5-2004

Effect of Raw Ingredient Surface Area, Storage Time and Antioxidants on Color and Oxidative Stability of Ground Beef in 80% Oxygen Modified Atmosphere Packaging

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EFFECT OF RAW INGREDIENT SURFACE AREA, STORAGE TIME, AND ANTIOXIDANTS ON COLOR AND OXIDATIVE STABILITY OF GROUND BEEF IN 80% OXYGEN MODIFIED ATMOSPHERE PACKAGING.

by

Avanthi Vissa

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

2004

ABSTRACT

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Utah State University, 2004

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Fresh beef packaged in high-oxygen modified atmosphere packaging (MAP) has longer red color stability than beef in oxygen-permeable polyvinyl chloride (PVC) film. However, fresh beef in high oxygen becomes rancid by 10 days storage at 2°C. Thus the objective of this study was to evaluate the effectiveness of various antioxidants (milk mineral, MM; sodium tripolyphosphate, STP; vitamin E, E) on color and thiobarbituric acid (TBA) values of ground chuck stored in 80% oxygen MAP for 14 days at 1° C. A preliminary experiment was also done to determine the effect of raw meat history (surface area during storage and storage temperature) on stability of ground beef in 80% oxygen MAP.

For the preliminary experiment, select beef clods (48 hrs postmortem) were cut into trim or coarsely ground and stored frozen or at 2°C in vacuum packaging (VP) for 30 days. Raw meat was then finely ground and wrapped in PVC film or in 80% oxygen. For experiment 2, fresh beef clods were coarsely ground and antioxidants (0.75 or 1.5% MM;

0.25 or 0.5% STP; 50 or 100 ppm vitamin E) were added, followed by fine grounding and packaging in 80% oxygen MAP.

Thiobarbituric acid assay was performed as a measure of rancidity. Hunter color L*, a*, b* values were measured on raw samples through the packaging film. Trim history greatly affected stability of beef in 80% oxygen MAP. VP refrigerated trim yielded ground beef with low oxidative and color stability compared to frozen trim. In comparison of antioxidants, 0.75% MM gave highest redness values (13-15) and lowest TBA values (< 0.5) after storage of ground beef in 80% oxygen MAP for 14 days. STP-treated beef also had low TBA values (< 0.5) at 14 days storage but samples were less red (a* of 10-12) than MM- treated samples. Samples with E were slightly better than controls, with redness values of 7.9 and 10.8, respectively. Thus, iron-chelating agents (MM and STP) were very effective for preventing rancidity and improving color stability in ground beef packaged in a high oxygen atmosphere.

(87 pages)

ACKNOWLEDGMENTS

I would like to take this opportunity to thank some people who have helped me to reach this stage. I would first like to thank Dr. Daren P. Cornforth for all his help and support throughout the course of my work. I would also like to thank my committee members Dr. Ilka Nemere and Dr. Donald McMahon for their help and guidance. I am thankful for the financial support received from Glanbia Foods (Twin Falls, ID) and The Utah Agricultural Experiment Station.

I would sincerely like to thank Karin Allen and Mihir Vasavada for their help with this research. I would also like to express my gratitude to my friends without whose encouragement and support I couldn't have realized my dreams in such a short time.

Finally I would like to thank my parents, Kashyap Vissa and Lalitha Vissa, for their support and encouragement through the course of my studies.

Avanthi Vissa

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LIST OF SYMBOLS,

ABBREVIATIONS, AND DEFINITIONS

BHT	Butylated hydroxyl toluene
C	Celsius
CO_2	Carbon dioxide
d	Day
FDA	Food and Drug Association
MAP	Modified atmosphere packaging
MM	Milk mineral
O2	Oxygen
PVC	Polyvinyl chloride
STP	Sodium tripolyphosphate
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
VE	Vitamin E in oil
VEOH	Vitamin E in ethanol
VP	Vacuum packaged

CHAPTER I

INTRODUCTION

One of the biggest challenges for the food industry is keeping foods fresh as much as possible for long periods of time. Lipid oxidation is common problem with foods and has great economic impact. Lipid oxidation of foods, especially foods containing oils and fats, leads to the development of off flavors and odors making the food rancid. Oxidative rancidity, as its also known as, renders the food unacceptable to the consumers and also leads to decreased nutritional quality of foods. Oxidation is the reaction of oxygen and lipids with formation of free radicals, which then continue this reaction, ultimately leading to deterioration of the foods.

Antioxidants delay onset lipid oxidation by minimizing free radical formation. Antioxidants are found naturally in foods. They are added in some processed foods, which are more susceptible to lipid oxidation. Generally 2 types of antioxidants are used in the food industry: natural or synthetic. The antioxidant of choice will be dependent on the food it is to be used in, and its potency. Other factors also have to be considered before deciding which antioxidant is to be used, as they can be sensitive to pH, heat and other factors. Vitamin E, tocopherols, BHA/BHT, rosemary, and propyl gallate are some of the more commonly used antioxidants. Antioxidants can be classified as food additives and are regulated by FDA. Antioxidants can be added only up to 0.02% of the weight based on the fat content of the foods.

The use of antioxidants can only help delay lipid oxidation and not stop it altogether. Lipid oxidation is a major problem in meats. Various antioxidants have been added to meats to help combat this problem and increase the shelf life of meats and meat

products. Meat quality is also affected by the way it has been packaged. In recent years newer methods have been developed to ensure quality and increased shelf life of meat and meat products. Meats are now increasingly being packaged in modified atmosphere packaging (MAP) as it keeps the meat looking more red. Rancidity problems have been studied in meats packaged in high oxygen MAP, where meats tend to go rancid faster than when packaged under a vacuum.

CHAPTER 2

LITERATURE REVIEW

Lipid Oxidation in Foods

Animal fats mainly contain large amounts of C16 and C18 fatty acids, medium amounts of unsaturated fatty acids, and small amounts of odd numbered acids (Fennema 1996). Lypolysis is the hydrolysis of ester bonds in lipids, which can be caused by heat, moisture or enzymes, resulting in the release of free fatty acids. Lypolysis occurs during deep fat frying of food, as there are large amounts of water from the foods that come in contact with the fat and also due to the high temperatures used for frying (Fennema 1996). Lipid oxidation can be defined as when the lipids in the foods undergo oxidation and become rancid. Lypolysis need not occur during lipid oxidation.

Lipid oxidation is a major concern for food manufacturers as it can lead to the development of off-flavors and odors due to the edible oils present in foods. Oxidation of foods could also decrease the nutritional value of food and some oxidation products are toxic (Fennema 1996). Lipid oxidation mainly occurs in 3 main steps: initiation, propagation and termination. Oxygen usually exists in the stable triplet state, but when oxygen is exposed to light or heat, it is converted to a singlet, excited state. In this excited state, oxygen abstracts hydrogen atoms from the carbon adjacent to the fatty acid double bonds, producing free radicals ($R^{\bullet}$) (Fennema 1996). Iron-oxygen complexes can catalyze this initiation reaction as follows: (Morrissey and others 1998).

$RH + HO^{\bullet} \rightarrow R^{\bullet}\ H_2O.$

Peroxyl radical is then formed when the fatty acyl radical reacts with oxygen.

$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$ (Morrissey and others 1998).

The $ROO^{\bullet}$ is highly oxidized and oxidizes other unsaturated fatty acids propagating the chain reaction.

$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$ (Morrissey and others 1998).

The reaction products from the propagation step, ROOH, further react with Fe^{2+} or Cu^{+} to give $ROO^{\bullet}$ and alkyl radicals ($RO^{\bullet}$) (Morrissey and others, 1994b).

$$Fe^{2+} + ROOH \xrightarrow{\text{Fast}} Fe^{3+} + RO^{\bullet} + OH^{-}$$

Termination is usually brought about by an antioxidant, such as Vitamin E, by donating an electron. Superoxide dismutase, work to convert singlet oxygen to hydrogen peroxide to water thereby limiting the formation of $OH^{\bullet}$ (Morrissey and others 1998). Vitamin E (α-tocopherol) is considered an important antioxidant, as it is present at 15 times higher concentration than any other antioxidants in the plasma lipids (Burton and others 1983). α-Tocopherol donates an electron to the peroxyl radical to form a hydroperoxide and a stable tocopheroxyl radical ($TO^{\bullet}$) (Packer 1993):

$$TOH + ROO^{\bullet} \rightarrow ROOH + TO^{\bullet}$$

Rhee, Anderson and Sams, (1996) found that chicken samples tended to have the lowest TBA values as compared to pork and beef. Beef samples had the highest TBA values. Pork samples tended to have higher TBA values when frozen rather than refrigerated.

Presence of iron catalyzes lipid oxidation at a much faster rate (Fennema 1996). Major catalyst for lipid oxidation was reported to be heme iron in raw meats and non-heme iron in cooked meats (Karabudak 2003). Lipid oxidation is generally faster in cooked meats than raw meats (Karabudak 2003). Faroouk and others (1991) reported that raw and cooked ground beef added with Fe^{2+} had higher TBA values than the controls. Decker and Welch (1990) have reported that iron release from ferritin is higher in the presence of ascorbate, than cysteine, when the pH is between 5.6-6.9. This study also reported that iron release was greater with ascorbate at concentrations of 0.001-1mM. Kanner and others (1988) noted that turkey and chicken had increased free iron ions when stored 4°C for 3 or 7 days and suggested that the increased free iron concentration led to increased lipid oxidation in chicken and turkey during storage. A study done by Richards and Rong (2004) showed that low molecular weight iron contributed little towards hemoglobin-mediated lipid oxidation in washed cod muscle. Another study (Osborn and Akoh 2003) reported that iron catalyzed lipid oxidation was higher in emulsions of pH 3.0 as compared to emulsions at a pH of 7.0. Gorelik and Kanner (2001) have shown that the addition of up to 50 μM ferric ions increased oxymyoglobin and lipid oxidation significantly. Low levels of Fe^{2+} have the ability to significantly increase lipid oxidation, suggesting that low levels of iron are needed near the lipid droplet surface to promote peroxide breakdown (Mei and others 1998).

It is common belief that metals, especially iron, play an important role in lipid oxidation (Harel and Kanner 1985). It has not been definitive as to which forms of iron, play the most important role in lipid oxidation (Love 1983). Liu and Watts have

suggested that heme and nonheme aid in the catalysis of lipid oxidation, where heme iron has more effect than nonheme iron. Other studies have shown that nonheme iron plays the major role in lipid oxidation in cooked meats (Sato and Hegarty 1971; Love and Pearson 1974). Boccia and others (2002) have reported that heme iron concentration was different in different cuts of poultry meats. The breast cuts had lower heme iron than legs and wings and after cooking the concentration of heme iron decreased in the leg and wing cuts. Igene and others (1979) found that heme pigments released iron during cooking and the increased level of nonheme iron was responsible for rapid catalysis of lipid oxidation in cooked meats.

Myoglobin was shown to be more pro-oxidant in heated muscle residues than in raw muscle residues (Tichivagana and Morrissey 1985). This was in agreement with the findings of Igene and others (1979). Some others studies have suggested that heme iron acts more like a pro-oxidant rather than nonheme iron (Johns and others, 1989). Verma and others (1985) have demonstrated, in egg albumin and water, that ferric heme irons increased lipid oxidation to a greater extent than ferrous pigments. Monahan and others (1993) reported that water washed pork muscle residues with added pro-oxidants hemoglobin or myoglobin oxidized faster than muscle residue containing iron-sulphate over a 6-day storage period. Johns and others (1989) also reported that when hemoglobin and iron-sulphate are added as pro-oxidants at similar levels, then hemoglobin catalyzes lipid oxidation at a faster rate than iron-sulphate. Monahan and others (1993) have also reported that in heated pork muscle residues, hemoglobin and myoglobin acted more as pro-oxidants than iron-sulphate, and TBA values were lower in treatments with iron-sulphate. This work was done in emulsion systems, where hemoglobin and myoglobin

may not have been present within the emulsion droplet and therefore acted as pro-oxidants.

Food Antioxidants

Food antioxidants can be classified as Type I or Type II antioxidants and as natural or synthetic. Naturally occurring antioxidants include retinoids (vitamin A) and tocopherols (vitamin E), found in many animals and plants; ascorbic acid (vitamin C), found in citrus and other fruits and vegetables; and beta carotene, found in deep orange and dark green vegetables. These may play a significant role in the prevention of cancer, heart disease, immune-deficiency diseases, and aging. Synthetic antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate. Natural and synthetic antioxidants are added to food to prevent undesirable deterioration. Foods preserved with antioxidants include vegetable oils, bread, and cheese. Antioxidants are also frequently applied to the packaging materials of cereals and nuts.

Type I antioxidants are those that donate an electron during lipid oxidation to terminate the oxidation process. Examples of Type I antioxidants are Vitamin E and C, BHA and BHT, and flavanoids like eugenol. Houlihan and others (1985) have shown that the antioxidant property may exist in phenolic compounds. Phenolic compounds exhibit antioxidant properties by interrupting free radical chain mechanism (Houlinan and Ho 1985). BHA/BHT were shown to be effective antioxidants in cooked pork, when used at a level of 0.01% of the meat weight (McCarthy and others 2001). BHT was shown to be effective as a chain breaking antioxidant in the lipid oxidation process and an effective radical scavenger (Fujisawa and others 2004). Wadhwa and others (1991) have reported

that the use of BHA/BHT at a level of 0.02% enhanced the shelf life of flavored butters. Studies by Vasavada and Cornforth (2003) have shown that a minimum of 0.01% of BHT based on meat weight was required for effectively lowering lipid oxidation in cooked ground pork.

Rosemary extract and ascorbic acid were used as natural antioxidants to sea bream fillets to see their effects on shelf life. The study concluded that ascorbic acid proved to be a good antioxidant, when the samples were displayed under low UV color balanced lights, as compared to the fluorescent lamps used in the super markets. Rosemary extract was effective under both the kinds of lighting (Gimenez and others 2004). Dietary feeding of steers with supplemental vitamin E has been shown to lower lipid oxidation in meats (Faustman and others 1993). Rosemary extract and other antioxidants were tested for effectiveness in irradiated bologna. Rosemary extract was effective in decreasing the TBA values, while erythrobate increased TBA values. Rosemary extract and nitrite were also effective in reducing the color changes caused by irradiation (Xuetong and others 2004). Vasavada and Cornforth (2003) showed that the use of rosemary oil at 0.05%-0.2% of raw meat weight was not effective in lowering lipid oxidation in cooked ground pork. However, ground rosemary at 0.4-0.8% was effective in lowering lipid oxidation and TBA values were at 1 or lower after 15 days of storage at 2° C. This study concluded that rosemary extract in oil was not as effective an antioxidant as ground rosemary.

Carnosine is another type I antioxidant that donates an electron to free radicals (Kohen and others 1988). Decker and Crum (1991) reported that carnosine had a color-protecting effect on salted ground pork. Its effectiveness was greater than that of BHT

and α-tocopherol. Carnosine effectively reduced TBA values in frozen pork stored for 6 months at levels of 0.5 and 1.5% (Decker and Crum 1991).

Vitamin E is one of the most widely used antioxidants in the meat industry. Arnold and others (1989) have shown that supplemental feeding of steers with vitamin E lowered lipid oxidation. Chan and others (1995) also reported that beef color stability was affected by endogenous tocopherol. The study reported that supplementation with vitamin E at 2000 international units prevented surface metmyoglobin formation. Hasty and others (2002) evaluated vitamin E supplementation on pork quality in 2 genotypes with distinct differences in pork quality. The study concluded that vitamin E supplementation had no effect on fresh pork quality in these different genotypes. Mitsumoto and others (1991) reported that the addition of vitamin E or vitamin C to ground beef improved pigment and lipid stability. vitamin E + C showed greater pigment and lipid stability than vitamin E or C alone. The use of vitamin C resulted in higher pigment stability, compared to control and vitamin E treatments. Tappel and others (1961) showed that vitamin C could act synergistically with vitamin E to lower lipid oxidation. Mitsumoto and others (1993) evaluated endogenous and exogenous supplementation of vitamin E. They reported that endogenous vitamin E improved pigment and lipid stability to a greater extent than exogenous addition of vitamin E to trim.

Type II antioxidants are those that reduce lipid oxidation by chelating metal ions. Tims and Watts (1958) have shown that polyphosphates act as antioxidants by chelating metals such as ferrous iron in cooked meats. Sodium tripolyphosphate has been added to meats as an antioxidant. Stoick and others (1991) have shown that the use of STP, in

cooked refrigerated restructured beef and frozen raw restructured beef, lowered lipid oxidation. Phosvitin is an iron binding egg yolk protein that has strong antioxidant activity in linoleate emulsions both in the presence and absence of iron (Yamamoto and others 1990). Liu and Baker (1986) have shown that phosvitin could inhibit iron-catalyzed oxidation in phospholipid emulsions. Lee and others (2002) reported that with increasing concentrations of phosvitin, increased inhibition of lipid oxidation was noted, at pH 6.0 in a phosphatidylcholine liposome model. Lipid oxidation was not further inhibited above 40 μM concentration of phosvitin. Phosvitin was a more effective antioxidant in cooked ground pork rather than raw ground pork (Lee and others 2002). Cho and others (2003) have shown that metal chelators like EDTA and STP can inhibit lipid oxidation in oil in water emulsions.

Lee and others (1998a) have shown that phytic acid was an effective antioxidant in cooked beef samples. Their study showed that phytic acid had greater inhibition of lipid oxidation than carnosine in cooked beef samples. They also compared sodium phytate, sodium pyrophosphate and sodium tripolyphosphate on extent of lipid oxidation in restructured beef (Lee and others 1998b). The results showed that all three chelators effectively lowered metmyoglobin formation, but the inhibitory effect of sodium phytate was stronger than the other 2 treatments.

More and more natural antioxidant compounds are being used in foods, although there will be continued use of synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytouluene (BHT). Consumer concerns regarding synthetic chemical antioxidants can be addressed by adding natural antioxidants to foods. Extracts containing high levels of phenolic compounds from grape seed and green tea extracts are

being used in food products to retard lipid oxidation (Rababah and others 2004). Sesemol, a substance found in sesame lignans, has a free phenolic group and has been shown to have antioxidant properties (Ghafoorunissa and others 2004). Sesame lignan extract added to cooked ground pork has been shown to be a better antioxidant than butylated hydroxyanisole (BHA) (Cho and others 2004).

The demand for the use of natural antioxidants over synthetic ones has increased over the past few years. More effort is placed on finding antioxidant properties in foods. Oleoresin rosemary has been shown to contain several compounds including rosmanol and carnosol that have antioxidant activity similar to or greater than BHA (Houlinan and Ho 1985). Korczak (1988) found that rosemary in precooked, minced products had a pronounced antioxidant activity. Stoick and others (1991) evaluated the use of oleoresin rosemary in cooked restructured beef during refrigerated storage and frozen storage of raw restructured beef. They reported use of rosemary alone was not an effective antioxidant under refrigerated storage conditions. For frozen samples increased concentrations of oleoresin rosemary increased oxidative stability. Wong and others (1995), reported that the use of rosemary extract and sage extract with vitamin E proved to decrease lipid oxidation in ground beef samples. No synergistic effect was seen when rosemary and sage extracts were added together.

Synthetic antioxidants are still used in the industry, even though there is an increasing demand for natural antioxidants. Tertiary butyl hyroquinone has been used in addition with STP and was shown to reduce lipid oxidation considerably (Ho and others 1995). Propyl gallate was as effective as rosemary extract or BHT/BHA in fresh pork sausages during 16 weeks of frozen storage. Less surface discoloration was observed

when propyl gallate or BHA/BHT were used as antioxidants (Ho and others 1995). Baowu and others (1999) have reported that propyl gallate in restructured beef surimi enhanced flavor by 7%. Controls without propyl gallate developed rancid off-flavors. Chen (1989) has shown that the use of propyl gallate or BHA reduced the mutagenicity caused when frying beef patties, but BHT increased mutagenicity.

Meat Color and Packaging

Packing is an important factor to consider when improving shelf life of products. Different methods have been used for packaging meats over the years. The newest method of packaging in the meat industry is the use of modified atmosphere packaging (MAP), where different combinations of gasses can be used to obtain the desired end product. High oxygen atmospheres are used to increase the redness of the meats. Stabilization of color is one of the main reasons that meat is packaged (Seidman and others 1984). Packaging under a vacuum extended the shelf life of products but raw beef had purple color that was not acceptable to consumers. Packaging with oxygen permeable polyvinyl chloride (PVC) allows the meat to bloom and look red, but the shelf life in PVC packaging is short (3-5 days at 2° C). Oxidation of myoglobin to brown metmyoglobin occurs with meats packaged in PVC within 5 days of storage at 3.3° C (Pierson and others 1970). Issanchou (1996) reported that consumers usually used color and packaging to determine meat purchases. The slightest brown color in meats reduces its sales (Hood 1994). Meats that exceed levels of 40% or more metmyoglobin (brown) are usually downgraded and rejected for purchase (Greene and others 1971).

High oxygen atmospheres maintain red color stability for 3-10 days. Daun and others (1971) reported that fresh meat developed metmyoglobin at a slower rate, after 10 days of packaging in 90% O_2 atmospheres, compared with meat packaged in air permeable packages. Bartkowski and others (1982) found that steaks packaged in 40-75% O_2 maintained acceptable color for 9 days, but had more moisture loss than the samples that were packaged under a vacuum. Krala (1998) reported that meat color was improved by 4 days when packaged in 80% O_2 MAP, rather than atmospheric air. Drip loss was also less in 80% O_2 MAP. Huffman and others (1975) reported that meat packaged in 100% CO_2 had lower aerobic bacterial growth, but the color was less desirable than meat packaged in 100% O_2. Ground beef patties packed in 80% O_2, had lower microbial growth and improved color stability as compared with beef patties packaged under a vacuum or PVC during 3 days of retail display (Ho and others 2003). Jakobsen and Bertelsen (2000) and Jayasingh and others (2002) found that high oxygen MAP causes meat to go rancid more rapidly. Jensen and others (1998) have also reported that pork loins packaged in 80% O_2 had increased lipid oxidation as compared to loins that were vacuum packaged.

Incident light, usually used for display in supermarkets, contributes to meat discoloration. Light induced changes in meat pigments have been hypothesized to precede lipid oxidation (Lynch and others, 1986). Brewer and Harbers (1991a) reported that packaging treatments that excluded oxygen delayed lipid oxidation, and packaging treatments that excluded both oxygen and light prevent loss in red color. Another study by Brewer and Harbers (1991b) evaluated the effects of ground pork in different packaging treatments that excluded light and/or oxygen over time in frozen storage. The

results showed that vacuum packaged pork maintained original sensory and physical characteristics for the longest period of time and showed very little change in 39 weeks of storage.

Postmortem handling of meats also affect the quality and shelf life. Reid (1983) has reported that freezing of meats leads to increased drip losses. Ground pork packaged in PVC had the greatest drip losses as compared to pork packed in Cryovac bags (Brewer and Harbers 1991a). Another factor that affects meat quality is the diet. If the diet is rich in vitamin E, then lipid oxidation is delayed. Arnold and others (1993) have shown that supplementation of steers with 500 IU or more vitamin E lowered lipid oxidation and improved color stability.

Dairy Constituents as Antioxidants in Meat Products

Many researchers have been looking at dairy constituents to act as antioxidants. Allen and Wrienden (1981) have reported that whey, α-lactalbumin and β-lactoglobulin have weak antioxidant activity. Whey is a cheap by-product of cheese making. Colbert and Decker (1991) evaluated the antioxidant activity of ultra-filtered permeate from acid whey. They tested the antioxidant potential in a phosphatidylcholine liposome model. Increased concentrations of acid whey increased inhibition of lipid oxidation. Twenty percent of acid whey was effective in lowering lipid oxidation but it increased the formation of conjugated fatty acids. Different fractions of the acid whey had different antioxidant potentials. Acid whey and its permeate inhibited iron, lipoxidase and hydrogen peroxide-activated, metmyoglobin-catalyzed lipid oxidation. The ability of permeates to retard iron catalyzed lipid oxidation ranged from 75-93%. Browdy and

Harris (1997) showed that whey powder suppressed the formation of TBARS and hydroperoxides in model systems and can be used as an antioxidant in some processed foods. Coronado and others (2002) have demonstrated that the use of whey powder in wiener sausages, during a 10 month frozen storage, had lower TBARS values but they were not significantly different from the other antioxidants used in the study. Whey protein isolate (WPI) was applied to king salmon as an edible coating and its effects on moisture loss and lipid oxidation were noted (Stuchell and Krochta 1995). The rate of moisture loss was reduced by 42-65% by WPI and lipid oxidation was also lower with WPI coating.

Tong and others (2000) investigated the antioxidant properties of whey in a Tween-20 stabilized salmon emulsion system. Whey fractions inhibited TBARS and lipid peroxides in a 10% salmon oil emulsion. Browdy and Harris (1997) reported that whey improves oxidative stability of soybean oil. TBARS and hydroperoxides were inhibited by whey and it also lowered oxygen uptake in model systems. Unheated whey and whey heated for 24 hours or more were the most effective in inhibiting lipid oxidation. McGookin and Augustin (1991) have shown that casein has antioxidant properties, and heating of casein in the presence of glucose or lactose enhanced the antioxidant activity. Whey protein isolates (WPI) hydrolytes and soy protein isolates (SPI) were used in cooked pork. The pork was cooked to 70° C and then stored for 4 days. WPI and SPI both lowered lipid oxidation, SPI had a greater effect than WPI (Pena-Ramos and Xiong 2003). Ellekjaer and others (1996) tested skim milk powder, sodium caseinate and whey protein in cooked sausages to determine any changes in quality and sensory attributes.

The results showed that milk protein was most similar to the controls in sensory qualities. A 1:1 blend of milk powder and whey protein had the lowest cooking losses.

Milk mineral (MM) is the dried permeate of ultra-filtered whey. Table 1 gives the composition of milk mineral available in the market by Glambia Foods (Twin Falls, ID). Jayasingh and others (2003) have shown that the use of MM in ground cooked pork lowered lipid oxidation. Cornforth and West (2002) reported that 2% of MM was required to decrease lipid oxidation in raw beef and pork, and only 1% was required to maintain oxidative stability in ground turkey. The phosphates in MM were suggested to maintain low TBARS values. Their results suggested that MM was chelating iron and thereby lowering lipid oxidation. Thus MM could be classified as a type II antioxidant.

Lipid Oxidation Methods

Different methods are used for the measurement of lipid oxidation: peroxide value, thiobarbituric acid assay and Kreis test. Peroxides are the main initial products of autoxidation. This technique uses iodine liberated from potassium iodide, or oxidation of ferrous to ferric ions. This method is highly empirical and the accuracy is questionable and the results vary with the details of the procedure used. Also this procedure is very sensitive to temperature. Thiobarbituric acid (TBA) assay is commonly used to determine the amount of lipid oxidation in foods. It is a very simple test and is highly correlated with sensory test scores. TBA reaction give a pink colored malonaldehyde-TBA complex, which is the measured spectrophotometrically at 530 nm (Rhee 1978). Kreis test was one of the first tests used to commercially to evaluate oxidation of fats. It has been difficult to get consistent results from different labs using this method.

Table 1. Composition of Milk Mineral

Constituent	Proportion
Mineral	80.2%
Inorganic Mineral (ash)	71.2%
Organic Mineral (citrate)	9.0%
Calcium	24.0%
Phosphorus	13.5%
Lactose	10.0%
Protein	5.0%
Free Moisture	4.0%
Fat	0.5%

TBA can be performed on a steam distillate from a sample (Tarladgis and others 1960). It can also be done on aqueous or acid extractions of the sample (Witte and others 1970). The distillation procedure described by Tarladgis and others (1960) requires 10 g of sample and 24 h for distillation. The aqueous extraction method also requires 10 g of sample and it has to be heated in a water bath for 1 h and cooled for 10 min (Witte and others1970). Pikul and others 1983) first extracted the lipids from the meat tissues and then performed TBA analysis on the lipids. For all the 3 methods the absorbance was read at 532nm. Pikul and others (1989) compared all of the above 3 methods for lipid oxidation determination. They found that the distillation method was 1.3 times lower than lipid extraction method, which in turn was 1.4 times higher than the aqueous extraction method.

The method described by Buege and Aust (1978) requires only 0.5 g of sample and only 10 min of heating in the water bath. The results are highly correlated with sensory scores. This would be the method of choice as it is fast, simple and very accurate. As a very small sample size is required to do this test and also it is very fast, this test is performed in my studies as a measure of lipid oxidation in raw ground beef.

Hypotheses and Objectives

Experiment 1 has the following hypotheses: Ground beef will be more prone to rancidity and color problems if made from raw materials with high surface area (coarse ground). Also ground beef will be more prone to rancidity and color problems if made from refrigerated raw materials, compared to frozen raw materials. The hypothesis for experiment 2 is: antioxidants such as milk mineral, sodium tripolyphosphate, or vitamin E will increase color stability and decrease rancidity in ground beef packaged in 80% oxygen MAP.

The objectives are as follows:

- To determine the effects of raw material surface area and storage time (30 days at – 20 C or 1°C) on TBA values and color of hamburger in PVC or 80% O_2.
- To determine the effects of MM; 0.75 & 1.5%, STP; 0.25 & 0.5% and VE; 50 & 100 ppm on TBA values and color of ground beef in 80% oxygen modified atmosphere packaging.

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CHAPTER 3

EFFECT OF STORAGE TEMPERATURE AND MEAT PARTICLE SIZE ON TBA VALUES AND COLOR OF GROUND BEEF IN 80% OXYGEN MODIFIED ATMOSPHERE PACKAGING OR POLYVINLY CHLORIDE OVER-WRAP

Abstract

Ground beef in 80% oxygen modified atmosphere packaging (MAP) has variable color stability, possibly due to variable ingredient quality. Ingredient quality is dependent upon storage history (temperature, time) and extent of meat surface oxidation, which is influenced by meat particle size and surface area. The objective of this study was to determine effects of ingredient storage temperature and particle size on color and thiobarbituric acid (TBA) levels of ground beef packaged in polyvinyl chloride (PVC) film or 80% oxygen-MAP. The raw material storage treatments (in vacuum bags) were: frozen intact or coarse ground, and refrigerated intact or coarse ground. After 30 days at 1°C, samples were finely ground and packaged in PVC or 80% oxygen and held at 1°C for 14 days. Storage temperature, ingredient particle size, and their interactions affected ($p < 0.05$) color stability. Ground beef from frozen ingredients maintained red color and a* values > 10 for 7 days. For refrigerated treatments, only samples prepared from intact trim and packaged in 80% oxygen maintained red color for 7 days. TBA values were unacceptably high for ground beef in 80% oxygen MAP, prepared from coarsely ground, refrigerated trim.

Introduction

Food packaging is designed to ensure that the quality and organoleptic characteristics of food are preserved. Pyschotrophic bacteria are the most common spoilage microorganisms on fresh red meats, their growth can be inhibited by vacuum packaging or using an oxygen barrier film. However vacuum packaged meats have a dark color and therefore are not commonly used for retail fresh beef. In the USA retail fresh beef has been packaged in oxygen permeable polyvinyl chloride (PVC) film allowing oxymyoglobin formation and red color development (bloom).

A more recent development is case ready modified atmosphere packaging (MAP). The most commonly used atmosphere is 80% oxygen and 20% CO_2. The 80% oxygen MAP system typically gives 3-7 days more red color stability than PVC film. The use of modified atmosphere packages prepared at the processing facility makes it possible to have case ready retail meat cuts, reducing the labor costs at the retail store.

Appearance (color and package features) affects consumer purchase decisions (Issanchou, 1996). Carpenter, Cornforth, and Whittier (2001) investigated whether consumer preferences for beef color and packaging affected cooked hamburger taste panel scores. They found that color and packaging method influenced consumers' buying decisions but did not influence panel scores. Oxygen permeable films and high oxygen atmosphere are used to increase meat red color stability. Krala (1998) reported that meat color was improved by 4 days when packaged in 80% O_2 MAP, rather than atmospheric air. Drip loss was also less in 80% O_2 MAP.

Jakobsen and Bertelsen (2000) and Jayasingh, Cornforth, Brennand, Carpenter, Whittier (2002) found that high oxygen MAP causes meat to go rancid more rapidly. Jensen, Flensted-Jensen, Skibsted, Bertelsen (1998) also reported that pork loins packaged in 80% O_2 had increased lipid oxidation, compared to vacuum packaged loins. Muscle quality and composition also affect meat quality and shelf life. Arnold, Scheller, Arp, Williams, Schaefer (1993) found that ground beef from animals on vitamin E supplemented diets was more color stable and had less lipid oxidation than controls without vitamin E supplementation. Meat storage temperature and packaging method also affects retail beef quality and shelf life. Brewer and Harbers (1991) found that ground pork frozen and stored in PVC had lower moisture holding capacity than meat frozen in Cryovac bags.

Coarsely ground beef held for 21 days before final grinding had lower color values than the same samples supplemented with vitamin E (Zerby, Belk, Sofos, McDowell, and Smith (1999). Ground meat particle size affects meat acceptability. Suman and Sharma (2003) reported that sensory scores were significantly higher for low fat ground buffalo patties prepared using a grind size of 3mm than those made with a grind size of 4 or 6 mm. No mention was made of color stability related to ground meat particle size. The objective of this study was to determine effects of ingredient storage temperature and surface area (intact trim or coarsely cround) on color and thiobarbituric acid (TBA) levels of ground beef packaged in polyvinyl chloride (PVC) film or 80% oxygen-MAP.

Materials and Methods

Experimental design and statistical analysis

The experiment was a nested factorial design to examine the effects of 2 ingredient storage temperatures (-20 and 1°C), 2 raw material ingredients (intact trim and coarse ground trim) and 2 packaging types (PVC and 80%O_2) on ground beef lipid and color stability. Days after retail storage (1, 4, 7, and 14) were nested within the interaction of storage temperature x raw material surface area x packaging methods. After 30 days storage of raw materials, the meat was finely ground and packaged in either PVC or 80% oxygen MAP. Packaged ground beef samples were analyzed on 1, 4, 7, and 14 days for TBA and color. TBA measurements were also taken on both interior and exterior portions of ground beef in PVC packaging. Treatments were analyzed for statistical significance using proc GLM/MIXED function in Statistical Analysis Software (SAS) version 9.0 (SAS Institute Inc., Cary, NC). Analysis of variance was used to identify statistically significant differences at the 95% confidence level. Post-hoc mean comparisons were made based on p-values ($\alpha = 0.05$) using the Tukey-Kramer adjustment to obtain differences of least square means. The entire experiment was done in two replicates.

Sample preparation and storage

Select grade boneless beef shoulder clods were purchased from E.A Miller and Sons, Inc. (Hyrum, UT). Within 48 h postmortem, beef (27.2 kg) was cut into 2.5 x 2.5 cm strips of variable length, and another 27.2 kg beef was coarsely ground through a 0.60 cm plate Four storage treatments (13.6 kg each) were prepared as follows:

1) Strips, vacuum packaged, frozen 30 d at –20° C.

2) Strips, vacuum packaged, refrigerated 30 days at 1° C.

3) Coarsely ground, vacuum packaged, frozen 30 d at –20° C.

4) Coarsely ground, vacuum packaged, refrigerated 30 days at 1° C.

After the 30 d storage period, meat from all treatments was finely ground through a 0.32 cm plate. From each treatment, four portions (1 kg each) were placed in a Styrofoam tray and over-wrapped with PVC film for analysis at 1, 4, 7, and 14 days retail storage. Another four portions (130 g each) from each treatment were placed in modified atmosphere packages of 80% oxygen + 20% carbon dioxide. The pouches (25 x 35 cm; Koch, Kansas City, MO) used for packaging were 3 mil thickness (0.75 gauge nylon, 2.25 gauge polyethylene), with an oxygen permeability of 0.6 cm^3 / 100 m^2 / 24 hrs at 0°C and a water vapor transmission rate of 0.6 g / 100 m^2 / 24 hrs at 38°C and 100% relative humidity. The gas cylinder containing 80% oxygen and 20% carbon dioxide was obtained from Praxair Distribution (Salt Lake City, UT) and certified to be within ± 0.5% of the indicated mixture. A final portion (100 g) from each treatment was placed in a vacuum bag and frozen at –20° C for later determination of fat content. The ground beef samples had mean fat content of 12.1 ± 1.2 % (AOAC, 1990).

Thiobarbituric acid assay (TBA) and color readings were taken after 1, 4, 7 and 14 d in retail packaging.

TBA analysis

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978). Duplicate samples (0.5g) were mixed with 2.5 ml

of stock solution containing 0.375% TBA (Sigma Chem. Co., St. Louis), 15% TCA (Mallinckrodt Baker, Inc., Paris, Kentucky) and 0.25 N HCl. The mixture was heated for 10 min in a boiling water bath (100°C) to develop a pink color, cooled in tap water and then centrifuged (Sorvall Instruments, Model RC 5C, DuPont, Wilmington, Delaware) at 6000 rpm for 10 min. The absorbance of the supernatant was measured spectrophotometrically (Spectronic 21D, Milton Roy, Rochester, NY) at 532 nm against a blank that contained all the reagents minus the meat. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ (Sinnhuber and Yu, 1958). The MDA concentration was converted to TBA number (mg MDA / Kg sample) as follows:

1) TBA# (mg / kg) = Sample A_{532} X (1 M TBA chromagen / 1.56×10^5) X [(1 mole / L) / M] X (0.003 L / 0.5 g meat) X (72.07 g MDA / mole MDA) X (1000 mg / g) X (1000 g / Kg), or 2) TBA No. (ppm) = sample A_{532} X 2.77

Hunter Color Measurements

The L*, a* and b* values were measured using a Hunter lab Miniscan portable colorimeter (Reston, VA), standardized using a white and black standard tile. Two measurements were taken through the packaging film per sample per treatment. The hue angle was calculated using the formula: hue angle = $\tan^{-1}$ (b*/a*). Larger hue angles are associated with less red color (Van Laack, Berry and Solomon 1996), where hue angle 0 = red and hue angle 90 = yellow. Raw samples were scored for color, where 1= purple, 2= reddish purple, 3= bright red, 4= tan and 5= brown.

Results and Discussion

The main effects of raw material surface area storage temperature and packaging method on color and TBA values of ground beef packaged in PVC film or 80% oxygen MAP are shown in table 2 (See Appendix A for detailed statistics). Raw material surface area (coarse ground or 2.5 x 2.5 x 12 cm strips) significantly affected TBA values but had no effect on Hunter color values of ground beef in retail packaging. Raw material storage temperature (-20 or 2° C) significantly affected TBA values and all Hunter color measurements except b* values of ground beef in retail packaging. The main effect of packaging (PVC or 80% O_2-MAP) significantly affected TBA values, b* and hue angle values but not L* and a* values (Table 2). The interaction of raw material surface area with storage temperature significantly affected TBA values and all Hunter color values except a* values (redness) of ground beef in retail packaging (Table 2). The means and standard deviations for L*, b* and Hue angle values for samples in PVC and 80% oxygen MAP are given in Tables 3-4.

Fig. 1 shows the increase in TBA values of ground beef in PVC packaging when the raw materials were coarsely ground and stored in vacuum bags for 30 days. TBA values remained low (> 1.0) if the raw materials were stored as strips rather than coarsely ground (Fig. 1). These results clearly show that grinding of raw materials followed by extended storage is detrimental to the oxidative stability of ground beef in retail PVC packaging. The effects of storage history (raw material surface area and storage temperature) on TBA values of ground beef in 80% oxygen MAP are sown in Fig. 2. For ground beef in 80% O_2 MAP storage temperature was the main factor affecting TBA values. Refrigerated raw ingredients were associated with higher TBA values than frozen

raw ingredients. The effect of raw material surface area (coarse ground or strips) had less effect on TBA values of ground beef in 80% O_2 MAP (Fig. 2). The difference in temperatures could be attributed to the fact that lipid oxidation occurs more slowly at lower temperatures, while higher temperatures lead to quicker oxymyoglobin oxidation (Yin and Faustman 1993).

Table 2. Main effects of raw material surface area (SA), storage temperature, and packaging method on color[1] and TBA values of raw ground beef packaged in PVC or 80% oxygen MAP.

Effect	L*	a*	b*	Hue Angle	TBA Value (ppm)
SA[2]	NS	NS	NS	NS	*
Temp[3]	*	*	NS	*	*
SA x Temp	*	NS	*	*	*
Packaging[4]	NS	NS	*	*	*
SA x Packaging	NS	NS	NS	NS	NS
Temp x Packaging	*	NS	NS	*	*
SA x Temp x Packaging	NS	*	NS	*	NS

* Significant at $p < 0.05$, NS = not significant.

1 L* = lightness; a* = redness; b* = yellowness; hue angle = aractan (b*/a*).

2 Raw samples were stored for 30 days in vacuum bags as strips or coarsely ground.

3 Raw samples in vacuum bags were either frozen (-20° C) or refrigerated (1° C).

4 After 30 days storage, meat was finely ground and packaged with polyvinyl chloride film or modified atmosphere packaged with 80% oxygen and 20% carbon dioxide.

Table 3. Means ± standard deviation for the interaction of raw material surface area (SA) [1], storage temperature[2], packaging method[3] and storage time on color of raw ground beef packaged in PVC film or 80% oxygen MAP. Mean color values were pooled over time in retail packaging (1, 4, 7, 14 d at 1° C).

Raw material	Storage temp	Packaging	L*	B*	Hue
G	F	O2	39.2 ± 3.4	14.6 ± 2.4	48.3 ± 1.6
G	F	PVC	39.1 ± 4.8	14.8 ± 1.9	41.6 ± 1.6
G	R	O2	45.8 ± 5.4	14.7 ± 2.1	62.7 ± 2.3
G	R	PVC	43.5 ± 2.9	15.9 ± 2.5	59.6 ± 1.5
T	F	O2	40.6 ± 3.8	15.1 ± 1.9	53.5 ± 2.5
T	F	PVC	43.2 ± 4.23	15.9 ± 2.3	54.3 ± 2.8
T	R	O2	42.9 ± 4.63	14.1 ± 2.4	55.2 ± 2.2
T	R	PVC	40.4 ± 2.76	14.8 ± 1.9	60.1 ± 1.8

1 Raw material surface area G = coarse ground; T = 2.5 x 2.5 x 12 cm Strips.
2 Storage temperature F = frozen; R = refrigerated.
3 Packaging method O2 = 80% oxygen, 20% carbon dioxide; PVC = polyvinyl chloride film.

Interestingly, sample location also affected TBA values of ground beef in PVC packaging (Fig. 3). Regardless of storage temperature or raw material surface area all ground beef samples taken from the anaerobic interior of beef chubs had low TBA values (< 1.0) after 14 days in PVC packaging. TBA values were always higher for ground beef samples taken from the exterior of chubs in PVC packaging and especially so for ground beef that had been prepared from refrigerated, rather than frozen raw materials (Fig. 3). This result is predictable because PVC film is oxygen permeable, so samples taken from the surface just below the PVC film would have been exposed to oxygen during retail storage. The interior samples had low TBA numbers, as the myoglobin was mainly present in the non-oxidized deoxymyoglobin form (Fennema, 1996). In agreement with these results, Brewer and Harbers (1991) noted that packing fresh ground pork in PVC

Table 4. Means ± standard deviation for the interaction of raw material surface area (SA) [1], storage temperature[2], packaging method[3] and storage time on color of raw ground beef packaged in PVC film or 80% oxygen MAP, in retail packaging (1, 4, 7, 14 d at 1° C).

SA	Storage temp	Packaging	Day	L*	B*	Hue
G	F	O2	1	35.93 ± 2.02	16.94 ± 2.65	44.57 ± 1.58
G	F	O2	4	40.34 ± 1.36	15.09 ± 2.03	48.97 ± 1.29
G	F	O2	7	40.04 ± 5.31	14.54 ± 1.26	46.32 ± 2.03
G	F	O2	14	40.60 ± 2.14	11.98 ± 0.80	50.35 ± 2.23
G	F	PVC	1	37.19 ± 3.20	15.76 ± 2.26	47.25 ± 1.84
G	F	PVC	4	40.86 ± 4.01	16.18 ± 0.58	54.06 ± 2.34
G	F	PVC	7	41.01 ± 7.05	13.77 ± 2.14	56.39 ± 1.77
G	F	PVC	14	37.65 ± 5.00	12.87 ± 1.04	61.46 ± 0.47
G	R	O2	1	41.30 ± 1.31	17.40 ± 0.84	45.05 ± 2.72
G	R	O2	4	45.32 ± 5.87	13.85 ± 1.04	49.77 ± 1.82
G	R	O2	7	38.72 ± 5.37	13.88 ± 2.22	56.79 ± 4.84
G	R	O2	14	39.39 ± 5.85	13.36 ± 1.78	59.45 ± 1.47
G	R	PVC	1	42.46 ± 1.94	18.65 ± 1.38	72.11 ± 2.03
G	R	PVC	4	44.88 ± 2.50	14.78 ± 0.82	70.69 ± 1.82
G	R	PVC	7	44.84 ± 1.08	16.29 ± 1.01	80.5 ± 1.66
G	R	PVC	14	42.79 ± 4.58	14.10 ± 3.12	64.12 ± 0.56
T	F	O2	1	41.66 ± 4.06	16.84 ± 1.38	46.76 ± 2.37
T	F	O2	4	40.85 ± 3.92	15.89 ± 0.66	50.18 ± 2.82
T	F	O2	7	34.98 ± 2.29	13.26 ± 1.11	53.13 ± 4.20
T	F	O2	14	42.30 ± 1.67	13.64 ± 1.88	48.63 ± 1.53
T	F	PVC	1	44.87 ± 4.02	17.72 ± 1.61	43.32 ± 3.79
T	F	PVC	4	41.38 ± 4.32	16.34 ± 2.96	51.25 ± 2.10
T	F	PVC	7	39.44 ± 2.62	15.27 ± 0.43	66.77 ± 2.96
T	F	PVC	14	45.56 ± 3.91	13.99 ± 1.88	65.63 ± 2.48
T	R	O2	1	42.35 ± 2.06	15.91 ± 1.36	48.48 ± 2.83
T	R	O2	4	39.09 ± 1.56	15.70 ± 1.85	47.81 ± 1.05
T	R	O2	7	42.46 ± 5.84	14.03 ± 1.33	47.1 ± 4.21
T	R	O2	14	47.91 ± 3.59	10.90 ± 1.03	50.66 ± 0.81
T	R	PVC	1	40.07 ± 2.61	16.28 ± 0.60	50.22 ± 1.66
T	R	PVC	4	38.72 ± 2.13	16.48 ± 0.33	65.58 ± 1.42
T	R	PVC	7	41.22 ± 1.90	12.71 ± 0.65	74.83 ±2.49
T	R	PVC	14	41.93 ± 3.90	13.82 ± 1.89	76.38 ± 1.67

1 Raw material surface area G = coarse ground; T = 2.5 x 2.5 x 12 cm Strips.
2 Storage temperature F = frozen; R = refrigerated.
3 Packaging method O2 = 80% oxygen, 20% carbon dioxide; PVC = polyvinyl chloride film.

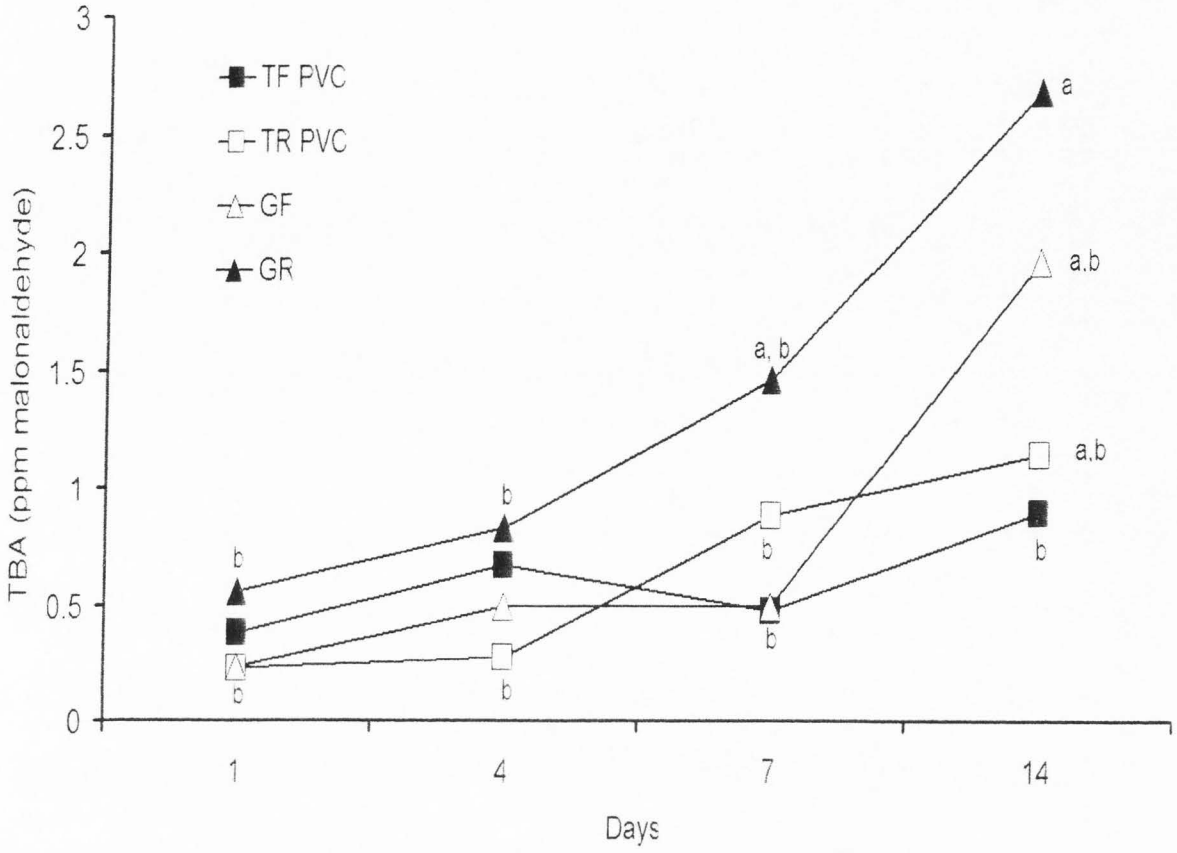

Fig. 1 Effect of raw ingredient storage history (raw ingredient surface area and storage time) on TBA values of raw ground beef packaged in oxygen-permeable polyvinyl chloride (PVC) film pooled over time (1, 4, 7 and 14 d). G = coarse ground, F = frozen, R = refrigerated, T = 2.5 x 2.5 x 12 cm strips.

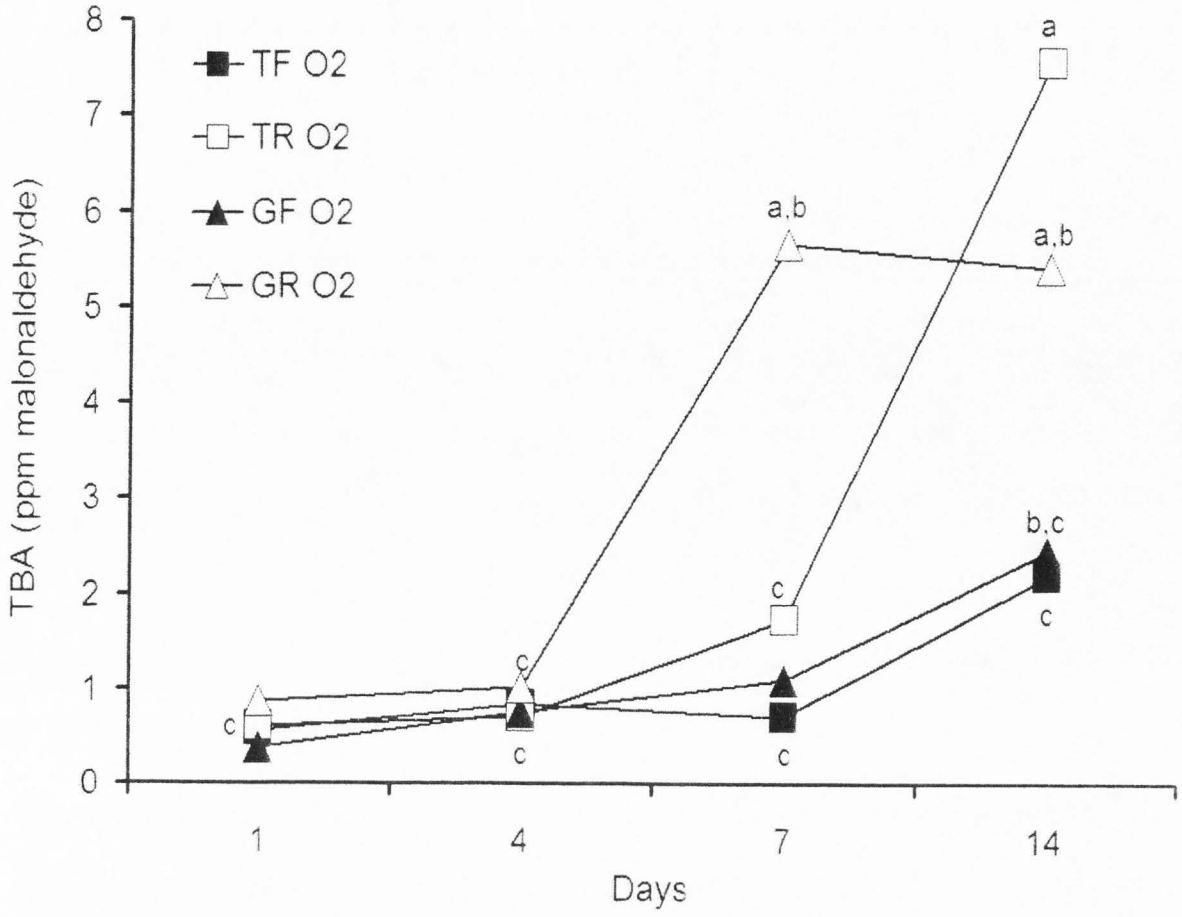

Fig. 2 Effect of raw ingredient storage history (raw ingredient surface area and storage time) on TBA values of raw ground beef packaged in 80% oxygen modified atmosphere packaging. G = coarse ground, F = frozen, R – refrigerated, T = 2.5 x 2.5 x 12 cm strips.

wrap and freezing for 39 weeks, gave higher TBA values, compared to samples packaged in vacuum or Saran Wrap. Redness (a* values) remained high (> 10) for 7 days for ground beef in PVC packaging if raw materials had been stored frozen but redness values were less than 7 which is indicative of brown color development if raw materials had been stored at 1° C for 30 days (Fig. 4). Ground beef stored in 80% oxygen MAP remained red for 7 days for all raw material storage treatments except for the treatment where raw materials were coarsely ground and then stored at 1° C for 30 days (Fig. 5). Regardless of storage history all ground beef samples had low redness values by 14 days of retail storage (Fig. 5). The mean values for L*, b* and hue angle of ground beef in retail packaging for 1-14 days at 1° C are shown in table 2-3. Brewer and Wu (1993) have shown that ground beef packaged in PVC had the most intense brown color in the exterior as compared to vacuum packaged ground beef. This study also indicated that exterior redness scores decreased after the samples were placed in display cases. Lanari, Schaefer, Cassens, and Scheller (1995) have noted that meat with increased oxygen blooms faster and retains the color longer, when not under illuminated conditions. Daly and Acton have shown that meat packaged in high oxygen MAP, lost their redness faster, by day 3 when stored at 4.4°C, then when stored at 0°C.

Conclusion

Trim history most significantly affected the stability of beef in 80% O_2 MAP stored for 14 days. Ground beef packaged in high oxygen atmosphere packaging was most susceptible to lipid oxidation. Ground beef made from trim, whether frozen or

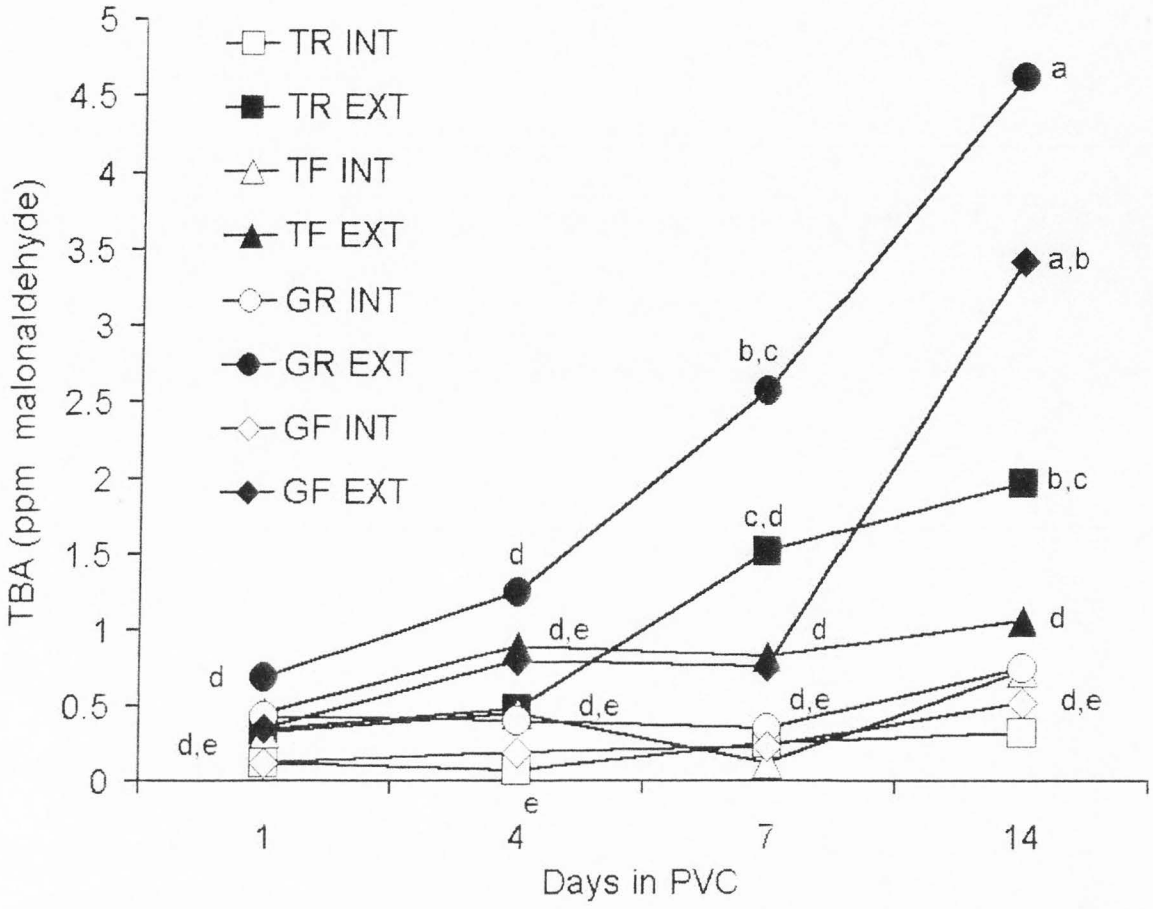

Fig. 3 Effect of raw ingredient storage history (raw ingredient surface area and storage time) on TBA values, taken from the interior and exterior of raw ground beef packaged in oxygen-permeable polyvinyl chloride (PVC) film. G = coarse ground, F = frozen, R = refrigerated, T = 2.5 x 2.5 x 12 cm strips.

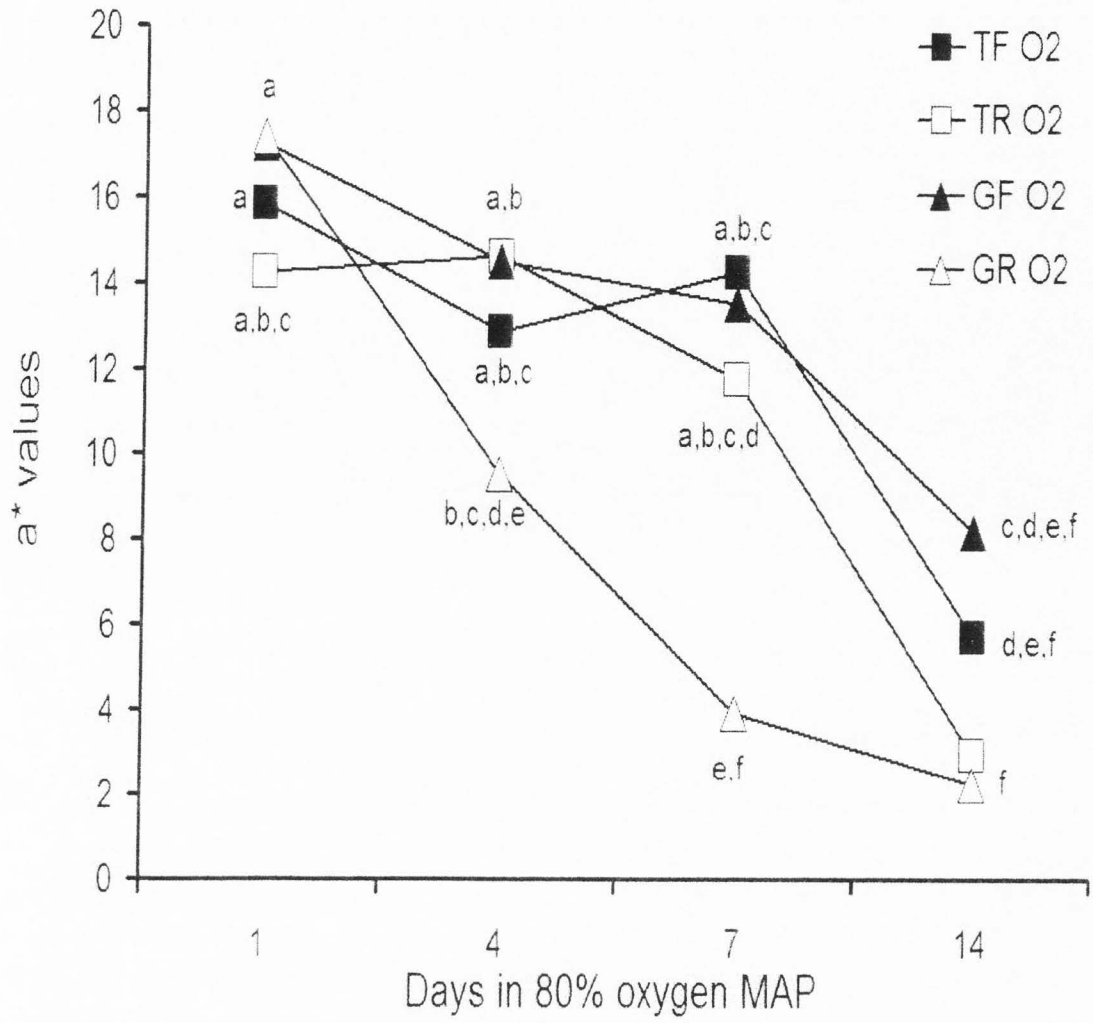

Fig. 4 Effect of raw ingredient storage history (raw ingredient surface area and storage time) on color (a*) values of raw ground beef packaged in oxygen-permeable polyvinyl chloride (PVC) film. G = coarse ground, F = frozen, R = refrigerated, T = 2.5 x 2.5 x 12 cm strips.

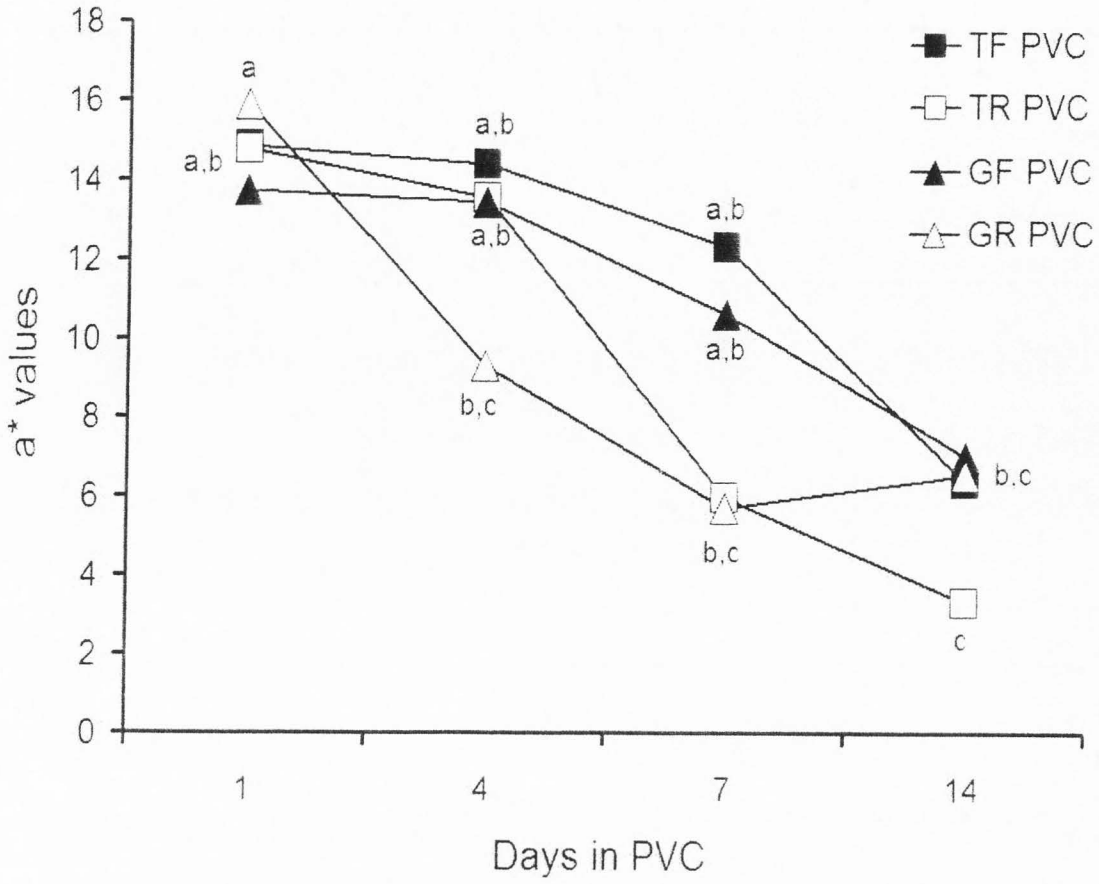

Fig. 5 Effect of raw ingredient storage history (raw ingredient surface area and storage time) on color (a*) values of raw ground beef packaged in 80% oxygen modified atmosphere packaging. G = coarse ground, F = frozen, R = refrigerated, T = 2.5 x 2.5 x 12 cm strips

refrigerated was the most resistant to color deterioration and lipid oxidation. In this study, ingredient storage methods, from most to least resistant to lipid oxidation for samples in PVC, were ranked as follow: Strips, Frozen > Strips, Refrigerated > Coarse-Ground, Frozen > Coarse-Ground, Refrigerated. For samples in high oxygen MAP, from most to least resistant to lipid oxidation were ranked as follows: Strips, Frozen > Coarse-Ground, Frozen > Coarse-Ground, Refrigerated > Strips, Refrigerated.

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CHAPTER 4

COMPARISON OF MILK MINERAL, SODIUM TRIPOLYPHOSPHATE, AND VITAMIN E AS ANTIOXIDANTS IN GROUND BEEF PACKAGED IN 80% HIGH-OXYGEN MODIFIED ATMOSPHERE PACKAGING

ABSTRACT The antioxidant effects of milk mineral, sodium tripolyphosphate and vitamin E were tested in raw ground beef packaged in 80% O_2 modified atmosphere packaging. Two levels of each of the antioxidants were tested and all were compared to a control. Vitamin E samples were either mixed with mineral oil or ethanol before being mixed with meat samples. A low and high level for each antioxidant was used; milk mineral: 0.75 and 1.5%; sodium tripolyphosphate: 0.25 and 0.5% and vitamin E: 50 or 100 ppm. Thiobarbituric acid (TBA) values were greatest in samples with vitamin E mixed with ethanol. The main effects and interactions were significantly different at $p < 0.05$ level. Lowest TBA numbers (> 1) were seen for samples treated with the either 0.75% or 1.5% of milk mineral. Milk mineral was also the most effective in maintaining high a* values even by day 14.

Introduction

Modified atmosphere packaging (MAP) is increasingly used in the meat industry in the United States. The main advantage of MAP is that the products are retail case-ready (prepared by the processor). Thus retailers avoid the expense of in-store meat processing facilities. Another advantage of MAP is increased shelf life and meat color stability depending upon the atmosphere used. Most commonly meats are packaged in

80% O_2 and 20% CO_2. The elevated oxygen level prolongs red color stability for several days at retail while the CO_2 functions to inhibit microbial growth (Manu-Tawiah and others, 1991). However high oxygen MAP increases lipid oxidation and accelerates rancid flavor development in cooked ground beef (Jakobsen and Bertelsen, 2000; Jayasingh and others 2002). To minimize lipid oxidation and rancid flavor development, a number of different antioxidant treatments have been investigated.

Arnold and others (1993) were the first to report the benefits of vitamin E supplementation in livestock diets for improvement of meat quality. Chan and others (1995) found that the addition of 2000 mg α-tocopherol acetate for 126 days before harvest in the diets of feedlot cattle reduced lipid oxidation in ground beef. Faustman and others (1989) confirmed the benefit of vitamin E supplementation to improve meat stability. Although dietary supplementation works well to reduce lipid oxidation in meat it is not done uniformly done in practice because of the expense of the supplementation procedure. Thus it is desirable to add antioxidants directly to beef trim or coarsely ground beef immediately before the final grind. Mitsumoto and others (1993) compared color stability of ground beef after application of vitamin E by either supplementation in the feed of the live animals or addition during grinding of the hamburger. They found that color stability was higher and lipid oxidation was lower if the vitamin E was given as a dietary supplement to the live animals. Benedict and others (1975) added 50 mg α-tocopherol/kg ground beef and reported increased color stability compared to controls.

A variety of other antioxidants have also been investigated for application in ground meats. Lee and others (1998a) reported that phytic acid decreased lipid oxidation and improved color stability in a ground beef model system. Milk mineral, the dried

ultra-filtered permeate of whey has been shown to have strong antioxidant activity in cooked ground beef and pork (Cornforth and West 2002; Jayasingh and Cornforth 2003). Cornforth and West (2002) concluded that MM was an iron chelating, type II antioxidant with colloidal calcium phosphate as the iron-chelating agent. Phytic acid and sodium tripolyphosphate are also known to be potent type II phosphate compounds capable of inhibiting lipid oxidation on cooked meats (Empson and others 1991; Lee and others 1998b). New antioxidants are needed to prevent lipid and color deterioration in ground beef packaged in high oxygen MAP environment. Thus the objective of this study was to evaluate MM, STP, and vitamin E as antioxidants in ground beef packaged in 80% O_2 MAP.

Materials and Methods

Experimental Design and Statistics

The experiment was designed to compare the effect of milk mineral (0.75 and 1.5%), sodium tripolyphosphate (0.25 and 0.5%) and vitamin E (50 and 100 ppm) on TBA values and color of ground beef in 80% oxygen modified atmosphere packaging. The experiment was a 3 X 3 X 4 factorial design, with three antioxidants (MM, STP, Vitamin E), 3 dosage levels (control w/o antioxidant, low and high antioxidant concentration), and 4 package storage times (1, 4, 7 and 14 days at 2° C). The experiment was done in 3 replications. All measurements were done in duplicate. Treatments were analyzed for statistical significance using proc GLM or MIXED function in Statistical Analysis Software (SAS) version 9.0 (SAS Institute Inc., Cary, NC). Analysis of variance was used to identify statistically significant differences at the 95% confidence level. Post-

hoc mean comparisons were made based on p-values ($\alpha = 0.05$) using the Tukey-Kramer adjustment to obtain differences of least square means.

Sample Preparation

Fresh beef trim was first coarsely ground through a Hobart grinder model 4125 (Hobart Mfg. Co., Troy, OH) with a 0.60 cm plate, and then finely ground using a 0.32 cm plate. The MM, vitamin E or STP was manually mixed with the meat and the meat chubs (130 gm/treatment) were placed in vacuum pouches. The pouches (25 x 35 cm; Koch, Kansas City, MO) used for packaging were 3 mil thickness (0.75 gauge nylon, 2.25 gauge polyethylene), with an oxygen permeability of 0.6 cm^3 / 100 m^2 / 24 hrs at 0°C and a water vapor transmission rate of 0.6 g / 100 m^2 / 24 hrs at 38°C and 100% relative humidity. They were then packaged in 80% oxygen MAP and held for 14 days at 2° C. The gas cylinder containing 80% oxygen and 20% carbon dioxide was obtained from Praxair Distribution (Salt Lake City, UT) and certified to be within ± 0.5% of the indicated mixture. Oxygen concentration in MAP was directly measured using an oxygen gas analyzer (Model 3500; Illinois Instruments, Ingleside, IL) to assure that oxygen concentration was 80 ± 2% in high oxygen packaging. A final portion (100 g) from each treatment was placed in a vacuum bag and frozen at –20° C for later determination of fat content. The ground beef samples had mean fat content of 12.1 ± 0.12 (AOAC, 1990). Thiobarbituric acid assay (TBA) and color readings were taken after 1, 4, 7 and 14 d in retail packaging.

TBA Analysis

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978). Duplicate samples (0.5g) were mixed with 2.5 ml of stock solution containing 0.375% TBA (Sigma Chem. Co., St. Louis, MO), 15% TCA (Mallinckrodt Baker, Inc., Paris, KY) and 0.25 N HCl. The mixture was heated for 10 min in a boiling water bath (100°C) to develop a pink color, cooled in tap water and then centrifuged (Sorvall Instruments, Model RC 5C, DuPont, Wilmington, DE) at 6000 rpm for 10 min. The absorbance of the supernatant was measured spectrophotometrically (Spectronic 21D, Milton Roy, Rochester, NY) at 532 nm against a blank that contained all the reagents minus the meat. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ (Sinnhuber and Yu 1958). The MDA concentration was converted to TBA number (mg MDA / Kg sample) as follows:

1) TBA# (mg / kg) = Sample A_{532} X (1 M TBA chromagen / 1.56 X 105) X [(1 mole / L) / M] X (0.003 L / 0.5 g meat) X (72.07 g MDA / mole MDA) X (1000 mg / g) X (1000 g / Kg), or 2) TBA No. (ppm) = sample A_{532} X 2.77

Interior and exterior samples were taken for the samples in PVC wrap and analyzed for rancidity using TBA analysis.

Hunter Color Measurements

The L*, a* and b* values were measured through the packaging film using a Hunter lab Miniscan portable colorimeter (Reston, VA), standardized using a white and black standard tile. The hue angle was calculated using the formula: hue angle = $\tan^{-1}$ (b*/a*). Larger hue angles are associated with less red color (Van Laack and others 1996)

where hue angle 0 = red and hue angle 90 = yellow. The raw samples were scored for color, where 1= purple, 2= reddish purple, 3= bright red, 4= tan and 5= brown.

Results and Discussion

The main effects of antioxidant treatments and storage time on color and lipid stability of ground beef stored in 80% oxygen MAP are shown in Table 5. The main effects of treatment and storage time and their interactions all significantly affected color and TBA values. Only the interaction for treatment and storage time for a* values was not significant (Table 5). (For detailed statistics see appendix B) Comparative p-values for TBA values for all the treatments except vitamin E in ethanol treatments, pooled over time (1, 4, 7, 14 d) are shown in table 6. It was seen that control and vitamin E treatments were significantly different from all the other antioxidant treatments (Table 6). Vitamin E in ethanol treatment was done only in 2 replicates. Vitamin E in ethanol treatments were not different from either the controls or vitamin E in oil treatments (Table 7). The means and standard deviations for L*, b* and hue angle are given in Table 8.

Table 5. Main effects of antioxidant treatments on color[1] and TBA values of raw ground beef packaged in 80% oxygen MAP.

Effect	TBA	L*	A*	B*	Hue angle
Treatment[2]	*	*	*	*	*
Day[3]	*	*	*	*	*
Treatment* Day	*	*	NS	*	*

1 L* = Lightness, a* = redness, b* = yellowness and Hue angle = aractan(b*/a*).

2 Treatments = control, MM 0.75%, MM 1.5%, STP 0.25%, STP 0.5%, VE5, VE10, VEOH5 and VEOH10.

3 Day = days in retail packing and stored for 1, 4, 7, or 14 d.

Treatments formulated with type II antioxidants (MM and STP) showed lower ($p < 0.05$) TBA values than treatments with vitamin E, a type I antioxidant (Fig. 6). Control and all vitamin E treatments had TBA values of 3.3- 3.5 by day 14 (Fig. 6). TBA values above 1 are associated with rancid flavors (Tarladgis and others 1960; Jayasingh and others 2003).

Table 6. Comparative p-values of TBA values for all the antioxidants treatments pooled for storage time (1, 4, 7, and 14 d).

	Cntrl	MM.75	MM1.5	STP.25	STP.5	VE5	VE10
Cntrl							
MM.75	<. 0001						
MM1.5	<.0001	0.9858					
STP.25	<. 0001	<. 0001	<. 0001				
STP.5	<. 0001	0.7882	0.9954	<. 0001			
VE5	0.1262	<. 0001	<. 0001	<. 0001	<. 0001		
VE10	0.6195	<. 0001	<. 0001	<. 0001	<. 0001	0.9651	

Table 7. Comparative p-values of TBA values for vitamin E in ethanol* treatments as compared to the other antioxidant treatments pooled for storage time (1, 4, 7, and 14 d).

	Cntrl	MM.75	MM1.5	STP.25	STP.5	VE5	VE10	VEOH5	VEOH10
VEOH5	1.0	<.0001	<.0001	<.0001	<.0001	0.23	0.68		0.98
VEOH10	0.99	<.0001	<.0001	<.0001	<.0001	0.84	0.99	0.98	

Note: * Vitamin E in ethanol treatments were done only for the 2nd and 3rd replicates.

Treatments with either level of MM maintained the lowest TBA values even after 14 days of retail storage. Treatments formulated with the highest level of STP (0.5%) also had TBA values less than 1 after 14 days of storage, but the lower level of STP was not as effective (Fig. 6). This is in agreement with Vara-Ubol and Bowers (2002) who showed that STP was a more effective antioxidant than α-tocopherol for inhibition of lipid oxidation and off flavors in raw ground beef packaged in 80% oxygen MAP. In

Table 8. Means ± standard deviation for the interaction of antioxidant treatment[1], and storage time[2] on color of raw ground beef packaged in 80% oxygen MAP, in retail packaging (1, 4, 7, 14 d at 1° C).

Treatment	Day	L*	B*	Hue Angle
Cntrl	1	46.5 ± 2.6	17.0 ± 0.8	51.17 ± 2.05
Cntrl	4	40.9 ± 4.1	14.6 ± 1.1	48.81 ± 4.24
Cntrl	7	44.2 ± 5.9	14.8 ± 1.7	50.49 ± 2.43
Cntrl	14	43.1 ± 8.2	12.6 ± 0.7	61.61 ± 2.74
MM0.75	1	41.2 ± 3.5	16.2 ± 1.6	47.21 ± 2.96
MM0.75	4	37.9 ± 1.5	17.3 ± 1.0	45.52 ± 1.65
MM0.75	7	40.8 ± 2.8	15.8 ± 1.1	45.91 ± 3.77
MM0.75	14	38.0 ± 2.1	14.7 ± 2.3	46.86 ± 1.34
MM1.5	1	39.8 ± 3.1	17.1 ± 1.7	47.10 ± 2.70
MM1.5	4	41.3 ± 2.2	15.3 ± 1.4	47.71 ± 2.59
MM1.5	7	40.6 ± 3.1	14.7 ± 1.1	46.93 ± 1.24
MM1.5	14	44.3 ± 5.1	14.9 ± 1.5	51.76 ± 3.51
STP0.25	1	37.9 ± 3.7	15.9 ± 1.2	47.08 ± 0.46
STP0.25	4	40.6 ± 2.7	17.2 ± 1.1	50.39 ± 1.83
STP0.25	7	37.3 ± 2.5	14.9 ± 1.2	47.10 ± 2.74
STP0.25	14	42.0 ± 4.6	12.1 ± 1.3	57.87 ± 1.94
STP0.5	1	37.8 ± 1.9	15.1 ± 1.9	43.80 ± 1.81
STP0.5	4	40.1 ± 2.4	16.8 ± 1.1	50.92 ± 2.54
STP0.5	7	38.0 ± 2.1	13.8 ± 1.3	45.92 ± 1.23
STP0.5	14	41.1 ± 6.0	13.9 ± 1.3	55.55 ± 3.48
VE10	1	43.3 ± 3.1	17.1 ± 1.5	45.99 ± 1.19
VE10	4	42.9 ± 4.7	14.2 ± 0.8	48.36 ± 2.10
VE10	7	46.1 ± 5.2	13.9 ± 1.4	54.35 ± 1.62
VE10	14	44.7 ± 6.1	12.8 ± 0.6	61.30 ± 9.33
VE5	1	39.2 ± 2.7	16.3 ± 0.6	50.83 ± 2.76
VE5	4	41.2 ± 5.1	14.3 ± 1.0	50.30 ± 2.02
VE5	7	45.3 ± 7.1	14.5 ± 2.1	55.06 ± 1.10
VE5	14	47.8 ± 5.9	13.5 ± 3.1	60.81 ± 3.56
VEOH10	1	40.3 ± 2.3	16.1 ± 0.7	53.38 ± 3.71
VEOH10	4	42.0 ± 2.7	14.8 ± 0.8	53.36 ± 5.31
VEOH10	7	41.5 ± 2.1	14.2 ± 1.9	54.11 ± 0.66
VEOH10	14	46.1 ± 3.9	13.0 ± 1.3	59.94 ± 3.17
VEOH5	1	44.0 ± 1.7	16.2 ± 1.1	51.23 ± 1.47
VEOH5	4	42.8 ± 3.1	15.7 ± 0.8	50.98 ± 1.37
VEOH5	7	44.9 ± 1.8	13.8 ± 0.9	51.74 ± 1.89
VEOH5	14	46.9 ± 3.6	14.1 ± 0.6	62.66 ± 3.56

contrast with our results, St. Angelo and others (1990) showed that type II, metal chelating antioxidants, were less effective than type I, free radical scavenging antioxidants. Mitsumoto and others (1993) showed that exogenous addition of vitamin E to ground beef was not as effective as an antioxidant as vitamin E supplementation in the diet. The Hunter color (L* and b*), hue angle values and TBA values on 1, 4, 7 and 14 days of retail storage in 80% oxygen MAP are given in table 8 for the interaction effect of treatment and storage time. Redness values (a*) were maintained by type II antioxidants better than type I antioxidants. The highest level of vitamin E treatment in ethanol had the lowest a* values by 14 days of retail storage (Fig. 7). Of the two type II antioxidants, MM at 0.75 or 1.5% levels maintained redness very well with a* values > 12 even after 14 days of retail storage (Fig. 7). It appears that iron plays a catalytic role in the browning of raw meat pigments. Current models of metmyoglobin formation (the brown fresh meat pigment) make no mention of iron (Wallace and others 1982; Shikama 1990). These models only show the role of anions for metmyoglobin formation.

Milk mineral can be a good source of calcium when added to meats. It has 24% calcium, so when 0.75% is added to 100 gm of meat, it will have 180 mg of calcium, equivalent to 20% of the recommended RDA (1200 mg Ca/day, USDA 1981). A serving size of 3 ounces (85 gm) of meat will have 150 mg Ca. from milk mineral.

Conclusions

Results of the present study indicate that the use of milk mineral in raw ground beef, packaged in high oxygen modified atmosphere packaging, not only lowered TBA values but also maintained red color stability for 14 days. Type II, metal chelating

antioxidants (STP and MM) more effective than type I antioxidants (vitamin E) for inhibition of lipid oxidation in raw ground beef in 80% oxygen MAP. Our results have shown that type II, metal- chelating antioxidants, (MM more than STP) dramatically increased the color stability of raw ground beef. It appears that iron plays a catalytic role in the browning of raw meat pigments.

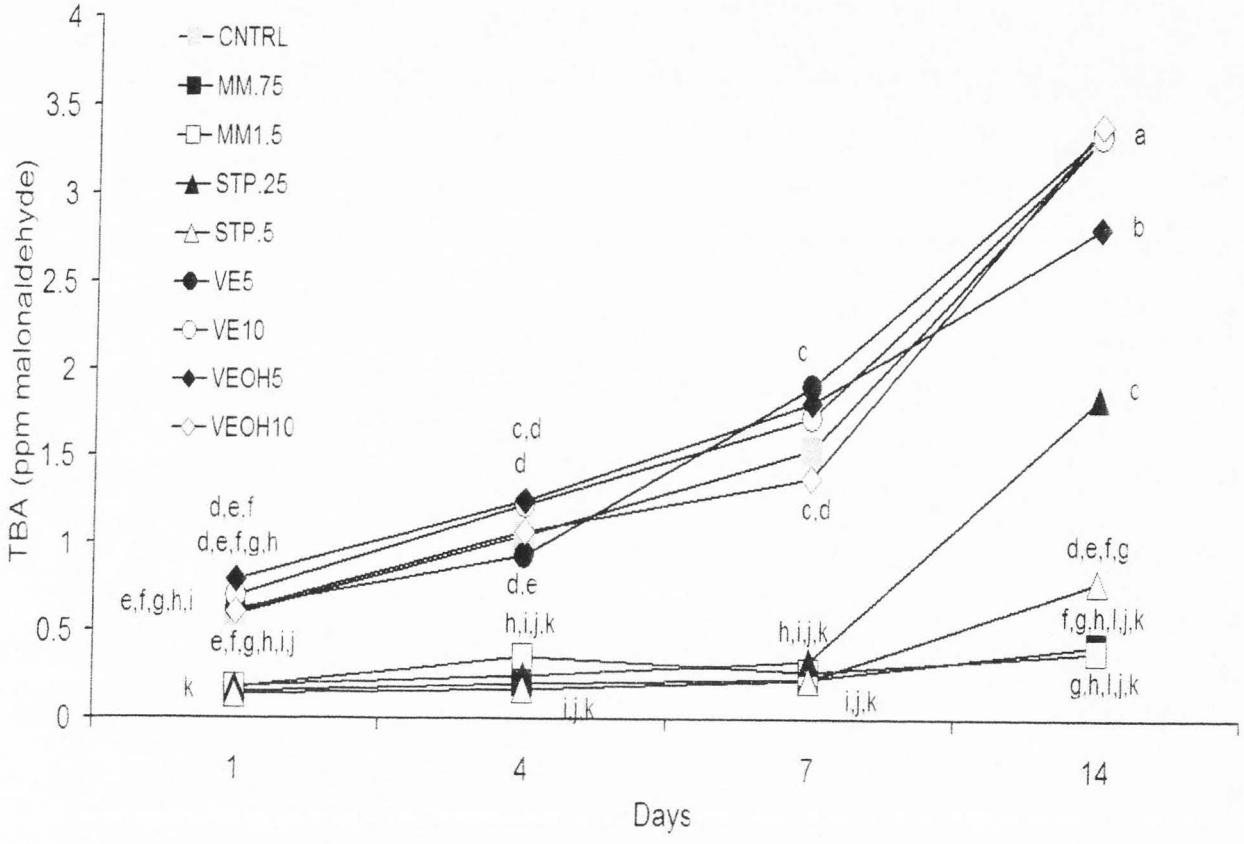

Fig. 6 Effect of Antioxidants on TBA values of Ground Beef Packaged in 80% oxygen MAP. Cntrl = control, MM.75 = samples treated with 0.75% MM, MM1.5 = samples treated with 1.5% MM, STP.25 = samples treated with 0.25% STP, STP.5 = samples treated with 0.5% STP, VE5 = samples treated with 5 ppm vitamin E in oil, VE10 = samples treated with 10 ppm vitamin E in oil, VEOH5 = samples treated with 5 ppm vitamin E in ethanol, VEOH10 = samples treated with 10 ppm vitamin E in ethanol.

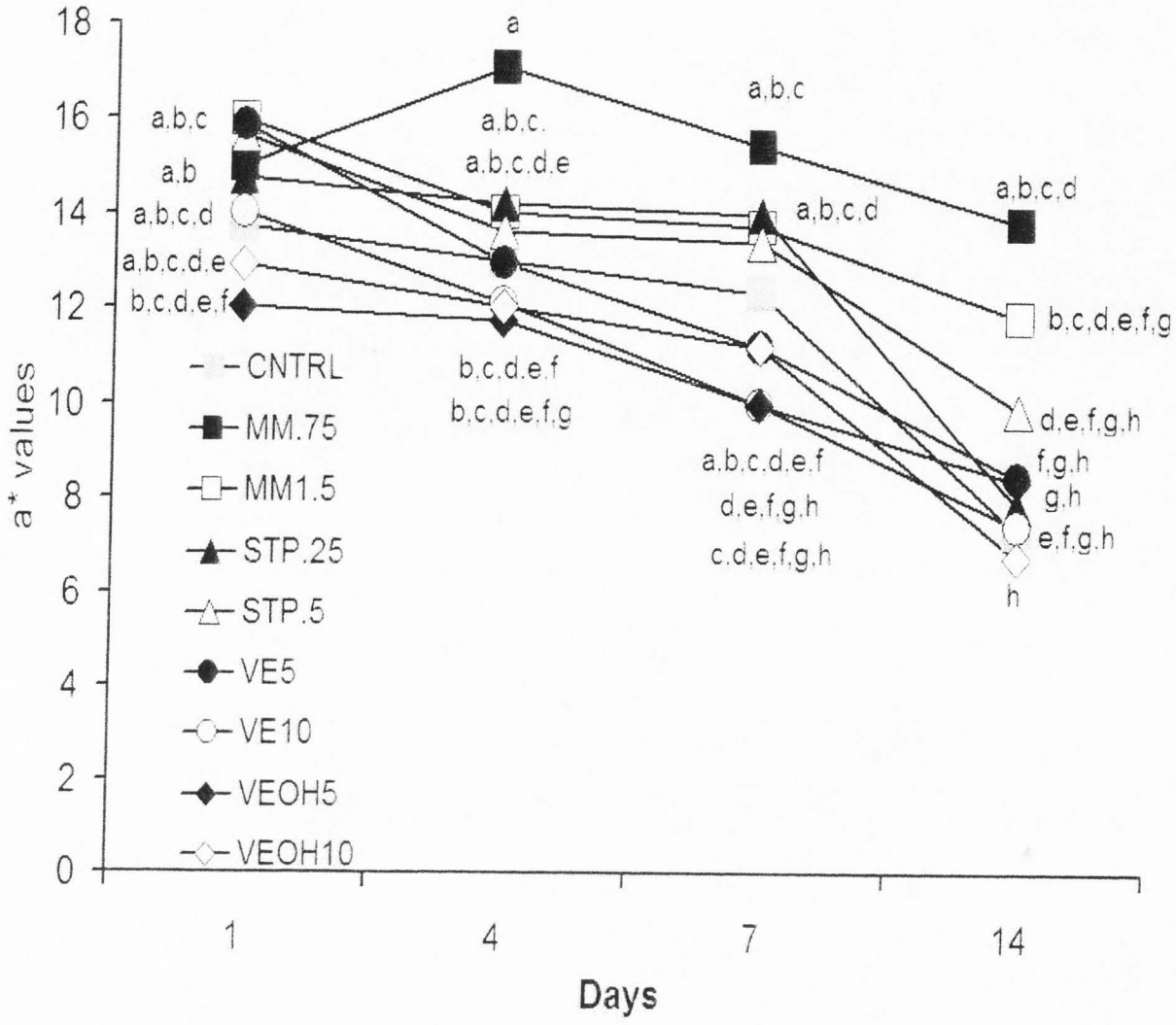

Fig. 7 Effect of Antioxidants on color (a*) values of Ground Beef Packaged in 80% oxygen MAP. Cntrl = control, MM.75 = samples treated with 0.75% MM, MM1.5 = samples treated with 1.5% MM, STP.25 = samples treated with 0.25% STP, STP.5 = samples treated with 0.5% STP, VE5 = samples treated with 5 ppm vitamin E in oil, VE10 = samples treated with 10 ppm vitamin E in oil, VEOH5 = samples treated with 5ppm vitamin E in ethanol, VEOH10 = samples treated with 5ppm vitamin E in ethanol.

Current models of metmyoglobin formation (the brown fresh meat pigment) make no mention of iron (Wallace and others 1982; Shikama 1990).

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CHAPTER 5

SUMMARY

Summary

The use of high oxygen modified atmosphere packaging has increased over the past few years. Recent studies have shown that, although the meat remains red for longer periods in high oxygen MAP, the meat tends to go rancid more rapidly. Using different antioxidants can retard lipid oxidation in meats that are packaged in high oxygen MAP. Our results show that the use of milk mineral in high oxygen MAP not only retards lipid oxidation but also maintains bright red color for 14 days. Another factor to consider while looking at retarding lipid oxidation is the meat history. Pre retail handling and storage temperatures also have an impact on the rate of lipid oxidation in meats. Raw material storage conditions greatly affected retail stability of ground beef. Ground beef was much more stable if trim was frozen rather than coarsely ground, vacuum packaged, and held at 2° C.

Trim history most significantly affected the stability of beef in 80% O_2 MAP stored for 14 days. Ground beef packaged in high oxygen atmosphere packaging was most susceptible to lipid oxidation. The addition of antioxidants of MM, in high oxygen MAP can help in delaying lipid oxidation and also maintain a bright red fresh meat color. Current models of metmyoglobin formation make no mention of iron. Further work needs to be done on how iron affects metmyoglobin. Also more research needs to be done on the color maintaining and antioxidant mechanism of milk mineral.

APPENDICES

Appendix A

Data for Chapter 3

Table A1. Analysis of Variance of Hue angle values for Ground Beef Packaged in PVC or 80% oxygen MAP.

Effect	Num DF	Den DF	F value	Pr > F
Size	1	88	2.03	0.1579
Temp	1	88	54.89	<0.0001
Size*Temp	1	88	10.22	0.0019
Packing	1	88	3.85	0.0529
Size*Temp*Packing	3	88	3.17	0.0283
Day(Size*Temp*Packing)	24	88	13.08	<0.0001

Table A2. Analysis of Variance of a* values for Ground Beef Packaged in PVC or 80% oxygen MAP.

Effect	Num DF	Den DF	F value	Pr > F
Size	1	88	1.68	0.1983
Temp	1	88	38.41	<0.0001
Size*Temp	1	88	3.05	0.0845
Packing	1	88	2.51	0.1167
Size*Temp*Packing	3	88	2.69	0.0514
Day(Size*Temp*Packing)	24	88	16.83	<0.0001

Table A3. Analysis of Variance of L* values for Ground Beef Packaged in PVC or 80% oxygen MAP.

Effect	Num DF	Den DF	F value	Pr > F
Size	1	88	0.56	0.4577
Temp	1	88	18.26	<0.0001
Size*Temp	1	88	14.43	0.0003
Packing	1	88	0.53	0.4665
Size*Temp*Packing	3	88	2.97	0.0363
Day(Size*Temp*Packing)	24	88	1.77	0.0292

Table A4. Analysis of Variance of b* values for Ground Beef Packaged in PVC or 80% oxygen MAP.

Effect	Num DF	Den DF	F value	Pr > F
Size	1	88	0.11	0.7358
Temp	1	88	0.35	0.5529
Size*Temp	1	88	5.33	0.0233
Packing	1	88	6.81	0.0106
Size*Temp*Packing	3	88	0.54	0.6542
Day(Size*Temp*Packing)	24	88	5.17	<0.0001

Table A5. Analysis of Variance of TBA values for Ground Beef Packaged in PVC or 80% oxygen MAP.

Effect	Num DF	Den DF	F value	Pr > F
Size	1	176	8.85	0.0033
Temp	1	176	61.10	<0.0001
Size*Temp	1	176	3.71	0.0557
Packing	1	176	74.48	<0.0001
Size*Temp*Packing	3	176	10.36	<0.0001
Day(Size*Temp*Packing)	24	176	16.37	<0.0001

Table A6. Paired t-tests for exterior and interior samples of Frozen Strip Raw Materials on Day 1.

t-Test: Two-Sample Assuming Equal Variances		
	TF Day 1	
	Interior	*Exterior*
Mean	0.313772	0.449433
Variance	0.012753	0.016331
Observations	4	4
Pooled Variance	0.014542	
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.59093	
P(T<=t) one-tail	0.081365	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.162729	
t Critical two-tail	2.446914	

Table A7. Paired t-tests for exterior and interior samples of Frozen Strip Raw Materials on Day 4.

t-Test: Two-Sample Assuming Equal Variances		
	TF Day 4	
	Variable 1	*Variable 2*
Mean	0.445208	0.893464
Variance	0.075301	0.019557
Observations	4	4
Pooled Variance	0.047429	
Hypothesized Mean Difference	0	
df	6	
t Stat	-2.91084	
P(T<=t) one-tail	0.013475	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.026951	
t Critical two-tail	2.446914	

Table A8. Paired t-tests for exterior and interior samples of Frozen Strip Raw Materials on Day 7.

t-Test: Two-Sample Assuming Equal Variances		
	TF Day 7	
	Interior	*Exterior*
Mean	0.117033	0.828646
Variance	0.000543	0.025608
Observations	2	2
Pooled Variance	0.013076	
Hypothesized Mean Difference	0	
df	2	
t Stat	-6.22319	
P(T<=t) one-tail	0.012431	
t Critical one-tail	2.919987	
P(T<=t) two-tail	0.024862	
t Critical two-tail	4.302656	

Table A9. Paired t-tests for exterior and interior samples of Frozen Strip Raw Materials on Day 14

t-Test: Two-Sample Assuming Equal Variances		
	TF Day 14	
	Interior	*Exterior*
Mean	0.729133	1.061741

Variance	0.188121	0.008576
Observations	4	4
Pooled Variance	0.098348	
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.4999	
P(T<=t) one-tail	0.092152	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.184305	
t Critical two-tail	2.446914	

Table A10. Paired t-tests for exterior and interior samples of Refrigerated Strip Raw Materials on Day 1

t-Test: Two-Sample Assuming Equal Variances		
	TR Day 1	
	Interior	*Exterior*
Mean	0.118902	0.336832
Variance	0.000535	0.042266
Observations	4	4
Pooled Variance	0.0214	
Hypothesized Mean Difference	0	
df	6	
t Stat	-2.10678	
P(T<=t) one-tail	0.039863	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.079726	
t Critical two-tail	2.446914	

Table A11. Paired t-tests for exterior and interior samples of Refrigerated Strip Raw Materials on Day 4

t-Test: Two-Sample Assuming Equal Variances		
	TR Day 4	
	Interior	*Exterior*
Mean	0.071674	0.474224
Variance	5.42E-05	0.158314
Observations	4	4
Pooled Variance	0.079184	
Hypothesized Mean Difference	0	

df	6
t Stat	-2.02309
P(T<=t) one-tail	0.044758
t Critical one-tail	1.943181
P(T<=t) two-tail	0.089516
t Critical two-tail	2.446914

Table A12. Paired t-tests for exterior and interior samples of Refrigerated Strip Raw Materials on Day 7

t-Test: Two-Sample Assuming Equal Variances		
	TR Day 7	
	Interior	*Exterior*
Mean	0.246322	1.519137
Variance	0.000677	2.217685
Observations	4	4
Pooled Variance	1.109181	
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.70915	
P(T<=t) one-tail	0.069141	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.138282	
t Critical two-tail	2.446914	

Table A13. Paired t-tests for exterior and interior samples of Refrigerated Strip Raw Materials on Day 14

t-Test: Two-Sample Assuming Equal Variances		
	TR Day 14	
	Interior	*Exterior*
Mean	0.314672	1.97224
Variance	0.007631	0.162356
Observations	4	4
Pooled Variance	0.084993	
Hypothesized Mean Difference	0	
df	6	
t Stat	-8.0407	
P(T<=t) one-tail	9.89E-05	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.000198	
t Critical two-tail	2.446914	

Table A14. Paired t-tests for exterior and interior samples of Frozen Coarse Ground Raw materials on Day 1

t-Test: Two-Sample Assuming Equal Variances		
	GF Day 1	
	Interior	*Exterior*
Mean	0.120841	0.345973
Variance	0.00136	0.006461
Observations	4	4
Pooled Variance	0.003911	
Hypothesized Mean Difference	0	
df	6	
t Stat	-5.0913	
P(T<=t) one-tail	0.00112	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.00224	
t Critical two-tail	2.446914	

Table A15. Paired t-tests for exterior and interior samples of Frozen Coarse Ground Raw materials on Day 4

t-Test: Two-Sample Assuming Equal Variances		
	GF Day 4	
	Interior	*Exterior*
Mean	0.190507	0.79312
Variance	0.010204	0.010511
Observations	4	4
Pooled Variance	0.010357	
Hypothesized Mean Difference	0	
df	6	
t Stat	-8.37395	
P(T<=t) one-tail	0.000789	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.000158	
t Critical two-tail	2.446914	

Table A16. Paired t-tests for exterior and interior samples of Frozen Coarse Ground Raw materials on Day 7

t-Test: Two-Sample Assuming Equal Variances		
	GF Day 7	
	Interior	*Exterior*

Mean	0.233165	0.755518
Variance	0.02992	0.123361
Observations	4	4
Pooled Variance	0.076641	
Hypothesized Mean Difference	0	
df	6	
t Stat	-2.66839	
P(T<=t) one-tail	0.018553	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.037106	
t Critical two-tail	2.446914	

Table A17. Paired t-tests for exterior and interior samples of Frozen Coarse Ground Raw materials on Day 14

t-Test: Two-Sample Assuming Equal Variances		
	GF Day 14	
	Interior	*Exterior*
Mean	0.513697	3.418803
Variance	0.06997	4.322443
Observations	4	4
Pooled Variance	2.196206	
Hypothesized Mean Difference	0	
df	6	
t Stat	-2.7723	
P(T<=t) one-tail	0.016162	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.032324	
t Critical two-tail	2.446914	

Table A18. Paired t-tests for exterior and interior samples of Refrigerated Coarse Ground Raw materials on Day 1

t-Test: Two-Sample Assuming Equal Variances		
	GR Day 1	
	Interior	*Exterior*
Mean	0.430458	0.68606
Variance	0.040438	0.02777

Observations	4	4
Pooled Variance	0.034104	
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.95739	
P(T<=t) one-tail	0.049025	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.09805	
t Critical two-tail	2.446914	

Table A19. Paired t-tests for exterior and interior samples of Refrigerated Coarse Ground Raw materials on Day 4

t-Test: Two-Sample Assuming Equal Variances		
	GR Day 4	
	Interior	*Exterior*
Mean	0.398534	1.255295
Variance	0.030155	0.106367
Observations	4	4
Pooled Variance	0.068261	
Hypothesized Mean Difference	0	
df	6	
t Stat	-4.63754	
P(T<=t) one-tail	0.001775	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.00355	
t Critical two-tail	2.446914	

Table A20. Paired t-tests for exterior and interior samples of Refrigerated Coarse Ground Raw materials on Day 7

t-Test: Two-Sample Assuming Equal Variances		
	GR Day 7	
	Interior	*Exterior*
Mean	0.34348	2.574577
Variance	0.001692	0.001019
Observations	2	2
Pooled Variance	0.001356	
Hypothesized Mean Difference	0	
df	2	
t Stat	-60.5974	

P(T<=t) one-tail	0.000136
t Critical one-tail	2.919987
P(T<=t) two-tail	0.000272
t Critical two-tail	4.302656

Table A21. Paired t-tests for exterior and interior samples of Refrigerated Coarse Ground Raw materials on Day 14

t-Test: Two-Sample Assuming Equal Variances		
	GR Day 14	
	Interior	*Exterior*
Mean	0.745615	4.626039
Variance	0.009098	0.83094
Observations	4	4
Pooled Variance	0.420019	
Hypothesized Mean Difference	0	
df	6	
t Stat	-8.46759	
P(T<=t) one-tail	7.41E-05	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.000148	
t Critical two-tail	2.446914	

Appendix B

Data for chapter 4

Table B1. Analysis of variance of antioxidant main effects for Hue values of ground beef packaged in 80% oxygen MAP.

Source	DF	Type 1 SS	Mean Square	F Value	Pr > F
Trt	8	1190.40	148.80	13.42	<.0001
Day	3	1826.48	608.82	54.89	<.0001
Trt*day	24	647.29	26.97	2.43	0.0020

Table B2. Analysis of variance of antioxidant main effects for a* values of ground beef packaged in 80% oxygen MAP.

Source	DF	Type 1 SS	Mean Square	F Value	Pr > F
Trt	8	330.05	41.25	14.64	<.0001
Day	3	585.82	195.27	69.30	<.0001
Trt*day	24	105.77	4.40	1.56	0.0755

Table B3. Analysis of variance of antioxidant main effects for b* values of ground beef packaged in 80% oxygen MAP.

Source	DF	Type 1 SS	Mean Square	F Value	Pr > F
Trt	8	29.98	3.74	2.60	0.0149
Day	3	166.68	55.56	38.52	<.0001
Trt*day	24	73.24	3.05	2.12	0.0079

Table B4. Analysis of variance of antioxidant main effects for L* values of ground beef packaged in 80% oxygen MAP.

Source	DF	Type 1 SS	Mean Square	F Value	Pr > F
Trt	8	599.83	74.97	8.32	<.0001
Day	3	173.20	57.73	6.41	0.0007
Trt*day	24	385.53	16.06	1.78	0.0318

Table B5. Analysis of variance of antioxidant main effects for TBA values of ground beef packaged in 80% oxygen MAP.

Source	DF	Type 1 SS	Mean Square	F Value	Pr > F
Trt	8	60.31	7.53	475.41	<.0001
Day	3	48.39	16.13	1017.16	<.0001
Trt*day	24	22.80	0.95	59.91	<.0001