OXIDATIVE STABILITY AND TEXTURE OF MEAT AS AFFECTED BY SALTS AND HAEM PIGMENTS

MAHINDA WETTASINGHE

OXIDATIVE STABILITY AND TEXTURE OF MEAT AS AFFECTED BY SALTS AND HAEM PIGMENTS

BY

©MAHINDA WETTASINGHE, B.Sc. (Agric.)

A thesis submitted to the School of Graduate

Studies in partial fulfilment of the

requirements for the degree of the

Master of Science

Department of Biochemistry

Memorial University of Newfoundland

July, 1995

St. John's Newfoundland Canada

National Library of Canada
Bibliothèque nationale du Canada

Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

0-612-25897-1

Canada

ABSTRACT

Due to concerns over the role of nitrite and high sodium chloride concentrations in cured meat products, nitrite-free alternative meat curing systems consisting of a colorant, the pre-formed cooked cured-meat pigment (CCMP) and antioxidant/chelator mixtures with or without an antimicrobial agent and other usual curing adjuncts have been developed. The present study was conducted to examine the antioxidative efficacy of CCMP (at 2.2, 6.2 and 10 μM), alone or in combination with sodium ascorbate (SA) and/or sodium tripolyphosphate (STPP), in a β-carotene/linoleate model system. For comparative properties, pro- or anti-oxidative effects of metmyoglobin (MMb), nitrosylmyoglobin (NOMb) and butylated hydroxyanisole (BHA), were also investigated in the same model system at the same concentrations. CCMP exhibited an antioxidative effect at 6.2 and 10 μM concentrations and its activity was greater than that of NOMb, but less than that of BHA, whereas MMb exhibited a prooxidative effect. Moreover, the antioxidative properties of CCMP in the presence of both SA and STPP (at 550 and 500 ppm, respectively) was marginally enhanced when compared to that of CCMP alone.

Sodium chloride, generally regarded as a prooxidant, is always used in curing mixtures. In order to investigate whether this prooxidant effect as well as the influence of salt on water-binding capacity (WBC) and texture originates from the sodium ion or the chloride ion of the molecule, a number of halides and sulphates of alkali and alkali-earth metals (at 100 and 200 meq/kg meat) were examined in a meat model system.

Similar studies were carried out using Pan®-salt (52% NaCl + 28% KCl + 12% $MgSO_4$ + 3% Lysine.HCl), a commercially-available low-sodium salt, at 1, 2 and 3%. Results were compared with those for 1, 2 and 3% NaCl. Lipid oxidation was monitored over a 7-day storage period at 4°C using the 2-thiobarbituric acid-reactive substances (TBARS) test. For systems exhibiting an antioxidative effect, further studies were carried out to determine their content of hexanal. Furthermore, salts with antioxidant activity were tested in a β-carotene/linoleate model system. Fluorides and iodides of alkali metals inhibited lipid oxidation in meat model systems as reflected in TBARS values and hexanal contents and in a β-carotene/linoleate model system. Meanwhile, chloride and bromide salts of alkali and alkali-earth metals had generally a minor prooxidative influence on lipid oxidation in both systems examined. Fluorides of alkali-earth metals did not exhibit an antioxidant activity, presumably due to an ion-pairing mechanism, while their iodide analogues remained effective. Pan®-salt showed a slight prooxidative effect in meat model systems similar to that of NaCl.

Halides and sulphates of alkali and alkali-earth metals (except MgF_2, $MgBr_2$, MgI_2 and CaF_2) increased the cook yield (ie., WBC) of treated samples whereas most salts (except LiF, NaF and LiCl) imparted a firm texture (ie., high shear force values) to meat. The effect of Pan®-salt on increasing the cook yield and improving the texture of meat was generally less than that of NaCl at the same concentrations examined.

ACKNOWLEDGEMENTS

My sincere gratitude is conveyed to Dr. F. Shahidi for providing me with financial support and the supervision of this thesis. Many thanks to my supervisory committee members, Drs. P.J. Davis and R. Hoover, for the guidance provided throughout this study. I would like to extend my appreciation to Dr. R.B. Pegg for his invaluable assistance and suggestions. I also thank Mr. Pat Mansfield for his assistance. Thanks to all colleagues in Dr. Shahidi's research group for creating a pleasant environment to work.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACS	- American Chemical Society
ADP	- Adenosine diphosphate
AOAC	- Association of Official Analytical Chemists
ATP	- Adenosine triphosphate
B.C.	- Before Christ
BHA	- Butylated hydroxyanisole
BHT	- Butylated hydroxytoluene
CCMP	- Cooked cured-meat pigment
DHA	- Docosahexaenoic acid
DNA	- Deoxyribonucleic acid
EDTA	- Ethylenediaminetetraacetic acid
EPA	- Eicosapentaenoic acid
GC	- Gas chromatography
HPLC	- High performance liquid chromatography
MA	- Malonaldehyde
MMb	- Metmyoglobin
NOMb	- Nitrosylmyoglobin
PG	- Propyl gallate
ppb	- Parts per billion

ppm	- Parts per million
PUFA	- Polyunsaturated fatty acids
SA	- Sodium ascorbate
SAPP	- Sodium acid pyrophosphate
SHMP	- Sodium hexametaphosphate
SPP	- Sodium pyrophosphate
STPP	- Sodium tripolyphosphate
TBA	- 2-Thiobarbituric acid
TBARS	- 2-Thiobarbituric acid-reactive substances
TBHQ	- *tertiary*-butyl hydroquinone
TCA	- Trichloroacetic acid
v/v	- Volume by volume
WBC	- Water-binding capacity
WOF	- Warmed-over flavour
w/v	- Weight by volume
w/w	- Weight by weight

CHAPTER 1. INTRODUCTION

Curing of meat with salt, containing sodium nitrate as an impurity, has long been known to man as a preservation method. With time, the curing techniques were developed and perfected. The cured products possess a characteristic cured-meat colour and a pleasant cured flavour as well as a prolonged shelf-life. Although a variety of ingredients are used in the curing mixtures, modern methods involve the addition of sodium nitrite, sodium chloride, sucrose, sodium ascorbate or erythrobate, polyphosphates and sometimes spices prior to thermal processing. Sodium nitrite, the key ingredient in the curing formulations, is responsible for the development of the characteristic colour and flavour and is also capable of prevention of the growth and toxin production of *Clostridium botulinum*, thereby lowering the risk of botulism. The reaction of nitric oxide, which is generated from nitrite upon the action of microorganisms and the other additives, with myoglobin gives rise to the formation of nitrosylmyoglobin (NOMb) which upon heating is converted to a pigment responsible for the pink coloration of cured meats.

Use of nitrite in meat and meat products has been a concern among consumers and researchers because of the formation of carcinogenic N-nitrosamines upon its reaction with amines present in the meat itself and in the gastric fluid. Considerable research has been carried out in order to find means to reduce or to eliminate the use of nitrite in meat curing. As a result, the addition level of nitrite to meat has been restricted to 156-200 ppm and the incorporation of ingredients such as ascorbate that are capable of lowering the nitrite requirement, has been recommended. Unfortunately, no single compound has yet been found to duplicate the role of nitrite in meat curing.

The concept of alternative meat-curing systems is aimed at complete elimination of nitrite from cures and use of multi-functional curing mixtures. One of the biggest challenges in formulating such a curing mixture is to find pigments capable of reproducing the characteristic cured-meat colour in meat. Certain natural and synthetic compounds have been used to impart a pink coloration to meat, but such compounds were unacceptable to consumers who were used to a nitrite cured-meat colour or because of hazards of chronic intoxication. An innovative technique to preform the cooked cured-meat pigment (CCMP) in the mid 1980's, added a new dimension to modern curing practices. Promising results were obtained in many aspects indicating its potential as a colorant in alternative meat-curing mixtures, but little is known about the effects CCMP on lipid oxidation.

The main role of sodium chloride in the curing process is to provide a salty flavour to the meat. However, the development of the desirable texture of cured-meat and meat products by sodium chloride cannot be underestimated. In addition, sodium chloride prevents the growth of common spoilage and pathogenic microorganisms and thereby extends the shelf-life of the products.

Sodium chloride in foods also plays some negative roles such as its possible connection to hypertension or high blood pressure in human subjects. Furthermore, sodium chloride may act as an antioxidant or prooxidant in meat, depending on the concentration and system in which it is added. Information on the role of sodium and chloride ions in lipid oxidation is controversial. Some researchers have demonstrated that

it is the sodium ion which is responsible for the anti- or pro-oxidative activity of sodium chloride while others have demonstrated that chloride ion is responsible for these effects.

The objectives of this study were to investigate (1) the effect of cooked cured-meat pigment (CCMP) on lipid oxidation (2) the effects of halides and sulphates of alkali and alkali-earth metals on lipid oxidation, cook yield and texture of meat and (3) the effect of Pan®-salt, a commercially available low-sodium salt mixture, on lipid oxidation, cook yield and texture of meats.

CHAPTER 2. LITERATURE REVIEW

2.1 Curing of meat

Preservation of fish and meat by salt curing has been known to man since 3500 B.C. The practice of meat preservation took place in the saline deserts of Hilther Asia and its coastal regions. Rock salt containing nitrates and borax as impurities was in abundance in this area (Pierson and Smoot, 1982). The art of meat preservation by salt and smoke was well known by 900 B.C. in Greece and was later passed on to the Romans. The reddening effect of these contaminants was not noticed until later by the Romans. They became skilled in the curing and pickling of a variety of meats including pork. Salts containing saltpetre were then intentionally added to meat to obtain the desired red colour and the distinctive flavour. With time, use of nitrate addition became a regular practice, and curing techniques were developed. Scientists have recognized that nitrate was reduced by naturally-occurring bacteria present in the post-mortem muscle tissues to nitrite and nitric oxide, which then could react with meat pigments during heating to produce the cured meat colour. However, scientific principles were not applied to meat curing until the latter half of the 19th century (Kramlich *et al.*, 1973). Today a wide selection of cured meat products is available. These products include bacon, sausages, salami and ham (Pierson and Smoot, 1982). Although a variety of compounds may be used in curing mixtures, current meat curing practice involves the addition of nitrite and salt, sugar, ascorbate, polyphosphates and/or spices (Shahidi, 1991). The effect of each curing ingredient is discussed in the following sections.

2.1.1 Sodium nitrite

The use of sodium nitrite ($NaNO_2$) has been regulated since 1925. Sodium nitrite has multi-functional properties in cured meats including the inhibition of *Clostridium botulinum*, development of cured meat flavour and colour, and prevention of warmed-over flavour (WOF) development by controlling lipid oxidation (Hadden *et al.*, 1975; Shahidi *et al.*, 1985; Shahidi and Pegg, 1992). The development of the cured meat colour results from the reaction of sodium nitrite with haem pigments in the muscle and exhibits the most obvious effect of adding nitrite to meat (Howard *et al.*, 1973). The modification of flavour is another important change produced in meat by the addition of nitrite. Nitrite influences the flavour of cured meats by virtue of its antioxidative properties and stabilization of microsomal lipids and haem pigments (Hadden *et al.*, 1975; Shahidi and Pegg, 1992). The concentration of carbonyl compounds produced from autoxidation of meat lipids is markedly reduced by the addition of nitrite (Shahidi, 1989a, b).

The preservative or bactericidal effect of nitrite in meat products is another reason for its use. The problem of botulism, the toxicity caused by *Clostridium botulinum*, is closely associated with uncured meat and meat products. The inhibition of growth and toxin production of *Clostridium botulinum* by nitrite is not a function of nitrite alone. It needs acidulants and common salt to act as an antimicrobial agent (Vösgen, 1992). The mechanism by which nitrite inhibits the outgrowth of spore and the growth of vegetative cells of microorganisms is not fully understood. It appears, however, that a reaction between nitrite and iron-containing enzymes is involved (Shahidi and Pegg, 1992).

2.1.2 Sodium chloride

A typical curing mixture contains 20-30% sodium chloride (NaCl). The final product however, contains 2-3% NaCl (Sofos, 1986; Knight and Parsons, 1988). Sodium chloride contributes to the flavour, binding properties and extended shelf-life of cured products by retarding the growth of microorganisms (Barbut and Mittal, 1989). Salts inhibit the growth of a wide range of spoilage and pathogenic microorganisms by dehydration and alteration of osmotic pressure (Kramlich *et al.*, 1973). Sodium chloride concentrations below 2% shorten the shelf-life of meat products. Whiting *et al.* (1984) have observed reduced shelf-life of frankfurters treated with 1.5% NaCl. In addition, salt plays an important role in the solubilization and extraction of myofibrillar proteins, especially the actomyosin complex. This functional property of salt is important in the production of sausages and other processed meat products. Solubilization and extraction of these muscle proteins contribute to meat its water and fat binding properties and consequently, reduces cook losses. Coagulation of solubilized actomyosin forms a protein network upon cooking, entrapping fat, water and other ingredients within the protein network. This results in a product with appealing texture and juiciness (Sofos, 1986).

2.1.3 Polyphosphates

The use of polyphosphates for processing of ham and other cured products is a common industrial practice. Sodium tripolyphosphate (STPP), sodium pyrophosphate

(SPP), disodium phosphate, sodium hexametaphosphate (SHMP) and sodium acid pyrophosphate (SAPP), individually or in combination are allowed in amounts not exceeding 0.5% (w/w) of the finished product (Sofos, 1986). The purpose of using polyphosphate is to increase the water holding capacity of cooked meat through increasing pH and ionic strength and by unfolding muscle proteins. In addition, polyphosphates have the ability to chelate metal ions and subsequently prevent off-flavour and off-odour development through lipid oxidation (Wierbicki *et al.*, 1976). Polyphosphates increase the binding properties of cured meats by increasing the solubility of muscle proteins, namely actomyosin and myosin, particularly in the presence of sodium chloride. There is some evidence for antimicrobial activity of certain polyphosphates in meat products and other foods (Sofos, 1986).

2.1.4 Sodium ascorbate

Sodium ascorbate (or its isomer sodium erythrobate) is added at a minimum level of 550 ppm when curing meat products (Brown *et al.*, 1974). It accelerates the rate of curing by acting as a reductant for the conversion of metmyoglobin to myoglobin and also it reacts with nitrite to increase the yield of nitric oxide from nitrous acid. Furthermore, excess ascorbate acts as an antioxidant (Kramlich *et al.*, 1973). Sodium ascorbate also participates in the inhibition of N-nitrosamine formation in cured meat products. Izumi *et al.* (1989) have shown an increased loss of nitrite from the curing mixtures containing ascorbate. These authors concluded that the reaction product formed between nitrite and

ascorbate may be responsible not only for nitrosation reactions but also for the loss of nitrite during the curing process.

2.1.5 Sugar (Sucrose)

The addition of sucrose to cures is primarily for flavour enhancement of cured meat and meat products. In addition, it softens the meat and meat products by counteracting the hardening effects of salt. Sucrose can be inverted to glucose and fructose upon heating. Glucose so formed can react with the amino groups of proteins and, upon cooking, forms browning products which enhance the flavour of cured meat. Usually, the sugar content of cured meat is around 2% (Kramlich *et al.*, 1973).

2.1.6 Spices

The addition of spices to certain cured meat products is a common practice. Their key role in meats is to impart a spicy flavour to the product. The latter is caused by the volatile compounds present in spices. Allspice, clove, sage, oregano, rosemary, thyme and black pepper possess antioxidative properties due to the presence of certain phenolic compounds (Shahidi and Wanasundara, 1992).

2.2 Nitrite and carcinogenesis

Although nitrite and its precursor nitrate have been involved in the curing of meat for centuries, their use in meat curing is of concern because of their potential adverse health effects (Buege *et al.*, 1980). However, it should be mentioned that the main source of nitrite in the human diet is not cured meat. The consumption of certain vegetables and vegetable juices containing high levels of nitrate increases salivary nitrite levels to hundreds of parts per billion (ppb), many times higher than that permitted in any food product. The salivary nitrite is derived from the conversion of dietary nitrate to nitrite by the action of microorganisms in the mouth (Gray and Randall, 1979). Toxicity and carcinogenicity of nitrite *per se* has been reported (Sebranek, 1979).

Reaction of nitrite with free amino acids in meats followed by decarboxylation or direct reaction of nitrite with amines of meat, spices and gastric fluid results in the formation of N-nitrosamines (Gray and Randall, 1979; Shahidi and Pegg, 1990). Many of these compounds are carcinogenic and, in addition, some exhibit mutagenic, embryopathic or teratogenic properties (Walters, 1980; Shahidi *et al.*, 1985; Shahidi *et al.*, 1987; Vösgen, 1992). Although there is no direct evidence that N-nitroso compounds are carcinogenic to man, animal studies such as in monkeys, mice, rats, rabbits, guinea pigs and sheep would suggest the potential danger (Gray and Randall, 1979). It has been reported that nitrite enhances the carcinogenic action of N-nitroso-N-methylbenzylamine in the pathogenesis of oesophageal tumours (Schweinsberg and Burkle, 1985).

The food items of major concern are cured meat products, especially bacon. The factors important to potential formation of N-nitrosamines in bacon include cooking, nitrite concentration, salt concentration, pH and presence of ascorbic acid. Of these factors nitrite concentration plays the most important role in the formation of N-nitrosamines. Mirvish (1970) has shown that the rate of N-nitrosamine formation is directly proportional to the square of nitrite concentration. Although, there have been suggestions that it is the initial and not the residual nitrite that influences N-nitrosamine formation in bacon, there is evidence to indicate that the lowest residual nitrite gives the least probability of nitrosamine formation during frying (Sebranek, 1979).

Addition of α-tocopherol with ascorbate, has remarkably reduced the amount of N-nitrosamine formed in the final cured product (Izumi *et al.*, 1989; Cassens, 1990; Shahidi and Pegg, 1992). Shahidi and Pegg (1992) have pointed that the most reliable means of overcoming the problem of N-nitrosamine formation in cured meat, is the total elimination of nitrite from the curing process. The absence of volatile N-nitrosamines in cooked nitrite-free cured muscle foods has been reported (Shahidi *et al.*, 1994).

2.3 Alternative meat curing systems

Despite all of the desired effects of nitrite, objections have been raised to the use of nitrites because of the formation of carcinogens such as N-nitrosamines in cured meat products (Dymicky *et al.*, 1975). It is unlikely that a single compound will be found that can perform all functions of nitrite. Therefore, any alternative meat curing system would

contain a mixture of substances which include a colorant, antioxidants and antibotulinal agents (Shahidi *et al.*, 1988; Shahidi and Pegg, 1992).

Substitutes for nitrite in producing the desired colour have been reported. Early investigations for alternative colorants concentrated on the use of naturally-occurring red coloured plant pigments, especially betalains of beet. Betalains consist of two coloured substances namely betacyanine, which is red in colour, and betaxanthin, which is yellow-coloured. Since beet powder is permitted as a colour additive by regulation, it has been used to simulate cured meat colour in cooked, smoked, and semi-dry fermented sausages (von Elbe and Maing, 1973; von Elbe *et al.*, 1974a, b).

Howard *et al.* (1973) have investigated 24 nitrogenous compounds for their ability to form ferrohaemochromes with bovine myoglobin. They found that methyl and hexyl nicotinate and N,N-diethylnicotinamide have the ability to produce a stable pink pigment in cooked ground meat. Moreover, methyl or hexyl nicotinate or N,N-diethylnicotinamide worked synergistically when used with low levels of nitrite. However, none of these nitrogenous compounds were able to produce the typical cooked cured-meat colour in sausages. Dymicky *et al.* (1975) have studied more than 300 compounds for their performance in imparting characteristic cured meat colour to products. They found that pyridine compounds were capable of producing a pink colour in meat model systems. Furthermore, 3-acylpyridines imparted the most desirable colour to meat. Lin and Lai (1979) have shown that imidazole could be used as a colour-fixing agent. Imidazole was demonstrated to react with the haem moiety of haemoglobin and subsequently form a red

colour pigment . The application of these compounds to meat is, however, not practical mainly due to their poor stability, poor solubility in water and possible toxicity.

Preparation of the natural cooked cured meat pigment (CCMP) has attracted the interest of researchers. Shahidi *et al.* (1984) were able to synthesize CCMP from haemin, an iron porphyrin compound prepared from bovine red blood cells and sodium nitrite. The purity of the pigment so obtained was about 65-72% and, as such, it did not impart the cooked cured meat colour to meat effectively. Shahidi *et al.* (1985) later demonstrated a novel method for the synthesis of CCMP using haemin and nitric oxide as the nitrosating agent. The CCMP so prepared had a purity of >97% and was capable of imparting an excellent cured colour to meats.

The applicability of CCMP to meat as an alternative for nitrite has been thoroughly investigated. Successful application of CCMP to meat systems such as sausages and salami has been reported (Pegg and Shahidi, 1987; Shahidi and Pegg, 1990, O'Boyle *et al.*, 1990; Shahidi and Pegg, 1991a). Shahidi and Pegg (1990) demonstrated that the colour characteristics of cooked comminuted pork containing 12 ppm of the pre-formed CCMP, were similar to those of nitrite-cured meat prepared with 156 ppm of sodium nitrite. There has also been some evidence for the antioxidative nature of CCMP in meat model systems (Shahidi *et al.*, 1987; Shahidi *et al.*, 1988)

One of the main problems associated with CCMP in early studies was its instability when exposed to light and oxygen. This problem was successfully overcome by storing the pigment under a positive pressure of nitric oxide or by encapsulating it in

a food-grade starch (Shahidi and Pegg, 1991b). Shahidi and Pegg (1991b) and O'Boyle *et al.* (1992) have demonstrated prolonged stability of microencapsulated CCMP in a mixture of β-cyclodextrin and modified starches such as N-Lock or maltodextrin. Shahidi and Pegg (1991b) have shown the ability of encapsulated CCMP to remain stable even after one year of storage.

The absence of volatile N-nitrosamines in cooked nitrite-free cured meat has been revealed by Shahidi *et al.* (1994). This finding provides a strong basis to researchers who are working towards nitrite-free curing of meat products with the hope of producing N-nitrosamine-free processed muscle foods.

Several alternative antimicrobial agents for nitrite have been reported. Among these, parabens, hypophosphite, biological acidulants such as lactic acid bacterial cultures and sorbates are in the forefront. Application of paraben to meat products is unlikely due to its insolubility in water. Use of lactic acid bacterial cultures to lower pH of meat products, especially in bacon, may not be acceptable to consumers as it imparts a tangy flavour (Pierson and Smoot, 1982). The sorbates have been found useful in preserving various food items (Sebranek, 1979). Tompkin *et al.* (1974) have shown the ability of potassium sorbate to delay toxin production by *Clostridium botulinum* in cooked uncured sausages. The influence of sorbic acid or potassium sorbate on the flavour of meat products has been a concern. Flavour alterations of products treated with sorbate have been reported (Pierson and Smoot, 1982).

2.4 Oxidation of meat and meat products

Oxidation via a free radical chain mechanism, until the end of the 1960s, was almost exclusively an area of research in radical chemistry, polymer and food sciences. Recently, this area was expanded and now is one of the most important areas of research in biology, biochemistry and medicine (Kanner, 1994). When cells are injured, such as in muscle foods after slaughtering, oxidative processes are favoured. Disruption of the muscle membrane system by mechanical grinding, cooking and hydrolytic enzymes, causes the release of iron needed to catalyze lipid autoxidation (Sato and Hegarty, 1971; Love, 1987). These oxidative processes affect lipids, pigments, proteins, carbohydrates, vitamins and the overall quality of foods (Shahidi and Wanasundara, 1992).

Fresh meat as well as processed meat products are susceptible to autoxidation. Tim and Watts (1958) first observed that cooked meat was rapidly oxidized at refrigeration temperatures, which is in marked contrast to the slow onset of rancidity commonly encountered in raw or frozen meat. The rapid onset of autoxidation in cooked refrigerated meat is coined as warmed-over flavour (WOF) and is a serious flavour defect in cooked, refrigerated meat and becomes most apparent on rewarming of the product (Tims and Watts, 1958; Sato and Hegarty, 1971; Fooladi *et al.*, 1979). The intensity of the undesirable sensory notes is directly correlated with the content of carbonyl compounds formed through lipid autoxidation reactions. The decrease in the intensity of desirable sensory notes may be attributed to a decrease in the content of compounds that contribute to desirable flavour or to the masking of the desirable flavour compounds by an increased content of undesirable flavour compounds (Drumm and Spanier, 1991).

2.4.1 Mechanism of lipid autoxidation

The mechanism of lipid autoxidation in muscle foods has been studied by several researchers utilizing model systems of linoleate emulsion, microsome membranes, or water-extracted muscle residues (Kanner *et al.*, 1991). The main reaction involved in lipid autoxidation is that involving molecular oxygen and unsaturated lipids (LH) to form lipid hydroperoxides (LOOH). However, direct reaction of lipids with oxygen is spin-forbidden because the ground state of lipids is of singlet multiplicity whereas that of oxygen is of triplet multiplicity (Miller *et al.*, 1990). Lipid peroxidation must therefore occur via reactions that by-pass the spin barrier between lipids and oxygen. These reactions are promoted by some type of initiator ($I^{\bullet}$) that can overcome the dissociation energy of an allylic bond and thus cause hydrogen abstraction and formation of a lipid alkyl radical ($L^{\bullet}$). Lipid alkyl radicals can rapidly add oxygen to form lipid peroxyl radicals ($LOO^{\bullet}$) which eventually liberate LOOH via hydrogen abstraction from a neighbouring allylic bond (Minotti and Aust, 1992).

Lipid peroxidation is initiated by externally generated oxidants, but once started, the reaction is autocatalytic. Transition metals can catalyze the initiation and enhance the propagation of lipid peroxidation. For example Fe^{2+} will reductively cleave LOOH to highly reactive alkoxyl ($LO^{\bullet}$) radicals, which in turn abstract hydrogen from lipids to form new lipid alkyl radicals. This reaction is known as LOOH-dependent lipid peroxidation (Svingen *et al.*, 1979).

Hydroperoxides, the primary products of lipid autoxidation, are unstable and thus, enter into breakdown and interaction mechanisms responsible for the formation of off odours and off flavours. Figures 2.1 and 2.2 depict the three-step free radical scheme and the generalized autoxidation process of lipids, respectively.

2.4.2 Factors that affect lipid autoxidation

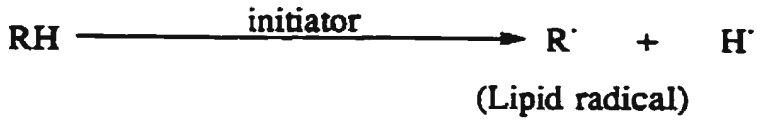
2.4.2.1 Ionizing radiation

The radiations of principle concern are those from charged particles such as electrons, protons, and α-particles, and electromagnetic waves or photons such as x-rays and γ-rays. Although each of these interacts with matter in a different manner, the primary event is the same, that is the ionization of atoms or molecules where an energetic electron is ejected and a positively charged species is formed in the parent compound. Electrons ejected in the ionization process may be sufficiently energetic to produce further ionization and excitation. If it is of less than 100 eV energy, the resulting secondary ionizations will be close to the primary ionization site, thus forming small clusters or spurs of excited and ionized species. More energetic electrons, called δ-rays, travel further from the initial site and form tracks of their own, similar to those of β-particles or other electrons with the same energy (Schaich, 1980).

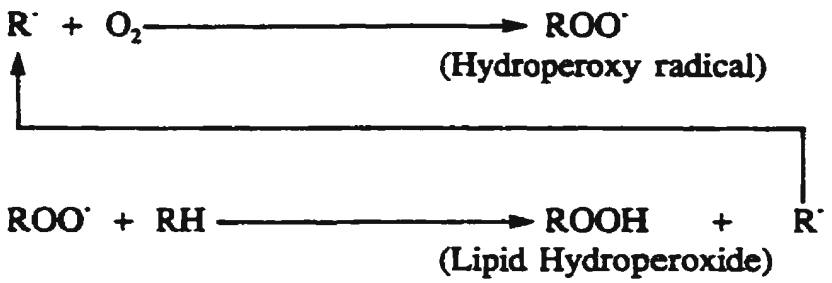
The major immediate consequence of the absorption of radiation is the production of free radicals. In biological systems, this may occur directly by deposition of energy within the molecule itself or indirectly through reactive species produced from the radiolysis of water (Schaich, 1980).

Figure 2.1 Three step free radical scheme. Adapted from Pegg (1993).

Initiation



Propagation



Termination

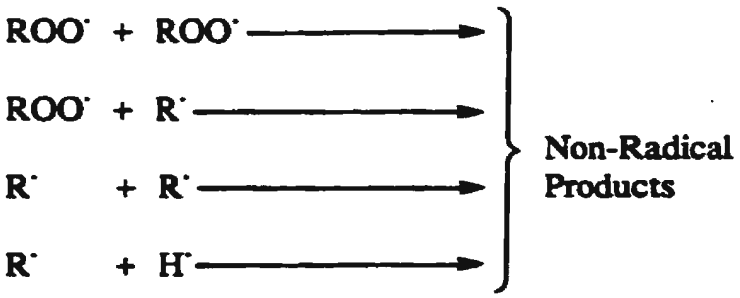
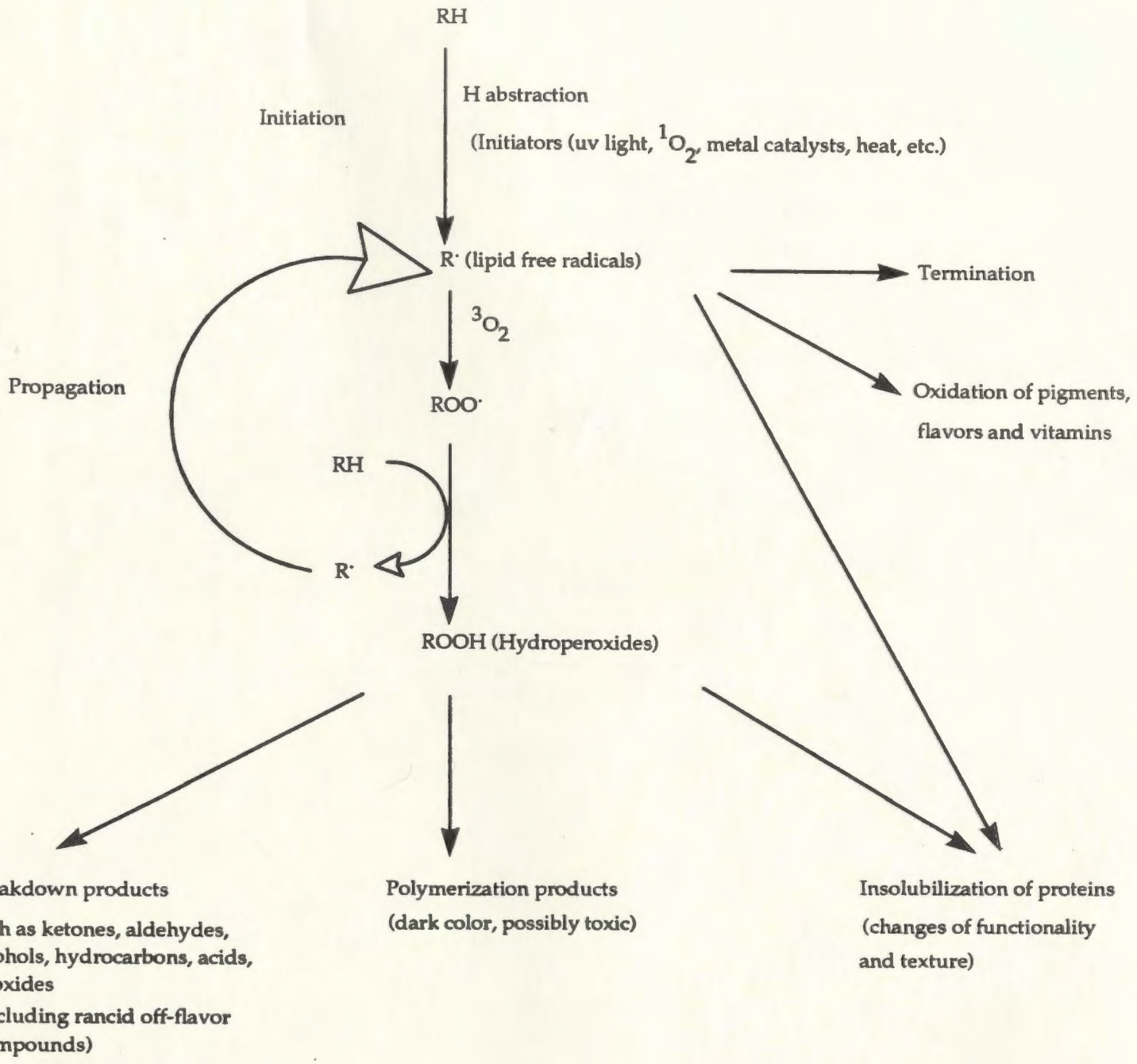


Figure 2.2 Generalized autoxidation process of lipids. Adapted from Shahidi and Wanasundara (1992).


RH
H abstraction
Initiation
(Initiators (uv light, $^1O_2$, metal catalysts, heat, etc.)
R· (lipid free radicals)
Termination
$^3O_2$
Propagation
ROO·
Oxidation of pigments,
flavors and vitamins
RH
R·
ROOH (Hydroperoxides)
Breakdown products
such as ketones, aldehydes,
alcohols, hydrocarbons, acids,
epoxides
(including rancid off-flavor
compounds)
Polymerization products
(dark color, possibly toxic)
Insolubilization of proteins
(changes of functionality
and texture)

2.4.2.2 Superoxide and perhydroxyl radicals

Under biological conditions, a significant amount of superoxide anion (O_2^-) can be generated. In meat tissues, there is no direct evidence that O_2^- is generated, however, the presence of O_2^- in other biological systems may suggest its presence in muscle tissues. The sources of O_2^- in muscle tissues may originate from membrane electron transfer systems, autoxidation of oxymyoglobin to metmyoglobin, and oxidation of ascorbic acid and other reducing components by free iron. Though O_2^- itself is not prooxidative, the loss of charge during formation of perhydroxyl radical ($HOO^\bullet$) allows the radical to penetrate into the membrane lipid region more easily, where it could initiate lipid peroxidation (Halliwell and Gutteridge, 1986; Kanner, 1994).

2.4.2.3 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is normally present as a byproduct of phagocytosis at low concentrations in aerobic cells. A system generating O_2^- would be expected to produce H_2O_2 by non-enzymatic dismutation or by superoxide dismutase catalyzed dismutation. Mitochondria, microsomes, peroxisomes and cytosolic enzymes have all been recognized as effective H_2O_2 generators when fully provided with their substrates. Hydrogen peroxide has limited reactivity and has not been shown to react directly with polyunsaturated fatty acids, however, it can cross biological membranes (Halliwell and Guttenridge, 1986).

2.4.2.4 Hydroxyl radicals

Hydroxyl radicals ($HO^{\bullet}$) are produced when water is exposed to high energy ionizing radiation. One-electron reduction of H_2O_2 decomposes it to HO^- and $HO^{\bullet}$, the latter being a highly reactive radical capable of oxidizing lipids and many other biological molecules (Bielski and Allen, 1977). Most of the $HO^{\bullet}$ is generated from the metal-dependant breakdown of H_2O_2, according to the following reaction:

$$M^{n+1} + H_2O_2 \text{ ---------> } M^{(n+1)+} + HO^{\bullet} + HO^-$$

in which M^{n+1} is a transition metal. Ferrous ion (Fe^{2+}) is known to promote the same reaction, which is also called the Fenton reaction.

2.4.2.5 Singlet oxygen

Singlet oxygen can be generated by both chemical and photochemical reactions. During propagation of lipid oxidation, haem proteins could accelerate the generation of peroxyl radicals, formation of singlet oxygen and the excitation of carbonyls. Lipid oxidation can be initiated by singlet oxygen, however no strong evidence of this pathway has been found in meats (Kanner *et al.*, 1987).

2.4.2.6 Ferryl ions

Regardless of their state of oxidation, myoglobin and haemoglobin are activated by H_2O_2, producing a short-lived intermediate of ferryl (Fe^{4+}) ion. Kanner and Harel (1985) have reported that H_2O_2-activated myoglobin and haemoglobin could initiate membrane lipid peroxidation.

2.4.2.7 Free metal ions

Transition metals, such as iron, manganese and copper, with their unstable d-electron system, are capable of catalyzing redox reactions. They may initiate lipid oxidation by the following mechanisms: (1) generation of unsaturated fatty acid radicals by single-electron transfer or hydrogen abstraction, (2) reaction with triplet oxygen to generate the superoxide radicals, (3) indirect generation of oxygen species by oxidizing flavin cofactors and (4) interaction with oxygen or peroxides or iron-containing enzymes and proteins to raise the oxidation state of metals from +3 to +5 (Kanner *et al.*, 1987).

Iron, an important catalyst in meats, is found mainly in haemoglobin and myoglobin. A small amount of iron is found bound to small molecules such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), organic acids and deoxyribonucleic acid (DNA). These compounds are capable of decomposing hydroperoxides (ROOH) to form free radicals (Kanner and Doll, 1991).

The main source of free iron or non-haem iron in cells is ferritin. Ferritin, a soluble iron storage protein found in liver, spleen, and skeletal muscle, has a molecular

mass of 450 kDa and contains 4500 iron atoms when fully loaded (Decker and Welch, 1990). Recently, it was found that O_2^- releases iron from ferritin and O_2^- is the primary reductant in the ascorbate-mediated ferritin iron release (Boyer and McCleary, 1987). During storage of muscle foods, ferritin loses iron at a significant rate, and this amount initiates membrane lipid peroxidation (Kanner and Doll, 1991).

Tichivangana and Morrissey (1985) indicated that Cu^{2+} catalyzed oxidation in a pattern similar to that of Fe^{2+} catalysis, but Cu^{2+} was slightly less effective as a prooxidant in the muscle. Moreover, these authors reported that the rate of prooxidant activity was in the order of: Fe^{2+}> Cu^{2+}> Co^{2+}, and that differences in activity between Fe^{2+} and Cu^{2+} as well as Fe^{2+} and Co^{2+} were significant in muscle systems. The susceptibility of raw and heated muscles to lipid oxidation catalyzed by the various prooxidants was in the order of: fish> turkey> chicken> pork> beef> lamb, which generally corresponds to the decrease of the polyunsaturated fatty acid (PUFA) content of the tissue (Salih *et al.*, 1989). The relative prooxidant activity of ions in fish muscle decreased in the following order: Cu^{2+}> Fe^{2+}> Co^{3+}> Cd^{2+}> Li^{+}> Ni^{2+}> Mg^{2+}> Zn^{2+}> Ca^{2+}> Ba^{2+} (Castell *et al.*, 1965). Shahidi and Hong (1991) reported that metal ions such as copper and iron ions can enhance lipid autoxidation to a greater extent at their lower valance states.

2.4.2.8 Haem pigments

Traditionally, lipid oxidation in meats has been attributed to haem catalysts such as haemoglobin, myoglobin and cytochromes (Tichivangana and Morrissey, 1985).

Brown *et al.* (1963) and Hirano and Olcott (1971) claimed that all haem pigments have prooxidant activity, although Brown *et al.* (1963) found that an induction period was sometimes necessary with ferrous haems. Furthermore, Brown *et al.* (1963) have reported that haems with iron in either the Fe^{2+} or Fe^{3+} states were effective catalysts of lipid oxidation. According to Shahidi and Hong (1991), haem pigments (iron-porphyrin compounds) possess a potent pro-oxidant effect in cooked meats.

It has been found that ferric haem pigments may only be effective catalysts in the presence of hydrogen peroxide. For example, Harel and Kanner (1985a) found that in the sarcosomal fraction isolated from turkey's dark meat, metmyoglobin or hydrogen peroxide alone had little influence on the rate of lipid peroxidation but together they accelerated the rate several hundred fold. Johns *et al.* (1989) have pointed out that ferric haem pigments are more powerful catalysts of lipid oxidation than inorganic iron compounds. They have also shown that hydrogen peroxide needs to be present for the ferric ions to be active catalysts.

Labuza (1971) has suggested that the protein portion of haemoprotein molecules may cause steric hindrance of the iron, preventing it from catalyzing oxidation. When meat is heated, denaturation of the protein portion of the molecule might facilitate exposure and/or release of iron for interaction with unsaturated fatty acids (Love, 1983).

Ben-Aziz *et al.* (1970a, b) have shown that cytochrome C is an effective prooxidant in a β-carotene/linoleate model system. Kanner *et al.* (1979) have demonstrated the prooxidant nature of metmyoglobin and oxymyoglobin towards β-

carotene bleaching and linoleate oxidation in β-carotene/linoleate model systems. Morrissey and Tichivangana (1985) have shown the antioxidant effect of nitrosylmyoglobin produced *in situ* in pork muscle systems containing myoglobin and metal ions as prooxidant catalysts. Shahidi *et al.* (1987) have demonstrated the antioxidant effect of cooked cured meat pigment, nitrosylferrohaemochrome in meat model systems. The antioxidative nature of this pigment was concentration-dependant and the effect was comparable to 200 ppm of α-tocopherol when used at 18-24 ppm.

Studies employing model systems of linoleate emulsions have shown that both haem and non-haem iron are important catalysts of muscle lipid peroxidation (Lin and Watts, 1970). Non-haem iron played a dominant prooxidant role in shrimp flesh whereas, in beef muscle, haem iron appeared to be the major catalyst (Liu, 1970a, b). Several other researchers have reported that haem pigments are not the principal prooxidants in meat model systems made with water-extracted muscle residues and that non-haem iron is the main catalyst (Sato and Hegarty, 1971; Love and Pearson, 1974; Tichivangana and Morrissey, 1985).

2.4.2.9 Enzymes

Lipid oxidation in meat can be initiated by tissue enzymes such as peroxidase, catalase, cytochrome C and lipoxygenase (Kanner and Kinsella, 1983). Ben-Aziz *et al.* (1970) have demonstrated the prooxidative nature of lipoxygenase in a β-carotene/ linoleate model system. Their studies show that the diene conjugation in linoleate

oxidation is proportional to the concentration of enzyme and haem proteins. Moreover, catalase, peroxidase and cytochrome C show a relatively weak catalytic activity in diene formation as compared to lipoxygenase.

The presence of a lipoxygenase that catalyses the insertion of oxygen into unsaturated fatty acids has been demonstrated in various animal tissues (Kanner and Kinsella, 1983). The possibility that other tissues in close proximity to muscles might also contain lipoxygenase capable of reacting with unsaturated fatty acids lead researchers to investigate skin tissues which contain lipoxygenase activity in mammals (German and Kinsella, 1985). The presence of lipoxygenase in chicken muscle has been reported (Grossman *et al.* 1988). German and Kinsella (1985) discovered a lipoxygenase activity in gill tissue of trout and suggested that the postmortem release of this enzyme could generate considerable quantities of reactive hydroperoxides. These in conjunction with metallic catalysts would serve as a potent source of initiating free-radical species for oxidation of lipids present in the tissues (German and Kinsella, 1985).

The pH of a muscle tissue plays an important role in enzymatically-activated lipid peroxidation. Kwoh (1971) has reported the enzymatic reducing activity of metmyoglobin increases with increasing the pH from 5.1 to 7.1. Presumably at higher pH values, the reducing enzymes are in a much more active state. Oxygen is utilized by way of the electron transport system and any metmyoglobin present is reduced (Kwoh, 1971).

2.4.2.10 Phospholipids

Most of the polyunsaturated fatty acids (PUFA) in meats are more likely in an esterified form with phospholipids rather than triacylglycerols (Igene *et al.*, 1980). Therefore, phospholipid fractions have been identified as primary substrates in the development of oxidative deterioration of muscle foods (Igene *et al.*, 1979). Pikul and Kummerow (1991) have pointed out the presence of high levels of arachidonic, docosatetraenoic, docosapentaenoic, and docosahexaenoic acids in phosphotidylinositol, phosphotidylethanolamine, phosphotidylserine, and phosphotidylcholine. Furthermore, phospholipids were found responsible for the generation of a major portion of TBA-reactive substances (TBARS). Igene *et al.* (1979) demonstrated compositional changes in the fatty acid profiles in total phospholipids of meat model systems after cooking and storage. A decrease in octadecadienoic, eicosatetraenoic, eicosapentaenoic and docosatetraenoic acids of phosphotidyl choline and ethanolamine in chicken dark muscle had been observed. Igene *et al.* (1979) concluded that hydrolysis and autoxidation of phospholipids upon cooking and storage were involved.

2.4.3 Effect of curing ingredients on lipid oxidation in meats

2.4.3.1 Effect of sodium chloride

Sodium chloride has been reported to act as a prooxidant (Kanner and Kinsella, 1983; Kanner *et al.*, 1991) or an antioxidant (Osinchak *et al.*, 1992), depending on the concentration and system in which it is added (Pearson and Gray, 1983). Coleman (1949)

has shown that sodium chloride accelerates oxidation of the ferrous ion of haemoglobin and myoglobin to the ferric form. Mabrouk and Dugan (1960) have shown that sodium chloride has no direct effect on the oxidation of lipids in the absence of other organic prooxidants. Recently, Kanner *et al.* (1991) have shown that the effect of sodium chloride seems, in part, to be attributed to its capability of displacing iron ions from binding macromolecules for oxidative reactions. They found that sodium chloride had increased the extraction of iron ions from muscle tissue, most of which were bound to molecules of a mass greater than 300 kDa. According to Love and Pearson (1974) and Igene *et al.* (1979), nonhaem iron released from haem pigments is the principle prooxidant in cooked meat. Arnold *et al.* (1991) have demonstrated that NaCl has a marked effect on the prooxidant activity of Cu^{2+} at temperatures below 0°C.

Castell *et al.* (1965) found that the prooxidant activity of NaCl in fish muscle was due to its sodium ion, and that cations of other salts such as lithium chloride and potassium chloride had a similar prooxidant effect. According to the studies of Osinchak *et al.* (1992), the prooxidative effect of NaCl is due mainly to its anion rather than the cation. Moreover, the inhibitory activity of other anions such as bromide, nitrate and particularly iodide was demonstrated.

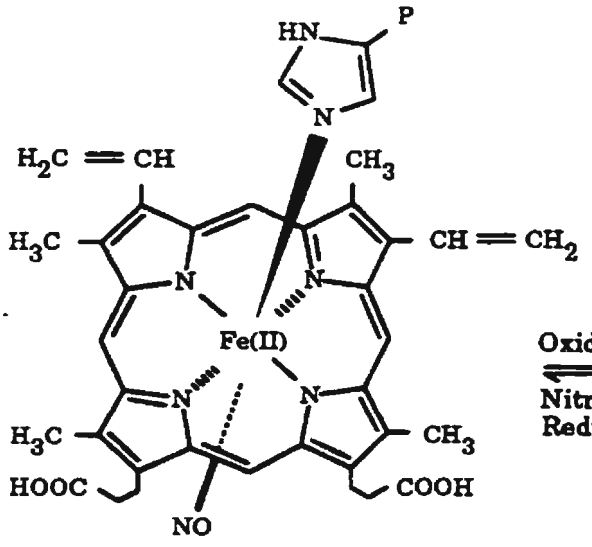
2.4.3.2 Effect of sodium nitrite

Woolford and Cassens (1977) and Kanner *et al.* (1979) have shown that the nitrite added to meat was accounted for as nitrosothiols, nitric-oxide myoglobin, protein-bound

nitrite, free nitrite and nitrate, and gaseous nitrogenous compounds. Igene *et al.* (1979) have reported that the addition of nitrite to meat can reduce TBARS values ninefold, sevenfold, and fivefold in beef, chicken white and dark meats, respectively. These authors suggested that nitrite converts the haem pigments to a catalytically inactive form, thus resulting in an inhibition of TBARS formation. Morrissey and Tichivangana (1985) have demonstrated a decrease in TBARS values of muscle systems with increasing levels of nitrite addition. While addition of low levels of nitrite (20 ppm) significantly ($p<0.01$) inhibited lipid oxidation, highly significant ($p<0.001$) reduction in oxidation of meats was noted at 50 ppm nitrite addition. According to the studies carried out by Zubillaga *et al.* (1984), sodium nitrite *per se* does not possess either prooxidative or antioxidative properties in a β-carotene/linoleate model system. These authors have suggested that the residual sodium nitrite in the tissue is probably not responsible for the antioxidative action, in the unlikely event that the nitrite became associated with the polar lipids.

The resistance of lipids of cooked nitrite cured meat to autoxidation is attributed to the cured meat pigment, nitrosylferrohaemochrome. The development of the cured meat colour is the result of the reaction of sodium nitrite with haem pigments in the muscle. The main compound formed is nitric-oxide myoglobin which is relatively unstable. During thermal processing, the protein moiety of the myoglobin is denatured and detached and a relatively stable haem pigment, nitrosylferrohaemochrome, is formed. Figure 2.3 exhibits reactions involved in the formation of the characteristic cooked cured meat pigment.

Figure 2.3 Some of the possible reactions during nitrite curing of meat. Adapted from Bard and Townsend (1971).

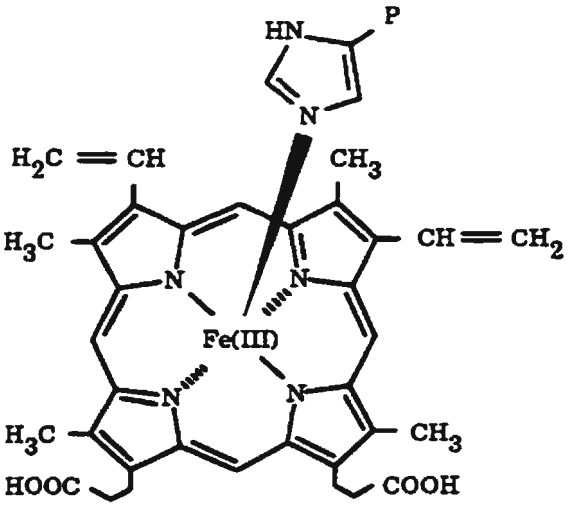


NITRIC OXIDE MYOGLOBIN

(NOMb, red, Fe^{+2})

Oxidation

Nitric Oxide + Reduction



METMYOGLOBIN

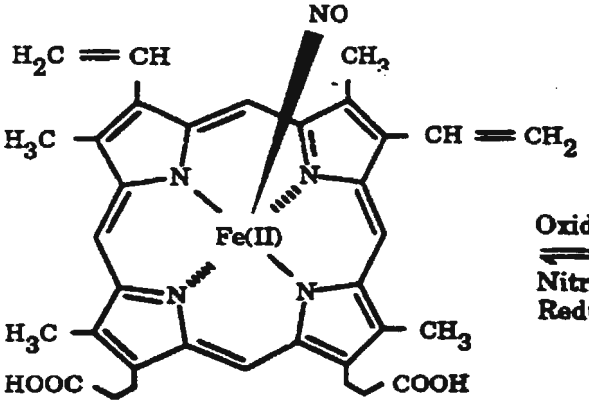
(metMb, brown, Fe^{+3})

Protein Denaturation and Detachment

(Heat)

Protein Denaturation and Detachment

(Heat)

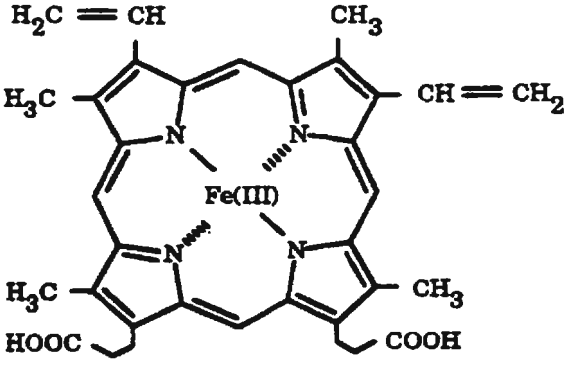


COOKED CURED-MEAT PIGMENT

(CCMP, pink, Fe^{+2})

Oxidation

Nitric Oxide + Reduction



DENATURED METMYOGLOBIN

(den. metMb, brown, Fe^{+3})

OXIDIZED PORPHYRINS

(green, yellow, colourless)

The antioxidative nature of nitric-oxide myoglobin in a β-carotene/linoleate model system has been demonstrated. Kanner *et al.* (1979) have shown the inhibitory effect of nitric-oxide myoglobin at a concentration of 10^{-5}M. Lower concentrations such as $3x10^{-7}$M and $4x10^{-7}$M, however, exhibited a definite prooxidative effect. Haem pigments can act both as inhibitors or catalysts of lipid oxidation, depending on their concentration. The catalytic effect of NOMb at low concentrations, therefore, bound to exceed inhibition (Kanner *et al.*, 1979).

2.4.3.3 Effect of ascorbates and phosphates

L-ascorbic acid can act as a prooxidant or an antioxidant depending on its concentration and medium of application. Mahoney and Graf (1986) have investigated the performance of L-ascorbic acid in a model system containing Fe^{3+}, Cu^{2+} and L-tryptophan. They observed a positive oxidation potential for low concentrations of L-ascorbic acid in the presence of Fe^{3+} or Cu^{2+} ions. Steinhart *et al.* (1993) have shown the prooxidative nature of L-ascorbic acid at concentrations ranging from 0.56 to 1.40 mmol/20 mL, in a model system containing Fe^{3+} and L-tryptophan, but acted as an antioxidant at concentrations higher than 1.40 mmol/20 mL.

Shahidi *et al.* (1987, 1988) have demonstrated the antioxidative nature of L-ascorbic acid and sodium ascorbate at 500 and 550 ppm addition levels in meat model systems, respectively. According to these authors, sodium ascorbate showed antioxidant properties in the presence of sodium chloride and sucrose, but the effect was

overshadowed by the prooxidative effect of sodium chloride after 3 days of storage. The antioxidative behaviour of fat-soluble analogues of ascorbic acid, such as ascorbyl palmitate and ascorbyl acetate, has also been revealed (Shahidi *et al.*, 1988).

The role of phosphates in retarding oxidative rancidity has been reported by Sato and Hegarty (1971). A reduction in TBARS values of meat treated with STPP was observed by Shahidi *et al.* (1988) and attributed to the ability of STPP to chelate metal ions. Crackel *et al.* (1988) have shown strong antioxidative effect of STPP in raw frozen restructured pork steaks.

2.5 Prevention of lipid oxidation

2.5.1 Physical methods

Vacuum packaging or packaging under an inert gas such as in atmospheres modified to exclude oxygen, as well as refrigeration or freezing can reduce the rate of autoxidation. However, these means are not always practicable because very little oxygen is needed to initiate and maintain the oxidation process. It is neither economical nor practical to remove traces of oxygen from foods (Shahidi and Wanasundara, 1992).

2.5.2 Antioxidants

Antioxidants are the major ingredients that protect the quality of lipid-containing foods by retarding autoxidation (Wanasundara *et al.*, 1994). Crackel *et al.* (1988) have shown that antioxidants are effective in retarding lipid oxidation in restructured beef

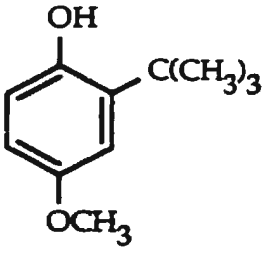
steaks. They have also shown that mixtures of natural antioxidants such as tocopherols, ascorbyl palmitate and citric acid are as effective as tertiary-butylhydroquinone (TBHQ). Combinations of physical methods and antioxidants have also been reported to preserve food quality (Shahidi and Wanasundara, 1992).

Labuza (1971) divided antioxidants into three major groups, namely type I, type II and type III. Type I antioxidants are primarily phenolic compounds such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and TBHQ. These are also known as free radical scavengers or primary antioxidants because they neutralize free radicals by donating a hydrogen atom or an electron to radicals. Type II antioxidants are mostly chelating agents such as ethylenediaminetetraacetic acid (EDTA) and citric acid which prevent the formation of free radicals, mainly by tying up transition metal ions, which act as prooxidants. Type III antioxidants such as bisulphite compounds regulate environmental factors such as redox compounds and water activity.

Currently, BHA, BHT, propyl gallate (PG) and TBHQ are commonly used as antioxidants in lipid-containing foods. Tocopherols and ascorbic acid and their derivatives are used as alternatives to BHA and BHT (Wanasundara *et al.*, 1994). Figure 2.4 depicts the chemical structures of some of the commonly used synthetic antioxidants in foods.

Phenolic antioxidants are excellent hydrogen or electron donors and their radical intermediates are relatively stable due to resonance delocalization and lack of suitable sites for attack by molecular oxygen. The phenoxy radical formed by the reaction of a phenol with a lipid radical is stabilized by delocalization of its unpaired electron around

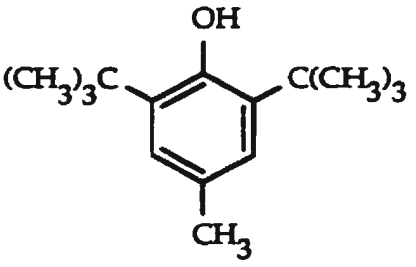
Figure 2.4 Chemical structures of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *t*-butylhydroquinone (TBHQ) and propyl gallate (PG).



BHA

3-tertiary-butyl-4-hydroxyanisole

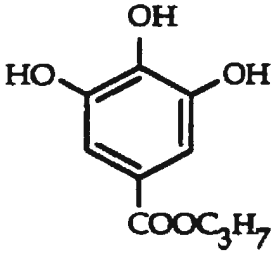
2-tertiary-butyl-4-methoxyphenol



BHT

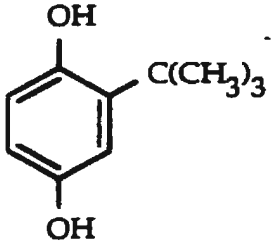
3,5-di-tertiary-butyl-4-hydroxytoluene

2,6-di-tertiary-butyl-4-methylphenol



PG

3,4,5-trihydroxypropylbenzoate



TBHQ

tertiary-butylhydroquinone

the aromatic ring (Shahidi and Wanasundara, 1992). Phenol itself is inactive as an antioxidant. Substitution of the hydrogen atoms in the ortho and para positions with alkyl groups increases the electron density of the OH moiety via an inductive effect and thus enhances its reactivity towards lipid radicals (Shahidi and Wanasundara, 1992).

Use of synthetic phenolic antioxidants has been a concern for consumers because of their possible adverse health effects and scientists over the years have been interested in natural products with antioxidant properties (Stoick *et al.*, 1991). Many spices and herbs have been shown to act as antioxidants in food systems and rosemary is among the most effective herbs. The antioxidative effect of spices and herbs comes from their polyphenolic compounds that occur in all parts of the plant (Houlihan *et al.*, 1984; Kramer, 1985; Lee and Ashmore, 1986). Plant phenolics are multifunctional and can act as free radical terminators, metal chelators and singlet oxygen quenchers. Examples of common plant phenolic antioxidants include flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and polyfunctional organic acids (Stoick *et al.*, 1991; Liu *et al.*, 1992; Shahidi and Wanasundara, 1992). Eugenol, found in the essential oil of clove, is a 2-methoxy phenolic derivative which has been reported to possess 90% of the antioxidant activity of BHA, whereas curcumin, the major phenolic pigment of turmeric, is reported to have 75% of the activity of BHT. The antioxidant activity displayed by spices, however, depends on several factors which include the chemical nature of the food or medium to which they are added (Al-Jalay *et al.*, 1987). Bracco *et al.* (1981) have revealed the ability of crude extracts of rosemary and sage to prolong the induction period

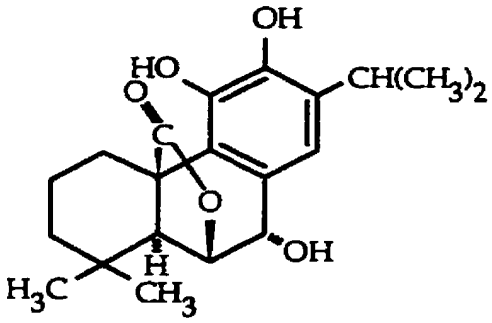
in chicken fat oxidation. They concluded that rosemary antioxidants retard degradation of linoleic acid, carotenoid loss and protect lipids from oxygen attack. Inatani *et al.* (1982) have isolated rosmanol, the major antioxidant in rosemary. Later, it was found that two other antioxidants namely epirosmanol and isorosmanol were also present in rosemary. The antioxidant activity of epirosmanol and isorosmanol in both lard and linoleic acid was examined and shown to be four times as effective as BHA and BHT (Nakatani and Inatani, 1984). Stoick *et al.* (1991) have shown that oleoresin rosemary had antioxidative properties when tested in restructured beef steaks. The effect was morepronounced when rosemary oleoresin was used together with sodium tripolyphosphate. Figure 2.5 depicts some of the natural antioxidants found in plant sources.

2.6 Methods of measuring lipid oxidation in meat

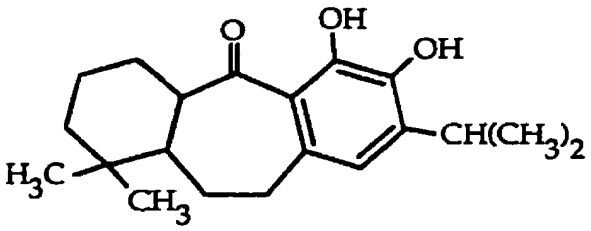
2.6.1 Thiobarbituric acid-reactive substances (TBARS) test

Kohn and Liversedge (1944) first observed the pink coloration of animal tissues which had been incubated with 2-thiobarbituric acid (TBA). The 2-thiobarbituric acid-reactive substances test has been commonly used to measure lipid oxidation in tissue samples ever since it was introduced in 1944. During autoxidation of PUFA of lipids, malonaldehyde (MA) is formed. This secondary oxidation product is highly reactive and remains bound to other food components such as proteins and nucleic acids (Shahidi and Hong, 1991). Tarladgis *et al.* (1964) have shown that heating of foods, under acidic

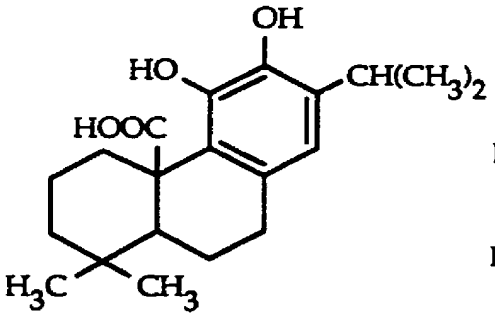
Figure 2.5 Chemical structures of some natural plant antioxidants. (A), Antioxidant components of rosemary and (B), antioxidant components of black pepper, capsicum and turmeric.



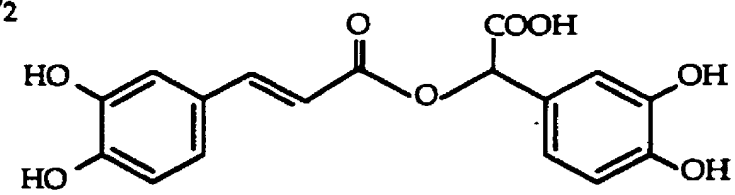
Rosmanol



Rosmaridiphenol

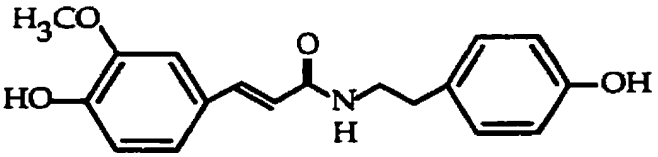


Carnosic acid

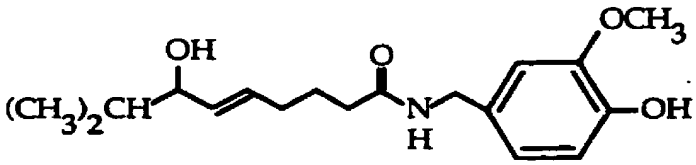


Rosmaric acid

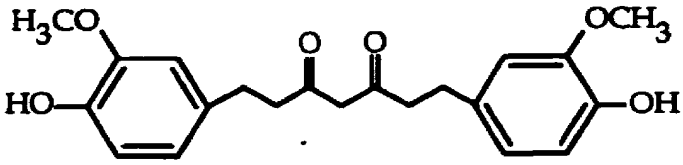
(A) Antioxidative compounds from rosemary



(i)



(ii)



(iii)

(B) Antioxidative compounds from (i) black pepper, (ii) capsicum and (iii) turmeric

conditions, would presumably release the bound MA from its adducts. Figure 2.6 depicts the steps involved in the formation of the pink-coloured TBA-MA adduct which has an absorption maximum at 532 nm (Kosugi *et al.*, 1989).

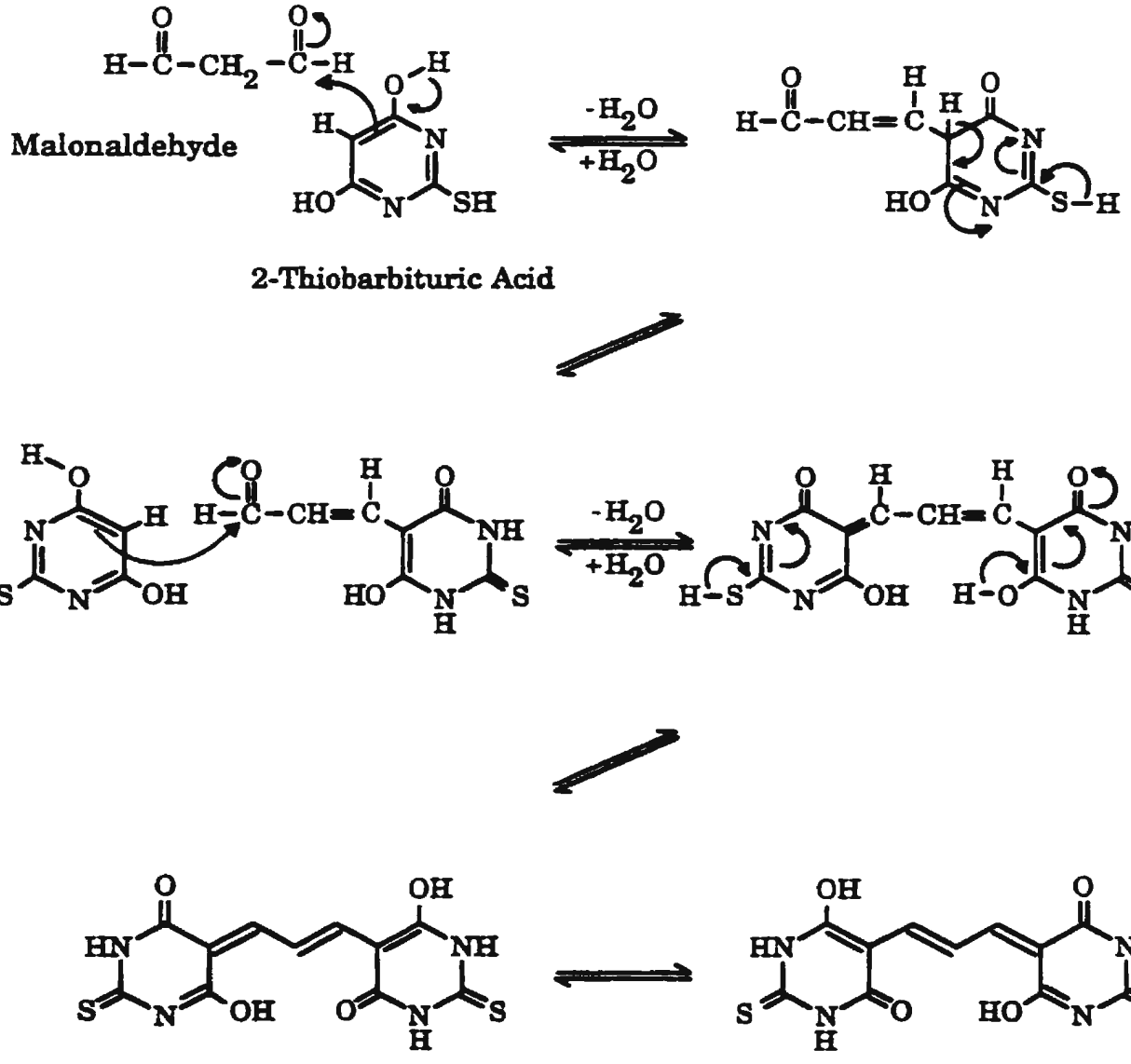
There are a variety of methods available for quantification of TBARS, depending on the nature of food to be investigated. Distillation (Tarladgis *et al.*, 1964) and extraction methods (Siu and Draper, 1978) are frequently used for assessing the oxidative state of muscle tissues.

2.6.1.1 Origin of malonaldehyde

Malonaldehyde (MA) is one of the most studied products of lipid peroxidation. It is believed to be a decomposition product of certain lipid hydroperoxides (Ichinose et al., 1989). Fatty hydroperoxides, labile primary oxidation products, readily involve in radical reactions which lead to their molecular transformation and degradation. The precise pathways for degradation of hydroperoxides and factors influencing it in the formation of malonaldehyde are poorly understood (Janero, 1990).

Malonaldehyde can be formed from PUFA with three double bonds (triene) or more (Raharjo and Sofos, 1993). Abstraction of a hydrogen atom from a triene fatty acid was envisioned to take place at one of the positions between two double bonds and subsequently with the presence of oxygen it would form conjugated peroxyl radicals. These radicals could also abstract a hydrogen atom from other PUFA to produce cyclic peroxides. It appears, however, more likely that the cyclic peroxyl radicals would react

Figure 2.6 Possible mechanism for the reaction of malonaldehyde with the TBA reagent in the classical TBA test for assessing lipid oxidation. Adapted from Pegg (1993).


Malonaldehyde
2-Thiobarbituric Acid
-H2O
+H2O

with oxygen to form an additional peroxyl group on the cyclic peroxides. The reason is that the reaction of alkyl radicals with oxygen is much faster, under autoxidation conditions, than the reaction with other PUFA. The peroxyl radical would ultimately abstract a hydrogen atom from PUFA to yield cyclic peroxide with an additional hydroperoxide group on the molecule. All cyclic peroxides could decompose under the conditions of the TBARS test to produce malonaldehyde (Frankel and Gardner, 1989; Raharjo and Sofos, 1993).

Pryor *et al.* (1976) proposed a modified pathway in which PUFA would form not only cyclic peroxides but also endoperoxides. The endoperoxide formation mechanism involves formation of a C-C bond. By this mechanism, the triene fatty acids could produce endoperoxide with an allyl radical which is subsequently transformed into prostaglandin-like endoperoxides. According to this mechanism, diene fatty acids would not give rise to endoperoxides because they are unable to produce allyl radicals. The inability of diene fatty acids to form prostaglandin-like endoperoxides has been proven experimentally. Hence, it is considered that the precursor of malonaldehyde is an endoperoxide with an allylic radical (Raharjo and Sofos, 1993).

2.6.2 Chromatographic methods

Both gas chromatography (GC) and high performance liquid chromatography (HPLC) have been used to measure the degree of lipid oxidation. Direct GC analysis of an extract of tissues is a time consuming process whereas GC with headspace analysis

represents a simple alternative to time consuming extraction procedures. HPLC is more suitable for isolation and quantification of non-volatile polymeric decomposition products and thermally-labile peroxides and hydroperoxides (Robards *et al.*, 1988).

Several GC methods have been reported for the analysis of volatile flavour compounds in oils and lipid-containing foods (Lamikanra and Dupuy, 1990; Ramarathnam *et al.*, 1991a,b; Drumm and Spanier, 1991; Ajuyah *et al.*, 1993). Headspace GC analysis is a simple technique that measures volatile compounds equilibrated with liquid or solid samples in a closed system. This method has been used to analyze hexanal as a lipid peroxidation product in cereal foods, vegetable and animal fats, and meats (Frankel, *et al.*, 1989; Umano and Shibamoto, 1987; Matiella and Hsieh, 1990).

A distinctive feature of the headspace analysis is that the volatile composition contained in the gas phase is used to determine the nature and composition of the condensed phase with which it is in contact (Ioffe and Vitenberg, 1984). The main drawback associated with headspace GC analysis is the size of sample that can be injected. Only those components that, by virtue of their concentration and relative volatility, are present in quantities sufficient to activate the detector will be detected. Relatively low-molecular weight and highly volatile compounds, such as C_2-C_8 esters, aldehydes and ketones, can be readily detected by the direct injection of a restricted quantity of headspace gas (Jennings, 1979).

2.6.2.1 Origin of hexanal and other volatiles

Carbonyl compounds, particularly volatile aldehydes, derived from fatty hydroperoxides, possess both desirable and undesirable effects in lipid-rich foods. The main volatiles produced from heated pork fat are pentanal, hexanal, heptanal (Yasuhara and Shibamoto, 1989). Most of these volatiles are the result of oxidation, usually of C_{18} PUFA such as linoleic and linolenic acids and the C_{20} arachidonic acid (Shahidi *et al.*, 1986). Hexanal, a volatile with a powerful fatty-green and grassy odour, is derived from linoleic acid and has been successfully used for evaluation of the oxidative state of red meats from different species as well as fish (Shahidi and Pegg, 1993). Although fatty ester hydroperoxides are the recognized precursors of volatile secondary products from lipids, the origin of many important degradation products remains obscure. Two major mechanisms of volatile production from lipid hydroperoxides include a homolytic scission through an alkoxyl radical and an acid-catalyzed heterolytic scission. The homolytic scission is also known as β-cleavage while the heterolytic scission is called Hock cleavage. Heterolytic cleavage occurs selectively between the carbon bearing the hydroperoxide group and the allylic double bond. This reaction produces hexanal and 12-oxo-10-dodecenoic acid from the 13-hydroperoxide of linoleic acid, and 2-nonenal and 9-oxononanoic acid from the 9-hydroperoxide. Volatiles formed by thermal decomposition are more diverse, and can usually be explained by homolytic cleavage on either side of the alkoxyl radical derived from hydroperoxides. This mechanism predicts the formation of pentane, hexanal, and 13-oxo-9,11-tridecadienoic acid from the 13-

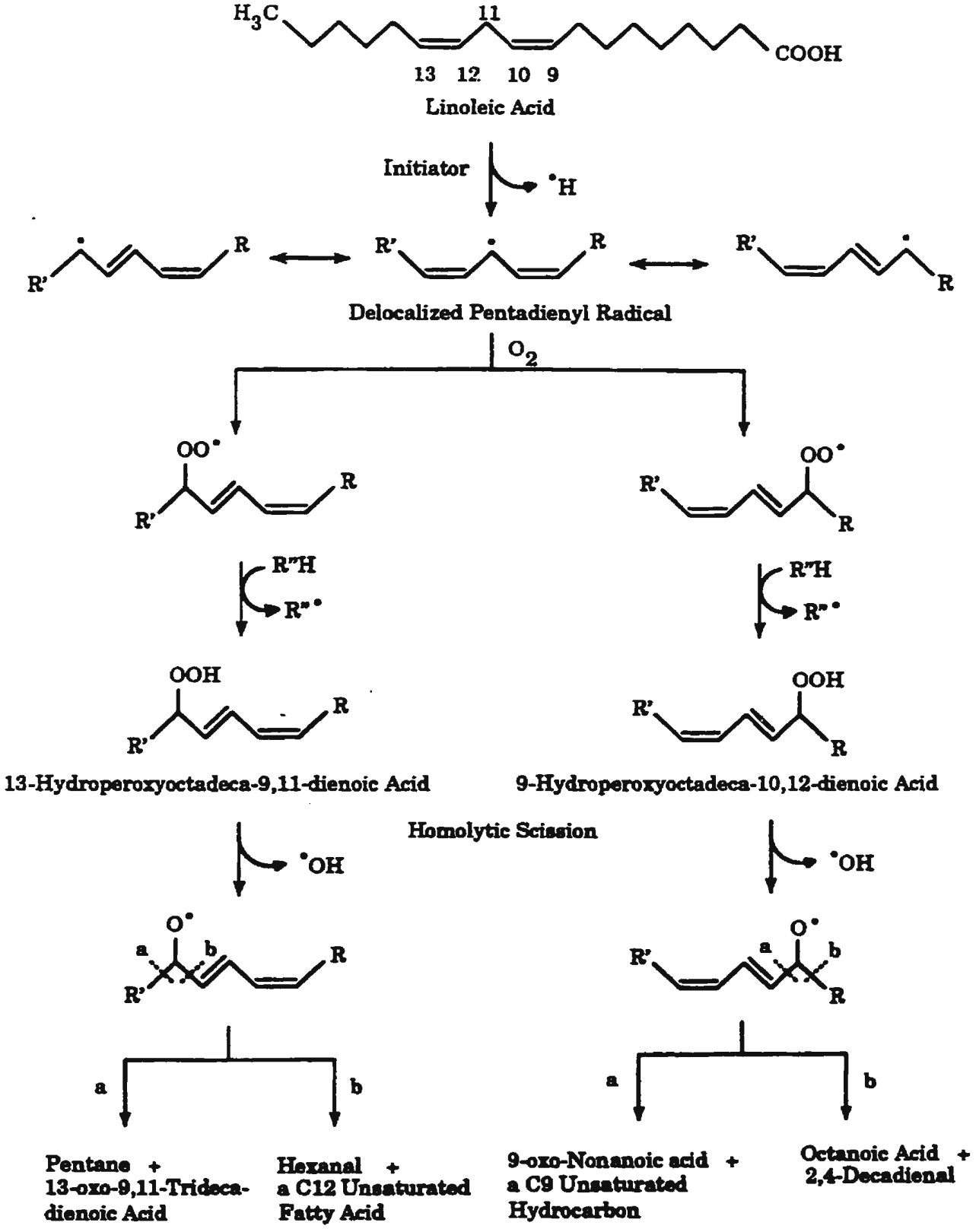
hydroperoxide of linoleic acid, and 2,4-decadienal, methyl octanoate, and methyl-9-oxononanoate from the 9-hydroperoxide. Other radical reactions lead to the formation of relatively minor amounts of additional volatiles (Frankel and Gardner, 1989). Figure 2.7 depicts the mechanism of hexanal formation from linoleic acid.

Hexanal has been used as an indicator to assess the oxidation status and subsequent flavour deterioration of fats from land animals (Frankel *et al.*, 1989; Shahidi and Pegg, 1993). However, propanal has been used as an indicator to assess the oxidation state of marine fats (Frankel, 1993; Frankel *et al.*, 1994). Propanal is generated from ω-3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)(Frankel, 1993).

2.7 Texture of restructured meat products

Texture is considered to be one of the most important sensory attributes of restructured products such as salami, pepperoni and sausages (Seideman and Theer, 1986; Fjelkner-Modig, 1986; Thiel *et al.*, 1986). Basically, the main factor responsible for the texture of restructured meat products is gelation of proteins. Gelation of proteins involves both intramolecular and intermolecular changes in proteins during thermal processing (Asghar *et al.*, 1985). Both NaCl and STPP play important roles in improvement ofgelling capacity (emulsifying capacity) and gel stability (emulsion stability) of restructured meat products (van Roon and Krol, 1985).

Figure 2.7 Autoxidation of linoleic acid and the production of hexanal. Adapted from Frankel *et al.* (1984) and Pegg (1993).


H3C
11
COOH
13 12 10 9
Linoleic Acid
Initiator
•H
R
R'
Delocalized Pentadienyl Radical
O2
OO•
R"H
R"•
OOH
13-Hydroperoxyoctadeca-9,11-dienoic Acid
9-Hydroperoxyoctadeca-10,12-dienoic Acid
Homolytic Scission
•OH
O•
a
b
Pentane + 13-oxo-9,11-Trideca-dienoic Acid
Hexanal + a C12 Unsaturated Fatty Acid
9-oxo-Nonanoic acid + a C9 Unsaturated Hydrocarbon
Octanoic Acid + 2,4-Decadienal

Water binding capacity (WBC), another important property that contributes to the texture of restructured meat products, represents the ability of muscle foods to bind water under a specific set of conditions (Trout, 1988). WBC is studied extensively because of its economic importance in terms of product yield (Trout, 1988). Although water is bound in muscle products by capillary action, the microstructure that produces the capillary action is the pores located between thick and thin filaments of the myofibrils, which are approximately 10 nm in diameter (Trout, 1988). Sodium chloride and pH regulators such as STPP contribute to the formation of the microstructure by solubilizing actomyosin complex and making intermolecular salt bridges with proteins. Solubilized actomyosin may denature upon cooking to form a dense fibrous protein network which holds fat, water and other ingredients (Acton *et al.*, 1983). It is therefore clear that NaCl and pH regulators are prime contributors to the texture of restructured meat. Moreover, nitrite may also have a considerable effect on the textural properties of cured, restructured meat products such as sausages (Randall and Voisey, 1977). Woolford *et al.* (1976) reported that nitrite can bind with myosin, a muscle protein, resulting in a modified myosin with increased emulsification capacity.

2.7.1 Impact of salt reduction on texture and other properties of restructured meat

Increasing concern with the association between dietary sodium intake and hypertension has promoted voluntary efforts by food processors to reduce the amount of sodium in processed foods (Rhee *et al.*, 1983a). This practice, however, introduces

adverse effects on the quality parameters of processed meats (Terrell, 1983). As NaCl levels are reduced, flavour, texture, water-binding capacity (WBC) and shelf-life of processed meat are also reduced (Terrell, 1983; Sofos, 1986; Barbut and Mittal, 1989; Bernthal *et al.*, 1991). Thiel *et al.* (1986) have reported a decrease in yield, breaking force and overall acceptability of chunked and formed ham when salt addition was reduced from 2% to 1.5% or below. Barbut and Mittal (1989) have demonstrated a decrease in the rigidity modulus (G) of meat homogenates with decreasing concentrations of NaCl.

Most of the adverse effects due to a reduction in salt content may be overcome by using a mixture of additives. Salt substitutes, partly or totally containing components instead of NaCl are widely used in the food industry (Lantinen, 1986). Use of other types of chloride salts such as KCl and $MgCl_2$ (Rhee *et al.*, 1983b) as well as polyphosphates (Sofos, 1986) as substitutes for NaCl has been reported. Nowadays, a variety of low-sodium salt mixtures are available in the market. Morton salt and Pan®-salt, examples of low-sodium salt formulations, are widely used in the diets of hypertensive patients (Lantinen, 1986; Puolanne *et al.*, 1988). Morton salt is a mixture of 65% NaCl, 25% KCl and 10% $MgSO_4.7H_2O$ (Lantinen, 1986) whereas Pan®-salt contains 52% NaCl, 28% KCl, 12% $MgSO_4$ and 1-3% lysine monohydrochloride (Puolanne *et al.*, 1988).

CHAPTER 3 MATERIALS AND METHODS

3.1 Materials

Three fresh pork shoulders were obtained and trimmed from most of their surface fat. Each pork shoulder was ground twice in an Omega (Type T 12) commercial meat grinder using a 0.79 and then a 0.48 cm plate. Ground pork shoulders were then vacuum packaged in separate plastic bags (Kapak Co., Minneapolis, MN) and stored in a deep freezer (Ultra Low, Revco, Inc., West Columbia, SC) at -60°C until used.

Food grade sodium tripolyphosphate (STPP) was obtained from Albright & Wilson, (A Division of Tenneco Canada Inc., Toronto, ON). Ethylenediaminetetraacetic acid (EDTA), sodium ascorbate, 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane, myoglobin, β-carotene, and BHA were obtained from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid (TCA) was obtained from Fisher Scientific (Nepean, ON). Reagent grade (purity 99 - 99.99%) lithium fluoride (LiF), lithium chloride (LiCl), lithium bromide (LiBr), lithium iodide (LiI), lithium sulphate (Li_2SO_4), sodium fluoride (NaF), sodium chloride (NaCl), sodium bromide (NaBr), sodium iodide (NaI), sodium sulphate (Na_2SO_4), potassium fluoride (KF), potassium chloride (KCl), potassium bromide (KBr), potassium iodide (KI), potassium sulphate (K_2SO_4), cesium fluoride (CsF), cesium chloride (CsCl), cesium bromide (CsBr), cesium iodide (CsI), cesium sulphate (Cs_2SO_4), magnesium fluoride (MgF_2), magnesium chloride ($MgCl_2$), magnesium bromide ($MgBr_2$), magnesium iodide (MgI_2) magnesium sulphate ($MgSO_4$), calcium fluoride (CaF_2), calcium chloride ($CaCl_2$), calcium bromide ($CaBr_2$), calcium iodide (CaI_2) calcium sulphate

($CaSO_4$), ferrous sulphate ($FeSO_4$), sodium nitrite ($NaNO_2$), sodium carbonate (Na_2CO_3), Tween 80, 2-heptanone, haemin and linoleic acid were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Methanol, ethanol and chloroform used in this study were ACS grade. Helium, hydrogen, nitrogen, nitric oxide and compressed air were obtained from Canadian Liquid Air Ltd. (St. John's, NF).

3.2 Sampling method

Proximate, thiobarbituric acid-reactive substances (TBARS), cook yield and texture analyses were carried out using three meat samples taken from three comminuted pork shoulders.

3.3 Proximate composition

3.3.1 Determination of moisture content

Approximately 3-4 g of pork was accurately weighed into a preweighed aluminium dish and placed in a forced-air convection oven (Fisher Isotemp 300, Fair Lawn, NJ) which was preheated to 105±1 °C. Samples were held at this temperature overnight or until a constant mass was obtained. The moisture content was calculated as the percent ratio of the weight difference of the samples before and after drying to that of the original material (AOAC, 1990).

3.3.2 Determination of crude protein content

Approximately 0.3-0.4 g of pork was accurately weighed on a nitrogen-free paper and placed in a digestion tube of a Büchi 430 digester (Büchi Laboratories, Flawil, Switzerland). The nitrogen content in different samples was determined by digestion in 20 mL of concentrated sulphuric acid in the presence of two Kjeltab catalyst tablets (Profamo, Dorval, PQ) in the digester until a clear solution was obtained. Digested samples were diluted with 50 mL of distilled water followed by addition of 150 mL of a 25% (w/v) solution of sodium hydroxide. Nitrogen in the samples was converted to ammonia which was steam-distilled (Büchi 321, Büchi Laboratories, Flawil, Switzerland) into a 50 mL solution of 4% (w/v) boric acid containing a few drops of end point indicator (EM Science, Gibbstown, NJ). Approximately 200 mL of distillate were collected and the content of ammonia in the distillate was determined by titrating it against a 0.1N standardized solution of sulphuric acid (AOAC, 1990). The crude protein content of pork was calculated as N% x 6.25.

3.3.3 Determination of total lipid content

Total lipids were extracted into a mixture of chloroform and methanol as described by Bligh and Dyer (1959). Approximately 25 g of sample were accurately weighed and then extracted with a mixture of 25 mL of chloroform and 50 mL of methanol (1:2, v/v) by homogenizing for 3 min with a Polytron homogenizer (Brinkmann Instruments, Rexdale, ON) at a speed of 4. A further extraction was done with the addition of 25 mL

of chloroform followed by homogenization. About 25 mL distilled water was added and the mixture was then filtered through a Buchner funnel using a Whatman No.3 filter paper (Fisher Scientific, Nepean, ON). The filtrate was allowed to separate overnight in a separatory funnel. Dilution with chloroform and water resulted in separation of homogenate layers and inclusion of lipids in the chloroform. A 10 mL aliquots of the lipid extract in chloroform, after drying over anhydrous sodium sulphate, was transferred into a tared 50 mL round bottom flask and the solvent was removed under vacuum using a Büchi RE 111 rotovapor (Büchi Laboratories, Flawil, Switzerland). The flask was then placed in a forced-air convection oven (Fisher Isotemp 300, Fair Lawn, NJ) at 80 °C for 1 h. After cooling in a desiccator, the lipid content was determined gravimetrically.

3.3.4 Determination of ash content

Approximately 3-4 g of pork was weighed into a cleaned porcelain crucible and then charred over a Bunsen burner and subsequently placed in a temperature controlled muffle furnace (Blue M Electric Co., Blue Island, IL) which was preheated to 550 °C. Samples were held at this temperature until a gray ash was produced and then cooled in a desiccator and weighed immediately. Ash content was calculated as percent ratio of the mass of the ash obtained after ignition to that of the original material (AOAC, 1990).

3.4 Preparation of meat model systems for thiobarbituric acid-reactive substances (TBARS) and headspace analyses

Meat model systems were prepared as described by Shahidi and Pegg (1990). Ground pork was mixed with 20% by weight of deionized water in Mason jars. Salts (LiF, LiCl, LiBr, LiI, Li_2SO_4, NaF, NaCl, NaBr, NaI, Na_2SO_4, KF, KCl, KBr, KI, K_2SO_4, CsF, CsCl, CsBr, CsI, Cs_2SO_4, MgF_2, $MgCl_2$, $MgBr_2$, MgI_2, $MgSO_4$, CaF_2, $CaCl_2$, $CaBr_2$, CaI_2, and $CaSO_4$) were added directly to meat at 100 and 200 meq/kg sample. The systems were then thoroughly homogenized and cooked at 85±2 °C (internal temperature of 72±2°C) in a thermostated water bath for 45 min while stirring occasionally with a glass rod. After cooling to room temperature, cooked meat samples were homogenized in a Waring blender (Model 33BL73) for 30 s and then stored for seven days at 4 °C.

Another set of similar model systems containing 1, 2 and 3% of Pan®-salt, a low-sodium salt mixture (52% NaCl, 28% KCl, 12% $MgSO_4$ and 3% lysine.HCl) were prepared. Model systems containing 1, 2 and 3% NaCl were also prepared for comparative studies.

3.5 Thiobarbituric acid-reactive substances (TBARS) test

Samples were analyzed for TBA-reactive substances (TBARS) over a 7-day period according to the method of Siu and Draper (1978). Two grams of each sample were placed in a centrifuge tube to which 5 mL of 10% TCA (Fisher Scientific, Nepean, ON) were added and vortexed (Fisher Vortex Genie 2, Fisher Scientific, Nepean, ON) at high

speed for 2 min. Five millilitres of a 0.02 M aqueous solution of 2-thiobarbituric acid was then added to each centrifuge tube which was further vortexed for 30 s. The samples were then centrifuged at 3000xg for 10 min and the supernatants were filtered through a Whatman No.3 filter paper. Filtrates were heated in a boiling water bath for 45 min, cooled to room temperature in ice, and the absorbance of the resulting pigment was read at 532 nm using a Hewlett Packard diode array spectrophotometer (Model 8452A, Hewlett Packard Co., Mississauga, ON). TBARS values were calculated by multiplying the absorbance readings by a factor of 3.4 which was obtained from a standard line prepared using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde (Figure A.1). Inhibition of TBARS formation (%) was calculated using the following equation:

$$\% \text{ Inhibition of TBARS formation} = \{(C - S)/C\}*100$$

where, C and S represent the TBARS values of the control and the treated sample, respectively.

3.6 Headspace analysis

A Perkin-Elmer 8500 gas chromatograph and an HS-6 headspace sampler (Perkin-Elmer Corp., Montreal, PQ) were used for volatile analysis of cooked pork samples. A high polarity Supelcowax 10 fused silica capillary column (30 m x 0.32 mm internal diameter, 0.10 μm film, Supelco Canada Ltd., Oakville, ON) was used. Helium was the

carrier gas employed at an inlet column pressure of 17.5 psig with a split ratio of 7:1. The oven temperature was maintained at 40 °C for 5 min and then ramped to 200 °C at 20 °C/min and hold at 200 °C for 5 min. The injector and flame ionization detector (FID) temperatures were adjusted to 280 °C and held at this temperature throughout the analysis (Shahidi and Pegg, 1993).

For headspace (HS) analysis, 4.0 g portions of homogenized pork samples were transferred to 5 mL glass vials. The vials were capped with teflon-lined septa, crimped and then frozen at -60 °C (Ultra Low, Revco, Inc., West Columbia, SC) until used. To avoid heat shock after removal from storage, frozen vials were tempered at room temperature for 30 min and then preheated in the HS-6 magazine assembly at 90 °C for a 45 min equilibration period. Pressurization time of the vial was 6 s, and the volume of the vapour phase drawn was approximately 1.5 mL. Chromatograph peak areas were expressed as integrator count units. Individual volatile compounds were tentatively identified by comparing relative retention times of GC peaks with those of commercially available standards. Quantitative determination of dominant aldehydes was accomplished using 2-heptanone as an internal standard (Shahidi and Pegg, 1993).

3.7 Preparation of meat model systems for texture analysis

Model systems containing LiF, LiCl, LiBr, LiI, Li_2SO_4, NaF, NaCl, NaBr, NaI, Na_2SO_4, KF, KCl, KBr, KI, K_2SO_4, CsF, CsCl, CsBr, CsI, Cs_2SO_4, MgF_2, $MgCl_2$, $MgBr_2$, MgI_2, $MgSO_4$, CaF_2, $CaCl_2$, $CaBr_2$, CaI_2, and $CaSO_4$ were prepared as described in Section

3.4, but the systems were not homogenized in a blender at any time. Systems were mixed thoroughly with a glass rod before cooking. After cooking, the excess water was decanted and the resultant meat crumbles were stored refrigerated at 4 °C overnight. The systems were brought back to the room temperature (25 °C) before Texture measurements. In addition, model systems were prepared with Pan®-salt as well as NaCl at 1, 2 and 3% levels.

3.8 Texture analysis

Texture analysis of meat samples, conditioned to room temperature, were performed using an Ottawa Texture Measuring System (Model MC 1061). Meat crumbles were stuffed into a four-blade cell of the texturemeter. The sample was compressed through the cell by the plunger at 6.2 cm/min. As the sample was pushed through the cell, the peak shear force (kg) was recorded (Daytronic, Model 9530A).

3.9 Determination of cook yield

Cook yield of comminuted pork treated with different salts at 100 or 200 meq/kg meat was determined as given below. A 10 g sample was transferred into a pre-weighed centrifuge tube along with an appropriate amount of salt and 5 g of deionized water. Tubes were covered with aluminium foil and then placed in a boiling water bath for 20 min. The tubes were cooled to room temperature and then centrifuged for 15 min using a clinical centrifuge (IEC Centra M5, International Equipment Co., Needham Heights.,

MA) at 3000xg. The liquid released was decanted and the sample was blotted on a Whatman No. 1 filter paper and transferred back into the tube. The percent cook yield was calculated using the following equation (Onodenalore, 1993):

$$\text{Cook Yield, \%} = (\text{Weight of cooked meat/Weight of fresh meat})*100$$

3.10 Preparation of metmyoglobin (MMb) solution

Myoglobin (18.8 mg) was weighed into a 10 mL volumetric flask and dissolved in a small volume of 0.05 M phosphate buffer (pH 4.5) and brought to the mark with the same phosphate buffer. The absorbance spectrum (490-650 nm) of the resultant pigment was used to ascertain that metmyoglobin had been formed (Figure A.3).

3.11 Synthesis of nitrosylmyoglobin (NOMb)

A solution of nitrosylmyoglobin (100 mM) was prepared according to the method of Fox and Thomson (1963). Sodium nitrite (700 µL of 1 mg/mL stock) and 35 mg of L-ascorbic acid were weighed into a 100 mL volumetric flask and filled up to the mark with a 0.05 M phosphate buffer (pH 4.5). Horse heart myoglobin (18.8 mg) was transferred into a 10 mL volumetric flask and filled up to the mark with the above solution. The concentrations of $NaNO_2$ and L-ascorbic acid in the reaction mixture were 7 and 350 ppm, respectively. The absorbance spectra (490-650 nm) of the mixture at different time intervals were recorded in order to ascertain that metmyoglobin was fully converted to nitrosylmyoglobin (Figure A.3).

3.12 Synthesis of the cooked cured meat pigment (CCMP)

Synthesis of CCMP was carried out according to the method of Shahidi *et al.* (1994). Haemin (25 mg) was weighed into a 10 mL volumetric flask and dissolved in 5 mL of 0.04 M sodium carbonate (Na_2CO_3) solution and then filled to the mark with the same solution. Haemin solution (1 mL) was added to a centrifuge tube containing about 150-153 mg of sodium tripolyphosphate (STPP), 300 mg of sodium ascorbate and 9 mL of 0.2 M acetate buffer (pH 6.5). Tubes were then transferred to an AtmosBag (Aldrich Chemical Co., Inc., Milwaukee, WI). Nitric oxide (NO) gas was bubbled into the solution for approximately 30 s and the tubes were then capped tightly so that the resulting nitrosylhaemochrome was maintained under a positive pressure of NO. Tubes were then centrifuged (ICE Centra M5, International Equipment Co., Needham Heights, MA) for 5 min at 4000xg. The supernatants were drained off and the precipitates were dissolved in 50 mL of a 0.04 M sodium carbonate solution followed by vortexing (Fisher Vortex Genie 2, Model G-560, Fisher Scientific, Bohemia, NY). Absorption spectrum of CCMP is shown in Figure A.3.

3.13 Preparation of β-carotene/linoleate model system

3.13.1 Preparation of aqueous linoleate solution

One millilitre of ethanolic linoleic acid (7.5% w/v), 0.3 mL of ethanolic Tween 80 (10%, v/v) and 5 mL of 0.5% (w/v) aqueous ethylenediaminetetraacetic acid (EDTA) were mixed in a 10 mL volumetric flask and the pH of the mixture was adjusted to 9.0

by dropwise addition of 0.1 N sodium hydroxide (NaOH). Finally, the volume was adjusted to 10 mL with distilled water (Ben-Aziz *et al.*, 1971).

3.13.2 Preparation of aqueous β-carotene solution

β-carotene (83.3 mg) and 0.9 mL of ethanolic Tween 80 (10%, v/v) were transferred into a 25 mL volumetric flask and the volume was adjusted to the mark with the addition of chloroform. One millilitre of this solution was evaporated to dryness under vacuum (Büchi RE 111 rotavapor, Büchi Laboratories, Flawil, Switzerland) and the residue was dissolved immediately in 10 mL of a 0.25% (w/v) solution of EDTA (Ben-Aziz *et al.*, 1971).

3.13.3 Preparation of aqueous buffered β-carotene/linoleate solution

Aqueous linoleate (1 mL) was mixed with aqueous β-carotene (1 mL) and the volume was adjusted to 10 mL with a 0.2 M citrate-phosphate buffer (pH 7). This solution, prepared immediately before use, contained 750 µg of linoleic acid, 33.3 µg of β-carotene, 0.66 µL of Tween 80 and 0.5 mg of EDTA per mL (Ben-Aziz *et al.*, 1971).

3.13.4 Assay procedure

The assay was carried out at room temperature (25 ± 2 °C) in a cuvette placed in a Hewlett Packard diode array spectrophotometer (Model 8452A). Buffered β-carotene/linoleate system (1.5 mL) and an appropriate volume of the compound to be

examined were transferred to the cuvette and the volume was then adjusted to 2 mL. The concentration of β-carotene, linoleate, EDTA and Tween in the assay medium was 25 µg, 562.5 µg, 375 µg, and 0.5 µL per mL, respectively (Ben-Aziz *et al.*, 1971). The absorbance was measured at 460 nm after every min for 5 min. Absorbance value of a blank sample containing all the ingredients except β-carotene was measured and substracted from absorbance value of sample containing β-carotene and all the other ingredients. Deionized water (0.5 mL) was used as a control sample. The amount of β-carotene bleached, in µg, was calculated using a factor obtained from a standard line (Figure A.2). The % β-carotene protection was calculated using the following equation:

$$\% \text{ β-carotene protection} = \{(C - S)/C\}*100$$

where, C and S represent the amount of β-carotene bleached by the control and the compound under investigation, respectively.

Metmyoglobin, nitrosylmetmyoglobin and CCMP were tested for their effects on β-carotene bleaching at 2.2, 6.2 and 10 µM levels. Sodium ascorbate was tested at 50, 100 and 550 ppm levels and STPP was tested at 50, 100 and 500 ppm levels, both in the presence and absence of CCMP (10 µM). The lower level of STPP used in these studies as compared to previous ones was necessary because of solubility limitations. In addition NaF, NaCl, NaI, KF, KI, CsF, CsI, CaF_2, CaI_2, and $FeSO_4$ were tested for their performance in a β-carotene/linoleate model system at 10 and 100 ppm levels. BHA was

used as a standard to compare the antioxidative/prooxidative properties of the aforementioned compounds.

3.14 Statistical test

Analysis of variance and Tukey's studentized range test (Snedecor and Cochran, 1980) were used to determine differences in mean values based on data collected from three determinations of various experiments. Significance was determined at a 95% level of probability.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Effect of haem pigments on lipid oxidation

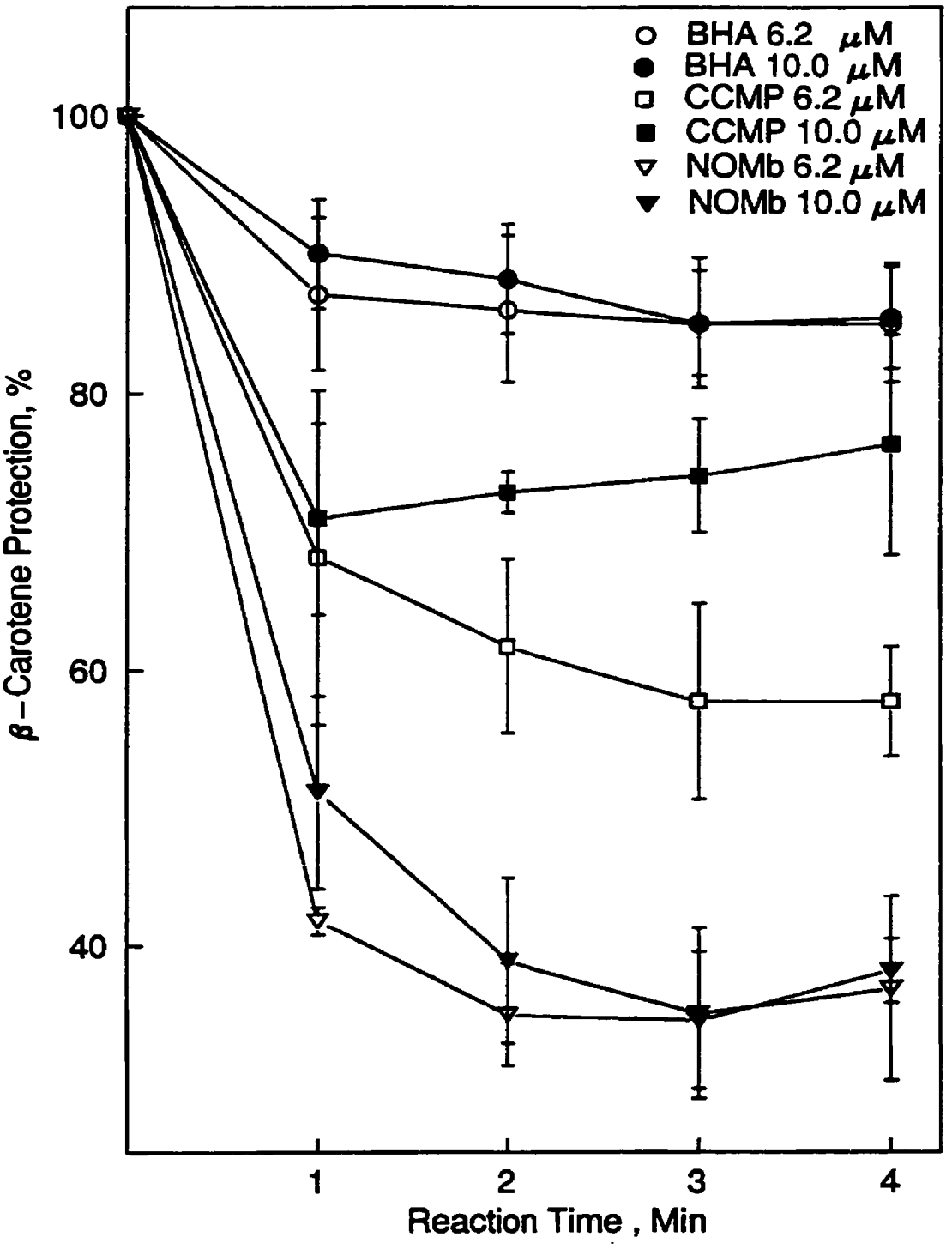
Assessing the pro- or anti-oxidative nature of organic and inorganic compounds by measuring the disappearance of β-carotene in the coupled oxidation of linoleate and β-carotene is commonplace (Ben-Aziz *et al.*, 1970a,b; Kanner *et al.*, 1979; Zubillaga *et al.*, 1984; Roozen *et al.*, 1994). The effect of MMb, NOMb and CCMP on β-carotene destruction in a β-carotene/linoleate model system is shown in Table 4.1 and Figure 4.1. The MMb exerted a prooxidative effect regardless of the concentration tested. A high extent of β-carotene destruction was observed during the 1st min of the reaction for all three concentrations examined. Over 72% of the total amount of β-carotene bleached by MMb occurred during the 1st min of the reaction. A decrease in the catalytic activity of MMb was observed with increasing of its concentration in the medium (Table A.1). Kanner *et al.* (1979) have also observed a similar trend for MMb when tested in a β-carotene/linoleate model system. These authors suggested that above a certain concentration of MMb, inhibition is bound to exceed initiation. They also pointed out that the chelated forms of metals act as hydroperoxide decomposers, but at the same time react with the free radicals generated in the process, thereby terminating free radical propagation. The prooxidative activity of metmyoglobin in model systems including β-carotene/linoleate models has been reported (Kanner *et al.*, 1979; Harel and Kanner, 1985b; Kanner and Harel, 1985; Tichivangana and Morrissey, 1985; Rhee and Ziprin, 1987). According to Love and Pearson (1974) and Igene *et al.* (1979), purified

Table 4.1 Effect of haem pigments and BHA on β-carotene destruction in a β-carotene/linoleate model system as reflected by cumulative loss of β-carotene (μg)[1].

Compound	Reaction Time, Min			
	1	2	3	4
Control (H_2O)	1.41±0.02^{c}	1.80±0.07^{c}	2.08±0.11^{c}	2.41±0.20^{c}
Concentration 2.2 μM				
BHA	0.20±0.05^{cde}	0.27±0.10^{cde}	0.30±0.07^{c}	0.34±0.03^{c}
MMb	9.18±0.07^{a}	10.71±0.86^{a}	11.28±0.90^{a}	11.54±0.65^{a}
NOMb	1.09±0.18^{c}	1.51±0.13^{c}	1.82±0.25^{c}	1.99±0.35^{c}
CCMP	5.68±0.45^{b}	7.05±0.38^{b}	7.85±0.35^{b}	8.40±0.34^{b}
Concentration 6.2 μM				
BHA	0.18±0.07^{cde}	0.25±0.11^{cde}	0.31±0.10^{c}	0.36±0.10^{c}
MMb	6.16±0.64^{b}	7.35±0.78^{b}	7.92±0.80^{b}	8.20±0.79^{b}
NOMb	0.82±0.01^{c}	1.17±0.13^{c}	1.36±0.11^{c}	1.49±0.10^{c}
CCMP	0.45±0.08^{cd}	0.49±0.01^{cd}	0.88±0.11^{c}	1.02±0.07^{c}
Concentration 10.0 μM				
BHA	0.11±0.01^{cde}	0.21±0.02^{cde}	0.31±0.10^{c}	0.35±0.08^{c}
MMb	4.74±0.12^{b}	5.96±0.11^{b}	6.54±0.11^{b}	6.52±0.35^{b}
NOMb	0.69±0.14^{c}	1.10±0.32^{c}	1.35±0.24^{c}	1.52±0.28^{c}
CCMP	0.41±0.04^{cd}	0.49±0.01^{cd}	0.54±0.03^{c}	0.57±0.06^{c}

[1] Results are mean values of three determinations ± standard deviation. Means sharing the same superscripts in a column are not significantly ($p<0.05$) different from one another.

Figure 4.1 Effect of butylated hydroxyanisole (BHA), nitrosylmyoglobin (NOMb), cooked cured-meat pigment (CCMP) and metmyoglobin (MMb) on β-carotene stability in a β-carotene/linoleate model system. Error bars represent standard deviations from means of three determinations.


BHA 6.2 μM
BHA 10.0 μM
CCMP 6.2 μM
CCMP 10.0 μM
NOMb 6.2 μM
NOMb 10.0 μM
100
80
60
40
β-Carotene Protection, %
1
2
3
4
Reaction Time , Min

metmyoglobin or EDTA-treated beef muscle extracts, did not accelerate lipid oxidation upon heating and subsequent storage, when added to a meat residue left after water extraction. According to Harel and Kanner (1985) and Kanner and Harel (1985), interaction of hydrogen peroxide (H_2O_2) with metmyoglobin can generate an active H_2O_2-metmyoglobin species that eventually initiates membrane lipid oxidation. This may not be the reason for prooxidative nature of metmyoglobin observed in the β-carotene/linoleate model system which is devoid of H_2O_2.

The effect of NOMb on β-carotene destruction was antioxidative for all concentrations tested (Figure 4.1). NOMb also exhibited an elevated catalytic activity during the 1st min of the reaction. The stabilizing effect of NOMb may be attributed to the fact that Fe^{2+} is tied by nitric-oxide (NO). Since NOMb has its iron atom in the ferrous oxidation state with all of its coordination sites occupied, it is more stable and does not release non-haem iron. The antioxidative effect of NOMb may also be attributed to the nitric-oxide group of NOMb. As the NO group has an unpaired electron, it can act as an antioxidant by free radical neutralization through direct coupling with an alkyl radical (Morrissey and Tichivangana, 1985). The strong antioxidative activity of NOMb observed in this study could therefore be attributed to (1) the involvement of all the coordination sites of the iron atom of NOMb, (2) quenching of free radicals by the NO group of NOMb, and (3) the hydroperoxide decomposing and quenching action of MMb produced from dissociation of NOMb.

The behaviour of CCMP in a β-carotene/linoleate model system, as shown in Table A.1 changed from prooxidative to antioxidative, depending on its concentration. Therefore, the critical concentration of CCMP which determines its role in lipid oxidation, lies within the concentration range tested in this study. Kanner *et al.* (1979) reported a similar phenomenon for NOMb. At lower concentrations, the ability of CCMP to quench free radicals may be inadequate to inhibit lipid oxidation. However, CCMP at a concentration of 10 μM showed a strong antioxidative property and the effect exceeded that of NOMb (Figure 4.1). Shahidi *et al.* (1987) also reported an antioxidative effect for CCMP in meat model systems. These authors have observed a negative correlation between TBARS values and the addition level of CCMP. As is the case for NOMb, CCMP (nitrisylferrohaemochrome) also has its iron atom in the ferrous oxidation state and the high stability of CCMP does not permit the release of non-haem iron ions which are powerful prooxidants. According to Kanner *et al.* (1979), iron porphyrin nitric oxide compounds can act in the early stages of the reaction to neutralize substrate-free radicals and subsequent inhibition of lipid oxidation. They also suggested that the NO group may interact with free radicals leaving iron porphyrin in the system. In the case of CCMP, nitrosylhaemochrome-radical may quench free radicals in the system while haemin, an iron porphyrin, which was generated upon the dissociation of nitrosylferrohaemochrome may act as a hydroperoxide decomposer. Furthermore, haemin may also act as a free radical-quencher at lower concentrations. Being a chelator, haemin may act as an initiator or a terminator with the latter overshadowing the degree of initiation.

4.2 Effect of CCMP on lipid oxidation in the presence of sodium ascorbate and/or STPP

Table 4.2 shows the effect of CCMP (at 10 μM) on β-carotene bleaching in the presence of sodium ascorbate at 50, 100 and 550 ppm levels. CCMP exhibited even a stronger antioxidative effect in the presence of sodium ascorbate when compared to that of CCMP alone (Figure 4.2(A)). This is not surprising since sodium ascorbate alone exhibited a strong antioxidative effect, at all concentrations tested (Figure 4.2(B)). CCMP and sodium ascorbate may have then acted synergistically to protect β-carotene against oxidation. Shahidi *et al.* (1987) have suggested that sodium ascorbate and CCMP may retard lipid oxidation probably by keeping the haem pigment in its catalytically inactive state. Sato and Hegarti (1971) and Pearson *et al.* (1977) have envisaged that sodium ascorbate may upset the balance between Fe^{2+} and Fe^{3+} or may- act as an oxygen scavenger. Being a metal binder, ascorbate can bind any iron ions which may originate from disassociation of CCMP.

The effect of CCMP (10 μM) on β-carotene destruction in a β-carotene/linoleate model system containing STPP at 50, 100 and 500 ppm, is shown in Table 4.2 and Figure 4.3(A). Presence of STPP had a counter effect on the antioxidative effect of CCMP. Moreover, the antioxidative effect of CCMP decreased with increasing concentration of STPP. According to Tims and Watts (1958), STPP can inhibit lipid oxidation in meat model systems by sequestering metal ions, especially non-haem irons. However, according to Trout (1990), STPP can affect the rate of MMb formation in beef model systems. A low rate of MMb formation at pH 5.5 and a sharp increase in the rate

Table 4.2 Effect of cooked cured-meat pigment (CCMP), sodium ascorbate (SA), sodium tripolyphosphate (STPP) and their combinations on β-carotene destruction in a β-carotene/linoleate model system as reflected by cumulative loss of β-carotene (μg)[1].

Experiment No.	Treatment	Reaction Time, Min			
		1	2	3	4
1	Control (H_2O)	1.41±0.02[a]	1.80±0.07[a]	2.08±0.11[a]	2.41±0.20[a]
2	CCMP 10 μM	0.41±0.04[b]	0.49±0.01[b]	0.54±0.03[c]	0.57±0.06[c]
3	SA 50 ppm	0.39±0.08[b]	0.51±0.00[b]	0.59±0.04[c]	0.67±0.06[c]
4	SA 100 ppm	0.30±0.04[b]	0.39±0.04[b]	0.48±0.01[c]	0.56±0.02[c]
5	SA 550 ppm	0.27±0.09[b]	0.33±0.07[b]	0.37±0.11[c]	0.39±0.08[c]
6	STPP 50 ppm	0.39±0.08[b]	0.62±0.01[b]	0.86±0.04[b]	1.09±0.01[b]
7	STPP 100 ppm	0.51±0.07[b]	0.77±0.04[b]	1.01±0.01[b]	1.20±0.01[b]
8	STPP 500 ppm	1.25±0.20[a]	1.78±0.09[a]	2.19±0.08[a]	2.72±0.16[a]
9	2+3	0.19±0.02[b]	0.28±0.05[b]	0.36±0.04[c]	0.41±0.03[c]
10	2+4	0.27±0.05[b]	0.35±0.04[b]	0.43±0.03[c]	0.48±0.01[c]
11	2+5	0.27±0.06[b]	0.36±0.08[b]	0.47±0.02[c]	0.53±0.02[c]
12	2+6	0.40±0.04[b]	0.52±0.06[b]	0.60±0.07[c]	0.65±0.03[c]
13	2+7	0.55±0.03[b]	0.65±0.03[b]	0.76±0.05[b]	0.80±0.03[b]
14	2+8	0.64±0.02[b]	0.77±0.05[b]	0.82±0.06[b]	0.86±0.02[b]
15	2+3+6	0.31±0.10[b]	0.41±0.06[b]	0.47±0.04[c]	0.50±0.06[c]
16	2+4+7	0.36±0.05[b]	0.51±0.04[b]	0.59±0.04[c]	0.60±0.07[c]
17	2+5+8	0.47±0.04[b]	0.56±0.03[b]	0.63±0.08[c]	0.70±0.03[c]

[1] Results are mean values of three determinations ± standard deviation. Means sharing the same superscripts in a column are not significantly ($p<0.05$) different from one another.

Figure 4.2 Effect of cooked cured-meat pigment (CCMP) on β-carotene stability in a β-carotene/linoleate model system containing sodium ascorbate (SA), (A) and the effect of sodium ascorbate alone, (B). Error bars represent standard deviations from means of three determinations.

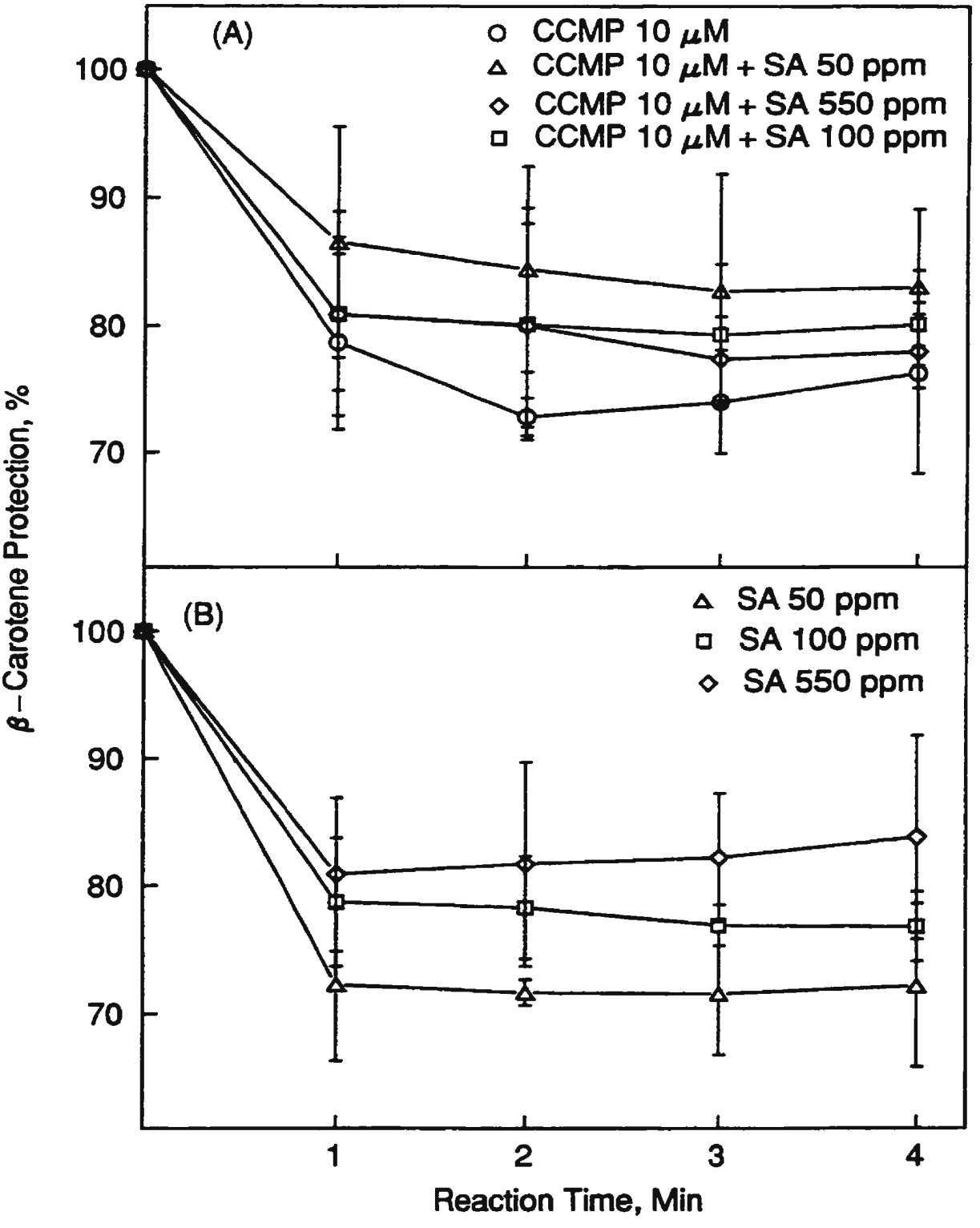
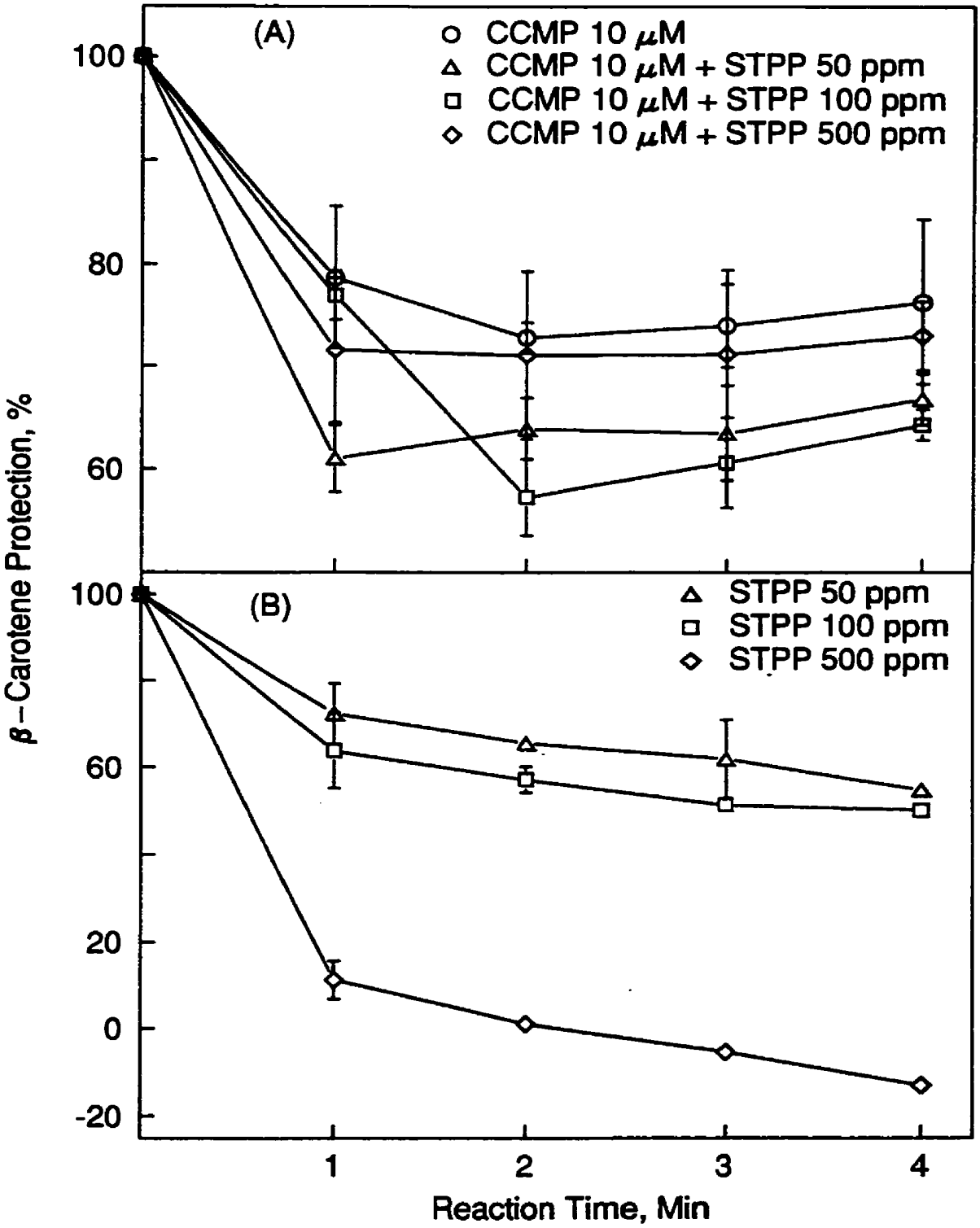

(A)
CCMP 10 μM
CCMP 10 μM + SA 50 ppm
CCMP 10 μM + SA 550 ppm
CCMP 10 μM + SA 100 ppm
(B)
SA 50 ppm
SA 100 ppm
SA 550 ppm
β−Carotene Protection, %
100
90
80
70
1
2
3
4
Reaction Time, Min

Figure 4.3 Effect of cooked cured-meat pigment (CCMP) on β-carotene stability in a β-carotene/linoleate model system containing sodium tripolyphosphate (STPP), (A) and the effect of STPP alone, (B). Error bars represent standard deviations from means of three determinations; where not shown, they were confined to the boundaries of symbols used.

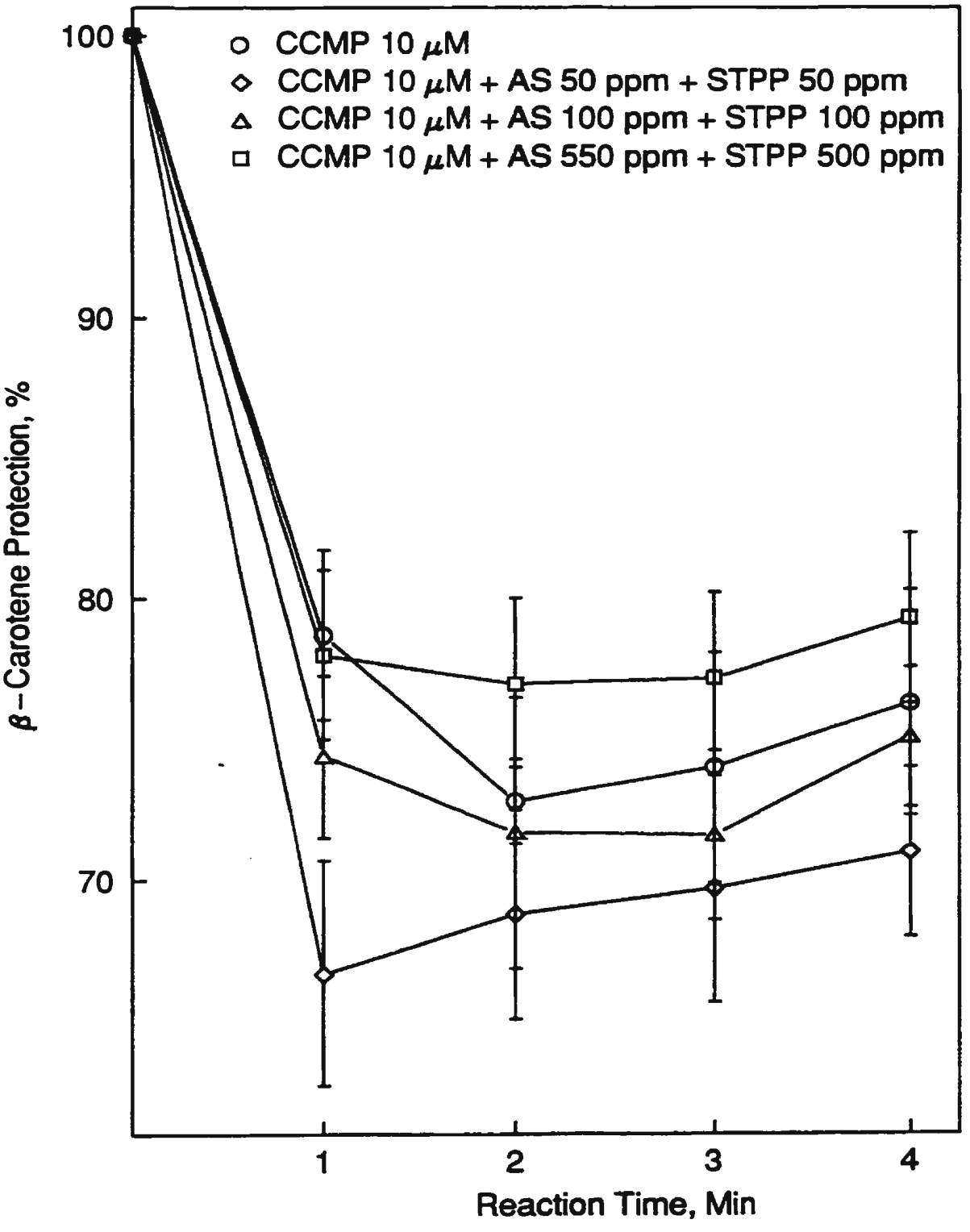

(A)
CCMP 10 μM
CCMP 10 μM + STPP 50 ppm
CCMP 10 μM + STPP 100 ppm
CCMP 10 μM + STPP 500 ppm
100
80
60
(B)
STPP 50 ppm
STPP 100 ppm
STPP 500 ppm
100
60
20
0
-20
β-Carotene Protection, %
1
2
3
4
Reaction Time, Min

between pH 5.5 and 6.5 were observed by this author. Based on these observations, it is suggested that the effect of CCMP is also pH-dependant. The iron (II) atom of CCMP may oxidize to the ferric form due to pH effects brought about by STPP. Since ferric haem ions are more powerful catalysts (Kanner and Harel, 1985), they may favour substrate free radical decomposition causing an increase in new free radical generation leading to enhanced β-carotene destruction.

At 500 ppm concentration, STPP alone showed a mild prooxidative effect while at 50 and 100 ppm showed an antioxidative effect (Table 4.2 and Figure 4.3(B)). At low concentrations, STPP may inhibit lipid oxidation by sequestering metal ions and at high concentration it may alter the pH buffering capacity of the reaction mixture to a point where free radical chain reactions are favoured causing a high β-carotene destruction which exceeds its sequestering effect.

Table 4.2 shows the effect of CCMP in the presence of both sodium ascorbate and STPP on β-carotene bleaching. As shown in Figure 4.4, CCMP in the presence of both sodium ascorbate and STPP, exhibited an antioxidative effect and the effect was not significantly ($p<0.05$) different from that of CCMP alone. This effect can be attributed to the antioxidative nature of CCMP backed up by sodium ascorbate but possibly counteracted by the presence of STPP. As discussed earlier, sodium ascorbate may act alone with CCMP to reduce the free radical concentration in the reaction mixture. Shahidi *et al.* (1987, 1988) have also reported a synergistic action between CCMP, sodium ascorbate and STPP towards inhibition of lipid oxidation in meat model systems.

Figure 4.4 Effect of cooked cured-meat pigment (CCMP) on β-carotene stability in a β-carotene/linoleate model system containing both sodium ascorbate (SA) and sodium tripolyphosphate (STPP). Error bars represent standard deviations from means of three determinations.


CCMP 10 μM
CCMP 10 μM + AS 50 ppm + STPP 50 ppm
CCMP 10 μM + AS 100 ppm + STPP 100 ppm
CCMP 10 μM + AS 550 ppm + STPP 500 ppm
100
90
80
70
β-Carotene Protection, %
1
2
3
4
Reaction Time, Min

4.3 Effect of anion and cation of different salts on lipid oxidation in a meat model system.

Tables 4.3 and 4.4 show the TBARS values of cooked comminuted pork (Moisture, crude protein, total lipid and ash contents were 71.84±0.20, 19.83±0.41, 7.16±0.01 and 1.13±0.30%, respectively) containing different salts at a concentration of 100 or 200 meq/kg meat. Both LiCl and NaCl, showed a prooxidative effect only at a concentration of 100 meq/kg meat, as reflected by % inhibition of TBARS formation (Figures 4.5 and 4.6). KCl, CsCl, $MgCl_2$, and $CaCl_2$ did not result in high TBARS values when compared to those of untreated controls (Tables 4.3 and 4.4).

Sodium chloride did not exhibit a prooxidative effect until the day-3 of the experiment (Figure 4.6(A)). Takiguchi (1989) have reported similar results for NaCl. According to this author the lower the NaCl content, the higher is the degree of lipid oxidation. Kanner *et al.* (1991) have also demonstrated the prooxidative effect of NaCl in a comminuted muscle system and suggested that NaCl may favour the displacement of iron ions from binding sites of haem compounds by disturbing the iron-protein interactions. The free iron ions so formed may catalyze lipid peroxidation. Kanner *et al.* (1988) have reported that the initiation of lipid peroxidation in turkey muscle tissue is enhanced by non-haem iron ions. Although no information on the role of LiCl in lipid oxidation is available, based on the literature data on NaCl, it is suggested that LiCl also acts in a manner similar to that of NaCl.

Table 4.3 TBARS values (mg malonaldehyde equivalents/kg meat) of cooked comminuted pork treated with different salts at a concentration of 100 meq/kg meat stored at 4°C[1].

Salt	Storage Period, Days				
	0	1	3	5	7
Control (No salt)	2.20±0.12^{c}	4.85±0.34^{b}	7.21±0.17^{c}	9.00±0.24^{b}	9.72±0.19^{d}
LiF	2.18±0.15^{cd}	4.44±0.12^{c}	7.64±0.10^{b}	7.73±0.02^{d}	8.98±0.07^{ef}
LiCl	2.24±0.03^{cd}	5.43±0.14^{a}	8.73±0.12^{a}	9.27±0.16^{b}	9.96±0.28^{d}
LiBr	2.48±0.14^{bc}	5.09±0.09^{b}	6.38±0.00^{de}	8.21±0.28^{c}	7.85±0.11^{g}
LiI	1.81±0.03^{e}	3.76±0.03^{e}	4.73±0.05^{i}	5.72±0.20^{f}	5.53±0.14^{k}
Li_2SO_4	2.30±0.04^{c}	5.00±0.25^{b}	7.19±0.38^{c}	7.38±0.49^{d}	8.32±0.27^{f}
NaF	1.10±0.04^{h}	2.61±0.00^{h}	5.15±0.01^{h}	5.46±0.00^{f}	5.79±0.65^{k}
NaCl	2.23±0.07^{cd}	4.21±0.05^{d}	7.13±0.11^{c}	10.92±0.12^{a}	8.84±0.14^{f}
NaBr	1.98±0.07^{e}	4.57±0.02^{c}	8.37±0.46^{b}	8.43±0.06^{c}	7.61±0.02^{g}
NaI	1.53±0.08^{f}	3.31±0.05^{f}	5.50±0.01^{f}	6.39±0.14^{e}	5.41±0.18^{k}
Na_2SO_4	1.94±0.02^{e}	4.53±0.10^{c}	7.07±0.02^{c}	8.81±0.26^{bc}	8.37±0.04^{f}
KF	1.32±0.26^{f}	0.72±0.05^{j}	1.04±0.08^{m}	1.49±0.00^{h}	1.36±0.01^{m}
KCl	2.86±0.15^{a}	4.97±0.07^{b}	6.26±0.01^{e}	8.70±0.36^{bc}	11.43±0.03^{a}
KBr	3.09±0.02^{a}	4.40±0.08^{c}	4.77±0.05^{i}	6.31±0.01^{e}	9.52±0.08^{de}
KI	2.35±0.04^{c}	2.78±0.20^{h}	4.62±0.00^{i}	4.37±0.02^{g}	4.93±0.01^{l}
K_2SO_4	3.15±0.02^{a}	4.41±0.12^{cd}	5.42±0.09^{f}	8.34±0.07^{c}	10.48±0.19^{c}

....continued on next page

..... continued from previous page.

CsF	1.41±0.06^{f}	2.58±0.02^{h}	4.23±0.07^{j}	5.40±0.12^{f}	6.29±0.00^{k}
CsCl	1.67±0.03^{f}	4.96±0.06^{b}	6.55±0.03^{d}	9.46±0.12^{b}	10.66±0.04^{bc}
CsBr	1.87±0.02^{e}	4.57±0.05^{c}	5.03±0.03^{h}	5.81±0.01^{e}	9.40±0.01^{d}
CsI	1.21±0.02^{g}	3.10±0.00^{g}	5.50±0.08^{f}	5.92±0.07^{e}	9.83±0.10^{d}
Cs_2SO_4	1.01±0.07^{h}	4.65±0.03^{c}	7.81±0.19^{b}	7.36±0.04^{d}	7.63±0.03^{g}
MgF_2	2.35±0.11^{c}	5.30±0.05^{a}	8.93±0.04^{a}	8.94±0.10^{b}	10.15±0.47^{bcd}
$MgCl_2$	2.05±0.02^{e}	4.95±0.18^{b}	7.38±0.12^{c}	8.10±0.26^{c}	10.97±0.13^{b}
$MgBr_2$	2.02±0.00^{e}	4.16±0.01^{d}	6.41±0.07^{de}	7.73±0.13^{d}	7.10±0.22^{h}
MgI_2	1.25±0.01^{g}	2.39±0.01^{f}	4.08±0.01^{j}	6.10±0.02^{e}	5.64±0.02^{k}
$MgSO_4$	2.02±0.00^{e}	5.23±0.01^{a}	8.74±0.04^{a}	8.51±0.30^{c}	7.58±0.18^{gh}
CaF_2	2.19±0.01^{d}	3.73±0.02^{e}	5.31±0.03^{g}	7.74±0.02^{d}	7.61±0.14^{g}
$CaCl_2$	2.54±0.05^{b}	5.05±0.01^{b}	7.18±0.04^{c}	8.85±0.15^{bc}	8.88±0.46^{fed}
$CaBr_2$	2.64±0.00^{b}	5.16±0.01^{a}	7.25±0.17^{c}	8.83±0.01^{c}	8.40±0.17^{f}
CaI_2	1.61±0.00^{f}	1.96±0.01^{i}	2.11±0.01^{i}	0.84±0.09^{i}	0.92±0.05^{n}
$CaSO_4$	2.62±0.02^{b}	2.77±0.03^{h}	3.76±0.05^{k}	5.27±0.04^{f}	5.87±0.02^{j}

[1] Results are mean values of three determinations ± standard deviation. Means sharing the same superscripts in a column are not significantly ($p<0.05$) different from one another.

Table 4.4 TBARS values (mg malonaldehyde equivalents/kg meat) of cooked comminuted pork treated with different salts at a concentration of 200 meq/kg meat stored at 4°C[1].

Salt	Storage Period, Days				
	0	1	3	5	7
Control (No salt)	2.20±0.12^{bc}	4.85±0.34^{c}	7.21±0.07^{b}	9.00±0.24^{ab}	9.72±0.19^{cd}
LiF	1.55±0.09^{e}	4.21±0.05^{e}	6.45±0.03^{d}	6.76±0.14^{f}	7.59±0.52^{h}
LiCl	2.68±0.05^{a}	5.32±0.01^{b}	7.13±0.07^{b}	7.51±0.31^{de}	7.45±0.20^{h}
LiBr	2.22±0.17^{bcd}	5.18±0.02^{b}	6.89±0.01^{c}	7.76±0.32^{de}	8.08±0.22^{fg}
LiI	1.04±0.02^{f}	2.19±0.05^{i}	3.54±0.08^{h}	4.06±0.20^{j}	3.82±0.02^{m}
Li_2SO_4	2.21±0.02^{bc}	4.65±0.04^{d}	7.75±0.06^{a}	7.41±0.80^{def}	8.62±0.22^{fg}
NaF	0.24±0.02^{i}	0.35±0.00^{m}	0.44±0.03^{m}	1.28±0.09^{e}	1.15±0.01^{o}
NaCl	2.01±0.06^{d}	4.14±0.01^{ef}	7.00±0.04^{bc}	6.91±0.10^{ef}	7.80±0.11^{h}
NaBr	2.39±0.01^{b}	4.35±0.01^{e}	7.84±0.39^{a}	8.10±0.45^{cd}	8.68±0.13^{f}
NaI	1.04±0.04^{f}	2.67±0.04^{h}	4.92±0.04^{f}	5.30±0.48^{h}	5.26±0.05^{l}
Na_2SO_4	2.09±0.01^{d}	4.31±0.09^{e}	6.42±0.03^{d}	8.10±0.06^{d}	10.15±0.04^{c}
KF	1.16±0.00^{f}	0.41±0.01^{m}	0.57±0.14^{ml}	0.49±0.03^{n}	0.30±0.02^{p}
KCl	2.29±0.07^{bc}	4.12±0.18^{ef}	4.60±0.08^{g}	6.89±0.06^{de}	7.87±0.01^{f}
KBr	2.84±0.18^{a}	4.01±0.08^{ef}	5.19±0.01^{f}	6.03±0.08^{g}	9.51±0.01^{e}
KI	2.01±0.02^{d}	2.01±0.01^{j}	2.50±0.04^{j}	2.97±0.13^{k}	5.13±0.19^{l}
K_2SO_4	2.16±0.00^{bc}	4.42±0.12^{de}	5.39±0.02^{f}	6.82±0.03^{de}	10.88±0.07^{b}

....continued on next page

.....continued from previous page.

CsF	0.41±0.06^{h}	0.36±0.03^{m}	0.99±0.01^{l}	1.00±0.05^{lm}	1.36±0.01^{o}
CsCl	1.52±0.06^{e}	3.61±0.14^{fg}	6.04±0.01^{e}	6.63±0.05^{f}	11.28±0.01^{a}
CsBr	1.66±0.00^{e}	5.05±0.07^{bc}	7.51±0.24^{a}	8.98±0.14^{b}	11.37±0.02^{a}
CsI	0.66±0.02^{gh}	1.71±0.07^{k}	3.12±0.01^{i}	4.56±0.01^{j}	6.36±0.10^{j}
Cs_2SO_4	1.17±0.01^{f}	3.94±0.14^{f}	6.83±0.14^{d}	7.81±0.04^{d}	7.80±0.04^{h}
MgF_2	2.27±0.08^{bc}	5.24±0.06^{b}	7.90±0.01^{a}	9.52±0.17^{a}	9.71±0.08^{d}
$MgCl_2$	2.04±0.01^{d}	4.87±0.03^{c}	6.24±0.03^{e}	6.96±0.00^{e}	7.65±0.33^{h}
$MgBr_2$	2.07±0.02^{d}	3.37±0.07^{g}	5.11±0.05^{f}	6.19±0.03^{g}	6.78±0.04^{i}
MgI_2	0.70±0.06^{g}	2.14±0.10^{ij}	1.72±0.00^{k}	3.02±0.07^{k}	3.25±0.12^{n}
$MgSO_4$	2.79±0.04^{a}	5.14±0.07^{b}	6.58±0.04^{d}	7.75±0.22^{d}	7.14±0.26^{h}
CaF_2	2.31±0.09^{bc}	5.78±0.12^{a}	7.16±0.03^{b}	8.81±0.19^{b}	8.62±0.29^{fg}
$CaCl_2$	2.49±0.05^{b}	5.23±0.01^{b}	7.70±0.01^{a}	8.49±0.02^{c}	8.14±0.12^{g}
$CaBr_2$	2.72±0.05^{a}	5.05±0.02^{bc}	7.87±0.08^{a}	8.48±0.03^{c}	8.88±0.08^{f}
CaI_2	0.38±0.03^{h}	0.80±0.01^{l}	0.81±0.05^{l}	0.84±0.01^{m}	1.24±0.02^{o}
$CaSO_4$	2.14±0.00^{c}	2.75±0.04^{h}	3.93±0.06^{g}	5.56±0.05^{h}	5.80±0.11^{k}

[1] Results are mean values of three determinations ± standard deviation. Means sharing the same superscripts in a column are not significantly ($p<0.05$) different from one another.

Figure 4.5 Effect of LiF, LiCl, LiBr, LiI and Li_2SO_4, at a concentration of 100, (A) or 200, (B) meq/kg sample, on lipid oxidation as reflected by % inhibition of TBARS formation in cooked comminuted pork stored at 4 °C. Error bars represent standard deviations from means of three determinations; where not shown, they were confined to the boundaries of symbols used.

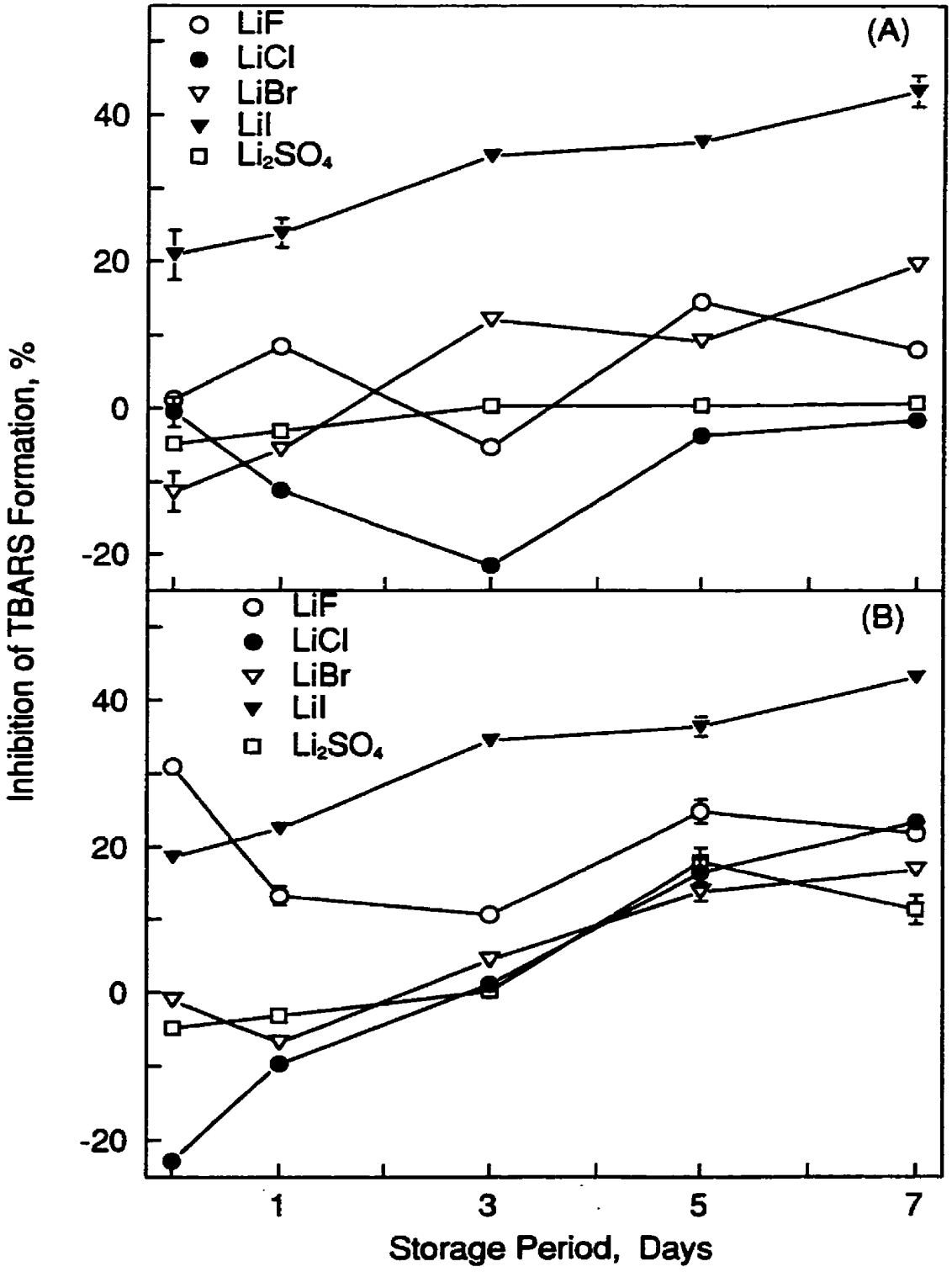
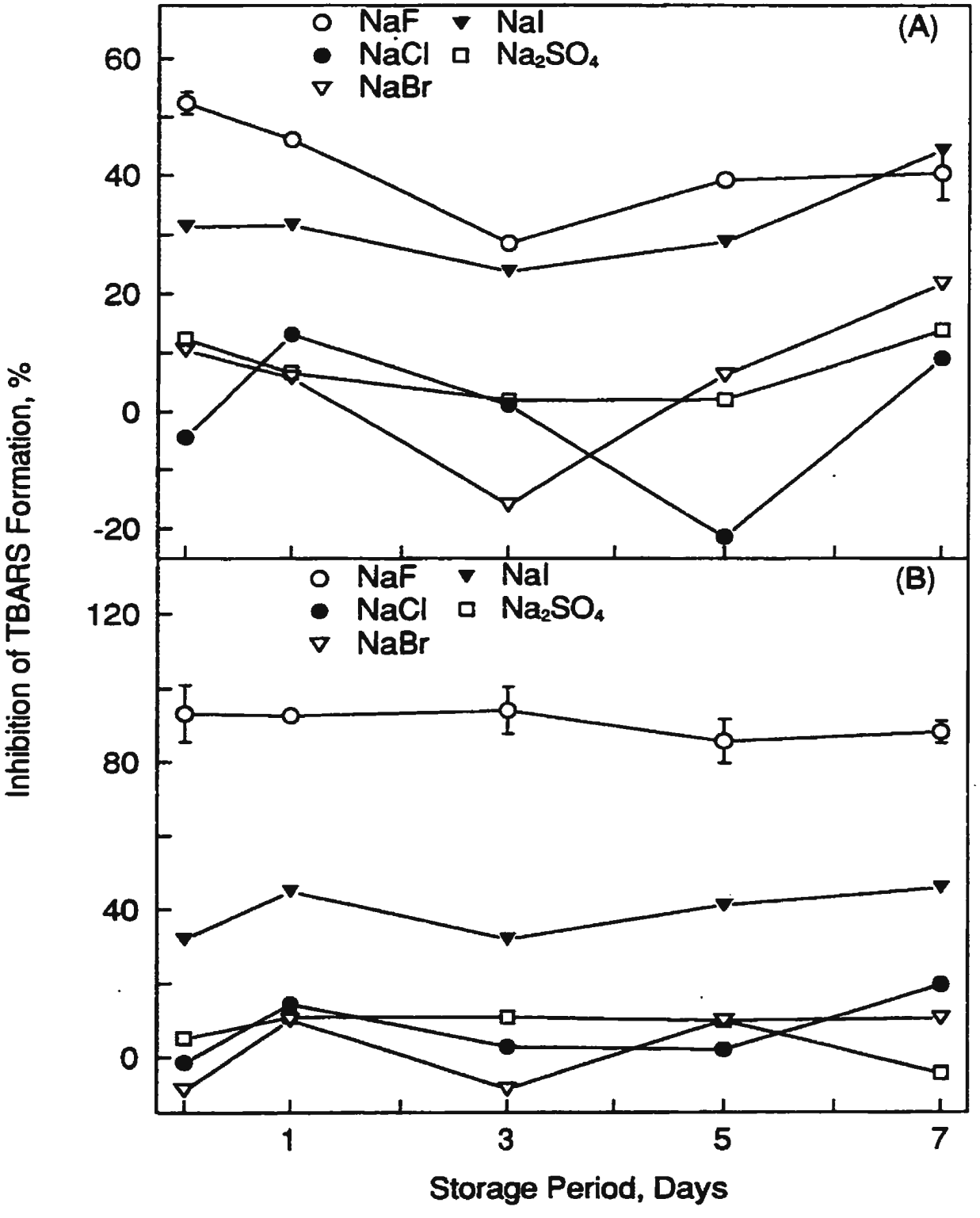

LiF
LiCl
LiBr
LiI
Li2SO4
(A)
(B)
40
20
0
-20
1
3
5
7
Inhibition of TBARS Formation, %
Storage Period, Days

Figure 4.6 Effect of NaF, NaCl, NaBr, NaI and Na_2SO_4, at a concentration of 100, (A) or 200, (B) meq/kg sample, on lipid oxidation as reflected by % inhibition of TBARS formation in cooked comminuted pork stored at 4 °C. Error bars represent standard deviations from means of three determinations; where not shown, they were confined to the boundaries of symbols used.


NaF
NaCl
NaBr
NaI
Na2SO4
(A)
(B)
60
40
20
0
-20
120
80
40
0
1
3
5
7
Inhibition of TBARS Formation, %
Storage Period, Days

All iodide salts tested inhibited the formation of TBARS at both 100 and 200 meq/kg levels, but the effect was more pronounced at the higher concentration (Figures 4.5, 4.6, 4.7, 4.8, 4.9 and 4.10). Osinchak *et al.* (1992) reported a similar effect for iodides on lipid oxidation in a phosphotidylcholine liposome model system. According to these authors, iodides may retard lipid oxidation by blocking free radical chain reactions and by preventing ferrous ions from oxidation. Moreover, iodide is a reducing agent towards ferric ions which are considered by some investigators as being more powerful than ferrous ions in free radical chain reaction initiation (Kanner and Harel, 1985; Kanner *et al.*, 1987). Furthermore, the iodine formed upon reaction with ferric ions, either free or bound to an organic substrate, is a good free radical scavenger. In addition, the weak C-I bond in organic iodides allows an alternative pathway for radical-initiated oxidative addition reactions which could have an antioxidative effect (Osinchak *et al.*, 1992).

Treatment of meat systems with NaF, KF and CsF resulted in low TBARS values at both concentrations compared to those of the control (Tables 4.3 and 4.4). Addition of the above salts to meat at a concentration of 200 meq/kg sample afforded much lower TBARS values as compared to the samples treated with 100 meq/kg sample. As shown in Figure 4.10, $CaSO_4$ also exhibited an inhibitory effect on lipid oxidation. Therefore, fluoride anion in NaF, KF, and CsF as well as sulphate anion of $CaSO_4$ may retard lipid oxidation by tightly binding any ferrous ion present in the system, thus eliminating the prooxidant activity of ferrous ion or preventing its oxidation to ferric ion.

Figure 4.7 Effect of KF, KCl, KBr, KI and K_2SO_4, at a concentration of 100, (A) or 200, (B) meq/kg sample, on lipid oxidation as reflected by % inhibition of TBARS formation in cooked comminuted pork stored at 4 °C. Error bars represent standard deviations from means of three determinations; where not shown, they were confined to the boundaries of symbols used.

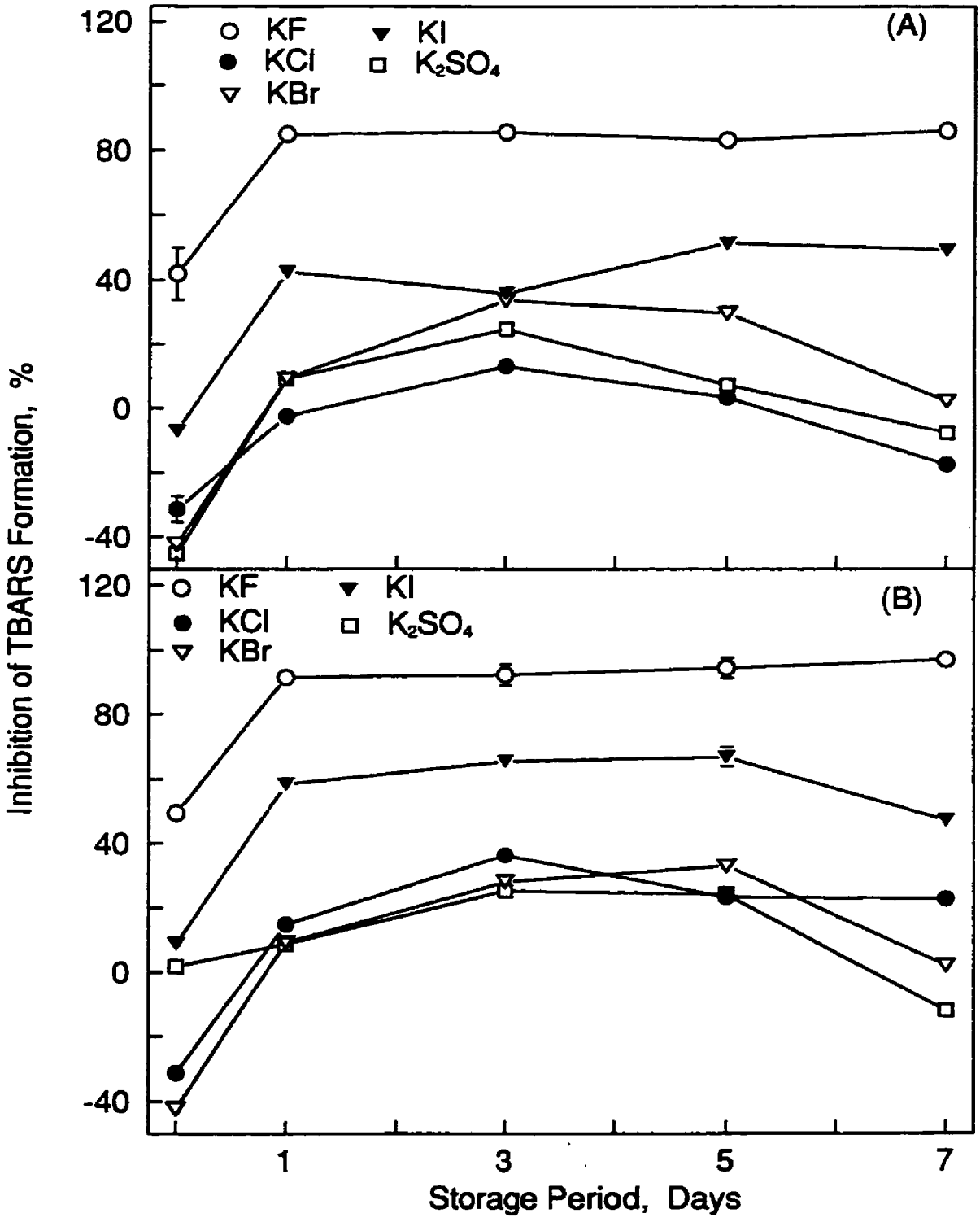

120
80
40
0
-40
KF
KCl
KBr
KI
K_2SO_4
(A)
(B)
Inhibition of TBARS Formation, %
1
3
5
7
Storage Period, Days

Figure 4.8 Effect of CsF, CsCl, CsBr, CsI and Cs_2SO_4, at a concentration of 100, (A) or 200, (B) meq/kg sample, on lipid oxidation as reflected by % inhibition of TBARS formation in cooked comminuted pork stored at 4 °C. Error bars represent standard deviations from means of three determinations; where not shown, they were confined to the boundaries of symbols used.

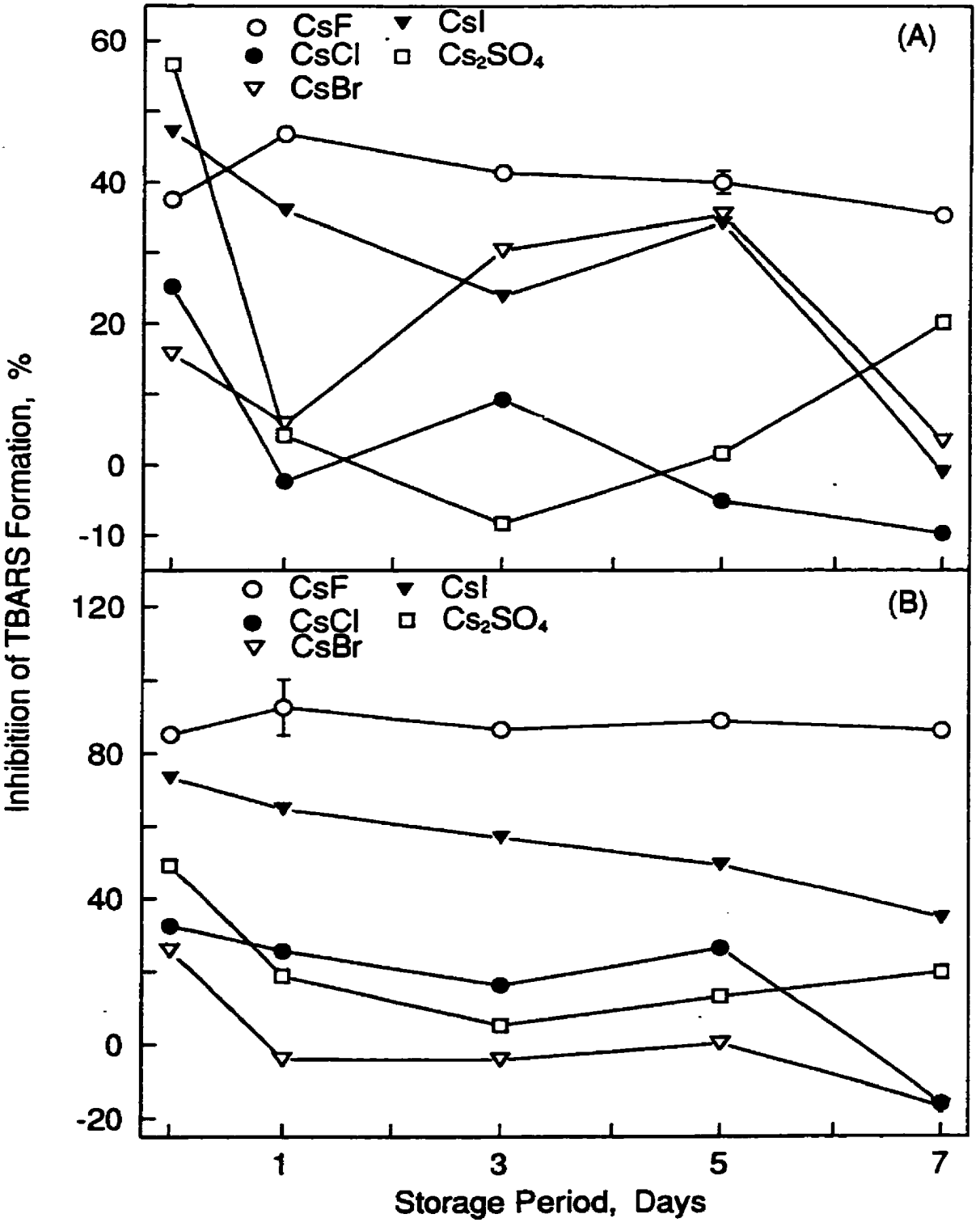

CsF
CsCl
CsBr
CsI
Cs_2SO_4
(A)
(B)
60
40
20
0
-10
120
80
40
0
-20
1
3
5
7
Inhibition of TBARS Formation, %
Storage Period, Days

Figure 4.9 Effect of MgF_2, $MgCl_2$, $MgBr_2$, Mg_2I and $MgSO_4$, at a concentration of 100, (A) or 200, (B) meq/kg sample, on lipid oxidation as reflected by % inhibition of TBARS formation in cooked comminuted pork stored at 4 °C. Error bars represent standard deviations from means of three determinations; where not shown, they were confined to the boundaries of symbols used.

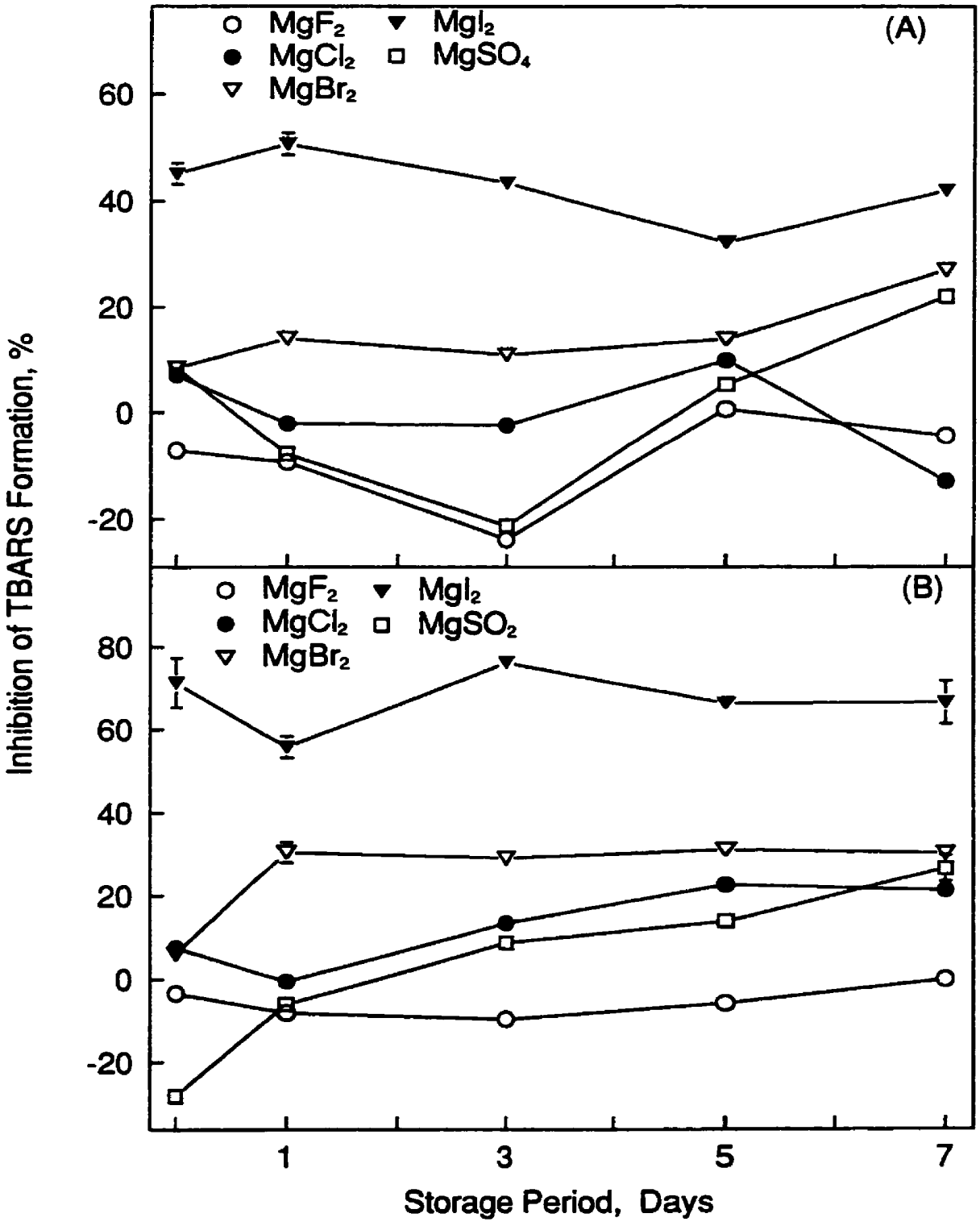
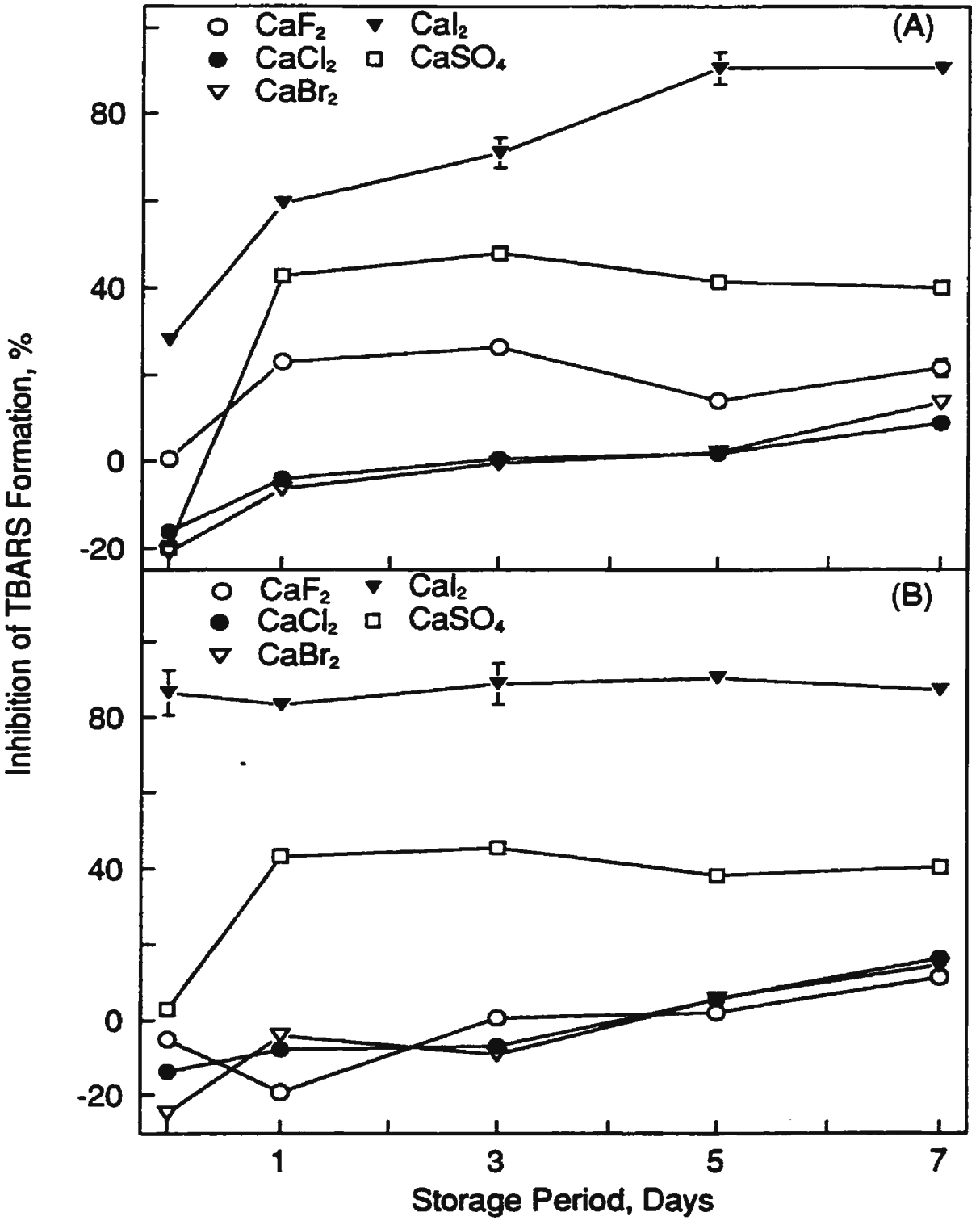

MgF2
MgCl2
MgBr2
MgI2
MgSO4
(A)
60
40
20
0
-20
MgF2
MgCl2
MgBr2
MgI2
MgSO2
(B)
80
60
40
20
0
-20
1
3
5
7
Storage Period, Days
Inhibition of TBARS Formation, %

Figure 4.10 Effect of CaF_2, $CaCl_2$, $CaBr_2$, CaI_2 and $CaSO_4$, at a concentration of 100, (A) or 200, (B) meq/kg sample, on lipid oxidation as reflected by % inhibition of TBARS formation in cooked comminuted pork stored at 4 °C. Error bars represent standard deviations from means of three determinations; where not shown, they were confined to the boundaries of symbols used.


CaF2
CaCl2
CaBr2
CaI2
CaSO4
(A)
(B)
80
40
0
-20
1
3
5
7
Inhibition of TBARS Formation, %
Storage Period, Days

The fluoride ion is known to inhibit the activity of numerous glycolytic enzymes such as enolase, succinic dehydrogenase, phosphoglucomutase, phosphatases and acetylcholine esterase (Newbrun, 1986) which are known to catalyze free radical initiation reactions (Kanner *et al.*, 1987). Fluoride ion can replace a hydrogen in an enzyme molecule which is involved in enzymatic transfer and irreversibly complex and block the normal function of the enzyme (Sheppard and Sharts, 1969). The inhibitory effect of fluoride ion on glycolytic enzymes may play an important role in lowering the TBARS formation in meat as evidenced by the present study. Furthermore, the strong inhibition of TBARS formation observed immediately after thermal processing suggests that the action of the fluoride ion had started from the point of its incorporation into meat. However, fluoride ion, in the presence of divalent cations such as magnesium and calcium, no longer exhibited any inhibitory effect on TBARS formation. This may be due to the strong pairing of fluoride ion with divalent cations which renders them unavailable for participation in reactions and/or interactions explained above.

The overall effect of halide and sulphate salts of alkali metals as well as magnesium and calcium, in a complex system like meat, may be a result of many interactions which are at the moment poorly understood.

Selected iodide and fluoride salts were also tested for their effect on bleaching of β-carotene in a β-carotene/linoleate model system (Tables 4.5 and 4.6). BHA, an antioxidant, and $FeSO_4$, a prooxidant were used for comparative purposes. The iodide and fluoride salts were antioxidative compared to the control containing deionized water.

Table 4.5 Effect of BHA, $FeSO_4$, NaCl, NaF, KF, CsF, CaF_2, NaI, KI, CsI and CaI_2, at a concentration of 10 ppm, on lipid oxidation as reflected by cumulative loss of β-carotene (μg) in a β-carotene/linoleate model system[1].

Salt	Reaction Time, Min			
	1	2	3	4
Control (H_2O)	1.41±0.02[a]	1.80±0.07[a]	2.08±0.11[a]	2.41±0.20[a]
BHA	0.15±0.06[d]	0.18±0.04[d]	0.19±0.03[d]	0.25±0.04[d]
$FeSO_4$	1.18±0.06[a]	1.71±0.13[a]	2.09±0.18[a]	2.48±0.19[a]
NaCl	0.62±0.12[b]	0.86±0.02[b]	1.06±0.03[b]	1.38±0.08[b]
NaF	0.26±0.06[c]	0.48±0.10[c]	0.75±0.06[c]	1.13±0.10[b]
KF	0.48±0.05[c]	0.63±0.06[c]	0.77±0.15[c]	0.89±0.04[c]
CsF	0.18±0.03[d]	0.35±0.02[c]	0.46±0.05[c]	0.68±0.06[c]
CaF_2	0.48±0.01[c]	0.74±0.05[c]	0.96±0.02[b]	1.10±0.01[c]
NaI	0.36±0.01[c]	0.64±0.04[c]	0.95±0.10[b]	1.24±0.09[b]
KI	0.42±0.03[c]	0.61±0.08[c]	0.74±0.07[c]	0.82±0.05[c]
CsI	0.54±0.10[c]	0.68±0.22[c]	1.07±0.11[b]	1.34±0.06[b]
CaI_2	0.78±0.03[b]	1.07±0.06[b]	1.38±0.05[b]	1.62±0.07[b]

[1] Results are mean values of three determinations ± standard deviation. Means sharing the same superscript in a column are not significantly ($p<0.05$) different from one another.

Table 4.6 Effect of BHA, $FeSO_4$, NaCl, NaF, KF, CsF, CaF_2, NaI, KI, CsI and CaI_2, at a concentration of 100 ppm, on lipid oxidation as reflected by cumulative loss of β-carotene (μg) in a β-carotene/linoleate model system[1].

Salt	Reaction Time, Min			
	1	2	3	4
Control (H_2O)	1.41±0.02[b]	1.80±0.07[b]	2.08±0.11[b]	2.41±0.20[b]
BHA	0.01±0.00[d]	0.05±0.01[d]	0.07±0.00[d]	0.09±0.01[d]
$FeSO_4$	2.77±0.17[a]	3.96±0.28[a]	4.99±0.60[a]	5.70±0.51[a]
NaCl	0.46±0.04[c]	0.71±0.03[c]	0.97±0.02[c]	1.35±0.02[c]
NaF	0.40±0.07[c]	0.67±0.13[c]	0.98±0.04[c]	1.15±0.10[c]
KF	0.27±0.02[c]	0.44±0.10[c]	0.59±0.05[c]	0.71±0.09[c]
CsF	0.15±0.03[c]	0.27±0.08[c]	0.52±0.05[c]	0.63±0.08[c]
CaF_2	0.30±0.01[c]	0.55±0.08[c]	0.65±0.09[c]	1.01±0.16[c]
NaI	0.36±0.11[c]	0.56±0.08[c]	0.74±0.06[c]	0.93±0.05[c]
KI	0.17±0.04[c]	0.34±0.06[c]	0.47±0.03[c]	0.58±0.01[c]
CsI	0.28±0.04[c]	0.33±0.06[c]	0.49±0.03[c]	0.58±0.06[c]
CaI_2	0.24±0.05[c]	0.40±0.01[c]	0.63±0.03[c]	0.74±0.04[c]

[1] Results are mean values of three determinations ± standard deviation. Means sharing the same superscript in a column are not significantly ($p<0.05$) different from one another.

The effect of iodide was concentration dependent and was more pronounced at 100 ppm addition level, similar to that observed in meat model systems. The effect of fluoride salts was independent of the concentrations tested. As expected, BHA exhibited a very strong antioxidant effect while $FeSO_4$ exhibited a prooxidant effect resulting in extensive bleaching of β-carotene destruction. The chemical energy generated upon oxidation/reduction reaction (Redox reaction) between ferrous and ferric forms may provide sufficient energy to accelerate free radical initiation reactions. Therefore, the behaviour of salts exhibiting antioxidative effects in a meat model system, may or may not correspond with their influence in an emulsion model system such as that of β-carotene/linoleate.

4.4 Effect of fluoride and iodide salts on volatile formation in cooked comminuted pork.

Cooked comminuted pork samples containing different salts were analyzed for their content of volatile off-flavour compounds over a 7-day storage period. Results for day 0, day 3 and day 7 are shown in Tables 4.7, 4.8, 4.9, 4.10, 4.11 and 4.12 and Figures 4.11, and 4.12 show the chromatograms of headspace volatiles of control and CaI_2 treated samples after 3 days of storage at 4°C. Of the identified volatiles, hexanal was the most prominent one. A highly significant correlation (r=0.9263) existed between hexanal content and TBARS values of cooked comminuted pork (Figure 4.13). Hexanal may be formed (1) from 13-hydroperoxide of linoleic acid through homolytic cleavage

Table 4.7 Volatile composition of cooked comminuted pork, containing different salts at a concentration of 100 meq/kg meat, before storage.

Salt	Volatile Content, mg/kg Meat								
	Ace	Pro	*i*-But	But	Pen	Hex	Hep	Oct	Hexdi
Control (No salt)	0.7	0.9	0.5	0.1	2.0	10.0	0.4	0.2	0.1
LiF	1.8	1.2	0.3	0.2	2.1	9.5	0.5	0.3	0.1
NaF	0.9	0.2	0.7	0.1	1.0	5.2	0.3	0.1	0.1
KF	1.5	0.2	0.5	0.0	0.6	1.7	0.1	0.1	0.0
CsF	2.0	0.8	0.3	0.1	1.4	6.8	0.4	0.2	0.1
MgF_2	1.8	1.0	0.3	0.1	1.7	7.8	0.3	0.2	0.1
CaF_2	3.1	1.1	0.2	0.2	2.2	9.6	0.6	0.3	0.1
LiI	1.4	0.6	0.1	0.1	1.0	5.5	0.3	0.2	0.1
NaI	1.6	0.9	0.2	1.0	1.5	7.1	0.5	0.3	0.1
KI	2.4	1.0	0.4	0.1	0.3	8.1	0.6	0.3	0.1
CsI	2.7	1.5	0.3	0.3	2.3	9.3	0.7	0.4	0.1
MgI_2	1.1	0.9	0.3	0.2	1.2	5.7	0.4	0.2	0.1
CaI_2	0.2	0.0	0.2	0.0	0.0	0.2	0.1	0.0	0.0

(Ace: Acetaldehyde, Pro: Propanal, *i*-But: *iso*-Butanal, But: Butanal, Pen: Pentanal, Hex: Hexanal, Hep: Heptanal, Oct: Octanal and Hexdi: Hexadienal).

Table 4.8 Volatile composition of cooked comminuted pork, containing different salts at a concentration of 200 meq/kg meat, before storage.

Salt	Volatile Content, mg/kg Meat								
	Ace	Pro	*i*-But	But	Pen	Hex	Hep	Oct	Hexdi
Control (No salt)	0.7	0.9	0.5	0.1	2.0	10.0	0.4	0.2	0.1
LiF	1.8	1.0	0.2	0.1	1.7	8.6	0.4	0.2	0.1
NaF	0.1	0.0	0.1	0.0	0.1	0.3	0.0	0.0	0.0
KF	1.4	0.0	0.7	0.0	0.0	0.1	0.0	0.0	0.0
CsF	0.6	0.0	0.2	0.0	0.0	0.1	0.0	0.0	0.1
MgF_2	1.8	1.0	1.8	0.1	1.5	7.3	0.3	0.1	0.1
CaF_2	2.7	1.0	0.2	0.1	2.3	9.2	0.7	0.3	0.1
LiI	1.5	0.7	0.2	0.1	0.9	4.9	0.3	0.2	0.1
NaI	1.3	1.0	0.2	0.1	1.0	5.9	0.4	0.2	0.1
KI	1.6	0.8	0.4	0.1	1.2	5.8	0.4	0.2	0.7
CsI	2.1	1.1	0.2	0.1	1.4	6.5	0.5	0.3	0.1
MgI_2	1.0	0.5	0.3	0.0	0.5	2.5	0.2	0.1	0.1
CaI_2	0.1	0.0	0.1	0.0	0.0	0.2	0.2	0.0	0.0

(Ace: Acetaldehyde, Pro: Propanal, *i*-But: *iso*-Butanal, But: Butanal, Pen: Pentanal, Hex: Hexanal, Hep: Heptanal, Oct: Octanal and Hexdi: Hexadienal).

Table 4.9 Volatile composition of cooked comminuted pork, containing different salts at a concentration of 100 meq/kg meat, after three days of storage at 4°C.

Salt	Volatile Content, mg/kg Meat								
	Ace	Pro	*i*-But	But	Pen	Hex	Hep	Oct	Hexdi
Control (No salt)	2.6	2.2	0.4	0.2	2.8	19.4	0.6	0.3	0.1
LiF	1.6	2.2	0.4	0.2	2.4	17.6	0.6	0.3	0.2
NaF	1.1	1.7	0.3	0.2	1.8	11.8	0.5	0.2	0.1
KF	1.2	0.6	0.4	0.1	0.7	3.6	0.2	0.2	0.2
CsF	1.0	1.4	0.3	0.0	1.2	9.2	0.3	0.2	0.1
MgF_2	1.7	2.7	0.4	0.2	2.3	14.8	0.4	0.1	0.1
CaF_2	1.7	2.2	0.4	0.2	2.2	13.8	0.4	0.3	0.1
LiI	1.2	1.5	0.4	0.2	1.8	11.4	0.6	0.3	0.1
NaI	1.5	2.0	0.3	0.2	2.2	12.7	0.7	0.1	0.1
KI	0.9	1.0	0.3	0.1	1.3	8.2	0.4	0.2	0.2
CsI	1.0	2.1	0.3	0.3	1.5	9.8	0.4	0.2	0.1
MgI_2	1.0	2.8	0.3	0.2	2.0	15.0	0.5	0.2	0.1
CaI_2	0.3	0.2	0.3	0.0	0.3	1.5	0.1	0.1	0.0

(Ace: Acetaldehyde, Pro: Propanal, *i*-But: *iso*-Butanal, But: Butanal, Pen: Pentanal, Hex: Hexanal, Hep: Heptanal, Oct: Octanal and Hexdi: Hexadienal).

Table 4.10 Volatile composition of cooked comminuted pork, containing different salts at a concentration of 200 meq/kg meat, after three days of storage at 4°C.

Salt	Volatile Content, mg/kg Meat								
	Ace	Pro	*i*-But	But	Pen	Hex	Hep	Oct	Hexdi
Control (No salt)	2.6	2.2	0.4	0.2	2.8	19.4	0.6	0.3	0.2
LiF	1.6	1.7	0.4	0.2	1.9	14.9	0.5	0.2	0.1
NaF	0.5	0.2	0.2	0.0	0.1	0.7	0.1	0.0	0.0
KF	0.3	0.0	0.2	0.0	0.1	0.4	0.0	0.0	0.0
CsF	0.3	0.1	0.2	0.0	0.1	0.2	0.0	0.0	0.0
MgF_2	1.6	2.6	0.3	2.1	0.3	13.8	0.4	0.1	0.1
CaF_2	1.7	2.4	0.3	0.2	2.5	16.9	0.5	0.2	0.1
LiI	1.4	1.5	0.3	0.2	1.8	11.8	0.5	0.2	0.1
NaI	1.2	2.0	0.3	0.2	1.9	11.5	0.7	0.3	0.1
KI	0.8	1.2	0.3	0.1	1.2	7.2	0.4	0.2	0.1
CsI	1.2	2.1	0.4	0.1	1.5	8.4	0.4	0.2	0.1
MgI_2	0.5	1.1	0.3	0.1	0.8	5.2	0.3	0.1	0.0
CaI_2	0.2	0.2	0.2	0.0	0.1	1.5	0.1	0.0	0.0

(Ace: Acetaldehyde, Pro: Propanal, *i*-But: *iso*-Butanal, But; Butanal, Pen: Pentanal, Hex: Hexanal, Hep: Heptanal, Oct: Octanal and Hexdi: Hexadienal).

Table 4.11 Volatile composition of cooked comminuted pork, containing different salts at a concentration of 100 meq/kg meat, after seven days of storage at 4°C.

Salt	Volatile Content, mg/kg Meat								
	Ace	Pro	*i*-But	But	Pen	Hex	Hep	Oct	Hexdi
Control (No salt)	4.0	2.8	3.0	0.3	3.8	32.0	1.1	0.6	0.3
LiF	3.0	3.8	4.0	0.3	3.8	30.9	1.3	0.6	0.3
NaF	2.0	3.5	3.5	0.3	0.2	22.0	1.1	0.5	0.2
KF	3.0	1.9	2.0	0.2	2.6	13.4	0.8	0.5	1.9
CsF	3.0	3.6	3.2	0.0	3.7	23.5	1.1	0.5	0.2
MgF_2	3.5	5.8	4.0	0.0	4.7	32.3	1.2	0.1	0.2
CaF_2	3.4	4.7	4.7	0.0	0.1	29.2	1.2	0.1	0.2
LiI	2.1	2.7	2.7	0.3	3.2	20.2	1.3	0.7	0.2
NaI	2.3	3.6	3.6	0.4	4.3	27.7	1.7	0.9	0.2
KI	2.0	2.9	3.1	0.3	3.8	23.6	1.4	0.7	0.2
CsI	2.0	5.1	4.9	0.5	4.8	29.2	0.3	0.7	0.3
MgI_2	2.1	4.7	4.6	0.0	4.8	30.6	1.5	0.1	0.3
CaI_2	0.6	1.2	5.1	0.0	1.5	7.4	0.7	0.1	0.1

(Ace: Acetaldehyde, Pro: Propanal, *i*-But: *iso*-Butanal, But: Butanal, Pen: Pentanal, Hex: Hexanal, Hep: Heptanal, Oct: Octanal and Hexdi: Hexadienal).

Table 4.12 Volatile composition of cooked comminuted pork, containing different salts at a concentration of 200 meq/kg meat, after seven days of storage at 4°C.

Salt	Volatile Content, mg/kg Meat								
	Ace	Pro	*i*-But	But	Pen	Hex	Hep	Oct	Hexdi
Control (No salt)	4.0	2.8	3.0	0.3	3.8	32.0	1.1	0.6	0.1
LiF	1.1	3.9	3.9	0.4	5.1	30.0	1.6	0.8	0.1
NaF	1.0	0.0	3.7	0.0	0.0	2.5	0.0	0.0	0.0
KF	1.0	0.3	0.3	0.0	0.3	0.6	0.1	0.1	0.0
CsF	1.9	0.0	1.9	0.0	0.2	1.3	0.0	0.0	0.0
MgF_2	1.3	3.6	4.3	0.0	2.8	27.0	1.1	0.1	0.1
CaF_2	3.6	4.5	5.4	0.0	4.7	33.9	1.4	0.1	0.1
LiI	1.7	2.0	2.0	0.2	2.7	19.2	1.2	0.6	0.1
NaI	1.9	4.2	4.2	0.4	4.1	26.9	0.6	1.7	0.1
KI	1.6	2.3	2.3	0.2	2.6	17.9	1.1	0.6	0.7
CsI	2.0	4.7	4.7	0.4	4.2	25.3	1.4	0.1	0.1
MgI_2	4.3	2.8	4.1	0.0	3.7	17.0	1.0	0.1	0.0
CaI_2	0.6	0.9	5.3	0.0	1.1	4.9	0.5	0.1	0.0

(Ace: Acetaldehyde, Pro: Propanal, *i*-but: *iso*-Butanal, But: Butanal, Pen: Pentanal, Hex: Hexanal, Hep: Heptanal, Oct: Octanal and Hexdi: Hexadienal).

Figure 4.11 Gas chromatogram of the headspace volatiles of an untreated cooked comminuted pork sample (control) after 3 days of storage at 4°C. (1) acetaldehyde, (2) propanal, (3) isobutanal, (4) butanal, (5) pentanal, (6) hexanal, (7) heptanal, (8) octanal, (9) 2,4-hexadienal.

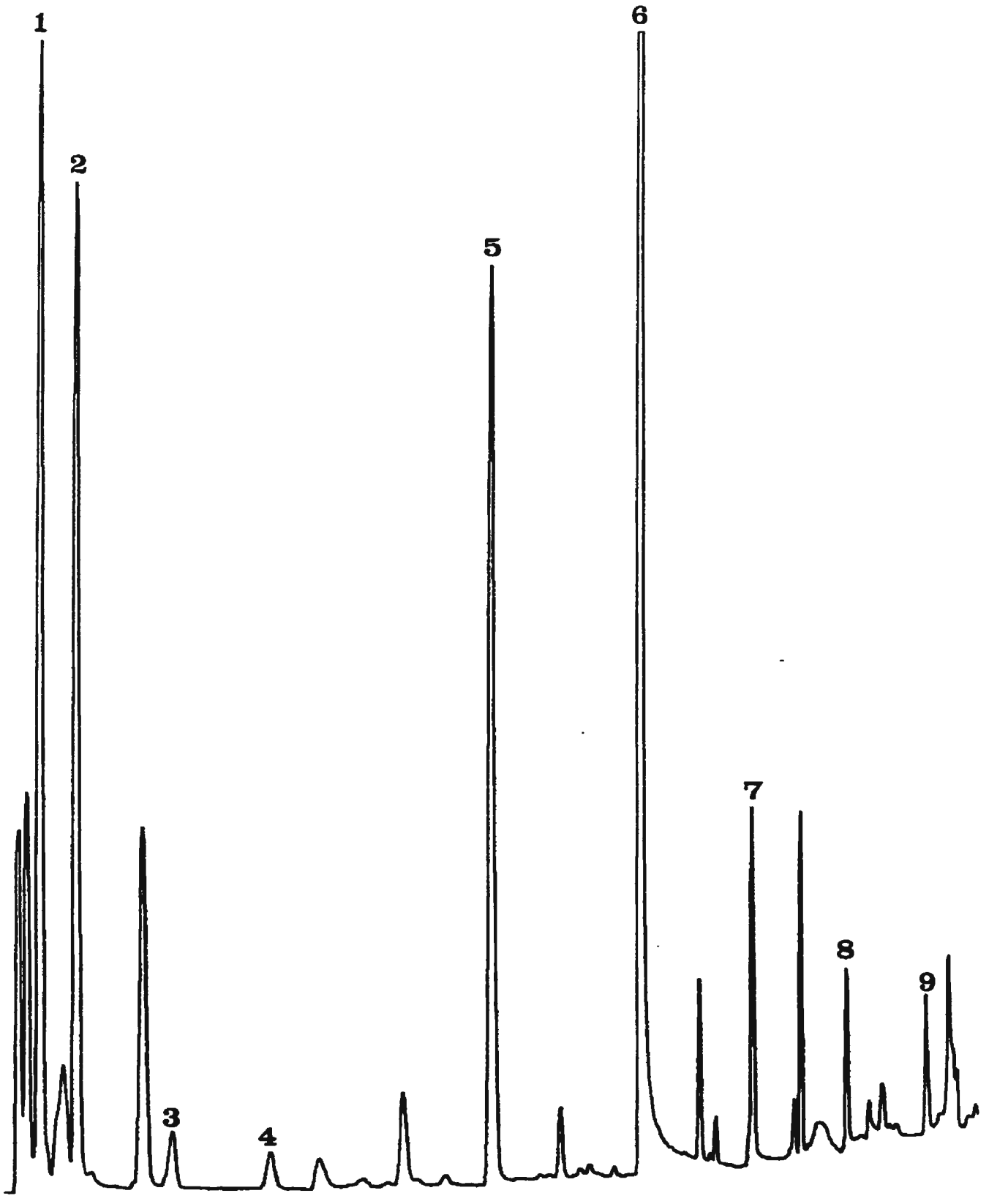
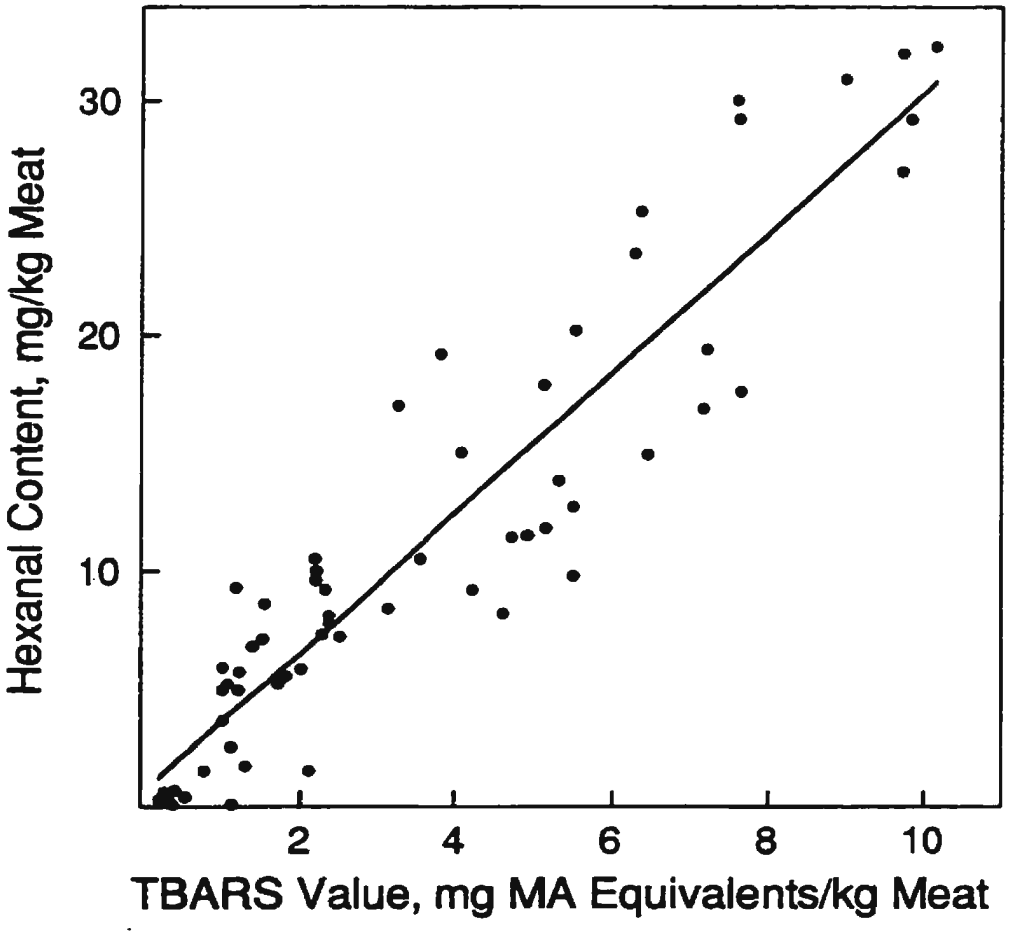

1
2
3
4
5
6
7
8
9

Figure 4.12 Gas chromatogram of the headspace volatiles of a cooked comminuted pork sample containing calcium iodide (CaI_2) at 200 meq/kg sample after 3 days of storage at 4°C. (1) acetaldehyde, (2) isobutanal, (3) butanal, (4) and (5) unidentified volatiles, (6) pentanal, (7) hexanal, (8) heptanal.


5
7
4
6
1
2
3
8

Figure 4.13 Relationship between hexanal contents and TBARS values of cooked comminuted pork (r=0.9263).


Hexanal Content, mg/kg Meat
30
20
10
2
4
6
8
10
TBARS Value, mg MA Equivalents/kg Meat

or (2) from the rearrangement of the 9-hydroperoxide of linoleic acid to the 13-hydroperoxide or (3) from oxidative decomposition of 2,4-decadienal (Frankel and Gardner, 1989).

The hexanal content of the control sample on day 0, was 10 mg/kg meat and increased to about 19 mg/kg meat by day 3. It further increased to 32 mg/kg meat on day 7 which is about a 3-fold increase over the 7 days of storage (Figure 4.14). Contents of acetaldehyde, propanal and pentanal of the control sample were also increased over the entire storage period, but all were present at a much lower concentration when compared to that of hexanal. These volatiles, however, may not adequately represent the oxidative status of meat. The minor volatiles such as acetaldehyde, propanal, isobutanal, butanal, pentanal, heptanal, octanal and 2,4- hexadienal are some of the important flavour notes that are also responsible for the warmed-over flavour development and meat flavour deterioration in heat-processed samples (Shahidi *et al.*, 1986).

As shown in Figures 4.14(A) and 4.15(A), hexanal formation was affected, to different extents, by LiF, NaF, KF, CsF, CaF_2 and MgF_2. These salts, except CaF_2, exhibited an inhibitory effect on lipid oxidation throughout the entire storage period (Figure 4.14). CaF_2, at a concentration of 200 meq/kg sample, however, enhanced hexanal generation after day 5. The inhibitory effect of the aforementioned salts, at a concentration of 200 meq/kg sample, on hexanal formation decreased in the following order: KF>CsF>NaF>MgF_2>LiF>CaF_2. KF, the strongest inhibitor, decreased the hexanal content by 99%, 98% and 98% on day 0, day 3 and day 7, respectively, when tested at

Figure 4.14 Effect of selected fluorides, (A) and iodides, (B) at a concentration of 100 meq/kg meat, on hexanal content of cooked comminuted pork stored at 4°C.

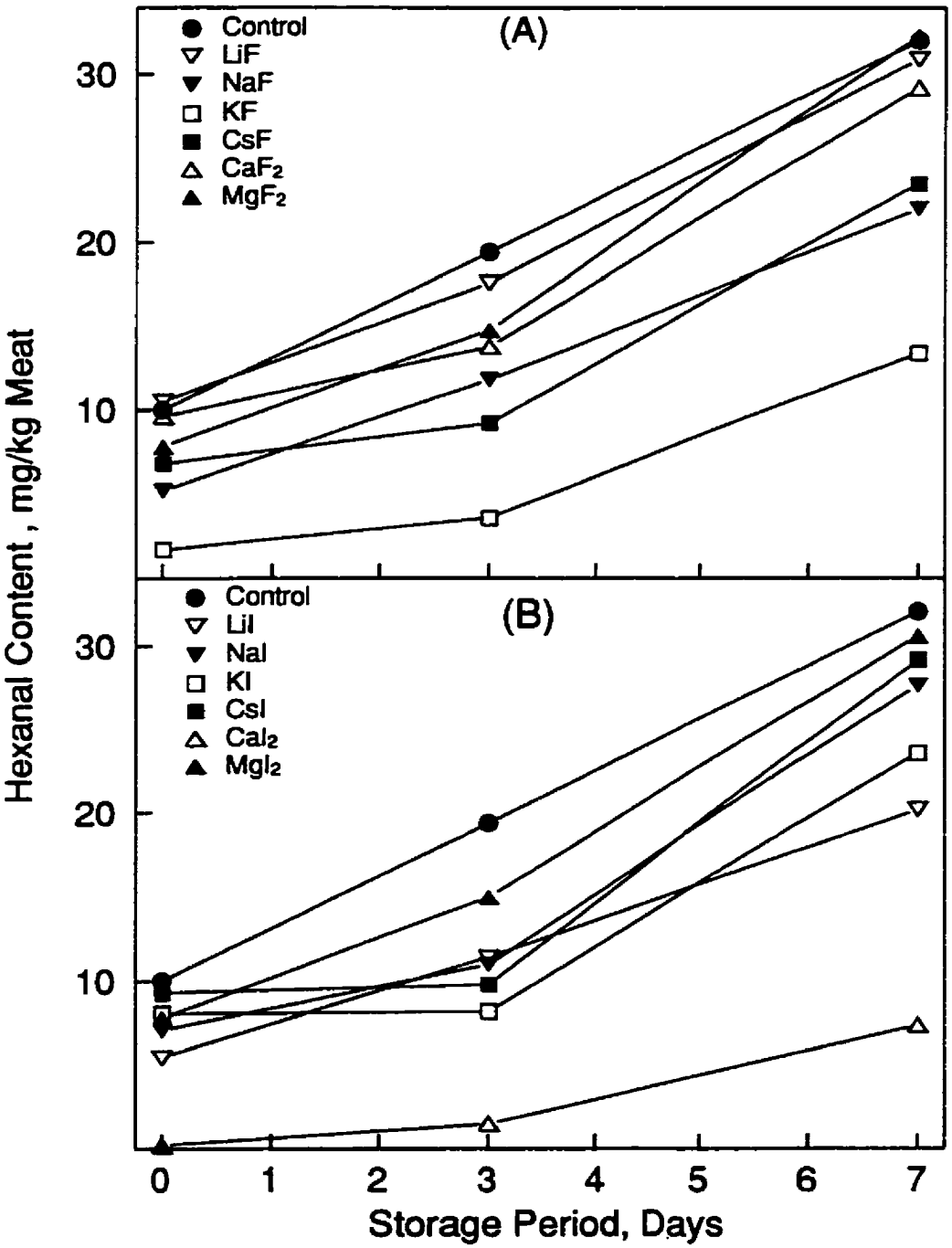
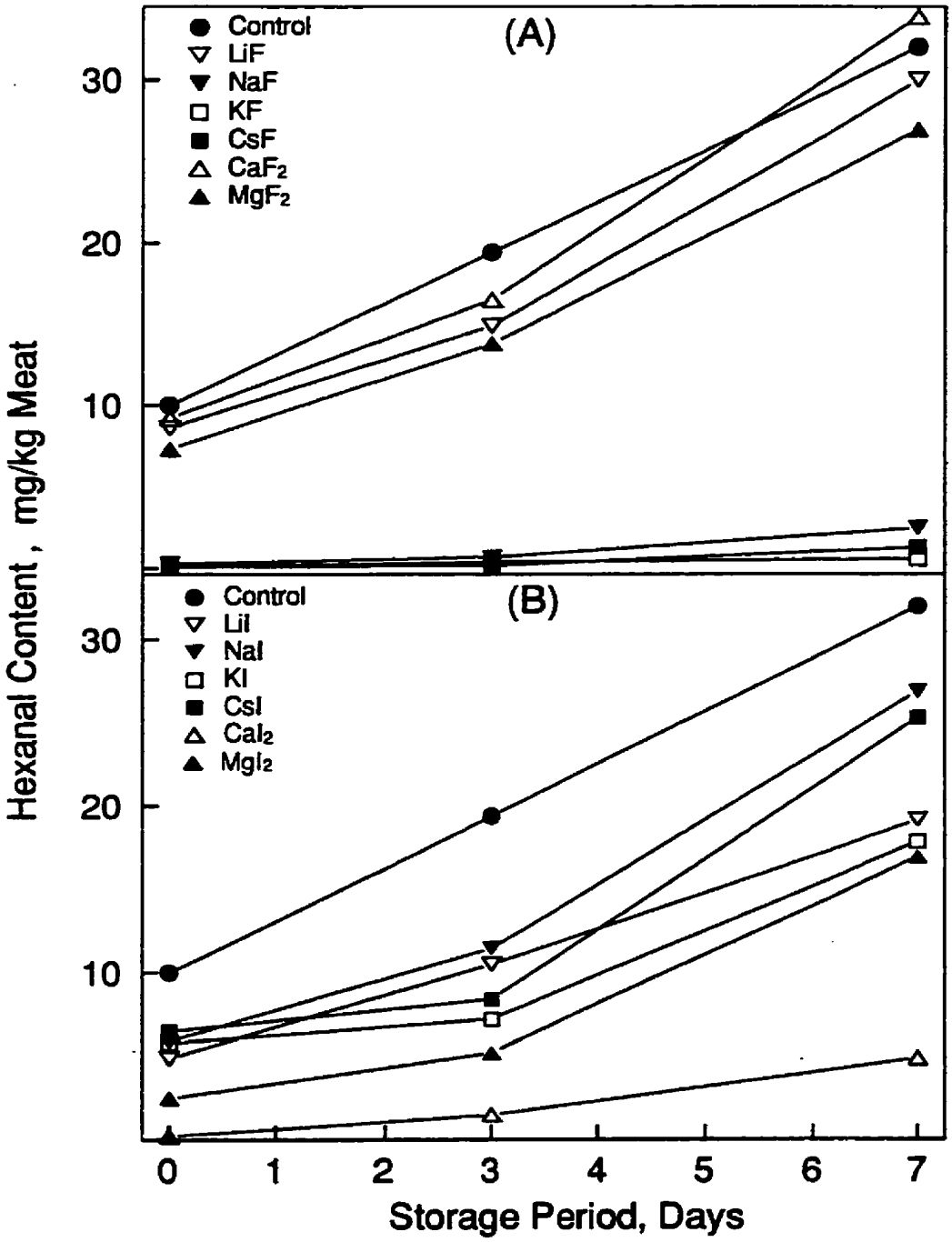

(A)
Control
LiF
NaF
KF
CsF
CaF2
MgF2
(B)
Control
LiI
NaI
KI
CsI
CaI2
MgI2
Hexanal Content , mg/kg Meat
10
20
30
0
1
2
3
4
5
6
7
Storage Period, Days

Figure 4.15 Effect of selected fluorides, (A) and iodides, (B) at a concentration of 200 meq/kg meat, on hexanal content of cooked comminuted pork stored at 4°C.


(A)
Control
LiF
NaF
KF
CsF
CaF2
MgF2
(B)
Control
LiI
NaI
KI
CsI
CaI2
MgI2
Hexanal Content, mg/kg Meat
10
20
30
0
1
2
3
4
5
6
7
Storage Period, Days

200 meq/kg meat. Being highly reactive, fluorides may interfere with free radical chain reactions probably through binding with free metal catalysts or by inhibiting certain catalytic enzymes such as lipoxygenases.

Effect of LiI, NaI, KI, CsI, MgI_2 and CaI_2 on hexanal formation is shown in Figures 4.14(B) and 4.15(B). Iodides also exhibited an inhibitory effect on lipid oxidation in meat systems regardless of their addition level. The inhibitory effect of iodides, at a concentration of 200 meq/kg sample, increased in the following order: CaI_2>MgI_2>KI>CsI>LiI>NaI. CsI was less effective after day 3 and thus the order changed as: CaI_2>MgI_2>KI>LiI>CsI>NaI. CaI_2, which caused the strongest inhibition among iodides, decreased the hexanal content by 98%, 92% and 85% on day 0, day 3 and day 7, respectively. The iodine formed upon reaction with ferric ions is a good free radical scavenger and could reduce the generation of 9- and 13-hydroperoxides which are the precursors of hexanal.

The literature on decomposition of lipid hydroperoxides does not yet provide a clear understanding of the pathway by which the majority of volatile products are formed from different hydroperoxides and how these pathways are affected by antioxidants. According to Frankel and Gardner (1987), various tocopherols and BHA can affect the stability of linoleic acid hydroperoxides. BHA and α-tocopherol are thought to stabilize unsaturated aldehyde against further oxidation. Similarly, fluorides and iodides may affect not only the free radical initiation reactions but also the hydroperoxide decomposition reactions.

4.5 Effect of Pan®-salt on lipid oxidation

Table 4.13 shows the TBARS values of comminuted pork treated with Pan®-salt or NaCl. Both salts resulted in a somewhat higher TBARS values when compared to those of untreated controls. The reduced prooxidative effect of Pan®-salt can be attributed to its KCl and $MgSO_4$ components which are less prooxidative than NaCl (Rhee *et al.*, 1983b). However, Pan®-salt may accelerate lipid oxidation by freeing iron ions which are bound to macromolecules and haem compounds. The free irons so formed are known to catalyze lipid peroxidation especially, in the presence of hydrogen peroxide in muscle tissues (Kanner and kinsella, 1983; Kanner and Doll, 1991).

4.6 Effect of different salts on the cook yield and texture of comminuted pork

Different salts affected the cook yield of meat to different degrees as shown in Table 4.14. MgF_2, $MgBr_2$, MgI_2 and CaF_2, even at 200 meq/kg meat addition level, did not significantly ($p<0.05$) change the cook yield when compared to that of the untreated sample (control). However, all other salts, at 200 meq/kg meat resulted in significantly ($p<0.05$) higher cook yields when compared to that of the control. The effect of halides of Li, Na and K decreased the cook yield of meat in the order of: $I^- > Br^- > Cl^- > F^-$. The sulphate anion had a lesser effect than that of the fluoride anion on enhancing the cook yield of the sample. Cations (as bromide or iodide) decreased the cook yield in the order of: $Na^+ > Li^+ > Cs^+ > K^+ > Mg^{2+} \geq Ca^{2+}$.

Table 4.13 Effect of Pan®-salt and NaCl on lipid oxidation in meat model systems as reflected in their TBARS values (mg malonaldehyde equivalents/kg meat)[1].

Treatment	Storage Period, Days				
	0	1	3	5	7
Control (No additive)	2.34±0.02^{b}	3.30±0.01^{c}	5.57±0.16^{a}	5.95±0.46^{c}	6.84±0.02^{c}
NaCl, 1%	2.36±0.02^{b}	3.14±0.12^{c}	5.45±0.25^{a}	6.80±0.32^{b}	7.16±0.14^{b}
NaCl, 2%	2.47±0.01^{b}	3.35±0.03^{c}	5.66±0.31^{a}	7.08±0.01^{ba}	7.20±0.05^{b}
NaCl, 3%	2.92±0.01^{a}	3.83±0.03^{b}	5.96±0.02^{a}	7.24±0.17^{a}	7.92±0.06^{a}
Pan®-salt, 1%	2.17±0.04^{c}	3.37±0.01^{c}	5.90±0.12^{a}	6.35±0.04^{c}	7.01±0.07^{bc}
Pan®-salt, 2%	1.97±0.04^{d}	3.07±0.04^{c}	5.60±0.12^{a}	6.01±0.30^{c}	6.80±0.04^{c}
Pan®-salt, 3%	2.23±0.03^{bc}	3.28±0.04^{c}	5.75±0.10^{a}	6.71±0.04^{b}	7.36±0.05^{b}

[1] Results are mean values of three determinations ± standard deviation. Means sharing the same superscript in each column are not significantly ($p<0.05$) different from one another.

Table 4.14 Effect of different salts on cook yield (%) of comminuted pork[1].

Salt	Concentration, meq/kg Sample	
	100	200
Control (No salt)	73.58±0.87^{c}	73.58±0.87^{c}
LiF	75.89±1.50^{c}	77.34±0.45^{b}
NaF	82.08±1.18^{b}	85.62±1.05^{a}
KF	76.78±0.33^{d}	78.28±0.37^{b}
CsF	77.73±0.63^{d}	79.93±0.78^{b}
MgF_2	71.26±0.80^{c}	71.23±1.60^{c}
CaF_2	72.28±0.07^{c}	71.85±2.09^{c}
LiCl	80.13±0.71^{b}	81.92±0.42^{b}
NaCl	79.86±1.07^{bd}	85.66±1.47^{a}
KCl	74.25±0.54^{c}	79.85±0.86^{b}
CsCl	74.73±0.52^{c}	79.97±0.79^{b}
$MgCl_2$	70.96±1.36^{c}	75.84±1.36^{b}
$CaCl_2$	72.76±0.35^{c}	75.13±0.95^{b}
LiBr	84.71±2.35^{a}	84.53±1.75^{a}
NaBr	77.70±1.65^{d}	82.39±0.51^{b}
KBr	73.67±1.05^{c}	79.86±0.53^{b}
CsBr	77.05±0.28^{d}	81.18±1.21^{b}
$MgBr_2$	71.65±0.35^{c}	73.98±1.41^{bc}
$CaBr_2$	72.68±0.83^{c}	76.63±0.84^{b}
LiI	81.33±1.80^{b}	88.53±1.27^{a}
NaI	85.47±0.86^{a}	89.51±1.47^{a}
KI	74.85±1.10^{c}	82.51±0.21^{b}
CsI	79.84±1.63^{b}	87.61±0.86^{a}
MgI_2	71.83±1.15^{c}	74.66±1.13^{bc}
CaI_2	73.25±0.44^{c}	76.50±0.85^{b}
Li_2SO_4	74.59±0.79^{c}	75.00±0.04^{b}
Na_2SO_4	81.58±1.03^{b}	85.08±0.96^{a}
K_2SO_4	74.28±0.52^{c}	75.41±1.94^{b}
Cs_2SO_4	80.95±0.23^{b}	83.50±1.08^{b}
$MgSO_4$	72.92±0.69^{c}	77.74±1.08^{b}
$CaSO_4$	74.69±0.81^{c}	77.24±1.00^{b}

[1] Results are mean values of three determinations ± standard deviation. Means sharing the same superscripts in a column are not significantly ($p<0.05$) different from one another.

Based on the results given in Table 4.14, it appears that monovalent cations and anions are more effective in increasing cook yield of restructured meat. Cook yield is a function of protein extraction and subsequent water binding. Addition of salts to meat can disassociate actomyosin complex to release a considerable amount of myosin which eventually forms a firm protein network upon thermal processing. This has a positive effect on moisture retention of cooked meats. Moreover, water-binding capacity of myofibrillar proteins is increased during extraction of proteins by salts. Salts may also shift the isoelectric point of the myofibrillar proteins in a manner that creates a larger net negative charge by ionizing carboxyl groups of protein molecules. Repulsion between these negatively charged groups may cause the protein to open up its spatial arrangement, thus increasing the degree of hydration. In addition, hydrated halide ions can attract the positively charged groups of the proteins, thus breaking the inter- and intra-protein salt bridges. This may result in further increase of the negatively charged species and in turn may enhance water-binding.

Results in Table 4.15 indicate that salts generally increase the texture of restructured pork to different degrees and that the effect is more pronounced at 200 meq/kg meat. Of the salts tested, NaCl, LiBr, NaBr, LiI and Na_2SO_4 did not exhibit a considerable effect on texture when tested at a concentration of 100 meq/kg meat. Moreover, salts such as LiF, NaF and LiCl had a very little effect on texture even at a concentration of 200 meq/kg meat. The other salts, regardless of their concentration, gave a firmer texture as reflected in maximum shear force data (Table 4.15). Magnesium salts,

Table 4.15 Effect of selected salts on texture of restructured meat as reflected in their maximum shear force (kg) data[1].

Salt	Concentration, meq/kg Sample	
	100	200
Control (No salt)	8.4±0.2^{d}	8.4±0.2^{d}
LiF	8.7±0.1^{d}	8.7±0.2^{d}
NaF	9.5±0.1^{d}	9.4±0.2^{d}
KF	11.4±0.2^{c}	11.5±0.1^{c}
CsF	11.1±0.2^{c}	13.8±0.2^{c}
MgF_2	13.3±0.1^{c}	13.7±0.1^{bc}
CaF_2	11.1±0.1^{c}	14.5±0.1^{c}
LiCl	8.7±0.1^{d}	8.7±0.3^{d}
NaCl	9.1±0.1^{d}	11.0±0.3^{c}
KCl	11.4±0.2^{c}	14.2±0.2^{bc}
CsCl	12.9±0.2^{c}	15.4±0.2^{bc}
$MgCl_2$	16.0±0.2^{b}	20.3±0.1^{a}
$CaCl_2$	14.8±0.2^{bc}	15.6±0.2^{b}
LiBr	9.8±0.2^{d}	11.3±0.2^{c}
NaBr	8.7±0.1^{d}	11.9±0.1^{c}
KBr	12.9±0.2^{c}	13.5±0.1^{c}
CsBr	13.3±0.1^{c}	14.0±0.2^{bc}
$MgBr_2$	19.8±0.2^{a}	21.1±0.1^{a}
$CaBr_2$	16.7±0.2^{b}	17.5±0.1^{b}
LiI	8.9±0.1^{d}	12.0±0.2^{c}
NaI	11.1±0.2^{c}	16.0±0.2^{b}
KI	13.1±0.1^{c}	17.6±0.2^{b}
CsI	16.8±0.2^{b}	16.9±0.2^{b}
MgI_2	19.2±0.2^{a}	20.8±0.2^{a}
CaI_2	15.7±0.1^{bc}	20.0±0.2^{a}
Li_2SO_4	11.0±0.2^{c}	11.0±0.3^{c}
Na_2SO_4	9.7±0.2^{d}	11.6±0.1^{c}
K_2SO_4	17.1±0.1^{b}	12.4±0.2^{c}
Cs_2SO_4	13.4±0.2^{c}	16.1±0.3^{b}
$MgSO_4$	17.7±0.1^{b}	20.3±0.1^{a}
$CaSO_4$	14.4±0.2^{c}	20.1±0.1^{a}

[1] Results are mean values of three measurements ± standard deviation. Means sharing the same superscripts in a column are not significantly ($p<0.05$) different from one another.

except MgF_2, resulted in a greater shear force when compared to the samples containing other types of salt. Calcium salts, except CaF_2 and $CaCl_2$, also caused higher shear force, thus indicating a higher degree of firmness. Both magnesium and calcium salts exhibited similar effects on texture and shear force data and these salts were not significantly ($p<0.05$) different from one another.

In general, salt-soluble myofibrillar proteins such as myosin, actomyosin and actin have been shown to play an important role in the formation of a firm protein network in the restructured meat products (Acton *et al.*, 1983). Solubilized proteins coagulate upon thermal processing to form a liquid-in-solid emulsion (Asghar *et al.*, 1985). In muscles, actin and myosin filaments are kept in phase by binding with Z- and M-lines, respectively, and thus exert a constraint on solubilization. Moreover, cross-bridges between actomyosin units decrease the protein solubilization. Addition of neutral salts affects the structural stability of macromolecules through electrostatic and lyotropic changes. The electrostatic effect of ions depends mainly on the sign and magnitude of their ionic charge while lyotropic effect originates from other properties of ions, such as ionic radii and electronic features (Acton *et al.*, 1983). According to Asghar *et al.* (1985), as explained in the previous paragraph, the addition of salts brings about a shift in the isoelectric point of the myofibrillar proteins and formation of a larger proportion of negatively charged carboxyl groups which repel each other and causes the protein to open up, thus increasing its hydration.

Cations of salts, at a concentration of 200 meq/kg meat, decreased the shear force in the following order: $Ca^{2+} \geq Mg^{2+} \geq Cs^{+} \geq K^{+} > Na^{+} \geq Li^{+}$ (for fluorides); $Mg^{2+} \geq Ca^{2+} \geq Cs^{+} \geq K^{+} \geq Na^{+} > Li^{+}$ (for chlorides and bromides); $Mg^{2+} \geq Ca^{2+} > K^{+} \geq Cs^{+} \geq Na^{+} > Li^{+}$ (for iodides) and $Mg^{2+} \geq Ca^{2+} > Cs^{+} > K^{+} \geq Na^{+} \geq Li^{+}$ (for sulphates). The effect of Ca^{2+} and Mg^{2+} ions can be attributed to solubilization of myofibrillar proteins. Usually, muscle contains an enzyme called calpain which is capable of disintegrating the Z-discs of myofibrils causing the release of actin molecules (Taylor and Ethrington, 1991). According to Taylor and Ethrington (1991), calpain requires Ca^{2+} ions in order to act upon Z-discs. However, the enhancement of the action of calpain by Ca^{2+} ions is not the only reason for the high protein solubilization caused by Ca^{2+}. Taylor and Ethrington (1991) observed that both Ca^{2+} and Mg^{2+} could increase the protein extraction in a myofibrillar protein model system containing calpain inhibitors. Moreover, it was demonstrated that Mg^{2+} ions are equally as effective as Ca^{2+} ions in terms of protein extraction. It is therefore thought that Ca^{2+} and Mg^{2+} may increase the solubilization of myofibrillar proteins by destabilization of protein structure.

The effect of monovalent cations on textural properties of heat processed meats may be attributed to their ability to neutralize electrostatic charges causing destabilization of protein structure. Cations are capable of lessening noncovalent interactions required to maintain the structure of myosin. In this regard divalent cations are more effective than monovalent cations (Goodno and Swenson, 1975). Furthermore, hydrated cations can interact with destabilized proteins making the peptide chains farther apart. There may be

a relationship between the hydrated ionic radii of cations and their performance in protein solubilization. The hydrated ionic radius of Li^+, Na^+, K^+ and Cs^+ ions are 3.4, 2.76, 2.32 and 2.28 Å, respectively (Cotton and Wilkinson, 1976). Results suggest that the shorter the hydrated ionic radii, the greater the protein solubilization. Lithium ion, which has the largest hydrated ionic radius, was the least effective for protein solubilization whereas cesium ion, which has the shortest hydrated ionic radius, was most effective.

The anions of salts did not follow a uniform trend in improving firmness of restructured meat. Their effects could depend upon the associated cations. Anions of salts, at an addition level of 200 meq/kg meat, decreased the firmness of restructured meat in the following orders: $I^- > Br^- \geq SO_4^{2-} > Cl^- \geq F^-$ (for lithium or sodium salts); $I^- > Cl^- > Br^- > SO_4^{2-} > F^-$ (for potassium salts); $I^- > SO_4^{2-} > Cl^- > Br^- > F^-$ (for cesium salts); $Br^- \geq Cl^- > I^- > SO_4^{2-} > F^-$ (for magnesium salts) and $I^- \geq SO_4^{2-} > Br^- > Cl^- > F^-$ (for calcium salts). Hydrated anions can strongly attract the positively charged groups of a destabilized protein and consequently the inter- and intra-protein salt bridges are broken down thus resulting in a further increase in the availability of myofibrillar proteins for gelation. According to the aforementioned orders, iodide ion imparted the highest firmness to meat in most cases whereas fluoride ion always did the least. The exact reasons for this phenomenon remain unknown.

The varying effects of anions and cations of salts on meat texture may arise from numerous factors. Any factor that affects the structural conformation of myofibrillar proteins can eventually cause either protein solubilization (salting-in) or insolubilization

(salting-out). The higher the degree of protein solubilization, the greater the firmness of the thermally processed comminuted meat and *vise-versa*.

4.7 Effect of Pan®-salt on the cook yield and texture of restructured meat

Table 4.16 shows the effect of Pan®-salt and NaCl on cook yield of restructured pork. An increase in cook yield was observed with increasing concentration of Pan®-salt. Treatment of meat with salts decreased the cook yield in the following order: 3% NaCl>3% Pan®-salt>2% NaCl≥2% Pan®-salt≥1% NaCl>1% Pan®-salt. The cook yield of meat treated with 1% Pan®-salt was not significantly ($p<0.05$) different from that of control sample. Furthermore, the effect of 2% Pan®-salt was not significantly ($p<0.05$) different from that of 1% and 2% NaCl. Since cook yield is a function of water-binding capacity, muscle protein solubilization plays an important role in cook yield. The higher the protein solubilization the greater the water retention upon thermal processing.

The effect of Pan®-salt on textural property of restructured pork is shown in Table 4.17 alone with data for NaCl-treated samples for comparison. Pan®-salt significantly ($p<0.05$) increased the firmness of restructured pork, regardless of its level of addition. Shear force data of samples containing 1 or 2% Pan®-salt were not significantly different from one another, but 3% addition caused a significantly higher shear force when compared to those containing 1 or 2% Pan®-salt. Furthermore, Pan®-salt, up to 2% addition level, had an effect similar to that of 1% NaCl, while 3% Pan®-salt resulted in a significantly ($p<0.05$) lower shear force value when compared to that

Table 4.16 Effect of Pan®-salt and NaCl on cook yield (%) of restructured pork[1].

Treatment	Cook Yield, %
Control (No additive)	69.54±1.02[c]
NaCl, 1%	74.32±0.84[b]
NaCl, 2%	76.48±0.51[b]
NaCl, 3%	81.29±1.79[a]
Pan®-salt, 1%	69.78±0.94[c]
Pan®-salt, 2%	74.67±0.94[b]
Pan®-salt, 3%	78.67±0.87[a]

[1] Results are mean values of three determinations ± standard deviation. Means sharing the same superscript are not significantly ($p<0.05$) different from one another.

Table 4.17 Effect of Pan®-salt and NaCl on the texture of restructured pork[1].

Treatment	Maximum Shear Force, kg
Control (No additive)	8.4±0.2[e]
NaCl, 1%	19.1±0.1[d]
NaCl, 2%	21.9±0.1[c]
NaCl, 3%	27.0±0.2[a]
Pan®-salt, 1%	18.9±0.1[d]
Pan-®salt, 2%	19.1±0.1[d]
Pan-®salt, 3%	24.8±0.2[b]

[1] Results are mean values of three measurements ± standard deviation. Means bearing the same superscripts are not significantly ($p<0.05$) different from one another.

of samples containing 3% NaCl. Addition of 1, 2 or 3% NaCl or Pan®-salt to meat increased the firmness of thermally processed samples in the following order: 3% NaCl>3% Pan®-salt>2% NaCl>1% NaCl = 2% Pan®-salt>1% Pan®-salt.

Samples containing Pan®-salt exhibited lower shear force values perhaps due to the presence of smaller amounts of NaCl in the mixture. Barbut and Mittal (1989) have also demonstrated a reduction in the rheological and gelation properties of pork, beef and poultry meat batters containing low levels of NaCl. Moreover, Thiel *et al.* (1986) reported a decrease in firmness of restructured ham with decreasing NaCl concentration. Partial replacement of NaCl with KCl and $MgSO_4$ definitely improved the textural properties of meat compared with the control, but the effect was still slightly lower than that of NaCl alone. Magnesium ions are known to improve the firmness of meat (Taylor and Etherington, 1991; Whipple *et al.*, 1994). However, the amount of magnesium ions (3%, w/w) present in Pan®-salt may not be sufficient to improve the texture of meat to any great extent.

CONCLUSIONS

The effect of cooked cured-meat pigment (CCMP), at 2.2, 6.2 and 10 μM concentrations, on lipid oxidation was examined in a β-carotene/linoleate model system. For comparison, metmyoglobin (MMb), nitrosylmyoglobin (NOMb) and butylated hydroxyanisole (BHA) were also examined in the same system. CCMP, at 6.2 and 10 μM, exhibited a concentration-dependent antioxidative effect, but showed prooxidative activity at 2.2 μM. NOMb and MMb exhibited an antioxidant effect and a prooxidant effect, respectively, for all concentrations tested. The antioxidative effect of CCMP was in between that of BHA and NOMb, while BHA exhibited the highest antioxidative effect. Combinations of CCMP (10 μM), sodium ascorbate (SA; 50, 100 and 550 ppm) and sodium tripolyphosphate (STPP; 50, 100 and 500 ppm) showed a similar antioxidant activity when compared to CCMP alone in the above model system.

Effects of halides and sulphates of alkali and alkali-earth metals (at 100 and 200 meq/kg meat) on lipid oxidation in meat and β-carotene/linoleate model systems were investigated. Lipid oxidation in meat model systems was monitored using both the 2-thiobarbituric acid-reactive substances (TBARS) test and hexanal analysis. Fluoride salts of alkali metals inhibited the formation of the TBARS and hexanal in a meat model system. Furthermore, alkali fluorides showed an antioxidant effect in a β-carotene/linoleate model system. The antioxidative properties of alkali halides can be attributed to the ability of fluoride anion to inhibit the activity of catalytic enzymes and also to its strong pairing with non-haem iron ion. Fluorides of alkali-earth metals (magnesium and calcium) were not antioxidative in a meat model system due to ion-

pairing of fluoride anions with divalent magnesium and calcium. Iodide salts of alkali and alkali-earth metals inhibited both the TBARS and hexanal formation in meat model systems and also exhibited antioxidative effects in a β-carotene/linoleate model system. The antioxidative effect of iodide anion can be attributed to its ability to alter the balance between ferrous and ferric forms of the redox system. Chlorides and bromides of alkali and alkali-earth metals were either prooxidative or possessed no considerable influence on lipid oxidation in meat model systems. Of sulphate salts tested, only calcium sulphate exhibited an antioxidative effect. Based upon the evidence presented above, it is concluded that pro- or anti-oxidative effects of salts are primarily due to their anions but also depended on the cation and its ion-pairing strength. Meanwhile, Pan®-salt (52% NaCl + 28% KCl + 12% $MgSO_4$ and 3% lysine.HCl), a low-sodium salt, exhibited a slight prooxidant effect in meat as assessed by the TBARS test.

In another experiment, the effects of halides and sulphates of alkali and alkali-earth metals on the cook yield and texture of meat were examined. Enhanced cook yields were evident in the samples containing monovalent cations (ie., alkali metal ions) when compared to those of divalent cations (ie., alkali-earth metal ions). Divalent cations were more effective than monovalent cations in imparting a firm texture to meat, although they were unable to influence the moisture retention of the system as much as the monovalent cations. Generally, fluorides had the least and iodides had the greatest effect on both cook yield and texture of meat. Results for chlorides, bromides and sulphates fall in between. The cook yield and shear force of Pan®-salt treated meats were somewhat lower than those of NaCl treated meats at the same concentration.

REFERENCES

Acton, J.C., Ziegler, G.R. and Burge, D.L., Jr. (1983). Functionality of muscle constituents in the processing of comminuted meat products. *CRC Crit. Rev. Food Sci. Nutr.* **18**, 99-121.

Ajuyah, A.O., Fenton, T.W., Hardin, R.T. and Sim, J.S. (1993). Measuring lipid oxidation volatiles in meats. *J. Food Sci.* **58**, 270-273.

Al-Jalay, B., Blank, G., McConnell, B. and Al-khayat, M. (1987). Antioxidant activity of selected spices used in fermented meat sausage. *J. Food Protec.* **50**, 25-27.

AOAC (1990). Official Methods of Analysis, 15th ed. Association of Official analytical Chemists, Washington, D.C.

Arnold, A.R., Bascetta, E. and Gunstone, F.D. (1991). Effect of sodium chloride on prooxidant activity of copper (II) in peroxidation of phospholipid liposomes. *J. Food Sci.* **56**, 571-578.

Asghar, A., Samejima, K. and Yasui, T. (1985). Functionality of muscle proteins in gelation mechanisms of structured meat products. *CRC Crit. Rev. Food Sci. Nutr.* **22**, 27-106.

Barbut, S. and Mittal, G.S. (1989). Effect of salt reduction on the rheological and gelation properties of beef, pork and poultry meat batters. *Meat Sci.* **26**, 177-191.

Bard, J. and Townsend, W.E. (1971). Cured meats: Meat Curing. In *The Science of Meat and Meat Products, Second Edition*, ed. J.F. Price and B.S. Schweigert. W.H. Freeman and Company, San Francisco, CA, pp.452-483.

Ben-Aziz, A., Grossman, S., Ascarelli, I. and Budowski, P. (1970a). Carotene-bleaching activities of lipoxygenase and haem proteins as studied by a direct spectrophotometric method. *Phytochemistry* **10**, 1445-1452.

Ben-Aziz, A., Grossman, S., Ascarelli, I. and Budowski, P. (1970b). Linoleate oxidation induced by lipoxygenase and haem proteins: A direct spectrophotometric assay. *Analytical Biochem.* **34**, 88-100.

Bernthal, P.H., Booren, A.M. and Gray, J.I. (1991). Effect of reduced sodium chloride concentration and tetrasodium pyrophosphate on pH, water-holding capacity and extractable protein of prerigor and postrigor ground beef. *Meat Sci.* **29**, 69-82.

Bielski, B.H.J. and Allen, A.O. (1977). Mechanism of the disproportionation of superoxide radicals. *J. Phys. Chem.* **11**, 1048-1050.

Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **32**, 911-917.

Boyer, R.F. and McCleary, C.J. (1987). Superoxide ions as a primary reductant in ascorbate-mediated ferritin iron release. *Free Rad. Biol. Med.* **3**, 389-395.

Bracco, U., Löliger, J. and Viret, J-L. (1981). Production and use of natural antioxidants. *J. Am. Oil Chem. Soc.* **58**, 686-690.

Brown, W.D., Harris, L.S., and Olcott, H.S. (1963). Catalysis of unsaturated lipid oxidation by iron protoporphyrin derivatives. *Arch. Biochem. Biophys.* **101**, 14-

Brown, C.L., Hendrick, H.B. and Bailey, M.E. (1974). Characteristics of cured ham as influenced by levels of sodium nitrite and sodium ascorbate. *J. Food Sci.* **39**, 977-979.

Buege, D.E., Birdsall, J.J., dryden, F.D., Judge, M.D., Kemp, J.D., Rogers, R.W., Rust, R.E., Sofos, J.N., Solberg, M., Tompkin, R.B., Wasserman, A.E. and Will, P.A. (1980). Update: Nitrite-Free Processed Meats. *Reciprocal Meat Conference Proceedings* **33**, 122-135.

Castell, C.H., Maclean, J. and Moore, B. (1965). Rancidity in lean fish muscle. IV. Effect of sodium chloride and other salts. *J. Fish Res. Bd. Canada* **22**, 929-944.

Cassens, R.G. (1990). Scientific accomplishments. In *Nitrite-cured Meat: A Food Safety Issue in Perspective*. Food & Nutrition Press, Inc. Trumbull, CT, pp 145-161.

Coleman, H.M. and Steffen, A.H. (1949). Treatment of animal materials. US Patent 2,491,646

Cotton, F.A. and Wilkinson, G. (1976). *Basic Inorganic Chemistry*. John Wiley & Sons, New York.

Crackel, R.L., Gray, J.I., Booren, A.M., Pearson, A.M. and Buckley, D.J. (1988). Effect of antioxidants on lipid stability in restructured beef steaks. *J. Food Sci.* **53**, 656-657.

Decker, E.A. and Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* **38**, 674-677.

Dymicky, M., Fox, J.B. and Wasserman, A.E. (1975). Colour formation in cooked model meat systems with organic and inorganic compounds. *J. Food Sci.* **40**, 306-309.

Drumm, T.D. and Spanier, A.M. (1991). Changes in the content of lipid autoxidation and sulphur-containing compounds in cooked beef during storage. *J. Agric. Food Chem.* **39**, 336-343.

Fjelkner-Modig, S. (1986). Sensory properties of pork, as influenced by cooking temperature and breed. *J. Food Quality* **9**, 89-105.

Fooladi, M.H., Pearson, A.M. Coleman T.H. and Merkel, R.A. (1979). The role of nitrite in preventing development of warmed-over flavour. *Food Chem.* **4**, 283-292.

Frankel,E.N., Neff, W.E. and Selke, E. (1984). Analysis of autoxidized fats by gas chromatography-mass spectrometry. IX. Homolytic vs. heterolytic cleavage of primary and secondary oxidation products. *Lipids* **19**, 790-800.

Frankel, E.N. and Gardner, H.W. (1989). Effect of α-tocopherol on the volatile thermal decomposition products of methyl linoleate hydroperoxides. *Lipids* **24**, 603-608.

Frankel, E.N., Hu, M-L. and Tappel, A.L. (1989). Rapid headspace gas chromatography of hexanal as a measure of lipid peroxidation in biological samples. *Lipids* **24**, 976-981.

Frankel, E.N. (1993). Formation of headspace volatiles by thermal decomposition of oxidized fish oils vs oxidized vegetable oils. *J. Am. Oil Chem. Soc.* **70**, 767-772.

Frankel, E.N., Parks, E.J., Xu, R., Schneeman, B.O., Davis, P.A. and German, J.B. (1994). Effect of n-3 fatty acid-rich fish oil supplementation on the oxidation of low density lipoproteins. *Lipids* **29**, 233-236.

German, J.B. and Kinsella, J.E. (1985). Lipid oxidation in fish tissue: enzymatic initiation *via* lipoxygenase. *J. Agric. Food Chem.* **33**, 680-683.

Goodno, C.C. and Swenson, C.A. (1975). Thermal transition of myosin and its helical fragments. II. Solvent-induced variations in conformational stability. *Biochem.* **14**, 873-878.

Gray, J.I. and Randall, C.J. (1979). The nitrite/N-nitrosamine problem in meats: An update. *J. Food Protec.* **42**, 168-179.

Grossman, S., Bergman, M. and Sklan, D. (1988). Lipoxygenase in chicken muscle. *J. Agric. Food Chem.* **36**, 1268-1270.

Hadden, J.P., Ockerman, H.W., Cahill, V.R. and Parrett, N.A. (1975). Influence of sodium nitrite on the chemical and organoleptic properties of comminuted pork. *J. Food Sci.* **40**, 626-630.

Halliwell, B. and Gutteridge, M.C. (1986). Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Arch. Biochem. Biophys.* **246**, 501-514.

Harel, S. and Kanner, J. (1985a). Hydrogen peroxide generation in ground muscle tissues. *J. Agric. Food Chem.* **33**, 1186-1188.

Harel, S. and Kanner, J. (1985b). Muscle membranal lipid peroxidation initiated by H_2O_2-activated metmyoglobin. *J. Agric. Food Chem.* **33**, 1188-1192.

Hirano,J. and Olcott, H.S. (1971). Effect of hame compounds on lipid oxidation. *J. Am. Oil Chem. Soc.* **48**, 523-524.

Houlihan, C.M., Ho, C.-T. and Chang, S.S. (1984). Elucidation of the chemical structure of a novel antioxidant, rosmaridiphenol, isolated from rosemary. *J. Am. Oil. Chem. Soc.* **61**, 1036-1039.

Howard,A., Duffy, P., Else, K. and Brown, D. (1973). Possible substitutes for nitrite for pigment formation in cured meat products. *J. Agric. Food Chem.* **21**, 894-898.

Ichinose, T., Miller, M.G. and Shibamoto, T. (1989). Gas chromatographic analysis of free and bound malonaldehyde in rat liver homogenates. *Lipids* **24**, 895-898.

Igene, J.O., Pearson, A.M., Merkel, R.A. and Coleman, T.H. (1979). Effect of frozen storage time, cooking, and holding temperature upon extractable lipids and TBA values of beef and chicken. *J. Anim. Sci.* **49**, 701-707.

Igene, J.O., Pearson, A.M., Dugan, L.R. and Price, J.F. (1980). Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. *Food Chem.* **5**, 263-276.

Inatani, R., Nakatani, N., Fuwa, H. and Seto, H. (1982). Structure of a new antioxidative phenolic diterpene isolated from rosemary (*Rosmarinus officinalis* L.). *Agric. Biol. Chem.* **46**, 1661-1666.

Ioffe, B.V. and Vitenberg, A.G. (1984). Head-Space Analysis and Related Methods in Gas Chromatography. John Wiley & Sons, Inc., New York, pp 2-7.

Izumi, K., Cassens, R.G. and Greaser, M.L. (1989). Reaction of nitrite with ascorbic acid and its significant role in nitrite free-cured food. *Meat Sci.* **26**, 141-153.

Janero, D.R. (1990). Malonaldehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Rad. Biol. Med.* **9**, 515-540.

Jennings, W. (1979). Foreword. In *Analysis of Foods and Beverages: Headspace Techniques*. ed. G. Charalambous. Academic Press, Inc., New York, pp. xi-xii.

Johns, A.M., Birkinshaw, L.H. and Ledward, D.A. (1989). Catalysts of oxidation in meat products. *Meat Sci.* **25**, 209-220.

Kanner, J., Ben-Gera, I. and Berman, S. (1979). Nitric-oxide myoglobin as an inhibitor of lipid oxidation. *Lipids* **15**, 944-948.

Kanner, J. and Kinsella, J.E. (1983). Lipid deterioration initiated by phagocytic cells in muscle foods: β-carotene destruction by a myoloperoxidase-hydrogen peroxide-halide system. *J. Agric. Food Chem.* **31**, 370-376.

Kanner, J. and Harel, S. (1985). Initiation of membranal lipid peroxidation by activated metmyoglobin and methaemoglobin. *Arch. Biochem. Biophys.* **237**, 314-321.

Kanner, J., German, J.B. and Kinsella, J.E. (1987). Initiation of lipid peroxidation in biological systems. *CRC Crit. Rev. Food Sci. Nutr.* **25**, 317-346.

Kanner,J., Shegalovich, I., Harel, S. and Hazan, B. (1988). Muscle lipid peroxidation dependent on oxygen and free meatal ions. *J. Agric. Food Chem.* **36**, 409-412.

Kanner, J. and Doll, L. (1991). Ferritin in turkey muscle tissue: A source of catalytic iron ions for lipid peroxidation. *J. Agric. Food Chem.* **39**, 247-249.

Kanner, J., harel, S. and Jaffe, R. (1991). Lipid peroxidation of muscle food as affected by NaCl. *J. Agric. Food Chem.* **39**, 1017-1021.

Kanner, J. (1994). Oxidative processes in meat and meat products: Quality implications. *Meat Sci.* **36**, 169-189.

Knight, P. and Parsons, N. (1988). Action of NaCl and Polyphosphates in meat processing: Responses of myofibrils to concentrated salt solutions. *Meat Sci.* **24**, 275-300.

Kohn, H.I. and Liversedge, M. (1944). On a new aerobic metabolite whose production by brain is inhibited by apomorphine, emetine, epinephrine and menadione. *J. Pharmacol.* **82**, 292-297.

Kosugi, H., Kojima, T. and Kikugawa,K. (1989). Thiobarbituric acid-reactive substances from peroxidized lipids. *Lipids* **24**, 873-881.

Kramer, R.E. (1985). Antioxidants in clove. *J. Am. Oil Chem. Soc.* **62**, 111-113.

Kramlich, W.E., Pearson, A.M. and Tauber, F.W. (1973). *Processed Meats.* The Avi Publishing Company, Inc., Westport, CT.

Kwoh, T.L. (1971). Catalysts of lipid peroxidation in meats. *J. Am. Oil Chem. Soc.* **48**, 550-555.

Labuza, T.P. (1971). Kinetics of lipid oxidation in foods. *CRC Crit. Rev. Food Sci. Nutr.* **2**, 355-405

Lamikanra, V.T. and Dupuy, H.P. (1990). Analysis of volatiles related to warmed over flavour of ccoked chevon. *J. Food Sci.* **55**, 861-862.

Lantinen, S. (1986). Research note: masking of the bitter taste of salt substitutes with lactose in food emulsions. *J. Food Quality* **9**, 199-204.

Lee, Y.B., Kim, Y.S. and Ashmore, C.R. (1986). Antioxidant property in ginger rhizome and its application to meat products. *J. Food Sci.* **51**, 20-23.

Love, J.D. and Pearson, A.M. (1974). Metmyoglobin and non-haem iron as prooxidant in cooked meat. *J. Agric. Food Chem.* **22**, 1032-1034.

Love, J.D. (1983). The role of haem iron in the oxidation of lipids in red meats. *Food Technol.* **37** (3), 117-120, 129.

Love, J. (1987). Mechanism of iron catalysis of lipid autoxidation in warmed-over flavour of meat. In *Warmed-over Flavour of Meat.* eds. A.J. St. Angelo and M.E. Bailey. Academic Press, Orlando, FL, pp. 19-39.

Lin, C.-W. and Lai, D.-J. (1979). I. Studies on the utilization of haem pigment from animal blood as a food colorant. II. The effect of imidazole on colouring of haemoglobin. *J. Chinese Agric. Chem. Soc.* **17**, 54-66.

Liu, H.-P. (1970a). Catalysts of lipid peroxidation in meats. 1. Linoleate peroxidation catalyzed by MetMb or Fe(II)-EDTA. *J. Food Sci.* **35**, 590-592.

Liu, H.-P. (1970b). Catalysts of lipid peroxidation in meats. 2. Linoleate oxidation catalyzed by tissue homogenates. *J. Food Sci.* **35**, 593-595.

Liu, H.-P. and Watts, B.M. (1970). Catalysts of lipid peroxidation in meats. 3. Catalysts of oxidative rancidity in meats. *J. Food Sci.* **35**, 596-598.

Liu, H.F., Booren, A.M., Gray, J.I. and Crackel, R.L. (1992). Antioxidant efficacy of oleoresin rosemary and sodium tripolyphosphate in restructured pork steaks. *J. Food Sci.* **57**, 803-806.

Matiella, J.E. and Hsieh, T. C.-Y. (1990). Analysis of crab meat volatile compounds. *J. Food Sci.* **55**, 962-966.

Miller, D.M., Buettner, G.R. and Aust, S.D. (1990). Transition metals as catalysts of autoxidation reactions. *Free Rad. Biol. Med.* **8**, 95-108.

Minotti,G. and Aust, S.D. (1992). Redox cycling of iron and lipid peroxidation. *Lipids* **27**, 219-226.

Mirvish, S.S. (1970). Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. *J. Natl. Cancer Inst.* **44**, 633-639.

Morrissey, P.A. and Tichivangana, J.Z. (1985). The antioxidant activities of nitrite and nitrosylmyoglobin in cooked meats. *Meat Sci.* **14**, 175-190.

Nakatani, N. and Inatani, R. (1984). Two antioxidative diterpenes from rosemary (*Rosmarinus officinalis* L.) and a revised structure for rosmanol. *Agric. Biol. Chem.* **48**, 2081-2085.

Newbrun, E. (1986). Mechanism of fluoride action in caries prevention. In *Fluorides and Dental Caries: Contemporary Concepts for Practitioners and Students*. ed. E. Newbrun. Charles C. Thomas Publisher, Springfield, IL, pp 155-172.

O'Boyle, A.R., Rubin, L.J., Diosady, L.L., Aladin-Kassam, N., Comer, F. and Brightwell, W. (1990). A nitrite-free curing system and its application to the production of wieners. *Food Technol.* **44** (5), 88, 90-91, 93, 95-96, 98, 100, 102-104.

O'Boyle, A.R., Aladin-Kassam, N., Rubin, L.J. and Diosady, L.L. (1992). Encapsulated cured-meat pigment and its application in nitrite-free ham. **57**, *J. Food Sci.* 807-812.

Onodenalore, A.C. (1993). Aqueous Washing of Mechanically Deboned Chicken Meat: Effect on Chemical and Functional Characteristics. M.Sc. Thesis, Memorial University of Newfoundland, St. John's, NF, Canada.

Osinchak, J.E., Hultin, H.O., Zajicek, O.T., Kelleher, S.D. and Huang, C.H. (1992). Effect of NaCl on catalysis of lipid oxidation by the soluble fraction of fish muscle. *Free Rad. Biol. Med.* **12**, 35-41.

Pearson, A.M., Love, J.D. and Shorland, F.B. (1977). Warmed-over flavour in meat, poultry and fish. In *Advancements in Food Research Vol. 23*. eds. C.O. Chichester, E.M. Mark, and G.F. Stewart. Academic Press, New York, pp. 1.

Pearson, A.M. and Gray, J.I. (1983). Mechanisms responsible for warmed-over flavours in cooked meat. In *The Maillard Reactions in Food and Nutrition*. eds. G.R. Walter and M.S. Feather. ACS Symposium Series **215**, American Chemical Society, Washington, DC., pp 287-300.

Pegg, R.B. (1993). Development of Nitrite-Free Meat Curing Systems. Ph.D. Thesis, Memorial University of Newfoundland, St John's, NF, Canada.

Pegg, R.B. and Shahidi, F. (1987). Single step preparation of cooked cured-meat pigment and its application to meat. Abstract. *Can. Inst. Food Sci. Technol. J.* **20**, 323-325.

Pierson, M.D. and Smoot, L.A. (1982). Nitrite, nitrite alternatives, and the control of *Clostridium botulinum* in cured meats. *CRC Crit. Rev. Food Sci. Nutr.* **17**, 141-187.

Pikul, J. and Kummerow, F.A. (1991). Thiobarbituric acid reactive substances formation as affected by distribution of polyenoic fatty acids in individual polyphosphates. *J. Agric. Food Chem.* **39**, 451-457.

Pryor, W.A., Stanley, J.P. and Blair, E. (1976). Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandins-like endoperoxides. *Lipids* **11**, 370-378.

Puolanne, E., Saarela, E. and Ruusunen, M. (1988). The effect of NaCl-KCl-$MgSO_4$ (Pan®) on the quality of cooked sausages. In *Proceedings of 34th International Congress of Meat Science and Technology, Part B*, August 29-September 2, Brisbane, Australia, pp. 302-304.

Raharjo, S. and Sofos, J.N. (1993). Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: A review. *Meat Sci.* **35**, 145-169.

Ramarathnam, N., Rubin, L.J. and Diosady, L.L. (1991a). Studies on meat flavour. 1. Qualitative and quantitative differences in uncured and cured pork. *J. Agric. Food Chem.* **39**, 344-350.

Ramarathnam, N., Rubin, L.J. and Diosady, L.L. (1991b). Studies on meat flavour. 2. A quantitative investigation of the volatile carbonyles and hydrocarbons in uncured and cured beef and chicken. *J. Agric. Food Chem.* **39**, 1839-1847.

Randall, C.J. and Voisey, P.W. (1977). A method for measuring the texture of meat and the effect of nitrite and salt addition on the texture of cured meats. *J. Texture Studies* **8**, 55-66.

Rhee, K.S., Smith, G.C. and Terrell, R.N. (1983a). Effect of reduction and replacement of sodium chloride on rancidity development in raw and cooked ground pork. *J. Food Protect.* **46**, 578-581.

Rhee, K.S., terrell, R.N., Quintanilla, M. and Vanderzant, C. (1983b). Effect of addition of chloride salts on rancidity of ground pork inoculated with a *Moraxella* or a *Lactobacillus* species. *J. Food Sci.* **48**, 302-303.

Rhee, K.S. and Ziprin, Y.A. (1987). Lipid oxidation in retail beef, pork and chicken muscles as affected by concentrations of haem pigments and none-haem iron and microsomal enzymic lipid peroxidation activity. *J. Food Biochem.* **11**, 1-15.

Robards, K., Kerr, A.F. and Patsalides, E. (1988). Rancidity and its measurement in edible oils and snack foods. *Analyst* **113**, 213-224.

Roozen, J.P. (1987). Effect of Types I, II and III antioxidants on phospholipid oxidation in a meat model for warmed over flavour. *Food Chem.* **24**, 167-185.

Salih, A.M., Price, J.E., Smith, D.M. and Dawson, L.E. (1989). Lipid oxidation in turkey meat as influenced by salt, metal cations and antioxidants. *J. Food Sci.* **12**, 71-83.

Sato, K. and Hegarty, G.R. (1971). Warmed-over flavour in cooked meats. *J. Food Sci.* **36**, 1098-1102

Schweinsberg, F. and Burkle, V. (1985). Nitrite: A co-carcinogen? *J. cancer Res. Clin. Oncol.* **109**, 200-202.

Schaich, K.M. (1980). Free radical initiation in proteins and amino acids by ionizing and ultraviolet radiations and lipid oxidation. Part I: Ionizing radiation. *CRC Crit. Rev. Food Sci. Nutr.* **13**, 89-159.

Sebranek, J.G. (1979). Advances in the technology of nitrite use and consideration of alternatives. *Food Technol.* **33** (7), 58-62,93.

Seideman, S.C. and Theer, L.K. (1986). Relationships of instrumental textural properties and muscle fibre types to the sensory properties of beef. *J. Food Quality* **9**, 251-261.

Shahidi, F., Rubin, J.L., Diosady, L.L., Chew,V., and Wood, D.F. (1984). Preparation of the cooked cured-meat pigment, dinitrosyl ferrohaemochrome from haemin and sodium nitric oxide. *Can. Inst. Food Sci. Technol. J.* **17**, 33-37.

Shahidi, F., Rubin, L.J., Diosady, L.L. and Wood. D.F. (1985). Preparation of the cooked cured-meat pigment, dinitrosyl ferrohaemochrome, from haemin and nitric oxide. *J. Food Sci.* **50**, 272-273.

Shahidi, F., Rubin, L.J. and D'Souza, L.A. (1986). Meat flavour volatiles: A review of the composition, techniques of analysis, and sensory evaluation. *CRC Crit. Rev. Food Sci. Nutr.* **24**, 141-243.

Shahidi, F., Rubin, L.J. and Wood, J.F. (1987). Control of lipid oxidation in cooked ground pork with antioxidants and dinytrosyl ferrohaemochrome. *J. Food Sci.* **52**, 564-567.

Shahidi, F., Rubin, L.J. and Wood, D.F. (1988). Stabilization of meat lipids with nitrite-free curing mixtures. *Meat Sci.* **22**, 73-80.

Shahidi, F. (1989a). Flavour and cooked meats. In *Flavour Chemistry: Trends and Developments*. eds. R. Teranishi, R. Buttery and F. Shahidi. ACS Symposium Series 388. American Chemical Society, Washington, DC, pp. 188-201.

Shahidi, F. (1989b). Current status of nitrite-free meat curing systems. In *Proceedings of the 35th International Congress of Meat Science and Technology*. 20-25 August, Copenhagen, Denmark, Volume III, 897.

Shahidi, F. and Pegg, R.B. (1990). Colour characteristics of cooked cured-meat pigment and its application to meat. *Food Chem.* **38**, 61-68.

Shahidi, F. (1991). Developing alternative meat-curing systems. *Trends Food Sci. Technol.* 219-222.

Shahidi, F. and Hong, C. (1991). Role of metal ions and haem pigments in autoxidation of heat-processed meat products. *Food Chem.* **42**, 339-346.

Shahidi, F. and Pegg, R.B. (1991a). Effect of the preformed cooked cured-meat-pigment (CCMP) on colour parameters of muscle foods. *J. Muscle Foods* **2**, 279-304.

Shahidi, F. and Pegg, R.B. (1991b). Encapsulation of the pre-formed cooked cured-meat pigment. *J. Food Sci.* **56**, 1500-1504.

Shahidi, F. and Pegg, R.B. (1992). Nitrite-free meat curing systems: update and review. *Food Chem.* **43**, 185-191.

Shahidi,F. and Wanasundara, P.K.J.P.D. (1992). Phenolic antioxidants. *CRC Crit. Rev. Food Sci. Nutr.* **32**, 67-103.

Shahidi, F. and Pegg, R.B. (1993). Hexanal as an indicator of meat flavour deterioration. *J. Food Lipids* **1**, 177-186.

Shahidi, F., Pegg, R.B. and Sen, N.P. (1994). Absence of volatile-N-nitrosamines in cooked nitrite-free cured muscle foods. *Meat Sci.* **37**, 327-336.

Sheppard, W.A. and Sharts, C.M. (1969). Organic fluorine chemistry. W.A. Benjamin Inc., NY.

Siu, G.M. and Draper, H.H. (1978). A survey of the malonaldehyde content of retail meats and fish. *J. Food Sci.* **43**, 1147-1149.

Snedecor, G.W. and Cochram, W.G. (1980). *Statistical Methods, 7th ed.* The Iowa State University Press, Ames, IA.

Sofos, J.N. (1986). Use of phosphates in low-sodium meat products. *Food Technol.* **40**,(9),52,54-58,60,62,64,66,68-69.

Steinhart, H., Vollmar, M. and sailer, C. (1993). Pro- and antioxidative effect of ascorbic acid on L-triptophan in the system Fe^{3+}/ascorbic acid/O_2. *J. Agric. Food Chem.*, **39**, 1017-1021.

Stoick, S.M., Gray, J.I., Booren, A.M. and Buckley, D.J. (1991). Oxidative stability of restructured beef steaks processed with oleoresin rosemary, tertiary butylhydroquinone and sodium tripolyphosphate. *J. Food Sci.* **56**, 597-600.

Svingen, B.A., Buege, J.A., O'Neal, F.O. and Aust, S.D. (1979). The Mechanism of NADPH-dependant lipid peroxidation: the propagation of lipid peroxidation. *J. Biol. Chem.* **254**, 5892-5899.

Takiguchi, A. (1989). Effect of NaCl on the oxidation and hydrolysis of lipids in salted sardine fillets during storage. *Nippon Suisan Gakkaishi* **55**, 1649-1654.

Tarladgis, B.G., Pearson, A.M. and Dugan, L.R., Jr. (1964). Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II - Formation of the TBA-malonaldehyde complex without acid-heat treatment. *J. Sci. Food Agric.* **15**, 602-607.

Taylor, M.A.J. and Etherington, D.J. (1991). The solubilization of myofibrillar proteins by calcium ions. *Meat Sci.* **29**, 211-219.

Terrell, R.N. (1983). Reducing the sodium content of processed meats. *Food Technol.*, **37** (7), 66-71.

Tichivangana, J.Z. and Morrissey, P.A. (1985). Metmyoglobin and inorganic metals as prooxidants in raw and cooked muscle systems. *Meat Sci.* **15**, 107-116.

Tims, M.J. and Watts, B.M. (1958). Protection of cooked meats with phosphates. *Food Technol.* **12** (5), 240-243.

Thiel, L.F., Bechtel, P.J., Mckeith, F.K. and Brady, P. (1986). The influence of reduced salt on the yield breaking force, and sensory characteristics of chunked and formed ham. *J. Food Quality* **9**, 355-364.

Tompkin, R.B., Christiansen, L.N., Shaparis, A.B. and Bolin, H. (1974). Effect of potassium sorbate on *Salmonella, Staphylococcus aureus, Clostridium perfringens and Clostridium botulinum* in cooked uncured sausage. *Appl. Microbiol.* **28**, 262-

Trout, G.R. (1988). Techniques for measuring water-binding capacity in muscle foods - A review of methodology. *Meat Sci.* **23**, 235-252.

Trout, G.R. (1990). The rate of metmyoglobin formation in beef, pork and turkey meat as influenced by pH, sodium chloride and sodium tripolyphosphate. *Meat Sci.* **28**, 203-210.

Umano, K. and Shibamoto, T. (1987). Analysis of headspace volatiles from overheated beef fat. *J. Agric. Food Chem.* **35**, 14-18.

van Roon, P.S. and Krol, B. (1985). A factorial analysis of water-binding properties and firmness of heated, comminuted pork liver and pork loin products as influenced by addition of sodium chloride and pH regulators. *Meat Sci.* **13**, 65-80.

von Elbe, J.H. and Maing, I-Y. (1973). Betalains, possible colorants of meat substitutes. *Cereal Sci. Today* **18**, 263-264, 316-317.

von Elbe, J.H., Klement, J.T., Amundson, C.H., Cassens, R.G., and Lindsay, R.C. (1974a). Evaluation of betalain pigments as sausage colorants. *J. Food Sci.* **39**, 128-132.

von Elbe, J.H., Maing, I-Y. and Amundson, C.H. (1974b). Colour stability of betanin. *J. Food Sci.* **39**, 334-337.

Vösgen, W. (1992). Curing: Are nitrite and nitrate necessary or superfluous as curing substances. *Fleischwirtschaft* **72**, 1675-1678.

Walters, C.L. (1980). Nitrosamines in meat products. In *Developments in Meat Science-1*. ed. R. Lawrie. Applied Science Publishers Ltd., London, pp. 195-217.

Wanasundara, U., Amarowicz, R. and Shahidi, F. (1994). Isolation and identification of an antioxidative component in canola meal. *J. Agric. Food Chem.* **42**, 1285-1290.

Whipple, G., Koohmaraie, M. and Arbona, J.R. (1994). Calcium chloride *in vitro* effects on isolated myofibrilar proteins. *Meat Sci.* **38**, 133-139.

Whiting, R.C., Benedict, R.C., Kunsch, C.A. and Woychick, T.H. (1984). Effect of sodium chloride levels in frankfurters on the growth of *Clostridium sporogens* and *Staphylococcus aureus*. *J. Food Sci.* **49**, 351-355.

Wierbicki, E., Howker, J.J. and Shults, G.W. (1976). Effects of salt, phosphates and other curing ingredients on shrinkage of lean pork meat and the quality of smoked processed ham. *J. Food Sci.* **41**, 1116-1121.

Woolford, G., Cassens, R.G., Greaser, M.L. and Sebranek, J.G. (1976). The fate of nitrite: reaction with protein. *J. Food Sci.* **41**, 585-588

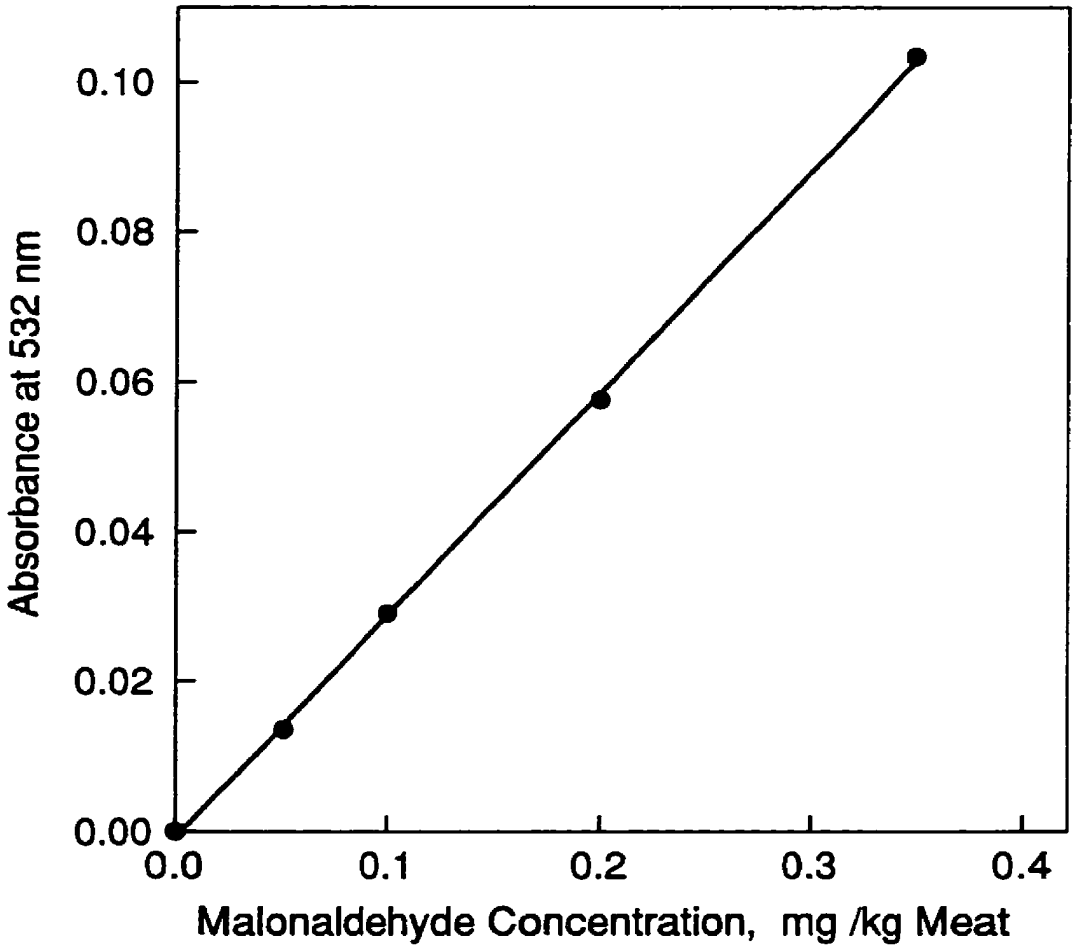
Woolford, G. and Cassens, R.G. (1977). The fate of sodium nitrite in bacon. *J. Food Sci.* **42**, 586-589.

Yasuhara, A. and Shibamoto, T. (1989). Analysis of aldehydes and ketones in the headspace of heated pork fat. *J. Food Sci.* **54**, 1471-1472, 1484.

Zubillaga, M.P., Maerker, G. and Foglia, T.A. (1984). Antioxidant activity of sodium nitrite. *J. Am. Oil Chem. Soc.* **61**, 772-776.

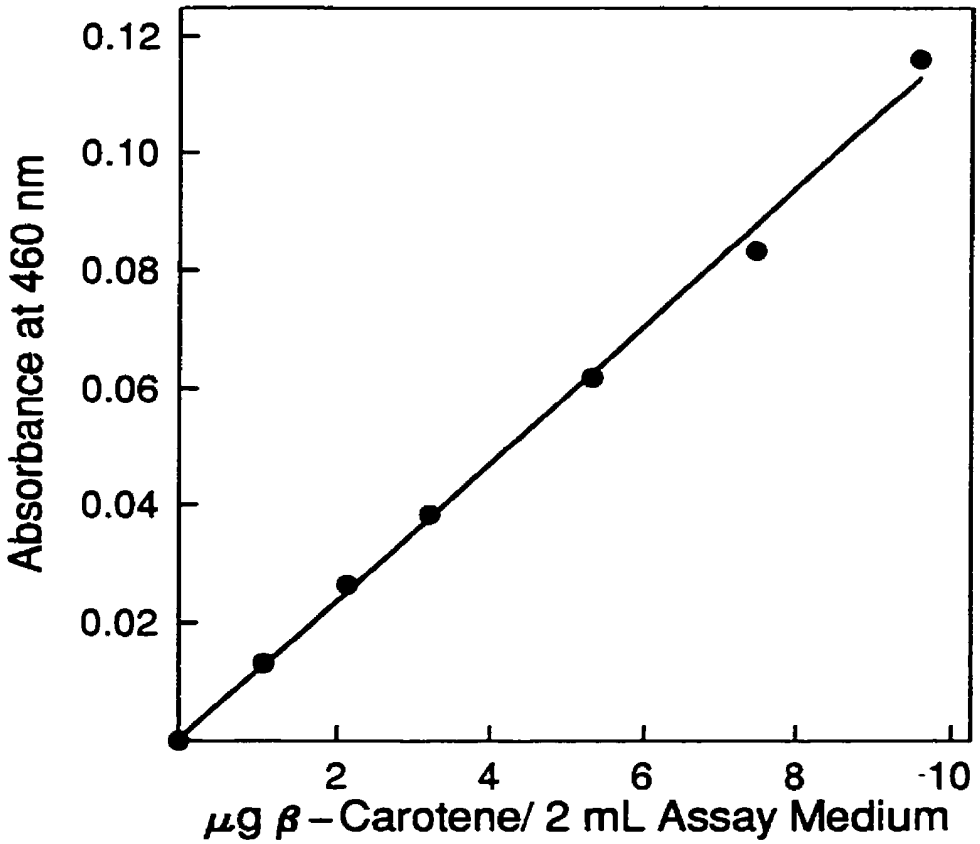
APPENDIX

Figure A.1 Dependence of the absorbance of malonaldehyde (MA)-TBA complex at 532 nm on the concentration of MA.



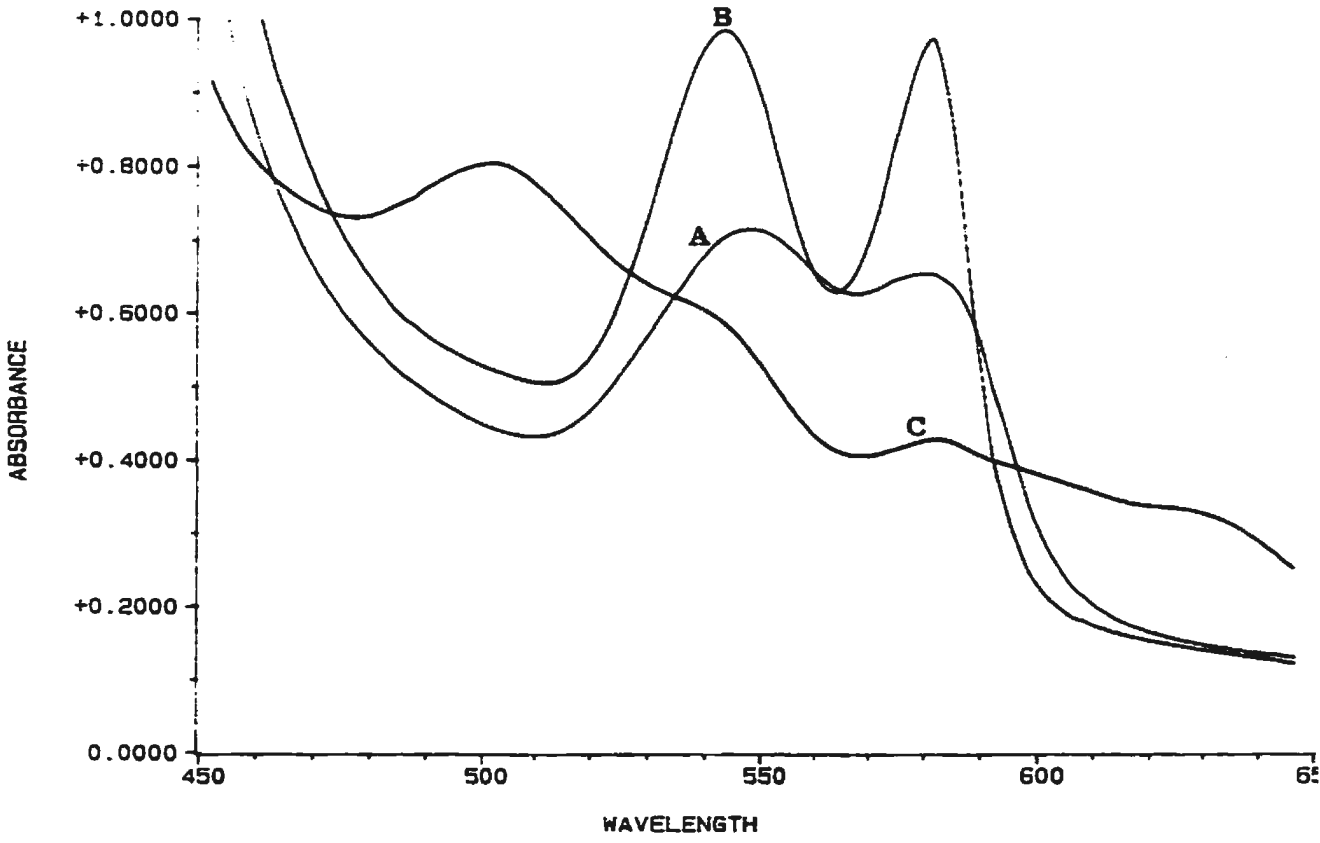
Regression coefficient (r) = 0.9996
Equation of the line was Y = aX + b where,
Y = absorbance at 532 nm
X = Concentration of malonaldehyde (MA) in mg MA equivalents/kg meat
a = 0.293
b = 0
Therefore, X = 3.4*Y

Figure A.2 Concentration dependence of the absorbance of β-carotene at 460 nm.



Regression coefficient (r) = 0.996685
Equation of the line was Y = aX + b where,
Y = absorbance at 460 nm
X = Concentration of β-carotene (μg/2 mL)
a = 0.0118
b = 0
Therefore, X = 85.02*Y

Figure A.3 Absorption spectra of (A); metmyoglobin (MMb), (B); nitrosylmyoglobin (NOMb), and (C); cooked cured-meat pigment (CCMP).


+1.0000
+0.8000
+0.6000
+0.4000
+0.2000
0.0000
ABSORBANCE
450
500
550
600
WAVELENGTH
A
B
C