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Oxidative Stability of Restructured Beef Steaks Processed with an Oleoresin Rosemary

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Susan Stoick

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J. I. Gray
Major professor

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OXIDATIVE STABILITY OF RESTRUCTURED BEEF STEAKS PROCESSED WITH AN OLEORESIN ROSEMARY

By

Susan Marie Stoick

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ABSTRACT

OXIDATIVE STABILITY OF RESTRUCTURED BEEF STEAKS PROCESSED WITH AN OLEORESIN ROSEMARY

By

Susan Marie Stoick

The antioxidant activity of an oleoresin rosemary (OR) was evaluated in raw restructured beef steaks stored at -20 C for six months and in cooked steaks stored at 4 C for six days. During refrigerated storage, thiobarbituric acid-reactive substances (TBARS) and sensory scores indicated that sodium tripolyphosphate (STPP) provided significant ($p<0.01$) protection against warmed-over flavor development, while the effects of OR were not significant. No significant differences existed between OR (0.10%)/STPP and TBHQ/STPP; however, this was most likely due to the presence of STPP. During frozen storage, a linear concentration effect of OR existed ($p<0.01$) for both TBARS values and sensory scores indicating that OR was an effective antioxidant. OR combined with STPP produced an additive protective effect during frozen storage. Hexanal content and phospholipid fatty acid profiles also underwent significant changes during frozen storage. Correlation coefficients (pooled within treatments and storage times) between TBARS values, sensory scores and hexanal levels were generally low and non-significant.

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INTRODUCTION

In recent years, the demand for pre-cooked and cooked, ready-to-eat, portion controlled meat entrees in the marketplace and in fast food franchises has grown, providing an expanding potential for consumer exposure to warmed-over flavor (WOF) (National Livestock and Meat Board, 1988). The development of rancidity and WOF in restructured meat products has been a major deterrent in their gaining acceptability by processors and consumers. The technology for restructuring meat was developed as a way to effectively utilize low value meat cuts and trimmings to produce a product with a higher perceived value at a reasonable cost (Seideman and Durland, 1983). During the manufacture of restructured meats, particle size reduction, exposure to oxygen, and addition of salt are a few of the processes which have been shown to decrease the stability of the lipids (Schwartz and Mandigo, 1976; Huffman et al., 1981). Miles et al. (1986) have shown that packaging conditions during frozen storage can affect both lipid and pigment oxidation. The cooking process also has a large influence on lipid stability and WOF development in restructured meat products (Sato and Hegarty, 1971; Willemot et al., 1985). The high susceptibility of restructured meats to lipid oxidation makes them a good system to test the stabilization of lipids by antioxidants.

Synthetic antioxidants such as butylated hydroxyanisole, (BHA), butylated hydroxytoluene (BHT), and tertiarybutyl hydroquinone (TBHQ) have been widely used for many years to help retard lipid oxidation in food systems, including meat products (Marion and Forsythe, 1964; Greene, 1969; Chastain et al., 1982; Miles et al., 1986; Crackel et al., 1988). In recent years, however, there has been renewed interest in natural antioxidants. Rosemary is one of many spices and herbs that has been shown to impart antioxidant activity in food systems (Chipault et al., 1952; Houlihan and Ho, 1985).

The major objective of this study was to evaluate the effectiveness of an oleoresin rosemary (OR) as an antioxidant during refrigerated and frozen storage of restructured beef steaks. A commercial OR (Kalsec Inc., Kalamazoo, MI) was tested at two levels with and without sodium tripolyphosphate (STPP). Its effectiveness was compared to that of salt, salt/STPP and TBHQ/STPP. A second objective was to evaluate alternative methods of hexanal analysis as an indicator of WOF development. Finally, it was desired to establish a relationship between thiobarbituric acid-reactive substances (TBARS) values, sensory scores, hexanal levels and changes in fatty acid profiles as a means for assessing lipid oxidation or WOF.

REVIEW OF THE LITERATURE

Lipid oxidation is one of the major causes of deterioration in the quality of meat and meat products, and it affects the color, flavor, nutritive value and even safety of foods (Tims and Watts, 1958; Pearson et al., 1983). Changes in consumer lifestyles have resulted in increased demand for portion-controlled, pre-cooked convenience products such as restructured beef steaks. This has also resulted in increased consumer exposure to warmed-over flavor (WOF). Restructured meats are highly susceptible to lipid oxidation due to the methods used during processing.

Flavor is a major criterion for judging acceptability of meat products and consequently this review will address the role of lipids in the development of WOF in beef. The distribution, composition and susceptibility of animal lipids to autoxidation will be discussed, followed by an overview of the mechanism of lipid oxidation and the resulting flavor compounds. Factors influencing WOF such as the restructuring process, additives and antioxidants will be addressed as will the potential of rosemary as a natural antioxidant. Finally, methods for assessment of lipid oxidation will be reviewed.

ANIMAL LIPIDS

Distribution of animal lipids

Lean muscle tissue in beef is generally composed of 70-75% water, 20-22% protein, 4-8% lipid and 1% ash. The term animal fats usually encompasses all of the lipid species, including triacylglycerols, phospholipids, sterols and sterol esters, and other lipids in minute quantities (Dugan, 1987). For the purpose of this discussion, the lipid composition of meat can be divided into two categories: lipids from muscle tissue (intramuscular lipids) and lipids from adipose tissue (depot lipids) (O'Keefe et al., 1968; Pearson et al., 1977). Depot lipids are generally localized in subcutaneous deposits, although significant amounts may be located in the thoracic and abdominal cavities and as intermuscular deposits. Also, Eichhorn et al. (1985) found that intramuscular fat (marbling) is similar to subcutaneous adipose tissue in fatty acid composition. Triacylglycerols are the major component of this fraction. The amount and fatty acid composition of the triacylglycerols may vary according to species, sex, age, diet, location in the body and environment (Eichhorn et al., 1985; Hornstein et al., 1967; Kuchmak and Dugan, 1965; Terrell and Bray, 1969).

In contrast, tissue lipids contain significant amounts of membrane-bound phospholipid material. These tissue lipids vary much less in proportion and fatty acid composition than do the depot lipids. The phospholipid (PL) content remains

constant and ranges from 0.5 - 1.0% when expressed as a concentration of total tissue weight (Dugan, 1987). Igene et al. (1979a) confirmed this by showing that in chicken and beef the phospholipids ranged from 0.54 - 0.82%. O'Keefe and coworkers (1968) found that the phospholipid fraction in beef cattle showed only slight variation from animal to animal within each muscle and did not differ significantly with regard to muscle location. Variation of animal age and diet produced little change in the PL content of a given muscle (Hornstein et al., 1967). Terrell and Bray (1969) reported that there were more fatty acid differences due to muscle location than the combined effects of weight and sex.

Composition of meat lipids

The triacylglycerol:phospholipid (TG:PL) ratio is a major factor that determines the composition of total lipid fatty acids in muscle. It also reflects the fat:lean ratio which is strongly influenced by the degree of marbling in muscle (Eichhorn et al., 1985). The TGs of animal fats are largely composed of palmitic, stearic and oleic acids and contain smaller amounts of other straight chain even numbered carbon fatty acids (Dugan, 1987; Eichhorn et al., 1985). Some researchers have reported C15:0, C17:0 and C17:1 in the fatty acid profiles of beef lipids (O'Keefe et al., 1968; Igene et al., 1981). Typically the TG fatty acids contain only one or two double bonds (Igene et al., 1981), thus

resulting in less than two percent of polyunsaturated fatty acids (PUFAs) (Pearson et al., 1977).

Conversely, the PLs are a very rich source of polyunsaturated and diunsaturated fatty acids. Over 50% of the PL fatty acids have two or more double bonds versus 10% in the TG (neutral lipid) fraction (O'Keefe et al., 1968). Eichhorn and coworkers (1985) reported that C20:5 and C22:5 make up 2-4% of the muscle PLs in beef while only being present in trace amounts in the total lipid extract. Terrell and Bray (1969) also reported that the PL fraction of bovine lipids contains a larger percentage of C18:2 and C18:3 and has C22:0 and C20:4 which are not found in the TG fraction.

Susceptibility of meats to rancidity and WOF development

WOF in meat, which was first recognized by Tims and Watts (1958), is a form of oxidative rancidity that develops within 12-48 hours in contrast to common rancidity which requires months to fully develop during frozen storage (Pearson et al., 1977; Igene et al., 1979a, 1980). WOF occurs most readily in cooked meats but can also occur in raw meats subjected to membrane disruption. Therefore, restructured meats are very vulnerable to WOF development since the membranes are subjected to disruption by the restructuring process and by cooking. Many researchers have implicated the phospholipids as the major contributors to

WOF in poultry, beef, pork and lamb (Younathan and Watts, 1960; Wilson et al., 1976; Willemot et al., 1985). Wilson et al. (1976) found that correlation coefficients between TBA numbers and PL levels were higher than those for TBA numbers and total lipids. Working with a model meat system of lipid-extracted muscle fibers with PLs, TGs and total lipids added back, Igene and Pearson (1979) verified that the PLs are the major contributors to WOF. The TGs did enhance WOF development in an additive manner when combined with the PLs as total lipid. Awad and coworkers (1968) correlated the loss of phospholipids with an increase in free fatty acids in bovine muscle over eight weeks of frozen storage. Igene and coworkers (1981) and Keller and Kinsella (1973) noted that no PLs were present in the drip loss of cooked meat. This meant that the polyunsaturated fatty acids were not liberated during cooking, but non-heme iron from heme pigments was released during cooking creating a potential for extreme susceptibility to lipid oxidation.

Phospholipids present in animal tissues include phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), sphingomyelin (SP), phosphatidyl inositol (PI), lysophosphatidyl choline (LPC) and other minor components (Pearson et al., 1977). Working with a model meat system with added PE, Igene and Pearson (1979) found that in beef, the C20:2 and C22:4 fatty acids of PE decreased by 74.5% and 55.1% from the original values, respectively, after cooking to 70 C and holding at

4 C for 48 hours. They concluded that polyunsaturated fatty acids associated with PE tend to be more labile to heat than those of PC resulting in a greater contribution of PE to WOF development. This conclusion is in agreement with Body and Shorland (1974) who reported that PE contained 17 to 43% PUFA versus 7 to 25% for PC and 1 to 4% for SP.

The high PUFA content, particularly linoleic and arachidonic acids, contributes to the lability of the PLs. Phospholipids from beef are approximately 15% more unsaturated than the triacylglycerols. Phospholipids may also be more susceptible to oxidation because of their close physical association in membranes with tissue catalysts of oxidation. Grinding and chopping disrupt the membranes and expose the PLs to oxygen, enzymes, heme pigments and metal ions causing the rapid development of rancidity even in fresh raw meat (Sato and Hegarty, 1971; Asghar et al., 1988).

Lipid stability during frozen storage of meat

Some early researchers initially found that tissue lipids were stable during frozen storage, however, numerous studies have now shown that lipid oxidation of the TGs and PLs will occur during frozen storage of meats (Awad et at., 1968; Keller and Kinsella, 1973; Bremner et al., 1976; Igene et al., 1979b). The degree of fatty acid unsaturation determines the stability of frozen meats. Wong and Hammond

(1977) reported that methyl linoleate oxidized about ten times faster than methyl oleate. Based on TBA analyses, Wilson and coworkers (1976) demonstrated that turkey meat is most susceptible to WOF development followed closely by chicken, pork, beef and mutton in that order.

There are conflicting reports as to whether the PLs or TGs undergo changes in frozen storage. Using intact muscle, Igene et al. (1979b) found that changes in total lipids of raw beef and chicken (light and dark) were mainly due to losses in the TG fraction. The PL fraction remained relatively constant through thirteen months of frozen storage. Keller and Kinsella (1973), on the other hand, determined that the total lipids of hamburger remained relatively constant, whereas there were significant losses in the PLs. TBA numbers also increased significantly during frozen storage of ground beef indicating oxidative reactions. The major reason for the conflicting reports may be due to the grinding of the beef which would release the membrane-bound PLs and promote oxidation. In a second study with intact beef and poultry, light and dark muscles, Igene et al. (1981) revealed that only minor changes occurred in the fatty acid profiles of the TGs during either freezer storage of the raw meat or subsequent storage after cooking. However, there was a significant decline in the amount of PE and PC, and total unsaturation of the raw tissues declined from 67.12% to 58.40% after eight months of frozen storage,

indicating the occurrence of lipid oxidation during frozen storage.

Frozen storage of cooked beef patties for up to 4.5 months resulted in a significant loss of C15:1 and C18:2 from the TG fraction, and an even more important loss of C18:3 and C20:4 from the PL fraction (Gokalp et al., 1983). High correlations between PL total saturated and PL unsaturated fatty acids, sensory panel rancidity scores and TBARS values were also reported. Since the oxidation of the PLs was more advanced at three months compared to the neutral lipid fraction between three months and four and one half months it was concluded that tissue lipid oxidation occurs in two stages. The PLs are oxidized first followed by the TGs, in agreement with El-Gharbawi and Dugan (1965).

Lipid stability during refrigerated storage of cooked meat

It is generally accepted that cooking triggers the rapid development of WOF within a few hours by liberating iron (ferrous) from various proteins (Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979b). Nonheme iron is the main catalyst of lipid oxidation in cooked meat, although, heme iron (activated methemyoglobin) may also initiate the oxidative process (Rhee, 1988). The heme pigments serve as a source of free iron as they are readily broken down during cooking. The increase in nonheme iron after cooking may also be due to release of iron from the

heme-iron complex by oxidative cleavage of the porphyrin ring (Schricker et al., 1982). Cooking also disrupts meat tissues, thus bringing the lipid substrates and catalysts in closer proximity. Membrane-bound phospholipids, the major lipid component contributing to WOF, are released during cooking. Cooking methods, temperatures and times can affect the level of nonheme iron released and hence the degree of lipid oxidation (Zisper and Watts, 1961; Huang and Greene, 1978; Chen et al., 1984a; Gros et al., 1986; Arganosa et al., 1989).

Willemot and coworkers (1985) examined cooked, lean ground pork stored at 4 C for up to sixteen days. They noted a rapid increase in TBA numbers correlated with sensory scores for WOF aroma and with a decrease in PLs and in PL-PUFAs. In the PLs, 57% of the linoleate was lost and 76% of the arachidonate versus 14% and 50% for the TG's. The main source of PUFA oxidation was the PLs. An increase in free fatty acids was not revealed, but it was suggested that the liberation of free fatty acids matched their oxidation (Willemot et al., 1985). Free fatty acids are more susceptible to oxidative breakdown than when esterified (Labuza, 1971).

LIPID OXIDATION IN MEATS

Mechanism of lipid oxidation

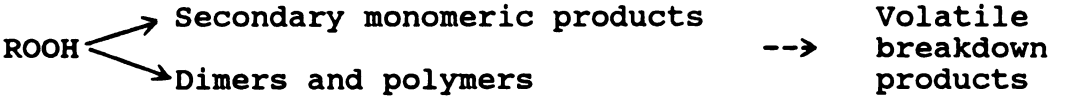
Lipids can participate in three types of oxidation reactions: autoxidation (thermal), photooxidation (light catalyzed) and lipoxidation (enzyme catalyzed). The focus of this discussion will be on autoxidation of unsaturated fatty acids. The reaction has been studied extensively, and it is generally accepted that it proceeds via a free radical chain mechanism, resulting in an autocatalytic system. The susceptibility of fatty acids to autoxidation and their subsequent rate of deterioration are affected by their degree of unsaturation and exposure to environmental factors such as oxygen concentration, temperature, heat, light, metal catalysts and enzymes. It is not the total fat content that is important but the amount of unsaturated fatty acid moieties (Labuza, 1971). The reaction of oxygen with unsaturated lipids (RH) involves free radical initiation, propagation and termination processes (Figure 1). During initiation a labile hydrogen is abstracted from a reactive methylene group adjacent to a double bond in the presence of trace metals, light or heat. The resulting lipid free radical (R.) reacts with oxygen to form a peroxy radical (ROO.). The propagation step also involves reaction of ROO. with RH to form a lipid hydroperoxide (ROOH) and another free radical (Frankel, 1984). Termination reactions result in formation of non-radical products.

Initiation:	$RH \rightarrow R^{\cdot} + H^{\cdot}$
Propagation:	$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$
	$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$
Termination:	$ROO^{\cdot} + ROO^{\cdot} \rightarrow ROOR + O_2$
	$R^{\cdot} + R^{\cdot} \rightarrow R_2$
	$R^{\cdot} + ROO^{\cdot} \rightarrow ROOR$
	$2RO^{\cdot} + 2ROO^{\cdot} \rightarrow 2ROOR + O_2$

Figure 1. Generalized mechanism for the autoxidation reaction (Paquette et al., 1985).

Decomposition of hydroperoxides

Hydroperoxides are the primary products of autoxidation. They can undergo homolytic cleavage to produce an alkoxy radical which decomposes to secondary monomeric products, or they can condense into dimers and polymers which further decompose to produce volatile materials as shown below (Frankel, 1984).



Hydroperoxides will decompose to a variety of volatile and non-volatile secondary products as shown in Figure 2. Step one involves homolytic cleavage on the oxygen-oxygen bond to yield the alkoxy and hydroxy free radicals. Carbon-carbon scission on either side of the alkoxy radical can result in formation of an aldehyde and a new free radical (2).

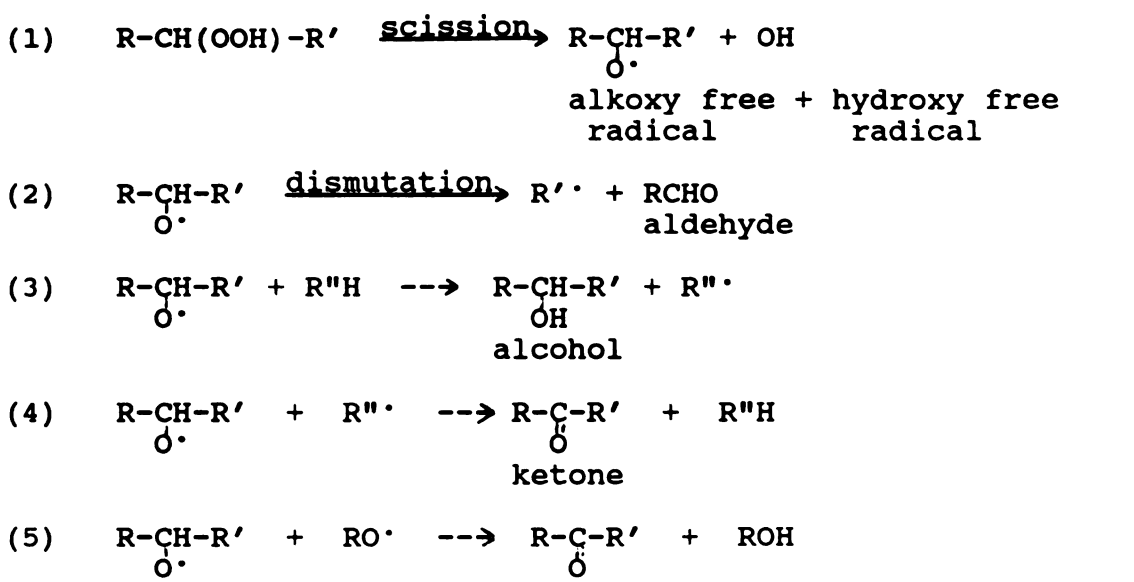


Figure 2. The general decomposition of hydroperoxides into aldehydes and ketones (Paquette et al., 1985).

Abstraction of a hydrogen atom from another molecule can produce an alcohol and a new free radical (3). Free radicals formed from reactions (2) and (3) may participate in the propagation of the chain reaction, or react with each other to form non-radical products which terminate the chain reaction. Ketones are formed from this type of reaction between an alkoxy radical and another simple free radical (4) and (5) (Paquette et al., 1985). Hydroperoxides themselves are odorless, but their decomposition to low molecular weight aldehydes, ketones, alcohols and acids may result in rancid odors and flavors. Many of the small molecular weight compounds formed have very low odor threshold values making them objectionable in the parts per billion (ppb) range. The development of fat rancidity in

complex food systems is also greatly affected by the interactions of proteins and amino acids with lipid oxidation products (Frankel, 1984).

Major products of lipid oxidation in beef

Hexanal is one of the major secondary products formed during the oxidation of linoleic acid (Gaddis et al., 1961; Frankel et al., 1981). It contributes a coarse, green plant-like aldehyde aroma note (Barbut et al., 1985). Initially linoleic acid oxidizes to two major hydroperoxides, 9-hydroperoxy-10,12-octadecadienoic acid (9-HPOD) and 13-hydroperoxy-9,11-octadecadienoic acid (13-HPOD). The 9-HPOD and 13-HPOD account for 95-98% of the peroxides formed due to the stabilization of the conjugated system, and they are present in equal amounts. The 13-HPOD breaks down to form hexanal and 9-HPOD to 2,4-decadienal (Figure 3). Recent studies by Schieberle and Grosch (1981) have revealed that not only the 13-HPOD, but also all compounds with a double bond in the w-6 position, such as 9-HPOD, 2-octenal and 2,4-decadienal, produce hexanal during autoxidation. Their results also showed that in a mixture of 2,4-decadienal and hexanal, hexanal degraded significantly slower than the unsaturated aldehyde. These results help explain why hexanal predominates among the volatile aldehydes arising from linoleic acid autoxidation.

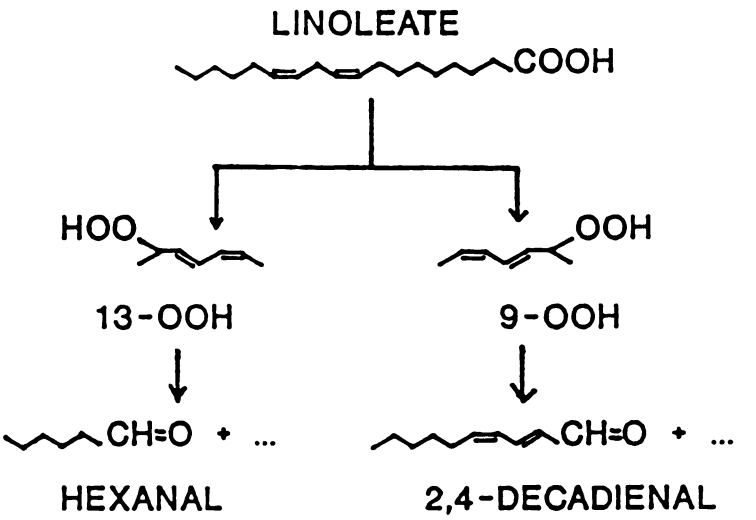


Figure 3. Autoxidation of linoleic acid.

Hexanal production has also been studied in meat systems. Cross and Ziegler (1965) measured both hexanal and pentanal in cooked, uncured pork. Hexanal detected in headspace samples from oxidizing PE and cooked meat increased with increasing oxidation (Love and Pearson, 1976). Shahidi and coworkers (1987) found that hexanal content, sensory scores and TBA numbers of cooked ground pork were linearly related. St. Angelo and coworkers (1987) reported a similar correlation of hexanal content with TBA numbers and sensory scores in cooked beef samples. Of more than 150 compounds identified in roast beef volatiles by gas chromatography/ mass spectrometry (GC/MS), hexanal was the one which always had the highest intensity (St. Angelo et al., 1987). These results indicate that hexanal is a major component of uncured meat volatiles, and may be useful as an indicator of the degree of lipid oxidation.

Several other secondary reaction products have been associated with the cardboard, rancid, stale, metallic flavor descriptors associated with WOF. St. Angelo et al. (1987) reported that 2,3-octanedione and total volatiles as well as hexanal, were significantly correlated to sensory scores and TBA values of roast beef. They also identified 2,4-decadienal and pentanal as major volatiles. Heptanal and n-nona-3,6-dienal were reported as the major flavor compounds related to WOF in turkey (Ruenger et al., 1978). Formation of short chain hydrocarbons by lipid oxidation,

especially pentane, has also been demonstrated (Evans et al., 1969; Seo, 1976; Seo and Joel, 1980).

Malonaldehyde is another major aldehyde produced during the autoxidation of polyunsaturated fatty acids. A mechanism for the formation of malonaldehyde, a three carbon dialdehyde, was proposed by Dahle et al. (1962) and modified by Pryor et al. (1976). The revised scheme involves a prostaglandin-like endoperoxide mechanism in which malonaldehyde is formed via the cleavage of a cyclic endoperoxide. Malonaldehyde is claimed to be an important biological breakdown product because of its ability to crosslink with the amino groups of proteins, enzymes and deoxyribonucleic acid (DNA) (Frankel, 1984). Malonaldehyde formation during autoxidation is also the basis for the thiobarbituric acid (TBA) test, a chemical method of monitoring oxidation. The TBA test involves the condensation of two molecules of thiobarbituric acid with malonaldehyde to form a pink chromagen which can be quantitated by measuring its absorbance at 532 nm. Many variations of this method have been developed for various food systems, however, criticism has been expressed concerning the specificity of the reaction for malonaldehyde. These concerns will be addressed in more detail later in this review.

Along with flavor deterioration, color loss is a major concern to meat processors. Color is the primary attribute used by the consumer to judge both fresh and cured meats before purchase (Fox, 1987). Myoglobin, the major meat pigment, exists in three interchangable states: reduced myoglobin (purple), oxymyoglobin (red) and metmyoglobin (brown). The brown metmyoglobin (Metmb) is the predominant color form where low oxygen tensions are present and is caused by the oxidation of the iron of heme in myoglobin (Seideman et al., 1984). In the oxidation reaction of reduced myoglobin to Metmb, the iron molecule in the heme goes from the ferrous (Fe^{2+}) state to the ferric (Fe^{3+}) state. Ferric pigments are catalysts for lipid oxidation (Younathan and Watts, 1960) while free radical products of lipid oxidation are known to damage pigments. Thus, an interrelationship exists between lipid and pigment oxidation. Factors which influence the reaction rate by reducing the oxygen tension or causing dissociation of the oxygen from oxymyoglobin include high temperature, low pH, ultraviolet light, salt, low oxygen atmoshperes, aerobacteria and low oxygen permeability of packaging film. Several researchers have found that the use of antioxidants to prevent lipid oxidation also slows down the conversion of myoglobin to Metmb (Greene, 1969; Greene et al., 1971; Greene and Price, 1975; Benedict et al., 1975).

FACTORS INFLUENCING WOF DEVELOPMENT

Heme compounds as catalysts of lipid oxidation

Fox and Benedict (1987) provide a detailed history of the role of heme pigment catalysis of oxidation reactions beginning with the initial work by Robinson (1924). Early researchers (prior to the 1970's) believed Metmb (heme iron) was the major catalyst of lipid oxidation in cooked red meat (Younathan and Watts, 1959; Watts, 1962; Tappel, 1962; Liu, 1970a; Liu and Watts, 1970). The decomposition of hydroperoxides to generate free radicals is thought to be the most important mechanism involved in heme-catalyzed lipid oxidation. Tappel (1962) proposed that the most probable mechanism of catalysis involves the formation of a coordinate complex between a hematin compound and lipid hydroperoxide. The step is a one electron transfer (ferrous to ferric), and it is facilitated by delocalization of the electron on the porphyrin ring structure. Homolytic scission of the hydroperoxide bond (ROOH) then forms a free radical which can abstract a labile hydrogen from an unsaturated fatty acid and propagate the lipid oxidation reaction. This mechanism does not involve a change in the valence of the heme iron.

The valency of iron in the proposed catalytic species has been widely disputed. Younathan and Watts (1958) and Kaschnits and Hatefi (1975) reported that Fe^{3+} hemes were the active catalysts while Hirano and Olcott (1971) and

Greene and Price (1975) reported that both ferrous or ferric compounds may function as catalysts but the Fe^{3+} hemes may be necessary for rapid catalysis. Labuza (1971) disagreed with the theory that only Fe^{3+} hemes are capable of catalyzing lipid oxidation in meat and expressed the need for knowlege of the electron orbital structures. He suggested that the protein portion of hemoprotein molecules may cause steric hindrance of the iron, preventing it from catalyzing oxidation. The rapid oxidation in cooked meat would result from the denaturation of the protein portion of myoglobin and subsequent exposure of the iron to unsaturated fatty acids.

As demonstrated above, data regarding heme catalyzed lipid oxidation is often contradictory and confusing. Love (1983) attributes this to the different types of model systems and techniques for following lipid oxidation. She states the need for carefully designed and controlled experiments to clarify the role of heme compounds in lipid oxidation. Also, it must be remembered that results obtained from model systems may not be representative of the reactions and mechanisms found in meat systems.

Non-heme iron and other metal catalysts

Sato and Hegarty (1971) were the first to propose that non-heme iron plays a major role in accelerating lipid oxidation in cooked meat, concluding that myoglobin is not directly

responsible for WOF development. They exhaustively extracted meat to remove the heme pigments then added back various heme fractions or pigments and found no catalysis of lipid oxidation. Love and Pearson (1974) and Igene et al. (1979b), working with similar model systems, confirmed the results of Sato and Hegarty (1971) and found that nonheme iron added to the muscle residue accelerated oxidation. In addition, EDTA added to a pigment extract before addition to a cooked beef residue resulted in reduced TBA values in the cooked beef residue. Igene and coworkers (1979b) also found that more than 90% of the iron in the fresh meat extract used was heme iron, but heating released some of the iron from its heme forms. The nonheme iron content increased from 8.7% of total iron in unheated extract to 27.0% in extracts heated to 70 C. Schricker and Miller (1983) and Chen et al. (1984a) have also reported the effects of final temperature, rate of heating and cooking method. Slow heating results in release of more nonheme iron than fast heating (Chen et al., 1984a). Braising and roasting methods cooked meat to a higher internal temperature and resulted in higher nonheme iron levels than a microwave method (Schricker and Miller, 1983). This result is consistent with the idea that microwave meat suffers less from WOF than meat cooked by slower conventional methods.

Fox and Benedict (1987) raised some questions with regard to the work of Sato and Hegarty (1971), Love and Pearson (1974)

and Igene et al. (1979b). Extraction and/or soaking periods up to twenty-four hours were required to completely extract the heme pigments, but apparently when the pigments were added back to the washed meat fibers the mixture was immediatly cooked. Fox and Benedict (1987) questioned whether or not sufficient time was allowed for the pigments to redistribute through the fibers. If the pigments were denatured on the surface of the fibers by cooking, the local concentrations of heme pigments would have been within the inhibitory concentration range. Also, in some of their experiments they added back heme pigments well above the minimum inhibitory range. Factors such as type of surfactant, surface tension, ionic strength and hydration spheres may also contribute to changes in oxidation rates at different pH values and may vary from system to system (Love, 1983).

Work by Wills (1965) showed that both heme and nonheme iron may act as prooxidants but that nonheme iron was the more active prooxidant at acid pH, while heme compounds were less pH sensitive. Liu (1970a,b) and Liu and Watts (1970) confirmed these results. They reported that in unsaturated fatty acid emulsions, ferrous iron and EDTA (1:1) accelerated oxidation at lower pH values but no catalysis took place above pH 6.4. Metmb was an active prooxidant in the pH range of 5.6 to 7.8. Ascorbate accelerated iron-catalyzed reactions at all pH values but inhibited heme

catalysis at pH 7.8 and had no effect at pH 5.6. Chelating agents inhibited Fe^{2+} catalysis, but not myoglobin catalysis (Liu, 1970a).

Rhee (1988) investigated the effects of adding Metmb and Metmb-hydrogen peroxide to water-extracted muscle residues. The rate of lipid oxidation was slow in the cooked muscle samples initially treated with Metmb alone, but was rapid in those treated with Metmb-hydrogen peroxide. These results support the theory that the lipid oxidizing activity of Metmb-hydrogen peroxide in cooked beef muscle residue system may be due largely to the nonheme iron released from Metmb by the action of hydrogen peroxide. However, they did not rule out the possibility of Metmb (heme iron) playing a role. In conclusion, it can be stated that more work needs to be done to clarify the roles of heme and nonheme iron in the catalysis of lipid oxidation, but in general, both forms can act as prooxidants with nonheme iron playing a greater role in cooked meat than in uncooked meat.

Sodium chloride (salt)

Sodium chloride (salt) is added to processed meats for its sensory, functional and preservation properties. In restructured meats, salt is critical for extracting the salt-soluble proteins which provide the cohesiveness of the meat particles in the finished product. However, it is widely recognized that sodium chloride may initiate

undesirable flavor and color changes in meat, and the mechanism of action remains poorly understood. Many researchers have reported the promotion of lipid oxidation by sodium chloride in restructured and ground meat systems (Schwartz and Mandigo, 1976; Govindarajan et al., 1977; Booren et al., 1981; Huffman et al, 1981; Chen et al., 1984b).

Ellis and coworkers (1968) investigated the mechanism of salt-induced rancidity and reported that increasing levels of salt accelerated autoxidation but did not alter the decomposition of hydroperoxides to monocarbonyls. A lower conversion of hydroperoxides to monocarbonyls was observed in samples containing high proportions of lean tissue. They postulated that salt may activate a component in the lean which results in a change in the oxidation characteristics of adipose tissue.

Studies on salt are complicated by the fact that salt may contain traces of metal impurities which could serve as catalysts. However, Olson and Rust (1973) reported that rancidity (taste panel scores) may still develop in the fat of dry cured hams processed with a purified low-metal salt; but, the use of antioxidants with salt improved flavor scores. Further confusion results from reports that sodium chloride can act as an antioxidant under certain conditions. Mabrouk and Dugan (1960) observed that autoxidation of

aqueous emulsions of methyl linoleate was suppressed by increasing concentrations of dissolved salt in the system. They suggested that the inhibition might result from decreased solubility of oxygen in the emulsions.

Rhee (1988) reported that lipid oxidation-catalyzing activity varies among different chloride salts and summarized the work of several researchers. Results of these studies (Watts and Peng, 1947; Zisper et al., 1964; Rhee et al., 1983) clearly indicate that replacement of NaCl by KCl is an effective means for reducing NaCl-catalyzed lipid oxidation in processed meats. However, the amount of KCl that can be added is limited to low concentrations (less than 0.5%) because of its bitter taste. From the above observations it is evident that more work is needed to fully understand the mechanism of salt-catalyzed lipid oxidation (WOF development) in processed meat systems.

Processing/particle size reduction

In general, any process that disrupts muscle membrane integrity enhances WOF development. Particle size reduction by grinding, chopping, flaking or emulsifying all lead to membrane disruption of the meat tissue and subsequent incorporation of air or oxygen into the tissues. Membrane disruption also may result in release of free iron, enzymes, and membrane bound lipids. The membrane bound lipids are high in phospholipids and, because of their high content of

PUFAs, are especially susceptible to oxidative deterioration (Igene et al., 1980). The vulnerability of the membrane-bound PLs also explains why lean meat suffers from WOF, even though it has a low fat content.

Other processes and additives are also known to affect the development of WOF. Packaging can play a major role in extending the shelf-life of processed meats. Miles and coworkers (1986) compared vacuum packaging to conventional oxygen permeable wrapping (PVC overwrap) of restructured pork chops during refrigerated storage at 3 C for sixteen days. Vacuum packaging reduced discoloration scores and lowered TBA values on days 8, 12 and 16 indicating that vacuum packaging is an effective method of extending the shelf-life of restructured pork. Crackel and coworkers (1988) stored vacuum packaged restructured beef steaks at -20 C for up to 12 months and reported TBA values of 0.75 or lower, regardless of antioxidant treatment. This TBA value is near the sensory threshold for WOF. The effects of raw meat composition, frozen and refrigerated storage and cooking have been discussed previously.

PREVENTION OF LIPID OXIDATION AND WOF DEVELOPMENT

Restructured meat products have been shown to be highly susceptible to WOF development. Many studies have indicated that autoxidation can be retarded by the addition of compounds possessing antioxidant activity. These compounds

may function as free radical scavengers, chelating agents, oxygen scavengers or by stabilizing otherwise catalytic species. A great deal of research has been conducted on the effects of synthetic and natural antioxidants, phosphates, ascorbates, nitrites and Maillard reaction products in meat systems.

Synthetic phenolic antioxidants

Phenolic antioxidants can be divided into two groups, naturally occurring and synthetic (Pearson and Gray, 1983). The phenolic antioxidants work primarily by interrupting the free radical chain mechanism of lipid oxidation. They are able to donate a hydrogen atom to the free radical yet are themselves stable due to resonance forms as shown below:

$$ROO^{\cdot} + AH \rightarrow ROOH + A^{\cdot} \quad \text{or}$$
$$R^{\cdot} + AH \rightarrow RH + A^{\cdot} \quad \text{or}$$
$$RO^{\cdot} + AH \rightarrow ROH + A^{\cdot} \quad \text{and}$$
$$A^{\cdot} + X \rightarrow \text{non-radical}$$

where AH is the antioxidant and X is some other moiety, (Labuza, 1971). Protection is provided due to an increase in the induction period and a slowing of the reaction rate. The concentration of added antioxidant and the time of addition both are key factors in their effectiveness.

The most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiarybutyl hydroquinone (TBHQ) and propyl gallate (PG).

As early as 1964, Marion and Forsythe demonstrated the effectiveness of BHA in delaying autoxidation (TBA values) in raw, ground turkey meat stored at 4 C. Greene (1969) and Greene et al. (1971) demonstrated that BHA and PG inhibited color degradation in raw ground beef, and in combination with ascorbic acid were effective in preventing lipid and pigment oxidation in ground beef for up to eight days of refrigerated storage. Chen and coworkers (1984b) reported the effectiveness of BHA/BHT in inhibiting lipid oxidation in cooked meat both during cooking and upon subsequent storage at 4 C for two days.

Chastain and coworkers (1982) studied restructured beef-pork steaks processed with 0.75% salt and BHA, TBHQ, or BHA/TBHQ at the 0.02% level based on a fat content of 20%. Cooked steaks were held up to twenty weeks in frozen storage. The addition of antioxidants alone or in combination decreased TBA numbers, increased sensory scores for flavor and acceptibility, and decreased discoloration of the raw meat compared to salt control samples. BHA was best for preventing color degradation while TBHQ was more effective in retarding off-flavor development. A combination of the two was recommended for maximum protection against both types of oxidative changes. Miles and coworkers (1986) found similar results in restructured pork chops formulated to contain 25% fat, 0.5% salt, 0.03% BHA, BHT and citric acid, and stored for sixteen days at 4 C in the dark. Low

TBA values and discoloration scores in comparison to a salt control indicated that the antioxidants were effective in protecting refrigerated restructured pork chops from lipid oxidation.

Natural phenolic antioxidants

Current research has focused heavily on natural antioxidants due to growing concerns about the safety and carcinogenic potential of synthetic antioxidants and an overall consumer perception that natural is better. Also, the levels of synthetic antioxidants permitted in foods are tightly regulated by the USDA, and they are approved for use in only a limited number of meat products.

Tocopherols are natural phenolic antioxidants present in many plants and oil seeds. Their antioxidant activity has been investigated in restructured beef steaks and ground beef (Chen et al., 1984b; Miles et al., 1986; Crackel et al., 1988) and in fats and oils and model systems (Cort, 1974; Cort et al., 1978; Roozen, 1987) with mixed results. Chen and coworkers (1984b) demonstrated that alpha-tocopherol-coated salts provided only marginal antioxidant properties, perhaps because of the low level used. Miles et al. (1986) and Crackel et al. (1988) have both shown that tocopherols do provide significant antioxidant activity in restructured meat products. Tocopherols can act as both

prooxidants and antioxidants, depending on concentration (Labuza, 1971).

A number of plant extracts have also been shown to provide antioxidant activity through their flavanoid constituents (a major group of plant phenols). Flavanoids have been studied in plant extracts, soybean flour, and soy protein hydrolyzates by Pratt and Watts (1964), Pratt (1965), Hayes et al. (1977), and Pratt et al. (1981). Similarly, proteins from other plant sources have also been shown to contain natural antioxidants. Rhee and coworkers (1981, 1983) have extensively studied the antioxidative potential of food ingredients prepared from glandless cottonseed, peanut and soy proteins. When added to ground beef or incorporated into a covering gravy (3%), improved oxidative stability was noted.

Rosemary as a natural antioxidant

Many spices and herbs have been shown to possess antioxidant activity. The early pioneering efforts of Chipault and coworkers (1952, 1955, 1956) demonstrated that rosemary (Rosmarinus officinalis, L.) and sage (Salvia officinalis, L.) were particularly effective when added to prime steam lard held at 98 C. Gerhardt and Blat (1984) performed a comprehensive evaluation of the antioxidant activity of spices in pork fat (Table 1), once again, showing rosemary as having optimal activity. The use of whole spices and

herbs is limited by their sensory compatibilty with specific food products. Spice extracts and distillates are being developed and deodorized for use in a wide range of foods, and process patents have been filed for production of a rosemary extract (Berner and Jacobson, 1973; Chang et al., 1977; Bracco et al., 1981).

At least 45 compounds are present in a molecular distillate of rosemary (Loliger, 1983). Only about half of them have been identified. Many of the active compounds are very unstable in pure form making analytical work on identification of the key antioxidant components very complicated (Loliger, 1983). The first important antioxidant compound isolated from rosemary leaves was carnosol, a phenolic diterpene (Brieskorn et al., 1964). Wu and coworkers (1982) also isolated and identified carnosol from rosemary leaves and reported that it had activity similar to BHT when added at 0.02% to lard. Further research has resulted in the isolation of several other phenolic compounds as shown in Figure 4: carnosic acid, rosmanol, rosmarinic acid, rosmariquinone, and rosmaridiphenol (Inatani et al., 1983; Houlihan et al., 1984, 1985). Houlihan and coworkers (1984, 1985) reported that rosmaridiphenol and rosmariquinone were better antioxidants than BHA when tested in lard at 0.02%, with the former showing slightly greater activity and approaching the effectiveness of BHT.

Table 1. Antioxidant activity of various spices added to pork fat and subjected to an accelerated oxidation test[a]

Spice	Protection Factor[b]	Antioxidant Activity Group Rating[c]
Mace	4.10	Optimal
Rosemary	4.30	Optimal
Sage	4.00	Optimal
Thyme	4.60	Optimal
Nutmeg	2.59	Very Good
Ginger	2.11	Good
Clove	1.95	Good
Marjoram	1.72	Good
Turmeric	2.16	Good
Paprika	1.51	Good
Garlic	1.11	Neutral to slight
White Pepper	1.15	Neutral to slight
Cumin	1.19	Neutral to slight
Coriander	1.10	Neutral to slight

[a] Adapted from Gerhardt and Blat (1984).

[b] Protection factor is ratio of induction period of sample (pork fat + spice) to the induction period of the blank (pork fat).

[c] Optimal antioxidant effect, protection factor > 3.5; very good 2.5 - 3.5; good 1.5 - 2.5; neutral to slight 1.0 - 1.5.

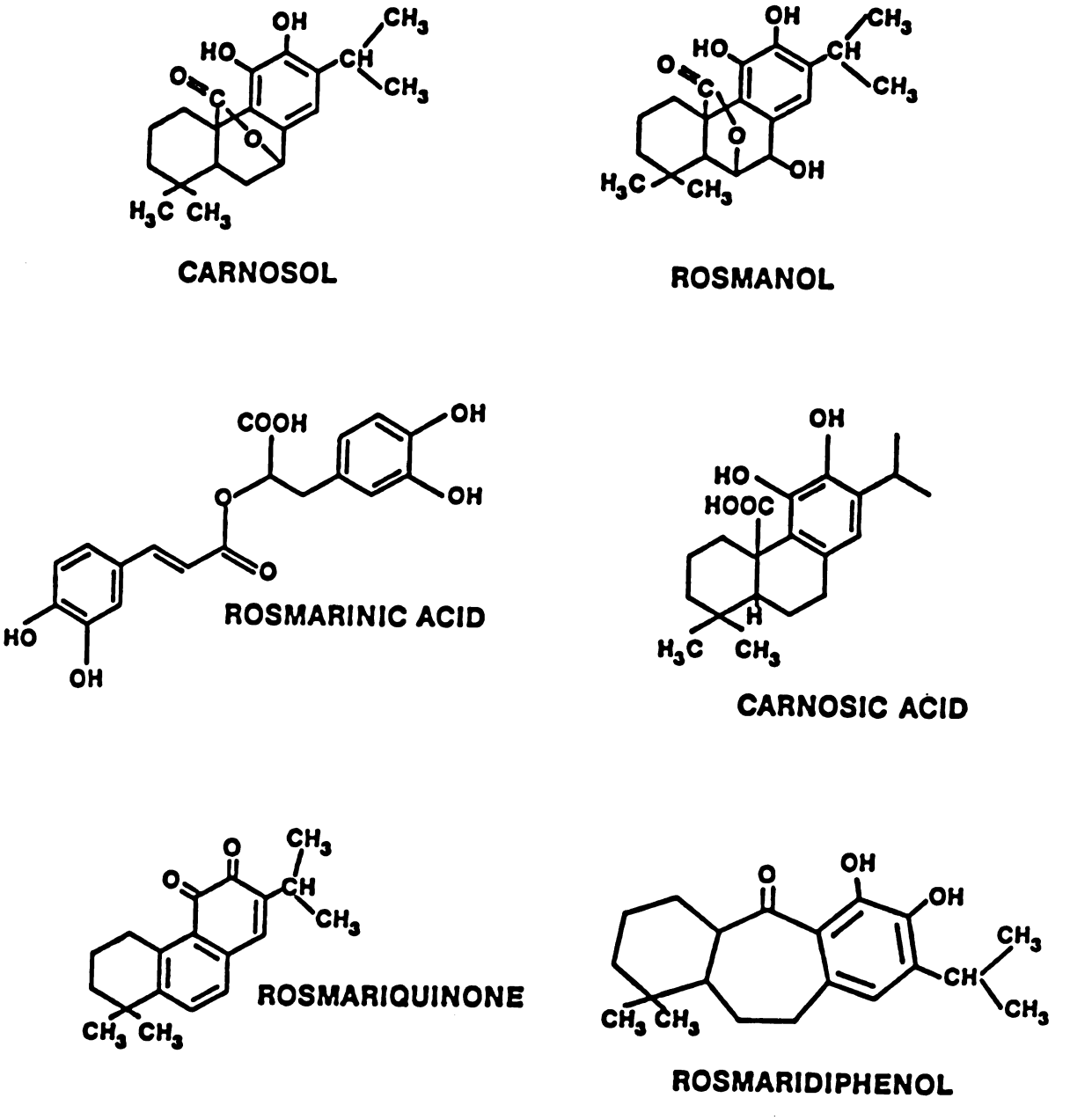


Figure 4. Structures of some antioxidant compounds in rosemary extracts.

The antioxidant activity of rosemary extracts has been evaluated in several food products as well as in accelerated oxidation tests using chicken fat and lard as substrates. Rosemary antioxidants (700 mg/kg) added to potato flakes provided satisfactory protection against oxidation as determined by pentane production, carotenoid loss and organoleptic evaluation (Loliger, 1983). Barbut and coworkers (1985) added 20 parts per million (ppm) rosemary oleoresin to turkey breakfast sausage prepared with 75% hand deboned turkey and 25% mechanically deboned turkey meat. Sensory aroma and taste scores and TBA numbers for samples stored at 4 C up to sixteen days demonstrated that oleoresin rosemary was comparable to a commercial blend of BHA/BHT/citric acid in suppressing lipid autoxidation. Korczak et al. (1988) tested several spices for antioxidant activity in precooked minced meat products stored under refrigerated and frozen conditions. Rosemary and sage used at levels of 0.1% and 0.5% based on fat content exhibited a pronounced antioxidant effect, while marjorum behaved as a prooxidant. In cooked restructured chicken nuggets stored at 4C for six days the addition of an oleoresin rosemary (0.1%) and sodium tripolyphosphate (0.3%) appeared to provide significant protection compared to a salt control but was somewhat less effective than a TBHQ/phosphate treatment (Lai, 1989).

Phosphates and other chelating agents

Phosphates perform several functions in processed meats. Their primary function is to increase the water binding capacity, thus resulting in higher cook yields and improved juiciness. Phosphates have also been shown to delay or prevent lipid oxidation in cooked meats (Tims and Watts, 1958; Marion and Forsythe, 1964; Sato and Hegarty, 1971; Shahidi et al., 1987). Phosphates act by chelating heavy metal ions such as iron and copper which are known prooxidants. This chelating ability is critical for eliminating the catalytic properties of Fe^{2+} which has been shown to be the major catalyst of lipid oxidation in cooked meats (Love and Pearson, 1974; Igene et al., 1979b).

Ethylenediamine tetraacetic acid (EDTA) is another chelating agent that presumably functions because it can sequester ferrous iron and copper. Liu and Watts (1970) demonstrated that EDTA prevented Fe^{2+}-catalyzed oxidation in raw beef, while Sato and Hegarty (1971) showed that EDTA (2.5 mg/g) suppressed lipid oxidation in cooked ground beef. Later, Igene and coworkers (1979a,b) found that the catalytic activity in a cooked meat pigment extract was significantly reduced by addition of 2% EDTA. These researchers concluded that EDTA effectively chelated free iron and thereby significantly reduced lipid oxidation in cooked meat. However, EDTA has not yet been approved for commercial use in meat products.

Citric acid has also been evaluated as an antioxidant in meat systems, but was found to be much less effective than phosphates or EDTA (Benedict et al., 1975). It is most commonly used along with other phenolic antioxidants to provide a synergistic effect. Sato and Hegarty (1971) reported minimal inhibition of lipid oxidation in cooked ground beef when sodium citrate was added to the level of 5 mg/g.

Other antioxidant compounds

Sodium nitrite is added to cured meat products to produce the characteristic cured meat color and flavor and to provide antibotulinal activity (Gray and Pearson, 1984). Nitrite is also an effective antioxidant, and four possible mechanisms of action have been proposed. Regardless of the exact mechanism of nitrite and/or nitrate in preventing oxidation and WOF development in meat, there is little doubt about its effectiveness in decreasing oxidation (Sato and Hegarty, 1971; Sato et al., 1973; Igene et al., 1979b, 1985b; Pearson et al., 1983).

Ascorbic acid at low levels (up to 100 mg/kg) catalyzes development of WOF (Tims and Watts, 1958; Sato and Hegarty, 1971). However, at high levels (1000 mg/kg and up) ascorbic acid retards autoxidation. Sato and Hegarty (1971) theorized that it shifted the balance between ferrous and

ferric iron or else acted as an oxygen scavenger. Several researchers have reported a synergistic activity between ascorbic acid and phosphates (Chang and Watts, 1949; Tims and Watts, 1958; Sato and Hegarty, 1971).

The antioxidant activity of Maillard reaction products (MRP) is well established. Zisper and Watts (1961) initially observed an antioxidant effect in over-cooked beef (110 C) and suggested that diluted slurries could be used to prevent rancidity development in normally cooked meat. However, the use of extracts from over-cooked, retorted or pressure-cooked meat may not be economically feasible unless low quality, inexpensive animal parts are used (Rhee, 1987). A comprehensive review on the role of MRP in WOF development has been written by Bailey et al. (1987).

Restructured meats

Restructuring refers to a group of procedures that reduces the particle size of the meat and then reforms the meat particles back into a shape that resembles an intact muscle (Seideman and Durland, 1983). The process allows effective use of trimmings and low-value primal cuts to produce a product of higher perceived value at a reasonable cost to consumers. Other advantages of restructured products are portion control, uniform quality of meat products and control of fat content. However, a severe drawback to the production of restructured meats is their increased

susceptibility to WOF development due to membrane disruption, exposure to oxygen, addition of salt and cooking.

Many methods for producing restructured meats have been developed and well summarized in the literature (Breidenstein, 1982; Seideman and Durland, 1983). Three basic steps are involved in restructuring: particle size reduction, blending the particles with other ingredients, and reforming to the desired shape.

Size reduction can be achieved by grinding, flaking, chunking or sectioning or combinations of these methods. The basic difference is the size of the particle achieved which in turn affects the textural properties of the finished products. It has been reported that flaking produces restructured products with improved texture, cohesiveness, juiceness and tenderness over those produced by grinding. Sectioning and forming uses larger muscle pieces and results in steaks having textural properties resembling intact muscle steaks (Seideman and Durland, 1983). The disadvantages to this process are that a lower fat content must be used because fat particles will be much more noticable, and uniform tenderness cannot be achieved due to the larger muscle sections.

Particle size reduction is followed by mixing or blending. Mixing is necessary for achieving uniform distribution of the additives and uniformity of lean/fat distribution. The mixing step is also critical for extraction of the salt-soluble muscle proteins needed for the binding of meat particles. Myofibrillar proteins, primarily myosin, are the major proteins responsible for binding (Macfarlane et al., 1977). Salt addition enhances and expedites the extraction of myosin and is generally regarded as essential for achieving the proper bind (Breidenstein, 1982). Salt is also known to improve the flavor, texture and/or juiciness ratings of restructured steaks and chops (Cross and Stanfield, 1976; Schwartz and Mandigo, 1976; Huffman et al., 1981). Generally, salt is expected to have an adverse effect on meat color, but there is some evidence that salt and/or sodium tripolyphosphates (STPP) added to meat may have a beneficial effect on color (Breidenstein, 1982). STPP also serves to reduce cooking losses and increase juiciness. Schwartz and Mandigo (1976) reported a synergism between salt and STPP on cooking loss, raw color scores, and several sensory characteristics. They conclude that 0.75% salt and 0.125% STPP were most desirable for use in producing restructured pork.

In the final processing step after blending, the meat-protein exudate mixture is stuffed into casings or extruded into the preliminary shapes referred to as logs. The logs

are frozen, then tempered to -3 C to -5 C and hydraulically pressed into the final shape. Individual portion control servings can then be sliced from the shaped log.

METHODOLOGY FOR MEASUREMENT OF LIPID OXIDATION

Many chemical, physical and sensory methods have been developed for following oxidation in oils and lipid-containing foods. Comprehensive reviews by Gray (1978) and Melton (1983) cover a wide range of methods. This review focuses on several methods widely used for measuring lipid oxidation in meat products.

TBA test The 2-thiobarbituric acid (TBA) test has become the most widely used method for monitoring the extent of oxidative deterioration in meat products (Gray, 1978; Melton, 1983). Malonaldehyde (MA) is a naturally occurring secondary oxidation product found in meat products containing oxidizing unsaturated fatty acids. The test is based on the condensation reaction between two moles of TBA and one mole of MA to form a red complex (Sinnhuber and Yu, 1958). Spectrophotometric measurement of the red color at 530 to 532 nm allows the extent of lipid oxidation to be reported as mg malonaldehyde/kg of sample or TBA number.

Although the TBA test provides a good general indicator of lipid oxidation, several problems and concerns about the specificity and reproducibility of the method exist. There

is evidence that the reaction is not specific for malonaldehyde; other products such as alka-2,4-dienals and 2-alkanals also react with TBA to form a red complex with the same absorption maximum (532 nm) (Jacobson et al., 1964; Marcuse and Johansson, 1973). Also, the amount of MA in an oxidizing sample depends on the fatty acid profile since PUFAs with three or more double bonds produce more MA than mono or diunsaturated fatty acids (Pryor et al., 1976; Pearson et al., 1983). A second problem is the ability of MA to crosslink with amino acids through vinylogous amidine linkages (Nair et al., 1986). Additionally, MA may crosslink amino acids and proteins with nucleic acid bases and other compounds or may transfer the MA moiety from one to another (Pearson et al., 1983; Nair et al., 1986). These crosslinking reactions are primarily of concern in relation to the reported toxicity, carcinogenicity and mutagenicity of MA (Nair et al., 1986).

A third problem with the TBA assay involves sample autoxidation during preparation, extraction or distillation, and heating steps which can produce artificially high TBA numbers (Melton et al., 1983; Pikul et al., 1983). Rhee (1978) reported that chilled blending and addition of propyl gallate (PG) and EDTA to distillation mixtures substantially reduced the TBA numbers of catfish samples but did not show any significant effect on beef, pork or chicken samples. Pikul and coworkers (1983) reported that fat extracted from

chicken breast and leg meat without added BHT during the TBA analysis had six times higher MA content than those treated with BHT during extraction. Therefore, these workers (Rhee, 1978; Pikul et al., 1983) recommended the addition of suitable antioxidants during sample preparation or before the critical heating step of any TBA assay to prevent sample autoxidation and erroneously high TBA numbers.

A final factor which affects results of the TBA test is the use of various methods to do the test. The test can be performed directly on the product followed by extraction of the red pigment (Sinnhuber and Yu, 1958) or on a portion of the steam distillate of the food (Tarladgis et al., 1960) or on an extract of the food (Witte et al., 1970). The steam distillation method is most frequently used in poultry, beef and pork products (Melton, 1983). In general, the TBA numbers obtained by the distillation method are higher than those determined by extraction methods (Witte et al., 1970; Siu and Draper, 1978). The original TBA distillation method has been modified by several researchers. Tarladgis et al. (1964) demonstrated that the usual acid and heat treatment was not necessary for the condensation reaction of TBA with MA or for maximum color development. These results were confirmed by Crackel et al. (1988) who developed a distillation method using an aqueous TBA solution. The aqueous method helps to reduce the appearance of an interfering peak at 450 nm (Tarladgis et al., 1964). Many

other modifications exist for specific food systems and make comparison of results from different laboratories difficult.

When using TBA numbers, results should only be used to assess the extent of lipid oxidation and establish trends rather than to quantitate malonaldehyde specifically. For this reason several researchers are now referring to the results as TBARS values (thiobarbituric acid-reactive substances) rather than just TBA numbers (Csallany et al., 1984; Igene et al., 1985a). This nomenclature will be used in the present study.

Attempts have been made to make the TBA test specific for MA. Kakuda et al., (1981) used high performance liquid chromatography (HPLC) to quantitate MA in aqueous distillates from freeze dried chicken samples. They reported a correlation coefficient of 0.946 between TBA absorbance values at 532 nm and the HPLC peak height of MA. Also, the HPLC method was faster, more sensitive and less affected by other side reactions. Csallany and coworkers (1984) developed another HPLC method to quantitate MA in rat livers and beef, pork and chicken muscle. They found MA levels measured by the TBA method were four to five times higher than those obtained by the HPLC method. Once again this was attributed to excess MA formed during the heating steps and/or to interfering color-reactive compounds. More recently, Schmedes and Holmer (1989) reported a new method

for selectively determining MA. They partitioned the MA and hydroperoxides in a Bligh and Dryer extraction. The MA was present in the methanol-water phase exclusively while the hydroperoxides were found in the chloroform phase. Therefore, the TBA test on both phases determined MA directly present in the sample versus MA formed during a ferric ion-catalyzed cleavage of hydroperoxides.

Gas chromatographic analysis

Many methods for analyzing volatile flavor compounds and secondary oxidation products using gas chromatography (GC) have been developed for a wide variety of oils and food systems. As discussed earlier, hexanal, a major secondary product of linoleate oxidation, is the predominant aldehyde identified in oxidizing meat systems. Many researchers have attempted to quantify hexanal levels using GC methods and relate them to other methods of analysis such as TBARS levels and sensory scores (Boggs et al., 1964; Cross and Ziegler, 1965; Fritsch and Gale, 1977; Barbut et al., 1985; Shahidi et al., 1987; St. Angelo et al., 1987).

Several GC methods have been developed for following lipid oxidation. A direct headspace gas-sampling technique was one of the first methods used. Volatiles are released from the food product by adding boiling water, tightly sealing the sample, and withdrawing headspace gas for GC analysis. Fritsch and Gale (1977) utilized this method for cereals

while Boggs et al. (1964) applied the technique to potato granules. With headspace gas analysis there is often insufficient concentration and only major peaks are identified.

A second GC method involves the solvent extraction of volatiles using a Likens-Nickerson extraction apparatus (Bailey et al., 1980). Volatiles released by boiling an aqueous homogenate of the sample are extracted into a suitable solvent in a closed, continuous extraction system. Conventional GC methods are then used to analyze the solvent containing the volatiles. Bailey and coworkers (1980) monitored lipid oxidation in roast beef stored at 4 C and observed an increase in hexanal, 2-pentyl furan and pentanal over time.

The development of porous polymer traps such as Tenax GC (p-2,6-diphenyl-p-phenylene oxide) for trapping volatiles has allowed successful collection of volatiles. Purge and trap methods are widely used. Basically, the sample is heated in a glass tube through which a stream of carrier gas flows to carry the volatiles onto a Tenax trap. The volatiles are then thermally desorbed from the Tenax and swept directly into the GC injection port. This method has been successfully used by Shahidi et al. (1987) for the hexanal analysis of cooked ground pork and by Selke and Frankel (1987) for the analysis of soybean oil volatiles. However

one weakness of the procedure is that some materials present are heat labile, and at the temperatures (120 C or higher) used to drive the volatiles onto the GC column there may be some formation of a more complex mixture (Waltking and Goetz, 1983).

A third method involves collection of volatiles on Tenax traps by either a purge and trap method (Olafsdottir et al., 1985; Barbut et al., 1985) or a vacuum extraction procedure (Vercellotti et al., 1987). Following collection, the volatiles are eluted with diethyl ether and injected into a GC to resolve the volatiles. Using this method, Barbut and coworkers (1985) identified hexanal as the compound primarily responsible for the aroma of oxidizing turkey sausage.

A direct GC method as an alternative to solvent extractions was developed by Dupuy et al. (1977). Many modifications of this external closed inlet device (ECID) (Scientific Instruments, River Ridge, LA) have been made making it suitable for nearly any application. Volatiles are first collected on Tenax traps. For oils, the oil can be directly applied to traps packed with glass wool. The trap containing the sample is then placed into the ECID which is interfaced to the GC injection port. The carrier flow is diverted through a heated chamber containing the trap, and the volatiles are thermally desorbed for 3-5 minutes (Dupuy

et al., 1985) St. Angelo and coworkers (1987) have successfully utilized a vacuum extraction system followed by ECID injection to study WOF development in beef. Hexanal and 2,3-octanedione (0.19 to 11.16 ppm) as well as total volatiles showed a highly significant degree of correlation when compared to sensory scores (2.1 to 5.0 on a 10 cm line) and TBARS values (3.75 to 14.71).

Tenax GC is widely used as the adsorbent in meat flavor studies (Galt and MacLeod, 1984). It has a very high affinity for organic compounds and adsorbs them reversibly. It is relatively hydrophobic, which is important in view of the large volume of water vapor produced on heating meat. It is preferred for applications where high boiling compounds are of interest, and it has a high temperature limit (375 C) compared to other porous polymers such as Porapaks and Chromosorbs. Also, it has been reported to give very high recoveries upon desorption. Artifactual formation must also be considered. The temperature employed for regeneration of, concentration on, and desorption from a porous polymer should not induce the development of artifacts which may be incorrectly identified as constituents of the product. Lewis and Williams (1980) observed compounds such as benzaldehyde, acetophenone, isopropylbenzyl alcohol, phenol and furans when polymers were held above their normal temperatures.

Sensory evaluation

WOF is a sensory phenomenon and any chemical and physical methods to quantify it must be conducted in combination with sensory analysis. TBARS values are not specific for WOF but are indicative only of oxidation (Sato and Hegarty, 1971; Sato et al., 1973; Pearson et al., 1977). However, many previous studies using trained panelists have shown a significant correlation between TBA values and off-flavor formation in cooked meats (Tarladgis et al., 1960; Zisper et al., 1964; Wilson et al., 1976; Igene et al., 1979a; Shahidi et al., 1987; St. Angelo et al., 1987).

Tarladgis et al. (1960) reported a high correlation between TBA numbers of ground pork and the sensory scores from panelists trained to detect rancid odors. They also reported a threshold level at which rancid odors were first perceived by panelists to be between a TBA value of 0.5 and 1.0. This range is still widely used in referring to a sensory threshold.

Igene et al. (1985a) investigated the relationship between TBA numbers and trained panel scores for WOF in cooked chicken white and dark meat. A statistically significant correlation coefficient of 0.87 was reported, and their results showed that TBA numbers accounted for over 75% of the variation in WOF. These and other researchers have concluded that TBA values can be used to indicate WOF aroma.

A study with untrained panelists produced different results. Greene and Cumuze (1981) used 52 inexperienced sensory panelists to rate a series of ground beef samples for intensity of oxidized flavor. Correlation coefficients between TBA values and sensory scores were reported as significant but low, and variability in panelist scoring was cited as a cause. Correlation coefficients were higher for the 28 out of 52 panelists who were determined statistically to be consistent in their scoring of the samples. No threshold level of detection could be established for the entire group, but for the discriminating panelists it was 0.6 to 2.0 which is fairly close the the range of 0.5 to 1.0 reported by Tarladgis et al. (1960).

Although there is agreement over the need for a trained sensory panel to evaluate WOF, there is a wide variation in reported methods. Some of the variations and inconsistencies include: use of 4 to 6 category scales and 3 to 10 trained or untrained panelists; evaluation of flavor, aroma or both; lack of precise procedural details; and terminology variation, ie. WOF/odor, rancid flavor/odor or off-flavor/odor (Poste et al. 1986). In many studies a great deal of panelist to panelist variation can also be found. Another difficulty encountered is the ability to discriminate between rancid flavors/odors and WOF/odor. Most studies report sensory scores for intensity of WOF or

rancidity as a single attribute. If sensory panels are to be effectively used in elucidating the mechanism of WOF more consistency in training and more detailed descriptors must be used.

Development of appropriate descriptors is a problem common to all sensory studies in which intensity is evaluated by trained panelists (Love, 1988). Poste and coworkers (1986) noted three phases of flavor changes discerned by a sensory panel during training to evaluate precooked stored pork. These were described as fresh-cooked, warmed-over (an intermediate stage, not yet rancid) and rancid (extremely oxidized flavor).

Johnson and Civille (1986) developed a descriptive language for evaluating WOF in meat. They proposed a list of terms and definitions for describing flavor changes in precooked, stored and reheated beef. The list includes character notes describing fresh, intermediate and extreme off-flavor in beef. The study was limited to beef because in preliminary work panelists found that WOF was equally identifiable in meat from different species (beef, pork, turkey and chicken) or different treatments (grilling, steaming, baking) within species, although the samples varied in intensity (Johnson and Civille, 1986). The descriptors used were cooked beef lean, cooked beef fat, browned, serum/bloody, grainy/cowy, cardboard, oxidized/rancid/painty and fishy as well as

sweet, sour, salty and bitter. Johnson and Civille (1986) also described a general pattern involved in WOF: the disappearance of the fresh flavors; the appearance then disappearance of the cardboard flavor; and the final dominance of other flavors by the oxidized/rancid note. They believe that a common descriptive vocabulary would benefit studies to examine causes and prevention of WOF by providing an established protocol that would allow comparison and evaluation of data from different labs.

Lyon (1988) investigated the sensory flavor change in precooked, stored, reheated chicken. A ten member highly trained panel (42 hours of training) evaluated samples for the following twelve attributes: chickeny, meaty, brothy, liver/organy, browned, burned, cardboard, warmed-over, rancid/painty, sweet, bitter, and metallic. Results indicate a loss of the chickeny, meaty, brothy, sweet and liver/organy notes in reheated samples on day zero. By day one there was a significant increase in cardboard, warmed-over and rancid/painty notes. Several multivariate statistical methods were used to further characterize the changes. They point out the importance of focusing at storage times of one day or less to direct chemical/analytical studies to uncover causal factors in flavor change at very early times in refrigerated storage of precooked meat with subsequent reheating.

Attempts have also been made to correlate data from GC analysis with flavor scores. In vegetable oils, the presence and concentration of certain volatiles have been correlated to flavor scores (Evans, 1969; Dupuy et al., 1977; Raghavan et al., 1989). Working with low fat foods such as cereals Fritsch and Gale (1977) reported the onset of rancid odor to occur when hexanal concentration increased to between 5 and 10 ppm. Bailey and coworkers (1980) found that hexanal and 2-pentyl furan were excellent indices of oxidation in meat products, but their contribution to WOF was not addressed.

More recently, researchers have attempted to correlate TBARS values, sensory scores and volatile aroma/hexanal levels. Shahidi et al. (1987) indicated that these parameters were linearly interrelated in cooked ground pork stored at 4 C. The correlation coefficients reported between TBARS values and relative hexanal levels, between sensory scores and relative hexanal levels and between TBARS values and sensory scores were 0.995, 0.981, and 0.986, respectively. However, the statistical methods used to calculate these correlations were not specified. Lai (1989) pointed out that due to strong interactions between treatments and storage times, correlation coefficients should be calculated by pooling information within treatments and storage times. This resulted in statistically significant but much lower correlation coefficients in restructured chicken nuggets

than when calculations were done by simply pairing the individual observation over treatments and storage times.

St. Angelo and coworkers (1987) conducted a detailed study to try and relate fundamental chemical and sensory changes observed in meat with character notes that may contribute to WOF. The trained panel used the descriptors outlined by Johnson and Civille (1986) and found that the character notes most used to describe WOF were cardboardy, rancid, stale and metallic. Total volatiles were analyzed using the ECID method previously discussed (Dupuy et al., 1977) with a GC coupled to a mass spectrometer. They found that of the many compounds which appear to be markers for following WOF development, hexanal and 2,3-octanedione as well as total volatiles showed a highly significant degree of correlation when compared to sensory scores and TBA numbers ranging from 0.8 to 0.92.

From the results discussed above it is apparent that a multidisciplinary approach consisting of chemical and instrumental analyses coordinated with sensory evaluation is necessary to study WOF development. Further reasearch is needed to identify the volatiles responsible for WOF and to establish threshold levels for their detection by sensory panelists. Finally, work must continue in establishing a universal set of flavor descriptors for use by trained

sensory panels so that results can be compared between laboratories.

MATERIALS AND METHODS

MATERIALS

Beef Beef rounds, 168 NAMP, (1984) and armbone chucks, 126 NAMP (1984) were purchased from Ada Beef Inc. (Ada, MI) and used as lean and fat fractions respectively. The meat was purchased vacuum packaged within 48 hours of slaughter and processed within 24 hours of purchase. Replicate experiments were done over three consecutive months.

Antioxidants and other ingredients Tenox TBHQ was supplied by Eastman Chemical Products Inc. (Kingport, TN). The rosemary-coated salts (Curox 100 and Curox 200) were obtained from Diamond Crystal Salt Company (St. Clair, MI), the oleoresin rosemary being produced by Kalsec Inc., (Kalamazoo, MI). The sodium tripolyphosphate (STPP) was purchased from Stauffer Chemical Co. (Washington, PA).

Fatty acid methyl esters Individual fatty acid methyl esters and standard mixtures were purchased from Supelco (Bellefonte, PA) and Alltech Associates, Inc. (Deerfield, IL).

Reagents and solvents All reagents and solvents used in this study were reagent grade and/or HPLC grade with the

exception of propylene glycol (Fischer Scientific) which met USP/FCC specifications.

EXPERIMENTAL DESIGN

The primary objective of this study was to evaluate the effectiveness of an oleoresin rosemary (OR) as an antioxidant during refrigerated and frozen storage of restructured beef steaks. It was also desired to compare several methods of hexanal analysis and to establish relationships between TBARS values, sensory scores, hexanal levels and changes in fatty acid profiles.

Three replications of seven treatments were used in this study. Table 2 lists the content of each treatment and its code. WOF development in the restructured steaks was tested during refrigerated storage of cooked steaks and during frozen storage of raw steaks. One set of steaks was cooked to an internal temperature of 70 C (158 F) in a convection oven set at 177 C. The temperature was monitored by copper constantan thermocouples placed in the center of individual steaks. After cooking, the steaks were stored at 4 C until analysis on days 0, 2, 4 and 6 for TBARS levels and sensory scores. A group of steaks was also refrigerated raw for five days, cooked and analyzed for TBARS levels and sensory scores. For the frozen study, raw steaks were held at -20 C +/- 7 C and analyzed after 0, 3 and 6 months. At each sampling period, representative steaks were thawed, cooked

as described above and analyzed for TBARS values and sensory scores. Raw steaks were analyzed for TBARS values, hexanal levels and fatty acid profiles. Samples to be analyzed were ground through a 3/16" plate, mixed thoroughly and analyzed within 30 minutes.

TABLE 2. Seven Experimental Treatments

	TREATMENT[1]	CODE
1.	SALT 0.75%	SALT
2.	SALT + STPP 0.3%	STPP
3.	SALT + ROSEMARY 0.05%	OR1
4.	SALT + ROSEMARY 0.10%	OR2
5.	SALT + STPP + ROSEMARY 0.05%	OR1/STPP
6.	SALT + STPP + ROSEMARY 0.10%	OR2/STPP
7.	SALT + STPP + TBHQ 0.02%	TBHQ/STPP
8.	VACUUM PACKAGE # 7	REFERENCE

[1] Antioxidant concentrations were based on the fat content.

Manufacture of restructured steaks The beef rounds were trimmed of all exterior fat and connective tissue, vacuum packaged, and frozen overnight. The fat fraction from the lower rib portion of the chuck was crust frozen, run through a Hobart Steakmaster Tenderizer, model 403, then sliced into 1/4" X 1/8" X 1/2" cubes using a Hobart Food Slicer, model 410, (Hobart Mfg. Co., Troy, OH) and stored at -30 C overnight. On the day of processing, the lean fraction was ground through a 24 X 48 mm kidney plate. The lean and fat fractions were estimated to contain 7% and 65% fat, respectively. The meat was formulated into 20 pound meat blocks containing 15% fat. The lean fraction was placed

into a water-jacketed, pre-chilled Keebler mixer (Keebler, Inc., Chicago, IL) and mixed for eight minutes. The salt, STPP and antioxidants, either coated on salt or dissolved in propylene glycol, were added to the lean beef during the first thirty seconds of mixing. The fat fraction was then added and mixed in for an additional three minutes under vacuum. The blended samples were stuffed into fibrous, moisture-proof casings 3.45" in diameter (Viskase Corp., Chicago, IL) and frozen at -30 C. After freezing, the logs were portioned into 9 X 1.5 cm steaks with a meat saw. Each raw steak weighed approximately 95-100 g. A portion of the steaks was then cooked as described previously. The cooked or raw steaks were placed on styrofoam trays (2 or 6 per tray) and heat sealed but not vacuum sealed in polyethylene-laminated nylon pouches (3 mil). These bags have an oxygen transmission rate of 9 $ml/m^2/24$ hr at 4 C and were obtained from Koch (Kansas City, MO). After packaging, the raw steaks were stored for 6 months at -20 C and the cooked steaks were stored for 6 days at 4 C. It is important to note that these processing and packaging conditions were chosen to stress the system to obtain a good test of the antioxidant effectiveness. A similar study of restructured beef steaks packaged under vacuum revealed only minor changes in TBARS values and sensory scores over 12 months of freezer storage, and the effects of various antioxidants were not apparent (Crackel et al., 1988).

METHODS OF ANALYSIS

Proximate analysis Proximate analysis of the raw steaks was done after processing and included moisture, protein and fat content. Moisture content was determined using AOAC procedure 24.002 (1975). Duplicate 5 g samples were weighed into pre-dried aluminum dishes, then dried for 24 hours in a vacuum oven (25 psi). Protein content of triplicate samples was determined using a micro Kjeldahl unit consisting of a model 1016 Tecator Digestion System, a model 322 Buchi Distillation Unit and a Model 342 Buchi Control Unit (Brinkmann Instruments Inc., Westbury, NY). Total fat was determined using the dry column method of Marmer and Maxwell (1981). A 2 ml aliquot of the lipid extract was quantitatively transferred to a dry, pre-weighed vial and placed in a drying oven for 24 hours to drive off all the solvent. Samples were reweighed and the amount of total fat was calculated.

Thiobarbituric acid test (TBA test) The TBA distillation method of Tarladgis et al. (1964), as modified by Crackel et al. (1988) was used to measure the development of oxidative rancidity. The modified method uses an aqueous solution of TBA in place of the acetic acid/water reagent. Spectrophotometric measurements were made with a Bausch and Lomb Spectronic 2000. A conversion factor of 6.2 was used to calculate TBARS values as mg malonaldehyde/kg meat (Crackel et al., 1988). To help prevent further lipid

oxidation during analysis, TBHQ dissolved in propylene glycol was added to the meat at the 0.01% level during mixing.

Lipid extraction and fractionation The dry column method of Marmer and Maxwell (1981) was used for simultaneous extraction and class separation of the raw steak lipids into neutral and phospholipid fractions. Duplicate 5g meat samples from each of the 7 treatments were analyzed after 3 and 6 months of frozen storage. At month 0, all treatments were considered equal and only one sample from each was analyzed and used to calculate an average.

Columns were prepared by inserting a glass wool plug into the tip of a glass column (3.5 X 50 cm) and adding a bed of 10 g of a $CaHPO_4 \cdot 2H_2O$/Celite 545 (9:1) mixture and tamping it down firmly. A 5g ground meat sample was added to 20g of anhydrous Na_2SO_4 and ground with a mortar and pestle. This mixture was reground with 15g of Celite 545 into a uniform free-flowing powder and quantitatively transferred to the column and tamped into place. The mortar, pestle and other utensils were rinsed with dichloromethane to remove trace amounts of lipids. The rinsings were added to the column along with enough dichloromethane to wet the entire column bed (approximately 40 ml). The column was then charged with 150 ml of dichloromethane, and the neutral lipid fraction was collected in 500 ml round bottom flasks. When the last

of the dichloromethane reached the top of the column packing, the column was charged with 150 ml of dichloromethane/methanol (9:1) and the receiving flasks were changed to collect the phospholipid fraction. This fraction was collected until the column was stripped of solvent. Both fractions were concentrated to 25 ml, quantitated and used for fatty acid determinations.

Fatty acid analysis The neutral lipid fractions were methylated according to the boron trifluoride-methanol procedure of Morrison and Smith (1964). The phospholipid fractions were methylatd using a room temperature KOH/MeOH procedure to help prevent formation of dimethylacetal (DMA) derivatives of long chain aldehydes (Maxwell and Marmer, 1983). The methylated samples were analyzed on a HP 5840A gas chromatograph equipped with a flame ionization detector and a HP 5840A integrator. The injection port temperature was 200 C, the detector temperature was 300 C and the nitrogen carrier gas flow rate was 25 ml/min. The fatty acid methyl esters were separated on a glass column (3m X 2mm id) packed with 10% SP-2330 on 100/120 Supelcoport (Supelco, Bellefonte, PA). The GC was programmed from an initial temperature of 150 C held for 1 minute, then increased at 1.5 C/min to 225 C for 10 minutes. The component fatty acids were identified by comparing retention times with those of known fatty acid methyl ester standards

assayed under identical conditions. Results were calculated and reported as percent of total.

Volatiles analysis Analysis of hexanal content in the restructured beef steaks was done using a purge and trap method after 3 and 6 months of frozen storage. A Tekmar Dynamic Headspace Concentrator, model 4000, with a Capillary Interface, model 1000. (Tekmar Company, Cincinnati, OH) was used for the isolation of direct vapor or headspace volatile constituents. One gram of a 10% meat slurry was weighed into a 25 ml sample holder along with an additional 5 ml of water and 2-heptanone as the internal standard. The sample holder was attached to the Tekmar and placed in an 80 C water bath. The sample was purged for 12 minutes with purified helium gas at a flow rate of 40 ml/min, and volatiles were collected on a Tenax trap (12 X 1/8 in) held at room temperature. To desorb the volatiles, the Tenax trap was heated to 180 C and held for 6 minutes. The desorbed volatiles were concentrated at the top of the capillary column at the capillary interface which was cooled to -150 C with pressurized liquid nitrogen. Following desorption, the interface was flash-heated for 10 seconds to inject the volatile components directly onto the capillary column. Sample lines and valves were heated to 120 C during the analysis. Between samples the Tenax trap was reconditioned by heating to 225 C for 10 minutes.

A HP model 5890 GC equipped with a flame ionization detector was used with a HP 101 25m X 0.2mm id fused silica capillary column to separate the volatiles. The oven temperature was initially cooled to 15 C for 15 minutes with dry ice then increased at a rate of 2.5 C/min to 40 C, then increased at 10 C/min to a final temperature of 215 C for 10 minutes. The flow rate of the helium carrier gas was 0.5 ml/min. Because the capillary column was connected directly to the capillary interface, the septum purge flow rate was not applicable. Injector and detector temperatures were set at 200 C and 300 C, respectively. Retention times and peak areas were integrated using a HP 3392A Integrator. Retention times of samples and standards were compared to identify hexanal. The hexanal content of the samples was estimated based on detector response to the internal standard, with corrections for 100% recovery of the internal standard.

Sensory evaluation

At each sampling period during refrigerated and frozen storage, cooked samples were evaluated by sensory panelists for degree of WOF. Volunteers participated in five or six initial training sessions which utilized triangle tests, group discussions and sample rating to familiarize them with WOF and oxidized flavor in beef and chicken and the rating method to be used. Panelists were selected for participation based on their performance and hence are

referred to as a trained panel. Mid-way through the storage study a reinforcement training session was conducted and a discussion held. A total of 11 panelists were used for the refrigerated study and 12 for the frozen study.

The samples were cooked, as previously described, approximately one hour before each taste panel. The seven treatments were randomly divided for morning and afternoon taste panels consisting of 4 and 3 samples respectively. The cooked steaks were cut into approximately 1.5 cm cubes and placed in 1 oz plastic cups with lids (Solo Cup Company, Urbana, IL) which could be reheated in a microwave oven immediately before serving.

At each session, panelists were presented with three or four coded samples and a reference sample (vacuum packaged TBHQ/STPP treatment) and asked to rate the intensity of WOF using a scale of six descriptors from no WOF to very strong WOF. Panelists were also asked to identify the presence or absence of non-meat flavors (ie. spice/rosemary) and comment on the perceived flavor. A sample form is shown in appendix A. Panelists were instructed not to rank the samples since the various antioxidant treatments could exhibit the same degree of oxidation. The six intensity descriptors were then assigned numbers from 0 (no WOF) to 5 (very strong WOF).

Hexanal analysis by vacuum collection/solvent extraction

Several problems were encountered in using the Tekmar headspace sampling unit for hexanal analysis. Therefore, alternative methods were evaluated. A vacuum collection/solvent extraction method was developed and evaluated during a study on restructured turkey rolls stored at 4 C for 48 hours. Results of the hexanal analysis were compared to TBARS values collected on the same samples.

Glass traps (84 X 9 mm) were packed with 200 mg of Tenax TA (60/80mesh) (Tekmar Company, Cincinnati, OH) between 1/2" plugs of silane treated glass wool. The traps were conditioned at 210 C under a stream of nitrogen (20- 30 ml/min) for 20-24 hours then capped and stored in a desiccator until used.

Ten grams of cooked restructured turkey were weighed into a 500 ml flat bottom flask to which 4 ml of aqueous 2-heptanone internal standard solution (66.4 ng) and 36 ml of distilled water were also added. The internal standard was prepared by adding 5 ul of 2-heptanone to 10 ml of methanol which was then brought to volume with distilled water to 1000 ml. The flask was fitted to a 24/40 Pyrex condenser topped with a rubber stopper fitted with a glass tube down the center. The Tenax trap was held in place between the glass tube and a 3-way stopcock connected to a vacuum pump with tygon tubing to maintain glass contact and prevent

interaction between volatiles and the plastic. Samples were held at 60 C +/- 4 C for 1.5 hours at 0.35 kg/sq cm.

The Tenax trap was charged with 2 ml of 2-methyl butane and centrifuged at approximately 500 rpm for 2 minutes to elute the volatiles. The traps were then re-extracted with 1 ml of 2-methyl butane and centrifuged for another 2 minutes. The extract was evaporated under nitrogen to 0.5 ml and a 2 ul sample was immediately injected onto the GC column using a pre-chilled 10 ul syringe.

A HP 5890 GC equipped with a flame ionization detector and a 50m X 0.32mm id Carbowax 20M fused silica capillary column (Hewlett-Packard Co., Avondale, PA) operated with helium carrier gas at 0.5 ml/min was used. A split ratio of 3:1 and a temperature program of 40 C for 10 minutes followed by 3 C/min to 80 C and 10 C/min to 200 C for 5 minutes were used. Injector and detector temperatures were 225 C and 275 C, respectively. Hexanal was identified and quantitated as discussed for the dynamic headspace volatile system, with the exception that all hexanal standards as well as samples were corrected to 100% recovery based on the internal standard due to variation caused by the vacuum system.

<u>Statistical analysis</u> The experiments were designed as either three-factor (treatment X time X replication) or four-factor (treatment X time X panelist X replication)

completely randomized blocks with balanced data. Means, standard errors and correlation coefficients were calculated using the MSTATC microcomputer statistical program (Michigan State University, 1989). Bonferroni t-statistics were used to make specific contrasts between treatments. Interactions of main effects and correlations between sensory scores, TBARS values and hexanal levels were performed as described by Gill (1978).

RESULTS AND DISCUSSION

LIPID STABILITY IN RESTRUCTURED BEEF STEAKS

The major objective of this study was to evaluate the effectiveness of an oleoresin rosemary (OR) as an antioxidant during refrigerated and frozen storage of restructured beef steaks. Second, it was desired to establish a relationship between TBARS values, sensory scores, hexanal levels and changes in fatty acid profiles as a means for assessing WOF development. Finally, alternative methods for quantitative analysis of hexanal levels as an indicator of lipid oxidation were evaluated.

Proximate analysis

Restructured beef steaks were manufactured as previously discussed. Moisture, fat and protein contents of each treatment and replication were not affected by the antioxidant treatments (Table 3). Moisture and fat content remained relatively constant throughout the study, however, the target lipid content of 15% was not always achieved. One reason for this was that the meat blocks were formulated based on estimated lipid contents of the lean and fat fractions. For the second and third replications of the experiment, slightly larger fat fractions were used, therefore, explaining the apparent increase in fat content for the second two replications. Also, unavoidable loss of

Table 3. Chemical composition of restructured beef steaks.

Treatment[1]		Component		
		Moisture (%)[2]	Fat (%)[2]	Protein (%)[3]
Salt	1	67.05	11.61	20.56
	2	67.09	13.08	18.09
	3	66.21	16.40	18.54
STPP	1	67.30	11.52	19.31
	2	66.60	13.60	18.64
	3	64.75	14.66	18.15
OR1	1	67.55	11.97	19.26
	2	67.45	12.07	18.63
	3	68.29	13.16	19.90
OR2	1	67.35	12.73	19.67
	2	66.88	12.98	19.55
	3	64.08	16.39	19.57
OR1/STPP	1	68.00	11.14	19.90
	2	65.17	16.01	18.95
	3	65.64	13.40	19.02
OR2/STPP	1	68.38	10.01	19.69
	2	66.12	13.07	19.77
	3	65.32	15.94	18.55
TBHQ/STPP	1	65.92	14.02	21.40
	2	65.70	13.42	19.25
	3	67.19	14.11	19.33
Mean		66.57	13.39	19.32
S.E.		1.17	1.76	0.79

[1] Samples were taken from each of three replications within each treatment group.

[2] Analyses were performed in duplicate.

[3] Analyses were performed in triplicate.

small amounts of fat on the sides and blades of the vacuum mixer could contribute to low fat levels.

The fatty acid composition of neutral lipid (NL) and phospholipid (PL) fractions immediately after processing are summarized in Tables 4 and 5. The values shown in the tables were calculated as area percentages of total fatty acids identified with lipid standards including the unknown peak between C17:0 and C18:0. This peak may be C17:1 but no standards were available to confirm its identity.

The triacylglycerol fatty acid profiles obtained were similar to those found in the literature (Hornstein et al., 1967; Eichhorn et al., 1985), and especially to those reported by Marmer et al. (1984). The results of this study and that of Marmer et al. (1984) who also used the dry column method for lipid extraction and class separation, demonstrate the high reproducibility of dry column methodology. The discrepancies that did occur involved the presence of C15:0, C15:1, C16:2 and C17:1, none of which were found in this study. Igene et al. (1981) found a higher proportion of C14:1 and no C14:0, while the current data indicate approximately 3.3% C14:0 and only 1.1% C14:1. When comparing these data it is important to remember that the restructured beef steaks analyzed in the present study were formulated to 15% fat while the literature values reported are for intact muscle pieces. This will result in

Table 4. Fatty acid composition of the neutral lipid fraction from restructured beef steaks after processing.[1]

Fatty Acid[2,3,4]	Sample						
	SALT	STPP	OR1	OR2	OR1/ STPP	OR2/ STPP	TBHQ/ STPP
14:0	3.24	3.19	3.21	3.32	3.22	3.22	3.36
14:1	1.15	1.18	0.97	1.10	1.21	1.22	1.03
16:0	25.23	25.06	25.68	25.72	25.30	25.37	26.45
16:1	5.78	5.88	5.30	5.76	5.85	5.94	5.72
17:0	1.37	1.36	1.37	1.32	1.31	1.30	1.32
U	1.25	1.19	1.29	1.23	1.15	1.15	1.16
18:0	12.36	12.06	12.14	11.92	12.12	12.05	12.22
18:1	45.30	46.05	45.03	44.98	45.54	45.70	44.71
18:2	3.15	3.29	3.72	3.41	3.23	3.23	3.26
18:3	1.16	0.73	1.28	1.24	1.07	0.82	0.77
% SAT FA	42.20	41.68	42.40	42.28	41.94	41.94	43.35
% MUFA	52.24	53.10	51.30	51.84	52.60	52.87	51.45
% PUFA	4.31	4.03	5.01	4.65	4.30	4.05	4.03
% TUFA	56.55	57.13	56.31	56.49	56.90	56.92	55.48

[1] Values are expressed as percent of total and represent the mean of three experimental replications.

[2] U = unknown compound.

[3] PUFA includes the di-unsaturated fatty acids.

[4] SAT FA = saturated fatty acid MUFA = mono-unsaturated fatty acid
PUFA = polyunsaturated fatty acid TUFA = total unsaturated fatty acid

Table 5. Fatty acid composition of the phospholipid fraction from restructured beef steaks after processing.[1]

Fatty Acid[2,3,4]	Sample						
	SALT	STPP	OR1	OR2	OR1/ STPP	OR2/ STPP	TBHQ/ STPP
14:0	0.28	tr	0.32	0.23	tr	tr	tr
16:0	13.96	14.88	14.76	14.41	13.69	14.00	14.83
16:1	1.76	1.88	1.67	1.67	1.63	1.49	1.81
17:0	0.61	0.54	0.45	0.43	0.41	0.44	0.54
U	0.90	0.74	0.65	0.66	0.66	0.57	0.66
18:0	10.77	10.99	10.87	11.19	10.62	10.90	10.67
18:1	22.43	23.05	21.51	22.12	20.99	20.72	22.36
18:2	20.92	21.48	23.95	23.67	23.98	24.87	23.26
18:3	2.98	2.40	1.74	1.54	1.94	1.68	1.63
20:3	5.01	4.34	4.78	4.64	5.14	4.92	4.47
20:4	14.39	14.09	14.84	15.14	15.99	15.82	14.51
20:5	1.66	1.40	0.88	0.89	1.00	0.91	1.25
22:4	1.55	1.47	1.77	1.70	1.94	1.74	1.55
22:5	2.79	2.53	1.81	1.74	1.92	1.82	2.24
% SAT FA	25.61	26.65	26.40	26.26	24.85	25.50	26.28
% MUFA	24.19	24.93	23.18	23.79	22.61	22.20	24.17
% PUFA	49.29	47.70	49.76	49.31	51.89	51.74	48.90
% TUFA	73.48	72.62	72.94	73.09	74.50	73.94	73.07

[1] Values are expressed as percent of total and represent the mean of two experimental replications.

[2] tr = trace amounts detected and U = unknown compound.

[3] PUFA includes the di-unsaturated fatty acids.

[4] SAT FA = saturated fatty acid MUFA = mono-unsaturated fatty acid
PUFA = polyunsaturated fatty acid TUFA = total unsaturated fatty acid

some variation between results.

The PL fatty acid profiles shown in Table 5 are also very representative of previously reported values (Hornstein et al., 1967; Marmer et al., 1984; Eichhorn et al., 1985). Greater discrepancies were found between the results of Igene et al. (1981) and those of the present study. The C16:0, C16:1 and C18:1 contents reported by Igene and coworkers were lower than those in the present study. Slight variability was found among literature values depending on muscle type analyzed, animal sex and age at the time of slaughter, and animal feeding history. Eichhorn et al. (1985) reported that bull muscles contained about 5% more polyunsaturated fatty acids (PUFA) than steers and attributed this to differences in the fat to lean ratios. In the present study, large variation was found between the results of the three experimental replications. Hornstein et al. (1967) also reported a large variability in PL profiles from different animals. Discrepancies between the first replication and the second and third replications tended to negate any statistically significant changes over time, so only two of the three replications of the PL data were used for analysis.

Despite slight discrepancies between literature and experimental values for individual fatty acids, the proportions of saturated and unsaturated component fatty

acids in each lipid fraction are in close agreement with data from the literature (Igene et al., 1981; Marmer et al., 1984; Eichhorn et al., 1985). These researchers reported approximately 45% saturated and 55% unsaturated fatty acids in the NL fraction compared to 42.3% and 56.5% reported in the present study. The PL fraction was reported to have approximately 33% saturated and 67% unsaturated fatty acids in comparison to 25.9% and 73.4% reported in the present study. A larger proportion of PUFAs were found in the present study, especially C20:4. These findings were not unusual as Hornstein et al. (1967) and Terrell and Bray (1969) reported 6 to 16% arachidonic acid in beef PLs. This high degree of unsaturation partially accounts for the lability of beef PLs to oxidation, and their role in WOF development (Igene and Pearson, 1979).

Weight loss in restructured beef steaks due to cooking can be a serious problem, as seen in Table 6. The presence or absence of STPP is a major determinant in cook loss, a 29.6% loss being obtained in steaks processed without STPP compared to a 22.1% loss in steaks containing STPP. STPP is known to increase water binding capacity which in turn increases yield and improves juiciness scores. The mechanism by which phosphates improve water retention appears to involve two actions. First, the pH of the meat is increased and second, solubilization of muscle proteins occurs. In addition to increased water binding, other

Table 6. Weight loss of restructured beef steaks due to cooking.[1,2,3]

No STPP		STPP	
Treatment	Cook loss	Treatment	Cook loss
Salt	30.05 (1.25)	STPP	22.94 (1.15)
OR1	29.94 (2.79)	OR1/STPP	22.03 (1.68)
OR2	28.68 (2.62)	OR2/STPP	21.70 (1.70)
		TBHQ/STPP	21.80 (0.73)
Mean	29.56		22.11

[1] Values are expressed as % weight loss after cooking.

[2] Each value represents the average of six steaks/treatment/time period.

[3] Values in parentheses are standard deviations.

benefits associated with the use of STPP include improved flavor of meat as a result of retention of natural juices, decreased degree of WOF of reheated meat, reduction of oxidative rancidity, and improved color retention.

REFRIGERATED STORAGE OF COOKED STEAKS

Three replications of seven treatments (Table 2) were used in this study. Restructured beef steaks were cooked to an internal temperature of 70 C and stored at 4 C for analysis after 0, 2, 4 and 6 days. Packaging in oxygen permeable bags was designed to stress the system during storage. The influence of antioxidants on the stability of the lipids was evaluated using the TBA procedure and sensory evaluation.

TBA test and sensory scores

TBARS values were measured in duplicate using the distillation method of Tarladgis et al. (1964), as modified by Crackel et al. (1988). A sensory panel consisting of eleven trained members evaluated the restructured beef steaks at each sampling period for degree of WOF (0 = no WOF; 5 = very strong WOF). Analyses of variance (ANOVAs) were initially performed using data from all sampling points to determine the significance of treatment, time, and time/treatment interaction. It was found that the effects of treatments, time and time/treatment were all significant. Therefore, the effects of treatments were analyzed separately for each sampling period. A set of designed

contrasts was developed, and Bonferroni t-tests were used to establish if significant differences in TBARS values or sensory scores existed between treatments. TBARS values and sensory scores are shown in Table 7 and Figures 5 and 6. After 6 days of refrigerated storage, the control steaks (salt only) had the largest TBARS values and sensory scores. These observations support the widely recognized catalytic effect of salt on lipid oxidation (Schwartz and Mandigo, 1976; Booren et al., 1981; Huffman et al., 1981; Chen et al., 1984b). The effects of added STPP were significant ($p<0.01$) for days 2, 4 and 6 as is clearly demonstrated in Figures 5 and 6 (significance levels are summarized in appendix B). The four treatments processed with STPP showed very little increase in TBARS levels or sensory scores over 6 days while the other treatments processed without STPP, regardless of the presence of OR, oxidized very rapidly. These results agreed with those of Shahidi et al. (1987) who found STPP to be an effective antioxidant in cooked pork stored at 4 C. They reported that although the addition of BHA or TBHQ at 30 ppm had a further effect in reducing TBARS values and hexanal levels, the major effect was due to STPP and sodium ascorbate used in combination. STPP acts as an antioxidant by chelating metal ions in the system which catalyze the oxidation reaction. However, addition of STPP will also increase the pH of the meat system, possibly creating a more oxidatively stable system (Judge and Aberle, 1980). The presence of OR

Table 7. Mean TBARS values and sensory scores for cooked, restructured beef steaks during refrigerated storage.[1,2,3]

Treatment	Day 0		Day 2		Day 4		Day 6	
	TBA	Sensory	TBA	Sensory	TBA	Sensory	TBA	Sensory
SALT	1.34	1.19	5.67	3.27	10.40	4.30	11.30	4.31
STPP	0.29	0.31	0.66	0.68	0.58	1.05	0.84	1.42
OR1	0.76	1.06	5.30	3.26	8.65	4.43	9.73	4.00
OR2	0.56	0.86	5.00	3.49	6.60	4.19	9.90	4.36
OR1/STPP	0.27	0.47	0.38	0.83	0.47	1.05	0.40	1.31
OR2/STPP	0.27	0.42	0.47	0.73	0.64	1.36	0.97	1.36
TBHQ/STPP	0.28	0.45	0.27	0.59	0 40	1.12	0.37	1.06

[1] All values represent means of three replicated experiments.

[2] TBARS values (TBA) expressed as mg malonaldehyde/kg meat.

[3] Sensory scores: 0 = no WOF; 5 = strong WOF.

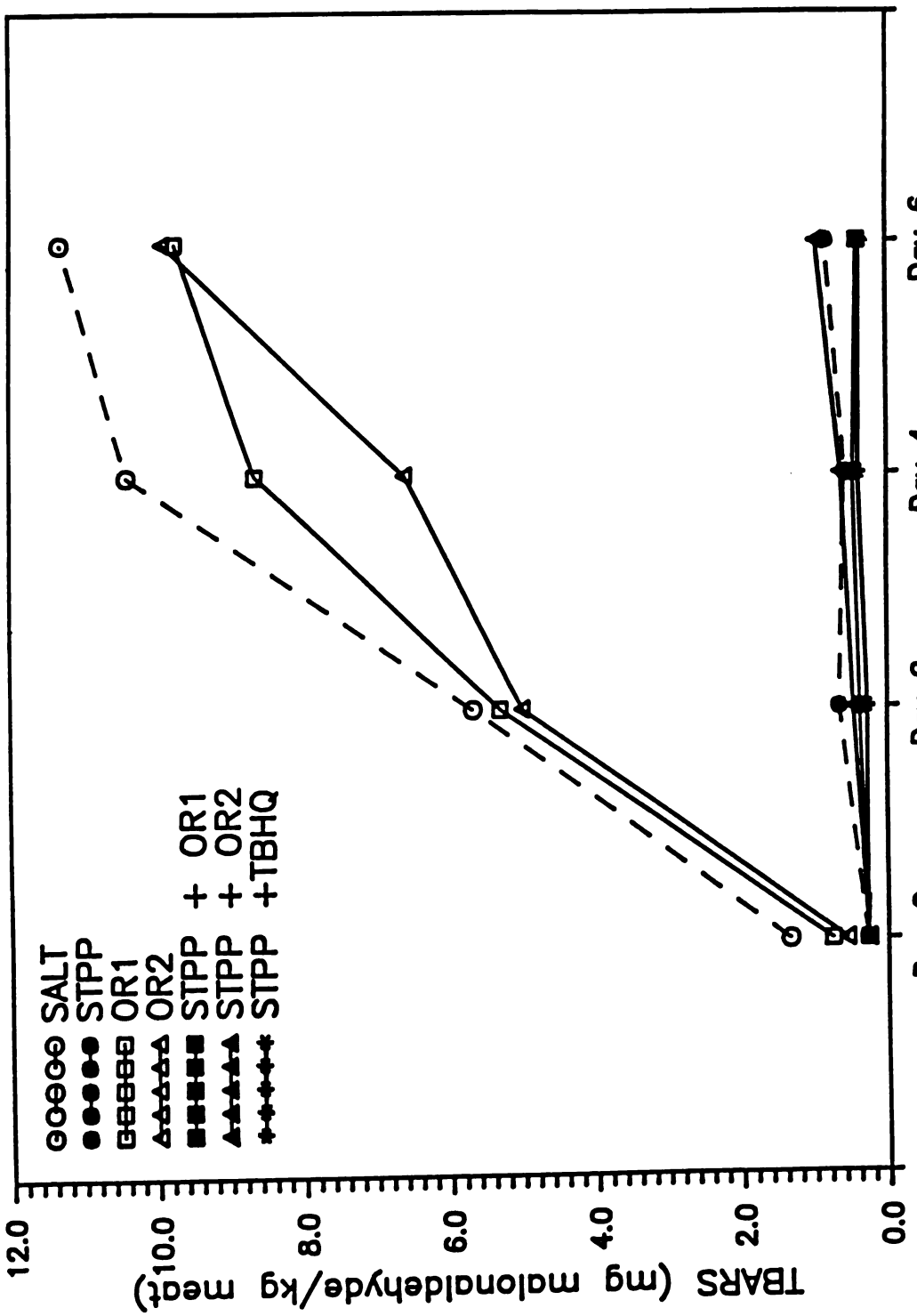


Figure 5. TBARS values of cooked, restructured beef steaks stored at 4°C.

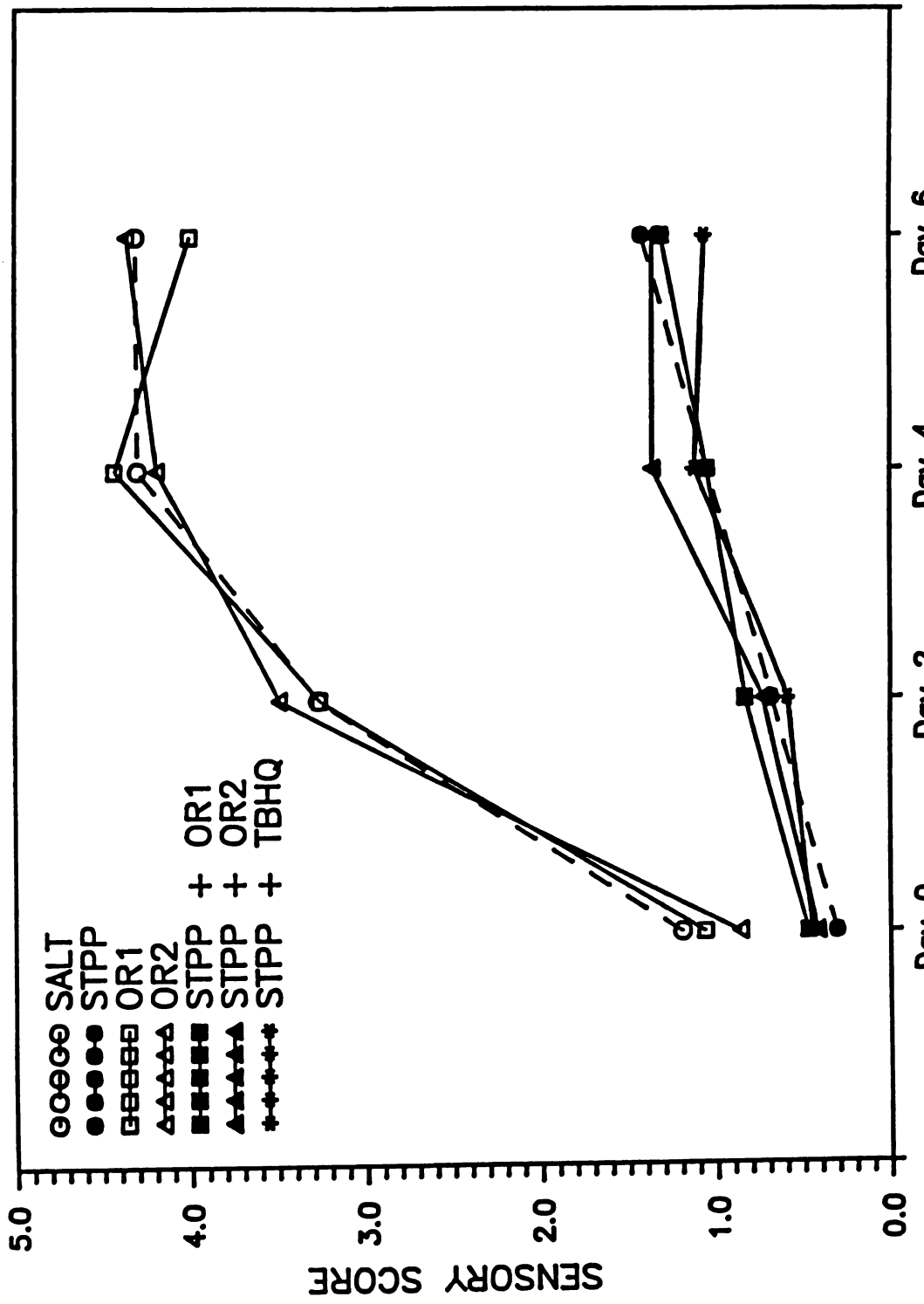


Figure 6. Sensory scores of cooked, restructured beef steaks stored at 4°C.

as well as any interactions between OR and STPP were not found to be significant at any storage time ($P>0.05$). There was no significant difference between the OR1/STPP or OR2/STPP treatments and TBHQ/STPP. However, since the OR alone was ineffective the antioxidant activity in these treatments is most likely due to the presence of STPP.

These results were somewhat unexpected since OR has been previously reported to provide antioxidant activity in meat systems. Korczak et al. (1988) reported that precooked pork balls processed with rosemary and stored at 4 C for 48 hours did not show signs of oxidation or flavor changes, while the control sample developed an undesirable, stale taste. Barbut et al. (1985) also showed the effectiveness of OR as an antioxidant in turkey sausage during refrigerated storage, however, these samples were not cooked. It is generally accepted that cooking triggers the rapid development of WOF by liberating iron from various heme proteins and disrupting tissue membranes, thus, bringing lipid substrates and catalysts into closer proximity.

Data were also collected for restructured steaks which were held raw at 4 C for 5 days, then cooked and analyzed (Table 8). Results indicate that OR1 and OR2 alone both provide antioxidant activity superior to that of the salt control and when used along with STPP an additive effect was observed. However, the TBHQ/STPP treatment provided the

Table 8. Mean TBARS values and sensory scores for raw restructured beef steaks during refrigerated storage.[1,2,3]

	Raw		Cooked			
	Day 0	Day 5	Day 0		Day 5	
Treatment	TBA	TBA	TBA	Sensory	TBA	Sensory
SALT	0.80	5.34	1.34	1.19	5.01	3.17
STPP	0.21	2.07	0.29	0.31	2.76	2.38
OR1	0.43	2.70	0.76	1.06	2.69	1.88
OR2	0.39	1.56	0.56	0.86	2.10	1.62
OR1/STPP	0.20	1.23	0.27	0.47	1.30	1.15
OR2/STPP	0.19	0.82	0.27	0.42	1.43	0.82
TBHQ/STPP	0.17	0.28	0.28	0.45	0.36	0.76

[1] All values represent means of three replicated experiments.

[2] TBARS values (TBA) expressed as mg malonaldehyde/kg meat.

[3] Sensory scores: 0 = no WOF; 5 = strong WOF.

best protection, the meat remaining well below the generally accepted TBA threshold of 0.5 to 1.0 for off-flavor detection (Tarladgis et al., 1960). These results implied that the cooking process somehow affects the OR in such a way that it can no longer function as an antioxidant. An alternative thought would be that there was so much potential for WOF development in the cooked steaks that the OR cannot quench all of the free radicals generated due to a lower concentration of antioxidant species. Oleoresin rosemary would be expected to contain lower concentrations of antioxidant species than found in TBHQ since it consists of both volatile and non-volatile compounds, some of which would not be expected to possess typical antioxidant properties. Although identification of some of the phenolic compounds in OR has been accomplished (Loliger, 1983), quantitation of the levels present is still not clear and may depend on the extraction method used.

A severe limitation to increasing the level of OR in meats is the spice flavor it imparts to the meat. Panelists in the current study were asked to identify the presence of non-meat flavor which they were previously trained to associate with rosemary. In freshly cooked steaks processed with 0.1% OR (OR2) and OR2/STPP, 15 of 33 and 12 of 33 responses correctly identified non-meat flavors. By the sixth day of refrigerated storage only 6 of 33 and 2 of 33 responses identified non-meat flavors in the two treatments.

Apparently the strong WOF and oxidized flavor masked the spice flavor because also during this sampling period, 9 of 33 incorrectly identified the salt control as having non-meat flavor.

FROZEN STORAGE OF RESTRUCTURED BEEF STEAKS

The restructured beef steaks used for this study were stored at -20 C and analyzed after 0, 3 and 6 months. Analyses included TBARS values, sensory scores, hexanal levels and fatty acid profiles.

TBA test and sensory scores

Analysis of TBARS values and sensory scores was done as previously described using ANOVAs and Bonferroni t-tests to make specific comparisons between treatments within each sampling period. The effects of time, treatment and time/treatment interactions were significant for both TBARS values and sensory scores.

Results of the frozen study differ substantially from those of the refrigerated study (Table 9, Figures 7 and 8). Although the salt control developed the highest TBARS levels and sensory scores at each sampling period, the trends are less clear cut for the remaining data. Significance levels of the designed contrasts for the treatment effects during frozen storage are given in Table 10. Based on TBARS values, the effect of STPP was significant at 3 and 6 months

TABLE 9. Mean TBARS values and sensory scores for restructured beef steaks during frozen storage. [1,2,3,4]

Treatment	Month 0		Month 3		Month 6	
	TBA	Sensory	TBA	Sensory	TBA	Sensory
SALT	1.34	1.19	3.93	3.08	5.29	3.83
STPP	0.29	0.31	2.14	2.58	3.04	3.00
OR1	0.76	1.06	2.52	2.06	3.22	2.92
OR2	0.56	0.86	1.61	1.67	2.41	2.58
OR1/STPP	0.27	0.47	1.36	1.17	2.37	2.86
OR2/STPP	0.27	0.42	1.18	1.13	1.77	2.20
TBHQ/STPP	0.28	0.45	0.78	1.17	1.89	2.11

[1] All values represent means of three replicated experiments.

[2] TBARS values (TBA) are expressed as mg malonaldehyde/kg meat.

[3] Representative steaks were thawed after 3 and 6 months at -20 C, cooked, and analyzed for TBARS values and sensory scores.

[4] Sensory scores: 0 = no WOF; 5 = strong WOF.

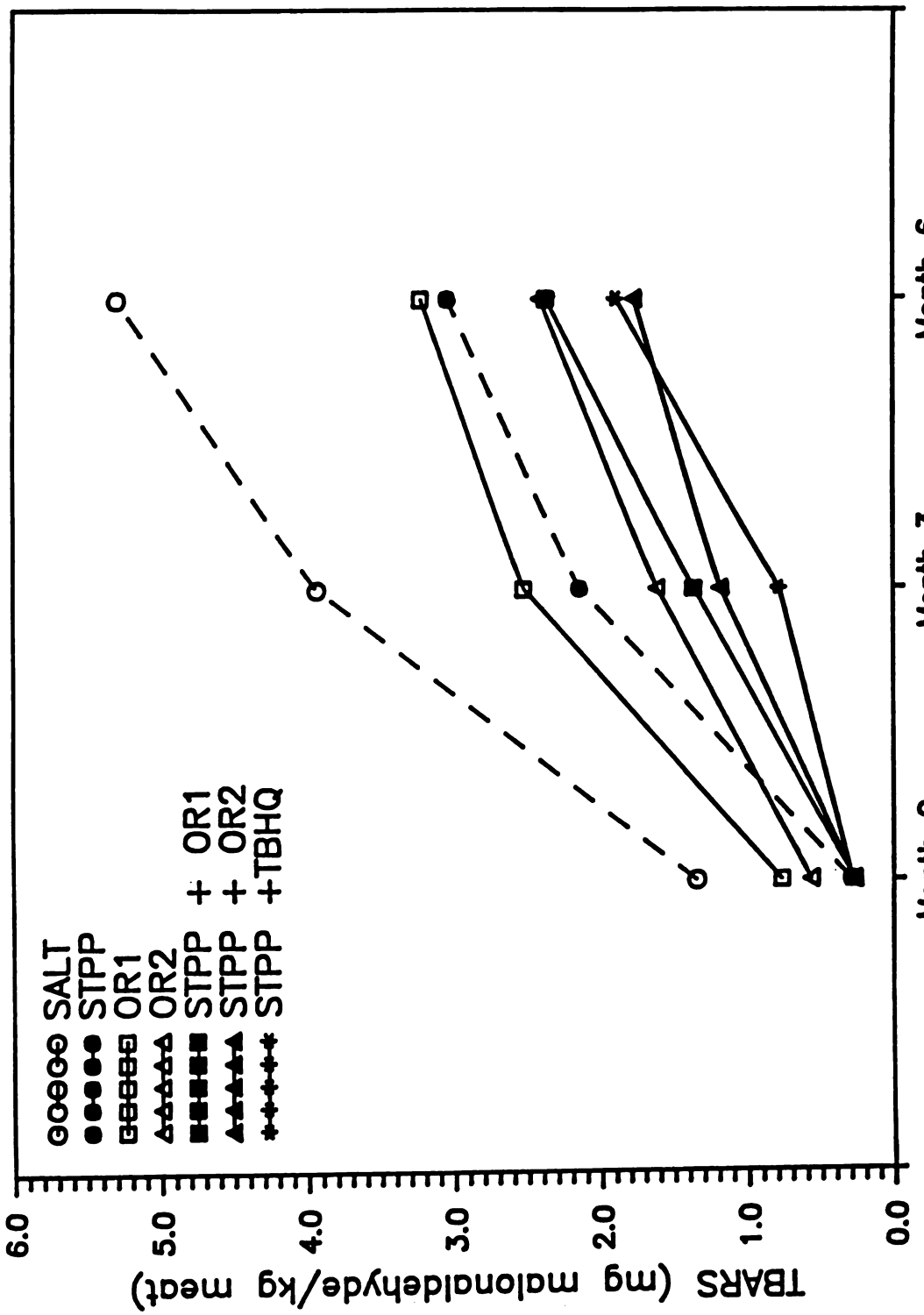


Figure 7. TBARS values of restructured beef steaks stored at -20°C.

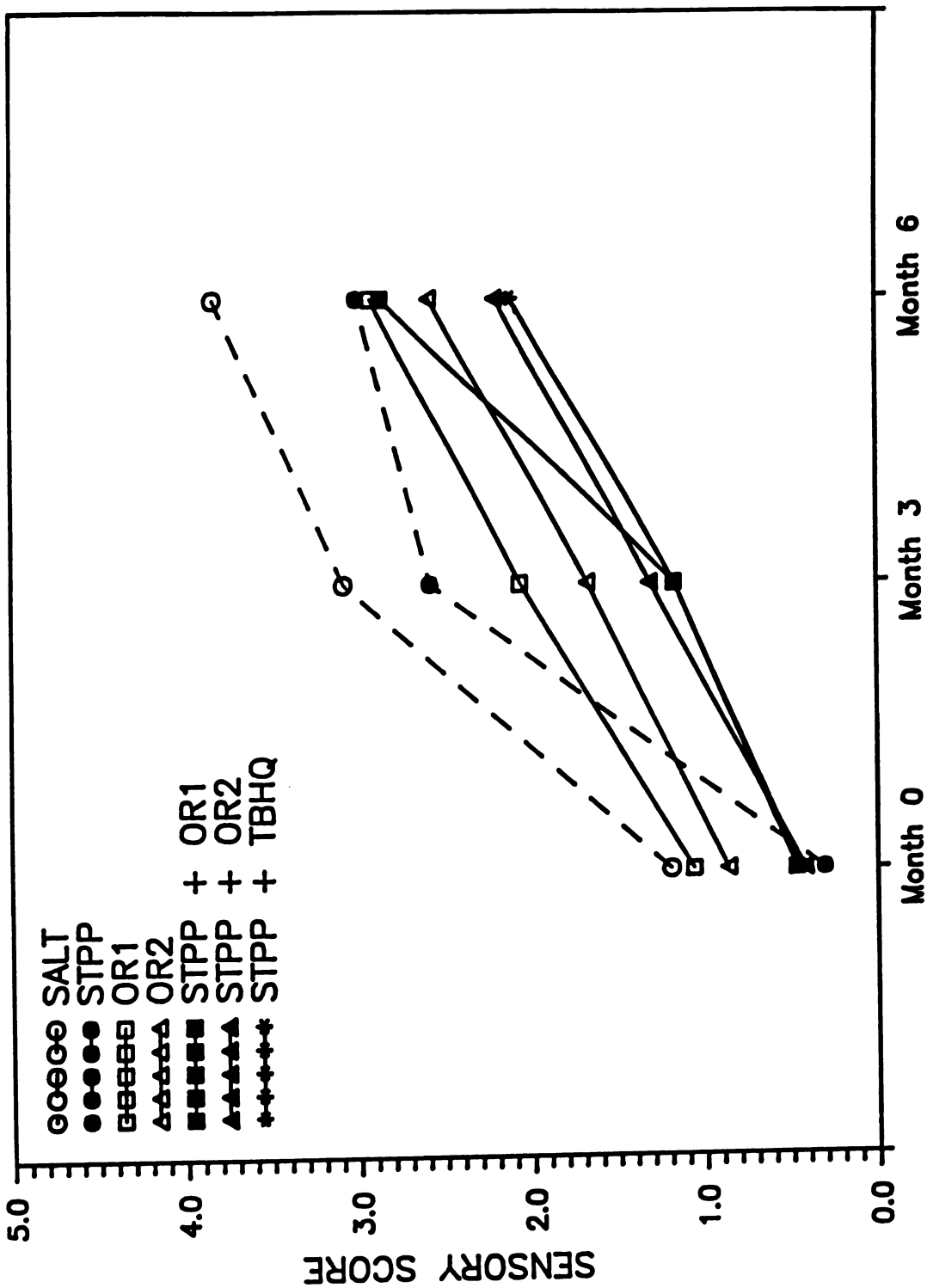


Figure 8. Sensory scores of restructured beef steaks stored at -20°C.

Table 10. Significance levels of designed contrasts for treatment effects on TBARS values and sensory scores during frozen storage of restructured beef steaks.

Contrast	Test	Month of storage		
		0	3	6
(1) STPP vs no STPP	TBA	ns	0.05	0.01
	Sensory	0.10	0.10	ns
(2) OR1/STPP vs TBHQ/STPP	TBA	ns	ns	ns
	Sensory	ns	ns	ns
(3) OR2/STPP vs TBHQ/STPP	TBA	ns	ns	ns
	Sensory	ns	ns	ns
(4) OR Linear Effect (OR_L)	TBA	ns	0.01	0.01
	Sensory	ns	0.01	0.01
(5) OR Quadratic Effect (OR_Q)	TBA	ns	ns	ns
	Sensory	ns	ns	ns
(6) Interaction STPP & OR_L	TBA	ns	ns	ns
	Sensory	ns	ns	ns
(7) Interaction STPP & OR_Q	TBA	ns	ns	ns
	Sensory	ns	ns	ns

ns: not significant

of storage, while sensory data showed only slight significance at 0 and 3 months. However, the main protective factor is no longer the STPP as during refrigerated storage. A linear concentration effect of OR existed ($p<0.01$) for both TBARS values and sensory scores. As the concentration of added OR increased, the oxidative stability of the steaks increased. There was no significant interaction between STPP and OR either in a linear or quadratic fashion, implying that no synergistic effects occurred. It appears to be more of an additive effect. STPP alone and OR1 or OR2 alone each provided some protection against oxidation, but when used in combination, OR1/STPP or OR2/STPP, the protective effects were greater. This additive effect is probably due to the different mechanisms involved. STPP functions as an antioxidant by chelating prooxidant metals such as ferrous iron in cooked meats, while the phenolic components in the OR act by interrupting the free radical chain mechanism. These results agreed with those reported by Lai (1989) in a similar study on breaded chicken nuggets processed with OR. TBHQ when used in combination with STPP exhibited the best protective effect. Both OR (0.10%) and STPP when used alone reduced the extent of salt-catalyzed lipid oxidation, and in combination an additive effect was observed.

In the present study the OR1/STPP and OR2/STPP treatments were not statistically different from the TBHQ/STPP

treatment ($p>0.05$). Therefore, it can be assumed that synthetic antioxidants can be replaced by OR in raw restructured beef steaks to increase shelf-stability during frozen storage. It is important to note that after 6 months of frozen storage, all the treatments reached TBARS values greater than 1.77 which exceeds the sensory threshold of 0.5 to 1.0 given by Tarladgis *et al*. (1960) for trained panelists. This threshold was reported for unprocessed beef, while the present steaks all contain salt which would tend to mask some of the off-flavors and result in a higher sensory threshold in the current study. Also, the steaks in the present study were purposely packaged in oxygen permeable bags to create an environment conducive to lipid oxidation. Vacuum packaging would increase the shelf-life of restructured beef steaks (Crackel *et al*., 1988), and this effect was seen in the vacuum packaged reference samples used in this study. TBARS values of these samples remained below 0.35 after six months. Sensory scores were all greater than 2.11 (on a scale of 0 to 5) which equates to detection of slight WOF. However, untrained panelists or typical consumers often cannot discriminate WOF until TBARS values reach 0.6 to 2.0 or higher (Greene and Cumuze, 1981) which would still make the OR2/STPP and TBHQ/STPP steaks in the present study acceptable to some consumers up to 6 months.

Detection of spice flavor from the added OR was a concern during frozen storage as well as during refrigerated storage. Twelve panelists participated in the frozen storage study. In the fresh steaks, 15 of 36 and 12 of 36 panelists detected non-meat flavor in the OR2 and OR2/STPP treatments, respectively. After three months, 12 of 36 and 9 of 36 samples were correctly identified and after 6 months 7 of 36 and 6 of 36 samples were identified. It is not known whether the spice flavor is lost over time or if it is masked by the increasing rancid flavors.

Hexanal content

During frozen storage, changes in hexanal content were measured using a Tekmar Dynamic Headspace Sampling Unit. Hexanal is a major degradation product of linoleic acid oxidation and has been used as a marker for following lipid oxidation in food systems. The amount of hexanal in the salt control (corresponding to the area under the curve) was arbitrarily set at 100 and values are reported as a percentage of the salt control. The data in Figure 9 for 5 of the 7 treatments were taken from only one experimental replication due to problems encountered with the equipment (original data are given in appendix C). It is clear from the data, that after 3 months of frozen storage the salt control had more than twice the hexanal content of the other treatments, a trend which agrees with the TBARS values and sensory scores.

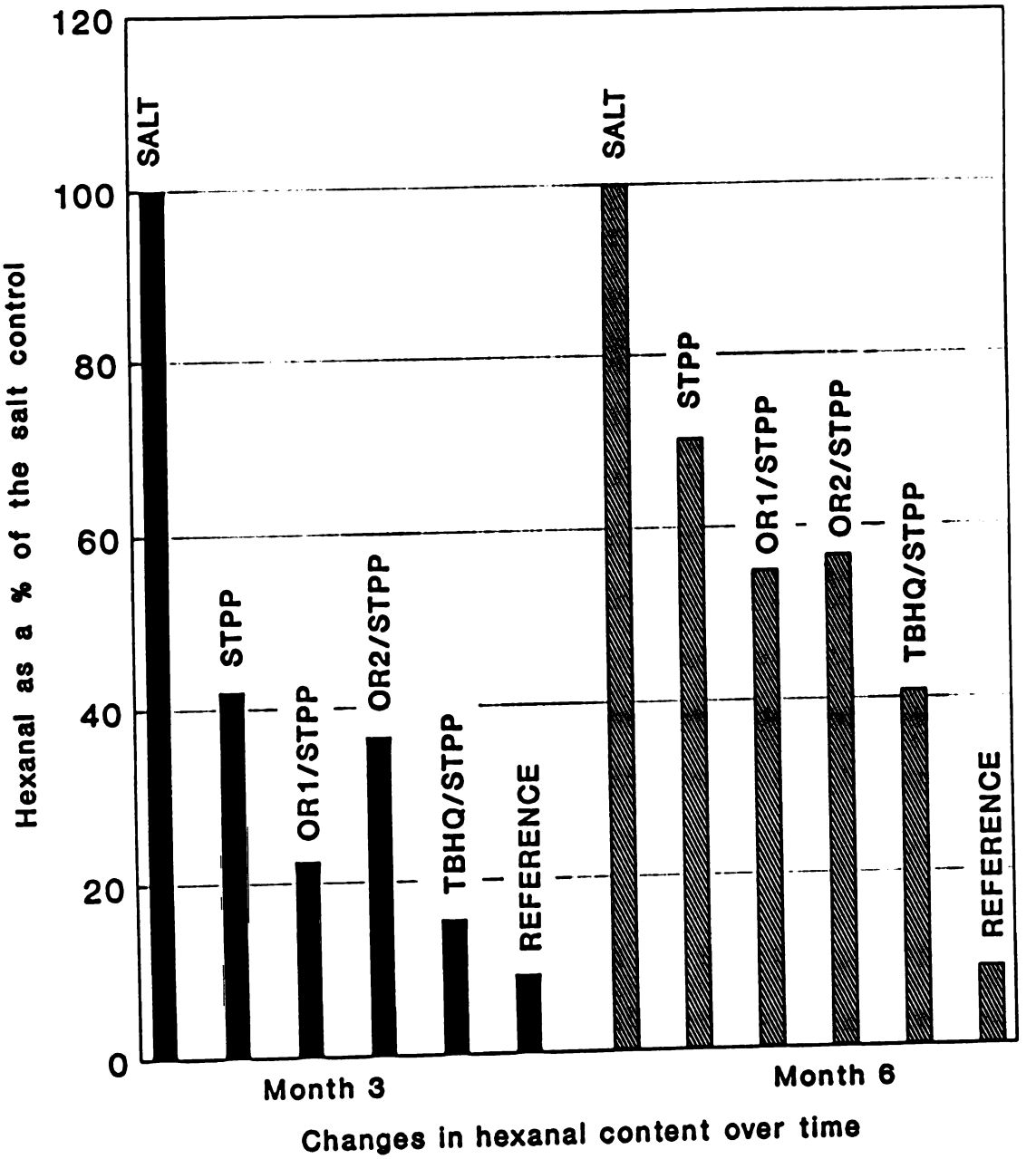


Figure 9. Changes in hexanal content during frozen storage.

Note: Data represent means of duplicate samples from one rep of the experiment.

After 6 months the hexanal levels for the salt control nearly doubled, and increased levels in all the treatments were also observed. These results are generally in agreement with those of Shahidi et al. (1987) who demonstrated that hexanal levels in cooked ground pork could be controlled by adding various antioxidants. STPP retarded hexanal levels to 50% of a salt control after 48 hours of refrigerated storage while TBHQ provided more complete protection, keeping hexanal levels at 3% of the salt control. Love and Pearson (1976) also reported that the addition of STPP caused a 50% decrease in the hexanal concentration in a model system. In turkey sausage, Barbut et al. (1985) reported that the addition of oleoresin rosemary or BHA/BHT substantially reduced measurable TBA-reactive substances and decreased the presence of oxidatively-derived carbonyls such as pentanal, hexanal, heptanal and 2,3-octanedione.

The reference sample (REF) consisted of the TBHQ/STPP treatment which was vacuum packaged to serve as a fresh-tasting reference sample for the sensory panelists to use in rating WOF. This sample remained relatively stable over the 6 months of storage while the non-vacuum packaged TBHQ/STPP exhibited a substantial increase in TBARS values and hexanal levels over time. These results are in agreement with Miles et al. (1986) who reported that vacuum packaging reduced TBA

values and discoloration scores for restructured pork chops compared to conventional oxygen permeable wrapping (PVC).

Although hexanal contributes to off-flavor and off-odor development during storage of meats, it is not solely responsible for WOF development. WOF results from a complex set of reactions which are not yet fully understood. No single compound has been identified as being uniquely responsible for cooked beef aroma as meat flavor is composed of an extremely complex collection of chemicals. Cooked beef flavor is generally thought to be composed of heteroatomic compounds such as thioethers, furans, pyrroles, pyridines, pyrazines, thiophenes and thiazoles (St. Angelo et al., 1988). It was suggested that many of these compounds resulted from Maillard reactions that occur during cooking of the meat. Reactions involving protein degradation and heteroatomic compounds have been linked with deterioration of the desirable beefy flavor notes. Many of these reactions involve free radicals. Johnson and Civille (1986) developed a descriptive language for evaluating WOF in meat that includes not only off-flavor descriptors, but fresh meaty flavor descriptors as well. St. Angelo et al. (1988) used cooked beef brothy (CBB), painty (PTY) and cardboardy (CBD) as descriptors of cooked beef during refrigerated storage. They found that in every case the CBB flavor note decreased in the WOF patties stored for 48 hours, while the PTY and CBD values increased from frozen

controls. However, use of appropriate antioxidants could retard off-flavor development. Recently, Spanier et al. (1988) referred to WOF as meat flavor deterioration (MFD) because of the complexity of the chemical reactions that take place during WOF development. This may be a more appropriate term for future work.

The hexanal data were presented as percent of the salt control for only one replication because several problems with the method prevented accurate quantitation of the data. The major problem was that the Tekmar headspace sampling unit did not give consistent results. Sensitivity to standards and samples changed from day to day as well as month to month, thus making comparisons between replicated samples and time periods very difficult. Attempts to find and repair gas leaks in the system which could cause fluctuations in sensitivity were most often unsuccessful.

Hexanal was often chromatographed as a split peak, again making quantitation difficult. It was not determined if the split peaks resulted solely from hexanal or if other interfering compounds were present. One possible explanation for the split peaks could be failure of the flash heating unit to deliver a pure, concise injection of the sample onto the column. It was found that the timing unit was inaccurate; a setting of 10 seconds only corresponded to 6 seconds when timed with a stop watch.

Artifactual formation must also be considered. The high temperatures used to heat the sample, concentrate it on, and desorb it from the Tenax trap could result in artifacts which may be incorrectly identified as constituents of the beef flavor profile. Roozen (1987) found that various heating steps during sample preparation contributed predominantly to the areas of the more volatile compounds in the flavor profile. It may also be possible that the Tenax trap was not fully reconditioned between samples, although, frequent replacement of the trap did not improve results. Another observation was that variation between duplicate samples increased as the level of oxidation increased. The system could not handle high levels of hexanal, and very small sample sizes were used. Finally, errors in quantitation also resulted because in the more highly oxidized samples a new peak in the chromatogram appeared and interfered with the internal standard, 2-heptanone. These problems led to a search for an improved method for hexanal analysis which is discussed later.

FATTY ACID PROFILES

The oxidative breakdown of NLs and PLs has been observed in frozen muscle held at various temperatures for various storage times (Hornstein et al., 1967; Awad et al., 1968; Keller and Kinsella, 1973; Igene et al., 1979b; Willemot et al., 1985). It is now generally accepted that the PLs are

the major lipids involved in WOF development while the TGs are mainly involved in oxidation during long term frozen storage (Pearson and Gray, 1983).

Differences in fatty acid content between selected treatments after six months of frozen storage were minimal for the NL fraction (Table 11). Appendix D contains 3 and 6 month NL fatty acid profiles. No significant differences between treatments were found for C18:3, C18:2, C18:1 or C16:0 ($p>0.05$). Total unsaturated fatty acid (TUFA) and saturated fatty acid (SAT FA) content were significantly different between the salt control and OR2 ($p<0.05$). However, the differences were minor and could easily have resulted from natural variation in the meat samples used. Neutral lipid changes over the six month storage period were also small (Table 12). No significant changes in TUFA, SAT FA, C18:1 or C16:0 were detected ($p>0.05$) for the SALT and OR2/STPP treatments. Only two treatments were analyzed because they were thought to be representative of high and low WOF development. Significant changes in C18:3 and C18:2 were observed for both treatments reinforcing the idea that the more highly unsaturated fatty acids oxidize most rapidly. These results are in agreement with those of Igene et al. (1981) and Willemot et al. (1985) who observed little change in the fatty acid profile of the TG fraction during frozen storage of beef or chicken.

Table 11. Differences in neutral lipid content between treatments after 6 months of frozen storage.[1,2]

	TREATMENT				
FATTY ACID	SALT	STPP	OR2	OR2/STPP	TBHQ/STPP
TUFA	55.95^{b}	56.20^{ab}	56.68^{a}	56.37^{ab}	56.26^{ab}
SAT FA	43.02^{a}	42.73^{ab}	42.34^{b}	42.62^{ab}	42.71^{ab}
18:3	0.41^{a}	0.50^{a}	0.55^{a}	0.41^{a}	0.42^{a}
18:2	2.71^{a}	2.64^{a}	2.76^{a}	2.77^{a}	2.60^{a}
18:1	45.62^{a}	45.70^{a}	46.28^{a}	45.89^{a}	45.87^{a}
16:0	26.10^{a}	25.89^{a}	25.99^{a}	26.14^{a}	25.93^{a}

[1] Values with the same superscript in the same row are not significantly different ($p<0.05$).

[2] Fatty acid content is expressed as percent of total.

Table 12. Significance levels for changes in fatty acid content over 6 months of frozen storage.

	Neutral lipids		Phospholipids	
Fatty acid	SALT	OR/STPP	SALT	OR/STPP
TUFA	ns	ns	-	-
PUFA	-	-	0.01	ns
SAT FA	ns	ns	0.05	ns
20:4	-	-	0.01	ns
18:3	0.01	0.05	ns	ns
18:2	0.05	0.01	ns	ns
18:1	ns	ns	0.01	ns
16:0	ns	ns	ns	0.05

ns: not significant

The PL fatty acid profiles for each of the seven treatments after six months of storage are presented in Table 13 (6 month PL data are found in appendix D). Significant differences in PLs existed between treatments after six months of frozen storage (Table 14). The PUFA content (including C18:2) dropped from 43.6% of total (OR2/STPP) to 35.9% (SALT). The SALT control was significantly lower in C20:4 than either the OR2/STPP or TBHQ/STPP ($p<0.05$). Little change in C18:3 was observed, however, this may be due to natural variation and to the low levels initially present. In Table 14, visual observation suggested that the SALT control contained less C18:2 than OR2, OR2/STPP and TBHQ/STPP, however, the differences were not statistically significant ($p>0.05$). A slight increase in C18:1 for the salt control compared to the phenolic antioxidant treatments was observed. Although no differences in total SAT FA are observed between treatments, the C16:0 content was significantly greater ($p<0.05$) in the more highly oxidized SALT and STPP treatments. Also changes in SAT FA over the 6 month storage period for the SALT control were significant ($p<0.05$) (Table 13). These results demonstrated that as the more highly unsaturated fatty acids are oxidized, the percentage of saturated and monounsaturated fatty acids increases.

Significant changes occurred over time in the PL fractions. Table 12 gives significance levels for the SALT and OR2/STPP

Table 13. Fatty acid composition of the phospholipid fraction from restructured beef steaks after six months of frozen storage.[1]

Fatty Acid[2,3,4]	Sample						
	SALT	STPP	OR1	OR2	OR1/ STPP	OR2/ STPP	TBHQ/ STPP
14:0	0.58	0.58	0.47	0.36	0.55	0.32	0.49
16:0	18.77	18.64	17.76	17.46	17.46	16.75	16.97
16:1	2.65	2.90	2.00	2.47	2.64	2.17	2.17
17:0	tr	tr	tr	tr	tr	tr	tr
U	0.77	0.65	0.56	0.46	0.49	0.45	0.50
18:0	11.67	11.68	11.72	11.30	11.61	11.66	11.73
18:1	29.69	29.21	27.32	27.76	27.45	25.02	25.84
18:2	20.18	19.57	23.42	23.00	22.98	25.18	23.56
18:3	1.35	1.29	0.77	0.79	0.78	1.04	1.10
20:3	2.47	2.59	2.84	3.02	2.80	3.00	3.11
20:4	9.19	9.95	11.02	11.19	11.00	12.00	11.69
20:5	0.87	0.93	0.47	0.45	0.52	0.51	0.83
22:4	0.74	0.78	0.95	1.03	0.93	1.02	0.92
22:5	1.14	1.17	0.77	0.77	0.84	0.84	1.11
% SAT FA	31.01	31.00	29.94	29.12	29.62	28.82	29.19
% MUFA	32.34	32.11	29.32	30.23	30.09	27.19	28.01
% PUFA	35.93	36.27	40.23	40.23	39.84	43.58	42.31
% TUFA	68.27	68.37	69.54	70.46	69.93	70.76	70.32

[1] Values are expressed as percent of total and represent the mean of two experimental replications.

[2] tr = trace amounts detected and U = unknown compound.

[3] PUFA includes the di-unsaturated fatty acids.

[4] SAT FA = saturated fatty acid MUFA = mono-unsaturated fatty acid
PUFA = polyunsaturated fatty acid TUFA = total unsaturated fatty acid

Table 14. Differences in phospholipid content between treatments after 6 months of frozen storage.[1,2]

	TREATMENT				
FATTY ACID	SALT	STPP	OR2	OR2/STPP	TBHQ/STPP
PUFA	35.93^{b}	36.26^{ab}	40.23^{ab}	43.58^{a}	42.31^{ab}
SAT FA	31.01^{a}	31.00^{a}	29.12^{a}	28.81^{a}	29.18^{a}
20:4	9.19^{b}	9.95^{ab}	11.19^{ab}	11.99^{a}	11.69^{a}
18:3	1.35^{a}	1.29^{a}	0.79^{a}	1.04^{a}	1.10^{a}
18:2	20.18^{a}	19.57^{a}	23.00^{a}	25.17^{a}	23.56^{a}
18:1	29.68^{a}	29.20^{ab}	27.76^{abc}	25.01^{c}	25.84^{bc}
16:0	18.76^{a}	18.64^{a}	17.46^{ab}	16.75^{b}	16.97^{b}

[1] Values with the same superscript in the same row are not significantly different ($p<0.05$).

[2] Fatty acid content is expressed as percent of total.

treatments. For the SALT control, significant changes in PUFA, SAT FA, C20:4 and C18:1 occurred while in the OR2/STPP treatment, only C16:0 showed significant change at the 95% confidence level. For the SALT control the SAT FA content increased by 19.6% over six months while the PUFA and TUFA contents decreased by 27.1% and 7.1%, respectively. In contrast, for the OR2/STPP treatment the SAT FA content increased 13.0% and the PUFA and TUFA contents decreased by only 15.8% and 4.3%, respectively. The increase in the percent SAT FA results from loss of the PUFAs. These results support the previous observation that the phospholipid fraction is very susceptible to oxidation. Gokalp et al. (1983) reported that in frozen beef patties stored for three months the total unsaturated fatty acids had decreased 10.1% in the PL fraction, and after 4.5 months of storage the NL unsaturated fatty acids had decreased 6.8%. In the NL fractions, reduction in C16:1 and C18:2 made significant contributions to increased rancidity scores and TBA values, but in the PL fraction changes in C18:3 and C20:4 were of greatest importance. Igene et al. (1980) also reported that oxidation of the TGs begins only after a prolonged storage period and that PLs make the greatest contribution to rancidity development.

CORRELATION OF TBARS VALUES, SENSORY SCORES AND HEXANAL CONTENT

One of the objectives of this study was to correlate sensory scores, TBARS values and hexanal levels. WOF is a sensory phenomenon and any chemical and physical methods to quantify it are best conducted in combination with sensory analysis. TBARS values are not specific for WOF but are indicative only of oxidation (Sato and Hegarty, 1971; Sato et al., 1973; Pearson et al., 1977). As discussed earlier, several concerns about the specificity of the TBA reaction limit its use as a quantitative indicator of WOF development. Limitations on the usefulness of sensory data depend on factors such as panel size, extent of training, specificity of descriptors, individual panelist variation, and procedural details. Sensory panelists must be trained to differentiate between WOF and other autoxidative changes. The quantitation of hexanal and other volatile compounds provides another measure of WOF development which has been shown to correlate well with sensory scores and TBARS values. High correlations between methods of analysis lend credibility to the results obtained by the individual methods.

High correlation coefficients between sensory scores and TBARS values ranging from 0.50 to 0.986 have been reported (Igene and Pearson, 1979; Chastain et al., 1982; Igene et al., 1985a; Shahidi et al., 1987; St. Angelo et al., 1987).

However, not all researchers have found statistically significant correlations between these two measures. The variability among literature values may be due to the different methods used to calculate the r value (pooled within groups, across groups, mean values only) and to the number of factors involved in the experiments. Many "significant" correlations of TBARS values and sensory scores which have been previously reported were calculated on individual observations over groups differing in mean values of TBARS values and sensory scores and were reported without comment.

Due to strong effects of treatments and storage times, the correlations between TBARS values and sensory scores in this study were calculated by pooling information within treatments and storage times (Lai, 1989). Statistically significant correlation coefficients of $r = 0.37$ ($p<0.10$) and $r = 0.49$ ($p<0.05$) were found between sensory scores and raw and cooked TBARS values, respectively, for restructured beef steaks during frozen storage for six months. During refrigerated storage, the correlation coefficient between the two factors was only 0.10. The relationship between TBARS values and sensory scores is very weak indicating they may not be useful for studying WOF development. In contrast, the intra-treatment, intra-storage time correlations for the refrigerated and frozen (raw TBARS) studies, calculated by simply pairing the individual

observations over treatments and storage times were 0.93 and 0.87, respectively. The correlation computed from samples from heterogeneous populations may be almost worthless as information about the relationship in a specific subpopulation, because the measure is highly dependent on the range of variables sampled, i.e., correlation over groups differing in mean values of x and y is necessarily higher than the correlation within a group (Gill, 1978).

Possible explanations for the low correlation coefficients were investigated. The present study involved more experimental factors and more treatments within a factor compared to other studies in which high correlation coefficients were reported. To investigate the effect of individual factors on the correlation of TBARS values and sensory scores, correlation within each factor (treatments and storage time) were calculated separately (Tables 15 and 16). The within-time correlations of the salt control and OR2-treated samples were very low during frozen and refrigerated storage. For the salt control this may be due to the panelists inability to detect variation over time because the sample developed very strong WOF during the early stages of storage. Poste et al. (1986) reported that WOF scores for cooked meat increased up to 3 days of storage, then either increased or decreased, depending on the panelist's threshold for rancidity. For the two treatments (OR2 and OR2/STPP) containing the highest level

Table 15. Within-time correlation coefficients between TBARS values and sensory scores of restructured beef steaks for each treatment.

Treatment	Refrigerated Storage	Frozen Storage	
	Cooked TBARS	Raw TBARS	Cooked TBARS
SALT	0.00	-0.15	0.40
STPP	0.37	0.57	0.69*
OR1	0.79*	0.66*	0.75*
OR2	-0.27	0.24	0.02
OR1/STPP	0.20	0.84*	0.74*
OR2/STPP	0.14	0.07	0.15
TBHQ/STPP	0.11	0.48	0.53

* Correlation coefficient is significant at $p<0.05$.

[1] Correlations calculated using TBARS values from raw or cooked steaks.

Table 16. Within-treatment correlation coefficients between TBARS values and sensory scores for restructured beef steaks at each sampling period.[1]

Refrigerated Storage		Frozen Storage		
Days	Cooked TBARS	Months	Raw TBARS	Cooked TBARS
0	-0.18	0	-0.10	-0.18
2	0.23	3	0.09	0.10
4	0.07	6	0.53*	0.64*
6	0.10			

* Correlation coefficient is significant at $p<0.05$.

[1] Correlations calculated using TBARS values from raw or cooked steaks.

of oleoresin rosemary (0.10%) the WOF may have been masked by the spice flavor resulting in inaccurate panel assessment for WOF.

In general, the correlations for the refrigerated storage study were extremely low. During refrigerated storage of cooked steaks, it was determined that the main antioxidative effect was due to the STPP. All the treatments without STPP oxidized very rapidly which, as previously mentioned, could cause panelists to be unable to detect changes in WOF over time. Many of the panelists gave scores at or near the top of the six point rating scale after only 48 hours of storage. This resulted in a severe leveling off of the sensory scores for the remaining four days of the storage period while the TBARS values continued to rise. The four treatments processed with STPP showed almost total inhibition of lipid oxidation in restructured beef steaks as indicated by the TBA test. The negative and low correlations obtained for refrigerated storage may be attributed to the inability of the sensory panel to detect very low levels of WOF. Thus, the different sensitivities of the TBA test and sensory panel evaluation to samples which oxidize very rapidly or very slowly may be responsible for the low correlations obtained.

Within-treatment correlations between TBARS values and sensory scores (Table 16) were also very low throughout both

the frozen and refrigerated studies. One would expect non-significant correlations at day zero and month zero and early in the study due to little variance in the degree of oxidation among samples. In the frozen study, the correlation became significant by month 6. These low correlations result for the same reasons as the low within-time correlations. It was also observed that for the more highly oxidized samples, especially during refrigerated storage when TBARS values reached levels up to 11.3, there was a lot of variance in TBARS values between the three experimental replications. The three replications were processed on three consecutive months using different beef each time which may account for some of the variation. This variation in TBARS values between replications combined with the plateau effect of the sensory scores could easily cause lower correlations.

Although some earlier data have shown significant correlations between TBARS values and sensory scores, absolute TBARS values of various tissues may not represent the status of oxidation nor can they accurately indicate sensory assessment. Ang (1988) suggests that rate of change in TBA numbers may be a more accurate measure of oxidation rate and flavor changes. Correlation coefficients reported in this manner for cooked broiler tissues stored at 4 C for five days related well to but were consistently slightly lower than those r values calculated in the normal fashion.

This method may provide an alternative when experimental replications show similar trends over time but poor correlation due to quantitative differences.

Quantitative analysis of hexanal and other volatiles provides another means for assessing the rate of WOF development as well as a means of identifying the compounds contributing to WOF. Correlation coefficients between hexanal content and TBARS values and sensory scores in restructured beef steaks after six months of frozen storage were calculated to determine their effectiveness in monitoring lipid oxidation. Both correlation coefficients (pooled within treatments) were low and non-significant; the r value between hexanal content and TBARS values was 0.24 while between hexanal content and sensory scores the r value was -0.20. Both of these values are much lower than the r value of 0.49 reported between TBARS values and sensory scores. These poor correlations are a direct result of the inconsistencies reported earlier concerning collection of the hexanal data. It was not possible to compare values month to month due to changes in sensitivity of the headspace sampling equipment, so it is only logical that correlation coefficients using data collected over several months of storage would be low and non-significant.

Even though results of this study showed little relationship among chemical and sensory analyses other studies have

reported significant relationships. St. Angelo et al. (1987) investigated WOF development in raw, freshly cooked, and stored and recooked beef muscle samples. Correlation coefficients between sensory scores and instrumental values (hexanal, 2,3-octanedione, and total volatiles) and between TBARS values and instrumental values ranged from 0.80 to as high as 0.92. These values were calculated over two storage periods for only one treatment unlike the present study which involved seven treatments. Also, the procedure for statistical analyses were not provided by these investigators.

In a study of cooked, ground pork, Shahidi et al. (1987) reported that the hexanal content, sensory scores and TBARS values of treated meats were linearly interrelated. The correlation coefficients between hexanal content and TBARS values and between hexanal content and sensory scores were reported to be 0.995 and 0.981 ($p<0.05$), respectively. TBARS values and sensory scores were also linearly related ($r=0.986$). However, the measurements were not performed at the same time, i.e., hexanal content and sensory evaluation were determined after two days of storage while the TBARS values were collected after thirty-five days of refrigerated storage. They observed that the hexanal and sensory values were more indicative of WOF while the TBARS values represented oxidized flavor and, as expected, these two off-flavors, although different, were interrelated. It must

also be noted that an untrained sensory panel consisting of nineteen members was used. These panelists may not have been distinguishing between WOF and rancidity. Although the method of analysis was not specified, the results may have been computed on the means of replicates for each treatment, leading to higher correlation coefficients.

Although many of the correlation coefficients were low, the data from this study indicate that sensory evaluation is a useful means of assessing WOF development in restructured beef steaks during storage. The panelists who participated in this study did not receive extensive training although most had prior experience in evaluating meat products for WOF. There was a threshold for detection of and sensitivity to WOF which limited the ability of the panel to discriminate between varying degrees of oxidation among the samples. Some panelists consistently limited their sample rating to a restricted range and never used the full range of six scale units. This may be due to the different thresholds of WOF perception experienced by individuals. Proper selection and training of the sensory panel may improve the consistency and accuracy in rating intensity of WOF. As discussed earlier, proper design of scoring scales and descriptors for specific flavors such as cooked beef brothy, cardboardy, and painty will result in improved sensory analysis.

AN ALTERNATIVE METHOD FOR HEXANAL ANALYSIS

The final objective of the present study was to evaluate alternative methods of hexanal analysis as an indicator of WOF. Several problems were encountered with the Tekmar headspace sampling unit used in the present study as discussed previously. The equipment was inconsistent over time and subject to frequent mechanical problems. However, gas chromatographic analysis of volatile flavor compounds and secondary oxidation products is still the preferred method for identifying and quantitating volatiles. Variation between methods occurs during sample extraction and volatile collection. Several of these methods were discussed previously.

A vacuum collection/solvent extraction method for analyzing hexanal levels was successfully developed. The method involves collecting the meat volatiles onto a Tenax trap by heating the sample to 60 C and pulling a 0.35 kg/sq cm vacuum on the entire system. After collection of the volatiles, the Tenax trap was charged with 2-methyl butane and centrifuged to elute the volatiles into the solvent. The solvent extract was then evaporated to a specified volume and injected onto a GC column suitable for separation of meat volatiles. Reproducibility and recovery studies were conducted using hexanal standards and 2-heptanone as the internal standard. The reproducibility was good and recoveries averaged approximately 90%. One problem that was

encountered was injecting a constant volume of solvent onto the GC. The 2-methyl butane is very volatile, and the syringe had to be pre-cooled before injections were made. Also, the standards were dissolved in water and had to be prepared fresh daily, so accurate preparation was necessary to ensure consistency from day to day.

This method was tested using cooked turkey rolls stored at 4 C. Hexanal and TBA analyses were conducted at 0, 24 and 48 hours for three treatments, SALT control, STPP and OR(0.10%)/STPP. The results are given in Table 17. All three treatments underwent oxidative deterioration over the 48 hour storage period as seen by the increasing TBARS values. The hexanal content also showed a steady increase over time for the OR/STPP and STPP treatments. The SALT control exhibited a slight decline in hexanal between 24 and 48 hours. This may result from a breakdown of hexanal to other oxidation products or from natural variation between samples having such high TBARS value. After 24 hours, all of these samples had TBARS values greater than 1.53 which is well above the generally accepted threshold range of 0.5 to 1.0. These results support the theory that WOF develops within the first 48 hours of refrigeration at 4 C.

The correlation coefficient between TBARS values and hexanal content was calculated by pooling information within treatments and storage times, and one pair of data for the

Table 17. Changes in hexanal content in turkey rolls over 48 hours at 4 C.[1,2]

	Storage Time (hours)					
	0		24		48	
Treatments	Hexanal	TBA	Hexanal	TBA	Hexanal	TBA
OR/STPP	26.94 (9.13)[3]	0.50	37.17 (6.27)	1.53	49.71 (1.13)	3.03
STPP	33.53 (3.65)	0.68	37.83 (2.93)	1.85	47.00 (6.47)	3.71
SALT	48.65 (2.09)	2.59	83.43 (3.52)	9.03	74.77 (9.09)	10.26

[1] All values represent the mean of duplicate samples from two replications of the experiment.

[2] Values are expressed as ng hexanal/g of meat.

[3] Values in parentheses are standard deviations.

STPP treatment at 48 hours was omitted as an outlier. A statistically significant r value of 0.65 was found indicating that the new hexanal method is useful for predicting WOF development. Correlations within each factor (treatments and storage time) were also calculated separately to investigate the effects of the individual factors. At time zero the correlation was low (r=0.25) while after 24 hours the r value was 0.95. At 48 hours the missing STPP data made calculation of the correlation difficult due to the small sample size. Within-time correlations for the SALT and OR/STPP treatments were high, 0.71 and 0.68, respectively. These results confirm the usefulness of instrumental analyses (hexanal) in studying WOF development.

The vacuum collection/solvent extraction method is very versatile. Volatiles other than hexanal could easily be identified and quantitated to establish a flavor profile analysis system. Other researchers have investigated compounds such as 2,3-octanedione, pentanal, 2,4-decadienal, heptanal and 2-pentyl furan as potential markers for WOF development (Bailey et al., 1980; Barbut et al., 1985; St. Angelo et al., 1987). This method of analyses could easily be coupled with mass spectrometry to aid in identifying unknown compounds in the samples.

Another method for hexanal analysis was examined briefly. An instrument called an external closed inlet device (ECID) has been developed which allows for direct injection of the volatiles from the Tenax traps with no solvent extraction step required (Dupuy et al., 1977). Heat and carrier gas flow are used to drive the sample onto the GC column. Sample collection techniques (purge and trap or vacuum distillation) are the same for this method, however, the sensitivity should be improved because the sample volatiles are not diluted in solvent before injection. This method was not pursued due to electrical malfunctions in the equipment and time constraints.

SUMMARY AND CONCLUSIONS

The effectiveness of an oleoresin rosemary as an antioxidant during refrigerated and frozen storage of restructured beef steaks was evaluated. Relationships between TBARS values, sensory scores, hexanal levels and fatty acid profiles were investigated. During refrigerated storage of cooked restructured beef steaks, the SALT control had the largest TBARS values and a poor sensory score after six days at 4 C. The effect of added STPP was significant ($p<0.01$) at days 2, 4, and 6, while the presence of OR as well as any interaction between OR and STPP were not significant at any storage period ($p>0.05$). No significant difference existed between the OR1/STPP, OR2/STPP treatments and TBHQ/STPP. However, since OR alone was ineffective the antioxidant activity in these treatments was most likely due to the presence of STPP not OR. Analysis of raw steaks refrigerated for five days indicated that OR1 and OR2 without STPP provided protection greater than in the SALT control. It is possible that in the cooked steaks the OR cannot quench all the free radicals generated due to a lower concentration of antioxidant species.

Results of the frozen storage study of raw steaks differed substantially from those of the refrigerated study. The SALT control developed the highest TBARS values and sensory

scores at each sampling period. A linear concentration effect of OR existed ($p<0.01$) for both TBARS values and sensory scores, and the main protective factor was no longer STPP as during refrigerated storage. STPP and OR1 or OR2 alone each provided some protection against oxidation, but when used in combination an additive effect was observed. The OR1/STPP and OR2/STPP treatments did not significantly differ from the TBHQ/STPP treatment ($p<0.05$). Therefore, it appears that synthetic antioxidants can be replaced by OR/STPP in raw restructured beef steaks to increase shelf-stability during frozen storage. At the high level of OR detection of spice flavor may be of concern.

Hexanal content was measured during six months of frozen storage. After three months of storage the SALT control developed more than twice the hexanal content of the other treatments in agreement with the TBARS values and sensory scores. Similar trends continued after six months with the TBHQ/STPP treatment showing the lowest level of hexanal.

Oxidative breakdown of NLs and PLs was observed during frozen storage. Neutral lipid changes over the six month storage period were small. Significant changes occurred over time in the PL fractions and significant differences existed between treatments after six months of frozen storage. The fatty acids most responsible for these changes were C20:4, C18:2, C18:1, C16:0, PUFA and SAT FA.

A statistically significant but moderate correlation coefficient (pooled within treatments and storage times) of r = 0.49 was found between TBARS values (cooked) and sensory scores during frozen storage while during refrigerated storage the correlation was very low (r=0.10). High variability between experimental replications and a leveling off of sensory scores over time may have contributed to the low correlations. Correlation coefficients between hexanal content and TBARS values and sensory scores were also low and non-significant.

An alternative method for hexanal analysis was investigated. A vacuum distillation collection/solvent extraction was found to be reproducible and exhibited good recoveries. Cooked turkey rolls stored at 4 C for 48 hours were evaluated. Hexanal content and TBARS values increased over time. A correlation coefficient of 0.65 was reported between these two methods of analysis indicating that the new hexanal method is useful for measuring WOF development.

From the results of this study it can be concluded that OR is effective in retarding lipid oxidation in restructured beef steaks during frozen storage. It is somewhat less effective in cooked products where STPP provides the predominant effect. Hexanal concentration could prove to be an excellent index of oxidative deterioration if

experimental conditions are closely controlled. Correlations between TBARS values, sensory scores and hexanal content may be improved by choosing similar beef for replications, expanding the sensory evaluation scale and increasing the training level of panelists and using improved methods of hexanal analysis. Changes in fatty acid profiles may also be used to monitor oxidative changes.

APPENDICES

Appendix A

SENSORY EVALUATION OF WARMED OVER FLAVOR IN RESTRUCTURED BEEF STEAKS

Name _____
Date _____

You will be given a series of coded samples and a reference sample. Consider the reference sample to have no warmed over flavor (WOF). Smell and taste each sample and carefully evaluate the degree of warmed over flavor. Place a mark by the descriptor you feel accurately describes the flavor of the sample. Also, indicate with yes or no the presence of other non-meat flavors. Please eat a portion of cracker and have a drink of water between samples to clear your mouth. More than one sample may have the same flavor intensity.

Descriptor	Code			
	_____	_____	_____	_____
No WOF	_____	_____	_____	_____
Very slight WOF	_____	_____	_____	_____
Slight WOF	_____	_____	_____	_____
Moderate WOF	_____	_____	_____	_____
Strong WOF	_____	_____	_____	_____
Very Strong WOF	_____	_____	_____	_____
Non-meat flavor	_____	_____	_____	_____

COMMENTS:

Figure 10. Sensory evaluation form used to evaluate warmed-over flavor in restructured beef steaks.

Appendix B

Table 18. Significance levels of designed contrasts for treatment effects on TBARS values and sensory scores during refrigerated storage of restructured beef steaks.

Contrast	Test	Day of storage			
		0	2	4	6
(1) STPP vs no STPP	TBA	ns	0.01	0.01	0.01
	Sensory	0.05	0.01	0.01	0.01
(2) OR1/STPP vs TBHQ/STPP	TBA	ns	ns	ns	ns
	Sensory	ns	ns	ns	ns
(3) OR2/STPP vs TBHQ/STPP	TBA	ns	ns	ns	ns
	Sensory	ns	ns	ns	ns
(4) OR Linear Effect (OR_L)	TBA	ns	ns	ns	ns
	Sensory	ns	ns	ns	ns
(5) OR Quadratic Effect f(OR_Q)	TBA	ns	ns	ns	ns
	Sensory	ns	ns	ns	ns
(6) Interaction STPP & OR_L	TBA	ns	ns	ns	ns
	Sensory	ns	ns	ns	ns
(7) Interaction STPP & OR_Q	TBA	ns	ns	ns	ns
	Sensory	ns	ns	ns	ns

ns: not significant

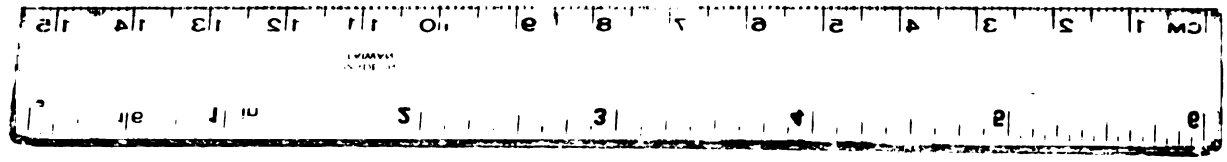


Table 19. Hexanal content of raw, restructured beef steaks stored at -20 C.[1,2]

Treatment	Month 3		Month 6		
	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
SALT	1939 (1931-1945)	1826 (1105-2546)	3186 (2965-3406)	3082 (3407-2756)	2320 (1975-2496)
STPP	1998 (1246-2750)	783 (668-898)	1454 (1454)	2163 (2093-2233)	1955 (1419-2490)
OR1	1641 (1019-2263)	1175 (1175)	2700 (2700)	1308 (1136-1480)	2067 (1823-2312)
OR2	1843 (1405-2280)	410 (288-531)	1283 (1209-1367)	1695 (1521-1868)	1140 (699-1404)
OR1/STPP	1127 (1112-1143)	614 (460-768)	1858 (1510-2205)	1498 (1474-1522)	1691 (1645-1737)
OR2/STPP	1044 (876-1212)	666 (627-706)	1826 (1687-2500)	1745 (1705-1785)	2883 (2799-2966)
TBHQ/STPP	618 (496-740)	283 (181-385)	2103 (2008-2648)	1260 (1238-1282)	723 (659-786)

[1] Values are expressed as ng of hexanal per gram of meat.

[2] Values in parentheses represent the range of one to four determinations per sample for each experimental replication (Rep).

Appendix D

Fatty Acid Profiles

Table 20. Fatty acid comoposition of the neutral lipid fraction from restructured beef steaks after three months of frozen storage [1]

Fatty Acid [2,3,4]	Sample						
	SALT	STPP	OR1	OR2	OR1/ STPP	OR2/ STPP	TBHQ/ STPP
14:0	3.33	3.25	3.22	3.15	3.22	3.25	3.30
14:1	1.14	1.11	1.25	1.39	1.19	1.21	1.17
16:0	25.83	25.50	25.32	25.45	25.28	25.54	25.48
16:1	5.96	5.69	6.00	6.31	5.86	6.04	5.97
17:0	1.42	1.46	1.41	1.30	1.35	1.37	1.36
U	1.19	1.20	1.23	1.14	1.17	1.18	1.18
18:0	12.17	13.15	11.80	11.41	12.07	11.77	12.06
18:1	45.40	44.61	45.61	46.12	45.58	45.75	45.40
18:2	2.90	3.05	3.28	2.97	3.31	3.09	3.07
18:3	0.68	1.00	0.88	0.80	0.99	0.83	1.04
% SAT FA	42.75	43.36	41.76	41.30	41.92	41.93	42.20
% MUFA	52.49	51.41	52.87	53.82	52.63	53.00	52.54
% PUFA	3.59	4.05	4.16	3.77	4.30	3.91	4.11
% TUFA	56.08	55.46	57.03	57.59	56.93	56.91	56.65

[1] Values are expressed as percent of total and represent the means of three experimental replications.

[2] tr = trace amounts detected and U = unknown compound.

[3] PUFA includes the di-unsaturated fatty acids.

[4] SAT FA = saturated fatty acid MUFA = mono-unsaturated fatty acid
PUFA = polyunsaturated fatty acid TUFA = total unsaturated fatty acid

Table 21. Fatty acid content of the neutral lipid fraction from restructured beef steaks after six months of frozen storage.[1]

Fatty Acid[2,3,4]	Sample						
	SALT	STPP	OR1	OR2	OR1/ STPP	OR2/ STPP	TBHQ/ STPP
14:0	3.28	3.24	3.23	3.18	3.29	3.30	3.28
14:1	1.23	1.40	1.30	1.18	1.29	1.38	1.46
16:0	26.10	25.89	26.09	25.99	26.26	26.14	25.93
16:1	5.98	5.95	6.08	5.91	5.95	5.92	5.92
17:0	1.35	1.35	1.29	1.25	1.30	1.31	1.32
U	1.06	1.07	1.04	1.00	1.08	1.03	1.05
18:0	12.29	12.25	11.65	11.91	11.96	11.87	12.17
18:1	45.62	45.70	46.16	46.28	45.43	45.89	45.87
18:2	2.71	2.64	2.66	2.76	2.84	2.77	2.60
18:3	0.41	0.50	0.52	0.55	0.61	0.41	0.42
% SAT FA	43.02	42.73	42.26	42.34	42.82	42.62	42.71
% MUFA	52.83	53.05	53.54	53.37	52.67	53.19	53.25
% PUFA	3.12	3.15	3.18	3.31	3.46	3.18	3.01
% TUFA	55.95	56.20	56.73	56.68	56.12	56.37	56.26

[1] Values are expressed as percent of total and represent the mean of three experimental replications.

[2] U = unknown compound.

[3] % PUFA includes the di-unsaturated fatty acids.

[4] SAT FA = saturated fatty acid MUFA = mono-unsaturated fatty acid
PUFA = polyunsaturated fatty acid TUFA = total unsaturated fatty acid

Table 22. Fatty acid composition of the phospholipid fraction from restructured beef steaks after three months of frozen storage[1]

Fatty Acid[2,3,4]	Sample						
	SALT	STPP	OR1	OR2	OR1/ STPP	OR2/ STPP	TBHQ/ STPP
14:0	0.68	0.38	0.50	0.74	0.56	0.59	0.47
16:0	16.84	16.03	16.94	16.75	15.74	15.82	15.24
16:1	2.52	2.01	1.93	3.26	1.89	2.19	1.82
17:0	0.64	0.55	0.56	0.57	0.47	0.54	0.50
U	0.84	0.76	0.76	0.71	0.65	0.63	0.74
18:0	12.29	12.10	12.91	10.99	11.63	11.32	11.91
18:1	27.71	25.92	26.59	28.52	25.59	25.21	25.34
18:2	18.07	20.23	21.93	20.09	21.73	22.28	21.51
18:3	2.33	2.57	0.64	0.87	1.36	1.21	2.65
20:3	3.75	4.16	3.68	3.45	4.60	4.01	4.37
20:4	10.81	11.60	11.02	11.37	12.88	13.27	11.87
20:5	1.28	1.23	0.58	0.63	0.73	0.71	1.29
22:4	0.91	1.10	1.13	1.19	1.19	1.22	0.93
22:5	1.39	1.43	0.89	0.93	1.03	1.05	1.43
% SAT FA	30.44	29.05	30.91	29.04	28.40	28.27	28.10
% MUFA	30.23	27.93	28.52	31.78	27.48	27.40	27.16
% PUFA	38.52	42.30	39.86	38.52	43.51	43.74	44.04
% TUFA	68.75	70.23	68.38	70.29	70.99	71.14	71.20

[1] Values are expressed as percent of total and represent the means of two experimental replications.

[2] U = unknown compound.

[3] PUFA includes the di-unsaturated fatty acids.

[4] SAT FA = saturated fatty acid MUFA = mono-unsaturated fatty acid
PUFA = polyunsaturated fatty acid TUFA = total unsaturated fatty acid

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