This is to certify that the

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STABILITY OF LIPIDS IN RESTRUCTURED BEEF STEAKS

presented by

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has been accepted towards fulfillment
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James Jan Gray
Major professor

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STABILITY OF LIPIDS IN RESTRUCTURED BEEF STEAKS

By

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ABSTRACT

STABILITY OF LIPIDS IN RESTRUCTURED BEEF STEAKS

By

Rhonda Lynn Crackel

The effect of antioxidants on the stability of lipids in restructured beef steaks was investigated. Two natural formulations--containing mixed tocopherols, ascorbyl palmitate and citric acid--and TBHQ were evaluated. Lipid oxidation was monitored over 12 months of frozen storage by a modified TBA procedure, gas chromatographic analysis of volatiles and sensory evaluation.

TBHQ significantly ($p < 0.05$) lowered TBA numbers in raw and freshly cooked samples and in samples that were cooked and held 4 hours at $4^{o}C$. Natural antioxidants provided significant protection in freshly cooked meat. Hexanal concentration in control samples was positively correlated with TBA numbers, but only at individual sampling periods. Hexanal was not detected in TBHQ-treated samples. Mean sensory scores from all treatments did not differ significantly ($p < 0.05$) at any sampling period. The correlation coefficients between sensory scores and TBA numbers were generally low and not significant ($p < 0.10$) until TBA numbers approached 2.0.

To my husband, Dan, for his patience, kindness and enduring love.

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Appreciation is extended to Dr. A.M. Booren, Dr. B.R. Harte and Dr. A.M. Pearson for serving on the guidance committee and for their critical review of this thesis. I also wish to thank the Diamond Crystal Salt Company for supplying antioxidant-coated salts and Dr. W. Wilkens for his technical advice. Further acknowledgement is due the Ralston Purina Company for financial assistance during this study.

Special thanks are extended to all my friends and coworkers in Lab 311 and the MSU Meats Laboratory for their assistance with this research project but mostly for their moral support and humorous outlook on life.

Finally, my deepest gratitude to my family and my husband, Dan, for their continual love and support of this and all endeavors.

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INTRODUCTION

The technology for restructuring meat was developed as a way to effectively utilize low-value cuts and trimmings to manufacture a product that has satisfactory sensory properties and a reasonable unit cost (Seideman and Durland, 1983). A major factor limiting the widespread acceptance of these products is the changes in quality which occur as the result of lipid oxidation.

The stability of lipids in restructured products is largely influenced by the procedures used in manufacture, especially reduction of particle size and the addition of sodium chloride. Sato and Hegarty (1971) demonstrated that comminution of fresh meat and subsequent exposure to air could cause the development of rancidity within one hour. Schwartz and Mandigo (1976) and Huffman et al. (1981b) have reported that added salt increases TBA numbers of restructured pork.

The use of antioxidants has been shown to reduce lipid oxidation in both restructured and ground meat (Greene et al., 1971; Chastain et al., 1982; Miles et al., 1986) during refrigerated storage and up to 20 weeks of frozen storage.

The major objective of this study was to determine the effect of antioxidants on lipid stability in restructured beef steaks over 12 months of frozen storage. Two natural

antioxidants and TBHQ were evaluated. A secondary objective was to examine the correlation between TBA numbers, gas chromatographic quantitation of volatiles and sensory evaluation as means for assessing lipid oxidation. Finally, in this study the TBA method of Tarladgis et al. (1960) and a modified TBA method (Tarladgis et al., 1964) were evaluated for use with muscle products.

REVIEW OF LITERATURE

The development of oxidative rancidity in meat products is a serious concern particularly in light of the increased consumption of pre-cooked meat items and restructured products. It is well known that lipid oxidation is associated with the development of undesirable flavors and odors in meat and meat products (Tims and Watts, 1958; Younathan and Watts, 1960; Greene, 1969; Greene and Price, 1975). Consequently, the role of lipids in the development of warmed-over flavor (WOF) in meats and the factors influencing their susceptibility to oxidation will be addressed in this review. These factors include the composition and location of lipids, the presence of pro-oxidative compounds and the processing parameters utilized in restructuring meats. Additionally, methods of assessing lipid oxidation and the use of antioxidants in meat products are discussed. A brief overview of meat restructuring is also provided.

Distribution of Animal Lipids

The lipids comprising animal fats are commonly classified as depot or adipose tissue and as intramuscular or tissue lipids (Watts, 1962; 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. These lipids consist mainly of triglycerides which may vary in amount and fatty acid composition according to species, diet, sex, age, environment and depot location within the animal (Deuel, 1955).

In contrast, tissue lipids vary much less in proportion and fatty acid composition. These lipids consist largely of membrane-bound phospholipids and lipoproteins of the cell wall (Kono and Colowick, 1961), mitochondria (Holman and Widmer, 1969) and other cellular structures. Although the amount of phospholipid varies inversely with the lipid content of meat, when expressed as a percentage of total tissue weight, levels of phospholipids remain fairly constant and range from 0.5 to 1.0 percent (Dugan, 1971). Confirming work by Igene et al. (1979a) showed the triglyceride content of fresh chicken light and dark meat and fresh beef to range from 2.0 to 12.9 percent, while the phospholipid fractions ranged only from 0.54 to 0.82 percent.

Composition of Meat Lipids

Animal fats, or triglycerides, typically contain mainly C_{14}, C_{16} and C_{18} fatty acids although Hansen et al. (1958) found beef tallow contained trace amounts of even-numbered carbon fatty acids from C_{10} to C_{26} and odd-numbered chains from C_{11} to C_{25}. Generally, the predominant fatty acids of

meat triglycerides are saturated or contain only one or two double bonds (Igene et al., 1981). Polyunsaturates comprise less than 2 percent of the triglyceride fatty acids found in beef or sheep (Pearson et al., 1977).

Species differences in the composition and structure of adipose tissue are well documented (Hilditch and Lovern, 1936; Shorland, 1952; Hilditch and Williams, 1964). These differences are shown in Table 1. Comparison of beef, lamb, chicken, pork and fish lipids shows fish fat to contain the least amount of saturated fatty acids and the highest amount of C_{20} unsaturated and other polyunsaturated acids. Sheep triglycerides contain the highest amount of saturates followed by beef, pork and chicken lipids. The oxidative stability of these meats is related to their degree of unsaturation with fish being the most susceptible to rancidity followed by chicken, pork, beef and lamb (Wilson et al., 1976).

Within a single species, the location of depot fat in the body may influence its fatty acid composition. It has been shown that the internal fats of beef, sheep and pig are richer in $C_{18:0}$ but lower in $C_{18:1}$ than the subcutaneous fats (Shorland, 1962). However, the composition of depot fats of poultry does not vary much regardless of location.

It has also been shown that within a single species, especially nonruminants, the composition of dietary fat may largely influence the composition of depot fat. Work by Ellis and Isbell (1926) showed that depot fat from pigs

Table 1. Fatty acid composition of beef, lamb, chicken, pork and fish fats expressed as percentage of total.[1]

Fatty Acid	Species				
	Beef[2]	Lamb[2]	Chicken[3]	Pork[4]	Fish[5]
12:0		0.8	0.1		
14:0	3.2	5.2	0.7	1.2	6.7
15:0	0.5	0.6	0.2		
16:0	24.8	24.6	22.8	22.7	11.5
17:0	1.8	1.0	0.2		
18:0	13.7	22.9	6.5	13.7	1.4
20:0		0.8			
14:1	0.9		0.2		
16:1	4.4	1.6	5.7	4.1	8.6
18:1	46.9	38.7	37.0	47.8	14.4
18:2	1.9	1.2	23.7	10.5	1.2
18:3	0.2	0.6	1.3		0.9
18:4					1.45
20:1		0.2	0.2		14.3
20:2			0.7		
20:3			0.1		
20:4			0.2		0.86
20:5					5.62
22:1					22.00
22:5					1.08
22:6					3.35

[1]Data from Pearson *et al.* (1977)

[2]Subcutaneous fat

[3]Depot fat

[4]Backfat

[5]Herring oil

fattened on rations containing high levels of unsaturated lipids contained degrees of unsaturation varying with the degree of unsaturation of the feed.

In contrast to triglyceride fractions, phospholipids in muscle are altered only slightly by dietary changes (Igene, 1976). Additionally, phospholipids contain a much larger proportion of C_{20} and C_{22} unsaturated fatty acids. Polyunsaturation of the phospholipid fraction is about 15 times greater than that of the triglyceride fraction (Igene and Pearson, 1979; Igene et al., 1981).

The major component phospholipids identified from animal tissues are phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), sphingomyelin (SP), phosphatidyl inositol (PI), lysophosphatidyl choline (LPC) and other minor components (Pearson et al., 1977). Each component phospholipid has a generally characteristic fatty acid composition. Higher levels of polyunsaturated fatty acids, especially C_{20}, have been reported for PE (17 to 43%) than for PC (7 to 25%) and SP (1 to 4%) (Body and Shorland, 1974).

The proportion of each component phospholipid varies between species and even between muscles within the same species. Keller and Kinsella (1973) reported the composition of beef phospholipids as 53 to 58% PC, 23 to 25% PE, 5 to 7% SP, 5 to 7% PI, 1 to 4% PS and 1 to 6% other components. Peng and Dugan (1965) reported the differences in component phospholipids in chicken dark and light meats.

Dark meat contained 52 to 58% PC, 24 to 30% PE, 7 to 9% PS and 3 to 4% SP while light meat contained 58 to 62% PC, 15 to 16% PE, 9 to 10% PS and 2 to 4% SP.

There is also significant variation in total phospholipid content among species (Kaucher et al., 1944; Igene et al., 1979b) and between muscle locations in the same animal (Gray and Macfarlane, 1961; Dugan, 1971). Poultry and fish muscle are known to be higher in phospholipids than red meats (Younathan and Watts, 1960; Igene et al., 1979b). There is also evidence which suggests red, oxidative muscles have a higher proportion of phospholipids than white, glycolytic muscles taken from the same species (Wilson et al., 1976; Igene et al., 1979b) although Katz et al. (1966) reported that chicken breast (white) muscle contained more phospholipids than leg (red) muscle.

Not only does the proportion of phospholipids vary with muscle type, work by Luddy et al. (1968) suggests that the fatty acid composition of phospholipids may also vary according to muscle type. White muscles were found to have a higher content of monoenes, while polyunsaturates predominated in the phospholipids from dark muscle.

Role of Lipids in Rancidity and WOF Development

Tims and Watts (1958) first postulated that warmed-over flavor (WOF) resulted from the rapid development of lipid oxidation. Younathan and Watts (1960) later showed phospholipids to be the lipid component most rapidly oxidized in

cooked meat. By compairing correlation coefficients between TBA numbers and total lipid levels versus those between TBA numbers and phospholipids, Wilson et al. (1976) suggested that phospholipids play a major role in the development of WOF in turkey, chicken, beef and lamb, but that total lipids are the major contributor to WOF in pork. Working with model systems of lipid-extracted muscle fibers, Igene and Pearson (1979) confirmed total phospholipids as the major contributors to WOF in cooked meat. Triglycerides were found to enhance the development of WOF only when combined with the phospholipids as total lipids. The role of phospholipids in the development of WOF in fish is not clear, but the relatively high content of unsaturated fatty acids in the triglyceride fraction would suggest that both triglycerides and phospholipids could contribute to the rapid development of oxidative rancidity (Pearson et al., 1977).

The development of oxidative rancidity has been found to vary with species, most likely because of differences in phospholipid content and fatty acid composition. Poultry and fish are known to have a higher content of phospholipids than red meats (Younathan and Watts, 1960; Igene et al., 1979b). They also contain a higher proportion of di- and polyunsaturated fatty acids (Igene et al., 1981). This could explain why rancidity occurs more rapidly in fish and poultry than beef or pork (Wilson et al., 1976; Igene et al., 1979b).

The lability of phospholipids is at least partially due to their high content of unsaturated fatty acids (Lea, 1957) particularly linoleic and arachadonic acids. The phospholipids from beef are approximately 15% more unsaturated than the triglycerides. Polyunsaturated fatty acids account for 16% and 22% of the total unsaturation of phospholipids from chicken white and dark meat, while the triglyceride fractions contain only 3% and 1.5% respectively (Igene and Pearson, 1979). Loss of polyunsaturated fatty acids (PUFAs) and decreases in total phospholipids have both been linked to the development of WOF in cooked meat (Keller and Kinsella, 1973; Igene and Pearson, 1979).

Phospholipids may also be more susceptible to oxidation due to their close association in membranes with tissue catalysts of oxidation. Processes which disrupt the membrane such as grinding, chopping or emulsifying expose the phospholipids to oxygen, enzymes, heme pigments and metal ions which can cause the rapid development of rancidity even in fresh, raw meat (Sato and Hegarty, 1971).

Igene and Pearson (1979) noted that certain individual component phospholipids are more significant in the development of WOF than others. Addition of PE to a meat model system significantly increased TBA numbers and lowered sensory scores, while addition of PC did not alter either TBA or sensory scores. This confirms the reports of Corliss and Dugan (1970) and Tsai and Smith (1971) that the ethanolamine moiety of PE exerts a greater prooxidant effect than

does the choline of PC and suggests that PE is the most important phospholipid component contributing to the development of WOF. The lability of PE has been attributed to its higher concentration of PUFAs, especially $C_{20:4}$ (Keller and Kinsella, 1973; Igene and Pearson, 1979) and to the ethanolamine moiety which has been found to exert a greater prooxidative effect than choline (Corliss, 1968; Tsai and Smith, 1971). Tsai and Smith (1971) proposed that the NH_3^+ group of ethanolamine may form hydrogen bonds with lipid hydroperoxides and promote their decomposition to prooxidant free radicals.

Lipid Stability During Frozen Storage of Meats

Numerous studies have shown that lipid oxidation occurs during freezer storage of meats (Keller and Kinsella, 1973; Bremner et al., 1976; Igene et al., 1979b). The stability of frozen meats depends essentially on the degree of fatty acid unsaturation (Greene, 1969; Igene 1976). Work by Igene et al. (1979b) showed the stability of various meats to be: beef> chicken white meat> chicken dark meat. These trends were indicated by increased TBA numbers and decomposition of constituent lipids.

Evidence suggests changes occurring in the total lipid content of raw meat during frozen storage are due mainly to losses in the triglyceride fraction whereas the levels of phospholipids remain relatively constant even over extended storage periods (Igene et al., 1979b). Contradictory data

from Keller and Kinsella (1973) indicated negligible decreases in total lipids but significant losses of phospholipids during frozen storage of hamburger. These trends may have been caused by the grinding of the meat which promoted oxidation of the membrane-bound phospholipids (Sato and Hegarty, 1971).

Lipid Oxidation in Meats

Mechanism of Lipid Oxidation

The autoxidation of unsaturated fatty acids is generally accepted to proceed according to a free radical chain mechanism. The susceptibility and rate of oxidation of fatty acids varies according to their degree of unsaturation and the presence of activating agents such as heat, light, enzymes or metal catalysts or inhibiting substances. The autocatalytic theory was extensively reviewed by Dugan (1961) and Lundberg (1962) and may be summarized as follows:

I. Initiation

$$RH + O_2 \text{ ------> } R^{\cdot} + OH^{\cdot}$$

RH represents an unsaturated fatty acid containing a labile hydrogen on a reactive methylene group adjacent to a double bond. $R^{\cdot}$ is the free radical formed by abstraction of the labile hydrogen.

II. Propagation

$$R^{\cdot} + O_2 \text{ ------> } ROO^{\cdot}$$

$ROO^{\cdot} + RH \text{ ------> } ROOH + R^{\cdot}$

Reaction of the lipid free radical with oxygen yields a peroxy radical which may abstract a labile hydrogen from another unsaturated fatty acid. A hydroperoxide, ROOH, and another free radical, $R^{\cdot}$, which is capable of perpetuating the chain reaction are formed.

Free radicals may also combine to form stable non-reactive products which terminate the chain reaction.

III. Termination

$R^{\cdot} + R^{\cdot} \text{ ------> } RR$

$R^{\cdot} + ROO^{\cdot} \text{ ------> } ROOR$

$ROO^{\cdot} + ROO^{\cdot} \text{ ------> } ROOR + O_2$

Products of Lipid Oxidation

Hydroperoxides, ROOH, are the primary initial products of lipid oxidation. Many secondary products are formed through subsequent reactions as shown in Figure 1 (Gray, 1978). The decomposition of hydroperoxides may produce alcohols, ketones, acids and lactones via either of two pathways. At low peroxide concentrations, hydroperoxide decomposition is mainly monomolecular (Dugan, 1961; Lundberg, 1962).

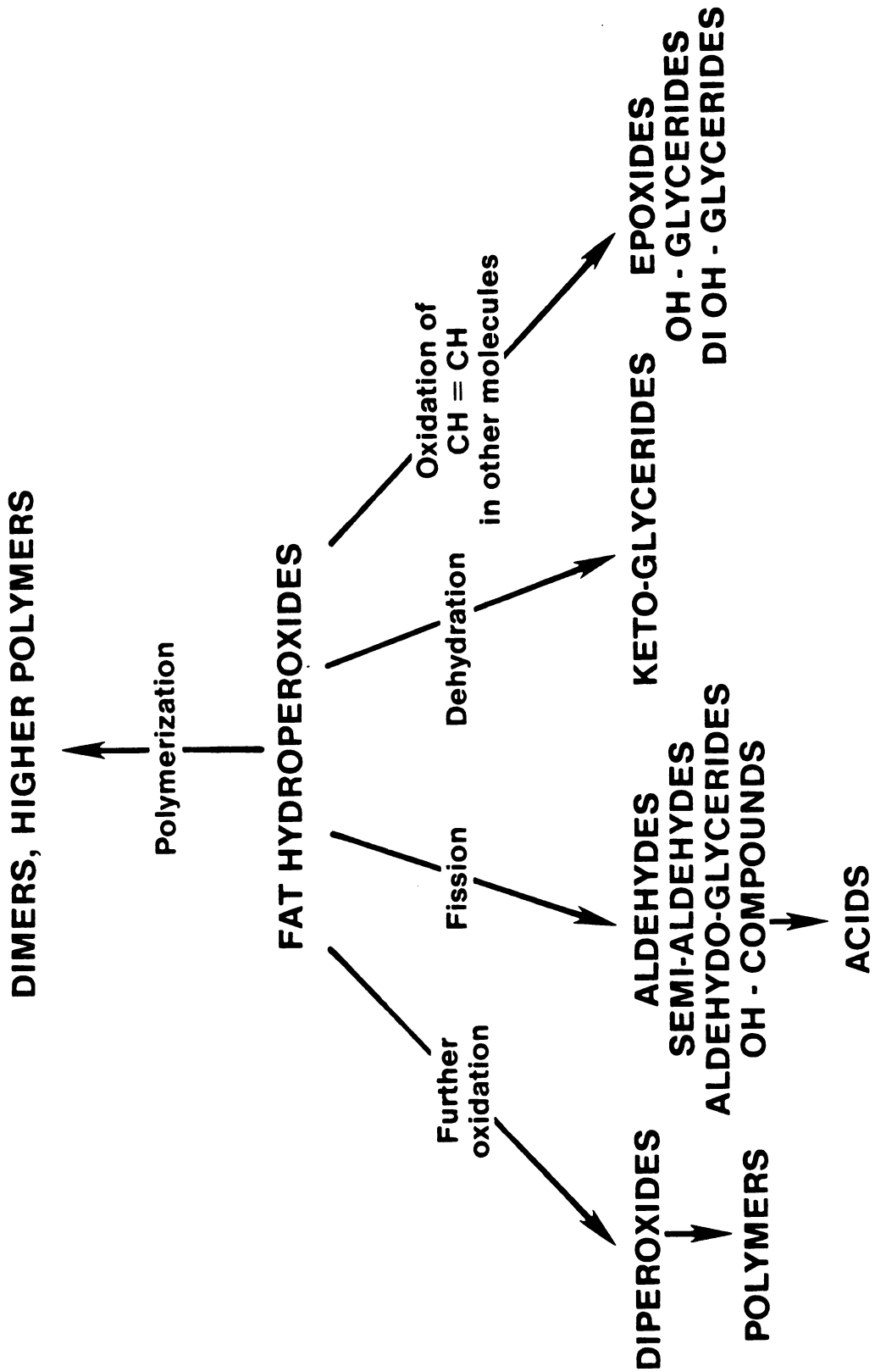
$ROOH \text{ ------> } RO^{\cdot} + OH^{\cdot}$

At higher concentrations, bimolecular decomposition occurs.

$2ROOH \text{ ------> } ROO^{\cdot} + RO^{\cdot} + H_2O$

The decomposition of hydroperoxides is of great importance in the development of WOF. Hydroperoxides themselves are odorless; however, their breakdown to aldehydes, ketones

Figure 1. Some routes of decomposition of fat hydroperoxides.


DIMERS, HIGHER POLYMERS
Polymerization
FAT HYDROPEROXIDES
Oxidation of
CH = CH
in other molecules
Dehydration
KETO-GLYCERIDES
EPOXIDES
OH - GLYCERIDES
DI OH - GLYCERIDES
Fission
ALDEHYDES
SEMI-ALDEHYDES
ALDEHYDO-GLYCERIDES
OH - COMPOUNDS
ACIDS
Further
oxidation
DIPEROXIDES
POLYMERS

and acids may result in rancid off-odors and flavors. While many of these secondary products are very reactive and may not accumulate appreciably in an oxidized product, concentrations as low as 1 ppb may exceed flavor thresholds (Sato et al., 1973).

Hexanal has been cited as one of the major secondary products formed during the oxidation of linoleic acid (Gaddis et al., 1961; Frankel et al., 1981). El-Gharbawi and Dugan (1965) reported the formation of hexanal during the storage of freeze-dried beef, while Cross and Ziegler (1965) measured both hexanal and pentanal in cooked, uncured pork. Love and Pearson (1976) detected hexanal in headspace samples taken from oxidizing PE and cooked meat and reported higher concentrations with increasing oxidation. Work by Fritsch and Gale (1977) suggests that hexanal production is indicative of lipid oxidation in dry cereals and may be related to the development of rancid odors.

Other aldehydes have also been linked to WOF. Ruenger et al. (1978) reported that heptanal and n nona-3-6-dienal are major flavor compounds related to WOF in turkey. Bailey et al. (1980) identified 2,4 decadienal along with hexanal in cooked beef. Alone and in combination, these two compounds have been highly correlated with flavor scores of oxidized oils (Dupuy et al., 1976; Rayner et al., 1978).

Another aldehyde of major importance in oxidizing foods is malonaldehyde, a three-carbon dialdehyde produced during autoxidation of polyunsaturated fatty acids. A mechanism

for the formation of malonaldehyde was proposed by Dahle et al. (1962) and modified by Pryor et al. (1976). The revised scheme suggests a prostaglandin-like endoperoxide mechanism in which malonaldehyde is formed via the cleavage of a cyclic endoperoxide. The formation of malonaldehyde during lipid oxidation is the basis for several chemical methods of monitoring oxidation. One such method, the thiobarbituric acid (TBA) test, involves the condensation of two molecules of thiobarbituric acid with malonaldehyde to form a pink pigment which can be quantitated by measuring absorbance at 532 nm. This method has been criticized, however, as being nonspecific for malonaldehyde and therefore an unreliable indicator of oxidation.

Consequences of Lipid Oxidation

As shown in Figure 1 (Gray, 1978) the hydroperoxides from lipid oxidation may undergo any of several secondary reactions. Although the formation of off-flavor compounds is a major determinant of product quality, several other reaction may affect a product's safety and acceptability. Secondary reactions may lead to the formation of potentially toxic compounds, result in a loss of nutrients or promote further oxidative reactions.

Pearson et al. (1983) reviewed the safety implications of lipid oxidation in muscle foods. They reported that evidence of the direct toxicity and mutagenicity of malonaldehyde is conflicting and inconclusive although data suggest that hydrolysis products may be mutagenic.

Additionally, malonaldehyde has been implicated as a catalyst of the formation of carcinogenic N-nitrosamines. It has also been reported that malonaldehyde and other lipid oxidation products can react with amino acids to form Schiff bases or may crosslink with proteins thus reducing the nutritional quality of a muscle food (Chio and Tappel, 1969; Braddock and Dugan, 1973; Gardner, 1979).

Another consequence of lipid oxidation is the loss of the desirable red color of raw meat due to the formation of metmyoglobin (MetMb). Haurowitz et al. (1941) demonstrated in model systems that free radicals from oxidizing lipids could destroy heme pigments. A positive correlation between metmyoglobin accumulation and lipid oxidation in raw meat was reported by Hutchins et al. (1967). Many other studies confirmed the interdependence of the two reactions (Greene, 1969; Greene et al., 1971; Greene and Price, 1975; Benedict et al., 1975). Through the use of antioxidants these researchers were able to prevent lipid oxidation and the conversion of myoglobin to metmyoglobin.

Work with enzymic lipid peroxidation of microsomal fractions produced similar results (Lin and Hultin, 1977); the oxidation of oxymyoglobin to metmyoglobin was prevented by BHA or glutathione peroxidase, which functions to decompose lipid hydroperoxides to non-reactive compounds. From his work with glutathione peroxidase, Hultin (1980) concluded that lipid oxidation precedes pigment oxidation

since no metmyoglobin was formed in a system treated with this enzyme.

Catalysis of Lipid Oxidation

Heme Compounds

The catalytic effect of iron porphyrins--hemoglobin, myoglobin and cytochromes--on lipid oxidation was first cited by Robinson (1924). Numerous studies have confirmed that heme compounds function as prooxidants when in contact with purified lipids (Tappel, 1952; Banks et al., 1961; Tappel, 1962; Liu, 1970; Hirano and Olcott, 1971), and it has been suggested they might function as prooxidants in meats (Younathan and Watts, 1958; Liu and Watts, 1970; Greene and Price, 1975).

The valency of iron in the proposed catalytic species has been widely disputed. Comparing cured and uncured cooked meats, Younathan and Watts (1958) reported that Fe^{3+} hemes were the active catalysts rather than the ferrous form of nitric oxide hemochromogen. However, Brown et al. (1963) reported that hemes containing either Fe^{2+} or Fe^{3+} exhibited catalytic activity. This was confirmed by Hirano and Olcott (1971) who also reported that rates of oxidation did not differ when Fe^{2+} or Fe^{3+} hemes were added to linoleate emulsions. Work by Greene and Price (1975) demonstrated that either ferrous or ferric compounds might function as catalysts, but that the Fe^{3+} is more active.

Labuza (1971) suggested that the ionic state of the metal is not as important in catalysis as is the ring structure of the heme. He proposed that the iron in heme may be sterically hindered by the large protein portion of the molecule. The rapid rate of oxidation in cooked meat would result from the denaturation of myoglobin and subsequent exposure of the iron. He also suggested that heating may release either the pigments or the lipids from protected compartments, causing more intimate contact between the two compounds.

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 close proximity and high electronegativity of the two oxygens would favor scission of the ROOH to form a free radical. The radical could abstract a labile hydrogen from an unsaturated fatty acid and propagate lipid oxidation. In this mechanism there would be no change in the valence of the heme iron.

Kanner and Harel (1985) reported the initiation of membranal lipid oxidation by "activated" metmyoglobin and methemoglobin (MetHb)--heme pigments combined with hydrogen peroxide. Heme pigments or H_2O_2 alone did not promote oxidation. However, the interaction of H_2O_2 with MetMb led very rapidly to the production of an activated species which initiated lipid peroxidation. They proposed that the autoxidation of oxyhemoglobin and oxymyoglobin leads to the

formation of methemeproteins and the superoxide radical, O_2^-, which dismutes to H_2O_2. A reactive porphyrin cation radical, $P^{\cdot+}$--Fe^{IV+}=O, results from the reaction of MetMb or MetHb with H_2O_2. This radical may then react to produce lipid free radicals which can initiate lipid oxidation.

In addition to their catalytic activity, heme compounds may also exhibit antioxidative properties. This depends largely on the proportion of heme and lipid in the system (Lewis and Wills, 1963). Kendrick and Watts (1969) examined the critical linoleate:heme ratios necessary for maximum catalysis of lipid oxidation. As the concentration of heme compounds was increased to optimal levels, lipid oxidation increased to a maximum. At heme concentrations exceeding the critical levels, oxidation decreased to zero. At these inhibitory heme concentrations, a red pigment, believed to be a stable heme-hydroperoxide complex, was formed. They also discovered that breakdown products from the reaction of hemes with H_2O_2 exhibited antioxidant properties. Similar work by Hirano and Olcott (1976) demonstrated that high concentrations of heme compounds inhibit lipid oxidation, while heme compounds at lower concentrations function as prooxidants. They proposed that compounds formed at high heme concentrations may act as free radical sinks thus decreasing lipid oxidation.

Much of the data regarding heme catalyzed lipid oxidation is confusing and contradictory. Love (1983)

explained that this is most likely due to the use of different types of model systems and experimental techniques for monitoring lipid oxidation. Additionally, results obtained from a model system may not be representative of the reactions and mechanisms found in meat systems. She suggests carefully designed and controlled studies are needed to clarify the role of heme compounds in lipid oxidation in meat.

Metals and Non Heme Iron

Heavy transition metals such as cobalt, copper, iron, manganese and nickel possessing two or more valency states generally increase the rate of oxidation of food lipids (Ingold, 1962). They may alter the rates of initiation, propagation and termination reactions as well as the rate of hydroperoxide decomposition. Their basic function is to increase the rate of formation of radical species.

Heaton and Uri (1961) reported that metals in their higher valency state may initiate lipid oxidation directly via the following reaction:

$$M^{(n+1)+} + RH \text{ ------> } M^{(n+1)+} H + R^{\cdot}$$

Metals in lower valency states may also initiate oxidation directly although they are thought to activate oxygen first with subsequent reactions producing free radicals.

$$M^{n+} + O_2 \text{ <------> } (M^{(n+1)+} \cdots O_2^{-}) \text{ ------> free radicals}$$

Ingold (1962) suggests that while these direct metal-lipid reactions may be significant in the early stages of oxidation, at later stages other reactions are more

important. The metal catalysed decomposition of hydroperoxides occurs much more rapidly that thermal decomposition and is the source of many free radicals as shown below.

$$M^{n+} + ROOH \text{ ------> } M^{(n+1)+} + RO^{\cdot} + OH^{-}$$

$$M^{(n+1)+} + ROOH \text{ ------> } M^{n+} + ROO^{\cdot} + H^{+}$$

Iron is the heavy metal most prevalent in meats. While most of the iron in meats is found in heme compounds, a number of compounds contain nonheme iron. Feritin, hemosiderin and transferrin function in the storage and transport of nonheme iron (Pearson et al., 1977), and several enzymes of the electron transport system contain nonheme iron. Several researchers have proposed that nonheme iron may also be a catalyst of lipid oxidation (Wills, 1965; Liu, 1970; Liu and Watts, 1970; Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979a).

Work by Wills (1966) showed that both heme and nonheme iron may catalyze lipid oxidation but that nonheme iron was a more active prooxidant at acid pH while heme compounds were less pH sensitive. This was confirmed by Liu (1970) and Liu and Watts (1970) who demonstrated that nonheme iron exhibited catalytic activity below pH 6.4 while MetMb was active over the pH range 5.6 to 7.8.

Sato and Hegarty (1971) presented evidence that nonheme iron is the major catalyst of lipid oxidation in cooked meat. Beef muscle was extracted with water prior to cooking to remove any potential prooxidants. Addition of myoglobin

or hemoglobin to the cooked, extracted fibers had little effect on lipid oxidation. However, $FeCl_2$ and $FeCl_3$ both promoted lipid oxidation with the ferrous form being more active. Love and Pearson (1974) reported similar results. Addition of nonheme Fe^{2+} to extracted muscle residue, at levels as low as 2ppm, resulted in increased TBA numbers while MetMb added at 1 to 10 mg/g did not promote oxidation. They also found, as did Sato and Hegarty (1971), that low levels (5 ppm) of ascorbic acid added with the Fe^{2+} enhanced its catalytic effect presumably by maintaining the nonheme iron in the ferrous state.

Igene et al. (1979a) also concluded that myoglobin is not the principal prooxidant in cooked meat. They reported that addition of a pigment extract to cooked beef muscle residue enhanced lipid oxidation. However, treatment of the extract with EDTA lessened its prooxidant activity. Treatment of the extract with H_2O_2 to destroy hemes increased its catalytic activity, which could also be suppressed by EDTA. Igene et al. (1979a) reported that over 90% of the iron in the fresh meat pigment was present as bound heme iron. Cooking destroyed the heme molecule and increased the concentration of nonheme iron in the extract from 8.72% to 27.0%. Thus, they concluded that the increased rate of lipid oxidation in cooked meat is due to the release of nonheme iron during cooking which catalyzes oxidation rather than the meat pigments per se.

Microsomal Enzymes

While it is generally accepted that lipid oxidation in muscle foods is essentially nonenzymatic in nature, there is evidence of enzymatic lipid peroxidation systems associated with muscle microsomes. Lin and Hultin (1976) demonstrated that microsomes from chicken leg and breast muscle produced malonaldehyde in the presence of ADP, NADPH, ferric chloride and oxygen. Further studies (Lin and Hultin, 1977) showed that peroxidizing microsomal fractions could oxidize myoglobin in vitro, and that inhibition of microsomal oxidation inhibited pigment oxidation. Hultin (1980) reported that addition of glutathione and glutathione peroxidase to an oxidizing microsomal system could decrease myoglobin oxidation. Since glutathione/glutathione peroxidase are known to decompose lipid hydroperoxides without the production of free radicals, Hultin concluded that lipid peroxidation may precede pigment oxidation.

Earlier studies of the oxidation of myoglobin (Govindarajan et al., 1977) showed that addition of lipase to ground beef increased pigment oxidation while addition of phospholipase A inhibited both pigment and lipid oxidation. The latter observation was unexpected since free fatty acids oxidize faster than the esterified form. Govindarajan et al. (1977) suggested that phospholipase A might inactivate an enzymatic system which destabilizes myoglobin. Hultin (1980) reported similar data but suggested that phospholipase A inhibition may be caused by free fatty acid

inhibition of the oxidative enzyme system in the microsomal membrane. He also proposed that, under proper conditions, native phospholipases might be used to produce a natural antioxidant system.

Sodium Chloride (Salt)

It is widely recognized that sodium chloride may initiate color and flavor changes in meat although the mechanism remains unclear. Early work suggested that NaCl catalyzed oxidation by lipoxidase (Lea, 1937) or by myoglobin (Tappel, 1952; Banks, 1961). Chang and Watts (1950) reported that salt had no greater effect on rancidity in the presence of hemoglobin or muscle extract than in their absence. They also demonstrated that the catalytic effect of NaCl depended on its concentration and the amount of moisture in the system. Aqueous salt solutions were prooxidative only at concentrations of NaCl above 15% while dry NaCl readily promoted oxidation of lard.

The mechanisms of NaCl-induced rancidity in pork were examined by Ellis et al. (1968). They reported that increasing levels of NaCl accelerated autoxidation but did not alter the decomposition of hydroperoxides to mono-carbonyls. However, in samples containing high proportions of lean, lower conversion of peroxides to monocarbonyls was observed. They postulated that NaCl may activate a component in the lean which results in a change in oxidation characteristics of the adipose.

Other researchers have proposed that salt catalyzed rancidity may be related to traces of metal impurities in the salt. Olson and Rust (1973) reported, though, that using a purified low-metal salt to cure hams did not improve taste panel scores over those for hams cured with conventional salt. They did find, however, that hams cured with salt containing antioxidants were preferred over the control samples and hams cured with low-metal salt.

Sodium chloride has also been reported to inhibit lipid oxidation under certain conditions. Mabrouk and Dugan (1960) observed that autoxidation of aqueous emulsions of methyl linoleate was suppressed by increasing concentrations of dissolved NaCl in the system. They suggested that the inhibition might result from decreased solubility of oxygen in the emulsions.

Measurement of Lipid Oxidation

Numerous chemical and physical methods have been developed for monitoring oxidation in oils and lipid-containing foods. These have been reviewed extensively by Gray (1978) and Melton (1983). In this review, several methods commonly used for measuring lipid oxidation in meat systems will be addressed.

TBA Test

One of the most commonly used methods for monitoring lipid oxidation in meat products is the 2-thiobarbituric acid test or TBA test (Gray, 1978; Melton, 1983). The

premise of this method is the condensation of two molecules of TBA with one molecule of malonaldehyde to produce a red complex which is quantitated spectrophotometrically in the region of 530 to 532 nm (Sinnhuber et al., 1958). The extent of lipid oxidation is expressed in terms of a TBA number having units of mg of malonaldehyde per kg of sample. The malonaldehyde found in oxidized meat is a secondary oxidation product formed mainly from polyunsaturated fatty acids containing three or more double bonds (Dahle et al., 1962; Pryor et al., 1976).

There are several ways in which the TBA test can be performed on muscle foods: 1) directly on the product, followed by extraction of the red pigment (Sinnhuber et al., 1958); 2) on a portion of the steam distillate of the food (Tarladgis et al., 1960) or 3) on an extract of the food (Witte et al., 1970). The most frequently used method involves steam distillation of volatiles--namely malonaldehyde--from the food and reaction of a portion of the distillate with TBA reagent to produce the characteristic red complex. This method has been widely used to monitor lipid oxidation and the development of WOF in poultry, beef and pork products (Melton, 1983).

The TBA test initially was reported to be specific for malonaldehyde (Sinnhuber et al., 1958). However, several researchers have reported that TBA can react with other oxidation products such as 2,4 alkadienals and 2 alkanals to produce compounds with the same absorption maximum as the

malonaldehyde-TBA complex (Jacobson et al., 1964; Marcuse and Johansson, 1973). Additionally, the amount of malonaldehyde in an oxidized sample depends on the fatty acid profile since oxidizing PUFAs produce more malonaldehyde than mono or diunsaturated fatty acids (Pryor et al., 1976; Pearson et al., 1983). Thus, the TBA method may be best used to assess the extent of lipid oxidation in general and to compare samples of the same composition at different stages of oxidation (Gray, 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 malonaldehyde, nor for maximum color development. The acid-heat treatment was found to alter the E_m^{530} value without accelerating the condensation reaction any more that heat alone. Use of an acidic TBA reagent was also found to contribute to the appearance of an absorption peak at 450 nm. These researchers proposed that acid may be responsible for the degradation of TBA which, when reacted with extracts or distillates from samples, produces peaks at 390 and 452 nm. To minimize these interfering reactions, Tarladgis et al. (1964) recommended a modified distillation method in which distillates of samples are reacted with TBA without the use of any acid.

Zipser and Watts (1962) modified the TBA method for analysis of nitrite-cured meats. Residual nitrite may react

with malonaldehyde during distillation to form oximes which result in lower TBA values. Sulfanilamide, added prior to distillation, reacts with the nitrite to form diazonium salts which do not interfere with the malonaldehyde-TBA reaction. Shahidi et al. (1985) have pointed out, however, that the use of sulfanilamide when residual nitrite is not present may also lead to erroneously low TBA numbers.

In general, the TBA numbers obtained by the distillation method are higher than those determined utilizing the extraction method. According to Witte et al. (1970) the distillation method produces TBA numbers which may be twice as large as those produced by an extraction method. Data from Siu and Draper (1978) showed that TBA numbers from the distillation procedure were approximately 1.5 times higher than those obtained from muscle extracts. They suggested the discrepancies in TBA values may result from: a) incomplete extraction of malonaldehyde using the filtration method, b) the release of bound malonaldehyde from proteins and amino acids during distillation or c) the formation of malonaldehyde during distillation.

The possibility of lipid oxidation and malonaldehyde formation during sample distillation was further investigated by Rhee (1978). She 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 and chicken samples. Based on these findings,

Rhee (1978) recommended the addition of PG and EDTA to samples, particularly fish, to minimize further lipid oxidation during the TBA distillation procedure.

Pikul et al. (1983) evaluated the influence of butylated hydroxytoluene (BHT) on the TBA assay of fat extracted from chicken breast and leg meat. Samples analyzed without added BHT reportedly yielded six times higher malonaldehyde concentrations than those treated with BHT during extraction and 75μg BHT/mg of fat during the TBA analysis. Thus, these workers recommended that BHT or other suitable antioxidant should be added during sample preparation or before the critical heating step of any TBA assay to prevent sample autoxidation and erroneously high TBA values.

The TBA test has also been modified to make it specific for malonaldehyde. Kakuda et al. (1981) used high performance liquid chromatography (HPLC) to quantitate malonaldehyde in aqueous distillates from freeze dried chicken samples having TBA numbers ranging from 3.96 to 16.60. They reported a linear relationship, with an r^2 of 0.946, between the TBA absorbance at 532 nm and the HPLC peak height of malonaldehyde. Kakuda et al. (1981) also reported that the HPLC method required less time than the standard TBA method, was more sensitive and was not affected by the presence of other TBA reactive substances or side reactions.

Another HPLC method was developed by Csallany et al. (1984) to quantitate malonaldehyde in rat liver and beef, pork and chicken muscle. They found that malonaldehyde levels as measured by the TBA method were four to five times higher than those obtained by the HPLC method. This was attributed to the artifactual formation of malonaldehyde during heating steps or to the presence of interfering color-reactive compounds.

Gas Chromatographic Analyses

Gas chromatography (GC) has been extensively used to monitor the products of lipid oxidation in model systems in order to elucidate the mechanisms of oxidation (Gray, 1978). Methods have also been developed which utilize secondary oxidation products as indices of lipid oxidation in foods.

Hexanal, a major secondary product of linoleate oxidation, and other aldehydes have been identified in oxidizing food systems including freeze dried beef (El-Garbawi and Dugan, 1965), cooked pork (Cross and Ziegler, 1965), cooked beef (Love and Pearson, 1976; Bailey et al., 1980), dry cereal (Fritsch and Gale, 1977) and cooked turkey (Ruenger et al., 1978). These volatile compounds have been used successfully to follow lipid oxidation in vegetable oils (Dupuy et al., 1976: Waltking and Zmachinski, 1977) and to predict flavor scores of various oils (Dupuy et al., 1977; Jackson and Giacherio, 1977; Warner et al., 1978).

Several GC methods have been developed for following lipid oxidation. Fritsh and Gale (1977) utilized a

headspace gas-sampling technique to measure hexanal in cereals. Volatiles were released from the cereal by the addition of boiling water, the system was sealed and a sample of headspace gas was withdrawn for GC analysis.

A direct GC method for the analysis of vegetable oils was developed by Dupuy et al. (1976) and later modified for aqueous and nonaqueous systems by Legendre et al. (1979). With this technique, the food to be analyzed is placed in an external inlet assembly where the volatiles are stripped from the sample by heat and an inert carrier gas. The volatiles condense onto a column which is then temperature programmed in an appropriate manner for GC analysis. Legendre et al. (1979) reported that this procedure is rapid, efficient and sensitive enough that sample volatiles can be analyzed without the need for prior enrichment. Bailey et al. (1980) used this direct sampling method to analyze volatile flavor components from cooked Boston butts. Data obtained from freshly cooked samples compared to that from meat stored at $4^{o}C$ for one day following cooking indicated that concentrations of hexanal and 2-pentyl furan increased significantly making them excellent indices of oxidative changes. Other aldehydes increased in concentration over the storage period, but not as substantially.

A third GC method, summarized by Bailey et al. (1980), involves the solvent extraction of volatiles using a Likens-Nickerson extraction apparatus. Volatiles released by boiling an aqueous homogenate of the sample were extracted

into a suitable organic solvent in a closed continuous extraction system. The solvent containing the extracted volatiles was concentrated and analyzed using conventional GC methods. This method was utilized by Bailey et al. (1980) to monitor lipid oxidation in roast beef stored at 4°C. Again, they observed an increase in the concentration of hexanal and 2-pentyl furan over time. Pentanal also increased during the storage period. From this work, Bailey et al. (1980) concluded that gas chromatographic methods are more informative than the TBA test since selected individual compounds can be quantitated.

Secondary lipid oxidation products other than aldehydes have been used to follow lipid oxidation. Saturated hydrocarbons are produced during the early stages of oxidation when aldehydes are either absent or not detectable (Horvat et al., 1964; Selke et al., 1970). Warner et al. (1974) reported significant correlations between the concentration of pentane in headspace samples and the sensory scores from trained panelists for both aged vegetable oils and potato chips.

Pentane formation has also been used to follow lipid oxidation in muscle foods. Seo and Joel (1980) monitored lipid oxidation in freeze dried pork by measuring levels of hydrocarbons in the headspace over the samples. The samples were then evaluated by a sensory panel to assess the degree of rancid odor. The overall pattern of pentane production was found to be significantly correlated with rancidity

scores. Additionally, 5µl pentane/10 g meat appeared to be the threshold for rancid odor. The authors suggested that while pentane was not directly responsible for rancid odors, pentane levels were indicative of the degree of lipid oxidation.

Sensory Evaluation

The most critical test of a food product's quality is its acceptance by the consumer. Consequently, many researchers have attempted to correlate data obtained from chemical measurements of lipid oxidation with the development of off-flavors and odors. 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. Additionally, they reported the range of TBA numbers at which rancid odors were first perceived by panelists to be between 0.5 and 1.0.

Other researchers have shown that TBA numbers are related to sensory scores of oxidized and warmed over flavors in meat (Watts, 1962; Igene and Pearson, 1979; Igene et al., 1979a). Greene and Cumuze (1981) examined the relationship between TBA numbers of cooked beef and the sensory score for oxidized flavor obtained from untrained panelists. They reported significant but low correlation coefficients for sensory scores versus TBA numbers and attributed this, in part, to variability in panelist scoring. When the panel was reduced to those statistically shown to be consistent in their scoring, correlation coefficients increased. The TBA

threshold range for the "discriminating" panelists was 0.6 to 2.0 which Greene and Cumuze considered in close agreement with the results of Tarladgis et al. (1960).

Similar studies by Igene et al. (1985a) examined the relationship between TBA numbers and sensory scores from a trained panel for WOF in cooked chicken white and dark meat. The meat was treated with chelators and/or antioxidants to produce samples having a wide range of TBA numbers. These researchers reported that TBA values were closely related to panel scores and that changes in TBA numbers accounted for over 75% of the variation in WOF scores.

Attempts have also been made to correlate data from GC analyses with flavor scores. The presence and concentration of certain volatiles have been correlated to flavor scores of vegetable oils (Evans, 1969; Dupuy et al., 1976; Warner et al., 1974), while Fritsch and Gale (1977) reported that hexanal in dry cereal was indicative of lipid oxidation and could be responsible for off flavors. Ruenger et al. (1978) reported that heptanal and n-nona-3,6-dienal are related to WOF in turkey. Seo and Joel (1980) correlated pentane concentrations with rancid odor in freeze dried pork, although they reported that pentane was not responsible for the off odors. Bailey et al. (1980) found that hexanal and 2-pentyl furan were excellent indices of oxidation in meat products but did not address their contribution to warmed over flavor. Clearly, further research is needed to identify

those volatiles responsible for warmed-over flavor and to establish threshold values for their detection by sensory evaluation.

Lipid Stability in Restructured Meats

Manufacture of Restructured Meats

The technology for manufacturing restructured meats was developed in the 1970's as a means for utilizing lower grades and cheaper cuts of meats that would normally be processed into roasts or ground meat (Booren et al., 1981). The goal was to manufacture a product which: (i) resembles an intact muscle in textural properties, (ii) is uniform, (iii) has desirable color and (iv) is completely edible. The methods for producing restructured meats have been well summarized and reported by several sources (Breidenstein, 1982; Seideman and Durland, 1983). The basic procedure of restructuring requires a reduction of particle size of the meat, blending of these particles and reforming them to the desired final shape.

Flake-cutting, slicing and sectioning are three comminution methods often used in manufacturing restructured products. Textural properties and other sensory characteristics of the finished product depend largely on the comminution method used and final particle size. It has been reported that flaking yields restructured products with improved texture, cohesiveness, juiciness and tenderness over those produced by grinding. Additionally, products

made with small and medium size flakes receive higher sensory scores than those made with large flakes (Seideman and Durland, 1983).

Slicing has also been used to produce desirable restructured meat products (Ockerman and Organisciak, 1979). Nobel et al. (1982) compared intact steaks and restructured steaks made from beef sliced 2.5, 5.0 or 7.5mm in thickness. Slice thickness had little effect on sensory attributes although restructured steaks were judged more tender and palatable than intact muscle steaks.

A procedure utilizing sectioning involves the cutting of large muscles into chunks of uniform or varying size. This method yields steaks having textural properties more nearly resembling those of intact muscle steaks than does the flaking process. Products manufactured by this procedure must have a lower fat content than flake-cut products, however, because fat particles are much larger and more noticable. Additionally, mechanical tenderization of the larger muscles may be necessary prior to sectioning to assure the desired tenderness of the finished product (Seideman and Durland, 1983).

Following particle reduction, the meat is mixed or blended. This serves to extract muscle proteins which promote binding between the meat pieces. Myofibrillar proteins, especially myosin, are the major proteins responsible for binding (Macfarlane et al., 1977). The binding of meat pieces depends upon the presence of a

protein-containing exudate at the particle surfaces (Booren et al., 1982). The mechanical action of tumbling, massaging or vacuum mixing disrupts the muscle cells thereby releasing the protein which acts as the binding agent.

The extraction of myofibrillar protein is enhanced by the addition of NaCl alone or with phosphates (Pepper and Schmidt, 1975; Siegel et al., 1978;). According to Theno et al. (1978a,b) salt functions to solubilize proteins while phosphates cleave actomyosin thus increasing the surface area so that greater amounts of myofibrillar proteins may be extracted.

Salt and phosphates have also been reported to act synergistically to increase the water holding capacity of restructured meat (Shults et al., 1972). It has been proposed that polyphosphates cleave the actomyosin complex formed at rigor into components with increased water holding capacity (Shults and Wierbicki, 1973; Theno et al., 1978b). In addition, phosphates alter the ionic strength of the sarcoplasm which increases the electrostatic repulsions between muscle filaments thus increasing the amount of space available for water binding. This increased water binding results in improved processing yields, reduced cooking losses, increased juiciness and enhanced texture and palatability scores (Schwartz and Mandigo, 1976; Neer and Mandigo, 1977; Huffman et al., 1981a).

Salt has also been found to improve the flavor of restructured meats. Several studies on both beef and pork

products have reported consumer preference for samples containing between 0.5% and 2.0% added NaCl over samples without added salt (Cross and Stanfield, 1976; Schwartz and Mandigo, 1976; Huffman et al., 1981b).

After mixing or blending, the meat particles and protein exudate are stuffed into casings or extruded into the preliminary shapes commonly referred to as logs, which are then frozen to increase the binding of particles. Meat logs are usually tempered to -3°C to -5°C then hydraulically pressed into the final shape. Logs can then be cleaved into individual servings with very close control of portion size possible.

Catalytic Factors

Restructured meat products are susceptible to lipid oxidation as evidenced by increased TBA numbers and pigment oxidation (Schwartz and Mandigo, 1976; Huffman et al., 1981b). Because of their manufacture, restructured meats may be less stable than intact muscle products. Two major factors influencing the development of WOF in restructured meat are reduction of particle size and the use of additives, especially NaCl.

Comminution of raw meat both disrupts the membranal structures and incorporates oxygen into the tissue. Membrane-bound lipids consist largely of phospholipids which, because of their high degree of unsaturation are especially susceptible to lipid oxidation (Igene et al., 1980). Grinding, chopping or emulsifying exposes these

labile phospholipids not only to oxygen but to other tissue catalysts such as enzymes, heme pigments and metal ions. Sato and Hegarty (1971) demonstrated that chopping of meat and exposure to air caused the development of rancidity in fresh meat within one hour.

Salt has also been shown to initiate undesirable reaction in restructured meat products. Schwartz and Mandigo (1976) reported that increased salt levels in flaked and formed pork produced increased TBA numbers and decreased raw color scores. Work by Huffman et al. (1981b) demonstrated that TBA values of restructured pork chops increased linearly in response to increasing salt levels. This trend was accompanied by decreasing raw color scores especially in samples held for 30 days at -15^{o}C.

Despite its detrimental effects, salt may actually inprove certain characteristics of restructured meats. Small amounts of added salt are necessary to assure proper binding of the meat particles in the finished product. Consumer evaluation of restructured beef steaks (Cross and Stanfield, 1976) showed that steaks containing 0.75% added salt were preferred over the controls containing 0% salt. Schwartz and Mandigo (1976) reported that added salt improved the aroma, flavor and eating texture of flaked and formed pork. Neer and Mandigo (1977) reported similar trends which were enhanced synergistically by the use of tripolyphosphate. Data from Huffman et al. (1981b) showed the addition of salt to restructured pork chops increased

cohesiveness and improved flavor, juciness and textural properties. Based on these observations, the addition of salt to restructured meat products at levels between 0.5 and 1.0% has been recommended (Schwartz and Mandigo, 1976; Huffman et al., 1981b).

Prevention of Lipid Oxidation in Meat

As previously noted, the lipids in muscle foods, especially restructured meat products, are susceptible to oxidation. Many studies have indicated that oxidation can be controlled by the use of compounds possessing antioxidant activity. These compounds may function as free radical scavengers, chelating agents, oxygen scavengers or by stabilizing otherwise catalytic species. Labuza (1971) reviewed these various types of antioxidants and the mechanisms by which they function. Several other researchers have investigated the effects of antioxidants, phosphates, ascorbate and nitrites on lipid stability in meats.

Antioxidants

In food systems, the most effective antioxidants function by interrupting the free radical chain mechanism of lipid oxidation. An antioxidant, AH, apparently reacts with radicals according to the following scheme (Dugan, 1976):

$$R^{\cdot} + AH \longrightarrow RH + A^{\cdot}$$
$$RO^{\cdot} + AH \longrightarrow ROH + A^{\cdot}$$
$$ROO^{\cdot} + AH \longrightarrow ROOH + A^{\cdot}$$
$$R^{\cdot} + A^{\cdot} \longrightarrow RA$$
$$RO^{\cdot} + A^{\cdot} \longrightarrow ROA$$

The free antioxidant radicals thus produced are thermodynamically unable to initiate other autoxidative chain reactions (Pokorny, 1971).

Antioxidants may be classified as either synthetic or naturally occurring (Pearson and Gray, 1983). Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and several other synthetic phenolic antioxidants have been widely studied in meat systems and, in general, have been shown to be effective in retarding lipid oxidation. Greene (1969) reported that BHA and propyl gallate (PG) offered substantial protection to fresh meat pigments and effectively inhibited lipid oxidation in raw ground beef. Greene et al. (1971) demonstrated that BHA or PG prevented lipid oxidation and reduced pigment oxidation in ground beef for up to 8 days of refrigerator storage. When the combination of antioxidants and ascorbic acid was used both lipid and pigment oxidation were effectively retarded. Olson and Rust (1973) used an antioxidant salt mixture containing BHA, BHT, citric acid and propylene glycol in the dry curing of hams. Improved flavor scores and decreased rancidity were noted in hams cured with the antioxidant mix over those cured using regular flake salt or low metal salt.

Chastain et al. (1982) utilized synthetic phenolic antioxidants in the manufacture of restructured beef/pork steaks formulated to contain 20% fat and 0.75% salt. The antioxidants BHA and tertiarybutyl hydroquinone (TBHQ) were added at the 0.02% level based on the fat content of the

steaks. The addition of antioxidants alone or in combination decreased TBA numbers, increased sensory scores for flavor and acceptability and decreased discoloration of the raw meat relative to control (NaCl only) samples. BHA was more effective in inhibiting discoloration while TBHQ offered greater protection against rancidity. These researchers suggested that a combination of antioxidants offers the best possibility for protection against both types of oxidative changes.

Chen et al. (1984) examined the effects of salt and antioxidants on the TBA numbers of beef. They reported that salt coated with Tenox 4--BHA, citric acid, propylene glycol--and a mixture of BHA and BHT in salt completely inhibited lipid oxidaiton in cooked meat. However, α-tocopherol coated on salt demonstrated only marginal antioxidant properties. This ineffectiveness could be related to the concentration used as α-tocopherol has been reported to act as a prooxidant at high concentrations (Cillard et al., 1980; Labuza, 1971). Earlier work by Cort (1974) showed, however, that in pork and beef fats α-tocopherol was as effective as BHT in preventing lipid oxidation while γ-tocopherol was more effective than either BHT or BHA.

Several researchers have investigated the antioxidant activity of other naturally occurring substances. Pratt and Watts (1964) demonstrated that a number of plant extracts prevented lipid oxidation in cooked meat. The antioxidant

activity was found to be related to the content of flavonoids, a major group of plant phenols. Flavonoids have also been cited as antioxidative constituents in soybean flour and protein isolates (Hayes et al., 1977). Pratt et al. (1981) identified three isoflavones and several phenolic acids which exhibited antioxidant activity from soy protein hydrolyzates.

Proteins from other plant sources have also been shown to contain natural antioxidants. Rhee and Ziprin (1981) reported that glandless cottonseed, peanut or soy proteins and aqueous extracts of these proteins effectively retarded lipid oxidation in beef. They suggested that small amounts of oilseed protein ingredients could be added to sauces and gravies to improve the oxidative stability of precooked meat products. Similar research by Rhee and Smith (1983) demonstrated that defatted glandless cottonseed flour added at a level of 2 to 3% effectively inhibited salt-catalyzed lipid oxidation and discoloration in raw ground beef. These results suggest that glandless cottonseed flour could be added to meat products containing <0.5% NaCl to prevent oxidative deterioration.

Phosphates and Other Chelating Agents

Phosphates are usually added to processed meats because they increase the water holding capacity and yield of the finished product. The addition of phosphates to cooked meats has also been shown to delay or prevent lipid oxidation (Tims and Watts, 1958; Sato and Hegarty, 1971).

Pyrophosphate, tripolyphosphate and hexametaphosphate all offer protection while orthophosphates do not (Sato and Hegarty, 1971). Tims and Watts (1958) proposed that the mechanism by which phosphates prevent oxidation in meat products is related to their ability to sequester heavy metal ions which are known prooxidants (Uri, 1961).

Other chelating agents have been shown to prevent lipid oxidation in meat presumably because of their ability to chelate nonheme iron and copper. Liu (1970) and Liu and Watts (1970) demonstrated that ethylenediaminetetraacetic acid (EDTA) eliminated Fe^{2+} catalyzed oxidation in a linoleate model system and in raw beef. Sato and Hegarty (1971) found that EDTA at a concentration of 2.5 mg/g suppressed lipid oxidation in cooked ground beef. Igene et al. (1979a) reported that the addition of 2% EDTA to meat pigments extracted from cooked beef significantly reduced their catalytic activity. Treatment of the pigment extract with H_2O_2 to release free irom from heme compounds increased its prooxidant activity which again could be lessened by EDTA. Igene et al., (1979a) concluded that EDTA effectively chelated nonheme iron and thereby significantly reduced lipid oxidation in cooked meat. Although EDTA has been proven effective in model systems, it has not 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. Sato and Hegarty (1971) reported

minimal inhibition of lipid oxidation in cooked ground beef when sodium citrate was added at the level of 5mg/g. Benedict et al. (1975) also reported only a slight decrease in lipid oxidation in meat treated with 0.0005% citric acid. MacDonald et al. (1980) demonstrated that 1000mg/kg levels of citric acid could reduce TBA numbers in refrigerated hams but that 50 mg/kg nitrite was more effective.

MATERIALS AND METHODS

Materials

Beef

Choice Yield Grade 2 three piece chucks 126 (NAMP, 1981) were purchased from Ada Beef Inc. (Ada, MI). The subprimals were purchased vacuum packaged within 48 hours of slaughter. Replicates were established using three consecutive slaughter weeks. Each replicate was processed within 24 hours of purchase.

Vacuum Packaging

Polyethylene-laminated nylon pouches (3 mil) were obtained from Koch (Kansas City, MO). These pouches have an oxygen transmission rate of 9 $ml/m^2/24$ hours at 4^oC (32^oF).

Antioxidants

Tenox TBHQ was supplied by Eastman Chemical Products Inc. (Kingsport, TN). Antioxidant-coated salts were supplied by Diamond Crystal Salt Company (St. Clair, MI). The two formulations used in the manufacture of restructured steaks are shown in Table 2.

Table 2. Composition[1] of antioxidant-coated salts[2] used in the manufacture of restructured beef steaks.

Component	Antioxidant System APMT-1	APMT-2
NaCl	93.8	93.60
AP[3]	3.0	1.70
Mixed Tocs[4]	0.6	1.75
Veg. Oil	0.3	0.75
Citric Acid	0.3	0.28
Silicon Dioxide[5]	2.0	1.92

[1] Expressed as percentage of total

[2] Added to yield a final concentration of 0.06% (fat basis) AP + mixed tocopherols

[3] Ascorbyl palmitate

[4] Mixed tocopherols

[5] Added as an anticaking agent

Fatty Acid Methyl Esters

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

Reagents and Solvents

All reagents and solvents utilized in this study were reagent grade and/or HPLC grade with the exception of propylene glycol (Fisher Scientific) which met USP/FCC specifications.

Experimental

Manufacture of Restructured Steaks

Restructured beef steaks were manufactured using the method of Booren et al. (1981). The cuts were trimmed of excess fat and connective tissue. The trim fat was used as the fat fraction for the restructured steaks. This was estimated visually to contain 90 to 95% fat. The fat content of the lean fraction (8 to 10%) was determined using a Hobart F101 Fat Tester.

The fractions were wrapped in plastic film to exclude as much air as possible and crust frozen for 4 hours at -30^{o}C. The fat fraction was then sliced into 1.5mm slices using a Hobart Food Slicer. The lean fraction was ground through a 24x48mm kidney plate. The fractions were formulated into 35 lb meat blocks containing 18% fat. Antioxidants, either coated on salt or dissolved in propylene glycol, were dispersed in the fat fraction

immediately prior to vacuum blending. Salt was added to yield a final concentration of 0.75% in the meat block. Each meat block was placed in a Keebler mixer (Keebler Inc., Chicago, IL) and vacuum mixed for 12 minutes. The blended samples were stuffed into fibrous, moisture-proof casings (5 inch diameter) and frozen overnight at -30°C. The following morning, the logs were unwrapped and portioned with a meat saw into approximately 12 ounce steaks. Individual steaks were vacuum packaged and placed in frozen storage for further analysis. Freezer temperatures ranged from approximately -20 to -24°C during the 12 month storage period.

Measurement of Lipid Oxidation

Lipid oxidation occurring in restructured beef steaks during frozen storage was quantitated by the TBA test, GC analysis of volatiles and sensory evaluation. A major objective of this study was to determine the correlation among the data obtained from each method of analysis. Raw and cooked samples from all treatments were analyzed by the TBA method (Tarladgis et al., 1960, 1964). For all analyses requiring cooked samples, steaks were prepared using a modified oven broiling technique described by Paul et al. (1956) to an internal temperature of 70°C (158°F). Temperature was monitored by copper constantan thermocouples placed in the center of individual steaks. Samples to be analyzed were ground twice through a 3/16 inch plate, mixed thoroughly and analyzed within 30 minutes.

Cooked samples containing NaCl only or NaCl plus TBHQ were utilized for GC analysis of volatiles. Volatiles were extracted with ethyl ether in a Likens-Nickerson apparatus, concentrated and analyzed with a Hewlett Packard 5840A gas chromatograph as described under "Methods of Analysis".

Cooked samples were also evaluated by trained sensory panelists. Panelists were selected and trained using the following procedures. Volunteers were selected for training based on their performance in triangle tests. Initial training sessions utilized triangle tests, group discussions, sample ranking and sample rating to familiarize the panelists with restructured steak and oxidized flavor (WOF). A reinforcing training session was conducted approximately mid-way through the storage study.

Research samples were evaluated using a rating procedure. Steaks were cooked approximately one hour before each morning tasting session. At each session panelists were presented with four coded samples representing the four experimental treatments and were asked to rate them on a 15 cm scale with left and right anchor points of "No WOF" and "Very Strong WOF". 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 degrees of oxidation. Portions of steaks from each treatment were held at $4^{o}C$ for four hours, reheated and evaluated in afternoon sessions. A panelist's score for each sample was determined by measuring the

distance from the left end of the scale to the vertical mark made by the panelist.

Evaluation of the TBA Test

A study was designed to evaluate the applicability of the TBA test for assessing lipid oxidation in meat systems. This involved two phases in which: 1) the use of acetic acid TBA reagent (Tarladgis et al., 1960) was compared with aqueous TBA reagent (Tarladgis et al., 1964) and 2) the use of antioxidants during assays was evaluated.

Several modifications of the traditional distillation TBA method were suggested by Tarladgis et al. (1964), one of which was the use of an aqueous TBA solution rather than one containing 90% glacial acetic acid. To compare these two methods the following procedures were used. Standard curves were established using solutions of tetramethoxypropane (TMP) ranging from 0 to $2x10^{-8}$ moles/5 ml. TMP was used because it undergoes hydrolysis to form malonaldehyde when treated with heat and/or acid. The malonaldehyde thus produced reacts with TBA to form the characteristic pink pigment. Both acidic and aqueous TBA reagents were reacted with aliquots of TMP solutions, and absorbance was measured at 532 nm.

The recovery of malonaldehyde during distillation was also determined. Aliquots of TMP were distilled and collected using the traditional TBA method (Tarladgis et al., 1960). Aqueous TBA reagent was used to develop the pink color. Percent recovery was determined using the

calibration curve for aqueous TBA. Using this recovery value and the proper standard curves, the multiplicative factors for acid and aqueous TBA assays were determined.

The second phase of this study was designed to examine the potential formation of TBA reactive substances (TBARS) during the TBA assay. Several researchers have suggested that further lipid oxidation may occur during heating steps of the TBA method (Siu and Draper, 1978; Rhee, 1978; Pikul et al., 1983). To prevent this, they advocated using anti-oxidants during any heating step and possibly during sample blending and preparation. The methods of Rhee (1978) and Pikul et al. (1983) were modified and applied to beef, fish, and chicken light and dark meat.

Prior to sample homogenization 1.0 ml of various ethanolic TBHQ solutions was added to give a final anti-oxidant concentration of 0.01% based on the fat content of the meat. Samples were then distilled in the usual manner. Distillates were developed using both acidic and aqueous TBA reagents.

Methods of Analysis

Proximate Analysis

Moisture content was determined using AOAC procedure 24.002 (1975). A variation of AOAC procedure 24.005 (1975) was used for crude fat determination.

Thiobarbituric Acid Test (TBA Test)

The TBA distillation method of Tarladgis et al. (1960) was modified (Tarladgis et al., 1964) and used to measure the development of oxidative rancidity. The modified method utilized an aqueous solution of TBA in place of the acetic acid/H_2O reagent.

Lipid Extraction and Fractionation

Total lipid was extracted from raw samples containing TBHQ using the procedure of Bligh and Dyer (1959). The dry column method of Marmer and Maxwell (1981) was used for simultaneous extraction and class separation, into neutral and phospholipid fractions, of lipids from raw and cooked steaks.

Columns were prepared by pouring a bed of 5.0g of a $CaHPO_4 \cdot 2H_2O$/Celite 545 (9:1) mixture into 11x300 mm glass columns. Sample size and solvent volume were decreased by one half due to the size of the chromatographic columns used. A 2.5g meat sample was added to 10g anhydrous Na_2SO_4 and ground with a mortar and pestle. This mixture was reground with 7.5g Celite 545 to a uniform free-flowing powder. This powder was quantitatively transferred to the column and tamped (moderately) into place. The mortar, pestle and other utensils were rinsed with methylene chloride to remove trace amounts of lipids. The rinsings were added to the column along with enough methylene chloride to wet the entire column bed. The column was then charged with 75 ml methylene chloride, and the neutral

lipids were eluted. When the last of the methylene chloride reached the top of the column, the column was charged with 75 ml methylene chloride/methanol (9:1) to elute the phospholipids. This fraction was collected until the column was stripped of solvent. The two fractions were concentrated to 50 ml, quantitated and used for fatty acid determinations.

Fatty Acid Analysis

Neutral and phospholipid fractions from raw and cooked beef steaks were methylated according to the boron trifluoride-methanol procedure of Morrison and Smith (1964). The methylated samples were analyzed using a Hewlett Packard 5840A gas chromatograph equipped with a flame ionization detector and a Hewlett Packard 5840A GC integrator. The fatty acid 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 operated using a temperature program with the initial temperature at 150^{o}C, held for one minute then increased at 1.5^{o}C/min to a final temperature of 225^{o}C which was maintained for 10 minutes. The injection port temperature was 200^{o}C, the flame ionization detector temperature 300^{o}C and the nitrogen carrier gas flow rate was 25 ml/min. The component fatty acids were identified by comparing retention times to those obtained from lipid standards assayed under identical conditions.

GC-MS Analysis of Fatty Acid Methyl Esters

Methyl esters of fatty acids from the phospholipid fraction of restructured beef steaks were analyzed by GC-MS. The gas chromatographic conditions were as previously described. Effluent from the GC passed into a Hewlett Packard 5985A mass spectrophotometer operated under the following conditions: electron impact voltage, 70eV; electron multiplier voltage, 2400 eV; threshold 0.6; source temperature, 200^{o}C; analog/digital measurements, 3/sec and ion detection in the positive mode.

Extraction of Volatiles from Cooked Steaks

Two hundred grams of ground, cooked meat were weighed into a 2000 ml boiling flask, 750 ml of water were added, and the mixture was attached to a Likens-Nickerson extraction apparatus. Ethyl ether (25 ml) was used as the extracting solvent. The system was allowed to reflux for 6 hours followed by concentration of the ether fraction which contained the extracted volatiles. The ether was first dried over anhydrous sodium sulfate then concentrated under a stream of nitrogen to a final volume of 0.5 ml. Extracts were stored in screw-top vials at -20^{o}C until analysis.

Chromatographic Analysis of Extracted Volatiles

The extracts were analyzed for hexanal using a Hewlett Packard 5840A gas chromatograph fitted with a 3m x 2mm (i.d) glass column packed with 10% Carbowax 20M TPA on Chromosorb WHP, 80/100 mesh (Supelco, Bellefonte, PA). Injection

volume was 0.5 µl. The GC was operated isothermally at 50°C, with an injection port temperature of 200°C, a detector temperature of 300°C and N_2 carrier gas at 25 ml/min. Hexanal was tentatively identified by comparing the retention time of the suspect peak to that obtained from a hexanal standard assayed under identical conditions. The GC analyses were terminated after hexanal eluted (approximately 15 minutes), and the oven temperature was raised to 190°C and held for 15 minutes to drive off the remaining volatiles from the column.

Sensory Evaluation

Freshly cooked steaks and cooked meat held 4 hours at 4°C were evaluated by trained sensory panelists. At each session, all panelists were presented with four coded samples representing the four experimental treatments. All samples were reheated in sealed containers and served warm. Samples were evaluated under red light to mask any differences in appearance. Panelists were asked to rate the intensity of warmed-over flavor using a 15 cm scale with anchor points 1 cm from each end. The descriptors at the left and right anchor points were "No WOF" and "Very Strong WOF", respectively.

Statistical Analysis

Data from TBA tests were analyzed by the ANOVA and Scheffe procedures of the Statistical Package for the Social Sciences, SPSS (Nie et al., 1975). SPSS/PC--SPSS for the

IBM PC/XT--was used for all computations (Norusis, 1984). Application and interpretation of these procedures was in accordance with Gill (1978). Sensory scores were analyzed by the ANOVA procedures outlined by Larmond (1977).

RESULTS AND DISCUSSION

Evaluation of the TBA Test

As stated in the review of literature, the original TBA distillation procedure has been modified and refined by several researchers. Tarladgis et al. (1964) found that the usual acid-heat treatment was not necessary for maximum color development but, rather, that it altered the spectral properties of the pigment and contributed to the appearance of an absorption peak at 450 nm. In the preliminary stage of the current study, a similar phenomenon--the presence of a yellow pigment absorbing at 450 to 452 nm--was observed during TBA assays of muscle products. In light of these observations, a study was designed to compare the original acetic acid-heat TBA assay (Tarladgis et al., 1960) to the modified aqueous TBA system (Tarladgis et al., 1964).

Malonaldehyde calibration curves were established using solutions of tetramethoxypropane (TMP) containing 0 to $2x10^{-8}$ moles/5 ml. Aliquots of these TMP solutions were reacted with both acidic and aqueous TBA solutions. The concentrations used and their respective absorbance values are

shown in Table 3. Plots of these data show a linear response with r^2 values of 0.999 for both acidic and aqueous TBA (Figure 2).

The recovery of malonaldehyde during distillation was subsequently determined. Aliquots of TMP were distilled and collected using the traditional TBA method. Aqueous TBA reagent was used to develop the pink pigment. The TMP concentrations utilized and corresponding absorbance values are found in Table 4. From the appropriate calibration curve recovery was found to range from 68.6 to 77.2 percent with an average of 73.1 percent. This value is slightly higher than that established by Tarladgis et al. (1960). Using the equations of Tarladgis et al. (1960) shown in Appendix B, the multiplicative factors for acidic and aqueous TBA assays were calculated to be 7.1 and 6.2, respectively. To express TBA numbers, with units of mg malonaldehyde/1000 g sample, absorbance values are multiplied by the above factors.

The appearance of a yellow pigment was not observed in any reactions utilizing TMP as the source of malonaldehyde. However, distillates from meat samples often produced the yellow pigment when developed with acetic acid TBA reagent. This was in agreement with the findings of Tarladgis et al. (1962), Tarladgis et al, (1964) and Igene et al. (1985a).

To further investigate this, distillates from beef, fish and chicken were divided into two lots, and identical samples were reacted with both acidic and aqueous TBA

Table 3. Absorbance of TMP standards developed with acidic and aqueous TBA reagents.

10^{-9} TMP moles/5 ml	Absorbance[1] at 532 nm	
	Acidic TBA	Aqueous TBA
0.00	0.000	0.000
0.92	0.010	0.016
1.85	0.025	0.029
4.62	0.060	0.073
9.24	0.127	0.147
18.48	0.264	0.295

[1] Analyses were performed in triplicate.

Table 4. Recovery of malonaldehyde during TBA distillation.[1,2]

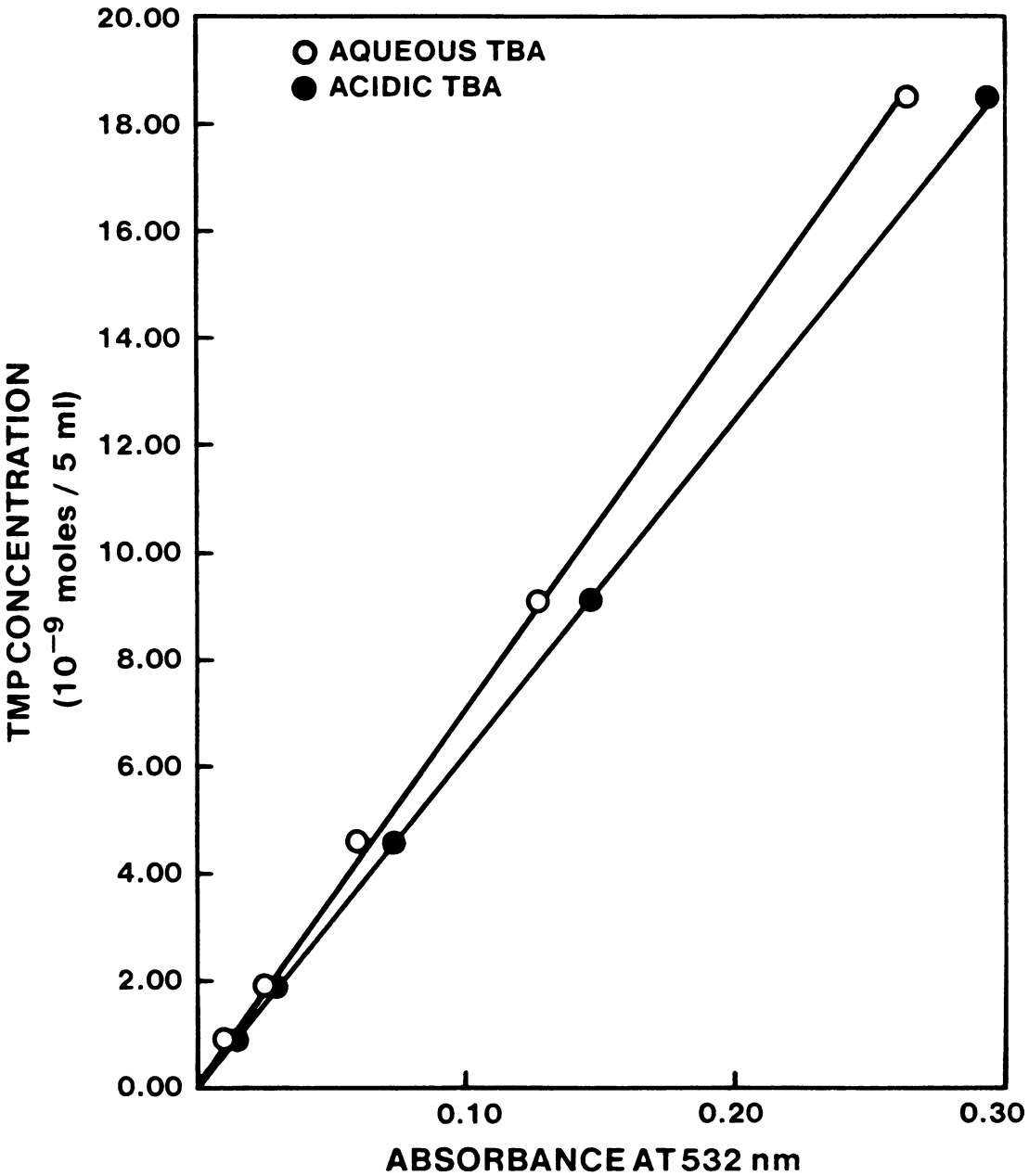
10^{-9} TMP moles/5 ml	Absorbance[3]	%Recovery
6.09	0.075	77.2
7.31	0.080	68.6
9.09	0.106	73.1
9.14	0.107	73.4

[1] Color was developed using aqueous TBA reagent.

[2] Analyses were performed in duplicate.

[3] Measured at 532 nm

Figure 2. Calibration curves for malonaldehyde-TBA complexes developed under aqueous and acidic conditions.


AQUEOUS TBA
ACIDIC TBA
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
TMP CONCENTRATION
(10^{-9} moles / 5 ml)
0.10
0.20
0.30
ABSORBANCE AT 532 nm

reagents. The sample type, treatments and absorbance values are summarized in Tables 5 and 6. Absorbance at 532 nm was consistently higher for the aqueous samples while absorbance at 450 nm was generally greater in samples developed with acidic TBA reagent. These trends were apparent in both raw and cooked samples.

Figure 3 shows the spectral scans of a distillate from beef developed with both types of TBA reagents. In the sample developed with aqueous reagent a very sharp absorption peak was noted at 532 nm while only a small peak was present at ca. 450 nm. However, in the sample developed with acidic TBA reagent, a substantial peak appeared at 450 nm and the absorbance at 532 was suppressed.

The appearance of this yellow pigment has been noted by several other researchers. Tarladgis and Watts (1960) reported the formation of a pigment absorbing at 450 nm during the TBA assay of oxidized oleic, linoleic, linolenic and arachidonic acids but found none from the reaction of acidic TBA reagemt with pure malonaldehyde solutions. They also noted that absorption at 450 nm increased over time when the TBA chromagen was held at room temperature but were unable to identify the compound responsible for the peak.

In further studies, Tarladgis et al. (1962) investigated some of the side reactions which occur during the acid-heat portion of the TBA test. They reported that the visible, UV and IR spectra of acetic acid TBA reagent were altered by heat and oxidizing agents. Absorbance at 450 nm

Table 5. Absorbance values[1] of distillates[2] from various raw muscle products developed with acidic and aqueous TBA reagents.

Sample	TBA Reagent	Absorbance 451 nm	Absorbance 532 nm
Chicken breast	H_2O	0.107	0.071
Chicken breast	acid	0.222	0.024
Chicken thigh	H_2O	0.064	0.061
Chicken thigh	acid	0.120	0.021
Beef	H_2O	0.082	0.108
Beef	acid	0.401	0.052
Fish	H_2O	0.048	0.112
Fish	acid	0.055	0.099

[1] Analyses were performed in duplicate.

[2] Distillates from each muscle type were subdivided and developed with each type of TBA reagent.

Table 6. Absorbance values[1] of distillates[2] from various cooked muscle products developed with acidic and aqueous TBA reagents.

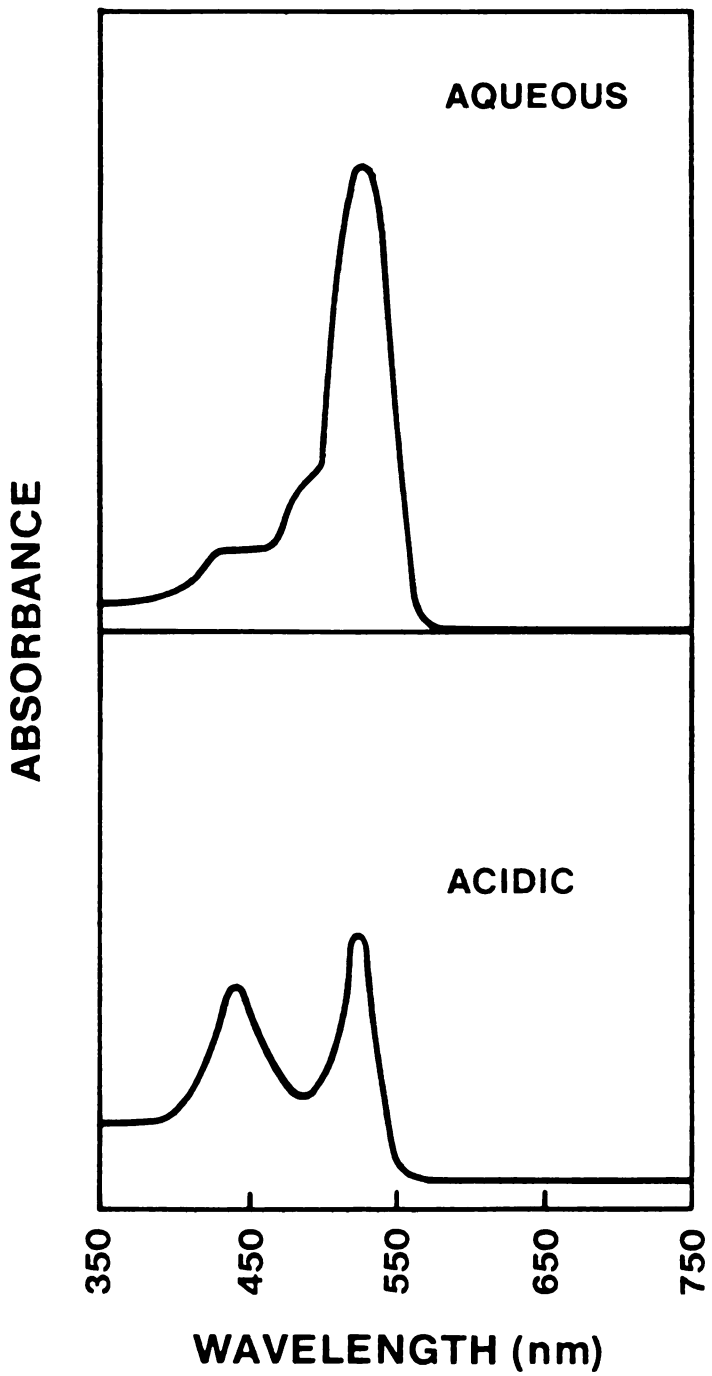
Sample	TBA Reagent	Absorbance 451 nm	Absorbance 532 nm
Chicken breast	H_2O	0.109	0.138
Chicken breast	acid	0.227	0.073
Chicken thigh	H_2O	0.133	0.386
Chicken thigh	acid	0.051	0.196
Beef	H_2O	0.075	0.082
Beef	acid	0.228	0.050
Fish	H_2O	N.A	N.A
Fish	acid	N.A	N.A

[1] Analyses were performed in duplicate.

[2] Distillates from each muscle type were subdivided and developed with each type of TBA reagent.

N.A = not available

Figure 3. Spectra of distillates from beef developed with aqueous and acidic TBA reagents.


AQUEOUS
ACIDIC
ABSORBANCE
350
450
550
650
750
WAVELENGTH (nm)

increased substantially upon heating the reagent 5 to 35 minutes. The addition of H_2O_2 to the TBA reagent magnified this increase of the 450 nm peak. Data from UV and IR spectra presented further evidence that the structure of TBA was altered by acid-heat treatment and H_2O_2. These researchers postulated that TBA may undergo partial hydrolysis or may be oxidized at the C-SH or CH_2 groups although they were unable to identify the specific compounds produced in the acetic acid TBA system. These researchers did suggest, however, that TBA should not be heated in the presence of acid or oxidizing agents. They also suggested that hydroperoxides present in oxidized samples could affect TBA in the same manner as does hydrogen peroxide.

Jacobson et al. (1964) modified the TBA test to correlate absorbance at 452 nm with flavor scores of various fats and oils. They used a moderate temperature for color development which favored formation of the yellow pigment. They also found, though, that recrystallization of TBA and redistillation of solvents markedly decreased artifactual absorbance at 452 nm. However, they did not address the possible interaction of the 452 nm and 532 nm absorbance peaks.

Marcuse and Johansson (1973) studied the production of a 450 nm absorbance peak from the reaction of TBA with alkanals, alkenals and 2,4 alkadienals. Maximum production of the yellow pigment was promoted by heating the reaction mixture at 50°C for 2 hours. Conditions similar to those of

the traditional TBA assay--95°C for 35 minutes--produced very little absorbance at 450 nm. As with Jacobson et al. (1964), this research was designed to study the applicability of utilizing the absorbance at 450 nm as an index of rancidity rather than to study the interference of a 450 nm peak with the 532 nm absorbance peak. It does, however, support the findings of the current study that formation of the yellow pigment is minimal when aqueous TBA reagent is heated in the presence of TBA reactive substances (TBARS) under the conditions normally used during TBA assays.

While preliminary data from the current study shown in Table 7 indicate that the yellow pigment interferes with TBA assays of fresh products, the data in Table 8 show that interference is not as substantial as TBA absorbance values increase. Distillates from fresh beef which produced the yellow pigment yielded lower absorption values at 532 nm than those possessing only minor absorption peaks at 450 nm (Table 7). In oxidized samples, absorbance at 532 nm changed little despite large differences in absorbance at 450 nm (Table 8). This demonstrates that at high concentrations of TBARS absorbance at 532 is not suppressed by the 450 pigment. This also suggests that results of the acetic acid TBA assay are less reproducible for fresh products than for more highly oxidized samples. In light of this evidence, and since formation of the yellow pigment is decreased when aqueous TBA reagent is used regardless of the

Table 7. Absorbance values[1] of distillates from fresh raw beef developed with acetic acid TBA reagent.

Sample[2]	Visual Appearance	Absorbance[3]
1a	Orange	0.010
b	Pink	0.015
2a	Orange	0.045
b	Pink	0.060
3a	Orange	0.073
b	Pink	0.091

[1] Values represent the mean of three measurements.

[2] Samples from a single source were analyzed in quadruplicate on successive days. Values from two replications were chosen for illustrative purposes.

[3] Measured at 532 nm

Table 8. Absorbance values[1] of distillates from various muscle products developed with acetic acid TBA reagent.

Sample[2]	Absorbance 451 nm	Absorbance 532 nm
Chicken breast	0.032	0.075
Chicken breast	0.227	0.073
Chicken thigh	0.051	0.196
Chicken thigh	0.301	0.200
Fish	0.023	0.150
Fish	0.111	0.149

[1] Values represent the mean of three measurements.

[2] Samples of each muscle type are from a single source and were analyzed in quadruplicate. Values from two replications were chosen for illustrative purposes.

degree of sample oxidation, all further TBA assays of the current study utilized aqueous TBA reagent.

Effect of TBHQ on Formation of TBARS During the TBA Assay.

The ability of TBHQ to prevent artifactual formation of TBARS during the TBA assay was evaluated. The effects of TBHQ added prior to sample blending on the 532 nm absorbance values of various raw and cooked muscle samples are presented in Table 9. The addition of 0.01% TBHQ (fat basis) to the distillation mixtures did not have any significant effect ($p < 0.05$) on the TBA results of beef or cooked chicken thigh. However, TBA absorbance values of fish and chicken breast were significantly lower when TBHQ was added prior to blending and distillation of both raw and cooked samples. TBHQ also significantly lowered the TBA absorbance value of raw chicken thigh meat. These data from the current study along with those of Rhee (1978) and Pikul et al. (1983) show that chicken and fish appear to be susceptible to oxidative changes during the TBA distillation procedure.

Rhee (1978) reported that addition of propyl gallate (PG) and EDTA prior to sample distillation significantly reduced the TBA numbers of both raw and cooked catfish. No effect was noted for beef, pork or chicken breast. Further experimentation demonstrated that the addition of PG and EDTA during the blending process was the most effective one-step method for minimizing further oxidation of fish lipids

Table 9. Absorbance values[1,2] of distillates from various muscle samples distilled with and without added TBHQ.

Sample	Absorbance at 532 nm -TBHQ	Absorbance at 532 nm +TBHQ
Beef		
Raw	0.083[a] (0.009)	0.090[a] (0.021)
Cooked	0.082[a] (0.003)	0.084[a] (0.016)
Chicken breast		
Raw	0.096[a] (0.007)	0.073[b] (0.003)
Cooked	0.184[a] (0.004)	0.155[b] (0.017)
Chicken thigh		
Raw	0.068[a] (0.005)	0.046[b] (0.003)
Cooked	0.335[a] (0.049)	0.300[a] (0.017)
Fish		
Raw	0.138[a] (0.027)	0.089[b] (0.012)
Cooked	0.318[a] (0.033)	0.270[b] (0.009)

1 Analyses were performed in triplicate.

2 Standard deviations are shown in parentheses.

[a,b] Values in the same row bearing the same superscript are not significantly different from each other at $p < 0.05$.

during the TBA test. Similar, but not as marked, results were observed for other meat samples.

Pikul et al. (1983) evaluated the influence of BHT on the TBA assay of fat extracted from chicken breast and leg meat. Samples analyzed without added BHT yielded 6 times higher TBA numbers than samples treated with BHT during lipid extraction and TBA assay. These researchers advised using antioxidants to prevent artifactually high TBA results.

This protective effect of antioxidants is significant for fish and chicken most likely because of the lability of their component lipids. Poultry and fish are known to contain proportionately more phospholipids than red meats (Igene et al., 1979b), and these phospholipids are highly unsaturated. Additionally, the depot fats of these species are unsaturated with poultry containing substantial amounts of C_{18} and C_{20} polyunsaturates while fish contain pentaenoic and hexaenoic acids (Pearson et al., 1977). In contrast, beef contains a smaller amount of phospholipids, and polyunsaturated fatty acids comprise less that 3 percent of its triglyceride fatty acids.

Evaluation of Restructured Steaks

Proximate Analysis

Restructured beef steaks were manufactured as detailed previously. The moisture and fat contents of each treatment and replication are shown in Table 10. As evident from the

Table 10. Moisture and lipid content of restructured beef steaks.

Sample[1]		%Moisture[2]	% Lipid Goldfisch[3]	% Lipid Dry[3] Column
Control	1	65.6	12.3	
	2	63.0	16.4	
	3	63.1	15.0	
APMT-1	1	65.7	14.6	
	2	63.3	14.9	
	3	62.3	15.5	
APMT-2	1	64.8	14.6	
	2	61.3	17.7	
	3	60.6	16.6	
TBHQ	1	61.9	16.4	18.1
	2	62.4	19.2	20.3
	3	60.8	18.0	18.1

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

[2] Analyses were performed in triplicate.

[3] Analyses were performed in duplicate.

table, the target lipid content, 18%, was not always achieved. This was due to the unavoidable loss of a small amount of fat on the sides and blades of the vacuum mixer. Additionally, lipids contents determined by the dry column procedure were slightly higher than those obtained by the AOAC method. This is in agreement with the results of Maxwell et al. (1980) who reported more complete extraction of phospholipid by the dry column method.

The fatty acid composition of neutral and phospholipid fraction of samples taken from each replication are summarized in Tables 11 and 12. Because these analyses were not performed at the initial stage of the study, TBHQ-treated samples were later chosen for analysis since their composition would have changed the least over time. The values shown in the tables were calculated as area percentages of total fatty acids identified with lipid standards.

Two peaks in the chromatograms of neutral lipid fatty acids could not be identified. One, eluting shortly after $C_{14:1}$, may have been $C_{15:0}$; the other, eluting between $C_{17:0}$ and $C_{18:0}$, may have been $C_{17:1}$. Nevertheless, the fatty acid profiles obtained were very similar to those found in the literature (Hornstein and Crowe, 1967; Igene et al., 1981), and especially to those reported by Marmer et al. (1984) who also used the dry column method for lipid extraction and class separation. This clearly demonstrates the high reproducibility of dry column methodology.

Table 11. Fatty acid composition of the neutral lipid fraction from restructured beef steaks.

Fatty Acid	Sample[1] TBHQ-1	TBHQ-2	TBHQ-3
	Area Percent		
14:0	3.2	3.1	3.0
14:1	0.7	1.1	1.0
16:0	25.6	25.3	24.6
16:1	5.5	5.5	5.2
17:0	1.3	1.2	1.4
18:0	13.5	13.4	13.9
18:1	46.4	46.7	47.2
18:2	3.0	2.6	2.9
18:3	0.8	0.9	0.6
20:0	tr	0.2	0.2
% Sat.	43.6	43.2	43.1
% Monounsat.	52.6	53.3	53.4
% Di and polyunsat.	3.8	3.5	3.5
% Total Unsat.	56.4	56.8	57.0

[1] Duplicate samples were taken from each of three replications containing TBHQ.

tr = trace amounts

Table 12. Fatty acid composition of the phospholipid fraction from restructured beef steaks.

Fatty Acid	Sample[1] TBHQ-1	TBHQ-2	TBHQ-3
	Area Percent		
14:0	0.2	tr	tr
14:1	tr	tr	tr
16:0	16.7	15.5	15.7
16:1	1.8	2.1	2.0
17:0	0.2	tr	0.3
18:0	14.6	14.6	14.4
18:1	23.4	26.5	25.4
18:2	25.1	20.3	23.1
18:3	0.9	1.1	0.8
20:0	tr	tr	tr
20:2	0.7	0.8	0.6
20:4	12.8	13.1	11.8
22:4	3.6	6.0	5.9
22:6	tr	tr	tr
% Sat.	31.7	30.1	30.4
% Monounsat.	25.2	28.6	27.4
% Diunsat.	25.8	21.1	23.7
% Polyunsat.	17.3	20.2	18.5
% Total unsat.	68.3	69.9	69.6

[1] Duplicate samples were taken from each of three replications containing TBHQ.

tr =trace amounts

Notable discrepancies were found, however, between experimental data and the values Igene et al. (1981) reported for $C_{14:0}$, $C_{14:1}$ and $C_{16:2}$. Igene et al. found a higher proportion of $C_{14:1}$ and no $C_{14:0}$ while the current data indicate approximately 3 percent $C_{14:0}$ and only 1 percent $C_{14:1}$. Additionally, they reported approximately 1 percent $C_{16:2}$ in beef triglycerides. Attempts were made to substantiate this claim, but no commercial source of $C_{16:2}$ could be located.

The fatty acid profiles of phospholipids shown in Table 12 are very representative of previously reported values (Hornstein and Crowe, 1967; Terrell and Bray, 1969; Marmer et al., 1984). Slight variability was found even among literature values depending on muscle type analyzed, animal feeding history, animal sex and age at time of slaughter. However, experimental data again agreed closely with those of Marmer et al. (1984) although the phospholipids extracted from restructured steaks were richer in $C_{20:4}$ and $C_{22:4}$. These findings were not unusual, though, as Hornstein and Crowe (1967) and Terrell and Bray (1969) reported 6 to 16 percent arachadonic acid in beef phospholipids.

The chromatograms obtained for the phospholipid fraction contained 3 peaks, comprising approximately 10% of the total peak area, which could not be identified. The major peak eluted between $C_{14:0}$ and $C_{16:0}$, while the two lesser peaks eluted near $C_{18:0}$ and $C_{20:4}$, respectively. Mass

spectroscopy of the major peak indicated that the compound most likely was not a fatty acid methyl ester. Other researchers have reported the presence of compounds identified as dimethylacetals (DMAs) of long chain aldehydes in the fatty acid profiles of polar lipids esterified by acid-catalyzed procedures (Maxwell and Marmer, 1983; Wood, 1983). DMAs arise from the hydrolysis of plasmalogens which contain a vinyl ether linkage in addition to the usual ester linkages. Since bovine polar lipid fractions may contain up to 30% plasmalogen, DMAs would be major components in chromatograms of phospholipid fatty acids (Maxwell and Marmer, 1983). Wood (1983) reported up to 8 percent $C_{16:0}$ DMA and 4 percent $C_{18:0}$ DMA in the total phospholipids of beef.

To prevent the acid-catalyzed hydrolysis of plasmalogen lipids, modified esterification methods have been utilized. Christopherson and Glass (1969) used methanolic KOH or sodium methoxide to prepare milk fat methyl esters. Maxwell and Marmer (1983) modified this method for use with phospholipid concentrates and reported that room temperature alkaline transesterification converts phospholipids to methyl esters without generating DMAs from plasmalogens.

Further examination of the data in Tables 11 and 12 reveals that, 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 typical and 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% saturates and 55% unsaturated fatty acids in the neutral lipids and 33% saturates and 67% unsaturates in the phospholipid fraction. A more significant difference in composition between the two fractions is evident in the di- and polyunsaturated fatty acids. Beef neutral lipids contain only about 4% di- and polyunsaturated fatty acids while the phospholipids contain approximately 40%. This high degree of unsaturation accounts, in part, for the lability of beef phospholipids to oxidation and their role in the development of WOF (Igene and Pearson, 1979).

Lipid Stability

The influence of antioxidants on the stability of lipids in restructured beef steaks was evaluated using the TBA procedure, quantitation of hexanal and sensory evaluation. The effectiveness of two natural antioxidants and TBHQ was monitored over 12 months of frozen storage.

TBA Test

The modified distillation procedure of Tarladgis et al. (1964) was used to quantitate TBARS in restructured steaks during the storage period. Analysis of variance (ANOVA) and Scheffe's test were used to determine if any significant differences existed among the TBA numbers from the various samples. Initially, ANOVAs were performed using data from

all sampling points to determine the significance of treatment, time and the time/treatment interaction. For raw and freshly cooked samples both treatment and time were significant factors. For cooked and stored samples, only treatment was significant. In all cases the time/treatment interaction was not significant.

Additional ANOVAs were performed, and Scheffe's procedure was used to determine which treatments were significantly different at each sampling period. In this manner, changes in the effectiveness of each antioxidant could be monitored over the course of the storage period.

As shown in Figure 4 and Table 13, the TBA numbers for all raw samples increased over time with the salt-only control oxidizing most rapidly and to the greatest extent. TBA values for all antioxidant-treated samples were lower than control values throughout the entire study, however, only TBHQ-treated samples had TBA numbers which were significantally lower ($p < 0.05$) than control samples over the major portion of the storage period. Additionally, the natural antioxidants were effective, statistically, only at 0 and 4 months. At all other sampling periods, TBA numbers from natural antioxidant samples were not different from those of control samples. There was also no significant difference in the effectiveness of the two natural anti-oxidants at any sampling period.

By the 12^{th} month of storage there were no statistical differences among the TBA numbers from any of the four

Figure 4. Changes in TBA numbers of raw restructured beef steaks over 12 months of storage at -20°C.

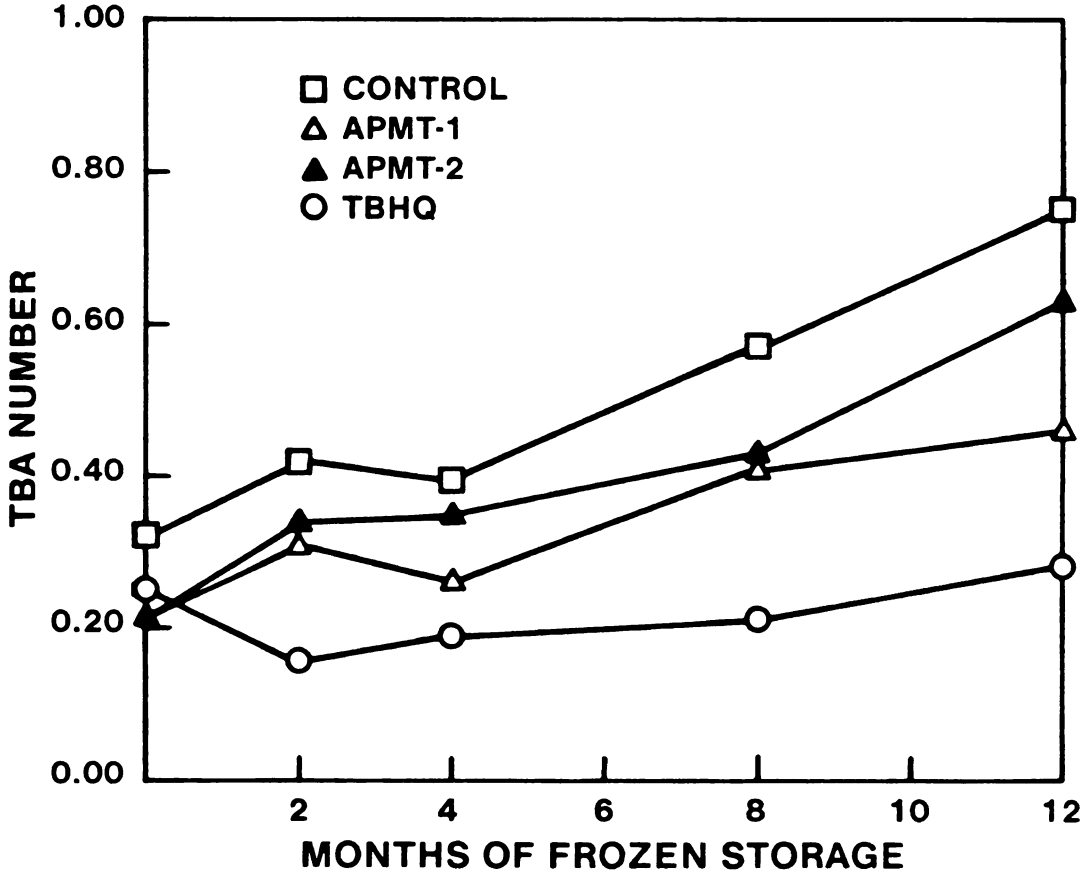

1.00
0.80
0.60
0.40
0.20
0.00
TBA NUMBER
CONTROL
APMT-1
APMT-2
TBHQ
2
4
6
8
10
12
MONTHS OF FROZEN STORAGE

Table 13. TBA numbers[1,2] of raw restructured beef steaks after 0, 2, 4, 8 and 12 months at -20°C.

Months of Storage	Treatment			
	Control	APMT-1	APMT-2	TBHQ
	TBA Number[3]			
0	0.32[a] (0.03)	0.22[b] (0.02)	0.22[b] (0.04)	0.25[ab] (0.02)
2	0.42[a] (0.08)	0.32[ab] (0.04)	0.34[a] (0.08)	0.16[b] (0.02)
4	0.39[a] (0.03)	0.26[bc] *	0.35[ac] *	0.19[b] (0.02)
8	0.57[a] (0.09)	0.41[a] (0.01)	0.43[a] (0.07)	0.21[b] (0.07)
12	0.75[a] (0.28)	0.46[a] (0.10)	0.63[a] (0.26)	0.28[a] (0.06)

[1] Values represent means of three replications which were analyzed in duplicate.

[2] Standard deviations are shown in parentheses.

[3] mg malonaldehyde/kg sample

* Value is from one replication only.

[a,b,c] Values in the same row bearing the same superscript do not differ significantly at $p < 0.05$.

treatments. However, as shown in Figure 4, TBA numbers from raw antioxidant-treated samples were substantially lower than that of the control sample. This apparent contradiction is readily explained by the data in Table 13. Because of the large variances associated with the data at this sampling period, statistical differences among the treatments were not evident.

Data for freshly cooked samples were analyzed in a similar manner (Table 14 and Figure 5). At all sampling periods, except month 4, all antioxidant treatments yielded significantly lower TBA numbers than did the control sample. At month 4, only TBHQ-treated samples had statistically lower TBA numbers. In addition, there were no differences among the various antioxidants; hence the natural antioxidants were just as effective as TBHQ in retarding lipid oxidation in freshly cooked meat that had been previously frozen for up to 12 months.

The protection offered by the various antioxidant treatments may be more evident in the cooked meat system than in raw samples due to the high degree of oxidation which occurs in the salt-only control during and immediately following cooking. TBA numbers from the control samples were increased approximately two to three fold by the cooking process while values from antioxidant-treated steaks increased only approximately 50 percent. This demonstrates that all three antioxidants have good, or at least adequate, carry-through and are capable of retarding lipid oxidation

Table 14. TBA numbers[1,2] of restructured beef steaks cooked after 2, 4, 8 and 12 months at -20°C.

Months of Storage	Treatment			
	Control	APMT-1	APMT-2	TBHQ
	TBA Number[3,4]			
2	1.04[a] (0.23)	0.45[b] (0.08)	0.42[b] (0.03)	0.27[b] (0.07)
4	0.73[a] (0.11)	0.53[ab] (0.15)	0.48[ab] (0.10)	0.30[b] (0.02)
8	1.17[a] (0.28)	0.70[b] (0.09)	0.58[b] (0.05)	0.33[b] (0.06)
12	1.16[a] (0.28)	0.62[b] (0.07)	0.49[b] (0.10)	0.32[b] (0.05)

[1] Values represent means of three replications which were analyzed in duplicate.

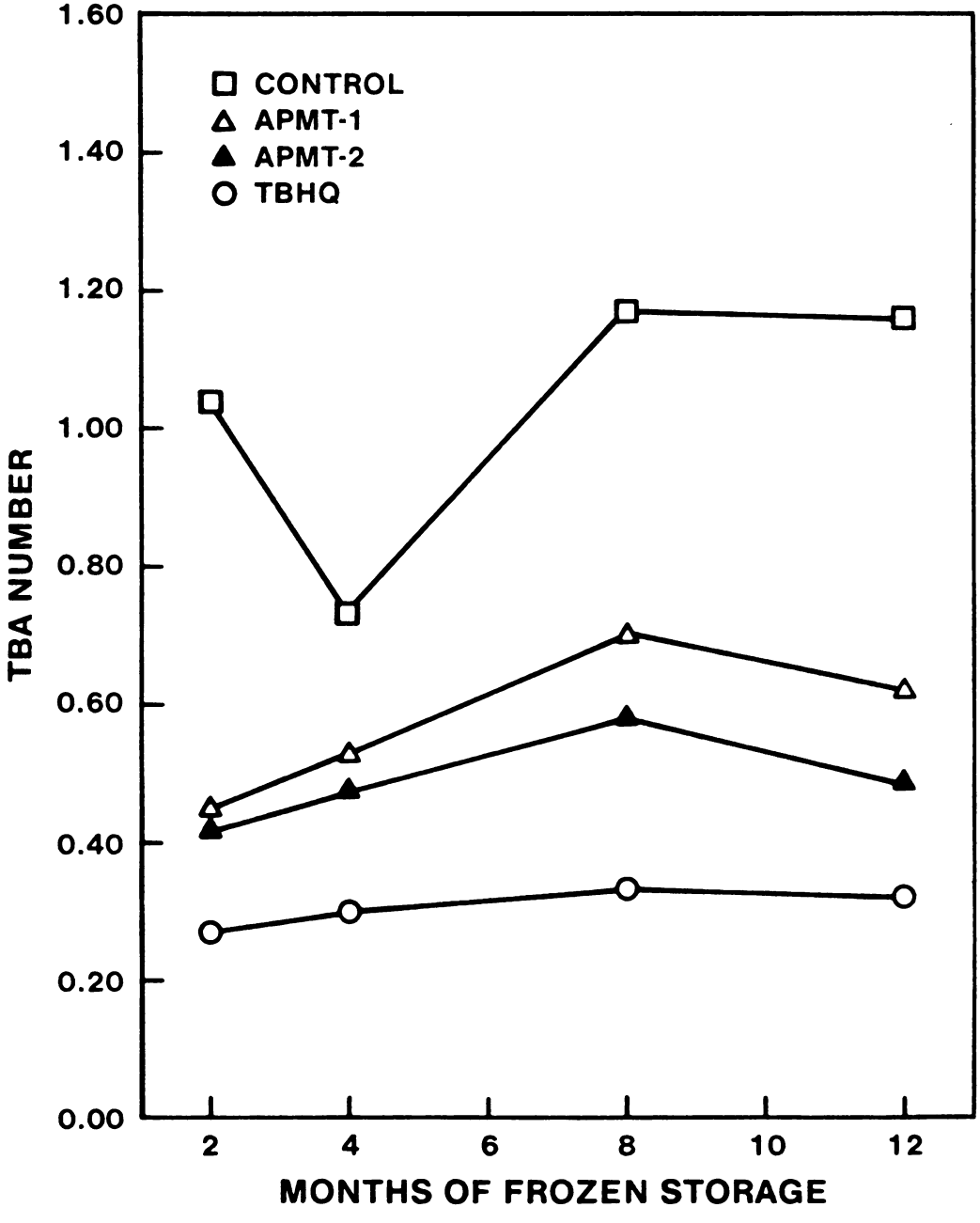
[2] Standard deviations are shown in parentheses.

[3] mg malonaldehyde/kg sample

[4] Corrected for loss of fat during cooking

[a,b,c] Values in the same row bearing the same superscript do not differ significantly at $p < 0.05$.

Figure 5. TBA numbers of restructured beef steaks cooked after 2, 4, 8 and 12 months at -20°C.


1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
TBA NUMBER
CONTROL
APMT-1
APMT-2
TBHQ
2
4
6
8
10
12
MONTHS OF FROZEN STORAGE

catalyzed by cooking. This catalysis of lipid oxidation could be related to the release of non-heme iron during cooking as postulated by Igene et al. (1979a).

Cooked samples were held for 4 hours at 4°C, reheated and analyzed. This procedure was used to simulate a pre-cook or cook-and-hold situation which could be found in the food service industry. As shown in Table 15 and Figure 6, TBA numbers of antioxidant-treated samples were lower than those of control samples at all sampling periods. The protective effect of TBHQ was statistically significant throughout the entire storage study. The TBA numbers for both APMT-treated samples were significantly lower than control values at 4 months, while values from APMT-2 samples were lower at 8 months. Again, the efficacies of the two natural antioxidants were not different ($p < 0.05$) from that of TBHQ.

Comparison of the magnitude of the TBA numbers from the various treatments is one way of determining the effectiveness of the antioxidants. Examination of the rates of lipid oxidation during the 4 hours of refrigerator storage may also demonstrate the effectiveness of the antioxidants in relation to each other. Figure 7 graphically shows the lipid oxidation which occurred in the various samples after they were cooked and held 4 hours at 4°C. The greater the slope of the line connecting the points representing TBA numbers, the more rapidily lipid oxidation occurred.

Table 15. TBA numbers[1,2] of restructured beef steaks cooked and held 4 hours at 4°C after 2, 4 and 8 months at -20°C.

Months of Storage	Treatment			
	Control	APMT-1	APMT-2	TBHQ
	TBA Number[3,4]			
2	1.84[a] (0.68)	0.79[ab] (0.24)	0.85[ab] (0.27)	0.38[b] (0.10)
4	1.49[a] (0.35)	0.67[b] (0.10)	0.74[b] (0.08)	0.35[b] (0.02)
8	2.04[a] (0.74)	0.98[ab] (0.14)	0.79[b] (0.13)	0.44[b] (0.05)

[1] Values represent means of three replications which were analyzed in duplicate.

[2] Standard deviations are shown in parentheses.

[3] mg malonaldehyde/kg sample

[4] Corrected for loss of fat during cooking

[a,b] Values in the same row bearing the same superscript do not differ significantly at $p < 0.05$.

Figure 6. TBA numbers of restructured beef steaks cooked and held at 4°C after 2, 4 and 8 months at -20°C.

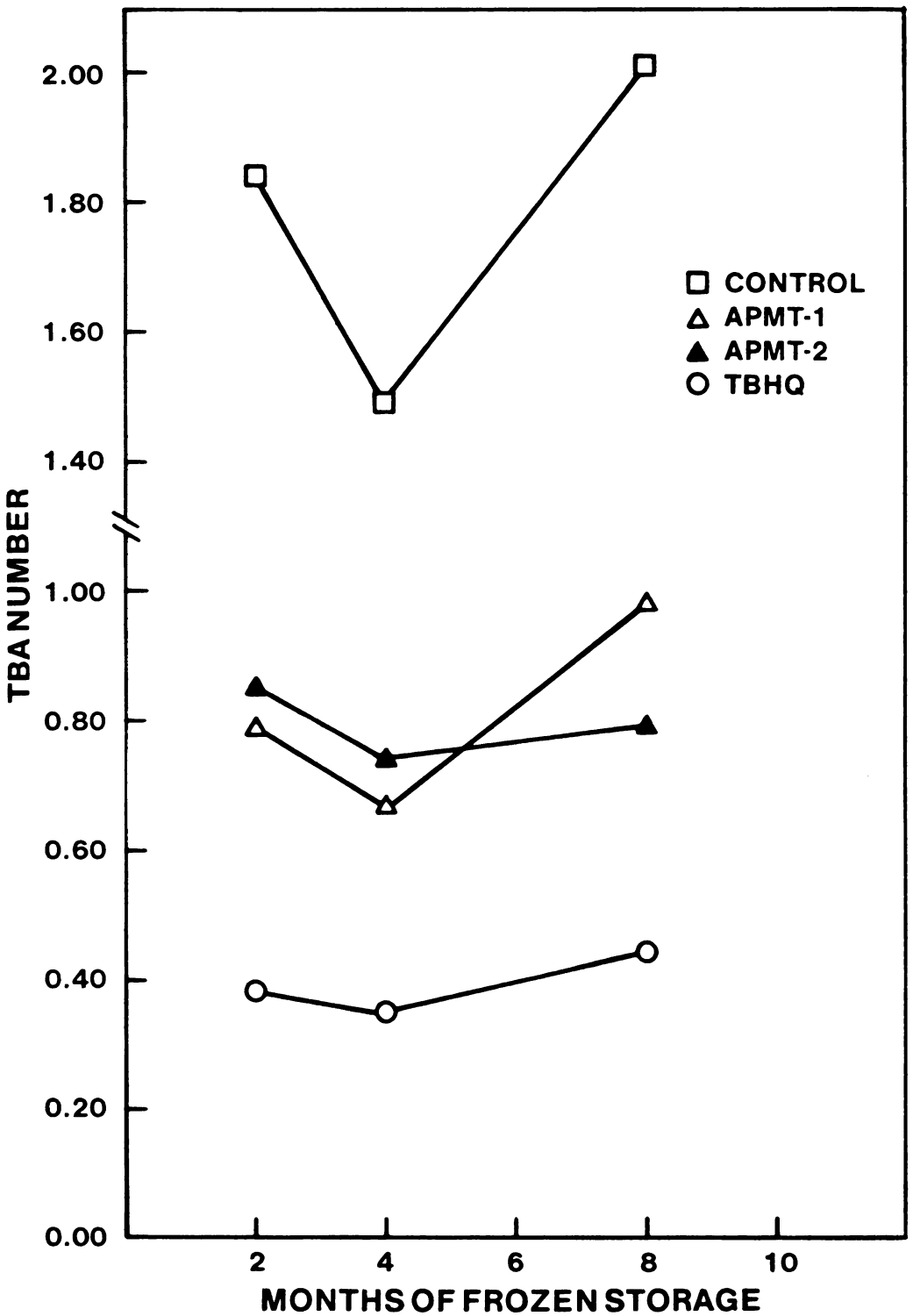
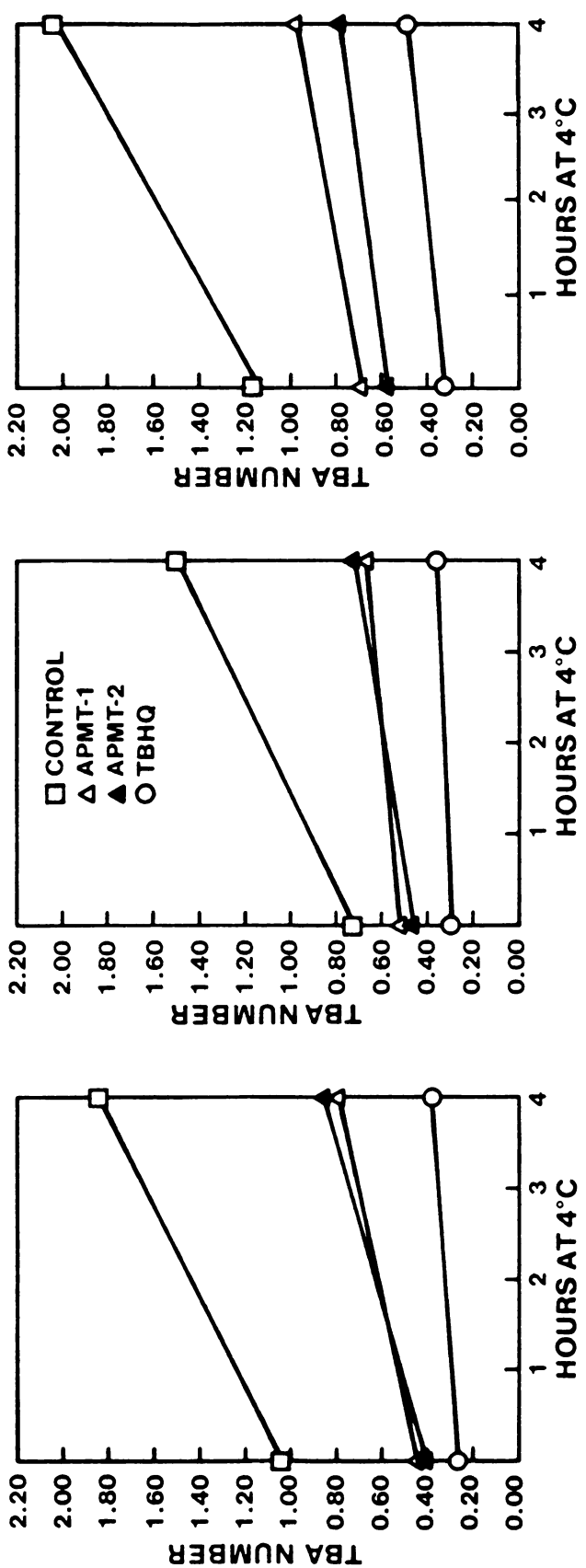

2.00
1.80
1.60
1.40
1.00
0.80
0.60
0.40
0.20
0.00
TBA NUMBER
CONTROL
APMT-1
APMT-2
TBHQ
2
4
6
8
10
MONTHS OF FROZEN STORAGE

Figure 7. Changes in TBA numbers of cooked restructured beef steaks over 4 hours of storage at 4°C after 2, 4 and months at -20°C.


TBA NUMBER
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
HOURS AT 4°C
1
2
3
4
□ CONTROL
△ APMT-1
▲ APMT-2
○ TBHQ

At all sampling periods, the salt-only control oxidized most rapidly. Each antioxidant offered protection, but TBHQ was the most effective in retarding oxidation as shown by the nearly horizontal lines in Figure 7. The two natural antioxidants were similar in effectiveness with APMT-1 being slightly more effective at 2 and 4 month sampling.

These data show that although there were no differences among the TBA numbers from the various antioxidant treatments in freshly cooked restructured steaks, TBHQ was more effective in slowing the rate of oxidation during subsequent refrigerator storage of the samples than were the natural antioxidants. It is possible that TBHQ had better carry-through and was therefore present in sufficient concentration to retard lipid oxidation to a greater extent, or, simply, that it was a more effective H donor and could slow the propagation steps of oxidation more readily than could the natural antioxidants.

The effects of antioxidants on lipid stability in restructured meat products have been evaluated by other researchers. Chastain et al. (1982) added BHA and TBHQ, alone and in combination, to beef-pork steaks formulated to 20% fat and 0.75% salt. When added at the level of 0.02% (fat basis), all antioxidants significantly ($p < 0.05$) decreased TBA numbers relative to those of the salt-only control both immediately after processing and over 20 weeks of frozen storage. Although there was no significant

difference among antioxidant treatments, TBA numbers for TBHQ-treated samples were consistently the lowest.

Miles et al. (1986) reported the effects of various antioxidants--both natural and synthetic--on lipid oxidation in restructured pork. In chops formulated to 25% fat and 0.5% salt, TBA numbers from samples containing 0.5% sodium tripolyphosphate, 0.03% BHA/BHT/citric acid (BBC) or 0.02% ascorbyl palmitate/ α tocopherol were significantly lower than those from salt-only controls (SC) after 12 days of refrigerator storage. α -Tocopherol (toc) also resulted in lower TBA numbers but was not as effective as the other antioxidants.

The TBA numbers for a no salt control (NSC) did not differ significantly ($p < 0.05$) from those of the other antioxidant treatments on day 12 and were higher than only the BBC treatment by day 16. This seems to indicate that the use of antioxidants would be unnecessary or would offer only minimal advantage in a system not containing salt. After only four days, however, the TBA number for the SC was nearly double that of the NSC, which clearly demonstrates the prooxidative effect of sodium chloride in restructured meats.

Schwartz and Mandigo (1976) examined the effect of salt on restructured pork and reported that TBA numbers increased as salt was increased from 0 to 2.25%. TBA numbers increased more than four-fold between 0 and 0.75% added salt

but to a lesser extent as salt levels were increased to 2.25%. Huffman et al. (1981b) reported similar results from their work with restructured pork chops. The addition of salt at 0.5, 1.0 and 1.5% produced a linear increase in TBA numbers both in freshly manufactured chops and in chops held at -15°C for 30 days.

GC Quantitation of Hexanal

A second method used to assess lipid oxidation in restructured steaks during frozen storage was gas chromatographic quantitation of hexanal extracted from the meat. As outlined previously, volatiles were extracted from cooked control and TBHQ-treated samples at 2, 4 and 8 months. TBA tests were also run on portions of each sample to determine if a correlation existed between TBA numbers and hexanal concentration. TBA values and corresponding hexanal peaks for control and TBHQ-treated samples at each sampling period are presented in Table 16. Hexanal could not be detected in the TBHQ-treated samples under the experimental conditions of the study which confirms the antioxidant's effectiveness in preventing lipid oxidation. For the individual sampling periods shown in Table 16, TBA numbers and hexanal concentration appear to be positively correlated in a linear manner; that is, hexanal concentration increased with increasing TBA numbers. The correlation coefficients for 2, 4 and 8 month sampling are 0.80, 0.99 and 0.94, respectively.

Increases in hexanal concentration as lipid oxidation occurs have been previously reported by several researchers.

Table 16. TBA numbers and relative hexanal concentration in volatiles extracted from cooked restructured beef steaks after 2, 4 and 8 months at -20°C.

Sample		Sampling Month	TBA[1,2] Number	Hexanal[3]	Cor.[4]
Control	a[5]	2	0.90	37,290	
	b	2	0.70	47,940	0.80
	c	2	1.10	119,699	
Control	a	4	0.54	40,610	
	b	4	0.62	56,970	0.99
	c	4	0.73	72,840	
Control	a	8	0.73	34,530	
	b	8	1.11	60,770	0.94
	c	8	1.20	87,520	
TBHQ	a	2	0.16	ND	
	b	2	0.27	ND	
	c	2	0.28	ND	
TBHQ	a	4	0.25	ND	
	b	4	0.25	ND	
	c	4	0.28	ND	
TBHQ	a	8	0.26	ND	
	b	8	0.35	ND	
	c	8	0.26	ND	

[1] Analyses were performed in duplicate.

[2] mg malonaldehyde/kg sample

[3] Values represent peak areas as determined by GC analysis.

[4] Correlation coefficient

[5] a,b,c = replications 1, 2 and 3.

ND = none detected

El-Gharbawi and Dugan (1965) found that hexanal, steam distilled from freeze-dried beef, increased greatly in concentration during storage of the meat. Cross and Ziegler (1965) reported that hexanal was always a major constituent of the volatiles from uncured ham but was absent or barely detectable in nitrite-cured ham. Similar trends were found when cured and uncured beef or chicken were compared. This they attributed to the more extensive lipid oxidation in the uncured samples.

Love and Pearson (1976) monitored hexanal concentration in the headspace over oxidizing PE and cooked meat. They concluded that hexanal concentration was related to the degree of sample oxidation since addition of tripoly-phosphate (TPP), which may act as an antioxidant, resulted in a 50% decrease in hexanal while samples treated with 5 ppm of Fe^{2+} produced twice as much hexanal as no-additive control samples.

Cooked, uncured meats were analyzed by Bailey et al. (1980). Hexanal was found to increase significantly in both Boston butts and roast beef during refrigerator storage so much so that these researchers suggested using hexanal as an indicator of lipid oxidation rather than the more traditional methods.

As shown in Table 16, hexanal concentration in the volatiles extracted from restructured steaks generally increased linearly with increasing sample oxidation as expressed by higher TBA numbers. This was true, however,

only when sampling periods were examined individually. Compilation of the data over the entire storage period shows high variability in the experimental results with a correlation of only 0.56.

It is evident from Table 16 that samples taken at different times did not yield the same concentration of hexanal even though they produced identical TBA numbers. At 2 months, the hexanal response from the sample with a TBA value 1.11 was 119,600 units; at 8 months a hexanal value of 60,770 was associated with the TBA number 1.11.

A possible source of this variability could be the samples themselves. Since the extraction of volatiles destroyed the sample, different steaks were used for each determination. These may have varied slightly in composition or were exposed to different conditions during storage if, for example, a leak developed in the vacuum sealed package.

It is also possible that an unrecognized source of variability was introduced into the experimental procedures at certain sampling periods. If the extraction or recovery of volatiles was not consistent over time, the hexanal concentrations would also vary. It is advisable, then, that for future research utilizing this extraction technique an internal standard should be used to indicate possible variability in the procedure.

Despite the variability in data collected in the current study, they do suggest that hexanal could be used as

an indicator of lipid oxidation. At individual sampling periods, hexanal concentration and TBA numbers are highly correlated. In addition, hexanal was not detected in antioxidant-treated samples, which had TBA numbers below 0.35 throughout the storage study. As lipid oxidation was retarded, so was the production of hexanal. With further research it may be possible to establish threshold TBA numbers and relate them to threshold levels for hexanal detection.

Sensory Evaluation

Lipid oxidation in restructured steaks was also assessed by sensory evaluation. A major objective of this portion of the study was to determine the correlation between sensory scores and TBA values as oxidation progressed during frozen storage of the samples.

Scores from the 8 panelists for the various treatments at each sampling period are shown in Appendix C. Values are shown for both freshly cooked meat and for samples that were held 4 hours at refrigerator temperature. ANOVAs were performed to determine if the judges could detect differences in rancidity among the various samples. In all cases, the mean sensory scores for all treatments were not significantly different ($p < 0.05$). In most instances, there were significant differences among the judges and their perception of WOF which is not unusual, though, since the panelists did not receive extensive training. Presumably these differences would decrease with a highly trained panel

that is very familiar with WOF and can accurately judge the intensity of WOF in a sample.

Correlation coefficients between sensory scores and TBA numbers were calculated on individual observations made by panel members at each sampling period. As evident from the data in Table 17, r values were generally low, and scores from very few panelists were correlated significantly with TBA numbers except at 8 month sampling. At this sampling period, scores from 75% of the panelists were correlated significantly ($p < 0.10$) with TBA numbers.

As shown in Table 18, mean TBA numbers at 8 month sampling were the highest, ranging from 0.33 to 2.04. Mean TBA numbers at 2 and 4 month sampling, also summarized in Table 18, ranged from 0.27 to 1.84 and from 0.30 to 1.49, respectively. Based on these data, it appears that the panel was more accurate in their scoring--their scores correlated significantly with TBA numbers--as mean TBA numbers increased over the 8 month period and when the differences in TBA numbers among the various treatments were largest.

At all sampling periods, mean sensory scores were higher for samples that had been held 4 hours at refrigerator temperature. These samples also had higher mean TBA numbers. This suggests that, although sensory scores and TBA numbers for the individual treatments were not always significantly correlated, panelists did indeed respond to

Table 17. Correlation coefficients between TBA numbers and sensory scores at 2, 4 and 8 month sampling periods.

Panelist	Month 2	4	8
		Coefficients	
1	0.229	-0.170	0.384*
2	0.389	0.119	0.466*
3	0.398*	0.102	0.412*
4	0.594*	0.594*	0.432*
5	0.281	0.585*	0.720*
6	0.163	0.181	0.346*
7	-0.332	-0.147	0.180
8	0.029	-0.276	-0.095

* Correlation coefficient is significant at $p < 0.10$.

Table 18. Mean TBA numbers and sensory scores for restructured beef steaks after 2, 4 and 8 months at -20°C.

Sample	Freshly Cooked		Cooked/Held	
	TBA[1,2] Numbers	Sensory[3,4] Scores	TBA Numbers	Sensory Scores
2 month				
Control	1.04	4.98	1.84	6.97
APMT-1	0.45	5.59	0.79	5.16
APMT-2	0.42	4.59	0.85	5.88
TBHQ	0.27	4.99	0.38	7.43
4 month				
Control	0.73	4.34	1.49	5.60
APMT-1	0.53	4.47	0.67	4.66
APMT-2	0.48	4.42	0.74	5.28
TBHQ	0.30	3.99	0.35	6.93
8 month				
Control	1.17	6.55	2.04	9.88
APMT-1	0.70	4.50	0.98	8.08
APMT-2	0.58	5.00	0.80	6.62
TBHQ	0.33	5.92	0.44	6.67

[1] Values represent mean of three replications which were analyzed in duplicate.

[2] mg malonaldehyde/kg sample

[3] Values represent mean of three observations made by each of 8 panelists.

[4] Sensory scores were assigned using the range 1 = no WOF to 14 = very strong WOF.

increased rancidity. With the exception of month 2, sample APMT-1, panelists consistently assigned slightly higher scores to samples from all treatments after the samples had been refrigerated and reheated. Thus, it appears that panelists were able to recognize that oxidation had occurred in samples that had been cooked and held, but were generally unable to accurately assess the varying degrees of oxidation among the samples, either freshly cooked or cooked and held.

It is possible, however, that panelists were not responding to increased rancidity, but rather to textural changes which occurred in the samples during refrigerator storage. Although samples were stored and rewarmed in sealed containers, they may have been slightly dehydrated and tougher after reheating than when freshly cooked. Panelists were instructed to consider only flavor, not texture, when evaluating samples, but textural differences in the samples may have introduced bias into their scores.

The data from this study support the conclusions of other researchers that there is a threshold for the detection of oxidized flavors and odors. Tarladgis et al. (1960) found that trained panelists first perceived rancid odor in cooked ground pork when the meat had TBA numbers between 0.5 and 1.0. Greene and Cumuze (1981) used untrained panelists to evaluate cooked ground beef and, because of variability among panelists, could not determine a threshold TBA value for the detection of rancid flavors. When the panel was narrowed to the "discriminating" members,

a TBA range of 0.6 to 2.0 was found to be the initial range of flavor differentiation.

The panel used for the current study might best be described as semi-trained. Although they were familiarized with the product and with WOF, they still had difficulty differentiating varying degrees of oxidation as demonstrated by the r values in Table 17. Perhaps the panelists were unable to accurately assess oxidation because TBA numbers were below the threshold of detection. As previously stated, panelists were more responsive to oxidation as TBA numbers approached 2.0. Because there were no significant differences among the mean sensory scores from all treatments, a threshold TBA range could not be determined. It seems probable, though, for this study a TBA number near 2.0 could represent the threshold for detection of WOF in restructured steaks.

Another possible explanation for the panelists' inability to ascertain varying degrees of lipid oxidation is the level of salt used for the manufacture of the restructured steaks. The previously mentioned studies used unsalted samples for sensory evaluation while samples in the current study contained 0.75% added sodium chloride. It is possible that this level of salt masked off-flavors in the steaks or otherwise influenced the panelists' perception of WOF.

Cross and Stanfield (1976) found that consumers preferred restructured steaks containing added salt (0.75%)

over no-salt steaks despite, as Schwartz and Mandigo (1976) demonstrated, the increased rancidity due to the addition of salt. Trained panel and consumer sensory evaluations conducted by Huffman et al. (1981a) showed improved flavor in flaked and formed hamburger patties which contained salt or salt and tripolyphosphate (TPP) relative to no-salt samples. They also reported that an increase in TBA numbers from 0.42 to 1.27 over 60 days of frozen storage did not significantly ($p < 0.05$) affect the sensory properties of the beef patties.

These data firmly support the findings of the current study that: 1) the addition of salt to restructured steaks is not necessarily detrimental to flavor despite its catalytic effect on lipid oxidation and 2) when sample oxidation is below a certain level, sensory scores are not necessarily affected by increased TBA numbers. It appears that, in the current study, either the TBA threshold for detection of rancidity was not reached, or that the added salt effectively masked WOF.

SUMMARY AND CONCLUSIONS

The effect of antioxidants on lipid stability in restructured beef steaks was investigated. TBA numbers from antioxidant-treated samples were consistently lower than those from control samples throughout the 12 month storage period. TBHQ significantly ($p < 0.05$) lowered TBA numbers in raw samples in up to 8 months of frozen storage. Samples containing TBHQ that were freshly cooked or cooked and held 4 hours at $4^{o}C$ had significantly lower TBA numbers than did control samples at all sampling periods. The natural antioxidants were most effective in freshly cooked samples. At all sampling periods, except month 4, they were statistically as effective as TBHQ in retarding lipid oxidation.

Control and TBHQ-treated samples were analyzed for hexanal content after 2, 4 and 8 months of frozen storage. Hexanal concentration in control samples was positively correlated with TBA numbers but only at individual sampling periods. Compilation of the data over the entire storage period showed high variability in the experimental results and a low correlation coefficient. Hexanal was not detected in TBHQ-treated samples which had TBA numbers below 0.35 throughout the storage period.

Analysis of sensory scores from the 8 member panel showed that the panelists did not detect differences in

rancidity among the various treatments at any sampling period. Additionally, the correlation coefficients between sensory scores and TBA numbers were generally low and non-significant until TBA numbers increased to the range 0.33 to 2.04.

From the results of this study, it can be concluded that antioxidants are effective in retarding lipid oxidation in restructured beef steaks. Under certain conditions, natural antioxidants containing mixed tocopherols, ascorbyl palmitate and citric acid may be as effective as TBHQ. Hexanal concentration could prove to be an excellent index of oxidative rancidity if experimental conditions and variability are closely controlled. There appears to be a positive, linear correlation between hexanal concentration and TBA numbers. Sensory data indicate that the addition of 0.75% sodium chloride to restructured beef steaks does not adversely affect flavor despite its prooxidative action. Based on these data it also appears that, when samples are below a threshold level of oxidation, sensory scores and TBA numbers are not necessarily correlated.

APPENDICES

Appendix A

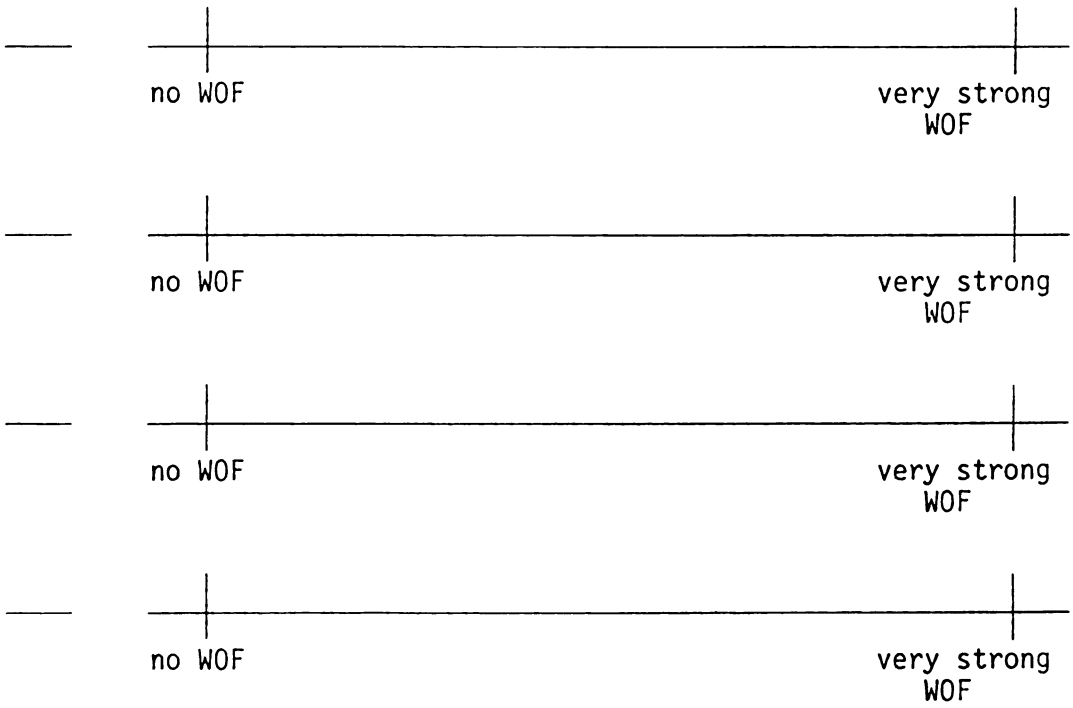
Taste panel form for the evaluation of restructured beef steaks for WOF.

NAME _____

DATE _____

Evaluate each sample carefully. Be certain you record the code numbers. Place a vertical line on the scale at the point you feel accurately describes the flavor of the sample. More than one sample may have the same flavor intensity. Additionally, you may mark beyond the anchor points if necessary.

CODE



This form has been reduced in size for illustrative purposes.

Appendix B

Calculation of the distillation constant, K, for both aqueous and acidic TBA assays.

To express experimental results from the TBA distillation method as "TBA Numbers" with units of mg malonaldehyde/ kg sample, absorbance values are multiplied by a constant, K, which is obtained from the standard curves and known dilutions using the following equation:

$$K = \frac{\text{conc. TMP/5 ml distillate}}{\text{optical density}} \times \text{MW of malonaldehyde} \times \frac{10^7}{\text{g of sample}} \times \frac{100}{\text{\% recovery}}$$

MW of malonaldehyde=72
g of sample=10
% recovery=73.1

The concentration of TMP for any given optical density (absorbance) can be determined using the equation for the standard curve. The equations for both aqueous and acidic TBA reagents are given below:

Aqueous: conc. = -0.0019 + 62.72(abs.)

Acidic: conc. = 0.2045 + 69.72(abs.)

If optical density is chosen as 0.100, the constants are calculated as follows:

$$K_{aq} = \frac{6.27 \times 10^{-9}}{0.100} \times 72 \times \frac{10^7}{10} \times \frac{100}{73.1} = 6.2$$

$$K_{ac} = \frac{7.18 \times 10^{-9}}{0.100} \times 72 \times \frac{10^7}{10} \times \frac{100}{73.1} = 7.1$$

Appendix C

Mean sensory scores[1] for restructured beef steaks freshly cooked after 2, 4 and 8 months at -20°C.

	Treatment			
Panelist	Control	APMT-1	APMT-2	TBHQ
2 months				
1	7.57	5.80	7.38	8.37
2	5.33	9.37	7.20	7.23
3	5.80	4.10	3.65	1.50
4	7.13	7.07	3.23	5.77
5	4.07	4.60	2.57	3.77
6	2.70	1.85	1.30	2.10
7	5.17	5.20	5.30	3.67
8	2.03	6.73	7.27	7.50
4 months				
1	6.90	6.33	8.67	8.37
2	7.70	10.75	5.45	6.70
3	1.43	2.57	5.03	2.23
4	6.40	1.55	2.80	1.25
5	5.15	4.35	5.40	3.20
6	1.93	2.87	2.97	1.90
7	4.43	4.10	2.10	5.03
8	0.95	3.35	2.95	3.95
8 months				
1	10.10	9.83	7.30	8.50
2	8.75	2.30	4.35	5.40
3	6.50	6.20	3.63	5.67
4	2.74	6.07	3.80	5.57
5	5.93	3.73	9.00	6.33
6	11.80	3.50	5.20	4.83
7	1.70	1.60	3.53	2.53
8	4.87	2.27	3.20	8.50

[1] Values represent the mean of three observations/panelist.

Mean sensory scores[1] for restructured beef steaks cooked and held 4 hours at 4 C after 2, 4 and 8 months at -20°C.

	Treatment			
Panelist	Control	APMT-1	AMPT-2	TBHQ
2 months				
1	9.40	4.50	7.60	6.07
2	8.97	9.37	8.57	12.20
3	4.55	4.45	3.10	3.95
4	10.20	7.10	7.47	7.30
5	7.97	4.72	4.13	4.90
6	5.55	3.75	5.40	8.05
7	5.67	2.27	6.70	5.47
8	3.43	5.10	4.10	11.47
4 months				
1	5.67	6.60	7.23	9.60
2	3.30	3.70	7.60	13.00
3	7.80	6.60	8.40	9.75
4	4.30	2.25	4.55	2.30
5	8.50	4.00	7.95	6.90
6	5.80	5.65	3.25	3.40
7	5.40	5.25	2.25	3.30
8	4.00	3.25	1.00	7.15
8 months				
1	9.83	8.23	8.40	9.40
2	5.17	9.27	9.00	7.43
3	12.87	11.03	8.90	9.73
4	12.25	10.30	2.00	3.15
5	11.43	9.83	7.07	8.50
6	12.23	6.40	6.03	5.70
7	4.83	5.43	4.20	3.33
8	10.43	4.13	7.33	8.53

[1] Values represent the mean of three observations/panelist.

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