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ROLE OF MEAT LIPIDS AND MEAT PIGMENTS IN DEVELOPMENT OF RANCIDITY AND WARMED-OVER FLAVOR IN FROZEN AND COOKED MEAT

By

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ABSTRACT

ROLE OF MEAT LIPIDS AND MEAT PIGMENTS IN DEVELOPMENT OF RANCIDITY AND WARMED-OVER FLAVOR IN FROZEN AND COOKED MEAT

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Experiments were designed to study the influence of total lipids, total phospholipids, individual phospholipids (PC and PE) and triglycerides on lipid oxidation and off-flavor development in frozen meat and cooked meat model systems. Experiments were also undertaken to explore the effect of length of frozen storage of whole meat on the stability of its constituent lipids and on development of TBA values. The effect of meat pigments, nitrite and non-heme iron on development of warmed-over flavor (WOF) in cooked meat was also studied.

The 2-thiobarbituric acid (TBA) test and or taste panel scores were used to assess the extent of lipid oxidation in frozen stored and cooked meat samples. The compositional changes in the lipid content of the samples were evaluated using GLC and TLC techniques. The levels of heme and non-heme iron in extracted meat pigments were determined using atomic absorption spectrophotometry.

Studies with frozen model meat systems showed that both triglycerides and phospholipids contribute to development of rancidity, although phospholipids make the greatest contribution. The influence of triglycerides in the development of rancidity in frozen meat model systems was shown to depend upon the degree of unsaturation and the length of frozen storage. The relationship between rancidity and oxidation of the polyunsaturated fatty acids (PUFAs) was confirmed, particularly in the phospholipids.

Evidence is presented showing that both total phospholipids and PE are major contributors to development of WOF in cooked meat model systems. The triglycerides enhanced development of WOF in the model meat system only when combined with the phospholipids. Phosphatidyl choline (PC) did not influence WOF in the model system. Changes in the PUFAs of the phospholipids were shown to be directly related to development of WOF, especially in PE.

Results revealed that changes in total lipids during frozen storage of raw meat were largely due to losses in the triglyceride fraction. The phospholipid content of raw meat was relatively constant, irrespective of the length of freezer storage. Cooking significantly ($P<0.01$) elevated the percentage of phospholipids in relation to total lipids and accounted for a significant ($P<0.001$) increase in the rate of lipid oxidation. Cooked meat held at 4°C for 48 hrs after cooking was more susceptible to development of WOF than similar samples held at -18°C

for 48 hrs. PE, PC, total phospholipids and their PUFAs were significantly less stable in cooked than in raw frozen meat. Thus, involvement of PUFAs in the development of WOF was verified. The stability of the different types of meat, either raw frozen or cooked was in the order of: beef > chicken white meat > chicken dark meat.

Both removal of meat pigments and addition of 156 ppm of nitrite significantly ($P<0.001$) inhibited the development of TBA values in cooked meat. Taste panel evaluation confirmed the beneficial effects of removal of heme pigments and addition of nitrite to meat for controlling the development of WOF. Thus, results suggested that heme pigments may catalyze lipid oxidation.

The percentage of bound heme iron in fresh meat pigment extract was slightly over 90% while the level of free non-heme iron was less than 10%. Cooking, however, released a significant amount of non-heme iron from bound heme pigments, which accelerated lipid oxidation in cooked meat. Thus, the rate of lipid oxidation in cooked meat is due in part to release of non-heme iron during cooking which then catalyzes lipid oxidation. Although earlier studies have suggested that myoglobin may catalyze lipid oxidation, this study showed that pigments *per se* do not greatly accelerate the development of WOF, but serve as a source of non-heme iron in cooked meat. Thus, results showed that non-heme iron was the major pro-oxidant in the development of WOF in cooked meat. Addition of 2.0% EDTA

effectively chelated the non-heme iron, and thus, significantly reduced lipid oxidation.

To my elder brother, Benedy O. Igene,

his wife, Mrs. D. Igene and their children,

Obayando

Victor

Ideyenmi

Ebosereme

Oiyimhebedan ("Mom")

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INTRODUCTION

In recent years, the marketing of precooked or partially cooked meat and meat products and frozen raw meats for consumer convenience has become an accepted procedure. An inherent problem associated with precooked or partially cooked meat products is development of oxidized flavors. The development of oxidized flavors associated with cooked, uncured meat and meat products becomes apparent in a matter of hours and is principally due to oxidative degradation of lipids. Control of the resulting off-flavors, which have been aptly called warmed-over flavor (WOF) by Tims and Watts (1958), is related to the degree of lipid unsaturation.

Even though lipid degradation has been associated with the development of undesirable flavors, a thorough understanding of the role of meat lipids in the production of off-flavors, and particularly for WOF has been lacking. Although phospholipids have been indirectly implicated as being the major contributors to WOF (Wilson, *et al.*, 1976; Younathan and Watts, 1960), the relative contributions of total and individual phospholipids and of total lipids and triglycerides to WOF have not been fully studied.

It is well known that raw fresh frozen meats gradually become oxidized during freezer storage, with the slow development of off-flavor being described as rancid (Greene,

1969). The essential difference between rancidity and WOF is that the latter becomes apparent within a few hours following cooking and storage, and especially following reheating, as compared to months or even years for normal rancidity. Keller and Kinsella (1973) have suggested that oxidation of the triglycerides may be the major factor involved in the deterioration of meat during freezer storage. On the other hand, Cadwell *et al*. (1960) and Greene (1971) have reported that changes in the phospholipids during frozen storage of raw meats results in rancidity and browning.

Thus, the primary objective of this research was to determine the role of meat lipids in the development of rancidity during freezer storage and of WOF in cooked meats. First, it was necessary to develop a model meat system consisting of lipid-free muscle fibers as base material to which each lipid component could be added for testing their role in the development of off-flavors associated with rancidity or WOF in both fresh and cooked meat. Knowledge gained from the model system should provide a good understanding of the behavior of lipids in intact meats.

In addition, it was deemed desirable to evaluate how variation in the composition of the triglycerides, total lipids and phospholipids influences oxidative changes in fresh and cooked meats. To this end, beef, chicken dark

meat and chicken white meat were stored at -18°C. At designated time intervals, packages of meat were removed from the freezer and analyzed to determine the role of different lipid components in development of off-flavors.

Specifically, the study was designed to determine the role of the following treatments on lipid oxidation in meats:

(a) To evaluate the changes that occur in the lipids of intact raw meats stored at -18°C;

(b) To test the stability of raw meats previously stored at -18°C and then cooked and stored at -18°C or at +4°C for 48 hours;

(c) To determine the influence of frozen storage of raw meats held at -18°C in relation to development of WOF; and

(d) Finally, to evaluate the effect of meat pigments and nitrite on the development of WOF.

LITERATURE REVIEW

Introduction

The flavor of meat and meat products is greatly influenced by the lipid constituents (Ory and St. Angelo, 1975). No other characteristic of meat, except possibly tenderness, is so important to consumer acceptance as flavor (Doty, 1961). It is well established that lipid degradation is associated with the development of undesirable flavors and odors in meat and meat products (Watts, 1954; Hornstein *et al.*, 1961). Thus, the present review will discuss the composition of lipids in meat and poultry and their role in flavor problems associated with heat processed meats. In addition, pro-oxidants and antioxidants will be reviewed in relationship to development of rancidity and warmed-over flavor (WOF).

Distribution of Animal Fats

Watts (1962) and Love and Pearson (1971) have reviewed the composition of animal fats. They classified meat lipids as depot or adipose tissue and as intramuscular or tissue lipids. They pointed out that depot fats are largely localized as subcutaneous deposits, although large quantities may be present in the thoracic and abdominal cavities and between the muscles as intermuscular deposits. In

addition, the depot fats consist largely of triglycerides and are deposited essentially as fat globules within the individual cells (Watts, 1962). Moreover, the triglycerides vary greatly in amount and composition according to the species, ration, environment, sex and other factors (Watts, 1962). In contrast, tissue lipids (mainly phospholipids) are relatively constant in proportion and are an integral part of various cellular structures, such as the cell wall (Kono and Colowick, 1961), the mitochondria (Holman and Widmer, 1969) and the sarcoplasmic reticulum (Newbold *et al*., 1973).

Fatty Acid Composition

Natural animal fats are composed principally of the straight chain even numbered carbon fatty acids, typically containing 16 and 18 carbon atoms (Dugan, 1971). According to Hilditch and Williams (1964), the most abundant and commonly occurring fatty acid in animal fat is oleic acid. They also stated that other unsaturated fatty acids that are widely distributed, though not uniformly among animals, include linoleic and palmitic. They also reported that saturated fatty acids constitute about one third of all fatty acids in animal fats, with palmitic acid being the most abundant and seldom being absent. Stearic acid is the next most abundant fatty acid and is also seldom

absent (Hilditch and Williams, 1964).

In monogastric animals, dietary fatty acids are usually reflected in the composition of the depot fat (Cook *et al.*, 1971). In ruminants, on the other hand, the depot fats are not influenced to any great extent by diet (Shorland *et al.*, 1957). Most diets of animals usually contain linoleic and linolenic acids (Gunstone, 1967). By elongation and desaturation, these two fatty acids provide the C_{20} and C_{22} polyunsaturated fatty acids of animal phospholipids (Gunstone, 1967; Poukka and Oksanen, 1972). Sprecher (1977) has pointed out that linoleate, linolenate, oleate and palmitoleate each serve as the initial unsaturated fatty acid precursor in biosynthesis of an independent family of polyunsaturated fatty acids. He concluded there is no direct crossover between unsaturated metabolites from one sequence to the other as shown below:

18:2 → 18:3 → 20:3 → 20:4 → 22:4 → 22:5

18:3 → 18:4 → 20:4 → 20:5 → 22:5 → 22:6

18:0 → 18:1 → 18:2 → 20:2 → 20:3

16:0 → 16:1 → 16:2 → 18:2 → 18:3 → 20:3 → 20:4

Minor amounts of odd numbered fatty acids, especially of saturated C_{15} and C_{17} as well as $C_{15:1}$ and $C_{17:1}$ and branched chain fatty acids, have been demonstrated to occur in animal fats, especially in those from ruminants

(Shorland, 1962).

Phospholipids in Animal Tissues

Small quantities of phospholipids are present as proteolipids and are found in animal tissues (Dugan, 1971). Lea (1957) and Younathan and Watts (1960) concluded that phospholipids may play an important role in the flavor and stability of meat and meat products during storage. In spite of the growing interest in the study of meat phospholipids, much remains to be known about their contribution to flavor deterioration in muscle tissue.

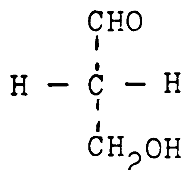
Phospholipids are mixed esters of fatty acids and phosphoric acid combined with either glycerol or sphingosine (Eibl, 1977). Lehninger (1970) and Gurr and James (1971), have comprehensively reviewed the composition and structure of the phospholipids. They stated that a wide spectrum of chemical species is made possible, firstly, by considerable variation in the types and combinations of fatty acids, and secondly, by esterification of different organic bases, amino acids and alcohols to the phosphate group. They further pointed out that phospholipids not only derive their lipid properties from long chain fatty acid moieties, but also have polar characteristics donated by ionization of the phosphate and base groups. They reported that biosynthesis of the complete phospholipid

molecule (denovo) occurs by two main pathways. One involves the transfer of a phosphate base from a water soluble nucleotide (cytidine diphosphobase) to a diglyceride. The other pathway involves transfer of a phosphatidic acid from a lipid soluble nucleotide (cytidine diphosphatidiglyceride) to the base.

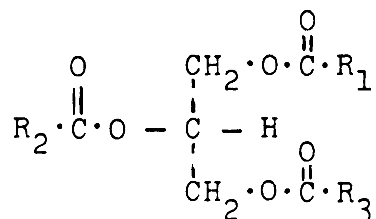
Pearson et al. (1977) have further reviewed the structure and composition of meat phospholipids. They presented the structure of diacyglycerophospholipids as is shown in Figure 1. They further emphasized the fact that most of the phospholipids in muscle are present as phosphoglycerides with the base esterified to the phosphate usually being choline, ethanolamine or serine.

Not all phospholipids have their hydrocarbon residues linked exclusively by an ester to glycerol (Lehninger, 1970). Gurr and James (1971) stated that plasmalogens possess a vinyl ether linkage, which is probably located at the 1-position. Dugan (1971) has reviewed a less abundant, but nevertheless important group of phospholipids, in which sphingosine is the alcoholic moiety. He pointed out that when sphingosine is esterified to phosphoryl choline it is usually referred to as sphingomyelin.

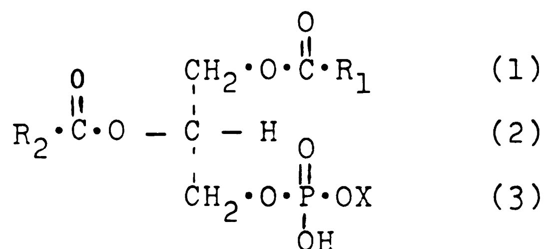
The level of phospholipids (0.5-1.0%) remains nearly constant when expressed as a percentage of muscle, but varies inversely as a function of total lipid (Dugan, 1971). There is, however, considerable variation in the



(A) L-Glyceraldehyde



(B) Triglyceride



(c) 1,2-Diacyl-Sn-glycerol-3-phosphoryl-X

R_1, R_2, and R_3 represent alkyl groups corresponding to different fatty acid residues. In structure C, Sn stands for stereochemical numbering and X for one of the following substituents:

Substituent	Phospholipid
H	Phosphatidic acid
$HO \cdot CH_2 \cdot CH_2 \cdot NH_2$	Phosphatidyl ethanolamine
$HO \cdot CH_2 \cdot CH_2N^+(CH_3)_3$	Phosphatidyl choline
$HO \cdot CH_2 \cdot CH \cdot NH_2$ COOH	Phosphatidyl serine

Figure 1. Structure of the diacylglycerophospholipids.

phospholipid content between species (Kaucher et al., 1943), and from location to location within the same species (Peng and Dugan, 1965; Acosta et al., 1966). Pearson et al. (1977) stated that poultry meat and fish are higher in phospholipids than red meats. When expressed as percentage of raw muscle tissue, Peng and Dugan (1965) and Acosta et al. (1966) have shown that dark meat from poultry contains more phospholipids than white meat. In contrast, Katz et al. (1966) have reported that chicken dark meat contains only about half as much phospholipids as white meat. Campbell and Turkki (1967) and Fooladi (1977) have shown that the phospholipid concentration is higher in cooked meat than in raw meat, regardless of whether it is expressed as percentage of fat or as percentage of total tissue.

The phospholipid components that have been isolated and identified from most skeletal tissues are somewhat similar (Peng and Dugan, 1965; Braddock and Dugan, 1972; Keller and Kinsella, 1973; Body and Shorland, 1974). The main components of phospholipids are phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), sphingomyelin (SP), phosphatidyl inositol (PI), lysophosphatidyl choline (LPC) and other minor components (Pearson et al., 1977).

Hornstein et al. (1961) and Dugan (1971) have reported the composition of beef phospholipids to be 50-60% PC, 30-40% PE and 10% SP. More recently, Keller and Kinsella

(1973) have reported the composition of beef phospholipids as being 53-58% PC, 23-25%, PE, 5-7% SP, 5-7% PI, 1-4% PS and 1-6% others.

Peng and Dugan (1965) have reported the quantitative amounts of phospholipids present in chicken dark meat and white meat. In the dark meat, they found 52-58% PC, 24-30% PE, 7-9% PS and 3-4% SP. On the other hand, the levels of the corresponding components in the white meat were 58-62%, 15-16%, 9-10% and 2-4%, respectively. The data presented above indicate non-uniformity in the values for phospholipid components reported by different workers for the same tissues. Perhaps the reason for this paradox is the fact that phospholipids are labile and difficult to handle (Younathan and Watts, 1960).

Fatty Acid Composition of the Phospholipids

Phospholipids from animal sources contain fatty acids mostly with a chain length between 16 and 20, in which palmitic, stearic, oleic, linoleic and arachidonic acids are predominant (Gurr and James, 1971). The most common arrangement for phosphoglycerides is for the saturated fatty acids to be located at the α-position and unsaturated fatty acids at the β-position (Peng and Dugan, 1965). Irrespective of species, the phospholipids are characterized by their high levels of polyunsaturated fatty acids (Hornstein *et al.*, 1961). The phospholipids from a

particular tissue are appreciably more unsaturated than triglycerides from the same source (Lea, 1957). However, Braddock and Dugan (1972) concluded that differences in fatty acid composition of the triglyceride and phospholipid fractions from fish are negligible.

The components of phospholipids tend to have a characteristic fatty acid composition (Hornstein et al., 1961; Peng and Dugan, 1965; Body and Shorland, 1974). PE has a higher proportion of unsaturated fatty acids than PC (Hornstein et al., 1961; Keller and Kinsella, 1973). Body and Shorland (1974) reported a higher content of polyunsaturated fatty acids (PUFAS) in PE (17-43%) as compared to PC (7-25%) and SP (1-4%). However, the proportion of PUFAS reported for PE and PC between and within muscle tissues seems to vary a great deal.

According to Body and Shorland (1974), the fatty acid profile of a given phospholipid component varies with the feeding regime of the animal and the conditions of analysis. In spite of the variable results reported for the fatty acid composition of the phospholipids, the levels of stearic and arachidonic acids seem to be markedly higher in PE than in any other component (Hornstein et al., 1961; Keller and Kinsella, 1973). Phospholipids from both chicken dark and white meat have a similar degree of unsaturation (Peng and Dugan, 1965; Katz et al., 1966).

The Role of Lipids in the Development of WOF

Hornstein (1967) concluded that fat influences meat flavor through autoxidative degradation of the unsaturated fatty acids. He then stated that the resulting carbonyl compounds may contribute desirable or undesirable flavors, depending on their concentration. The development of WOF in cooked meat is generally accepted to be the result of autoxidation of tissue lipids (Younathan and Watts, 1960; Ruenger *et al.*, 1978). Among tissue lipids, the phospholipids have been implicated as the lipid component most readily susceptible to oxidation in cooked meat (Younathan and Watts, 1960) or in freeze-dried beef (El-Gharbawi and Dugan, 1965; Chipault and Hawkins, 1971).

The phospholipids are more complex than the neutral lipids and tend to oxidize very rapidly, at least partially due to their high content of PUFAS, which are very labile (Lea, 1957). Oxidation of tissue lipids seems to occur in two stages, that is, the phospholipids are oxidized first and the neutral lipids later (El-Gharbawi and Dugan, 1965; Chipault and Hawkins, 1971). Hornstein *et al.* (1961) concluded that phospholipids do not contribute to desirable meat flavor, especially in lean meat.

Corliss and Dugan (1970) and Tsai and Smith (1971) reported that the nature of the nitrogenous components bound in ester linkage to the phosphoric acid moiety may influence the oxidation of the unsaturated fatty acids in

the phospholipid molecule. Tsai and Smith (1971) studied the effects of phosphorylated and non-phosphorylated bases, such as ethanolamine, choline and serine, on the oxidation of methyl linoleate in an aqueous system. Only the phosphorylated and non-phosphorylated bases of ethanolamine exerted a pro-oxidant effect. Corliss and Dugan (1970) also reported that the ethanolamine moiety exerted a greater pro-oxidant effect than the choline portion.

Recently, Igene and Pearson (1978) have provided convincing evidence that total phospholipids are principally responsible for the development of WOF in cooked beef and poultry. The triglycerides are much less susceptible to oxidation than the phospholipids (Younathan and Watts, 1960; Love and Pearson, 1971). Hence, the triglycerides appear to exert only a minor influence on development of WOF (Igene and Pearson, 1978).

Role of Lipids on Storage Stability of Frozen Meats

The storage stability of frozen meats, just like cooked meats, depends essentially on the composition of their lipid constituents and more especially on the degree of unsaturation (Watts, 1954; Greene, 1969; Igene *et al*., 1976). Thus, mutton, beef, pork, poultry and fish can be arranged in order of decreasing stability, reflecting their degree of increasing lipid unsaturation (Wilson *et al*., 1976). It is often assumed that tissue lipids are quite

stable in frozen storage (Kimoto _et al._, 1974). Some research workers (Sulzbacher and Gaddis, 1968; Bratzler _et al._, 1977) have concluded that autoxidation of the triglycerides, principally in the adipose tissue, is responsible for the development of rancidity of raw frozen meats. This view is contrary to that held by others (Cadwell _et al._, 1960; Watts, 1962; Greene, 1969), who have concluded that oxidative changes in tissue lipids are primarily due to autoxidation of the phospholipids.

Microbial growth does not occur in meat stored below -9°C (Kimoto _et al._, 1974). However, lipolytic degradation of the phospholipids during freezer storage has been attributed as the cause of rancidity in beef (Awad _et al._, 1968), poultry (Davidkova and Khan, 1967) and fish (Braddock and Dugan, 1972).

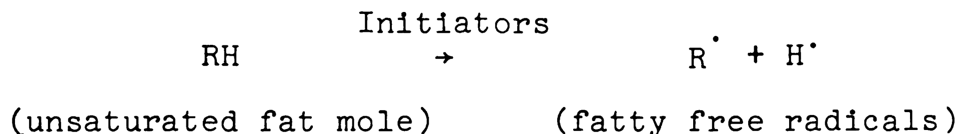
Mechanism of Lipid Oxidation

Autoxidation (uptake of oxygen) of food lipids is promoted by heat, light and trace metal catalysts, especially copper and iron (Ingold, 1967; Waters, 1971). The rate and degree of autoxidative degradation of lipids is directly related to the amount of unsaturation (Love and Pearson, 1971). Dugan (1961) and Lundberg (1962) have reviewed the mechanisms involved in autocatalytic oxidation of lipids. The free radical chain theory of autoxidation (Farmer and Sutton, 1943; Bolland and Koch, 1945)

has been widely accepted as the mechanism of oxidation in non-conjugated unsaturated fatty acids, which form the great bulk of food fats of both animal and vegetable origin (Lea, 1953).

According to the autocatalytic theory as explained by Dugan (1961), oxidation takes place at a reactive methylene group adjacent to a double bond. This results in production of a hydroperoxide (ROOH) which still retains the original degree of unsaturation. At the same time, a resonance system set up by the free radical leads to the production of conjugated isomers. The hydroperoxides may then decompose to yield more free radicals, which can initiate new reaction chains. The steps involved are illustrated in the modified scheme taken from Sato and Herring (1973) and are shown below:

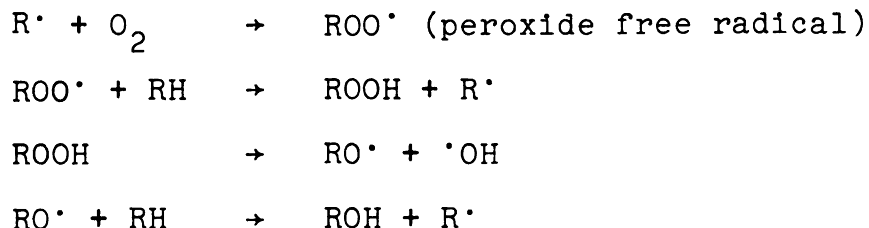
I. Initiation:



$$RH \xrightarrow{\text{Initiators}} R^{\cdot} + H^{\cdot}$$

(unsaturated fat mole) (fatty free radicals)

II. Propagation:



$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$ (peroxide free radical)

$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$

$ROOH \rightarrow RO^{\cdot} + {}^{\cdot}OH$

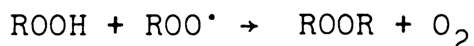
$RO^{\cdot} + RH \rightarrow ROH + R^{\cdot}$

According to Sato and Herring (1973), the free radicals formed can combine with atmospheric oxygen to form peroxide free radicals, which can then react with the substrate to form more free radicals that then propagate the reaction. They theorized that termination occurs as shown below:

III. Termination:

$R^{\cdot} + R^{\cdot} \rightarrow R{-}R$

$R^{\cdot} + ROO^{\cdot} \rightarrow ROOR$

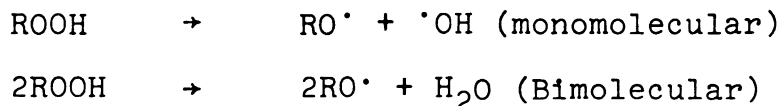


$ROOH + ROO^{\cdot} \rightarrow ROOR + O_2$

They concluded that these reactions lead to the formation of inactive stable non-reactive end products.

Products of Lipid Oxidation

The hydroperoxides are the primary products of the reaction of oxygen with unsaturated lipids (Sato and Hegarty, 1973). The secondary products of lipid oxidation (alcohols, ketones, acids, lactones, etc.) are believed to be formed largely through the decomposition of the hydroperoxides (Herz and Chang, 1970). The mechanism of further breakdown of the hydroperoxides has been reported to be monomolecular as well as bimolecular (Mabrouk and Dugan, 1960; Lundberg, 1962) as shown below:



$$ROOH \rightarrow RO^{\cdot} + {}^{\cdot}OH \text{ (monomolecular)}$$

$$2ROOH \rightarrow 2RO^{\cdot} + H_2O \text{ (Bimolecular)}$$

The catalytic and thermal decomposition of the hydroperoxides is of great importance to flavor in general and to WOF in particular. The most numerous members of any class of compounds identified in meat flavor concentrates are the carbonyls (Herz and Chang, 1970). Although meat flavor *per se* resides in the water soluble meat extract (Kramlich and Pearson, 1958), Hornstein and Crowe (1960, 1963) have suggested that the characteristic species flavor is due to the carbonyls. Although there are both water and fat soluble carbonyls, Sanderson *et al*. (1966) reported that those involved in meat flavor are primarily lipid soluble.

Hexanal has been reported to be a product of lipid oxidation of linoleate (Gaddis *et al*., 1961). El-Gharbawi and Dugan (1965) found the concentration of hexanal to increase during the storage of freeze-dried beef, while Cross and Ziegler (1965) demonstrated that hexanal occurred in greater quantities in uncured than in cured ham. Love and Pearson (1976) also reported that hexanal is one of the principal products associated with lipid oxidation and is implicated as a component of WOF. Recently, Ruenger *et al*. (1978) reported that heptanal and n-nona-3-6-dienal were related to the development of WOF in turkey meat. The

identification of these undesirable flavor compounds, which are typical end products of lipid oxidation, further supports the fact that WOF is due to lipid oxidation.

Catalysts of Lipid Oxidation

Robinson (1924) first implicated the porphyrins (hemoglobins, myoglobin and cytochromes) as the catalysts of lipid oxidation in meats, and attributed the catalysis to their iron content. It is generally accepted that hemoglobin and other iron porphyrins accelerate lipid oxidation, with the hemoproteins being implicated as the major pro-oxidants in meat products (Tappel, 1952; Younathan and Watts, 1958). Watts (1954) indicated that the reaction between lipid and hemoproteins brings about destruction of the pigment as well as oxidation of the fat. Banks (1944) suggested that the active catalyst results from a combination of a fatty peroxide with an iron porphyrin. Maier and Tappel (1959) proposed that catalytically active hemes form unstable compounds with fat peroxides, which then decompose to give two free radicals, each of which is capable of initiating an oxidative chain.

Although it has been suggested that lipid and myglobin oxidation are intimately related (Watts, 1954), it has yet to be conclusively demonstrated whether the oxidation of the lipid occurs first and causes the oxidation of the

pigment or vice-versa. Tappel (1955) showed that hematin compounds catalyze the oxidation of unsaturated fatty acids, and that the catalytic activity is dependent on the presence of iron. Younathan and Watts (1959) also reported the catalytic activity of myoglobin in tissue oxidation. They found that uncured cooked meat, containing ferric globin hemochromogen showed greater oxidation shortly after cooking. They concluded that it was the ferric form of the pigment, which is the active catalyst in tissue rancidity.

According to Labuza (1971), the rapid rate of oxidation in cooked meat may be due to the denaturation of myoglobin during the cooking process. He suggested that unfolding of the protein allows greater exposure and access of the iron to the previously formed peroxide. Haurowitz *et al.* (1941) reported that the pro-oxidant effect of hemin or hemoglobin on linoleic or linolenic acid was due to the destruction of the pigment and the subsequent release of inorganic iron.

Non-heme iron and other heavy transition metals have been reported to function as catalysts of lipid oxidation in cooked meats (Kwoh, 1970). The mechanism of metal catalysis was reviewed by Ingold (1967) and Waters (1971). Wills (1966) showed that both non-heme iron and hemoproteins are involved as catalysts in lipid peroxide formation. Sato and Hegarty (1971), Kwoh (1970) and Love and Pearson (1974) have presented data showing that non-heme

iron is the major pro-oxidant in cooked meats. They concluded that meat pigments *per se* have no catalytic effects on lipid oxidation in cooked meats.

Hirano and Olcott (1971) and Kendrick and Watts (1969) have demonstrated that heme compounds may act as either accelerators or inhibitors of lipid oxidation, their action depending on the ratio of heme to unsaturated fatty acids. There is now strong evidence that ferrous iron is a more active catalyst of lipid oxidation than ferric iron (Smith and Dunkley, 1962; Waters, 1971; Love and Pearson, 1974).

Influence of Grinding and Cooking

It has been postulated that any process causing disruption of the muscle membrane system, such as grinding or cooking, results in exposure of the labile lipid components to oxygen and thus accelerates development of oxidative rancidity (Pearson *et al*., 1977). Sato and Hegarty (1971) have reported a very rapid increase in TBA values, and hence of WOF, for raw meats one hour after grinding and exposure to air at room temperature. They suggested that any catalysts of lipid oxidation present in the muscle system are brought into contact with the oxidation-susceptible lipids and contribute to the rapid development of WOF.

It is well known that heating accelerates the development of oxidized flavor (rancidity) in meat and meat

products (Younathan and Watts, 1959; 1960; Keller and Kinsella 1973; Wilson et al., 1976; Fooladi, 1977). The rapid oxidation of lipids in cooked meat has been attributed to the conversion of ferrous iron of the porphyrin to the ferric form during heating (Younathan and Watts, 1959). According to Yamauchi (1972 a,b) as quoted by Pearson et al. (1977), the development of rancidity is most rapid in meat that is heated at 70°C for one hour. He demonstrated that the TBA value of cooked meat decreased as the cooking temperature was increased above 80°C. Recently, Huang and Greene (1978), confirmed that meat subjected to high temperatures and or long periods of heating developed lower TBA numbers than similar samples subjected to lower temperatures for shorter periods of time. They postulated that antioxidant substances produced during the browning reaction exert TBA retarding activity; and which progresses as the meat is heated. Similarly, Zipser and Watts (1961) and Sato and Herring (1973) have presented evidence for antioxidant activity in retorted beef. They attributed the decreased amount of oxidation to development of the browning reaction, which takes place during the heating process. According to Hamm (1966), the Maillard reaction in meats begins at about 90°C and increases with further increases in temperatures and heating times.

Control of Lipid Oxidation

By using various chemical compounds, such as antioxidants and chelating agents as well as by exclusion of oxygen, it is possible to control lipid oxidation in cooked meats with some degree of success (Sato and Herring, 1973). Antioxidants may interfere with or delay the onset of oxidative breakdown in fats and fatty foods (Blanck, 1955). According to Shelton (1959), primary or phenolic antioxidants, which include the tocopherols, butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) in combination with or without chelating or synergistic agents, may break the oxidative reaction chains. Uri (1961) has reviewed the mechanism of antioxidants in foods. It is necessary to ensure that the antioxidants used in meat and meat products are not harmful to the consumer (Sato and Herring, 1973).

There is evidence that potent natural antioxidants (the tocopherols) are present in meats (Igene _et al_., 1976), and that antioxidant substances can be produced in meats by high temperature treatment (Pearson _et al_., 1977). Zipser and Watts (1958) and Huang and Greene (1978) have found that production of antioxidant substances by prolonged heat treatment can stabilize uncured canned meats against oxidative rancidity. The use of extracts of soybean and soy products, which contain natural antioxidant substances (flavonoids), has been found to retard oxidation

in sliced cooked beef (Watts, 1962; Pratt, 1972). The inhibition of lipid autoxidation by adding nitrite, phosphate, ascorbate and other curing ingredients has been reviewed by Pearson et al. (1977). Sato and Hegarty (1971) showed that nitrite will completely eliminate WOF at 220 ppm and will inhibit development at 50 ppm. Recently, Fooladi (1977) and Igene et al. (1978) have demonstrated that 156 ppm of nitrite effectively prevents WOF in cooked meat and poultry. Zipser et al. (1964) proposed that nitrite forms a stable complex with iron porphyrins in heat denatured meat, thereby inhibiting the development of WOF.

EXPERIMENTAL

Materials

Solvents and Chemicals

All chemicals and solvents utilized in this investigation were of reagent grade. All solvents were freshly redistilled before use unless otherwise specified.

Methyl Ester Mixtures

Standard mixtures for the determination of relative retention times by GLC were obtained from Applied Science Lab; (State College, PA) and Supelco, Inc. (Bellefonte, PA). The standard mixtures contained a wide range of fatty acids, starting with C_{10} through $C_{22:6}$ and included $C_{24:0}$.

TLC Standard Mixture

Polar lipid mixtures for TLC containing cholesterol, phosphatidyl ethanolamine, lecithin and lysolecithin were obtained from Supelco Inc. In addition, pure bovine lecithin, phosphatidyl ethanolamine and a serum lipid mixture containing specified amounts of various phospholipid components were also obtained from Supelco Inc.

Thin-Layer Plates

Precoated 20 x 20 cm silica gel G thin layer plates (0.5 mm thick) were obtained from Fisher Scientific Co. (St. Louis, MO) and used for all TLC separations.

Gas-liquid Chromatography Column Packings

Acid-washed and salinized (80/100 mesh) supelcoport coated with 10% diethylene glycol sucinate (DEGS) was obtained from Supelco Inc. and used for the GLC separations.

Source of Meat

Beef, chicken dark meat and chicken white meat were used in these studies. The beef and chicken (old layers) were obtained from the Michigan State University Meat and Poultry Processing Laboratories. Portions of longissimus dorsi (LD) muscle were exercised from beef carcasses at 24 hrs postmortem. Thigh (dark meat) and breast (white meat) meat were removed from the chicken carcasses at 24 hrs postmortem.

Methods

Meat for Evaluating the Effect of Frozen Storage on Lipids and Their Role in the Development of Oxidized Flavor

In order to establish a basis for changes during frozen storage, fresh bone-in samples of beef (LD) muscle were cut into identical thicknesses (2 inch) and sizes (2 lb lots), wrapped with freezer paper, numbered and stored at -18°C. In the same manner, fresh cuts of unskinned thighs and breast meat from chicken carcasses were randomly wrapped in packages of 4 lb each and stored at -18°C. The packaged meat was held at -18°C for 13 months. At designated storage periods, samples were removed from the freezer and held at 4°C for 18-24 hrs to allow thawing, after which the meat was tested for both rancidity and WOF.

Preparation of Meat for Rancidity or WOF Evaluation

External fat was trimmed from the beef while any adhering skin was removed from the chicken samples. The meat was then cut into pieces after removing the bones and ground twice through a 3/16 inch plate (except where otherwise specified) using a Hobart meat grinder.

For assessment of rancidity, portions of the ground meat were removed for TBA evaluation and lipid analysis.

For evaluation of WOF, about 400 g of meat were packed into each of two retortable pouches and heat sealed. The bags were cooked in boiling water to an internal temperature of 70°C. Immediately after cooking, the bags were opened, the drippings collected and the meat thoroughly mixed. Prior to storing the cooked meat at 4°C or -18°C, the TBA values of the freshly cooked meat were measured. Then equal amounts of the cooked meat were held at 4°C and at -18°C for 48 hrs in unsealed retortable pouches. At the end of 48 hrs, the TBA values as well as comprehensive lipid analyses of the cooked meat were determined.

Methods of Analyses

TBA Test

The distillation method of Tarladgis *et al*. (1960) was utilized to measure the development of oxidative rancidity by the TBA test. However, a modification in the distillation procedure was made for nitrite treated samples. Hougham and Watts (1958), Younathan and Watts (1959) and Zipser and Watts (1962) have shown that nitrite interferes with the distillation step by nitrosation of malonaldehyde. Hence the modified TBA test of Zipser and Watts (1962), in which sulfanilamide is added to the sample, was utilized for all preparations containing nitrite. TBA numbers were expressed as mg malonaldehyde/kg meat.

Taste Panel Evaluation of WOF in Cooked Meat

Sensory evaluation was carried out by trained panelists after 48 hrs storage of the cooked meat at 4°C. At each setting, all panelists were presented with four different coded samples representing different treatments. A control sample consisting of freshly cooked meat was also presented along with the treated samples. All experimental samples were reheated and served while hot. The panel scoring system was as follows: 1 = very pronounced WOF; 2 = pronounced WOF; 3 = moderate WOF; 4 = slight WOF; and 5 = no WOF.

Extraction of Lipid from Muscle Tissue

Total lipid was extracted from fresh or cooked meat including the experimental treatments involving meat model systems by the procedure of Folch *et al*. (1957). This procedure involves the use of chloroform-methanol (2:1) by homogenizing the tissues several times in a Waring blender followed by filtration. The combined filtrate was transferred to a separatory funnel to allow the chloroform and aqueous layer to separate. The chloroform layer was evaporated to a constant weight of lipid under reduced pressure at 30-40°C using a Rotavapor-R (Büchi, Switzerland).

Separation of the total lipids into triglycerides and phospholipids was achieved by the method of Choudhury *et al*.

(1960). This method involves the separation of total lipids on activated silicic acid in which neutral lipids are preferentially removed by washing with chloroform. The phospholipids combine with the activated silicic acid and are solubilized and extracted with methanol. The extracted total lipids, triglycerides and phospholipids in raw and cooked meat were expressed as the percent of fresh or cooked tissue.

Drippings from Cooked Meat

Following cooking, the drippings were collected, cooled and the volume determined using a graduated cylinder. Then the lipid present in the drippings was extracted using the procedure of Folch *et al*. (1957). The levels of triglycerides and phospholipids were determined by the method of Choudhury *et al*. (1960). The amount of lipids present in the drippings were expressed as percentage of original tissue.

Thin-Layer Chromatography (TLC)

Separation of Phospholipid Components - The total phospholipids extracted from fresh or cooked meat were separated into their components using preparative TLC. Exactly 50 mg/ml of total phospholipids (equivalent to 0.475 mg phospholipid/spot) were spotted on each plate under a stream

of nitrogen gas using a Hamilton microsyringe equipped with a Chaney adaptor. A standard mixture of authentic phospholipids (Supelco, Inc.) was simultaneously spotted on the left hand side of the plate. The plates were developed in chloroform: methanol:water (65:25:4, v/v). After the plates were dried in a stream of nitrogen gas, 50 mg/ml of the same sample representing total phospholipids (for determination of total phosphorus) were spotted on the right hand side of the plate for determination of phosphorus. The spots were identified by spraying the plates with potassium dichromate/sulfuric acid and charring for 10-15 minutes at 175°C using a forced air oven.

Quantitation of Phospholipids (Phosphorus Determination) - After the TLC separation of lysolecithin (LPC), sphingomyelin (SP), phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) from the total phospholipids (including the spots representing total phospholipids), they were scraped from the TLC plates into 30 ml micro-Kjeldahl flasks for digestion with perchloric acid as described by Rouser *et al*. (1966). A standard curve was prepared using aliquots of monobasic potassium phosphate solution containing 1-10 μg of phosphorus. Absorbance of 820 nm was multiplied by a factor of 11.0 (after correcting for the blank), which was calculated from a standard curve to convert the readings to μg of phosphorus. The values for

phosphorus in µg were subsequently converted to mg phosphorus/g phospholipid, and expressed as such in this study.

Purification of PC and PE - Plates for the determination of fatty acids in PC and PE were prepared as described earlier herein except they were sprayed with iodine vapors. Spots containing PC and PE were immediately recovered and eluted with chloroform-methanol (4:1, v/v), evaporated to dryness under nitrogen gas and redissolved in chloroform. Before converting them to methyl esters, PC and PE were checked for purity by TLC.

Preparation of Methyl Esters - Total lipids, triglycerides, total phospholipids, PC and PE from all meat samples used for fatty acid analysis were converted to methyl esters by the Boron-trifluoride/methanol procedure as described by Morrison and Smith (1964).

Gas-Liquid Chromatography (GLC)

GLC analysis of all fatty acid methyl esters was performed using a Perkin-Elmer model 900 gas chromatograph equipped with a hydrogen flame ionization detector (FID). The column, 6 ft x 2 mm (i.d.) stainless steel, was packed with 10% (w/w) diethylene glycol succinate (DEGS) on supelcoport (Supelco, Inc.). The column was set at 185°C,

the injection port at 220°C and the detector at 250°C. The carrier gas was helium and the flow rate was maintained at 30-40 ml/min while hydrogen gas and air were adjusted to 30 and 285 ml/min, respectively.

Quantitative identification of the emerging peaks was done using retention times of standard mixtures of known fatty acid methyl esters (Applied Science Lab., Inc., State College, PA; Sulpelco, Inc.). Peak areas were calculated quantitatively as the product of peak height and width at half height. Results were expressed as percent of the total area.

Statistical Treatment

Statistical analysis was calculated using the STAT SERIES developed by the Michigan State University Agricultural Experimental Station and was raw on control Data Corporation (CDC) 6500 computer. Alternately, some statistical analyses were carried out using a Wang programmable computer. Analysis of variance for TBA values, taste panel scores and phosphorus changes were also computed. Standard deviations, correlation and regression coefficients were also calculated. The significance of the computed correlation coefficients was determined by the "r" distribution table from Snedecor and Cochran (1973). The significance between treatments was determined using either Tukey's test for multiple comparisons or the student "t" test for differences

between two means. Graphs were plotted using SPSS (Statistical Package for the Social Science) version 7.0 (MSU, March 18, 1978).

Experimental Systems

Four broad groups of experiments were conducted to ascertain the role of meat lipids and meat pigments on the development of rancidity and WOF in frozen and in cooked meat, respectively.

Experiment A

The effects of type of lipids on the development of rancidity in model meat systems during frozen storage at -18°C were determined.

Experiment B

The effects of type of lipids on the development of warmed-over flavor (WOF) were measured in cooked model meat systems.

Experiment C

The effect of length of frozen storage in relation to its effect on the stability of meat lipids as well as on the production of off-flavors was determined.

Experiment D

The role of meat pigments, nitrite, heme and non-heme-iron on development of WOF was assessed.

Preparation of Model Meat System

For Experiments A and B, a model meat system was developed to study the relative contributions of triglycerides, total lipids and phospholipids to the development of rancidity during freezer storage and of WOF following cooking and storage at 4°C.

Lipid-free muscle fibers were used as the matrix for the model system. Fresh raw beef, chicken dark meat or white meat were each ground once through a 3/16 inch plate using a Hobart meat grinder. Then total lipids contained in the ground meat were extracted by the method of Folch *et al.* (1957). The phospholipids were separated from total lipids using the procedure of Choudbury *et al.* (1960).

The solvent was removed from the residue (protein matrix) by drying under vacuum and later in a stream of nitrogen gas at room temperature. Following removal of the solvent, the model systems were either used immediately or packed in cryovac bags, frozen and stored at -18°C. The preparation of the model system is outlined schematically in Figure 2.

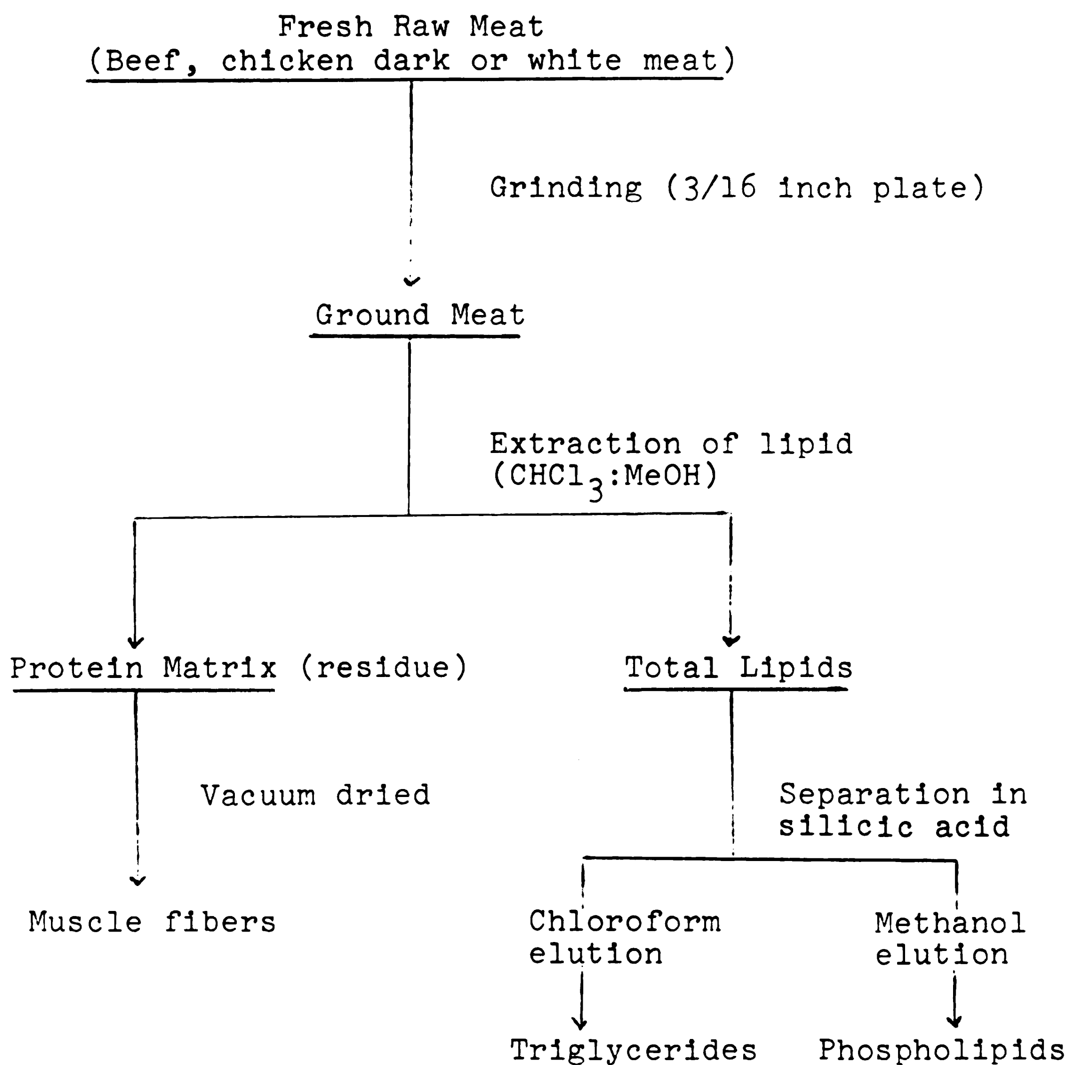


Figure 2. Preparation of model meat system.

Design of Experimental Treatments Involving Model Systems

Experiments were conducted to test the influence of triglycerides, total lipids and total phospholipids on the development of rancidity during freezer storage. In addition, the development of WOF was studied using the same experimental treatments. The design of the treatments is shown in Table 1.

The levels of total lipids, triglycerides and phospholipids added back to the model systems closely corresponded to that removed during the extraction process. Total phospholipids were used in the beef and chicken meat model systems at levels of 0.8 and 0.7%, respectively. Total lipids and triglycerides were added to the beef model system at levels of 10.0 and 9.2%, respectively. On the other hand, total lipids and triglycerides from chicken dark meat were added to the model system at concentrations of 5.0 and 4.3%, respectively. Total lipids and triglycerides were also added to the chicken white meat model system at concentrations of 5.0 and 4.3%.

Experiment A

The primary objectives of Experiment A were to study the influence of triglycerides, total lipids and total phospholipids on the development of rancidity during frozen storage of meat model systems. The experimental model

Table 1. [a]Design of Model Meat Experimental Treatments.

Meat Source	Code Number	Composition of Model Meat Systems
Beef	B_1	0.8% beef phospholipids in beef muscle fibers
	B_2	9.2% beef triglycerides in beef muscle fibers
	B_3	10% beef total lipids in beef muscle fibers
	B_4	Beef muscle fibers only (control)
*Chicken dark meat	D_1	0.7% dark meat phospholipids in dark meat muscle fibers
	D_2	4.3% dark meat triglycerides in dark meat muscle fibers
	D_3	5.0% dark meat total lipids in dark meat muscle fibers
	D_4	Dark meat muscle fibers only (control)
*Chicken white meat	W_1	0.7% white meat phospholipids in white meat muscle fibers
	W_2	4.3% white meat triglycerides in white meat muscle fibers
	W_3	5.0% white meat total lipids in white meat muscle fibers
	W_4	White meat muscle fibers only (Control)

[a]Muscle fibers without added lipids served as controls. Each experiment was replicated 4 times.

*The same amount of lipids were applied to chicken dark meat and white meat fibers in order to eliminate variations due to lipid levels.

systems (Table 1) were prepared, stored in polyethylene bags and were held at -18°C for 8 months. Prior to frozen storage, the initial TBA values of the model systems were determined. At the same time, the composition of the fresh lipids in the model systems were also measured. TBA values of the frozen model systems were also determined at 1, 4 and 8 months of storage. At the end of 8 months storage, fatty acid analysis of the triglycerides and total phospholipids, including that of PC and PE, were carried out. The composition of the phospholipid components was also determined at the end of 8 months storage.

Experiment B

Experiment B was designed to test the influence of triglycerides, phospholipids and total lipids on the development of WOF in the cooked meat model systems. Thus, the meat model systems, including the control samples (Table 1), were packed in 6-1/4 inch x 8-1/2 inch retortable pouches (Continental Diversified Industries, Chicago, IL) and heat sealed with a Multi-Vac sealing machine (Busch-W. Germany). The bags containing the treated samples were cooked to an internal temperature of 70°C in boiling water.

Following cooking, the bags were opened, and the contents thoroughly mixed. The meat was then stored at 4°C for 48 hours. TBA values, taste panel scores and lipid composition of the different model systems were then

determined. A trained panel of 12 individuals consisting of members of faculty and graduate students in the Department of Food Science and Human Nutrition served as judges for taste panel and odor evaluations. The scoring system used was as described earlier under methods of analyses.

Experiment C

The principal objectives of this experiment were to study how length of frozen storage influences the composition and stability of meat lipids as well as its effect on the production of rancidity and WOF.

Fresh cuts of beef, chicken dark and white meat were used for this study and were frozen and stored at -18°C for over one year. Basically, the idea was to measure the content of extractible lipids at 0, 8 and 13 months of frozen storage while at the same time determining the levels of malonaldehyde produced by oxidative rancidity in fresh and/or frozen meat as well as in cooked meat. Prior to frozen storage (0 day), randomly selected samples of fresh beef, chicken dark or white meat were analyzed for their initial TBA numbers in the fresh as well as in the cooked state. At the same time, the concentration of total lipids, triglycerides, total phospholipids, including the component phospholipids (LPC, SP, PE and PC), was determined in both the fresh and cooked meat. In addition, the initial fatty

acid composition of the triglycerides, total phospholipids, PE and PC associated with both fresh and cooked meat were also measured.

At 8 and 13 months of frozen storage, randomly selected packages of frozen meat were also removed from the freezer, thawed and evaluated for TBA numbers as well as for extractible lipids and fatty acid composition before and after cooking. This procedure was adopted to effectively relate variations in lipid composition and stability to the development of rancidity in frozen as well as in cooked meat.

Experiment D

The role of meat pigments, nitrite and non-heme iron on the development of WOF was investigated. The study was divided into two stages. The first stage involved the effects of total meat pigments and nitrite on the development of WOF, while Stage II was designed to compare the relative contributions of heme and non-heme iron on the development of WOF.

In Stage I, lean cuts of fresh beef, chicken dark meat and white meat were used. The meat was cut into pieces, ground first through a 3/8 inch and later through a 3/16 inch plate. Each kind of meat was divided into two groups. Muscle pigments from one group were removed by extraction with deionized water at 4°C for 24 hrs with several volumes of water. Then the meat was filtered through cheese cloth

until it was virtually devoid of pigments. The unextracted group of samples was used as controls.

Four experimental treatments were designed for each type of meat as shown in Table 2. Each experimental treatment consisted of 200 g of meat that was mixed with 100 ml of distilled deionized water. In the nitrite treated samples, the level of nitrite was 156 ppm.

In Stage II, total pigments were removed from 2.0 kg of fresh beef (LD) as described in Stage I. The extracted meat pigments were concentrated in a Stokes freeze-drier. Then the concentrated extract was divided into lots A and B as shown in Figure 3. The extract from lot A was further subdivided into two equal parts. One part was cooked and the other part used as an uncooked control. Half of both the cooked and uncooked extracts was treated with 2.0% EDTA in order to chelate the non-heme iron. Extract B was treated with 30% H_2O_2 to destroy the pigment and release non-heme iron (Wills, 1966; Kwoh, 1970). One part of the H_2O_2 treated extract was again chelated with 2% EDTA. The unchelated extracts served as controls. Each extract was added back to the residue and cooked in retortable pouches. The design of the experimental treatments is shown in Table 3.

Table 2. Design and Formulation of Treatments.

Meat Type	Code Number	Treatments and Formulations
Beef	A	Beef with pigment, no nitrite
	B	Beef with pigment, plus nitrite
	C	Beef without pigment, no nitrite
	D	Beef without pigment plus nitrite
Chicken dark meat	A	Dark meat with pigment, no nitrite
	B	Dark meat with pigment plus nitrite
	C	Dark meat without pigment, no nitrite
	D	Dark meat without pigment plus nitrite
Chicken white meat	A,B,C,D	Same Treatments were used as for dark meat

Nitrite was added at a level of 156 ppm. 200 g of meat was used for each treatment. Samples were cooked and stored at +4°C for 48 hrs. There were 4 replicates for each treatment.

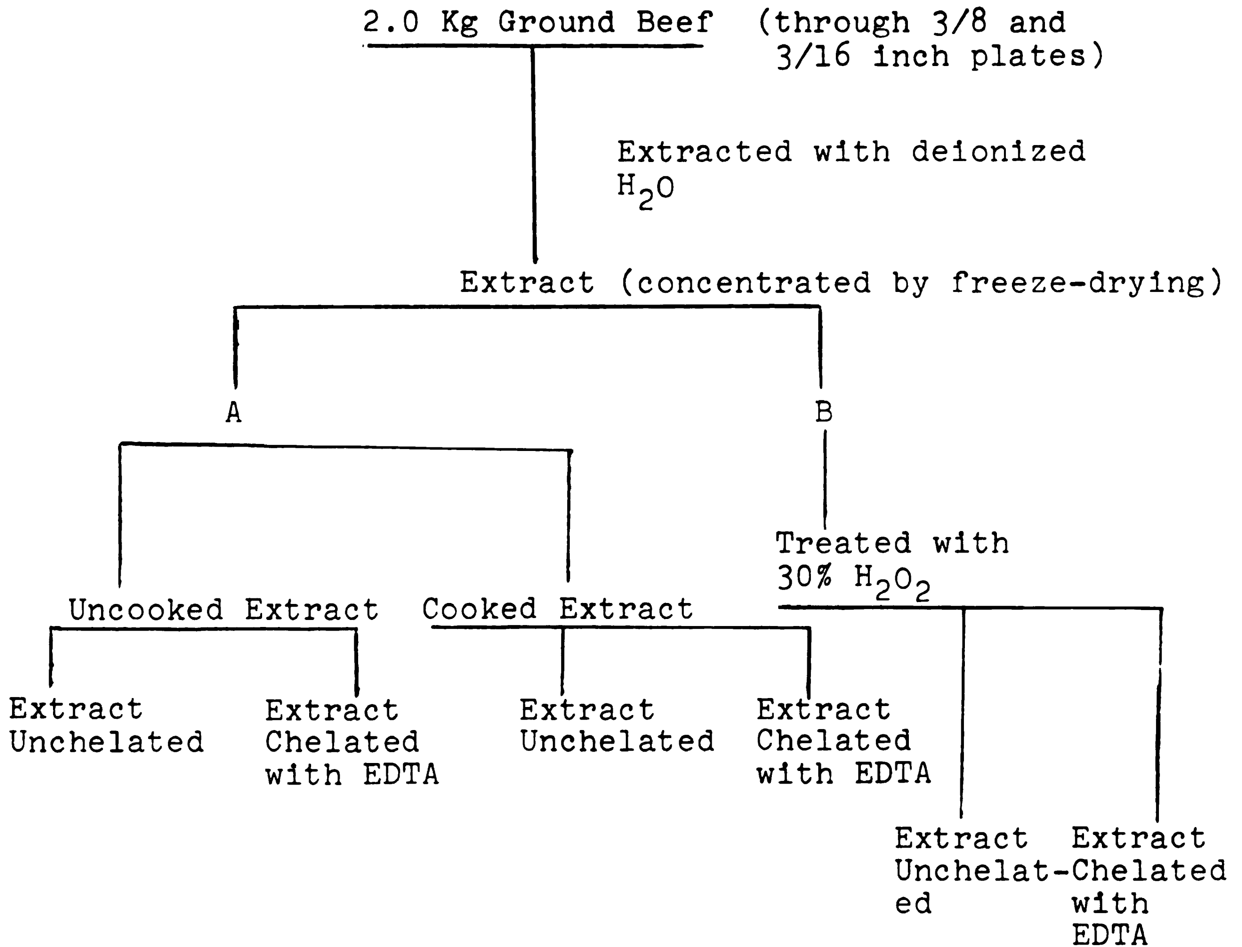


Figure 3. Preparation and design of experiment to compare the effect heme and non-heme-iron on the development of WOF.

Table 3. Experimental Treatments to Compare the Effect of Heme and Non-heme Iron on the Development of WOF.

Treatment #	Preparation of Experimental Treatments
1	Residue + total meat pigment
2	Residue + total meat pigment (chelated*)
3	Residue + total cooked meat pigment
4	Residue + total cooked meat pigment (chelated)
5	Residue + H_2O_2 treated meat pigment
6	Residue + H_2O_2 treated meat pigment (chelated)
7	Residue + deionized water

*Non-heme iron was chelated using 2% EDTA.

Cooking

The treated samples were packed in retortable pouches, cooked and stored at 4°C for 48 hrs as was described for Experiments B and C. TBA numbers and/or taste panel evaluation (Stage I) of the samples were used to determine the extent of lipid oxidation as influenced by either nitrite, total pigment or non-heme iron.

RESULTS AND DISCUSSION

Role of Meat Lipids on the Development of Rancidity

The role of triglycerides, total lipids and total phospholipids on the development of rancidity during frozen storage of meat was studied by adding them back to lipid-free muscle fibers alone and in various combinations. The experimental design of this study is shown in Table 1.

The samples were prepared and packed in polyethylene bags and stored at -18°C for up to 8 months. Prior to storage (0 time), however, the initial TBA values as well as a comprehensive fatty acid analysis of the triglycerides, total phosphilipids and of PC and PE were determined. The samples were tested for development of oxidative rancidity by the TBA test after 1, 4 and 8 months of frozen storage. TBA numbers and a final comprehensive lipid analysis were carried out at the conclusion of 8 months in frozen storage.

Beef Lipids

The TBA values presented in Table 4 show the effects of beef lipids on development of rancidity during frozen storage. In the control samples (containing no added lipids), the TBA numbers were only 0.4, 0.42, 0.89 and 1.05 at 0, 1, 4 and 8 months, respectively. The low TBA values indicate that the control samples, which were composed of

Table 4. The effect of adding beef triglycerides, total phospholipids and total lipids on TBA values of model beef meat systems stored at -18°C.

Time in Frozen Storage (Months)	Experimental Treatments			
	Control (B_4)	Triglycerides (B_2)	Total Phospholipids (B_1)	Total Lipids (B_3)
0	0.40	0.38	0.65	0.66
1	0.42	0.82	13.01	20.84
4	0.89	6.33	15.14	21.52
8	1.05	8.31	15.74	20.30

TBA numbers = mg malonaldehyde/kg meat.

B_1 = 0.8% phospholipids; B_2 = 9.2% triglycerides; B_3 = 10% total lipids; B_4 = muscle fibers only.

the extracted meat fibers, did not undergo any appreciable amount of oxidation. Thus, the validity of using the lipid extracted muscle fibers as the basis of the meat model system was verified. In the samples containing added triglycerides, the initial TBA value at 0 time was 0.38. It then rose slowly to 0.82 after 1 month in frozen storage but increased rapidly to 6.33 and 8.31 after 4 months and 8 months in freezer storage, respectively. The TBA numbers of the triglycerides increased most rapidly between 1 and 4 months of freezer storage and then more slowly between 4 and 8 months. This indicates a slowing down in the rate of lipid oxidation after a rapid acceleration between 1 and 4 months storage.

The TBA values for the samples containing added phospholipids were 0.65, 13.01, 15.14 and 15.74 at 0, 1, 4 and 8 months of freezer storage, respectively. On the other hand, a combination of triglycerides and total phospholipids, which represents the total lipids in the original meat sample, gave TBA values of 0.66, 20.84, 21.52 and 20.38 at 0, 1, 4 and 8 months of freezer storage, respectively. Thus, total phospholipids and total lipids behaved similarly except that the latter gave much higher TBA values, suggesting that the triglycerides further accelerated the rate of oxidation. These results would indicate that the phospholipids make the greatest contribution to rancidity, but the triglycerides also contribute to the development of

rancidity in frozen storage, either alone or in combination with the phospholipids.

The increase in TBA numbers during freezer storage (Table 4) suggest that unlike the triglycerides, total lipids and total phospholipids do not exhibit a noticeable induction period before lipid oxidation. This is consistent with data reported by El-Gharbawi and Dugan (1965) and Chipault and Hawkins (1971). They observed that oxidation of tissue lipids occurred in two stages. The phospholipids are oxidized first and their initial rate of oxidation decreases with time, while after a period of low oxygen absorption, the triglycerides show a rapid rate of oxidation. The trend is shown graphically in Figure 4.

Analysis of variance indicates that differences between treatments, storage periods and interactions between treatments and length of storage were all highly significant ($P < 0.001$). This suggests that triglycerides, total lipids and total phospholipids are all important in the development of oxidized flavor during freezer storage. However, the results of this study clearly indicate that total phospholipids would contribute more to the development of oxidized flavor during frozen storage than the triglycerides. This supports the suggestion of Cadwell _et al_. (1960), Watts (1962), and Greene (1969), who have concluded that oxidative changes during freezer storage are primarily due to autoxidation of the phospholipids.

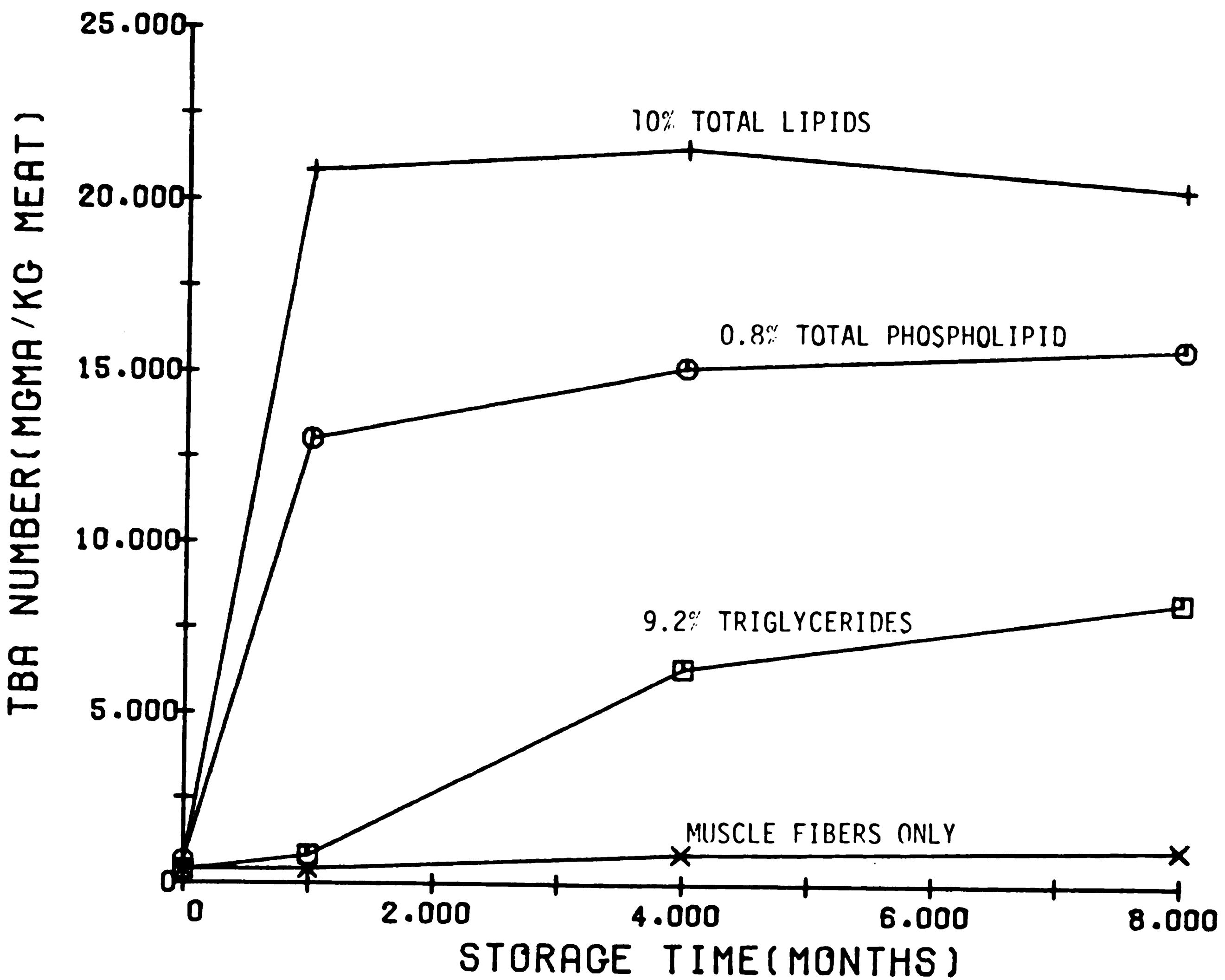


Figure 4. Effect of adding beef triglycerides, total phospholipids and total lipids on TBA values of model beef meat systems stored at -18°C.

The role of beef triglycerides in the development of rancidity may not be important when meat is stored frozen for a short period of time. However, beef triglycerides could play an important role when meat is stored frozen for longer periods. Several researchers (Sulzbacher and Gaddis, 1968; Bratzler et al., 1977) have concluded that autoxidation of the triglycerides, principally in the adipose tissue, is responsible for development of rancidity during frozen storage of meat.

Chicken Dark Meat Lipids

The TBA values for samples containing chicken dark meat triglycerides, total lipids and total phospholipids are presented in Table 5. The control sample (muscle fibers alone) showed consistently greater TBA values than control beef samples, with values of 1.13, 3.08, 3.23 and 3.48 at 0, 1, 4 and 8 months of frozen storage, respectively. The higher TBA values for chicken dark meat indicate that the extracted muscle fibers probably contain some unextracted bound lipids. However, the residual lipids in the extracted fibers were not determined.

The samples containing triglycerides had TBA values of 1.40, 5.52, 10.75 and 10.36 at 0, 1, 4 and 8 months of frozen storage, respectively. Thus, the added triglycerides substantially increased the TBA values, indicating that

Table 5. Effect of adding chicken dark meat tryglycerides, total phospholipids and total lipids on TBA values of chicken dark meat model meat systems stored at -18°C.

Time in Frozen Storage (Months)	Experimental Treatments			
	Control (D_4)	Triglycerides (D_2)	Total Phospholipids (D_1)	Total Lipids (D_3)
0	1.13	1.40	1.63	2.05
1	3.08	5.52	11.52	15.05
4	3.23	10.75	12.20	16.86
8	3.48	10.36	13.33	17.33

TBA Numbers = mg malonaldehyde/kg meat.

D_1 = 0.7% phospholipids; D_2 = 4.3% triglycerides; D_3 = 5% total lipids; D_4 = muscle fibers only.

they contributed considerably to lipid oxidation. Chicken dark meat triglycerides did not exhibit the long induction period that was observed for beef triglycerides. This could be related to the higher degree of unsaturation in chicken triglycerides (Katz et al., 1966).

When total phospholipids were added back to the extracted muscle fibers and frozen, the TBA values were 1.63, 11.52, 12.20 and 13.33 at 0, 1, 4 and 8 months, respectively. In the samples containing total lipids, on the other hand, TBA numbers were 2.05, 15.05, 16.86 and 17.33 at 0, 1, 4 and 8 months of freezer storage, respectively. Thus, total phospholipids and total lipids from chicken dark meat behaved in a similar way to those of beef. Thus, like beef the TBA values for chicken dark meat total lipids were higher than for the total phospholipids alone. Further more, analysis of variance for TBA values show that treatments, length of frozen storage and the interaction of treatment x length of storage were all highly significant ($P < 0.001$).

These results indicate that triglycerides, total lipids and total phospholipids are all important in development of rancidity in chicken dark meat.

The results also demonstrated that triglycerides contribute almost as much to development of rancidity as phospholipids, although the TBA values for total phospholipids were consistently greater than those for triglycerides.

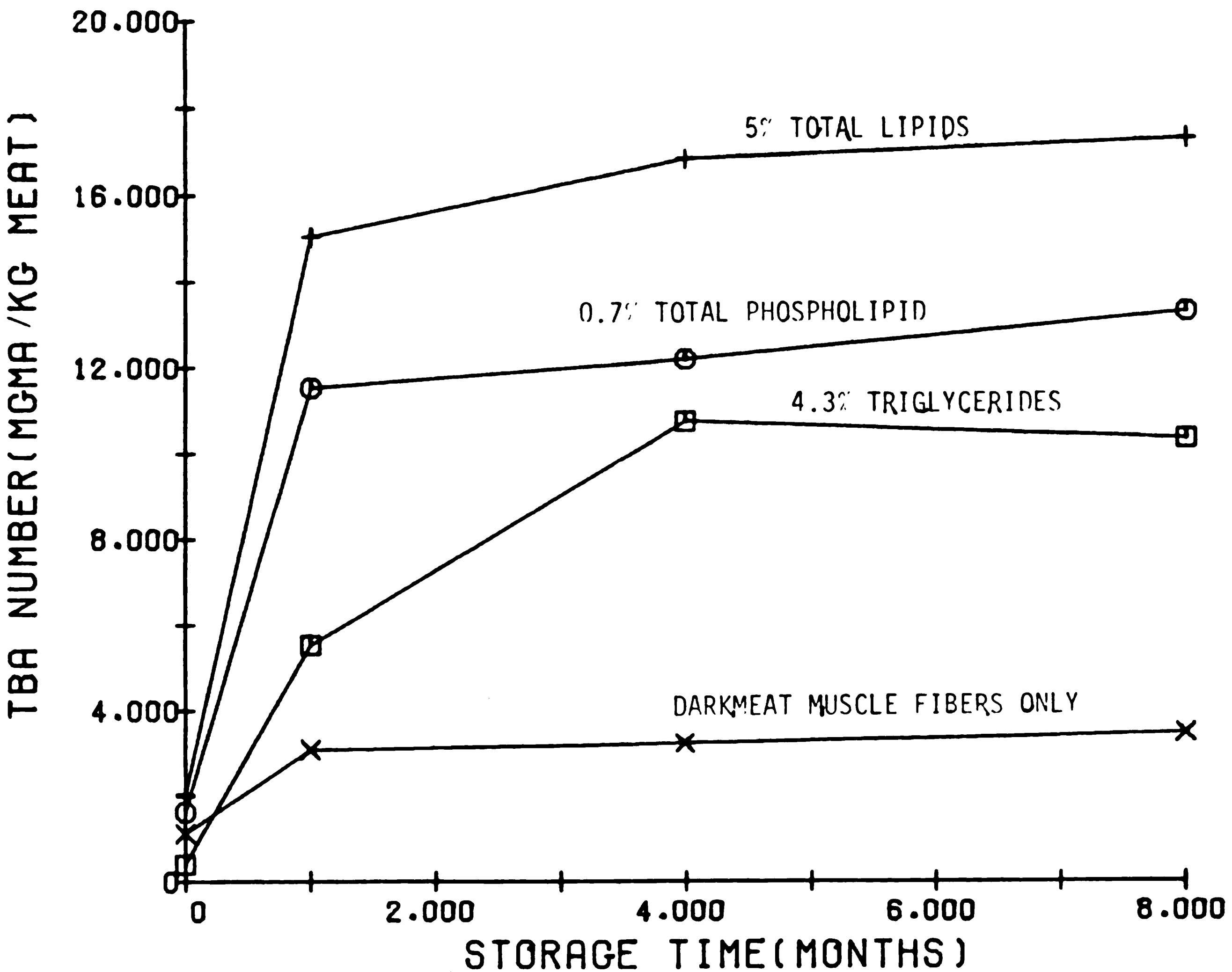


Figure 5. Effect of adding chicken dark meat triglycerides, total phospholipids and total lipids on TBA values of chicken dark meat model meat systems stored at -18°C.

Hornstein et al. (1961) observed that the phospholipid fraction and total lipids from pork and beef become rancid quickly when exposed to air. The triglyceride fraction developed off-flavors less readily, leading them to conclude that phospholipids make the greatest contribution to off-flavor.

Chicken White Meat Lipids

TBA numbers for the chicken white meat model systems containing triglycerides, total lipids and total phospholipids are presented in Table 6. TBA numbers for the control samples were 0.42, 0.57, 1.39 and 1.78 at 0, 1, 4 and 8 months of frozen storage. In the samples containing triglycerides, the TBA values were not significantly different from those of the control, being 0.70, 0.83, 1.30 and 1.46 at 0, 1, 4 and 8 months, respectively. Similarly, the TBA values for samples containing total phospholipids were 0.78, 0.94, 1.98 and 1.95 at 0, 1, 4 and 8 months of freezer storage, respectively. Again the differences in TBA values in comparison to the model system alone were not statistically significant ($P > 0.05$).

Although the TBA values were slightly higher in the samples containing added phospholipids, the values were all low in comparison to those for beef and chicken dark meat. The rapid increase in TBA numbers observed for beef and

Table 6. Effect of adding chicken white meat triglycerides, total phospholipids and total lipids on TBA values of chicken white meat model meat systems stored at -18°C.

Time in Frozen Storage (Months)	Experimental Treatments			
	Control (W_4)	Triglycerides (W_2)	Total Phospholipids (W_1)	Total Lipids (W_3)
0	0.42	0.70	0.78	0.95
1	0.57	0.83	0.94	1.15
4	1.39	1.30	1.98	1.48
8	1.78	1.46	1.95	1.74

W_1 = 0.7% total phospholipids; W_2 = 4.3% triglycerides; W_3 = 5% total phospholipids; W_4 = muscle fibers only.

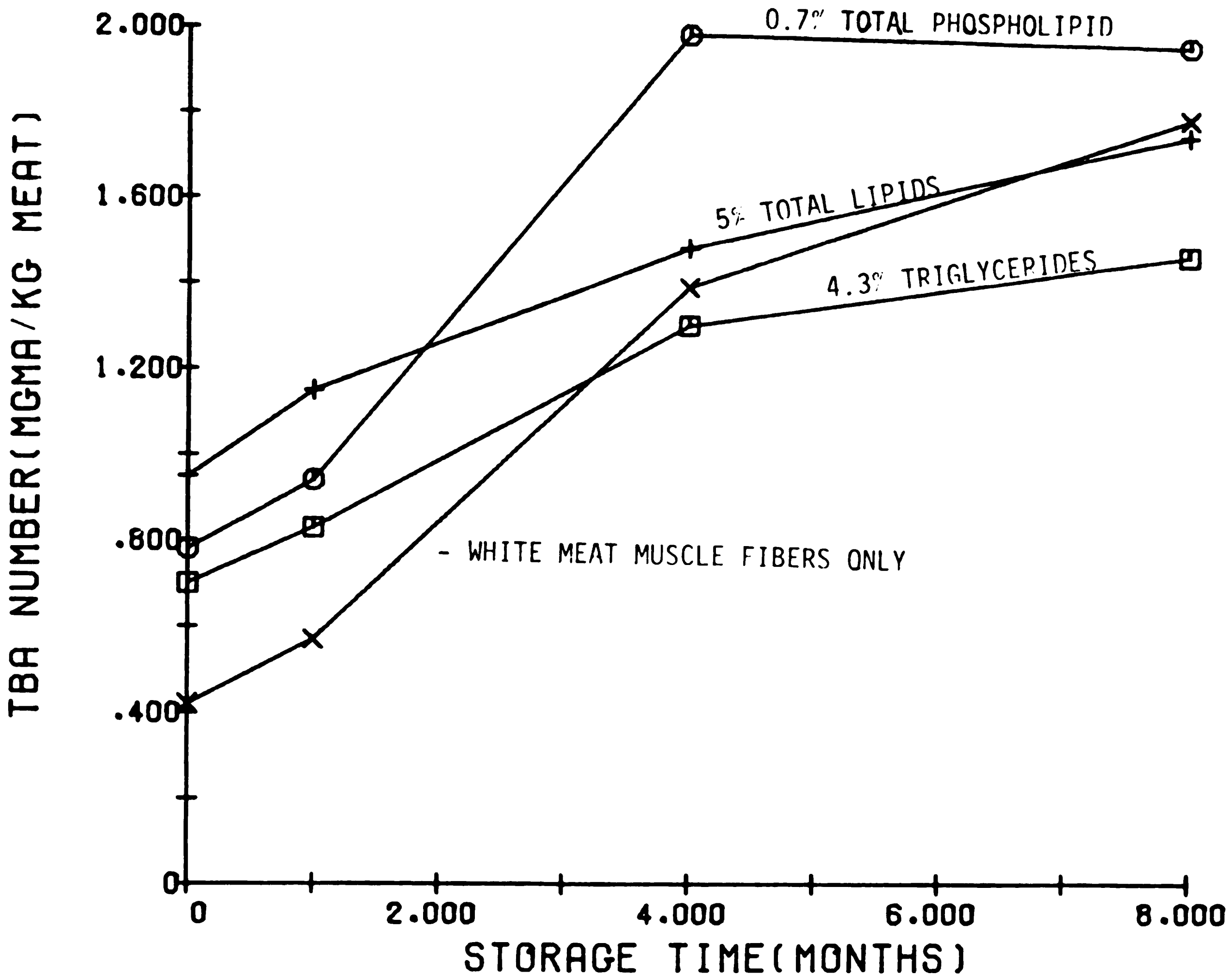


Figure 6. Effect of adding chicken white meat triglycerides, total phospholipids and total lipids on TBA values of chicken white meat model meat systems stored at -18°C.

chicken dark meat lipids were not exhibited by chiekcn white meat lipids. The greater stability exhibited by chicken white meat lipids was unexpected. However, the low TBA values observed in the chicken white meat samples are closely related to their lipid composition. The fatty acid profiles for white meat triglycerides and total phospholipids, including PC and PE will be discussed later herein.

Stability of Phosphilipids During Frozen Storage

The levels of LPC, PE, PC and total phospholipid phosphorus were measured prior to frozen storage (0 time) and again after 8 months in freezer storage, using the phosphorus assay procedure of Rouser *et al*. (1966). Changes in individual and total phospholipids during frozen storage are presented in Table 7 and in Figure 7.

Phosphatidyl Ethanolamine (PE)

Data presented in Table 7 and analysis of variance indicate significant ($P < 0.01$) losses in the level of PE during frozen storage in all meat model systems containing added phospholipids. At the end of 8 months, PE had decreased by 55% in beef, 83% in chicken dark meat and by 34% in chicken white meat. Corliss and Dugan (1970) and Tsai and Smith (1971) reported that PE is the most reactive component in the phospholipids and have thus suggested that

Table 7. Changes in individual and total phospholipids during frozen storage (-18°C) of beef and chicken model meat systems.[1,2]

Length of Frozen Storage (month)	Beef				Chicken Dark Meat				Chicken White Meat			
	LPC	PE	PC	TP	LPC	PE	PC	TP	LPC	PE	PC	TP
0	1.87[a]	3.57	13.00[a]	19.34[a]	2.55	5.81	15.48	31.51	1.62[a]	7.91	18.81	33.16
	±0.43	±1.14	±0.29	±0.20	±0.59	±0.19	±1.10	±1.41	±0.22	±0.36	±0.44	±0.91
8	1.95[a]	1.60	10.62[a]	18.82[a]	1.35	1.00	4.52	13.65	1.78[a]	5.24	8.91	20.02
	±0.41	±0.37	±0.32	±0.44	±0.13	±0.36	±0.54	±1.06	±0.27	±0.48	±0.53	±0.59

[1]Results represent mean of 4 determinations.

[2]Mg phosphorus/g phospholipid
LPC = lysolecithin; PE = phosphatidyl ethanolamine; PE = phosphatidyl choline; TP = total phospholipid
All numbers in same column within a given meat type bearing same superscript are not significant at 5% level.

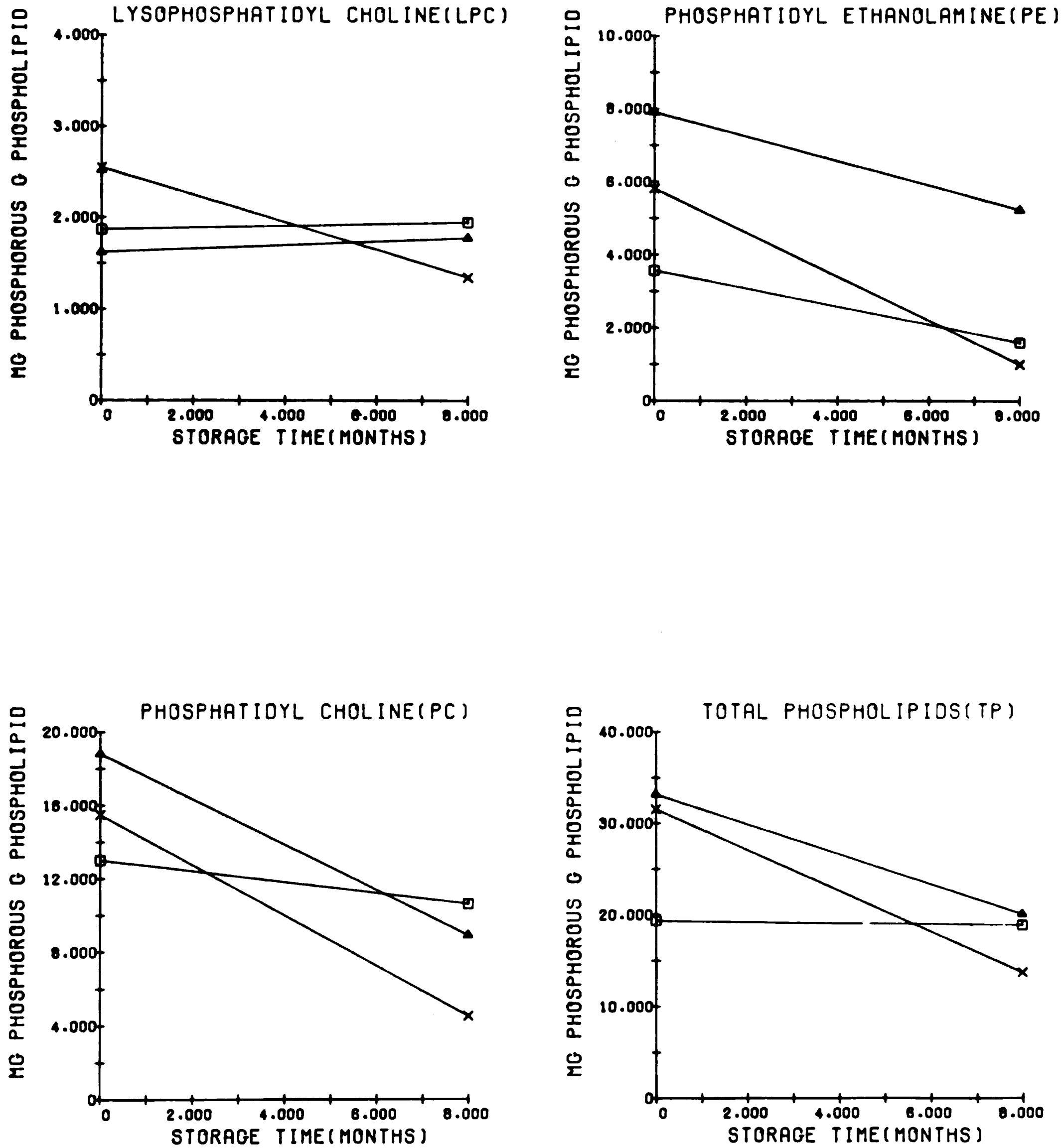


Figure 7. Influence of storage at -18°C on the stability of phospholipids in meat model systems.

Legend: □ Beef X chicken dark meat

△ chicken white meat

changes in PE appear to be indicative of the extent of lipid oxidation. The greater stability of PE in chicken white meat phospholipids may thus contribute to the low TBA values observed herein. The fatty acid profiles presented later herein verifies this conclusion.

Phosphatidyl Choline (PC)

Highly significant ($P < 0.01$) decreases (Table 7 and Figure 7) were observed during freezer storage for PC in chicken dark and white meat, but the decrease observed for beef was not significant ($P > 0.05$). The concentration of PC declined 70.8% in chicken dark meat, 52.6% in chicken white meat and 18.3% in the beef during freezer storage.

Corliss and Dugan (1970) and Tsai and Smith (1971) have reported that phosphatidyl choline (PC) does not exert any pro-oxidant effect. However, Acosta *et al*. (1966) reported that of the phospholipid fraction, PC was most active in early stages of autoxidation. It is well known (Hornstein *et al*., 1961; Keller and Kinsella, 1973, Body and Shorland, 1974) that PE is much more highly unsaturated than PC, and therefore, would be expected to be more susceptible to autoxidative degradation (Tsai and Smith, 1971).

Total Phospholipids (TP)

The concentrations of total phospholipid phosphorus at 0 time and at the end of 8 months freezer storage are

also presented in Table 7 as well as in Figure 7. Total phospholipids did not change in beef during 8 months frozen storage, but decreased by 56.7 and 39.6% in dark meat and chicken white meat.

The apparent stability of PC and total phospholipids observed in beef model meat systems containing added phospholipids were not related to the composition of their fatty acid profiles as can be seen later herein. Thus, PC and total phospholipids from beef were more stable during freezer storage than would be predicted from changes in their fatty acid profiles.

Changes in Fatty Acid Composition Due to Storage

It has been aptly demonstrated by Lea (1953) and Keller and Kinsella (1973) that changes in the fatty acid composition of lipids provide a good indirect measure of the extent of lipid oxidation. Thus, the composition of the fatty acids in triglycerides, total phospholipids and individual phospholipids (PC and PE) were determined prior to frozen storage and after 8 months storage.

Beef Triglycerides

The composition of fatty acids in beef triglyceride is presented in Table 8. Prior to frozen storage the levels of saturated, mono- di- and poly-unsaturated fatty acids

Table 8. Changes in the fatty acid composition of triglycerides during frozen (-18°C) storage of model meat systems (0-8 months).[a]

Fatty Acid	Beef		Chicken Dark Meat		Chicken White Meat	
	Prior to Storage	Frozen Storage (8 mo.)	Prior to Storage	Frozen Storage (8 mo.)	Prior to Storage	Frozen Storage (8 mo.)
12:0	----	----	----	----	----	----
14:0	3.15	2.63	0.54	0.98	0.84	0.82
14:1	----	----	----	----	----	----
15:0	----	0.33	----	----	0.80	----
16:0	25.26	24.06	22.46	24.80	25.14	21.94
16:1	3.62	4.34	3.70	6.15	2.77	3.27
16:2	----	1.68	----	----	----	0.39
17:0	1.02	0.89	----	1.18	----	0.45
18:0	22.64	22.08	7.57	8.96	8.30	8.65
18:1	42.19	39.93	39.07	42.91	34.32	39.07
18:2	1.27	2.63	25.03	14.27	24.93	21.52
$18:3w_6$	----	0.13	----	----	----	0.34
$18:3w_3$	0.85	1.30	1.63	0.74	2.90	1.20
20:1	----	----	----	----	----	----
20:2	----	----	----	----	----	0.22
20:3	----	----	----	----	----	0.17
20:4	----	----	----	----	----	1.96
% Saturated	52.07	49.99	30.57	35.92	35.08	31.86
% Monoenoic	45.81	44.27	42.77	49.06	37.09	42.34
% Dienoic	1.27	4.31	25.03	14.27	24.93	21.91
% Polyenoic	0.85	1.43	1.63	0.74	2.90	3.89
Total Unsaturation	47.93	50.01	69.43	64.07	64.92	68.14

[a]As percent of total fatty acids.

were 52.07, 45.81, 1.27 and 0.85%, respectively. These values are in good agreement with data presented by Hornstein et al. (1967). They reported that beef triglycerides varied from 40.09-60.1% for the saturated fatty acids, 37.53-50.96% for monounsaturated, 1.14-3.42% for the diunsaturated and 0.10-2.61% for the polyenoic fatty acids.

Some changes in the fatty acid profile of the triglycerides were observed at the end of 8 months freezer storage. The dienoic acids increased from 1.27 to 4.31% during freezer storage, while the polyenoic acids increased from 0.85 to 1.43%. Consequently, there was a corresponding increase of 4.5% in total unsaturation from the original value at the end of frozen storage.

Although there was some increase in unsaturation of the fatty acids during frozen storage of the beef triglycerides, they were relatively stable in comparison to the phospholipid fraction (Table 9). It is known that beef triglycerides are highly saturated and as such are slow to oxidize (El-Gharbawi, 1965; Chipault and Hawkins, 1971). This probably explains the long induction period observed (Figure 4) in beef samples containing added triglycerides.

Chicken Dark Meat and White Meat Triglycerides

The initial levels of saturated, mono-, di- and polyunsaturated fatty acids (Table 8) in chicken dark meat triglycerides were 30.57, 42.77, 25.03 and 1.63%, respectively.

The corresponding levels of the same fatty acids in chicken white meat were 35.08, 37.09, 24.93 and 2.90%, respectively. Hence chicken dark meat and white meat triglycerides have a similar composition of fatty acids except for a slightly greater amount of saturated fatty acids in the white meat. Katz _et al_. (1966) reported that the fatty acid composition of the triglycerides in chicken dark meat or white meat varied from 30-32% for saturated, 41-43% for monounsaturated, 25-26% for diunsaturated and 2-3% for the polyunsaturated fatty acids, respectively.

Unlike the beef triglycerides, $C_{18:2}$ and $C_{18:3}$ fatty acids decreased in chicken dark meat and white meat triglycerides during storage. The dienoic acids (principally $C_{18:2}$) decreased from the original value by 43 and 14% in the dark and white meat triglycerides, respectively. In addition, polyenoic fatty acids decreased from the original value by 56% in dark meat but increased by 34% in the white meat triglycerides during 8 months frozen storage. At the end of storage, total unsaturation had decreased from the original value by 7.72% in the dark meat but increased by nearly 5% in the white meat.

Since the unsaturated fatty acids in chicken white meat triglycerides were stable during storage, they probably accounted for the low TBA values (Table 6) obtained for samples containing added white meat triglycerides. The observed decreases in di- and polyenoic fatty acids in

chicken dark meat triglycerides, on the other hand, seem to reflect the high TBA values (Table 5) observed in the triglyceride treated samples.

Stability of Fatty Acids in Total Phospholipids

The fatty acid composition of total phospholipids was measured prior to frozen storage and again at the end of storage. The composition of the fatty acid profiles of beef, chicken dark and white meat samples containing added phospholipids is presented in Table 9.

The initial levels of saturated, mono-, di- and polyenoic fatty acids in beef total phospholipids were 34.98, 37.57, 11.95 and 15.50%, respectively. Hornstein et al. (1967) reported the amount of corresponding fatty acids in beef phospholipids to be 28.32-39.59, 23.87-40.32, 11.94-27.74 and 8.15-20.77%, respectively. Thus, the data presented in Table 9 are in accord with these values.

The initial values of the saturated, mono-, di-, and polyenoic fatty acids obtained in chicken dark meat phospholipids were 34.27, 21.48, 21.51 and 22.74%, respectively. The initial values for the corresponding fatty acids in the chicken white meat samples were 35.67, 27.11, 20.93 and 16.28%, respectively. Thus, the white meat was 5.63% higher in monounsaturates but approximately 6.5% lower in the polyunsaturates than the dark meat. These values generally agree with data reported by Katz et al.

Table 9. Changes in the fatty acid composition of total phospholipids during frozen storage (-18°C) of model meat systems (0-8 months).[a]

	Beef		Chicken Dark Meat		Chicken White Meat	
Fatty Acid	Prior to Storage	Frozen Storage (8 mo.)	Prior to Storage	Frozen Storage (8 mo.)	Prior to Storage	Frozen Storage (8 mo.)
12:0	----	----	----	----	----	----
14:0	1.46	1.03	0.14	1.63	1.54	0.70
14:1	0.52	0.67	---	0.45	----	0.16
15:0	1.16	2.80	0.37	0.24	1.94	0.20
16:0	18.49	26.15	15.60	24.93	21.94	16.55
16:1	3.61	3.83	1.05	3.66	1.49	2.21
16:2	0.74	1.09	---	1.22	----	0.20
17:0	0.92	3.47	----	2.44	----	0.86
18:0	12.95	14.41	18.16	18.93	10.25	15.29
18:1	33.44	34.05	20.43	35.82	25.62	27.01
18:2	10.52	7.60	21.51	6.92	20.32	21.54
18:3w_6	0.37	0.36	0.37	0.37	----	0.24
18:3w_3	1.29	1.95	0.41	0.98	0.57	0.70
20:1	----	0.24	----	0.24	----	----
20:2	0.69	---	----	----	0.61	0.30
20:3	2.77	0.64	0.53	0.20	1.20	0.72
20:4	8.51	1.22	17.41	1.97	11.26	9.67
20:5	0.76	0.49	----	----	0.43	0.18
22:3	----	----	----	----	----	----
22:4	0.88	----	1.23	----	----	1.37
22:5w_6	----	----	----	----	2.22	0.54
22:5w_3	0.92	----	----	----	0.60	0.54
22:6	----	----	2.79	----	----	1.02
% Saturated	34.98	47.86	34.27	48.17	35.67	33.60
% Monoenoic	37.57	38.79	21.48	40.17	27.11	29.38
% Dienoic	11.95	8.69	21.51	8.14	20.93	22.04
% Polyenoic	15.50	4.66	22.74	3.52	16.28	14.98
Total Unsaturation	65.02	52.14	65.73	51.83	64.32	66.40

[a]Percent of total fatty acids.

(1966). In the present study, the amount of total unsaturation prior to frozen storage was 65.02, 65.73 and 64.32% in beef, chicken dark and white meat, respectively. Thus, there was a close similarity in the fatty acid profiles for the phospholipids associated with different types of muscle, which is in agreement with reports by Body *et al*. (1966), Ansell and Hawthorne (1964) and Body and Shorland (1974).

Significant losses were observed in the PUFAS associated with the phospholipids (Table 9). During frozen storage of beef and chicken dark meat samples containing added phospholipids, the $C_{18:2}$ fatty acid decreased by 28.0 and 68.0% from the original value, respectively. On the other hand, it increased by 6.0% in chicken white meat. Arachidonic acid ($C_{20:4}$) decreased from the original value by 86% in beef, 89% in chicken dark meat but by only 14% in white meat. Total PUFAS decreased from the original values by 69.94, 84.52 and 7.98% in beef, chicken dark meat and white meat, respectively. Consequently, the levels of total unsaturation at the end of 8 months freezer storage were 52.14, 51.83 and 66.40% in beef, chicken dark meat and white meat, respectively. Although both beef and chicken dark meat showed a dramatic decline in the amount of PUFAS during 8 months frozen storage, indicating extensive oxidation, chicken white meat showed little change. These results verify the relative stability of white meat

during freezer storage and support the observed instability of beef and chicken dark meat samples.

The changes observed in the fatty acid profiles of the phospholipids provide good evidence for the involvement of PUFAS in the development of oxidized flavors. In general, significant decreases were observed in the polyenoic acids of the phospholipids in beef and chicken dark meat samples which would reflect their high TBA values (Tables 4 and 5). On the other hand, the low TBA values obtained for chicken white meat samples (Table 6) can be attributed to the greater stability of their PUFAS.

Lea (1953, 1957), Younathan and Watts (1960), Hornstein *et al*. (1961) and Keller and Kinsella (1973) have concluded that changes in PUFAS of the phospholipids, and especially of arachidonic acid, result in serious flavor problems. Thus, the results of this study provide convincing evidence for the involvement of phospholipids in development of oxidized flavor during storage of frozen meat, particularly in beef and chicken dark meat.

Changes in the Fatty Acid Profiles of PC and PE

In studying the mechanisms of autoxidative degradation of lipids during frozen storage of meat, the composition of the fatty acid profiles in PC and PE associated with total phospholipids was also determined before and following 8 months of frozen storage. Results are presented

in Tables 10 and 11 for PC and PE, respectively.

The most remarkable changes in the fatty acids of PC and PE occurred in the polyenes in general, and in the $C_{20:4}$ and $C_{22:4}$ fatty acids in particular. The levels of $C_{20:4}$ in PC and PE for beef declined from 20.34 to 0.24% and from 32.75 to 1.23%, respectively. Thus, a loss of 99 and 96% of $C_{20:4}$ from the original value occurred during freezer storage for PC and PE, respectively. Similarly, $C_{20:4}$ decreased by 96 and 74% in the PC and PE of chicken dark meat. For chicken white meat the levels of $C_{20:4}$ in PC and PE also declined during frozen storage, the decreases being 76.0 and 50.23% from the original values, respectively. The initial amount of $C_{22:4}$ had disappeared from both PC and PE by the end of frozen storage in all meat samples.

In the beef samples, the levels of PUFAS declined by 59.22 and 64.04% from the original values in PC and PE during freezer storage, respectively. In the chicken dark meat, PUFAS also decreased, with declines of 61.22 and 29.00% in the PC and PE during frozen storage, respectively. Polyenoic acids also declined in the chicken white meat samples during storage with losses of 27.95 and 42.80% from the original values for PC and PE, respectively.

The data presented in Tables 10 and 11 clearly indicate that polyenoic acids are not stable and undergo autooxidative degradation. Lea (1953) reported that unsaturation

Table 10. Changes in the fatty acid composition of PC during frozen storage (-18°C) of model meat systems (0-8 months).[a]

	Beef		Chicken Dark Meat		Chicken White Meat	
Fatty Acid	Prior to Storage	Frozen Storage (8 mo.)	Prior to Storage	Frozen Storage (8 mo.)	Prior to Storage	Frozen Storage (8 mo.)
12:0	6.05	----	----	----	----	----
14:0	5.14	0.36	----	----	0.34	----
14:1	----	0.16	----	-----	1.38	----
15:0	1.00	1.49	0.93	0.43	1.55	----
15:1	----	0.52	----	1.28	----	2.10
16:0	21.97	26.39	19.70	26.04	17.84	18.76
16:1	0.73	1.90	----	1.03	----	1.35
16:2	----	0.79	----	0.51	----	0.36
17:0	1.94	1.43	3.35	0.69	1.72	1.45
18:0	10.53	10.46	17.14	18.50	12.07	17.79
18:1	23.73	23.78	15.45	25.70	14.94	18.76
18:2	4.48	7.77	10.78	10.79	10.11	11.54
$18:3w_6$	----	0.48	0.42	0.90	0.52	----
$18:3w_3$	0.27	1.39	0.35	1.03	0.40	2.40
20:1	----	0.28	----	----	----	----
20:2	0.78	15.03	2.76	3.43	5.52	3.00
20:3	----	----	0.24	----	----	0.84
20:4	20.34	0.24	17.84	0.72	16.66	3.97
20:5	----	7.13	0.28	8.95	11.89	17.67
22:3	----	----	----	----	----	----
22:4	3.03	----	10.78	----	5.06	----
$22:5w_6$	----	----	----	----	----	----
$22:5w_3$	----	0.40	----	----	----	----
22:6	----	----	----	----	----	----
% Saturated	46.63	40.13	41.12	45.66	33.52	38.00
% Monoenoic	24.46	26.36	15.45	28.01	16.32	22.21
% Dienoic	5.26	23.87	13.54	14.73	15.63	14.90
% Polyenoic	23.64	9.64	29.91	11.60	34.53	24.88
Total Unsaturation	53.36	59.87	58.90	54.34	66.48	61.99

[a]Percent of total fatty acids; PC = phosphatidyl choline.

Table 11. Changes in the fatty acid composition of PE during frozen storage (-18°C) of model meat systems (0-8 months).[a]

	Beef		Chicken Dark Meat		Chicken White Meat	
Fatty Acid	Prior to Storage	Frozen Storage (8 mo.)	Prior to Storage	Frozen Storage (8 mo.)	Prior to Storage	Frozen Storage (8 mo.)
12:0	0.48	----	----	----	----	----
14:0	1.55	1.23	0.25	0.49	1.80	0.34
14:1	----	0.34	0.33	0.16	1.56	0.14
15:0	0.33	0.45	1.60	0.65	4.14	0.63
15:1	----	0.92	----	0.72	----	4.62
16:0	13.96	21.48	15.73	13.28	13.85	16.60
16:1	1.65	2.01	----	0.65	----	1.44
16:2	----	1.68	----	1.23	----	0.58
17:0	1.49	2.24	2.70	1.08	2.25	2.98
18:0	14.89	17.01	23.23	22.68	13.64	21.36
18:1	19.50	24.84	16.39	22.88	15.92	21.17
18:2	4.80	8.39	11.84	12.82	11.08	10.97
$18:3w_6$	----	----	0.33	1.15	0.28	0.07
$18:3w_3$	0.37	1.96	0.28	1.32	0.33	0.96
20:1	----	----	----	0.26	----	0.58
20:2	1.06	4.92	1.06	3.94	5.97	1.56
20:3	0.37	----	0.23	0.79	0.28	0.38
20:4	32.75	1.23	18.68	4.83	17.52	8.72
20:5	----	10.74	----	9.73	7.58	3.46
22:3	----	0.56	0.35	----	----	----
22:4	6.80	----	5.94	0.99	3.79	1.44
$22:5w_6$	----	----	----	----	----	0.36
$22:5w_3$	----	----	0.57	----	----	0.58
22:6	----	----	0.49	0.35	----	1.06
% Saturated	32.70	42.41	43.51	38.18	35.68	41.91
% Monoenoic	21.15	28.11	16.72	24.67	17.48	27.95
% Dienoic	5.86	14.99	12.90	17.99	17.05	13.11
% Polyenoic	40.29	14.49	26.87	19.16	29.78	17.03
Total Unsaturated	67.30	57.59	56.49	61.82	64.31	58.09

[a]Percent of total fatty acids; PE = phosphatidyl ethanolamine.

rapidly disappears at advanced stages of autoxidation. Therefore, the PUFAS appear to be principally responsible for development of rancidity, which is in agreement with earlier reports by Lea (1953, 1957), Hornstein *et al*. (1961) and Keller and Kinsella (1973). Results indicate that PC and PE both play an important role in the development of rancid flavors in stored meat and meat products.

Data presented for TBA values (Tables 4 and 5) and the changes in unsaturation of the PUFAS (Tables 9, 10 and 11) confirm the positive relationship between rancidity and oxidation of the fatty acids. It is well known that polyenoic acids are extremely reactive and through oxidative degradation give rise to a number of carbonyl compounds, which greatly influence oxidized flavor (Lea, 1953, 1957; Younathan and Watts, 1960).

Summary of Results

The role of triglycerides, total lipids and total phospholipids on the development of rancidity during frozen storage of meat was studied using lipid-free muscle fibers in combination with added triglycerides, total lipids or total phospholipids, respectively. The experimental model systems were held at -18°C for 8 months.

Results of this study indicated that added total lipids and total phospholipids greatly increased the TBA values. Oxidation took place in two stages. The total lipids and

total phospholipids were the first to oxidize and their rate of oxidation decreased with time. Oxidation of the triglycerides only began after a prolonged induction period.

Lipids from chicken white meat were more stable to autoxidative degradation during frozen storage than those from beef or chicken dark meat. It is postulated that the greater amount of oxidation in beef and chicken dark meat may be due to residual meat pigments.

Results showed that both triglycerides and phospholipids contribute to development of rancidity, but the phospholipids make the greatest contribution. The importance of the triglycerides in development of oxidation depends on the degree of unsaturation and the length of frozen storage. The study confirmed the positive relationship between rancidity and oxidation of the PUFAS in both the phospholipids and triglycerides.

EXPERIMENT B

Role of Triglycerides, Total Lipids and Total Phospholipids on Development of WOF in Cooked Meat Model Systems

The experiments to test the effect of triglycerides, total lipids and total phospholipids on development of WOF in cooked meat involved the use of lipid-free muscle fibers. The preparation of the lipid-free muscle fibers was outlined earlier herein, while the treatments are shown in Table 1.

The prepared samples were cooked in retortable pouches to 70°C internal temperature in boiling water. After storage for 48 hours at 4°C, the samples were tested for TBA values and were also judged by a trained panel of 12 individuals.

Mean TBA values and their corresponding mean sensory scores are presented in Table 12. Results showed that the model systems containing added total lipids gave the highest TBA values, followed by samples containing total phospholipids, triglycerides, and the lipid-free muscle fibers (control), respectively. Although the samples containing total lipids exhibited the highest TBA values, they did not consistently show the lowest flavor ratings. This is consistent with the report by Love and Peerson (1971) suggesting that neutral lipids in association

Table 12. TBA numbers and sensory scores for cooked beef chicken dark meat and white meat model systems.[1,2]

Meat Type	Composition of Treatments	Mean TBA Numbers	Mean Sensory Score[3]
	B_1=0.8% phospholipids	5.76[b]	2.69[a]
Beef	B_2=9.2% triglycerides	1.88[a]	3.84[b]
	B_3=10% total lipids	6.81[b]	2.64[a]
	B_4=control	1.16[a]	3.75[b]
Chicken	D_1=0.74% phospholipids	8.48[b]	2.97[a]
Dark Meat	D_2=4.3% triglycerides	6.30[a,b]	3.65[a,b]
	D_3=5% total lipids	11.74[c]	3.25[a]
	D_4=control	5.78[a]	3.88[a,b]
Chicken	W_1=0.7% phospholipids	5.03[b]	2.80[a]
White Meat	W_2=4.3% triglycerides	2.99[a]	3.35[a]
	W_3=5% total lipids	5.53[b]	3.09[a]
	W_4=control	2.18[a]	4.02[b]

[1]There were 4 replicates for each treatment.

[2]Taste panel score were from 1-5, where 1=very pronounced WOF and 5=No WOF.

[3]All numbers in same column within a meat type bearing the same superscript are not significant at the 5% level.

with polar lipids may trap volatile decomposition products of polar lipids and thus reduce their effect on flavor.

Analyses of variance for TBA values and sensory scores revealed that the experimental treatments were significantly ($P < 0.001$) different. Tukey's test for multiple comparisons clearly showed that total lipids and total phospholipids significantly ($P < 0.01$) increased TBA values while significantly ($P < 0.05$) reducing flavor scores. Thus, total lipids and total phospholipids are major contributors to development of WOF in cooked meat.

Results showed that neither the model system (control) nor the triglycerides alone significantly influenced development of WOF in cooked meat. The results of this study clearly provide evidence for the involvement of total phospholipids in development of WOF in cooked meat. Thus, this study confirms the work of Wilson et al. (1976), who first implicated total phospholipids as being responsible for development of WOF in cooked meat. Although triglycerides alone did not significantly influence the development of WOF in cooked meat, they did have an additive effect in combination with phospholipids (Table 12).

Statistically significant ($P < 0.05$) correlation coefficients of $r = -.57$ and $r = -.51$ were found between TBA values and sensory scores for beef and chicken white

meat model systems, respectively. These results confirm the existence of a relationship between WOF and panel scores. They are in agreement with the data presented by Zipser *et al*. (1964), who reported a close relationship between TBA values and development of oxidized flavor in cooked meat.

In the case of chicken dark meat, however, the correlation between TBA values and sensory scores was not statistically significant. The reason for this discrepancy may be due to the apparent retention of lipids by the extracted muscle fibers. Chicken dark meat fibers (control) had a higher mean TBA value of 5.78 in comparison to values of 1.16 and 2.18 for beef and chicken white meat, respectively. The high TBA value for the chicken dark meat control indicates that the extracted muscle fibers probably contain some unextracted bound lipids. Any residual bound lipids may cause a discrepancy in sensory scores for the control samples. There is, however, no evident discrepancy in sensory scores, which suggests the major effect is upon TBA values. However, the relatively high TBA value for the control would decrease the relationship between TBA values and panel scores.

Changes in Phospholipid Components Due to Cooking and Storage at 4°C

Quantitative and compositional changes in the components of the total phospholipids were determined before and after cooking using the phosphorus assay procedure of Rouser *et al.* (1966). Phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) combined constitute over 75% of the total phospholipids in meat, in addition to being highly unsaturated (Peng and Dugan, 1965; Body and Shorland, 1974). Hence, the changes in PC and PE will be discussed in detail in order to elucidate the role of phospholipids in development of WOF in cooked meat.

The compositional changes in the phospholipid components as a result of cooking are shown in Table 13. The level of PE relative to total phospholipids prior to cooking was 18.5% for both beef and chicken dark meat but was 23.9% in chicken white meat. These values are in close agreement with data reported by several workers (Lea, 1957; Peng and Dugan, 1965; Lee and Dawson, 1976). The initial proportion of PC relative to total phospholipids was 54.7% in beef, 49.1% in chicken dark meat and 56.7% in white meat. These values are in good agreement with those presented by Peng and Dugan (1965), David Kova and Khan (1967) and Keller and Kinsella (1973).

Results indicated that cooking caused significant ($P < 0.01$) decreases in total phospholipids. Analysis

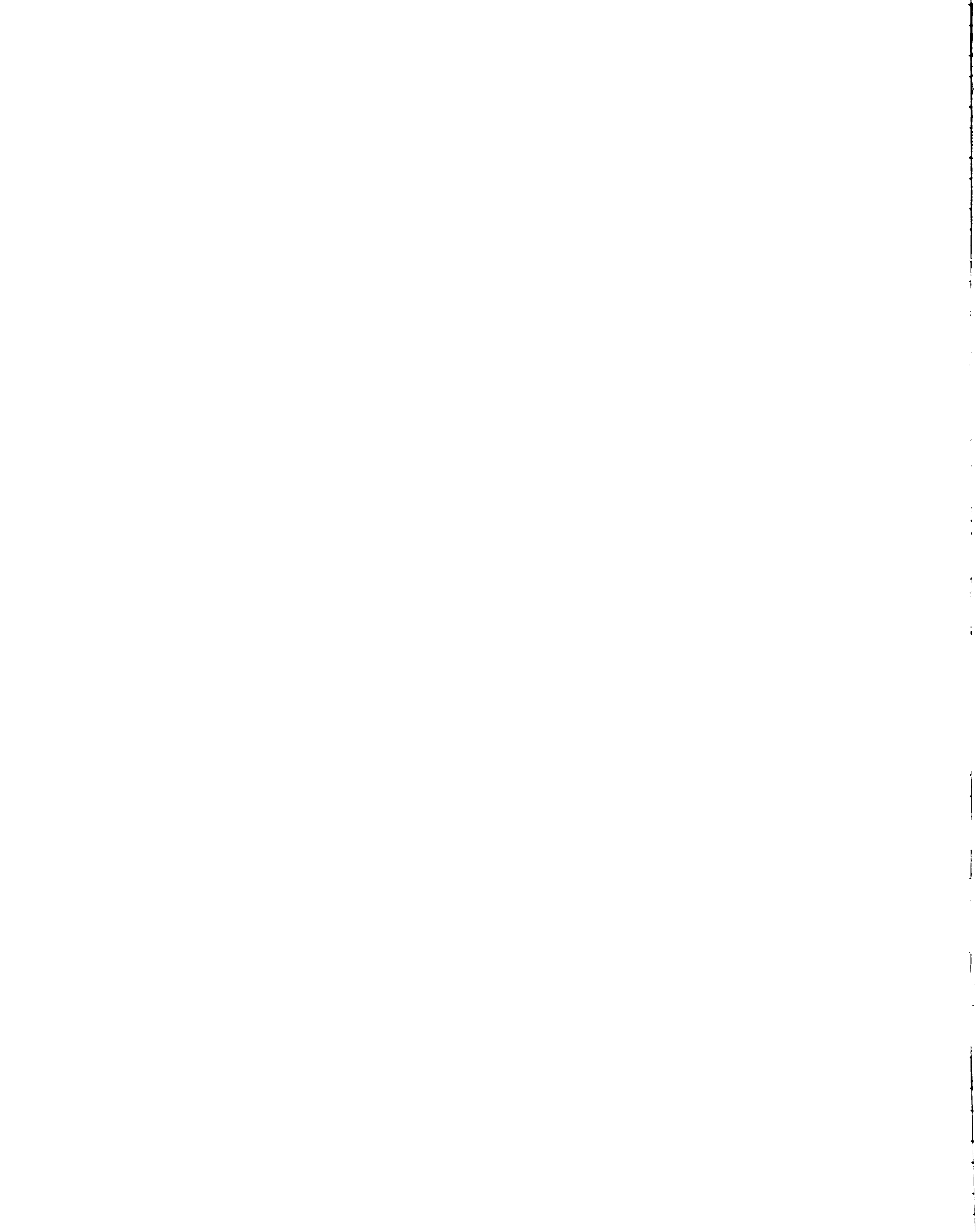


Table 13. Mean changes in phospholipids due to cooking and storage at 4°C.[1,2,3]

Classes of Phospholipids	Beef Model System		Chicken Dark Meat Model System		Chicken White Meat Model System	
	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked
LPC[4]	2.23	0.97	2.55	1.44	1.86[a]	1.15[a]
PE[5]	3.57[a]	2.78[a]	5.81	2.55	7.91	4.00
PC[6]	10.57	5.73	15.48[a]	13.48[a]	18.81	10.86
Others[7]	3.0	1.16	7.67	10.24	4.58	7.97
Tp[8]	19.34	10.64	31.51	28.14	33.16	23.98

[1]Values given are mg phosphorus/g phospholipid.

[2]There were 4 replicates for each component.

[3]All numbers in same column within a given meat type bearing the same superscript are not significant at 5% level.

[4]LPC = Lysophosphatidyl choline.

[5]PE = phosphatidyl ethanolamine.

[6]PC = Phosphatidyl choline.

[7]Other phospholipids were calculated by difference.

[8]Total phospholipids.

of variance shows highly significant ($P < 0.01$) interactions in the content of total phospholipids between the fresh and cooked state. The significant interactions indicate that PC and PE did not consistently behave in the same manner during cooking. While a significant decrease in PE was not observed in beef; PC was found to decrease by 45.0% after cooking. Changes in PE and PC in chicken dark meat were opposite to that for beef, i.e., PE significantly decreased while PC was stable during cooking and storage. In chicken white meat, highly significant ($P < 0.01$) losses occurred in both PE and PC during cooking and storage.

Results showed that total phospholipids from all types of meat significantly decreased during cooking and storage. Phosphatidyl ethanolamine (PE) was less stable to cooking and storage than phosphatidyl choline (PC). This is further verified by the fatty acid composition for PC and PE as will be discussed later herein. Decreases in the component phospholipids may be due to either autooxidation, hydrolytic decomposition, lipid browning reactions or to lipid-protein co-polymerization as outlined by Lea (1957). Products of lipid autoxidative degradation have been associated with the development of off-flavors, principally with WOF (Ruenger et al., (1978).

Changes in Fatty Acid Composition as a Result of Cooking

Triglycerides

The fatty acid profiles of the samples containing added triglycerides were determined prior to cooking and again following cooking and storage. The values are presented in Table 14. For beef samples, the saturated fatty acids decreased from 52.07 to 49.47%, while the monoenes increased from 45.81 to 49.29% to give a net increase of 5.42% in total unsaturation.

In the chicken samples containing added triglycerides, total unsaturation increased from 69.42 to 71.56% in dark meat, and from 64.92 to 68.99% in the white meat. These results are consistent with reports showing that only minor changes take place in the fatty acid content of the triglycerides as a result of cooking (Chang and Watts, 1952; Giam and Dugan, 1965; Campbell and Turkki, 1967). Thus, the stability of the triglycerides is consistent with the low TBA values (Table 12).

Total Phospholipids

Compositional changes in the fatty acid profiles in total phospholipids of meat model systems due to cooking and storage are shown in Table 15. In beef the levels of saturated fatty acids were unchanged. The monoenes increased slightly, the dienes from 11.95 to 13.12%, while

Table 14. Mean fatty acid composition of triglycerides in model meat systems.[a]

Fatty Acid	Beef		Chicken Dark Meat		Chicken White Meat	
	Prior to cooking	After Cooking	Prior to Cooking	After Cooking	Prior to Cooking	After Cooking
14:0	3.15	1.86	0.54	0.58	0.84	0.63
14:1	----	0.80	----	----	----	----
15:0	----	----	----	----	0.80	----
16:0	25.26	32.92	22.46	19.94	25.14	23.08
16:1	3.62	4.20	3.70	4.08	2.77	3.85
17:0	1.02	0.53	----	0.69	----	----
18:0	22.64	14.16	7.57	7.23	8.30	7.30
18:1	42.19	44.29	39.07	41.27	34.32	39.49
18:2	1.27	1.24	25.03	24.48	24.93	23.94
$18:3w_6$	----	----	----	----	----	1.20
$18:ew_3$	0.85	----	1.63	1.73	2.90	0.51
% Sat.	52.07	49.47	30.57	28.44	35.08	31.01
% Mono.	45.81	49.29	42.77	45.35	37.09	43.34
% Dienoic	1.27	1.24	25.03	24.48	24.93	23.94
% Polyenoic	0.85	----	1.63	1.73	2.90	1.71
Total Unsat.	47.93	50.53	69.43	71.56	64.92	68.99

[a]Percent of total fatty acids.

Table 15. Changes in the fatty acid composition of total phospholipids of meat model systems due to cooking and storage at 4°C.[a]

	Beef		Chicken Dark Meat		Chicken White Meat	
Fatty Acid	Prior to Cooking	After Cooking	Prior to Cooking	After Cooking	Prior to Cooking	After Cooking
14:0	1.46	0.72	0.14	0.17	1.54	0.87
14:1	0.52	----	----	----	----	----
15:0	1.16	1.86	0.37	0.57	1.94	0.69
16:0	18.49	18.73	15.60	16.52	21.94	18.98
16:1	3.61	3.67	1.05	1.26	1.49	2.76
16:2	0.74	0.25	----	----	----	----
17:0	0.92	0.84	----	----	----	1.07
18:0	12.95	12.83	18.16	21.03	10.25	10.74
18:1	33.44	34.81	20.43	19.74	25.62	33.87
18:2	10.52	12.66	21.51	19.22	20.32	16.65
$18:3w_6$	0.37	----	0.37	0.23	----	----
$18:3w_3$	1.29	0.69	0.41	0.43	0.57	0.85
20:2	0.69	0.21	----	----	0.61	0.56
20:3	2.77	1.92	0.53	0.52	1.20	0.62
20:4	8.51	9.32	17.41	18.88	11.26	6.90
20:5	0.76	----	----	----	0.43	1.62
22:4	0.88	0.42	1.23	0.57	----	0.36
$22:5w_6$	----	----	----	----	2.22	2.15
$22:5w_3$	0.92	1.08	----	----	0.60	1.30
22:6	----	----	2.79	0.86	----	----
% Saturated	34.98	34.98	34.27	38.29	35.67	32.35
% Monoenoic	37.57	38.48	21.48	21.00	27.11	36.63
% Dienoic	11.95	13.12	21.51	19.22	20.93	16.65
% Polyenoic	15.50	13.42	22.74	20.83	16.28	14.37
Total Unsaturated	65.02	65.02	65.73	61.05	64.32	67.65

[a]Percent of total fatty acids.

the polyenes decreased from 15.50 to 13.42%; a loss of 13.42% in unsaturation from the original value. This indicates that the polyenes are important in the development of oxidized flavor.

The changes observed in the fatty acid profiles in chicken dark and white meat phospholipids are also shown in Table 15. The level of $C_{18:2}$ fatty acid decreased in both chicken dark and white meat during cooking. Arachidomi acid ($C_{20:4}$) increased from 17.41 to 18.88% in chicken dark meat but decreased from 11.26 to 6.90% in white meat. The level of polyenoic acids decreased from 22.74 to 20.83% and from 16.28 to 14.37% in chicken dark meat and white meat, respectively.

Although total unsaturation was not drastically affected by cooking and storage, nevertheless, the moderate changes that occurred in both the dienoic and the polyenoic fatty acids may be important in development of WOF. Several workers (Lea, 1957; Younatham and Watts, 1960; Keller and Kinsella, 1973) have reported that the PUFAS may be involved in the heat induced degradation of lipids which results in off-flavors. Recently, Ruenger _et al_. (1978) have reported that WOF is a result of autoxidation of tissue lipids. Thus, the present study demonstrates that decreases in total phospholipids as well as in PUFAS are related to the development of WOF in cooked meat.

Changes in PC and PE in Beef Phospholipids

The fatty acid profiles for PC and PE in beef phospholipids are presented in Table 16. Following cooking, total unsaturation in PC increased from 53.36 to 65.44%, mainly at the expense of the saturated fatty acids. Significant increases were also evident in the dienoic and polyenoic acids following cooking and storage. Specifically, $C_{18:2}$, $C_{20:4}$ and $C_{22:4}$ fatty acids increased from the original value by 106.0, 34.4 and 59.1%, respectively.

In PE, however, total unsaturation decreased from 67.30 to 64.67%. Similar to PC, however, the $C_{18:2}$ and $C_{20:4}$ fatty acids increased by 103.1 and 14.0% from the original value, respectively. In PE, however, $C_{20:2}$ and $C_{22:4}$ decreased by 74.5 and 55.1% from the original value, respectively.

These results clearly show that PE is less stable than PC, and therefore, it probably contributes more to development of WOF. Keller and Kinsella (1973) provided data showing that PE was much more highly unsaturated and much less stable than PC. Results of this study clearly revealed that PE is significantly more unsaturated than PC. The levels of PUFAS were 40.29 and 23.64% for PE and PC, respectively. This is in agreement with the data presented by Body and Shorland (1974) showing that the levels of PUFAS were from 17 to 43% and 7-25% in PE and PC, respectively.

Table 16. Changes in fatty acid composition of phosphatidyl choline and phosphatidyl ethanolamine of beef model system.[a]

	Phosphatidyl Choline (PC)		Phosphatidyl Ethanolamine (PE)	
Fatty ACid	Prior to Cooking	After Cooking	Prior to Cooking	After Cooking
12:0	6.05	0.38	0.48	0.95
14:0	5.14	0.44	1.55	1.04
14:1	----	----	----	1.08
15:0	1.00	0.32	0.33	2.50
16:0	21.97	14.81	13.96	10.42
16:1	0.73	0.95	1.65	----
17:0	1.94	0.76	1.49	2.78
18:0	10.53	17.85	14.89	16.79
18:1	23.73	20.25	19.50	11.56
18:2	4.48	9.23	4.80	9.75
$18:3w_6$	----	----	----	0.33
$18:3w_3$	0.27	----	0.37	0.28
20:1	----	----	----	----
20:2	0.78	2.47	1.06	0.27
20:3	----	0.38	0.37	0.78
20:4	20.34	27.34	32.75	37.35
20:5	----	----	----	0.76
22:4	3.03	4.82	6.80	3.05
22:5	----	----	----	0.31
% Saturated	46.63	34.56	32.70	34.48
% Monoenoic	24.46	21.20	21.15	12.64
% Dienoic	5.26	9.23	5.86	10.02
% Polyenoic	23.64	35.01	40.29	42.56
Total Unsaturation	53.36	65.44	67.30	64.67

[a]Percent of total fatty acids.

Changes in PC and PE in Chicken Dark Meat

The fatty acid profiles of PC and PE for chicken dark meat phospholipids are presented in Table 17. Total unsaturation in PC increased from 58.90 to 64.99% following cooking and storage. A slight increase in total unsaturation of PE (1.59%) also occurred. Unlike beef, linoleic acid ($C_{18:2}$) decreased in both PC and PE of chicken dark meat. Arachidonic acid increased from 17.84 to 21.10% in PC but decreased from 18.68 to 13.95% in PE, representing a loss of 25.32% from the original value. This is in agreement with the work of Keller and Kinsella (1973) who reported a decrease of about 25% in $C_{20:4}$ fatty acid following cooking of beef.

Changes in PC and PE of Chicken White Meat

The fatty acid profiles recorded for PC and PE in chicken white meat are presented in Table 18. In PC, $C_{18:2}$, $C_{20:4}$, $C_{20:5}$ and $C_{22:4}$ fatty acids decreased by 61.30, 53.60, 27.75 and 23.52% from the original value, respectively.

For PE, $C_{18:2}$ decreased from 11.08 to 8.92%, $C_{20:2}$ from 5.97 to 1.22% and $C_{20:4}$ decreased from 17.52 to 3.36% during cooking and storage. The significant loss of $C_{20:4}$ underlines its vulnerability to cooking, especially in PE. On the other hand, $C_{20:5}$ increased from 7.58 to 9.11%,

Table 17. Changes in fatty acid composition of phosphatidyl choline and phosphatidyl ethanolamine of dark meat model system.[a]

	Phosphatidyl Choline (PC)		Phosphatidyl Ethanolamine (PE)	
Fatty Acid Fatty Acid	Prior to Cooking	After Cooking	Prior to Cooking	After Cooking
14:0	----	----	0.25	0.22
$14:1w_6$	----	0.53	0.33	----
15:0	0.93	0.92	1.60	1.53
16:0	19.70	15.56	15.73	16.70
$16:1w_7$	----	----	----	----
16:2	----	----	----	----
17:0	3.35	1.98	2.70	2.74
18:0	17.14	15.75	23.23	20.95
$18:1w_9$	15.45	14.56	16.39	23.00
$18:2w_6$	10.78	8.39	11.84	6.41
$18:3w_6$	0.42	0.53	0.33	0.60
$18:3w_3$	0.35	0.27	0.28	1.70
$20:2w_6$	2.76	3.94	1.06	0.75
$20:3w_6$	0.24	0.24	0.23	0.26
$20:4w_6$	17.84	21.10	18.68	13.95
$20:5w_3$	0.28	2.32	----	4.43
$22:3w_6$	----	----	0.35	0.75
$22:4w_6$	10.78	13.91	5.94	5.45
$22:5w_6$	----	----	0.57	0.56
$22:6w_3$	----	----	0.49	----
% Saturated	41.12	34.21	43.51	41.92
% Monoenoic	15.45	15.09	16.72	23.22
% Dienoic	13.54	12.33	12.90	7.16
% Polyenoic	29.94	37.57	26.87	27.70
Total Unsaturation	58.90	64.99	56.49	58.08

[a]Percent of total fatty acids.

Table 18. Changes in fatty acid composition of phosphatidyl choline and phosphatidyl ethanolamine of white meat model system.[a]

	Phosphatidyl Choline (PC)		Phosphatidyl Ethanolamine	
Fatty Acid	Prior to Cooking	After Cooking	Prior to Cooking	After Cooking
14:0	0.34	----	1.80	----
14:1	1.38	----	1.56	----
15:0	1.55	0.97	4.14	0.47
16:0	17.84	16.54	13.85	15.42
16:1	----	1.72	----	3.39
16:2	----	----	----	----
17:0	1.72	4.47	2.25	3.34
18:0	12.07	9.45	13.64	14.02
18:1	14.94	20.79	15.92	23.13
18:2	10.11	3.91	11.08	8.92
$18:3w_6$	0.52	----	0.28	----
$18:3w_3$	0.40	6.44	0.33	6.68
20:2	5.52	9.71	5.97	1.22
20:3	----	4.12	0.28	3.36
20:4	16.66	7.73	17.52	3.36
20:5	11.89	8.59	7.58	9.11
22:3	----	----	----	----
22:4	5.06	3.87	3.79	7.24
$22:5w_6$	----	----	----	0.35
$22:5w_3$	----	1.68	----	----
22:6	----	----	----	----
% Sat.	33.52	31.43	35.68	33.25
% Monoenoic	16.32	22.51	17.48	26.52
% Dienoic	15.63	13.62	17.05	10.14
% Polyenoic	34.53	32.43	29.78	30.10
Total Unsat.	66.48	68.56	64.31	66.76

[a]Percent of total fatty acids.

while $C_{22:4}$ increased from 3.79 to 7.24% and $C_{18:3}$ increased from 0.33 to 6.68% during cooking and storage.

The Importance of Phospholipids on Development of WOF

Holman and Elmer (1947) attributed the tendency of the phospholipids to oxidize to their high content of $C_{20:4}$, $C_{22:3}$, $C_{22:4}$, $C_{22:5}$ and $C_{22:6}$ fatty acids. Thus, the significant changes in some specific PUFAS, which occurred in total phospholipids (Table 15) and in PC and PE (Tables 16, 17 and 18), are of great significance to development of WOF.

Greater losses in PUFAS occurred in PE than in PC. Furthermore, PE was found to be less stable to cooking than PC (Table 13). Most of the losses in fatty acids were observed in the $C_{18:2}$, $C_{20:4}$ and $C_{22:4}$ associated with chicken dark and white meat. Love and Pearson (1971) and Keller and Kinsella (1973) indicated that the loss of $C_{20:4}$ fatty acids was consistent with its greater propensity to undergo autoxidation, especially when associated with PE. Thus, results of this study confirm that PUFAS associated with PE tend to be more labile to heat than those of PC. Hence, PE may be more significant in the development of WOF. Decreases in other PUFAS in addition to arachidonic acid, including that of $C_{18:2}$ fatty acid, also appear to accelerate development of WOF.

The observation that PUFAS associated with PE are less

stable to cooking than those of PC led to another experiment. The objective of the second experiment was to study the relative contributions of PC, PE and serum phospholipids in development of WOF using beef muscle fibers as the base material. Pure bovine PC, PE and serum phospholipids were added back to the model system at a level of 0.0375% either with or without 156 ppm of nitrite. Each of the 8 experimental treatments was prepared using 50 ml deionized water. The experimental design is shown in Table 19.

The samples were cooked in retortable pouches to 70°C internal temperature and stored for 48 hrs at 4°C. After storage, TBA analyses as well as taste panel evaluation of the samples were conducted. A panel of 4 trained, high skilled individuals served as the judges.

A plot of TBA numbers and sensory scores is shown graphically in Figure 8. A highly significant ($P < 0.01$) "r" value of -.62 was found between TBA numbers and flavor scores.

Analysis of variance for treatment effect upon TBA numbers and sensory scores are presented in Table 20. Differences between experimental treatments were statistically significant ($P < 0.001$) for both TBA numbers and sensory scores. Addition of PE and serum phospholipids significantly ($P < 0.01$) increased TBA values while decreasing sensory scores. The addition of PC did not

Table 19. The influence of PC, PE, total serum phospholipid and nitrite on development of WOF using beef model system.[1]

Treatment	Preparation of Samples
1	Model system only (control)
2	Model system + nitrite
3	Model system + PC
4	Model system + PC + nitrite
5	Model system + PE
6	Model system + PE + nitrite
7	Model system + serum phospholipids
8	Model system + serum phospholipid + nitrite

[1]Phospholipids were added to the model system at a level of 0.0375% and nitrite at 156 ppm.
PC = Phosphatidyl choline.
PE = Phosphatidyl ethanolamine.
Serum phospholipids (lysophosphatidyl choline, 8%; sphingomyelin, 24%; PC; 53%; phosphatidyl serine, 1%; phosphatidyl inositol, 4%; PE, 6%; phosphatidyl glycerol, 2%; cardiolipin, 1% and phosphatidic acid, 1%).

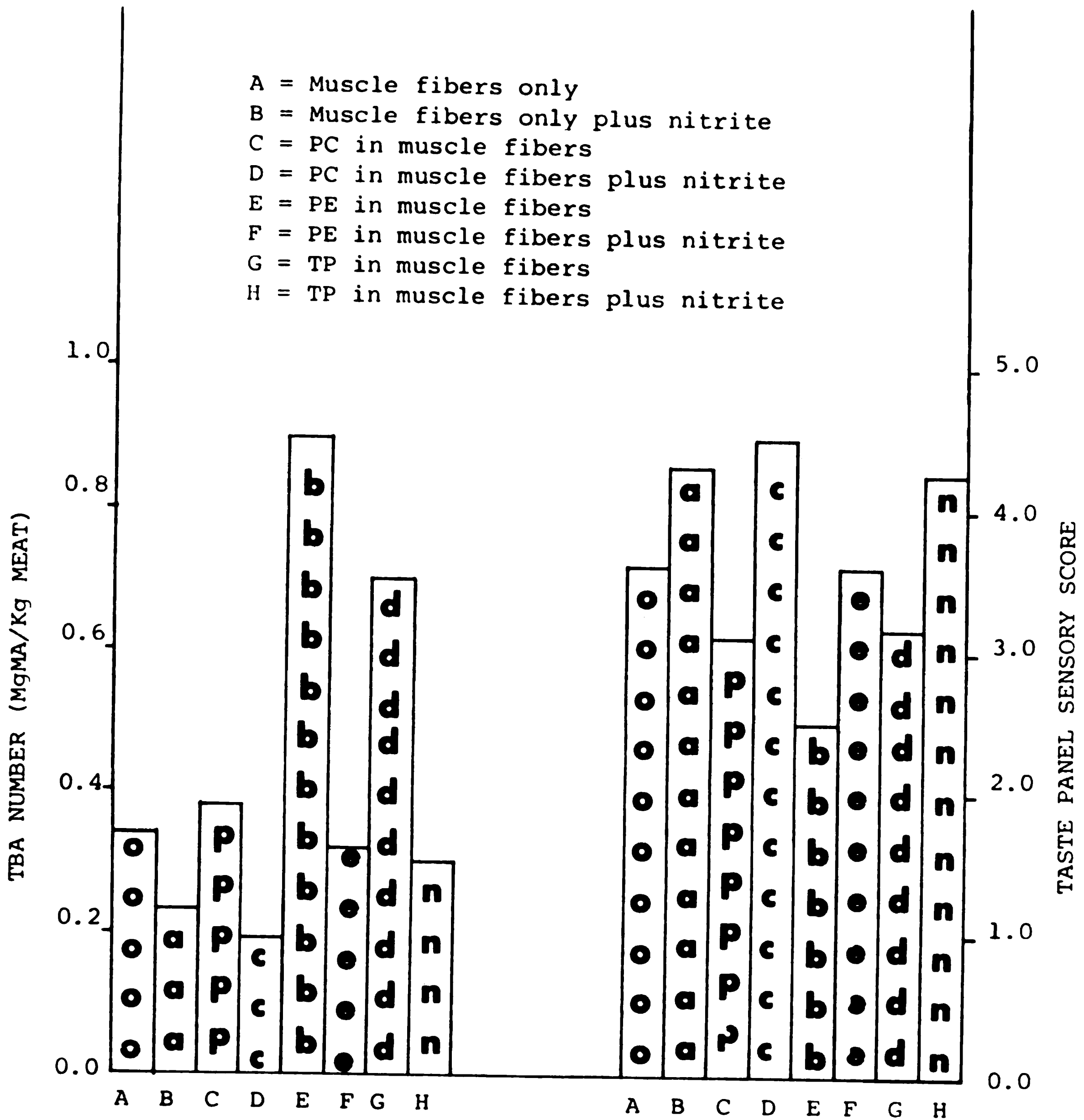


Figure 8. TBA number and sensory scores in cooked beef model meat systems containing purified phospholipids and nitrite.

Table 20. TBA numbers and sensory scores for cooked meat model systems containing added PC, PE, serum phospholipids or nitrite.[1,2]

Experimental Treatment	Mean TBA Number	Mean Sensory Score
1. Model system only	0.36±0.07[b]	3.33±0.42[a,b]
2. Model system + nitrite	0.26±0.06[a]	4.11±0.44[b,c]
3. Model system + PC	0.34±0.03[a,b]	3.36±0.51[a,b]
4. Model system + PC + nitrite	0.29±0.07[a]	4.52±0.27[c]
5. Model system + PE	0.81±0.13[d]	2.64±0.58[a]
6. Model system + PE + nitrite	0.36±0.07[b]	3.61±0.23[b]
7. Model system + TP	0.62±0.12[c]	3.29±0.49[a,b]
8. Model system + TP + nitrite	0.34±0.05[a,b]	4.04±0.43[b,c]

[1]Each treatment was replicated 4 times.

[2]A significant ($P<0.01$) "r" value of -.62 was found between TBA numbers and sensory score. Numbers in same column bearing same superscript are not significant at 5% level.

significantly influence either TBA numbers or sensory scores. Addition of 156 ppm of nitrite significantly ($P < 0.01$) reduced TBA numbers and improved WOF scores for all samples, except for the ones with added PC, which were not significantly altered.

Sato and Hegarty (1971) showed that nitrite will completely eliminate WOF at 220 ppm and will inhibit development at 50 ppm. Recently, Fooladi (1977) showed that 156 ppm of nitrite effectively prevents WOF in cooked meat and poultry. Zipser et al. (1964) proposed that nitrite forms a stable complex with iron porphyrins in heat denatured meat, thereby inhibiting the development of WOF. The inhibition of lipid oxidation in model systems containing only residual pigments suggests that nitrite converted the pigments to catalytically inactive forms and may have also directly reacted with unsaturated fatty acids. Thus, the results are in agreement with the report of Cassens et al. (1976) indicating that nitric oxide reacts with unsaturated fatty acids perhaps by chelation.

Results of this study have demonstrated that PE is the most important phospholipid component contributing to development of WOF. However, the other phospholipid components also have an additive effect. Thus, results are in excellent agreement with the data presented by Corliss and Dugan (1970) and Tsai and Smith (1971). They reported that PE exerted a much greater pro-oxidant

effect than PC. Hence, the present study confirms the involvement of phosphatidyl ethanolamine (PE) in development of off-flavors, and especially of WOF in cooked meat and meat products.

Summary of Results

The effects of triglycerides, total lipids and total phospholipids on the development of WOF in cooked meat were studied using lipid-free muscle fibers in combination with added triglycerides, total lipids and total phospholipids. In addition, pure samples of bovine PC, PE and serum phospholipids were also added to the model system in a second part of this study.

Preparations were heated to 70°C in retortable pouches, stored at 4°C for 48 hrs and evaluated by the TBA test and sensory panel scores. Changes in the fatty acids of the triglycerides, phospholipids, PC and PE were measured using GLC and TLC methods.

Results showed that adding total phospholipids to the model system significantly ($P < 0.01$) increased TBA, while significantly ($P < 0.05$) decreasing flavor scores. Addition of triglycerides to the model system did not significantly ($P > 0.05$) influence development of WOF in cooked meat. The triglycerides, however, when combined with phospholipids as total lipids did have a significant

influence ($P < 0.01$) on TBA numbers, while reducing flavor scores. Significant ($P < 0.05$) correlation coefficients of $r = -.57$ and $r = -.51$ were found between TBA values and sensory panel scores for beef and chicken white meat model systems, respectively.

Addition of PE and serum phospholipids significantly ($P < 0.01$) increased TBA values while decreasing sensory panel scores. The use of PC did not significantly influence either TBA values or sensory scores. Addition of 156 ppm of nitrite significantly ($P < 0.01$) reduced TBA numbers and prevented development of WOF.

Cooking caused significant ($P < 0.01$) decreases in PE of meat phospholipids while PC was rather more stable. In addition, PUFAS in PE were less stable to cooking and storage than those of PC.

Thus, total phospholipids are major contributors to development of WOF in cooked meat. The triglycerides enhance WOF development only when combined with phospholipids. In addition, PE is the most important component of total phospholipids contributing to WOF in cooked meat. Changes in the content of PUFAS, especially in those of PE, appear to be directly related to development of WOF. However, nitrite at a level of 156 ppm prevented WOF in cooked meat.

EXPERIMENT C

Effect of Length of Frozen Storage Time on the Stability of Meat Lipids and on Production of Off-Flavors

The principal objectives of this experiment were to study how length of frozen storage of raw meat influences the composition and stability of meat lipids as well as its effect on the production of rancidity in frozen meat and WOF in frozen-cooked meat.

Fresh cuts of beef (L-D), chicken dark meat and chicken white meat were frozen and stored at -18°C for over one year. The levels of lipids were measured at 0, 8 and 13 months during frozen storage. At the same time periods, the levels of malonaldehyde produced during oxidation of fresh and/frozen meat as well as in cooked meat held at 4°C or -18°C for 48 hrs were measured.

Levels of Extracted Lipids From Muscle Tissues

Total lipids were extracted from the meat by the procedure of Folch *et al*. (1957) while the levels of triglycerides and phospholipids were determined using the method of Choudhury *et al*. (1960).

Beef Lipids

The levels of triglycerides, total lipids and total phospholipids are presented in Table 21. Prior to frozen storage, fresh raw beef contained 13.72% total lipids, which decreased to 9.52 and 9.82% at 8 and 13 months of frozen storage, respectively. The rather high total lipid (13.72%) content of beef may be due to a high level of marbling. Wilson et al. (1976) reported a value of 14.79% for total lipids in a highly marbled beef cut. The results also support the report by Pearson et al. (1977) that total lipids in beef are highly variable, ranging from lower than 2.0 to over 12.0%.

Prior to frozen storage (0 time), the levels of total lipids and triglycerides in the cooked beef held at 4°C and at -18°C were slightly lower than the amount in the fresh uncooked meat. At 8 and 13 months of frozen storage, however, the concentrations of both triglycerides and phospholipids were higher in the cooked than in the fresh meat. Results showed that both total lipids and triglycerides significantly ($P < 0.01$) decreased during frozen storage, regardless of whether it was fresh or cooked.

The content of total phospholipids in raw meat were constant during frozen storage, being 0.71, 0.71 and 0.70% at 0, 8 and 13 months, respectively. Results verify the report by Pearson et al. (1977) that the phospholipid

Table 21. Effect of length of frozen storage, cooking and storage temperature upon beef lipids.

Lipids[1]	Storage Temp.	Storage Time (Months)		
		5	8	13
Total Lipids	Fresh/frozen	13.72±0.06[a]	9.52±0.60[b]	9.82±0.93[b]
	4°C[2]	13.01±0.04[a]	10.01±0.17[b]	10.45±0.21[b,c]
	-18°C[3]	13.26±0.20[a]	10.01±0.22[b]	10.95±0.42[b,c]
Triglycerides	Fresh/frozen Cooked and Held	12.89±0.12[a]	8.70±0.42[c]	9.09±0.83[c]
	+4°C[2]	11.98±0.05[b]	8.97±0.11[c]	9.33±0.04[c]
	-18°C[3]	12.18±0.23[b]	9.01±0.19[c]	9.85±0.43[d,c]
Phospholipids	Fresh/Frozen	0.71±0.03[a]	0.71±0.09[a]	0.70±0.13[a]
	+4°C[2]	0.93±0.02[b]	0.99±0.02[b]	0.94±0.01[b]
	-18°C[3]	0.95±0.01[b]	0.95±0.01[b]	0.98±0.01[b]

[1]Expressed as percentage of total tissue.

[2]Meat was cooked and held at 4°C for 48 hrs.

[3]Meat was cooked and held at -18°C for 48 hrs.

[4]Values within the same lipid group and in same row bearing the same superscript are not significantly different at P<.05.

fraction of tissue is relatively constant although the fat content is highly variable. The results are also in close agreement with the report by Dugan (1971) that the level of phospholipids in meat ranges from 0.5 to 1.0%.

Results also showed that cooking significantly ($P<0.01$) increased the amount of phospholipids in cooked beef. However, the differences in the content of phospholipids for cooked meat held at 4°C and -18°C were not statistically significant. Results of this study agree with the data presented by Campbell and Tutkki (1967) and Fooladi (1977), who found that the phospholipid concentration was higher in cooked than in raw meat. A graphic illustration of the variations in beef lipids due to freezing and cooking is shown in Figure 9.

Chicken Dark Meat Lipids

The levels of total lipids, triglycerides and phospholipids in chicken dark meat are presented in Table 22 and Figure 10. The amounts of total lipids in the raw meat were 9.12, 6.06 and 7.27% during frozen storage at 0, 8 and 13 months, respectively. When cooked, the levels of total lipids, triglycerides and phospholipids were significantly ($P < 0.05$) higher than the corresponding amounts in the uncooked meat, regardless of the length of freezer storage. Similar to beef, total lipids and triglycerides significantly ($P < 0.01$) decreased during

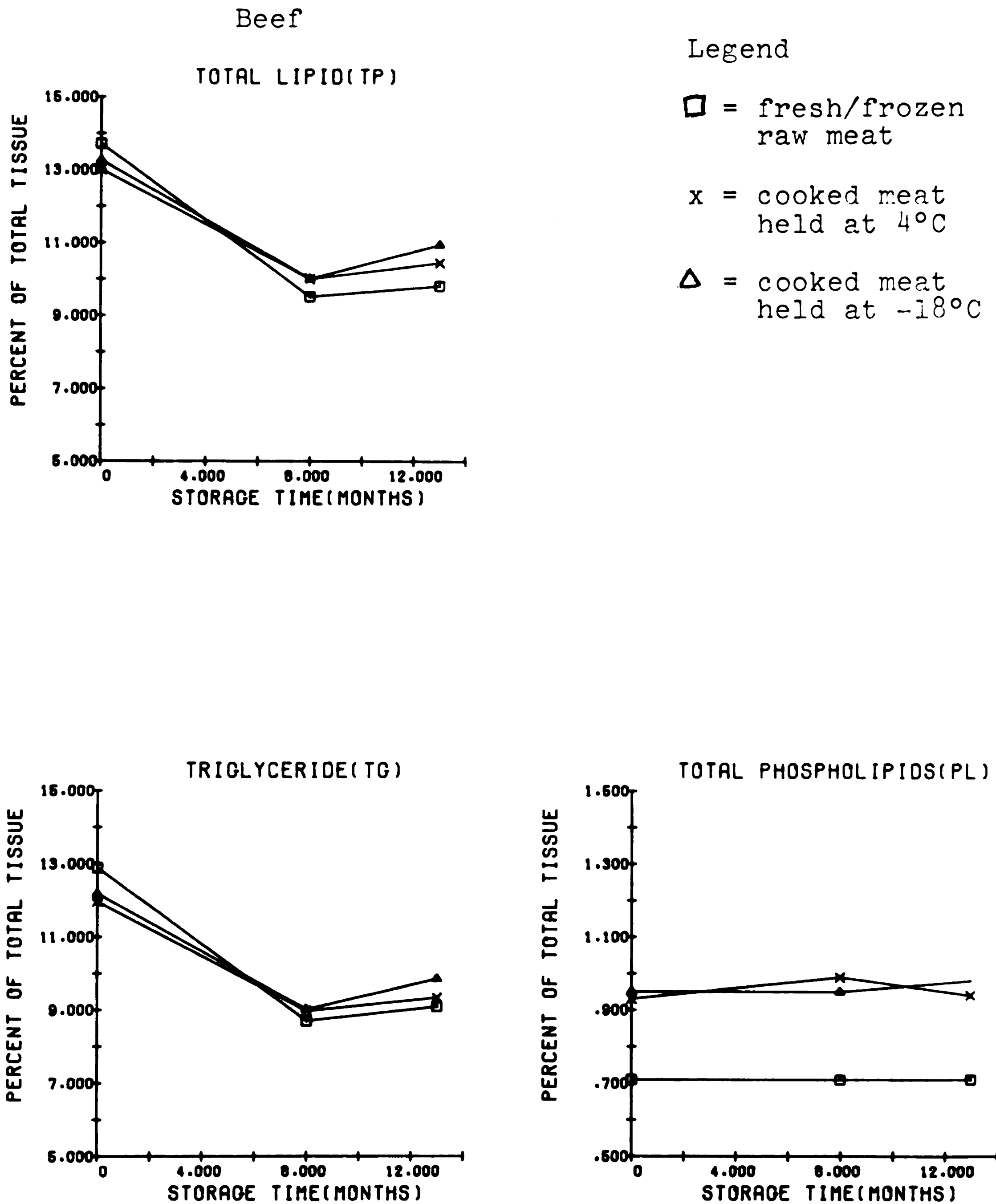


Figure 9. Influence of length of frozen storage and cooking on the levels of beef lipids.

Table 22. Effect of length of frozen storage, cooking and storage temperature upon chicken dark meat lipids.

Lipids[1]	Storage Temp.	Storage Period (Months)[4]		
		0	8	13
Total Lipids	Fresh/frozen	9.12±0.47[a]	6.06±0.21[c]	7.27±0.06[d]
	+4°C[2]	10.66±0.20[b]	7.00±0.19[d]	8.61±0.05[e]
	-18°C[3]	10.73±0.42[b]	7.18±0.07[d]	9.17±0.04[f]
Triglycerides	Fresh/frozen	8.20±0.43[a]	5.15±0.21[c]	6.42±0.06[e]
	+4°C[2]	9.53±0.16[b]	5.89±0.02[d]	7.55±0.07[f]
	-18°C[3]	9.64±0.35[b]	6.03±0.11[d]	7.99±0.01[g]
Phospholipids	Fresh/Frozen	0.82±0.03[a]	0.83±0.02[a]	0.85±0.01[a]
	+4°C[2]	0.96±0.01[b]	1.01±0.13[b]	0.98±0.00[b]
	-18°C[3]	0.99±0.01[b]	0.98±0.02[b]	0.99±0.01[b]

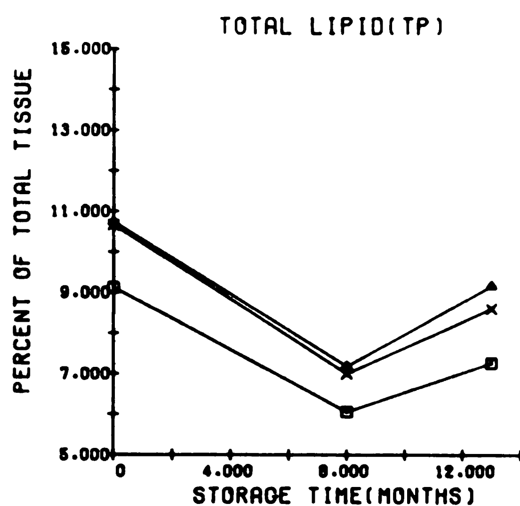
[1]Expressed as percentage of total tissue.

[2]Meat was cooked and held at 4°C for 48 hrs.

[3]Meat was cooked and held at -18°C for 48 hrs.

[4]Values within the same lipid group and in the same row bearing the same superscript are not significantly different at P<.05.

Chicken Dark Meat

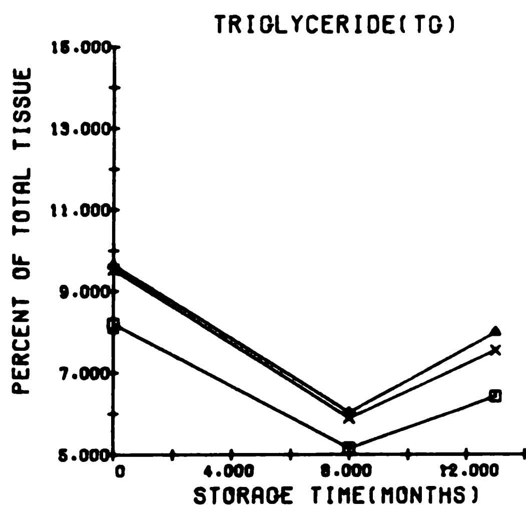


Legend:

□ = fresh/frozen raw meat

x = cooked meat held at 4°C

Δ = cooked meat held at -18°C



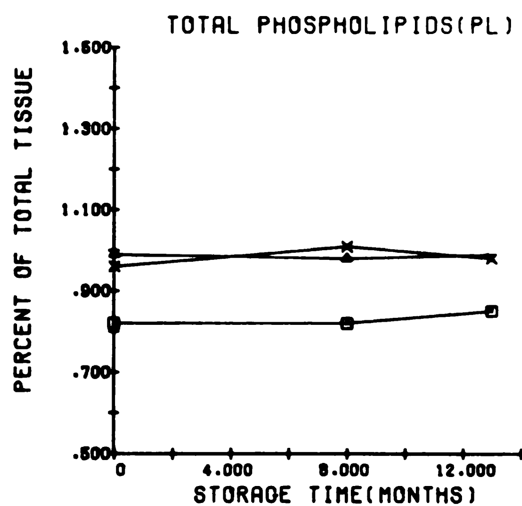


Figure 10. Influence of length of frozen storage and cooking on the levels of chicken dark meat lipids.

frozen storage, while the phospholipids remained relatively constant. The amount of phospholipids in raw chicken dark meat were 0.82, 0.82 and 0.85% at 0, 8, and 13 months, respectively. Hence, the levels of phospholipids in the raw dark meat were higher than those found in raw beef. Watts (1954) and Acosta et al. (1966) have also reported that the levels of phospholipids are lower in the red meats than in poultry meat.

Chicken White Meat Lipids

The contents of lipids extracted from chicken white meat are presented in Table 23 and in Figure 11. In the raw meat the amounts of total lipids were 2.58, 1.93 and 1.85% at 0, 8 and 13 months of frozen storage, respectively. The levels of total lipids and triglycerides in raw chicken white meat also decreased during frozen storage as was found in beef and chicken dark meat. Similar to beef and chicken dark meat, the amount of total lipids, triglycerides and phospholipids significantly ($P < 0.05$) increased during cooking, regardless of the length of freezer storage.

The phospholipids in raw chicken white meat amounted to 0.54, 0.53 and 0.52% at 0, 8 and 13 months of frozen storage, respectively. Phospholipids were higher in both the raw and cooked chicken dark meat (Table 22) than in the white meat (Table 23). This is in agreement with

Table 23. Effect of length of frozen storage, cooking and storage temperature upon chicken white meat lipids.

Lipids[1]	Storage Temp.[2]	Storage Period (Months)[4]		
		0	8	13
Total Lipids	Fresh/frozen	2.58±0.21[a]	1.93±0.00[d]	1.85±0.09[d]
	+4°C[2]	2.94±0.22[b]	2.25±0.15[e]	2.39±0.13[e]
	-18°C[3]	3.24±0.14[c]	2.31±0.01[e]	2.30±0.14[e]
Triglycerides	Fresh/frozen	2.00±0.14[a]	1.34±0.01[c]	1.31±0.06[c]
	+4°C[2]	2.66±0.25[b]	1.45±0.13[c]	1.54±0.06[c,d]
	-18°C[3]	2.48±0.11[b]	1.56±0.04[c,d]	1.61±0.09[c,d]
Phospholipids	Fresh/frozen	0.54±0.03[a]	0.53±0.01[a]	0.52±0.04[a]
	+4°C[2]	0.64±0.01[b]	0.67±0.06[b]	0.62±0.02[b]
	-18°C[3]	0.66±0.01[b]	0.67±0.02[b]	0.60±0.01[c]

[1]Expressed as percentage of total tissue.

[2]Meat was cooked and held at 4°C for 48 hrs.

[3]Meat was cooked and held at -18°C for 48 hrs.

[4]Values in the same lipid group and in same row and bearing the same superscript are not significantly different at $P<.05$.

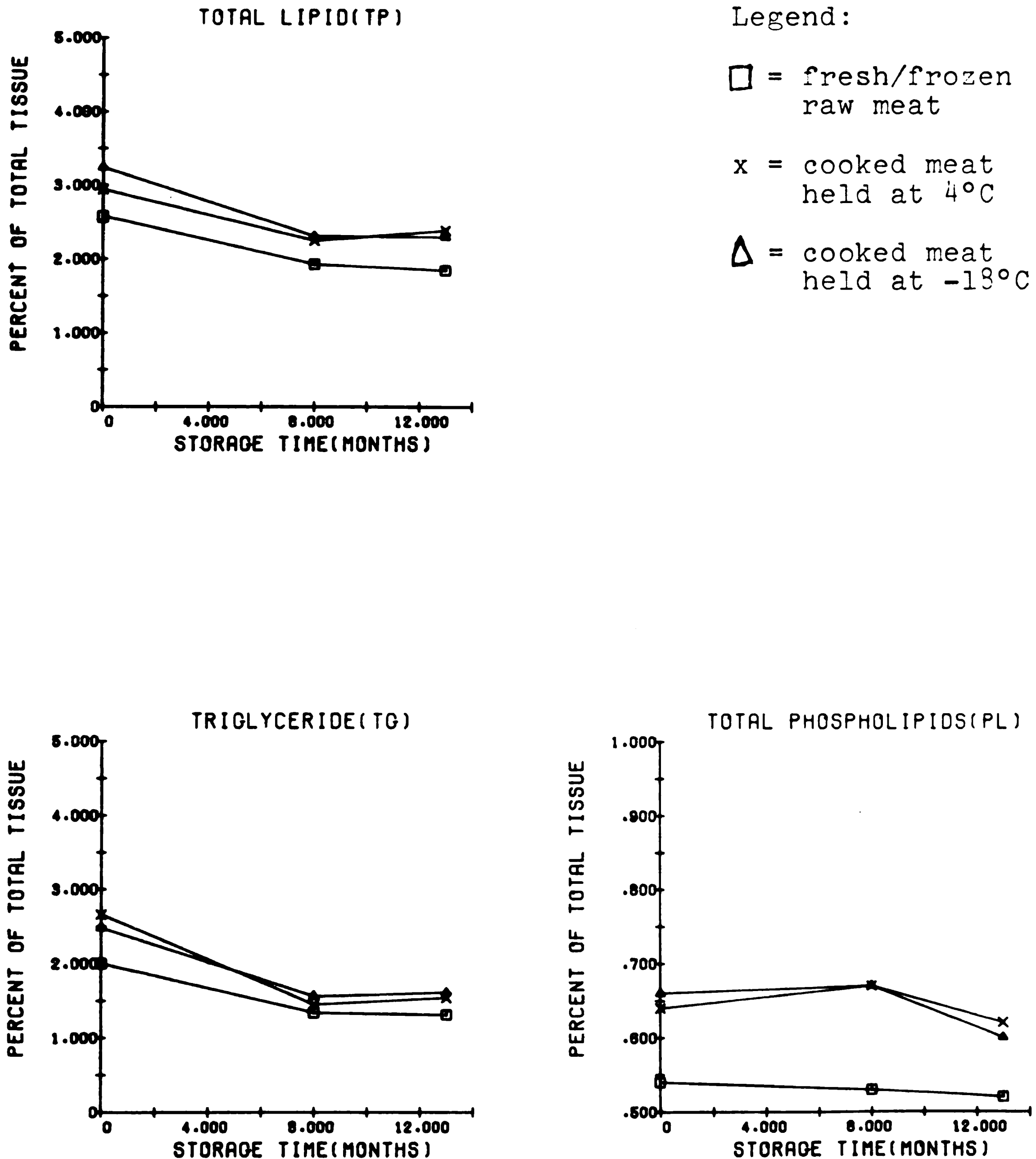


Figure 11. Influence of length of frozen storage and cooking on the levels of chicken white meat lipids.

Peng and Dugan (1965) and Acosta *et al*. (1966), who have reported that chicken dark meat contains higher levels of phospholipids than chicken white meat.

Effect of Frozen Storage on the Levels of Extractable Lipids

The data presented in Tables 21, 22 and 23 showed that significant ($P < 0.01$) losses occurred in both total lipids and triglycerides during frozen storage of raw beef, chicken dark meat and white meat. On the other hand, the levels of phospholipids in raw meat were not significantly changed during frozen storage.

The results of this study showing decreasing levels of total lipids during frozen storage are in agreement with the work of Zipser and Watts (1962). They reported a 21% decrease in total lipids of oxidizing mullet tissue during five-days of refrigerated storage. They did not, however, report any loss in gross phospholipid content during storage. On the contrary, Acosta *et al*. (1966) reported an apparent decrease in the phospholipid content of turkey tissues frozen for 180 days at -25°C. They also reported increased levels of total lipids in all tissues except the liver.

Results of this study showed that losses observed in total lipids during frozen storage were principally due to changes in the triglycerides, since the phospholipids

were relatively constant. Thus, results of this study are in agreement with the report by Campbell and Turkki (1967), who indicated that neutral lipids, being mostly intercellular, were more rapidly released by the tissue than the intracellular phospholipids.

It is not fully understood why total lipids decreased during frozen storage of meat, especially in the raw tissues. It is well known, however, that the phospholipids unlike the neutral lipids, are an integral part of the tissue membranes (Kono and Calowick, 1961) and are firmly held in an electrostatic interaction with the proteins (Gurr and James, 1971). According to Gurr and James (1971), the neutral lipids are loosely held within the tissues by Van der Waals forces and as such are easily eluted.

It will be seen later herein that the variation in the amount of total lipids appear to be positively related to the variation in the levels of malonaldehyde as measured by the TBA test.

Composition of Lipids in the Drippings

The lipids contained in the drippings of cooked meat were measured immediately after cooking and are presented in Table 24. The total lipids in the drippings were 1.67, 1.37 and 0.05% for beef, chicken dark meat and white meat, respectively. The concentration of

Table 24. Composition of Lipids in Cooked Drippings.

Lipids	Beef	Chicken Dark Meat	Chicken White Meat
Total Lipids	1.67[a]	1.37[a]	0.053[a]
	2.07[b]	1.77[b]	0.063[b]
Triglycerides	1.65[a]	1.36[a]	-----
	2.05[b]	1.76[b]	-----
Phosphospids	0.0094[a]	0.0089[a]	-----
		0.012[b]	-----

[a]Expressed as percentage of fresh tissue.

[b]Expressed as percentage of cooked tissue.

phospholipids in the drippings in comparison to the triglycerides was extremely low (about 0.01%).

Results of this study agree with the report by Campbell and Turkki (1967) indicating that the low concentration of phospholipids in the drippings is probably because the phospholipids are firmly held within the tissues. They also indicated that the high levels of triglycerides in the drippings can be accounted for by the fact they are mainly intercellular, and as such loosely bound by the tissues.

Composition and Stability of Phospholipid Components

The concentrations of LPC, SP, PE, PC and total phospholipids were measured in both the raw and cooked meat during frozen storage, using the phosphorus assay procedure of Rouser *et al*. (1966). The data representing combined mean values from beef, chicken dark meat and white meat are presented in Table 25 and in Figure 12. The combined data were used since the species values were not discordant.

Lysophosphatidyl Choline (LPC)

The concentrations of LPC in raw meat were 1.25, 1.43 and 1.93 mg phosphorus/g phospholipid at 0, 8 and 13 months of frozen storage, respectively. These values for LPC

Table 25. Effect of cooking, storage temperature and length of frozen storage on the stability of the individual phospholipid components in beef, chicken dark and white meat (combined).[1]

Phospholipids[2]	Storage Temp.	Storage Time (Months)		
		0	8	13
LPC[3]	Fresh/frozen	1.25±0.69	1.43±0.52	1.93±0.66
	+4°C[8]	1.54±0.67	1.80±0.39	1.09±0.56
	-18°C[9]	1.91±0.71	1.37±0.85	1.14±0.24
SP[4]	Fresh/frozen	2.34±0.59	1.88±0.88	1.45±0.60
	+4°C[8]	1.69±0.54	1.74±0.57	1.87±0.78
	-18°C[9]	1.95±0.66	1.67±0.54	0.92±0.59
PE[5]	Fresh/frozen	6.68±1.08	4.89±0.80	3.77±0.64
	+4°C[8]	2.55±1.06	2.64±1.03	2.58±0.63
	-18°C[9]	4.09±1.85	3.69±0.99	3.17±0.60
PC[6]	Fresh/frozen	16.70±0.99	14.39±1.94	12.21±1.82
	+4°C[8]	12.89±0.73	11.46±1.61	10.80±2.09
	-18°C[9]	15.48±0.99	11.60±2.84	12.23±2.40
TP[7]	Fresh/frozen	29.26±1.92	24.17±2.82	22.39±1.45
	+4°C[8]	24.85±0.98	20.52±2.00	20.62±2.01
	-18°C[9]	26.76±1.84	21.25±3.38	22.28±2.29

[1]Mean value of 12 determinations; four each from beef, chicken dark and white meat.
[2]Values expressed as mg phosphorus/g phospholipid.
[3]LPC = lysophosphatidyl choline.
[4]SP = Shingomyelin.
[5]PE = phosphatidyl ethanolamine.
[6]PC = Phosphatidyl choline.a
[7]TP = Total phospholipid phosphorus.
[8]Meat was cooked and held at 4°C for 48 hrs.
[9]Meat was cooked and held at -18°C for 48 hrs.

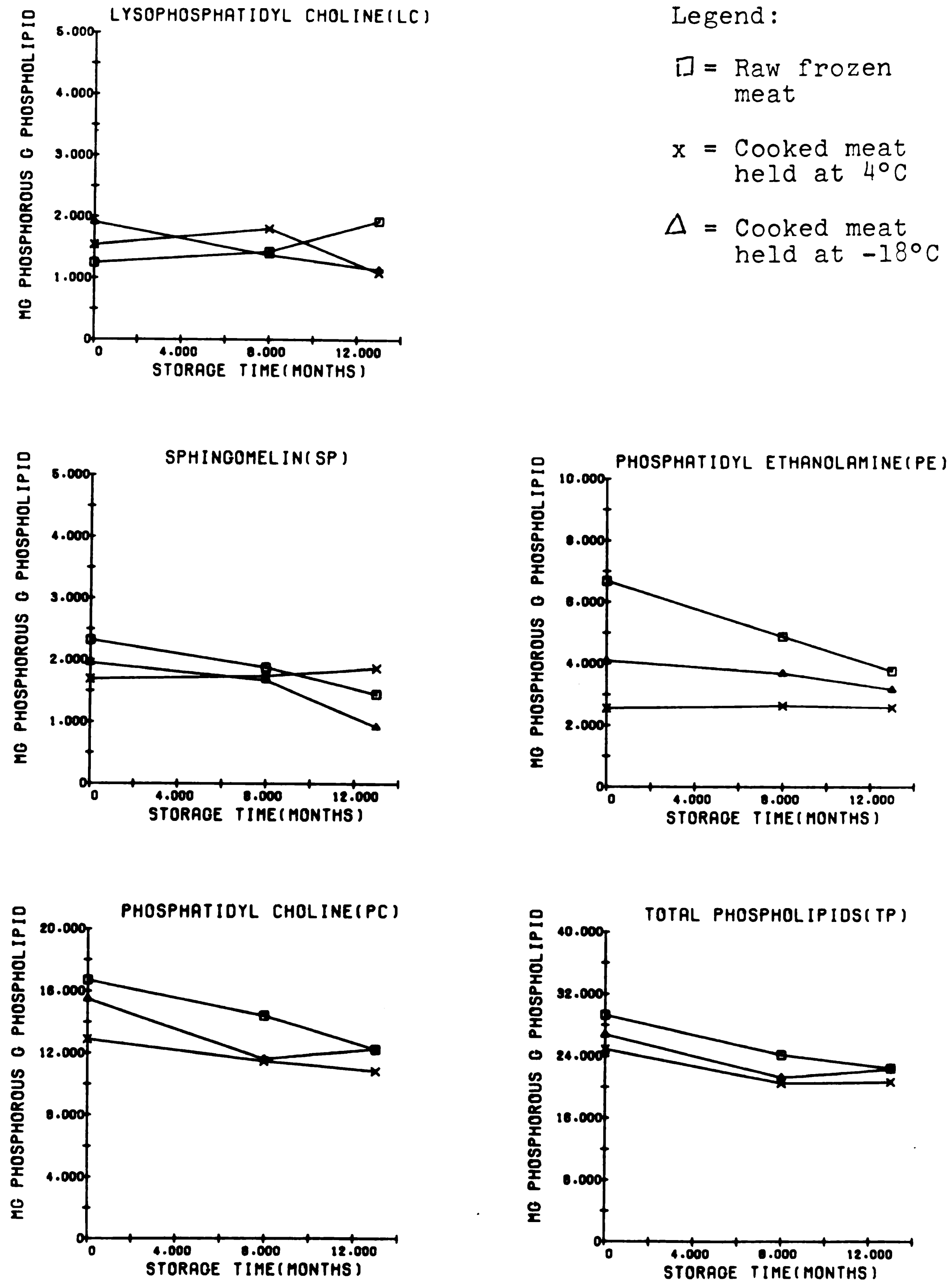


Figure 12. Influence of frozen storage and cooking on the composition and stability of LPC, SP, PE, PC and total phospholipid phosphorus (TP) in meat.

comprised 4.27, 4.90 and 6.60% of total phospholipids. The levels of LPC in fresh tissue seem to be quite variable. For instance, Davidkova and Khan (1967) reported a value of 1.5% in fresh chicken tissues, while Keller and Kinsella (1973) and Lee and Dawson (1976) reported values of 0.60 and 6.8% for LPC, respectively. The increasing levels of LPC (Table 25) during frozen storage suggest that lipolysis occurred. Several researchers (Awad et al., 1968; Braddock and Dugan, 1972) have reported increasing levels of LPC during frozen storage of meat, which is indicative of lipolytic activity.

Phosphatidyl Ethanolamine (PE)

Results presented for PE in Table 25 showed that the levels of PE decreased in the raw meat at 8 and 13 months during frozen storage. PE also decreased in the cooked meat held at -18°C for 48 hrs following cooking. The initial concentration of PE in fresh tissue (6.68 mg phosphorus/g phospholid) was 22.83% of the total phospholipids. This is in good agreement with values reported by Davidkova and Khan (1967) and Keller and Kinsella (1973). The levels of PE in raw tissues were 4.89 and 3.77 mg phosphorus/g phospholipid at 8 and 13 months of frozen storage, respectively. These values accounted for a loss of 26.80 and 43.56%, respectively, from the initial concentration of PE at 0 time.

The levels of PE were significantly ($P < 0.05$) higher in fresh/raw frozen than in cooked meat, regardless of the length of freezer storage. However, PE was relatively higher in the cooked meat held at -18°C for 48 hrs than in the cooked meat held at 4°C for 48 hrs. Thus, results indicated a higher rate of lipid autoxidation in the cooked meat held at 4°C for 48 hours than either that of the cooked meat held at -18°C for 48 hours or in the raw frozen meat stored at -18°C from 0 to 13 months.

Phosphatidyl Choline (PC)

The initial level of PC in fresh tissue at 0 time was 57.07% of the total phospholipids. This value is in good agreement with those reported for PC by Peng and Dugan (1965) and Keller and Kinsella (1973). The concentration of PC in raw meat was found to decline during frozen storage, the levels being 16.70, 14.39 and 12.21 mg phosphorus/g phospholipid at 0, 8 and 13 months, respectively. The values at 8 and 13 months amounted to losses of 13.83 and 26.88% of PC from the original value at 0 time, respectively.

Levels of PC also consistently declined in the cooked meat held at 4°C for 48 hrs. Although the levels of PC in cooked meat held at -18°C for 48 hrs declined, the rate of decline was not consistent, the concentrations being 15.48, 11.60 and 12.23 mg phosphorus/g phospholipid at

0, 8 and 13 months, respectively. Results showed that like PE, the lowest concentrations of PC were found in the cooked meat held at 4°C for 48 hrs, regardless of the length of freezer storage. Thus, a higher rate of lipid oxidation occurred in the cooked meat held at 4°C for 48 hrs. than for the other two treatments.

Total Phospholipids (TP)

The concentrations of total phospholipids in the raw meat were 29.26, 24.17 and 22.39 mg phosphorus/g phospholipid at 0, 8 and 13 months of frozen storage, respectively. In the cooked meat held at 4°C for 48 hrs, the levels of TP were lower, being 24.85, 20.52 and 20.62 mg phosphorus/g phospholipid at 0, 8 and 13 months, respectively. Similarly, the levels of TP for the cooked meat held at -18°C for 48 hrs were 26.76, 21.25 and 22.28 mg phosphorus/g phospholipid, respectively, at the same storage periods.

Thus, the levels of phospholipids declined consistently during frozen storage of raw meat. This is in agreement with the report of Zipser _et al_. (1962) showing a progressive loss in total phospholipids during refrigerated storage of mullet tissues.

Analysis of variance showed that total phospholipids were significantly ($P < 0.05$) higher in raw than in cooked meat. However, TP tended to be higher in the cooked held at -18°C for 48 hrs following cooking in comparison

to that held at 4°C. This indicates that a higher rate of lipid autoxidation occurred in cooked meat held at 4°C for 48 hrs following cooking.

Results of this study (Table 25) have shown that PE, PC and TP were more stable in raw frozen meat than in that cooked and held either at 4°C or at -18°C for 48 hrs following cooking. Results also showed that PE was less stable than PC, especially for the cooked meat held at 4°C for 48 hrs. Thus, results further verify the instability of PE, especially in cooked meat. Furthermore, results have demonstrated that phospholipid components were less stable in the cooked meat held at 4°C for 48 hrs following cooking, thus, indicating a higher rate of lipid autoxidation than for the other treatments.

The effect of cooking on changes in the amounts of PC and PE is shown in Figure 13. Plate A represents phospholipid components from fresh beef tissue while plate B shows the same components following cooking and storage of the tissue. Quantitative differences were evident in three unidentified components (X, Y and Z) and also in PC and PE. This can be seen by comparing plates A and B, which show a reduction in the amount of the unidentified components as well as in PC and PE in the cooked sample. Thus, results of this study further verify the involvement of the phospholipids in development of WOF in cooked meat, since they were shown to be less stable to cooking.

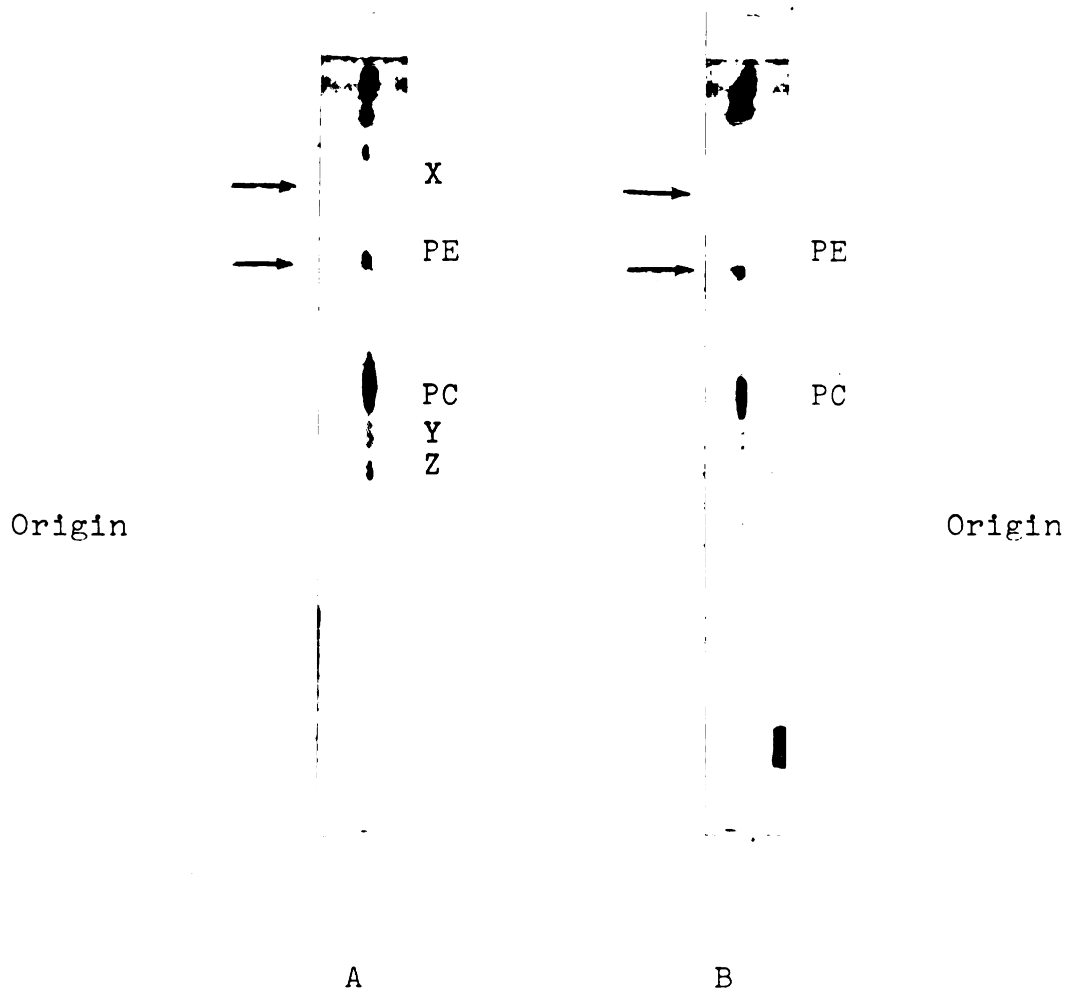


Figure 13. Effect of cooking on quanitative changes of PC and PE on thin layer plates. A represents total phospholipids from fresh uncooked beef tissue, while B shows the total phospholipids from cooked beef. Note the changes in size of the spots for PC, PE, and the unidentified spots X, Y and Z.

Phospholipid Components in the Drippings

Analysis of the composition of the phospholipids recovered from beef drippings is shown in Figure 14. Samples A and C represent drippings from beef, while B is from a standard mixture of known phospholipid components. Phosphatidyl ethanolamine (PE) was completely absent in the cooked drippings (Figure 14). The absence of PE in the drippings indicates that PE is more tightly bound to the membrane than PC or the phospholipid components. Thus, the level of PE in the meat would increase during cooking and more PE could be available to react with atmospheric oxygen. The results indicate that PE is more important in development of oxidized flavor than previously believed.

Changes in Fatty Acid Composition of Lipids During Frozen Storage and Cooking

The fatty acid composition of the triglycerides, total lipids, PC and PE were analyzed in fresh and in frozen raw meat as well as in the cooked meat held at 4°C and at -18°C for 48 hrs after cooking.

Triglycerides

Changes in the fatty acid composition of beef triglycerides during frozen storage and cooking is presented

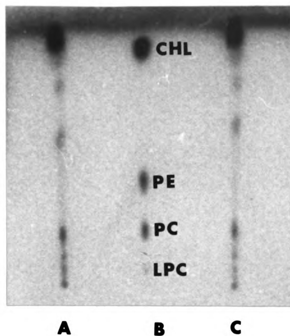


Figure 14. Thin layer plate showing the composition of the phospholipid components in the drippings of cooked meat. A and C represent total phospholipids from cooked beef drippings. B shows a standard mixture containing LPC, PC, PE and cholesterol (CHL). X indicates position of the missing PE in the cooked drippings.

in Table 26. The percentages of total unsaturation in raw frozen beef were 54.77, 52.24 and 53.52% at 0, 8 and 13 months, respectively. In the cooked meat held at 4°C, total unsaturation was slightly less, being 53.17, 51.53 and 53.20% at 0, 8 and 13 months, respectively. Similarly, the levels of total unsaturates in the cooked meat held at -18°C for 48 hrs after cooking were 54.89, 52.31 and 51.09% at 0, 8 and 13 months, respectively. Although total unsaturation slightly decreased during frozen storage of beef, the difference in unsaturation between the raw frozen and cooked meat does not appear to be significant. The small changes in total unsaturation reflected the stability of the saturated, mono- and dienoic fatty acids during frozen storage and cooking. Thus, the data provide further verification of the stability of fatty acids in the triglycerides in either frozen or cooked meat.

The fatty acid composition in chicken dark meat triglycerides are presented in Table 27. The percentages of total unsaturation in raw meat gradually increased during frozen storage, the values being 69.47, 71.81 and 73.29% at 0, 8 and 13 months, respectively. In the cooked chicken dark meat held at 4°C, the levels of total unsaturates at 0, 8 and 13 months, were 71.03, 71.52 and 70.25%, respectively. Similarly, the corresponding values for cooked meat held at -18°C for 48 hrs following

Table 26. Changes in the fatty acid composition of beef triglycerides during frozen storage and cooking.[a]

Fatty Acids	0 Month			8 Months			13 Months		
	Fresh Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C
12:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
14:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
14:1	5.76	3.76	4.16	3.44	4.03	4.01	3.77	3.48	3.16
15:0	1.87	1.60	2.00	0.70	0.64	0.92	0.99	0.93	0.56
15:1	0.49	0.72	0.50	0.35	0.48	0.33	0.47	0.30	0.42
16:0	27.99	30.04	28.30	33.48	33.82	33.40	28.24	28.35	33.59
16:1	4.94	5.04	5.83	5.28	5.56	6.02	4.14	3.87	3.65
16:2	1.10	0.96	0.82	0.70	1.29	1.34	1.13	0.77	0.71
17:0	0.83	0.80	0.83	0.53	0.48	0.67	0.94	0.58	0.63
18:0	14.54	14.39	13.98	13.05	13.53	12.70	16.31	16.94	14.13
18:1	40.50	40.45	40.23	41.41	39.05	39.26	41.99	42.69	41.05
18:2	1.98	1.44	2.46	1.06	1.12	1.35	1.13	1.36	1.26
$18:3w_6$	-----	-----	-----	-----	-----	-----	0.14	-----	-----
$18:3w_3$	-----	00.80	0.89	-----	-----	-----	0.56	0.46	0.84
20:1	-----	-----	-----	-----	-----	-----	0.19	0.27	-----
% Sat	45.23	46.83	45.11	47.76	48.47	47.69	46.48	46.80	48.91
% Mono	51.69	49.97	50.72	50.48	49.12	49.62	50.56	50.61	48.28
% di & Mono–	3.08	3.20	4.17	1.76	2.41	2.69	2.96	2.59	2.81
% Total Unsat	54.77	53.17	54.89	52.24	51.53	52.31	53.52	53.20	51.09

[a]Calculated as percent of total fatty acids.

cooking were 67.69, 72.94 and 70.69%. These results showed that the fatty acids in the triglycerides of chicken dark meat are not greatly altered during frozen storage and cooking.

The fatty acid composition of chicken white triglycerides are presented in Table 28. While the levels of total unsaturation slightly increased during frozen storage of raw chicken dark meat (Table 27), the reverse was the case during frozen storage of raw chicken white meat (Table 28). The levels of total unsaturation in frozen chicken white meat were slightly less than the values in cooked white meat, although the amount of unsaturates in the latter also slightly declined during frozen storage. Only minor variations were found in saturated, mono-, di-, or polyenoic fatty acids during frozen storage and cooking.

Results showed that only minor changes occurred in the fatty acid profiles of triglycerides during frozen storage and cooking of either beef (Table 26), chicken dark meat (Table 27) or chicken white meat (Table 28). Thus, the results are in agreement with the work of Chang and Watts (1952), Campbell and Turkki (1967) and Igene and Pearson (1978) showing that only slight changes occurred in the fatty acid composition of triglycerides during cooking of meat.

Table 27. Changes in the fatty acid composition of chicken dark meat triglycerides during frozen storage and cooking.[a]

	0 Month			8 Months			13 Months		
		Cooked and Stored			Cooked and Stored			Cooked and Stored	
Fatty Acids	Fresh Meat	+4°C	-18°C	Frozen Meat	+4°C	-18°C	Frozen Meat	+4°C	-18°C
14:1	0.68	0.72	0.61	0.89	0.90	0.62	0.81	0.90	0.78
15:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
15:1	-----	-----	-----	-----	-----	-----	-----	-----	-----
16:0	25.26	23.82	26.15	22.22	22.25	21.30	21.04	23.88	21.18
16:1	5.04	5.18	5.44	6.03	6.07	5.81	4.07	4.62	4.11
16:2	-----	-----	-----	0.47	0.43	0.35	-----	0.16	0.26
17:0	-----	0.38	0.38	0.30	0.48	0.13	-----	-----	-----
18:0	5.26	4.77	5.78	5.67	5.75	5.63	5.67	5.87	8.13
18:1	41.68	44.63	40.79	40.78	39.90	41.81	42.78	43.17	38.98
18:2	20.63	19.38	19.24	22.34	22.35	22.72	23.72	19.74	23.95
$18:3w_6$	0.26	0.08	0.18	-----	0.27	0.18	0.29	0.20	0.29
$18:3w_3$	1.18	1.04	1.43	1.30	1.60	1.45	1.40	1.23	1.62
20:1	-----	-----	-----	-----	-----	-----	-----	-----	0.35
20:4	-----	-----	-----	-----	-----	-----	0.22	0.23	0.35
% Sat	30.53	28.97	32.31	28.19	28.48	27.06	26.71	29.75	29.31
% Mono	47.40	50.53	46.84	47.70	46.87	48.24	47.66	48.69	44.22
% Di	20.63	19.38	19.24	22.81	22.78	23.07	23.72	19.90	24.21
% Poly	1.44	1.12	1.61	1.30	1.87	1.63	1.91	1.66	2.26
Total Unsat	69.47	71.03	67.69	71.81	71.52	72.94	73.29	70.25	70.69

[a]Calculated as percent of total fatty acids.

Table 28. Changes in the fatty acid composition of chicken white meat triglycerides during frozen storage and cooking.[a]

	0 Month			8 Months			13 Months		
		Cooked and Stored			Cooked and Stored			Cooked and Stored	
Fatty Acids	Fresh Meat	+4°C	-18°C	Frozen Meat	+4°C	-18°C	Frozen Meat	+4°C	-18°C
14:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
14:1	0.98	0.66	0.68	0.41	0.54	0.45	0.85	0.87	0.82
15:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
15:1	0.52	1.05	0.43	-----	0.32	-----	0.37	0.39	0.33
16:0	18.84	18.40	17.85	22.04	18.25	19.73	22.21	22.00	19.78
16:1	4.31	4.15	4.21	5.23	4.45	4.21	4.27	5.24	4.09
16:2	-----	-----	-----	0.42	0.36	0.41	0.37	0.28	0.22
17:0	-----	-----	-----	0.34	0.36	0.36	0.27	0.37	0.16
18:0	7.63	6.50	7.12	6.42	6.93	6.88	6.49	6.88	6.95
18:1	43.17	43.35	42.15	41.84	44.37	43.89	37.32	39.12	38.39
18:2	22.59	21.67	23.67	22.18	22.29	22.53	24.68	21.83	25.88
18:3w_6	0.26	0.33	-----	0.28	0.25	0.18	0.30	-----	0.33
18:3w_3	1.70	1.70	1.08	0.84	1.88	1.36	1.71	1.75	1.96
20:1	-----	1.39	2.16	-----	-----	-----	0.09	0.17	-----
20:2	-----	0.80	0.65	-----	-----	-----	-----	-----	-----
20:3	-----	-----	-----	-----	-----	-----	0.21	0.30	-----
20:4	-----	-----	-----	-----	-----	-----	0.86	0.80	1.09
% Sat	26.47	24.90	24.97	28.80	25.54	26.97	28.97	29.25	26.89
% Mono	48.98	50.60	49.63	47.48	49.68	48.55	42.90	45.79	43.63
% Di	22.59	22.47	24.32	22.60	22.65	22.94	25.05	22.11	26.10
% Poly	1.96	2.03	1.08	1.12	2.13	1.54	3.08	2.85	3.38
Total Unsat	73.53	75.10	75.03	71.20	74.46	73.03	71.03	70.75	73.11

[a]Calculated as percent of total fatty acids.

Changes in the Fatty Acid Composition of Total Phospholipids

Beef Phospholipids

The fatty acid composition of beef phospholipids is presented in Table 29. The proportions of saturated, mono-, di- and polyenoic fatty acids in the fresh uncooked beef tissue at 0 time were 32.88, 39.67, 11.95 and 15.50%, respectively. These values are in good agreement with those reported by Hornstein *et al.* (1967) and O'Keefe *et al.* (1968).

Significant changes occurred in the unsaturated fatty acids in the phospholipids (unlike the beef triglycerides) during frozen storage and cooking. The losses in unsaturation could be largely accounted for by changes in $C_{18:2}$, $C_{20:3}$ and $C_{20:4}$ fatty acids. In the raw frozen meat, the level of dienoic acid, which was 11.95% at 0 time, decreased to 6.49 and 7.82% at 8 and 13 months of frozen storage, respectively. Similarly, the initial level of 15.50% polyenoic acids in the fresh beef declined to 6.80 and 7.39% at 8 and 13 months of frozen storage, respectively. Thus, total unsaturation in the raw tissues declined during frozen storage, the levels being 67.12, 58.41 and 58.40% at 0, 8 and 13 months, respectively. The observed losses in unsaturation would indicate the occurrence of lipid oxidation during frozen

Table 29. Changes in the fatty acid composition of beef phospholipids during frozen storage and cooking.[a]

Fatty Acids	0 Month			8 Months			13 Months		
	Fresh Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C
12:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
14:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
14:1	1.46	0.72	1.85	2.52	1.27	2.42	1.84	2.03	2.10
15:0	0.52	-----	0.82	0.50	0.67	0.55	0.53	0.34	0.71
15:1	1.16	1.86	2.06	0.95	0.53	0.62	0.62	0.30	0.98
16:0	18.49	18.73	20.03	27.73	22.65	25.27	26.87	23.93	23.13
16:1	3.61	3.67	4.33	5.29	4.00	5.59	3.32	3.38	3.58
16:2	0.74	0.25	0.41	0.76	0.60	0.62	0.71	0.85	0.46
17:0	0.92	0.84	0.62	0.76	0.80	0.91	0.70	0.81	0.75
18:0	12.95	12.83	13.19	12.60	14.51	13.26	13.50	16.56	13.76
18:1	33.44	34.81	35.66	35.92	36.98	38.07	37.41	36.80	35.57
18:2	10.52	12.66	10.10	5.29	7.19	5.39	6.81	6.39	8.95
18:3w_6	0.37	-----	-----	-----	1.20	0.78	-----	-----	0.13
18:3w_3	1.29	0.68	0.91	0.57	-----	-----	0.70	1.35	0.59
20:1	-----	-----	-----	0.44	0.67	0.28	-----	0.20	0.16
20:2	0.69	0.21	0.25	0.44	0.47	0.17	0.30	0.25	0.39
20:3	2.77	1.92	1.73	0.76	1.60	1.14	1.07	1.08	1.46
20:4	8.51	9.32	6.59	3.02	6.33	3.87	4.56	4.83	6.25
20:5	0.76	-----	-----	-----	0.53	-----	-----	-----	0.18
22:2	-----	-----	-----	-----	-----	-----	-----	-----	-----
22:3	-----	-----	-----	-----	-----	-----	-----	-----	0.13
22:4	0.88	0.42	0.63	-----	-----	0.38	0.59	0.24	0.39
22:5w_6	-----	-----	-----	1.57	-----	0.06	-----	-----	0.33
22:5w_3	0.92	1.08	0.82	0.88	-----	0.62	0.47	0.66	-----
22:6	-----	-----	-----	-----	-----	-----	-----	-----	-----
% Sat	32.88	32.40	34.66	41.59	38.63	39.99	41.60	41.64	38.35
% Mono	39.67	41.06	43.90	45.12	43.45	46.98	43.19	42.71	42.39
% Di	11.95	13.12	10.76	6.49	8.26	6.18	7.82	7.49	9.80
% Poly	15.50	13.42	10.68	6.80	9.66	6.85	7.39	8.16	9.46
Total Unsat	67.12	67.60	65.34	58.41	61.37	60.01	58.40	58.36	61.65

[a]Calculated as percent of total fatty acids.

storage.

Significant losses occurred in both the dienoic and polyenoic fatty acids of the cooked meat held at either 4°C or at -18°C for 48 hrs at all periods of frozen storage. In the cooked meat held at 4°C, the initial level of total unsaturation was 67.60%, but it declined to 61.37 and 58.36% at 8 and 13 months of frozen storage, respectively. Similarly, the corresponding values for the cooked meat held at -18°C for 48 hrs following cooking were 65.34, 60.01 and 61.65% at 0, 8 and 13 months, respectively. Thus, results demonstrated the occurrence of lipid oxidation in the cooked meat throughout storage.

Chicken Dark Meat Phospholipids

The fatty acid composition for chicken dark meat phospholipids is presented in Table 30. The levels of saturated, mono-, di- and polyenoic fatty acids in the fresh unfrozen tissues were 33.76, 21.99, 21.51 and 22.74%, respectively. Most of the changes in unsaturation during frozen storage and cooking were due to alterations in the levels of $C_{18:1}$, $C_{18:2}$, $C_{20:4}$ and $C_{22:4}$ fatty acids. Although the concentration of mono- and dienoic fatty acids gradually increased during storage of the raw frozen tissues, the level of polyenoic fatty acids declined. The initial concentration of PUFAS was 22.74% at 0 time, but it declined to 19.69 and 11.06% at 8 and 13 months,

Table 30. Changes in the fatty acid composition of chicken dark meat phospholipids during frozen storage and cooking.[a]

Fatty Acids	0 Month			8 Months			13 Months		
	Fresh Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C
12:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
14:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
14:1	0.14	0.17	0.16	-----	-----	0.22	-----	-----	0.28
15:0	-----	-----	-----	0.54	0.86	-----	-----	-----	-----
15:1	0.37	0.57	0.31	-----	-----	0.88	-----	-----	0.23
16:0	15.60	16.52	16.44	12.63	18.44	15.65	17.99	23.27	19.85
16:1	1.05	1.03	0.79	1.61	2.20	2.88	2.57	2.00	2.15
16:2	-----	0.23	0.17	0.21	0.39	0.19	0.34	0.28	0.23
17:0	-----	-----	-----	0.27	0.43	0.51	-----	0.38	-----
18:0	18.16	21.03	20.83	18.19	18.44	15.51	12.47	17.27	15.31
18:1	20.43	19.74	19.34	23.94	26.07	28.53	31.75	26.27	29.48
18:2	21.51	19.22	20.11	21.83	20.95	23.26	23.63	18.00	18.82
13:3w_6	0.37	0.23	0.35	0.24	0.12	0.19	0.28	0.13	0.35
18:3w_3	0.41	0.43	0.53	0.88	0.45	0.64	1.21	0.88	0.68
20:1	-----	-----	0.26	0.13	1.08	-----	0.19	-----	0.23
20:2	-----	-----	-----	0.96	0.97	1.42	-----	-----	-----
20:3	0.53	0.52	0.57	0.44	-----	0.22	0.34	0.75	0.60
20:4	17.41	18.88	15.28	13.86	8.44	8.97	7.90	8.76	10.43
20:5	-----	-----	-----	-----	-----	-----	-----	-----	-----
22:3	-----	-----	-----	0.29	-----	-----	-----	-----	-----
22:4	1.23	0.57	1.84	1.12	0.60	0.42	0.42	0.88	0.74
22:5w_6	-----	-----	-----	0.21	-----	-----	-----	-----	-----
22:5w_3	2.79	0.86	0.33	0.24	-----	-----	-----	-----	-----
22:6	-----	-----	2.69	2.41	0.56	0.51	0.91	1.13	0.62
% Sat.	33.76	37.55	37.27	31.63	38.17	31.67	30.46	40.92	35.16
% Mono-	21.99	21.51	20.86	25.68	29.35	32.51	34.51	28.27	32.37
% Di-	21.51	19.45	20.28	23.00	22.31	24.87	23.97	18.28	19.05
% Poly	22.74	21.49	21.59	19.69	10.17	10.95	11.06	12.53	13.42
Total Unsat	66.24	62.45	62.73	68.91	61.83	68.33	69.54	59.08	64.84

[a]Calculated as percent of total fatty acids.

respectively. Results indicate the involvement of the PUFAS in development of rancidity during frozen storage of meat. Nevertheless, total unsaturation in the raw frozen tissues increased gradually from 66.24% at 0 time to 68.91 and 69.54% at 8 and 13 months of frozen storage, respectively. The increasing levels of monounsaturated fatty acids during frozen storage of raw tissues largely accounted for the increased total unsaturation during frozen storage.

While the concentration of monoenoic fatty acids rose significantly in the cooked meat, the level of polyenoic fatty acids declined drastically. The dienoic fatty acids were relatively unchanged during cooking. The polyenoic fatty acids in cooked chicken dark meat held at 4°C for 48 hrs amounted to 21.49, 10.17 and 12.53% at 0, 8 and 13 months, respectively. Similarly, the corresponding levels in the cooked meat held at -18°C for 48 hrs after cooking were 21.59, 10.95 and 13.42% at the same time intervals, respectively. Results showed that most of the losses in unsaturation occurred in $C_{20:4}$ in particular, and in the PUFAS in general. Thus, the involvement of the PUFAS in the development of WOF in cooked meat is verified.

Chicken White Meat Phospholipids - Most of the changes in the fatty acid profiles of chicken white meat (Table 31) phospholipids occurred in the polyenoic fatty acids.

Table 31. Changes in the fatty acid composition of chicken white meat phospholipids during frozen storage and cooking.[a]

Fatty Acids	0 Month			8 Months			13 Months		
	Fresh Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C
12:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
14:0	-----	-----	-----	-----	-----	-----	-----	-----	-----
14:1	0.65	0.15	-----	-----	-----	-----	-----	-----	-----
15:0	-----	-----	0.26	-----	-----	-----	-----	-----	-----
15:1	2.01	0.57	0.61	0.95	0.67	1.58	-----	-----	-----
16:0	14.26	18.25	20.45	19.98	20.86	17.85	20.61	22.18	20.13
16:1	0.70	0.84	1.02	1.14	0.94	0.85	0.79	0.80	0.83
16:2	-----	0.23	-----	0.19	0.27	0.28	-----	0.23	0.08
17:0	-----	-----	-----	0.43	0.36	0.45	-----	0.17	-----
18:0	14.67	14.78	14.82	13.13	16.36	14.91	17.98	14.84	18.80
18:1	21.44	21.99	21.90	19.26	25.40	22.59	21.17	20.93	19.46
18:2	16.30	17.78	16.44	14.27	15.37	13.56	16.61	18.47	16.70
18:3w_6	2.85	0.23	-----	-----	-----	-----	-----	-----	-----
18:3w_3	-----	0.57	0.15	0.14	0.27	9.55	0.28	0.60	0.50
20:1	-----	-----	-----	-----	-----	-----	-----	-----	-----
20:2	0.39	0.34	0.38	4.56	1.35	0.76	-----	0.23	-----
20:3	0.79	1.45	1.23	0.48	0.81	0.68	0.51	0.91	0.67
20:4	17.23	15.77	16.34	11.09	13.85	13.44	16.89	15.70	15.47
20:5	-----	0.44	-----	0.36	-----	-----	0.42	-----	0.37
22:3	1.96	1.59	-----	-----	-----	-----	-----	-----	-----
22:4	0.52	0.28	1.17	0.52	0.45	0.45	1.61	1.19	1.50
22:5w_6	-----	-----	-----	-----	-----	1.02	0.79	-----	0.42
22:5w_3	1.46	0.95	0.64	11.65	1.02	-----	0.31	1.02	0.83
22:6	4.77	3.79	4.59	0.95	2.02	2.03	2.04	2.73	4.24
% Sat	28.93	33.03	35.53	33.54	37.58	33.21	38.59	37.19	38.93
% Mono	24.80	23.55	23.53	21.35	27.01	25.02	21.96	21.73	20.29
% Di-	16.69	18.35	16.82	19.02	16.99	14.60	16.61	18.93	16.78
% Poly	29.58	25.07	24.12	26.09	18.42	27.17	22.84	22.15	24.00
Total Unsat	71.07	66.93	64.47	66.46	62.42	66.79	61.41	62.81	61.07

[a]Calculated as percent of total fatty acids.

The level of PUFAS in the fresh tissue was 29.58% at 0 time, 26.09% at 8 months but only 22.84% at 13 months. Lower levels of PUFAS were found in the cooked meat. In the cooked meat held at 4°C for 48 hours the PUFAS comprised 25.07, 18.42 and 22.15% at 0, 8 and 13 months, respectively. The corresponding levels in the cooked meat held at -18°C for 4- hrs did not change consistently, being 24.12, 27.17 and 24.00% at 0, 8 and 13 months, respectively. Results showed that the PUFAS were less stable in the cooked meat held at 4°C for 48 hours than in either the raw frozen meat or in that cooked and held at -18°C for 48 hours.

Results of this study for beef (Table 29), chicken dark meat (Table 30) and for chicken white meat (Table 31) verify the involvement of unsaturated fatty acids, in general, and of PUFAS, in particular, in the development of oxidized flavor in frozen or cooked meat. PUFAS were less stable than either the unsaturated, mono- or dienoic fatty acids, especially in the cooked meat. Thus, the results confirm those obtained in the earlier studies herein (experiments A and B) involving model meat systems, which show that the PUFAS of the phospholipids are major contributors in the development of rancidity during freezer storage or in development of WOF in cooked meat.

Fatty Acid Composition of Lipids from Drippings

The fatty acid profiles of the triglycerides and phospholipids in the cooked drippings of beef and chicken dark meat are presented in Table 32. The levels of saturated, mono-, di- and polyenoic fatty acids in the triglycerides from beef drippings were 49.85, 47.25, 2.68 and 0.22%, respectively. Similarly, the levels of the same fatty acid in the drippings from beef phospholipids were 45.34, 49.66, 3.98 and 1.02%, respectively.

The concentrations of saturated fatty acids in the triglycerides and phospholipids of chicken dark meat drippings were 35.97 and 35.27%, respectively. The percentage of monomeric acids in the chicken dark meat drippings were similar to that found in the beef (Table 32). However, much higher levels of dienoic fatty acids were recovered in the drippings from chicken dark meat than from beef. It is interesting to note that extremely low levels of PUFAS (0.36 and 2.15%) were found in both the triglycerides and phospholipids from chicken dark meat drippings.

The low levels of PUFAS in the phospholipids of the drippings suggest that the more highly unsaturated phospholipid components are firmly held within the membranes. Results verify the conclusion that phosphatidyl ethanolamine (PE) is firmly bound to the membranes, and thus is not released during cooking, thereby elevating the levels

Table 32. Fatty acid composition of the triglycerides and phospholipids obtained in the cooked drippings from beef and chicken dark meat.[a]

	Beef Drippings		Chicken Dark Meat Drippings	
Fatty Acid	Triglycerides	Phospholipids	Triglycerides	Phospholipids
12:0	----	----	1.16	----
14:0	----	----	1.52	0.69
14:1	6.03	2.07	----	----
15:0	1.79	0.95	----	----
15:1	0.45	0.30	----	----
16:0	34.21	27.43	26.92	21.93
16:1	5.86	4.96	6.51	6.85
16:2	1.34	0.71	----	----
17:0	0.89	0.59	----	1.60
18:0	12.96	16.37	6.37	11.05
18:1	34.91	41.86	40.52	43.40
18:2	1.34	3.27	15.63	10.65
18:3	----	----	0.22	0.23
18:3	0.22	0.59	0.14	1.28
20:1	----	0.47	1.01	1.71
20:2	----	----	----	----
20:3	----	----	----	0.64
20:4	----	0.43	----	----
% Saturated	49.85	45.34	35.97	35.27
% Monoenoic	47.25	49.66	48.04	51.96
% Dienoic	2.68	3.98	15.63	10.62
% Polyenoic	0.22	1.02	0.36	2.15
Total Unsaturation	50.15	54.66	64.03	64.73

[a]Calculated as percentage of total fatty acids.

of unsaturation in cooked meat. Thus, the development of WOF in cooked meat is enhanced by the high levels of PE retained during cooking.

Results of this study (Tables 21, 22 and 23) demonstrated that there were significantly ($P < 0.01$) higher levels of phospholipids in the cooked meat in comparison to the uncooked meat. Thus, the higher rate of lipid autoxidation in cooked meats is at least in part due to elevated levels of phospholipids.

Changes in the Fatty Acid Profiles of PC and PE During Frozen Storage and Cooking of Meat

The fatty acid composition of PC and PE in total phospholipids was analyzed in the raw meat prior to freezer storage at 0 time and also following 13 months of frozen storage. At the same time periods, the fatty acid composition of PC and PE was also measured in the cooked meat after being held at 4°C and -18°C for 48 hrs following cooking.

Beef - The fatty acid profiles of PC of raw and cooked beef are presented in Table 33. The composition of PC in fresh raw beef (at 0 time) comprised of 36.62, 41.63, 10.83 and 10.92% of the saturated mono-, di-, and polyenoic fatty acids, respectively. These values are in close agreement with the data presented by Keller and

Table 33. Changes in the fatty acid composition of phosphatidyl choline (PC) in beef phospholipids during frozen storage and cooking.[a]

	0 Months			13 Months		
	Fresh Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C
Fatty Acids						
12:0	-----	-----	0.82	-----	0.99	-----
14:0	0.46	0.65	0.78	-----	-----	0.29
14:1	0.86	0.65	0.89	-----	0.58	-----
15:0	0.51	-----	-----	0.69	0.39	0.23
15:1	1.92	1.59	3.48	0.92	1.55	0.75
16:0	20.86	11.67	16.70	21.11	21.86	18.64
16:1	3.04	3.36	3.70	2.20	3.13	1.73
16:2	0.91	0.75	0.43	0.83	0.93	0.69
17:0	2.13	0.60	0.85	2.06	1.39	0.78
18:0	12.66	19.60	8.88	14.50	13.97	14.39
18:1	34.90	25.66	33.76	28.91	29.60	30.79
18:2	9.92	9.71	9.45	11.56	10.83	8.63
$18:3w_6$	2.63	0.42	0.32	-----	-----	0.75
$18:3w_3$	0.53	1.40	1.28	0.55	0.81	0.86
20:1	0.91	-----	-----	0.73	0.70	-----
20:2	-----	1.87	1.60	1.10	0.35	1.44
20:3	1.22	1.96	0.96	1.37	0.54	0.69
20:4	5.32	14.19	7.04	10.28	6.50	4.03
20:5	1.22	5.92	9.06	2.06	4.33	14.10
22.3	-----	-----	-----	-----	0.35	0.36
22:4	-----	-----	-----	0.32	0.85	0.22
$22:5w_6$	-----	-----	-----	0.46	-----	-----
$22:5w_3$	-----	-----	-----	0.35	0.35	0.26
22:6	-----	-----	-----	-----	-----	0.37
% Sat.	36.62	32.52	28.03	38.36	38.60	34.33
% Monoenoic	41.63	31.26	41.83	32.76	35.56	33.27
% Dienoic	10.83	12.33	11.48	13.49	12.11	10.76
% Polyenoic	10.92	23.89	18.66	15.39	13.73	21.64
Total Unsat.	63.38	67.48	71.97	61.64	61.40	65.67

[a]Calculated as percent of total fatty acids.

Kinsella (1973). The levels of dienoic and polyenoic fatty acids in the PC of raw meat increased during frozen storage, while the monenoic fatty acids in PC decreased by almost 9.0% during frozen storage.

At 0 time, the levels of dienoic and polyenoic fatty acids in PC were much higher in cooked meat than in fresh meat. At 13 months of frozen storage, the polyenoic fatty acids of PC in the cooked meat held at 4°C for 48 hrs decreased by 42.53% from the original value at 0 time. On the other hand, the level of polyenoic fatty acids in PC of the raw frozen and in the cooked meat held at -18°C for 48 hrs increased. The variations in the levels of polyenoic fatty acids in PC were principally due to changes in $C_{20:4}$ and $C_{20:5}$ fatty acids. The percentage of total unsaturation in PC decreased in both the raw and cooked meat as the length of frozen storage increased. Results showed that the PUFAS of PC were least stable in the cooked meat held at 4°C for 48 hrs, thus indicating much higher rate of autoxidation.

The initial levels of saturated, mono-, di- and poly-enoic fatty acids in PE of fresh beef (Table 34) were 35.46, 25.47, 15.66 and 23.42%, respectively. Following cooking (0 time), significant losses occurred in the dienoic and polyenoic fatty acids in PE of the cooked meat. Most of the polyenoic fatty acids were severely affected during cooking, thus greatly decreasing total

Table 34. Changes in the fatty acid composition of phosphatidyl ethanolamine (PE) in beef phospholipids during frozen storage and cooking.[a]

Fatty Acids	0 Month			13 Months		
	Fresh Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C
12:0	-----	0.33	0.80	-----	-----	-----
14:0	-----	0.72	1.70	-----	-----	0.38
14:1	-----	0.74	1.11	-----	-----	-----
15:0	0.70	-----	-----	0.60	1.35	0.40
15:1	2.55	1.12	-----	0.35	-----	0.22
16:0	9.28	31.84	19.15	14.99	17.30	17.59
16:1	1.39	2.65	1.28	1.95	3.05	2.00
16:2	1.74	-----	0.32	2.40	1.35	1.44
17:0	2.65	0.72	0.64	1.70	2.40	0.72
18:0	22.83	9.51	23.94	20.95	22.26	12.47
18:1	21.53	36.17	29.84	22.38	23.77	28.78
18:2	9.05	8.40	9.31	11.09	8.59	13.91
$18{:}3w_6$	0.37	-----	-----	-----	1.05	0.20
$18{:}3w_3$	1.14	-----	-----	0.75	0.79	2.20
20:1	-----	-----	-----	-----	-----	-----
20:2	4.87	0.41	-----	2.00	0.08	1.80
20:3	1.16	0.43	-----	2.00	1.50	0.60
20:4	15.08	4.96	11.91	13.99	8.12	4.50
20:5	0.70	2.00	-----	3.50	7.22	12.23
22:3	1.30	-----	-----	0.35	0.79	0.16
22:4	0.65	-----	-----	0.65	0.38	0.08
$22{:}5w_6$	-----	-----	-----	-----	-----	0.16
$22{:}5w_3$	1.86	-----	-----	0.35	-----	0.16
22:6	1.16	-----	-----	-----	-----	-----
% Sat.	35.46	43.12	46.23	38.24	43.31	31.56
% Monoenoic	25.47	40.68	32.23	24.68	26.82	31.00
% Dienoic	15.66	8.81	9.64	15.49	10.02	17.15
% Polyenoic	23.42	7.39	11.91	21.59	19.85	20.29
Total Unsat.	64.55	56.88	53.78	61.76	56.69	68.44

[a]Calculated as percent of total fatty acids.

unsaturation. Both the dieonic and polyenoic fatty acids in the PE of cooked meat were not as severely altered at 13 months of frozen storage as was the case at 0 time. However, the dienoic and polyenoic fatty acids of PE were found to be least stable in the cooked meat held at 4°C for 48 hrs. This indicated a greater rate of oxidation in the cooked meat held at 4°C.

Chicken Dark Meat - The fatty acid profiles in PC of raw and cooked chicken dark meat are presented in Table 35. Levels of saturated, mono-, di-, and polyenoic fatty acids in the PC of the raw meat decreased during frozen storage. The PUFAS decreased from the initial level of 18.79% at 0 time to only 8.29% at 13 months. Thus, there was a high rate of PC oxidation in the raw chicken dark meat during frozen storage.

The changes in the fatty acids of the PC in the cooked meat were not consistent. However, the dienoic and polyenoic fatty acids were less stable in the cooked chicken dark meat held at 4°C for 48 hrs than in that held at -18°C for 48 hrs following cooking.

Results of the changes in the fatty acids of PE for chicken dark meat (Table 36) also clearly showed that the unsaturated fatty acids were less stable in the cooked meat held at 4°C than in the other two treatments, which indicate a higher rate of autoxidation in

Table 35. Changes in the fatty acid composition of phosphatidyl choline (PC) in chicken dark meat phospholipids during frozen storage and cooking.[a]

	0 Month			13 Months		
Fatty Acids	Fresh Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C
12:0	-----	0.57	0.26	-----	-----	-----
14:0	0.41	0.86	0.91	0.34	-----	-----
14:1	-----	0.86	0.25	-----	-----	-----
15:0	0.48	-----	0.91	0.68	-----	-----
15:1	0.61	-----	-----	1.08	0.92	0.75
16:0	16.42	29.93	12.11	28.47	18.78	18.19
16:1	0.92	0.77	-----	1.42	1.15	0.93
16:2	0.61	0.29	3.28	1.02	0.69	0.75
17:0	0.54	0.57	-----	0.81	0.46	0.93
18:0	20.36	20:19	13.24	25.76	26.87	20.12
18:1	23.62	22.21	11.30	20.44	15.27	12.90
18:2	16.63	18.62	14.63	9.76	12.37	12.34
$18:3w_6$	-----	-----	-----	0.34	-----	0.44
$18:3w_3$	0.61	-----	10.82	0.95	0.31	0.75
20:1	-----	-----	-----	0.51	-----	-----
20:2	0.61	-----	11.30	1.42	3.05	13.08
20:3	0.31	-----	-----	-----	-----	-----
20:4	10.96	5.13	11.80	3.75	7.33	5.23
20:5	2.88	-----	0.63	3.25	12.80	12.21
22:3	-----	-----	6.81	-----	-----	1.38
22:4	1.49	-----	-----	-----	-----	-----
$22:5w_6$	-----	-----	-----	-----	-----	-----
$22:5w_3$	0.78	-----	1.75	-----	-----	-----
22:6	1.76	-----	-----	-----	-----	-----
% Sat.	38.21	52.12	27.43	56.06	46.11	39.24
% Monoenoic	25.15	23.84	11.55	23.45	17.34	14.58
% Dienoic	17.85	18.91	29.21	12.20	16.11	26.17
% Polyenoic	18.79	5.13	31.81	8.29	20.44	20.01
Total Unsat.	61.79	47.88	72.57	43.94	53.89	60.76

[a]Calculated as percent of total fatty acids.

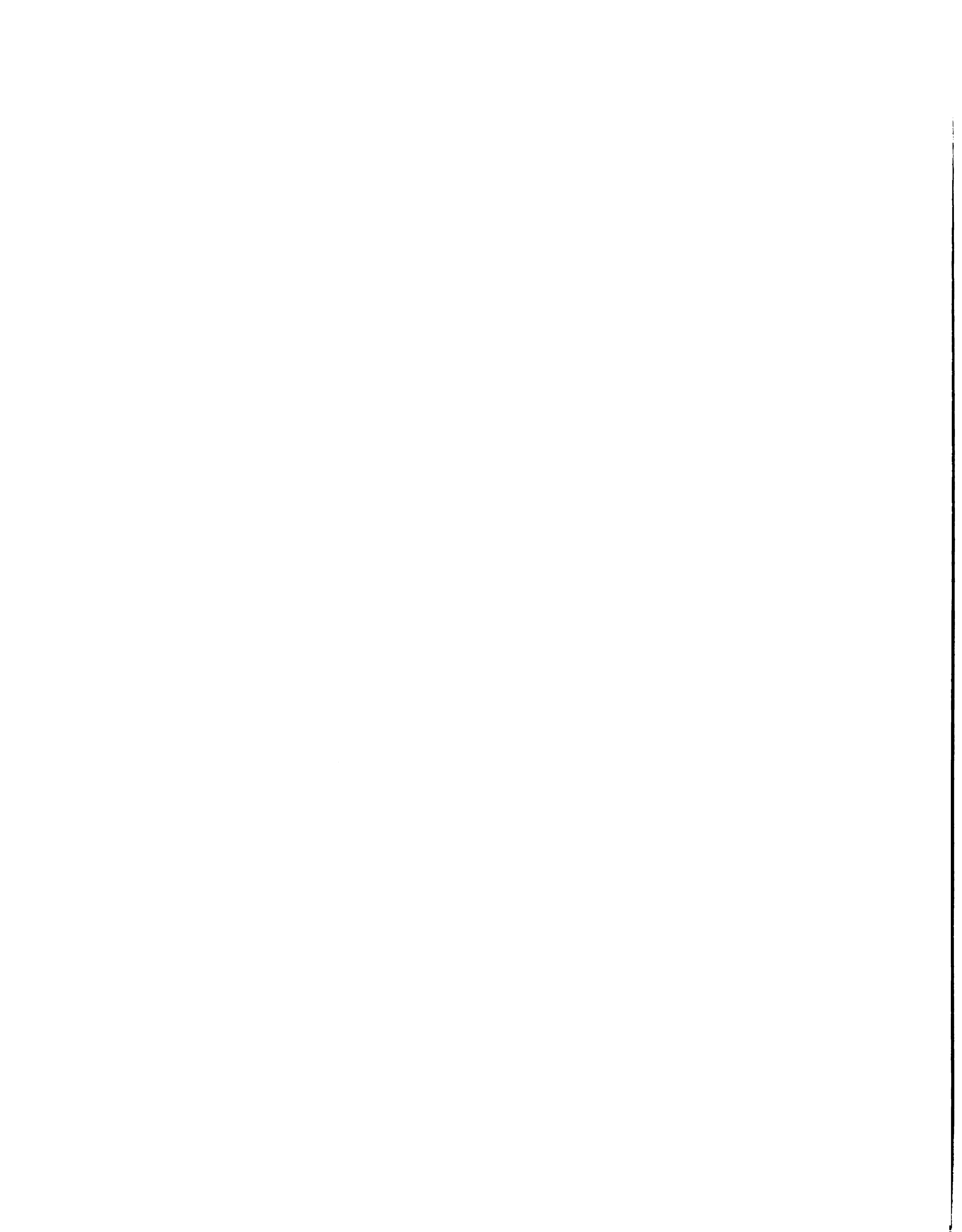


Table 36. Changes in the fatty acid composition of phosphatidyl ethanolamine (PE) in chicken dark meat phospholipids during frozen storage and cooking.[a]

Fatty Acids	0 Month			13 Months		
	Fresh Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C
12:0	-----	4.49	-----	-----	-----	-----
14:0	-----	0.82	0.88	0.41	-----	-----
14:1	-----	-----	0.42	-----	-----	-----
15:0	0.99	0.22	-----	-----	-----	-----
15:1	1.29	-----	0.55	0.47	-----	-----
16:0	11.91	21.02	14.52	17.39	13.23	10.83
16:1	0.99	-----	1.26	1.24	0.59	0.36
16:2	1.32	0.88	0.67	0.44	0.44	0.59
17:0	1.49	-----	0.84	0.53	0.74	0.59
18:1	15.45	10.32	16.41	14.80	11.03	10.87
18:2	14.56	2.46	9.72	10.07	10.00	9.96
$18:3w_6$	-----	-----	0.38	0.27	0.29	0.55
$18:3w_3$	-----	-----	0.29	1.04	0.69	0.59
20:1	0.74	-----	0.25	0.21	0.39	-----
20:2	0.59	2.30	2.78	11.46	4.90	5.93
20:3	-----	-----	0.55	-----	-----	-----
20:4	19.56	10.95	12.46	9.24	12.74	15.45
20:5	1.65	-----	9.09	12.44	11.76	8.86
22:3	0.15	-----	0.27	0.41	0.66	0.55
22:4	1.32	-----	1.39	0.30	0.33	0.51
$22:5w_6$	0.17	-----	-----	-----	-----	-----
$22:5w_3$	0.33	-----	0.34	0.18	-----	-----
22:6	1.22	-----	2.19	1.07	0.93	2.57
% Sat.	40.66	73.09	40.97	36.36	45.25	43.21
% Monoenic	18.47	10.32	18.89	16.72	12.01	11.23
% Dienoic	16.47	5.64	13.17	21.97	15.34	16.48
% Polyenoic	24.40	10.95	26.96	24.95	27.40	29.08
Total Unsat.	59.34	26.91	59.03	63.64	54.75	56.79

[a]Calculated as percent of total fatty acids.

the former.

Chicken White Meat - The fatty acid composition of PC in the raw and cooked chicken white meat phospholipids is presented in Table 37. The level of polyenoic fatty acids as well as of total unsaturation of PC increased during frozen storage of raw chicken white meat. The level of polyenoic fatty acids in PC was lower in the cooked than in the raw meat, irrespective of the length of frozen storage. Results, thus, verify the involvement of PUFAS in the development of WOF in cooked meat.

The involvement of PUFAS in the development of oxidized flavor is clearly demonstrated in Table 38, which presents the fatty acid profiles of PE for raw and cooked chicken white meat. At each storage interval, the levels of polyenoic fatty acids of PE in raw chicken white meat were significantly higher than the corresponding levels in the cooked meat. However, the PUFAS were more stable in the PE of the cooked meat held at -18°C for 48 hours after cooking than in that held at 4°C for 48 hrs. Arachidonic acid ($C_{20:4}$) was the fatty acid most severely affected in the cooked meat held at 4°C for 48 hrs.

Results of fatty acid analyses for PC and PE clearly demonstrate that the PUFAS were less stable in cooked than in raw meat. However, the PUFAS were less stable in the cooked meat held at 4°C than that held at -18°C

Table 37. Changes in the fatty acid composition of phosphatidyl choline (PC) in chicken white meat phospholipids during frozen storage and cooking.[a]

	0 Month			13 Months		
		Cooked and Stored			Cooked and Stored	
Fatty Acids	Fresh Meat	+4°C	-18°C	Frozen Meat	+4°C	-18°C
12:0	0.22	-----	0.69	-----	-----	-----
14:0	1.19	0.67	0.80	0.66	0.32	0.71
14:1	0.15	0.49	0.57	-----	-----	-----
15:0	-----	-----	0.15	0.44	0.57	-----
15:1	5.03	1.09	0.31	2.05	1.07	1.23
16:0	18.84	26.15	22.90	21.07	21.10	19.72
16:1	0.43	1.28	0.46	2.64	2.14	1.54
16:2	0.44	0.18	-----	0.92	0.70	0.42
17:0	1.31	1.46	0.20	1.03	3.41	0.34
18:0	14.00	8.51	13.55	11.65	19.95	16.61
18:1	18.07	27.00	26.64	24.47	24.78	23.49
18:2	12.20	15.75	20.04	7.88	7.13	9.79
18:3w_6	0.33	-----	-----	0.38	-----	0.22
18:3w_3	0.32	-----	-----	1.32	1.0	0.42
20:1	0.35	-----	-----	-----	0.86	0.34
20:2	2.95	1.82	1.53	0.59	0.86	0.49
20:3	-----	-----	-----	0.51	0.14	0.28
20:4	13.68	8.76	9.77	6.15	5.99	13.09
20:5	7.61	3.19	2.39	15.92	9.98	6.26
22:3	0.06	-----	-----	0.37	-----	1.17
22:4	2.49	0.49	-----	0.26	-----	0.28
22:5w_6	-----	-----	-----	-----	-----	-----
22:5w_3	-----	-----	-----	1.10	-----	0.92
22:6	0.66	3.16	-----	0.59	-----	2.68
% sat.	35.56	36.79	38.29	34.85	45.35	37.38
% monoeonic	24.03	29.86	27.98	29.16	28.85	26.60
% dienoic	15.59	17.75	21.57	9.39	8.69	10.70
% polyenoic	24.82	15.60	12.16	26.60	17.11	25.32
Total Unsat.	64.44	63.21	61.71	65.15	54.65	62.62

[a]Calculated as percent of total fatty acids.

Table 38. Changes in the fatty acid composition of phosphatidyl ethanolamine (PE) in chicken white meat phospholipids during frozen storage and cooking.[a]

Fatty Acids	0 Month			13 Months		
	Fresh Meat	Cooked and Stored +4°C	Cooked and Stored -18°C	Frozen Meat	Cooked and Stored +4°C	Cooked and Stored -18°C
12:0	-----	0.93	-----	-----	-----	-----
14:0	-----	1.16	1.55	0.41	0.53	-----
14:1	-----	2.89	0.54	-----	-----	-----
15:0	-----	0.62	0.59	0.22	0.47	-----
15:1	4.48	-----	0.67	-----	1.05	1.09
16:0	11.30	36.08	20.53	15.08	24.73	19.55
16:1	0.37	-----	1.01	1.22	1.89	1.14
16:2	0.47	-----	1.13	0.40	0.63	0.33
17:0	2.13	-----	3.35	0.81	1.42	0.33
18:0	22.40	26.72	28.70	21.53	17.05	22.14
18:1	15.83	10.40	12.57	16.88	16.84	18.25
18:2	10.41	-----	6.16	11.53	10.70	10.86
$18:3w_6$	0.50	-----	-----	0.24	-----	0.33
$18:3w_3$	0.42	1.54	1.26	1.08	0.74	0.57
20:1	0.22	-----	1.13	-----	-----	-----
20:2	0.84	18.50	1.26	00.65	1.42	1.09
20:3	0.45	-----	-----	0.73	-----	0.68
20:4	22.21	1.16	12.91	12.31	8.84	12.25
20:5	1.57	-----	3.14	12.99	12.52	8.55
22:3	0.41	-----	-----	0.43	-----	0.49
22:4	1.49	-----	0.89	0.27	-----	0.27
$22:5w_6$	0.48	-----	-----	-----	-----	-----
$22:5w_3$	0.21	-----	-----	1.19	0.37	0.46
22:6	3.81	-----	2.62	2.03	0.80	1.63
% Sat.	35.83	65.51	54.72	38.05	44.20	42.02
% Monoenoic	20.90	13.29	15.92	18.10	19.78	20.48
% Dienoic	11.72	18.50	8.55	12.58	12.75	12.28
% Polyenoic	31.55	2.70	20.82	31.27	23.27	25.23
Total Unsat.	64.17	34.49	45.29	61.95	55.80	57.99

[a]Calculated as percent of total fatty acids.

for 48 hrs following cooking. In addition, the di- and polyenoic fatty acids associated with PC were relatively more stable during freezer storage and cooking than those of PE. The results for beef (Tables 33 and 34), chicken dark meat (Tables 35 and 36) and chicken white meat (Tables 37 and 38) clearly verify the involvement of the unsaturated fatty acids, and particularly of the PUFAS autoxidation of both PC and PE. Thus, the results confirm the previous studies involving model meat systems (experiments A and B), which clearly demonstrated that PUFAS are involved in the development of rancidity in frozen meat and of WOF in cooked meat.

There is currently no available data on the effect of freezing and cooking on the fatty acid composition of PC and PE in either chicken dark meat or white meat. In addition, the current information available for beef (Keller and Kinsella, 1973) is not comprehensive.

Thus, this study provides a detailed and comprehensive analysis of fatty acid composition of PC and PE in fresh raw/frozen and cooked meat, which is not currently available in the published literature.

Effect of Frozen Storage of Meat in the Raw State on its TBA Value Following Cooking and Holding at 4°C or -18°C for 48 Hrs

According to deFremery *et al*. (1977) extensive consumer surveys have indicated that 3/4 of all consumers prefer fresh to frozen meat, yet 2/3 of these consumers froze the meat after purchasing it. The authors cited another study which showed that 63% of consumers froze their meat after purchase, even when held for only a few days. This practice is not uncommon in commercial meat processing establishments.

The present work rose out of a need to explore the stability of meat during frozen storage, which is of particular relevance in long distance transporting of meat by land or sea. There is currently no information on the effect of frozen storage of meat in the raw state upon development of WOF after cooking. Thus, the main objective of this study was to examine the effect of frozen storage of meat in the raw state on its TBA values following cooking and holding at either 4°C or -18°C for 48 hrs.

Lipid antoxidation in raw and cooked meat was measured using the 2-thiobarbituric acid (TBA) test of Tarladgis *et al*. (1960). The TBA numbers of the fresh raw meat were measured prior to frozen storage at 0 time, and

also at 8 and 13 months of frozen storage. At the same time periods, the TBA numbers of the cooked meat held at either 4°C or -18°C for 48 hrs after cooking were also determined.

Beef

The TBA numbers for raw frozen and cooked beef are presented in Table 39 and Figure 15. In the raw frozen beef, the TBA values rose slowly from 0.27 at 0 time to 0.31 at 8 months and to 0.41 at 13 months of frozen storage. Thus, raw beef was very stable during frozen storage. The TBA numbers were still well below the threshold levels for rancidity of 1-2 as outlined by Watts (1962).

When beef was cooked and held at -18°C for 48 hrs after cooking, the TBA values were 1.63, 2.64 and 0.79 after 0, 8 and 13 months of frozen storage, respectively. The values were significantly ($P < 0.01$) different from each other. Although the reason for the drop in TBA value at 13 months is unknown, Buttkus (1967) has postulated that a reaction between myosin and malonaldehyde may take place during frozen storage and cause a decline in TBA numbers.

When beef was cooked and held at 4°C for 48 hrs after cooking, the TBA values were significantly ($P < 0.001$) higher than those obtained in the other treatments. The

Table 39. Effect of length of frozen storage at -18°C and cooking on the level of TBA numbers in beef (LD)[1,2,3]

Storage Time (Months)	Raw Meat Stored at -18°C	Cooked Meat	
		Held at -18°C for 48 hrs	Held at 4°C for 48 hrs
0	0.27±0.02[a]	1.63±0.08[d]	7.26±0.20[h]
8	0.31±0.03[a]	2.64±0.21[e]	6.09±0.16[f]
13	0.41±0.01[b]	0.79±0.03[c]	6.55±0.07[g]

[1]TBA number is expressed as mg malonaldehyde/kg meat.

[2]Values in the same column or row bearing the same letter are not significantly different at $P < .05$.

[3]Each value represents a mean of 4 replicates.

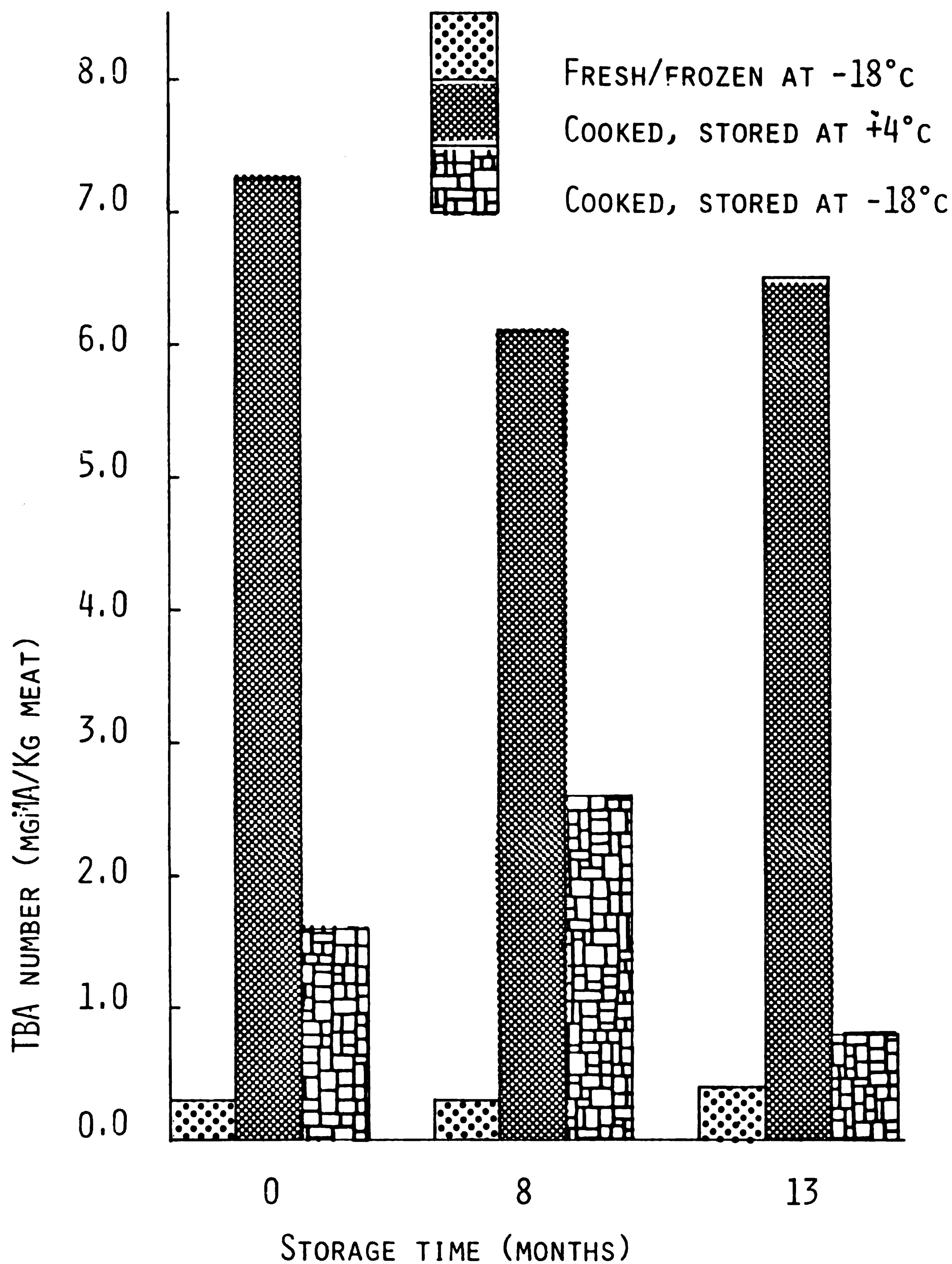


FIG. 1[illegible]. Influence of the Length of Storage Time at -18°C on the TBA Number of Beef Muscle.

values were 7.26, 6.09 and 6.55 after 0, 8 and 13 months of frozen storage in the fresh state, respectively. The variation in TBA values is closely related to changes in the amount of total lipids (Table 21) in the cooked meat, i.e., high TBA values and high lipid content were directly related and vice versa.

Chicken Dark Meat

TBA values for fresh/raw frozen and cooked chicken dark meat are presented in Table 40 and Figure 16. In the raw meat, the TBA numbers rose gradually during frozen storage, the values being 0.36, 1.78 and 2.44 at 0, 8 and 13 months, respectively. Thus, the TBA numbers for raw chicken dark meat would exceed the threshold values for acceptability after 8 months in freezer storage. Results showed that a higher rate of lipid oxidation took place in raw chicken meat (Table 40) than in the raw beef (Table 39). The differences in the rate of oxidation can be largely explained on the basis of a greater amount of lipid unsaturation in chicken dark meat as compared to beef.

In cooked chicken dark meat held at -18°C for 48 hrs after cooking, the TBA values were 6.80, 5.42 and 5.73 at 0, 8 and 13 months of frozen storage, respectively. Similarly, the corresponding values in the cooked meat held at 4°C for 48 hrs after cooking were 16.65, 12.22

Table 40. Effect of length of frozen storage at -18°C and cooking on the level of TBA numbers in chicken dark meat.[1,2,3]

Storage Time (Months)	Raw Meat Stored at -18°C	Cooked Meat Held at -18°C for 48 hrs	Cooked Meat Held at 4°C for 48 hrs
0	0.36±0.06[a]	6.80±0.15[f]	16.65±0.36[k]
8	1.78±0.34[b]	5.42±0.14[d]	12.22±0.50[g]
13	2.44±0.51[c]	5.73±0.10[e]	13.34±0.55[h]

[1]TBA number is expressed as mg malonaldehyde/kg meat.

[2]Values in the same column or row, bearing the same letter are not significantly different at $P < .05$.

[3]Each value represents a mean of 4 replicates.

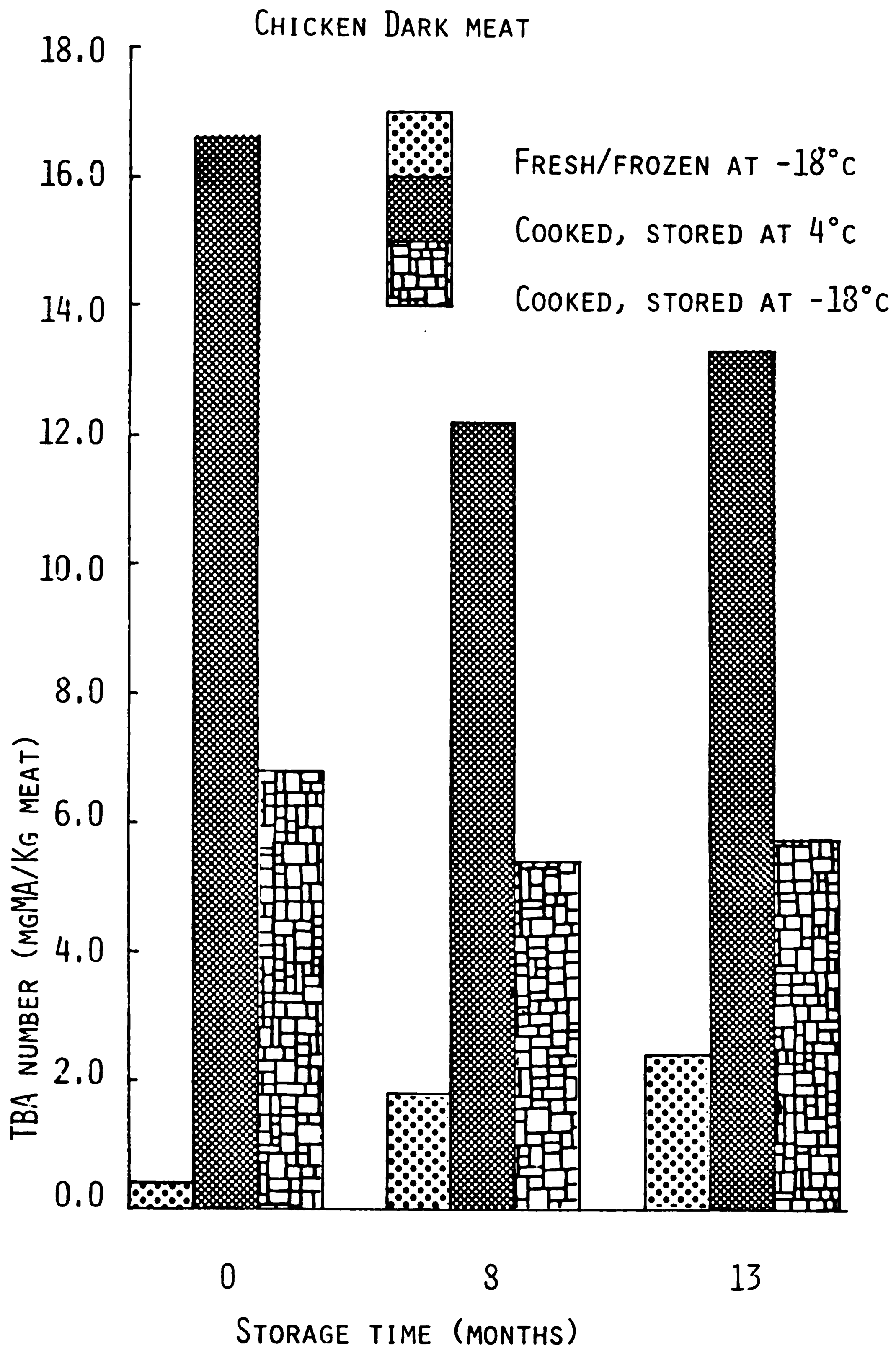


FIG. 16. Influence of the Length of Storage Time at -18°C on the TBA Number of Chicken Dark Meat Muscle.

and 13.34 at 0, 8 and 13 months, respectively. Thus, cooked chicken dark meat held at -18°C for 48 hrs after cooking was considerably more stable to lipid autoxidation than that held at 4°C for 48 hrs following cooking. In addition, results showed that the variation in the TBA values for cooked meat was closely related to differences in the concentration of total lipids (Table 22) for cooked chicken dark meat. This further underlines the relationship between lipid composition and the development of lipid oxidation in cooked meat (Experiment B).

Chicken White Meat

The TBA numbers of raw frozen chicken white meat were 0.37, 1.45 and 1.09 after 0, 8 and 13 months of frozen storage, respectively. Thus, raw chicken white meat was more stable than raw chicken dark meat but was less stable than beef. The greater oxidative stability of raw chicken white meat in comparison to the dark meat was largely due to the lower lipid content (Table 23) of the former.

When chicken white meat was cooked and held at -18°C for 48 hrs following cooking, the TBA numbers were 5.56, 3.58 and 4.15 after 0, 8 and 13 months of frozen storage, respectively. Similarly, the corresponding values for the cooked white meat held at 4°C for 48 hrs following cooking

Table 41. Effect of length of frozen storage at -18°C and cooking on the level of TBA numbers in chicken white meat.[1,2,3]

Storage Time (Months)	Raw Frozen Meat at -18°C	Cooked Meat	
		Held at -18°C for 48 hrs	Held at 4°C for 48 hrs
0	0.37 0.02[a]	5.56 0.09[f]	12.59 0.35[k]
8	1.45 0.05[c]	3.58 0.19[d]	6.58 0.11[g]
13	1.09 0.04[b]	4.15 0.02[e]	8.89 0.09[h]

[1]TBA number is expressed as mg malonaldehyde/kg meat.

[2]Values in the same column or row, bearing the same letter are not significantly different $P < .05$.

[3]Each value represents a mean of 4 replicates.

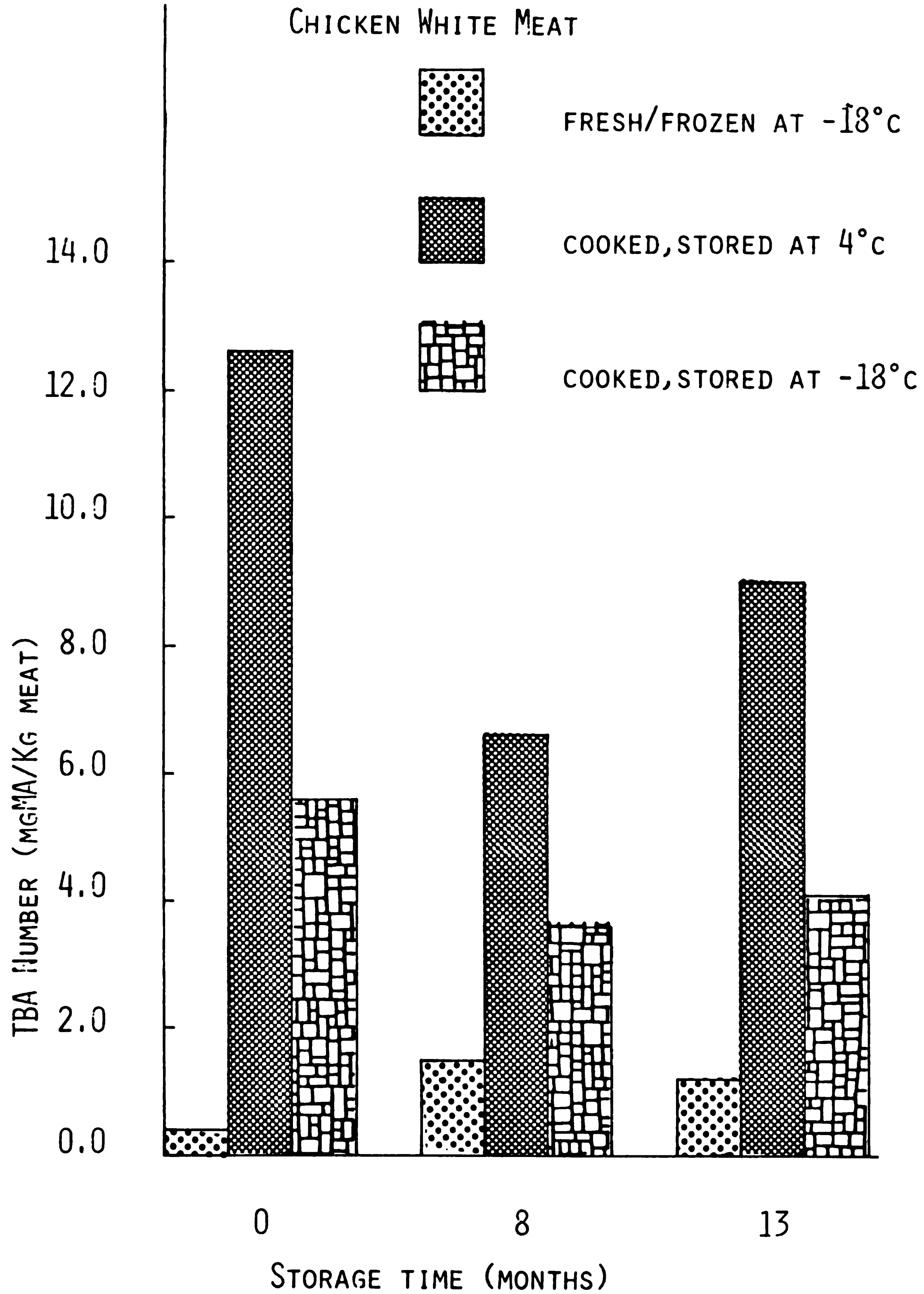


FIG. 17. Influence of the Length of Storage Time at -18°C on the TBA number of Chicken White Meat Muscle.

were 12.59, 6.58 and 8.89 at 0, 8 and 13 months of frozen storage, respectively. Thus, lipid oxidation was considerably higher in cooked chicken white meat held at 4°C for 48 hrs in comparison to the same meat held at -18°C for 48 hrs following cooking. Results also showed that the TBA values for the cooked meat are closely related to the amount of total lipids (Table 23).

Table 42 and Figure 18 give the TBA values of cooked meat immediately following cooking and prior to holding either at 4°C or -18°C for 48 hrs. Prior to frozen storage of the raw meat at 0 time, a portion of it was cooked and assessed for TBA numbers immediately following cooking. After 8 months of freezer storage, a portion of the raw frozen meat was cooked and again analyzed for its TBA value. The TBA value for the freshly cooked beef at 0 time was 0.60. After 8 months of frozen storage of the raw beef, the TBA value immediately following cooking was 0.26. Lower values (Table 42) were also found in both chicken dark meat and white meat analyzed after frozen storage. Results showed that meat stored frozen in the raw state is more stable to development of WOF when cooked than the previously unfrozen raw meat.

These results were unexpected since it is usually believed that frozen raw meat is less stable to autoxidative degradation than fresh unfrozen meat. The TBA test does not fulfill all the requirements of a reproducible

Table 42. Influence of frozen storage of meat in the raw state on TBA values of the cooked meat prior to holding at 4°C or -18°C for 48 hrs.[1,2,3]

Time of Raw Meat in Frozen Storage	Beef	Chicken Dark Meat	Chicken White Meat
0 Month	0.60	7.71	5.02
8 Months	0.26	2.10	1.52

[1]Fresh/frozen raw meat samples were cooked and the TBA number measured immediately after cooking.

[2]TBA number is expressed as mg malonaldehyde/kg meat.

[3]Each value represents a mean of 4 replicates.

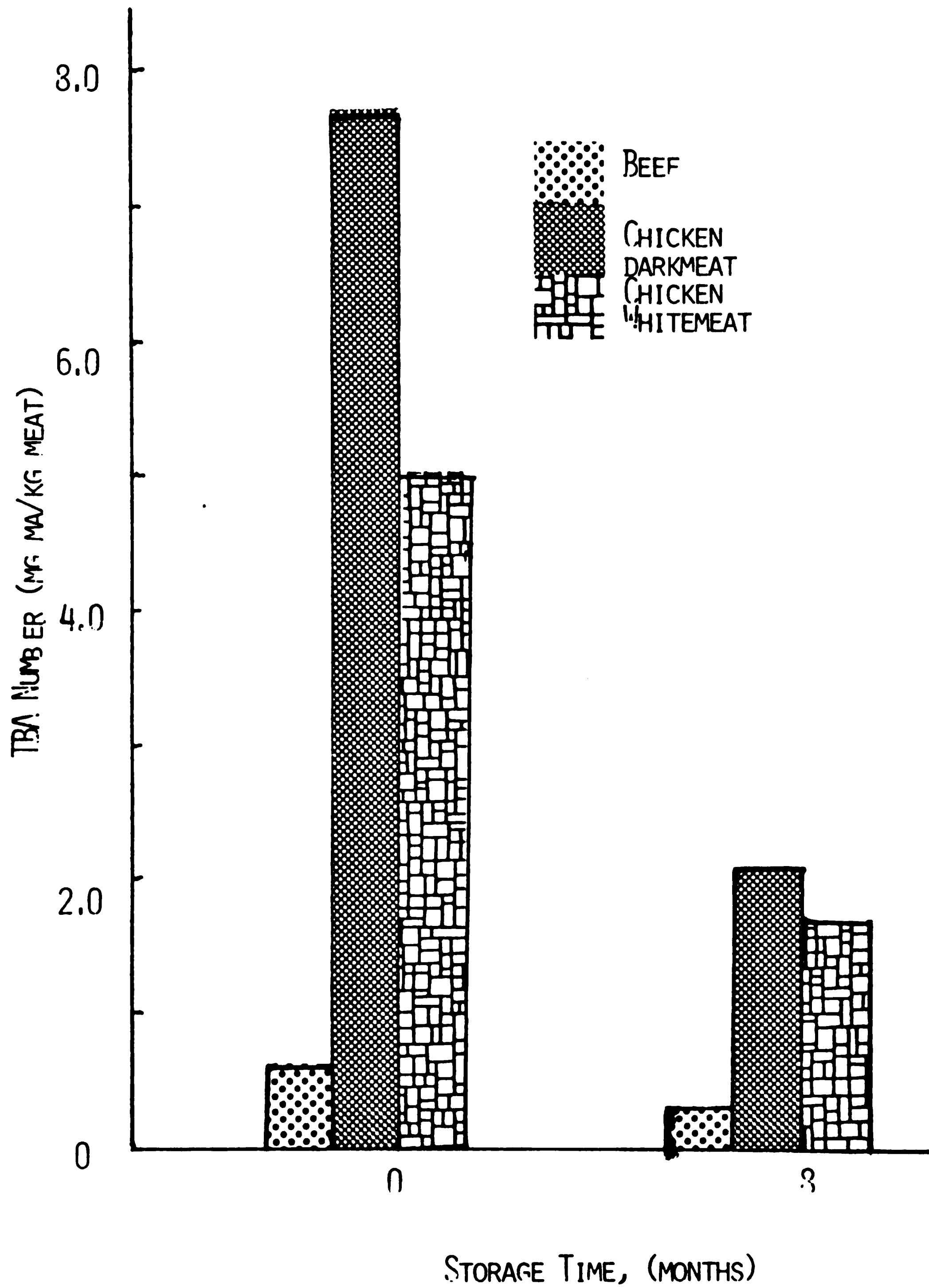


FIG. 1[illegible]. TBA Number of Cooked Meat Measured Within 1 Hour Following Cooking.

technique and has been criticized on several points (Lea, 1962; Gray, 1978). Although the determination of malonaldehyde (MA) can be used to follow oxidative deterioration of food and food products, the relationship between oxidative food deterioration and MA production is not simple (Arata and Chen, 1976). Since foods are complex systems (Lea, 1962), the oxidation of fat in food is expected to be a complex process in which food constituents play an unclear role. This underlines the need for a sensory measurement along with the TBA evaluation of test products. In this present study, it was not practicable to conduct a taste panel evaluation along with the TBA test.

Comparison of the TBA values for beef (Table 39), chicken dark meat (Table 40) and chicken white meat (Table 41) clearly showed that a higher rate of lipid oxidation took place in the cooked meat held at 4°C than in that held at -18°C for 48 hrs after cooking. The higher rate of lipid oxidation in the cooked meat held at 4°C is further verified by the lower stability of its phospholipid components (Table 25). In addition, results of the fatty acid analyses for total phospholipids, PC and PE clearly demonstrated that both dienoic and polyenoic fatty acids were least stable in the cooked meat held at 4°C for 48 hrs following cooking. Thus, the higher levels of TBA numbers for cooked meat held at 4°C support the possible

involvement of the PUFAS in development of WOF in cooked meat.

These results suggest that it is not advisable to hold precooked meat at 4°C for any length of time. However, precooked meat can be held at -18°C on a short term basis without any great loss of quality. Most commercial enterprises freeze precooked meat and meat products at -18°C, which would help to minimize development of WOF.

Malonaldehyde measurement is used to determine whether or not foods are stable or rancid (Tarladgis _et al_. (1960). Recently, malonaldehyde has been implicated as a cause of stomach cancer (Shamberger, 1978), especially in countries with high meat consumption. Thus, the fact that lower levels of malonaldehyde were found in cooked meat that had been previously frozen in the raw state (Tables 39, 40, 41 and 42) may be of public health significance. However, further research on this controversial subject will be needed to ascertain if this is true.

The reason for the decreasing level of malonaldehyde during freezer storage is not actually known. Chang _et al_. (1961) suggested that the formation of carbonyl addition products may possibly account for the loss of malonaldehyde during frozen storage. A decline in TBA values was observed during frozen storage of cooked meat and fishery products by Tarladgis and Watts (1960).

Buttkus (1967) has postulated that myosin and malonaldehyde may interact and result in a decrease of TBA numbers.

Results of this study indicate that the decreasing concentration of TBA numbers in cooked meat previously frozen in the raw state may be due to the differences in the lipid content of the frozen meat. In this study, there was an inconsistent but decreasing concentration of total lipids and triglycerides during frozen storage of the raw meat as well as in the cooked meat (Tables 21, 22 and 23).

The reason for the decreasing concentration of total lipids and of the triglycerides in particular is not fully known. The procedure of Folch _et al_. (1957) for the quantitative removal of total lipids from animal tissues, involving the use of chloroform-methanol as solvents was demonstrated to remove phospholipids and proteolipids as well as triglycerides from various fresh tissues of animals. However, Zipser _et al_. (1962) have indicated that chloroform-methanol did not remove all the TBA reactive materials from cooked or oxidizing fish tissue, probably due to peroxidation or oxidative scission of fatty acids, which decreased lipid solubility in fat solvents. They believe that changes in the solubility of the oxidized lipid material interfere drastically with its extraction and estimation. These authors also speculated that although the oxidation undoubtedly occurs

mainly in the polyenes, their oxidation could affect the solubility of the larger triglyceride molecules of which they are a part. This could probably account for the decreased level of triglycerides observed in this study for both raw frozen and cooked meat. Acosta *et al.* (1966) suggested that molecular changes, such as the formation of polymers and complexes, may reduce the extent of lipid extractability in solvents such as chloroform and methanol.

The variation in TBA values of the cooked meat can be partially explained on the basis of the differences in the amount of extractable lipids, i.e., high TBA values are positively related to high lipid content and vice-versa. This verifies the involvement of lipids and particularly of the PUFAS in the development of off-flavors in meat and meat products.

Summary of Results

The main objectives of this study were two fold: (1) To examine the effects of frozen storage of meat in the raw state on the stability of its constituent lipids, and (2) to assess the influence of frozen storage of meat in the raw state on its TBA values following cooking and holding at either 4°C or -18°C for 48 hrs. following cooking.

Fresh cuts of beef, chicken dark meat and white meat were frozen and stored at -18°C for over one year. At 0, 8 and 13 months storage periods, the lipid composition of the fresh/frozen raw meat was measured. In addition, TBA numbers were determined. At the same time periods, a portion of the raw frozen meat was also cooked and held at either 4°C or -18°C for 48 hrs., after which it was analyzed for constituent lipids and malonaldehyde (TBA numbers).

Results showed that total lipids and triglycerides in the raw meat significantly ($P < 0.001$) decreased during frozen storage while the level of phospholipids was constant, irrespective of the time in freezer storage Levels of triglycerides, total lipids and phospholipids were significantly ($P < 0.05$) elevated in the previously fresh/frozen raw meat after cooking and holding at either 4°C or -18°C for 48 hrs. The lipid fraction in the cooked drippings consisted largely of the triglycerides, whereas, PE was essentially absent.

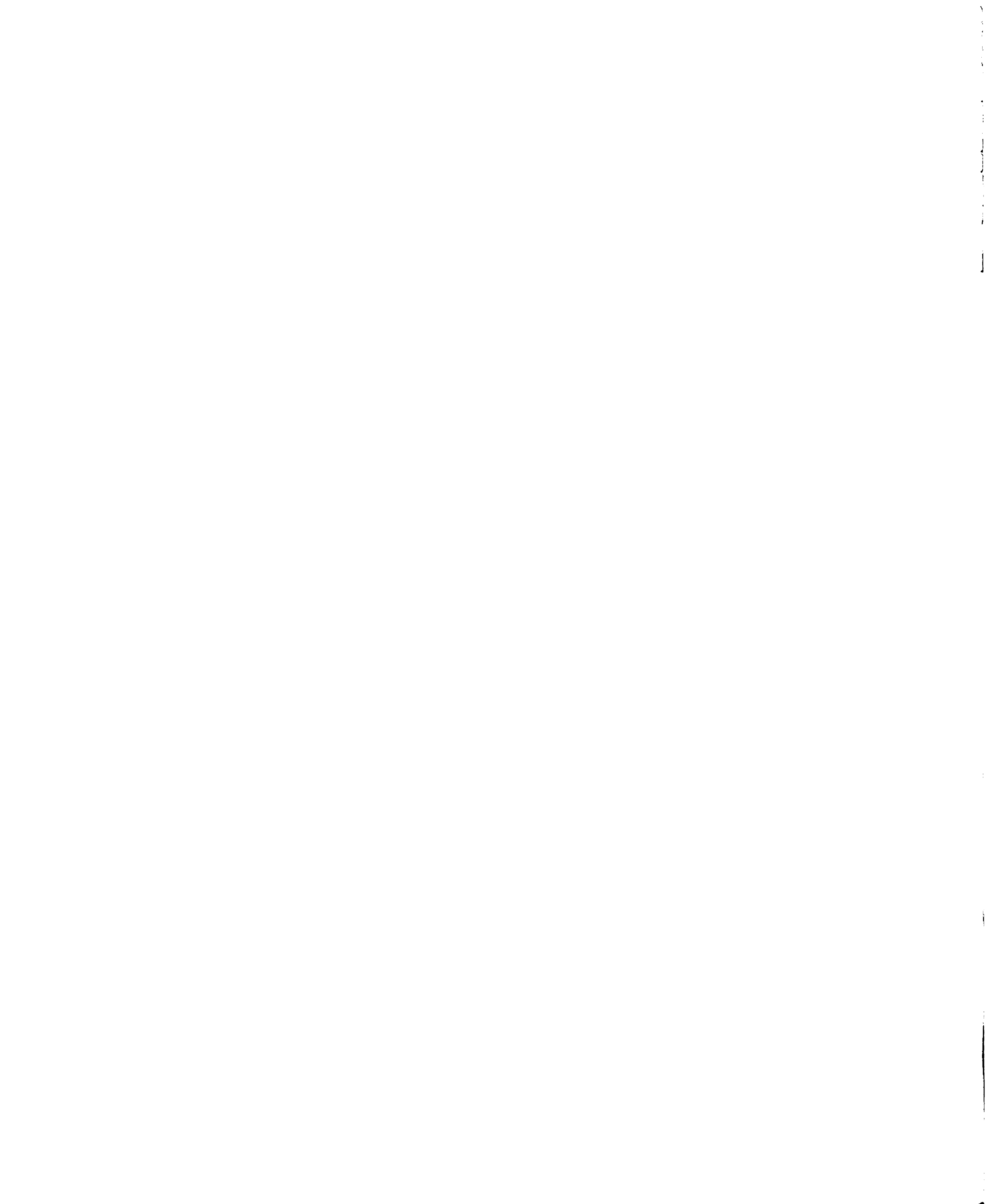
Phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and total phospholipid (phosphorus) were significantly ($P < 0.01$) higher in the fresh/frozen raw than in the cooked meat. The phospholipid components were least stable in the cooked meat held at 4°C for 48 hrs.

Only minor changes occurred in the fatty acid profiles of the triglycerides in either the raw or cooked meat

during storage, thus reflecting the stability of the triglycerides to autoxidative degradation. The dienoic and polyenoic fatty acids in total phospholipids, in PC and in PE were more stable in the raw frozen meat than in the cooked meat. The PUFAS were least stable to autoxidation in cooked meat held at 4°C for 48 hrs following cooking. Fatty acids in PC were less unsaturated than those of PE and were also more stable than those of PE, either in the raw frozen meat or in the meat cooked and held for 48 hrs. The stability of unsaturated fatty acids in the total phospholipids, in PC and in PE from different types of meat were in the following order: Beef > chicken white meat > chicken dark meat.

The stability of different types of raw meat during freezer storage was in the following order: Beef > chicken white meat > chicken dark meat. Higher TBA numbers were found in the cooked meat held at 4°C than in the cooked meat held at -18°C for 48 hrs following cooking, irrespective of the type of meat. Non-consistent but decreasing levels of malonaldehyde were found in the previously frozen raw meat after it was cooked and held for 48 hrs following cooking.

Results showed that changes in total lipids during frozen storage of raw meat were largely due to losses in the triglycerides. The phospholipid content of raw meat was relatively constant irrespective of the time



of freezer storage. The concentrations of triglycerides, total lipids and phospholipids were significantly elevated in the cooked meat. PE, PC, total phospholipids and their PUFAS were less stable in the cooked than in the raw frozen meat. Cooked meat held at 4°C for 48 hrs was more susceptible to development of WOF than similar meat held at -18°C for 48 hrs. after cooking. Results suggest that its constituent lipids were not as stable to auto-oxidation. Results verified the involvement of PUFAS in the development of WOF, the stability of different types of meat being in the order of: Beef > chicken white meat > chicken dark meat.

Experiment D

Influence of Heme Pigments, Non-heme Iron and Nitrite on Development of WOF in Cooked Meat

The role of meat pigments, nitrite and non-heme iron on development of WOF in cooked meat was investigated. The study was divided into two stages. The first part was designed to study the effect of meat pigments and nitrite on development of WOF in beef, chicken dark meat and white meat. The second part was designed to compare the relative contributions of heme and non-heme iron on the development of WOF.

The first part of this study was conceived to investigate the effect of removal of meat pigments and/or the addition of nitrite to control the development of oxidized flavor in cooked meat. The design of the experiment and preparation of samples are presented in Table 2.

The proximate composition of lipids and moisture in both the extracted and non-extracted meat samples was determined. This was done to enable a meaningful assessment of the effect of pigments on development of WOF. The tissue lipids were left intact while the only variables were removal of the meat pigments and the addition of nitrite.

The proximate composition of the meat in terms of total lipids, triglycerides, phospholipids and moisture are presented in Table 43. Total lipids and triglycerides tended to be slightly lower in the pigment extracted meat than in that containing pigment, except for chicken white meat. The amounts of phospholipids were 0.58 and 0.60% in beef with and without pigments, respectively. The corresponding levels of total phospholipids in chicken dark meat were 0.86 and .80%, respectively. The concentration of total phospholipids was considerably lower in chicken white meat than in chicken dark meat, with values of 0.49 and 0.54% in the chicken white meat and with and without pigments, respectively. The concentration of moisture was somewhat higher in all samples after extraction of the pigments. There were only minor differences in the levels of total lipids, triglycerides, phospholipids and moisture between samples with and without pigments. Thus, the only variable was the presence or absence of the meat pigments.

The fatty acid composition of the triglycerides and total phospholipids in meat with and without pigments is presented in Tables 44 and 45. The pattern of fatty acid composition in both the triglycerides and total phospholipids is in good agreement with the data presented by Hornstein _et al_. (1961, 1967), Katz _et al_. (1960) and O'Keefe _et al_. (1968). Total unsaturation in the triglycerides (Table 44) and phospholipids (Table 45) was

Table 43. Levels of lipids and moisture in meat samples (% Fresh tissue).[1]

Component	Beef		Dark Meat		White Meat	
	With Pigment	Without Pigment	With Pigment	Without Pigment	With Pigment	Without Pigment
Total Lipids	4.39	3.23	4.80	3.77	1.63	1.87
Triglyceride	3.76	3.61	3.90	2.97	1.14	1.30
Phospholipid	0.58	0.60	0.86	0.80	0.49	0.54
Moisture	76.01	78.57	74.13	78.23	80.22	80.51

[1]Results represent duplicate determinations.

Table 44. Summary of fatty acid composition of the triglycerides in beef, chicken dark and white meat.[1]

Fatty Acid	Beef		Dark Meat		White Meat	
	With Pigment	Without Pigment	With Pigment	Without Pigment	With Pigment	Without Pigment
% Saturated	47.64	43.96	25.99	28.59	31.41	29.39
% Monoenoic	48.33	50.69	49.70	48.78	45.00	49.08
% Dienoic & Polyenoic	3.20	5.35	24.31	22.63	23.59	21.53
Total Un-saturated	51.53	59.04	74.01	71.41	68.59	70.61

[1]As percentage of total fatty acids.

Table 45. Summary of fatty acid composition of the total phospholipids in beef, chicken dark and white meat.[1]

Fatty Acid	Beef		Dark Meat		White Meat	
	With Pigment	Without Pigment	With Pigment	Without Pigment	With Pigment	Without Pigment
% Saturated	41.54	41.20	32.50	32.04	36.02	32.30
% Monoenoic	33.53	38.93	29.18	28.78	27.37	31.83
% Dienoic	11.88	11.45	26.32	23.91	17.96	22.45
% Polyenoic	13.05	8.42	12.00	15.27	18.65	13.42
Total Unsaturated	58.46	58.80	67.50	67.96	63.90	67.70

[1] As percentage of total fatty acids.

considerably higher in both chicken dark meat and white meat than in beef. Furthermore, there were no consistent and significant differences in the fatty acid composition of tissues with and without pigments. Thus, any differences in the rate of lipid oxidation (TBA values) between experimental treatments should be related to the effect of pigments and/or nitrite, which will be discussed later herein.

Changes in TBA Numbers and Taste Panel Scores

Mean TBA numbers and the corresponding mean taste panel scores for cooked meat are presented in Table 46. Analyses of variance indicated that highly significant ($P < 0.001$) differences occurred among treatments for both TBA values and taste panel scores. Results showed that the samples without removal of the pigments and without added nitrite (A) had the highest TBA values, and consequently the lowest taste panel ratings. The samples without pigments and without added nitrite (C) had significantly ($P < 0.01$) lower TBA values than the samples with pigments containing no added nitrite (A). However, taste panel scores were significantly ($P < 0.01$) higher for the former than in the latter. Thus, meat pigments were clearly the major pro-oxidant in the development of WOF in cooked meat.

Table 46. Mean TBA numbers and sensory scores in cooked beef, chicken dark meat and white meat (4 replicates for each treatment).[1,2,3]

Treatments	Beef		Chicken Dark Meat		Chicken White Meat	
	TBA No.	Taste Panel Score	TBA NNo.	Taste Panel Score	TBA No.	Taste Panel Score
A: Cooked meat with pigment, no nitrite	1.93^{c}	2.42^{a}	11.19^{c}	1.92^{a}	9.52^{c}	2.10^{a}
B: Cooked meat with pigment plus nitrite	0.21^{a}	4.42^{b}	2.32^{a}	3.84^{b}	1.37^{a}	4.77^{c}
C: Cooked meat without pigment, no nitrite	0.61^{b}	3.50^{b}	4.20^{b}	3.42^{b}	4.41^{b}	3.17^{b}
D: Cooked meat without pigment plus nitrite	$0.42^{a,b}$	4.31^{b}	1.42^{a}	4.50^{b}	2.12^{a}	$4.15^{b,c}$

[1]Taste panel score was from 1-5, with 1 being pronounced WOF and 5 no WOF.

[2]All numbers in same column followed by same superscript are not significant at $P<.5$.

[3]Significant ($P<0.01$) "r" value between TBA numbers and panel scores are: Beef = -.74; chicken dark meat = -.91 and chicken white meat = -.87.

Results showed that the removal of pigments caused a 2-fold reduction in the TBA numbers for chicken white meat and a 3-fold decrease for beef and chicken dark meat. These results further underline the importance of meat pigments as pro-oxidants of lipid oxidation. Addition of nitrite also reduced TBA numbers, with a 9-fold, 7-fold and 5-fold reduction for beef, chicken white meat and dark meat, respectively.

Taste panel evaluation confirmed the beneficial effects of both the removal of heme pigments and the addition of nitrite. However, the addition of nitrite was significantly ($P < 0.05$) more beneficial as a means of controlling oxidized flavor in cooked meat than the removal of meat pigments. These results support the contention that nitrite converts the pigments to the catalytically inactive form (Zipser et al. (1964).

Significant ($P < 0.01$) correlation coefficients were found between TBA numbers and taste panel scores with "r" values of -.74, -.91 and -.87 for beef, chicken dark meat and chicken white meat, respectively. These values confirm the existence of a relationship between WOF and sensory panel scores. They are in essential agreement with those of Zipser et al. (1964), who reported a significant relationship between TBA values and sensory scores for cooked meat.

Results showed that both removal of meat pigments

and addition of nitrite effectively improved the flavor of cooked meat by inhibiting the development of WOF. The effect of nitrite in protecting against autoxidation of lipids, and thus, in preventing development of WOF is consistent with the results presented by Sato and Hegarty (1971) and Fooladi (1977).

This study suggests that meat pigments are major pro-oxidants in the development of WOF in cooked meat. Younathan and Watts (1959, 1960) studied the catalytic activity of myoglobin in tissue oxidation. They found that uncured meat, containing ferric globin hemochromogen, showed greater oxidation shortly after cooking. They concluded that it was the ferric (metmyoglobin) form of the pigment, which is the active catalyst in tissue rancidity. In contrast, Sato and Hegarty (1971), Kwoh (1970) and Love and Pearson (1974) have presented data indicating that non-heme iron is the major pro-oxident in cooked meat. They concluded that meat pigments *per se* have no catalytic effects on lipid oxidation in cooked meat.

Role of Heme and Non-heme Iron as Pro-oxidants of Lipid Oxidation

Thus, the second stage of the experiment was conducted using beef (LD) to assess the relative contribution of heme and non-heme iron on development of WOF in cooked meat.

This study (Table 3) consisted of 6 experimental treatments. Basically, the total pigments were extracted from beef (Figure 3) and concentrated by freeze-drying in a Stokes freeze-drier. The concentrate was then divided into three lots: fresh pigments, cooked pigments and hydrogen peroxide (H_2O_2) treated pigments. Half of each lot was then treated with 2.0% EDTA to chelate the non-heme iron. The 6 different treatments (pigments) were added back separatley to a constant weight of meat residue and cooked in boiling water to an internal temperature of 70°C. The cooked meat was held at 4°C for 48 hrs, after which it was analyzed for TBA numbers.

The mean TBA values obtained in this study are presented in Table 47. The TBA value on addition of total fresh meat pigment to the residue was 5.00. When the total fresh meat pigment was treated with 2.0% EDTA, however, the TBA value dropped to 1.55. After the fresh meat extract was heated to destroy the pigment and the filtrate was added back to the residue, the TBA value was 4.35. On treating part of this filtrate with 2.0% EDTA and adding it back to the meat residue, the TBA value dropped to 1.46. Thus, results indicate that the chelated non-heme iron was unavailable and lipid oxidation was inhibited.

When the heme pigment was destroyed by treatment with 30.0% H_2O_2 and the filtrate was added back to the residue,

Table 47. Role of heme and non-heme iron on the development of TBA numbers in cooked beef.[1,2]

Treatment #	Preparation of Experimental Treatments	Mean TBA Numbers
1	Residue + total raw meat pigments	5.00
2	Residue + total raw meat pigment (chelated)	1.55
3	Residue + total cooked free meat pigment	4.35
4	Residue + total cooked free meat pigment (chelated)	1.46
5	Residue + H_2O_2 treated total meat pigment	6.02
6	Residue + H_2O_2 treated meat pigment (chelated)	1.54

[1]Each experimental treatment consisted of 100 g beef residue in addition to 50 ml of the concentrated extract.

[2]EDTA was used to chelate the inorganic or free iron at concentration of 2.0%.

the TBA number of the cooked meat was 6.02. This indicates that non-heme iron was released, and subsequently increased the rate of lipid oxidation. After treating part of this filtrate with 2.0% EDTA and adding it back to the residue, the TBA number dropped to 1.54. The results reflect the effectiveness of chelating iron as a means of preventing lipid oxidation in meat.

The levels of heme and non-heme iron in the extracted meat pigments were determined using an atomic absorption spectrophotometer (Instrumentation Laboratory Inc., Lexington, MA). The samples were prepared by nitric and perchloric acid digestion before determining the iron content in the spectrophotometer. Non-heme iron was separated from heme iron first by chelation with EDTA and then by precipitating the bound heme iron using 12.5% of trichloroacetic acid (TCA). After centrifugation, the supernatant was removed for the determination of free non-heme iron. The difference between total iron and free non-heme iron represented the amount of heme iron in the extract.

The levels of heme and non-heme iron in the different meat extracts are presented in Table 48. The concentration of total iron in the fresh meat pigment extract was 20.64 μg/g of meat. The level of non-heme iron in the meat pigment extract was 1.80 μg/g meat, while the amount of bound iron was 18.84 μg/g of meat. Thus, the level

of bound iron in the meat was slightly above 90% and the free non-heme iron comprised only 8.72%.

When the total fresh meat pigment extract was heated to destroy the heme molecule and release the iron, the concentration of non-heme iron in the filtrate was 5.51 μg/g meat or about 27% of the total iron. Results showed that cooking released a significant amount of the free iron, which would account for the increased rate of lipid oxidation on adding the cooked filtrate back to the meat residue (Table 47).

The level of non-heme iron in H_2O_2 treated fresh meat extract after filtration was 13.59 μg/g meat. Thus, the H_2O_2 treatment released approximately 60% of the total iron in the pigment extract. The increased level of free iron would explain the high TBA value observed for the meat containing the H_2O_2 treated pigment extract (Table 47).

Results demonstrate that non-heme iron is the major pro-oxidant of lipid autoxidation in cooked meat and meat products. These results confirm the reports of Sato and Hegarty (1971) and Love and Pearson (1974), that non-heme iron and not myoglobin is the principal pro-oxidant in cooked meat. Results also show that non-heme iron is released from the heme pigments as a result of cooking or destruction by treatment with H_2O_2, thus accelerating lipid oxidation. Support is also obtained for the theory

Table 48. Concentrations of total iron, heme iron and free non-heme iron in treated and untreated meat pigment extract.

	Experimental Treatments	Fe^{++} µg/g Meat
1	Total iron in fresh meat pigment extract	20.64
2	Non-heme iron in fresh meat pigment extract	1.80
3	Heme iron in fresh meat pigment extract	18.84
4	Total iron in cooked meat pigment filtrate	5.51
5	Free non-heme iron in cooked pigment filtrate	4.18
6	Total iron in H_2O_2 treated meat pigment extract	13.59
7	Free iron in H_2O_2 treated meat pigment extract (chelated)	12.33

that mechanical treatments, such as grinding, may also release non-heme iron from meat pigment and accelerate lipid oxidation.

These results verify the report of Haurowitz et al. (1941) that the pro-oxidant effect of hemin or hemoglobin on linoleic and linolenic acid is due to release of inorganic iron. Results show that cooking releases a considerable proportion of bound iron, which then catalyzes lipid oxidation in cooked meat. Addition of 2.0% EDTA effectively chelated the non-heme iron, and thus, significantly reduced lipid oxidation.

Summary of Results

The role of meat pigments, nitrite and non-heme iron on the development of WOF in cooked meat was investigated. The first part of the experiment was designed to study the effect of total meat pigments and nitrite on development of WOF. The second part of the study was designed to determine the relative contribution of heme and non-heme iron to development of WOF. In addition, the concentrations of total iron, free non-heme iron and heme iron in meat pigment extracts were determined. The effects of heating and H_2O_2 treatment of the free iron content of the pigment extracts were also assessed.

Results showed that removal of the heme pigments caused a 2-fold reduction in TBA numbers for chicken

white meat and a 3-fold decrease for both beef and chicken dark meat. Addition of nitrite also reduced TBA numbers with a 9-fold, 7-fold and a 5-fold reduction for beef, chicken white meat and dark meat, respectively. Taste panel evaluation confirmed the beneficial effects of removal of the heme pigments and the addition of nitrite. Significant correlation coefficients were found between TBA numbers and taste panel scores with "r" values of -.74, -.87 and -.91 for beef, chicken white meat and dark meat, respectively. Thus, results suggested that heme pigments may catalyze lipid oxidation in cooked meat.

The proportion of bound iron in the fresh meat pigment extract was slightly above 90% of the total iron, while the level of free non-heme iron was about 8.72%. Cooking, however, released a significant amount of free iron from bound heme pigments, which accelerated lipid oxidation in cooked meat. Treatment of the pigment extracts with H_2O_2 also caused destruction of the pigment and the release of about 60% of the bound heme iron. Although earlier studies have suggested that myoglobin may catalyze lipid oxidation, this study showed that pigments *per se* do not greatly influence the development of WOF, but serve as a source of non-heme iron in cooked meat. Thus, the rapid rate of lipid oxidation in cooked meat is due in part to release of non-heme iron, which then catalyzes lipid oxidation. Results showed that non-heme

iron was the major pro-oxidant in the development of WOF in cooked meat. Addition of 2.0% EDTA effectively chelated non-heme iron, and thus, significantly reduced lipid oxidation.

SUMMARY AND CONCLUSIONS

Four broad groups of experiments were conducted to ascertain the role of meat lipids and meat pigments on the development of rancidity and WOF in frozen and in cooked meat. In the first phase of the study, a model meat system consisting of lipid-free muscle fibers was developed. The model meat system was the base material to which lipid components (triglycerides, total lipids and phospholipids) were added for testing their role in the development of rancidity or WOF in both fresh and cooked meat.

Results showed that both triglycerides and phospholipids contribute to development of rancidity during frozen storage of meat, but phospholipids make the greatest contribution. The relationship between rancidity and oxidation of the PUFAS was confirmed, particularly in the phospholipids.

Evidence is presented showing that both total phospholipids and PE are major contributors to WOF development in cooked meat. The triglycerides enhanced WOF only if combined with the phospholipids. Phosphatidyl choline (PC) did not influence WOF. Changes in the PUFAS of the phospholipids were directly related to development of WOF, especially to those of PE.

Results showed that changes in total lipids during

frozen storage of raw meat were largely due to losses in the triglyceride fraction. The phospholipid content of raw meat was relatively constant, irrespective of the length of freezer storage. Cooking significantly elevated the levels of phospholipids in meat. The cooked meat held at 4°C for 48 hrs after cooling was more susceptible to development of WOF than similar meat held at -18°C for 48 hrs. The involvement of PUFAS in the development of WOF was verified. The stability of the different types of meat, either raw frozen or cooked was in the order of: beef > chicken white meat > chicken dark meat.

Results demonstrated that removal of meat pigments and addition of 156 ppm of nitrite significantly ($P < 0.01$) inhibited the development of TBA values. Taste panel evaluation confirmed the beneficial effects of removal of the heme pigments and the addition of 156 ppm nitrite to meat for controlling the development of WOF. Thus, results suggested that heme pigments may catalyze lipid oxidation.

The percentage of bound iron in fresh meat pigment extract was slightly over 90% while the level of free non-heme iron was less than 10%. Cooking, however, released a significant amount of non-heme iron from bound heme pigments, which accelerated lipid oxidation in cooked meat. Thus, the rate of lipid oxidation in cooked meat is due in part to release of non-heme iron during cooking,

which then catalyzes lipid oxidation. Although earlier studies have suggested that myoglobin may catalyze lipid oxidation, this study showed that pigments *per se* do not greatly accelerate the development of WOF, but serve as a source of non-heme iron in cooked meat. Thus, results showed that non-heme iron was the major pro-oxidant in the development of WOF in cooked meat. Addition of 2.0% EDTA effectively chelated the non-heme iron, and thus, significantly reduced lipid oxidation.

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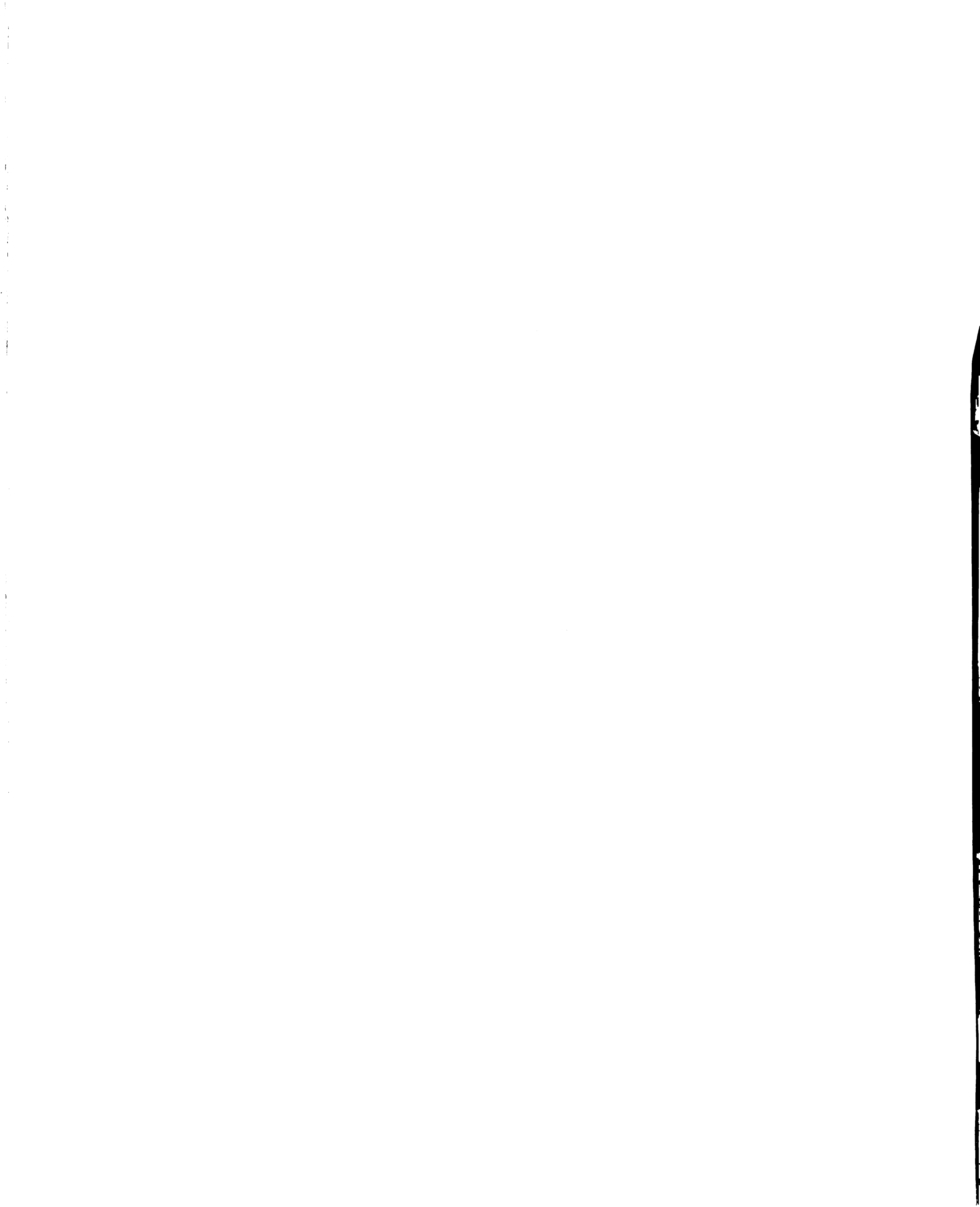

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