5-2006

Use of Natural Antioxidants to Control Oxidative Rancidity in Cooked Meats

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USE OF NATURAL ANTIOXIDANTS TO CONTROL OXIDATIVE RANCIDITY IN COOKED MEATS

by

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

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

UTAH STATE UNIVERSITY
Logan, Utah

2006

ABSTRACT

Use of Natural Antioxidants to Control Oxidative Rancidity in Cooked Meats

by

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Utah State University, 2006

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The research in this dissertation focused on determining antioxidant effects of various natural antioxidants in cooked meat systems. Milk mineral (MM), spices, and raisin paste were used in cooked meat systems to verify their potential antioxidant properties.

The MM study determined the antioxidant activity of 1.5% MM added to uncured cooked beef meatballs, and possible additive effects of MM in combination with 20-ppm or 40-ppm sodium nitrate in cooked beef sausages. There was no additive inhibition of lipid oxidation in samples containing 20-ppm or 40-ppm sodium nitrite plus 1.5% MM. Cooked meat yield was not different between control meatballs and those containing MM. As expected, treatments containing nitrite had higher redness (a^*) values than samples without nitrite. The MM at 1.5% was a very effective antioxidant as compared to controls.

The Garam Masala (GM) study determined the antioxidant effects and sensory attributes of the individual spices in an Indian spice blend GM in cooked ground beef,

and possible additive antioxidant effects between Type I and Type II antioxidants. All spices had antioxidant effects on cooked ground beef, compared to controls without spices, with cloves being the most effective. All spices at their lowest effective recommended level effectively lowered the perception of rancid odor and rancid flavor in cooked ground beef as compared to control samples. As expected, most spices also imparted distinctive flavors to the cooked ground beef. Type II antioxidants (iron binding phosphate compounds) were more effective than individual Type I antioxidants (spices and butylated hydroxytoluene; BHT) in cooked ground beef. There was a positive additive antioxidant effect seen with rosemary + MM and rosemary + sodium tripolyphosphate (STPP) treatments as compared to individual rosemary treatment. There was no additive antioxidant effect observed for other combinations of spices with phosphate antioxidants.

The raisin study was done to determine the antioxidant activity of raisin paste added to cooked ground beef, pork, and chicken. Thiobarbituric acid (TBA) values were measured using the distillation method, on the distillates, to avoid interference from sugar in the raisins. Beef, pork, and chicken flavor intensity, rancid flavor intensity, and raisin flavor intensity were evaluated by a trained sensory panel ($n = 6$). Addition of 2% raisin paste effectively inhibited rancid flavor development for 14 days after cooking in cooked ground beef, pork, and chicken. Sugar added at levels equivalent to that contributed by the raisins inhibited rancidity, probably due to antioxidant effects of Maillard browning products, suggesting that the antioxidant effect of raisins was due to their sugar content.

(216 pages)

ACKNOWLEDGMENTS

I wish to extend sincere and heartfelt thanks and gratitude to my major professor and mentor, Dr. Daren P. Cornforth. Without his continuous encouragement, guidance, and assistance, completion of this degree would not have been possible. I would also like to thank my committee members, Dr. Deloy Hendricks, Dr. Donald McMahon, Dr. Marie Walsh, and Dr. Steven Aust, for all their help and technical guidance during this work.

I wish to thank my labmates and friends Karin Allen, Saumya Dwivedi, Avanthi Vissa, Preetha Jayasingh, and Liza John, Jeff Wu, and Paul Joseph for all their help and support during my stay in Logan. I would also like to thank all my other friends in Logan for their support and love over these years. I gratefully acknowledge financial support received from the Agricultural Experiment Station at Utah State University, Dairy Management Incorporated, Glanbia Foods, and the California Raisin Board during the course of this project.

I wish to thank my brother Amit for his love and support through the years. Finally I would like to thank my parents, Mr. Narendra Vasavada and Mrs. Kalpana Vasavada, for teaching me the importance of knowledge and for their encouragement and support throughout my career.

Mihir Vasavada

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LIST OF SYMBOLS, NOTATIONS, AND DEFINITIONS

Abbreviation Key

ANOVA	Analysis of variance
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
C	Celsius
CIE	Commission Internationale De L'Eclairage
Ctrl	Control
CFU	Colony forming units
d	Day
DPPH	1,1-diphenyl-2-picrylhydrazyl
GAE	Gallic acid equivalents
GM	Garam Masala
LSD	Least significant difference
MM	Milk mineral
MDA	Malonaldehyde
MRP	Maillard reaction products
$NaNO_2$	Sodium nitrite
Nit	Nitrite
ORAC	Oxygen radical absorbance capacity
PG	Propyl gallate
PUFA	Polyunsaturated fatty acids

RBC	Rancid beef control
RM	Rosemary
STPP	Sodium tripolyphosphate
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tertiary butylated hydroxyquinone
TE	Trolox equivalents
USDA	United States Department of Agriculture
WOF	Warmed-over flavor

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Lipid oxidation is one of the major processes occurring during food deterioration. It is of great economic concern to the food industry because it leads to the development of various off-flavors and off-odors in oils and fat-containing foods. The development of rancid off-flavors renders these foods less acceptable and decreases their nutritional quality.

Spices have been used for many years for various applications in the food industry. It is assumed that the spices mask, rather than prevent rancid off-flavor. In the U.S.A., butylated hyrdroxyanisole (BHA), BHT, STPP, and nitrite are the main additives used to control lipid oxidation in cooked meats. Nitrite is the main "cure" ingredient for cured cooked meats such as ham, bacon and cured sausges. For uncured cooked meats BHA, BHT, or STPP are the main antioxidants.

Hypothesis

My hypothesis is that there are a number of alternative antioxidants (MM, individual spices of GM and raisin paste) that have equal or greater antioxidant effects compared to BHT or STPP in cooked ground meats. I also hypothesize that the Type II iron chelating antioxidants (MM, STPP, nitrites) will have greater antioxidant effects in an iron-rich system (cooked meats) than oxygen-radical scavenging Type I antioxidants, such as BHT or rosemary extract.

Preventing spoilage will always remain of great interest to the meat industry. The main problem concerned with the chemical deterioration in meats is the oxidative

spoilage resulting due to the reaction of oxygen and lipids, and formation of free radicals. Oxidative deterioration of meats occurs rapidly after cooking meats. Oxidation of unsaturated fatty acids in cooked meats during storage and reheating results in stale or rancid flavors known as warmed-over flavor (WOF) (Sato and Hegarty 1971). The development of WOF is an undesirable sensory characteristic reminiscent of the smell of paint or wet cardboard (St. Angelo and others 1988).

Lipid oxidation occurs to a great extent in ground beef stored in a high oxygen atmosphere (Jayasingh and others 2002). Lipid oxidation in meats prior to cooking affects the flavor and color of meat products (McMillin 1996). After cooking, lipid oxidation mainly involves the greater availability of oxidation promoters, due to the release of non-heme iron, and of phospholipids from disrupted cell membranes (Younathan 1985).

The increased demand for convenience foods and the evolving markets for precooked meats call for more options to prevent lipid oxidation in meat products after cooking. The WOF problem of cooked meat has assumed much greater significance in recent years due to a 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, which can cause it to have off-flavors.

Antioxidants are substances that can delay onset, or slow the rate of oxidation. There are two kinds of antioxidants, natural and synthesized. The main lipid-soluble antioxidants currently used in foods are monohydric or polyhydric phenols with various aromatic substitutions. The choice of an antioxidant in a food system depends on factors

such as potency of an antioxidant in a particular application, ease of incorporation in the food, carry-through characteristics, sensitivity to pH, tendency to discolor or produce off flavors, availability, and cost (Nawar 1996). For maximum efficiency, primary antioxidants such as BHA, BHT, propyl gallate (PG), and tertiary butylhydroquinone (TBHQ) are often used in combination with other phenolic antioxidants or with various metal sequestering agents.

Antioxidants are considered food additives and their use is subject to regulation under the Federal Food Drug and Cosmetic Act. Antioxidants for food products are also regulated under the Meat Inspection Act, the Poultry Inspection Act, and various state laws. In most instances the total concentration of authorized antioxidants, added singly or in combination, must not exceed 0.02% by weight based on the fat content of food (Nawar 1996).

The general public concern with the safety of chemical additives has stimulated a continuing search for new antioxidants that may occur naturally in food or may form inadvertently during processing. Compounds with antioxidant properties have been found in spices, oil seeds, citrus pulp and peel, cocoa shells, oats, soybean, hydrolyzed plants, animal and microbial proteins, and in products that have been heated and / or have undergone non-enzymatic browning.

Generally lipid oxidation is faster in cooked meat than in raw meat (Tichivangana and Morrissey 1985). 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 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). Jayasingh and Cornforth (2003) compared the antioxidative activity of 0.5% to 2.0% MM with that of BHT and STPP in raw and cooked pork mince during frozen (-20°C) or cold (2°C) storage. Cooked samples with MM or STPP had significantly lower TBA values than were observed for the treatment with BHT. Nitrites and nitrates function as antioxidants by binding to heme iron, which upon reduction forms NO-heme complexes that stabilize the heme group during cooking. The non-heme iron released by cooking is the primary prooxidant in cooked meats (Igene and others 1979). Thus, the first objective of this dissertation was to evaluate possible additive antioxidant effects of MM and sodium nitrite to reduce TBA values of cooked beef samples during storage at 2°C for 15 d.

According to the American Spice Trade Association (ASTA 2001) U.S. consumption of spices exceeds 1 billion lb / year. The U.S. per capita consumption has continued to grow from 2.1 lb in 1980 to approximately 3.6 lb in 2000. Spices such as cloves, cinnamon, black pepper, turmeric, ginger, garlic, and onions exhibit antioxidant properties in different food systems (Younathan and others 1980; Al-Jalay and others 1987; Jurdi-Haldeman and others 1987). Spices have antioxidant properties due to the presence of compounds such as flavanoids, terpenoids, lignans, and polyphenolics (Craig 1999).

Antioxidative effects have been investigated for dried and ethanolic extracts of spices (marjoram, wild marjoram, caraway, peppermint, clove, nutmeg, curry powder, cinnamon, sage, basil, thyme, and ginger) on the oxidative stability of fresh minced

chicken meat, and fresh and microwave cooked pork patties pretreated with NaCl, and subjected to either refrigerated or frozen storage (4°C and -18°C, respectively). Application of dried spices to chicken meat inhibited lipid oxidation in frozen samples, and dried marjoram, wild marjoram and caraway had the highest antioxidative activity (Abd-El-Alim and others 1999). Although the individual components of Garam Masala have been shown to have antioxidant activity in model systems, my second objective was to determine the optimum level of each spice for antioxidant properties in cooked ground beef and to carry out sensory evaluation on cooked ground beef containing spices at their recommended level. Tests were also conducted to determine which antioxidant type (I or II) was most effective in cooked meat products and to evaluate possible additive antioxidnat effects of Type I (cinnamon, clove, BHT, ground rosemary) and Type II (MM, STPP) antioxidants used together in cooked ground beef.

Raisins are recognized as a good source of dietary antioxidants. According to the USDA, raisins are second only to prunes in the ability to resist oxidation as measured by the oxygen radical absorbance capacity (ORAC) test. Grapes and raisins have been shown to contain various antioxidant compounds, including bioflavanoids (Shalashvili and others 2002) proanthocyanidins (Foster 1997; Murga and others 2000), catechin monomers (Katalinic 1999), procyanidin dimers (Yamakoshi and others 2002) and other polyphenolic antioxidants (Meyer and others 1997; Frankel 1999). Bower and others (2003) have reported that beef jerky formulated to contain 15% (w/w) raisin puree produced conditions inhibitory to pathogenic bacteria by decreasing the pH to 5.4 and water activity to 0.64, and increasing the antioxidant activity by > 600%. Although raisins contain antioxidant compounds, their possible antioxidant effectiveness in cooked

ground meat systems has not been previously studied. Thus, the final objective of this dissertation was to evaluate the possible antioxidant effects of raisin paste on TBA values and trained panel rancidity scores in cooked ground beef, pork, and chicken.

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CHAPTER 2

LITERATURE REVIEW

The physical and chemical changes in muscle foods during the conversion of muscle to meat and post-mortem storage and utilization may alter the quality, amount, nutritional value, healthfulness, and safety of meat. Changes in product or external conditions during storage, result in deterioration of quality, including discoloration, development of off-flavors, loss of nutrients, textural changes, and progression of spoilage and / or pathogenicity (Skibsted and others 1994). Metabolic reactions resulting from biological membrane disruption (Stanley 1991), and biochemical oxidative processes (Xiong and Decker 1995) are major influences on deteriorative changes. Muscle foods are susceptible to oxidative activity because of their lipid, protein, pigment, vitamin and carbohydrate composition (Kanner 1994). Lipid oxidation has also been shown to be one of the major causes of quality deterioration of processed meat, imposing an adverse effect on flavor, color, and texture as well as nutritional value (Byrne 2000).

Lipid Oxidation

The muscle food components that are most influenced by oxidative processes included unsaturated fatty acids in lipids, amino acids in proteins, heme groups in pigments, and the structural elements of vitamins with conjugated double bonds. There are many free radical forms of atomic species with one or more unpaired electrons that are involved in oxidation reactions, including, hydrogen atoms (H•), tricholoromethyl (CCl_3•) from liver metabolism of CCl_4, superoxide (O_2•$^-$), hydroxyl (OH•), thiyl (RS•)

with unpaired electrons residing on sulfur, peroxyl ($RO_2\bullet$), alkoxyl ($RO\bullet$), and oxides of nitrogen ($NO\bullet$, $NO_2\bullet$), (Foote 1985; Kanner 1994; Halliwell and others 1995; Thomas 1995). These radicals come in contact with other non-radical molecules in the biological systems, and generate new radicals through reactions of addition, reduction or electron donation, oxidation by electron acceptance, or oxidation by hydrogen atom transfer (Halliwell and others 1995). These reactive radicals may initiate free-radical chain reactions, such as lipid peroxidation, pigment discoloration, or interactions between lipids and heme pigments (Foote 1985; Kanner and others 1987; Frankel 1991; Thomas 1995).

The perhydroxyl and hydroxyl radicals, ferryl iron (IV), and lipid free radicals are the primary activators for participation of oxygen and metal compounds in one-electron reduction processes (Kanner 1994). Johnson and others (1992) have reviewed the several important reactions between the iron in myoglobin and oxygen derivatives and mentioned the Fenton reaction ($Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH\bullet$), superoxide reaction ($Fe^{+3} + O_2\bullet^- \rightarrow Fe^{+2} + O_2$), and Haber-Weiss reaction ($H_20_2 + O_2\bullet^- \rightarrow OH^- + OH\bullet + O_2$) to be the main reactions involved.

Lipid peroxidation occurs in unsaturated fatty acids in lipid depots and in phospholipids in membranes through enzymatic and nonenzymatic autocatalytic mechanisms (Rhee 1988; Stanley 1991). The enzymatic reactions in several animal tissues occur due to the presence of enzymes such as lipoxygenase, peroxidase and microsomal enzymes, which catalyze insertion of oxygen into polyunsaturated fatty acids with unconjugated dienes (Kanner and Kinsella 1983a, b; Hsieh and Kinsella 1989; Stanley 1991). Phospholipases hydrolyze phospholipids to create conditions less

favorable for chain propogation, by releasing free fatty acids from the membrane surface (Shewfelt and others 1981).

The nonenzymic autocatalytic pathway of free-radical chain reaction for lipid peroxidation is categorized into initiation, propogation and termination phases (Kanner and others 1987; Hamilton 1989; Shahidi 1994). Lipid oxidation is terminated when free radicals combine to give stable, non-propagating reactions or by reduction of a donor that cannon propogate.

Oxygen usually exists in the stable triplet state, but when oxygen is exposed to light or heat, it may convert to a singlet, excited state. In this excited state, oxygen abstracts hydrogen atoms from the carbon adjacent to the fatty acid double bonds, producing free (R•) radicals (Nawar 1996). The role of iron-oxygen complexes has been reviewed by Morrissey and others (1998) as follows:

$RH + HO\bullet \rightarrow R\bullet + H_2O$

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

$R\bullet + O_2 \rightarrow ROO\bullet$

The ROO• is highly reactive with other unsaturated fatty acids, thus propagating the chain reaction as follows:

$ROO\bullet + RH \rightarrow ROOH + R\bullet$

Morrissey and others (1998) reviewed that the lipid peroxides (ROOH) further react with Fe^{2+} or Cu^{+} to give peroxide free radicals (ROO•) and alkyl radicals (RO•) as follows:

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

$Fe^{3+} + ROOH \xrightarrow{Fast} Fe^{2+} + ROO\bullet + H^{+}$

According to a review of lipid oxidation by Morrissey and others (1998), termination can be brought about by an antioxidant, such as vitamin E, donating an electron to peroxide free radical, or by $2R\bullet \rightarrow R\text{-}R$ for many large molecular weight complexes between fatty acids, or fatty acid + proteins. These reactions are explained in a diagram format by Shahidi (1994).

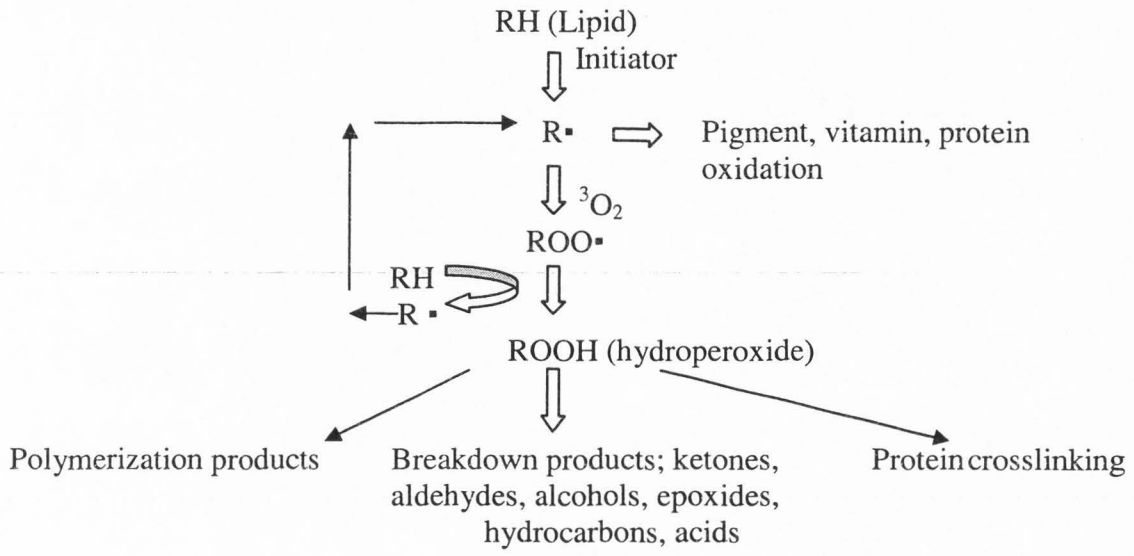


Figure 1 - Flow diagram of muscle food lipid oxidation (adapted from Shahidi 1994).

Lipid oxidation in meat products

Lipid oxidation is a major cause of deterioration in the quality of meat and meat products (Asghar and others 1988; Ladikos and Lougovois 1990). Lipid oxidation leads to the production of malondialdehyde (Shamberger and others 1974). Lipid oxidation may also decrease nutritional value by forming potentially toxic products during cooking and processing (Shahidi and others 1992a).

The factors affecting lipid peroxidation in animal tissues include species, anatomical location, diet, environmental temperature, sex, age, phospholipids content, and body composition (Gray and Pearson 1987). Processing factors that influence rate of lipid peroxidation include composition of raw materials, time post-mortem, heating, comminution or particle size reduction, and added ingredients such as salt, spices, and antioxidants (Kanner 1994).

The role of lipids in the development of WOF

The development of WOF in cooked meat is generally regarded to be the result of oxidation of tissue lipids (Ruenger and others 1978), with phospholipids being implicated as the lipid component most readily susceptible to oxidation in cooked meat (Younathan and Watts 1960). The phospholipids generally contain more poly unsaturated fatty acids (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. The triglycerides are much less susceptible to oxidation than the phospholipids (Love and Pearson 1971), and hence the triglycerides appear to exert only a minor influence on development of WOF. The rate and degree of oxidative degradation has been directly related to the degree of unsaturation of the lipids present (Igene and Pearson 1979; Tichivangana and Morrissey 1985) and degree of oxygen exposure (O'Grady and others 2000; Jayasingh and others 2002). Oxidation of unsaturated fatty acids in cooked meats during storage and reheating, results in stale or rancid flavors known as WOF (Sato and Hegarty 1971). The heme pigment content in conjunction with catalase activity may provide an indication of lipid

oxidation potential in raw meat, with PUFA amounts being a major determinant in inter-species oxidation rate differences (Rhee and others 1996).

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 through micro-capillaries that open up through the center of the material, and as a result greatly increases exposure to atmospheric oxygen.

Lipid oxidation is generally faster in cooked meats as compared to raw meat (Tichivangana and Morrissey 1985). Cooked meat develops rancid flavor more rapidly than uncooked meat during refrigerated storage, resulting in WOF (Tims and Watts 1958). Heating accelerates development of oxidized flavor (rancidity) in meat and meat products (Younathan and Watts 1960). According to Yamauchi (1972a, b), the development of rancidity is most rapid in meat that is heated at 70°C for 1 h, and the TBA value of cooked meat decreases as the cooking temperature increases above 80°C. Huang and Greene (1978) confirmed that meat subjected to high temperatures and / or

long periods of heating developed lower TBA numbers than similar samples subjected to lower temperatures for shorter periods of time. They postulated that antioxidant substances produced during the browning reaction exert TBA-retarding activity, which progresses as the meat is heated. Cooked (71°C) ground pork patties could be kept warm for 60 min at 71°C without significantly increasing TBA number (Jayasingh and Cornforth 2003). According to Hamm (1966), the Maillard reaction in meats begins at about 90°C and increases with further increases in temperatures and heating times. Catalase, which is present in uncooked meat and destroyed by heating, inactivates hydrogen peroxide and could provide an explanation for the more rapid development of lipid oxidation in cooked than in raw muscle foods (Harel and Kanner 1985a).

Sato and Hegarty (1971) have reported a very rapid increase in TBA values, and hence of WOF, for raw meats 1 h 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 lipids and contribute to the rapid development of WOF.

Role of iron in lipid oxidation

Iron is a trace element of considerable concern due to its role as a prooxidant in lipid oxidation in meat and meat products. Nawar (1996) has reviewed that the presence of iron catalyzes lipid oxidation. The oxidation of biomolecules has been shown to occur significantly only in the presence of catalytic metals, such as copper and iron (Miller and others 1990). Many different iron complexes, including low molecular weight compounds, heme compounds, and storage forms such as ferritin and hemosiderin are

found in meat (Hazell 1982; Stryer 1988). Han and others (1995) have reviewed that all forms of iron present in beef contribute to development of lipid oxidation. There are reports that heme and non-heme iron catalyze oxidation in both raw and cooked meat systems (Liu and Watts 1970). However, the concept that the greater propensity of WOF in cooked and comminuted products is due to the release of non-heme iron during cooking and grinding (Igene and others 1979), makes the most sense.

Non-heme iron has been identified as a prooxidant in cooked meat, with little oxidative activity of myoglobin (Love and Pearson 1974). Sato and Hegarty (1971) reported that non-heme iron was the active catalyst in cooked meats. The heme iron content decreases in ground beef with cooking and during storage. Cooking destroys the porphyrin rings of heme pigments resulting in non-heme iron release from heme pigments (Buchowski and others 1988; Lee and others 1998). Lee and others (1998) also showed an inverse relationship between heme iron content and TBA number of cooked beef, supporting the view that non-heme iron in cooked meat is responsible for catalyzing lipid peroxidation resulting in WOF. Both final temperature and rate of heating influence release of non-heme iron from meat pigment extracts. Slow heating results in release of more non-heme 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 non-heme iron during cooking catalyzing oxidation (Chen and others 1984). It is believed that microwaved meat suffers less from WOF then meat cooked by the slower conventional methods of cooking (Schriker and Miller 1983).

Robinson (1924) suggested that iron porphyrins cause oxidative deterioration of PUFAs. Heme compounds have been shown to accelerate lipid oxidation (Pearson and

others 1977). Younathan and Watts (1959) proposed that only ferric forms of heme compound pigments are effective catalysts and this suggestion was supported by Fishwick (1970) and Verma and others (1985). Recent work by Harel and Kanner (1985a, b) and Rhee (1988) suggested that ferric heme pigments work as effective catalysts only in presence of hydrogen peroxide. Rate of peroxidation accelerated several hundred fold when isolated sarcosomal fraction from turkey dark meat metmyoglobin and hydrogen peroxide were evaluated together.

Rhee (1988) explained that the combined catalytic effect was partially due to release of iron from heme by hydrogen peroxide. However, Harrel and Kanner (1985b) claim that hydrogen peroxide leads to formation of an activated heme (ferryl) complex with iron in the quadrivalent state which initiates lipid peroxidation.

Heme proteins like hemoglobin and myoglobin convert to met (+3) and ferryl (+4) oxidation states during storage, which also promote lipid oxidation (Barron and others 1997). Concentration of copper, iron and heme increases with storage, and accelerates oxidation (Decker and Hultin 1990). Heating results in more non-heme iron, and treatment with heat and hydrogen peroxide destroys the iron-porphyrin complex in ground beef extracts (Schricker and Miller 1983).

Although the non-heme iron 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). In another study, it has been shown that iron was released from ferritin by both cysteine and ascorbate at the pH found in muscle foods (5.5 to 6.9), and the rate of Fe release from ferritin was influenced by temperature, ferritin and reducing agent

concentration. Physiological concentration of ferritin-catalysed lipid oxidation in-vitro, and heating ferritin increased the rate of lipid oxidation. Thus, ferritin could be involved in the development of off-flavor in both cooked and uncooked muscle foods (Decker and Welch 1990).

Another study suggests that the iron source that is important in the catalysis of lipid oxidation is the Fe^{2+} ion. Neither the iron bound to transferrin or ferritin nor the central iron component in heme pigments had significant effects on the oxidation of lipids in the oil emulsion system (Kim and others 1996). These results may be useful in the development of strategies to prevent lipid oxidation in meat (Kim and others 1996). Iron sources identified as important in catalysis of lipid oxidation were Fe^{2+} and Fe^{3+} ions, whereas hemoglobin was a very weak catalyst (Kim and others 1998).

In another study by Han and others (1995), it was shown that heating increased TBA and peroxide values in both cooked and uncooked muscle food systems. All forms of iron catalysed lipid oxidation in aqueous systems, with greatest oxidation by heme and low molecular weight iron fractions. Oxidation in lipid extracts was not increased by ferritin, $FeCl_2$ or $FeCl_3$, but haem iron was the major oxidation catalyst. Lipid stability decreased with addition of any iron forms inherent in beef or with increased heating, which helps the understanding of the rapid oxidation of meat during refrigerated storage or after cooking.

Factors affecting lipid oxidation

The rate of lipid oxidation is dependent on the oxygen concentration at low concentrations of oxygen (Nawar 1985). The rate of lipid oxidation has been shown to increase with an increase in temperature (Nawar 1985). Rate of lipid oxidation is directly proportional to the surface area exposed to the air and so comminution or disruption of muscle tissues increases rate of lipid oxidation. Lipid oxidation increases in foods with lower water activity ($a_w < 0.1$), and decreases when water activity reaches $a_w = 0.3$. This effect is due to the reduced catalytic activity of metal catalysts and by quenching of free radicals. At water activity of 0.55 to 0.85 the rate of lipid oxidation increases due to mobilization of catalysts and oxygen (Nawar 1985).

Tests to determine lipid oxidation

The thiobarbituric acid (TBA) test is the most frequently used method for assessing lipid oxidation in meat. Sensory panelists describe the extent 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. Consumer panelists not only detect rancid flavor in cooked pork samples with TBA values > 1.0, but also preferred samples with TBA values < 0.4 (Jayasingh and Cornforth 2003).

Food Antioxidants

The use of antioxidants retards the rate of lipid oxidation by minimizing formation or propagation of free radicals. Food antioxidants can be classified into Type I or Type II antioxidants and also as natural or synthetic antioxidants. Natural antioxidants include retinoids (vitamin A) and tocopherols (vitamin E) found in many animals and plants; ascorbic acid (vitamin C) found in citrus fruits and many vegetables, and betacarotene, found in deep green vegetables. Spices (cloves, cinnamon, black pepper, turmeric) ginger, garlic and onions exhibit antioxidant properties in different food systems (Younathan and others 1980; Al-Jalay and others 1987; Jurdi-Haldeman and others 1987). The total antioxidant capacity of ground cinnamon and ground cloves has been reported to be as high as 2675 and 3144 umol TE (trolox equivalents) / g sample. Wu and others (2004) showed that these values are the highest among various food, vegetables, spices, and other foods as measured by the oxygen absorbance capacity (ORAC) test. Grape seed and green tea extracts possess antioxidant properties (Rababah and others 2004). Some of the commonly used antioxidants are α- tocopherol, ascorbic acid, BHA and BHT. The criterion for choosing an antioxidant depends upon the kind of food, the potency of the antioxidant, storage temperature of the food, and the fat content.

Type I antioxidants

Type I 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 reactions with lipid radicals, forming lipid-antioxidant complexes. These include vitamin C, vitamin E, BHA, BHT, TBHQ, and PG.

Many of the naturally occurring phenolic compounds like flavonoids, eugenol, vanillin and rosemary antioxidant are classified as Type I antioxidants. Their antioxidant role has been suggested to be due to the presence of phenolic compounds (Houlihan and others 1985). Phenolic compounds from plants also possess antioxidant activity (Pokorny 1991; Shahidi 2000). Such activity has been mainly attributed to flavonoids and ascorbic acid in citrus fruits (hesperidin, neohesperidin, and eriocitrin) and to carnosol and rosmarinic acid in rosemary (Schwarz and others 2001).

All tocopherols contain contain a phenolic structure which scavenges lipid and oxygen radicals throught the formation of tocopheryl quinone radical whose energy is 2 to 3 times lower than most fatty acid radicals (Buettner 1993). Formation of the lower energy tocopheryl quinine radical minimizes the chance that the free radical can further promote lipid oxidation. Compounds such as ascorbic acid and reduced glutathione, can reduce the tocopheryl radical, thus regenerating its antioxidant activity (Parker 1989).

The major antioxidant mechanism of carotenoids is through their ability to interact with singlet oxygen, thus not allowing the singlet oxygen to form lipid peroxides (Olson 1993). Carotenoids can also inhibit oxidation reactions by accepting or donating electrons (Bradley and Min 1992).

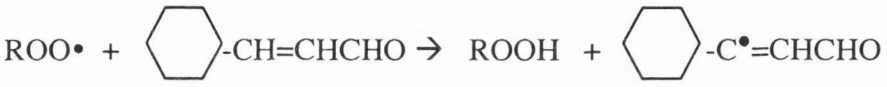
Synthetic phenolic antioxidants such as BHT are used to improve the stability of lipids in food products. McCarthy and others (2001) reported a significant antioxidant effect of BHT/BHA in cooked pork patties when added at a level of 0.01% of meat weight, which is about 7-fold more BHT than allowed by USDA (0.01% by fat weight; DeHoll 1981). They are quite volatile and easily decompose at high temperatures. BHT

was shown to inhibit the propagation step of chain of lipid oxidation by its action as a radical scavenger (Fujisawa and others 2004).

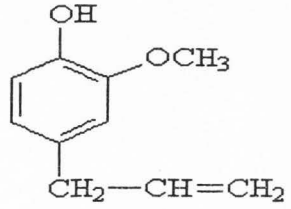
Consumer concern about the safety of synthetic food additives has led to renewed interest in natural products (Andres and Duxbury 1990). Rosemary, a natural antioxidant, has been reported to contain certain components (rosemanol, rosmariquinone, rosmaridiphenol, carnosol), which may be as effective as BHT as an antioxidant (Houlihan and others 1984, 1985; Nakatani and Intani 1984). Such compounds in rosemary extracts have been shown to exhibit antioxidant properties equal to or slightly less than BHT (Wu and others 1982; Houlihan and others 1985). Rosemary extracts at concentrations ranging from 0.02% to 0.05% of total weight, have been reported to inhibit lipid oxidation in beef (Wu and others 1994), pork (Decker and others 1993), and chicken (Lai and others 1991). Water soluble rosemary extracts at 500 ppm, have been shown to significantly decrease thiobarbituric acid reactive substances (TBARS) formation and to preserve red color in cooked turkey, up to 7 d refrigerated storage (Yu and others 2002). Rosemary extract has been shown to maintain sensory quality in processed pork products for up to 10 d refrigerated storage (Nissen and others 2004). However, results from our laboratory have shown that ground rosemary at 0.4% to 0.8% was very effective in significantly delaying onset of rancidity as compared to BHT (up to 0.02% of meat weight), and rosemary oil (up to 0.2% of meat weight) in cooked, ground pork (Vasavada and Cornforth 2003). The phenolic compounds present in rosemary break free radical chain reactions by hydrogen atom donation.

Mechanism of action of some common Type I antioxidants

Cinnamic aldehyde is a Type I antioxidant that can donate a hydrogen atom (H•) to an alkoxy free radical (ROO•) to form semi-stable hydroperoxides (ROOH), thus slowing the propagation step of lipid oxidation, as shown in the reaction sequence below. The hydrogen atom (H•) could be abstracted from 3 possible locations on the cinnamic aldehyde, at sites adjacent to double bonds, since hydrogen abstraction takes place easily from carbons adjacent to the double bonds. After donating the hydrogen atom, the unpaired electron on cinnamic aldehyde is stabilized by resonance delocalization on the benzene ring.

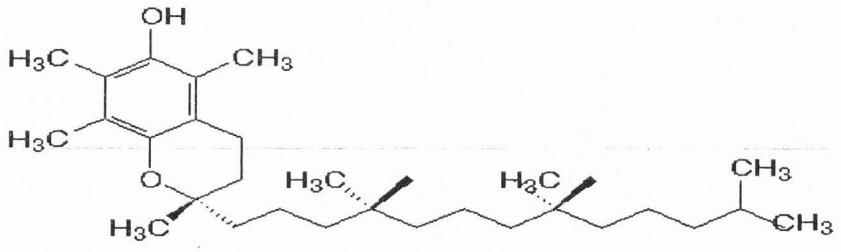


Eugenol, which is the main component of cloves, has been shown to inhibit lipid oxidation by 2 steps. Firstly, it interferes with the chain reactions by trapping the active oxygen, and secondly, it is metabolized to a dimer form (dieugenol), and this dimeric form inhibits lipid peroxidation at the level of propogation of free radical chain reaction (Ogata and others 2000).



Eugenol

Vitamin E (below) has been tested as an antioxidant in ground beef. Addition of vitamin E or vitamin C to ground beef improves lipid and color stability. Addition of both vitamin E and vitamin C showed greater pigment and lipid stability than vitamin E or C alone (Mitsumoto and others 1991). Vitamin E is a Type I antioxidant that inhibits lipid oxidation by donating a hydrogen atom from its phenol hydroxyl group producing stable radical intermediates due to resonance delocalization.



Vitamin E (α-tocopherol)

Ascorbate (vitamin C) is also a Type I antioxidant, capable of donating hydrogen atoms from positions 2 and 3 of the lactone ring. Ascorbate acts either as an antioxidant or pro-oxidant depending on concentration of lipid hydroperoxides, and lower molecular weight metals (Kanner and Mendel 1977; Yamamoto and others 1987). Ascorbate is capable of inhibiting lipid oxidation by inactivating free radicals and by regenerating α – tocopherol. Ascorbates can also act as pro-oxidants by reducing iron to its catalytic ferrous form. Hence, ascorbates should be used in combination with metal chelators to have antioxidant effects. Ascorbyl-palmitate has been shown to inhibit lipid oxidation in turkey (Calvert and Decker 1992).

Maillard reaction products

Cooking can cause the formation of antioxidants in food. Retorting treatment of meats has been shown to increase oxidative stability compared to less severe heat treatments (Sato and others 1973; Einerson and Reineccius 1978). The low molecular weight, water-soluble antioxidants in severely cooked meats were suggested to be Maillard reaction products (MRP), which are formed from amines and carbonyls at elevated temperatures. These MRP have been shown to be antioxidants (Yen and Hsieh 1995), by acting as reducing agents and free radical scavengers.

Antioxidant effect of spices used in Garam Masala spice blend

The Garam Masala spice blend has 13 different ingredients in varying levels. These include black pepper, caraway, cardamom, chili, cinnamon, clove, coriander, cumin, fennel, ginger, nutmeg, salt, and star anise. The approximate composition includes black pepper (10%), cardamom (30%), cinnamon (5%), cloves (5%), nutmeg (5%), coriander (25%), cumin (20%), and caraway, chili, fennel, ginger, salt, and star anise in variable proportions.

The total phenolic content of ground cinnamon and ground cloves was reported to be 157 and 113 mg GAE (gallic acid equivalents) / g (Wu and others 2004). Both these spices are components of the Chinese 5-spice blend and Garam Masala blend. This high concentration of phenolics in ground cinnamon and ground cloves is responsible for high antioxidant activity of these spices.

Black Pepper *(Piper nigrum)*

Jun and others (2000) reported that black pepper is found to be effective at 0.33% in providing desirable sensory properties to chicken feet (jokpyun, a traditional Korean gel-type delicacy). Black pepper derived from peppercorn has a sharp, woody, penetrating aroma and is hot and biting to taste because of its oleoresin content. Piperine is the active antioxidant compound present in black pepper (Badmaev and others 2000). Black pepper and piperine have been shown to reduce high fat diet induced oxidative stress in Wistar rats, as measured TBARS, conjugated dienes, and activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, and reduced glutathione (Vijayakumar and others 2004).

Caraway *(Carum carvi)*

Black caraway oil has been shown to have marked chelating activity against Fe^{2+} and also reduced lipid oxidation in human low density lipoproteins and TBARS (Yu and others 2005). Dried caraway has been shown to have high antioxidant activity, along with its ethanolic extract, in chicken meat (Abd-El-Alim and others 1999).

Cardamom *(Elletoria cardamomum)*

Investigation of antioxidant compounds in cardamom showed the presence of protocatechualdehyde, protocatechuic acid, 1,7-bis (3,4-dihydroxyphenyl) hepta-4E,6E-dien-3-one, and 2,3,7-trihydroxy-5-(3,4-dihydroxy-E-styryl)-6,7,8,9-tetrahydro-5H-benzocycloheptene (Kikuzaki and others 2001), with protocatechualdehyde and 1,7-bis (3,4-dihydroxyphenyl) hepta-4E,6E-dien-3-one having more antioxidant activity than alpha-tocopherol and L-ascorbic acid. Cardamom has been shown to increase oxidative

stability of lipids in cookies, with the sensory threshold for cardamom being 1.0% (Badei AZM and others 2002).

Chili *(Capsicum annuum)*

Peppers get their heat from a compound called capsaicin, a pungent ingredient of hot chili pepper that has been shown to protect against experimentally-induced mutagenesis and tumorigenesis, and to also induce apoptosis in various immortalized or malignant cell lines (Surh 1999). The majority of the naturally occurring phenolics retain antioxidative and anti-inflammatory properties, which appear to contribute to their chemopreventive or chemoprotective activity (Surh 1999).

Cinnamon *(Cinnamonum verum)*

Cinnamon has cinnamic aldehyde that gives it a distinct odor and flavor. Cinnamon essential oil has been found to have great antioxidant activity in Chinese-style sausage (Yong and others 1998). Cinnamon has been shown to be a better superoxide radical scavenger than mint, anise, BHA, ginger and BHT (Murcia and others 2004). Cinnamon has been shown to have a high concentration of antioxidants (> 75 mmol / 100g) (Dragland and others 2003).

Clove *(Syzygium aromaticum)*

Cloves had a strong antioxidant effect and gave good stability and effectively retarded flavor deterioration in frozen stored fish mince, at 0.05% for 28 wk, and 0.1% for 50 wk (Joseph and others 1992). Addition of clove powder (0.20% w/w) significantly reduced oxidative rancidity (measured as TBARS), and improved acceptability of oysters

for 278 d, as compared to 235 d and 237 d for BHT-treated and untreated samples, respectively (Abraham and others 1994). Clove oil is about 90% eugenol (Dorman and others 2000). Antioxidative activity of clove buds is partly due to the presence of aroma compounds such as eugenol and eugenyl acetate (Lee and Shibamoto 2001). Eugenol has been shown to act as an antioxidant on oleogenous foods (Farag and others 1989a). The activities of eugenol (200 ppm) and isoeugenol (200 ppm) in inhibiting malonaldehyde formation in cooked ground pork have been shown to be between 95-99% (Shahidi and others 1992b). These activities were higher than those of ascorbic acid, α-tocopherol and BHT (Shahidi and others 1992b). Activity of BHT, a known synthetic antioxidant, was 88% at 200 ppm level of addition to meat (Shahidi and others 1992). Clove and MRP have been shown to be very effective in arresting the build-up of secondary oxidation products, formed during refrigerator storage of cooked meat, and also affect the extent of release of non-heme iron during cooking of meat, which is believed to be the primary catalyst accelerating lipid oxidation (Jayathilakan and others 1997).

Coriander *(Coriandrum sativum)*

Supplementation of a high-fat, cholesterol-containing diet with 10% coriander seeds has been shown to protect tissues by preventing formation of unwanted free radicals in groups of female Sprague-Dawley rats (Chithra and Leelamma 1999). Coriander extract has been shown to demonstrate antioxidative activity alone and in synergism with BHT (Melo and others 2003). Linalool is the main antioxidative compound in coriander (Reddy and Lokesh 1992). The essential oil content of the dried fruit ranges from 0.5 to 1% and the oil contains d-linalool, camphor, d-pinene, camphene,

pinene, sabinene, myrcene, terpinene, limonene, and other constituents (Simon and others 1984). Linalool has been shown to have antioxidant properties, much the same as vitamin E and lipoic acid, to prevent lipid peroxidation in guinea pig brains (Celik and Ozkaya 2002) by limiting damage from oxidation reaction in unsaturated fatty acids.

Cumin *(Cuminum cyminum)*

Extracts of plants like cumin, clove, cinnamon and rosemary, originally having high levels of phenolic compounds, have been shown to exhibit strong H-donating activity and are effective scavengers of hydrogen peroxide and superoxide radicals (Lugasi and others 1995). Cumin aldehyde is the principal contributor in aroma and flavor and antioxidant properties (Reddy and Lokesh 1992). Addition of 2% *Acer rubrum L.* var. *trilobum* (a cumin-like spice) has been shown to improve the hygienic quality of koefte, especially when made with low-fat beef, and gave a storage life of about 6 days when stored at 7°C (Kivanc and Akguel 1991).

Fennel *(Foeniculum vulgare)*

Fennel has a slight sweet or licorice aromatic flavor similar to star anise but less intense. *Foeniculum vulgare* oils have been shown to have antioxidant properties comparable to α-tocopherol and BHT, as evaluated by TBARS assay and spectroscopic detection of hydroperoxy-dienes from linoleic acid in a micellar system (Ruberto and others 2000). Aqueous and ethanolic fennel seed extracts have been shown to display strong antioxidative activity, reducing power, and scavenging and metal chelating activities in comparison to standard antioxidants such as BHA, BHT and α -tocopherol (Oktay and others 2003). Eight antioxidant compounds (3-caffeoylquinic acid, 4-

caffeoylquinic acid, 1, 5-O-dicaffeoylquinic acid, rosmarinic acid, eriodictyol-7-O-rutinoside, quercetin-3-O-galactoside, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside) have been isolated and identified in fennel by Parejo and others (2004). A study of the antioxidative activities, including the radical scavenging effects, inhibition of hydrogen peroxide, and Fe^{2+} ion-chelating activity for fennel samples showed antioxidant activity for fennel as a radical scavenger in the experiment using the 1,1-dipehnyl-2-picryl-hydrazyl (DPPH) radical, and towards hydrogen peroxide at 0.2 g /ml concentration. The Fe^{2+} ion-chelating activities of the samples were shown to be greater than 70% (El and others 2003).

Ginger *(Zingiber officinale)*

Ginger is an effective tenderizing, antioxidative and antimicrobial agent used in meat and meat products. The antioxidative potential of the volatile oil fraction has been shown to be due to diverse groups of phenols (Naveena and Mendiratta 2001). Ginger extract at the level of 3% can improve the sensory quality and shelf life of mutton chunks (Mendiratta and others 2000).

Kim and Lee (1995) showed that ginger extract was effective in retarding the development of rancidity in precooked beef for a 47-d period and it was directly related to ginger concentration. Addition of freeze-dried ginger and fenugreek extracts to ground beef patties at 500 ppm has been shown to be effective in retarding odor generation, TBA increases and oxidative colour change (Mansour and Khalil 2000). Gingerol-related compounds and diarylheptanoids are the main antioxidant compounds seen in ginger (Nakatani 2003). About 5 antioxidants have been identified as 4, 6, 8, and 10-gingerol,

and 6-shogaol on the basis of their molecular weight as determined by LC-MS and by using DPPH free radicals it has been found that 6-gingerol is more efficient than BHT (Cho and others 2001). Zingerone is also an antioxidant compound found in ginger (Reddy and Lokesh 1992).

Nutmeg *(Myristica fragrans Houtt)*

Nutmeg has been shown to contain about 10% essential oil, which is primarily composed of terpene hydrocarbons (pinenes, camphene, p-cymene, sabinene, phellandrene, terpinene, limonene and myrcene, together 60 to 90%), terpene derivatives (linalool, geraniol and terpineol, together 5 to 15%) and phenylpropanes (myristicine, elemicine and safrol, together 2 to 20%) (Nakatani 2003). Addition of 2.5% nutmeg in a model salad dressing formula was found to have a slight antioxidant effect (McKee and others 1993). γ-terpinene, a monoterpene hydrocarbon present in nutmeg essential oils, has been shown to retard the peroxidation of linoleic acid. The retardation of linoleic acid peroxidation by γ-terpinene has been found to be due to rapid chain termination via a very fast cross-reaction between hydroperoxyl radicals and linoleylperoxyl radicals (Foti and Ingold 2003).

Salt

There have been contradictory findings related to salt content in meat and its possible role as an antioxidant or a pro-oxidant. Lipid oxidation monitored during refrigerated and frozen storage of raw and cooked turkey breast or thigh muscle by the TBA test, indicated that the most significant prooxidant effect was caused by salt + Cu^{2+} + Fe^{2+}, followed by salt + Fe^{3+} or Cu^{2+} alone (Salih and others 1989). Steaks with no salt

(pooled across antioxidant levels) were shown to have lower TBA values than steaks with any salt type (NaCl, KCl or a 65% NaCl + 35% KCl combination) after 85 d storage or either level of salt after 155 d storage at 0.375% or 0.750% salt level. Steaks with either level of added salt resulted in higher ratings for juiciness, saltiness and overall palatability than steaks with no added salt (Wheeler and others 1990). In ground pork, 0% to 2% NaCl showed an increase in TBARS concentration with increasing NaCl concentration. Peroxide values did not increase during storage of pork with 0% and 0.5% NaCl, but increased 22.5 times and 44 times with 1% and 2% NaCl, respectively (Lee and others 1997). In dry smoked beef the pH of the meat was shown to increase with increasing salt concentration, and in general the addition of salt significantly increased the degree of oxidation, while smoking produced antioxidant activity ($P < 0.05$) (Dzudie and others 2003). Phosphate buffer (25mM NaH_2PO_4 / Na_2HPO_4), high final pH (7.0) of surimi pellet, and the presence of salt (0.1M NaCl) were all inhibitory to both protein and lipid oxidation during storage of shelf-stable surimi (Subramanian and others 1996). In dry-cured Longissmus Dorsi meat, glutathione peroxidase activity and TBARS levels were shown to be significantly lower ($P < 0.05$) in samples produced with the salt and it is believed that salt acts as an enzyme inhibitor and antioxidant (Sarraga and others 2002).

Star Anise *(Pimpinella anisum)*

Star anise has carminative, stomachic, stimulant and diuretic properties. Anethole is the main compound present in star anise (Curtis and others 1996). In Chinese marinated pork shanks (with star anise as an ingredient in the marinade), antioxidant

effects were seen in the marinated pork shanks as compared to the controls (Wang and others 1997). Anethole has been also shown to have anti-inflammatory and antifungal activities (Karapinar and Aktug 1987; Curtis and others 1996). Star anise has been shown to have potent antimicrobial property due to the presence of anethole (De and others 2002).

Raisins as antioxidants in meat

Raisins have been recognized as a good source of dietary antioxidants. According to the USDA, raisins are second only to prunes in the ability to resist oxidation as measured by the ORAC test (http://www.ars.usda.gov/is/pr/1999/990208.htm). The total antioxidant capacity of raisins is about 90-100 times lower than those seen for dried cinnamon and clove powder. Grapes and raisins have been shown to contain various antioxidant compounds, including bioflavanoids (Shalashvili and others 2002), proanthocyanidins (Foster 1997; Murga and others 2000), catechin monomers (Katalinic 1999), procyanidin dimers (Yamakoshi and others 2002) and other polyphenolic antioxidants (Meyer and others 1997; Frankel 1999).

Antioxidative activity of beef jerky containing 15% w/w of raisin puree and measured by the ferric reducing antioxidant potential assay, was shown to increase by > 600% as compared to control samples. The product received favorable sensory ratings for appearance, texture and flavor, comparable to the non-raisin control (Bower and others 2003). In a study by Karakaya and others (2001), the highest total phenolic contents in beverages (on the basis of individual servings) were found in black tea, instant coffee, coke, and red wine, while highest phenolic contents in solid foods were

found in red grapes, raisins, tarhana and dried black plums. Antioxidative activity of golden raisins was shown to be significantly higher than in dipped and sun-dried Thompson raisins, suggesting that enzymatic browning negatively affected antioxidative activity (Yeung and others 2003). Raisins and juices were shown to have good potential in terms of inhibition of TBARS formation, probably due to the higher levels of polyphenols, which are powerful antioxidants (Agte and others 2003). Vasavada and Cornforth (2006; in press) have shown that raisins have antioxidant properties in cooked meats due to the MRP formed by heating of sugars in raisin.

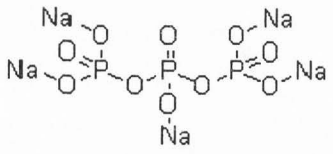
Type II antioxidants

Type II antioxidants are those that reduce lipid oxidation by chelating iron and copper ions, thus preventing metal-mediated lipid oxidation. Various Type II antioxidants such as polyphosphates, nitrites and nitrates, citric acid, phytic acid, and MM have been investigated for their antioxidant properties in meat systems.

Sodium tripolyphosphate as a Type II antioxidant

St. Angelo and others (1988) and Liu and others (1992) have reported that STPP 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 STPP is hypothesized to be due to its sequestering of metals (Watts 1950; Tims and Watts 1958), particularly iron which is the major pro-oxidant in meat systems (Igene and others 1979). Polyphosphates have been shown to chelate ferrous iron from cooked meats (Tims and Watts 1958).

According to the limits set by USDA (2000), STPP can be used in meat and poultry products as an antioxidant at a maximum level of 0.5%. In the figure below, the sodium ions (Na^+) dissociate in solution, allowing iron to bind to the negatively charged phosphate groups.



Structure of Sodium Tripolyphosphate

Polyphosphates have been shown to be effective antioxidants in cooked meat systems. Polyphosphates are less effective in inhibiting lipid oxidation in raw beef (Mikkelsen and others 1991), probably due to decreased antioxidant activity due to hydrolysis of polyphospates by endogenous skeletal muscle polyphosphatases during storage. In cooked meat systems, however, polyphosphates are very effective, partially due to the fact that phosphatases have been inactivated and also due to increased importance of iron as a lipid oxidation catalyst in cooked meats.

There have been conflicting reports regarding the efficacy of Type I and Type II antioxidants in inhibiting lipid oxidation. 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 (2001) indicate that STPP, 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 STPP was used in combination with rosemary oleoresin in cooked restructured pork steaks most of the antioxidant action was from STPP. Sodium tripolyphosphate 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% STPP when evaluated by trained taste panelists even after 4 d storage at 4°C (Vara-Ubol and Bowers 2001).

Nitrites and nitrates

Nitrites and nitrates function as antioxidants by converting heme proteins to inactive nitric oxide forms (Igene and others 1985), by chelating free iron (Kanner and others 1984), by stabilizing lipid membranes (Freybler and others 1993) and by forming nitrosated heme compounds which possess antioxidant activity (Morrissey and Tichivangana 1985). USDA regulations limit the addition of sodium nitrite in cured meats to 156 ppm. Sodium nitrate has been shown to reduce the oxidation rate, measured by TBARS and peroxide values, in a meat model system composed of minced pork and fat (Parolari 2000). Rosemary extract and sodium nitrite have been shown to lower TBARS values, independent of radiation dose or storage time in bologna processed from ground turkey meat (Fan and others 2004). Nitrite / nitrate / ascorbic acid blend (100 ppm / 200 ppm / 500 ppm, respectively) has been shown to be equally effective to spices such as paprika and garlic, in reducing lipid oxidation in ground, dry, ripened pork sausage (Aguirrezabal and others 2000).

Volatile N-nitrosamines in foods, such as meat products, have been reported to cause cancer. When the breakdown products of N-nitrosodimethylamine and N-

nitrosopyrrolidine after 5 kGy irradiation in distilled water were reacted in an in vitro representation of the human stomach, they both were reformed, but only in the presence of sodium nitrite (Ahn and others 2002). Links have been observed between the high incidence of stomach cancer in China and Japan and the intake of certain fish products and pickled/fermented vegetable products; N-nitroso-N-methylurea has been suggested to be a potential causative agent (Sen and others 2001). Incorporation of 200 to 2000 ppm of ascorbic acid in the fish sauce and other foods, prior to nitrosation, inhibited such NMU formation appreciably (Sen and others 2001). NMU formation could occur in fish sauce from the high-risk area for stomach cancer and in the fish sauce spiked human gastric juice during nitrosation under simulated gastric conditions (Deng and others 1998).

Phytic acid

Phytic acid is a naturally occurring Type II antioxidant found in high concentrations in barley, wheat and wild rice (Empson and others 1991). Phytic acid has greater antioxidant effects than carosine in cooked beef samples (Lee and others 1998). Sodium phytate, sodium pyrophosphate and STPP all lowered metmyoglobin formation in raw beef samples, but sodium phytate was most inhibitory to lipid oxidation (Lee and others 1998). Phytic acid more effectively inhibits lipid peroxidation in beef homogenates than other antioxidants, such as ascorbate, BHT and EDTA, by removing myoglobin-derived iron from negatively charged phospholipids, thus preventing their autoxidation and off-flavor formation (Lee and Hendricks 1995).

Milk mineral

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). Milk mineral is another phosphate-based Type II antioxidant and is used as a natural calcium source. It is the dried permeate of ultra filtered whey and contains 13% by weight phosphorus on dry weight basis (Cornforth and West 2002). Milk mineral (1.5%) was effective for maintenance of low TBA numbers (< 1.0) of cooked, ground pork for 14 d refrigerated storage (Jayasingh and Cornforth 2003). Cooked ground beef and pork have been shown to require 2.0% milk mineral to maintain TBARS values < 1.0 after 14 d of storage, compared to 1% MM for ground turkey (Cornforth and West 2002). Among MM components (phosphate, calcium and citrate), polyphosphates most effectively maintained low TBARS levels during storage. Results suggest that milk mineral chelates soluble Fe to colloidal calcium phosphate particles, thus removing Fe as a catalyst for lipid oxidation (Cornforth and West 2002).

Spices as possible Type II antioxidants

Eugenol compounds have been shown to inhibit low-density lipoprotein oxidation by forming complexes with reduced metals. Potent inhibitory effects of isoeugenol may be related to the decreased formation of perferryl ion on the iron-oxygen chelate complex as the initiating factor of lipid peroxidation, by keeping iron in a reduced state. Inhibition of lipid oxidation by eugenol compounds is due to the suppression of free radical cascade

of lipid peroxidation in low-density lipoproteins by reducing copper iron (Ito and others 2005).

Consumer concerns regarding synthetic antioxidants have contributed to the increased use of natural antioxidants. BHA has toxic effects including liver swelling and it influences the liver enzyme activities (Halladay and others 1980). Usage of synthetic antioxidants has been a safety concern (Wurtzen and others 1986; Farag and others 1989b). However, the use of synthetic antioxidants such as BHA/ BHT still continues in the food industry.

My research focuses on the use of some natural antioxidants like various spices, MM, and raisin paste in cooked meats, to control lipid oxidation. The effectiveness of these natural antioxidants in cooked meats can be a viable alternative to the use of synthetic antioxidants in the meat industry in future.

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CHAPTER 3

EVALUATION OF MILK MINERAL ANTIOXIDANT ACTIVITY IN BEEF MEATBALLS AND NITRITE-CURED SAUSAGE

Abstract

The objectives of this study were to determine the antioxidant activity of 1.5% milk mineral (MM) added to uncured cooked beef meatballs and to evaluate possible additive antioxidant effects of MM in combination with 20 or 40-ppm sodium nitrite in beef sausages. All treatments were also formulated with 1.5% salt and 10% added water. Thiobarbituric acid (TBA) values and Hunter color values were determined at 1 d, 8 d, and 15 d of storage at 2°C. Meatball cooked yield was also measured and was not different ($P < 0.05$) between control meatballs and those containing MM. As expected, treatments containing nitrite had higher redness (Commission Internationale de l'Eclairage; *CIE* a*) than samples without nitrite. Redness values increased with storage time in sausages containing 40-ppm nitrite. However, redness values decreased ($P < 0.05$) during storage of control meatballs, associated with increased lipid oxidation (higher TBA values). Lipid oxidation was lower ($P < 0.05$) in samples containing 1.5% MM with TBA values < 1.2 after 15 d storage compared with 6.1 for control samples. There was no additive inhibition of lipid oxidation in samples containing 20 or 40-ppm sodium nitrite plus 1.5% MM. Milk mineral alone at 1.5% of meat weight was sufficient for inhibition of lipid oxidation in cooked beef samples.

Reprinted from Vasavada MN, Cornforth DP. 2005. Evaluation of milk mineral antioxidant activity in beef meatballs and nitrite-cured sausage. J Food Sci 70(4):C250-3.

Introduction

Lipid oxidation is a major cause of deterioration in the quality of meat and meat products (Asghar and others 1988; Ladikos and Lougovois 1990). Lipid oxidation leads to production of malonaldehyde, a potent mutagen and / or carcinogen (Shamberger and others 1974). Lipid oxidation is faster in heated meat than in raw meat tissues (Tichivangana and Morrissey 1985). The rate and degree of oxidative degradation has been directly related to the degree of unsaturation of the lipids present (Igene and Pearson 1979; Tichivangana and Morrissey 1985) and degree of oxygen exposure (O'Grady and others 2000; Jayasingh and others 2002). Oxidation of unsaturated lipids in cooked meats during storage and reheating results in stale or rancid flavors known as warmed-over flavor (WOF) (Sato and Hegarty 1971).

The greater propensity of WOF in cooked and comminuted products is due to the release of non-heme iron during cooking and grinding (Igene and others 1979). Unsaturated lipids, especially those of the membrane phospholipids fraction, are the compounds undergoing autoxidation (Younathan and Watts 1960; Igene and Pearson 1979). The development of WOF in cooked meat is generally accepted to be the result of autoxidation of tissue lipids (Younathan and Watts 1960; Ruenger and others 1978).

Cooked meat develops rancid flavor more rapidly than uncooked meat during refrigerated storage, resulting in WOF (Tims and Watts 1958). The thiobarbituric acid test (TBA) is the most frequently used test to assess lipid oxidation in meat. Sensory panelists describe the extent of lipid oxidation in terms of rancid odor or taste. Tarladgis and others (1960) found that TBA numbers (milligrams of TBA reactive substances /

kilogram of 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 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.

Nitrites and nitrates function as antioxidants by binding to heme iron, which upon reduction form NO-heme complexes that stabilize the heme group during cooking. The ionic iron released by cooking is the primary prooxidant in cooked meats (Igene and others 1979). Milk mineral (MM) is the mineral fraction of skim milk. It works as an antioxidant in cooked meats by iron-chelation to colloidal calcium phosphate (Cornforth and West 2002). The objective of this study was to evaluate possible additive effects of MM and sodium nitrite to reduce TBA values of cooked beef samples during storage at 2°C for 15 d.

Materials and Methods

Experimental design and statistics

The study was a factorial design with 4 treatments (control, 1.5% MM, 1.5% MM + 20 ppm sodium nitrite, 1.5% MM + 40 ppm sodium nitrite), 3 cooked meat storage times (1, 8, and 15 d), and 3 replicates of the experiment. The treatment means were calculated by analysis of variance (ANOVA) using STATISTICA™ software (Statsoft Inc., Tulsa, Okla., U.S.A.). Significant differences among means were determined by

calculation of Fisher's least significant difference (LSD) values. Significance was defined at $P < 0.05$.

Sample preparation

Milk mineral (MM) is a dried, white, free flowing powder obtained from Glanbia Foods (Twin Falls, Id., U.S.A.). The composition of MM is shown in Table 1.

Table 1 - Composition of milk mineral[a]

Constituent	% of Total Weight
Mineral	80.2%
Inorganic mineral (ash)	71.2%
Organic mineral (citrate)	9.0%
Calcium	24.0%
Phosphorus	13.5%
Water	4.0%
Lactose	10.0%
Protein	5.0%
Fat	0.5%
Typical Particle Size	< 7-μm dia

[a] Source – TruCal™ specifications – Glanbia Foods Inc., Twin Falls, Idaho.

The treatments were formulated as described in Table 2. All 4 treatments had 10% water and 1.5% salt, based on meat weight. The samples (500 g each) were formulated by manually mixing the ingredients in the amounts listed.

Meatballs (treatments 1 and 2) were cooked in a boiling water bath to an internal temperature of 85°C as measured with a Versatuff 396 digital thermometer with micro-needle probe (Atkins Technical Inc., Gainesville, Fla., U.S.A.).

Table 2 - Formulation of beef meatballs and beef sausage

Treatment	Constituents (% meat weight)
Control	Ground beef, 10.0% water, 1.5% salt, made into meatballs
Milk mineral	Ground beef, 10.0% water, 1.5% salt, 1.5% milk mineral, made into meatballs
Milk mineral + sodium nitrite 20 ppm	Ground beef, 10.0% water, 1.5% salt, 1.5% milk mineral, 20-ppm sodium nitrite, made into sausage
Milk mineral + sodium nitrite 40 ppm	Ground beef, 10.0% water, 1.5% salt, 1.5% milk mineral, 40-ppm sodium nitrite, made into sausage

Nitrite cured sausages (Treatments 3 and 4) in fibrous cellulose casings were cooked to an internal temperature of 74°C. After cooking, products were placed in resealable plastic bags (S.C. Johnson and Son Inc., Racine, Wis., U.S.A.), cooled for 10 to 15 min at room temperature and stored for 1, 8, or 15 d at 2°C.

Cooked yield

Raw meatballs were weighed. After cooking, meatballs were held at room temperature for 10 min. The fluid exudate (drip) was drained off, and the samples were reweighed. Cooked yield was calculated as follows:

Cooked yield (%) = [(drained weight after cooking) / (weight before cooking)] x 100

Hunter color measurement

Hunter color lightness, redness, and yellowness (*CIE L*, a*, b**) values were measured on the meatballs and sausage samples using a Hunter Lab Miniscan portable colorimeter with a 5 mm aperture (Reston, Va., U.S.A.). The instrument was set for illuminant D-65 and 10° observer angle, and standardized using black and white standard plates.

TBA value

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978). Duplicate meat samples (0.5 g) for all the treatments were mixed with 2.5 mL of stock solution containing 0.375% TBA (Sigma Chem. Co., St. Louis, Mo., U.S.A.), 15% TCA (Mallinkrodt Baker, Inc., Paris, Ky., U.S.A.) and 0.25 N HCl.

The mixture was heated for 10 min in a boiling water bath to develop a pink color. It was then cooled in tap water and centrifuged (Sorvall Instruments, Model RC 5C, DuPont, Wilmington, Del., U.S.A.) at 6000 rpm for 10 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 other reagents of the test minus the meat.

The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 156000 M^{-1} cm^{-1} (Sinnhuber and Yu 1958). The MDA concentration was then converted to TBA number (milligrams of MDA / kilogram of meat sample) using the following equation:

TBA no. (mg/ kg) = Sample A_{532} x (1 M TBA Chromagen / 156000) x [(1 mole / L) / M] x (0.003 L / 0.5 g meat) x (72.07 g MDA / mole MDA) x (1000 g / Kg) (1)

or

TBA nr (ppm) = Sample A_{532} x 2.77 (2)

Results and Discussion

Control meatballs had a cooked yield of 65.8%, which was not different ($P < 0.05$) from the mean cooked yield of 68.7% for the meatballs containing 1.5% MM. Treatments (control, 1.5% MM, 1.5% MM + 20 ppm sodium nitrite, 1.5% MM + 40 ppm sodium nitrite) significantly affected the Hunter color redness (a^*) and yellowness (b^*) values but had no effect on lightness (L^*) values (Table 3). The redness values (pooled over storage time) from highest to lowest were 1.5% MM + 40 ppm nitrite > 1.5% MM + 20 ppm nitrite > 1.5% MM > control (Table 4). Storage days after cooking significantly affected Hunter color b^* values but had no effect on L^* or a^* values (Table 3).

Table 3 - Summary of significance ($P < 0.05$) as determined by analysis of variance (ANOVA)

	TBA	L^*	a^*	b^*
Treatment	*	NS	*	*
Storage time	NS	NS	NS	*
Treatment x storage time	*	NS	*	NS

* = significant at $P < 0.05$; NS = not significant at $P < 0.05$.

Yellowness (b^*) values were higher ($P < 0.05$) after 8 d or 15 d storage compared with day 1 samples (Table 4). The treatment x storage time interaction was significant ($P < 0.05$) for Hunter color a^* values but not for L^* or b^* values (Table 3). Control samples (without MM or nitrite) had a significant decrease in redness (a^*) values during storage, from 4.6 on day 1 to 1.3 on day 15 (Table 4). The MM and both treatments with sodium nitrite had a protective effect on color during storage, and no change was observed in cooked samples during storage (Table 4). As expected, nitrite-cured samples had a pink

color and higher redness values than control or MM samples. Redness values exhibited a concentration dependent response with higher redness values for samples with the higher level of added nitrite (40 ppm; Table 4). It was also noted that redness values significantly increased during 15 d of storage for samples treated with 1.5% MM + 40-ppm nitrite (Table 4).

Table 4 - Pooled means for treatment main effects, storage time main effects, and their interactions on Hunter color *L, *a** and *b** values[a]**

Treatment	*L**	*a**	*b**
Control	53.4	2.7[a]	13.6[b]
MM	52.1	4.7[b]	13.3[b]
Nitrite 20 ppm	53.3	7.3[c]	11.1[a]
Nitrite 40 ppm	52.9	9.0[d]	10.6[a]
$LSD_{0.05}$	NS	0.7	1.1
Storage time (d)	L*	a*	b*
1	52.3	6.3	11.4[a]
8	53.1	5.6	12.5[b]
15	53.4	5.9	12.6[b]
$LSD_{0.05}$	NS	NS	0.9
Treatment x storage time (d)	L*	a*	b*
Control x day 1	52.8	4.6[b]	13.2
Control x day 8	53.0	2.2[a]	14.5
Control x day 15	54.4	1.3[a]	13.2
MM x day 1	53.0	4.8[b]	13.1
MM x day 8	51.7	4.4[b]	13.1
MM x day 15	51.7	4.9[b]	13.8
Nitrite 20 x day 1	52.6	7.2[c]	10.4
Nitrite 20 x day 8	53.9	7.0[c]	11.1
Nitrite 20 x day 15	53.4	7.8[cd]	11.7
Nitrite 40 x day 1	50.6	8.5[de]	8.9
Nitrite 40 x day 8	53.8	8.9[de]	11.4
Nitrite 40 x day 15	54.2	9.6[e]	11.5
$LSD_{0.05}$	NS	1.3	NS

[a]LSD = least significant difference; MM = milk mineral; NS = not significant at $P < 0.05$; $LSD_{0.05}$ = significant at $P < 0.05$; means within a column with the same letter are not significant ($P < 0.05$).

With regard to TBA values, treatment main effects and the 2-way interaction of treatment x storage time were highly significant (Table 3; for detailed statistics see Appendix B). The main effect of storage time (d) did not affect TBA values, because TBA values did not change significantly with time for 3 of the 4 treatments (those containing MM; Table 3).

Figure 2 shows the 2-way interaction for treatment x day effects on TBA values of cooked products. TBA values of control meatballs increased to > 6.0 during 15 d of refrigerated storage (Figure 2). Meatballs with 1.5% MM had lower ($P < 0.05$) TBA values than the control meatballs. Sausages with 1.5% MM and 20-ppm or 40-ppm sodium nitrite also had TBA values lower than control samples but not significantly different from the treatment with MM alone (Figure 2).

Cornforth and West (2002) previously reported that cooked ground beef and pork required 2% MM to maintain TBARS values < 1.0 after 14 d of storage, compared with 1% MM for ground turkey. TBARS values of cooked ground beef were lower ($P < 0.05$) when MM was added in water suspension, rather than as a dry powder. Among MM components (phosphate, Ca, and citrate), polyphosphates most effectively maintained low TBA values during storage. The authors concluded that MM chelates soluble iron to colloidal calcium phosphate particles, thus removing iron as a catalyst for lipid oxidation (Cornforth and West 2002). Lactoferrin is a milk protein that binds iron and thus may possibly contribute to antioxidant effects of MM. However, the antioxidant contribution of lactoferrin in MM is small. Lactoferrin in TruCal™ MM is non-detectable by immunoassay. MM contains only 5% protein consisting entirely of α-lactalbumin (MW 14000) and β-lactoglobulin (MW 18500) (Bastian 2005).

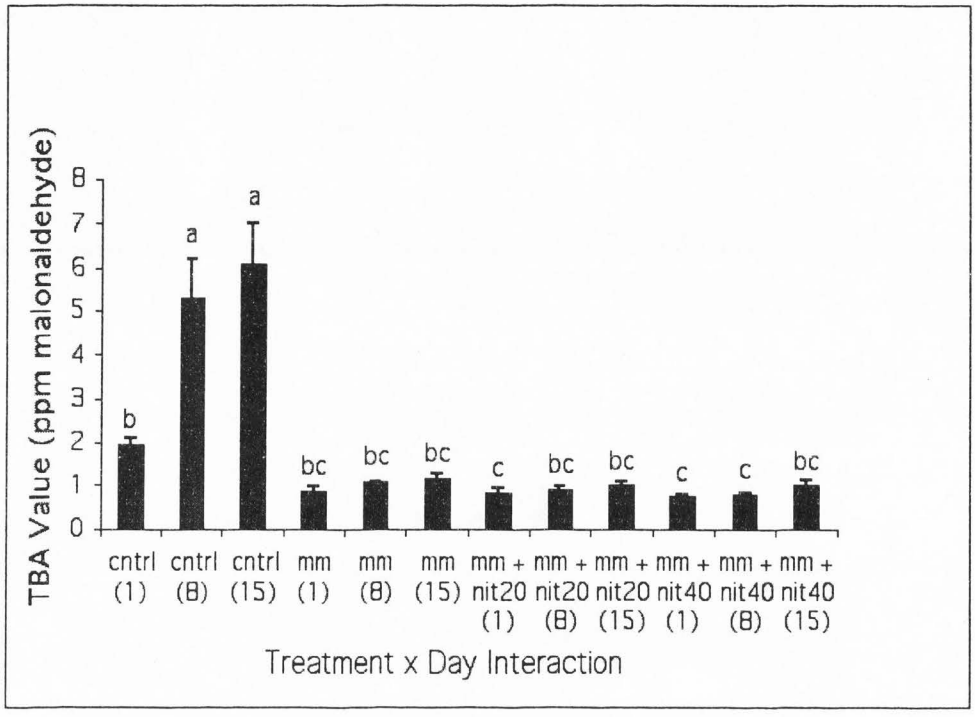

Figure 2 - Mean thiobarbituric acid (TBA) values + standard error of the mean (SEM) for treatment X storage time interactions (1, 8, or 15 d storage at 2°C). Treatments were control without antioxidants (cntrl), 1.5% milk mineral (mm), 1.5% MM + 20 ppm sodium nitrite (mm + nit 20), and 1.5% MM + 40-ppm sodium nitrite (mm + nit 40). Mean values (bars) with the same superscript letter are not different ($P < 0.05$).

Jayasingh and Cornforth (2003) compared the antioxidative activity of 0.5% to 2.0% MM with that of butylated hydroxytoluene (BHT) and sodium tripolyphosphate (STPP) in raw and cooked pork mince during frozen (-20°C) or cold (2°C) storage. In addition, effects of holding time before serving were investigated on the TBA values of pork patties, and the impact of TBA values on sensory acceptability was determined. The different treatments had no effect on the oxidative stability of raw meat (Jayasingh and Cornforth 2003). However, cooked samples with MM or STPP had significantly lower

TBA values than were observed for the treatment with BHT. TBA values of cooked patties did not significantly increase during 0 to 60 min of holding time, but TBA values were significantly higher after 90 or 120 min. Sensory panelists preferred patties with TBA values < 0.5, compared with patties with TBA values > 1.4 (Jayasingh and Cornforth 2003).

In the United States, sausages are typically formulated with 156 ppm sodium nitrite. However, cured pink color development occurs with as little as 14 ppm sodium nitrite in beef rounds or 4 ppm in pork shoulder cuts (Heaton and others 2000). The USDA-FSIS permits nitrite levels as low as 40 ppm in bacon, in combination with sugar and starter cultures, so that fermentation occurs (USDA 1999). Inhibition of *Clostridium botulinum* is achieved by product acidification during fermentation.

In the present study, sausages with 20 ppm or 40 ppm sodium nitrite were both pink, but pink color was most intense in sausages with 40 ppm nitrite after 15 d of storage. There were no additional antioxidant effects of 1.5% MM with sodium nitrite on TBA values during storage of beef sausages. MM (1.5%) alone was sufficient to maintain low TBA values during storage. Addition of 20 ppm or 40 ppm nitrite to samples containing 1.5% MM did not decrease the TBA values during storage, compared with samples with MM alone.

Conclusions

Milk mineral (1.5%) was very effective for inhibition of oxidation in cooked meatballs during 15 d of refrigerated storage. Thus, MM has potential application as an antioxidant for addition to ground meatballs before cooking. Addition of 20 ppm or 40

ppm sodium nitrite to sausages containing 1.5% MM did not result in lower TBA values. Thus, there was no additional antioxidant effect between 1.5% MM and sodium nitrite for improving oxidative stability of cooked beef sausages.

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CHAPTER 4

EVALUATION OF GARAM MASALA SPICES AND PHOSPHATES AS ANTIOXIDANTS IN COOKED GROUND BEEF

Abstract

This study determined antioxidant effects and sensory attributes of individual ingredients (black pepper, caraway, cardamom, chili powder, cinnamon, cloves, coriander, cumin, fennel, ginger, nutmeg, salt, star anise) in an Indian spice blend (Garam Masala), in cooked ground beef. Thiobarbituric acid (TBA) values were measured as an indicator of rancidity for cooked samples on 1, 8, or 15 d refrigerated storage. Cooked samples were evaluated by a trained panel (n = 13) for intensity of rancid odor / flavor, beef flavor, and spice flavor and correlated with TBA values of same day samples. We also investigated possible additive effects between spice antioxidants and iron binding (Type II) antioxidants on lipid oxidation by measuring TBA values. All spices had antioxidant effects on cooked ground beef, compared to controls. Among spices, cloves were the most effective in controlling lipid oxidation, with TBA values of 0.75, after 15 d refrigerated storage. All spices at their recommended levels lowered rancid odor and flavor in cooked ground beef, compared to controls. As expected, most spices also imparted distinctive flavors to cooked ground beef. There was a positive correlation (0.77) between TBA values on 15 d and rancid odor / flavor. Type II antioxidants (such as iron-binding phosphate compounds) were more effective than individual Type I antioxidants (such as spices and butylated hydroxytoluene), for maintenance of low TBA values in cooked ground beef during storage. Additive effects were observed with

rosemary + milk mineral or sodium tripolyphosphate (STPP), compared to rosemary alone.

Introduction

According to the American Spice Trade Association, a spice is "any dried plant product used primarily for seasoning purposes." This includes herbs, spice seeds, roots, spice blends and other plant-based elements. Spices such as cloves, cinnamon, black pepper, turmeric, ginger, garlic and onions exhibit antioxidant properties in different food systems (Younathan and others 1980; Al-Jalay and others 1987; Jurdi-Haldeman and others 1987). Antioxidative effects of dried and ethanolic extracts of spices (marjoram, wild marjoram, caraway, peppermint, clove, nutmeg, curry powder, cinnamon, sage, basil, thyme, and ginger) on the oxidative stability of fresh minced chicken meat, and fresh and microwave cooked pork patties pretreated with NaCl, and subjected to either refrigerated or frozen storage (4 and -18°C, respectively) have been investigated, and results show that application of dried spices to chicken meat inhibited lipid oxidation in frozen samples, with dried marjoram, wild marjoram and caraway having the highest antioxidative activity (Abd-El-Alim and others 1999).

Retail Garam Masala spice blends have up to 13 different ingredients including black pepper, caraway, cardamom, chili, cinnamon, cloves, coriander, cumin, fennel, ginger, nutmeg, salt, and star anise in varying levels. One example composition includes black pepper (10%), cardamom (30%), cinnamon (5%), cloves (5%), coriander (25%), cumin (20%), and nutmeg (5%; http://www.labellecuisine.com).

Previous work has identified numerous antioxidant compounds in the various spices of Garam Masala. Black pepper *(Piper nigrum)* has piperine as the active antioxidant compound (Badmaev and others 2000). Caraway *(Carum carvi)* has been shown to have high antioxidant activity in cooked, frozen chicken meat (Abd-El-Alim and others 1999). The ethyl acetate-soluble fraction of cardamom *(Elletoria cardamomum)* has been shown to have a high radical-scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Kikuzaki and others 2001). Chili peppers *(Capsicum annuum)* contain capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide), which is an antioxidant (Surh 1999). Antioxidant activity in fresh chili peppers has also been attributed to presence of ascorbic acid, flavonoids, and phenolic acids (Jimenez and others 2003). However, ascorbic acid content is undoubtedly lower in dried chili powder compared to fresh fruit. Cinnamon *(Cinnamonum verum)* contains cinnamic aldehyde, which gives it a distinct flavor and odor. Cinnamon was a better superoxide radical scavenger than mint, anise, BHA, ginger and BHT (Murcia and others 2004). Cloves *(Syzygium aromaticum)* have been shown to have antioxidant activity partly due to the presence of aroma compounds such as eugenol and eugenyl acetate (Lee and Shibamoto 2001). Cloves have the strongest antioxidative activity among Chinese 5-spice ingredients, in cooked ground beef during refrigerated storage (Dwivedi and others 2006).

Linalool is the main antioxidative compound in coriander *(Coriandrum sativum)* (Reddy and Lokesh 1992). Coriander extract demonstrates antioxidative activity alone and in synergism with BHT and thus an aqueous extract of coriander has potential for use as an antioxidant preparation in foods (Melo and others 2003). Cumin *(Cuminum*

cynicum) has cumin aldehyde as the principal contributor to aroma, flavor, and antioxidant properties (Reddy and Lokesh 1992). Extracts of plants like cumin having high levels of phenolic compounds exhibited strong H-donating activity and are effective scavengers of hydrogen peroxide and superoxide radicals (Lugasi and others 1995). Fennel *(Foeniculum vulgare)* has been shown to have in-vitro antioxidant activity (Oktay and others 2003). The antioxidant compounds in fennel include 3-caffeoylquinic acid, rosamirinic acid, and quercetin-3-O-galactoside (Parejo and others 2004). Ginger *(Zingiber officinale)* has been shown to have gingerol-related compounds and diarylheptanoids as the main antioxidant fractions (Nakatani 2003). Nutmeg *(Myristica fragrans Houtt)* contains about 10% essential oil, which is primarily composed of terpene hydrocarbons (pinenes, camphene, p-cymene, sabinene, phellandrene, terpinene, limonene and myrcene; 60 to 90%), terpene derivatives (linalool, geraniol and terpineol; 5 to 15%) and phenylpropanes (myristicine, elemicine and safrol; 2 to 20%; Nakatani 2003). These compounds are responsible for the antioxidant properties of nutmeg.

Salt has been shown to have both pro-oxidant and antioxidant effects in meat products. TBA values increased with increasing salt concentration (0-2%) in frozen ground pork during 10 wk storage (Lee and others 1997). On the other hand, phosphate buffer (25mM NaH2PO4/Na2HPO4), high final pH (7.0) of surimi pellet, and the presence of salt (0.1M NaCl) all inhibited both protein and lipid oxidation during storage. (Subramanian and others 1996). Salaeh and Muangwong (2001) have found that protocatechuic acid had more antioxidant activity than BHT in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, and could be used as a marker for radical scavenging activity of star anise *(Illicium Verum).*

Although the individual components of Garam Masala have been shown to have antioxidant activity in model systems, our first objective was to compare individual spices of Garam Masala spice blend for their antioxidant effect in cooked ground beef. Sensory evaluation was also done on cooked ground beef containing the Garam Masala individual spices. Finally, tests were also conducted to evaluate possible additive effects of Type I antioxidants (cinnamon, clove, BHT, ground rosemary), and Type II iron-chelating antioxidants (milk mineral, STPP), when used together in cooked ground beef.

Materials and Methods

Comparison of TBA values during storage

To compare TBA values during storage, a factorial design was used with 14 ground beef treatments (black pepper, caraway, cardamom, chili powder, cinnamon, cloves, coriander, cumin, fennel, ginger, nutmeg, retail Garam Masala, salt and star anise), at four levels (0, 0.1, 0.5, or 1.0 % of raw meat weight), 3 storage times (1, 8, or 15 d), and 3 replicates of the entire experiment. Thiobarbituric acid values (duplicates for each sample) were measured as an indicator of rancidity at 1, 8, or 15 d storage of cooked ground beef crumbles at 2°C. The lowest effective (recommended) spice level among the levels tested in this study (0.1, 0.5, or 1%), for each individual spice was determined as the lowest spice concentration that resulted in TBA values significantly lower than the controls (0% spice), or other spice levels.

Ground cardamom, cinnamon, chili powder, cloves, cumin, fennel, star anise, ginger (McCormick & Co. Inc., Hunt Valley, Md., U.S.A.), ground black pepper (Inter-American Foods Inc., Cincinnati, Oh., U.S.A.), ground coriander (Spice Islands Trading

Co., San Francisco, Calif., U.S.A.), ground nutmeg (Pacific Foods, Kent, Wa., U.S.A.), ground caraway (Philips Foods Inc., San Francisco, Calif., U.S.A.), non-iodized salt (Morton Intl. Inc., Chicago, Ill., U.S.A.), retail Garam Masala blend (MDH Garam Masala blend, Mahashian Di Hatti Ltd, New Delhi, India), and lean ground beef chuck (20% fat) were purchased locally. Each spice was manually mixed with ground beef (100 g / treatment) at 0.1, 0.5, and 1.0% levels. Mixed samples were thoroughly cooked at 163°C for 5 min on a Teflon coated electric skillet (West Bend Co, West Bend, Wis., U.S.A.), with intermittent stirring to avoid burning. Cooking was done to achieve a final internal temperature of 82°C to 85°C, as measured using a VersaTuff Plus 396 digital thermometer (Atkins Technical, Inc, Gainesville, Fla., U.S.A.) with a thin probe for fast response. Portions (10 g) of cooked beef crumbles were placed in ziploc bags, and temperature of the crumbles was measured. The cooked ground beef crumbles were placed in re-sealable plastic ziploc bags (S.C. Johnson and Son, Inc., Racine, Wis., U.S.A.), cooled for 10 to 15 min at room temperature and stored for 1, 8 or 15 d at 2°C. Thiobarbituric acid values were measured in duplicate at 1, 8 or 15 d on the cooked samples as an indicator of oxidative rancidity. For each ingredient spice the experiment was replicated 3 times. Duplicate sample analysis was performed. Duplicates were not averaged prior to data entry. Thus, there were 6 observations per treatment (3 replicates x 2 duplicates = 6 observations per treatment).

The thiobarbituric acid assay was performed as described by Buege and Aust (1978). Duplicate samples (0.5 g) for all treatments 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 to develop a pink color, cooled in tap water and then centrifuged (Sorvall Instruments, Model RC 5C, DuPont, Wilmington, Del., U.S.A.) at 6000 rpm for 10 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 except the meat. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 156,000 / mole / cm for the pink TBA-MDA pigment (Sinnhuber and Yu 1958). The absorbance values were converted to ppm malonaldehyde by using the following equations:

1) TBA # (mg/ kg) = Sample A_{532} x (1 M TBA Chromagen / 156,000) x [(1 mole / L) / M] x (0.003 L / 0.5 g meat) x (72.07 g MDA / mole MDA) x (1000 g / Kg), or

2) TBA # (ppm) = Sample A_{532} x 2.77 (where MDA = malonaldehyde).

Sensory evaluation

Cooked beef samples made with spices at their lowest effective levels determined previously were evaluated for intensity of rancid odor, rancid flavor, beef flavor and spice flavor. A total of 19 treatments were evaluated with 0.1% level for cinnamon, cloves, retail Garam Masala, and salt; 0.5% level for black pepper, chili powder, coriander, cumin, fennel, ginger, nutmeg and star anise; 1.0% level for caraway and cardamom; fresh control, 15 d rancid control, 1.5% milk mineral (dried, white, free-flowing powder, consisting primarily of colloidal calcium phosphate particles; Cornforth and West 2002), 0.4% ground rosemary, and 0.5% level for STPP control. The rancid control was cooked ground beef without added spices and held 15 d at 2°C. The fresh control was cooked ground beef without spices, prepared on the day of the panel. Trained

panelists (n = 13) evaluated samples after 15 d of storage at 2°C. TBA values were measured in duplicate on the samples that were served to the panelists on the same day as the panel evaluation (15 d storage time). Duplicates were not averaged prior to data entry. Thus, for TBA values, there were 6 observations per treatment (3 replicates x 2 duplicates = 6 observations per treatment). Spice treatments and 3 control samples were cooked, packaged and stored as previously described.

All panelists had previous sensory panel experience with cooked beef products. The panelists were trained in two sessions. In the first session, panelists were familiarized with the 5-point intensity scale and its usage. Panelists were also familiarized with cooked beef flavor (both fresh and rancid samples) and cooked ground beef with each individual added spice and Garam Masala spice blend at low (0.1%) and high (1.0%) spice concentrations. Group discussion was conducted regarding sample attributes. In the second session, panelists again evaluated the same samples. The most consistent panelists (n = 13) were included in the final sensory panel.

The 16 treatment samples at recommended concentrations as determined previously, and 3 controls of cooked beef crumbles were evaluated in 5 sessions. A set of 6 or 7 samples (6 g each) were served to each panelist in each session, consisting of 3 or 4 spice-treated samples and 3 controls. Samples were coded and microwave re-heated for 25 s to attain a temperature of 80°C to 85°C immediately before serving. Samples were evaluated in individual booths under red lights. The serving order was randomized to avoid positional bias. Panelists were asked to evaluate samples for intensity of rancid odor, rancid flavor, beef flavor, and spice flavor on a 5- point scale, where 1 = no flavor or odor, 2 = slightly intense, 3 = moderately intense, 4 = very intense, and 5 = extremely

intense flavor or odor. Panelists were also asked to provide additional qualitative comments for each sample. Before evaluating the next sample, ballot instructions specified that the previous sample be expectorated into cups provided for that purpose. Panelists were instructed to rinse their mouths with tap water. Unsalted crackers were also provided to cleanse the palate.

Comparison of Type I and Type II antioxidant effectiveness

Four Type I antioxidants (ground cinnamon 0.5%, ground cloves 0.1%, ground rosemary 0.4%, and BHT 0.01% of meat weight) and two Type II antioxidants (1.5% milk mineral, Cornforth and West 2002; 0.5% sodium tripolyphosphate, USDA maximum) and various combinations of each at half their lowest effective levels, were evaluated for antioxidant effects after 1, 8, or 15 d refrigerated storage at 2°C. The effective levels for cinnamon (0.5%) and cloves (0.1%) were obtained by previous work (Dwivedi and others 2006). The effective level for ground rosemary (0.4%) was found by preliminary experiments in this lab. A total of 17 treatments were evaluated as follows: control, BHT 0.01% of meat weight, cinnamon 0.5%, cloves 0.1%, rosemary 0.4%, MM 1.5%, STPP 0.5%, BHT 0.005% + MM 0.75%, cinnamon 0.25% + MM 0.75%, cloves 0.05% + MM 0.75%, rosemary 0.2% + MM 0.75%, BHT 0.005% + STPP 0.25%, cinnamon 0.25% + STPP 0.25%, cloves 0.05% + STPP 0.25%, rosemary 0.2% + STPP 0.25%, BHT 0.005% + cinnamon 0.25% + cloves 0.05% + rosemary 0.2%, and MM 0.75% + STPP 0.25%.

All 17 treatments were prepared by mixing the various levels of Type I and Type II antioxidants in 300 g ground beef. Mixed samples were thoroughly cooked at 163°C

for 5 min on a grill, with stirring to avoid burning. Cooking was done to achieve a final internal temperature of 82°C to 85°C, as measured using a VersaTuff Plus 396 digital thermometer (Atkins Technical, Inc, Gainesville, Fla., U.S.A.) with a thin probe for fast response. The cooked ground beef crumbles were placed in re-sealable plastic bags, cooled for 10 to 15 min at room temperature and stored for 1, 8, or 15 d at 2°C. Two sampling methods were compared for their effects on TBA values of cooked samples during storage. In method 1, samples were obtained 3 times (1, 8, or 15 d) from the same bag (100 g ground beef per treatment). In method 2, sample bags (100 g each) were prepared separately for sampling after storage at 1, 8, or 15 d. Comparison of TBA values between method 1 and 2 allowed determination of the possible higher TBA values in method one, from the repeated opening and closing of the same bag during 15 d storage. Thiobarbituric acid (TBA) values were measured in duplicate at 1, 8, or 15 d on the cooked samples as an indicator of oxidative rancidity. The entire experiment was replicated 3 times. Duplicate sample analysis was performed.

Statistical analysis

Mean TBA values for various spices of Garam Masala spice blend were calculated and compared by analysis of variance using the proc GLM function in SAS version 9.0 (SAS Institute Inc., Cary, N.C., U.S.A.). Statistical significance was identified at the 95% confidence level, and post hoc means comparisons were made based on P-values obtained using the Tukey-Kramer adjustment. Treatment means for sensory values and TBA values were also calculated using the SAS program. Correlation coefficients were calculated among sensory panel scores and TBA values. Significance

was defined at $P < 0.001$ for correlation coefficients. To compare type I and type II antioxidant effectiveness, treatment means were calculated by ANOVA using Statistica™ software (Statsoft Inc, Tulsa, Okla., U.S.A.). Significant differences among means were determined by calculation of Fisher's least significant difference (LSD) values. Significance was defined at $P < 0.05$ for ANOVA and LSD values.

Results and Discussion

Comparison of TBA values during storage

The main effects of spice treatment, storage time (1, 8, or 15 d), spice level (0, 0.1, 0.5, or 1.0%), the two-way interactions between spice treatment * storage time, spice treatment * spice level, and spice treatment * spice level were all significant at $P < 0.05$. The three-way interaction between spice treatment * storage time * spice level was not significant at $P < 0.05$.

Table 5 shows the spice treatment * spice level interaction mean for TBA values for various individual spices of Garam Masala and for the retail Garam Masala spice blend. The 5 spices of Chinese 5-spice (black pepper, cinnamon, cloves, fennel, and star anise) are also ingredients of the Garam Masala spice blend. In Table 5, the mean TBA values for cooked ground beef + individual Chinese 5-spice ingredients were the same as recently published from this laboratory (Dwivedi and others 2006; Appendix A). These values are included here in order to statistically compare all 13 ingredients of Garam Masala. For cinnamon, cloves, and retail Garam Masala, the lowest effective level was 0.1% (Table 5). For each spice treatment, the lowest effective spice level among levels tested in this study (0.1, 0.5, or 1%) was defined as the lowest spice concentration that

resulted in TBA values significantly lower than the controls (0% spice), or other spice levels. For black pepper, chili, coriander, cumin, fennel, ginger, nutmeg, and star anise, the lowest effective level was found to be 0.5% (Table 5). Caraway and cardamom were found to have a lowest effective level of 1.0% (Table 5).

Table 5 - Mean TBA ± standard deviation values pooled over storage time, for the 2-way interaction of treatment x spice level (0, 0.1, 0.5, or 1.0% of raw meat wt)

Spice	0.0% level	0.1% level	0.5% level	1.0% level
Black Pepper	3.43 ± 1.32 a	2.87 ± 1.37 a	1.28 ± 0.42 b	1.26 ± 0.41 b
Caraway	3.58 ± 1.69 a	2.40 ± 0.99 b	2.66 ± 1.25 ab	1.26 ± 0.74 c
Cardamom	3.43 ± 1.32 a	2.70 ± 1.46 ab	2.21 ± 1.02 b	1.11 ± 0.21 c
Chili Powder	3.58 ± 1.69 a	2.33 ± 0.92 b	1.13 ± 0.58 c	1.08 ± 0.26 c
Cinnamon	4.15 ± 2.29 a	1.66 ± 1.30 b	0.76 ± 0.44 b	0.78 ± 0.40 b
Cloves	3.58 ± 1.69 a	0.76 ± 0.22 b	0.97 ± 0.32 b	0.88 ± 0.28 b
Coriander	3.45 ± 1.41 a	2.39 ± 1.20 b	1.61 ± 0.63 bc	1.03 ± 0.19 c
Cumin	3.45 ± 1.41 a	2.75 ± 1.20 a	1.08 ± 0.33 b	1.04 ± 0.21 b
Fennel	2.84 ± 1.59 a	2.32 ± 1.40 ab	1.40 ± 1.07 bc	0.99 ± 0.74 c
Ginger	4.29 ± 2.25 a	2.51 ± 2.19 b	0.88 ± 0.25 c	1.33 ± 0.99 c
Nutmeg	3.43 ± 1.32 a	2.16 ± 0.81 b	0.97 ± 0.24 c	1.04 ± 0.19 c
Retail Garam Masala	3.15 ± 1.34 a	1.73 ± 0.83 b	1.29 ± 0.51 b	0.82 ± 0.13 b
Salt	3.45 ± 1.41 a	2.89 ± 1.39 ab	1.92 ± 0.91 b	2.27 ± 1.00 b
Star Anise	3.18 ± 1.76 a	2.55 ± 1.42 a	0.97 ± 0.55 b	0.71 ± 0.38 b

a-c - means with the same letter within a row are not significantly different ($p < 0.05$).

Figure 3 compares TBA values of spice treatments after 15 d storage, in order to determine which spices have greatest antioxidant effect over time at their lowest effective tested level. The TBA values after 15 d storage were as high as 4.00 for 0.1% salt, and as low as 0.75 for 0.1% clove samples and 0.89 for 0.5% ginger samples (Figure 3; for detailed statistics see Appendix C). Thus, cloves were the most potent antioxidant spice of Garam Masala. Even the lowest clove level of 0.1% was sufficient to maintain TBA values < 1.0 for cooked ground beef after refrigerated storage for 15 d, where TBA

values > 1.0 are usually associated with the perception of rancid flavor (Tarladgis and others 1960; Jayasingh and Cornforth 2003). Cloves were also the most potent antioxidant spice in Chinese 5-spice (Dwivedi and others 2006; Appendix A).

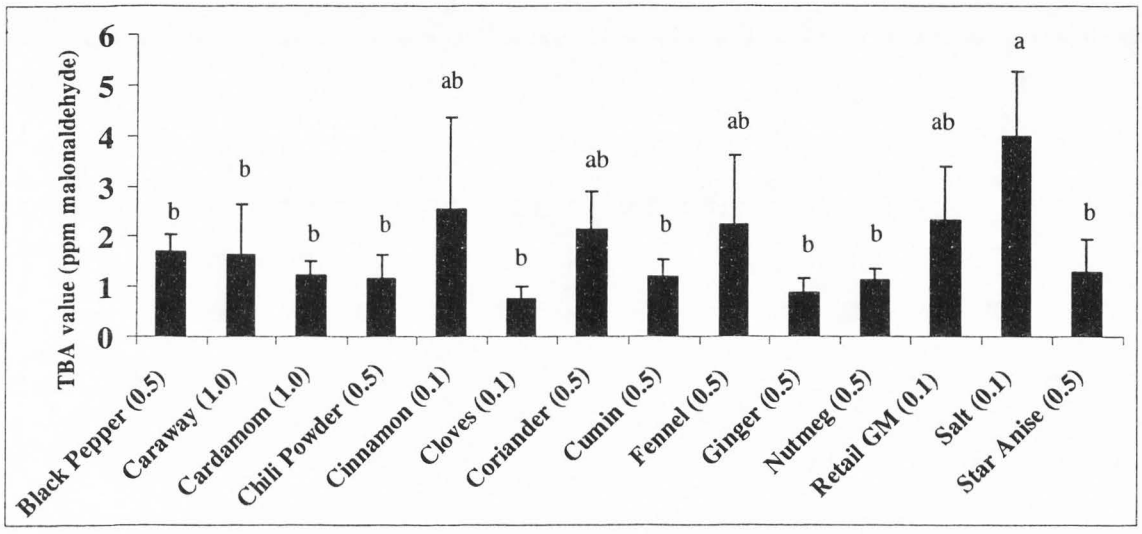

Figure 3 - Comparison of mean TBA values after 15 d storage for cooked ground beef formulated with spices used in Garam Masala, at their recommended levels as determined in experiment 1.The Y-axis error bars show standard deviation from the mean. a-b – mean TBA values with the same letters are not significantly different (P < 0.05).

Most previous studies of antioxidant effects of spices have been conducted in model systems, however a few antioxidant studies have been conducted in food systems. Cloves at 0.05% enhanced the storage stability and acceptability of frozen fish mince for about 28 wk and for 50-wk storage, an addition rate of 0.1% was optimal (Joseph and others 1992). Clove powder at 0.2% w/w significantly reduced oxidative rancidity and improved acceptability of oysters. The oysters remained acceptable for 278 d when treated with cloves as compared to 235 and 237 d for BHT-treated and untreated samples, respectively (Abraham and others 1994). In Chinese marinated pork shanks, antioxidant

effects were observed compared to controls, and attributed to star anise as a marinade ingredient (Wang and others 1997). Ground black pepper oleoresin extracted by supercritical carbon dioxide was more effective in reducing lipid oxidation of cooked ground pork than oleoresin extracted by conventional methods (Tiprisukond and others 1998). Cinnamon essential oil has been shown to have great antioxidant activity in Chinese-style sausages (Ying and others 1998).

In the present study, 0.5% ginger also maintained TBA values < 1.0 for 15 d refrigerated storage. In agreement with this finding, ginger extract (3%) has been effectively used for improving the sensory quality and shelf life of cooked mutton chunks (Mendiratta and others 2000). Fresh pork treated with 5% ginger extract in combination with lactic acid (1%), liquorice (1%), acetic acid (1%) and garlic extract (4%) has been shown to maintain freshness for 144 h as compared to control pork that remained fresh for 24 to 48 h (Zhang and others 1996).

Sensory evaluation results

Trained panel sensory evaluation was done for cooked ground beef with individual Garam Masala spices at their previously determined recommended levels (Figure 3) compared to various control samples, after 15 d refrigerated storage. Table 6 shows the rancid odor / flavor scores for all treatments. The 15-d rancid control sample (without added spices) and the salt sample had the highest scores for rancid odor (3.3 and 2.7 respectively; Table 6), where a score of 3.0 indicates moderately intense rancid odor. These 2 samples were significantly higher than others for rancid odor intensity (Table 6).

Table 6 – Mean trained panel sensory scores and thiobarbituric acid (TBA) values of spice-treated, cooked ground beef crumbles after 15 d storage at 2°C. Recommended spice levels were used as determined from Table 5

Treatment	Use level (% meat weight)	Rancid odor	Rancid flavor	Beef flavor	Spice flavor	TBA value	Qualitative comments
Black Pepper	0.5	1.4 b	1.2 b	2.1 bc	3.2 ab	1.6 fg	Peppery, hot
Caraway	1.0	1.8 b	1.6 b	2.1 bc	2.6 b-d	3.9 c	Spicy, dill like flavor
Cardamom	1.0	1.4 b	1.1 b	2.1 bc	2.6 b-d	3.2 cd	Spicy, Mexican spice flavor
Chili Powder	0.5	1.4 b	1.4 b	2.0 c	1.9 c-g	1.7 e-g	Bland, pizza spice like flavor
Cinnamon	0.1	1.1 b	1.1 b	1.7 c	2.9 a-c	1.6 fg	Cinnamon flavor, spicy
Cloves	0.1	1.0 b	1.1 b	2.2 bc	3.1 ab	0.4 fg	Strong clove flavor, smells like dentist's office
Coriander	0.5	1.4 b	1.3 b	2.0 c	2.2 b-f	3.4 c	Spicy
Cumin	0.5	1.6 b	1.7 b	1.9 c	2.5 b-e	4.4 bc	Spicy, taco style spice, licorice flavor
Fennel	0.5	1.5 b	1.6 b	1.9 c	3.1 ab	5.5 b	Licorice flavor, spicy
Ginger	0.5	1.4 b	1.4 b	2.6 a-c	1.6 d-g	1.0 fg	Weak spice flavor and odor
Nutmeg	0.5	1.3 b	1.2 b	1.6 c	2.5 b-e	3.1 c-e	Spicy, nutmeg like flavor
RGM	0.1	1.1 b	1.1 b	1.9 c	3.1 ab	0.7 fg	Spicy flavor
Salt	0.1	2.7 a	2.6 ab	2.2 bc	1.4 e-g	7.1 a	Salty flavor
Star Anise	0.5	1.2 b	1.0 b	1.8 c	3.9 a	1.9 d-f	Licorice flavor, spicy
Fresh Beef	0.0	1.5 b	1.5 b	3.2 a	1.1 fg	1.1 fg	Steak-like, oily, beefy
15 d RBC	0.0	3.3 a	3.4 a	2.0 c	1.0 g	7.2 a	Rancid, painty, stale

Treatment	Use level (% meat weight)	Rancid odor	Rancid flavor	Beef flavor	Spice flavor	TBA value	Qualitative comments
MM	1.5	1.4 b	1.6 b	2.3 a-c	1.1 fg	0.4 g	Bland flavor
Rosemary	0.4	1.1 b	1.1 b	2.1 bc	3.1 ab	0.8 fg	Rosemary like flavor
STPP	0.5	1.4 b	1.4 b	3.0 ab	1.1 fg	0.3 g	Beefy, salty

a-g - means with the same letters in a column are not significantly different ($P < 0.05$). Abbreviations; RGM = retail Garam Masala; 15 d RBC = 15 d rancid beef control; MM = milk mineral; STPP = sodium tripolyphosphate.

The lowest rancid odor intensity scores were for clove treated samples, with a mean score of 1.0, indicating that these samples had no rancid odor at all (Table 6).

Rancid flavor intensity scores were highest ($P < 0.05$) for the 15 d rancid control sample with a score of 3.4, followed by the salt treated sample at 2.6 (Table 6). The lowest rancid flavor intensity score was 1.0 for the star anise treated samples, showing that these samples had no detectable rancid flavor (Table 6).

As expected, the highest beef flavor intensity scores (3.2) were observed for fresh cooked beef control samples, corresponding to moderately (3.0) to very intense (4.0) beef flavor (Table 6). The lowest beef flavor intensity scores (1.6) were obtained for nutmeg samples, indicating that these samples had no beef flavor (1.0) to slightly intense beef flavor (2.0; Table 6).

All spices had antioxidant effects with 15 d storage TBA values significantly ($P < 0.05$) less than the rancid control sample (Table 6). Most spices also had a strong masking effect to reduce the perception of rancid flavor / rancid odor. For example, coriander treated sample had a 15 d TBA value of 3.4, which is directly associated with rancid flavor and odor as compared to fresh control beef sample with TBA value of 1.1.

However, the sensory scores for rancid odor (1.4) and rancid flavor (1.3) for coriander samples were low, and comparable to fresh control beef sample with scores of 1.5.

The highest spice flavor intensity was recorded for star anise treated samples, with values as high as 3.9, where a value of 4.0 corresponds to very intense spice flavor (Table 6). The lowest spice flavor intensity values were obtained for the 15 d rancid control beef sample with values of 1.0, and the fresh beef control (1.1). Controls with MM and STPP added as antioxidants also had low spice flavor intensity scores of 1.1, indicating that these samples had no detectable spice flavor, as expected (Table 6).

The 15 d rancid control sample and the salt treated sample had significantly higher TBA values (7.2 and 7.1, respectively), compared to other samples (Table 6). At their recommended level, several spices (black pepper, cinnamon, chili powder, cloves, ginger, and the retail Garam Masala blend) had 15 d TBA values as low as the control antioxidant treatments formulated with STPP or milk mineral (Table 6). Caraway, cardamom, coriander, cumin, fennel, nutmeg, and star anise at their recommended level, had less antioxidant activity as seen by TBA values, than the aforementioned spices (Table 6).

Panel comments indicated that all added spices imparted some type of spice flavor to the cooked ground beef samples (Table 6). For instance, sample with black pepper was described as peppery, and caraway treated samples had a dill-like flavor. Cardamom imparted a hot Mexican spice flavor. Samples containing chili powder had a pizza spice-like flavor. Samples with added cinnamon tasted cinnamony. Clove-treated samples were described as having a strong odor reminiscent of a dentist's office. Samples with coriander had a spicy flavor, while cumin imparted a taco-style spicy flavor. Fennel and

star anise both imparted a licorice flavor. Ginger treated ground beef had a weak odor and flavor. Nutmeg imparted its own characteristic nutmeg-like flavor. Ground beef samples containing retail Garam Masala were described as having a spicy flavor. Samples with added salt were described as salty. The 15 d rancid control samples were described as having a rancid, painty or stale flavor. The controls with STPP were described as beefy or salty, whereas the fresh control sample was described as beefy or oily. The milk mineral treated sample was described as having a bland flavor (Table 6).

The correlation coefficients between the various flavor intensity scores and TBA values showed that there was a high positive correlation of 0.81 between rancid odor and rancid flavor. There was also a relatively high positive correlation coefficient of 0.77 between TBA values and rancid odor or rancid flavor. There were negative correlations of –0.38, –0.36, and –0.26 between spice flavor and rancid odor, rancid flavor, and beef flavor. There was also a negative correlation (-0.42) between TBA values and beef flavor intensity. Thus, as lipid oxidation increases as shown by higher TBA values, the beef flavor intensity decreases.

Comparison of antioxidant effects between Type I and Type II antioxidants

The treatment effect and the treatment * storage time effect were found to be significant for various comparisons between Type I and Type II antioxidants. The effects of storage time, sampling method, treatment * sampling method, storage time * sampling method, and the 3 way interaction of treatment * storage time * sampling method were not significant ($P < 0.05$). Since there were no significant effects of sampling method

(method 1 or 2) or storage time on TBA values, the sampling method and storage time effects were pooled for calculation of means in the remaining tables.

Table 7 shows the mean TBA values for the various treatments of Type I and Type II antioxidants in cooked ground beef, pooled over storage time and sampling method. The control samples had mean TBA values significantly higher than all other treatments, with values of 2.50 (Table 7). All treatments with antioxidants were able to control lipid oxidation and maintain pooled mean TBA values of < 1.0 compared to controls (Table 7). Among individual antioxidants, rosemary was the least effective. Samples with rosemary had the highest pooled mean TBA value (0.84) among the individual antioxidants used, which was significantly higher than samples with BHT, cloves, MM, or STPP (Table 7).

In general, Type II antioxidants (MM and STPP), had significantly lower TBA values (0.48 and 0.42, respectively) than ground beef with Type I antioxidants, except cloves (Table 7), for control of lipid oxidation in cooked ground beef during 15 d refrigerated storage.

There was a positive additive antioxidant effect ($P < 0.05$) of rosemary + MM, and rosemary + STPP treatments to lower TBA values, compared to ground beef samples with rosemary alone (Table 7). The other combinations of Type I and Type II antioxidants were not significantly different than the individual Type I or Type II antioxidant treatments (Table 7). Thus, there was very limited additive antioxidant effects observed between Type I and Type II antioxidants, when combined at half their effective levels.

Table 7 - Treatment main effects on TBA values, pooled over time and sampling method, for cooked ground beef treated with Type I or Type II antioxidants, and their combinations

Treatment	TBA means ± SD
Control	2.50 ± 1.10 a
BHT (0.01% of meat wt; Type I)	0.61 ± 0.09 c-f
Cinnamon (0.5%; Type I)	0.75 ± 0.20 bc
Cloves (0.1%; Type I)	0.55 ± 0.09 e-g
Rosemary (0.4%; Type I)	0.84 ± 0.25 b
MM (1.5%; Type II)	0.48 ± 0.08 fg
STPP (0.5%; Type II)	0.42 ± 0.06 g
* BHT + MM	0.57 ± 0.10 d-f
* Cinnamon + MM	0.70 ± 0.14 b-d
* Cloves + MM	0.56 ± 0.09 d-g
* Rosemary + MM	0.52 ± 0.08 e-g
* BHT + STPP	0.49 ± 0.10 fg
* Cinnamon + STPP	0.61 ± 0.13 c-f
* Cloves + STPP	0.51 ± 0.10 e-g
* Rosemary + STPP	0.53 ± 0.11 e-g
* BHT + Cinnamon + Cloves + Rosemary	0.63 ± 0.14 c-e
* MM + STPP	0.51 ± 0.10 e-g

$LSD_{0.05} = 0.14$.

a-g - means with the same letters are not significantly different ($p < 0.05$).

* - to test possible additive effects, combined antioxidant treatments were used at 1/2 the concentration of the individual treatments alone, as listed above.

Abbreviations; BHT = butylated hydroxytoluene; MM = milk mineral; STPP = sodium tripolyphosphate.

Conclusions

All individual spices of Garam Masala were effective to maintain low TBA values in cooked ground beef during refrigerated storage compared to controls but imparted characteristic spice flavor. Among Garam Masala spices, only cloves could be used at 0.1% and still maintain TBA values < 1.0 for 15 d refrigerated storage. All the spices at their recommended level were able to significantly reduce the perception of rancid odor

and rancid flavor, as compared to 15 d rancid control samples. When used individually at their lowest effective levels, Type II antioxidants (MM and STPP) worked significantly better than all Type I antioxidants except cloves, for control of lipid oxidation in cooked ground beef during refrigerated storage. At lower use levels, there was an additive effect of rosemary + MM or STPP, for maintaining low TBA values during refrigerated storage of cooked ground beef.

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CHAPTER 5

EVALUATION OF ANTIOXIDANT EFFECTS OF RAISIN PASTE IN COOKED GROUND BEEF, PORK AND CHICKEN

Abstract

The objective of this study was to evaluate the possible antioxidant activity of raisin paste added to raw ground beef, pork or chicken before cooking to 163°C. Samples were held at 2°C for up to 14 days. Thiobarbituric acid values were measured using a distillation method, to avoid yellow color interference found in "wet" TBA methods. Sample meat flavor intensity, rancid flavor intensity and raisin flavor intensity were evaluated by a trained panel ($n = 6$). Addition of raisin paste lowered ($P < 0.05$) TBA values and decreased panel scores for rancid flavor scores of all meat samples in a concentration dependent responsive manner. Highest antioxidant effects were obtained with a minimum of 1.5%, 2.0%, or 2.0% raisin paste in cooked ground beef, pork or chicken, respectively. There was a high correlation (0.93, 0.94, or 0.94) between TBA values and sensory rancid flavor scores in beef, pork and chicken samples, respectively.

Addition of a reducing sugar (glucose) was nearly as effective as raisins for maintenance of low TBA values and rancid flavor scores, probably due to antioxidant effects of Maillard browning products. There was no detectable raisin flavor in cooked ground beef samples with added raisins.

Reprinted from Vasavada MN, Cornforth DP. 2006. Evaluation of antioxidant effects of raisin paste in cooked ground beef, pork, and chicken. J Food Sci 71(4):

However, all meats with added glucose had a higher raisin flavor intensity score than controls, indicating that panelists associated sweetness with raisin flavor. Maillard browning (sample darkening) was evident after cooking of ground chicken with either raisins or glucose.

Introduction

Lipid oxidation is a major cause of deterioration in the quality of meat and meat products (Asghar and others 1988; Ladikos and Lougovois 1990). Lipid oxidation leads to production of malonaldehyde, a mutagen and/or carcinogen (Shamberger and others 1974). Lipid oxidation is faster in heated meat than in raw meat tissues (Tichivangana and Morrissey 1985; Tims and Watts 1958). The rate and degree of oxidative degradation has been directly related to the degree of unsaturation of the lipids present (Igene and Pearson 1979; Tichivangana and Morrissey 1985) and degree of oxygen exposure (Jayasingh and others 2002; O'Grady and others 2000). Oxidation of unsaturated fatty acids in cooked meats during storage and reheating, results in stale or rancid flavors known as warmed-over flavor (Sato and Hegarty 1971).

The greater propensity of warmed-over flavor (WOF) in cooked and comminuted products is due to the release of non-heme iron during cooking and grinding (Igene and others 1979). Unsaturated lipids, especially those of the membrane phospholipids fraction, are the compounds undergoing oxidation (Igene and Pearson 1979; Younathan and Watts 1960).

The thiobarbituric acid (TBA) test is the most frequently used test to assess lipid oxidation in meat. Sensory panelists describe the extent 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 and 1.0. This "threshold" has served as a guide for interpreting TBA test results (Tarladgis and others 1960).

Raisins are recognized as a good source of dietary antioxidants. According to the USDA, raisins are second only to prunes in the ability to prevent oxidation as measured by the oxygen radical absorbance capacity (ORAC) test. Grapes and raisins have been shown to contain various antioxidant compounds, including bioflavanoids (Shalashvili and others 2002), proanthocyanidins (Foster 1997; Murga and others 2000), catechin monomers (Katalinic 1999), procyanidin dimers (Yamakoshi and others 2002) and other polyphenolic antioxidants (Meyer and others 1997; Frankel 1999).

There has been one previous study evaluating raisins as antioxidants in meat systems. Bower and others (2003) reported that beef jerky formulated to contain 15% (w/w) raisin puree produced conditions inhibitory to pathogenic bacteria by decreasing the pH to 5.4 and water activity to 0.64. Antioxidant activity of the beef jerky was increased by > 600%.

Although raisins contain antioxidant compounds, their possible antioxidant effectiveness in cooked ground meat systems has not been previously studied. Thus, the objective of this study was to evaluate the possible antioxidant effects of raisin paste on TBA values and trained panel rancidity scores in cooked ground beef, pork and chicken.

Materials and Methods

Sample preparation

Ground beef (15% fat), lean ground pork shoulder (20% fat), chicken breasts and whole dark raisins were purchased from local supermarkets. Raisin paste was prepared by blending 60 g raisin with 20 ml distilled water for 1 min in an Osterizer blender (Sunbeam Products, Inc. Boca Raton, Fla., U.S.A.). The raisin paste was manually mixed with ground beef (400 g) at 0.5%, 1.0%, 1.5%, and 2.0% of meat weight respectively. The raisin paste was manually mixed with ground pork (400 g) or ground chicken (400 g) at 1.0%, 2.0%, 3.0%, and 4.0% of meat weight, respectively. Control samples containing no raisins were also prepared. Total sugar content of retail seedless raisins was 59.2% by weight, with a standard deviation of 7.9 (USDA 2006). At 99.9% confidence level, the total sugar content was 48.2 to 70.2% (Hayter 1996). Thus, for later calculations of raisin sugar content, the highest value (70.2%) was used. Glucose and fructose are the two predominant sugars found in raisins (Pilandro and Wrolstad 1992). D-glucose undergoes the browning reaction faster than does D-fructose (BeMiller and Whistler 1996). For all 3 meats, a comparison treatment was prepared consisting of beef, pork, or chicken with glucose added at a level approximately equivalent to the sugar content of the highest level of added raisins (2.0% raisins in beef; 4.0% raisins in pork or chicken). Thus, the beef + glucose treatments were formulated to contain 1.45% glucose, and the pork or chicken + glucose treatments contained 2.9% glucose. Based on preliminary experiments, 2.0% raisins were sufficient to obtain antioxidant effects in cooked ground beef. However, higher levels (4.0% added raisins) were evaluated in cooked ground pork or chicken,

because these meats have higher poly-unsaturated fatty acid content (Paul and Southgate 1978), and thus might require higher levels of raisins for antioxidant effects.

The ground meat samples were thoroughly cooked to well done state on a grill at a temperature setting of 163°C. A small amount of water was added during cooking to prevent sticking and charring. After cooking, the ground meats were divided into 4 equal portions, placed in resealable plastic bags and cooled for 10 min at room temperature. Bags were then sealed and stored at 2°C for 1, 4, 7, or 14 d.

TBA test

Thiobarbituric acid reactive substances (TBARS) values were measured on duplicate 10 g samples at each storage period (1, 4, 7, or 14 d) using a distillation method (Tarladgis and others 1960; Koniecko 1979). Samples (10 g) were blended in a mixer with 47.5 ml distilled water and then the blender rinsed with 50 ml of water. Then, 2.5 ml of HCl (1:2 solution) was added, and the mixture was distilled through a condensing assembly to collect 50 ml of distillate. Five ml of the distillate was mixed with 5 ml TBA solution, boiled for 35 min, and duplicate absorbance readings were taken at 538 nm in a UV spectrophotometer. The absorbance values were multiplied by a factor of 7.8 to obtain TBA values (Koniecko 1979).

Sensory evaluation

A trained panel (n = 6) evaluated cooked samples at 1, 4, 7, and 14 d of refrigerated storage. Panelists were selected based on their sensitivity and reproducibility for detection of rancid samples (TBA Value > 1.5) in preliminary tests. All panelists had previous sensory panel experience with cooked beef products. The panelists were trained

in two sessions. In the first session, panelists were familiarized with the 5-point intensity scale and its usage. Panelists were also familiarized with cooked beef, pork or chicken flavors (fresh and rancid samples) and samples with added raisins at low (0.5%) and high (4%) raisin levels. Group discussions were conducted regarding sample attributes. In the second session, panelists again evaluated the same samples. The most consistent panelists (n = 6) were included in the final sensory panel.

At each panel session, panelists were served a total of 6 samples (6 g per sample), including the 4 raisin samples, the control without raisins, and the glucose treated sample. Samples were coded and microwave reheated for 25 s to attain a temperature of 80°C to 85°C immediately before serving. Samples were evaluated in individual booths. The serving order was randomized to avoid positional bias.

The panelists evaluated cooked samples for cooked beef, pork or chicken flavor intensity, rancid flavor intensity and raisin flavor intensity on a scale of 1 to 5; where 1 = no detectable flavor, 2 = slightly intense flavor, 3 = moderately intense flavor, 4 = very intense flavor, and 5 = extremely intense flavor, respectively. Before evaluating the next sample, ballot instructions specified that the previous sample be expectorated into cups provided for that purpose. Panelists were instructed to rinse their mouth with tap water. Unsalted crackers were also provided to cleanse the palate.

Hunter color measurements

The L^* (lightness), a^* (redness) and b^* (yellowness) values were measured for cooked ground chicken samples after 1 d of storage, using a Hunter lab Miniscan portable colorimeter (Reston, Va., U.S.A.) standardized using a white and black standard tile. In

preliminary tests, there was very little visual darkening of ground beef or pork after cooking, and therefore Hunter color measurements were not done on cooked ground beef or pork samples.

Experimental design

For beef, pork and chicken the TBA values and the sensory evaluation experiments were done in 3 separate replicates. The experiment was a completely randomized block design with 6 treatments (control, 1.45% glucose, 0.5%, 1.0%, 1.5%, or 2.0% raisin in ground beef; control, 2.9% glucose, 1.0%, 2.0%, 3.0%, or 4.0% raisin in ground pork and chicken), 4 storage times (1, 4, 7, and 14 d at 2°C) and 3 replicates of the whole experiment. Treatment means were calculated by analysis of variance (ANOVA) using StatisticaTM software (Statsoft Inc, Tulsa, Okla., U.S.A.). The error term was calculated to account for differences among blocks (replicates). Significant differences among means were determined by calculation of Fisher's least significance difference (LSD) values. Significance was accepted at $P < 0.05$.

Results

The main effects of raisin level (0 to 4%), storage time (1 to 14 d) and their interaction significantly ($P < 0.05$) affected TBA values of cooked ground beef, pork and chicken during refrigerated storage. Raisin level also significantly ($P < 0.05$) affected beef flavor intensity, rancid flavor intensity and raisin flavor intensity of cooked ground beef, pork and chicken. The main effect of storage time (1, 4, 7, or 14 d) significantly ($P < 0.05$) affected cooked ground beef flavor and rancid flavor intensity in cooked ground

beef. However, storage time had no significant ($P < 0.05$) effect on pork or chicken flavor intensity or rancid flavor intensity. The interaction of raisin level and storage time significantly ($P < 0.05$) affected rancid flavor intensity of cooked ground beef but not in cooked ground pork or chicken. The raisin level and storage time interaction did not affect beef flavor, pork flavor, chicken flavor, or raisin flavor in any of the meats.

Thiobarbituric acid value

TBA values of control cooked ground beef samples reached as high as 6.81 after 14 d storage at 2°C (Table 8). Addition of 0.5%, 1.0%, 1.5%, or 2.0% raisins significantly decreased TBA values to 3.34, 2.05, 1.48, and 0.98, respectively, after 14 d refrigerated storage (Table 8). Also after 14-d refrigerated storage, TBA values of cooked ground beef with 1.5% or 2.0% raisins were significantly lower than beef samples with 0.5% or 1.0% raisin (Table 8). In cooked ground beef, 1.45% glucose (equivalent to glucose content of 2.0% raisin level) was as effective as 1.5% or 2.0% raisin level for maintaining low TBA values (1.52) after 14 d refrigerated storage (Table 8).

In cooked ground pork samples, TBA values were also much higher in control samples than samples with added raisins or glucose, with values up to 15.43 after 14 d refrigerated storage (Table 9). TBA values were 7.09, 3.49, 3.01, and 2.30 for cooked ground pork with 1.0%, 2.0%, 3.0%, or 4.0% added raisins, respectively, after 14 d storage (Table 9). TBA values after 14 d storage for cooked ground pork with 2.0% to 4.0% added raisins were significantly lower than 1.0% level of added raisins (Table 9). Samples with 2.9% added glucose had TBA values (4.43) similar to samples with 2.0% to 4.0% added raisins after 14 d refrigerated storage (Table 9).

Table 8 - Interaction effects of treatment x storage time on TBA values (n = 6) of cooked ground beef formulated with raisin paste or glucose

Treatment	Storage Days at 2°C	TBA Value[1]
Control	1	2.43 ± 0.81 ef
Control	4	4.13 ± 0.64 c
Control	7	5.16 ± 0.28 b
Control	14	6.81 ± 0.34 a
0.5% Raisin	1	1.45 ± 0.46 hi
0.5% Raisin	4	2.34 ± 0.74 ef
0.5% Raisin	7	2.77 ± 0.72 e
0.5% Raisin	14	3.34 ± 0.78 d
1.0% Raisin	1	1.00 ± 0.28 i-k
1.0% Raisin	4	1.21 ± 0.12 h-j
1.0% Raisin	7	1.66 ± 0.24 gh
1.0% Raisin	14	2.05 ± 0.31 fg
1.5% Raisin	1	0.88 ± 0.28 jk
1.5% Raisin	4	1.16 ± 0.12 i-k
1.5% Raisin	7	1.32 ± 0.11 h-j
1.5% Raisin	14	1.48 ± 0.33 hi
2.0% Raisin	1	0.70 ± 0.25 k
2.0% Raisin	4	0.81 ± 0.14 jk
2.0% Raisin	7	0.88 ± 0.25 jk
2.0% Raisin	14	0.98 ± 0.25 i-k
1.45% Glucose	1	0.89 ± 0.49 jk
1.45% Glucose	4	1.20 ± 0.30 h-j
1.45% Glucose	7	1.53 ± 0.36 hi
1.45% Glucose	14	1.52 ± 0.38 hi

[1] Mean thiobarbituric acid (TBA) values ± standard deviation (SD).
Means with the same letter (a-k) are not different ($P < 0.05$); least significant difference among means ($LSD_{0.05}$) = 0.49.

Table 9 - Interaction effects of treatment x storage time on TBA values (n = 6) of cooked ground pork formulated with raisin paste or glucose

Treatment	Storage Days at 2°C	TBA Value[1]
Control	1	8.63 ± 2.89 c
Control	4	11.96 ± 1.81 b
Control	7	14.95 ± 1.84 a
Control	14	15.43 ± 2.99 a
1.0% Raisin	1	2.34 ± 0.60 g-j
1.0% Raisin	4	5.40 ± 2.09 d-f
1.0% Raisin	7	6.19 ± 3.59 c-e
1.0% Raisin	14	7.09 ± 2.94 cd
2.0% Raisin	1	1.26 ± 0.63 ij
2.0% Raisin	4	2.44 ± 1.78 g-j
2.0% Raisin	7	3.22 ± 2.16 f-j
2.0% Raisin	14	3.49 ± 2.53 f-i
3.0% Raisin	1	0.94 ± 0.40 j
3.0% Raisin	4	1.74 ± 1.03 h-j
3.0% Raisin	7	2.94 ± 2.36 f-j
3.0% Raisin	14	3.01 ± 2.33 f-j
4.0% Raisin	1	1.16 ± 0.72 ij
4.0% Raisin	4	1.60 ± 1.40 ij
4.0% Raisin	7	2.18 ± 1.44 g-j
4.0% Raisin	14	2.30 ± 1.56 g-j
2.9% Glucose	1	1.57 ± 0.98 ij
2.9% Glucose	4	2.67 ± 2.47 g-j
2.9% Glucose	7	4.24 ± 3.42 e-h
2.9% Glucose	14	4.43 ± 3.36 e-g

[1] Mean thiobarbituric acid (TBA) values ± standard deviation (SD). Means with the same letter (a-j) are not different ($P < 0.05$); least significant difference among means ($LSD_{0.05}$) = 2.50.

For cooked ground chicken samples, TBA values after 14 d refrigerated storage were high (9.27) for control samples as compared to TBA values of 2.96, 0.90, 0.45, and 0.33 for samples with 1.0%, 2.0%, 3.0%, or 4.0% added raisins respectively (Table 10).

There was no significant difference in 14-d TBA values among treatments with 2.0% to 4.0% added raisins and all had significantly lower TBA values than chicken samples with 1.0% added raisins (Table 10).

Samples with 2.9% added glucose (equivalent to glucose content of 4.0% raisin level) had TBA values (0.46) not different from samples with 2.0 to 4.0% added raisins after 14 d refrigerated storage (Table 10). For detailed statistics of raisin effects on all 3 meats, see Appendix D.

Table 10 - Interaction effects of treatment x storage time on TBA values (n = 6) of cooked ground chicken formulated with raisin paste or glucose

Treatment	Storage Days at 2°C	TBA Value[1]
Control	1	4.02 ± 1.19 d
Control	4	5.59 ± 1.29 c
Control	7	6.69 ± 1.20 b
Control	14	9.27 ± 0.78 a
1.0% Raisin	1	1.61 ± 0.71 gh
1.0% Raisin	4	2.18 ± 0.64 fg
1.0% Raisin	7	2.60 ± 0.92 ef
1.0% Raisin	14	2.96 ± 1.21 e
2.0% Raisin	1	0.74 ± 0.42 i
2.0% Raisin	4	0.76 ± 0.43 i
2.0% Raisin	7	0.82 ± 0.44 i
2.0% Raisin	14	0.90 ± 0.65 hi
3.0% Raisin	1	0.54 ± 0.23 i
3.0% Raisin	4	0.49 ± 0.13 i
3.0% Raisin	7	0.49 ± 0.12 i
3.0% Raisin	14	0.45 ± 0.16 i
4.0% Raisin	1	0.45 ± 0.14 i
4.0% Raisin	4	0.44 ± 0.10 i
4.0% Raisin	7	0.59 ± 0.16 i
4.0% Raisin	14	0.33 ± 0.04 i
2.9% Glucose	1	0.49 ± 0.14 i
2.9% Glucose	4	0.47 ± 0.11 i
2.9% Glucose	7	0.44 ± 0.13 i
2.9% Glucose	14	0.46 ± 0.13 i

[1] Mean thiobarbituric acid (TBA) values ± standard deviation (SD).
Means with the same letter (a-i) are not different ($P < 0.05$); least significant difference among means ($LSD_{0.05}$) = 0.72.

Sensory evaluation

Cooked ground beef without added raisins (control) had pooled mean rancid flavor intensity score of 3.40, where, 3.0 = moderately intense and 4.0 = very intense rancid flavor (Figure 4). Samples with 1.5% or 2.0% added raisins or 1.45% added glucose had significantly lower ($P < 0.05$) rancid flavor intensity scores than control samples or samples with 0.5% added raisins (Figure 4). Beef flavor intensity scores were lowest in control samples and were significantly higher ($P < 0.05$) in samples with 1.0% to 2.0% added raisins (Figure 4). There was no detectable raisin flavor in cooked ground beef samples with 0.5% to 1.5% added raisins. However, ground beef with 2.0% raisins or 1.45% added glucose had a significantly higher raisin flavor intensity score compared to samples without raisins (Figure 4). Thus, panelists apparently associated sweetness with raisin flavor.

The cooked ground pork sensory scores showed that, as with cooked ground beef, samples with added raisins or glucose had lower rancid flavor scores than controls (Figure 5). Samples with 2.0% to 4.0% added raisins or 2.9% added glucose had lower ($P < 0.05$) rancid flavor intensity scores than control samples or samples with 1.0% added raisins (Figure 5). Pork flavor intensity scores were higher ($P < 0.05$) for all samples with added raisins or glucose, compared to control samples (Figure 5). Cooked ground pork samples with 4.0% added raisins had higher raisin flavor scores than all other samples, with a mean score of 2.29, where a score of 2.0 was associated with a slightly intense raisin flavor. Raisin flavor scores were also higher ($P < 0.05$) in samples with added glucose, compared to controls (Figure 5), again indicating that panelists associated sweetness with raisin flavor.

Rancid flavor intensity scores of cooked ground chicken were rather high in control samples, with a value of 3.97, where, 4.0 equals very intense rancid flavor (Figure 6). All levels of added raisins or glucose had lower rancid flavor intensity scores as compared to controls (Figure 6). Chicken flavor scores were consistently higher ($P < 0.05$) for samples with 2.0% to 4.0% added raisins or 2.9% glucose, compared to samples with 1.0% raisin or control samples (Figure 6). Raisin flavor scores were generally higher for cooked ground chicken samples with 4.0% added raisin or 2.9% added glucose, again indicating that raisin flavor was associated with sample sweetness (Figure 6).

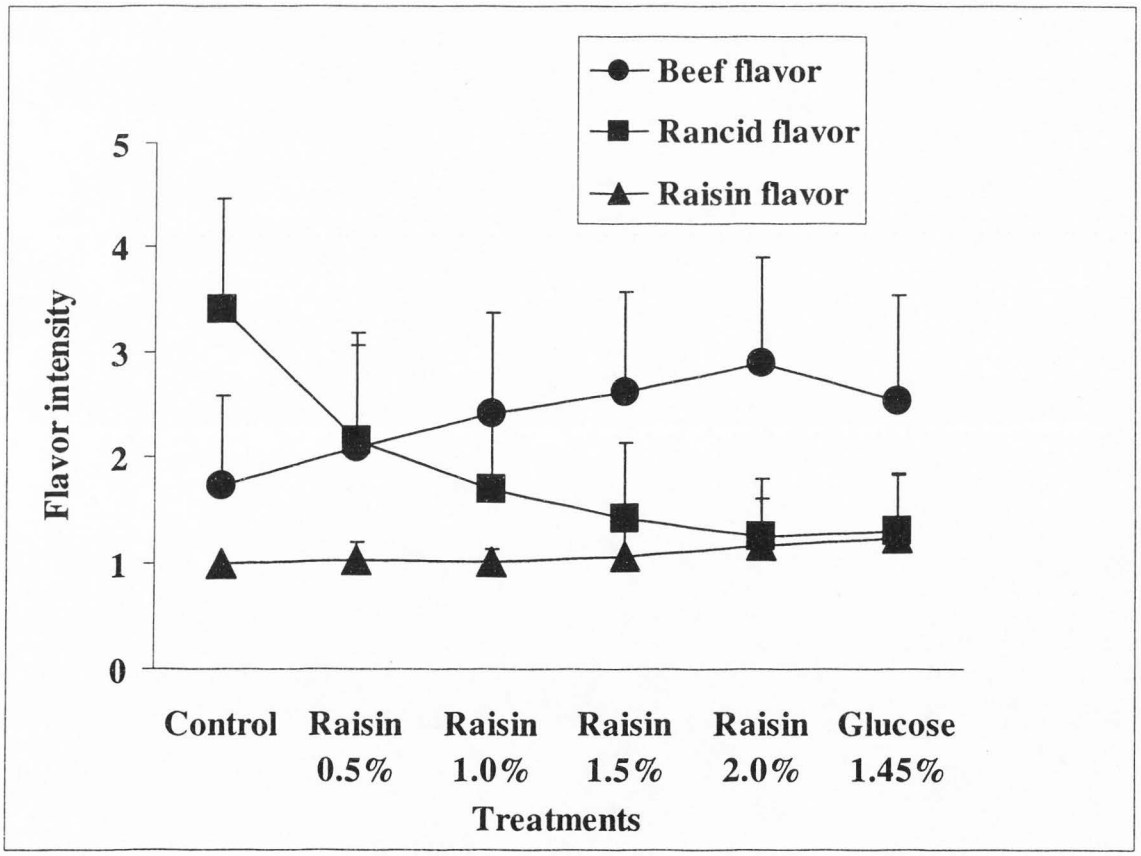

Figure 4 - Mean flavor intensity scores pooled over storage time for cooked ground beef with added raisins or glucose. Values are means pooled over storage time (1, 4, 7, 14 d at 2°C). Y- axis error bars represent standard deviation from the mean.

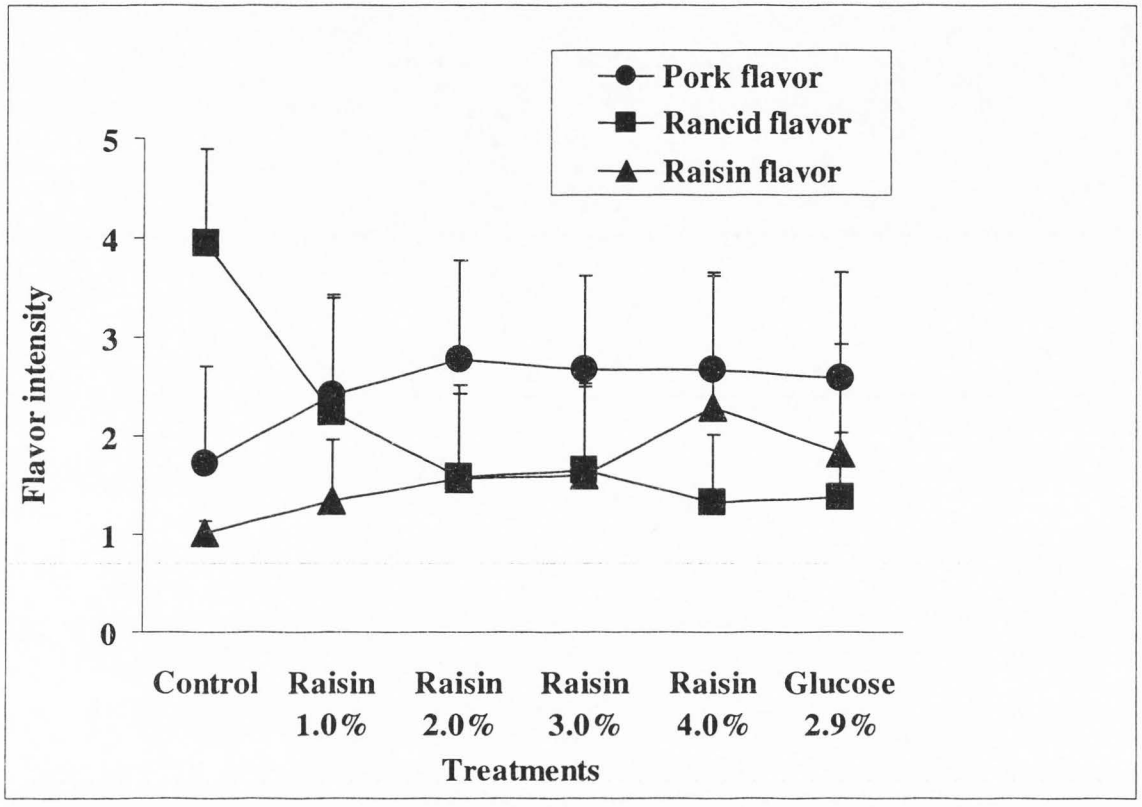

Figure 5 - Mean flavor intensity scores pooled over storage time for cooked ground pork with added raisins or glucose. Values are means pooled over storage time (1, 4, 7, 14 d at 2°C). Y- axis error bars represent standard deviation from the mean.

Correlation coefficients between TBA values and beef, pork or chicken flavor, rancid flavor and raisin flavor in cooked meat samples showed that there was a high correlation (0.93 to 0.94) between TBA values and rancid flavor intensity scores for all cooked meat samples, indicating a close association between lipid oxidation as measured by the TBA test and rancid flavor score as measured by the sensory evaluation panel.

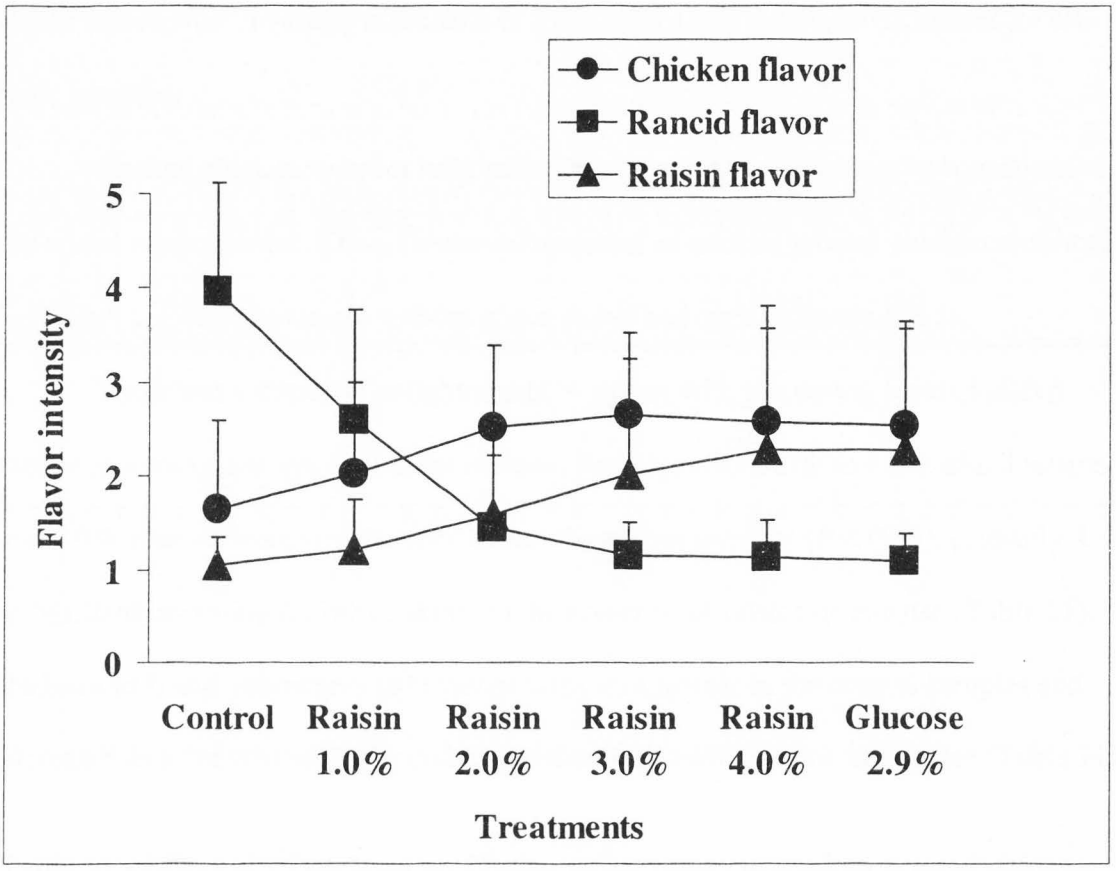

Figure 6 - Mean flavor intensity scores pooled over storage time for cooked ground chicken with added raisins or glucose. Values are means pooled over storage time (1, 4, 7, 14 d at 2°C). Y- axis error bars represent standard deviation from the mean.

The correlation coefficient between TBA values and beef flavor scores was -0.81, indicating that as lipid oxidation increased as measured by the TBA values, beef flavor intensity significantly decreased. This inverse relationship between meat flavor and TBA values was also seen in pork and chicken samples.

There was also a high inverse relationship between rancid flavor scores and beef, pork, or chicken flavor intensity scores (-0.88, -0.92, -0.93, respectively), indicating that as the rancid flavor scores increased, species-specific meat flavor scores decreased. Raisin flavor scores were moderately but significantly ($P < 0.05$) positively correlated with beef, pork, or chicken flavor scores (0.55, 0.64, 0.78, respectively). In general, raisin

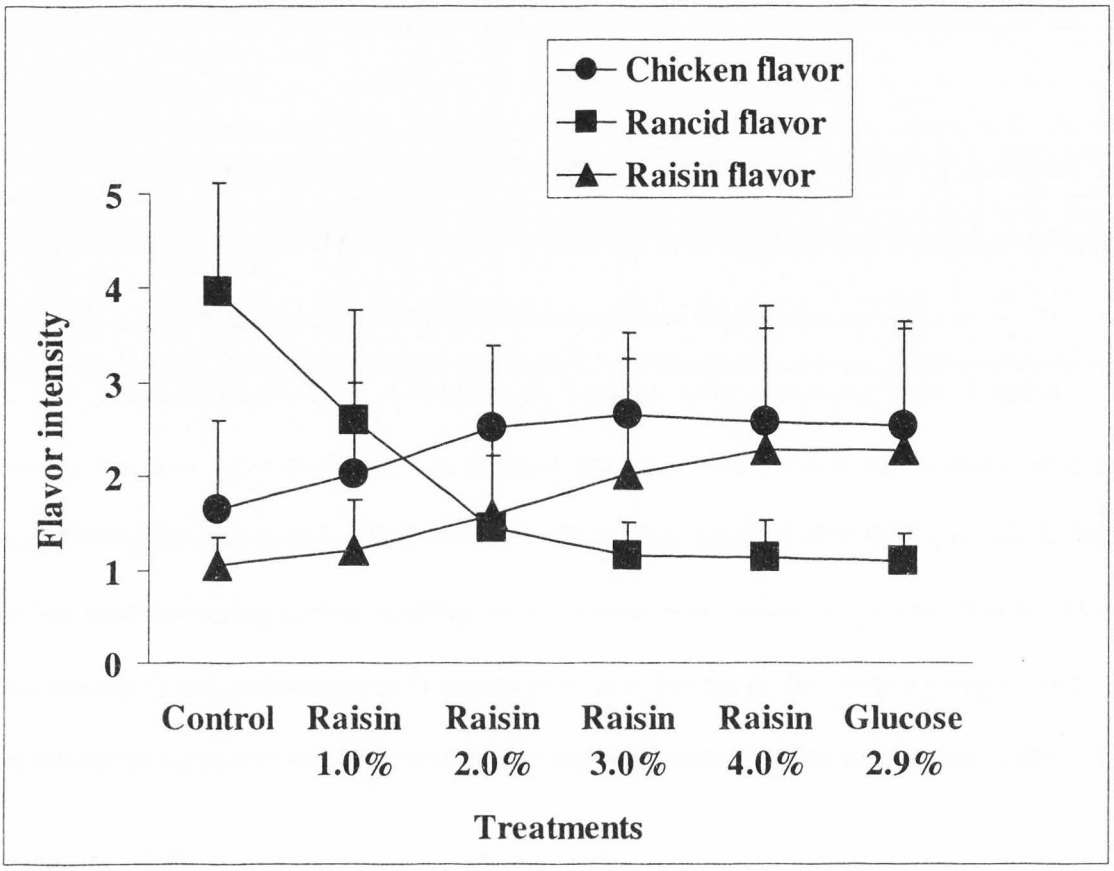

Figure 6 - Mean flavor intensity scores pooled over storage time for cooked ground chicken with added raisins or glucose. Values are means pooled over storage time (1, 4, 7, 14 d at 2°C). Y- axis error bars represent standard deviation from the mean.

The correlation coefficient between TBA values and beef flavor scores was -0.81, indicating that as lipid oxidation increased as measured by the TBA values, beef flavor intensity significantly decreased. This inverse relationship between meat flavor and TBA values was also seen in pork and chicken samples.

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Discussion

In preliminary experiments, addition of TBA reagent to cooked meat samples containing raisins resulted in development of a yellow rather than a pink chromagen. Other investigators have also reported the development of a yellow interfering chromagen when TBA reagent was added to samples containing sugars or aldehydes (Almandos and others 1986; Guzman-Chozas and others 1997; Sun and others 2001; Jardine and others 2002). Havens and others (1996) measured the absorbance of the yellow chromagen at 450 nm as a measure of lipid oxidation in freeze-dried ground beef patties. The yellow chromagen develops when TBA reagent is added to the meat sample in "wet" TBA methods (Witte and others 1970; Buege and Aust 1978). However, the development of a yellow chromagen in samples containing carbohydrates can be avoided using the distillation method of Tarladgis and others (1960), since the volatile TBA reactive substances can be separated from the less volatile sugar aldehydes by distillation. Thus, the Tarladgis distillation method was used in this study.

In the present study, addition of raisins to cooked ground meats resulted in lower TBA values of cooked ground meats during refrigerated storage, compared to control samples. Although, raisins contain a number of polyphenolic antioxidant compounds, added sugar (glucose) was nearly as effective as raisins for maintaining low TBA values during refrigerated storage. Similar results have been reported for addition of honey to chopped turkey meat (Antony and others 2002). Antony and others further reported that Maillard reaction products had antioxidant effects in turkey meat. The proposed mechanisms for antioxidant activity of Maillard reaction products include hydroperoxide

reduction, inactivation of free radicals formed during oxidative degradation of unsaturated fatty acids (Wijewickreme and Kitts 1997; Wijewickreme and others 1999), oxygen scavenging (Yen and Hsieh 1995) and chelating heavy metal ions (Wijewickreme and others 1997). Thus, the antioxidant effects of raisins in cooked ground meats were primarily due to the formation of Maillard reaction products during the heating of sugars with the amino groups of proteins, peptides, and / or free amino compounds in meats.

Conclusions

Compared to control samples, addition of raisin paste lowered ($P < 0.05$) TBA values and decreased the panel scores for rancid flavor of cooked ground beef, pork, and chicken in a concentration dependent responsive manner. In cooked ground beef, 1.5% to 2.0% raisin paste was more effective than 0.5% to 1.0% raisin paste. For pork, 2.0% to 4.0% raisin paste was more effective than the 1.0% raisin level. For chicken, 2.0% to 4.0% raisin paste was more effective than the 1.0% raisin levels for reducing panel rancid flavor scores and TBA values.

There was a high correlation between the TBA values and the sensory rancid flavor scores in all meat samples. Addition of sugar (glucose) was nearly as effective as raisins for maintenance of low TBA values and rancid flavor scores of cooked ground beef, pork, and chicken, probably due to antioxidant effects of Maillard browning products formed during heating of sugars and meat proteins. The development of Maillard browning products was especially evident during cooking of ground chicken with either raisins or glucose, resulting in a much darker product after cooking.

There was no detectable raisin flavor in cooked ground beef samples with added

raisins. However, for all meats the samples with added glucose had a higher raisin flavor intensity score than controls without raisins, indicating that panelists associated sweetness with raisin flavor.

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CHAPTER 6

OVERALL SUMMARY

The research in this dissertation focused on the use of natural additives to control oxidative rancidity in cooked meats. Twenty-two different natural additives were tested in various experiments for their ability to control lipid oxidation in different cooked meats. It was found that all the natural additives tested in cooked meats had antioxidant effects, and were effective in controlling lipid oxidation to some extent. Some of the additives such as milk mineral (MM), sodium tripolyphosphate (STPP) and cloves were highly effective, whereas some other additives were less effective.

The Type II antioxidants such as MM and STPP were observed to be more effective as compared to the Type I antioxidants such as different spices. The Type I antioxidants act by slowing the propagation of lipid oxidation, whereas the Type II antioxidants act to inhibit initiation as well as propagation of lipid oxidation by binding iron and making it unavailable as a potential catalyst for lipid oxidation.

Milk mineral (1.5%) was found to be very effective in controlling lipid oxidation in cooked beef meatballs and nitrite-cured sausages. It was found to be as effective as sodium nitrite (20 or 40 ppm) in controlling lipid oxidation. It also maintained the brown color of cooked meatballs, as compared to control samples. Thus, MM has potential application as an antioxidant for addition to ground meatballs before cooking. Also MM when added to cooked meat systems can be considered a good source of added calcium, and thus has nutritional importance. It would have great potential as an antioxidant in all cooked meats where a pink cured color from nitrite addition is undesirable. Milk mineral

in the future could have a significant role in replacing nitrite as an effective antioxidant in the cooked meat industry. Further work can be done to check possible synergistic effects of MM with various other natural antioxidants in cooked ground beef. Work can also be done to see antioxidant effects of MM in cooked meats from other species.

All spices had antioxidant effects in cooked ground beef, as compared to control samples. Among the various spices of Garam Masala spice blend, cloves were found to be very effective in controlling lipid oxidation in cooked ground beef. Most spices imparted distinctive flavors to the cooked ground beef. Also, trained panel sensory analysis showed that all spices reduced the perception of rancid odor / rancid flavor by a masking effect. The U.S. consumption of spices exceeds 1 billion lb/year and the world market for imported spices is worth over $2.3 billion (ASTA 2001). With such a wide spread and improving market for the spice trade, there are endless possibilities of using spices in various food items and cuisines. This work with spices of Garam Masala spice blend investigating 13 individual spices shows the potential application of using spices in cooked meats. There are also possible options of using spices in combination with various Type II antioxidants like MM or STPP to control lipid oxidation in cooked meats. Further work can be carried out to check the antioxidant effects of various spices in various other cooked meats from different species.

Raisin paste worked very well in controlling lipid oxidation in cooked ground beef, pork, and chicken. Raisin paste reduced thiobarbituric acid (TBA) values and rancid flavor scores in cooked meats. Raisins are rich in various antioxidant compounds such as bioflavanoids, proanthocyanidins and other polyphenolic antioxidants. The antioxidant

effect shown by glucose (added at the same levels as present in raisin) in beef, pork and chicken, suggested that Maillard browning products might contribute significantly to the antioxidant activity of raisins in cooked meats. It was also found that the distillation method for TBA analysis avoids the interference from sugars and prevents formation of a yellow chromogen. This yellow chromogen formation occurs in "wet" methods where TBA is directly added to the samples having high sugar content. The possible use of raisin paste as an antioxidant in cooked meats would provide a tasty alternative for consumers. Also, the use of glucose in meats would provide a cheap and viable antioxidant alternative in cooked meats. Further work can be carried out to check the use of different time-temperature combinations of cooking, to show the effect of Maillard browning in controlling lipid oxidation in cooked meats.

Thus, the results of this dissertation justify my hypothesis that for cooked ground meats there are a number of alternative antioxidant treatments (MM, Garam Masala spices and raisin paste) that have equal or greater antioxidant effects as compared to butylated hydroxytoluene (BHT) or STPP. The results also justify my hypothesis that the Type II iron-chelating antioxidants (MM, STPP and nitrites) have greater antioxidant effects in an iron-rich system (cooked meats) than oxygen radical scavenging Type I antioxidants (BHT and rosemary extract).

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APPENDICES

APPENDIX A

CHINESE 5 – SPICE PAPER

EVALUATION OF ANTIOXIDANT EFFECTS AND SENSORY ATTRIBUTES OF CHINESE 5-SPICE INGREDIENTS IN COOKED GROUND BEEF

Abstract

This study determined antioxidant and sensory characteristics of cinnamon, cloves, fennel, pepper, and star anise (Chinese 5-spice ingredients) in cooked ground beef. Total aerobic plate counts were also measured. Mean thiobarbituric acid (TBA) values were high (3.4 ppm) for control cooked ground beef samples. With 1% use level, all spice treatments had lower pooled mean TBA values than controls. At the lowest use level of 0.1% of meat weight, all spices except pepper had lower TBA values than controls. Treatments with 0.1% cloves had lower ($P < 0.05$) TBA values than 0.1% levels of other individual spices. Star anise, fennel, pepper, and cinnamon samples at 0.5% use level had lower mean TBA values than controls, but not different from 1.0% levels, respectively. Thus, the lowest effective spice level for cloves was 0.1% and 0.5% for the other spices. There was a high correlation ($P < 0.01$) between TBA values and panel scores for rancid odor and flavor (0.83 and 0.78, respectively). Spice flavor was inversely correlated ($P < 0.01$) with rancid odor and flavor (-0.57 and -0.61, respectively). The 5-spice blends did not affect microbial load of cooked samples compared with controls. In conclusion, all spices and blends had a dual effect, reducing rancid odor/flavor and imparting a distinctive flavor to cooked ground beef.

Reprinted from Dwivedi S, Vasavada MN, Cornforth D. 2006. Evaluation of antioxidant effects and sensory attributes of Chinese 5 – spice ingredients in cooked ground beef. J Food Sci 71(1):C012-017.

Introduction

The Chinese conceptualized the theory of '5 Elements' under which everything in our surroundings could be categorized into 5 basic elements. Chinese 5-spice is 1 application of the 5 elements theory. It was developed in an attempt to produce a powder that encompassed the 5 flavor elements; sweet, salty, sour, pungent, and bitter (Needham and Wang 1956). The traditional 5-spice mixture includes cinnamon, cloves, fennel, Szechwan pepper, and star anise. Today, however, Chinese 5-spice may also include ginger and nutmeg and can be easily obtained in any Asian market.

Lipid oxidation is 1 of the major causes of food deterioration. Lipid oxidation may also decrease nutritional value by forming potentially toxic products during cooking and processing (Shahidi and others 1992; Maillard and others 1996). Warmed-over flavor (WOF) is associated with cooked meat and intensifies during refrigerated storage (Tims and Watts 1958). Heating temperature affects the extent of lipid oxidation (Keller and Kinsella 1973). Ferric and ferrous iron ions catalyze the decomposition of lipid peroxides to more volatile aldehydes and ketones (McDonald and Hultin 1987). Early work showed that the meat pigment myoglobin had little or no catalytic effect on lipid oxidation in simple model systems or red meats (Sato and Hegarty 1971; Love and Pearson 1974). However, more recent work has shown lipid oxidation catalyzed by oxidized myoglobin species (Reeder and Wilson 2001), hemoglobin in fish muscle (Richards and Hultin 2002), and heme derived from myoglobin oxidation (Baron and Andersen 2002).

Compounds with antioxidant properties have been found in spices, oil seeds, citrus pulp and peel, and in products that have been heated and / or have undergone non-

enzymatic browning. In addition to imparting distinctive flavors, spices contain antioxidant properties and inhibit rancid flavor development associated with lipid oxidation (Chipault and others 1952, 1955; Namiki 1990). Spices such as cloves, cinnamon, turmeric, black pepper, ginger, garlic, and onions exhibit antioxidant properties in different food systems (Younathan and others 1980; Al-Jalay and others 1987; Jurdi-Haldeman and others 1987). Spices have antioxidant properties due to the presence of compounds such as flavanoids, terpenoids, lignans, and polyphenolics (Craig 1999). However, their use may be limited in some foods, due to their characteristic flavor and aroma. Use of un-sterilized spices and herbs also increases the possibility of bacterial contamination in high moisture foods (Garcia and others 2001).

Antioxidant compounds have been identified in all 5 components of Chinese 5-spice. Anise (*Pimpinella anisum* L.), nutmeg, and licorice all had strong hydroxyl radical (OH•) scavenging activity in deoxyribose assay (Murcia and others 2004). Fennel (*Foeniculum vulgare*) has in vitro antioxidant activity (Oktay and others 2003). The antioxidant compounds in fennel include 3-caffeoylquinic acid, rosamirinic acid, and quercetin-3-O-galactoside (Parejo and others 2004). Cloves (*Syzygium aromaticum*) contain eugenol and eugenyl acetate as the major aroma constituents. Both compounds inhibit hexanal formation (a product of lipid oxidation) in cod liver oil (Lee and Shibamoto 2001). Antioxidant activity in pepper (*Capsicum annum*) is due to presence of ascorbic acid, flavonoids, capasaicinoids, and phenolic acids (Jimenez and others 2003). Cinnamic aldehyde in cinnamon (*Cinnamomum aromaticum*) has potential antioxidant properties. Cinnamon and mint exhibited higher antioxidant properties than anise, ginger, licorice, nutmeg, or vanilla in a lipid peroxidation assay (Murcia and others 2004). A

concentration of 500 µg/ml cinnamon extract inhibited hexanal production by 5% (Lee and Shibamoto 2002).

Although the components of Chinese 5-spice have been shown to have antioxidant activity in model systems, our objective was to determine the lowest effective level of each spice for antioxidant properties in cooked ground beef. Sensory evaluation was also done on cooked ground beef containing various spices at their recommended (lowest effective) antioxidant level.

Materials and Methods

Experiment 1 - Thiobarbituric acid (TBA) assay

The experiment was a completely randomized block design with 6 ground beef treatments (cinnamon, cloves, fennel, pepper, star anise, and retail 5-spice blend), at 4 levels (0, 0.1, 0.5, and 1.0 % of meat weight), 3 storage days (1, 8, and 15 d), and 3 replications of the entire experiment. TBA values (duplicates for each sample) were measured as an indicator of rancidity at 1, 8, and 15 d storage of cooked ground beef crumbles at 2°C.

Treatment means were calculated by analysis of variance (ANOVA) using Statistica^{TM} software (Statsoft Inc, Tulsa, Okla., U.S.A.). Significant differences among means were determined by calculation of Fisher's least significant difference (LSD) values. Significance was defined at $P < 0.05$ for ANOVA and LSD values.

The recommended or lowest effective spice level (0.1%, 0.5%, or 1%) for each individual spice was determined as the lowest spice concentration that resulted in TBA values significantly lower than the controls (0% spice). The 5 spices at their lowest

effective levels were mixed to create the low clove 5-spice blend for sensory testing in experiment 2. The low clove 5-spice blend was created because cloves have strong flavor and odor that could be a concern with consumers. Thus, it was desirable to evaluate a blend with a clove proportion lower than 20% (the level if each of the 5 spices were present in equal proportions).

Experiment 2 - Sensory evaluation

Cooked beef samples made with spices at their lowest effective levels as described in experiment 1 were evaluated for intensity of cooked beef flavor, rancid flavor/odor, and spice flavor intensity. A total of 10 treatments were evaluated (0.5% cinnamon, 0.1% cloves, 0.5% fennel, 0.5% pepper, 0.5% star anise, 0.5% retail 5-spice blend, 0.5% optimal 5-spice blend, rancid control, 0.5% sodium tripolyphosphate (STPP) control, fresh control). The rancid control was cooked ground beef without added spices and held 15 d at 2°C, allowing time to observe the full extent of oxidation in the controls compared to spice-treated samples. The fresh control was cooked ground beef without spices prepared on the day of the panel evaluation. Trained panelists (n = 13) evaluated samples after 15 d of storage at 2°C. TBA values were measured on the samples that were served to the panelists. Treatment means were calculated by ANOVA as described in experiment 1. Correlation coefficients were calculated among sensory scores and TBA values. Significance was defined at $P < 0.01$ for correlation coefficients.

Experiment 3 - Aerobic plate count

A 10-g portion of ground beef was mixed with 90 mL of sterile peptone water (Difco, Detroit, Mich., U.S.A.) in a dilution bottle, and plate counts were done on serial

dilutions of cooked ground beef samples after 1, 8, or 15 d storage at 2°C, following standard procedures (Messer and others 1978). Standard methods agar (Difco) was used as growth media. Duplicate plates were counted after incubation at 37°C for 48h.

Sample preparation

Ground star anise, fennel, cloves, and cinnamon (McCormick & Co. Inc., Hunt Valley, Md., U.S.A.), black pepper (Inter-American Foods Inc., Cincinnati, Ohio, U.S.A.), and lean ground beef (15% fat) were purchased at a local grocery. Retail Chinese 5-spice blend (Dynasty, San Francisco, Calif., U.S.A.) was also purchased locally. In addition to the 5 traditional spices, the retail blend also contained ginger and licorice. Each spice was manually mixed with ground beef (100 g/ treatment) at 0.1%, 0.5%, and 1.0% levels. Mixed samples were thoroughly cooked at 163°C for 5 min, to a final temperature of 82°C to 85°C, as measured using a VersaTuff Plus 396 digital thermometer (Atkins Technical, Inc, Gainesville, Fla., U.S.A.) with a thin probe for fast response. The cooked ground beef crumbles were placed in resealable plastic bags, cooled for 10 to 15 min at room temperature, and stored for 1, 8, or 15 d at 2°C. Thiobarbituric acid (TBA) values were measured in duplicate at 1, 8, or 15 d on the cooked samples as an indicator of oxidative rancidity. For each ingredient spice, the experiment was replicated 3 times. Duplicate sample analysis was performed. Thus, there were 6 observations per treatment.

TBA value

Thiobarbituric acid-reactive substances (TBARS) assay was performed as described by Buege and Aust (1978). Duplicate samples (0.5 g) for all the treatments

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 5B, DuPont, Wilmington, Del., U.S.A.) at 4300 x g for 10 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 except the meat. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 1.56×10^5/M/cm for the pink TBA-MDA pigment (Sinnhuber and Yu 1958). The absorbance values were converted to ppm malonaldehyde by using the following equations:

TBA nr (mg/kg) = Sample A_{532} x (1 M TBA Chromagen /156000) x [(1 mole/L)/ M] x (0.003 L/ 0.5 g meat) x (72.07 g MDA/mole MDA) x (1000 g/kg) (1)

TBA nr (ppm) = Sample A_{532} x 2.77 (where MDA = malonaldehyde) (2)

Sensory evaluation

All panelists had previous sensory panel experience with cooked beef products. The panelists were trained in 2 sessions. In the 1st session, panelists were familiarized with the 5-point intensity scale and its usage. Panelists were also familiarized with cooked beef flavor (both fresh and rancid samples) and cooked ground beef with individual added spices (cinnamon, cloves, fennel, pepper, and star anise) and Chinese 5-spice blends at low (0.1%) and high (1%) spice concentrations. Group discussion was

conducted regarding sample attributes. In the 2nd session, panelists again evaluated the same samples. The most consistent panelists (n = 13) were included in the final panel.

Treatment samples were prepared with spice concentration at lowest effective levels of 0.5% for cinnamon, fennel, star anise, or black pepper, and 0.1% for clove (% raw meat weight) as determined in experiment 1 of this study. The low clove blend was 4.8% by weight cloves and 23.8% each of cinnamon, fennel, pepper, and star anise. Spice treatments were cooked, packaged, and stored as previously described. Three control cooked beef samples were also prepared. The controls were (1) fresh, (2) STPP, and (3) rancid. Fresh control samples were cooked immediately before serving, using lean ground beef (15% fat) purchased locally on the d of the panel. STPP controls were formulated with 0.5% STPP, cooked and refrigerated for 15 d. Rancid controls were cooked samples without STPP or spice and refrigerated for 15 d. TBA values were measured for all controls and treated samples on the same day as the panel evaluation.

The 7 treatment samples at optimal concentrations and 3 controls of cooked beef crumbles were evaluated in 3 sessions. A set of 5 or 6 samples (6 g each) was served to each panelist in each session, consisting of 2 or 3 spice-treated samples and 3 controls. Samples were coded and microwave reheated for 25 s to attain a temperature of 80°C to 85°C immediately before serving. Samples were evaluated in individual booths under red lights. The serving order was randomized to avoid positional bias.

Panelists were asked to evaluate samples for intensity of rancid odor, rancid flavor, beef flavor, and spice flavor on a 5- point scale, where 1 = no flavor or odor, 2 = slightly intense, 3 = moderately intense, 4 = very intense, and 5 = extremely intense flavor or odor. Panelists were also asked to provide additional qualitative comments for

each sample. Before evaluating the next sample, ballot instructions specified that the previous sample be expectorated into cups provided for that purpose. Panelists were instructed to rinse their mouth with tap water. Unsalted crackers were also provided to cleanse the palate.

Results and Discussion

Experiment 1 - TBA assay of cooked ground beef with individual spices

Main effects of treatment (cinnamon, cloves, fennel, pepper, star anise, retail 5-spice blend), spice level (0%, 0.1%, 0.5%, 1.0%) and day of refrigerated storage (1, 8, 15 d) significantly affected the TBA values of cooked ground beef (Table A1). All 2-way interactions also affected ($P < 0.05$) TBA values, but the 3-way interaction of treatment x spice level x day storage did not significantly affect TBA values (Table A1).

Table A1 - Summary of significance ($P < 0.05$) as determined by ANOVA

	n	TBA	P - level
Treatment	72	*	0.0001
Spice Level	108	*	0.0001
D of storage	144	*	0.0001
Treatment x level	18	*	0.0001
Treatment x day	24	*	0.0001
Level x day	36	*	0.0001
Treatment x level x day	6	NS	0.1065

* Significant at $P < 0.05$; NS = not significant at $P < 0.05$; n = nr observations per mean.

Cooked ground beef mean TBA values for the 2-way interaction of spice treatment x level are shown in Table A2. Mean TBA values were high (3.4) for control cooked ground beef samples. With 1% use level, all spice treatments had lower ($P < 0.05$) TBA values than controls. At the lowest use level of 0.1% of meat weight, all spices except pepper had lower TBA values than controls, and clove treatments had lower ($P < 0.05$) TBA values than other spices. Mean TBA value for the 0.1% clove treatment was 0.76, compared to 1.66, 2.32, 2.87, and 2.55 for 0.1% cinnamon, fennel, pepper, and star anise, respectively (Table A2).

Thus, the recommended or lowest effective spice level for cloves was 0.1% and 0.5% for the other spices, where lowest effective spice level was defined as the lowest spice weight/ 100 g meat (0.1, 0.5, or 1.0) that had significantly lower TBA values than other levels (Table A2). After 15 d refrigerated storage, TBA values were as high as 5.9 for controls without added spice, compared with 0.79, 0.75, 2.22, 1.70, 1.30, and 0.37 for 0.5% cinnamon, 0.1% cloves, 0.5% fennel, 0.5% pepper, 0.5% star anise, and 0.5% 5-spice blend (lowest effective levels respectively; Figure A1 to A6).

Table A2 - Mean thiobarbituric acid (TBA) values[a] for cooked ground beef formulated with the individual spices of Chinese 5-spice, at use levels of 0.1%, 0.5%, and 1.0% of raw meat weight[b]

TREATMENT	SPICE LEVEL (% meat wt.)	TBA (ppm MDA)
CONTROL	0.0	3.41 a
CINNAMON	0.1	1.66 cd
CINNAMON	0.5	0.76 e
CINNAMON	1.0	0.78 e
CLOVES	0.1	0.76 e
CLOVES	0.5	0.96 de
CLOVES	1.0	0.88 e
FENNEL	0.1	2.32 bc
FENNEL	0.5	1.39 de
FENNEL	1.0	0.99 de
PEPPER	0.1	2.87 ab
PEPPER	0.5	1.28 de
PEPPER	1.0	1.26 de
STAR ANISE	0.1	2.55 b
STAR ANISE	0.5	0.97 de
STAR ANISE	1.0	0.71 e
RETAIL 5- SPICE	0.1	0.99 de
RETAIL 5- SPICE	0.5	0.73 e
RETAIL 5- SPICE	1.0	1.00 de
$LSD_{0.05}$		0.76

[a] Mean TBA values with the same letter are not different ($P < 0.05$).
[b] Means were pooled for storage time (1, 8, and 15 d) after cooking ($n = 18$).

TBA values > 1.0 are usually associated with rancid flavor / odor by sensory panelists (Tarladgis and others 1960; Jayasingh and Cornforth 2003). Note that TBA values of clove-treated ground beef samples (Figure A2) remained less than 1.0 for the entire 15-d storage period as did the samples with 0.5 or 1.0 % retail 5-spice blend (Figure A6). Ground beef with 1.0% fennel or 0.5% to 1.0% pepper had TBA values < 1.1 for 8 d storage (Figure A3 and A4). Ground beef with 1.0% cinnamon or 1.0% star anise had TBA values <1.0 for 15 d storage (Figure A1 and A5). Thus, treatment with cloves was clearly the most effective among individual spices for maintenance of low TBA values of cooked ground beef during refrigerated storage.

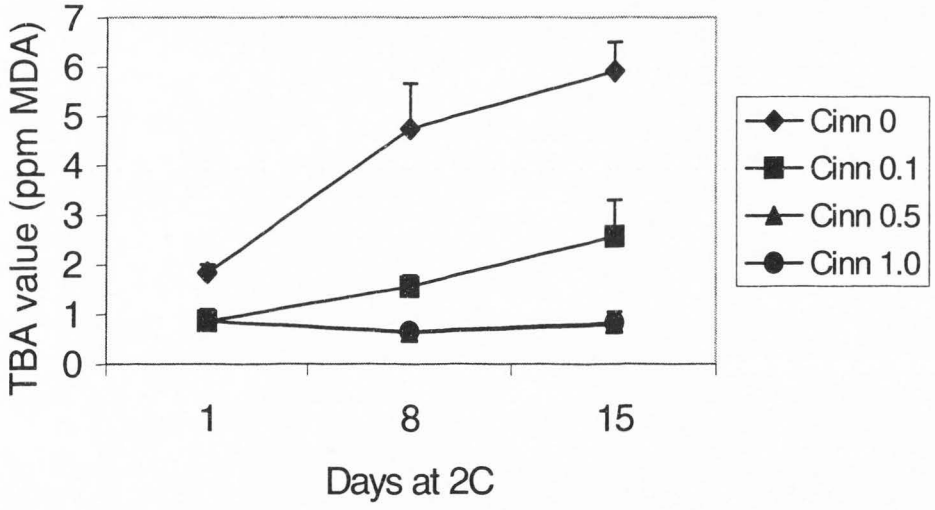

Figure A1 - Effect of cinnamon concentration (0%, 0.1%, 0.5%, 1.0% of meat wt) on thiobarbituric acid (TBA) values of cooked ground beef during refrigerated storage (ppm MDA = parts per million malonaldehyde). Mean values differing by more than 0.94 are significantly different. $LSD_{0.05}$ = 0.94.

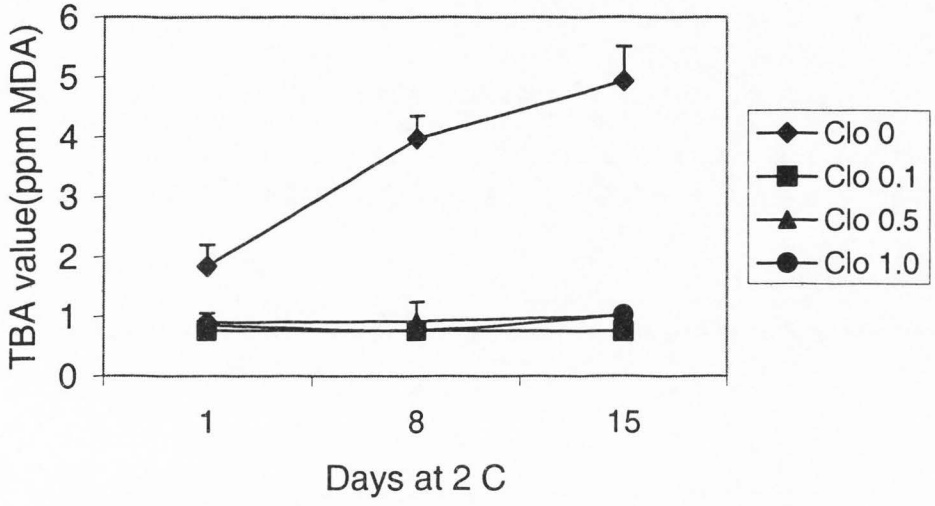

Figure A2 - Effect of clove concentration (0%, 0.1%, 0.5%, 1.0 % of meat wt) on thiobarbituric acid (TBA) values of cooked ground beef during refrigerated storage (ppm MDA = parts per million malonaldehyde). $LSD_{0.05}$ = 0.94.

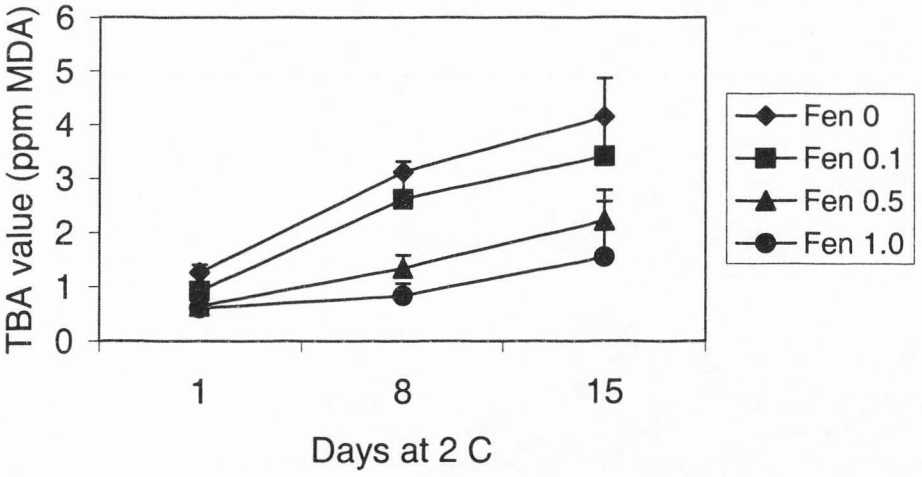

Figure A3 - Effect of ground fennel concentration (0%, 0.1%, 0.5%, 1.0 % of meat wt) on thiobarbituric acid (TBA) values of cooked ground beef during refrigerated storage (ppm MDA = parts per million malonaldehyde). $LSD_{0.05}$ = 0.94.

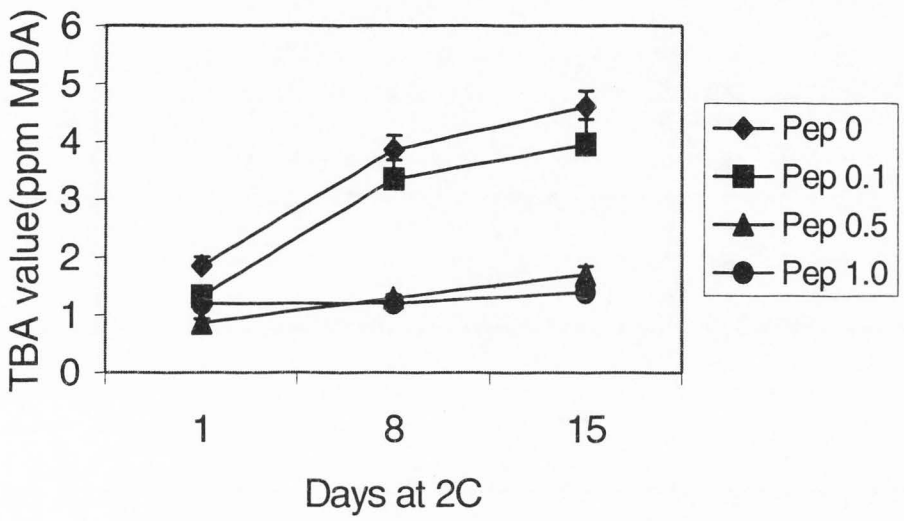

Figure A4 - Effect of pepper concentration (0%, 0.1%, 0.5%, 1.0 % of meat wt) on thiobarbituric acid (TBA) values of cooked ground beef during refrigerated storage (ppm MDA = parts per million malonaldehyde). $LSD_{0.05}$ = 0.94.

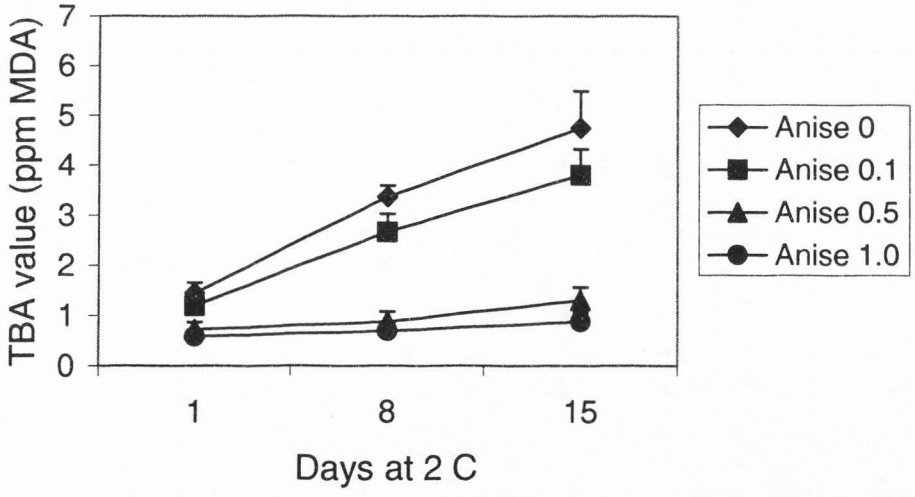

Figure A5 - Effect of star anise concentration (0%, 0.1%, 0.5%, 1.0 % of meat wt) on thiobarbituric acid (TBA) values of cooked ground beef during refrigerated storage (ppm MDA = parts per million malonaldehyde). $LSD_{0.05}$ = 0.94.

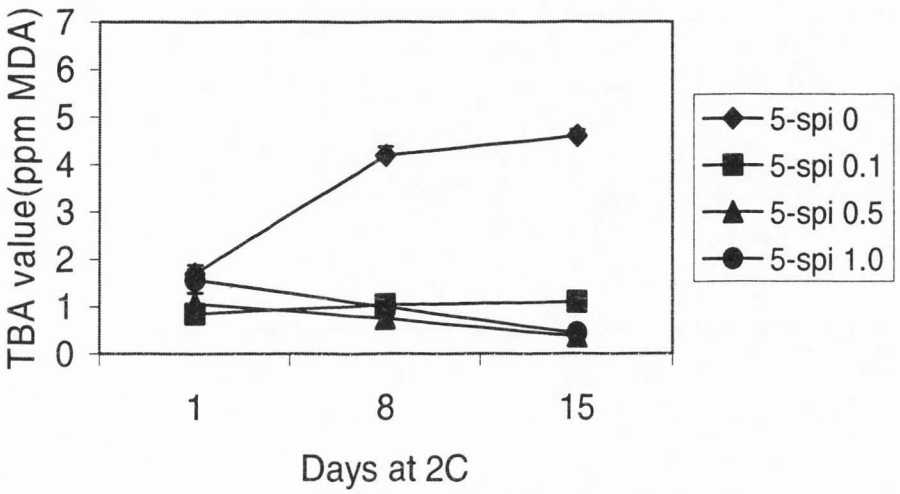

Figure A6 - Effect of retail Chinese 5-spice concentration (0%, 0.1%, 0.5%, 1.0 % of meat wt) on thiobarbituric acid (TBA) values of cooked ground beef during refrigerated storage (ppm MDA = parts per million malonaldehyde). $LSD_{0.05}$ = 0.94.

The antioxidant effects of cinnamon, clove, fennel, pepper, and star anise in this study are in agreement with previous findings by others. Cinnamon essential oil has been shown to have significant antioxidant activity in Chinese-style sausages (Ying and others 1998). Cloves at 0.05% were shown to enhance the storage stability and acceptability of frozen stored fish mince for about 28 wk. For 50-week storage, a use level of 0.1% was optimal (Joseph and others 1992). Clove powder at 0.2% w/w significantly reduced oxidative rancidity measured by TBARS, and improved acceptability of oysters. The oysters remained acceptable for 278 d when treated with cloves compared with 235 and 237 d for butylated hydroxytoluene (BHT)-treated and untreated samples, respectively (Jawahar and others 1994). Clove and Maillard reaction products have been shown to inhibit the increase of secondary oxidation products formed during refrigerated storage of cooked meat and to affect the extent of non-heme iron release during cooking, which is believed to be the primary catalyst accelerating lipid oxidation (Jayathikalan and others

1997). Black pepper was an effective sensory flavoring agent in chicken feet (Jokpyun, a traditional Korean gel type delicacy) at 0.33% level, based on response surface methodology (Mira and others 2000). Ground black pepper oleoresin extracted by supercritical carbon dioxide was more effective in reducing lipid oxidation of cooked ground pork than oleoresin extracted by conventional methods (Tiprisukond and others 1998). Star anise was effective at 0.5% level based on meat weight. Anise-treated samples had a TBA value of 0.97. Anise has also been shown to have antioxidant effects in Chinese marinated pork shanks as compared to controls (Tzu and others 1997).

Experiment 2 - Sensory evaluation

Mean trained panel sensory scores and thiobarbituric acid (TBA) values of spice-treated, cooked ground beef crumbles after 15 d storage at 2°C are shown in Table A3. The control samples without added spices (rancid control) had the highest scores for rancid odor and flavor intensity and also the highest TBA values (7.2). The control samples made with 0.5% sodium tripolyphosphate (STPP control) had low scores for rancid odor, flavor, and spice flavor intensity, and also had lowest TBA value of 0.3. This observation is in agreement with previous work showing that phosphate compounds such as sodium tripolyphosphate (STPP) or milk mineral are quite effective antioxidants in cooked ground meats, due to their ability to bind ionic iron and thus prevent iron catalysis of lipid oxidation (Cornforth and West 2002; Jayasingh and Cornforth 2003). The control samples without spices, and prepared on the day of the panel evaluation (fresh control) also had low scores for rancid odor, flavor, and spice flavor intensity, and had a relatively low TBA value of 1.0. All cooked ground beef samples made with spices (cinnamon,

cloves, fennel, pepper, and star anise) had lower ($P < 0.05$) scores for rancid odor and flavor, and lower ($P < 0.05$) TBA values, compared to the control without spices (rancid control), but similar to the low TBA values of the controls with STPP. Previous work on antioxidant mechanism of spices in model systems has identified various phenolic compounds that are type 1 antioxidants, capable of interruption of the initiation and propagation steps of lipid oxidation by donation of hydrogen (H•). However, one cannot rule out the possibility that the fiber component of spices may bind ionic iron in cooked meat systems, and thus behave as type 2 antioxidants such as STPP.

Beef samples made with spices also had lower ($P < 0.05$) beef flavor intensity scores, and higher ($P < 0.05$) spice flavor intensity, compared with fresh or STPP controls. Among the 5 individual spice treatments, samples made with star anise had a higher ($P < 0.05$) spice flavor intensity than samples with cinnamon, cloves, or fennel. 5-spice blends (retail or optimum) effectively lowered ($P < 0.05$) rancid odor, rancid flavor, and TBA values of stored, cooked ground beef samples compared with treatments without added spices (rancid controls). The retail 5-spice blend had significantly higher ($P < 0.05$) spice flavor intensity than the low clove blend, perhaps because the retail blend contained ginger and licorice in addition to the traditional 5 spices. Panel comments indicated that spices imparted characteristic flavors to the samples. The cinnamon-treated samples tasted cinnamony, and samples with black pepper tasted peppery. Controls without added spices were painty, stale, or rancid; control with STPP was beefy and salty; and fresh control was beefy or oily. The retail 5-spice treatment had licorice or spicy flavor, and low clove spice blend was spicy. In this study, the trained panel provided precise information on intensity of various flavors, with no indication of

acceptability. One may infer, however, that samples with high scores for rancid flavor would not be acceptable to most consumers. Conversely, samples with moderate spice flavor intensity would be acceptable to many people. Some panelists commented that some samples were "too hot", or "too spicy", indicating a dislike for higher spice levels (Table A3).

Table A3 - Mean trained panel sensory scores and thiobarbituric acid (TBA) values of spice-treated, cooked ground beef crumbles after 15 d storage at 2°C. Lowest effective spice levels were used as determined from Table 8. [a]

Treatment	Level (% meat wt)	Rancid odor	Rancid flavor	Beef flavor	Spice flavor	TBA value	Qualitative comments
Rancid ctrl	0.0	3.3 a	3.4 a	2.0 b	1.0 d	7.2 a	Rancid, painty, stale
STPP ctrl	0.5	1.4 b	1.4 b	3.0 a	1.1 d	0.3 f	Beefy, salty
Fresh ctrl	0.0	1.5 b	1.5 b	3.2 a	1.1 d	1.0 de	Steak- like, oily, beefy
Cinnamon	0.5	1.1 b	1.1 b	1.7 b	2.9 bc	1.6 cd	Cinnamon flavor, spicy
Cloves	0.1	1.0 b	1.1 b	2.2 b	3.1 bc	0.4 e	Strong clove flavor, like dentist office
Fennel	0.5	1.5 b	1.6 b	1.9 b	3.1 bc	5.5 b	Licorice flavor, spicy
Pepper	0.5	1.4 b	1.2 b	2.1 b	3.2 ab	1.6 cd	Peppery, hot
Star anise	0.5	1.2 b	1.1 b	1.8 b	3.9 a	1.9 c	Licorice flavor
Retail 5 spice blend	0.5	1.0 b	1.2 b	1.9 b	3.3 ab	0.7 ef	Strong spicy, black licorice
Low clove spice blend	0.5	1.3 b	1.2 b	2.1 b	2.4 c	1.0 de	Spicy
$LSD_{0.05}$		0.52	0.52	0.68	0.74	0.66	

[a]Mean values within a column with the same letter are not different ($P < 0.05$)

Correlation coefficients among mean trained panel sensory scores and thiobarbituric acid (TBA) values of spice-treated, cooked ground beef crumbles are shown in Table A4. There was a high correlation ($P < 0.01$) between TBA values and panel scores for rancid odor and flavor (0.83 and 0.78, respectively). Not surprisingly, a very high correlation (0.98) was observed between rancid flavor and rancid odor. There was a significant inverse relationship between spice flavor and beef flavor, indicating that samples with added spice did not retain a typical cooked ground beef flavor. Spice flavor was inversely correlated ($P < 0.01$) with rancid odor and flavor (-0.57 and –0.61, respectively). Thus, samples with added spice tended to lose their beef flavor but did not taste rancid.

Table A4 - Correlation coefficients (r) among mean trained panel sensory scores and thiobarbituric acid (TBA) values of spice-treated, cooked ground beef crumbles after 15 d storage at 2°C

	Rancid odor	Rancid flavor	Beef flavor	Spice flavor	TBA value
Rancid odor	1.00	-	-	-	-
Rancid flavor	0.98*	1.00	-	-	-
Beef flavor	0.03	0.05	1.00	-	-
Spice flavor	-0.57*	-0.61*	-0.60*	1.00	-
TBA value	0.83*	0.78*	-0.31	-0.21	1.00

* $P < 0.01$

Experiment 3 – Aerobic plate count

Aerobic plate counts were done after 1, 8, or 15 d storage at 1°C of cooked ground beef samples made with 0.5% retail 5-spice, 0.5% optimum 5-spice, or controls without spice. Log_{10} mean aerobic plate counts pooled over storage time were 4.1, 4.1, and 3.9, respectively, and were not significantly different, which is not unusual for

cooked samples. There was no significant treatment x time interaction for aerobic plate counts. Thus, addition of 0.5% spice blends had no antimicrobial effects during storage of cooked ground beef in this study. Spices and essential oils are known to exhibit antimicrobial effects in various food products or model systems (Yuste and Fung 2002; Guynot and others 2003; Ozkan and others 2003). The lack of antimicrobial effects in cooked ground beef during storage in this study may be due to heat inactivation or loss of antimicrobial components during cooking.

Conclusions

All spices imparted a distinctive flavor to the cooked ground beef and had marked antioxidant properties. These traditional spices do not simply mask the rancid off-flavors, but rather have antioxidant effects.

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APPENDIX B

DATA FOR CHAPTER 3

Table B1 – Main effects of treatment and storage time on TBA values of cooked meatballs and nitrite-cured sausage

Treatment	TBA
Control	4.44
MM	1.05
MM + Nit 20	0.92
MM + Nit 40	0.85

Day	TBA
1	1.11
8	2.03
15	2.31

Table B2 – Effect of treatment x replicate x storage time interaction on TBA value of cooked meatballs and nitrite-cured sausages

Treatment	Replicate	Day	TBA value
Cntrl	1	1	1.88
Cntrl	1	1	2.10
MM	1	1	0.60
MM	1	1	0.69
MM + Nit 20	1	1	0.61
MM + Nit 20	1	1	0.70
MM + Nit 40	1	1	0.65
MM + Nit 40	1	1	0.48
Cntrl	1	8	7.15
Cntrl	1	8	6.77
MM	1	8	1.02
MM	1	8	1.12
MM + Nit 20	1	8	0.83
MM + Nit 20	1	8	0.62
MM + Nit 40	1	8	0.84
MM + Nit 40	1	8	0.73
Cntrl	1	15	6.49
Cntrl	1	15	7.18
MM	1	15	0.84
MM	1	15	1.04
MM + Nit 20	1	15	0.87
MM + Nit 20	1	15	0.72
MM + Nit 40	1	15	0.71
MM + Nit 40	1	15	0.65
Cntrl	2	1	2.47
Cntrl	2	1	2.24
MM	2	1	0.91
MM	2	1	0.79
MM + Nit 20	2	1	0.86
MM + Nit 20	2	1	0.85
MM + Nit 40	2	1	0.67
MM + Nit 40	2	1	0.77
Cntrl	2	8	5.99
Cntrl	2	8	6.76
MM	2	8	1.05
MM	2	8	1.12
MM + Nit 20	2	8	1.02
MM + Nit 20	2	8	0.98
MM + Nit 40	2	8	0.78
MM + Nit 40	2	8	0.79

Cntrl	2	15	8.27
Cntrl	2	15	7.93
MM	2	15	1.49
MM	2	15	1.64
MM + Nit 20	2	15	1.31
MM + Nit 20	2	15	1.21
MM + Nit 40	2	15	1.22
MM + Nit 40	2	15	1.38
Cntrl	3	1	1.56
Cntrl	3	1	1.47
MM	3	1	1.16
MM	3	1	1.16
MM + Nit 20	3	1	1.11
MM + Nit 20	3	1	1.00
MM + Nit 40	3	1	0.90
MM + Nit 40	3	1	0.96
Cntrl	3	8	2.51
Cntrl	3	8	2.65
MM	3	8	1.12
MM	3	8	1.19
MM + Nit 20	3	8	0.92
MM + Nit 20	3	8	1.05
MM + Nit 40	3	8	0.83
MM + Nit 40	3	8	0.86
Cntrl	3	15	2.52
Cntrl	3	15	4.01
MM	3	15	1.14
MM	3	15	0.85
MM + Nit 20	3	15	0.96
MM + Nit 20	3	15	0.97
MM + Nit 40	3	15	1.15
MM + Nit 40	3	15	0.94

Table B3 – Effect of treatment x replicate x storage time on Hunter color values of cooked meatballs and nitrite-cured sausage

Treatment	Replicate	Day	*L** value	*a** value	*b** value
Cntrl	1	1	52.75	5.21	9.22
Cntrl	1	1	49.03	6.36	13.53
MM	1	1	52.03	6.36	14.66
MM	1	1	50.68	6.52	14.50
MM + Nit 20	1	1	47.85	7.43	8.87
MM + Nit 20	1	1	49.91	9.00	13.31
MM + Nit 40	1	1	45.96	11.41	9.31
MM + Nit 40	1	1	58.66	7.82	7.08
Cntrl	1	8	50.37	2.16	15.05
Cntrl	1	8	54.6	1.63	16.28
MM	1	8	47.97	4.97	13.61
MM	1	8	51.27	4.61	13.26
MM + Nit 20	1	8	55.08	8.00	11.53
MM + Nit 20	1	8	52.5	7.13	10.62
MM + Nit 40	1	8	51.35	10.54	11.83
MM + Nit 40	1	8	55.76	9.34	11.05
Cntrl	1	15	55.87	0.82	16.00
Cntrl	1	15	51.79	1.62	14.77
MM	1	15	47.55	5.01	14.25
MM	1	15	51.04	5.33	13.70
MM + Nit 20	1	15	50.56	8.22	13.20
MM + Nit 20	1	15	48.23	8.49	12.09
MM + Nit 40	1	15	50.26	11.15	12.77
MM + Nit 40	1	15	56.88	8.66	10.77
Cntrl	2	1	49.86	4.75	13.38
Cntrl	2	1	54.56	4.73	13.44
MM	2	1	54.08	3.56	10.50
MM	2	1	52.67	4.97	13.76
MM + Nit 20	2	1	52.13	7.51	10.89
MM + Nit 20	2	1	55.73	6.21	8.64
MM + Nit 40	2	1	55.33	8.42	9.89
MM + Nit 40	2	1	54.27	8.92	10.28
Cntrl	2	8	50.27	2.10	13.19
Cntrl	2	8	54.02	2.12	15.15
MM	2	8	52.02	4.32	12.44
MM	2	8	51.88	4.88	13.18
MM + Nit 20	2	8	56.45	6.09	11.16
MM + Nit 20	2	8	51.34	5.70	10.75
MM + Nit 40	2	8	54.56	7.16	10.11

MM + Nit 40	2	8	54.63	7.01	12.58
Cntrl	2	15	52.99	1.90	14.28
Cntrl	2	15	45.19	1.04	5.67
MM	2	15	50.06	4.70	12.48
MM	2	15	52.24	5.11	14.07
MM + Nit 20	2	15	51.43	8.76	12.12
MM + Nit 20	2	15	55.84	6.92	11.55
MM + Nit 40	2	15	55.17	10.27	12.58
MM + Nit 40	2	15	58.88	7.51	12.58
Cntrl	3	1	54.15	3.65	15.60
Cntrl	3	1	56.17	2.95	14.07
MM	3	1	55.36	3.77	13.01
MM	3	1	53.34	3.65	12.13
MM + Nit 20	3	1	58.13	6.48	9.32
MM + Nit 20	3	1	51.82	6.36	11.62
MM + Nit 40	3	1	44.51	6.46	8.25
MM + Nit 40	3	1	45.12	7.90	8.78
Cntrl	3	8	53.77	1.97	14.89
Cntrl	3	8	54.72	3.12	12.55
MM	3	8	53.64	3.95	13.94
MM	3	8	53.49	3.39	12.08
MM + Nit 20	3	8	54.26	7.71	12.00
MM + Nit 20	3	8	53.89	7.35	10.69
MM + Nit 40	3	8	55.38	8.94	11.64
MM + Nit 40	3	8	50.96	10.18	11.10
Cntrl	3	15	57.94	0.55	14.96
Cntrl	3	15	62.48	1.60	13.45
MM	3	15	53.50	4.30	13.62
MM	3	15	55.63	4.74	14.46
MM + Nit 20	3	15	61.39	5.63	10.06
MM + Nit 20	3	15	53.03	8.82	11.33
MM + Nit 40	3	15	50.74	10.06	11.55
MM + Nit 40	3	15	53.14	10.13	11.17

Table B4 - Data showing effect of treatment effect on cooked yield of meatballs with and without added milk mineral

Treatment	Replicate	Initial wt (g)	Final wt (g)	Drip wt (g)	% Cook yield	Average % cook yield
Control	1	220.76	162.74	58.3	73.7	65.8
Control	2	218.86	135.17	84.15	61.8	
Control	3	219.02	135.65	83.6	61.9	
Milk mineral	1	222.44	170.82	51.65	76.8	68.7
Milk mineral	2	220.06	145.04	73.94	65.9	
Milk mineral	3	218.54	138.48	80.18	63.4	

Table B5 - ANOVA table for MM cooked yield data

Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Treatment	1	12.50	4	48.92	0.26	0.64

Table B6 - ANOVA table for MM color data

Main Effect: Treatment				
Dependent Variable	Mean Square Effect	Mean Square Error	f (df1,2) 3,60	p-level
L	5.79	12.90	0.45	0.72
A	140.69	1.21	115.99	0.00
B	42.30	2.67	15.87	0.00
Main Effect: Day				
Dependent Variable	Mean Square Effect	Mean Square Error	f (df1,2) 2,60	p-level
L	8.55	12.90	0.66	0.52
A	2.69	1.21	2.22	0.12
B	10.01	2.67	3.76	0.03
Interaction: Treatment x Day				
Dependent Variable	Mean Square Effect	Mean Square Error	f (df1,2) 6,60	p-level
L	8.29	12.90	0.64	0.70
A	6.30	1.21	5.19	0.00
B	3.20	2.67	1.20	0.32

Table B7 - ANOVA table for MM TBA value data

Main Effect: Treatment					
Univariate Test	Sums of Squares	df	Mean Square	F	p-level
Effect	165.78	3	55.26	34.06	0.00
Error	110.33	68	1.62		
Main Effect: Day					
Univariate Test	Sums of Squares	df	Mean Square	F	p-level
Effect	19.03	2	9.52	2.55	0.09
Error	257.09	69	3.73		
Main Effect: Treatment x Day					
Univariate Test	Sums of Squares	df	Mean Square	F	p-level
Effect	39.01	6	6.50	7.46	0.00
Error	52.29	60	0.87		

APPENDIX C

DATA FOR CHAPTER 4

Table C1 - Summary of significance (P < 0.05) of treatment (each individual spice), storage time (1, 8, 15 d), spice levels (0, 0.1, 0.5, or 1.0% of meat weight), and their interactions on TBA values of cooked ground beef during refrigerated storage

Source	df	Sum of Squares	Mean Square	F	p-level
Treatment	13	85.86	6.60	10.76	<0.0001
Level	3	884.43	294.81	480.35	<0.0001
Treatment x Level	39	130.38	3.34	5.45	<0.0001
Day	2	416.25	208.13	339.11	<0.0001
Treatment x Day	26	24.72	0.95	1.55	0.04
Level x Day	6	188.31	31.38	51.14	<0.0001
Treatment x Level x Day	8	50.27	0.64	1.05	0.37

* = significant at p < 0.05, NS = not significant, n = nr of observations per mean, df = degrees of freedom.

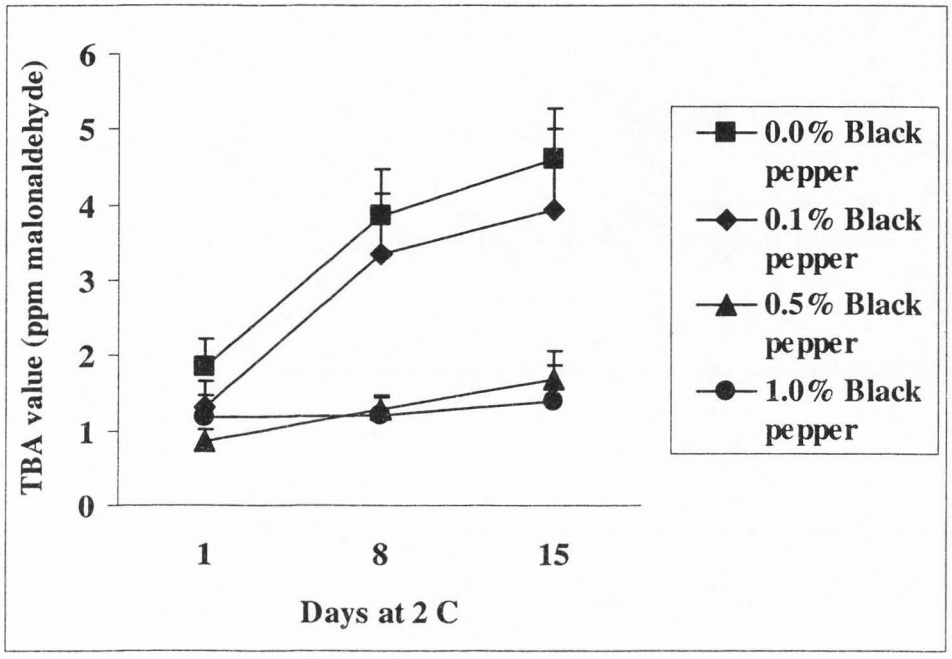

Figure C1 - Effect of black pepper concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

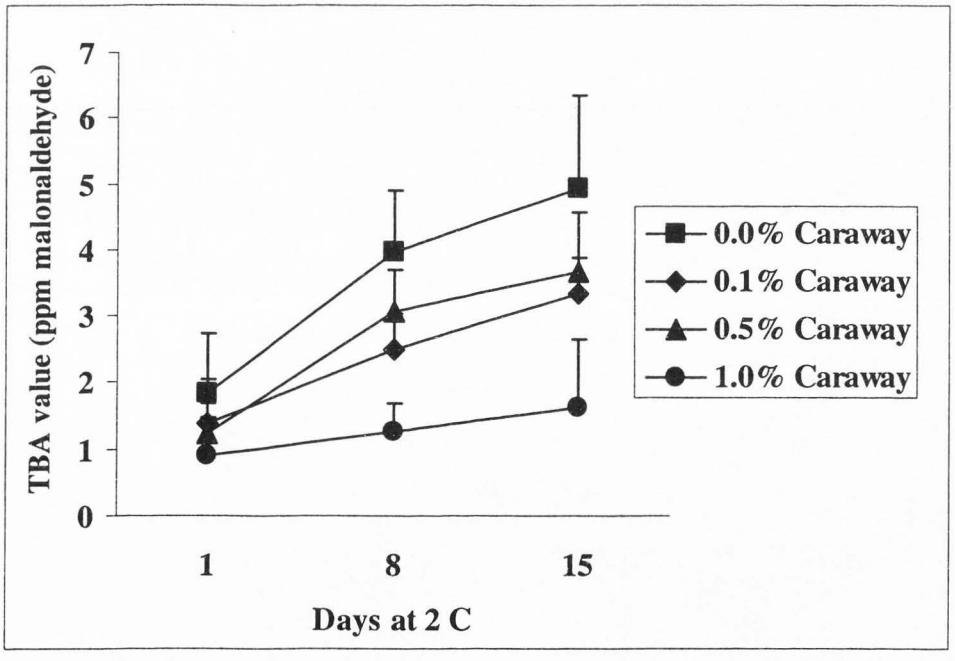

Figure C2 - Effect of caraway concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

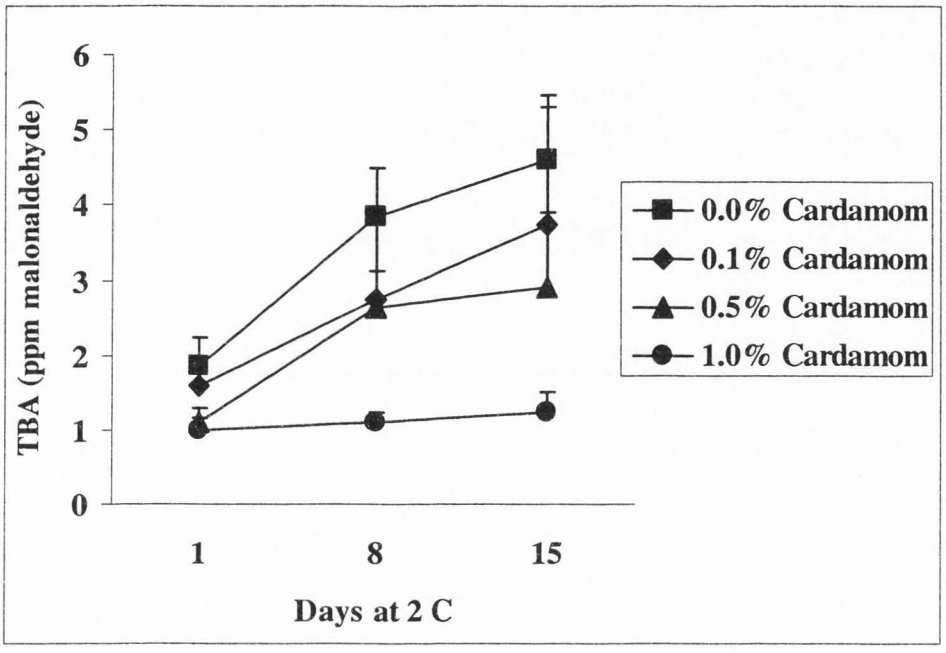

Figure C3 - Effect of cardamom concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

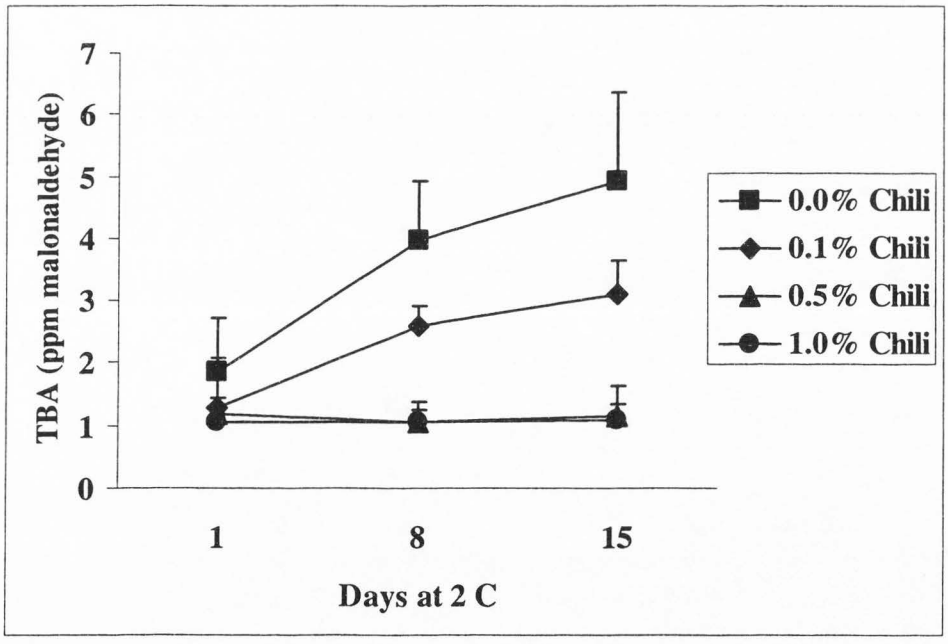

Figure C4 - Effect of chili powder concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

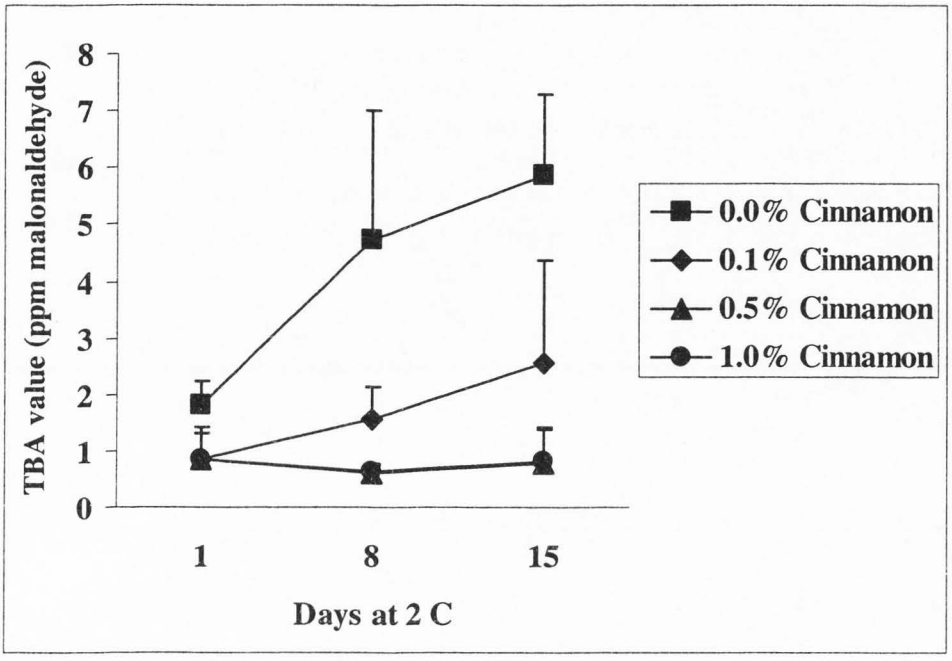

Figure C5 - Effect of cinnamon concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

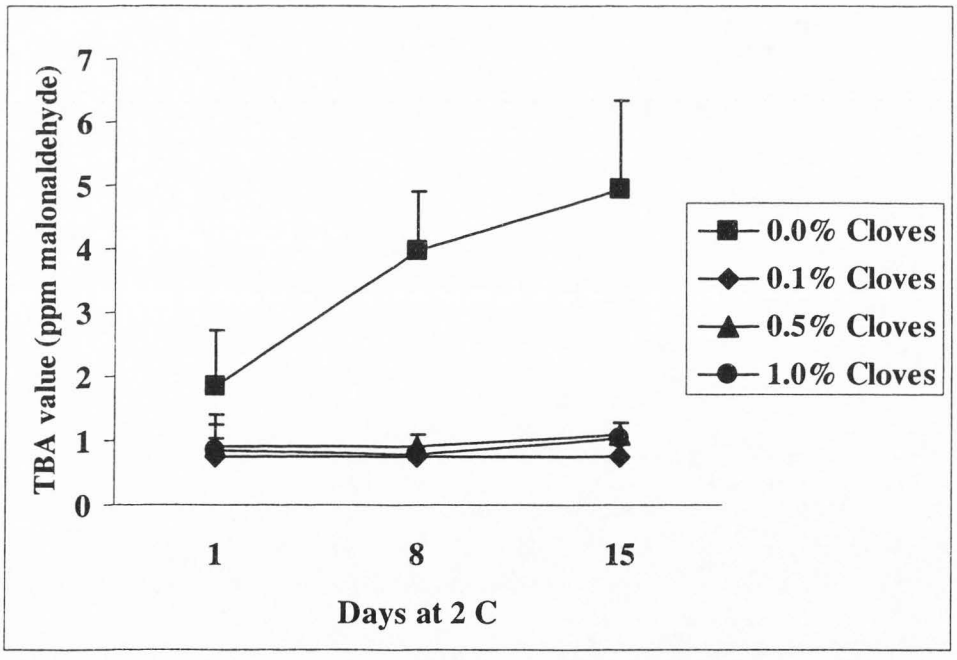

Figure C6 - Effect of clove concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

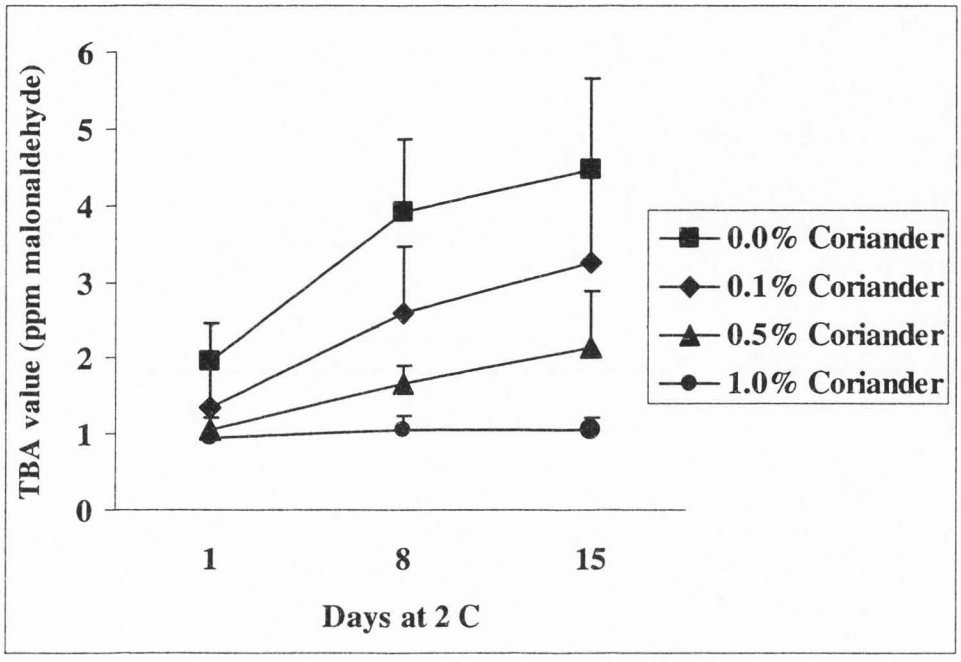

Figure C7 - Effect of coriander concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

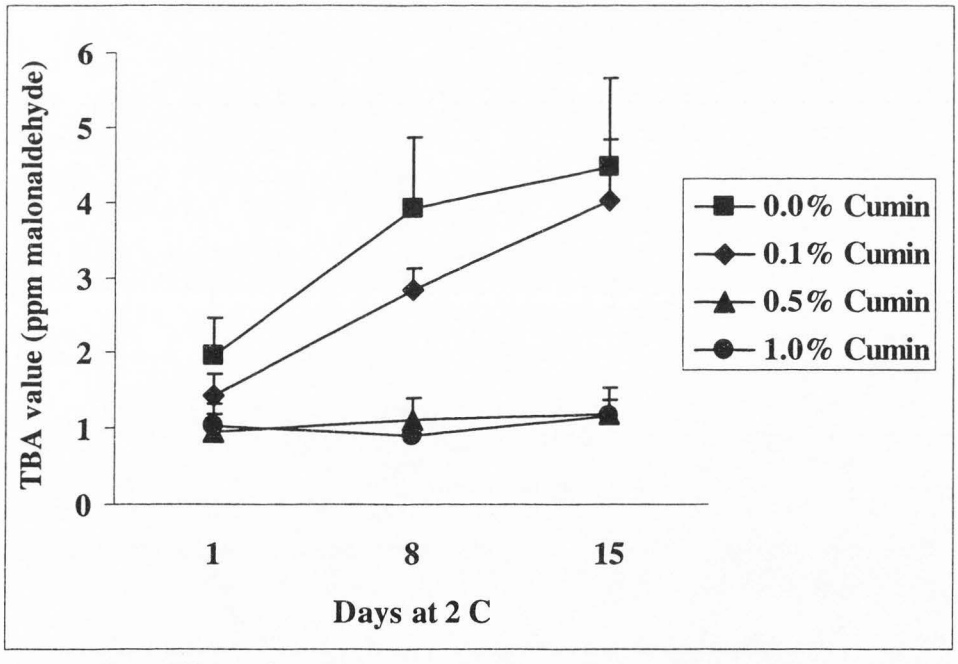

Figure C8 - Effect of cumin concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

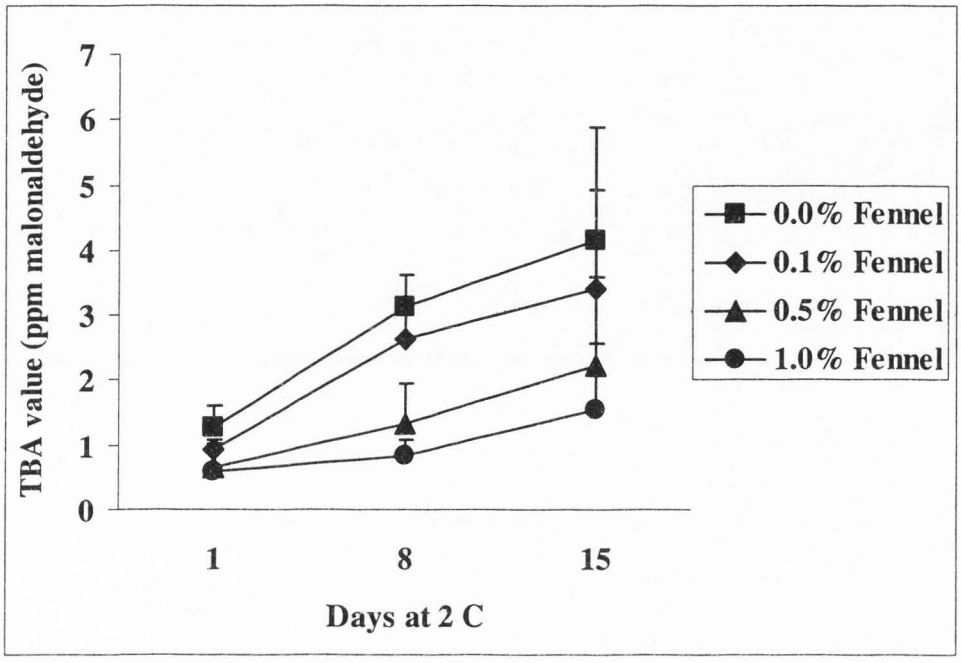

Figure C9 - Effect of fennel concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

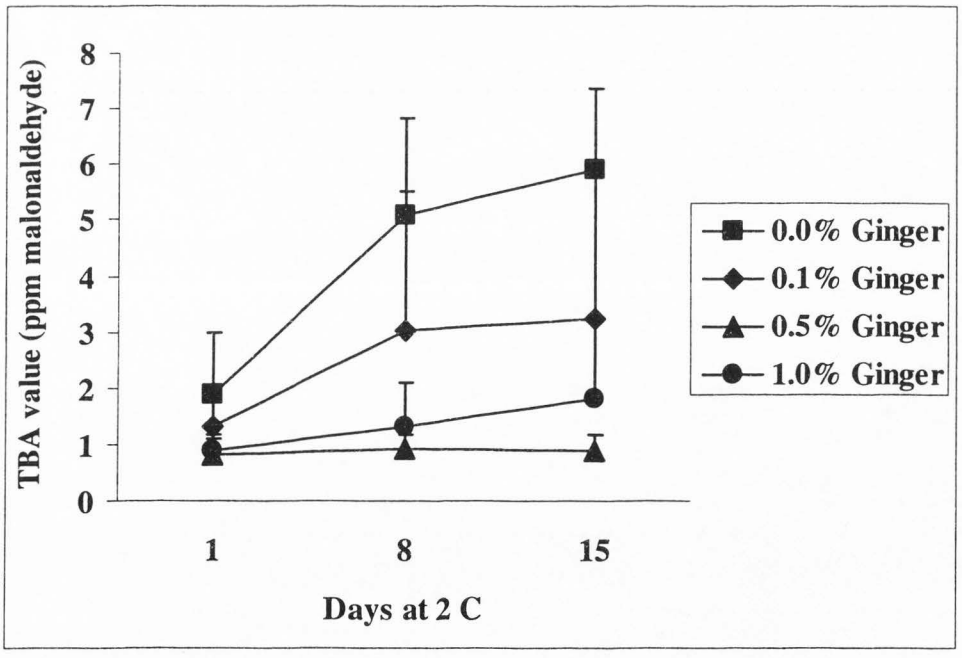

Figure C10 - Effect of ginger concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

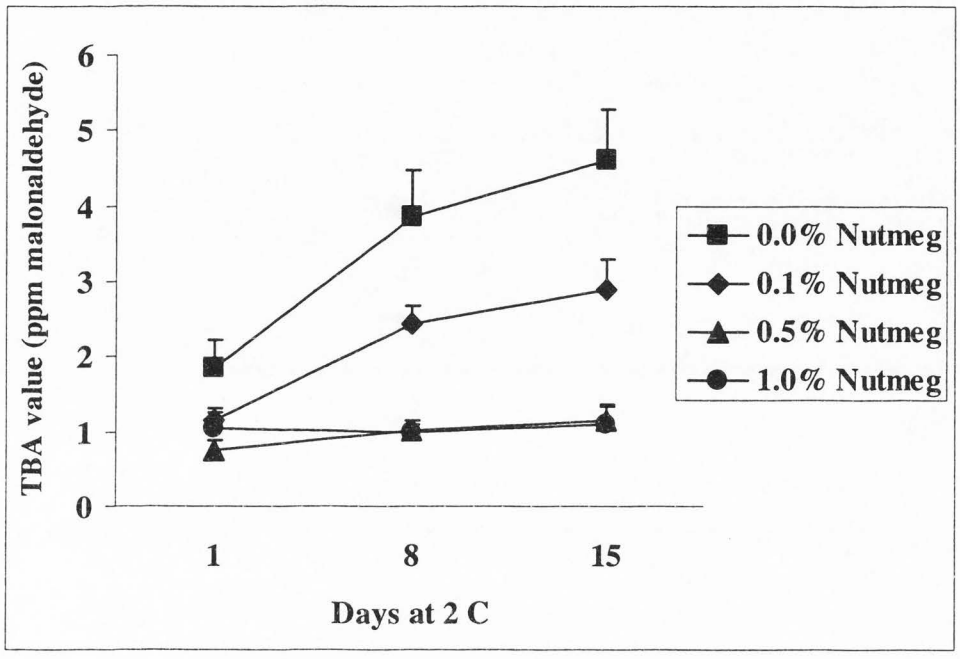

Figure C11 - Effect of nutmeg concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

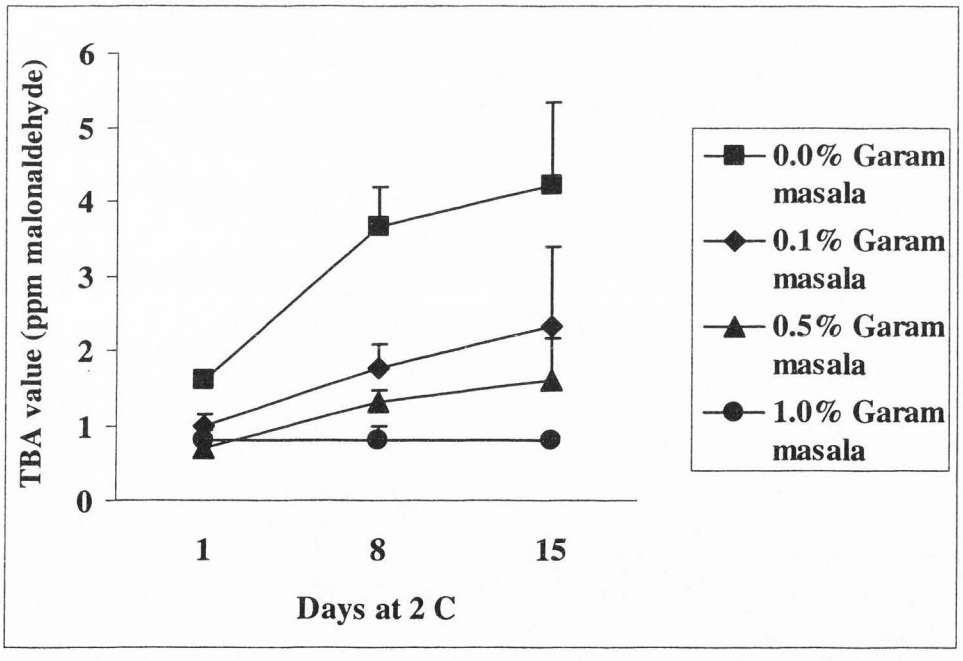

Figure C12 - Effect of retail Garam Masala concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

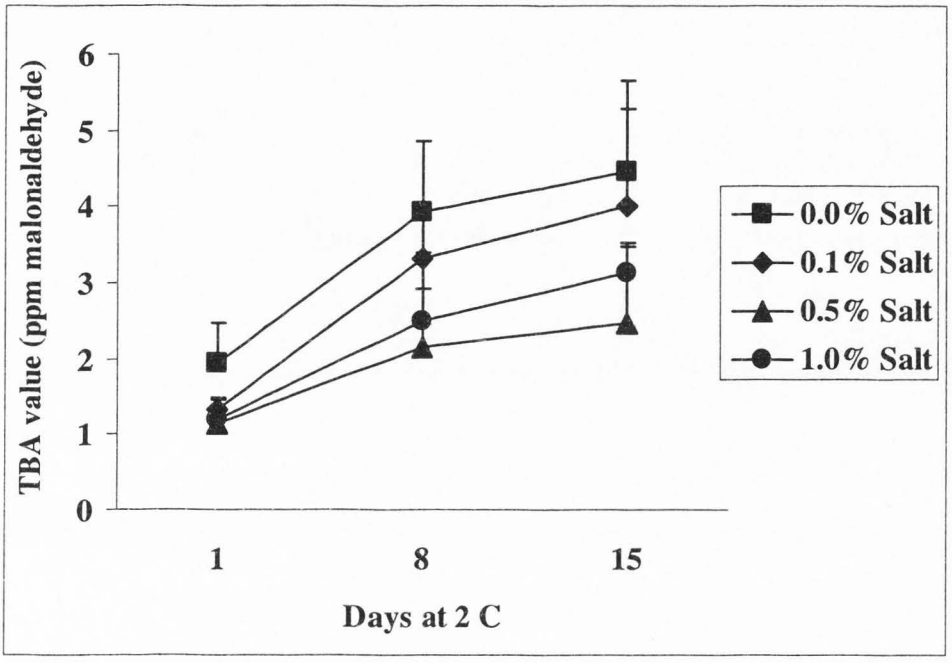

Figure C13 - Effect of salt concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

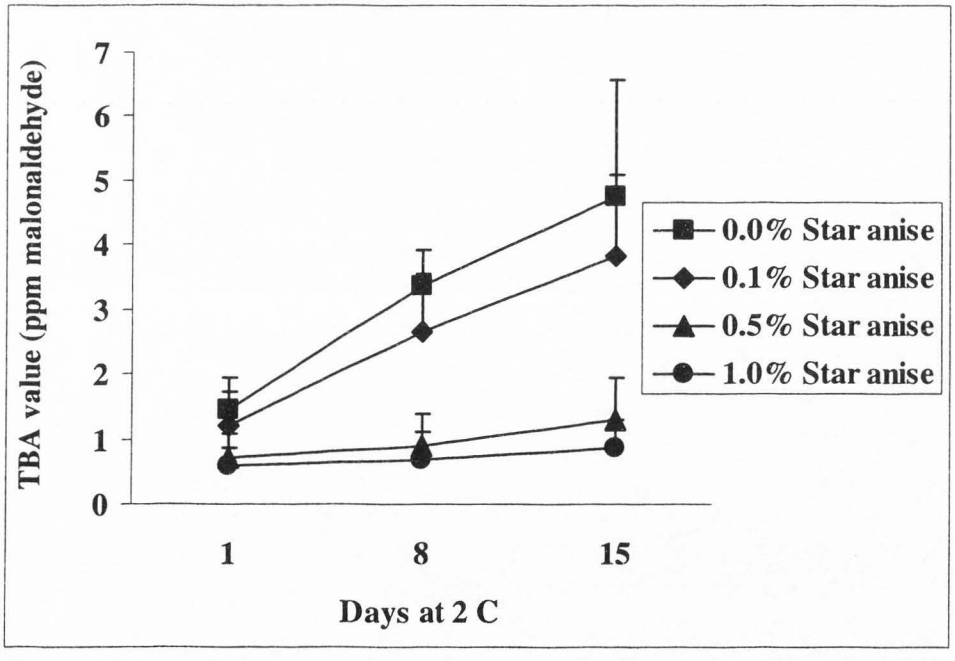

Figure C14 - Effect of star anise concentration (0, 0.1, 0.5, or 1.0% of meat wt.) on thiobarbituric acid values of cooked ground beef during refrigerated storage.

Table C2 - Correlation coefficients among mean trained panel sensory scores and thiobarbituric acid (TBA) values of spice-treated, cooked ground beef crumbles after 15 d storage at 2°C

	Rancid Odor	Rancid Flavor	Beef Flavor	Spice Flavor	TBA Value
Rancid Odor	1.00				
Rancid Flavor	0.81*	1.00			
Beef Flavor	-0.14	-0.13	1.00		
Spice Flavor	-0.38*	-0.36*	-0.26*	1.00	
TBA Value	0.77*	0.77*	-0.42*	-0.17	1.00

* $P < 0.001$.

Table C3 – Data for calculation of correlation coefficients between TBA value and sensory scores for various spices of Garam Masala spice blend

Treatment	TBA value	Rancid odor	Rancid flavor	Beef flavor	Spice flavor
Cinnamon	1.59	1.07	1.14	1.71	2.93
Star anise	1.86	1.21	1.00	1.79	3.93
Coriander	3.38	1.36	1.29	2.00	2.21
Cumin	4.41	1.64	1.71	1.93	2.50
Ginger	1.02	1.43	1.43	2.57	1.57
Fennel	5.47	1.50	1.64	1.93	3.07
Salt	7.07	2.71	2.64	2.21	1.36
Cardamom	3.18	1.36	1.14	2.07	2.57
Caraway	3.88	1.79	1.64	2.07	2.64
Black pepper	1.59	1.36	1.21	2.14	3.21
Nutmeg	3.06	1.29	1.21	1.64	2.50
Milk mineral	0.36	1.36	1.57	2.29	1.07
Chili powder	1.68	1.43	1.43	2.00	1.86
Cloves	0.42	1.00	1.07	2.21	3.07
Rosemary	0.83	1.14	1.07	2.07	3.14
Retail Garam Masala	0.69	1.07	1.14	1.93	3.07
Control15	7.19	3.31	3.44	1.97	1.00
Controlfresh	1.05	1.49	1.53	3.21	1.09
Controlstpp	0.32	1.40	1.43	2.99	1.14

Table C4 - Summary of significance ($p < 0.05$) of treatment (Type I or Type II antioxidants and their combinations), storage time (1, 8, 15 d), sampling method (method 1 or 2), and their various interactions on TBA values of cooked ground beef during refrigerated storage, as determined by analysis of variance (ANOVA)

Effect	n	TBA value
Treatment	36	*
Storage Time	204	NS
Sampling Method	306	NS
Treatment * Storage Time	12	*
Treatment * Sampling Method	18	NS
Storage Time * Sampling Method	102	NS
Treatment * Storage Time * Sampling Method	6	NS

* = significant at $p < 0.05$, NS = not significant, n = nr of observations per mean. Method 1 – samples at storage d (1, 8, 15) were from the same bag; Method 2 – samples at storage d (1, 8, 15) were from individual bags prepared for that d.

Table C5 – Main effects of sampling method and storage time on TBA values of cooked ground beef added with various Type I and Type II antioxidants

Sampling method	TBA
Open	0.72
Not open	0.66

Day	TBA
1	0.63
8	0.69
15	0.75

Table C6 – Treatment x storage time interaction effects on TBA value of cooked ground beef added with various Type I and Type II antioxidants

Treatment	Day	TBA
Control	1	1.47
Control	8	2.62
Control	15	3.42
BHT	1	0.59
BHT	8	0.63
BHT	15	0.59
Cinnamon	1	0.77
Cinnamon	8	0.70
Cinnamon	15	0.77
Cloves	1	0.59
Cloves	8	0.50
Cloves	15	0.55
Rosemary	1	0.69
Rosemary	8	0.84
Rosemary	15	1.00
MM	1	0.50
MM	8	0.44
MM	15	0.49
STPP	1	0.42
STPP	8	0.41
STPP	15	0.43
BHT + MM	1	0.60
BHT + MM	8	0.58
BHT + MM	15	0.55
Cinnamon + MM	1	0.75
Cinnamon + MM	8	0.63
Cinnamon + MM	15	0.73
Cloves + MM	1	0.55
Cloves + MM	8	0.54
Cloves + MM	15	0.57
Rosemary + MM	1	0.50
Rosemary + MM	8	0.54
Rosemary + MM	15	0.52
BHT + STPP	1	0.53
BHT + STPP	8	0.47
BHT + STPP	15	0.46
Cinnamon + STPP	1	0.57
Cinnamon + STPP	8	0.66
Cinnamon + STPP	15	0.59
Cloves + STPP	1	0.51

Cloves + STPP	8	0.54
Cloves + STPP	15	0.48
Rosemary + STPP	1	0.53
Rosemary + STPP	8	0.56
Rosemary + STPP	15	0.50
BHT + Cinnamon + Cloves + Rosemary	1	0.61
BHT + Cinnamon + Cloves + Rosemary	8	0.63
BHT + Cinnamon + Cloves + Rosemary	15	0.66
MM + STPP	1	0.52
MM + STPP	8	0.50
MM + STPP	15	0.49

Table C7 - ANOVA tabe for GM TBA value data

Dependent Variable: TBA Value					
Source	df	Sum of Squares	Mean Square	F	p-level
Model	167	1780.23	10.66	17.37	< 0.0001
Error	840	515.55	0.61		
Corrected Total	1007	2295.78			

Table C8 - ANOVA table for GM sensory data

Dependent Variable: Rancid Odor					
Source	df	Sum of Squares	Mean Square	F	p-level
Model	18	81.67	4.54	8.07	< 0.0001
Error	247	138.94	0.56		
Corrected Total	265	220.61			
Dependent Variable: Rancid Flavor					
Source	df	Sum of Squares	Mean Square	F	p-level
Model	18	89.52	4.97	9.26	< 0.0001
Error	247	132.69	0.54		
Corrected Total	265	222.21			
Dependent Variable: Beef Flavor					
Source	df	Sum of Squares	Mean Square	F	p-level
Model	18	40.00	2.22	2.61	0.0005
Error	247	210.30	0.85		
Corrected Total	265	250.30			
Dependent Variable: Spice Flavor					
Source	df	Sum of Squares	Mean Square	F	p-level
Model	18	199.97	11.11	11.01	< 0.0001
Error	247	249.21	1.01		
Corrected Total	265	449.18			

Table C9 - ANOVA table for Type I and Type II additive antioxidant effect data

Main Effect: Treatment					
Univariate Test	Sum of Squares	df	Mean Square	F	p-level
Effect	131.92	16	8.25	96.50	0.00
Error	50.84	595	0.09		
Main Effect: Sampling Method					
Univariate Test	Sum of Squares	df	Mean Square	F	p-level
Effect	0.55	1	0.55	1.84	0.18
Error	182.21	610	0.30		
Main Effect: Day					
Univariate Test	Sum of Squares	df	Mean Square	F	p-level
Effect	1.55	2	0.77	2.60	0.08
Error	181.21	609	0.30		
Main Effect: Treatment x Day					
Univariate Test	Sum of Squares	df	Mean Square	F	p-level
Effect	22.52	32	0.70	14.75	0.00
Error	26.77	561	0.05		
Main Effect: Treatment x Sampling Method					
Univariate Test	Sum of Squares	df	Mean Square	F	p-level
Effect	0.90	16	0.06	0.66	0.84
Error	49.39	578	0.09		
Main Effect: Sampling Method x Day					
Univariate Test	Sum of Squares	df	Mean Square	F	p-level
Effect	0.49	2	0.25	0.83	0.44
Error	180.17	606	0.30		
Main Effect: Treatment x Sampling Method x Day					
Univariate Test	Sum of Squares	df	Mean Square	F	p-level
Effect	1.32	32	0.04	0.89	0.64
Error	23.52	510	0.05		

APPENDIX D

DATA FOR CHAPTER 5

Table D1 – Mean TBA values for treatment and storage time main effects in cooked ground beef

Treatment	TBA
Control	4.63
Raisin 0.5	2.47
Raisin 1.0	1.48
Raisin 1.5	1.21
Raisin 2.0	0.84
Glucose 1.45	1.28

Day	TBA
1	1.22
4	1.81
7	2.22
14	2.70

Table D2 – Mean TBA values for treatment and storage time main effects in cooked ground pork

Treatment	TBA
Control	12.75
Raisin 1.0	5.26
Raisin 2.0	2.60
Raisin 3.0	2.16
Raisin 4.0	1.81
Glucose 2.9	3.23

Day	TBA
1	2.65
4	4.30
7	5.62
14	5.96

Table D3 – Mean TBA values for treatment and storage time main effects in cooked ground chicken

Treatment	TBA
Control	6.39
Raisin 1.0	2.34
Raisin 2.0	0.80
Raisin 3.0	0.49
Raisin 4.0	0.45
Glucose 2.9	0.46

Day	TBA
1	1.31
4	1.65
7	1.94
14	2.40

Table D4 – Mean sensory panel scores for storage time main effect in different cooked meats

Day	Beef flavor	Rancid flavor	Raisin flavor
1	2.74	1.56	1.08
4	2.21	1.92	1.04
7	2.27	1.90	1.08
14	2.31	2.11	1.09
	Pork flavor	**Rancid flavor**	**Raisin flavor**
1	2.61	1.86	1.56
4	2.51	1.95	1.80
7	2.44	2.11	1.52
14	2.32	2.16	1.56
	Chicken flavor	**Rancid flavor**	**Raisin flavor**
1	2.23	1.81	1.81
4	2.41	1.82	1.78
7	2.24	1.90	1.72
14	2.44	2.06	1.66

Table D5 - Interaction effects of treatment x storage time on sensory scores[1] (n = 18) of cooked ground beef formulated with raisin paste or glucose

Treatment	Day at 2°C	Beef flavor intensity[1]	Rancid flavor intensity[1]	Raisin flavor intensity[1]
Control	1	1.94 ± 0.94 h-j	3.00 ± 0.91 c	1.00 ± 0.00 b
Control	4	1.72 ± 0.83 ij	3.22 ± 0.88 bc	1.00 ± 0.00 b
Control	7	1.56 ± 0.78 j	3.56 ± 1.15 ab	1.00 ± 0.00 b
Control	14	1.67 ± 0.91 ij	3.83 ± 1.20 a	1.00 ± 0.00 b
0.5% Raisin	1	2.61 ± 1.20 b-g	1.44 ± 0.70 ef	1.00 ± 0.00 b
0.5% Raisin	4	1.72 ± 0.75 ij	2.44 ± 1.15 d	1.00 ± 0.00 b
0.5% Raisin	7	2.00 ± 0.91 g-j	2.28 ± 0.75 d	1.06 ± 0.24 ab
0.5% Raisin	14	2.06 ± 0.80 f-j	2.44 ± 1.15 d	1.06 ± 0.24 ab
1.0% Raisin	1	2.83 ± 0.92 a-d	1.22 ± 0.43 ef	1.06 ± 0.24 ab
1.0% Raisin	4	2.39 ± 0.92 b-h	1.72 ± 0.67 e	1.00 ± 0.00 b
1.0% Raisin	7	2.28 ± 0.96 c-i	1.50 ± 0.71 ef	1.00 ± 0.00 b
1.0% Raisin	14	2.17 ± 0.99 e-j	2.33 ± 0.97 d	1.00 ± 0.00 b
1.5% Raisin	1	3.00 ± 0.91 ab	1.17 ± 0.38 f	1.11 ± 0.47 ab
1.5% Raisin	4	2.22 ± 0.88 d-i	1.56 ± 1.04 ef	1.00 ± 0.00 b
1.5% Raisin	7	2.44 ± 0.86 b-h	1.56 ± 0.70 ef	1.00 ± 0.00 b
1.5% Raisin	14	2.83 ± 1.04 a-d	1.39 ± 0.61 ef	1.11 ± 0.32 ab
2.0% Raisin	1	3.28 ± 0.83 a	1.28 ± 0.57 ef	1.11 ± 0.32 ab
2.0% Raisin	4	2.67 ± 1.14 a-f	1.33 ± 0.77 ef	1.17 ± 0.38 ab
2.0% Raisin	7	2.89 ± 0.90 a-c	1.22 ± 0.43 ef	1.17 ± 0.51 ab
2.0% Raisin	14	2.72 ± 1.13 a-e	1.17 ± 0.38 f	1.22 ± 0.55 ab
1.45% Glucose	1	2.78 ± 0.73 a-e	1.22 ± 0.55 ef	1.22 ± 0.73 ab
1.45% Glucose	4	2.56 ± 1.10 b-h	1.22 ± 0.43 ef	1.28 ± 0.57 a
1.45% Glucose	7	2.44 ± 1.04 b-h	1.28 ± 0.46 ef	1.28 ± 0.67 a
1.45% Glucose	14	2.39 ± 1.14 b-h	1.50 ± 0.62 ef	1.17 ± 0.51 ab
$LSD_{0.05}$		0.62	0.51	0.23

[1] Mean flavor intensity scores ± standard deviation (SD), where 1 = no detectable flavor, 2 = slightly intense flavor, 3 = moderately intense flavor, 4 = very intense flavor and 5 = extremely intense flavor. Means within a column with the same letter are not different ($P < 0.05$).

Table D6 - Interaction effects of treatment x storage time on sensory scores[1] (n = 18) of cooked ground pork formulated with raisin paste or glucose

Treatment	Day at 2°C	Pork flavor intensity[1]	Rancid flavor intensity[1]	Raisin flavor intensity[1]
Control	1	1.89 ± 1.02 cd	3.83 ± 1.04 a	1.00 ± 0.00 f
Control	4	1.67 ± 0.97 d	3.94 ± 1.06 a	1.00 ± 0.00 f
Control	7	1.67 ± 0.97 d	3.94 ± 0.94 a	1.00 ± 0.00 f
Control	14	1.67 ± 1.03 d	4.06 ± 0.87 a	1.06 ± 0.24 ef
1.0% Raisin	1	2.83 ± 0.99 a	1.72 ± 0.83 d-f	1.28 ± 0.46 d-f
1.0% Raisin	4	2.28 ± 1.18 a-d	2.50 ± 1.04 bc	1.28 ± 0.57 d-f
1.0% Raisin	7	2.50 ± 0.86 a-c	2.22 ± 1.17 b-d	1.44 ± 0.78 c-f
1.0% Raisin	14	2.06 ± 0.94 b-d	2.56 ± 1.38 b	1.39 ± 0.61 c-f
2.0% Raisin	1	2.89 ± 0.90 a	1.44 ± 0.78 e-g	1.44 ± 0.70 c-f
2.0% Raisin	4	2.83 ± 0.92 a	1.39 ± 0.70 e-g	1.89 ± 1.23 a-c
2.0% Raisin	7	2.72 ± 1.13 ab	1.94 ± 1.26 c-e	1.39 ± 0.61 c-f
2.0% Raisin	14	2.67 ± 1.08 ab	1.56 ± 0.86 e-g	1.56 ± 0.78 c-f
3.0% Raisin	1	2.67 ± 0.97 ab	1.67 ± 0.97 d-g	1.44 ± 0.92 c-f
3.0% Raisin	4	2.72 ± 1.13 ab	1.22 ± 0.43 fg	1.94 ± 1.11 a-c
3.0% Raisin	7	2.61 ± 0.92 ab	1.94 ± 1.00 c-e	1.50 ± 0.86 c-f
3.0% Raisin	14	2.67 ± 0.91 ab	1.78 ± 0.94 d-f	1.50 ± 0.71 c-f
4.0% Raisin	1	2.72 ± 1.02 ab	1.39 ± 0.85 e-g	2.39 ± 1.42 a
4.0% Raisin	4	2.89 ± 0.90 a	1.39 ± 0.61 e-g	2.39 ± 1.50 a
4.0% Raisin	7	2.50 ± 0.92 a-c	1.11 ± 0.32 g	2.17 ± 1.15 ab
4.0% Raisin	14	2.56 ± 0.98 ab	1.39 ± 0.85 e-g	2.22 ± 1.44 a
2.9% Glucose	1	2.67 ± 1.03 ab	1.11 ± 0.32 g	1.83 ± 1.10 a-d
2.9% Glucose	4	2.67 ± 1.19 ab	1.28 ± 0.75 fg	2.28 ± 1.36 a
2.9% Glucose	7	2.67 ± 1.08 ab	1.50 ± 0.71 e-g	1.61 ± 0.85 b-e
2.9% Glucose	14	2.33 ± 1.08 a-d	1.61 ± 0.70 e-g	1.61 ± 0.98 b-e
$LSD_{0.05}$		0.66	0.58	0.60

[1] Mean flavor intensity scores ± standard deviation (SD), where 1 = no detectable flavor, 2 = slightly intense flavor, 3 = moderately intense flavor, 4 = very intense flavor and 5 = extremely intense flavor. Means within a column with the same letter are not different ($P < 0.05$).

Table D7 - Interaction effects of treatment x storage time on sensory scores[1] (n = 18) of cooked ground chicken formulated with raisin paste or glucose

Treatment	Day at 2°C	Chicken Flavor Intensity[1]	Rancid Flavor Intensity[1]	Raisin Flavor Intensity[1]
Control	1	1.61 ± 0.92 f	3.72 ± 1.32 a	1.06 ± 0.24 d
Control	4	1.78 ± 1.06 ef	3.83 ± 1.15 a	1.17 ± 0.51 d
Control	7	1.56 ± 0.86 f	4.11 ± 1.08 a	1.00 ± 0.00 d
Control	14	1.67 ± 1.03 f	4.22 ± 1.06 a	1.00 ± 0.00 d
1.0% Raisin	1	2.00 ± 0.97 c-f	2.33 ± 1.37 cd	1.28 ± 0.75 cd
1.0% Raisin	4	2.11 ± 1.02 b-f	2.56 ± 1.10 bc	1.22 ± 0.43 cd
1.0% Raisin	7	1.94 ± 1.00 d-f	2.56 ± 1.15 bc	1.17 ± 0.51 d
1.0% Raisin	14	2.06 ± 0.94 b-f	2.94 ± 1.06 b	1.17 ± 0.38 d
2.0% Raisin	1	2.44 ± 0.98 a-d	1.39 ± 0.70 ef	1.61 ± 0.98 b-d
2.0% Raisin	4	2.67 ± 0.84 ab	1.11 ± 0.32 f	1.56 ± 0.70 b-d
2.0% Raisin	7	2.44 ± 0.86 a-d	1.44 ± 0.78 ef	1.56 ± 0.86 b-d
2.0% Raisin	14	2.50 ± 0.86 a-d	1.83 ± 0.99 de	1.61 ± 1.04 b-d
3.0% Raisin	1	2.56 ± 0.86 a-d	1.22 ± 0.43 f	2.06 ± 1.26 ab
3.0% Raisin	4	2.67 ± 0.97 ab	1.11 ± 0.32 f	2.06 ± 1.26 ab
3.0% Raisin	7	2.61 ± 0.92 a-c	1.06 ± 0.24 f	2.11 ± 1.32 ab
3.0% Raisin	14	2.78 ± 0.81 a	1.22 ± 0.43 f	1.89 ± 1.13 a-c
4.0% Raisin	1	2.44 ± 1.10 a-d	1.11 ± 0.32 f	2.33 ± 1.53 a
4.0% Raisin	4	2.56 ± 0.92 a-d	1.22 ± 0.55 f	2.50 ± 1.42 a
4.0% Raisin	7	2.56 ± 1.10 a-d	1.11 ± 0.32 f	2.11 ± 1.57 ab
4.0% Raisin	14	2.78 ± 0.88 a	1.11 ± 0.32 f	2.22 ± 1.63 ab
2.9% Glucose	1	2.33 ± 1.14 a-e	1.11 ± 0.32 f	2.50 ± 1.69 a
2.9% Glucose	4	2.67 ± 0.91 ab	1.11 ± 0.32 f	2.17 ± 1.20 ab
2.9% Glucose	7	2.33 ± 0.97 a-e	1.11 ± 0.32 f	2.39 ± 1.42 a
2.9% Glucose	14	2.83 ± 1.10 a	1.06 ± 0.24 f	2.06 ± 1.21 ab
$LSD_{0.05}$		0.63	0.51	0.71

[1] Mean flavor intensity scores ± standard deviation (SD), where 1 = no detectable flavor, 2 = slightly intense flavor, 3 = moderately intense flavor, 4 = very intense flavor and 5 = extremely intense flavor. Means within a column with the same letter are not different ($P < 0.05$).

Table D8 – Data for determining correlation coefficients between mean TBA values and sensory scores in cooked ground beef

Treatment	Day	TBA	Beef flavor	Rancid flavor	Raisin flavor
Control	1	2.43	1.94	3.00	1.00
Control	4	4.13	1.72	3.22	1.00
Control	7	5.16	1.56	3.56	1.00
Control	14	6.81	1.67	3.83	1.00
Raisin 0.5	1	1.45	2.61	1.44	1.00
Raisin 0.5	4	2.34	1.72	2.44	1.00
Raisin 0.5	7	2.77	2.00	2.78	1.06
Raisin 0.5	14	3.34	2.05	2.44	1.06
Raisin 1.0	1	1	2.83	1.22	1.06
Raisin 1.0	4	1.21	2.39	1.72	1.00
Raisin 1.0	7	1.66	2.28	1.50	1.00
Raisin 1.0	14	2.05	2.17	2.33	1.00
Raisin 1.5	1	0.88	3.00	1.17	1.11
Raisin 1.5	4	1.16	2.22	1.56	1.00
Raisin 1.5	7	1.32	2.44	1.56	1.00
Raisin 1.5	14	1.48	2.83	1.39	1.11
Raisin 2.0	1	0.7	3.28	1.28	1.11
Raisin 2.0	4	0.81	2.67	1.33	1.17
Raisin 2.0	7	0.88	2.89	1.22	1.17
Raisin 2.0	14	0.98	2.72	1.17	1.22
Glucose 1.45	1	0.89	2.78	1.22	1.22
Glucose 1.45	4	1.2	2.56	1.22	1.28
Glucose 1.45	7	1.53	2.44	1.28	1.28
Glucose 1.45	14	1.52	2.39	1.50	1.17

Table D9 – Data for determining correlation coefficients between mean TBA values and sensory scores in cooked ground pork

Treatment	Day	TBA	Pork flavor	Rancid flavor	Raisin flavor
Control	1	8.63	1.89	3.83	1.00
Control	4	11.96	1.67	3.94	1.00
Control	7	14.95	1.67	3.94	1.00
Control	14	15.43	1.67	4.06	1.06
Raisin 1.0	1	2.34	2.83	1.72	1.28
Raisin 1.0	4	5.4	2.28	2.50	1.28
Raisin 1.0	7	6.19	2.50	2.22	1.44
Raisin 1.0	14	7.09	2.06	2.56	1.39
Raisin 2.0	1	1.26	2.89	1.44	1.44
Raisin 2.0	4	2.44	2.83	1.39	1.89
Raisin 2.0	7	3.22	2.72	1.94	1.39
Raisin 2.0	14	3.49	2.67	1.56	1.56
Raisin 3.0	1	0.94	2.67	1.67	1.44
Raisin 3.0	4	1.74	2.72	1.22	1.94
Raisin 3.0	7	2.94	2.61	1.94	1.50
Raisin 3.0	14	3.01	2.67	1.78	1.50
Raisin 4.0	1	1.16	2.72	1.39	2.39
Raisin 4.0	4	1.6	2.89	1.39	2.39
Raisin 4.0	7	2.18	2.50	1.11	2.16
Raisin 4.0	14	2.3	2.56	1.39	2.22
Glucose 2.9	1	1.57	2.67	1.11	1.83
Glucose 2.9	4	2.67	2.67	1.28	2.28
Glucose 2.9	7	4.24	2.67	1.50	1.61
Glucose 2.9	14	4.43	2.33	1.61	1.61

Table D10 – Data for determining correlation coefficients between mean TBA values and sensory scores in cooked ground chicken

Treatment	Day	TBA	Chicken flavor	Rancid flavor	Raisin flavor
Control	1	4.02	1.61	3.72	1.06
Control	4	5.6	1.78	3.83	1.17
Control	7	6.69	1.56	4.11	1.00
Control	14	9.27	1.67	4.22	1.00
Raisin 1.0	1	1.61	2.00	2.33	1.28
Raisin 1.0	4	2.18	2.11	2.56	1.22
Raisin 1.0	7	2.6	1.94	2.56	1.17
Raisin 1.0	14	2.96	2.06	2.94	1.17
Raisin 2.0	1	0.74	2.44	1.39	1.61
Raisin 2.0	4	0.76	2.67	1.11	1.56
Raisin 2.0	7	0.82	2.44	1.44	1.56
Raisin 2.0	14	0.9	2.50	1.83	1.61
Raisin 3.0	1	0.54	2.56	1.22	2.06
Raisin 3.0	4	0.49	2.67	1.11	2.06
Raisin 3.0	7	0.49	2.61	1.06	2.11
Raisin 3.0	14	0.45	2.78	1.22	1.89
Raisin 4.0	1	0.45	2.44	1.11	2.33
Raisin 4.0	4	0.44	2.56	1.22	2.50
Raisin 4.0	7	0.59	2.56	1.11	2.11
Raisin 4.0	14	0.33	2.78	1.11	2.22
Glucose 2.9	1	0.49	2.33	1.11	2.50
Glucose 2.9	4	0.47	2.67	1.11	2.17
Glucose 2.9	7	0.44	2.33	1.11	2.39
Glucose 2.9	14	0.46	2.83	1.06	2.06

Table D11 – Data for Hunter color values of cooked ground chicken

Treatment	Replicate	*L**	*a**	*b**
Control	1	82.34	0.16	15.43
Control	1	75.19	-0.25	14.81
Control	2	79.37	-0.05	15.55
Control	2	74.37	0.08	14.15
Control	3	79.88	1.08	17.00
Control	3	78.74	0.54	15.18
Raisin 1.0	1	60.11	6.47	19.56
Raisin 1.0	1	69.81	6.00	19.83
Raisin 1.0	2	74.74	4.96	17.57
Raisin 1.0	2	65.54	5.94	21.85
Raisin 1.0	3	72.91	5.27	19.50
Raisin 1.0	3	73.92	5.23	18.15
Raisin 2.0	1	60.66	7.49	21.80
Raisin 2.0	1	57.87	8.11	19.77
Raisin 2.0	2	61.38	7.53	21.36
Raisin 2.0	2	60.03	8.20	22.90
Raisin 2.0	3	64.03	7.80	23.99
Raisin 2.0	3	61.91	7.62	23.09
Raisin 3.0	1	55.04	8.83	25.61
Raisin 3.0	1	56.16	8.60	22.94
Raisin 3.0	2	59.67	8.97	24.03
Raisin 3.0	2	55.12	9.78	27.74
Raisin 3.0	3	57.28	8.38	23.07
Raisin 3.0	3	56.44	9.19	28.29
Raisin 4.0	1	57.18	8.62	23.42
Raisin 4.0	1	61.52	7.94	22.07
Raisin 4.0	2	55.49	9.42	22.93
Raisin 4.0	2	57.42	9.39	26.50
Raisin 4.0	3	60.62	8.48	24.43
Raisin 4.0	3	62.59	8.06	20.27
Glucose 2.9	1	58.16	9.15	23.53
Glucose 2.9	1	56.51	9.62	24.46
Glucose 2.9	2	55.00	10.68	27.75
Glucose 2.9	2	50.31	10.57	26.75
Glucose 2.9	3	54.66	10.19	24.53
Glucose 2.9	3	59.13	9.72	24.53

Table D12 - ANOVA table for beef raisin TBA data

Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Treatment	5	47.60	138	0.73	64.86	0.00
Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Day	3	14.03	140	2.12	6.61	0.00
Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Treatment	5	47.60	120	0.18	259.09	0.00
Day	3	14.03	120	0.18	76.38	0.00
Treatment x Day	15	2.48	120	0.18	13.48	0.00

Table D13 - ANOVA table for pork raisin TBA data

Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Treatment	5	414.80	138	6.52	63.64	0.00
Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Day	3	81.36	140	19.50	4.17	0.01
Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Treatment	5	414.80	120	4.76	87.19	0.00
Day	3	81.36	120	4.76	17.10	0.00
Treatment x Day	15	5.63	120	4.76	1.18	0.29

Table D14 - ANOVA table for chicken raisin TBA data

Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Treatment	5	132.91	138	1.03	129.13	0.00
Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Day	3	7.63	140	5.60	1.36	0.26
Effect	df Effect	Mean Square Effect	df Error	Mean Square Error	F	p-level
Treatment	5	132.91	120	0.40	333.34	0.00
Day	3	7.63	120	0.40	19.12	0.00
Treatment x Day	15	4.75	120	0.40	11.92	0.00

Table D15 - ANOVA table for beef raisin sensory data

Main Effect: Treatment				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 5,426	p-level
Beef Flavor	12.37	0.93	13.37	0.00
Rancid Flavor	48.51	0.66	73.22	0.00
Raisin Flavor	0.66	0.12	5.67	0.00
Main Effect: Day				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 3,428	p-level
Beef Flavor	6.34	1.02	6.21	0.00
Rancid Flavor	5.76	1.19	4.86	0.00
Raisin Flavor	0.01	0.12	0.05	0.99
Main Effect: Treatment x Day				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 15,**	p-level
Beef Flavor	0.47	0.90	0.52	0.93
Rancid Flavor	1.16	0.61	1.92	0.02
Raisin Flavor	0.04	0.12	0.31	0.99

Table D16 - ANOVA table for pork raisin sensory data

Main Effect: Treatment				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 5,426	p-level
Pork Flavor	10.76	1.00	10.76	0.00
Rancid Flavor	71.84	0.80	89.58	0.00
Raisin Flavor	13.55	0.84	16.16	0.00
Main Effect: Day				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 3,428	p-level
Pork Flavor	1.56	1.11	1.41	0.24
Rancid Flavor	2.05	1.62	1.26	0.29
Raisin Flavor	1.73	0.98	1.76	0.15
Main Effect: Treatment x Day				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 15,**	p-level
Pork Flavor	0.38	1.02	0.38	0.98
Rancid Flavor	0.96	0.79	1.22	0.25
Raisin Flavor	0.46	0.85	0.54	0.91

Table D17 - ANOVA table for chicken raisin sensory data

Main Effect: Treatment				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 5,426	p-level
Chicken Flavor	11.48	0.90	12.73	0.00
Rancid Flavor	97.49	0.61	160.16	0.00
Raisin Flavor	20.91	1.14	18.39	0.00
Main Effect: Day				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 3,428	p-level
Chicken Flavor	1.25	1.02	1.22	0.30
Rancid Flavor	1.45	1.73	0.83	0.48
Raisin Flavor	0.46	1.37	0.34	0.80
Main Effect: Treatment x Day				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 15,**	p-level
Chicken Flavor	0.17	0.93	0.18	1.00
Rancid Flavor	0.50	0.61	0.82	0.66
Raisin Flavor	0.22	1.18	0.19	1.00

Table D18 - ANOVA table for chicken raisin color data

Main Effect: Treatment				
Dependent Variable	Mean Square Effect	Mean Square Error	f(df1,2) 5,30	p-level
L	468.17	11.08	42.24	0.00
A	75.96	0.28	275.19	0.00
B	88.35	2.98	29.61	0.00

APPENDIX E

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I am preparing my dissertation in the Department of Nutrition and Food Sciences at Utah State University. I hope to complete my degree in the spring of 2006.

An article, Evaluation of antioxidant effects and sensory attributes of Chinese 5-Spice ingredients in cooked ground beef, of which I am a contributing author, and which appeared in your journal (Vol. 71(1), 2006, pp. no. C012-017), reports an essential part of my dissertation. I would like permission to reprint it as a chapter in my dissertation. (Reprinting the chapter may necessitate some revision). Please note that USU sends dissertation to Bell & Howard Dissertation Services to be made available for reproduction.

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Amanda Ferguson, Production Manager 3/20/06

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Phone:	435-512-1840
Journal Name:	Journal of Food Science
Journal Article:	Evaluation of Antioxidant Effects and Sensory Attributes of Chinese 5-Spice Ingredients in Cooked Ground Beef

Dear Ms. Saumya Dwivedi,

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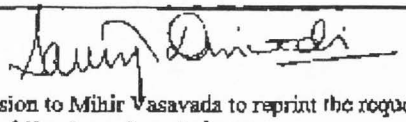

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Amanda Ferguson, Production Manager 3/20/06

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OBJECTIVE

- Seeking a challenging position in the food industry, that will utilize my diverse educational background and previous work experience in food science.

EDUCATIONAL BACKGROUND

- Ph.D in Food Science, Utah State University, Logan, UT 84322. May 2006. GPA 3.88.
- M.S. in Food Science, Utah State University, Logan, UT 84322. May 2004. GPA 3.85.
- Bachelors of Technology in Dairy Technology (1999), Anand, India.

WORK EXPERIENCE

Research Assistant at Utah State University (08/02 – 05/06)
- Working on use of natural antioxidants such as spices, raisin paste, and milk mineral in cooked meat systems, and on possible synergistic effects of various Type I and Type II antioxidants on prevention of oxidative rancidity, in cooked ground meats.

Laboratory Technician (01/06 – 04/06)
- Helping with standardization of processing steps for beef jerky to follow FSIS standards.

Laboratory Technician (10/05 – 11/05)
- Helped with making of Cheddar cheese for various laboratory projects and also with Kraft Mozzarella cheese project as a laboratory technician.

Research Assistant at Utah State University (08/00 – 07/02)
- Worked on comparing sodium lactate and sodium levulinate for their effects on microbiological and chemical properties of fresh pork and turkey sausages for my Masters thesis project.

Senior Quality Control Officer (Sumul Dairy, Surat, India, 02/00 – 06/00)
- Worked in the Quality Control laboratory on assessing the quality of milk and milk products, and also for the conception of HACCP plan in the dairy.

- Involved in managing 10-15 employees in a shift and to check the quality of products such as fluid milk, ice cream and butter, manufacture during a particular shift.
- Responsible for reporting shift operations to the QA Manager.

In-plant trainee (Vidya Dairy, Anand, India, 10/97 – 10/98).
- Worked as a trainee in various sections such as cheese, fluid milk, ice cream, butter, quality control, and processing of milk and milk products.
- Involved in trials for buttermilk, Swiss cheese, Mozarella cheese, and processed cheese and cheese spread, with the dairy.
- Involved in ISO 9000 and HACCP initial paperwork with the dairy.

Food Technology Trainee (Dudhsagar Dairy, Mehsana, India, 11/99 – 12/99).
- Training in various sections including condensed and dried milk and milk products.
- Involved in making plant layouts for ISO 9000 and HACCP certification with the dairy.

COMPUTER EXPERIENCE

- Experienced in use of statistical software such as SAS 8.02, STATISTICA, and SPSS.
- Experienced in statistical data analysis, interpretation, documentation, and presentation.
- Proficient in the use of MS Office, CA-Cricket III, basic internet skills, and making scientific posters and presentations.

CAREER RELATED PROJECTS

- Currently working on potential use of natural antioxidants in cooked meat systems and evaluating possible synergism between Type I and Type II antioxidants (Ph.D project).
- Worked on comparing sodium lactate and sodium levulinate as antimicrobials for improving quality of pork and turkey sausage (Masters project).

PROFESSIONAL AFFILIATIONS

- Member of The Institute of Food Technologists (IFT) since 2001.
- Member of American Meat Science Association (AMSA) since 2002.

AWARDS AND ACHIEVEMENTS

- Position of Research Assistant for Ph.D and M.S. Degrees at Utah State University from 08/00, and teaching assistant for Food Analysis (Spring 2003), Food Chemistry (Fall 2003-2005).
- Winner of the College of Agriculture Award and nominated finalist for Robins Research Assistant of the Year Award (2005), for excellence in research at Utah State University.
- Nominated for active participation in Institute of Food Technologists activities at Utah State University (2005).
- Member of "College Bowl" (IFT) team for Utah State University for the year 2001-03, 2005 and "Product Development" (IFT) team for Utah State University for the year 2003.

- Cleared All India Exam for Masters Degree in NDRI, Karnal, India in the Dairy Chemistry - Dairy Microbiology Division.
- Acknowledged as one of the most active participant in extra-curricular activities at the undergraduate level and actively involved in the activities of the Indian Student Association (ISA) at Utah State University as a member and as the Cultural Secretary for 2002-2003.

PUBLICATIONS

- M. Vasavada, C.E. Carpenter, D.P. Cornforth and V. Ghorpade. 2003. Sodium levulinate and sodium lactate effects on microbial growth and stability of fresh pork and turkey sausages. J Muscle Foods 14(2):119-129.
- M. Vasavada and D.P. Cornforth. 2005. Evaluation of milk mineral antioxidant activity in beef meatballs and nitrite-cured sausage. J Food Sci 70(4): 250-253.
- S. Dwivedi, M. Vasavada, and D. P. Cornforth. 2006. Evaluation of antioxidant effects and sensory attributes of Chinese 5-spice ingredients in cooked ground beef. J Food Sci 71(1):C012-017.
- M. Vasavada, D.P. Cornforth. 2006. Evaluation of antioxidant effects of raisin paste in cooked ground beef, pork, and chicken. (Accepted in J Food Sci).
- M. Vasavada, S. Dwivedi, and D.P. Cornforth. 2006. Evaluation of garam masala spices and phosphates as antioxidants in cooked ground beef. (Accepted in J Food Sci).

TECHNICAL PRESENTATIONS AND POSTERS

Posters at various conferences:
- **Reciprocal Meat Conference:** Use of levulinic acid in pork and turkey sausages (2002), Evaluation of various antioxidants including rosemary powder, rosemary oil and BHT (2003), Use of garam masala blend in ground beef for prevention of oxidative rancidity (2004).
- **International Conference of Meat Science and Technology:** Use of raisin paste in cooked ground beef and pork to prevent oxidative rancidity (2005).
- **Institute of Food Technologists Annual Meeting:** Use of milk mineral in cooked, ground beef for prevention of oxidative rancidity and comparison with sodium nitrite (2004).

REFERENCES

- Will be made available upon request.