGA Meat Science and Technology Center (Athens, GA).

### ***Muscle Sampling, Ground Beef Manufacture, and Sample Packaging***

After delivery, boxes were sorted by carcass and primal. Each carcass and primal were processed independently. Vacuum packaged round subprimals were further processed 24 h prior

to grinding to remove bone and prepare meat for grinding. Individually, cubed round muscles were placed in plastic-lined, dark storage boxes to maintain carcass identification and stored overnight ( $2 \pm 2^\circ\text{C}$ ). Round grinding occurred 21 d postmortem with each carcass processed separately. Meat was placed in a grinder (Daniels AMFG-50; Daniels Food Equipment, Parkers Prairie, MN), coarse ground (13 mm plate) and fine ground (6 mm plate) prior to patty formation. Ninety patties (Patty-O-Matic EZ Slider; Patty-O-Matic, In., Farmingdale, NJ) were produced from each carcass ( $113 \pm 2$  g, 13 mm thick) with carcasses processed by alternating treatments. Following processing of each batch, the grinder and patty machine were thoroughly cleaned to prevent treatment cross-contamination. Patties were placed on sausage trays, blast frozen ( $-40^\circ\text{C}$ ) for 20 min, vacuum packaged, and placed in frozen storage ( $-25^\circ\text{C}$ ) for later analysis. Patties per carcass were randomly assigned to proximate analysis, Allo-Kramer shear force, consumer sensory, and trained sensory evaluation.

Chucks were processed and allocated 23 d postmortem following the methods previously described for the Round.

### ***Proximate Analysis***

Patties were thawed for 12 h ( $2 \pm 2^\circ\text{C}$ ), removed from packaging, minced, flash frozen in liquid nitrogen, and homogenized using a Waring blender (Model 34BL97; Dynamics Corporation of America, New Hartford, CT). Samples ( $1 \pm 0.10$ g) were weighed into filter bags (ANKOM XT4; ANKOM Technology, Macedon, NY) and transferred to a drying oven ( $100^\circ\text{C}$ ) for 24 h to analyze moisture. Percent moisture was determined using the following equation:  $(\text{wet sample weight} - \text{dry sample weight}) / (\text{wet sample weight}) \times 100$ . Filter bags following moisture analysis were analyzed for total lipid content (Ankom XT15 Extraction System; Macedon, NY).

Homogenized samples (0.2 – 0.299 g) were placed in foil and nitrogen (protein) content was analyzed using a Leco Nitrogen Analyzer (Model FP268; Leco Corporation, St. Joseph, MI). Sample ash percentage was calculated by difference using the following equation: (100 - % lipid - % moisture - % protein).

### ***Shear Force Analysis***

Allo-Kramer shear force was performed using an Instron Universal Testing Machine (Model 1011; Instron Corporation, Norwood, MA, USA). Patties were thawed ( $2 \pm 2^\circ\text{C}$ ) for 24 h prior to testing and cooked to  $73^\circ\text{C}$  in a  $174^\circ\text{C}$  convection oven (DFG-100-3 Series; G.S. Blodgett Corporation, Burlington, VT) with patty endpoint temperature verified using a probe thermometer (model 450- ATT; OMEGA Engineering, Stamford, CT). A standardized specimen size ( $4 \times 3.5 \text{ cm}^2$ ) was cut, weighed, placed flat in a 5-blade Allo-Kramer shear cell attachment, and analyzed. A 5K N load cell with a 25 cm/min crosshead speed was used to record peak shear force (Bluehill software, Instron Corp.) and expressed as Kgf/g.

### ***Trained and Consumer Sensory Analyses***

Sensory analyses were conducted according to procedures outlined in the American Meat Science Association (AMSA) Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat (AMSA, 2016).

Two patties per carcass and primal were weighed, placed on poly trays with absorbent pads, covered with polyvinyl chloride film, allowed to thaw for  $24 \pm 2 \text{ h}$  ( $2 \pm 2^\circ\text{C}$ ), re-weighed, and cooked following the procedure previously described for shear force analysis. Cooked weights were collected, patties were cut into six equally sized wedges, wrapped in foil, and rested under a

heat lamp before serving to maintain temperature (maximum 10 min). Percent thaw loss and percent cook loss were calculated respectively using the following equations:  $\{[(\text{frozen weight} - \text{thaw weight}) / \text{frozen weight}] \times 100\}$  and  $\{[(\text{thaw weight} - \text{cook weight}) / \text{thaw weight}] \times 100\}$ .

Trained panelists ( $N = 8$ ) participated in evaluating 16 samples daily across two sessions with 4 h between each session and sample order determined by a random number generator (Microsoft Excel; Redmond, WA). All samples, along with a warm-up sample, were served to panelists unseasoned in warmed, numbered yogurt maker (Euro Cuisine, Inc.; Los Angeles, CA) glass jars. Water and unsalted crackers were utilized as palate cleansers. The sensory room contained 8 individual booths with positive airflow and red lighting to conceal differences in patty color. Panelists were given a paper ballot with an 8-point hedonic scale for cohesion/binding (1 = extremely fragile, 2 = very fragile, 3 = moderately fragile, 4 = slightly fragile, 5 = slight cohesion/bind, 6 = moderate cohesion/bind, 7 = very tight cohesion/bind, 8 = extreme cohesion/bind), beef flavor intensity (1 = extremely bland, 2 = very bland, 3 = moderately bland, 4 = slightly bland, 5 = slightly intense, 6 = moderately intense, 7 = very intense, 8 = extremely intense), and juiciness (1 = extremely dry, 2 = very dry, 3 = moderately dry, 4 = slightly dry, 5 = slightly juicy, 6 = moderately juicy, 7 = very juicy, 8 = extremely juicy) and a 6-point scale for off-flavor (1 = none detected, 2 = threshold off-flavor, 3 = slightly intense, 4 = moderate off-flavor, 5 = very strong off-flavor, 6 = extreme off-flavor).

Consumer panel samples were prepared and served as described for trained sensory analysis with modifications. Patties used for consumer sensory were thawed in vacuum bags, cooked, cut into four wedges, and served individually to panelists on paper plates. Consumer panelists ( $N = 112$ ) from Athens, GA and surrounding communities gathered at the UGA Animal and Dairy Science building (Athens, GA) for sensory panels. Prior to panel participation, panelists

were given a brief project overview and oriented to the survey design. Panelists could participate once and were monetarily compensated for their study participation.

Each consumer evaluated 8 samples and recorded ratings on an iPad (Apple, Cupertino, CA) running Qualtrics (Seattle, WA and Provo, UT) survey software. The survey section asked participants to provide demographic information about their gender, marital status, ethnicity, income, education level, weekly ground beef consumption, and most important beef palatability trait. Additionally, participants were asked to identify the specific attribute influence on their purchasing decisions using a 100-point sliding scale with anchors at 0, 20, 40, 60, 80, and 100, where 0 = no influence and 100 = extremely influential. Sample survey questions used 100-point continuous sliding scales to evaluate cohesion, juiciness, beef flavor, off-flavor, and overall palatability (0 = extremely fragile/dry/bland/no off-flavor/overall dislike and 100 = extreme cohesion/juiciness/beef flavor/off-flavor/overall like) with anchors placed at 0, 20, 40, 60, 80, and 100 for reference. Panelists were provided with plastic utensils, toothpicks, napkins, expectorant cup, water cup, and unsalted crackers to cleanse their palate between samples.

### ***Statistical Analysis***

Data were analyzed as a completely randomized design with carcass as the experimental unit and patty as the observational unit. Treatment served as the fixed effect. The PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc, Cary, NC) was utilized and pairwise comparisons between the least square means were computed using the PDIFF option of the LSMEANS statement. Differences were considered statistically significant at  $P \leq 0.05$ , with tendencies determined at  $0.05 > P \leq 0.10$ .

## RESULTS

### *Proximate Analysis, Shear Force Analysis, and Cooked Patty Measures*

There were no Treatment main effects for both Round proximate composition measures, Allo-Kramer shear force, percent thaw loss, and percent cook loss nor Chuck patty proximate composition measures, Allo-Kramer shear force, and percent thaw loss ( $P > 0.15$ ; Table 3.2). Chuck OMG patty percent cook loss tended ( $P = 0.10$ ) to be less than CON patty percent cook loss.

### *Sensory Analysis*

Demographic information for consumer sensory panels is summarized in Table 3.3. There were no Treatment main effects for Round trained sensory ratings of cohesion, juiciness, and off-flavor, nor for Chuck trained sensory scores of cohesion and off-flavor ( $P > 0.23$ ; Table 3.4); however, CON patties had decreased sensory scores for Round beef flavor and Chuck juiciness compared to OMG patties ( $P < 0.02$ ). Chuck CON beef flavor scores tended ( $P = 0.09$ ) to be less than OMG patty scores.

There were no Treatment main effects for Round consumer ratings of cohesion, juiciness, beef flavor, and overall palatability scores, nor for Chuck consumer ratings of off-flavor ( $P > 0.15$ ; Table 3.4); however, CON Chuck consumer cohesion, beef flavor, and overall palatability scores were smaller than OMG scores ( $P < 0.05$ ). Round OMG consumer off-flavor scores tended to be reduced compared to CON patty scores. Chuck OMG consumer juiciness scores tended to be greater than CON patty scores ( $P = 0.07$ ).

## DISCUSSION

Meat consumption trends vary among differing cultures and nations; however, with an expected 34% world population increase by 2030, it is predicted dietary demand for meat will reach 255,877 metric tons by 2027 (Fiala, 2008; OECD-FAO, 2018). Consumers are becoming increasingly more aware of where their food originates, and the nutritional value associated with it. Beef is a protein source containing many essential vitamins and minerals. A single, 85 g (3 oz) beef serving contains 25 g of protein as well as 101%, 53%, and 14% of daily recommended intake values for Vitamin B<sub>12</sub>, Zinc, and Iron, respectively (Zanovec et al., 2010). Despite being a nutritionally dense protein source, beef has been placed under public scrutiny due to containing elevated saturated fatty acid contents. The *Dietary Guidelines for Americans* recommends an individual to obtain 20 to 35% of daily calories from fat, with less than 10% derived from saturated fat sources (USDA and U.S. Department of Health and Human Services, 2020). The popularity of beef among consumers coupled with its undesirable fatty acid profile has directed research to explore nutritional regimens that enhance its PUFA content.

Studies utilizing microalgae and flaxseed are encouraging for beef product PUFA profile enhancement, but many often report negative palatability attributes of products originating from the supplemented cattle. Palatability is the term characterizing the overall eating experience of a food product. In beef, tenderness, juiciness, flavor, and their interaction influence product palatability (Smith and Carpenter, 1974; O'Quinn et al., 2018). Industry and research emphasis on tenderness has caused the modern beef supply to become more reliably tender via genetic selection and technological advancement. Flavor has been reported to be correlated to overall palatability success, once tenderness is deemed acceptable (Legako et al., 2015; Lucherker et al., 2016). Thus,

consumers have begun to place greater emphasis on product flavor and juiciness (Guelker et al., 2013).

Wistuba et al. (2006) found supplementing steers 3% fish oil during finishing did not influence Warner-Bratzler Shear Force or steak cook loss values, which is supported by the present study. Using two separate sensory panels, Vatansever et al. (2000) found sirloin steaks from steers fed fish oil had decreased tenderness scores when panelists evaluated the steaks using an 8-point hedonic scale, but observed no differences when panelists used a 100-mm line scale. In the current study, differences were observed regardless if panelists used an 8-point hedonic scale or 100-mm line scale for palatability attribute evaluation. Moreover, trained panelists reported increased beef flavor ratings for Round and Chuck OMG patties, as well as increased juiciness scores for OMG Chuck patties, translating OMG patties having a greater beef identity and juiciness perception. Phelps et al. (2016a and 2016b) fed increasing microalgae meal concentrations (*Schizochytrium limacinum* CCAP 4087/2) to beef heifers and found as supplementation increased, off-flavor prevalence increased and was deemed undesirable by panelists for both *Longissimus lumborum* steaks and ground beef patties, with the most common reported off-flavors characterized as fishy, grassy, or oxidized. Similarly, Wistuba et al. (2006) claimed *Longissimus dorsi* steaks originating from steers supplemented with fish oil had elevated fishy, abnormal flavors ratings. In the present study, Round OMG off-flavors scores tended to be less than CON patties in the present study and consumers found greater overall satisfaction from Chuck patties. In agreement but using dairy products, Franklin et al. (1999) found microalgae (*Schizochytrium* sp.) supplementation did not negatively alter milk flavor perception when evaluated by consumers.

Overall, greatOplus supplementation does not appear to adversely impact ground beef patty sensory perception like many other n-3 feed additives have in previous studies. Thus, patties

originating from steers supplemented with greatOplus may be more marketable to consumers due to observed palatability attribute enhancements.

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**Table 3.1.** Finishing diet composition (dry-matter basis) for steers fed a conventional feedlot diet (CON) or a conventional feedlot diet with greatOplus <sup>1</sup> (OMG)

Ingredient, %	CON	OMG
Steam-flaked corn	58.27	50.84
Wet corn gluten feed	20.00	20.00
Corn silage	15.00	15.00
greatOplus	0.00	10.00
Soybean meal, dehulled	2.57	0.00
Vitamin/mineral premix <sup>2</sup>	4.16	4.16
Nutrient composition		
Crude protein, %	14.00	14.00
NEg, Mcal/kg	1.46	1.48
Ether extract, %	3.18	5.19
Calcium, %	0.68	0.70
Phosphorus, %	0.38	0.42
Potassium, %	1.02	1.05
Sodium, %	0.35	0.35
Vitamin A (added), IU/kg	2,205.00	2,205.00
Vitamin E (added), IU/kg	22.00	22.00
Monensin, mg/kg <sup>3</sup>	33.00	33.00

<sup>1</sup>greatOplus is a proprietary extruded blend of flaxseed, *Nannochloropsis oculata* algal biomass, and wheat middlings.

**Table 3.2** Least squares means for proximate analysis, Allo-Kramer shear force values, and cooked measures of 90/10 Round and 80/20 Chuck beef patties originating from beef steers fed either a conventional feedlot diet (CON) or a conventional feedlot diet with greatOplus<sup>1</sup> (OMG)

	CON	OMG	SEM <sup>2</sup>	P-value
<b>Round</b>				
Protein, %	19.78	20.08	0.20	0.28
Lipid, %	7.38	6.98	0.44	0.53
Moisture, %	71.36	71.50	0.45	0.82
Ash, %	1.48	1.43	0.27	0.89
Allo-Kramer, Kgf/g	4.17	4.17	0.19	0.99
Thaw loss, %	4.07	4.26	0.88	0.88
Cook loss, %	30.35	31.38	1.37	0.59
<b>Chuck</b>				
Protein, %	19.22	19.43	0.32	0.65
Lipid, %	13.72	13.82	0.53	0.89
Moisture, %	65.76	66.02	0.41	0.65
Ash, %	1.30	0.73	0.30	0.19
Allo-Kramer, Kgf/g	3.43	3.57	0.15	0.49
Thaw loss, %	2.65	3.96	0.64	0.15
Cook loss, %	30.77	28.16	1.09	0.10

<sup>1</sup>greatOplus is a proprietary extruded blend of flaxseed, *Nannochloropsis oculata* algal biomass, and wheat middlings.

<sup>2</sup>Largest standard error of the least square means is presented.

**Table 3.3.** Consumer panelist ( $N = 112$ ) demographics for sensory panels evaluating 90/10 Round and 80/20 Chuck patties originating from beef steers fed either a conventional feedlot diet or a conventional feedlot diet with greatOplus<sup>1</sup>

Characteristic	Response	Consumer percentage
<b>Gender</b>	Male	46.43
	Female	53.57
<b>Marital Status</b>	Married	60.71
	Single	39.29
<b>Age</b>	20-29	29.46
	30-39	19.64
	40-49	17.86
	50-59	17.86
	60 and above	15.18
<b>Ethnic origin</b>	Asian	0.89
	Caucasian	89.29
	Hispanic	3.57
	Mixed race	4.46
	Other	1.79
<b>Annual income</b>	Under \$25,000	15.18
	\$25,000-\$34,999	6.25
	\$35,000-\$49,999	8.93
	\$50,000-\$74,999	16.07
	\$75,000-\$99,999	23.21
	\$100,000 and above	30.36
<b>Education level</b>	High school graduate	4.46
	Some college/technical school	16.96
	College graduate	49.12
	Postgraduate	29.46
<b>Weekly beef consumption</b>	1-3 times	66.96
	4-6 times	32.14
	7 or more times	0.90
<b>Most important beef palatability trait</b>	Flavor	66.08
	Juiciness	16.96
	Tenderness	16.96
<b>Attribute influence on purchasing decision</b>	Color	60.71
	Animal welfare	37.50
	Price	64.29
	Nutrient content	47.32
	Omega-3 content	21.43
	Eating satisfaction claims	45.54

<sup>1</sup>greatOplus is a proprietary extruded blend of flaxseed, *Nannochloropsis oculata* algal biomass, and wheat middlings.

**Table 3.4.** Least squares means for trained panel ( $N = 8$ ) and consumer panel ( $N = 112$ ) sensory ratings of 90/10 Round and 80/20 Chuck beef patties originating from beef steers fed either a conventional feedlot diet (CON) or a conventional feedlot diet with greatOplus (OMG)

	CON	OMG	SEM <sup>1</sup>	<i>P</i> -value
<b>Round trained sensory scores<sup>2</sup></b>				
Cohesion	4.23	4.19	0.16	0.86
Juiciness	3.39	3.48	0.18	0.71
Beef flavor	3.38	3.71	0.08	0.01
Off-flavor	1.31	1.39	0.07	0.50
<b>Round consumer sensory scores<sup>3</sup></b>				
Cohesion	48.99	45.98	2.42	0.39
Juiciness	38.66	35.91	2.78	0.49
Beef flavor	48.97	49.50	1.69	0.83
Off-flavor	23.02	18.87	1.57	0.07
Overall palatability	46.61	44.86	2.49	0.63
<b>Chuck trained sensory scores</b>				
Cohesion	3.86	3.65	0.12	0.23
Juiciness	4.03	4.42	0.11	0.02
Beef flavor	3.56	3.80	0.08	0.09
Off-flavor	1.13	1.21	0.05	0.30
<b>Chuck consumer sensory scores</b>				
Cohesion	59.25	65.85	1.85	0.02
Juiciness	56.92	63.55	2.40	0.06
Beef flavor	58.02	62.30	1.50	0.05
Off-flavor	17.39	14.36	1.44	0.15
Overall palatability	58.22	64.96	1.78	0.01

<sup>1</sup>Largest standard error of the least square means is presented.

<sup>2</sup>Cohesion: 1 = extremely fragile and 8 = extreme cohesion; Juiciness: 1 = extremely dry and 8 = extremely juicy; Beef flavor: 1 = extremely bland and 8 = extremely intense; Off-flavor: 1 = none detected and 6 = extreme off-flavor.

<sup>3</sup>Cohesion: 0 = extremely fragile and 100 = extreme cohesion; Juiciness: 0 = extremely dry and 100 = extremely juicy; Beef flavor: 0 = extremely bland and 100 = extremely intense; Off-flavor: 0 = none detected and 100 = extreme off-flavor; Overall palatability: 0 = extremely dislike and 100 = extremely like.

CHAPTER 4

FEEDING MICROALGAE AND FLAXSEED (*Nannochloropsis oculata*) TO  
BEEF STEERS II: EFFECTS ON 90/10 ROUND AND 80/20 CHUCK GROUND BEEF  
PATTY COLOR STABILITY<sup>1</sup>

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<sup>1</sup>Catrett, C.C., A.M. Stelzleni, J.S. Drouillard, S.A. Devane, G.A. McKinney, X. Li, K.A. Turner,

D.A. Alambarrio, and J.M. Gonzalez. To be submitted to *J. Anim. Sci.*

## ABSTRACT

The objective was to determine effects of feeding greatOplus on ground beef color stability. Black-hided steers ( $N = 700$ ) were weighed and allocated to 28 pens ( $N = 25$  head/pen), with pens randomly assigned to one of two treatments consisting of being fed either a conventional finishing diet (**CON**;  $n = 14$ ) or a conventional feedlot diet containing 10% (dry-basis) of an extruded proprietary flaxseed, wheat middling, and *Nannochloropsis oculata* algae blend (**OMG**;  $n = 14$ ). After 173 d on feed, one head per pen was randomly selected, transported, harvested, and fabricated at a commercial abattoir. Round and Chuck subprimals were vacuum packaged and shipped to University of Georgia Meat Science Technology Center. On d 21 and 23 postmortem, Round and Chuck muscles were coarse and fine ground, producing thirty-four ( $113 \pm 2$  g, 13-mm thick) patties per carcass and primal, with random allocation to objective and subjective color, thiobarbituric acid reactive substances (**TBARS**), metmyoglobin reduction activity (**MRA**), or oxygen consumption rate (**OCR**) analyses. Color data were collected every 12 h for 84 h, with TBARS, MRA, and OCR measured every 24 h. There were Time effects for all measurements consistent with ground beef discoloration ( $P < 0.01$ ). There were no Treatment  $\times$  Time interactions nor Treatment effects for  $a^*$ , chroma, worst point color, overall color, and discoloration ratings ( $P > 0.20$ ). Treatment  $\times$  time interactions occurred for Chuck  $L^*$ , as well as, Round hue angle, percent surface metmyoglobin, and percent surface deoxymyoglobin ( $P < 0.05$ ). At differing time points for each attribute, CON patties presented elevated  $L^*$ , hue angle, and percent surface metmyoglobin values and decreased percent surface deoxymyoglobin than OMG patties ( $P < 0.05$ ). There were no Treatment  $\times$  Time interactions ( $P > 0.24$ ) for TBARS; however, throughout display, CON patties had greater TBARS values than OMG patties ( $P < 0.05$ ). While CON patty surface metmyoglobin percentages and TBARS values indicated decreased color stability than

OMG patties, subjective color panelists indicated differences were not visually apparent. There were no Treatment  $\times$  Time interactions nor Treatment effects for MRA and OCR ( $P > 0.21$ ); however, there were time main effects for each assay consistent with discoloration ( $P < 0.05$ ). Overall, greatOplus supplementation did not adversely affect ground beef patty color stability as other omega-3 enriched feeding regimens have in previous literature.

**KEY WORDS:** color, finishing diet, ground beef, microalgae, omega-3 fatty acid

## INTRODUCTION

Since 1998, consumers have increased their beef spending habits by approximately \$113 per person (Speer et al., 2015). Ground beef is the largest fundamental beef product category, with consumption estimated to be approximately 12.3 kg per capita in the United States (Schulz, 2021). Beef is a nutritionally dense protein source containing many essential nutrients (Zanovec et al., 2010), but contains negligible amounts of essential omega-3 fatty acids due to ruminal polyunsaturated fatty acid (PUFA) biohydrogenation (Harfoot, 1978). Omega-3 fatty acids, namely  $\alpha$ -linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been acknowledged to promote health by reducing the risk of developing cardiovascular disease (Ruxton et al., 2004; Calder, 2016). Shahidi and Ambigaipalan (2018) recommended consuming two fish servings weekly to obtain 0.30 to 0.45 g of EPA and DHA; however, 9 out of 10 Americans do not meet seafood consumption recommendations (USDA and HHS, 2020). Givens and Gibbs (2006) reviewed beef's role as a potential PUFA source and concluded human omega-3 fatty acid intake could be enhanced via animal diet supplementation.

Literature reports beef PUFA content was enhanced via oilseed, forage, and fish-derived product feed supplementation (Woods and Fearon, 2009; Vahmani et al., 2015), but negative fresh quality attributes were often documented. Phelps et al. (2016b) reported feeding microalgae meal (*Schizochytrium limacinum* CCAP 4087/2) elevated ground beef omega-3 content; however, as microalgae supplementation increased, beef patty color measurements rapidly decreased below consumer acceptability thresholds (Mancini et al., 2022). Vatansever et al. (2000) noted decreased color stability and increased lipid oxidation in ground beef patties derived from cattle fed flaxseed and fish oil. Excluding price, meat color is the most important fresh quality attribute influencing consumer purchasing decisions (Killinger et al., 2004). When metmyoglobin (**MMb**) reaches 20% surface coverage, meat is discounted and after reaching 40% coverage, meat is rejected by consumers (McMillin, 2008). Globally, annual meat discoloration product and revenue losses are estimated to be 5.8 metric tons and \$14.2 billion (Maia Research Analysis, 2020). Ramanathan et al. (2022) reported in the United States 13.4 million kg of beef are discarded due to discoloration, translating to a \$3.73 billion annual economic loss.

Thus, the study objective was to examine the effects of feeding a microalgae and flaxseed blend (greatOplus; *Nannochloropsis oculata*; NBO3 Technologies, Manhattan, KS) to finishing steers on 90/10 Round and 80/20 Chuck ground beef patty color stability.

## **MATERIALS AND METHODS**

All experimental procedures involving animals were approved by the Kansas State University Institutional Animal Care and Use Committee and the University of Georgia (UGA) Institutional Review Board approved all procedures for human subject use in sensory panel evaluations.

### ***Live Cattle Management***

A more detailed description of steer management is described in Chapter 3. Briefly, black-hided yearling steers were purchased from sale barns in Kansas and Nebraska and transported to the Kansas State University Beef Cattle Research Center (Manhattan, KS), where they were acclimated, individually weighed, separated into 14 weight blocks, and allocated to 28 feedlot pens ( $N = 25$  head/pen), with pens randomly assigned to one of two treatments consisting of a control diet without supplemental omega-3 fatty acids (**CON**; Table 4.1) or a diet containing 10% (dry basis) of an extruded proprietary blend of flaxseed, wheat middling, and *Nannochloropsis oculata* algae (**OMG**; greatOplus; NBO3 Technologies, Manhattan, KS). Cattle were fed twice daily, *ad libitum* for a total of 175 d and on d 173, randomly one head per pen ( $n = 14$ /treatment) was weighed, transported to a commercial abattoir (Hertzog Meat Company, Butler, MO), harvested, and fabricated, with vacuum packaged Round and Chuck subprimals shipped 13 d postmortem and delivered 17 d postmortem to the UGA Meat Science and Technology Center (Athens, GA).

### ***Muscle Sampling, Ground Beef Manufacture, and Sample Packaging***

After delivery, boxes were sorted by carcass and primal. Each carcass and primal were processed independently. Vacuum packaged round subprimals were further processed 24 h prior to grinding to remove bone and cube meat for grinding. Individually, cubed round muscles were placed in a plastic-lined, dark storage boxes to maintain carcass identification and stored overnight ( $2 \pm 2^\circ\text{C}$ ). Round grinding occurred 21 d postmortem with each carcass processed separately. Meat was placed in a grinder (Daniels AMFG-50; Daniels Food Equipment, Parkers Prairie, MN), coarse ground (13 mm plate) and fine ground (6 mm plate) prior to patty formation (Patty-O-Matic EZ Slider; Patty-O-Matic, In., Farmingdale, NJ). Thirty-four patties ( $113 \pm 2$  g, 13 mm thick) were

produced from each carcass, with carcasses processed by alternating treatments. Following processing of each batch, the grinder and patty machine were thoroughly cleaned to prevent treatment cross-contamination. Patties per carcass were packaged on a white 2S foam tray with a Dri-Loc 50 absorbent pad (Cryovac Sealed Air Corp., Duncan, SC) and overwrapped with polyvinyl chloride film (**PVC**; AEP Industries Inc., South Hackensack, NJ) with an oxygen transmission rate of  $1,450 \text{ cm}^{-3} \cdot 645.2 \text{ cm}^{-2} \cdot 24 \text{ h}^{-1}$  and randomly assigned to objective and subjective color evaluation, metmyoglobin reducing activity (**MRA**), oxygen consumption rate (**OCR**), and thiobarbituric acid reactive substances (**TBARS**) analysis. Twelve trays were displayed per carcass and primal with two packages designated for daily color evaluation and ten packages for MRA, OCR, and TBARS assays, which were randomly removed and assigned.

Chucks were processed and allocated 23 d postmortem following the methods previously described for the Round.

### ***Simulated Retail Display***

Trays were placed in two coffin style retail display cases (M1X, Hussmann Corp.; zBridgeton, MO) for 84 h under 24 h continuous warm-white, fluorescent lighting (Octron/ECO; 30000K; F032/830/ECO; Sylvania Company, Versailles, KY; 1844 Lux). Retail case temperature was monitored and recorded using digital temperature loggers (TR-50U2; T & D Corp., Japan). The mean daily case temperature was  $2.6 \pm 1^\circ\text{C}$  and the case was defrosted twice daily (morning and evening) at  $11^\circ\text{C}$  for 30 min. Trays were rotated within the case every 12 h from left to right and front to back to account for variation in temperature and light intensity within the case. Randomly, every 24 h, two trays per carcass and primal were removed from display with one tray

allocated to fresh MRA and OCR analyses while the other was vacuum packaged, frozen ( $-25 \pm 2^\circ\text{C}$ ), and stored until TBARS analysis.

### ***Instrumental and Visual Color Evaluation***

Two trays containing two patties for each carcass and primal were used to collect Commission Internationale de l'Eclairage (International Commission on Illumination)  $L^*$ ,  $a^*$ , and  $b^*$  values with a MiniScan (Model 4500; HunterLab; Reston, VA) using Illuminant A, 31.8 mm aperture, and  $10^\circ$  observer. Daily, twelve measurements were taken across the two trays and averaged for each carcass. Initial measurements were collected after approximately 2 h of bloom time at  $2 \pm 2^\circ\text{C}$ . Measurements were taken every 12 h, beginning at 0 h through 84 h. Spectral reflectance values were used to calculate percent surface oxymyoglobin, deoxymyoglobin, and metmyoglobin proportions following American Meat Science Association (AMSA) Meat Color Evaluation Guidelines (AMSA, 2012) adapted from Krzywicki (1979). Values for  $L^*$ ,  $a^*$ , and  $b^*$  were used to calculate delta E (change in color), hue angle (color), and chroma (saturation index) using the following equations:  $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + \Delta b^*)^2]^{1/2}$ ; hue angle = arctangent ( $b^*/a^*$ ); chroma =  $(a^{*2} + b^{*2})^{1/2}$ .

Trays used for instrumental color evaluation were used for daily visual color panels. Panelists ( $N = 8$ ) were screened using the Farnsworth–Munsell 100 Hue Color Vision Test and oriented according to scales published by AMSA (2012). Panelists evaluated patties every 12 h from 0 h to 84 h for surface color and worst point color (0 = bright red or reddish-pink, 20 = dull red or reddish-pink, 40 = dark red or reddish-pink, 60 = slightly brownish red, 80 = moderately brownish red, 100 = brown/gray), as well as percent discoloration (0 = 0 – 4%, 20 = 5 – 20%, 40 = 21 – 40%, 60 = 41 – 60%, 80 = 61 – 80%, 100 = 81 – 100%) electronically using iPads (Apple,

Cupertino, CA) running Qualtrics (Seattle, WA and Provo, UT) survey software. Subjective color attributes were evaluated on 100-point continuous line scales with anchors at 0, 20, 40, 60, 80, and 100 (Phelps et al., 2016a).

### ***Lipid Peroxidation (TBARS) Analysis***

Daily during display, trays for TBARS analysis were randomly removed from the case, vacuum packaged, frozen ( $-25 \pm 2^{\circ}\text{C}$ ), and stored until later analysis. Lipid peroxidation analyses were performed using the rapid, wet TBARS method described by Buege and Aust (1978). Samples were thawed for 12 h, homogenized using a food processor (Model 70550; Hamilton Beach Manufacturing Company, Glen Allen, VA), and duplicate 0.5 g samples were placed in 15 mL screw-capped conical tubes. Thiobarbituric acid/trichloroacetic acid stock solution was added to each tube, loosely capped, and placed in a boiling water bath ( $100 \pm 2^{\circ}\text{C}$ ) for 10 min. Tubes were cooled in a water bath ( $25^{\circ}\text{C}$ ) for 10 min, centrifuged for 15 min at  $4,000 \times g$  at  $4^{\circ}\text{C}$ , with supernatant transferred into a  $12 \times 75$  mm culture tube and sample absorbance read at 532 nm (Jasco V-630 Spectrophotometer, Jasco Inc., Easton, MA). All samples were evaluated in duplicate and those with a coefficient of variation greater than 10% were re-analyzed. Thiobarbituric acid reactive substances values were expressed as mg of malondialdehyde per kg of muscle tissue.

### ***Metmyoglobin Reduction Activity and Oxygen Consumption Rate***

Methods described by Phelps et al. (2016a) were conducted for MRA and OCR with modifications. Daily, trays for MRA and OCR analyses were randomly removed from the case and a representative sample was cut from each patty using a 5.1 cm diameter  $\times$  1.3 cm culture dish.

The displayed surface was used for both MRA and OCR evaluation, with one culture dish randomly assigned to MRA and the other to OCR.

For MRA analysis, each dish was submerged for 20 min at room temperature in a 400-mL beaker containing 200 mL of 0.3% sodium nitrite solution. Following submersion, samples were blotted of excess solution and vacuum packaged in 15.2 cm × 30.5 cm vacuum bags (3 mil standard barrier, Prime Source Vacuum Pouches; Bunzl Processor Division, Koch Supplies, Kansas City, MO) that possessed an oxygen transmission rate of  $4.5 \text{ cm}^3 \cdot 100 \text{ cm}^{-2} \cdot 24 \text{ h}^{-1}$  at 30 °C and sealed using a GS Multivac (Model A-300-116; Multivac Inc., Kansas City, MO). Samples were placed in a Boekel incubator (Model 132000; Boekel Industries, Feasterville, PA) operating at 30°C and rescanned with reflectance measurements (400 to 700 nm) taken at 0, 1, and 2 h using a MiniScan (Model 4500; HunterLab; Reston, VA). Spectral reflectance values at 525, 572, and 700 were used to calculate percent MMb formation. Metmyoglobin reduction activity was calculated using the formula:  $[(\text{Initial \% MMb} - \text{Final \% MMb}) / \text{Initial \% MMb}] \times 100$  (AMSA, 2012).

The assigned culture dish for OCR analysis was placed in a vacuum bag, sealed, and scanned immediately after vacuum packaging with reflectance measurements (400 to 700 nm) recorded. Samples were placed in a Boekel incubator (Model 132000; Boekel Industries, Feasterville, PA) operating at 25°C and rescanned at 20 min intervals for 1 h. Oxygen consumption rate activity was calculated using the formula:  $[(\text{Initial \% Omb} - \text{Final \% Omb}) / \text{Initial \% Omb}] \times 100$  (AMSA, 2012).

### *Statistical Analyses*

Data were analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc, Cary, NC), with carcass as the experimental unit, patty as the observational unit, and the fixed effects of

treatment, time, and their interaction. Instrumental and visual color data were analyzed as a completely randomized design (**CRD**) with repeated measures, with time (hour of display) serving as the repeated measure, carcass as the subject, and compound symmetry as the covariance structure. Round data for MRA and OCR were analyzed as a CRD with a  $2 \times 4$  factorial arrangement, Chuck MRA and OCR data were analyzed as a CRD with a  $2 \times 2$  factorial arrangement, and both primal TBARS data were analyzed separately as a CRD with a  $2 \times 5$  factorial arrangement, each with the same aforementioned fixed effects. Pairwise comparisons between the least square means were computed using the PDIFF option of the LSMEANS statement. Differences were considered statistically significant at  $P \leq 0.05$ .

## **RESULTS**

### ***Instrumental and Visual Color Evaluation***

There were no Treatment  $\times$  Time interactions nor Treatment main effects for Round L\*, a\*, and b\* values ( $P > 0.15$ ; Fig. 4.1); however, there were Time main effects for each value ( $P < 0.01$ ). L\* values did not differ at 24, 36, 60, and 84 h ( $P > 0.10$ ), but were less than values recorded at 0, 12, and 48 h ( $P < 0.01$ ). Hours 0, 12, 48, and 72 differed in L\* values with 0 h being the greatest L\* value and 48 h the smallest recorded. A\* values differed and declined at all time points ( $P < 0.01$ ), except at 72 and 84 h where values did not differ ( $P > 0.99$ ). b\* values did not differ at 0 and 12 h ( $P > 0.66$ ) but differed and declined at all other time points ( $P < 0.01$ ).

No Treatment  $\times$  Time interactions occurred for Chuck a\* and b\* values ( $P > 0.15$ ; Fig. 4.2) but a Treatment  $\times$  Time interaction occurred for L\* ( $P = 0.05$ ). From h 0 to 72 h CON patties contained elevated L\* values than OMG patties ( $P < 0.01$ ) but did not differ at each time point. There were no Treatment effects on a\* and b\* values ( $P > 0.17$ ); however, there were Time main

effects for each value ( $P < 0.01$ ).  $a^*$  values differed and declined at all time points ( $P < 0.01$ ), excluding 48 and 60 h ( $P > 0.16$ ).  $b^*$  values did not differ from 48 to 84 h ( $P > 0.09$ ) but differed and declined from 0 to 36 h ( $P < 0.01$ ).

There were no Treatment  $\times$  Time interactions nor Treatment effects for Round chroma and delta E ( $P > 0.29$ ; Fig. 4.3); however, there was a Treatment  $\times$  Time interaction ( $P = 0.04$ ) for hue angle and Time main effects for chroma and delta E measurements ( $P < 0.01$ ). From 0 to 60 h, CON and OMG patties did not differ in hue angle ( $P > 0.21$ ), but CON patties exhibited greater hue angle values than OMG patties at 72 and 84 h ( $P < 0.01$ ). Round chroma values did not differ from 0 to 12 h and 72 to 84 ( $P > 0.08$ ) but differed from each other and declined at all other time points ( $P < 0.01$ ). Delta E values did not differ ( $P > 0.56$ ) at 72 and 84 h but differed and increased from 0 to 72 h ( $P < 0.01$ ).

There were no Treatment  $\times$  Time interactions nor Treatment effects for Chuck hue angle, chroma, and delta E ( $P > 0.18$ ; Fig. 4.4); however, there were Time main effects for each ( $P < 0.01$ ). Hue angle values did not differ from 0 to 12 h nor 36 to 48 h ( $P > 0.22$ ) but differed and declined at all other time points with the smallest value recorded at 84 h ( $P < 0.01$ ). Chroma values differed and declined at all time points ( $P < 0.01$ ), excluding 48 and 60 h ( $P > 0.42$ ). Delta E values did not differ ( $P > 0.34$ ) from 48 to 60 h but differed from 0 to 36 h and 72 to 84 h with the greatest value reported at 60 h ( $P < 0.01$ ).

Treatment  $\times$  Time interactions occurred for Round percent surface metmyoglobin and deoxymyoglobin ( $P < 0.05$ ; Fig. 4.5), but not for percent surface oxymyoglobin ( $P = 0.90$ ). From 0 to 60 h, CON and OMG percent surface metmyoglobin did not differ ( $P > 0.35$ ), but at 72 and 84 h, CON patties had elevated percent metmyoglobin and decreased percent deoxymyoglobin than OMG patties ( $P < 0.05$ ). There were no Treatment effects ( $P = 0.89$ ) for percent surface

oxymyoglobin; however, time main effects occurred ( $P < 0.01$ ). Surface oxymyoglobin values did not differ ( $P > 0.53$ ) from 0 to 12 h but differed and declined from 24 to 84 h consistent with ground beef discoloration ( $P < 0.01$ ).

There were no Treatment  $\times$  Time interactions nor Treatment effects for Chuck percent surface metmyoglobin, oxymyoglobin, and deoxymyoglobin ( $P > 0.22$ ; Fig. 4.6); however, there were Time main effects for each value ( $P < 0.01$ ). Surface metmyoglobin values did not differ from 0 to 12 h nor 24 to 48 h ( $P > 0.06$ ) but differed and declined at all other time points ( $P < 0.01$ ). From 60 to 72 h, surface oxymyoglobin values did not differ ( $P = 0.13$ ) but varied and declined at all other time points with the smallest value reported at 84 h ( $P < 0.01$ ). Surface deoxymyoglobin values did not differ ( $P = 0.93$ ) from 0 to 12 h but differed and increased at all other time points with the greatest percentage reported at 84 h ( $P < 0.01$ ).

There were no Treatment  $\times$  Time interactions nor Treatment main effects for Round overall color, worst point color, and discoloration ( $P > 0.20$ ; Fig. 4.7); however, there were Time main effects for each subjective color attribute consistent with ground beef discoloration ( $P < 0.01$ ). Overall color values did not differ from 0 to 36 h ( $P > 0.14$ ) but differed and increased at all other time points ( $P < 0.01$ ). Worst point color values did not differ from 0 to 12 h, 24 to 36 h, and 72 to 84 h ( $P > 0.09$ ) but differed from 48 to 72 h, with the smallest value reported at 0 h and the greatest value at 84 h ( $P < 0.01$ ). Discoloration values differed and increased from 48 to 60 h ( $P < 0.01$ ) but did not differ from 0 to 36 h and 72 to 84 h, with the smallest value at 12 h and the greatest value at 84 h ( $P > 0.12$ ).

There were no Treatment  $\times$  Time interactions nor Treatment main effects for Chuck overall color, worst point color, and discoloration ( $P > 0.09$ ; Fig. 4.8); however, there were Time main effects for each subjective color attribute ( $P < 0.01$ ). Overall color and discoloration values did not

differ from 0 to 12 h and 48 to 72 h ( $P > 0.08$ ) but differed and increased at remaining time points, with the greatest values reported at 48 h ( $P < 0.01$ ). Worst point color values differed from each other and increased from 0 to 48 h ( $P < 0.01$ ) but did not differ from 48 to 72 h, with the greatest value reported at 48 h ( $P > 0.07$ ).

### ***Lipid Peroxidation (TBARS) Analysis***

There were no Treatment  $\times$  Time interactions for Round patty TBARS values ( $P = 0.24$ ; Fig. 4.9); however, there were observed Time and Treatment main effects where d 0, 1, and 2 did not differ ( $P > 0.17$ ) but differed on d 3 and 4 ( $P < 0.01$ ) with the greatest TBARS value recorded on d 4. On each analysis day, CON patties presented elevated TBARS values than OMG patties with the greatest value reported on d 4 ( $P < 0.01$ ).

There were no Treatment  $\times$  Time interactions for Chuck patty TBARS values ( $P = 0.48$ ; Fig. 4.10); however, there were observed Time and Treatment main effects where d 0, 1, and 2 did not differ ( $P > 0.07$ ) but differed on d 3 and 4 ( $P < 0.04$ ) with the greatest TBARS value recorded on d 4. On each day of analysis, CON patties presented elevated TBARS values than OMG patties with the greatest value reported on d 4 ( $P < 0.01$ ).

### ***Metmyoglobin Reduction Activity and Oxygen Consumption Rate***

There were no Treatment  $\times$  Time interactions nor Treatment main effects for Round MRA and OCR ( $P > 0.21$ ; Fig. 4.11); however, there were Time main effects for each assay ( $P < 0.01$ ). Metmyoglobin reduction activity values did not differ at d 0, 1, and 4 ( $P = 0.34$ ) and were greater than d 2 MRA values. Day 2 MRA values differed ( $P = 0.02$ ) from d 0, 1, and 4, with d 2 presenting

the smallest values. Oxygen consumption rate values differed and declined each display day with the greatest OCR values observed on d 0 and the smallest values on d 4 ( $P < 0.01$ ).

There was no Treatment  $\times$  Time interaction, Treatment main effect, nor Time main effect for Chuck MRA values, as well as no Treatment  $\times$  Time interaction nor Treatment main effect for Chuck OCR ( $P > 0.13$ ; Fig. 4.12); however, there was a Time main effect for Chuck OCR values ( $P = 0.05$ ), with d 0 presenting increased OCR values than d 1.

## DISCUSSION

Excluding price, color is the most impressionable fresh quality attribute influencing consumer retail decisions accounting for approximately 66% of consumer purchasing decisions regardless of personal color preference (Killinger et al., 2004). As products are displayed for extended time, oxymyoglobin (bright-red color) decreases and metmyoglobin (brown/gray color) increases. When MMb reaches 20% surface coverage, meat is discounted and after reaching 40% coverage, meat is rejected by consumers (McMillin, 2008). Faustman et al. (1998) stated beef consumers prefer bright, cherry-red color beef products; thus, extending and maintaining product redness during retail display increases profitability and sustainability. Meat discoloration not only impacts consumer purchasing decisions, but also product discounts and discard. Ramanathan et al. (2022) reported \$1.18 billion is lost due to discounted meat and \$2.55 billion is lost due to discarded meat in the United States annually. Combined, these translate to a \$3.73 billion loss for the beef industry from meat discoloration (Ramanathan et al., 2022). Globally, meat discoloration product and revenue loss are estimated to be 5.8 metric tons and \$14.2 billion annually (Maia Research Analysis, 2020).

Previous studies utilizing flaxseed, microalgae, and marine-sourced supplements reported improvement in meat product fatty acid profiles, but with negative effects on product color stability. Fatty acids containing two or more double bonds in their chains are more susceptible to oxidation. Oxidation of EPA and DHA can cause myoglobin pigment oxidation, leading to beef products presenting elevated surface metmyoglobin formation (Jacobsen, 2008). An assay commonly used to evaluate oxidation and discoloration in meat is TBARS. Younathan and Watts (1959) reported consumers can detect product oxidization when a TBARS value greater than 1 mg malonaldehyde per kg of muscle tissue is recorded. Phelps et al. (2016a) evaluated the impact of supplementing beef heifers with increasing microalgae meal (*Schizochytrium limacinum* CCAP 4087/2) concentrations on *Longissimus lumborum* color stability and found as supplementation increased TBARS values increased quadratically by 0.1, 0.5, and 0.7 mg malonaldehyde/kg meat on retail d 0 and 0.5, 1, and 1.5 mg malonaldehyde/kg meat on display d 7. It should be noted on d 0 for algae100 and algae150 groups, TBARS values already exceeded the 1 mg TBARS threshold value and on d 7 all treatment groups surpassed the threshold value. In the present study, there were observed Time effects consistent with product discoloration and Treatment effects where at each time point CON patties contained elevated TBARS values on average by 0.28 mg malonaldehyde/kg meat for the Round and 0.15 mg malonaldehyde/kg meat for the Chuck than OMG patties. While there were significant Treatment effects in the present study, it should be noted TBARS values for both CON and OMG patties did not approach the 1 mg malonaldehyde/kg tissue threshold value established by Younathan and Watts (1959).

Evaluating and characterizing meat color can be achieved via objective color scans taken with a colorimeter and subjective visual panels. Modern colorimeters use Commission Internationale de l'Eclairage L\*a\*b\* values for color interpretation where L\* measures black to

white,  $a^*$  measures green to red, and  $b^*$  measures blue to yellow. Additionally, spectral reflectance is measured from 400 to 700 nm. These values can be used to calculate hue angle (color), chroma (color saturation),  $\Delta E$ , and surface myoglobin redox forms to further describe meat surface color objectively (AMSA, 2012). Visual panels are used to simulate retail setting consumer evaluations and can serve as a benchmark for objective color measurements. Objective color differences can be detected by trained color panelists at unit changes of 0.95, 0.99 and 0.50 for  $a^*$ ,  $b^*$ , and chroma values, respectively (Mancini et al., 2022). Chroma values 18 and below are indicative of an unacceptable surface color appearance when evaluated by consumers (Mancini et al., 2022). Vatansever et al. (2000) reported ground beef from steers fed linseed, linseed/fish oil, or fish oil respectively had decreased color saturation and increased metmyoglobin formation. LaBrune et al. (2008) reported at display d 5 and 7, *Longissimus Lumborum* steaks from heifers fed 10% flaxseed had reduced  $a^*$  values and increased hue angle than steaks from heifers fed conventional feedlot diets. Phelps et al. (2016a) reported as dietary microalgae meal (*Schizochytrium limacinum* CCAP 4087/2) supplementation increased, *Longissimus Lumborum*  $a^*$  values decreased linearly from display d 2 to 4 and quadratically from days 5 to 7 by 32%, 50%, and 113% respectively. Additionally, surface MMb percentages increased as supplementation increased by 47%, 57%, and 65% presenting an undesirable brown surface color. In the present study, color measurements followed patterns consistent with ground beef discoloration with minimal discernable treatment effects. Time effects were the greatest influence on color shown by the decrease in  $L^*$ ,  $a^*$ ,  $b^*$ , chroma, percent surface oxymyoglobin and increase in hue angle,  $\Delta E$ , and percent metmyoglobin for each primal and treatment over the 84 h display period. On average, Round  $a^*$  values decreased by 16 units and Chuck  $a^*$  values decreased by 7.9 units over the 84 h period which could be detected over time by a consumer, which is consistent with

discoloration throughout display; however, contrary to previous studies, OMG patties were unable to be discerned from CON patties throughout display indicating supplementation did not adversely affect ground beef color stability. Moreover, chroma values decreased throughout retail display for each primal, but did not fall below 18 which is considered the consumer acceptability threshold. The increased color stability of OMG patties could be attributed to greatOplus containing *Nannochloropsis oculata* or the dietary inclusion of Vitamin E.

Currently, there is a great interest in commercial production of PUFA, specifically EPA and DHA, because of their diverse health benefits. Besides fish oil, microalgae is a good source for EPA and DHA. Characterized as a rich PUFA, protein, and antioxidant source, *Nannochloropsis* sp. Have been used as a food source in aquaculture systems (Sukenik et al., 1989). The desirable fatty acid profile combined with a prolific growth pattern has differentiated *Nannochloropsis* sp. As the most promising photoautotrophic EPA producer for human consumption (Spolaore et al., 2006). Specifically, *Nannochloropsis oculata* is a green, unicellular marine algae with elevated oil and PUFA content commonly used in biodiesel (Chiu et al., 2009). Although highly regarded for its composition and applicability, research investigating *Nannochloropsis oculata* as an omega-3 animal feed source to enhance meat product fatty acid profiles is limited. In the present study, an extruded proprietary blend containing flaxseed, wheat middling, and *Nannochloropsis oculata* was fed to steers and no detrimental color stability nor oxidation effects were observed. The observed color stability may be attributed to the antioxidant content of the algae within greatOplus; however, an analysis of the algal biomass and supplement antioxidant content was not conducted in the present study. GreatOplus supplement composition coupled with Vitamin E dietary inclusion could have provided greater product color stability and reduce the negative effects observed in previous studies utilizing other algal biomasses.

Vitamin E or  $\alpha$ -tocopherol has been identified as a major fat-soluble antioxidant with the ability to decrease meat product oxidation (Wood and Enser, 1997). Vitamin E was supplemented to both treatments at  $85 \text{ IU}\cdot\text{animal}^{-1}\cdot\text{d}^{-1}$ . Research is limited on small-level antioxidant inclusion in diets containing PUFA sources like flaxseed and algal biomasses. While Albertí et al. (2014) found no color advantages when adding vitamin E to diets containing 5% flaxseed, Juárez et al. (2012) reported adding vitamin E ( $1,051 \text{ IU}\cdot\text{animal}^{-1}\cdot\text{d}^{-1}$ ) to feedlot steer diets containing 10% flaxseed produced steaks with reduced hue angles and surface metmyoglobin formation than steaks from cattle fed diets with 10% flaxseed and  $451 \text{ IU}\cdot\text{animal}^{-1}\cdot\text{d}^{-1}$  vitamin E. Phelps et al. (2017) found at retail display d 10, supplementing selenium, yeast, and vitamin E in feedlot diets containing microalgae meal (*Schizochytrium limacinum* CCAP 4087/2) produced steaks with 40% greater  $a^*$  values than diets that did not include antioxidants. Additionally, surface metmyoglobin percentages were decreased for steaks from steers fed antioxidants/microalgae than steaks from steers supplemented with only microalgae. Further research is warranted to understand effects of supplementing smaller Vitamin E amounts alongside PUFA feed sources on product color stability. Future studies using greatOplus (*Nannochloropsis oculata*; NBO3 Technologies, Manhattan, KS) should focus on understanding the effects of increasing dietary supplementation concentration on product composition and color stability.

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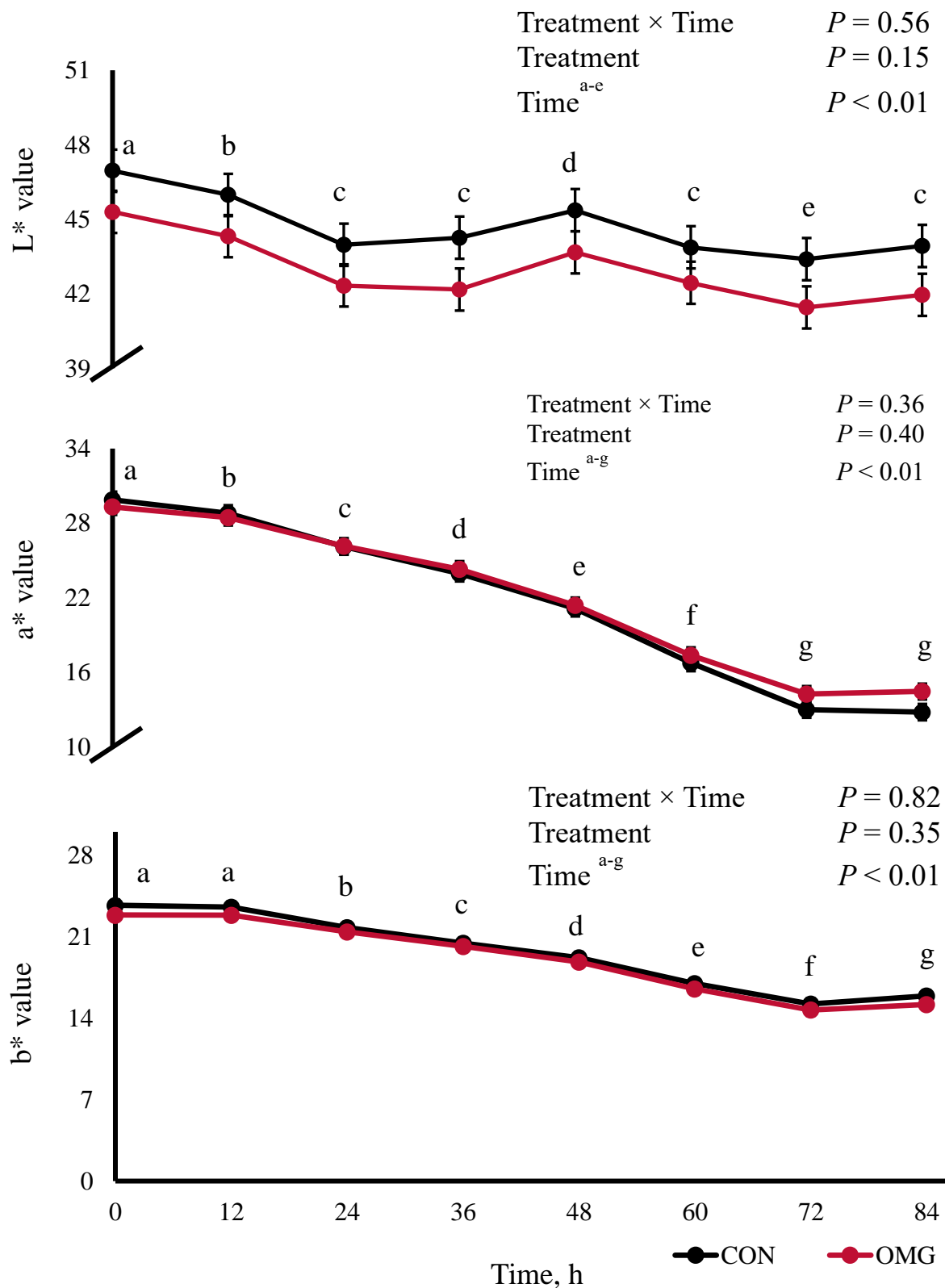
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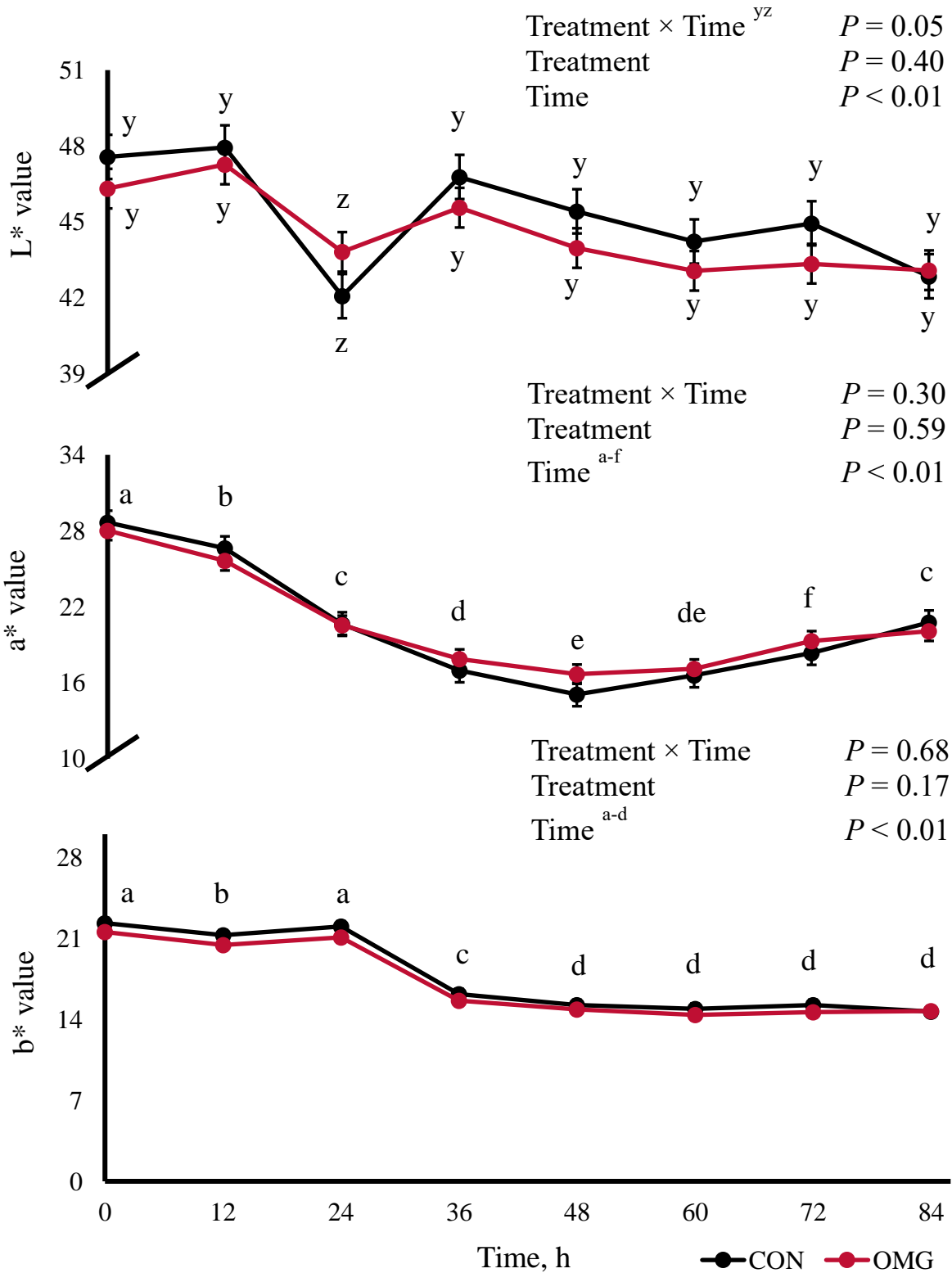
**Table 4.1.** Finishing diet composition (dry-matter basis) for steers fed a conventional feedlot diet (CON) or a conventional feedlot diet with greatOplus<sup>1</sup> (OMG)

Ingredient, %	CON	OMG
Steam-flaked corn	58.27	50.84
Wet corn gluten feed	20.00	20.00
Corn silage	15.00	15.00
greatOplus	0.00	10.00
Soybean meal, dehulled	2.57	0.00
Vitamin/mineral premix <sup>2</sup>	4.16	4.16
Nutrient composition		
Crude protein, %	14.00	14.00
Neg, Mcal/kg	1.46	1.48
Ether extract, %	3.18	5.19
Calcium, %	0.68	0.70
Phosphorus, %	0.38	0.42
Potassium, %	1.02	1.05
Sodium, %	0.35	0.35
Vitamin A (added), IU/kg	2,205.00	2,205.00
Vitamin E (added), IU/kg	22.00	22.00
Monensin, mg/kg <sup>3</sup>	33.00	33.00

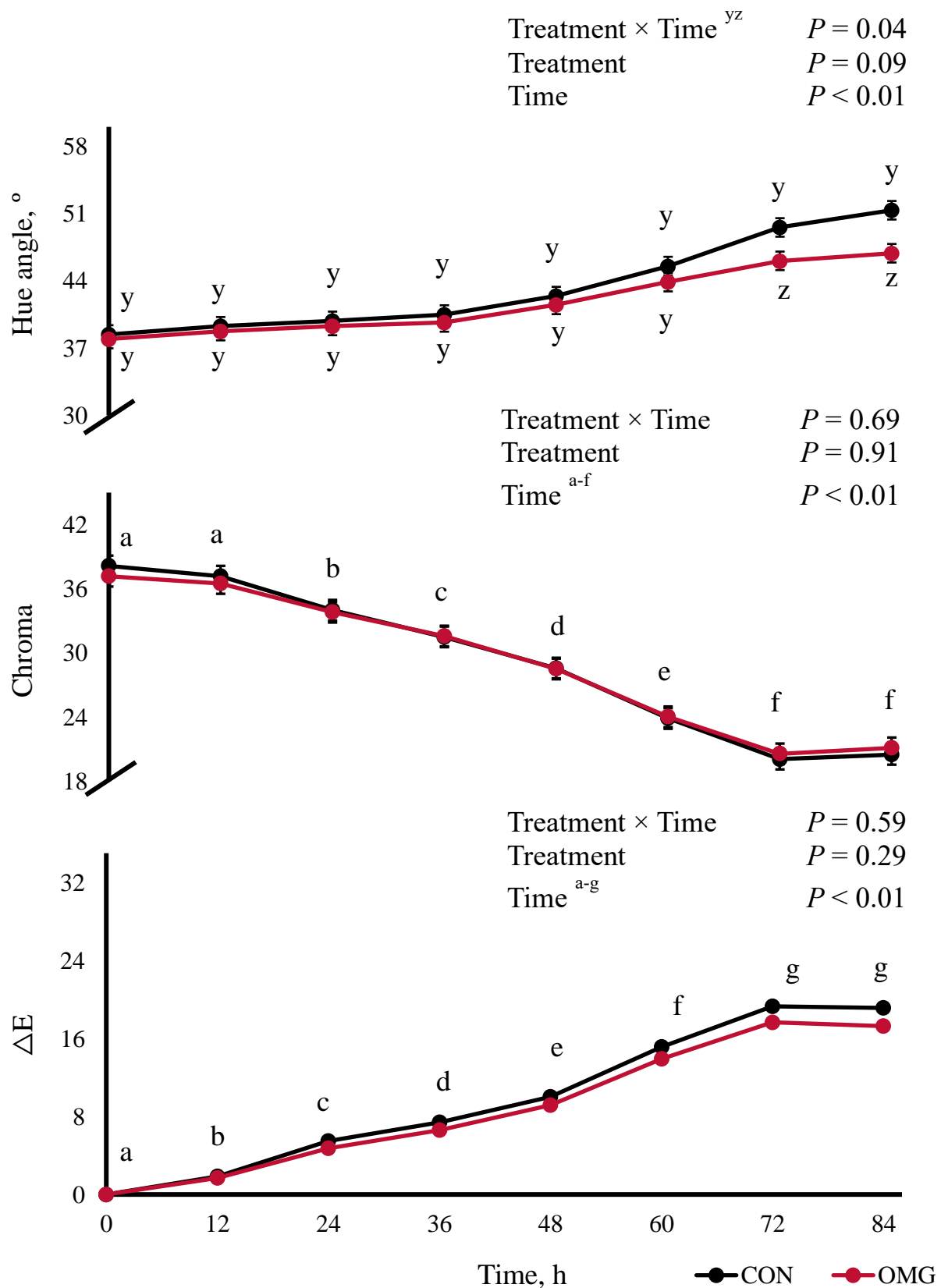
<sup>1</sup>greatOplus is a proprietary extruded blend of flaxseed, *Nannochloropsis oculata* algal biomass, and wheat middlings.



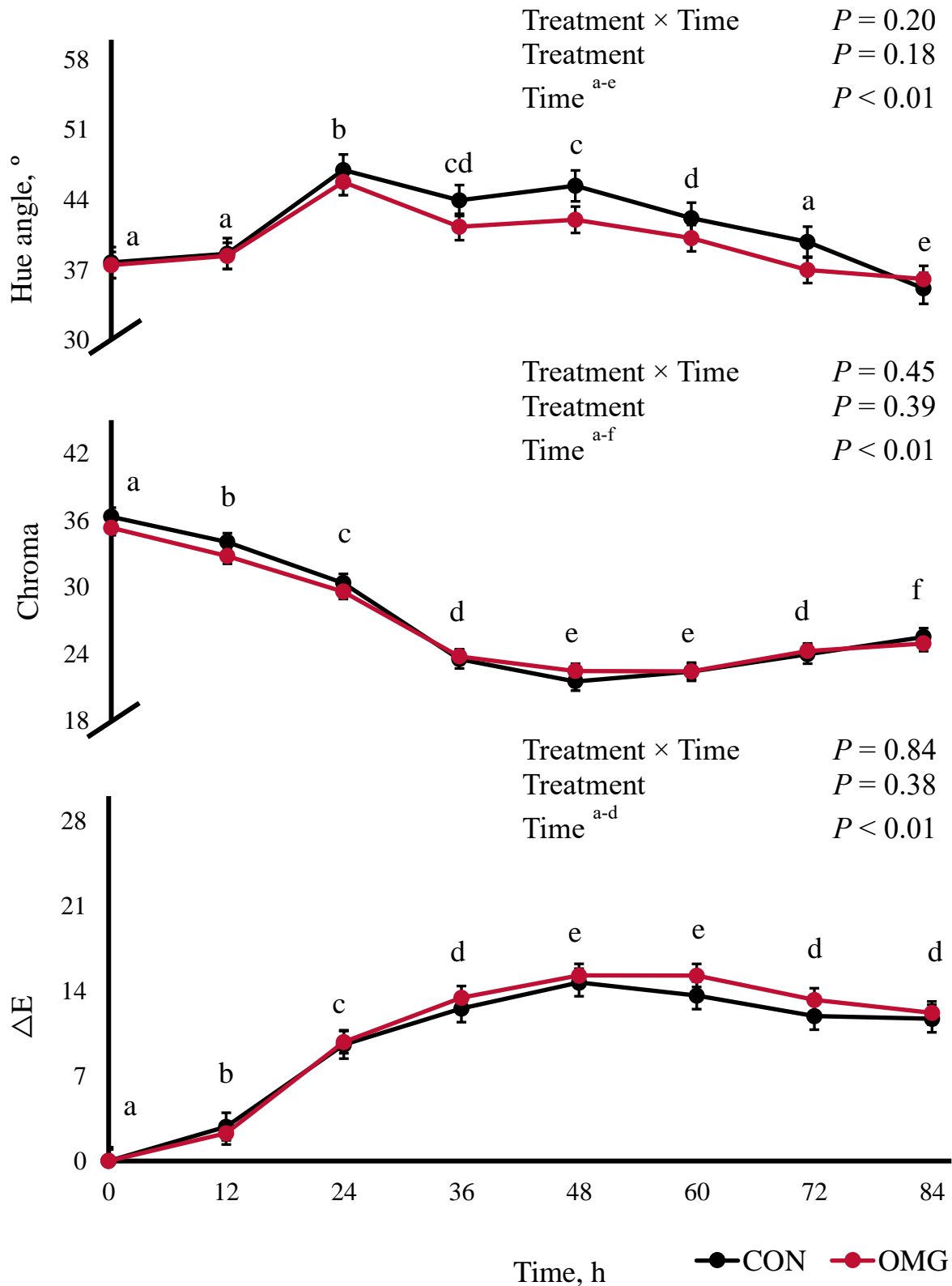
**Figure 4.1.** L\* (lightness), a\* (redness), and b\* (blueness) values for ground Round beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middling, and *Nannochloropsis oculata* algae blend (OMG; greatOplus; NBO3 Technologies). Patties were displayed under simulated retail conditions for 84 h. Significance was declared at  $P \leq 0.05$  with Time<sup>a-g</sup> main effect mean separations presented.



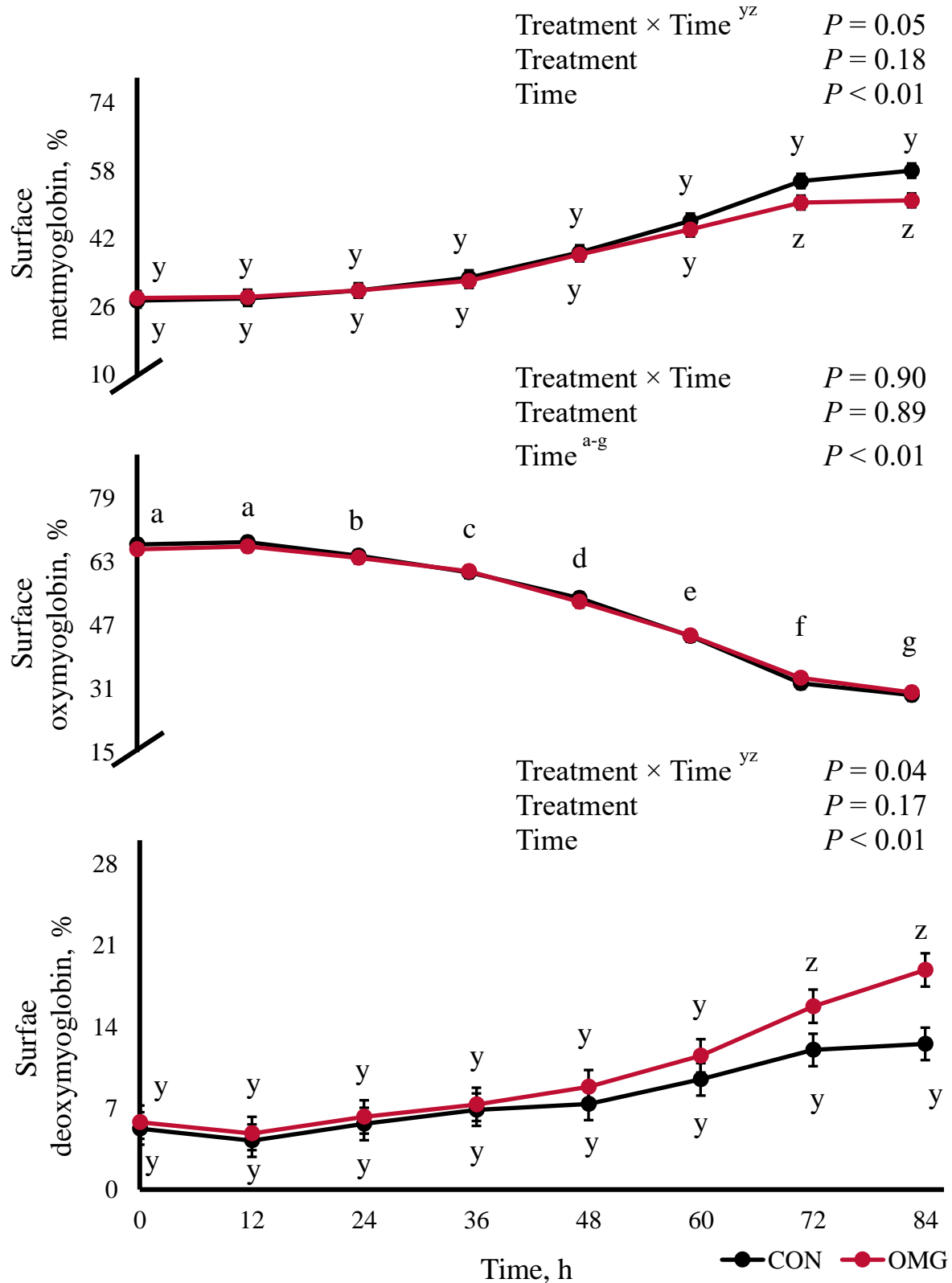
**Figure 4.2.** L\* (lightness), a\*(redness), and b\* (blueness) values for ground Chuck beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Patties were displayed under simulated retail conditions for 84 h. Significance was declared at  $P \leq 0.05$  with Treatment × Time<sup>yz</sup> interaction and Time<sup>a-f</sup> main effect mean separations presented.



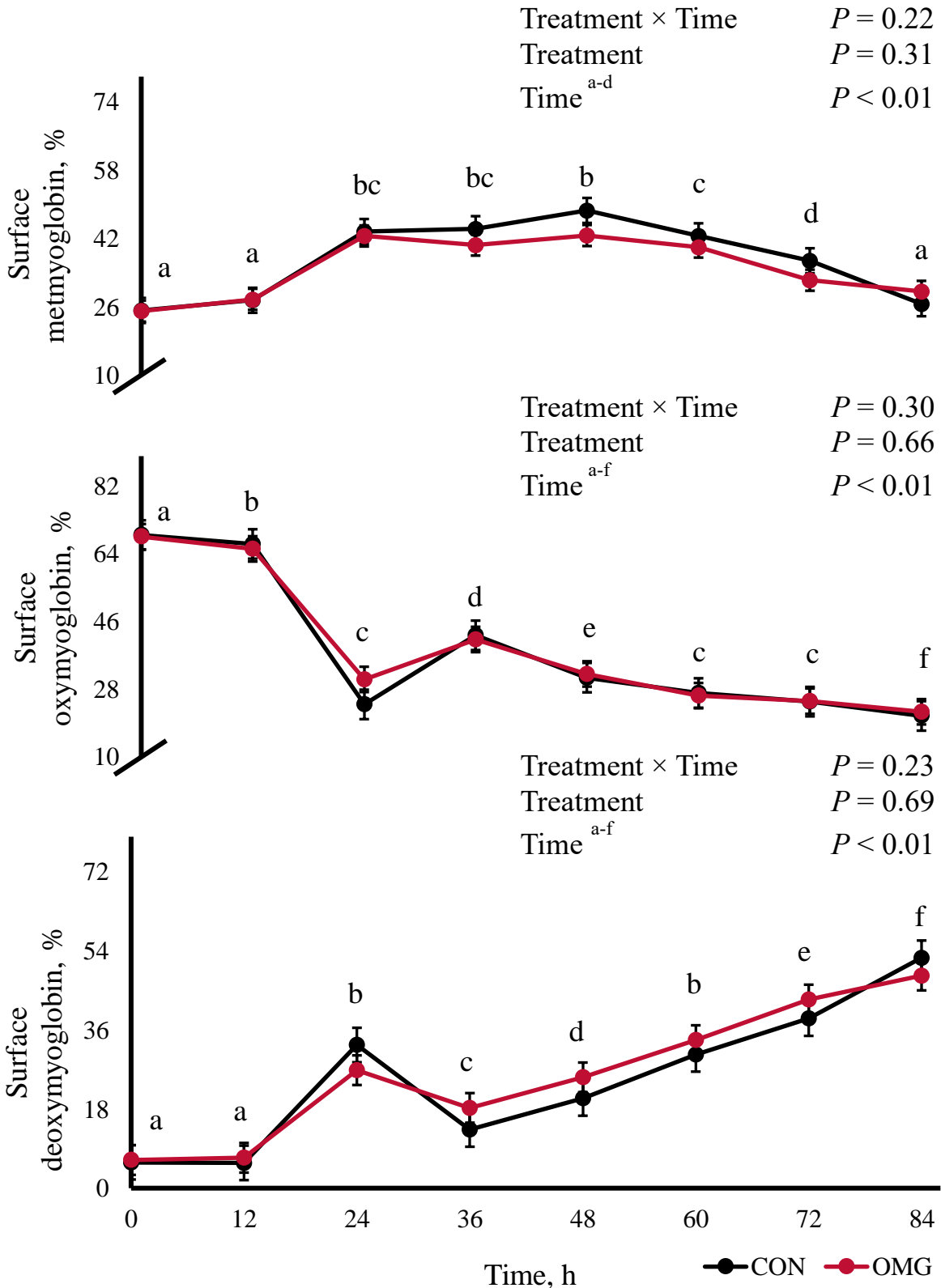
**Figure 4.3.** Hue angle (color), chroma (saturation), and delta E (total change in color) values for ground Round beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Patties were displayed under simulated retail conditions for 84 h. Significance was declared at  $P \leq 0.05$  with Treatment × Time<sup>yz</sup> interaction and Time<sup>a-g</sup> main effect mean separations presented.



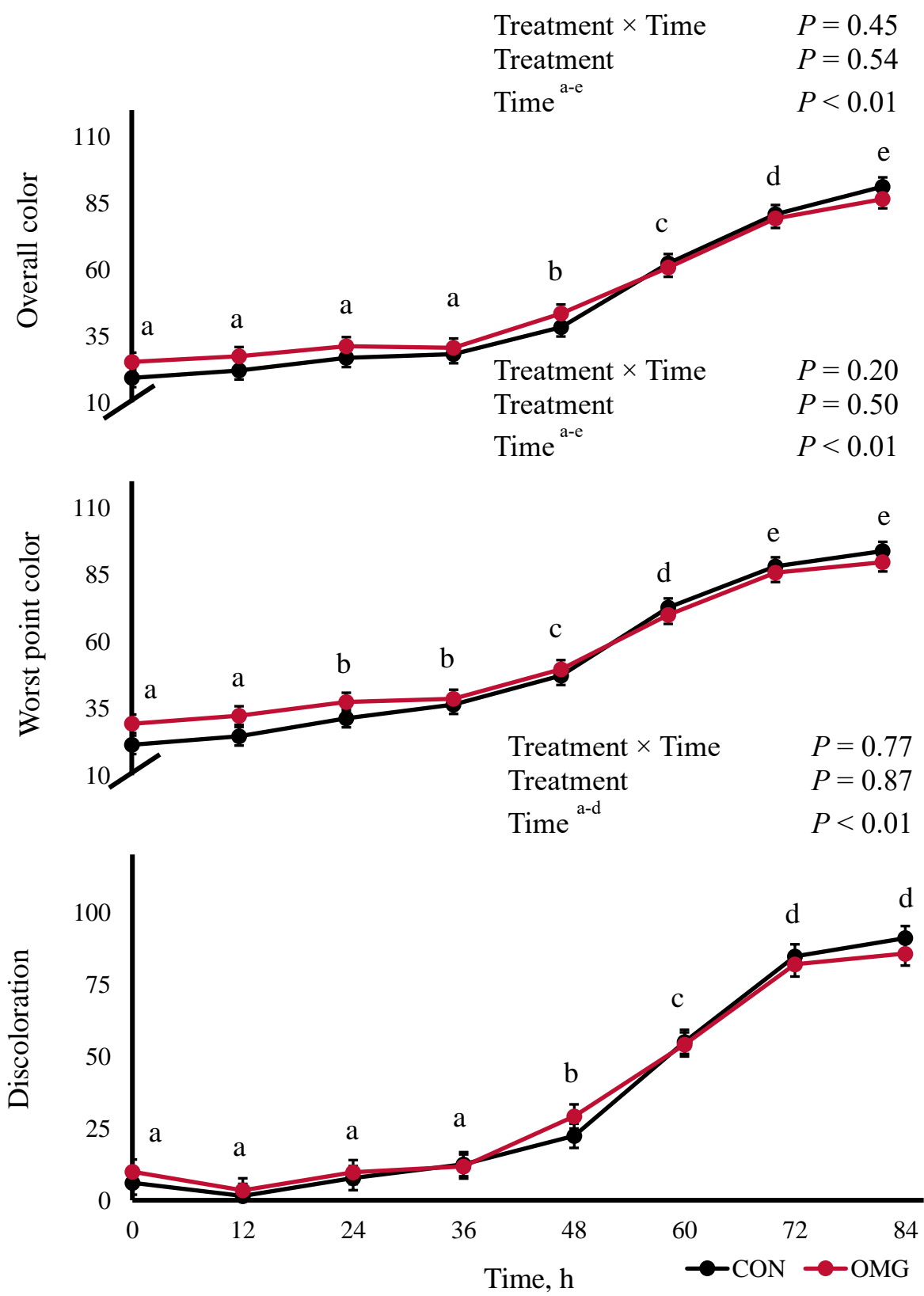
**Figure 4.4.** Hue angle (color), chroma (saturation), and delta E (total change in color) values for ground Chuck beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Patties were displayed under simulated retail conditions for 84 h. Significance was declared at  $P \leq 0.05$  with Time <sup>a-f</sup> main effect mean separations presented.



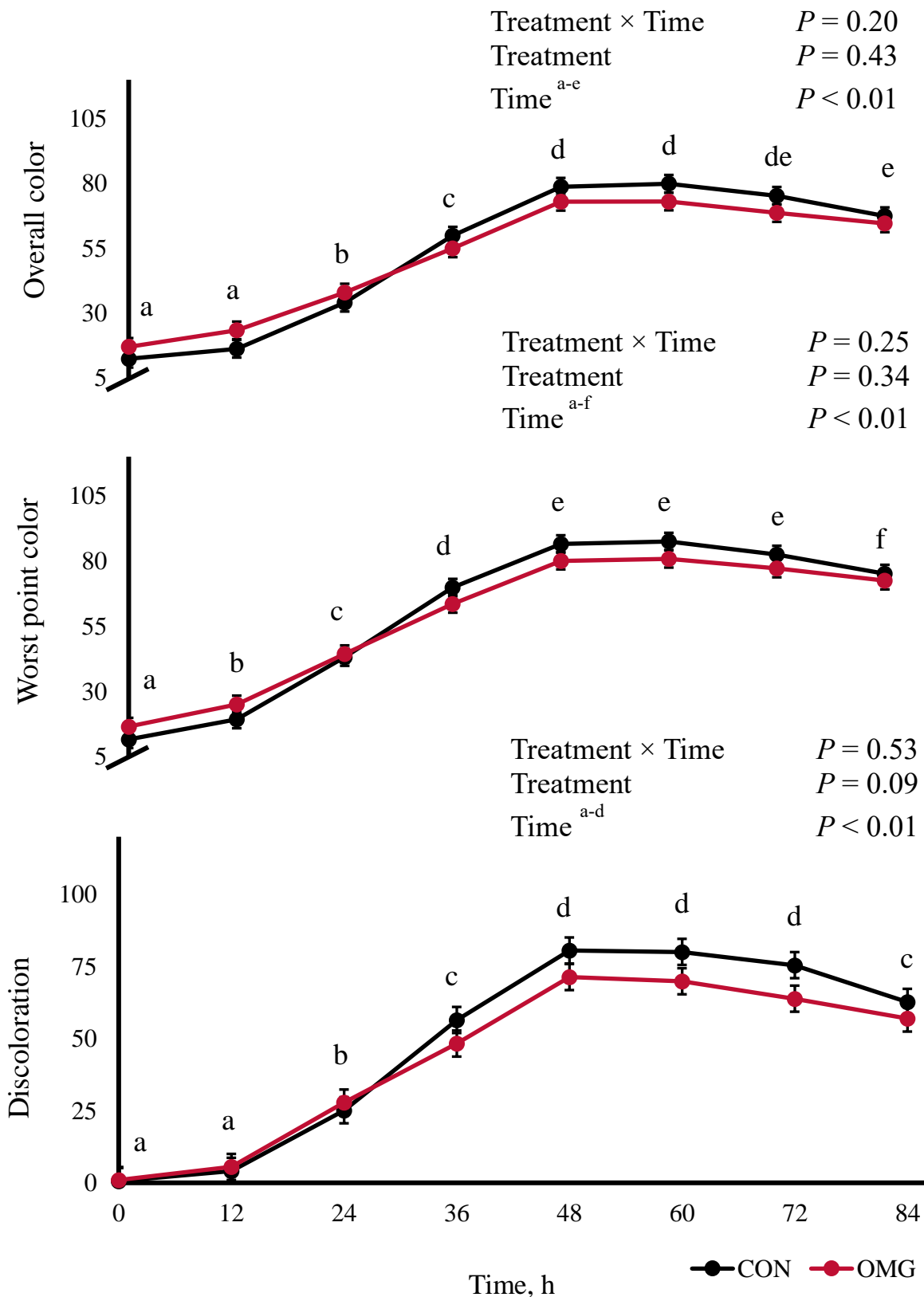
**Figure 4.5.** Surface metmyoglobin, oxymyoglobin, and deoxymyoglobin for ground Round beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Patties were displayed under simulated retail conditions for 84 h and percent myoglobin forms were calculated using the equations of Krzywicki (1979). Significance was declared at  $P \leq 0.05$  with Treatment × Time<sup>yz</sup> interactions and Time<sup>a-g</sup> main effect mean separations presented.



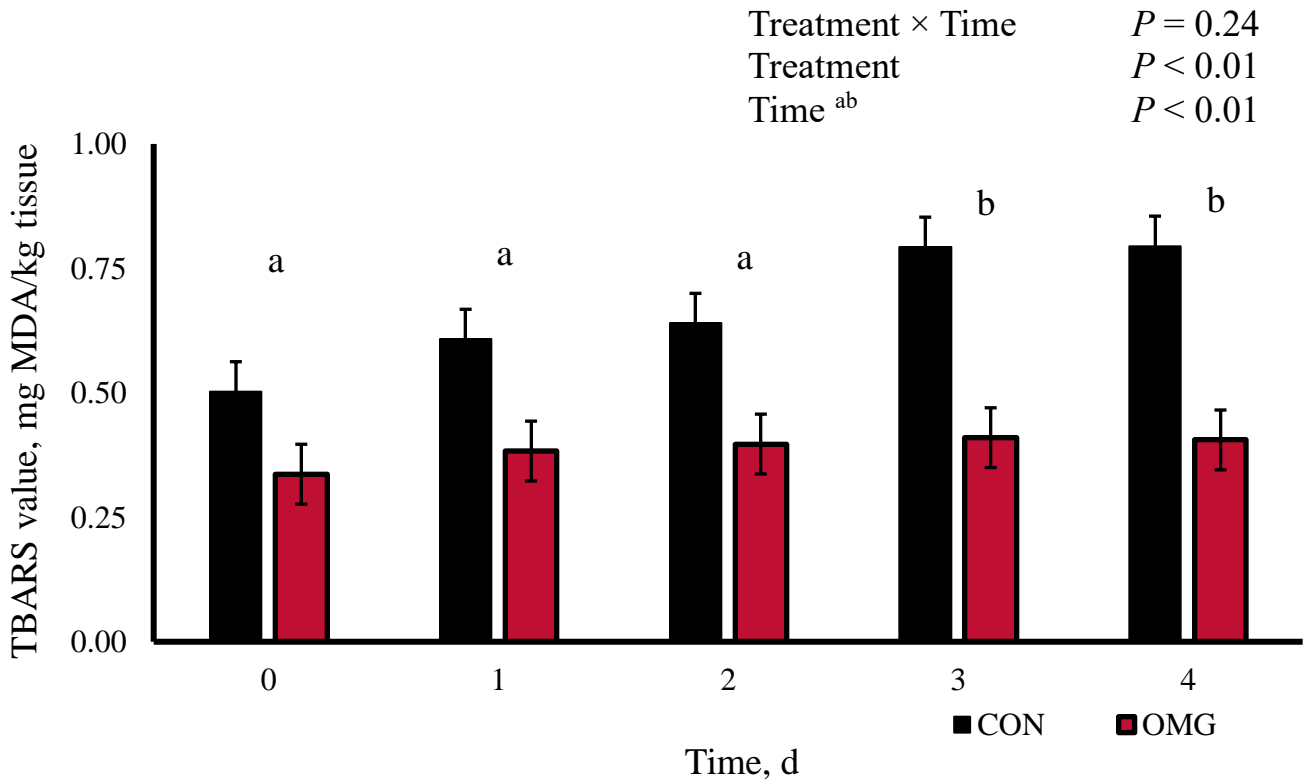
**Figure 4.6.** Surface metmyoglobin, oxymyoglobin, and deoxymyoglobin for ground Chuck beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Patties were displayed under simulated retail conditions for 84 h and percent myoglobin forms were calculated using the equations of Krzywicki (1979). Significance was declared at  $P \leq 0.05$  with Time<sup>a-f</sup> main effect mean separations presented.



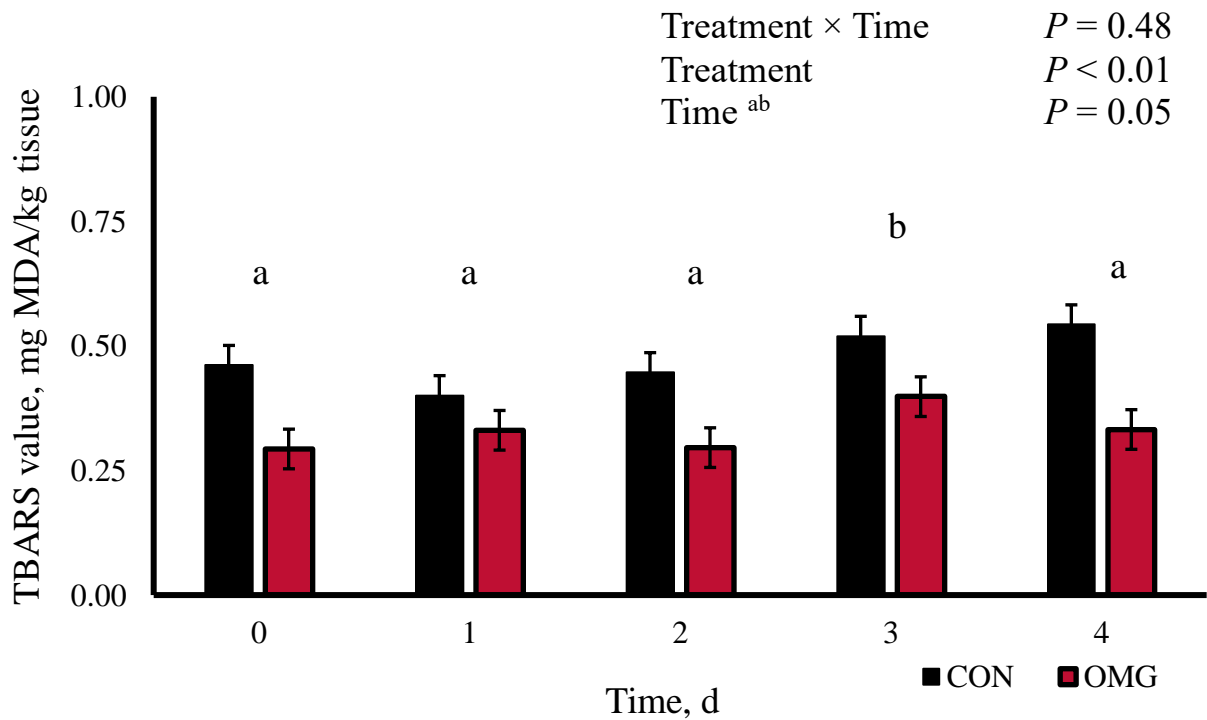
**Figure 4.7.** Subjective color scores of ground Round beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Patties were displayed for 84 h with evaluation every 12 h for surface color and worst point color (0 = bright red or reddish-pink and 100 = brown/gray) and discoloration (0 = 0 - 4% and 100 = 81 - 100%) electronically using iPads running Qualtrics survey software. Attributes were evaluated on 100-pt continuous line scales with anchors at 0, 20, 40, 60, 80, and 100 and significance was declared at  $P \leq 0.05$  with Time<sup>a-e</sup> main effect mean separations presented.



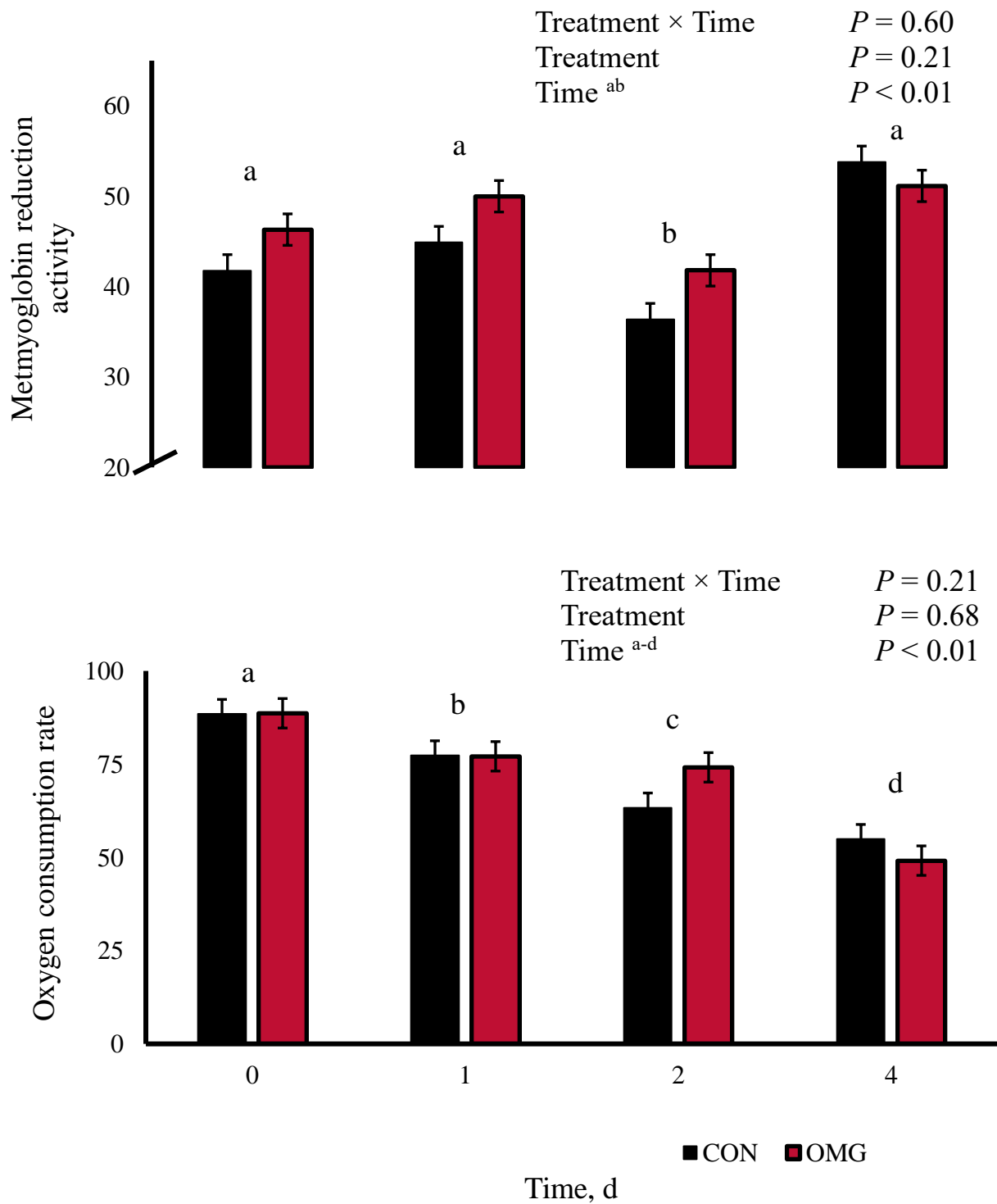
**Figure 4.8.** Subjective color scores of ground Chuck beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Patties were displayed for 84 h with evaluation every 12 h for surface color and worst point color (0 = bright red or reddish-pink and 100 = brown/gray) and discoloration (0 = 0 - 4% and 100 = 81 - 100%) electronically using iPads running Qualtrics survey software. Attributes were evaluated on 100-pt continuous line scales with anchors at 0, 20, 40, 60, 80, and 100 and significance was declared at  $P \leq 0.05$  with Time <sup>a-f</sup> main effect mean separations presented.



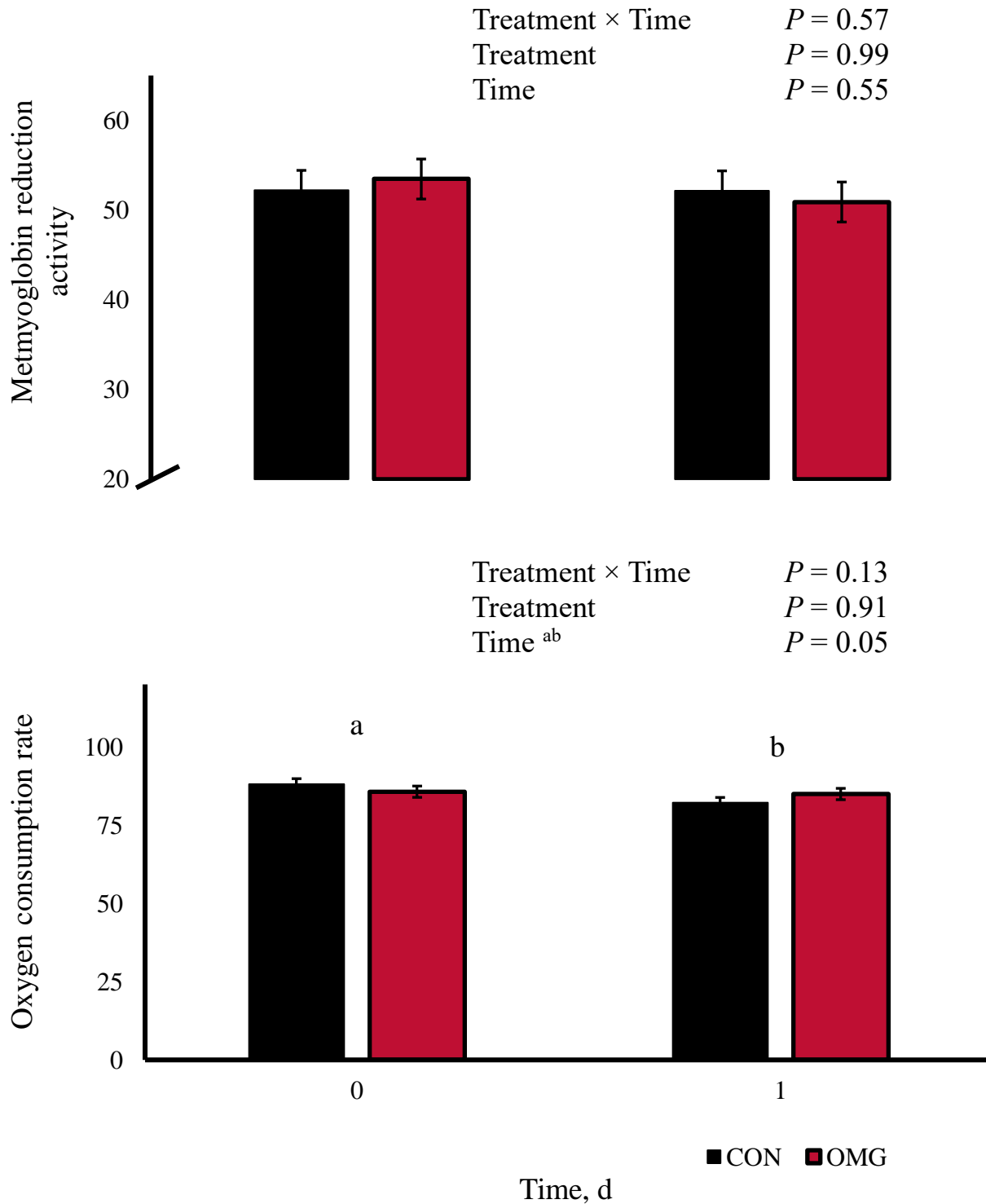
**Figure 4.9.** Thiobarbituric acid reactive substances (TBARS) values for ground Round beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Values were calculated using equations outlined by Buege and Aust (1978). Patties were displayed under simulated retail conditions for 84 h and removed every 24 h for TBARS analysis. Significance was declared at  $P \leq 0.05$ , with Time<sup>ab</sup> main effect separations presented.



**Figure 4.10.** Thiobarbituric acid reactive substances (TBARS) values for ground Chuck beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Values were calculated using equations outlined by Buege and Aust (1978). Patties were displayed under simulated retail conditions for 84 h and removed every 24 h for TBARS analysis. Significance was declared at  $P \leq 0.05$ , with Time<sup>ab</sup> main effect separations presented.



**Figure 4.11.** Metmyoglobin reduction activity (MRA) and Oxygen consumption rate (OCR) values for ground Round beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Values were calculated using equations described by Phelps (2017). Patties were displayed under simulated retail conditions for 84 h and removed on d 0, 1, 2, and 4 for analysis. Significance was declared at  $P \leq 0.05$  with Time<sup>a-d</sup> effect mean separations presented.



**Figure 4.12.** Metmyoglobin reduction activity (MRA) and Oxygen consumption rate (OCR) values for ground Chuck beef patties from steers fed a control feedlot diet (CON) or a diet containing an extruded proprietary flaxseed, wheat middlings, and *Nannochloropsis* algae blend (OMG; greatOplus; NBO3 Technologies). Values were calculated using equations described by Phelps (2017). Patties were displayed under simulated retail conditions for 84 h and removed on d 0 and 1 for analysis. Significance was declared at  $P \leq 0.05$  with Time<sup>ab</sup> effect mean separation presented.

## CHAPTER 5

### FEEDING MICROALGAE AND FLAXSEED (*Nannochloropsis oculata*) TO BEEF STEERS III: EFFECTS OF FROZEN STORAGE ON 90/10 ROUND AND 80/20 CHUCK GROUND BEEF PATTY OXIDATION AND PALATABILITY<sup>1</sup>

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<sup>1</sup>Catrett, C.C., A.M. Stelzleni, J.S. Drouillard, S.A. Devane, G.A. McKinney, X. Li, K.A. Turner,

D.A. Alambarrio, and J.M. Gonzalez. To be submitted to *J. Anim. Sci.*

## ABSTRACT

The objective was to determine effects of frozen storage on oxidation and palatability ratings of beef patties originating from steers fed greatOplus. Steers ( $N = 700$ ) were weighed, allocated to 28 pens ( $N = 25$  head/pen), with pens randomly assigned to being fed a conventional finishing diet (**CON**;  $n = 14$ ) or a conventional feedlot diet containing a 10% extruded flaxseed, wheat middling, and *Nannochloropsis oculata* algae blend (**OMG**;  $n = 14$ ). After 173 d on feed, one steer per pen was randomly selected and harvested at a commercial abattoir, with Round and Chuck subprimals vacuum packaged and shipped to University of Georgia Meat Science and Technology Center (Athens, GA). On d 21 and 23 postmortem, Round and Chucks were ground, producing one hundred twelve patties ( $113 \pm 2$  g, 13-mm thick) per carcass and primal. Patties were flash frozen ( $-40^{\circ}\text{C}$ ), placed in white, cardboard boxes, and stored for 0, 30, 60, or 90 d ( $-25^{\circ}\text{C}$ ). At each time point, samples were randomly removed, vacuum packaged, and allocated to thiobarbituric acid reactive substances (**TBARS**) and trained sensory analyses. There were no Treatment  $\times$  Time interactions nor Time effects for each primal TBARS values ( $P > 0.35$ ); however, there were Treatment effects where CON patties had elevated TBARS values than OMG patties ( $P < 0.01$ ). No Treatment  $\times$  Time interactions nor Treatment effects were observed for each primal cooked patty parameters and trained sensory analyses ( $P > 0.16$ ); however, there were Time effects observed ( $P < 0.01$ ). Chuck thaw loss and both primal cook loss presented a Time effect where d 0 patties had increased loss than OMG patties at each other time point ( $P < 0.01$ ), but values from d 30, 60, and 90 did not differ from each other ( $P > 0.14$ ). Time effects occurred for Round beef flavor and off-flavor ratings where d 0 patties had decreased beef flavor identity and reduced off-flavor prevalence than patties from all other time points ( $P < 0.01$ ), but did not differ from d 30 to 90 ( $P > 0.30$ ). Chuck beef flavor, cohesion, juiciness, and off-flavor ratings presented a Time

effect where d 0 patties had decreased beef flavor identity, cohesion, juiciness, and off-flavor prevalence compared to patties from d 30, 60, and 90 ( $P < 0.01$ ); however, ratings did not differ from d 30 to 90 for each attribute ( $P > 0.15$ ). Overall, greatOplus supplementation coupled with long-term frozen storage did not negatively affect ground beef patty oxidation levels and palatability attributes.

**KEY WORDS:** finishing diet, frozen storage, ground beef, microalgae, oxidation

## INTRODUCTION

Since 1998, consumers have increased their beef spending habits by approximately \$113 per person (Speer et al., 2015). Retailers have begun to prominently highlight ground beef in meat advertisements due to its competitive price point and have established it as a valuable product to consumers. In 2020, ground beef consumption was estimated to be around 12.3 kg per capita in the United States (Schulz, 2021). Chilled and frozen storage are commonly used to ensure the safe delivery of large volumes of beef to distant end-users (Holman et al., 2017). Freezing is a significant strategy used to preserve food quality and minimize product quality loss (Cullere et al., 2013). While frozen storage is used to minimize unwanted meat metabolic processes, ice crystal formation during the freezing process contributes to cell rupture and muscle fiber damage (Sebranek, 1982). Moreover, frozen storage may negatively alter physical and chemical properties including product texture, cook loss, oxidation, and product sensory attributes (Perez-Palacios et al., 2010). The main contributor to quality deterioration during frozen storage is the elevated presence of oxidation catalysts which inherently impacts product flavor and texture (Asghar et al., 1988). Ozer and Secen (2018) reported thiobarbituric acid reactive substances (**TBARS**) values of

frozen stored raw and cooked beef burgers steadily increased over a 3-month period. In an evaluation of cooked beef meatballs stored up to 90 d, Sun et al. (2020) found product acceptability decreased as storage time increased. Research regarding effects of frozen storage on boxed beef is limited and no previous studies have investigated the effects of frozen storage on omega-3 (n-3) enhanced beef.

Thus, the study objective was to evaluate the effects of long-term frozen storage on oxidation and palatability of 90/10 Round and 80/20 Chuck ground beef patties originating from steers fed a microalgae and flaxseed blend (greatOplus; *Nannochloropsis oculata*; NBO3 Technologies, Manhattan, KS) during finishing.

## **MATERIALS AND METHODS**

All experimental procedures involving animals were approved by the Kansas State University Institutional Animal Care and Use Committee and the University of Georgia (UGA) Institutional Review Board approved all procedures for human subject use in sensory panel evaluations.

### ***Live Cattle Management***

A more detailed description of steer management is described in Chapter 3. Briefly, black-hided yearling steers were purchased from sale barns in Kansas and Nebraska and transported to the Kansas State University Beef Cattle Research Center (Manhattan, KS), where they were acclimated, individually weighed, separated into 14 weight blocks and allocated to 28 feedlot pens ( $N = 25$  / pen), with pens randomly assigned to one of two treatments consisting of a control diet without supplemental omega-3 fatty acids (CON; Table 5.1) or a diet containing 10% (dry basis)

of an extruded proprietary blend of flaxseed, wheat middling, and *Nannochloropsis oculata* algae (OMG; greatOplus; NBO3 Technologies, Manhattan, KS). Cattle were fed twice daily, *ad libitum* for a total of 175 d and on d 173, randomly one head per pen ( $n = 14$  / treatment) was weighed, transported to a commercial abattoir (Hertzog Meat Company, Butler, MO) for harvest and fabricated, with round and chuck subprimals shipped 13 d postmortem and delivered 17 d postmortem to the UGA Meat Science and Technology Center (Athens, GA).

### ***Muscle Sampling, Ground Beef Manufacture, and Sample Packaging***

After delivery, boxes were sorted by carcass and primal. Each carcass and primal were processed independently. Vacuum packaged round subprimals were further processed 24 h prior to grinding to remove bone and cube meat for grinding. Individually, cubed round muscles were placed in a plastic-lined, dark storage boxes to maintain carcass identification and stored overnight ( $2 \pm 2^\circ\text{C}$ ). Round grinding occurred 21 d postmortem with each carcass processed separately. Meat was placed in a grinder (Daniels AMFG-50; Daniels Food Equipment, Parkers Prairie, MN), coarse ground (13 mm plate), and fine ground (6 mm plate) prior to patty formation (Patty-O-Matic EZ Slider; Patty-O-Matic, In., Farmingdale, NJ). One hundred twelve patties ( $113 \pm 2$  g, 13 mm thick) were produced from each carcass, with carcasses processed by alternating treatments. Following processing of each batch, the grinder and patty machine were thoroughly cleaned to prevent treatment cross-contamination. Patties were blast frozen ( $-40^\circ\text{C}$ ) on sausage trays for 20 minutes and once frozen, placed on parchment paper, and layered into a white, cardboard box (S-12770; ULINE, Pleasant Prairie, WI) and frozen ( $-25^\circ\text{C}$ ). Each carcass was randomly assigned to an HRI box with 4 carcasses per box. Samples were randomly removed on d 0, 30, 60, and 90 for

thiobarbituric acid reactive substances (**TBARS**) and trained sensory analyses, vacuum packaged, and stored until later analysis.

Chucks were processed and allocated 23 d postmortem following the methods previously described for the Round.

### ***Lipid Peroxidation (TBARS) Analysis***

On storage d 0, 30, 60, and 90, patties for TBARS were randomly removed from boxes, vacuum packaged, frozen ( $-25 \pm 2^{\circ}\text{C}$ ), and stored until later analysis. Lipid peroxidation analysis was performed using the rapid, wet TBARS method described by Buege and Aust (1978). Samples were thawed for 12 h, homogenized using a food processor (Model 70550; Hamilton Beach Manufacturing Company, Glen Allen, VA), and duplicate 0.5 g samples were placed in 15 mL screw-capped conical tubes. Thiobarbituric acid/trichloroacetic acid stock solution was added to each tube, loosely capped, and placed in a boiling water bath ( $100 \pm 2^{\circ}\text{C}$ ) for 10 min. Tubes were cooled in a water bath ( $25^{\circ}\text{C}$ ) for 10 min, centrifuged for 15 min at  $4,000 \times g$  at  $4^{\circ}\text{C}$ , with supernatant transferred into a  $12 \times 75$  mm culture tube and sample absorbance read at 532 nm (Jasco V-630 Spectrophotometer, Jasco Inc., Easton, MA). All samples were evaluated in duplicate and those with a coefficient of variation greater than 10% were re-analyzed. Thiobarbituric acid reactive substances values were expressed as mg of malondialdehyde per kg of muscle tissue.

### ***Trained Sensory Analysis***

Sensory analyses were conducted according to procedures outlined in the American Meat Science Association (**AMSA**) Research Guidelines for Cookery, Sensory Evaluation, and

Instrumental Tenderness Measurements of Meat (AMSA, 2016) with modifications chronicled in Chapter 3

Two patties per carcass and primal were weighed, placed on poly trays with absorbent pads, covered with polyvinyl chloride film, allowed to thaw for 24 h ( $2 \pm 2^\circ\text{C}$ ), re-weighed, and cooked following the methods described by Chapter 3. Cooked weights were collected, patties were cut into 6 equally sized wedges, wrapped in foil, and rested under a heat lamp before serving to maintain temperature (maximum 10 min). Percent thaw loss and percent cook loss were calculated respectively using the following equations:  $[(\text{frozen weight} - \text{thaw weight}) / \text{frozen weight}] \times 100$  and  $[(\text{thaw weight} - \text{cook weight}) / \text{thaw weight}] \times 100$ .

Trained panelists ( $N = 16$ ; 8/session) participated in evaluating 8 samples daily across 21 sessions with 24 h between each session and sample orientation determined by a random number generator (Microsoft Excel; Redmond, WA). All samples, along with a warm-up sample, were served to panelists unseasoned in warmed, numbered yogurt maker (Euro Cuisine, Inc.; Los Angeles, CA) glass jars. Water and unsalted crackers were utilized as palate cleansers. The sensory room contained 8 individual booths with positive airflow and red lighting to conceal differences in patty color. Panelists were provided an iPad (Apple, Cupertino, CA) running Qualtrics (Seattle, WA and Provo, UT) survey software utilizing an 8-point hedonic scale during each session rating cohesion/binding (1 = extremely fragile, 2 = very fragile, 3 = moderately fragile, 4 = slightly fragile, 5 = slight cohesion/bind, 6 = moderate cohesion/bind, 7 = very tight cohesion/bind, 8 = extreme cohesion/bind), beef flavor intensity (1 = extremely bland, 2 = very bland, 3 = moderately bland, 4 = slightly bland, 5 = slightly intense, 6 = moderately intense, 7 = very intense, 8 = extremely intense), and juiciness (1 = extremely dry, 2 = very dry, 3 = moderately dry, 4 = slightly dry, 5 = slightly juicy, 6 = moderately juicy, 7 = very juicy, 8 = extremely juicy) and a 6-point

scale for off-flavor (1= none detected, 2 = threshold off-flavor, 3 = slightly intense, 4 = moderate off-flavor, 5 = very strong off-flavor, 6 = extreme off-flavor).

### ***Statistical Analysis***

Each primal was analyzed independently as a completely randomized design with a  $2 \times 4$  factorial arrangement. Carcass served as the experimental unit and patty as the observational unit, with fixed effects of treatment, time, and their interaction. The PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc, Cary, NC) was utilized and pairwise comparisons between the least square means were computed using the PDIFF option of the LSMEANS statement. Differences were considered statistically significant at  $P \leq 0.05$ .

## **RESULTS**

### ***Lipid Peroxidation (TBARS)***

There was no Treatment  $\times$  Time interaction nor Time main effect for Round HRI TBARS values ( $P > 0.35$ ; Fig. 5.1); however, there was a Treatment main effect ( $P < 0.01$ ) where at each time point, CON patties had elevated TBARS values than OMG patties.

There was no Treatment  $\times$  Time interaction nor Time main effect for Chuck HRI TBARS values ( $P > 0.85$ ; Fig. 5.2); however, there was a Treatment main effect ( $P < 0.01$ ) where at each time point, CON patties had increased TBARS values than OMG patties.

### ***Cooked Patty Measures and Trained Sensory***

There was no Treatment  $\times$  Time interaction, nor Time or Treatment main effects for Round HRI thaw loss percentages ( $P > 0.17$ ; Fig. 5.3). There was no Treatment  $\times$  Time interaction nor

Treatment main effect for Chuck HRI thaw loss percentages ( $P > 0.26$ ; Fig. 5.4); however, there was a Time main effect ( $P < 0.01$ ) where d 0 patties had greater thaw loss percentages and differed from all other time points which did not differ from each other ( $P > 0.15$ ).

There were no Treatment  $\times$  Time interactions nor Treatment main effects for Round (Fig 5.5) and Chuck (Fig. 5.6) HRI patty cook loss percentages ( $P > 0.24$ ); however, there was a Time main effect for both where d 0 patties had greater cook loss percentages and differed from all other time points ( $P < 0.01$ ) which did not differ from each other ( $P > 0.58$ ).

There were no Treatment  $\times$  Time interactions nor Treatment main effects for Round beef flavor and off-flavor ratings ( $P > 0.19$ ; Fig. 5.7); however, there were Time main effects for both where d 0 patties had decreased beef flavor and off-flavor ratings which differed from all other time points ( $P < 0.01$ ). No Treatment  $\times$  Time interactions nor Treatment or Time main effects were observed for Round cohesion and juiciness ratings ( $P > 0.31$ ).

There were no Treatment  $\times$  Time interactions nor Treatment main effects for Chuck beef flavor, cohesion, juiciness, and off-flavor ratings ( $P > 0.16$ ; Fig. 5.8); however, there were Time main effects for each attribute where d 0 patties had decreased ratings which differed from all other time points ( $P < 0.01$ ).

## DISCUSSION

Consumers are becoming increasingly more aware of where their food originates, and the nutritional value associated with it. A single, 85 g (3 oz) beef serving contains 25 g of protein as well as 101%, 53%, and 14% of daily recommended intake values for Vitamin B<sub>12</sub>, Zinc, and Iron, respectively (Zanovec et al., 2010). Despite being a nutritionally dense protein source, beef has been placed under public scrutiny due to exhibiting elevated saturated fatty acids and negligible

amounts of essential omega-3 (**n-3**) fatty acids primarily due to rumen biohydrogenation of polyunsaturated fats (**PUFA**; Harfoot, 1978). In the last decade, consumers have grown concerned regarding saturated fat content and the omega-6 (**n-6**) to n-3 fatty acid ratio of their ground beef. Lusk and Parker (2009) reported consumers would prefer compositionally improved ground beef originating from cattle with decreased saturated fats and smaller n-6 to n-3 ratios. It is critical to understand consumer preferences since their concerns, demands, and purchasing decisions direct the entire industry. Thus, beef research has shifted focus to increasing product health perception specifically via n-3 fatty acid profile enhancement. Literature demonstrates PUFA content can be improved and n-6 to n-3 ratios can be decreased in beef products originating from cattle supplemented with flaxseed, fish oil, linseed, and other marine sources; however, many studies report detrimental effects on product oxidation and palatability. Contrarily, in Chapter 3 and 4 there were no significant differences in TBARS values nor in consumer and trained sensory analyses from conventionally stored patties originating from the cattle used in the present study.

Jones et al. (2018) reported meat consumption for each species has increased since 2012 representing an elevated demand for animal protein. Ground beef sales represent 63% and 49% of total food service and retail sales volume annually, respectively (Speer et al., 2015). In 2020, ground beef consumption was estimated to be around 12.3 kg per capita in the United States (Schulz, 2021). As beef prices increase, retailers have begun to prominently highlight ground beef in advertisements due to its competitive price point and establish it as a valuable product to consumers. Ground beef retail popularity has driven the meat industry to utilize bulk, long-term frozen storage solutions to meet consumer demand. Understanding the impact of long-term frozen storage on product oxidation and palatability is limited and research is non-existent investigating the impact of long-term frozen storage on n-3 enhanced meat.

Thiobarbituric-acid reactive substances quantification is an assay commonly used to evaluate oxidation in meat. Lipid oxidation has been thought to be a harmful process responsible for major changes to molecular chemical characteristics, such as functional loss and the formation of cytotoxic and genotoxic chemicals, namely oxidized lipid-derived aldehydes, and peroxides. Adduct formation through Schiff base arrangement may explain the loss of malonaldehyde activity during frozen storage. Utrera & Estevez (2013) reported protein and lipid oxidation products interact strongly to produce Schiff base arrangement via condensation. Lipid oxidation products, namely hydroperoxides and aldehydes, may establish several types of covalent bonds with proteins through hydrophobic interaction and hydrogen bonds, produce Schiff base arrangement, and promote polymerization. Malonaldehyde and 4-hydroxy-2-nonenal may cross-link with the lysine residue free amino groups thus decreasing their contents after a period of frozen storage (Viljanen et al., 2004). Research has established a strong correlation between TBARS values and sensory panel flavor scores (Mielche & Bertelsen, 1993; Spanier et al., 1993). Younathan and Watts (1959) reported consumers may be able to detect product rancidity and oxidized off-flavors when a TBARS value greater than 1 mg malonaldehyde per kg of muscle tissue is recorded; however, Zhang et al. (2019) reported TBARS value had no significant effect on sensory ratings, indicating untrained consumers cannot discern abnormal flavor development caused by increased lipid oxidation levels. Al-Dalali et al. (2022) found in marinated raw beef TBARS values increased as storage time increased with the greatest value reported at 2 months of frozen storage (39.11  $\mu\text{g}$  MDA/kg) which was considerably elevated than values obtained at 0 months (4.32  $\mu\text{g}$  MDA/kg). In the present study, TBARS values did not differ nor increase throughout the 90 d frozen storage period. And values were below the aforementioned research threshold value at each time point suggesting consumers would not be able to detect rancid or oxidized off-flavors in the samples.

The lack of treatment differentiation for TBARS could be attributed to greatOplus containing *Nannochloropsis oculata*.

Currently, there is a great interest in commercial production of PUFA, specifically EPA and DHA, because of their diverse health benefits. Besides fish oil, microalgae is a good source for EPA and DHA. Characterized as a rich PUFA, protein, and antioxidant source, *Nannochloropsis* sp. have been used as a food source in aquaculture systems (Sukenik et al., 1989). The desirable fatty acid profile combined with a prolific growth pattern has differentiated *Nannochloropsis* sp. as the most promising photoautotrophic EPA producer for human consumption (Spolaore et al., 2006). Specifically, *Nannochloropsis oculata* is a green, unicellular marine algae with elevated oil and PUFA content commonly used in biodiesel (Chiu et al., 2009). Although highly regarded for its composition and applicability, research investigating *Nannochloropsis oculata* as an omega-3 animal feed source to enhance meat product fatty acid profiles is limited. In the present study, an extruded proprietary blend containing flaxseed, wheat middling, and *Nannochloropsis oculata* was fed to steers and no detrimental oxidation concentrations were observed. The observed stability may be attributed to the antioxidant content of the algae within greatOplus; however, an analysis of the algal biomass and supplement antioxidant content was not conducted in the present study. Nonetheless, greatOplus supplementation seemed to mitigate negative product oxidation effects observed in previous studies utilizing other algal biomasses.

Palatability is the term characterizing the overall eating experience of a food product. In beef, tenderness, juiciness, flavor, and their interaction influence product palatability (Smith and Carpenter, 1974; Drey and O'Quinn, 2017). Lucherk et al. (2016) determined approximately 50% of consumers identify flavor as the most important trait with tenderness only ranked the most

important 39.3% of the time. Phelps et al. (2016a) evaluated the impact of supplementing beef heifers with increasing microalgae meal concentrations (0, 50, 100, 150) on *Longissimus lumborum* palatability. Trained panelists indicated no difference in steak juiciness, beef flavor intensity, connective tissue, nor overall tenderness ( $P > 0.18$ ); however, as microalgae meal supplementation increased off-flavor prevalence increased by 0.07, 0.36, 0.89 for each respective treatment (quadratic;  $P < 0.01$ ). Chapter 3 reported no detrimental treatment effects on ground round and chuck patty palatability ratings originating from beef steers supplemented with a greatOplus, with treatment patties tending to reduce off-flavor prevalence and increase beef flavor identity. For each palatability attribute evaluated in the present study, there were no Treatment effects; however, there were observed Time effects where d 0 patties had decreased sensory ratings than patties at d 30, 60, and 90. The observed Time effects suggest long-term frozen storage may not be as detrimental to palatability as previously reported in literature; however, a consumer sensory analysis should be conducted to better understand how untrained panelist may characterize product palatability attributes. Nevertheless, the present study provides data to support and validate the use of long-term frozen storage with n-3 enriched meat products without compromising product integrity.

## **ACKNOWLEDGEMENTS**

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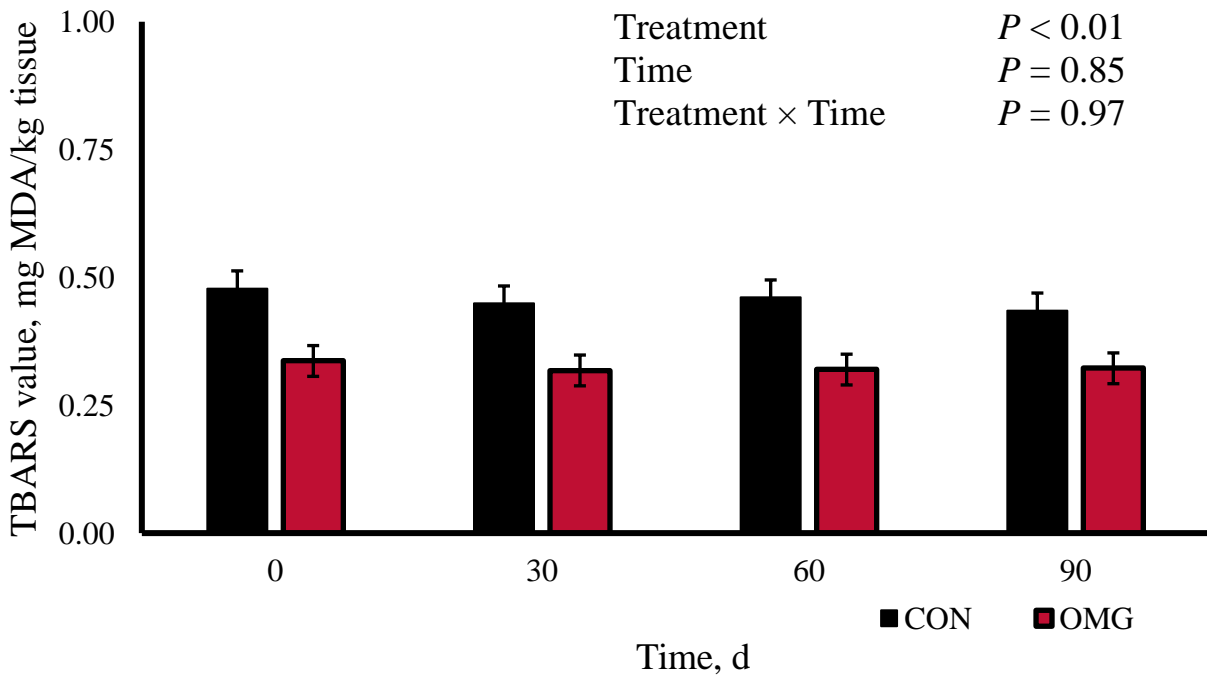
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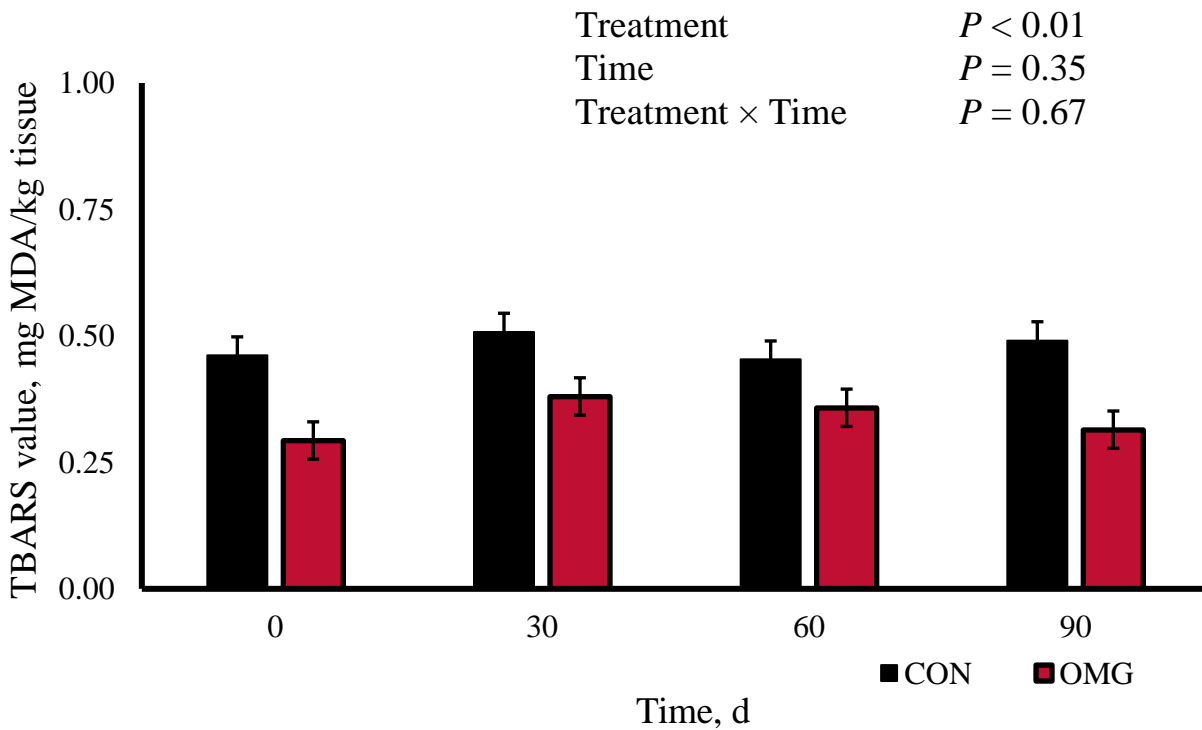
**Table 5.1.** Finishing diet composition (dry-matter basis) for steers fed a conventional feedlot diet (CON) or a conventional feedlot diet with greatOplus<sup>1</sup> (OMG)

Ingredient, %	CON	OMG
Steam-flaked corn	58.27	50.84
Wet corn gluten feed	20.00	20.00
Corn silage	15.00	15.00
greatOplus	0.00	10.00
Soybean meal, dehulled	2.57	0.00
Vitamin/mineral premix <sup>2</sup>	4.16	4.16
Nutrient composition		
Crude protein, %	14.00	14.00
NEg, Mcal/kg	1.46	1.48
Ether extract, %	3.18	5.19
Calcium, %	0.68	0.70
Phosphorus, %	0.38	0.42
Potassium, %	1.02	1.05
Sodium, %	0.35	0.35
Vitamin A (added), IU/kg	2,205.00	2,205.00
Vitamin E (added), IU/kg	22.00	22.00
Monensin, mg/kg <sup>3</sup>	33.00	33.00

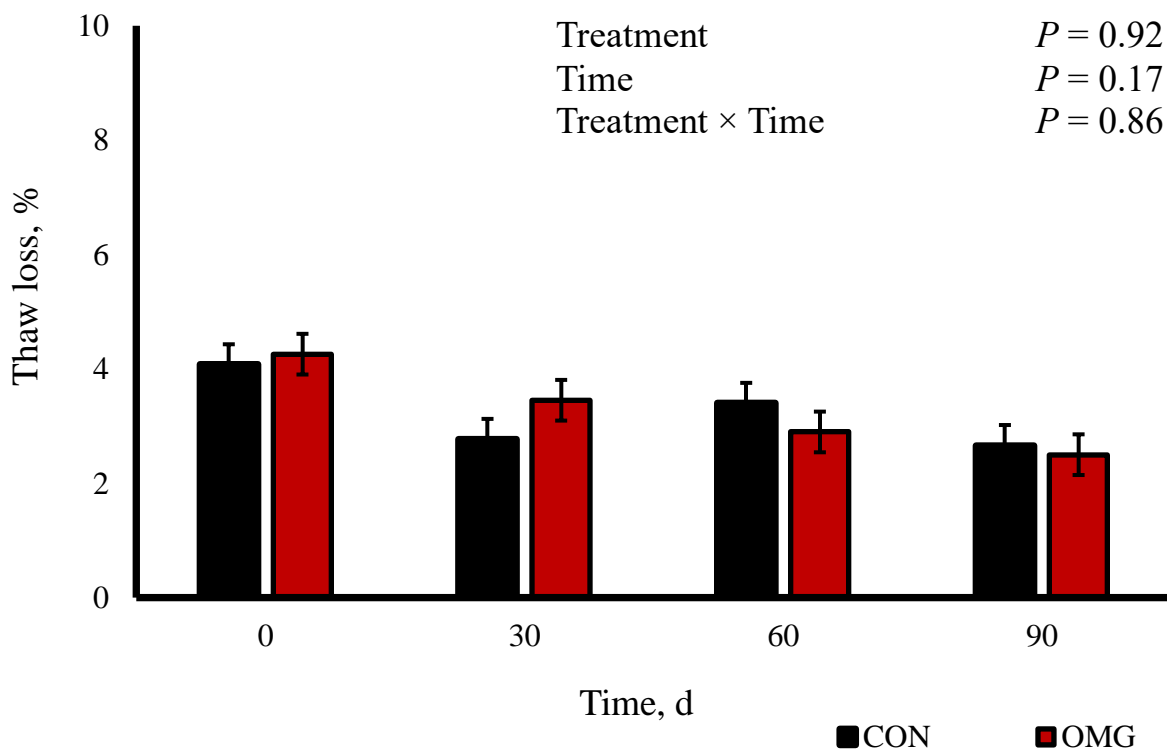
<sup>1</sup>greatOplus is a proprietary extruded blend of flaxseed, *Nannochloropsis oculata* algal biomass, and wheat middlings.



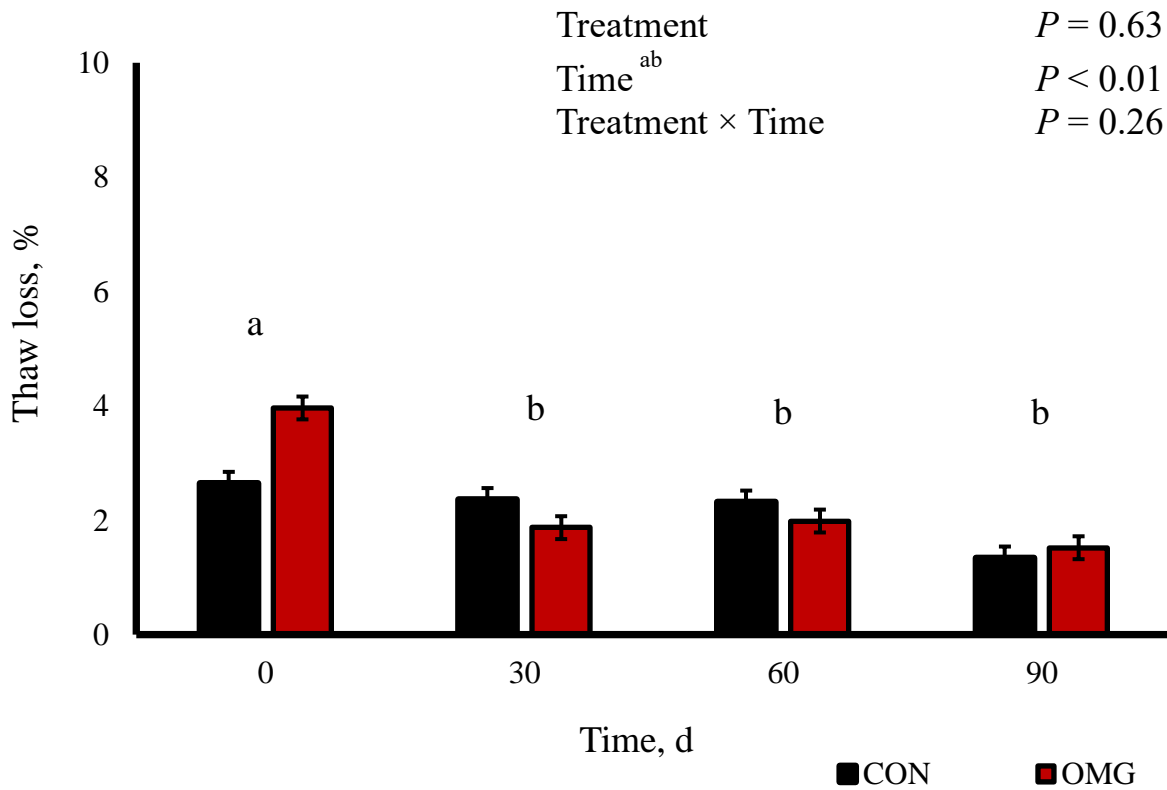
**Figure 5.1.** Thiobarbituric Acid Reactive Substances (TBARS) values for frozen-stored ground Round beef patties from steers fed a conventional feedlot diet (CON) or a diet containing 10% extruded proprietary blend of flaxseed, wheat middlings, and *Nannochloropsis* algae (OMG; greatOplus; NBO3 Technologies). Values were calculated using equations outlined by Buege and Aust (1978). Patties were stored at -20°C in HRI boxes and removed at 0, 30, 60, and 90 d for TBARS analysis. Significance was declared at  $P < 0.05$ .



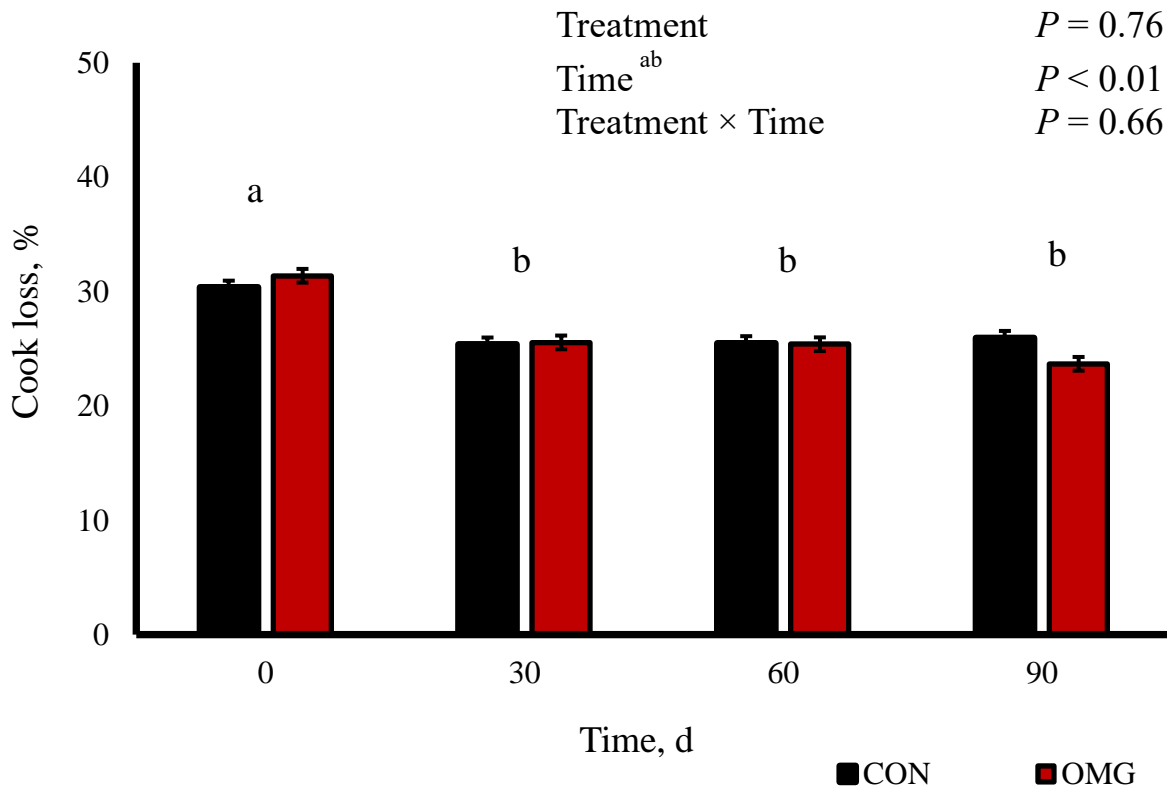
**Figure 5.2.** Thiobarbituric Acid Reactive Substances (TBARS) values for frozen-stored ground Chuck beef patties from steers fed a conventional feedlot diet (CON) or a diet containing 10% extruded proprietary blend of flaxseed, wheat middlings, and *Nannochloropsis* algae (OMG; greatOplus; NBO3 Technologies). Values were calculated using equations outlined by Buege and Aust (1978). Patties were stored at  $-20^{\circ}\text{C}$  in HRI boxes and removed at 0, 30, 60, and 90 d for TBARS analysis. Significance was declared at  $P < 0.05$ .



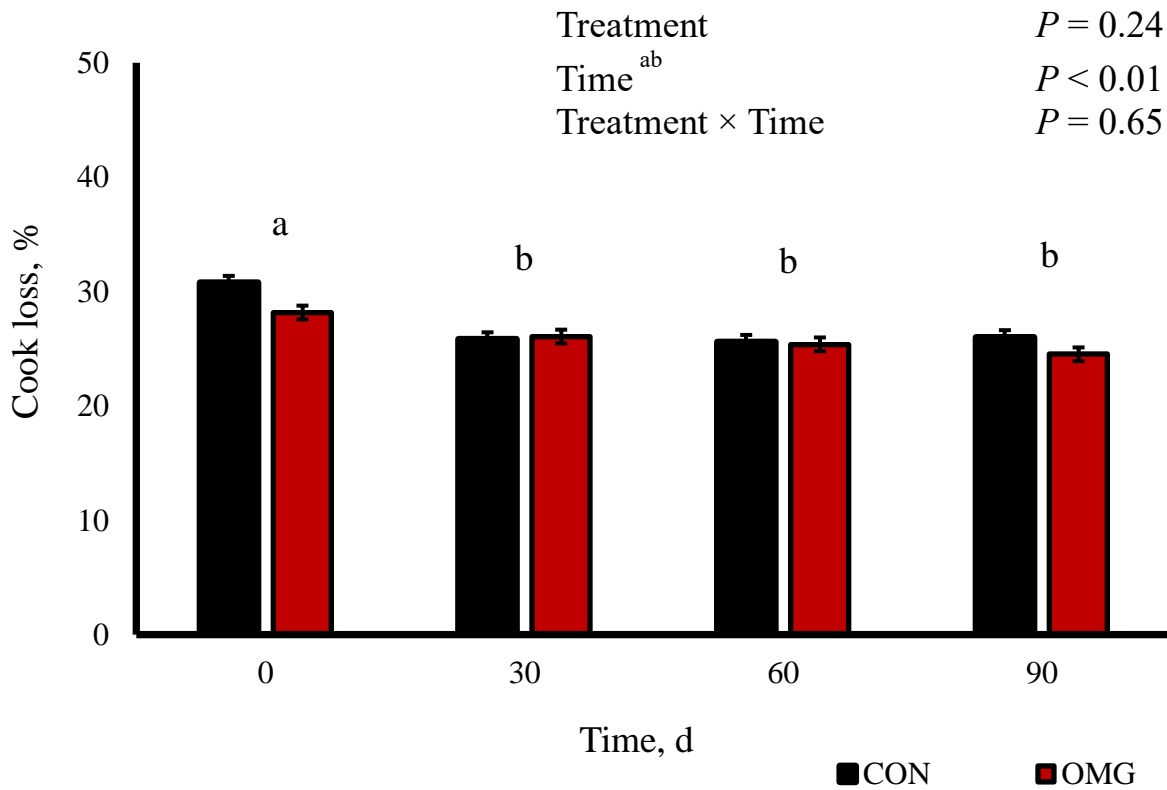
**Figure 5.3.** Thaw loss for frozen-stored ground Round beef patties from steers fed a conventional feedlot diet (CON) or a diet containing 10% extruded proprietary blend of flaxseed, wheat middlings, and *Nannochloropsis* algae (OMG; greatOplus; NBO3 Technologies). Patties were stored at -20°C in HRI boxes and removed at 0, 30, 60, and 90 d for analyses. Significance was declared at  $P \leq 0.05$ .



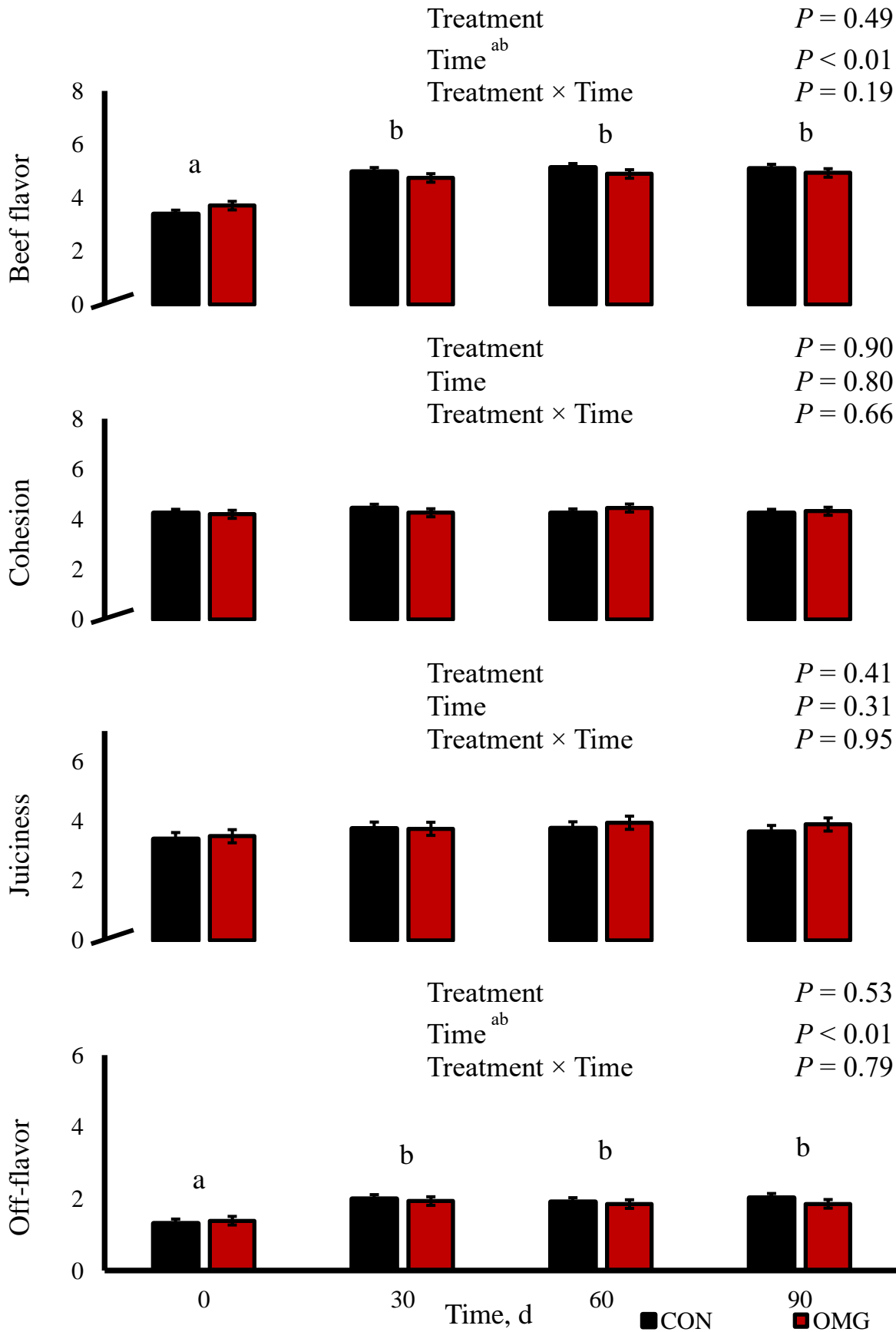
**Figure 5.4.** Thaw loss for frozen-stored ground Chuck beef patties from steers fed a conventional feedlot diet (CON) or a diet containing 10% extruded proprietary blend of flaxseed, wheat middlings, and *Nannochloropsis* algae (OMG; greatOplus; NBO3 Technologies). Patties were stored at -20°C in HRI boxes and removed at 0, 30, 60, and 90 d for analyses. Significance was declared at  $P \leq 0.05$ .



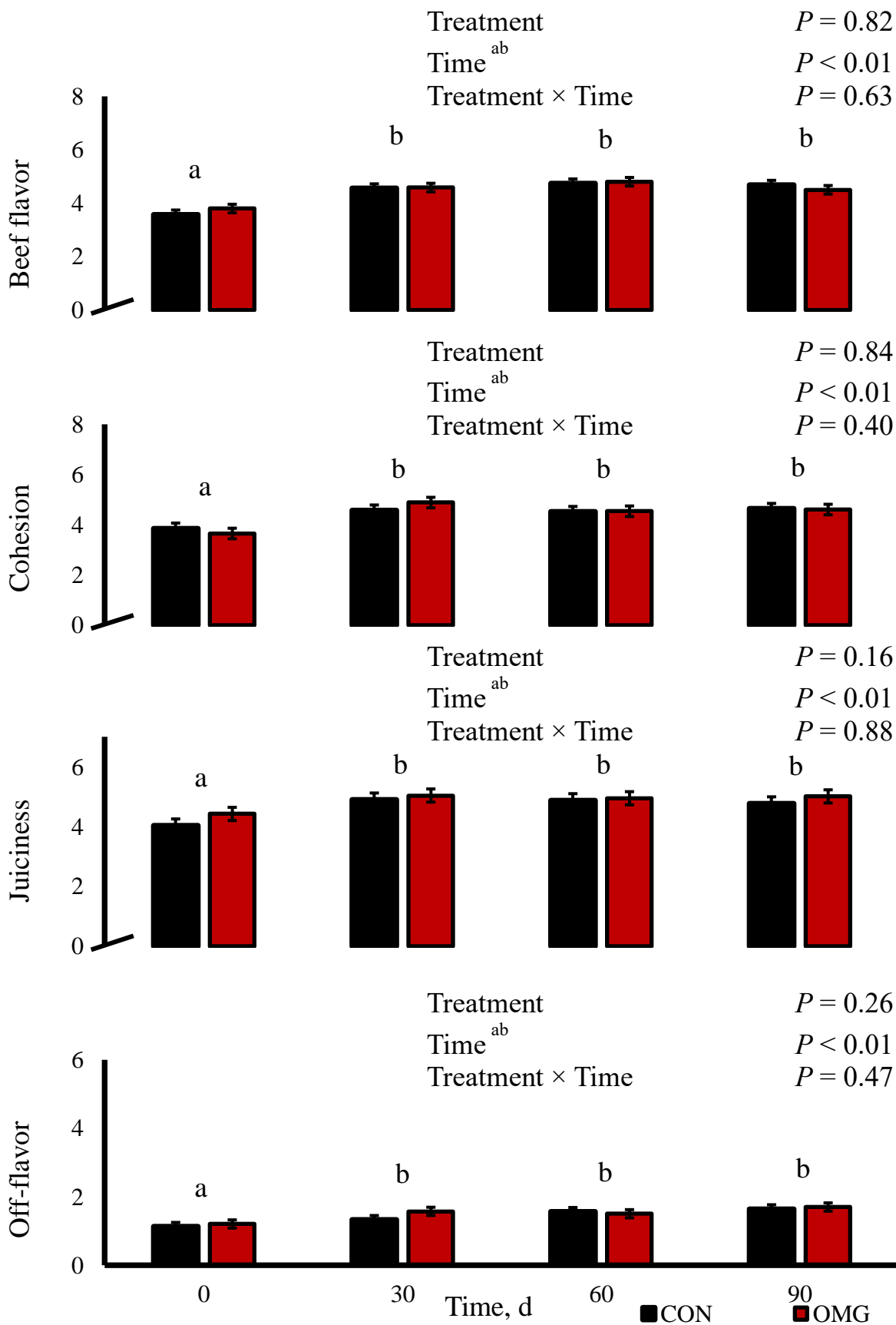
**Figure 5.5.** Cook loss for frozen-stored ground Round beef patties from steers fed a conventional feedlot diet (CON) or a diet containing 10% extruded proprietary blend of flaxseed, wheat middlings, and *Nannochloropsis* algae (OMG; greatOplus; NBO3 Technologies). Patties were stored at -20°C in HRI boxes and removed at 0, 30, 60, and 90 d for analyses. Significance was declared at  $P \leq 0.05$ .



**Figure 5.6.** Cook loss for frozen-stored ground Chuck beef patties from steers fed a conventional feedlot diet (CON) or a diet containing 10% extruded proprietary blend of flaxseed, wheat middlings, and *Nannochloropsis* algae (OMG; greatOplus; NBO3 Technologies). Patties stored at -20°C in HRI boxes and removed at 0, 30, 60, and 90 d for analyses. Significance was declared at  $P \leq 0.05$ .



**Figure 5.7.** Trained sensory ratings for frozen-stored ground Round beef patties from steers fed a conventional feedlot diet (CON) or a diet containing 10% extruded proprietary blend of flaxseed, wheat middlings, and *Nannochloropsis* algae (OMG; greatOplus; NBO3 Technologies). Patties evaluated using 8 pt hedonic scales (0 = no beef identity/bind/juice/off-flavor; 8 = very beefy/bound/juicy/off-flavor). Significance was declared at  $P \leq 0.05$ .



**Figure 5.8.** Trained sensory ratings for frozen-stored ground Chuck beef patties from steers fed a conventional feedlot diet (CON) or a diet containing 10% extruded proprietary blend of flaxseed, wheat middlings, and *Nannochloropsis* algae (OMG; greatOplus; NBO3 Technologies). Patties evaluated using 8 pt hedonic scales (0 = no beef identity/bind/juice/off-flavor; 8 = very beefy/bound/juicy/off-flavor). Significance was declared at  $P \leq 0.05$ .

## CHAPTER 6

### CONCLUSION

Overall, this research supports that ground beef patties originating from steers supplemented with greatOplus during finishing did not have adverse fresh quality attributes nor detrimental product palatability attributes which is contrary to previous omega-3 (**n-3**) enriched meat product studies. Further research utilizing increasing supplementation concentrations should be used to better understand how greatOplus may impact meat composition, color stability and palatability. If increasing in-feed supplementation further enhances product fatty acid profiles while continuing to mitigate negative fresh quality attributes, the resultant meat products may be able to gain more traction and provide an outlet for farmers, packers, and retailers to participate in a value-added beef program sought by consumers.

Additionally, the present study creates a foundation regarding the use of long-term frozen storage packaging with n-3 enhanced meat products. As the world population continues to grow and the demand for meat increases, the meat industry will become more dependent on bulk style, long-term frozen storage packaging to deliver large quantities of high-quality meat to consumers. Chapter 5 results validate the use of long-term frozen storage to deliver n-3 enhanced meat to consumers in great quantities without compromising product sensory and oxidation. Further research placing other n-3 enhanced products in long-term frozen storage should be conducted to better understand how packaging influence the final product and consumer sensory panels should be conducted understand the product palatability biological applicability.