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Prevention of Pigment Deterioration and Lipid Oxidation in Ground Beef and Pork

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PREVENTION OF PIGMENT DETERIORATION AND LIPID OXIDATION IN GROUND BEEF AND PORK

by

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ABSTRACT

Prevention of Pigment Deterioration and Lipid Oxidation in Ground Beef and Pork

by

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Fresh beef was modified-atmosphere packaged in carbon monoxide or oxygen to prolong red surface color. After comparison of several packaging method using carbon monoxide, steaks pretreated with 5% carbon monoxide for 24 hours and then vacuum packaged had the best combination of color and microbial stability (5 weeks), with the least potential for carbon monoxide inhalation.

In the evaluation of ground beef in high-oxygen, modified-atmosphere-packaging, thiobarbituric-acid numbers increased over time, and the flavor was disliked slightly after 6 or 10 days of storage at 2° Celsius.

The antioxidant effect of milk-mineral was tested in raw and cooked ground pork stored refrigerated or frozen. Thiobarbituric-acid numbers were low for all raw treatments. For cooked ground pork, thiobarbituric-acid numbers were lower for samples with milk-mineral or sodium-tripolyphosphate, compared to control or samples with

butylated-hydroxytoluene. Sodium-tripolyphosphate, a type 2 antioxidant (iron chelator), was also very effective in preventing heme degradation during refrigerated storage.

(163 pages)

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LIST OF SYMBOLS, NOTATIONS, AND DEFINITIONS

Abbreviation Key

BHT	Butylated hydroxy toluene
C	Celsius
CFU	Colony forming units
CO	Carbon monoxide
CO_2	Carbon dioxide
d	day
FDA	Food and Drug Association
MAP	Modified atmosphere packaging
MM	Milk mineral
O_2	Oxygen
N_2	Nitrogen
$NaNO_2$	Sodium nitrite
PVC	Polyvinyl chloride
RM	Rosemary
STP	Sodium tripolyphosphate
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
USDA	United States Department of Agriculture
VP	Vacuum packaging
WOF	Warmed-over flavor

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Pigment and lipid oxidation are major deteriorative reactions in meat and meat products during storage. They lead to significant loss in quality characteristics such as color, flavor, texture and nutritive value (Wilson and others 1976; Akamittath and others 1990). This dissertation will focus on prevention of loss of color due to pigment deterioration and loss of flavor due to lipid oxidation.

Hypothesis

My hypothesis is that the color loss and lipid oxidation occurring during storage of fresh and cooked meats may be prevented or retarded by alternative packaging and addition of antioxidants, respectively.

A primary factor determining consumer purchase of fresh meat is the "fresh meat" color. When meat is cut and the surface is exposed to air, deoxymyoglobin, which is purple, is rapidly oxygenated to cherry red oxymyoglobin; but over time myoglobin oxidizes to metmyoglobin to form a brown discoloration (Kropf 1993). Greene and others (1971) reported that consumers made a decision against purchasing when brown metmyoglobin reached 30-40% of total pigments on the surface of fresh retail beef. In the USA, most fresh retail beef is displayed in styrofoam trays overwrapped with oxygen-permeable polyvinyl chloride (PVC) films. Packaging by this method allows rapid surface pigment oxygenation and red color development (bloom), but brown discoloration occurs within 1-7 d, depending upon the muscle and cutting method (Madhavi and Carpenter 1993). However, the ideal packaging system should provide at

least 21 d color stability, including 7 d for packaging and distribution, 7 d retail display and 7 d at home. This goal can be achieved by employing modified atmosphere packaging (MAP) that includes 0.5% carbon monoxide (CO) to attain bloom in place of oxygen, 60% carbon dioxide (CO_2) and 39.5% nitrogen (N_2) (Sorheim and others 1999). CO binds strongly to myoglobin to form carboxymyoglobin, which has a bright red color (El-Badawi and others 1964), and an absorption spectrum nearly identical to oxymyoglobin (Cornforth 1994). Low levels of CO have little effect on the meat microflora (Clark and others 1976; Gee and Brown 1978; Luno and others 1998). Modified atmosphere packaging (MAP) systems with low levels of CO do not present a toxic threat to the consumer, and are widely used and accepted for beef packaging in Norway (Sorheim and others 1997). In the USA, MAP with 3-9% CO is used for vegetable processing (Luno and others 1998). After cooking, CO-treated meats turn brown (Vahabzadeh and others 1983), with 85% loss of CO (Watts and others 1978). However, CO may mask spoilage of fresh meat because the red color may persist beyond the microbial shelf life of the meat (Kropf 1980).

Short of freezing, vacuum packaging followed by refrigerated storage is the most effective method currently used for shelf life extension of uncooked meat. Vacuum packages are also compact and durable during distribution process. However, consumer acceptance of vacuum packaged retail beef has been low because of its dark reddish-purple color (Meischen and others 1987). Brewer and others (1994) obtained a red color in vacuum packaged beef by exposing the steaks to 100% carbon monoxide (CO) for 1 h before VP. The objective of the first study was to evaluate lower and thus safer levels of

CO for pretreatment before vacuum packaging, and to determine red color stability and microbial load of steaks and ground beef as affected by CO pretreatment time, concentration and pressure.

Alternatively, red surface color can be prolonged for 3-4 d on beef cuts packaged in modified atmospheres containing oxygen for bloom and carbon dioxide as an antimicrobial (Manu-Tawiah and others 1991; Zhao and others 1994). An advantage of high oxygen modified atmosphere packaging (MAP) is that the product is case-ready, while coarsely ground meat in bulk chubs must be finely ground and repackaged at the retail store. Although oxygen maintains the desirable red color of the fresh meat, it also promotes oxidation of lipid, especially in minced beef that has its cell structure disrupted, exposing labile lipid components to oxygen (Sato and Hegarty 1971). O'Grady and others (2000) reported that lipid oxidation of minced beef stored in 80% O_2 increased significantly between d 7 and 10 of storage. However, no sensory studies have been done to correlate lipid oxidation with palatability in ground beef stored in high-oxygen MAP. Processors implementing the high-oxygen MAP process have had consumer complaints about the flavor of the product. Thus, the second objective of this study was done to evaluate the palatability, color, and lipid oxidation status of ground beef packaged in a modified atmosphere package (80% O_2 and 20% CO_2) for up to 10 d at 2 ° C, compared with ground beef stored in oxygen-impermeable casing at the same temperature and time.

Lipid oxidation is a major cause of deterioration in the quality of meat and meat products (Asghar and others 1988). Generally lipid oxidation is faster in cooked meat than in raw meat (Tichivangana and Morrissey, 1985). The rate and degree of oxidation

has been directly related to the degree of unsaturation of the lipid present (Igene and Pearson 1979; Tichivangana and Morrissey 1985) and degree of oxygen exposure (O'Grady and others 2000).

The thiobarbituric acid (TBA) test is the most frequently used method for assessing lipid oxidation in meat. Tarladgis and others (1960) found that TBA numbers (mg TBA reactive substances/Kg tissue) were highly correlated with trained sensory panel scores for rancid odor in ground pork. The TBA number at which a rancid odor was first perceived was between 0.5 to 1.0. This "threshold" has served as a guide for interpreting TBA test results. According to Greene and Cumuze (1981) the range of oxidized flavor detection for inexperienced panelists was within a range of TBA numbers similar to the previously determined threshold level for trained panelists. However, these data did not provide any information regarding consumer preferences. Thus the third objective of this study was to determine if various levels of oxidized flavor expressed in terms of TBA number were preferable or not to consumer panelists.

Oxidation of unsaturated fatty acid in cooked meats during storage and reheating results in stale or rancid flavors known as warmed-over flavor (WOF) (Sato and Hegarty 1971). The warmed-over flavor problem of cooked meat has assumed much greater significance in recent years due to the rapid increase in fast food service facilities requiring the use of large quantities of precooked or partially cooked meats or meat products. In these facilities, cooked meat may be kept warm for a variable time prior to serving. The fourth objective of this study was to determine the degree of oxidative

degradation that occurs when cooked ground pork was warmed for up to 2 h after cooking.

The greater propensity for WOF in cooked and comminuted products is due to release of non-heme iron during cooking and grinding (Igene and others 1979). Recently, it has been reported that dried milk mineral (MM), the dried permeate of ultra-filtered whey, has antioxidant properties in cooked meats, apparently due to iron-chelation by colloidal phosphate (Cornforth and West 2002). Cooked ground pork required 2% MM to maintain TBA number <1.0 during storage at 2°C while samples with 1% MM maintained a TBA number <2.0 (Cornforth and West 2002). However, much precooked pork is frozen, as is the case for frozen pizza toppings. More information is needed on the possible antioxidant effects of milk mineral in frozen meats. Thus, the fifth objective was to determine the optimum level at which dried milk mineral can be used in raw and cooked ground pork stored at two temperatures (2 ° C or -20 ° C), as compared to other antioxidants (butylated hydroxy toluene and sodium tripolyphosphate).

Carpenter and Clark (1995) reported that nonheme iron levels in meat are accurately and rapidly determined by Ferrozine assay in HCl-TCA extracts. This method uses an extraction solution containing 50% 6N HCl following homogenization using a stainless steel probe-type mixer. However, continued use of this method in our labs resulted in higher than expected nonheme iron levels in meat samples. Thus, we investigated the possibility that iron was removed from the metal homogenizer probe during blending of the highly acidic samples, causing the increased iron levels. Thus the sixth objective of this study was to determine nonheme iron levels in samples blended

with a glass rod or a new stainless steel probe, compared to the old and worn blender probe.

Type 1 antioxidants such as vitamin E, Rosemary extract, and butylated hydroxytoluene (BHT) are electron donors capable of slowing the propagation step of lipid oxidation. Type 2 antioxidants such as phytate, sodium tripolyphosphate, or sodium nitrite bind iron, preventing iron catalysis of lipid oxidation. The objective of seventh study was to compare antioxidant effectiveness of BHT and Rosemary extract (Type 1 antioxidants) with type 2 antioxidants sodium tripolyphosphate (STP), milk mineral (MM; a natural phosphate source) and sodium nitrite in cooked ground pork during storage.

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CHAPTER 2

LITERATURE REVIEW

Color and flavor of meat are primary factors influencing consumers' purchase of meat. Consumers prefer a bright red color while purchasing raw meat and prefer cooked meat with no detectable rancid odor. Thus, loss of surface red color in raw meat and occurance of rancid odor in cooked meats are of prime concern to the meat industry.

Meat pigments

Myoglobin is the primary meat pigment. It is a complex molecule composed of a protein moiety (globin) and a heme prosthetic group (Kendrew and others 1960). It exists in three forms; purple-red deoxymyoglobin in fresh meat in the absence of air; bright-red oxymyoglobin formed in the presence of oxygen; and brown metmyoglobin, the result of myoglobin oxidation.

When meat is freshly cut and exposed to air, deoxymyoglobin, which is purple, becomes oxygenated into oxymyoglobin and the bright red color associated with freshness. In oxymyoglobin, heme iron is in the ferrous state (Fe^{2+}). Iron in heme can form six coordinate bonds, four with pyrrole groups of the porphyrin ring of heme and one with histidine F8, which connects heme to the globin. The sixth position is available for binding oxygen or other small ligand such as carbon monoxide (CO). The visible light absorption spectrum of CO-myoglobin is nearly identical to that of oxymyoglobin.

Oxymyoglobin has a relatively short shelf life and oxidizes to brown metmyoglobin (Kropf 1993). In meat, metmyoglobin formation occurs rather slowly (d) in presence of air (20% oxygen). Metmyoglobin formation occurs much more rapidly at 1% oxygen or less (George and Stratmann 1952). Carboxymyoglobin is more resistant to oxidation than is oxymyoglobin, owing to the stronger binding of CO. It is documented that when brown discoloration reaches 30-40% of total pigments on the surface of fresh retail beef, consumers make a no-purchase decision (Greene and others 1971). Thus, color is a primary factor affecting retail meat purchase decisions. Consumers associate bright-red color with freshness of raw meat, and gray or tan color with cooked meat.

Cured meat products have a characteristic pink color. Cooking causes denaturation (unfolding) of the globin protein, therefore the exposed heme is much more prone to oxidation. Thus in the presence of air, the gray-tan cooked meat pigment (denatured globin hemichome) is formed. Mononitrosylhemochome is the pink pigment of cured meats (Killday and others 1988).

Traditional vacuum packaging vs. modified atmosphere packaging

Traditionally coarsely ground beef is centrally packaged in vacuum for distribution to retail where it is reground and repackaged to obtain the bright red color of oxymyoglobin. An alternative method is to centrally package the meat ground to its final degree in a modified atmosphere packaging (MAP), and distribute this as retail ready.

In February 2002 the USDA and FDA jointly approved the distribution of fresh meats in a master bag system using an atmosphere of 0.4% CO, 30% CO_2 and a balance

as N_2. Modified atmosphere packaging (MAP) systems with low levels of CO do not present a toxic threat to the consumer, and are widely used and accepted for beef packaging in Norway (Sorheim and others 1997). CO binds strongly to myoglobin to form carboxymyoglobin, which has a bright red color (El-Badawi and others 1964), and absorption spectra nearly identical to oxymyoglobin (Cornforth 1994). Low levels of CO have little effect on the meat microflora (Clark and others 1976; Gee and Brown 1978; Luno and others 1998). However, CO may mask spoilage because the stable red color can last beyond the microbial shelf life of the meat (Kropf 1980).

Another method to achieve increased red color stability in case-ready meats is to package the product in a high-oxygen atmosphere (approximately 80% oxygen, 20% carbon dioxide), where it remains red for 7-14 d. This is the primary packaging method currently used with case-ready meats in the USA. An advantage of high-oxygen modified atmosphere packaging (MAP) is that the product is case-ready, while coarsely ground beef in bulk chubs must be finely ground and re-packaged at the retail store. Georgala and Davidson (1970) reported that optimum fresh beef color stability is obtained using a gas mixture of 80% O_2 and 20% CO_2. Oxygen functions to oxygenate myoglobin and CO_2 inhibit the growth of aerobic spoilage bacteria (Zhao and others 1994; Manu-Tawiah and others 1991). However, the high oxygen levels may promote rancidity (O'Grady and others 2000).

Lipid oxidation

Lipid oxidation is the major cause of flavor deterioration in meat and meat products (Asghar and others 1988). The rate and degree of lipid oxidation has been directly related to heating (Tichivangana and Morrissey 1985); degree of unsaturation of the lipid present (Igene and Pearson 1979; Tichivangana and Morrissey 1985) and level of oxygen exposure (O'Grady and others 2000). Mincing of beef facilitates oxygen penetration deep into the chub and facilitates myoglobin oxygenation and more pronounced color, but it also disrupts the muscle cell structure exposing the labile lipid components to oxygen (Sato and Hegarty 1971). All the above factors accelerate lipid oxidation in cooked meats during storage and reheating resulting in stale or rancid flavors known as warmed-over flavor (WOF).

The role of lipids in the development of WOF

The development of WOF in cooked meat is generally accepted to be the result of oxidation of tissue lipid (Younathan and Watts 1960; Ruenger and others 1978). Among tissue lipid, the phospholipid has been implicated as the lipid component most readily susceptible to oxidation in cooked meat (Younathan and Watts 1960). The triglycerides are much less susceptible to oxidation than the phospholipid (Love and Pearson 1971). Hence, the triglycerides appear to exert only a minor influence on development of WOF. The phospholipid generally contains more PUFAs, which are very labile (Lea 1957). Igene and Pearson (1979) have provided convincing evidence that total phospholipids are principally responsible for the development of WOF in cooked beef and poultry.

Influence of heating and grinding

Any process causing disruption of the muscle membrane system, such as grinding or cooking, results in exposure of the labile lipid components to oxygen and thus accelerates development of oxidative rancidity (Pearson and others 1977). Saturated fats are relatively stable at the temperatures used in conventional canning operations, but unsaturated fats deteriorate, under the conditions of oxygen and heat, to form a large number of volatile compounds, which give rise to both desirable and undesirable flavors (Pitcher 1993). Drying (dehydration) brings food component molecules into close proximity, thereby increasing the likelihood that they will interact (Horner 1993). Also, the removal of water from a food material increases its physical accessibility to atmospheric oxygen though micro-capillaries that open up though the center of the material, and as a result greatly increases exposure to atmospheric oxygen.

Heating accelerates development of oxidized flavor (rancidity) in meat and meat products (Younathan and Watts 1960). Huang and Greene (1978) showed that meat subjected to high temperatures and or long period of heating developed lower TBA numbers than similar samples subjected to lower temperatures for shorter period of time. They postulated that antioxidant substances produced during the browning reaction exert TBA retarding activity; and which progresses as the meat is heated. According to Hamm (1966), the Maillard reaction in meats begins at about 90 ° C and increases with further increases in temperatures and heating times.

Sato and Hegarty (1971) have reported a very rapid increase in TBA values, and hence of WOF, for raw meats one hour after grinding and exposure to air at room

temperature. They suggested that any catalysts of lipid oxidation present in the muscle system are brought into contact with the oxidation-susceptible lipid and contribute to the rapid development of WOF.

Iron

Iron is a trace element of considerable concern due to its role as a prooxidant in lipid oxidation in meat and meat products. Many different iron complexes, including low molecular weight compound, heme compound, and storage forms such as ferritin and hemosiderin have been found in meat (Hazell 1982; Stryer 1988). All forms of iron present in beef contribute to development of lipid oxidation (Han and others 1995). Although the nonheme storage protein, ferritin, is the second most abundant iron-containing compound in the adult human (Granick 1958), the amount in meat is generally low because most of the ferritin is located in the liver, spleen and bone marrow (Moore 1973). Heme and nonheme iron catalyze oxidation in both raw and cooked meat systems (Wills 1996; Liu and Watts 1970). However, Sato and Hegarty (1971) reported that nonheme iron was the active catalyst in cooked meats. The heme iron content decreases in ground beef with cooking during storage. Cooking destroys the porphyrin rings of heme pigments resulting in nonheme iron release from heme pigments (Lee and others 1998). Lee and others (1998) also showed a inverse relationship between heme iron content and TBA number of cooked beef, supporting the view that nonheme iron in cooked meat is responsible for catalyzing lipid peroxidation resulting in WOF. Both final temperature and rate of heating influence release of nonheme iron from meat pigment extracts. Slow heating results in release of more nonheme iron than fast heating. Since

cooking of meat generally involves slow heating, this may help explain the propensity of precooked meat for lipid oxidation, with release of nonheme iron during cooking catalyzing oxidation (Chen and others 1984). It is believed that micro-waved meat suffers less from WOF then meat cooked by the slower conventional method of cookery (Schiker and Miller 1983).

Lipid oxidation has become more important with increased consumption of precooked meat items in both institutional and home use setting. Cooked meat develops WOF more rapidly during refrigerated storage than uncooked meat (Tim and Watts 1958). The thiobarbituric acid (TBA) test is the most frequently used method for assessing lipid oxidation in meat. Sensory panelists describe the extend of lipid oxidation in terms of rancid odor or taste. Tarladgis and others (1960) found that TBA numbers (mg TBA reactive substances/Kg tissue) were highly correlated with trained sensory panel scores for rancid odor in ground pork. The TBA number at which a rancid odor was first perceived was between 0.5 to 1.0. This "threshold" has served as a guide for interpreting TBA test results. According to Greene and Cumuze (1981) the range of oxidized flavor detection for inexperienced panelists was within a range of TBA numbers similar to the previously determined threshold level for trained panelists.

Antioxidants

The rate of lipid oxidation in meat products can be effectively retarded by the use of antioxidants. Food antioxidants are classified as Type 1 or Type 2 antioxidants. Type 1 antioxidants can terminate the free-radical chain reaction of lipid oxidation by donating

hydrogen or electrons to free radicals and convert them to more stable products. They may also function by addition in reactions with lipid radicals, forming lipid-antioxidant complexes. Eg: Phenolics such as BHT, BHA, TBHQ and tocopherols. Many of the naturally occurring phenolic compounds like flavonoid, eugenol, vanillin and rosemary antioxidant are classified as Type 1 antioxidants. The antioxidant role was suggested to be due to the presence of phenolic compound (Houlihan and others 1985). Such compound break free radical chain reaction by hydrogen atom donation. Synthetic phenolic antioxidants such as BHT are used to improve the stability of lipid in food products. McCarthy and others (2001) reported a significant antioxidant effect of BHT/BHA in cooked pork patties when added at a level of 0.01% of meat weight. They are quite volatile and easily decompose at high temperatures. Consumers are concerned about the safety of synthetic food additives, which has led to renewed interest in natural products (Andres and Duxbury 1990). Rosemary, a natural antioxidant, has been reported to contain certain components (rosemanol, rosmariquinone, rosmaridiphenol, carnosol), which may be as effective as BHT as an antioxidant (Houlihan and others 1984, 1985; Nakatani and Intani 1984). Wu and others (1982) and Houlihan and others (1985) reported that naturally occurring compound in rosemary extracts exhibited antioxidant properties equal to or slightly less than BHT. Other researchers (Stoick and others 1991; Lai and others 1991; Liu and others 1992) have shown that rosemary oleoresin, either water-soluble or oil-soluble, have no particular advantage in restructured beef or pork steaks.

Type 2 antioxidants retard lipid oxidation by chelating metal ions, especially iron, preventing metal mediated lipid oxidation. St. Angelo and others (1988) and Liu and others (1992) reported that sodium tripolyphosphate (STP) at a level of 0.5% meat weight was very effective at inhibiting lipid oxidation and oxidative flavor changes in cooked meat during storage The antioxidant role of STP is hypothesized to be due to its sequestering of heavy metals (Watts 1950; Tim and Watts 1958), particularly iron which is the major pro-oxidant in meat systems (Igene and others 1979). Sodium tripolyphosphate can be used in meat and poultry products as an antioxidant at a maximum level of 0.5% (USDA 2000). St Angelo and others (1990) reported that metal chelators were less effective than antioxidants that function as free radical scavengers in inhibiting or minimizing the loss of desirable meat flavor. However, results by Vara-Ubol and Bowers (2002) indicate that STP, a metal chelator, was much more effective than a-tocopherol, a free radical scavenger, in inhibiting the loss of desirable meat flavor, as well as the development of oxidative off flavors.

Liu and others (1992) reported that when STP was used in combination with rosemary oleoresin in cooked restructured pork steaks most of the antioxidant action was from STP. STP alone at 0.3% level was as effective as 0.5% level in reducing oxidative flavor changes of cooked pork during storage. Stale aroma and flavor were almost non-existent in cooked pork containing 0.3 or 0.5% STP when evaluated by trained taste panelists even after 4 d storage at 4 degree C (Vara-Ubol and Bowers 2002).

Nitrites and nitrates function as antioxidants by converting heme proteins to inactive nitric oxide forms and by chelating the metal ions. Whey is another natural food

antioxidant (Colbert and Decker 1991; Browdy and Harris 1997), due to the presence of protein sulfhydryl groups with reducing abilities, and also due to iron chelation by whey proteins (Tong and others 2000). Recently, it has been reported that dried milk mineral, the dried permeate of ultra-filtered whey, has antioxidant properties in cooked meats, apparently by iron-chelation to colloidal phosphate (Cornforth and West 2000).

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CHAPTER 3

EVALUATION OF CARBON MONOXIDE TREATMENT IN MODIFIED ATMOSPHERE PACKAGING OR VACUUM PACKAGING TO INCREASE COLOR STABILITY OF FRESH BEEF

Abstract

My goal was to obtain > 21 d red color stability for carbon monoxide (CO)-treated beefsteaks in vacuum packaging (VP). In preliminary tests, pretreatment for 24 h in a 5% CO modified atmosphere package (MAP) was needed to maintain redness after re-packaging in VP. Pressure pretreatment with 5% CO for 2 h developed redness, but was impractical for large-scale application. Color stability and microbial load were then compared after treatment of steaks in 5% CO-MAP for 24 h, then VP; 100% CO-MAP for 1 h, then VP; steaks and ground beef in 0.5% CO-MAP; and steaks and ground beef in polyvinyl chloride (PVC) wrap. Steaks remained red for 5, 6, 8, and <1-week storage at 2°C, respectively. Steaks microbial load exceeded spoilage levels (>10^6cfu/cm^2) at 5, 6, 7, and <2-weeks, respectively. Thus, extended color stability in VP was achieved by pretreatment with 5% CO for 24 h or 100% CO for 1 h.

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Introduction

An important factor limiting fresh meat shelf life is the loss of red color stability. Thus, there is need for new packaging and processing method to increase fresh beef color stability. In the USA, most fresh retail beef is displayed in styrofoam trays overwrapped with oxygen-permeable polyvinyl chloride (PVC) films. Packaging by this method allows rapid surface pigment oxygenation and red color development (bloom), but brown discoloration occurs within 1-7 d, depending upon muscle and cutting method (Madhavi & Carpenter, 1993). Red surface color can be prolonged for 3-4 d on beef cuts packaged in modified atmospheres containing oxygen for bloom, carbon dioxide as an antimicrobial, and nitrogen as a filler (Manu-Tawiah, Ammann, Sebranek, & Molins, 1991). However, the ideal packaging system should provide at least 21 d color stability, including 7 d for packaging and distribution, 7 d retail display and 7 d at home. This goal can be achieved by employing MAP that includes 0.5% CO to attain bloom in place of oxygen, 60% carbon dioxide (CO_2) and 39.5% nitrogen (N_2) (Sorheim, Nissen, & Nesbakken, 1999). CO binds strongly to myoglobin to form carboxymyoglobin, which has a bright red color (El-Badawi, Cain, Samuels, & Angelmeier, 1964), and an absorption spectrum nearly identical to oxymyoglobin (Cornforth, 1994). Low levels of CO have little effect on the meat microflora (Clark, Lentz, & Roth, 1976; Gee & Brown, 1978; Luno, Beltran, & Roncales, 1998). MAP systems with low levels of CO do not present a toxic threat to the consumer, and are widely used and accepted for beef packaging in Norway (Sorheim, Aune, & Nesbakken, 1997). In the USA, MAP with 3-9% CO is used for vegetable processing (Luno, Beltran, & Roncales, 1998). After

cooking, CO-treated meats turn brown (Vahabzadeh, Collinge, Cornforth, Mahoney, & Post, 1983), with 85% loss of CO (Watts, Wolfe, & Brown, 1978). However, CO may mask spoilage because the stable red color can last beyond the microbial shelf life of the meat (Kropf, 1980).

Short of freezing, vacuum packaging followed by refrigerated storage is the most effective method currently used for shelf life extension of uncooked meats. Vacuum packages are also compact and durable during distribution processes. However, consumer acceptance of vacuum packaged retail beef has been low because of its dark reddish-purple color (Meischen, Huffman, & Davis, 1987). Brewer, Wu, Field, and Ray (1994) obtained a red color in vacuum packaged beef by exposing the steaks to 100% carbon monoxide (CO) for 1 h before VP. The objective of this study was to evaluate lower and thus safer levels of CO pretreatment before VP, and to determine red color stability and microbial load of steaks and ground beef as affected by CO pretreatment time, concentration, and pressure treatment.

Materials and Method

Apparatus

Equipment used in this study: Hobart grinder model 4152 (Hobart Mfg. Co., Troy, OH); Multivac packaging machine (model M855, Kansas City, MO); Hunter lab Miniscan portable colorimeter (Reston, VA); caliper (Fisher, Salt Lake City, UT); anaerobic jar (BBL Gas Pack System, Becton Dickinson Co., Cockeysville, MD.); Ultravac vacuum packaging machine (Sandy, UT); Pressure cooker (10 liters) (Mirromatic, Mirro Corp., Manitowoc, WI).

Gases

Gas cylinders were obtained from Praxair Distribution (Salt Lake City, UT) and certified to be within ± 0.5% of the indicated mixture: 100% CO; 5% CO, 60% CO_2 and 35% N_2; 0.5% CO, 60% CO_2 and 39.5% N_2.

Description of experiments

Preliminary test 1 was done to determine which low level of CO (0.5 or 5%) was sufficient for development of surface redness in MAP. Steaks were exposed to 0.5 or 5% CO in MAP for 48 h at 2°C. Hunter color and CO penetration depth values were taken periodically during storage.

Preliminary test 2 was done to determine if the CO-exposure time for red color stability could be shortened by using CO under pressure. Steaks (batch size = 4) were placed on a stainless steel wire rack in a pressure cooker modified by addition of a port to accept gas delivered in a stainless steel tube, and a shut off valve to maintain pressure. Pressure was regulated by adjustment of the CO pressure regulatory valve. After CO exposure for 30, 60, 90, or 120 min at 15 psig (pound per square inch relative to the gauge), steaks were removed from the chamber, vacuum packaged and stored at 2°C. Hunter color and CO penetration depth values were taken immediately before vacuum packaging and again after 24 h of storage in VP.

The optimum CO pretreatment method as determined in preliminary experiments (5% CO in MAP for 24 h then VP) was then compared to various other packaging treatments as follows:

- steaks in 5% CO in MAP for 24 h, then VP (5% CO-VP);

- steaks in 100% CO in MAP for 1 h, then VP (100% CO-VP);
- steaks in 0.5% CO in MAP (0.5% CO-MAP);
- steaks in oxygen-permeable polyvinyl chloride film (PVC);
- ground beef (15% fat) in 0.5% CO in MAP (0.5% CO-MAP);
- ground beef in PVC film (PVC).

Samples were stored at 2°C for up to 8 weeks. Hunter color measurements, CO penetration depth and microbial plate counts were conducted every week for 8 weeks or until the Hunter a* values dropped below 10, indicating loss of redness.

Three complete replications were done for each experiment. For Hunter color values, n=15 (3 replications and 5 readings per sample). For microbial analysis, n=6 (3 replications and duplicate readings per sample).

Statistical analysis

Treatment means were calculated by ANOVA, using Statistica (Statsoft, Inc., Tulsa, OK). Differences between means were determined by calculation of Fisher's least significant difference (L.S.D) values, when appropriate. Significance was defined at $P<0.05$. Figures were prepared using the curve-smoothing feature of the Cricket 1.01 graphics program (Computer Associates International, Islandia, NY).

Meat samples

Select grade vacuum-packaged boxed beef short loins were purchased and fabricated into 1-inch thick boneless top loin steaks in the USU meat lab. Thirty-six, 15, and 27 steaks were used per treatment in preliminary experiment 1, 2 and the final

experiment, respectively. For the ground beef used in the final experiment, nine 300g chubs/loin were obtained from thee separate loins (27 samples) by passing lean trim twice though a Hobart grinder with a fine (0.32 cm) plate. The ground beef samples had a fat content of 15% as determined by the AOAC soxhlet fat extraction method (AOAC, 1991).

Packaging

MAP packaging: Steaks were placed on porous mesh filter pad (by cutting commercial furnace filters to appropriate size, i.e., 7 cm wide and 12 cm long) for modified atmosphere treatment so that the bottom surface was also exposed to the gas atmosphere. Modified atmosphere packages (1 steak/package) were prepared with a Multivac packaging machine. Package headspace volume ≈ 1.5 liter. Ground meat was placed on Styrofoam trays for modified atmosphere treatment, and then packaged as described for steaks. The packaging films were Cryovac (W.R. Grace & Company, Duncan, SC) R169B film [O_2 transmission rate of 1 cm^3/(645 cm^2.24 hours) at 0% relative humidity and 23°C and water transmission rate of 0.4g/(645 cm^2.24 hours) at 100% relative humidity and 23°C] for the pocket and R665B film [O_2 transmission rate of 1 cm^3/(645 cm^2.24 hours) at 0% relative humidity and 23°C and water transmission rate of 0.5g/(645 cm^2.24 hours) at 100% relative humidity and 23°C] for the top.

Vacuum packaging: The steaks were vacuum packaged using an Ultravac vacuum packaging machine. The packaging film was clear nylon-polyethylene (O_2 permeability = 0.6 g O_2/625 cm^2/24h at 0°C; Koch, Kansas City, MO).

Polyvinyl chloride film wrap: Steaks and ground beef were placed on white styrofoam trays and over wrapped with an oxygen permeable PVC film (Anchor Packaging item no. SWM-518 select wrap purchased from Koch).

Hunter color measurements

After opening the package, surface color of raw steaks and ground beef was measured using the Hunter L*, a*, b* system with the Hunter lab Miniscan portable colorimeter (Reston, VA), standardized using a white and black standard plate. The hue-angle = $\tan^{-1}(b^*/a^*)$ was calculated. Larger hue angle values are associated with less red color (Van Laack, Berry, & Solomon, 1996.), where hue-angle 0 = red and hue-angle 90 = yellow.

Carbon monoxide penetration measurements

CO penetration depth (bright red band) was measured in mm with a caliper (Fisher, Salt Lake City, UT). Penetration depth values were the mean of two measurements per sample.

Aerobic and anaerobic plate counts

Steaks: A 25 cm^2 area was swabbed with moist, sterile cotton tipped applicator (Hardwood Products Company, Guilford, ME). The cotton tip was broken off inside a 99ml dilution bottle and plate counts were done by serial dilutions following standard procedures (Messer, Peeler, & Gilchrist, 1978). Standard method agar (Difco, Detroit, MI) was used as growth medium.

Ground beef: A 10g portion of ground beef was mixed with 90 ml sterile water, followed by serial dilution and plate counts as described above.

For anaerobic plate counts, plates were incubated in an anaerobic jar (BBL Gas Pack System, Becton Dickinson Co., Cockeysville, MD.). Duplicate plates were counted after incubation at 37° C for 48 h.

Sensory panel evaluation

Cooked meat patties were evaluated for possible sensory differences due to storage method (raw patties stored with or without CO in MAP).

Panel: The consumer panel consisted of 56 untrained panelists (students, faculty and staff members of Utah State University). The patties were evaluated using a Triangle test. Panelists were given thee samples. Two of the samples were identical and the third sample was different. One half of the panelists (23) received plates with 2 CO-treated samples and one control sample. The remaining 23 panelists received plates with 2 control samples and one CO-treated sample. The samples were coded with random numbers. There were 6 possible orders in which the samples could be tasted (MMC, MCM, CMM, MCC, CMC, CCM) where 'C' were the control samples and 'M' were the CO-treated samples. To avoid positional bias each of the 6 combinations were served to nine different panelists (6 X 9 = 54 + 2 extra = 56 panelists). Panelists were instructed to circle the odd sample of the three samples they were given. Panelists were not instructed to look for a specific attribute such as flavor or color. Thus any perceived difference would be unique to each panelist.

Patty preparation: Control patties (~112g each) were packaged in a MAP of 60% CO_2 and 40% N_2. The CO-treated patties were packaged in a MAP of 60% CO_2, 39.5% N_2 and 0.5% CO. All patties were held at 1°C for 5 d, then blast frozen at –29°C and held a 5 d before sensory evaluation. The patties were thawed at 1°C for 24 h and cooked till well done (internal temperature 77°C). Before cooking, the control patties were brown, but CO-treated patties were bright-red. Each cooked patty was cut into six sections while hot and used for different judges. Coded sections were randomly arranged on a partitioned dinner plate (3 sections per plate), covered with foil to prevent dehydration, and kept warm in a gas oven preheated to 93°C, and served within 10 min. Samples were evaluated in partitioned booths with red light to reduce color bias. Cold water was provided for drinking between samples.

The number of correct identifications required for significance ($p<0.05$) in the triangle test was determined using table 3b of the second edition of Sensory Testing Method (ASTM, 1996).

Results

Preliminary experiment

0.5% vs. 5% MAP pre-treatment: In preliminary experiment 1 it was observed that 0.5% CO produced lower Hunter a* values (redness values) in MAP steaks after 24 h than did 5% CO (Fig. 1). The CO penetration depth was 4 mm in 5% CO MAP compared to 2 mm in 0.5% CO MAP after 24 h (Fig. 2). Thus, the 5% CO concentration level for 24 h was used in the final experiment for pre-treating the steaks before vacuum packaging.

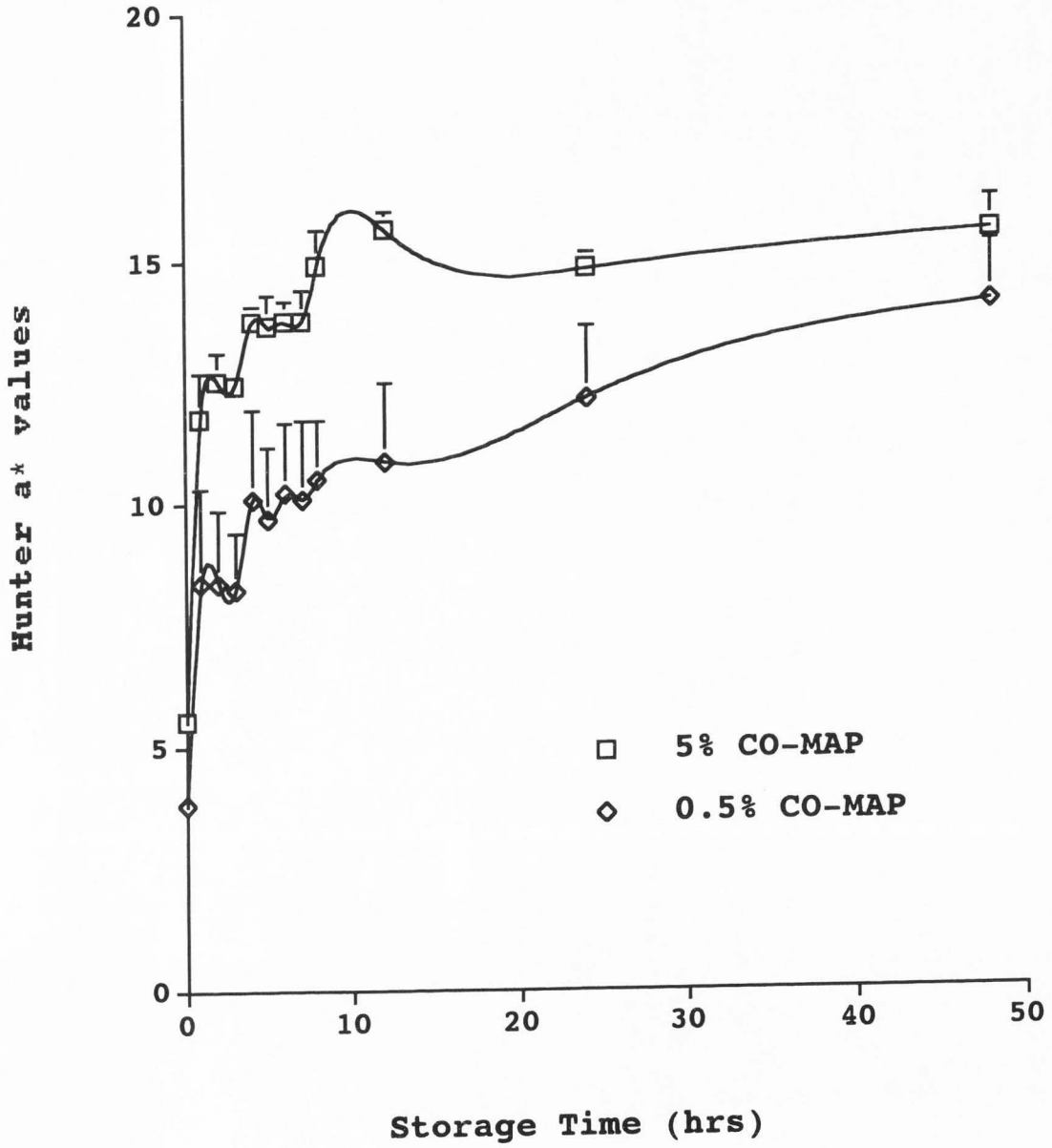

Fig. 1. Effect of CO concentration (5 or 0.5%) on Hunter color redness (a*) values of beef top loin steaks in a modified atmosphere package (MAP). In addition to CO, the MAP package contained 60% CO_2 and 35% or 39.5% N_2, respectively.

Pressure vs. MAP pretreatment with 5% CO: In preliminary experiment 2, all steaks developed a bright-red color after pressurized (15 psig with 5%CO, 60% CO_2 & 35% N_2) CO treatment, with Hunter color a* (redness) values > 12 (Fig. 3) and CO penetration depth of 1-2 mm (Fig. 4). However, after 24 h VP storage, only steaks pressure treated for 120 min retained a red color (Fig. 3), with Hunter redness values 11-13 and penetration depth of 2 mm (Fig. 4). In comparison, steaks pretreated with 5% CO in a modified atmosphere package (MAP) for 24 h had similar bright-red color, and greater CO penetration depth (4 mm) (Fig. 1 & Fig. 2). The 24 h CO-MAP pretreatment was also much less labor intensive for large quantities of steaks. Thus, CO-MAP pretreatment was used for VP steaks in the final experiment, instead of pressure pretreatment with 5% CO.

Final experiment

Steaks

Hunter color values: The 0.5% CO-MAP steaks remained red (a* > 13) for the full 8 week study (Table 1). However, red color was lost (a* ≤ 10) after 5-, 6-, and 1-week storage for 5% CO-VP, 100% CO-VP & PVC steaks, respectively. The 0.5% CO-MAP and the 100% CO-VP steaks had lower hue angle values compared to 5% CO-VP and PVC steaks (Table 1), where lower hue angle values indicate more red color and less yellow color.

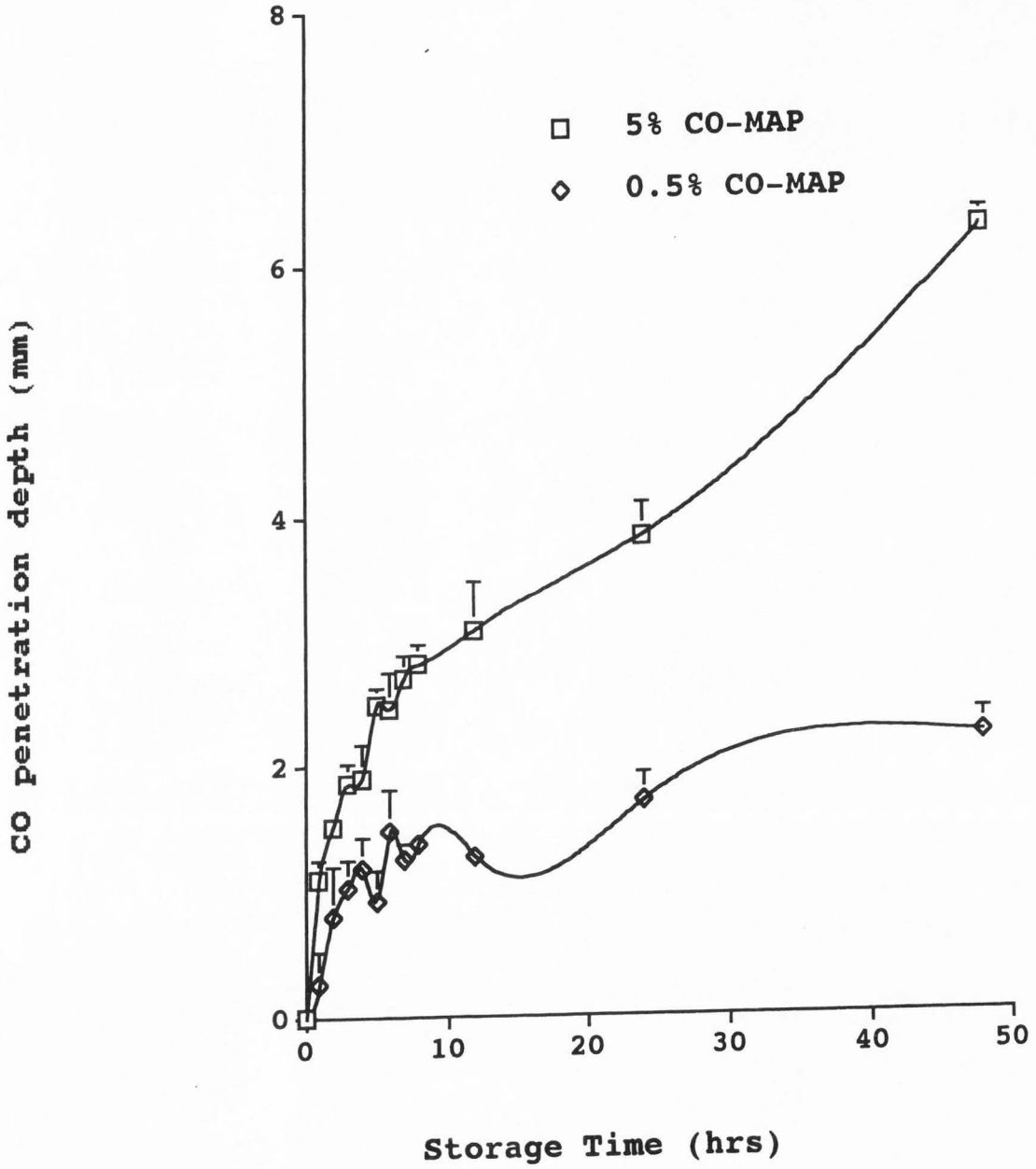

Fig. 2. Effect of CO concentration (5 or 0.5%) on CO penetration depth in beef top loin steaks in a modified atmosphere package (MAP). In addition to CO, the MAP package contained 60% CO_2 and 35% or 39.5% N_2, respectively.

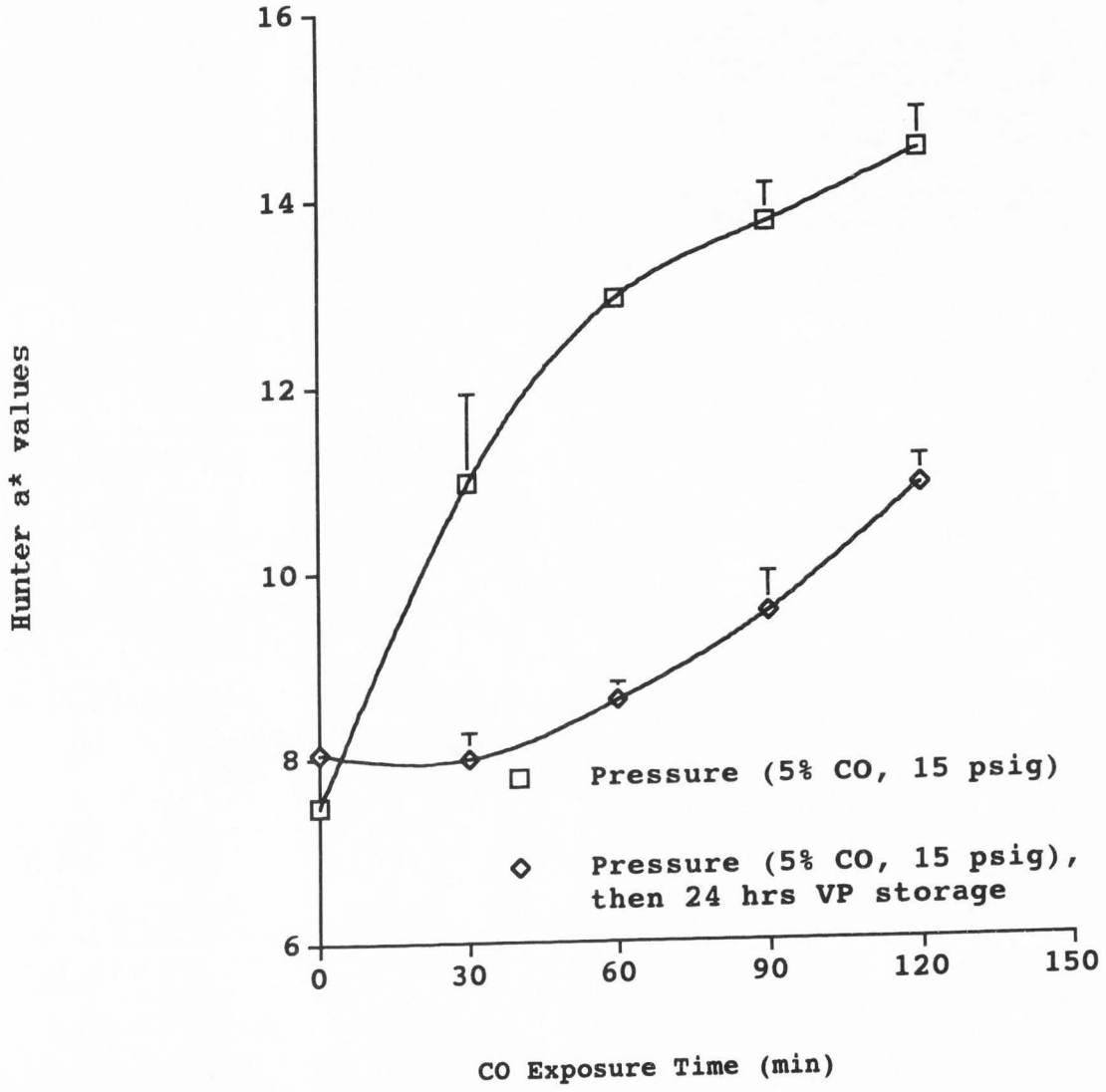

Fig. 3. Hunter color redness (a*) values of steaks pressure treated at 15 psig for 30-120 min with 5% CO, 60% CO_2 and 35% N_2. Color measurements were taken immediately after pressure treatment. Steaks were then vacuum packaged (VP) and color measurements were taken again after 24 h at 2°C.

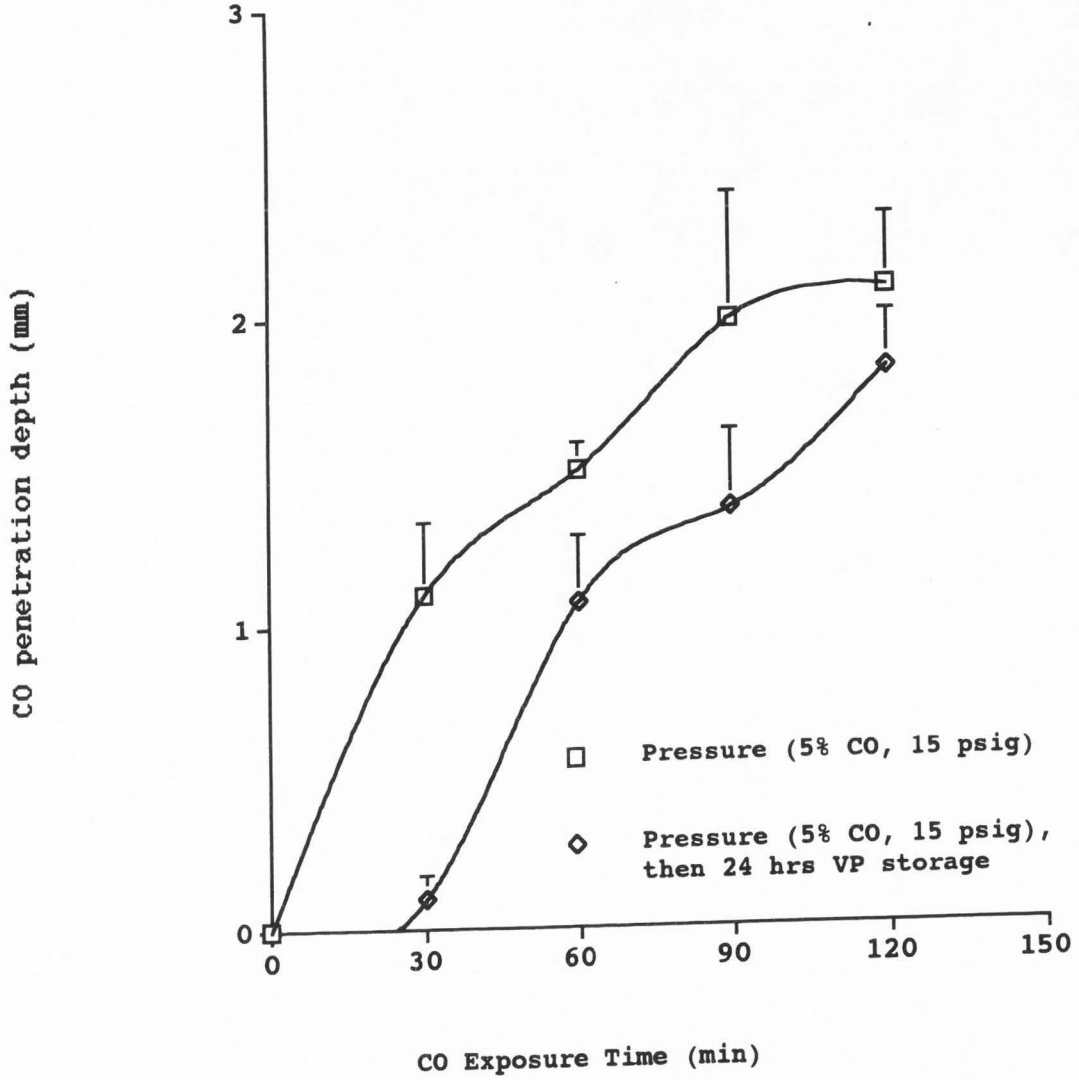

Fig. 4. CO penetration depth in steaks pressure treated at 15 psig for 30-120 min with 5% CO, 60% CO_2 and 35% N_2. CO penetration depth measurements were taken immediately after pressure treatment. Steaks were then vacuum packaged (VP) and CO penetration depth measurements were taken again after 24 h at 2°C.

CO penetration depth: For 0.5% CO-MAP steaks, CO penetration depth increased steadily with storage time in MAP, reaching 11 mm from both top and bottom surfaces of the 25 mm-thick steak by 8 weeks storage (Fig. 5). For 5% CO-VP steaks, CO penetration depth was 2 mm through 2 weeks storage, dropping to near zero (no visible boundary between surface red and underlying purple meat) by 3 weeks storage. For 100% CO-VP steaks, CO penetration depth was 8-11 mm through 4 weeks storage, dropping to 4 mm at 5 to 6 weeks storage (Fig. 5). The boundary between the red CO surface layer and the underlying purple meat was also much less defined after 3-4 weeks of storage.

Microbial analysis: Aerobic and anaerobic plate counts of PVC steaks reached spoilage levels ($>10^6$ cfu/cm^2) earlier than the 0.5% CO-MAP, 5% CO-VP, and 100% CO-VP steaks (Table 1). After 5 weeks of storage, the anaerobic plate count of steaks in 0.5% CO-MAP was lower than for steaks in 5% CO-VP or 100% CO-VP, indicating more anaerobic bacterial growth in VP than in MAP. The lower bacterial growth in MAP than VP was probably due to the antimicrobial effects of the CO_2 in MAP (Enfors, Molin, & Ternstrom, 1979).

Ground beef

Hunter color values: Ground beef in 0.5% CO-MAP remained red ($a^* > 14$) for the full 8-week study (Table 2). However, red color was lost ($a^* \leq 8$) within the first week of storage for ground beef in PVC. The ground beef in 0.5% CO-MAP had lower hue angle values than did ground beef in PVC, where lower hue angle values indicate more red color and less yellow color (Table 2).

CO penetration depth: Total CO penetration was observed in 0.5% CO-MAP ground beef by 1 week, and ground beef remained red ($a^* > 14$) throughout the 8 weeks of study (Fig. 5).

Microbial analysis: Aerobic and anaerobic plate counts of PVC ground beef reached spoilage levels ($>10^6$cfu/gm) by 2 weeks of storage at 2°C, compared to 3 weeks for aerobic and 5 weeks for anaerobic plate counts in 0.5% CO-MAP (Table 2). Thus, MAP gave significantly longer microbial stability to ground beef compared to PVC, due to the presence of 60% CO_2 in the MAP (Enfors et al, 1979). It was also noted that ground beef in 0.5% CO-MAP reached spoilage levels by week 5 of storage but the fresh bright-red appearance was maintained for the entire 8-week study. Thus in the final 3 weeks of storage the ground beef appeared fresh but was actually spoiled. This result is in agreement with previous findings of Kropf (1980) that CO may mask spoilage because the stable red color can last beyond the microbial shelf life of the meat.

Sensory analysis: Of the 56 panelists, 20 were able to point out the odd sample correctly while the rest (36) were not able to pick the odd sample. The number of correct identifications required for significance ($p<0.05$) in the triangle test was 25 (ASTM, 1996). Thus there were no significant sensory differences observed due to CO-treatment of ground beef.

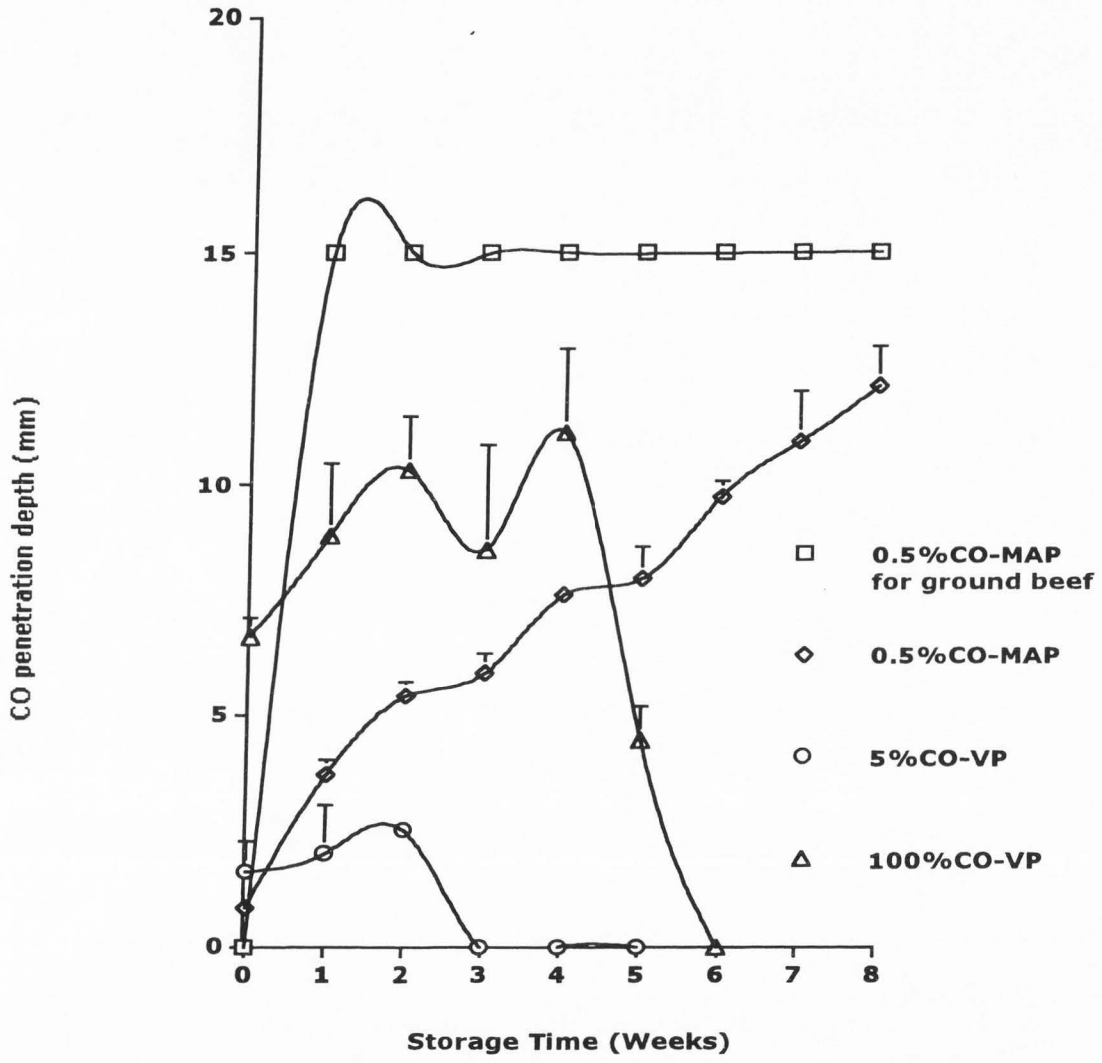

Fig. 5. CO penetration depth in steaks and ground beef as affected by CO concentration and packaging method. Ground beef was stored in a modified atmosphere package (MAP) of 0.5% CO, 60% CO_2 and 39.5% N_2. Some beef top loin steaks were stored in a modified atmosphere package (MAP) of 0.5% CO, 60% CO_2 and 39.5% N_2 (COMAP). Other beef steaks were pretreated in either 5% CO, 60% CO_2 and 35% N_2 (COVP5) or 100% CO (COVP100) in MAP for 24 h or 1 h, respectively, to develop surface redness, then vacuum packaged. All treatments were stored at 2°C for 8 weeks or until surface redness disappeared.

Table 1. Effect of CO pretreatment and packaging method on Hunter color values[1] and microbial load of beef top loin steaks stored at 2°C for 8 weeks.

Treatment	Week	L*	a*	b*	Hue angle	Saturation	APC[2]	AnPC[2]
0.5%CO-MAP[3]	0	35.1	8.8	7.3	39.6	11.4	0.69	0.48
0.5%CO-MAP	1	37.8	13.2	10.2	37.7	16.7	2.26	2.25
0.5%CO-MAP	2	38.3	13.9	11.0	38.3	17.7	2.67	2.68
0.5%CO-MAP	3	39.1	13.3	10.6	38.6	17.0	3.41	3.36
0.5%CO-MAP	4	39.1	13.5	10.8	38.7	17.3	4.48	3.5
0.5%CO-MAP	5	40.1	13.6	10.9	38.7	17.5	4.85	3.92
0.5%CO-MAP	6	39.0	13.2	9.8	36.7	16.5	5.47	4.84
0.5%CO-MAP	7	37.8	14.4	11.5	38.7	18.4	5.49	5.73
0.5%CO-MAP	8	39.0	13.9	11.6	39.9	18.1	6.64	5.97
5%CO-VP[4]	0	37.1	12.8	10.0	37.9	16.2	1.49	0.48
5%CO-VP	1	39.1	10.2	10.2	46.4	14.6	2.85	2.79
5%CO-VP	2	36.9	12.4	10.8	41.0	16.4	3.39	3.34
5%CO-VP	3	38.2	11.0	10.2	42.9	15.0	4.59	3.49
5%CO-VP	4	36.9	10.3	9.7	43.4	14.2	4.94	4.85
5%CO-VP	5	38.9	10.0	9.9	43.8	14.0	5.21	5.02
100%CO-VP[5]	0	37.5	13.2	9.5	35.7	16.3	0.48	0.48
100%CO-VP	1	38.0	13.0	9.6	36.5	16.2	1.43	1.4
100%CO-VP	2	38.7	12.7	9.7	37.4	15.9	2.61	2.5
100%CO-VP	3	37.9	13.0	10.2	38.0	16.5	3.41	2.77
100%CO-VP	4	38.2	11.1	9.9	41.5	14.9	4.56	4.74
100%CO-VP	5	37.2	12.1	9.5	38.3	15.4	5.78	5.36
100%CO-VP	6	38.5	10.5	8.8	40.0	13.7	5.89	5.47
PVC[6]	0	37.6	11.5	13.3	49.4	17.6	1.61	1.18
PVC	1	33.4	9.8	12.1	51.2	15.6	3.87	3.74
PVC	2	36.4	5.3	10.7	64.2	12.0	6.09	6.03
LSD[7]		**1.87**	**1.87**	**0.89**	**3.9**	**1.83**	**0.45**	**0.37**
Ave. SEM [8]		**0.69**	**0.61**	**0.33**	**1.12**	**0.62**	**0.13**	**0.1**

[1]L*=Lightness; a*=redness; b*=yellowness; Hue angle=$\tan^{-1}(b^*/a^*)$, where lower values indicate more redness ; Saturation index=$(a^{*2}+b^{*2})^{1/2}$.
[2]APC=$\log_{10}$ aerobic plate count/cm^2; AnPC=$\log_{10}$ anaerobic plate count/cm^2. [3]0.5%CO-MAP=0.5% CO, 39.5% N_2, 60% CO_2 in a modified atmosphere package.
[4]5%CO-VP=5% CO, 35% N_2, 60% CO_2 in a MAP for 24 h, then vacuum packaged.
[5]100%CO-VP=100% CO in a MAP for 1 h, then vacuum packaged.
[6]PVC=Standard oxygen-permeable polyvinyl chloride film wrap.
[7]LSD=Fisher's least significant difference among column means ($p<0.05$).
[8]Average standard error of the mean.

Table 2. Effect of CO pretreatment and packaging method on Hunter color values[1] and microbial load of ground beef stored at 2°C for 8 weeks.

Treatment	Week	L*	a*	b*	Hue angle	Saturation	APC[2]	AnPC[2]
0.5%CO-MAP[3]	0	43.3	15.6	13.7	41.4	20.8	3.98	1.74
0.5%CO-MAP	1	45.9	16.4	12.9	38.3	20.8	4.16	2
0.5%CO-MAP	2	45.6	16.4	13.4	39.2	21.2	4.8	4.56
0.5%CO-MAP	3	46.5	17.5	14.2	39.0	22.5	6.06	5.62
0.5%CO-MAP	4	47.3	16.5	14.2	40.9	21.8	6.66	5.83
0.5%CO-MAP	5	47.4	16.8	13.4	38.5	21.5	7.63	6.7
0.5%CO-MAP	6	45.3	16.5	13.0	38.4	21.0	7.72	7.56
0.5%CO-MAP	7	45.4	16.1	13.0	38.9	20.7	8.01	7.93
0.5%CO-MAP	8	47.6	14.7	13.5	42.5	20.0	8.27	8.29
PVC[4]	0	48.4	8.3	15.3	61.4	17.4	4.65	4.54
PVC	1	51.9	4.5	12.0	69.5	12.8	5.62	5.14
PVC	2	52.0	2.2	12.5	79.3	12.9	7.99	8.05
LSD[5]		**4.42**	**1.42**	**1**	**5.54**	**1.06**	**0.19**	**0.44**
Ave. SEM [6]		**1.49**	**0.5**	**0.37**	**1.59**	**0.42**	**0.07**	**0.14**

[1]L*=Lightness; a*=redness; b*=yellowness; Hue angle=$\tan^{-1}(b^*/a^*)$, where lower values indicate more redness ; Saturation index=$(a^{*2}+b^{*2})^{1/2}$.
[2]APC=$\log_{10}$ aerobic plate count/gm; AnPC=$\log_{10}$ anaerobic plate count/gm.
[3]0.5%CO-MAP=0.5% CO, 39.5% N_2, 60% CO_2 in a modified atmosphere package.
[4]PVC=Standard oxygen-permeable polyvinyl chloride film wrap.
[5]LSD=Fisher's least significant difference among column means ($p<0.05$).
[6]Average standard error of the mean.

Discussion

Brewer et al (1994) obtained 6 weeks red color stability by exposing the steaks to 100% CO for 1 h before VP. In our study, we also observed 6 weeks color stability for steaks exposed to 100% CO for 1 h before VP. The goal of the present study was to obtain > 21 d red color stability using a lower and thus safer level of CO than used by Brewer et al (1994). Using 5% CO for 24 h before VP, steaks were red color stable for 5 weeks. Thus it is feasible to obtain extended red color stability in VP steaks using a

lower, safer level of CO. The disadvantage of obtaining red color in VP is the need for two packaging steps; a pretreatment with CO in MAP to develop red color, then repackaging in VP.

Steaks and ground beef in 0.5% CO in modified atmosphere packages maintained their desirable red color for the entire 8-week study. This is the packaging procedure widely used in Norway. The major disadvantage of the MAP system is the bulk of the steak plus headspace gas, and the increased likelihood of package puncture during distribution, as compared to vacuum packaging.

Safety issues during packaging

Red color in VP steaks was achieved by pretreatment with 5% CO for 24 h, or 100% CO for 1 h. From a safety standpoint, the use of 5% CO would be preferred. The CO was purchased in pre-mixed gas cylinders, and pumped directly into the headspace of the MAP package. Thus, personnel were not exposed to dangerous levels of CO during MA packaging. This was verified by placement of several CO detectors in the room. When the CO-MAP packages were opened so that the bloomed steaks could be vacuum packaged, the residual CO in the MAP headspace was released. Even so, with 5% CO, the CO levels in the room atmosphere remained in the safe zone (<35 ppm continuous exposure over 8 h). With 100% CO-MAP, alarms were set off when packages were opened directly in front of a detector.

Conclusion

It was feasible to develop surface redness on beef steaks by MAP in 5% CO for 24 h, and to maintain redness for 21 d in a VP. It is predicted that steak redness in VP could be extended beyond 3 weeks if the 5% CO pre-treatment time was increased to greater than 24 h. Browning was completely prevented for at least 5 weeks by these treatments. Pretreatment with 5% CO followed by VP, if approved by the USDA, would allow for central packaging and distribution of VP beef steaks and retention of desirable red color for > 21 d, which is considerably longer than the average color stability of 3-10 d for beef in PVC film.

MAP of ground beef in 0.5% CO would dramatically improve color stability, but there is the possibility that after 5 weeks storage the product could be spoiled but still appear fresh. Thus, a regulation for expiration dates on the label is recommended.

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CHAPTER 4

SENSORY EVALUATION OF GROUND BEEF STORED IN HIGH-OXYGEN MODIFIED ATMOSPHERE PACKAGING

Abstract

The quality of ground beef stored in high-oxygen modified atmosphere packaging (MAP: 80% O_2; 20% CO_2), was evaluated and compared to controls stored in oxygen-impermeable chubs. Patties were formed from stored ground meat at d 1, 6, and 10. Color, microbial load, thiobarbituric acid (TBA) number, and sensory acceptability were measured. Patties from both treatments bloomed to red with a* values >16. Aerobic plate counts increased to 9 X 10^5 CFU/g by 10 d storage, but were not different ($p < 0.05$) between treatments. TBA number of high-oxygen samples increased to 2.1 after 10 d, compared to 0.8 for controls. Flavor of samples in high-oxygen were rated less desirable after 6 or 10 d.

Introduction

A primary factor determining consumer purchase of fresh meat is the "fresh meat" color. When meat is cut and the surface is exposed to air, deoxymyoglobin, which is purple, is rapidly oxygenated to cherry red oxymyoglobin; but over time myoglobin oxidizes to metmyoglobin to form a brown discoloration (Kropf 1993).

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Greene and others (1971) reported that consumers made a decision against purchasing when brown metmyoglobin reached 30-40% of total pigments on the surface of fresh retail beef. The bright red color associated with freshness can be obtained by packaging the meat in a modified atmosphere containing 80% oxygen with the remainder as carbon dioxide (CO_2). CO_2 is included to inhibit the growth of aerobic spoilage bacteria (Zhao and others 1994; Manu-Tawiah and others 1991). An advantage of high-oxygen modified atmosphere packaging (MAP) is that the product is case-ready, while coarsely ground beef in bulk chubs must be finely ground and repackaged at the retail store.

Although oxygen maintains the desirable red color of the fresh meat, it also promotes oxidation of lipid, especially in minced beef that has its cell structure disrupted, exposing labile lipid components to oxygen (Sato and Hegarty 1971). O'Grady and others (2000) reported that lipid oxidation of minced beef stored in 80% oxygen increased significantly between d 7 and d 10 of storage. However, no sensory studies have been done to correlate lipid oxidation with palatability in ground beef stored in high-oxygen MAP. Processors implementing the high-oxygen MAP process have had consumer complaints about the flavor of the product. Thus, this study was done to evaluate palatability, color, and lipid oxidation status of ground beef packaged in a modified atmosphere package (80% O_2 & 20% CO_2) for up to 10 d at 2° C, compared with ground beef stored in oxygen-impermeable casings at the same temperature and time.

Materials and Methods

Experimental design and statistical analysis

Finely ground beef in a high-oxygen MAP (80% O_2 & 20% CO_2) and coarsely ground beef chubs in oxygen-impermeable film (control) were stored at 2° C up to 10 d. Aerobic plate count was done every d for 10 d on MAP and control treatments. Other measurements (sensory analysis, thiobarbituric acid (TBA) test, pH and Hunter color) were done on patties formed after 1, 6, and 10 d of storage.

Treatment and storage time means were compared by analysis of variance (ANOVA), using Statistica (Statsoft, Inc., Tulsa, Okla., U.S.A.) for all measurements except sensory. Sensory analysis was done using Minitab (Minitab Inc, State College, Pa., U.S.A.). Differences between means were determined by calculation of Fisher's least significant difference (LSD) values when appropriate. Significance was defined at $p<0.05$. Figures were prepared using the Cricket 1.01 graphics program (Computer Associates International, Islandia, N.Y., U.S.A.).

Meat samples and packaging

Control. Coarsely ground beef chubs (1.3 kg) were prepared and packaged at Stone Meats, Inc. (Harrisville, Utah, U.S.A.). The chubs were prepared from USDA inspected, ungraded (no-roll) beef trim by mixing 90% and 50% lean beef trim to a final composition of 85% lean and 15% fat. The coarsely ground beef was then packaged in oxygen-impermeable chub packaging using a Kartridge Pack 44 chub machine (BWI Kartridge Pak, Davenport, Iowa, U.S.A.). Packages were placed in an insulated cooler

and delivered to the Utah State University (USU) meat lab on the same day they were prepared. Packages were then held in the USU meat lab cooler at 2° C for up to 10 d. The coarsely ground beef chubs were finely ground on d 1, 6, and 10 by passing the beef through a Hobart grinder model 4152 (Hobart Mfg. Co., Troy, Ohio, U.S.A) with a 0.32 cm plate, then manually formed into 112 g patties using a circular form from a Hollymatic patty machine (Hollymatic Corp., Park Forest, Ill., U.S.A.).

MAP. Finely ground beef (0.45 kg / package) was prepared and packaged at Stone Meats, Inc. (Harrisville, Utah, U.S.A.) from USDA-inspected, ungraded beef trim to a final composition of 85% lean and 15% fat as described for the control samples. Samples were then packaged in high-oxygen MAP to a target of 80% O_2 and 20% CO_2, using a Ross INPACK S45 modified atmosphere tray packaging machine (Robert Reiser and Co., Inc., Canton, Mass., U.S.A.). The actual headspace gas composition in the MAP was 78% O_2, 18% CO_2 and 4% residual air as measured with a Mocon PacCheck model 650 dual headspace analyzer (Minneapolis, Minn., U.S.A.). The packages were delivered to USU as previously described. The MAP package consisted of a foam tray and a clear top film that were both moisture and oxygen impermeable. The barrier foam tray had a maximum oxygen transmission rate of 0.1 cc/m^2 at 23° C, 0% relative humidity (RH) and maximum moisture vapor transmission rate of 2.0g/ (24 h. 254 cm^2) at 100% RH and 38°C (Cryovac Sealed Air Corporation, Duncan, S.C., U.S.A.). The oxygen-barrier film had a maximum oxygen transmission rate of 20 cc / (24 h, 254 cm^2) and moisture vapor transmission rate of less than 0.10 g / (24 h. 254 cm^2) at 100% RH and 4.4°C (Cryovac Lid 1050 Lidstock, Duncan, S.C., U.S.A.). MAP samples were stored at USU at 2° C for

10 d. On d 1, 6, and 10 meat from 3 packages was formed into patties (112 g) as previously described.

Hunter color measurements

After patty preparation on d 1, 6, and 10, the patties were held in the dark (meat lab cooler) for 1 h at 2° C to allow oxygenation and red color development. Color measurements were then taken using the Hunter L*, a*, b* system with a Hunter lab Miniscan portable colorimeter (Reston, Va., U.S.A.), standardized using a white and black standard plate. Three measurements were taken per patty on 3 patties per treatment (control and MAP). Patties were then blast frozen (-20° C) for later pH and TBA analyses.

pH determination

Duplicate samples (10 g) of the patties were blended with 90 mL distilled water for 1 min with a Polytron homogenizer (Brinkman Instruments, Westbury, N.Y., U.S.A.). The pH of filtrate was measured with a pH meter model 610 A (Fisher Scientific Company, Houston, Texas, U.S.A.) calibrated at pH 4.0 and 7.0.

TBA analysis

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978), as modified by Lee and others (1999). Duplicate meat samples (0.5g) were mixed with 2.5 ml of stock solution containing 0.375% TBA (Sigma Chemical Co., St. Louis, Mo.., U.S.A.), 15% TCA (Mallinckrodt Baker Inc., Paris, Ky., U.S.A.), 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, Del., U.S.A.) at 5500 rpm for 25 min. The absorbance of the supernatant was measured spectrophotometrically (Spectronic 21D, Milton Roy, Rochester, N.Y., U.S.A.) 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 meat sample) as follows;

1) TBA# = Sample A_{532} X (1 M TBA chromogen / 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

Aerobic plate counts

A 10g portion of ground beef was mixed with 90 ml sterile peptone water (Difco, Detroit, MI) in a dilution bottle and plate counts were done on serial dilutions following standard procedures (Messer and others 1978). Standard method agar (Difco, Detroit, Mich., U.S.A.) was used as growth media. Duplicate plates were counted after incubation at 37° C for 48 h.

Sensory evaluation

Hamburger patties were formed as previously described and evaluated at 1, 6, and 10 d of storage. In addition to the stored samples, 1-d old MAP samples (25 MAP

packages, 454g/package) were supplied by Stone Meats, Inc. (Harrisville, Utah, U.S.A.) on the morning of d 6 and 10. Therefore, on d 1, samples of MAP and control were evaluated, while on d 6 and 10, samples of stored MAP, stored control and 1-d MAP were evaluated. In the results all the 1-d MAP panel scores were pooled to give the 1-d MAP mean sensory scores.

Panel members were recruited by posting signs on the campus. The panel size was 81, 72 and 76 judges for d 1, 6, and 10 panels, respectively.

Patties were grilled on an institutional grill (General Electric Hotpoint electric grill model HG4, Chicago Heights, Ill., U.S.A.) set at 163°C. Patties were cooked on 1 side for approximately 2 min, flipped, and cooked to an internal temperature of 71 to 74°C, measured using a thermocouple thermometer model 91100-50 (Cole-Parmer Instrument Company, Vernon Hills, Ill., U.S.A.). Patties were freshly grilled throughout the 2-h sensory testing period to insure freshness. Cooked patties were placed on a ceramic plate, covered with foil and held under heat lamps for brief period (<5 min) to keep patties warm before serving. Each panelist received one-quarter portions of patties that were coded and served on warm ceramic plates. Rinse water was provided, and samples were evaluated in individual booths under white fluorescent lights. Sampling order was alternated to avoid positional bias. Panelists were asked to evaluate flavor, texture, juiciness and overall quality based on a standard 9-point hedonic scale where 9 = like extremely and 1 = dislike extremely. Panelists were also asked to comment on what they liked or disliked about the samples.

Results

Hunter color

Patties stored in MAP had higher ($p < 0.05$) redness (a*) value on d 1 but the a* values were not different from controls on d 6 and 10. Patties in MAP also had higher lightness (L*) and yellowness (b*) values than controls on 1 or 10 d storage, but no differences were observed between treatments for hue angle (Table 3). Both treatments had high redness values (a*>14) through 10 d storage. Thus, ground beef in both treatments produced patties with bright red color through 10 d of storage at 2°C.

pH determination

The MAP and control samples had significant pH differences (Table 3), especially after 10 d storage at 2°C. The pH of control samples decreased from 5.7 at d 1 to 5.3 at d 10. MAP samples had only a slight decrease from 5.8 to 5.7 over the same period.

TBA analysis

After 6 d of storage, MAP samples had much higher mean TBA number (1.8) than controls (0.6; Table 3). After 10 d MAP samples had TBA number of 2.1 compared to 0.8 for controls. Thus high oxygen atmosphere packaging was associated with increased TBA number during storage, in agreement with the previous study by O'Grady and others (2000).

Aerobic plate counts

No significant differences ($p < 0.05$) were observed in aerobic plate count (APC) between the control and MAP samples (Fig. 6). APC of ground beef in both treatments remained below spoilage levels during 10 d storage at 2° C, ie., $<10^7$ colony forming units (cfu) / gram of meat (Ayres 1960; Sofos 1994). APC were low (about 1000 cfu/gm) for both treatments at d 1 and increased to about 10^5 cfu/gm after 10 d storage.

Sensory evaluation

There was no significant difference in sensory scores for flavor between MAP and control samples on d 1 (Table 4). Flavor score of control samples was 6 to 7 over 10 d storage, where 6= like slightly and 7= like moderately. By d 6 the flavor score of MAP samples decreased to 4.8 (4 = dislike slightly and 5 = neither like nor dislike). The 10-d old MAP samples were considered the least desirable of all the samples tested with a mean flavor score of 4.5. Note that the average sensory score for the stored MAP samples is on the "dislike" side of the scale. In addition to lower flavor scores, the 6-and 10-d old MAP samples received significantly lower scores ($p<0.05$) than control samples for texture, juiciness and overall quality (Table 4). The decrease in texture and juiciness scores during storage of MAP samples is probably due to the "halo" or "horns" effect (Lawless and Heymann 1998), where high or low scores on one sensory characteristic can affect the score of another characteristic. In this case, the dislike for flavor of MAP samples probably affected the panels' attitudes toward texture and juiciness.

Table 3. Effect of packaging method and storage time on Hunter color values[a], pH and thiobarbituric acid number (TBA No.) of ground beef stored at 2°C for 1, 6, or 10 d.

Treatment	D	L*	a*	b*	Hue angle	pH	TBA No.
Control[b]	1	43.2±1.6	14.4±0.6	16.3±0.6	48.5±0.2	5.7±0.10	0.6±0.1
Control	6	41.3±1.3	16.4±0.4	17.8±0.6	47.4±1.4	5.7±0.04	0.6±0.1
Control	10	41.1±3.1	18.8±1.6	18.8±1.0	45.1±3.8	5.3±0.02	0.8±0.1
80% O_2 MAP[c]	1	46.9±0.4	17.6±0.4	20.0±0.7	48.7±0.6	5.8±0.02	0.9±0.1
80% O_2 MAP	6	42.1±0.9	16.3±0.8	17.8±0.8	47.6±0.3	5.8±0.03	1.8±0.2
80% O_2 MAP	10	48.7±1.0	17.3±0.6	20.6±0.5	49.9±1.4	5.7±0.03	2.1±0.1
LSD[d]		**2.89**	**1.55**	**1.30**	**N.S**	**0.06**	**0.2**

[a]L*=Lightness; a*=redness; b*=yellowness; Hue angle=$\tan^{-1}$(b*/a*), where lower values indicate more redness.
[b]Control = Coarsely ground beef packaged in a traditional oxygen-impermeable chub package. The coarsely ground beef chubs were finely ground on d 1, 6, and 10 by passing through a 0.32 cm Hobart grinder plate.
[c]80% O_2 MAP: Finely ground beef was packaged in a high-oxygen modified atmosphere package (MAP; 80% O_2 & 20% CO_2).
[d]LSD=Fisher's least significant difference among column means ($p<0.05$). N.S =not significant.

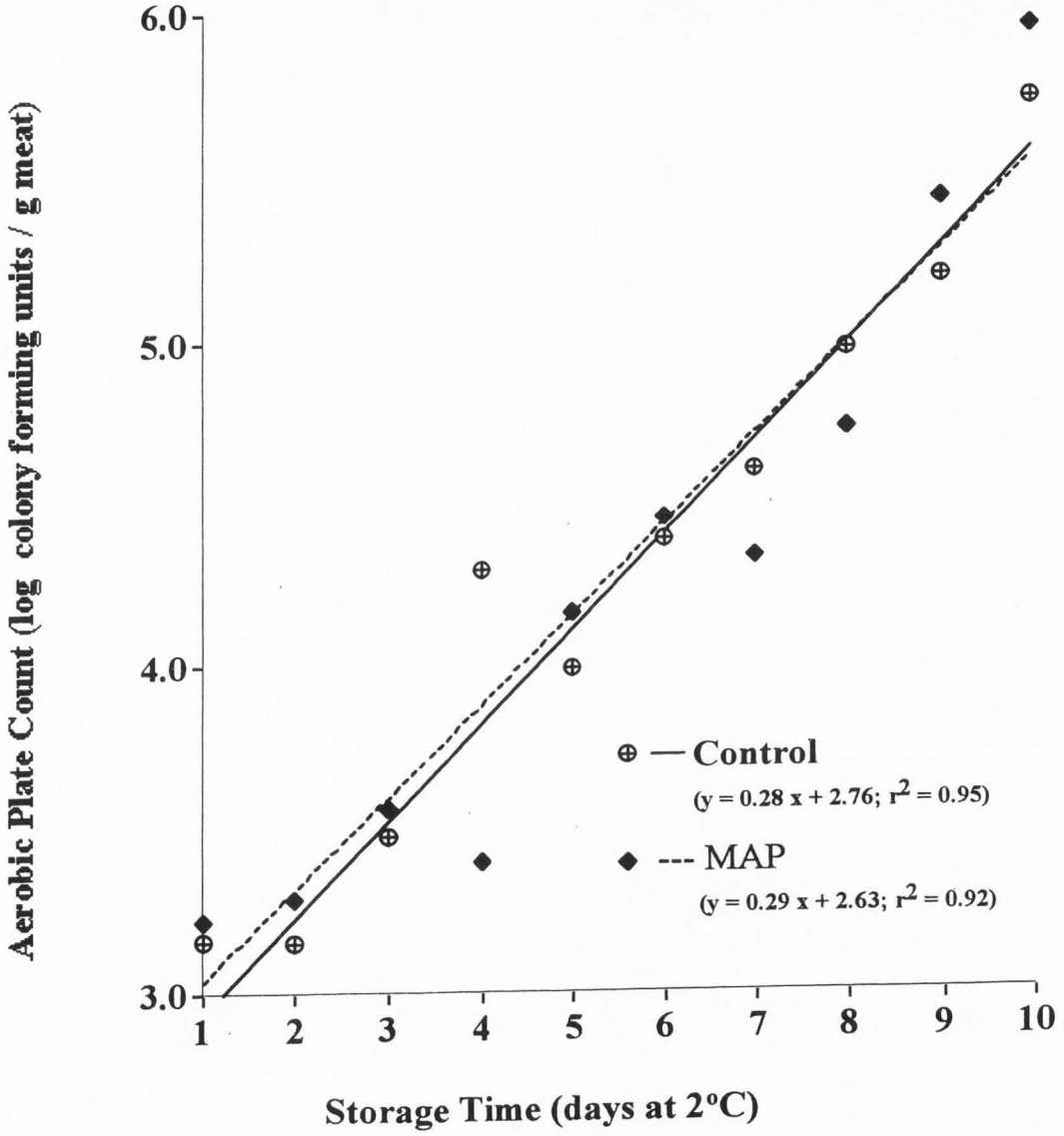

Fig. 6. Aerobic plate count of ground beef packaged in high oxygen modified atmosphere packaging (MAP; 80% oxygen and 20% carbon dioxide) compared to controls packaged in oxygen impermeable casings.

Table 4. Effect of packaging method and storage time on mean score for texture, juiciness and overall acceptability of ground beef stored at 2°C for 1, 6 or 10 d.

Treatment	D	Flavor	Texture	Juiciness	Overall acceptability
MAP	1	6.8[a]	6.7 [a]	6.7 [a]	6.7 [a]
Control	1	6.6[ab]	6.7 [a]	6.8 [a]	6.8 [a]
MAP	6	4.8[c]	5.6 [b]	6.0 [bc]	5.1 [b]
Control	6	6.2[b]	6.3 [a]	6.3 [ab]	6.1 [a]
MAP	10	4.5[c]	5.5 [b]	5.7 [c]	5.0 [b]
Control	10	6.8[a]	6.5 [a]	6.4 [ab]	6.6 [a]

Means scores in a column with the same superscript letter are not different ($p<0.05$). Hedonic score 9 = like extremely and 1 = dislike extremely.

Discussion

Ground beef in high oxygen MAP maintained a bright red color for 10 d. The coarsely ground beef that was stored for 10 d in oxygen-impermeable chubs also turned red (bloomed) after fine-grind and patty-making operations. The aerobic plate count did not show any significant difference between the two treatments. However, panelists rated the flavor of MAP ground beef as undesirable by 6 d storage. The undesirable flavor was not attributed to microbial spoilage since APC of both treatments were below spoilage levels after 10 d storage, and the undesirable flavor was described as rancid by several panelists. Low pH was not the source of off-flavor in 6- or 10-d old MAP samples since pH of MAP samples decreased only slightly during storage, from 5.8 to 5.7. The off-

flavor of 6 or 10 d MAP samples was due to oxidative rancidity in the high oxygen MAP, as indicated by the high TBA numbers of these samples. TBA number > 1 is usually associated with rancid flavor/odor by sensory panelists (Tarladgis and others 1960). This can clearly be seen in the low flavor scores of the MAP samples on d 6 and d 10, which were associated with TBA numbers > 1. O'Grady and others (2001) and Coventary and others (1998) have also reported higher TBA numbers for ground beef stored in 40% - 80% oxygen MAP. O' Grady and others (2001) further reported that addition of α-tocopherol reduced lipid oxidation. However, labeling would be an issue with the addition of α-tocopherol or other antioxidants. According to USDA regulations, ground beef with any added substance must be labeled with the term "pattie" rather than "ground beef".

Conclusions

High oxygen modified atmosphere packaging was effective in maintaining a desirable red color for 10 d refrigerated storage. However, the color of MAP samples was not significantly more red than ground meat from the chub (control) treatment. A significant development of rancid off-flavor was detected in the high oxygen MAP by the sixth d of storage.

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CHAPTER 5

EFFECT OF HOLDING TIME ON TBA VALUES OF COOKED PORK PATTIES

Abstract

This experiment was done to determine the effect of holding time before serving cooked patties on thiobarbituric acid (TBA) values as a measure of rancidity. Cooked pork patties held at 71°C for 90 or 120 min had higher ($p<0.05$) TBA values than patties held for 0-60 min. Thus ground pork patties could be held warm for 60 min after cooking without significantly increasing TBA number.

Introduction

Lipid oxidation is a major cause of deterioration in the quality of meat and meat products (Asghar, Gray, Buckley, Pearson, & Booren, 1988). Generally lipid oxidation is faster in heated meat than in raw meat tissues (Tichivangana & Morrissey, 1985). The rate and degree of oxidation degradation has been directly related to the degree of unsaturation of the lipid present (Igene & Pearson, 1979; Tichivangana & Morrissey, 1985). Oxidation of unsaturated lipid in cooked meats during storage and reheating results in stale or rancid flavors known as warmed-over flavor (Sato & Hegarty, 1971). The warmed-over flavor problem of cooked meat has assumed much greater significance in recent years due to the rapid increase in fast food service facilities requiring the use of

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large quantities of precooked or partially cooked meats or meat products. In these facilities, cooked meat may be kept warm for a variable time prior to serving. The objective of this study was to determine the degree of oxidative degradation that occurs when cooked ground pork was kept warmed for up to 2 h after cooking.

Materials and Methods

Experimental design and statistical analysis

TBA numbers were measured on cooked patties as affected by 30, 60, 90, or 120 min of warm storage (71°C). TBA measurements were done in duplicate and the entire experiment was replicated three times. Treatment means were calculated by ANOVA, using Statistica (Statsoft, Inc., Tulsa, OK). Difference between means was determined by calculation of Fisher's least significant difference (LSD) values, when appropriate.

Sample preparation

Pork trim was finely ground through a Hobart grinder model 4152 (Hobart Mfg. Co., Troy, OH) with a 0.32 cm plate, then manually formed into 112 g patties using a circular form from a Hollymatic patty machine (Hollymatic Corp., Park Forest, IL). Patties were grilled on an institutional grill (General Electric Hotpoint electric grill model HG4, Chicago Heights, IL) set at 163°C. Patties were cooked on one side for approximately 2 min, flipped, and cooked to an internal temperature of 71-74°C, measured using a thermocouple thermometer model 91100-50 (Cole-Parmer Instrument Company, Vernon Hills, IL). The patties were then maintained at 71°C for 30, 60, 90,

and 120 min in an oven. TBA test was done on these patties immediately after removal from the oven.

TBA analysis

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978), as modified by Lee, Hendricks and Cornforth (1999). Duplicate meat samples (0.5g) were mixed with 2.5 ml of stock solution containing 0.375% TBA (Sigma Chemical 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 5500 rpm for 25 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 & Yu, 1958). The MDA concentration was converted to TBA number (mg MDA / Kg meat sample) as follows;

1) TBA# (mg / kg) = Sample A_{532} X (1 M TBA chromogen / 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

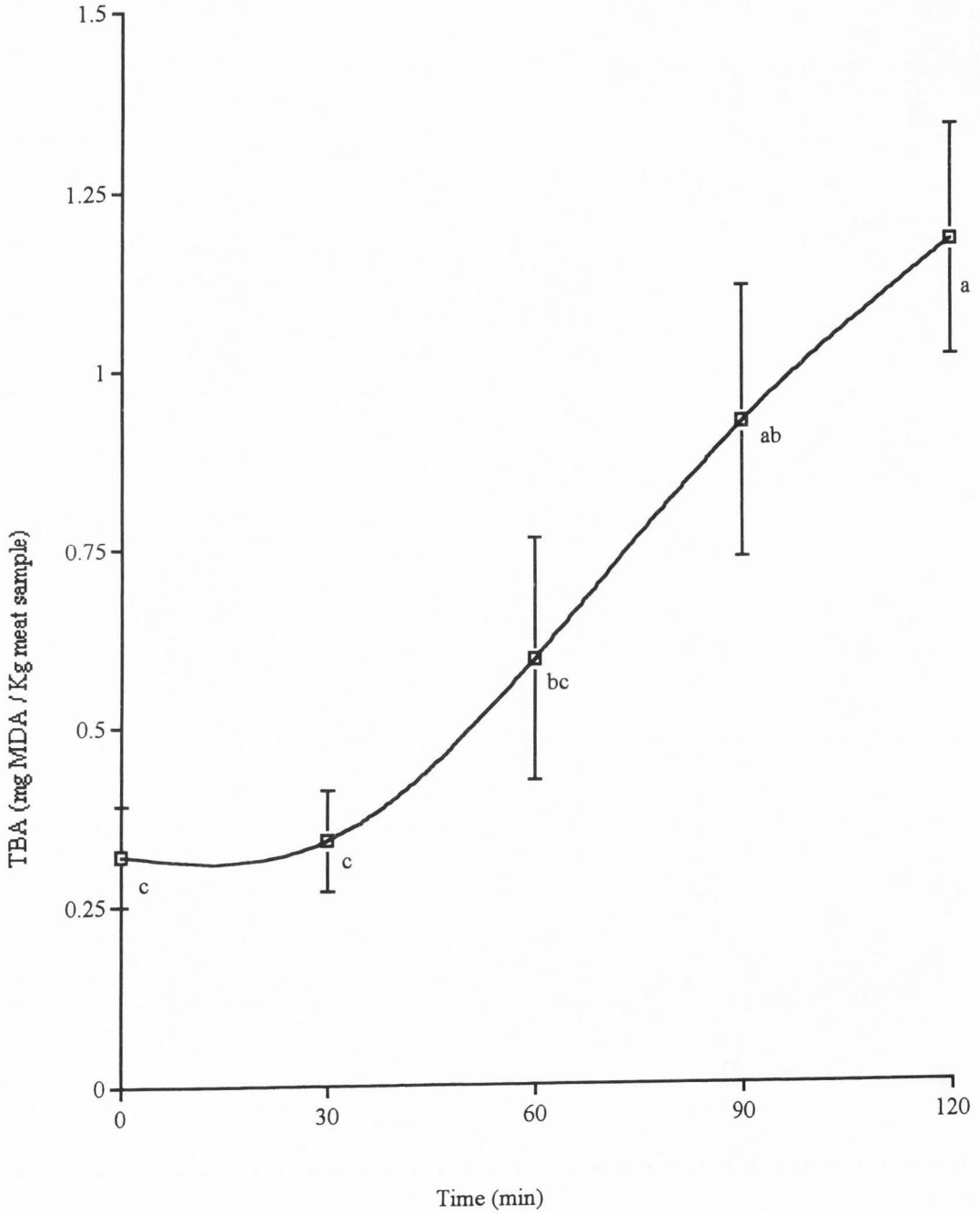

Fig. 7. TBA number of cooked patties kept warm at 71°C for various time after cooking. Values are means (n = 3) ± standard error of the mean.

Results and Discussion

Significant differences were obtained in the TBA No. for the different holding times (Fig. 7). TBA number increased from 0.3 at 0 min to 1.17 at 120 min. TBA numbers at 120 min were significantly higher than 0 to 60 min. There were no significant differences in TBA No. of samples held for 0, 30, or 60 min after cooking. Thus, patties could be kept warm for up to 60 min after cooking without significantly increasing TBA number.

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CHAPTER 6

CONSUMER PREFERENCE AMONG BEEF PATTIES WITH VARIOUS LEVELS OF OXIDATION, EXPRESSED IN TERMS OF TBA NUMBER

Abstract

This experiment was done to determine if various levels of oxidized flavor expressed in terms of thiobarbituric acid (TBA) number were preferred by consumer panelists or not. Pork patties were cooked and stored at 2°C for 0, 1, 2 and 3 d to obtain TBA numbers of 0.4, 1.5, 3.4 and 3.9, respectively. Paired-preference sensory testing indicated that panelists preferred ($p<0.001$) patties with TBA number of 0.4 compared to those patties with TBA numbers of 1.4 and greater.

Introduction

The thiobarbituric acid (TBA) test is the most frequently used method for assessing lipid oxidation in meat. Tarladgis, Watts, Younathan and Dugan (1960) found that TBA numbers (mg TBA reactive substances/ kg tissue) were highly correlated with trained sensory panel scores for rancid odor in ground pork. The TBA number at which a rancid odor was first perceived was between 0.5 and 1.0. This "threshold" has served as a guide for interpreting TBA test results. According to Green and Cumuze (1981) the range of oxidized flavor detection for inexperienced panelists was within a range of TBA numbers similar to the previously determined threshold level for trained panelists.

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However, these data did not provide any information regarding consumer preferences. Thus, the objective of this study was to determine if various levels of oxidized flavor were preferred or not by consumer panelists.

Materials and Methods

Experimental design and statistical analysis

The preference test (ASTM, 1996) was used as an appropriate test. Patties were prepared, cooked and stored at 2°C for up to 3 days to obtain different TBA numbers. These patties were served to the panelists (n = 64) and samples were also taken for analysis of TBA. Significance was accepted at $p < 0.001$ if 46 or more panelists (out of 64 total panelists) preferred one sample over the other in a pair (ASTM, 1996). Data were discarded if no preference was identified for a given pair, since the paired-preference test is a forced choice method, and consumers are not allowed to give a "no preference" response (ASTM, 1996).

Sample preparation

A single batch of pork trim was finely ground using a Hobart grinder, model 4152 (Hobart Mfg. Co., Troy OH) with a 0.32 cm plate, then manually formed into 112 g patties using a circular form from a Hollymatic patty machine (Hollymatic Corp., Park Forest, IL) and then frozen (-20°C). On d 3, 2, and 1 before the panel, patties were tempered at 22°C for 1 h before cooking. Patties were grilled on an institutional grill (General Electric Hotpoint electric grill model HG4, Chicago Heights, IL) set at 163°C. Patties were cooked on one side for approximately 2 min, flipped, and cooked to an

internal temperature of 71-74°C, measured using a thermocouple thermometer model 91100-50 (Cole- Parmer Instrument Company, Vernon Hills, IL). After cooking, patties were stored at 2°C for 1, 2, and 3 d to obtained different TBA values. Freshly cooked patties on the day of the panel were used as the control for the preference test. One-d old patties with 0.5% STP were also used in the preference test to obtain samples with TBA number of less than 0.5.

TBA analysis

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978), as modified by Lee, Hendricks and Cornforth (1999). Duplicate meat samples (0.5g) were mixed with 2.5 ml of stock solution containing 0.375% TBA (Sigma Chemical 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 5500 rpm for 25 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 $1.56 \times 10^5 M^{-1}cm^{-1}$ (Sinnhuber & Yu, 1958). The MDA concentration was converted to TBA number (mg MDA / Kg meat sample) as follows;

1) TBA# (mg / kg) = Sample A_{532} X (1 M TBA chromogen / 1.56 X 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

Sensory evaluation

The preference test was used as an appropriate test. Each panelist was served 6 pairs of cooked patties. Each pair consisted of a control sample and a test comparison sample of either patties with STP (low TBA), or 1-, 2-, or 3-d old patties. Freshly cooked patties were used as the control for the preference test. At d 3, 2, and 1 before the panel, pork patties were cooked and stored at 2°C as described in sample preparation, in order to obtain patties with different TBA values. On the day of the panel, fresh patties were grilled throughout the two-hour sensory testing period and used as control samples. On the day of the panel, precooked patties (STP patties, 1-, 2-, and 3-d old patties) were microwave heated to an internal temperature of 65 - 74°C. Cooked patties were placed on a ceramic plate, covered with foil and held at 71°C in an oven for brief period (<5 min) to keep patties warm before serving.

Panel members were recruited by posting signs on the campus. The panel size was 64 judges. Each panelist received 6 pairs of samples. Each panelist received 1/4 patty portions which were coded and served on warm ceramic plates. Order of sample presentation was balanced to avoid positional bias. Rinse water was provided and samples were evaluated in individual booths under white fluorescent lights. Panelists

Table 5. Sensory panel paired-preference test results. Significance was accepted at $P < 0.001$ if 46 or more panelists (out of 64 total panelists) preferred one sample over the other in a pair.

TBA No. comparison	Preference(64 panelists)
0.4 (Control) vs. 0.2 (STP)	No preference (33/64)
0.4 (Control) vs. 0.4 (Control)	No preference (32/64)
0.4 (Control) vs. 1.5 (1-d old)	Preferred 0.4 (53/64)
0.4 (Control) vs. 3.7 (2-d old)	Preferred 0.4 (55/64)
0.4 (Control vs. 4.0 (3-d old)	Preferred 0.4 (61/64)

were instructed to identify their preference within each pair and were also asked to comment on what they liked or disliked about the samples.

Results and Discussion

Panelists had no preference between freshly cooked pork patties (TBA No. 0.4) and 1-d old patties containing STP (TBA No. 0.2; Table 5). Panelists had a highly significant ($P<0.001$) preference for the freshly cooked samples (TBA No. 0.4), compared to all stored samples (1, 2, 3 d storage with TBA No. 1.5, 3.7, and 4.0, respectively; Table 5).

Conclusion

Previous studies have shown that both trained and untrained panelists can detect oxidized flavor in a TBA range of 0.5 to 1.0. This study showed that consumer panelists not only detected rancid flavor in samples with TBA>1 but also preferred samples with TBA<0.4.

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CHAPTER 7

COMPARISON OF ANTIOXIDANT EFFECTS OF MILK MINERAL, BUTYLATED HYDROXYTOLUENE AND SODIUM TRIPOLYPHOSPHATE IN RAW AND COOKED GROUND PORK

Abstract

The antioxidant effects of 0.5 - 2.0 % milk mineral (MM) was tested in raw and cooked ground pork stored at 2 ° or –20 °C, compared to butylated hydroxytoluene (BHT) and sodium tripolyphosphate (STP). TBA numbers were below 0.2 and not different between raw meat treatments. TBA numbers were lower ($p<0.01$) for cooked treatments with MM or STP compared to controls or treatments with BHT.

Introduction

The greater propensity for warmed-over flavor in cooked and comminuted products is due to release of non-heme iron during cooking and grinding (Igene, King, Pearson, & Gray, 1979). Recently, it has been reported that dried milk mineral (MM), the dried permeate of ultra-filtered whey, has antioxidant properties in cooked meats, apparently by iron-chelation to colloidal phosphate (Cornforth & West, 2002). Cooked ground pork required 2% MM to maintain TBA number <1.0 while samples with 1% MM maintained a TBA number < 2.0 (Cornforth & West, 2002). Samples in this study were stored at 2°C.

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However, much precooked pork is frozen, as is the case for frozen pizza toppings. More information is needed on the possible antioxidant effects of milk mineral in frozen meats. Thus, the objective of this study was to determine the optimum level at which dried milk mineral can be used in raw and cooked ground pork stored at two temperatures (2°C or -20°C), as compared to other antioxidants (butylated hydroxy toluene and sodium tripolyphosphate).

Materials and Methods

Experimental design and statistical analysis

The antioxidant effects of dried milk mineral (MM), BHT, and STP were compared in raw and cooked ground pork. The meat was stored at refrigerated temperature of 2°C for 8 d for raw meat and 15 d for cooked meat. The raw and cooked meat was also stored at frozen temperature of -20°C for 6 months. The concentration of MM used was 0 (control), 0.5, 1.0, 1.5 and 2% of meat weight. BHT was used at a level of 0.01% based on fat weight (DeHoll, 1981) and the STP was used at a level of 0.5% of meat weight. Product stability was measured using the TBA analysis described by Buege and Aust (1978). TBA measurements were taken on d 0, 1, 5, and 8 for the raw ground pork and d 0, 1, 4, 8, 12 and 15 for the cooked ground pork stored at refrigeration temperature (2°C). TBA measurements were made every month for 6 months for the frozen meats (-20°C). All measurements were performed in duplicate. The entire experiment was replicated three times. Treatment means were calculated by ANOVA, using Statistica (Statsoft, Inc., Tulsa, OK). Difference between means was determined by calculation of Fisher's least significant difference (LSD) values, when appropriate.

Figures were prepared using the curve-smoothing feature of the Cricket 1.01 graphics program (Computer Associates International, Islandia, NY).

Sample preparation

Ground pork (15% fat) was prepared by passing lean pork trim through a Hobart grinder model 4152 (Hobart Mfg. Co., Troy, OH) with a 0.32 cm plate. Fat content was determined by a solvent extraction method (AOAC, 1991). Dried milk mineral (Tru Cal) was obtained from Glanbia Ingredients (Richfield, Idaho). The MM was commercially prepared by drying permeate obtained from ultra-filtration of whey, and consisted of ~24% calcium, 13.5% phosphorous and 9% citrate. Samples were prepared by addition of 0.5, 1, 1.5, or 2% of MM to 100 g of raw meat.

Raw ground pork: The MM, BHT, and STP was manually mixed with meat and then placed in a sealed Ziplock plastic bag (S.C. Johnson & Son, Racine, WI) and held at 2°C for 8 d or -20°C for 6 months. The sealed bags contained headspace air, so oxygen was present during storage, but sample dehydration was minimized by bag closure.

Cooked ground pork: The MM, BHT and STP was manually mixed with meat and cooked at 163°C for 15 min. The cooked pork crumbles were then placed in sealed Ziplock plastic bags (S.C. Johnson & Son, Racine, WI) and held at 2°C for 15 d or -20°C for 6 months. As with raw samples, the sealed bag of cooked pork crumbles contained headspace air.

TBA analysis

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978), as modified by Lee, Hendricks and Cornforth (1999). Duplicate meat samples (0.5g) were mixed with 2.5 ml of stock solution containing 0.375% TBA (Sigma Chemical 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 5500 rpm for 25 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 $1.56 \times 10^5\ M^{-1}cm^{-1}$ (Sinnhuber & Yu, 1958). The MDA concentration was converted to TBA number (mg MDA / Kg meat sample) as follows;

1) TBA# (mg / kg) = Sample A_{532} X (1 M TBA chromogen / 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

Results and Discussion

Raw ground pork

Refrigerated storage (2°C for 8 d): No significant difference ($P>0.05$) was seen between the different treatments. TBA values for all the treatments in raw ground pork were < 0.2 (Table 6).

Frozen storage (-20°C for 6 months): No significant difference was seen between the different treatments. TBA values for all frozen raw ground pork treatments were < 0.5 (Table 7). In contrast to these results, McCarthy, Kerry, Kerry, Lynch, and Buckley (2001) recently reported significant benefits of BHT and other antioxidants added to raw pork patties. The difference between the two studies may be explained by differences in meat preparation and storage. In the study by McCarthy and others (2001), samples were exposed to more oxidizing processing conditions (4 weeks frozen storage of ground meat before patty-making) and higher patty storage temperature (4°C versus 2°C in the present study), so antioxidants had greater opportunity to demonstrate their effectiveness. Also, patties in the study by McCarthy and others (2001) were over-wrapped in oxygen-permeable cling film, while oxygen exposure in the present study was limited to the oxygen in the headspace of the Ziplock bag. Control patties (McCarthy and others, 2001) had TBA value of 2.44 after 9 d, while patties with BHT/BHA at 0.01% of meat weight had TBA value 0.27. Controls in the present study had low TBA values during storage (< 0.2 and <0.5 for refrigerated or frozen samples, respectively), so antioxidant addition did not improve storage stability of raw samples.

Table 6. Comparison of milk mineral (MM), STP (sodium tripolyphosphate) and BHT (butylated hydroxytoluene) effects on TBA number of raw and cooked ground pork stored at 2 °C for 8 and 15 d respectively.

Treatment	TBA Means* (Raw)	TBA Means* (Cooked)
Control	0.17 ± 0.09	5.0 ± 3.0[a]
BHT	0.16 ± 0.05	4.0 ± 3.0[a]
0.5% MM	0.13 ± 0.03	2.0 ± 2.0[b]
1% MM	0.17 ± 0.04	0.7 ± 0.4[bc]
1.5% MM	0.16 ± 0.06	0.6 ± 0.3[c]
2% MM	0.15 ± 0.04	0.5 ± 0.4[c]
0.5% STP	0.20 ± 0.10	0.18 ± 0.08[c]

* TBA means pooled for all storage times. Means scores in a column with the same superscript letter are not different (P<0.05).

Table 7. Comparison of milk mineral (MM), STP (sodium tripolyphosphate) and BHT (butylated hydroxytoluene) on TBA number of raw and cooked ground pork stored at -20°C for 6 months.

Treatment	TBA Means* (Raw)	TBA Means* (Cooked)
Control	0.40 ± 0.26	2.25 ± 0.80[a]
BHT	0.34 ± 0.28	2.01 ± 0.60[ab]
0.5% MM	0.42 ± 0.36	1.17 ± 0.82[b]
1% MM	0.48 ± 0.47	0.68 ± 0.34[c]
1.5% MM	0.33 ± 0.29	0.57 ± 0.26[c]
2% MM	0.36 ± 0.33	0.77 ± 0.56[c]
0.5% STP	0.35 ± 0.28	0.50 ± 0.40[c]

* TBA means pooled for all storage times. Means scores in a column with the same superscript letter are not different (P<0.05).

Cooked ground pork

Refrigerated storage (2°C for 15 d): Significant difference ($P<0.05$) was seen between the different treatments (Table 6). Control (TBA No. = 5.0) and BHT (TBA No. = 4.0) were significantly different from milk mineral treatments (TBA No. < 2.0) and sodium tripolyphosphate treatment (TBA No. = 0.18). The 0.5% MM concentration was significantly different from 1.5% MM, 2% MM, and STP treatments, which were not significantly different from each other. TBA values increased markedly with storage time for cooked pork controls and samples formulated with BHT or 0.5% MM. TBA values did not increase during refrigerated storage of samples formulated with 1.5 – 2% MM (Fig. 8). Since the pooled mean TBA values of 1.5% and 2% MM samples were not different (Table 6), the 1.5% level of MM is recommended as the minimum MM level adequate for optimum inhibition of lipid oxidation in refrigerated ground pork. Therefore 0.5% STP treatment or 1.5 % MM were most effective for maintaining low TBA values (<1.0) during storage at 2°C.

Frozen storage (-20°C for 6 months): TBA number was significantly lower ($P<0.01$) for treatments with sodium tripolyphosphate or 1, 1.5, and 2% milk mineral compared to 0.5% milk mineral, BHT and control treatments. The mean TBA numbers for STP, 1, 1.5, and 2% milk mineral treatment after 6 months storage at -20°C were < 0.8, compared to > 1.1 for 0.5% MM, BHT and control treatments (Table 7). No significant effect ($P >0.05$) was seen over time. Therefore STP treatment or 1-2% MM were effective for maintaining low TBA values in cooked ground pork during frozen storage.

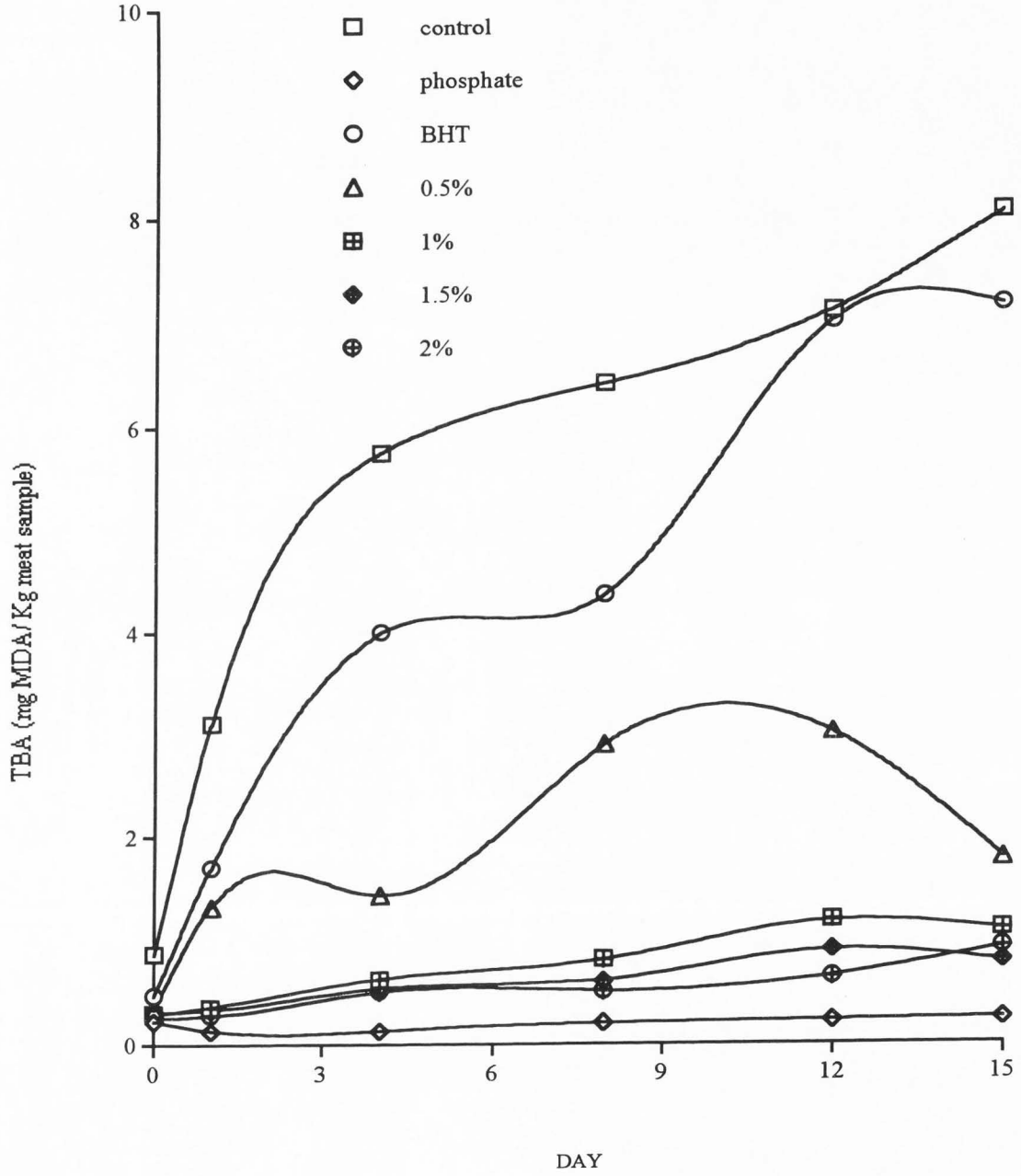

Fig.8. TBA number of cooked ground pork formulation with various levels of milk mineral (MM; 0.5 – 2%), sodium tripolyphosphate (STP; 0.5%) and butylated hydroxytoluene (BHT, 0.01% of fat) compared to control samples without antioxidants stored at 2°C for 15 d.

In contrast to results of the present study, McCarthy and others (2001) reported a significant antioxidant effect of BHT/BHA in cooked pork patties. In their study, BHT/BHA was added at a higher level of 0.01% of meat weight. In the present study, BHT was added at 0.01% of fat weight, as specified in USDA regulations for pork sausage (DeHoll, 1981), or only 0.0015% of meat weight, based on 15% fat content of the samples. Thus, McCarthy and others (2001) used 6.67-fold more BHT than used in the present study. Results of the present study indicate that the USDA- approved level of BHT in fresh pork sausage or "brown-and-serve" sausage (0.01% of fat weight) is too low to provide antioxidant activity in cooked pork crumbles. Further studies done by Vasavada and Cornforth (2003) indicated that a minimum of 0.01% BHT based on meat weight was needed to obtain antioxidant effects in cooked ground pork.

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CHAPTER 8

HOMOGENIZATION WITH A STAINLESS STEEL PROBE CAUSES IRON CONTAMINATION OF MEAT SAMPLES EXTRACTED IN ACID

Abstract

Determination of nonheme iron levels in meat is routinely determined by Ferrozine assay in hydrochloric acid - trichloroacetic acid (HCl-TCA) extracts because it is considered to be easy and accurate. This method uses an extraction solution containing 50% 6N HCl followed by homogenization using stainless steel probe (rotating rod). However, continued use of this method in our labs resulted in higher than expected nonheme iron levels in meat samples. We now report that the excess iron is due to iron contamination from the stainless steel probe used for sample blending. Less iron contamination was observed with a new stainless steel probe compared to the older and more worn probe. Mixing samples with a glass rod, rather than blending eliminated the iron contamination problem.

Introduction

Meat iron analysis is important for human nutrition research. Carpenter and Clark (1995) reported that nonheme iron levels in meat are accurately and rapidly determined by Ferrozine assay in HCl-TCA extracts. This method uses an extraction solution containing 50% 6N HCl followed by homogenization using a stainless steel probe-type mixer. However, continued use of this method in our labs resulted in higher than expected nonheme iron levels in meat samples. Thus, we investigated the possibility that iron was removed from the metal homogenizer probe during blending of the highly acidic

samples, causing the increased iron levels. The objective of this study was to determine nonheme iron levels in samples blended with a glass rod or a new stainless steel probe, compared to the old and worn blender probe.

Materials and Methods

Experimental design and statistical analysis

Nonheme iron levels of ground beef were determined in sample mixed using a glass rod, a stainless steel probe or an old stainless steel probe. Sample measurements were done in duplicate. The experiment was replicated 3 times. Treatment means were calculated by ANOVA, using Statistica (Statsoft, Inc., Tulsa, Okla., U.S.A.). Significance was defined at $p < 0.01$. Difference between means was determined by calculation of Fisher's least significant difference (LSD) values, when appropriate.

Meat samples

Ground beef samples were obtained from three separate carcasses processed at the Utah State University meat laboratory. Beef trim was finely ground through a Hobart grinder model 4152 (Hobart Mfg. Co., Troy, Ohio, U.S.A.) with a 0.32 cm plate.

Iron extraction

Duplicate two gram meat samples were accurately weighed into screw-cap culture tubes followed by addition of 15 ml 1:1 40%TCA: 6N HCl extraction solution. Then 0.1 ml 1% sodium nitrite was added. Homogenization was accomplished in approximately 15 second for each sample. All tubes were then capped and placed in a water bath for 18 h at

65° C. Samples were allowed to cool at room temperature and filtered through GF/A filter paper (Whatman, Maidtone, England).

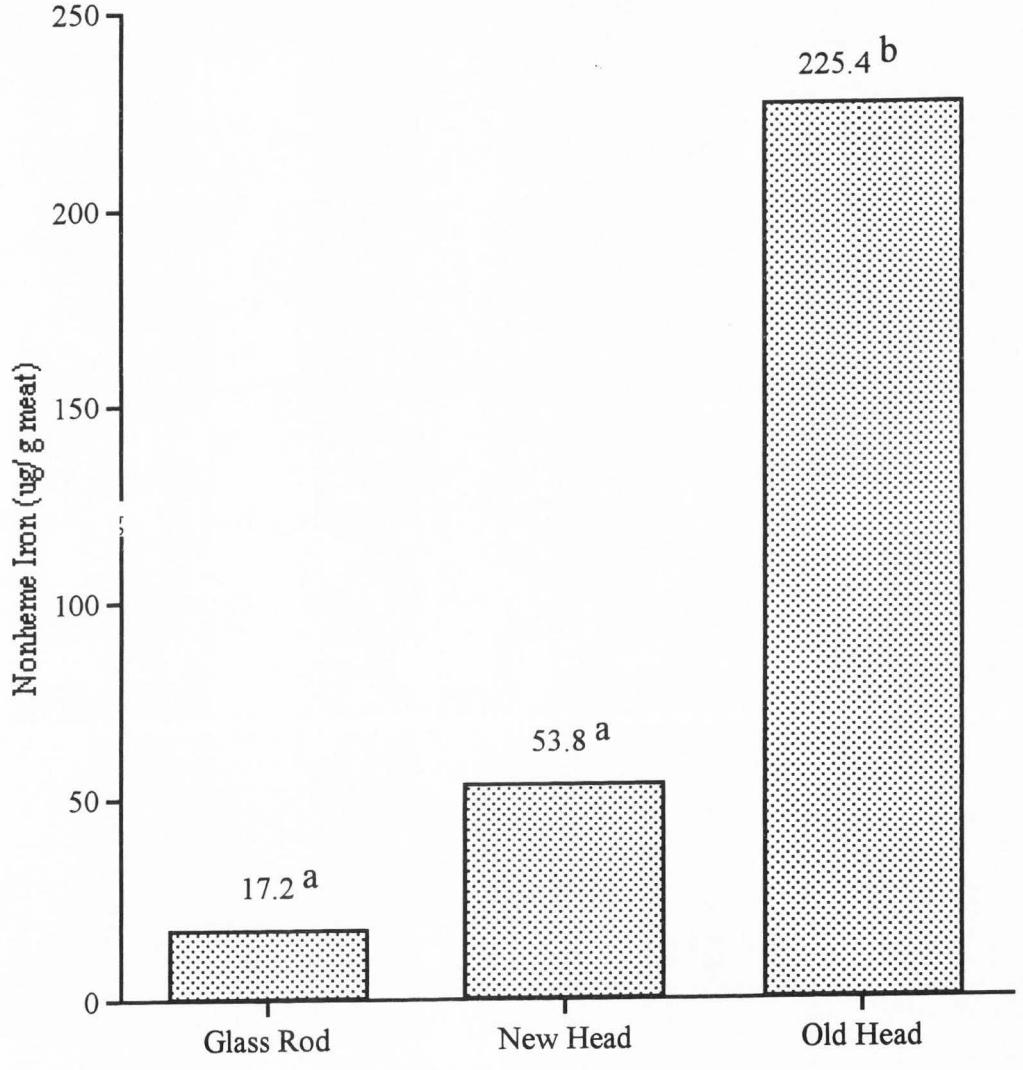

Fig. 9. Effect of blending treatment on nonheme iron content of ground beef. Means with different superscript letters are different ($P<0.01$).

Ferrozine assay for nonheme iron

A standard curve for iron concentration was made using stock solutions containing 10, 8, 6, 4, 2, and 0 micrograms iron/ mL in 0.1N HCl. A reference blank was made that contained all reagents used in both the extraction solution and the Ferrozine assay. Aliquots (0.5 mL) of reference solution or filtered samples were mixed by vortexing with 1.25 mL 0.02% acetic acid, 2.00 mL 30% ammonium acetate, and 1.25 mL of 1mM Ferrozine solution. Samples were then placed in the dark for 15 min. Absorbance was measured at 562 nm and iron content was determined from the standard curve, and adjusted for sample dilution (Micrograms nonheme iron/ g meat = Sample value from std curve X 8.55).

Results

Figure 9 shows the iron concentration observed for each treatment. Samples blended using the old stainless steel probe had significantly more nonheme iron than other samples.

Conclusion

Samples blended with the old metallic probe had high levels of nonheme iron due to iron extraction by the acidic sample solution. Less iron contamination was observed with a new stainless steel probe. Mixing samples with a glass rod eliminated the iron contamination problem.

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CHAPTER 9

COMPARISON OF TYPE 1 AND TYPE 2 ANTIOXIDANT EFFECTIVENESS IN COOKED GROUND PORK DURING REFRIGERATED STORAGE

Abstract

Antioxidant effectiveness of BHT and Rosemary extract (Type 1 antioxidants) were compared to type 2 antioxidants sodium tripolyphosphate (STP), milk mineral (MM; a natural phosphate source) and sodium nitrite in cooked ground pork during refrigerated (2°C) storage. TBA and nonheme iron values increased and heme iron levels decreased ($p<0.05$) during storage of controls and samples with type one antioxidants, but less so for samples containing phosphates or sodium nitrite. At currently recommended levels, type 2 antioxidants (STP, MM, sodium nitrite) were more effective antioxidants in cooked ground pork than the type 1 antioxidants (BHT or Rosemary oil extract).

Introduction

The rate of lipid oxidation in meat products can be effectively retarded by the use of antioxidants. Food antioxidants are classified as Type 1 or Type 2 antioxidants. Type 1 antioxidants can terminate the free-radical chain reaction of lipid oxidation by donating hydrogen or electrons to free radicals and convert them to more stable products. They may also function by addition in reactions with lipid radicals, forming lipid-antioxidant complexes. Eg: Phenolics such as BHT, BHA, TBHQ and tocopherols. Many of the naturally occurring phenolic compounds like flavonoid, eugenol, vanillin and rosemary antioxidant are classified as Type 1 antioxidants. The antioxidant role was suggested to be due to the presence of phenolic compounds (Houlihan and others 1985). Such

compounds break free radical chain reaction by hydrogen atom donation. Synthetic phenolic antioxidants such as BHT are used to improve the stability of lipid in food products. McCarthy and others (2001) reported a significant antioxidant effect of BHT/BHA in cooked pork patties when added at a level of 0.01% of meat weight. They are quite volatile and easily decompose at high temperatures. Consumers are concerned about the safety of synthetic food additives, which has led to the renewed interest in natural products (Andres and Duxbury 1990). Rosemary a natural antioxidant has been reported to contain certain components (rosemanol, rosmariquinone, rosmaridiphenol, carnosol), which may be as effective as BHT as an antioxidant (Houlihan and others 1984, 1985; Nakatani and Intani 1984). Wu and others (1982) and Houlihan and others (1985) reported that naturally occurring compounds in rosemary extracts exhibited antioxidant properties equal to or slightly less than BHT. Other researchers (Stoick and others 1991; Lai and others 1991; Liu and others 1992) have shown that rosemary oleoresin, either water-soluble or oil-soluble, have no particular advantage in restructured beef or pork steaks.

Type 2 antioxidants retard lipid oxidation by chelating metal ions, especially iron and preventing metal mediated lipid oxidation. St. Angelo and others (1988) and Liu and others (1992) reported that sodium tripolyphosphate (STP) at a level of 0.5% meat weight was very effective at inhibiting lipid oxidation and oxidative flavor changes in cooked meat during storage The antioxidant role of STP is hypothesized to be due to its sequestering of heavy metals (Watts 1950; Tim and Watts 1958), particularly iron which is the major prooxidant in meat systems (Igene and others 1979). St Angelo and others

(1990) reported that metal chelators were less effective than antioxidants that function as free radical scavengers in inhibiting or minimizing the loss of desirable meat flavor. However, results by Vara-Ubol and Bowers (2002) indicate that STP, a metal chelator, was much more effective than α-tocopherol, a free radical scavenger, in inhibiting the loss of desirable meat flavor, as well as the development of oxidative off flavors.

Liu and others (1992) reported that when STP was used in combination with rosemary oleoresin in cooked restructured pork steaks most of the antioxidant action was from STP. STP alone at 0.3% level was as effective as 0.5% level in reducing oxidative flavor changes of cooked pork during storage. Stale aroma and flavor were almost non-existent in cooked pork containing 0.3 or 0.5% STP when evaluated by a trained taste panelist even after 4 d storage at 4 degree C (Vara-Ubol and Bowers 2002).

Nitrites and nitrates function as antioxidants by converting heme proteins to inactive nitric oxide forms and by chelating the metal ions. Whey is another natural food antioxidant (Colbert and Decker 1991; Browdy and Harris 1997), due to the presence of protein sulfhydryl groups with reducing abilities, and also due to iron chelation by whey proteins (Tong and others 2000). Recently, it has been reported that dried milk mineral, the dried permeate of ultra-filtered whey, has antioxidant properties in cooked meats, apparently by iron-chelation to colloidal phosphate (Cornforth and West 2000).

The objective of this study was to compare antioxidant effectiveness of BHT and Rosemary extract (Type 1 antioxidants) with type 2 antioxidants sodium tripolyphosphate (STP), milk mineral (MM; a natural phosphate source) and sodium nitrite in cooked ground pork during storage.

Materials and Methods

Experimental design and statistical analysis

Cooked meat was stored at refrigerated temperature of 2°C for 13 d. Product stability was measured using the TBA analysis described by Buege and Aust (1978). TBA analysis, heme analysis and non heme analysis measurements were taken on d 0 and 13. All measurements were performed in duplicate. The entire experiment was replicated three times.

The data were analyzed using STATISTICA (Statsoft Inc., Tulsa, OK) software. Data were analyzed by MANOVA as a complete factorial (6) in a whole plot model. The whole plot factors (treatments) were control (no antioxidant), 0.01% butylated hydroxytoluene (percent of fat), 1.5% milk mineral, 0.5% sodium tripolyphosphate, 0.2% rosemary oil and 156 ppm sodium nitrite. Whole plot factors had n=3, reflecting independent observations from three separate batches of ground pork. Storage time in d (1 or 13) was the subplot factor. Differences between means were determined by calculation of Fisher's least significant difference (LSD) values, when appropriate.

Sample preparation

Raw ground pork (15% fat) was obtained by passing lean pork trim through a Hobart grinder model 4152 (Hobart Mfg. Co., Troy, Ohio, U.S.A.) with a 0.32 cm plate. Fat content was determined by a solvent extraction method (AOAC, 1991). Dried milk mineral (Tru Cal) was obtained from Glanbia Ingredients (Richfield, Idaho, U.S.A). The MM was commercially prepared by drying permeate obtained from ultra-filtration of whey, and consisted of ~ 24% calcium, 13.5% phosphorous, and 9% citrate.

Antioxidants were added to raw ground pork (20% fat) at recommended levels (0.01% of fat content for BHT, 0.2% of meat wt. for Rosemary oil, 0.5% of meat wt. for STP, 1.5% of meat wt. for MM, and 156 ppm sodium nitrite). The antioxidants were manually mixed with meat which was cooked at 163°C for 15 min. The cooked pork crumbles were then placed in Ziplock plastic bags (S.C. Johnson & Son, Racine, WI) and stored at 2°C for 13 d.

TBA analysis

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978), as modified by Lee, Hendricks and Cornforth (1999). Duplicate meat samples (0.5g) were mixed with 2.5 ml of stock solution containing 0.375% TBA (Sigma Chemical 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 5500 rpm for 25 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 $1.56 \times 10^5 M^{-1}cm^{-1}$ (Sinnhuber and Yu 1958). The MDA concentration was converted to TBA number (mg MDA / Kg meat sample) as follows;

1) TBA# (mg / kg) = Sample A_{532} X (1 M TBA chromogen/ 1.56 X 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

Heme iron analysis

Hemin was determined using the acidified acetone extraction of Hornsey (1956) as modified by Carpenter and Clark (1995). Samples of meat (ca. 5 g) were placed in 50 mL centrifuge tube, and 20 mL of acetone and 0.5 mL of HCl were added. Water was added so that the total water in the tube, both from the meat and from the added water, equaled 4.5 g. The mixture was macerated with a glass rod for 15s. The absorbance of the filtrate was measured at 640 nm, and heme iron in the sample calculated. Water content of the meat was determined by drying at 105 °C for 16 h (AOAC, 1991).

Nonheme iron analysis

Non heme iron analysis was determined as described by Carpenter and Clark (1995). 2 g meat sample was weighed into screw-cap tubes with 15 ml 1:1 40% TCA/6N HCl (Schicker and others 1982; Torrence and Bothwell 1968) extraction solution. Then 0.1 ml of 1% sodium nitrite was added. Samples were homogenized for 15 sec with glass rod, capped, heated 18 h at 65C, then cooled and filtered through GF/A filter paper (Whatman, London, Eng.). Soluble iron was measured by the ferrozine assay (Carter 1971). Iron stock solutions were prepared containing 10, 8, 6, 4, 2, and 0 ppm iron in 0.1N HCl. Aliquots (0.5 ml) of stock or filtered samples were vortexed with 1.25 ml

0.02% acetic acid, 2 ml 30% ammonium acetate, and 1.25 ml of 1mM ferrozine. Samples were held for 15 min, in the dark. Absorbance was measured at 562 nm. Iron content was determined from the standard curve regression equation, and adjusted for sample dilution.

Nonheme iron (ppm) / g meat = Sample value from standard curve X 8.05.

Results and Discussion

Compared to control, all treatments with antioxidants had lower TBA values at d 1 of storage (Fig. 10). However, cooked pork samples with Type 2 antioxidants (STP, MM, nitrite) had lower ($p<0.05$) TBA values than Type 1 samples (Rosemary, BHT) after 13 d storage (Fig. 10). TBA values above 1.0 are associated with rancid odor and flavor (Tarladgis and others 1960). The interaction of treatment and storage time affected ($p<0.05$) heme iron content of cooked ground pork. The heme iron content decreased ($p<0.05$) during refrigerated storage in all treatments except in samples treated with STP (Fig 11). On d 13 of storage heme iron levels were higher in all samples with antioxidants, compared to controls (Fig. 11). The non heme iron pooled mean (all treatments) on d 13 was 15 micrograms/ gram meat, which was significantly higher ($p<0.05$) than d 1 (9 micrograms/ gram meat; Fig 12). Non heme iron content increased in all treatments. Thus there were no significant treatment * storage time interaction effects.

A number of previous studies have shown the effectiveness of chelating agents as antioxidants (type 2) in meat and meat products. Igene and others (1979) reported that a meat model system containing 2% ethylene diamine-tetraacetic acid (EDTA) had lower TBA values than controls without antioxidant. STP and phytic acid are both iron

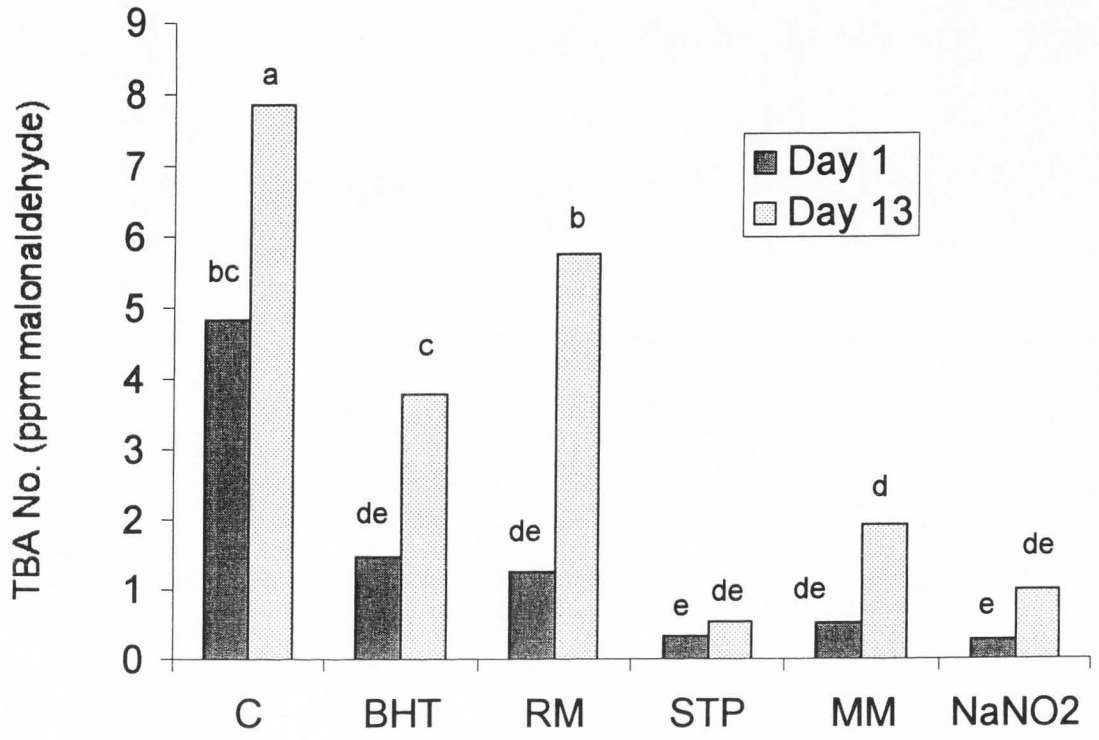

Fig. 10. Thiobarbituric acid (TBA) values during storage (2°C) of cooked ground pork formulated with various antioxidants. C = control; BHT = 0.02% butylated hydroxytoluene; RM = 0.2% rosemary oil; STP = 0.5% sodium tripolyphosphate; MM = 1.5% milk mineral; NaNO2 = 156 ppm sodium nitrite. Means with different letters are different ($p<0.05$).

chelating agents that have been shown to maintain low TBA values in cooked meat systems during storage (Lee and others 1998a, 1998b). Vara-Ubol and Bowers (2002) indicated that STP, a metal chelator, was much more effective than a-tocopherol, a free radical scavenger, in inhibiting the loss of desirable meat notes, as well as the development of oxidative off flavors. In contrast, St. Angelo and others (1990) reported that metal chelators (type 2) were less effective than antioxidants that function as free radical scavengers (type 1) in inhibiting or minimizing the loss of desirable meat flavor.

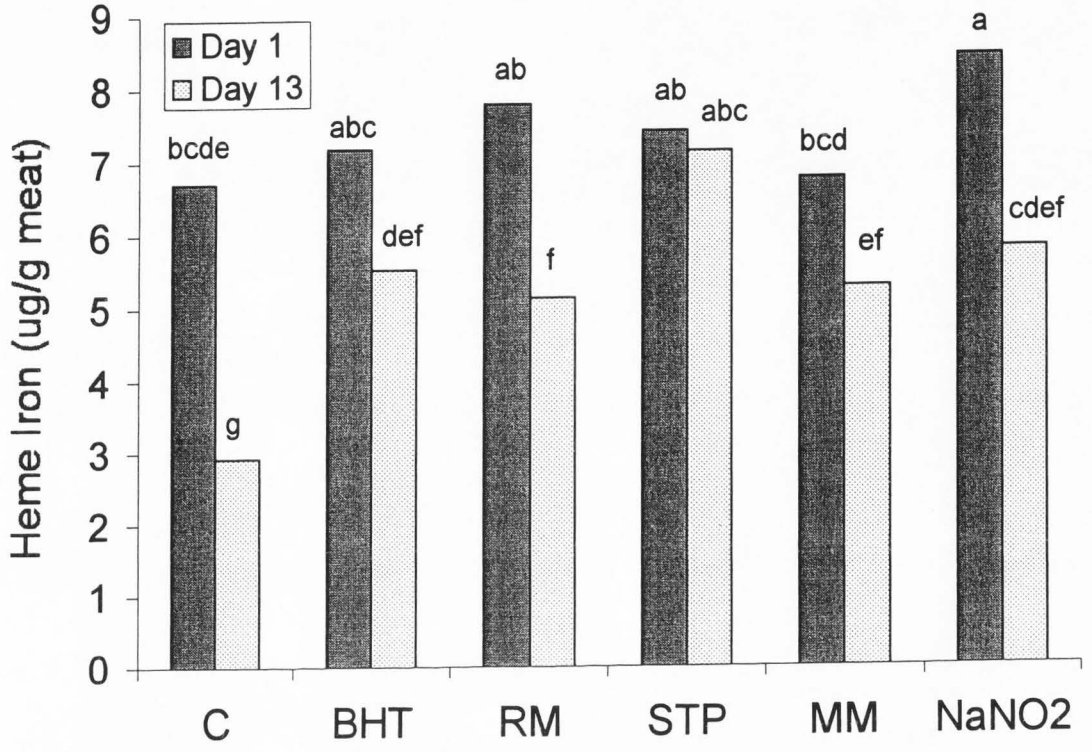

Fig. 11. Heme iron values during storage (2C) of cooked ground pork formulated with various antioxidants. C = control; BHT = 0.02% butylated hydroxytoluene; RM = 0.2% rosemary oil; STP = 0.5% sodium tripolyphosphate; MM = 1.5% milk mineral; NaNO2 = 156 ppm sodium nitrite. Means with different letters are different ($p<0.05$).

Milk mineral (MM) the dried permeate of ultra filtered whey is a newly discovered phosphate-based antioxidant. The active ingredient is colloidal calcium phosphate, which chelates soluble iron in cooked meat systems thereby inhibiting lipid oxidation (Cornforth and West, 2000; Jayasingh and Cornforth 2003). In this study MM was more effective than Type 1 antioxidants (BHT and rosemary oil) but less effective than STP and sodium nitrite at the levels used (Fig. 10).

Previous studies have indicated that nonheme iron content of meat increases after cooking and storage, with an associated increase in TBA values. Igene and others (1979) reported an increase in nonheme iron from 1.8 ppm in fresh meat pigment extract to 4.18 ppm in cooked meat pigment filtrate, and TBA values increased to 5.0 in absence of iron chelating agents. In a beef model system, the TBA value increased from 1.0 for control with no added ferrous iron to 5.4 for samples with 4.0 ppm of added ferrous iron (Love and Pearson 1974).

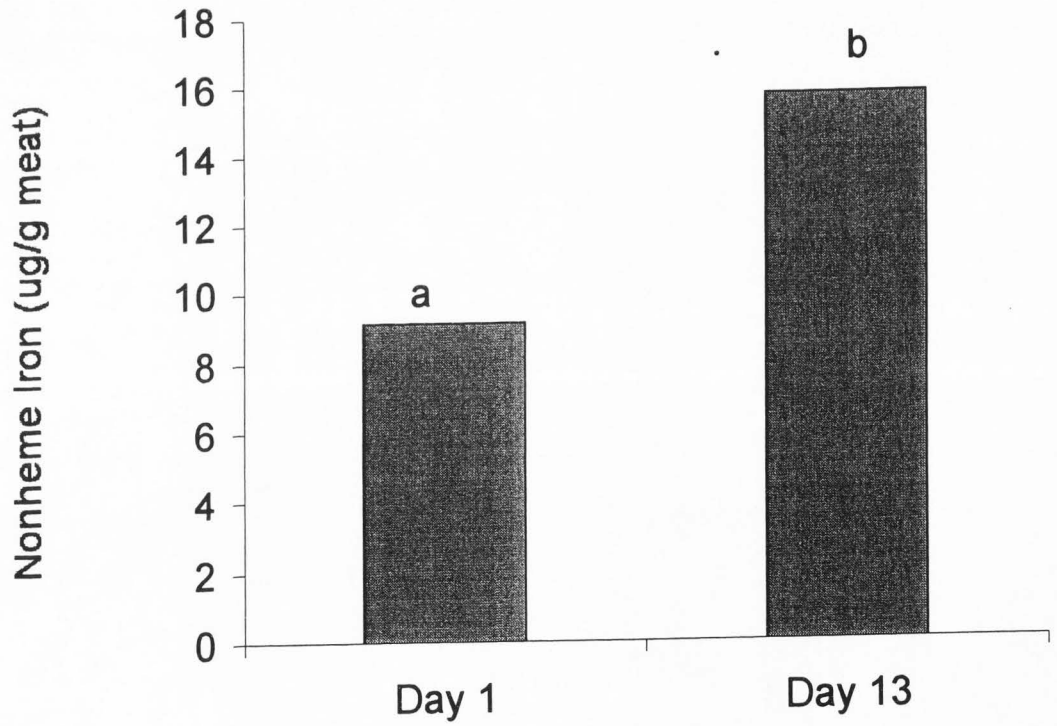

Fig 12. Means (pooled among treatments) for nonheme iron content of cooked ground pork after storage for 1 or 13 d at 2C. Means with different letters are different ($p<0.05$).

Nitrite is another metal chelator (type 2) used in cured meats. It is a very effective antioxidant for prevention of increase in TBA values after cooking and storage (Igene and others 1979; MacDonald and others 1980). Mahoney and others (1979) reported that 25 ppm of sodium nitrite was sufficient for antioxidant effects in bologna. In this study sodium nitrite was more effective than type 1 antioxidants (BHT and rosemary oil).

In our study, heme iron degradation was the source for the increase in nonheme iron after cooking and storage. The heme group was the major source of iron for subsequent iron catalyzed lipid oxidation. The heme group itself was also subject to further oxidative deterioration during storage. Previous studies have indicated that heme degradation is affected by cooking temperature and duration. Compared to fresh meat, heme iron levels decreased by 15% after boiling (30 min) and 10% after baking to 70°C (Jansuittivechalkul and others 1985). Heme iron levels decrease steadily as the cooking temperature increases. The heme iron levels decreased from 15.2 μg/g in unheated meat to 13.4 μg/g at 60°C to 10.4 μg/g at 97°C (Buchowski and others 1988). Lee and others (1998b) reported an increase in nonheme iron by 4.4 μg/g on cooking to 70°C corresponding to a decrease in heme iron by 30%, whereas in the presence of phytic acid, heme iron decreased by only 17% after cooking and remained at that level for 9 d. storage. In control without antioxidant, heme iron degradation continued during 9 d storage to 44% of fresh meat level.

In this study rosemary oil and BHT were the least effective antioxidants at the levels used. Vasavada and Cornforth (2003) also found that rosemary oil at 0.05 – 0.2% of raw meat weight was not effective for inhibition of lipid oxidation of cooked ground

pork. Ground rosemary at 0.4 – 0.8% was very effective in maintaining TBA values at 1 or less after 15 d of storage at 2 degree C. They also reported that a minimum of 0.01% BHT (based on meat weight) was needed to obtain antioxidant effects in cooked ground pork. And this study concluded that ground rosemary at 0.4-0.8% had a greater antioxidant effect than rosemary oil or BHT.

Conclusions

When used at currently recommended levels, the type 2 antioxidants (STP, MM, sodium nitrite) were more effective antioxidants in cooked ground pork than the type 1 antioxidants (BHT or Rosemary oil extract). Of the antioxidants studied, STP (type 2 antioxidant) was most effective for prevention of heme pigment degradation during storage of cooked ground pork.

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CHAPTER 10

OVERALL SUMMARY

It was feasible to develop surface redness on beef steaks by MAP in 5% CO for 24 h, and to maintain redness for 21 d in a VP. It is predicted that steak redness in VP could be extended beyond 3 weeks if the 5% CO pre-treatment time was increased to greater than 24 h. Browning was completely prevented for at least 5 weeks by these treatments. Pretreatment with 5% CO followed by VP, if approved by the USDA, would allow for central packaging and distribution of VP beef steaks and retention of desirable red color for > 21 d, which is considerably longer than the average color stability of 3-10 d for beef in PVC film. MAP of ground beef in 0.5% CO would dramatically improve color stability, but there is the possibility that after 5 weeks storage the product could be spoiled but still appear fresh. Thus, a regulation for expiration dates on the label is recommended. Recently, USDA approved distribution of fresh meats in a master bag system using 0.4% CO in an anaerobic MAP system along with 30% CO_2 and a balance as N_2.

High oxygen modified atmosphere packaging was effective in maintaining a desirable red color for 10 d refrigerated storage. However, the color of MAP samples was not significantly more red than ground meat from the chub (control) treatment. A significant development of rancid off-flavor was detected in the high oxygen MAP by the sixth d of storage, as indicated by the high TBA numbers of these samples, compared to control vacuum-packaged ground beef, which had higher more desirable flavor score and TBA values less than 1.0 after 6 or 10 d storage.

In the study on holding time after cooking, it was concluded that pork patties could be kept warm for up to 60 min after cooking without significantly increasing TBA number. TBA numbers at 120 min were significantly higher than 0 to 60 min and there were no significant differences in TBA No. of samples held for 0, 30, or 60 min after cooking.

In the paired preference sensory study, panelists preferred pork patties with TBA number < 0.5, compared to patties with TBA No. > 1.4, which is in agreement with studies done by Tarladgis and others (1960) where a TBA number >1 is usually associated with rancid flavor by sensory panelists.

In the experiment comparing antioxidant effects of milk mineral (MM) to other antioxidants, it was found that 0.5% sodium tripolyphosphate (STP) and 1, 1.5 or 2% MM were effective at maintaining low TBA numbers though 6 months storage at -20°C of cooked ground pork. For refrigerated storage (2°C) 1.5% MM was the minimum level for optimum antioxidant effect. 0.01% butylated hydroxytoluene (BHT; based on fat weight) was insufficient for antioxidant activity in cooked pork crumbles.

In the experiment done to determine nonheme iron contamination by the stainless steel probe during homogenization, it was concluded that samples blended with the old metallic probe had high levels of nonheme iron due to iron extraction by the acidic sample solution. Less iron contamination was observed with a new stainless steel probe. Mixing samples with a glass rod eliminated the iron contamination problem. Therefore, the non heme analysis in our next objective was determined by glass rod homogenization.

In the experiment comparing the effectiveness of Type 1 and 2 antioxidants, the type 2 antioxidants (STP, MM, sodium nitrite) were more effective antioxidants in cooked ground pork than the type 1 antioxidants (BHT or Rosemary oil extract) when used at currently recommended levels. STP (a Type 2 antioxidant) was especially effective for prevention of heme pigment degradation during storage of cooked ground pork.

Conclusions

Modified atmosphere packaging with CO or oxygen was effective in prolonging surface redness of meat. It was feasible to develop surface redness on beef steaks by MAP in 5% CO for 24 h, and to maintain redness for 21 d in a VP. MAP of ground beef in 0.5% CO would dramatically improve color stability, but there is the possibility that after 5 weeks storage the product could be spoiled but still appear fresh. High oxygen modified atmosphere packaging was effective in maintaining a desirable red color for 10 d refrigerated storage. However, a significant development of rancid off-flavor was detected in the high oxygen MAP by the sixth d of storage.

Lipid oxidation was faster in cooked meat as compared to raw meat, and the degree of lipid oxidation increased with oxygen exposure. Sensory panelist perceive TBA number>1 as rancid, and prefer cooked meat samples to have a TBA number <1. TBA number < 1 can be achieved by the addition of 1.5% MM or 0.5% STP to cooked pork patties. MM, STP and sodium nitrite (iron binding, Type 2 antioxidants) were more

effective than Type 1 antioxidants (BHT or rosemary oil extract) in maintaining a TBA number <1 for 15 d at 2°C or 6 months at –20 °C in cooked ground pork.

Results of my study confirm the initial hypothesis. Fresh meat color stability was prolonged substantially by alternative packaging method (carbon monoxide pre-treatment or high-oxygen, modified atmosphere packaging). In cooked meats lipid oxidation was significantly retarded by antioxidants, especially the type 2 iron chelators (sodium tripolyphosphate, sodium nitrite and milk mineral).

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APPENDICES

APPENDIX A.

DATA FOR CHAPTER 2

Table A1. Aerobic plate count (APC expressed in CFU/g of meat) of ground beef packaged in high oxygen modified atmosphere packaging (MAP; 80% oxygen and 20% carbon dioxide) compared to controls packaged in oxygen impermeable casings.

D	Rep	Control (APC)	MAP (APC)
1	1	1010	1640
1	2	1650	1720
1	3	1670	1640
2	1	1000	2000
2	2	1700	2500
2	3	1600	1300
3	1	2000	1300
3	2	3400	1700
3	3	3600	7900
4	1	22500	2500
4	2	16500	2000
4	3	20000	3000
5	1	12000	13000
5	2	8500	14000
5	3	8500	16000
6	1	41000	22000
6	2	19000	26500
6	3	13000	35500
7	1	44500	18500
7	2	41000	17000
7	3	33000	28000
8	1	93500	59000
8	2	101500	49000
8	3	82500	51500
9	1	195000	275000
9	2	150000	330000
9	3	115000	195000
10	1	510000	760000
10	2	465000	1255000
10	3	600000	610000

APPENDIX B.

DATA FOR CHAPTER 3

Table B1. TBA number of cooked patties kept warm at 71°C for various times after cooking. (TBA No. is expressed in mg of MDA/ g of meat).

Time (min)	Replicate	TBA No.
0	1	0.44
0	2	0.20
0	3	0.31
30	1	0.34
30	2	0.21
30	3	0.46
60	1	0.32
60	2	0.53
60	3	0.91
90	1	0.77
90	2	0.68
90	3	1.30
120	1	1.10
120	2	0.94
120	3	1.46

APPENDIX C.

DATA FOR CHAPTER 5

Table C1. Comparison of milk mineral (MM), STP (sodium tripolyphosphate) and BHT (butylated hydroxytoluene) effects on TBA number of raw ground pork stored at 2 °C for 8 d. (TBA No. is expressed in mg of MDA/ g of meat).

Replicate	Treatments	D	TBA No.
1	Control	0	0.13
2	Control	0	0.04
3	Control	0	0.11
1	Control	1	0.17
2	Control	1	0.14
3	Control	1	0.18
1	Control	5	0.20
2	Control	5	0.11
3	Control	5	0.19
1	Control	8	0.24
2	Control	8	0.14
3	Control	8	0.44
1	0.5% STP	0	0.08
2	0.5% STP	0	0.06
3	0.5% STP	0	0.09
1	0.5% STP	1	0.14
2	0.5% STP	1	0.13
3	0.5% STP	1	0.15
1	0.5% STP	5	0.13
2	0.5% STP	5	0.12
3	0.5% STP	5	0.31
1	0.5% STP	8	0.13
2	0.5% STP	8	0.18
3	0.5% STP	8	0.64
1	BHT	0	0.15
2	BHT	0	0.08
3	BHT	0	0.12
1	BHT	1	0.18
2	BHT	1	0.17
3	BHT	1	0.16
1	BHT	5	0.16
2	BHT	5	0.12
3	BHT	5	0.27

1	BHT	8	0.16
2	BHT	8	0.17
3	BHT	8	0.25
1	0.5% MM	0	0.09
2	0.5% MM	0	0.09
3	0.5% MM	0	0.09
1	0.5% MM	1	0.11
2	0.5% MM	1	0.17
3	0.5% MM	1	0.10
1	0.5% MM	5	0.14
2	0.5% MM	5	0.16
3	0.5% MM	5	0.14
1	0.5% MM	8	0.14
2	0.5% MM	8	0.16
3	0.5% MM	8	0.20
1	1% MM	0	0.12
2	1% MM	0	0.13
3	1% MM	0	0.14
1	1% MM	1	0.12
2	1% MM	1	0.18
3	1% MM	1	0.13
1	1% MM	5	0.24
2	1% MM	5	0.22
3	1% MM	5	0.15
1	1% MM	8	0.14
2	1% MM	8	0.24
3	1% MM	8	0.18
1	1.5% MM	0	0.05
2	1.5% MM	0	0.13
3	1.5% MM	0	0.14
1	1.5% MM	1	0.09
2	1.5% MM	1	0.17
3	1.5% MM	1	0.14
1	1.5% MM	5	0.13
2	1.5% MM	5	0.20
3	1.5% MM	5	0.26
1	1.5% MM	8	0.16
2	1.5% MM	8	0.23
3	1.5% MM	8	0.23

1	2% MM	0	0.07
2	2% MM	0	0.13
3	2% MM	0	0.13
1	2% MM	1	0.09
2	2% MM	1	0.18
3	2% MM	1	0.15
1	2% MM	5	0.17
2	2% MM	5	0.15
3	2% MM	5	0.16
1	2% MM	8	0.18
2	2% MM	8	0.18
3	2% MM	8	0.24

Table C2. Comparison of milk mineral (MM), STP (sodium tripolyphosphate) and BHT (butylated hydroxytoluene) effects on TBA number of cooked ground pork stored at 2 °C for 15 d. (TBA No. is expressed in mg of MDA/ g of meat).

Replicate	Treatment	D	TBA No.
1	Control	0	0.56
2	Control	0	1.33
3	Control	0	0.68
1	Control	1	2.75
2	Control	1	3.53
3	Control	1	3.05
1	Control	4	8.62
2	Control	4	4.20
3	Control	4	4.43
1	Control	8	7.32
2	Control	8	6.38
3	Control	8	5.54
1	Control	12	5.44
2	Control	12	7.36
3	Control	12	8.53
1	Control	15	4.18
2	Control	15	9.99
3	Control	15	9.97
1	0.5% STP	0	0.31
2	0.5% STP	0	0.30
3	0.5% STP	0	0.07
1	0.5% STP	1	0.13
2	0.5% STP	1	0.11
3	0.5% STP	1	0.15
1	0.5% STP	4	0.08
2	0.5% STP	4	0.17
3	0.5% STP	4	0.12
1	0.5% STP	8	0.16
2	0.5% STP	8	0.26
3	0.5% STP	8	0.18
1	0.5% STP	12	0.22
2	0.5% STP	12	0.28
3	0.5% STP	12	0.19

1	0.5% STP	15	0.39
2	0.5% STP	15	0.13
3	0.5% STP	15	0.17
1	BHT	0	0.40
2	BHT	0	0.57
3	BHT	0	0.41
1	BHT	1	1.50
2	BHT	1	1.77
3	BHT	1	1.84
1	BHT	4	3.93
2	BHT	4	2.97
3	BHT	4	5.07
1	BHT	8	5.78
2	BHT	8	1.93
3	BHT	8	5.37
1	BHT	12	7.19
2	BHT	12	5.68
3	BHT	12	8.14
1	BHT	15	4.60
2	BHT	15	8.48
3	BHT	15	8.39
1	0.5% MM	0	0.48
2	0.5% MM	0	0.33
3	0.5% MM	0	0.16
1	0.5% MM	1	0.61
2	0.5% MM	1	2.97
3	0.5% MM	1	0.39
1	0.5% MM	4	2.46
2	0.5% MM	4	0.68
3	0.5% MM	4	1.14
1	0.5% MM	8	6.13
2	0.5% MM	8	1.17
3	0.5% MM	8	1.40
1	0.5% MM	12	5.85
2	0.5% MM	12	1.33
3	0.5% MM	12	1.86
1	0.5% MM	15	2.01
2	0.5% MM	15	1.14
3	0.5% MM	15	2.19

1	1% MM	0	0.43
2	1% MM	0	0.26
3	1% MM	0	0.17
1	1% MM	1	0.22
2	1% MM	1	0.57
3	1% MM	1	0.29
1	1% MM	4	0.46
2	1% MM	4	0.83
3	1% MM	4	0.59
1	1% MM	8	0.56
2	1% MM	8	0.95
3	1% MM	8	0.91
1	1% MM	12	1.30
2	1% MM	12	0.66
3	1% MM	12	1.60
1	1% MM	15	0.55
2	1% MM	15	1.25
3	1% MM	15	1.46
1	1.5% MM	0	0.43
2	1.5% MM	0	0.24
3	1.5% MM	0	0.24
1	1.5% MM	1	0.23
2	1.5% MM	1	0.49
3	1.5% MM	1	0.26
1	1.5% MM	4	0.51
2	1.5% MM	4	0.55
3	1.5% MM	4	0.56
1	1.5% MM	8	0.59
2	1.5% MM	8	0.57
3	1.5% MM	8	0.66
1	1.5% MM	12	1.26
2	1.5% MM	12	0.67
3	1.5% MM	12	0.76
1	1.5% MM	15	0.46
2	1.5% MM	15	0.77
3	1.5% MM	15	1.11
1	2% MM	0	0.46
2	2% MM	0	0.16
3	2% MM	0	0.15

1	2% MM	1	0.18
2	2% MM	1	0.36
3	2% MM	1	0.27
1	2% MM	4	0.44
2	2% MM	4	0.61
3	2% MM	4	0.46
1	2% MM	8	0.31
2	2% MM	8	0.76
3	2% MM	8	0.45
1	2% MM	12	0.57
2	2% MM	12	0.85
3	2% MM	12	0.51
1	2% MM	15	0.31
2	2% MM	15	0.73
3	2% MM	15	1.71

Table C3. Comparison of milk mineral (MM), STP (sodium tripolyphosphate) and BHT (butylated hydroxytoluene) on TBA number of raw ground pork stored at -20°C for 6 months. (TBA No. is expressed in mg of MDA/ g of meat).

Month	Replicate	Treatment	TBA No.
0	1	Control	0.31
0	1	0.5% STP	0.19
0	1	BHT	0.31
0	1	0.5% MM	0.77
0	1	1% MM	0.74
0	1	1.5% MM	0.70
0	1	2% MM	0.58
0	2	Control	0.16
0	2	0.5% STP	0.27
0	2	BHT	0.27
0	2	0.5% MM	0.23
0	2	1% MM	0.26
0	2	1.5% MM	0.29
0	2	2% MM	0.31
0	3	Control	0.36
0	3	0.5% STP	0.22
0	3	BHT	0.23
0	3	0.5% MM	0.43
0	3	1% MM	0.32
0	3	1.5% MM	0.29
0	3	2% MM	0.35
1	1	Control	1.12
1	1	0.5% STP	1.47
1	1	BHT	1.43
1	1	0.5% MM	1.49
1	1	1% MM	2.24
1	1	1.5% MM	1.39
1	1	2% MM	1.12
1	2	Control	0.44
1	2	0.5% STP	0.41
1	2	BHT	0.40
1	2	0.5% MM	0.24
1	2	1% MM	0.55
1	2	1.5% MM	0.21

1	2	2% MM	0.19
1	3	Control	0.43
1	3	0.5% STP	0.31
1	3	BHT	0.46
1	3	0.5% MM	0.51
1	3	1% MM	0.60
1	3	1.5% MM	0.36
1	3	2% MM	0.45
2	1	Control	0.69
2	1	0.5% STP	0.58
2	1	BHT	0.63
2	1	0.5% MM	0.52
2	1	1% MM	0.57
2	1	1.5% MM	0.61
2	1	2% MM	1.34
2	2	Control	0.45
2	2	0.5% STP	0.30
2	2	BHT	0.27
2	2	0.5% MM	0.28
2	2	1% MM	0.34
2	2	1.5% MM	0.25
2	2	2% MM	0.46
2	3	Control	0.25
2	3	0.5% STP	0.27
2	3	BHT	0.25
2	3	0.5% MM	0.36
2	3	1% MM	0.36
2	3	1.5% MM	0.19
2	3	2% MM	0.18
3	1	Control	0.32
3	1	0.5% STP	0.13
3	1	BHT	0.24
3	1	0.5% MM	0.09
3	1	1% MM	0.21
3	1	1.5% MM	0.44
3	1	2% MM	0.57
3	2	Control	0.06
3	2	0.5% STP	0.08
3	2	BHT	0.05

3	2	0.5% MM	0.04
3	2	1% MM	0.08
3	2	1.5% MM	0.03
3	2	2% MM	0.09
3	3	Control	0.36
3	3	0.5% STP	0.29
3	3	BHT	0.23
3	3	0.5% MM	0.47
3	3	1% MM	0.31
3	3	1.5% MM	0.33
3	3	2% MM	0.23
4	1	Control	0.17
4	1	0.5% STP	0.27
4	1	BHT	0.11
4	1	0.5% MM	0.17
4	1	1% MM	0.12
4	1	1.5% MM	0.12
4	1	2% MM	0.13
4	2	Control	0.25
4	2	0.5% STP	0.20
4	2	BHT	0.19
4	2	0.5% MM	0.12
4	2	1% MM	0.10
4	2	1.5% MM	0.12
4	2	2% MM	0.16
4	3	Control	0.67
4	3	0.5% STP	0.24
4	3	BHT	0.22
4	3	0.5% MM	0.64
4	3	1% MM	0.36
4	3	1.5% MM	0.14
4	3	2% MM	0.18
5	1	Control	0.25
5	1	0.5% STP	0.30
5	1	BHT	0.20
5	1	0.5% MM	0.17
5	1	1% MM	0.22
5	1	1.5% MM	0.21
5	1	2% MM	0.15

5	2	Control	0.23
5	2	0.5% STP	0.36
5	2	BHT	0.29
5	2	0.5% MM	0.26
5	2	1% MM	0.27
5	2	1.5% MM	0.21
5	2	2% MM	0.22
5	3	Control	0.85
5	3	0.5% STP	0.24
5	3	BHT	0.36
5	3	0.5% MM	0.42
5	3	1% MM	0.52
5	3	1.5% MM	0.17
5	3	2% MM	0.14
6	1	Control	0.26
6	1	0.5% STP	0.54
6	1	BHT	0.28
6	1	0.5% MM	0.23
6	1	1% MM	0.46
6	1	1.5% MM	0.21
6	1	2% MM	0.13
6	2	Control	0.19
6	2	0.5% STP	0.28
6	2	BHT	0.20
6	2	0.5% MM	0.22
6	2	1% MM	0.29
6	2	1.5% MM	0.24
6	2	2% MM	0.23
6	3	Control	0.66
6	3	0.5% STP	0.33
6	3	BHT	0.47
6	3	0.5% MM	1.18
6	3	1% MM	1.10
6	3	1.5% MM	0.48
6	3	2% MM	0.41

Table C4. Comparison of milk mineral (MM), STP (sodium tripolyphosphate) and BHT (butylated hydroxytoluene) on TBA number of cooked ground pork stored at -20°C for 6 months. (TBA No. is expressed in mg of MDA/ g of meat).

Month	Replicate	Treatment	TBA No.
0	1	Control	2.39
0	1	0.5% STP	0.71
0	1	BHT	2.08
0	1	0.5% MM	1.43
0	1	1% MM	1.01
0	1	1.5% MM	0.30
0	1	2% MM	0.60
0	2	Control	1.62
0	2	0.5% STP	0.25
0	2	BHT	1.15
0	2	0.5% MM	1.09
0	2	1% MM	0.39
0	2	1.5% MM	0.44
0	2	2% MM	0.49
0	3	Control	3.44
0	3	0.5% STP	0.55
0	3	BHT	3.47
0	3	0.5% MM	1.37
0	3	1% MM	0.82
0	3	1.5% MM	0.78
0	3	2% MM	0.62
1	1	Control	3.07
1	1	0.5% STP	1.37
1	1	BHT	3.02
1	1	0.5% MM	1.48
1	1	1% MM	1.56
1	1	1.5% MM	1.21
1	1	2% MM	2.39
1	2	Control	2.70
1	2	0.5% STP	0.10
1	2	BHT	2.17
1	2	0.5% MM	0.87
1	2	1% MM	0.53

1	2	1.5% MM	0.48
1	2	2% MM	0.40
1	3	Control	1.53
1	3	0.5% STP	0.40
1	3	BHT	1.72
1	3	0.5% MM	0.66
1	3	1% MM	0.52
1	3	1.5% MM	0.43
1	3	2% MM	0.40
2	1	Control	2.68
2	1	0.5% STP	1.79
2	1	BHT	3.11
2	1	0.5% MM	3.88
2	1	1% MM	1.27
2	1	1.5% MM	0.91
2	1	2% MM	1.31
2	2	Control	3.47
2	2	0.5% STP	0.60
2	2	BHT	1.84
2	2	0.5% MM	0.88
2	2	1% MM	0.64
2	2	1.5% MM	0.51
2	2	2% MM	0.74
2	3	Control	1.71
2	3	0.5% STP	0.41
2	3	BHT	1.61
2	3	0.5% MM	0.64
2	3	1% MM	0.59
2	3	1.5% MM	0.37
2	3	2% MM	0.38
3	1	Control	0.98
3	1	0.5% STP	0.25
3	1	BHT	1.72
3	1	0.5% MM	1.95
3	1	1% MM	0.26
3	1	1.5% MM	0.83
3	1	2% MM	1.66
3	2	Control	2.38
3	2	0.5% STP	0.25

3	2	BHT	1.75
3	2	0.5% MM	0.40
3	2	1% MM	0.53
3	2	1.5% MM	0.26
3	2	2% MM	0.36
3	3	Control	1.95
3	3	0.5% STP	0.26
3	3	BHT	2.07
3	3	0.5% MM	0.58
3	3	1% MM	0.60
3	3	1.5% MM	0.77
3	3	2% MM	0.53
4	1	Control	1.14
4	1	0.5% STP	0.26
4	1	BHT	2.35
4	1	0.5% MM	0.14
4	1	1% MM	0.17
4	1	1.5% MM	0.19
4	1	2% MM	0.24
4	2	Control	2.53
4	2	0.5% STP	0.20
4	2	BHT	2.43
4	2	0.5% MM	2.28
4	2	1% MM	0.68
4	2	1.5% MM	0.33
4	2	2% MM	0.25
4	3	Control	2.98
4	3	0.5% STP	0.40
4	3	BHT	1.67
4	3	0.5% MM	0.99
4	3	1% MM	0.73
4	3	1.5% MM	0.68
4	3	2% MM	1.09
5	1	Control	1.23
5	1	0.5% STP	0.36
5	1	BHT	1.53
5	1	0.5% MM	0.80
5	1	1% MM	0.79
5	1	1.5% MM	0.49

5	1	2% MM	0.49
5	2	Control	2.56
5	2	0.5% STP	0.74
5	2	BHT	1.67
5	2	0.5% MM	1.10
5	2	1% MM	0.70
5	2	1.5% MM	0.51
5	2	2% MM	0.61
5	3	Control	2.93
5	3	0.5% STP	0.24
5	3	BHT	2.41
5	3	0.5% MM	1.50
5	3	1% MM	1.17
5	3	1.5% MM	0.95
5	3	2% MM	1.65
6	1	Control	1.42
6	1	0.5% STP	0.63
6	1	BHT	1.64
6	1	0.5% MM	0.60
6	1	1% MM	0.48
6	1	1.5% MM	0.39
6	1	2% MM	0.59
6	2	Control	3.20
6	2	0.5% STP	0.35
6	2	BHT	1.47
6	2	0.5% MM	1.43
6	2	1% MM	0.51
6	2	1.5% MM	0.62
6	2	2% MM	0.82
6	3	Control	1.30
6	3	0.5% STP	0.42
6	3	BHT	1.37
6	3	0.5% MM	0.43
6	3	1% MM	0.44
6	3	1.5% MM	0.40
6	3	2% MM	0.43

APPENDIX D.

DATA FOR CHAPTER 6

Table D1. Effect of blending treatment on nonheme iron content (micrograms/ gram of meat) of ground beef.

Treatment	Replicate	Nonheme Iron
Glass Rod	1	20.619
Glass Rod	2	20.443
Glass Rod	3	10.485
New Head	1	44.369
New Head	2	48.221
New Head	3	68.717
Old Head	1	118.82
Old Head	2	244.273
Old Head	3	313.007

APPENDIX E.

DATA FOR CHAPTER 7

Table E1. Thiobarbituric acid (TBA) values during storage (2°C) of cooked ground pork formulated with various antioxidants. Control; BHT = 0.01% butylated hydroxytoluene based on fat; RM = 0.2% rosemary oil; STP = 0.5% sodium tripolyphosphate; MM = 1.5% milk mineral; NaNO2 = 156 ppm sodium nitrite.

Treatment	Replicate	D	TBA No.
Control	1	1	6.01
BHT	1	1	1.30
STP	1	1	0.41
1.5% MM	1	1	0.67
RSMRY	1	1	1.06
NANO2	1	1	0.40
Control	1	13	8.34
BHT	1	13	3.61
STP	1	13	0.79
1.5% MM	1	13	3.48
RSMRY	1	13	7.05
NANO2	1	13	0.56
Control	2	1	4.88
BHT	2	1	1.01
STP	2	1	0.44
1.5% MM	2	1	0.75
RSMRY	2	1	1.89
NANO2	2	1	0.32
Control	2	13	8.22
BHT	2	13	2.75
STP	2	13	0.20
1.5% MM	2	13	1.69
RSMRY	2	13	6.38
NANO2	2	13	0.19
Control	3	1	3.65
BHT	3	1	2.10
STP	3	1	0.17
1.5% MM	3	1	0.17
RSMRY	3	1	0.88
NANO2	3	1	0.11
Control	3	13	7.00
BHT	3	13	4.99
STP	3	13	0.64

1.5% MM	3	13	0.61
RSMRY	3	13	3.88
NANO2	3	13	2.24

Table E2. Heme iron values expressed in micrograms / gram of meat, during storage (2°C) of cooked ground pork formulated with various antioxidants. Control; BHT = 0.01% butylated hydroxytoluene based on fat; RM = 0.2% rosemary oil; STP = 0.5% sodium tripolyphosphate; MM = 1.5% milk mineral; NaNO2 = 156 ppm sodium nitrite.

Treatment	Replicate	D	Heme Iron
Control	1	1	6.89
BHT	1	1	7.29
STP	1	1	7.05
1.5% MM	1	1	7.39
RSMRY	1	1	8.45
NANO2	1	1	8.82
Control	1	13	3.53
BHT	1	13	5.30
STP	1	13	6.04
1.5% MM	1	13	5.82
RSMRY	1	13	7.12
NANO2	1	13	6.82
Control	2	1	6.45
BHT	2	1	7.34
STP	2	1	8.03
1.5% MM	2	1	6.69
RSMRY	2	1	8.00
NANO2	2	1	8.36
Control	2	13	3.18
BHT	2	13	5.58
STP	2	13	7.49
1.5% MM	2	13	5.78
RSMRY	2	13	4.02
NANO2	2	13	6.48
Control	3	1	6.83
BHT	3	1	6.94
STP	3	1	7.23
1.5% MM	3	1	6.21
RSMRY	3	1	6.91
NANO2	3	1	8.06
Control	3	13	2.12
BHT	3	13	5.73

STP	3	13	7.93
1.5% MM	3	13	4.27
RSMRY	3	13	4.30
NANO2	3	13	4.05

APPENDIX F.

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14 June 2004

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Via email preejaya@cc.usu.edu

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Journal Name:	Meat Science
Journal Article:	Evaluation of carbon monoxide (CO) treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. 2001. Meat Science, 59:317-324.
Authors:	Jayasingh, P., Cornforth, D. P., Carpenter, C. E. and Whittier, D.

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Journal Address: Institute of Food Technologists,
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Authors:	P. Jayasingh, D.P. Cornforth, C.P. Brennand, C.E. Carpenter, and D.R. Whittier

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Authors:	P. Jayasingh, D.P. Cornforth, C.P. Brennand, C.E. Carpenter, and D.R. Whittier

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11 June 2004

Our ref: HW/smc/June 2004.jl262

Dr Preetha Jayasingh

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MEAT SCIENCE, Vol 66, No 1, 2003, Pages 83-89, Jayasingh and Cornforth, "Comparison of antioxidant effects on ..."

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PUBLICATIONS

1. Jayasingh, P., Cornforth, D. P., Carpenter, C. E. and Whittier, D. 2001. Evaluation of carbon monoxide (CO) treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. Meat Science, 59:317-324.
2. Jayasingh, P., Cornforth, D. P., Brennand, C. P., Carpenter, C. E. and Whittier, D. R. 2002. Sensory evaluation of ground beef stored in high-oxygen modified atmosphere packaging. J. Food Sci. 67:3493-3496.

3. Jayasingh, P. and Cornforth, D. P. 2003. Comparison of antioxidant effects of milk mineral, butylated hydroxytoluene and sodium tripolyphosphate in raw and cooked ground pork. Meat Sci. 66:83-89.
4. Cornforth, D. P and Jayasingh, P. 2004. Chemical and physical characteristics of meat color pigments, in Encyclopedia of Meat Sciences, Elsevier Science Ltd., London, England, In Press.
5. Jayasingh, P. and Cornforth, D. P. 2004. Comparison of antioxidant effects of milk mineral, butylated hydroxy toluene and sodium tripolyphosphate in raw and cooked ground pork. FeedInfo News Service (WorldWide Data Systems Inc.), http://www.feedinfo.com/Console/PageViewer.aspx?page=53970.

SCIENTIFIC PRESENTATIONS

1. Jayasingh, P., Cornforth, D. P., Carpenter, C. E. and Whittier, D. 2000. Evaluation of carbon monoxide (CO) treatment in modified atmosphere (MAP) or vacuum packaging (VP) to increase color stability of fresh beef. Recip. Meat Conf. Proc. 53:132.
2. Jayasingh, P. and Cornforth, D. P. 2001. Antioxidant effect of dried milk mineral in fresh and cooked ground pork. Recip. Meat Conf. Proc. 54:198.
3. Jayasingh, P., Cornforth, D. P., Brennand, C. P., Carpenter, C. E., and Whittier, D. R. 2002. Sensory evaluation of ground beef stored in high-oxygen modified atmosphere packaging. IFT Annual. Meeting Technical Prog. Abstract 46G-17, page 111-112.
4. Jayasingh, P. and Cornforth, D. P. 2002. Comparison of the antioxidant effects of milk mineral, butylated hydroxytoluene, and sodium tripolyphosphate in raw and cooked ground pork during frozen storage. Proc. 56th Recip. Meat Conf. www.meatscience.org/Pubs/rmcarchv/2002/posters.html.
5. Jayasingh, P., Carpenter, C. E. and Cornforth, D. P. 2003. Comparison of Type 1 and Type 2 antioxidant effectiveness in cooked ground pork during refrigerated storage. IFT Ann. Meeting Technical Prog. Abstract 76F-26, page 203.

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