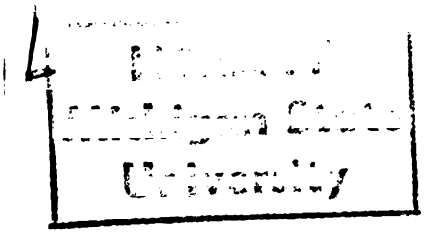

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LIPID STABILITY IN TURKEY MEAT AS INFLUENCED BY COOKING, REFRIGERATED AND FROZEN STORAGE, SALT, METAL CATIONS AND ANTIOXIDANTS

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Abdulwahab Mehdi Salih

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James F. Price
Major professor

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# LIPID STABILITY IN TURKEY MEAT AS INFLUENCED BY COOKING, REFRIGERATED AND FROZEN STORAGE, SALT, METAL CATIONS AND ANTIOXIDANTS

BY

Abdulwahab Mehdi Salih

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## ABSTRACT

LIPID STABILITY IN TURKEY MEAT AS INFLUENCED BY COOKING, REFRIGERATED AND FROZEN STORAGE, SALT, METAL CATIONS AND ANTIOXIDANTS

BY

Abdulwahab Mehdi Salih

Experiments were designed to study the effect of two types of pure salt, two types of rock salt, a broad spectrum antibiotic, iron, copper, magnesium and two types of antioxidants on the development of oxidative rancidity and off-flavor during refrigerated and frozen storage of raw and cooked ground turkey breast and thigh meat. Experiments were also undertaken to develop an improved extraction 2-thiobarbituric acid (TBA) method to assess lipid oxidation and warmed-over flavor in poultry products.

The improved extraction TBA method was compared with the traditional TBA procedure. The two methods were correlated with sensory scores. The compositional changes of lipids were evaluated by GLC and TLC procedures. Identification of dimethyl acetals (DMA) of hexa- and octadecanal in phospholipids was done by GLC-MS spectrophotometric analysis. The concentration of total iron and copper were

determined by atomic absorption spectrophotometry, nonheme iron was determined colorometrically. Aerobic plate counts were used to assess microbial population during storage.

The improved extraction TBA method was found to be fast, precise, accurate and easier to perform than the distillation method.

TBA results revealed that lipid oxidation was greater in the cooked than in the raw turkey meat ($P<0.001$). The concentration of phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) decreased after cooking and during frozen storage ($P<0.01$), which is another indication of lipid oxidation. Turkey thigh meat was more susceptible to lipid oxidation than turkey breast meat ($P<0.001$).

It has been shown that the relative prooxidant effect of divalent cations and salts on turkey meat were in the following order as measured by TBA values: $Fe^{3+}$> $Cu^{2+}$> fine flake salt (FFS) > control (CNT). Treatments had no detectable effect on lipid oxidation as monitored by changes in fatty acid profile, but the proportion of PE and PC was significantly affected ($P<0.01$) by iron and copper. The percent of nonheme iron of the total iron in turkey meat was 93 %. This is much higher than that reported in other species. The copper content in turkey thigh was found to be 3 times that in the breast meat. Tenox 6 (T6) was more effective in controlling the prooxidant effect of iron and

copper (P<0.01) than the other antioxidant which contained vitamin E, ascorbyl palmitate and citric acid (VE). Lipid oxidation began immediately after mixing Fe, Cu, VEFe, T6Fe and VECu with ground meat.

The decrease in proportion of unsaturated fatty acids during 12 months of storage was due to changes in 18:2, 20:2, 20:3, 20:4, 22:3 and 22:6 fatty acids. Since fatty acid profile changes were small and unconsistent prior to the 12 months period they were not good methods for monitoring lipid oxidation in stored poultry meat. Identification and quantitation of dimethyl acetals of hexa- and octadecanal in the phospholipid fraction of raw and cooked turkey breast meat was measured during 12 months of frozen storage.

Spoilage was detected in raw turkey breast meat refrigerated at 4 C after 21 days as measured by aerobic plate count. The number of the viable microorganisms decreased with frozen storage (P<0.01).

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

*In the name of Allah the most merciful and the most beneficient*

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# INTRODUCTION

Lipids are an integral part of muscle structure and are of interest to the food technologist because of their effect on meat product acceptance and quality. Lipids are prone to autoxidation. The unsaturated fatty acids oxidize readily by free radical chain reaction mechanisms. Autoxidation produces secondary decomposition by-products which develop rapidly in the meat and contribute to the rancid odors and flavors.

Meat products containing salt, but not nitrite cured, are often susceptible to oxidative flavor changes. Poultry products are particularly susceptible to rancidity development because of the high content of unsaturated fatty acids. Lipid oxidation in foods can be catalyzed by certain divalent cations, even when present in trace amounts. Metal cations, such as iron and copper, may come from packaging materials, processing equipment or added ingredients such as salt, spices or flavorings. Temperature abuse, prolonged storage and exposure to atmospheric oxygen also contributed to rancidity development.

Most of the research on the effect of cations on lipid oxidation was done on model systems or isolated components. Very few studies have been reported using low fat meat such

as poultry meat. The effect of salt purity and physical form in catalyzing the onset of rancidity has not been clearly established. It has been often stated that processors must be cautious not to use salt containing iron or copper cations. Sodium chloride has been reported to have a prooxidant effect. The extent to which meat rancidity can be attributed to pure salt or inadvertent metal ion contamination needs closer evaluation.

Specific objectives were to:

1. Evaluate the effectiveness of an improved extraction TBA test for monitoring lipid oxidation in poultry products.
2. Determine the effect of divalent metal cations on the development of rancidity in turkey meat.
3. Determine the effect of salt source and purity on the development of rancidity.
4. Evaluate the influence of cooking on the rate of rancidity development.
5. Determine the effect of refrigerated and frozen storage on oxidative rancidity.
6. Determine the changes in fatty acid profile of neutral and phospholipids that accompany progressive oxidation.
7. Examine the effectiveness of certain antioxidants in preventing lipid oxidation.

Three different research approaches would be utilized. Experiments were undertaken to develop an extraction 2-thiobarbituric acid (TBA) method to assess lipid oxidation and warmed-over flavor. Sensory evaluation was used to detect the accuracy of the improved extraction TBA method.

Turkey breast muscle, pectoralis minor, was used to evaluate the effect of selected levels of metal ions, an antibiotic and different types of salts on the lipid oxidation. Pectoralis minor was chosen because it is a lean tissue and has no external fat or tendons, thus the treatments could be reproduced with a minimum of change. Refrigerated and frozen storage were used for both the raw and cooked treatments. The third experiment was designed based on preliminary results obtained. Lipid oxidation in turkey breast and thigh muscles during refrigerated and frozen storage were evaluated. Treatments tested include metal ions, pure salt, antioxidants and their combinations. TBA values fatty acid profile, changes in the levels of lipid fractions and phospholipid classes were used as indicators of lipid oxidation.

# LITERATURE REVIEW

## Composition of Poultry Lipids

Lipid content and composition of different poultry tissues vary considerably and influence oxidation potential (Dawson and Gartner, 1983). The lipid content of turkey breast muscle is about half that in thigh muscle (Wilson, 1974). The variation is due largely to differences in triglyceride content. A less variable fraction is composed of cholesterol, phospholipids and glycolipids, collectively. These are referred to as essential lipids, of which phospholipids constitute the bulk of those present in muscle and organs (Acosta et al., 1966; Hornstein et al., 1967; O'Keefe et al., 1968). On a weight percentage basis, all muscle tissues contain about the same amount of phospholipids (Katz et al., 1966). Phospholipids are more labile to oxidation than neutral lipids (El-Gharbawi, 1964). Love and Pearson (1971) reported that even though the phospholipid content of meat is relatively small, the susceptibility of phospholipids to oxidation makes them important in determining meat quality. Phospholipids are unstable as a result of their high unsaturated fatty acid content. Phospholipids may also exist in closer contact with tissue components which catalyze oxidation than the

triglycerides, which increases their tendency for oxidation (El-Gharbawi and Dugan, 1965).

Variability in content and composition of muscle lipids within an animal depends upon the muscle function (Allen and Foegeding, 1981). Katz et al. (1966) reported that chicken white meat contained 1% lipid, of which 52% was neutral lipids and 48% phospholipids; chicken dark meat had 2.5% lipid, of which 79% was neutral lipids and 21% phospholipids. While Wangen et al., (1971) reported that turkey breast contained 1% lipid and thigh contained 3.5% lipid. Neutral lipids were 29% of the total from breast, and 74% from thigh. Phospholipids were 71% of the total in breast lipids and 26% in thigh lipids. They concluded that on an absolute basis thigh had the larger concentration of phospholipids, but as a percentage of total lipids the phospholipids comprised a larger proportion ($P < .01$) in the breast lipids. A similar trend was reported by Marion and Miller (1968) for chicken breast and thigh tissues. These reports are consistent with the findings of Pikul et al. (1984) who reported that the average fat content of chicken breast was 1.08% and 2.32% for leg meat. Fat from breast meat contained 62.6% phospholipids, 32.2% triglycerides and 4.8% cholesterol, whereas, fat from leg meat contained 32.6% phospholipids, 62.5% triglycerides and 4.6% cholesterol. In contrast to these findings, results of Acosta et

al. (1966) on turkey meat show that the phospholipids in white meat as a percent of the total lipid are less than in red meat.

The main components of phospholipids are phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), sphingomyelin (SP), phosphatidyl inositol (PI), lysophosphatidyl choline (LPC), phosphatidylserine (PS) and other minor components (Pearson et al., 1977).

Peng and Dugan (1965) have reported the composition of phospholipids of chicken white meat and dark meat. In the white meat they found 58-62% PC, 15-16% PE, 9-10% PS and 2-4% SP. The levels of the corresponding components in dark meat were 52-58%, 24-30%, 7-9% and 3-4%, respectively.

Fishwick (1968) reported that the phospholipids of turkey breast contained 60.9% PC+PI, 29.2% PE, 8.2% SP and 1.7% PS. The corresponding phospholipid levels in leg were 55.1, 36.0, 6.6 and 2.4%, respectively. Wangen et al. (1971) also reported the composition of phospholipids in turkey meat. In the turkey breast they found 51.90% PC, 22.84% PE, 10.42% SP, 9.81% PI+PS and 5% LPC, while they found in the thigh 50.14% PC, 27.71% PE, 9.66% SP, 8.41% PI+PS and 4.05% LPC.

## Effect of Chilling and Frozen Storage on Turkey Lipids

Turkey muscle after storage at -25 C for 0, 60 or 180 days has been reported to contain less extractable phospholipids than fresh tissue, the loss being tentatively attributed to the formation of polymers or other complexes of low solubilities (Acosta et al., 1966). The amount of extractable phospholipids decreased with time of storage at -10 C or below, up to a maximum loss of cd. 10%. This decrease appeared to be largely independent of the actual temperature (Davidkova and Khan, 1967; Fishwick, 1968).

Fishwick (1968) extracted the total lipid from tissue stored for various lengths of time at -60 C under an atmosphere of carbon dioxide. And reported that the losses of phospholipids during low-temperature storage were nonselective. He concluded that autoxidation is unlikely to be involved in loss of phospholipid from tissues stored at -60 C in an atmosphere of carbon dioxide. Turkey breast muscle stored for 4 months at -60 C still had a peroxide value of <1 micromole per gram lipid, although the amount of extractable phospholipid had fallen to 89% of initial amount. Davidkova and Khan (1967) reported that the phospholipid content of chicken muscle decreased and the free fatty acid and triglyceride content increased during frozen storage. They explained the decrease in phospholipid content by the

loss of lecithins and cephalins. Also they pointed out that lipolysis may have occurred during frozen storage, and that lipid hydrolysis and protein denaturation may be an interdependent phenomenon.

The nature of the mechanism that binds phospholipids to protein and makes them resistant to extraction by the single stage procedure used is not clear. It might be related to changes in salt concentration caused by the freezing out of water (Love, 1966). The increased yield of triglyceride, which is apparently a result of increased extractability after storage, was explained by Davidkova and Khan (1967) as resulting from the deteriorative biochemical changes which makes the association or binding between triglycerides and proteins less strong, permitting greater extraction.

## Effect of Cooking on Lipid Oxidation

Meat stored for a short time after cooking develops warmed-over flavor (WOF). The WOF problem has assumed greater significance in recent years due to the increased consumption of precooked meat items for both institutional and home use (Sato and Hegarty, 1971, Igene and Pearson, 1979).

Younathan and Watts (1960) proposed that the rapid development of WOF in cooked meat is related to the oxidation of protein bound lipids. Labuza (1971) suggested that

the rapid rate of oxidation in cooked meat may be due to denaturation of myoglobin during cooking. Nitrite strongly inhibits the development of WOF as cured meat does not develop this off flavor. Tarladgis (1961) suggested that heme compounds from muscle are active catalysts of lipid oxidation in the oxidized form ($Fe^{3+}$) and nitrite retains the heme in the reduced form ($Fe^{2+}$) in cured meat pigment. Younathan and Watts (1959) and Sato and Herring (1973) suggested that the rapid oxidation of lipids in cooked meat is initiated by the conversion of the iron in the porphyrin ring of myoglobin to the ferric form. During heating, the pigment, ferric hemochromogen, is an active catalyst for unsaturated fat oxidation. Pearson and Gray (1983) and Igene et al. (1985) have proposed that nitrite reacts with myoglobin and subsequent cooking leads to stabilization of the heme pigments, thereby preventing the release of free $Fe^{2+}$ ions. Zipser et al. (1964) and Igene et al. (1985) proposed that nitrite may inhibit lipid oxidation in cured meat by forming a stable complex with meat pigments after cooking.

Sato and Hegarty (1971) postulated that cooking disrupts the muscle membrane system, resulting in exposure of the labile lipid to oxygen and other reaction catalysts.

Keller and Kinsella (1973) showed that the cooking loss (i.e., drip) of total lipids was proportional to the amount

of fat initially present and changes in the major individual phospholipid classes were small but closely paralleled the respective changes in total phospholipids during cooking. They also indicated that the relative proportion of unsaturated fatty acids in PC and PE decreased during cooking.

### Effect of Meat Type (Light vs. Dark)

Heme and nonheme iron have been reported to act as catalysts of lipid oxidation in meats (Wills, 1966; Liu and Watts, 1970). Barron and Lyman (1938) reported that heme catalyzed oxidation was more active in red muscle because of the larger amount of myoglobin compared to white muscle. Lipid oxidation in raw turkey thigh meat was greater than in turkey breast meat (Keskinel et al., 1964). Marion and Forsythe (1964) showed that the thiobarbituric acid (TBA) value was higher for the turkey red meat stored at 4 C for 1 to 7 days than for turkey white meat. They attributed this difference to the higher total lipid content of red meat. Jacobson and Koehler (1970) found that the TBA value of the dark meat of both chicken and turkey were greater than the TBA value of light meat, both before and after storage.

## Lipid Oxidation

Lipid oxidation in cooked and stored meat, has been the subject of numerous studies since 1946. Younathan and Watts (1960) and Pearson et al. (1977) stated that chicken fat is particularly high in oleic and linoleic fatty acids, and because of this unsaturated nature, poultry meat is more susceptible to rancidity than red meats. Turkey meat is most susceptible to the development of oxidative rancidity followed by chicken, pork, beef and mutton (Wilson et al., 1976). Off-flavors caused by the decomposition products of oxidized lipids develop very rapidly in meat after cooking. Hornstein et al. (1961), Govindarajan et al. (1977), and Igene and Pearson (1979) confirmed that the lean-tissue lipids of muscle are primarily responsible for oxidative deterioration. Oxidative deterioration is accompanied by various secondary reactions having oxidative and non-oxidative characteristics (Pearson etal., 1983). The important fatty acids involved in oxidation are the unsaturated oleic, linoleic and linolenic. Labuza (1971) reported that the rate of oxidation increases geometrically with the degree of unsaturation.

## Mechanism of Lipid Oxidation

Unsaturated fatty acid autoxidation has been proposed to occur by a free radical chain reaction mechanism which involves three stages: initiation, the formation of free

radical; propagation, the free radical chain reactions; and termination, the formation of non-radical products.

Initiation takes place when a labile hydrogen is removed from a carbon atom adjacent to a double bond in an unsaturated fatty acid (RH), with the formation of a free radical ($R^{\bullet}$). Initiators in this reaction include light, heat, heavy metals and oxygen. The development of the free

$$R_1H \xrightarrow{\text{initiators}} R_1^{\bullet} + H^{\bullet} \text{ (free radicals)}$$

radical ($R^{\bullet}$) mechanism was described in detail by Swern (1961).

Propagation involves the combination of the first free radical with molecular oxygen to form peroxide free radical ($ROO^{\bullet}$). These compounds are then able to abstract another hydrogen from a non-oxidized unsaturated fatty acid ($R_2H$) to form further free radical and hydroperoxide which is capable of propagating the chain reaction. The hydroperoxide is one of the major oxidation products that decomposes to form compounds responsible for off-flavor (Gaddis et al., 1961; Horvat et al., 1969).

$$R_1^{\bullet} + O_2 \longrightarrow R_1OO^{\bullet} \text{ (peroxide free radical)}$$

$$R_1OO^{\bullet} + R_2H \longrightarrow ROOH + R_2^{\bullet}$$

(hydroperoxide)

Another important mechanism for the oxidation of unsaturated lipids which involves activated species of oxygen was reviewed by Frankel (1985). Singlet oxygen, produced by photo-oxidation in the presence of sensitizers such as chlorophyll, is an important reactant.

Singlet oxidation of methyl oleate takes an entirely different stereochemical course than free radical autoxidation. Singlet oxygen adds directly to either unsaturated carbon by an ene addition reaction, and results in a change of configuration of double bond from cis to trans.

As linoleate reacts with singlet oxygen at a rate at least 1500 times faster than triplet oxygen, it was suggested that this very fast reaction is important way of initiating the free radical autoxidation of unsaturated lipids. Hydroperoxides formed by singlet oxidation can decompose thermally or in the presence of metal catalysts into alkoxyl and peroxyl radicals that can accelerate free radical autoxidation.

Deactivation of free radical with the creation of non-radical end products is considered to be the termination of the chain reaction. Free radical inhibitors ($R_1$) include antioxidants that may form inert end products as a termination step.

$R^{\bullet} + R^{\bullet} \longrightarrow RR$

$R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$

$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$

$R^{\bullet} + R_1 \longrightarrow RR_1$

## Lipid Oxidation Products

Hydroperoxides are the primary products of unsaturated fatty acid oxidation. Hydroperoxides are colorless and odorless and do not contribute to the off-flavor associated with lipid oxidation (Watts, 1954; Sato and Herring, 1973). Degradation of hydroperoxides through a series of scission and dismutation reactions yields the secondary products of lipid oxidation. The secondary products include aldehydes, ketones, acids, lactones, alcohols and unsaturated hydrocarbons (Frankel, 1984). The secondary products are directly responsible for the off-flavor of oxidative rancidity (Lea, 1962; Sato and Herring, 1973). Herz and Chang (1970) reported that carbonyls are the predominant members in any class of compounds in the meat flavor concentrates. Fat soluble carbonyls are the ones primarily involved with meat flavor. Love and Pearson (1976) found that hexanal is one of the important products of lipid oxidation which contributes to the development of WOF. Heptanal and n-nano-3-6-dienal were found to be associated with WOF in turkey meat (Ruenger et al., 1978).

## Catalysis of Lipid Oxidation

Porphyrins of hemoglobin, myoglobin and the cytochromes are catalysts for unsaturated fat oxidation in meat because of their iron content (Robinson, 1924; Tappel, 1952; Younathan and Watts, 1959). The reaction between lipid and hemoprotein is believed to destroy the pigment and oxidize the fat (Watts, 1954). Tappel (1955) demonstrated that hematin compounds catalyze the oxidation of unsaturated fatty acids, and that iron is the active factor in catalytic activity. The ferric form of heme is the active catalyst of lipid oxidation in muscle (Younathan and Watts, 1959). However, Brown et al. (1963) reported that heme with iron in either the ferrous or ferric states were effective catalysts of lipid oxidation. Labuza (1971) disagreed with the theory that only ferric hemes are capable of catalyzing lipid oxidation in meat and stressed the importance of knowledge of the electron orbital structures in understanding the role of heme pigments in lipid oxidation. He suggested that the protein portion of hemoprotein molecules may cause steric hindrance of the iron, preventing it from catalyzing oxidation. When meat is heated, denaturation of the protein portion of the molecule might facilitate exposure of iron to unsaturated fatty acids. In a 1975 review, Green and Price concluded that either $Fe^{2+}$ or $Fe^{3+}$ hemes might function as catalysts

of lipid oxidation, but the $Fe^{3+}$ hemes may be necessary for rapid catalysis.

The most important mechanism involved in heme-catalyzed lipid oxidation has been considered to be the catalytic decomposition of hydroperoxides to generate free radicals.

However it was reported by Kanner and Harel (1985) that the interaction of hydrogen peroxide with MetMb or MetHb led very rapidly to the production of an activated catalysts which initiated membranal lipid peroxidation. The activity of this catalyst as a prooxidant decreased rapidly during the first 5 minutes, and within 10 minutes was down to 50% of the fresh preparation. Kanner and Harel (1985) have suggested that the prooxidant effect was obtained when the ratio between heme group and hydrogen peroxide is almost 1:1, consequently the maximum prooxidant effect was reached at 5 to 7 micromoles MetHb vs. 30 micromoles MetMb (keeping hydrogen peroxide at 30 micromoles), because MetHb molecule contains four heme groups instead of one in MetMb.

Tappel (1962) reported that the most probable mechanism involved the formation of a coordinate complex between the heme compound and lipid hydroperoxide, followed by hemolytic scission of the peroxide bond. In this mechanism there would be no changes in the valence of heme iron. The findings of Sato and Hegarty (1971) were surprising in that neither ferrous nor ferric iron at relatively high concen-

tration had much effect on TBA values while the addition of iron powder resulted in an approximate doubling of TBA values. Accordingly they thought that the concentration of ionic iron or a balance between ferrous or ferric ions was critical to the reaction. Their results also showed that ferrous iron could play an important role in WOF development. Hirano and Olcott (1971) and Kendrick and Watts (1969) have demonstrated that heme compounds may act as either accelerators or inhibitors of lipid oxidation. The action depends on the ratio of heme to unsaturated fatty acids. Waters (1971) and Love and Pearson (1974) reported that there is strong evidence that ferrous iron is a more active catalyst of lipid oxidation than ferric iron. Love and Pearson (1974) and Igene et al. (1979) have demonstrated that nonheme iron is the major prooxidant in cooked meat and is released from heme pigments during cooking. Schricker et al.(1982), Schricker and Miller (1983) and Chen et al. (1984) have confirmed these results. It has been demonstrated in a variety of systems that the hemoproteins are able to act as antioxidants with certain ratios of fatty acid to heme, but Liu and Watts (1970) suggested that the ratio in red meats should favor catalysis rather than an antioxidant effect. Kunsman et al. (1978) suggested that the polyunsaturated fatty acid / hemoprotein ratio in mechanically deboned red meat may be in the range where

hemes exert an antioxidant effect, thus explaining the observation that lipids in this product do not oxidize more than in ground beef. $Cu^{2+}$ has been reported as having an inhibitory effect on the oxidation of hydrocarbons (Ingold, 1962). The inhibitory properties of cupric salts in lipid oxidation was attributed to chain termination by the catalyst (Ingold, 1962; Sato and Hegarty, 1971). However, a complex formation of free radicals with $Cu^{2+}$ during propagation may also result in reduced rate according to Ingold (1962). The data reported by Tichivangana and Morrissey (1985) indicate that $Cu^{2+}$ catalyzed oxidation followed a pattern similar to that for $Fe^{2+}$ catalysis, but $Cu^{2+}$ was slightly less effective. The rates of oxidation show that prooxidant activity was in the order : $Fe^{2+}$> $Cu^{2+}$> $Co^{2+}$> myoglobin (Mb), and that differences in activity between $Fe^{2+}$ and $Cu^{2+}$, $Fe^{2+}$ and $Co^{2+}$ and $Fe^{2+}$ and Mb were significant at the $P< 0.05$, $P<0.01$ and $P<0.001$ levels, respectively in all muscle systems. The susceptibility of the raw and heated muscle systems to lipid oxidation catalyzed by the various prooxidants was in the order : fish> turkey> chicken> pork> beef> lamb, which is generally consistent with polyunsaturated fatty acid content of these tissues.

Between 1 and 12% sodium chloride the rate of lipid oxidation increased in proportion to the concentration of the salt added (Castell et al., 1965). Castell et al.

(1965) concluded that pure sodium chloride has no direct effect on the oxidation of lipids but acts only on non-lipid components which activate the lipid oxidation. Hills and Conochie (1946) proposed that fat peroxide reacts with chloride ions, resulting in the formation of chlorine. The free chlorine formed brings about further oxidation of the fat.

Of particular interest is the ability of salt to denature actomyosin and other proteins in the muscle. Salt probably disrupts the lipoproteins or other complexes containing lipids (Dyer, 1953). It was found by Castell et al. (1965) that the prooxidant activity of sodium chloride in fish muscle was the result of sodium ions, and that cations of other metal salts had a similar prooxidant effect. When they used equivalent concentrations, the relative activity was in the following order : $Cu^{2+} > Fe^{3+} > Co^{3+} > Cd^{2+} > Li^{+} > Ni^{3+} > Mg^{2+} > Zn^{2+} > Ca^{2+} > Ba^{2+}$. They found that sodium chloride and many other metal salts acted as a prooxidants, but they have a strong inhibitory effect on $Cu^{2+}$-induced rancidity.

## Antioxidants

Antioxidants are substances that slow down oxidation of fats or fat-containing foods to prolong their wholesomeness and palatability. The concentration of an antioxidant used is important for reasons of cost, safety, sensory

properties and functionality. Some antioxidants provide increased protection as their concentration increases, whereas others have optimal levels, and at higher levels may be prooxidant (Dugan, 1976). Dugan (1976) indicated that the most effective antioxidants in food systems function by interrupting the free radical chain mechanism. This group [which includes butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tocopherol] consists mainly of phenolic compounds, capable of donating a labile hydrogen ion.

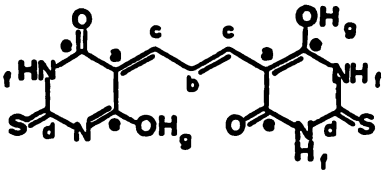
Acidic compounds such as citric acid, ascorbic acid and EDTA delay the onset of oxidative rancidity by inactivation of prooxidant metals, and are often used in combination with phenolic antioxidants. A combination of antioxidants is commonly used to obtain better effect than the various individual antioxidants (Dugan, 1960). Citric acid was more effective against iron and nickel while ascorbic acid and its derivatives were effective against copper, but not iron (Morris et al., 1950).

Sato and Hegarty (1971) showed that nitrite can completely eliminate WOF at 220 PPM and will inhibit its development at 50 PPM. Fooladi (1977) indicated that 156 PPM of nitrite prevents WOF in cooked meat and poultry. It has been reported by Kraybill et al. (1949) that BHA was very valuable in the stabilization of food which has been heat

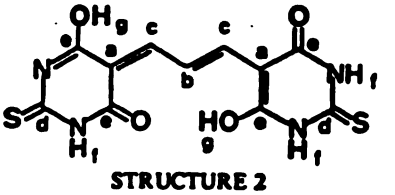
processed. According to Marion and Forsythe (1964), a significant delay of autoxidation of turkey lipids occurs with the addition of 0.04% BHA based on weight of lipids. Lower tocopherol content of turkey meat compared to chicken meat might be responsible for better storage stability of chicken meat (Mecchi et al., 1953).

### TBA, Sensory Scores and Bacterial Count

The reaction of malonaldehyde (MAL) with 2-thiobarbituric acid (TBA) has been widely used for measuring the extent of oxidative deterioration of lipids in muscle foods (Gray, 1978 and Rhee, 1978). The TBA test expresses lipid oxidation in mg MAL per Kg of meat sample. MAL is a secondary oxidation product of polyunsaturated fatty acids which have three or more double bonds (Dahle et al., 1962; Pryor et al., 1976). The structure of the adduct of TBA-MAL was reported by Nair and Turner (1984) who indicated that the combined spectroscopic data are totally consistent with two spectrally equivalent tautomeric structures 1 and 2.




STRUCTURE 1




STRUCTURE 2

TBA-MAL Adduct

They also concluded that formation of the 2:1 adduct of TBA and MAL probably is limited by neucleophilic attack involving carbon-5 of TBA onto carbon-1 of MAL followed by dehydration and similar subsequent reaction of the intermediate 1:1 adduct with second molecule of TBA.

Siu and Draper (1978) showed that the absorption spectrum of the pink solution in the visible range generated by adding the TBA reagent to meat distillates or filtrates was identical to that of the complex formed with the pure 1,3,3,- tetramethoxypropane (TEP). The meat extracts exhibited a single pink spot corresponding to that of the TBA-MAL complex when chromatographed on silica gel G (Rf=0.83). They concluded that these findings indicate that the TBA reaction is a valid indicator of the MAL present in meats.

It was reported by Kakuda et al. (1981) that there was a linear relationship, with an $r^2$ of 0.946, between the TBA absorbance at 532 nm and HPLC peak height of the MAL. The HPLC method measured MAL levels from $1x10^{-11}$ to $4x10^{-11}$ mole/10 microliters, compared to detection limits of $2 x 10^{-11}$ mole/10 microliters for the colored MAL-TBA complex.

The TBA test can be performed directly on the food product, followed by extraction of the colored complex (Sinnhuber and Yu, 1958), or on a portion of the distillate of the food sample (Tarladgis et al., 1960), or on an

extract of the food (Witte et al., 1970.) Although the distillate method is the most popular one, it doesn't necessarily mean that it is the most accurate or reproducible method. Witte et al. (1970) reported that the extraction method has the advantage of simplicity and ease of use. It also has the advantage of being more specific and selective for carbonyl compounds as described by Tarladgis et al. (1962) because it does not require heating. Witte et al. (1970) reported that TBA values determined by the distillate method were twice as large as those determined by extraction. The differences may be due to the incomplete extraction of malonaldehyde in the extraction method, since no heat was involved.

The heat of distillation may have increased the quantities of aldehyde from lipid precursors, or heat disrupted certain carbonyl addition products thought to occur by reaction between malonaldehyde and amino acids, pyrimidines or protein (Buttkus, 1967). Witte et al. (1970) concluded that it is unlikely that the low values obtained in the extraction method were due to color development with TBA at room temperature rather than by heating since it has been shown that the $E_m^{530}$ of the color developed at room temperature is greater than that developed by boiling (Tarladgis et al., 1964). A high

correlation between the two methods was observed by Witte et al. (1970).

There was a linear relationship between peroxide value and MAL concentrations during the oxidation of various classes of polyunsaturated fatty acids: linoleate, arachidonate, pentaenuate and hexaenuate (Sinnhuber and Yu, 1977).

Significant ($P<0.05$) correlation coefficients of $r=-0.57$ and $r=-0.51$ were found between TBA values and sensory scores for beef and chicken white meat model systems, respectively (Igene and Pearson, 1979). A correlation coefficient of -0.77 between flavor ratings and TBA number for turkey meat was reported by Jacobson and Koehler (1970).

Microbial and/or oxidative changes limit the acceptable shelf life of meat and result in significant flavor changes in the final products (Dawson et al., 1975). Lipid oxidation is accelerated and bacterial reproduction occur more rapidly in ground beef resulting in off-flavor.

Keskinel et al. (1964) reported that the TBA number increased in beef, pork, lamb and turkey meat after grinding. Freshly ground chicken and turkey have a bacterial count from 100,000 to 1,000,000 per gram (Maxey et al., 1973), and storage for 4 days at 5 C increases bacterial numbers to a level which is approximately

associated with unacceptable products. Dawson et al. (1975) concluded that a comparison of bacterial growth with panel scores indicates that the rapid bacterial growth the first 10 days of storage had little effect on the flavor of fried patties. This work seems to indicate that the grinding has a greater effect on TBA than the initial increase in bacterial population.

## Fatty acid profile

It has been reported by Lea (1953) and Keller and Kinsella (1973) that changes in fatty acid composition of lipids are important indirect indicators of lipid oxidation. Igene et al. (1981) demonstrated that the fatty acid profiles in the triglycerides of beef, chicken dark meat and chicken white meat were not significantly changed during 13 months of frozen storage or cooking. These findings are in agreement with the results of Chang and Watts (1952), and Campbell and Turkki (1967). However, Igene et al. (1981) reported that there were significant changes in fatty acid composition in phospholipids of beef and chicken dark and white meat. Unsaturated fatty acids particularly polyunsaturated fatty acids (PUFAS) participate in the development of oxidized flavor in frozen and/or cooked meat. Most of the losses in unsaturation were found in arachidonic acid. These results were verified by Igene and Pearson (1979) with model meat

systems. They have shown that the PUFAS of phospholipids were major contributors in the development of rancidity during frozen storage or in development of WOF in cooked meat.

Fishwick (1968) investigated changes in lipids of turkey muscle during chilled and frozen storage and reported that the plot of the loss of PE against the increase in free fatty acids (FFAS) was linear. The slopes of the lines calculated on a molar basis correspond to PE/FFA ratios of 36:100 and 38:100, respectively for leg and breast muscle. These values are of the same order of magnitude as the respective concentrations (38% and 32%) of PE in the total phospholipid, excluding SP. He has reported that the decrease in the PC content of the tissue during storage could not be followed directly because PE produced by enzyme hydrolysis was not separated from PC on thin layer plates. A linear relationship was, however, established between the increase in LPC and increase in FFA. The molar ratio of LPC/FFA was found to be 41:100 and 59:100 for leg and breast muscle respectively, compared with concentrations of PC in the total phospholipid, excluding SP, of 59% and 66%. The latter figures, however, are too high, probably by about 10 units because of the inclusion of PI in the PC fraction from the thin-layer plates. These findings indicate that lipid hydrolysis at -

10 C and -20 C was mainly due to the action of phospholipase A on the glycerophospholipids of the muscle to give corresponding lyso compounds and fatty acids. There was no evidence of any loss of SP. However Davidkova and Khan (1967) have published data on changes in the lipids of broiler chickens stored for two years at -10 C. The authors found that PC and PE decreased and FFAS and LPC increased. About 70% of the total increase in FFA content of the muscle was probably due to phospholipase B activity and the remaining 30% to breakdown of triglyceride.

The fatty acid composition as percent of the lipids in fresh turkey muscle has been reported by Fishwick (1968) as shown in Table 1. The data show that phospholipids were comparatively rich in polyunsaturated fatty acids, especially 20:4 and 22:6, whereas these acids were virtually absent from the triglyceride fraction.

Table 1. Fatty acid composition percent of the lipid of fresh turkey muscle *

| Fatty acid | Breast PL | Breast DG | Breast FFA | Breast TG | Leg PL | Leg DG | Leg FFA | Leg TG |
|---|---|---|---|---|---|---|---|---|
| 14:0 | tr | 0.5 | 0.6 | 1.2 | 0.2 | 1.4 | 0.6 | 1.2 |
| 16:A | 9.9 | 3.3 | 0.0 | 0.0 | 7.8 | 1.5 | 0.0 | 0.0 |
| 16:0 | 19.0 | 25.9 | 14.6 | 27.6 | 14.5 | 18.8 | 18.4 | 23.7 |
| 16:1 | 1.1 | 5.9 | 2.6 | 7.8 | 1.3 | 4.8 | 2.7 | 6.9 |
| 17:0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.7 |
| 18:A | 2.1 | 0.5 | 0.8 | 0.0 | 2.2 | 0.0 | 0.0 | 0.0 |
| 18.0 | 12.9 | 11.0 | 8.7 | 7.3 | 13.9 | 8.1 | 8.4 | 6.4 |
| 18:1 | 11.8 | 25.1 | 14.0 | 27.6 | 10.8 | 25.9 | 19.2 | 28.4 |
| 18:2 | 22.3 | 17.8 | 30.1 | 27.4 | 24.9 | 28.4 | 29.6 | 28.1 |
| 18:3 | 0.0 | 2.4 | 0.7 | 1.0 | 0.0 | 0.9 | 0.8 | 1.6 |
| 20:0 | 0.0 | 0.0 | 0.0 | 0.0 | tr | 0.0 | 0.0 | 0.0 |
| 20:3 | 0.2 | 0.2 | 0.0 | 0.0 | 0.7 | 0.9 | 11.7 | 0.0 |
| 20:4 | 7.8 | 2.0 | 12.4 | 0.0 | 9.8 | 4.5 | 5.2 | 0.4 |
| 20:5 | 2.9 | 2.7 | 6.0 | tr | 3.9 | 0.5 | 0.0 | 0.4 |
| 24:0 | 0.0 | 0.0 | 0.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22:5 | 1.9 | tr | 2.0 | 0.0 | 1.6 | 1.2 | 0.3 | 0.6 |
| 22:6 | 8.2 | 0.9 | 6.7 | tr | 8.3 | 3.0 | 3.1 | 1.6 |

* Phospholipid (PL); diglyceride (DG); free fatty acid (FFA); triglyceride (TG); saturated aldehyde (A)

From Fishwick (1968)

# EXPERIMENTAL PROCEDURE

## Materials

### Chemicals and Solvents

Chemicals and solvents used were of analytical or reagent grade. Fresh and glass distilled solvents were used directly, otherwise they were redistilled before use.

### Methyl Esters

Fatty acids and methyl ester mixtures used as standards for determination of the relative retention time of fatty acids in phospholipids and neutral lipids by gas liquid chromatography (GLS) were purchased from Supelco, Inc., Bellefonte, PA. and Applied Science Lab., State College, PA.

### TLC Standards

For the TLC analysis two types of standard mixtures were used. The polar lipid mixture contained cholesterol, phosphatidyl ethanolamine, phosphatidyl choline and lysophosphatidyl choline, while the serum lipid mixture contained specified quantities of lysolecithin, sphingomyeline, lecithin, phosphatidyl serine, phosphatidyl inositol,

phosphatidyl ethanolamine, phosphatidyl glycerol, cardiolipin and phosphatidic acids.

**Thin Layer Plates**

Redi-Coats-G, 20x20 cm thin layer silica gel (0.50 mm) glass plates were purchased from Supelco Inc. Plates were activated before use at 110C for 30 minutes, and used for phospholipid fractionation.

**Silicic Acid**

Silicic acid was obtained from Mallinckrodt, Inc. The fines were washed out with deionized distilled water followed by anhydrous methanol. Silicic acid was activated at 110 C for 24 hours before using for separation of polar lipids.

**Antioxidants**

Two types of antioxidants were used. An antioxidant coated salt (Diamond Crystal Salt Co. St. Clair, Michigan) was composed of 99.5% fine flake salt, 0.045% ascorbyl palmitate, 0.015% active vitamin E (mixed tocopherols, 0.0215% total), 0.0005% citric acid and 0.0425% silicone dioxide ( anti cake). At 2% fat in meat, 2% of this salt is equivalent to 0.06% active antioxidant in the fat. This salt was mixed with pure fine flake salt to achieve 0.02% antioxidant based on fat content of meat and 2% salt in the meat. The other antioxidant was Tenox 6 which contains

butylated hydroxyanisole(BHA) 10%, butylated hydroxytoluene (BHT) 10%, propyl gallate (PG) 6%, citric acid 6%, corn oil 28%, glyceryl monooleate 28% and propylene glycol 12%. (Eastman Kodak Co. Kingsport, Tennessee). Tenox 6 was mixed with salt not to exceed 0.02% antioxidant, based on the fat content of meat and 2% salt in the meat.

**Source of Salt**

Two types of pure salt (PS), calcium magnesium free salt (CMF) and fine flake salt (FFS), and two types of impure rock salt (RS), northern rock salt (NRS) and southern rock salt (SRS), were obtained from Diamond Crystal Salt Co. St. Clair, Michigan. Analytical composition of the CMF and FFS was obtained from the same Company. (Tables 1 and 2 , Appendix A).

**Metal Ions**

Ferric chloride, ferrous chloride, cupric chloride and magnesium chloride were purchased from Sigma Chemical Company, St. Louis, Missouri.

**Antibiotic**

Chlortetracycline hydrochloride was purchased from Sigma Chemical Company, St. Louis, Missouri.

**Plate Count Agar**

The BBL Standard Methods Agar with lecithin and polysorbate 80 was used for aerobic plate count and was purchased from BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, MD.

**TEP Standard Solution**

The 1,1,3,3-Tetraethoxypropane (TEP) used for making standards for thiobarbituric acid analysis was obtained from Sigma Chemical Company. St. Louis, Missouri.

**Source of Meat**

Turkey meat used in this study was obtained from Bil-Mar Foods, Inc., Zeeland, Michigan. The white meat was represented by pectoralis minor, breast muscle, and the red meat represented by the deboned thighs. Thoroughly chilled samples were brought to the laboratory in nylon bags in insulated ice boxes with a minimum of delay.

## Methods

**Extraction of Total Lipids**

Total lipids were extracted by the method of Folch et al. (1957) with some modifications. A specified weight of ground turkey meat was homogenized at 23,000 rpm for one minute in Virtis homogenizer ( The Virtis Co. Gardiner,

N.Y.) with 4 volumes chloroform:methanol (2:1). The homogenates were filtered through Whatman #1 filter paper using Buchner funnel. Four more volumes of chloroform:methanol (2:1) were used for reextraction of the residue. The combined filtrate was transferred to a separatory funnel and washed with 2 volumes of 0.74% potassium chloride solution and left at -25 C overnight to allow the chloroform and aqueous layer to separate. The chloroform layer was collected and evaporated under reduced pressure at 40 C using a Rotavopor-R ( Buchi, Switzerland). Traces of chloroform were removed by a stream of nitrogen and the crude lipid was redissolved to a constant volume in chloroform and transferred under nitrogen to vials with air tight teflon lined screw caps to be stored at -25 C for further analysis.

**Separation of Neutral and Phospholipids**

Total lipids were separated into neutral and phospholipids according to the method of Choudhury and Arnold (1960) with slight modifications. One half gram of crude lipid was added to 10 g of silicic acid in a 125 ml Erlenmeyer flask. Twenty five milliliters of chloroform was added, the mixture shaken and the flasks sealed under a stream of nitrogen and kept in the freezer 8 to 16 hours. The content of each flask was filtered through a sintered glass funnel under vacuum. The silicic acid remaining in

the funnel was washed with five 30 ml aliquots of chloroform. This filtrate contained the neutral lipids. The chloroform was evaporated and the neutral lipid sample dried and preserved in the same manner as total lipids. A known volume from the neutral lipid solution was heated in the oven to a constant weight at 105 C. The percentage of neutral lipids equals the weight of the extracted lipid over the sample weight times 100.

The phospholipid content was determined by washing the silicic acid residues with three 25 ml aliquots of methyl alcohol. The methyl alcohol was evaporated and the phospholipid sample redissolved in chloroform to a specified volume. Phospholipid was quantitated and preserved for further analysis as in neutral lipids.

**Classification of Phospholipids**

The TLC plates were heat activated, then cooled for 30 minutes. A five microliter aliquot of phospholipid sample (50 mg/ml) or standards were spotted with a microsyringe, The spots were dried with a stream of nitrogen and the plates were placed in the developing chamber. The chambers were lined on all sides with saturation paper saturated with solvent just before use in order to obtain reproducible results with straight solvent fronts. Two hundred ml

of fresh chloroform/methanol /water mixture (65/25/4, v/v) and new chamber liners were used for each run.

The developed chromatograms were nitrogen dried for several minutes. Chromatograms were visualized with sulfuric acid-potassium dichromate reagent prepared by dissolving 0.6 g of potassium dichromate in 200 ml of 55% reagent grade sulfuric acid. Spots were developed in an oven at 175 C for 30 minutes. Quantitation of phospholipid classes from the spots was accomplished according to the densitometric method described by Rouser et al. (1964). A Shimadzu Dual-Wavelength Thin Layer Chromato Scanner Model CS-930 and Data Recorder Dr-2 were used in this investigation.

**Preparation of Methyl Esters**

Boron-trifluoride/methanol was used for converting neutral and phospholipids to methyl esters according to the procedure described by Morrison and Smith (1964).

**Gas-Liquid Chromatography (GLC)**

A Varian (Model 3700) chromatograph equipped with hydrogen flame ionization detector (FID) and CDS 111 integrator was used for GLC analysis of fatty acid methyl esters. Two meter x 2mm i.d. glass columns packed with 10% SP-2330, 68% cyanopropyl on 100/120 supelcoport (Supelco, Inc.) were used. The carrier gas was nitrogen at 20 ml/ minute. The hydrogen flame was supplied with 300 ml air/-

of trace chloroform/methanol. Water mixture (65:25:4, v:v:v) and new chamber liners were used for each run.

The developed chromatograms were nitrogen dried for several minutes. Chromatograms were visualized with sulfuric acid-potassium dichromate reagent prepared by dissolving 9.6 g of potassium dichromate in 300 ml of 55% reagent grade sulfuric acid. Spots were developed in an oven at 175 C for 20 minutes. Quantitation of phospholipid classes from the spots was accomplished according to the densitometric method described by Mosser et al. (1984). A Shimadzu Dual-Wavelength Thin Layer Chromato Scanner Model CS-930 and Data Recorder DR-2 were used in this investigation.

Preparation of Methyl Esters

Boron-trifluoride/methanol was used for converting neutral and phospholipids to methyl esters according to the procedure described by Morrison and Smith (1964).

Gas-Liquid Chromatography (GLC)

A Varian (Model 3700) chromatograph equipped with hydrogen flame ionization detector (FID) and CDS III integrator was used for GLC analysis of fatty acid methyl esters. Two meter x 2mm i.d. glass columns packed with 10% SP-2330, 80% cyanopropyl on 100/120 supelcoport (Supelco, Inc.) were used. The carrier gas was nitrogen at 20 ml/minute. The hydrogen flow was supplied with 300 ml air/-

minute and 30 ml hydrogen/minute. The injection port temperature was 200 C and the detector temperature was 300 C. The column temperature was programmed at 150 C for 3 minutes, followed by an increase of 5 C/minute to a final temperature of 220 C.

Qualitative identification of the fatty acid peaks was done by comparison with the retention times of the known fatty acid methyl ester peaks. Peak areas were quantitated by the integrator, and the results were expressed as percentage of the total area.

**Identification of dimethyl acetal of Hexa- and Octadecanal**

Phospholipid samples prepared as previously described were esterified with either sodium methoxide or boron trifluoride-methanol ($BF_3$-MeOH). To verify that the compounds were not plasticide or other artifacts from the extraction procedures employed, phospholipid samples were also saponified with alcoholic KOH solution. The nonsaponifiable part was separated and washed out with petroleum ether. The free fatty acids were extracted from the saponifiable portion with hexane after acidification with HCl. The volume of the remaining solution was reduced with nitrogen and 4-16 mg were dried and esterified with $BF_3$-MeOH as previously described. GLC-MS spectrophotometric analysis for identification and quantitation of the two dimethyl

acetals was performed at Northern Regional Research Center (USDA, Peoria, Illinois, courtesy of Dr. Edwin Frankel).

**Thiobarbituric Acid Test (TBA)**

An improved extraction TBA method was used in this investigation (Salih et al., 1986). It is a modification of the extraction method of Witte et al. (1970) with further modification as recommended by personal communication with J. Bowers (1984).

Ten grams of ground meat were homogenized with 35 ml of 3.86% perchloric acid in a Virtis homogenizer at a speed of 13,800 rpm (speed setting at 60) for one minute. The blended sample was filtered through Whatman no. 2V filter paper into a 50 ml Erlenmeyer flask. The Virtis flask was washed with 5 ml distilled water and filtered. Two 5 ml portions of the filtrate were pipetted to 50 ml test tubes. Five ml of 0.02 M thiobarbituric acid in distilled water was added, the tubes were capped, the contents mixed thoroughly, and then incubated at room temperature in the dark for 15-17 hours. The absorbance was determined on Perkin-Elmer Spectrophotometer at 531 nm against a reagent blank in which 5 ml of distilled water was added in place of the filtrate.

The standard stock solution of 1,1,3,3-tetraethoxypropane (TEP) was prepared by dissolving) 0.233 g TEP in a

1000 ml of distilled water. This is a $10^{-3}$ M TEP solution. Ten ml of this solution were diluted to 500 ml in distilled water. This stock solution contained $10^{-7}$moles TEP/5ml (and the one used for standard each time). Both TEP solutions were kept refrigerated. With each group of meat samples a standard curve was made with concentrations of 1, 2, 4, 6 and 8 x $10^{-8}$ moles TEP/5ml. To 5 ml TEP in each reaction tube 5 ml TBA reagent were added. The contents were mixed and tubes were capped and stored for 15-17 hours.

TBA values were derived from the regression equation for the standard curve. The regression equation for the standard curve is: sample concentration = {[absorbance-intercept estimate]/concentration estimate}/$10^{-8}$. The $r^2$ should be not below 0.95 otherwise something may be wrong with the standard solution. Concentration is determined from the regression equation and multiplied by the constant (K), which is 0.774, to determine mg MAL/Kg meat which is the TBA value. K is derived from 93% recovery of TEP to MAL, which weighs 72 g/mole. The recovery of MAL was determined by adding known amounts of TEP to meat homogenates. The 5 ml filtrate analyzed was equivalent to 1g meat . Thus the sample concentration (A) x $10^{-8}$ moles of MAL is the same as A x $10^{-5}$ moles MAL/Kg meat. This is equivalent to 72 A x $10^{-5}$g MAL/Kg meat or 72 A x $10^{-2}$ mg MAL/Kg meat.

After correction for recovery (0.72 A x 100/93) the constant is 0.774.

**Nonheme Iron**

Nonheme iron was determined as described by Schricker et al. (1982) with some modifications. Modification of the Schricker method included centrifuging the sample after incubation for 10 minutes at 27,000 x g to precipitate interfering pigments. Calculation of nonheme iron was based on weight of the sample after 20 hours incubation at 60 C to correct for any evaporation which may have occurred.

**Total Iron and Copper**

The concentration of total iron and copper were determined using an atomic absorption spectrophotometer (Perkin-Elmer Model 2380). Iron and copper standards were used. A wet ashing procedure was used for preparation of samples. (Schricker et al. 1982).

**Aerobic Plate Count**

A pour plate method was used as described by Deibel and Lindquist (1981). Raw and cooked turkey breast meat treatments analyzed include control (CNT), rock salt (RS). antibiotics (ANT) and cupric + ferrous ions (CuFe). Maximum refrigeration and frozen storage times were 21 days and 12 months, respectively. Raw and cooked turkey thigh meat treatments included control (CNT), pure salt (PS), ferric

ions (Fe) and cupric ions (Cu). Maximum refrigeration and frozen storage times were 14 days and 3 months, respectively. Plates were incubated at 37 C for 48 hours.

**Moisture**

The A.O.A.C. (1975, 25.003b) procedure for the determination of moisture was used.

**Total Fat**

The total solids left after moisture determination were used for total fat determination. The Goldfisch ether fat extraction method (A.O.C.S., 1974, 24.005b) was used.

**Protein**

The Micro Kjeldhal procedure was used to determine the total protein (A.O.A.C., 1975, 23.009). Buchi distillation unit (model 322), Buchi control unit (342), Buchi titrator (model E 526) and an integrator were used for automatic distillation, titration and calculation.

**Cooking**

The samples were vacuum packaged in polyethylene mylar laminate bags and cooked in water bath at 75 to 78 C to an internal temperature of 71C.

## Experimental Design

### Experiment A

The objectives of this experiment were:

1. To evaluate the effectiveness of an improved extraction thiobarbituric acid test (TBA) for monitoring lipid oxidation in poultry products.
2. To determine the correlation between TBA methods and sensory scores.

For objective no.1, the TBA of cooked turkey breast meat, refrigerated at 4 C for 72 hours, was determinedby both the improved extraction and the distillation methods. The effect of BHA (125 micrograms/g fat) was comparedand the critical TBA value at which wormed-over flavor (WOF) could be recognized by sensory evaluation was determined for the two methods.

For objective no. 2, freshly deboned unfrozen chicken breast was ground by a single pass through the 7 mm plate of a food grinder (Kichen Aid Stand mixer KSA with plastic grinding attachment, Hobart Corp., Troy, OH). Sixty grams of ground meat were stuffed into 50 ml polystyrene centrifuge tubes and sealed with a screw cap. Ground meat was cooked in a water bath to different final temperatures (Table 2). The water bath and center product temperature during cooking were monitored using thermocouples and a Honeywell recording potentiometer (Model F 2157). When

final temperature was reached the tubes were cooled in ice for 30 minutes before analysis and storage. The cooked

Table 2. Experimental design for the heat treatment of ground chicken breast (1)

| | ground chicken breast | | |
|---|---|---|---|
| Sample number | Water bath temp.(C) | Final center temp. of sample (C) | Cooking time (min) |
| 1 | 95 | 56 | 5.6 |
| 2 | = | 65 | 7.2 |
| 3 | = | 70 | 8.7 |
| 4 | = | 88 | 19.9 |
| 5 | = | 90 | 28.0 |

(1) All heating treatments were evaluated in duplicate

meat samples were reground and mixed immediately before testing to eliminate variation within the sample. Lipid oxidation was measured using the extraction method described by Salih et al. (1986) and distillation method of Tarladgis (1960). TBA tests were performed within two hours of cooking and after 48 hours of storage at 4 C.

Cooked ground chicken was evaluated for warmed-over flavor (WOF) after 48 hours of storage at 4 C by sensory test. A panel composed of 8 males and 4 females, evaluated the samples. All panelists had previous training and experience in evaluating WOF in meat.

Ground chicken was served in covered one ounce plastic cups coded with random three digit numbers and presented in

a balanced block design. The ground meat was heated for 15 seconds to a temperature of 70 C in a microwave immediately before serving. Five samples were evaluated at each session. A freshly cooked sample was used as a reference. An unstructured 100 mm scale with ends of the scale labeled "no warmed-over flavor" and "strong warmed-over flavor" was used. A typical scoring form and instructions are shown in Table 2, Appendix B. Analysis of variance (ANOVA) was used for testing the significant differences attributable to main effects. Main effects tested were treatments, judges, replications and two-way interactions. Duncans new multiple range test was used to calculate significant differences between treatments (Steel and Torrie, 1960).

**Experiment B**

The primary objectives of experiment B were to study the effect of two types of pure salt, two types of rock salt, a broad spectrum antibiotic, iron, copper and magnesium on the development of oxidative rancidity during refrigerated and frozen storage of raw and cooked ground turkey breast meat.

**Turkey Breast Meat Processing:**

A 75.0 Kg batch of freshly separated pectoralis minor turkey breast muscle was trimmed of external fat and tendons and cut into two halves. Consequently two identical

portions of meat were obtained, each portion weighed approximately 36.0 Kg. All meat was packaged under vacuum in polyethylene mylar laminate bags (Koch Equipment Co. Kansas City, MO), approximately 4.0 Kg each, and frozen rapidly to -25 C for one day. Packages of one portion were thawed in the cold room within 6 to 8 hours and ground twice through 3/8 inch plate using a Hobart meat grinder. The ground meat was divided into 10 portions, 3.6 Kg per portion and each portion was mixed with one of the treatments indicated in Table 3 using a Hobart Kitchen Aid mixer for one minute. Each treated portion was divided into 9 portions, wrapped in PVC film and stored as described in Table 4.

The other 36.0 Kg in packages were thawed, ground, and mixed with the same treatments (Table 3), cooked, wrapped, and stored as in Table 4.

Before analysis, each wrapped sample was reground by a single pass through the 7 mm plate of a food grinder (Kichen Aid Stand mixer KSA with a plastic grinding attachment).

Table 3. Treatments of experiment B

| Code | Description |
|---|---|
| 1. CNT | Control |
| 2. CMF | 2% calcium magnesium free salt (CMF)[1] |
| 3. FFS | 2% fine flake salt (FFS)[2] |
| 4. NRS | 2% northern rock salt |
| 5. SRS | 2% southern rock salt |
| 6. ANT | 100 PPM chlortetracycline |
| 7. FeCu | 25 PPM ferrous ions + 25 PPM cupric ions + 2% CMF |
| 8. Cu | 50 PPM cupric ions + 2% CMF |
| 9. Fe | 50 PPM ferric ions + 2% CMF |
| 10. Mg | 50 PPM magnesium ions + 2% CMF |

[1]CMF type high grade salt (Diamond Crystal Salt Co., St. Clair, Michigan)

[2]Alberger Process Fine Flake Salt (Diamond Crystal Co., St. Clair, Michigan)

Table 4. Design of experiment B

| Raw or cooked turkey breast | |
|---|---|
| A sample (3.6 Kg) for each treatment shown in Table 3 was divided into nine equal subsamples, wrapped in PVC film and refrigerated or frozen | |
| Refrigerated at 4 C for the indicated storage times | Frozen at -25C for the indicated storage times |
| 1. 0-day | 1. 1-month |
| 2. 2-days | 2. 3-months |
| 3. 7-days | 3. 6-months |
| 4. 14-days | 4. 12-months |
| 5. 21-days | |

**Experiment B Analysis:**

1. TBA
2. Fat extraction
3. Fat separation into neutral and phospholipids
4. GC analysis of fatty acids
5. TLC phospholipid classification
6. Heme iron analysis
7. Total iron and copper monitoring by atomic absorption spectrophotometer
8. Fat, protein and moisture determination
9. Aerobic plate count

**Experiment C**

The objectives of experiment C were to study the effect of fine flake salt, iron, copper and two types of antioxidants on the development of oxidative rancidity during refrigerated and frozen storages of raw and cooked ground turkey breast and thigh meat.

**Turkey breast and thigh meat processing:**

A 36.Ø Kg batch of each of freshly separated pectoralis minor turkey breast muscle and deboned turkey thigh meat were trimmed from external fat and tendons. All meat was packaged under vacuum in polyethylene mylar laminate bags and frozen rapidly to -25 C. Each meat type was thawed in the cold room within 6 to 8 hours and ground twice through 3/8 inch plate using Hobart meat grinder and divided into two halves. One half was divided into 1Ø portions, 1.8 Kg per portion. Each portion was mixed with one of the treatments listed in Table 5 and divided into subsamples which were wrapped and stored as reported in Table 6. The second half was divided and treated like the first half, but the meat was cooked.

Table 5. Treatments of experiment C

| Code | Description |
|---|---|
| 1. CNT | Control |
| 2. FFS | 2% fine flake salt |
| 3. Fe | 50 PPM ferric ions + 2% FFS |
| 4. Cu | 50 PPM cupric ions + 2% FFS |
| 5. VE[1] | 2% FFS coated with 0.045% ascorbyl palmitate, 0.015 active vitamin E (mixed tocopherols, 0.0215 % total), 0.0005% citric acid |
| 6. VEFe | VE + 50 PPM ferric ions |
| 7. VECu | VE + 50 PPM cupric ions |
| 8. T6 | 2% pure salt coated with tenox 6 (BHA 10%, BHT 10%, PG 6%, citric acid 6%, corn oil 28%, glyceryl monooleate 28% and propylene glycol 12%) |
| 9. T6Fe | T6 + 50 PPM ferric ions |
| 10. T6Cu | T6 + 50 PPM cupric ions |

[1]An antioxidant coated salt prepared by Diamond Crystal Co.

Table 6. Design of experiment C

| Raw or cooked turkey meat<br>Breast or Thigh | |
|---|---|
| A sample (1.8 Kg) for each treatment shown in Table 5 was divided into seven equal subsamples (breast) or six subsamples (thigh), wrapped in PVC film and refrigerated orfrozen | |
| Refrigerated at 4 C for the indicatedstorage times | Frozen at -25Cfor the indicatedstorage times |
| 1. 0-day | 1. 1-month |
| 2. 2-days | 2. 3-months |
| 3. 7-days | 3. 6-months (breast) |
| 4. 14-days | |

**Experiment C Analysis:**

1. TBA
2. Fat extraction (for thigh meat only)
3. Fat separation into neutral and phospholipids
4. GC analysis of fatty acid
5. Heme iron analysis
6. Total iron and copper monitoring by atomic absorption spectrophotometer
7. Fat, protein and moisture determination
8. Aerobic plate count

**Statistical Analysis**

Statistical analyses were performed using Statistical Analysis Systems (SAS, 1985) for the five factor analysis of variance (ANOVA) with nested design of TBA results of experiments B and C together. Statistical Package for the Social Sciences (SPSS, 1984) was used for the 3 factor ANOVA of fatty acid profile in turkey breast neutral and phospholipids. MSTAT (1985) was used for ANOVA for aerobic plate count, neutral and phospholipid and phospholipid classes. Microstat (Ecosoft, 1984) was used for analysis of taste panel scores, and to obtain standard deviations, simple correlation and regression coefficients. The significance between treatments was determined using either the Tukey test or Boonferroni t-test for multiple comparison analysis, after a significant F was determined. Graphs were plotted using Plotit (Eisensmith, 1985).

## RESULTS AND DISCUSSION

### TBA Methods and Sensory Scores

The relationship between TBA measurements by an improved extraction method or the distillation TBA method of Tarladgis et al. (1960) and taste panel scores for monitoring lipid oxidation and WOF in poultry meat were investigated.

TBA numbers obtained using the distillation method are 1.4 to 2.0 times larger than those of the improved extraction method (Table 7). These results are comparable to the findings of Witte et al. (1970) who reported that the TBA values determined by the distillation method were approximately twice as large as those determined by the extraction

Table 7. Comparison between distillation and improved extraction TBA method[1]

| Meat type | Distillation | Extraction | Ratio |
|---|---|---|---|
| raw breast | 8.39 ± 0.30 | 4.25 ± 0.11 | 2.0 X |
| cooked breast | 10.07 ± 0.32 | 7.10 ± 0.26 | 1.4 X |
| raw thigh | 11.52 ± 0.31 | 6.10 ± 0.19 | 1.9 X |
| cooked thigh | 14.16 ± 0.37 | 7.89 ± 0.21 | 1.8 X |

[1]Means represent six determinations

method. The heat used in distillation may increase quantities of aldehydes, disrupt certain carbonyl products thought to occur by a reaction between malonaldehyde and amino acids, pyrimidine or protein (Buttkus, 1967). Heat used in distillation may speed up the oxidation process (Witte et al., 1970 and Siu and Draper, 1978). The latter was substantiated by the decrease in TBA number when adding BHA before blending. The decrease in TBA number was about 18 and 15% in distillation and in the improved TBA methods, respectively (Tables 8 and 9). These findings agree with results of Pikul et al. (1983) which demonstrate a significant decrease in TBA value when BHA was added.

Table 8. Effect of BHA on TBA values measured by the distillation method[1,2]

| Meat type | Control | + BHA | % Decrease |
|---|---|---|---|
| raw breast | 8.39 ± 0.30 | 6.44 ± 0.27 | 23.0 |
| cooked breast | 10.07 ± 0.32 | 8.76 ± 0.29 | 13.0 |
| raw thigh | 11.52 ± 0.30 | 9.14 ± 0.28 | 21.0 |
| cooked thigh | 14.16 ± 0.37 | 11.94 ± 0.30 | 15.7 |

[1]BHA: 125 micrograms/mg fat

[2]Values represent means of six determinations

Table 9. Effect of BHA on TBA values measured by the improved extraction method[1]

| Meat type | Control | +BHA | % Decrease |
|---|---|---|---|
| raw breast | 4.26 ± 0.11 | 3.45 ± 0.12 | 18.9 |
| cooked breast | 7.10 ± 0.25 | 6.35 ± 0.22 | 10.6 |
| raw thigh | 6.10 ± 0.19 | 5.06 ± 0.27 | 17.1 |
| cooked thigh | 7.89 ± 0.21 | 6.91 ± 0.22 | 12.3 |

[1]Values represent means of six determinations

Heat in the distillation method is used to free malonaldehyde from its bound state with protein, while perchloric acid is the releasing factor in the extraction method. The standard deviation in the distillation method is generally higher than that in the improved extraction method (Table 7).

The relationship between the improved extraction and distillation TBA methods is good. The correlation coefficient was higher when the cooked chicken breast meat was analyzed after 48 hours of refrigeration than when done 2 hours after cooking (r = 0.934 vs. 0.854, Figures, 1 and 2). Witte et al. (1970) reported that the correlation coefficient between TBA values determined by the extraction method and the distillation method was represented by an r value of 0.845.

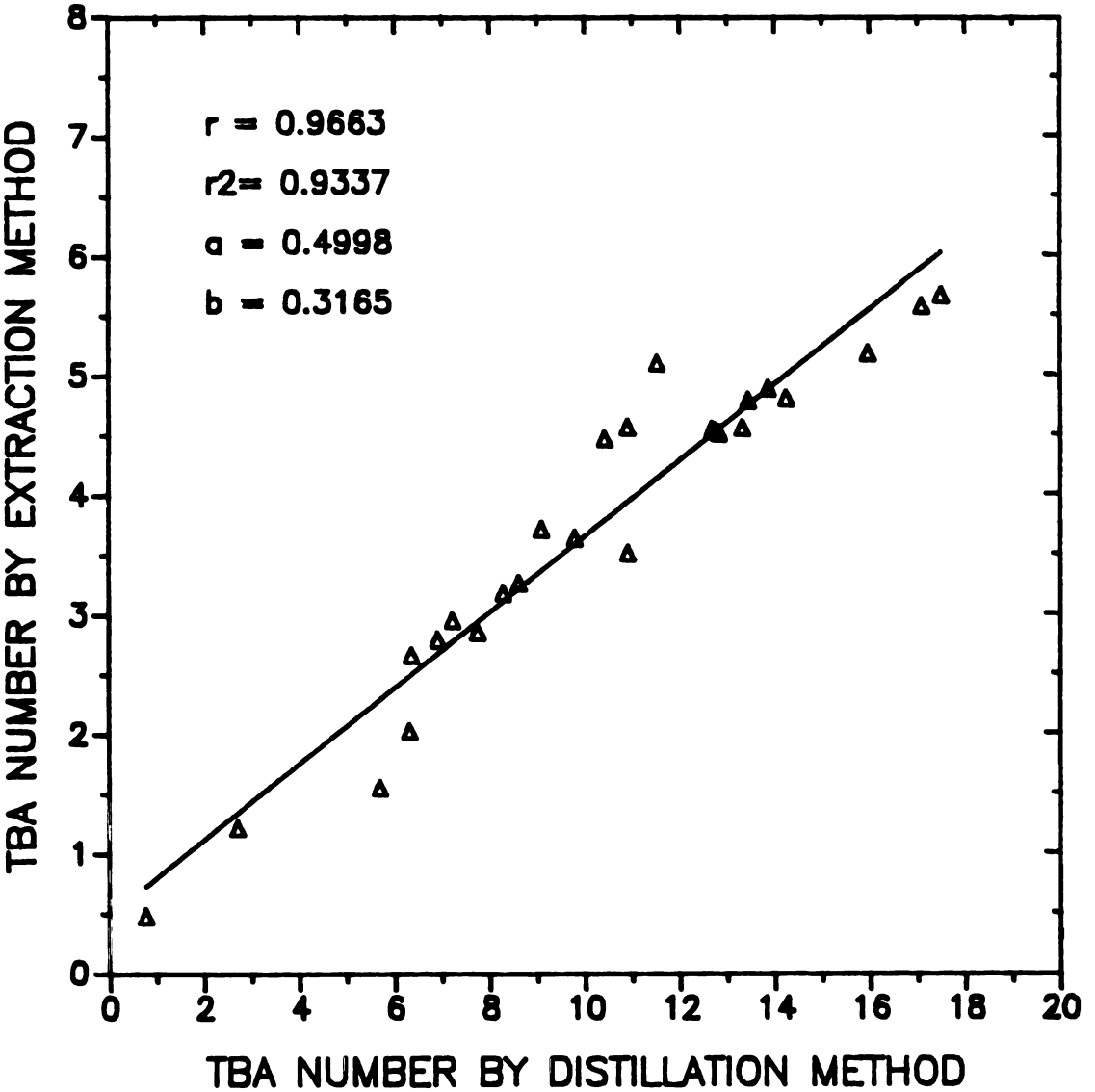



Figure 1. Relationship between the improved extraction and distillation TBA methods for chicken breast meat refrigerated at 4 C for 48 hours.

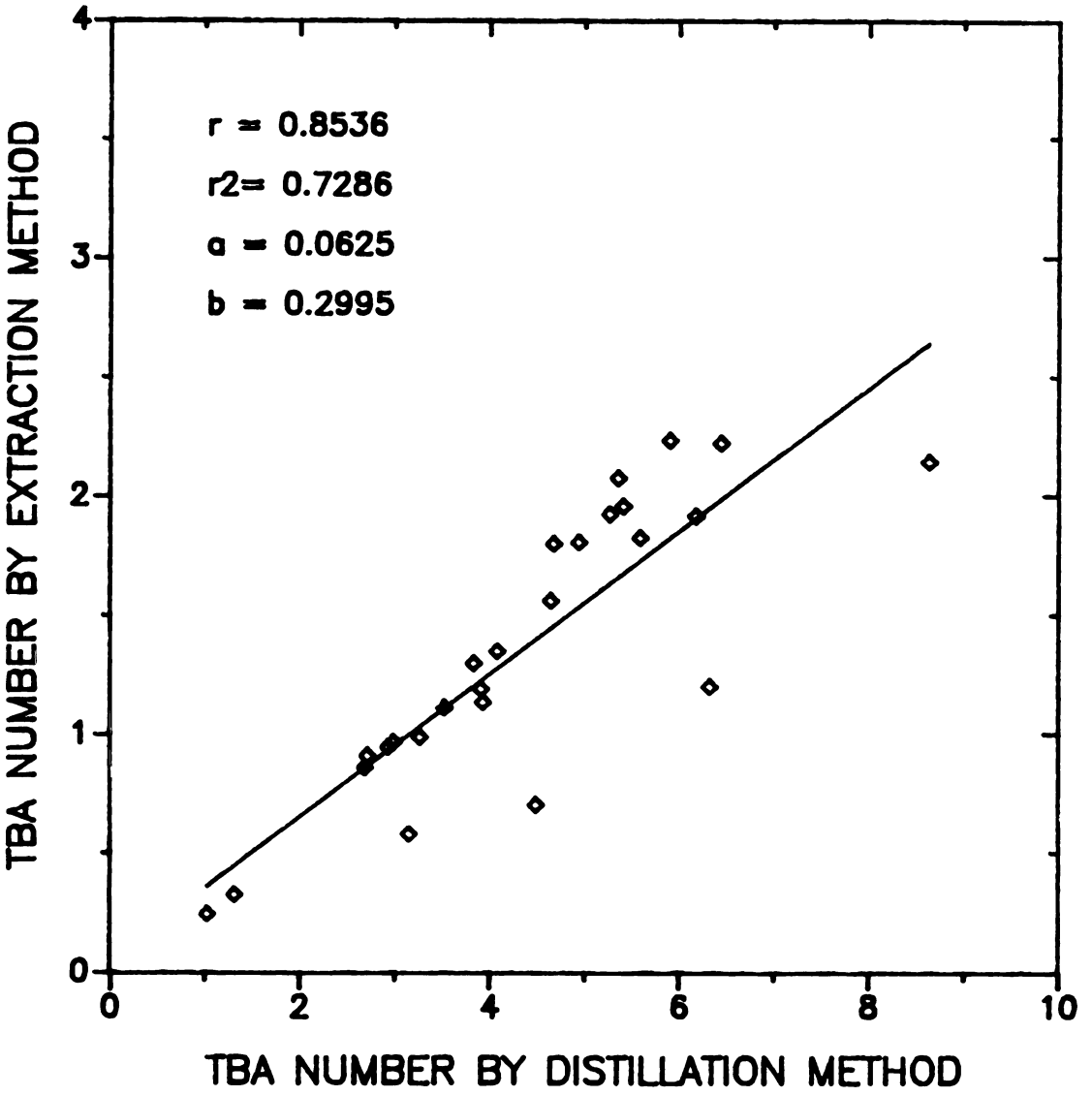



Figure 2. Relationship between the improved extraction and distillation TBA methods for chicken breast meat refrigerated at 4 C for 2 hours.

The correlation coefficient between the improved extraction or distillation TBA values and the panel scores is demonstrated by Figures 3 and 4 (r = 0.850 for the extraction and 0.835 for the distillation method). The relationship of both methods with panel scores was high indicating that TBA is a valid objective test used with sensory evaluation for monitoring warmed over flavor in poultry meat.

Data showed that the TBA values which could be considered as an indication of warmed over flavor development in cooked turkey breast meat as proved by the triangle test are 1.20 and 3.41 for the improved extraction and distillation methods, respectively (Table 10).

Table 10. Relationship between TBA methods and taste panel for cooked turkey breast[1]

| Meat type | TBA Values Extraction | TBA Values Distillation | Difference by Triangle test |
|---|---|---|---|
| freshly cooked | 0.32 ± 0.02 | 1.87 ± 0.07 | -(p<0.05) |
| 24 hr after cooking | 1.20 ± 0.01 | 3.41 ± 0.15 | +(p<0.01) |
| 48 hr after cooking | 4.31 ± 0.10 | 8.56 ± 0.17 | +(p<0.01) |

[1]Means are averages of 5 determinations

- = Difference is not significant at the indicated level.

+ = Difference is significant at the indicated level.

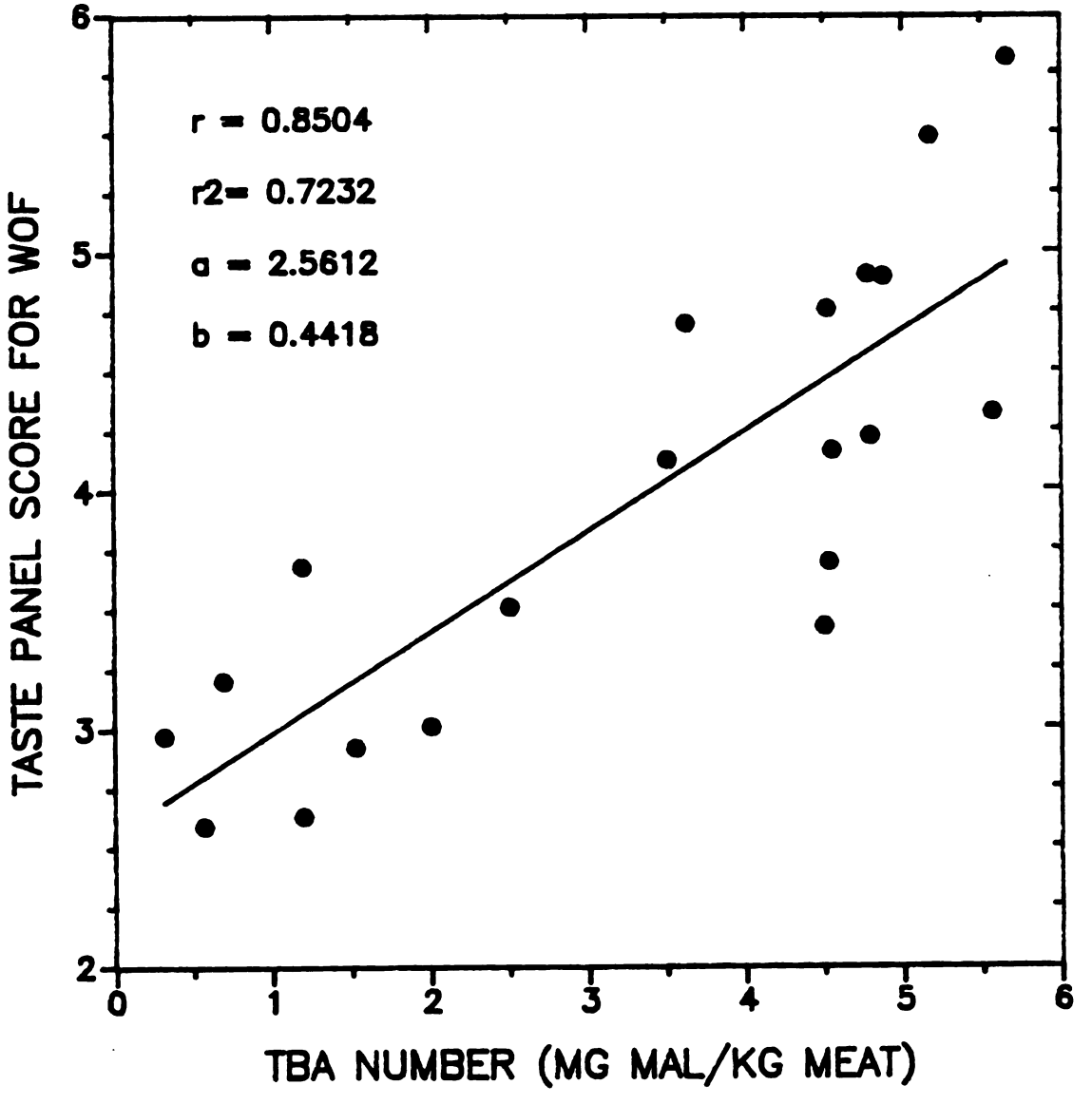



Figure 3. Relationship between taste panel scores and TBA numbers for the improved extraction method.

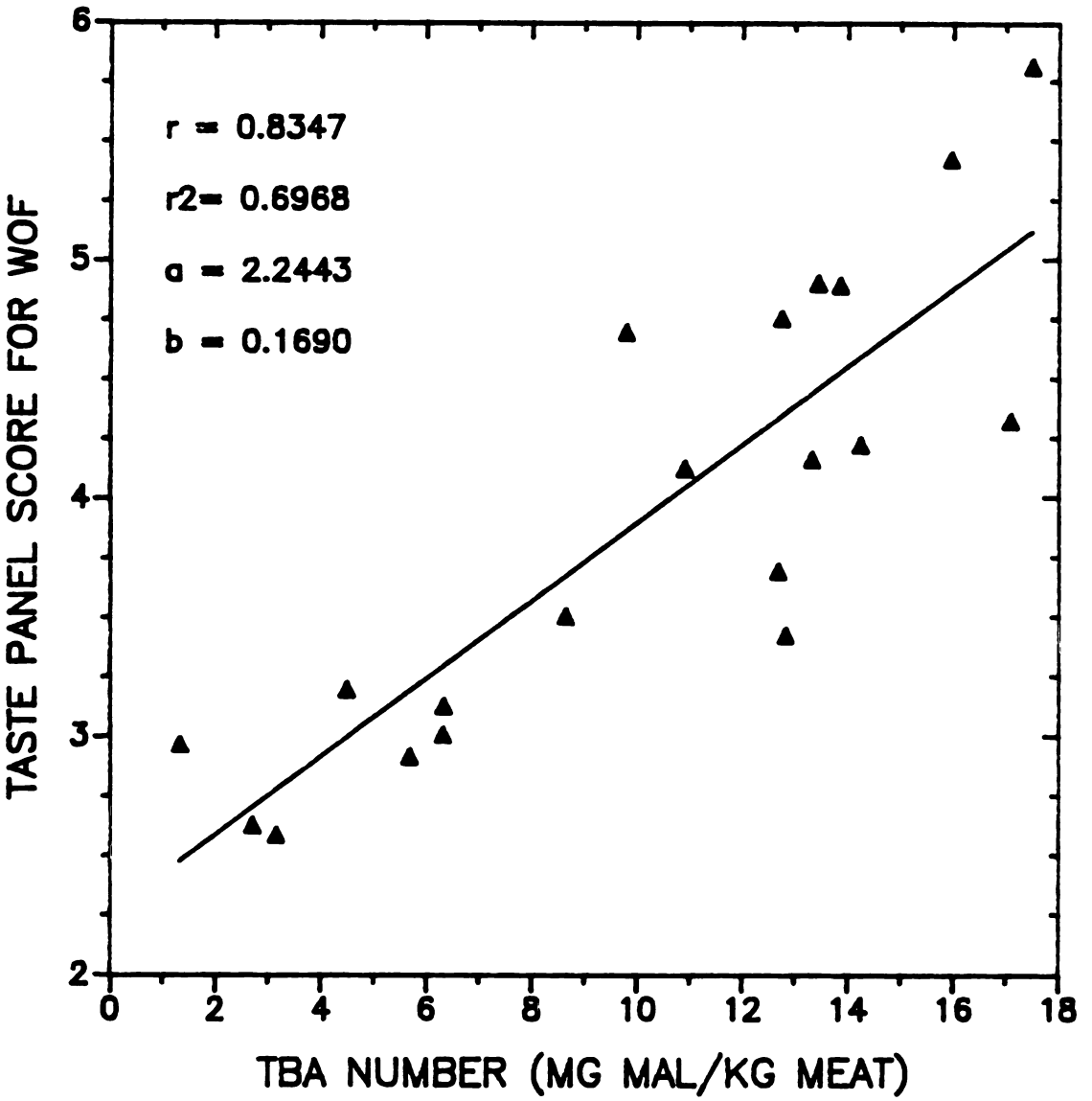



Figure 4. Relationship between taste panel scores and TBA numbers for the distillation method.

Dawson et al. (1975) assumed that TBA values above 2 (using distillation method of Tarladgis et al, 1960) would be associated with the development of rancid flavor in a turkey pattie.

In conclusion, the improved extraction TBA method was found to be fast, precise, easier to perform and as accurate as the distillation method. The following points should be considered when using the improved extraction procedure.

1. High fat meat or high levels of microbial contamination may cause a turbid filtrate.
2. Before the improved extraction method is used for new samples, an absorbance scan of TBA-MAL complex should be taken to check for interfering compounds.
3. Boiling to develop the TBA-MAL complex may cause the formation of an orange colored complex if carbohydrate is present in the sample.

## Lipid Oxidation, Factors and Variables For Experiments B and C Together

### Meat Type (Light vs. Dark)

Turkey thigh muscle was more susceptible to lipid oxidation than turkey breast muscle for each of the treatments (Figure 5) as measured by TBA test. Maximum TBA values are

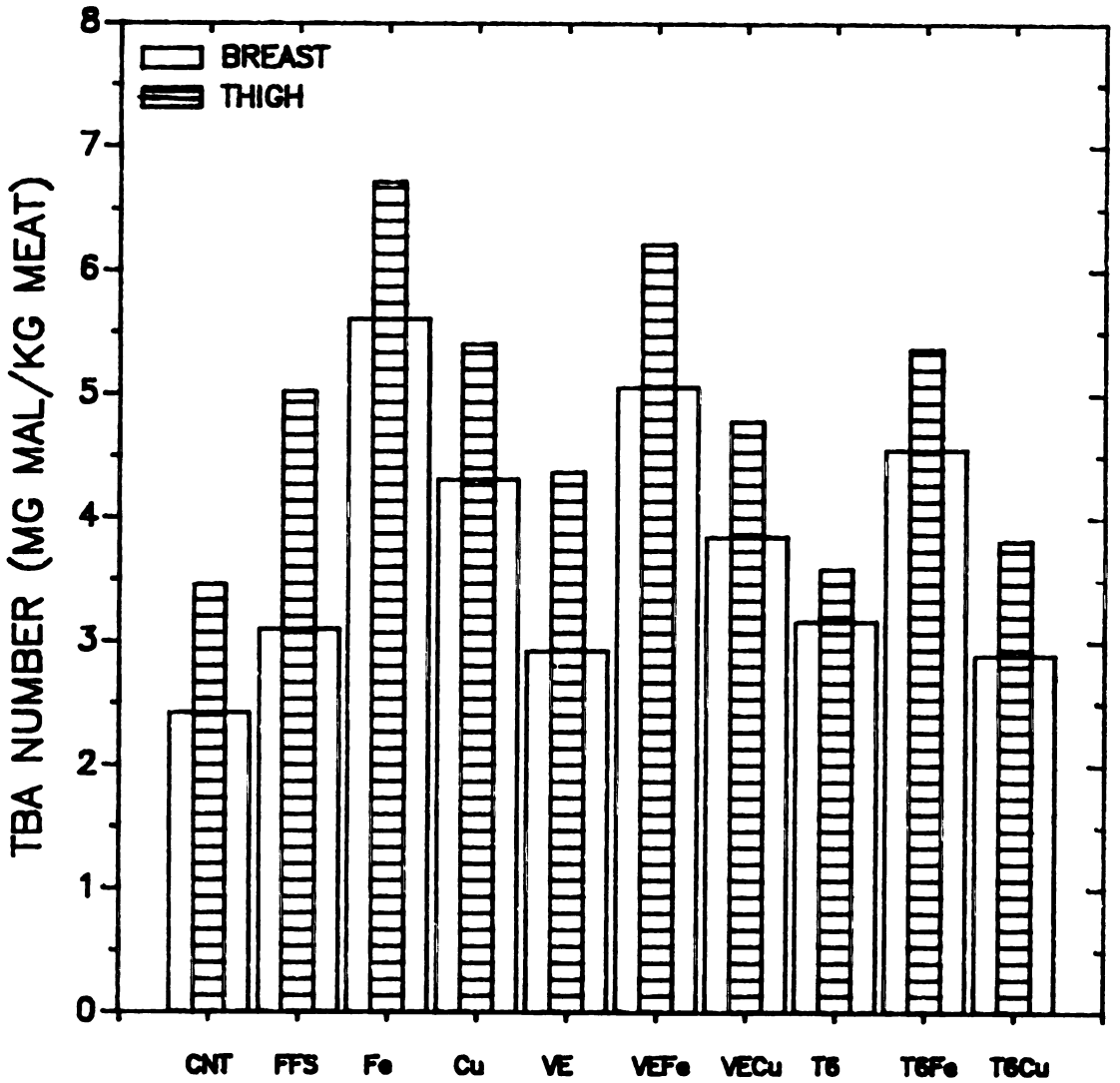



FIgure 5. Effect of treatments and turkey meat type on TBA values.

control (CNT); fine flake salt (FFS); ferric ions (Fe); cupric ions (Cu); vit. E, ascorbyl palmitate, citric acid (VE); tenox 6 (T6)

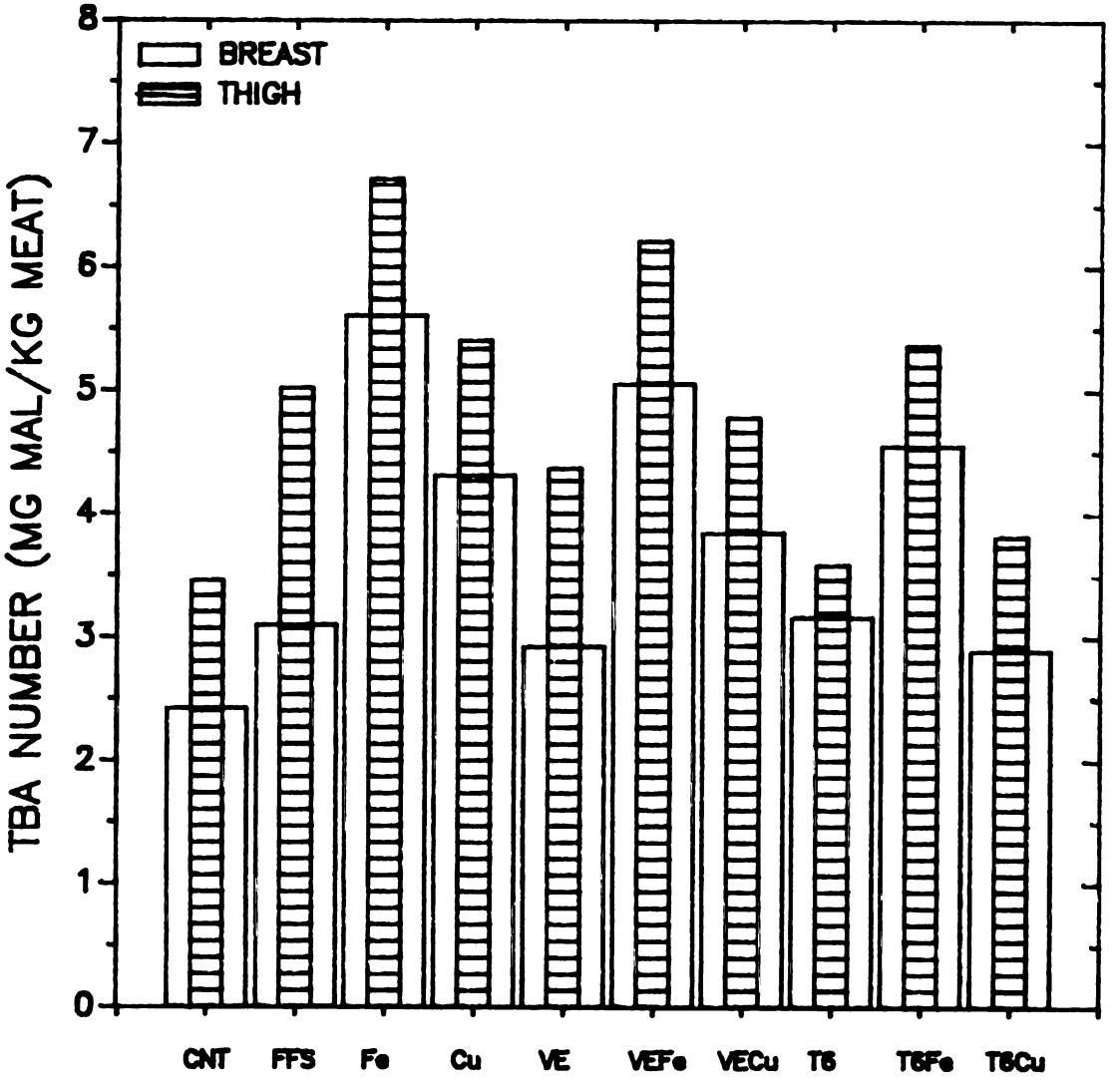



FIgure 5. Effect of treatments and turkey meat type on TBA values.

control (CNT); fine flake salt (FFS); ferric ions (Fe); cupric ions (Cu); vit. E, ascorbyl palmitate, citric acid (VE); tenox 6 (T6)

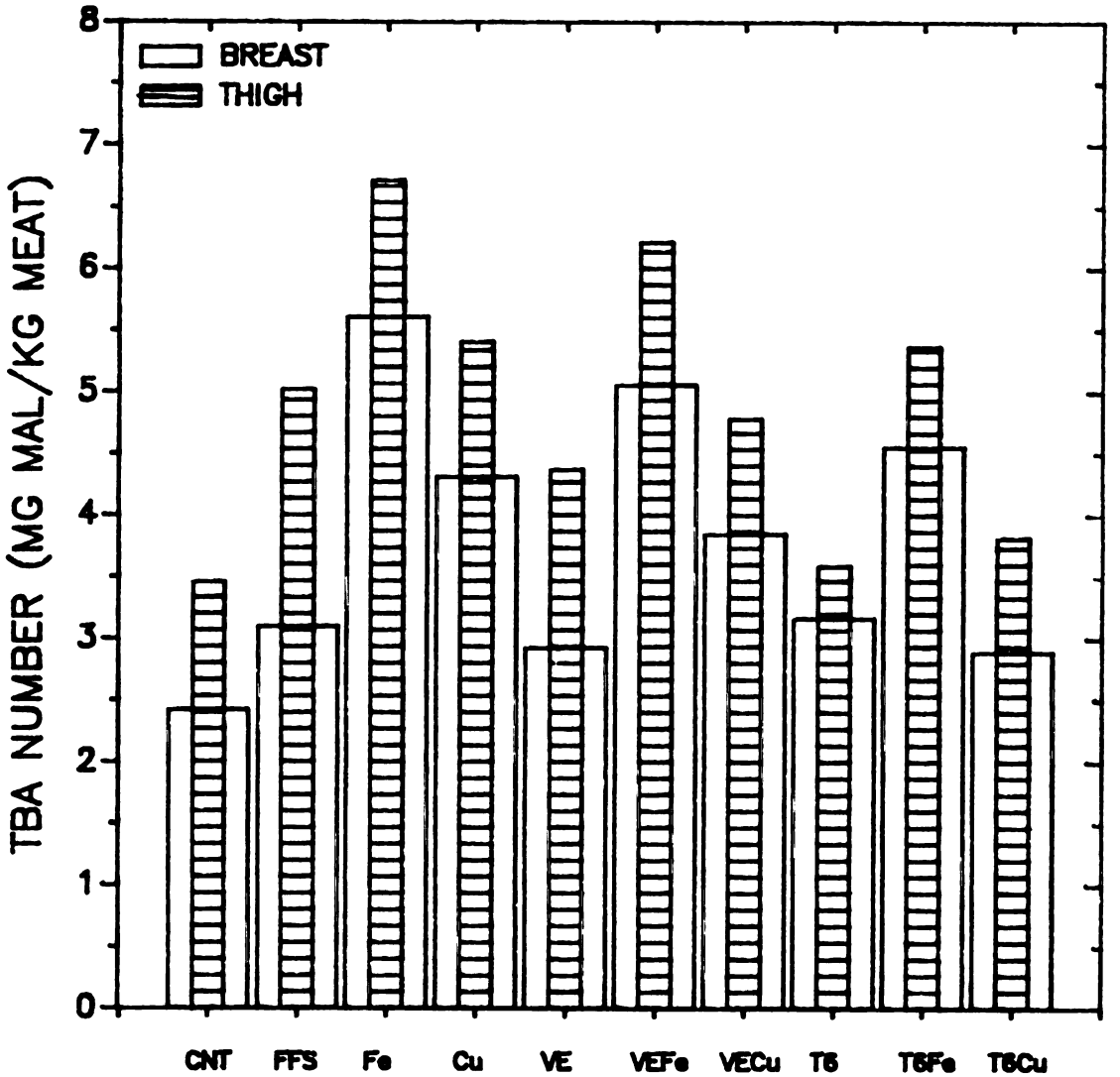



FIgure 5. Effect of treatments and turkey meat type on TBA values.

control (CNT); fine flake salt (FFS); ferric ions (Fe); cupric ions (Cu); vit. E, ascorbyl palmitate, citric acid (VE); tenox 6 (T6)

reached after 7 and 14 days of refrigeration at 4 C, and three months and six months of frozen storage at - 25 C. The difference in TBA values for the two types of meat is highly significant ($P<0.001$). These findings agree with Marion and Forsythe (1964) who reported that the TBA value was higher for the turkey red meat stored at 4 C for 1 to 7 days than for turkey white meat stored similarly. They attributed this difference to the higher total lipid content of red meat. In an earlier work Barron and Lyman (1938) attributed these differences to the larger amount of myoglobin in red muscle compared to the white muscle.

The changes of TBA values for both thigh and breast meat with storage time are shown in Figures 6 and 7 for refrigerated and frozen meat, respectively. The peak of lipid oxidation as measured by TBA is reached after 2 weeks of refrigeration (after which a decrease in TBA was noticed in a preliminary study) or 3 months of frozen storage.

**Cooking**

Meat stored for a short time after cooking develops warmed-over flavor much faster than uncooked meat. Labuza (1971) suggested that the rapid rate of oxidation in cooked meat is due to the denaturation of myoglobin during the cooking process. The increase in lipid oxidation as represented by TBA values was proportional to the storage time between 0 and 14 days of refrigeration at 4 C.

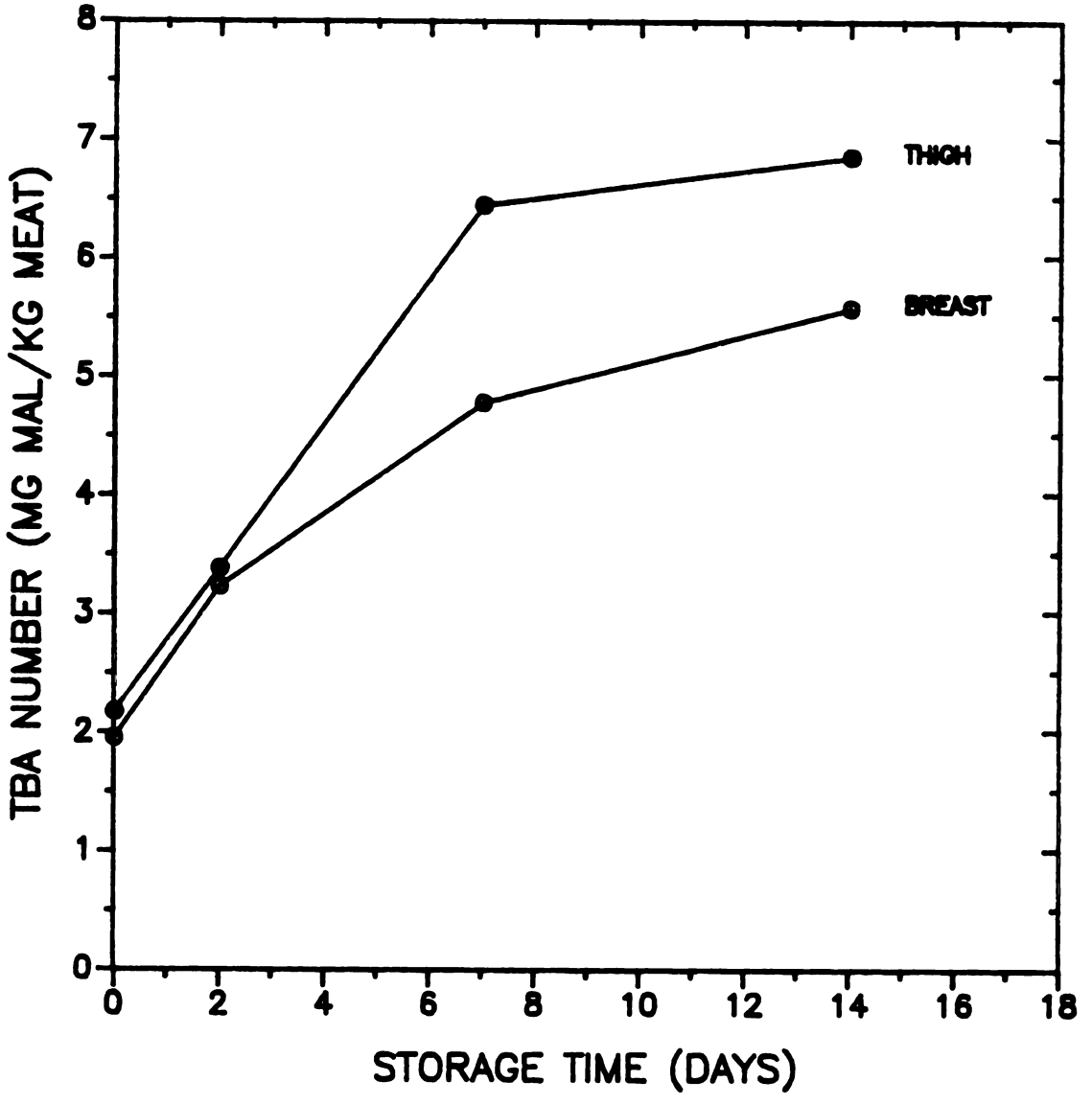



Figure 6. Effect of meat type and refrigerated storage on TBA values of turkey meat.

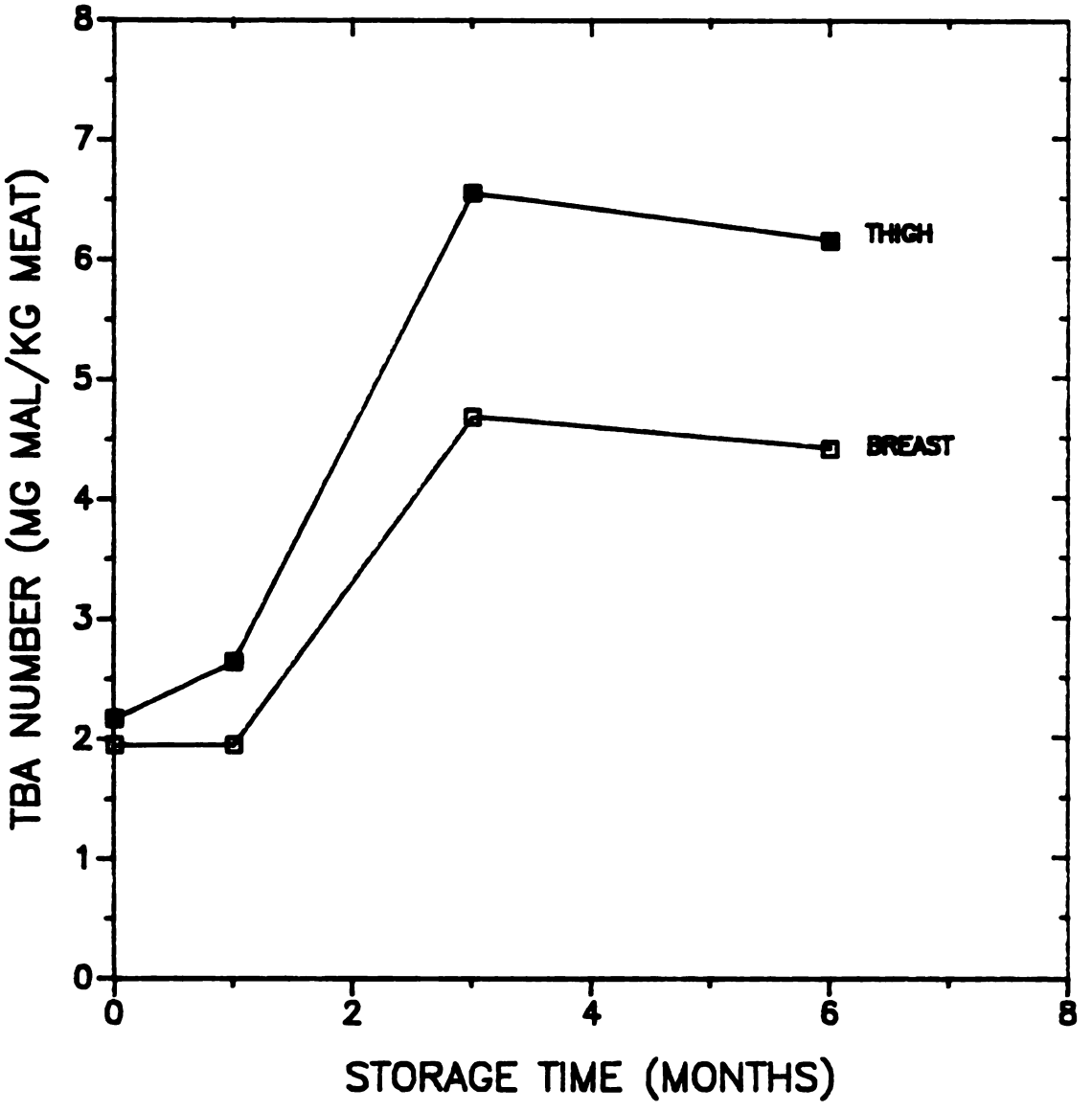



Figure 7. Effect of meat type and frozen storage on TBA values of turkey meat.

Lipid oxidation developed more in the cooked than in the raw turkey meat ($P<0.001$) as shown in Figure 8.

The TBA values for the cooked meat reached a maximum after 3 months of frozen storage at -25 C while the raw meat reached a maximum TBA value after 6 months (Figure 9). Lipid oxidation in cooked meat was higher than raw meat throughout storage (Figure 9). Sato and Hegarty (1971) proposed that cooking disrupts the muscle membrane and results in exposure of labile lipid components to oxygen and other catalysts. Igene et al. (1985) suggested that cooking might liberate most of the heme iron which acts as an active prooxidant. These assumptions might explain the higher TBA values after cooking in turkey red meat than in turkey white meat (Figure 10). TBA changes for raw and cooked turkey breast and thigh with storage for refrigerated and frozen storage are represented in Figures 11 and 12, respectively. An interaction between meat type, cooking and storage time could be observed from these Figures.

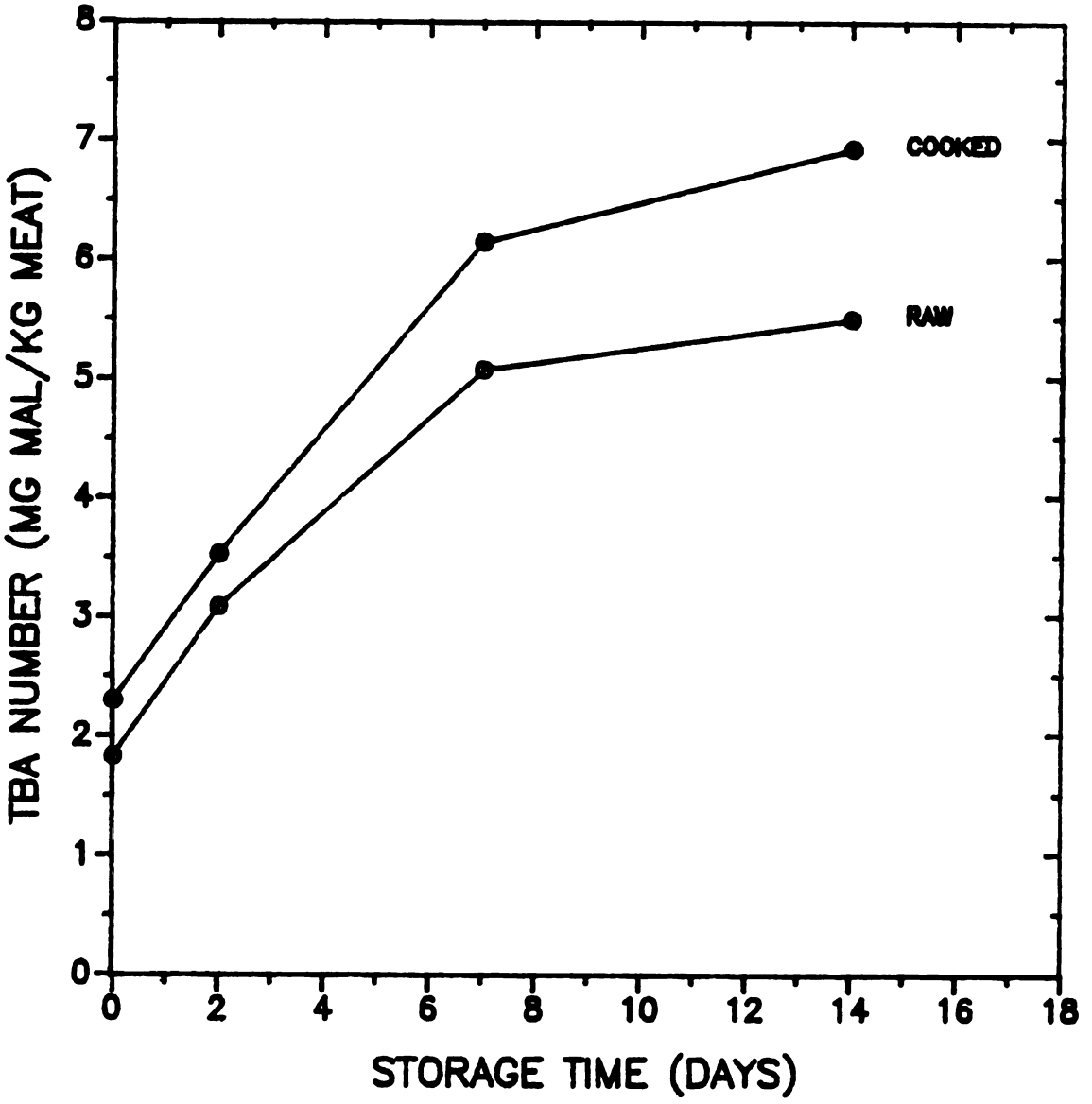



Figure 8. Effect of cooking and refrigerated storage on TBA values of turkey meat.

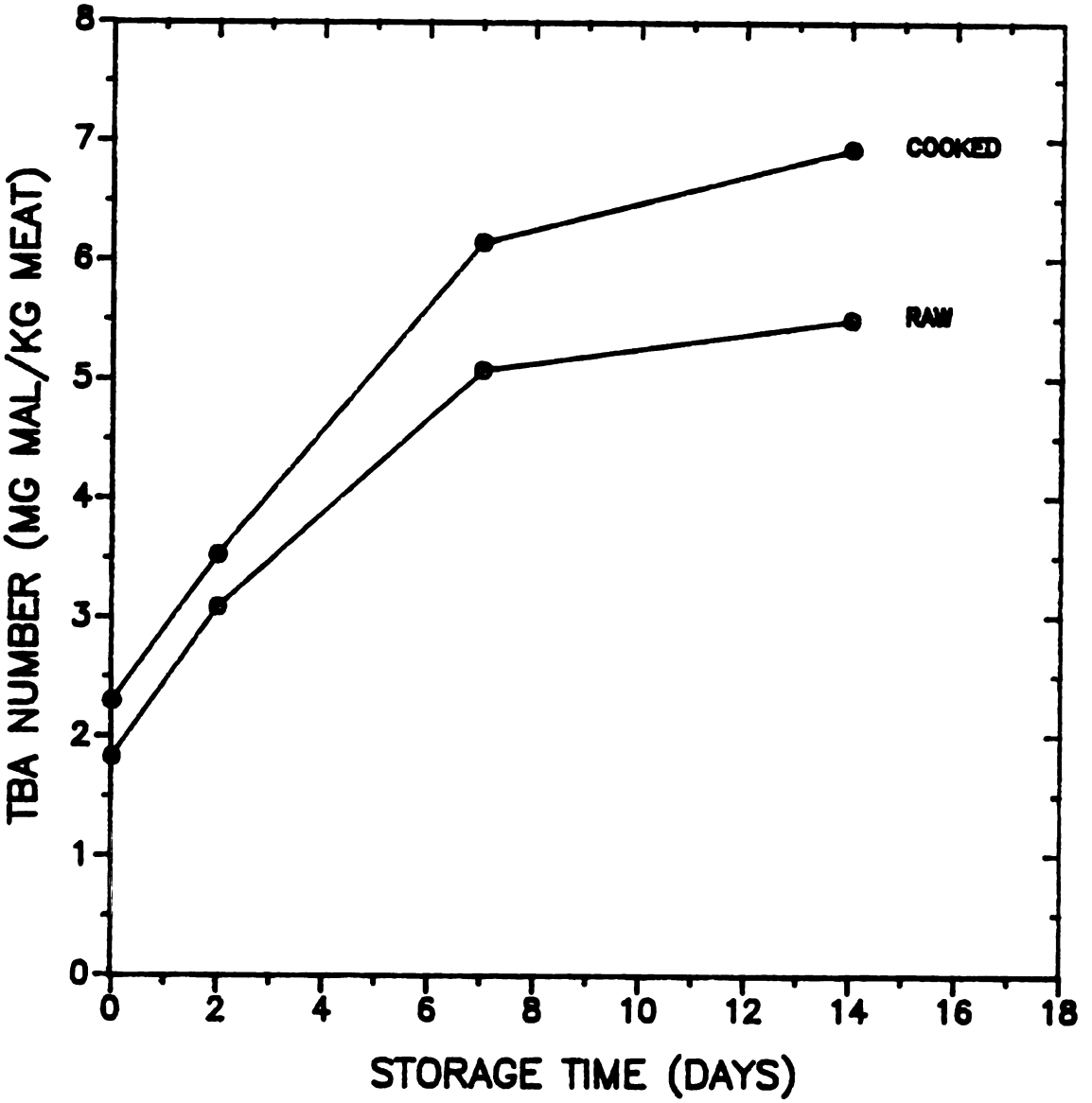



Figure 8. Effect of cooking and refrigerated storage on TBA values of turkey meat.

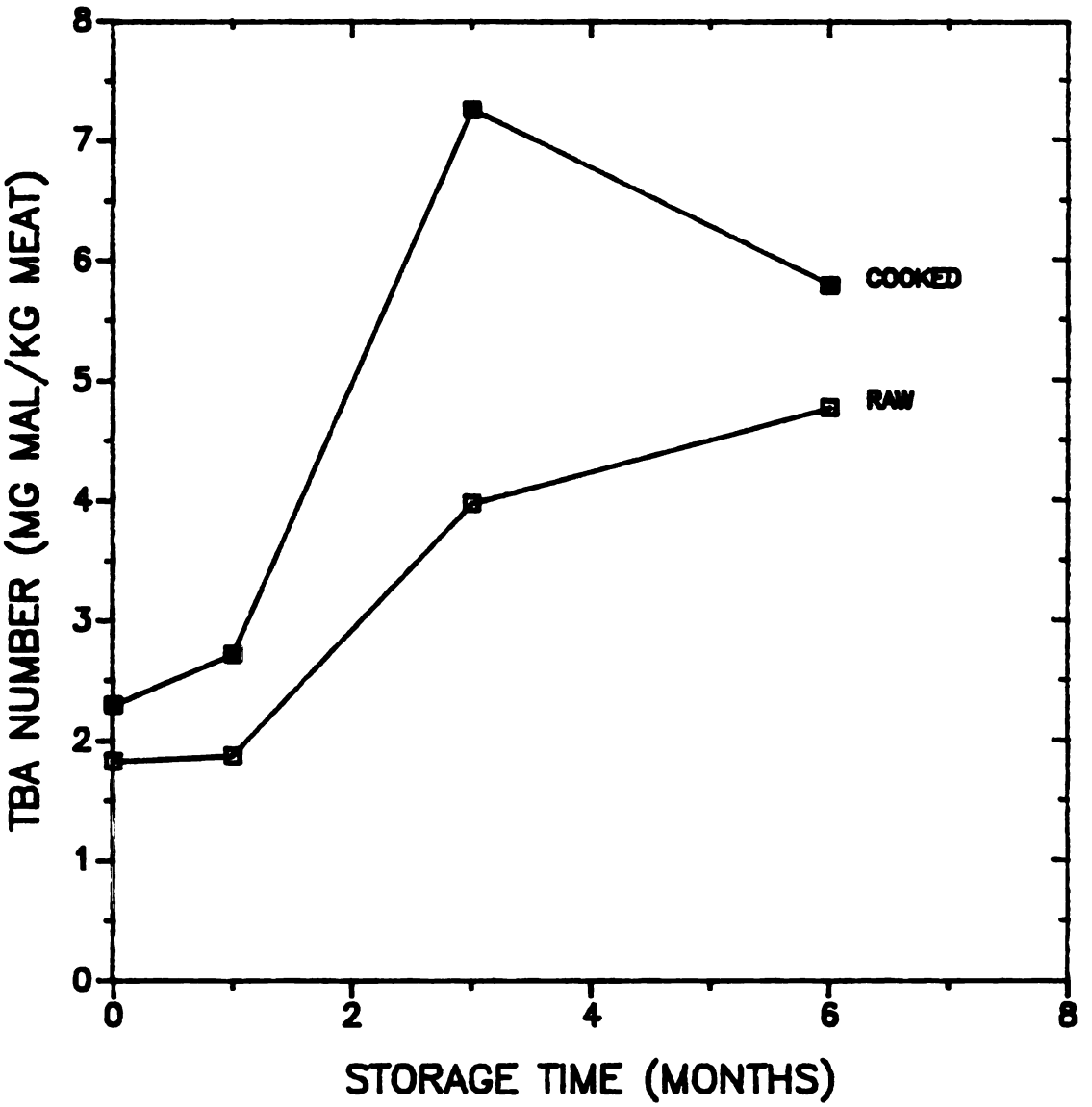



Figure 9. Effect of cooking and frozen storage on TBA values of turkey meat.

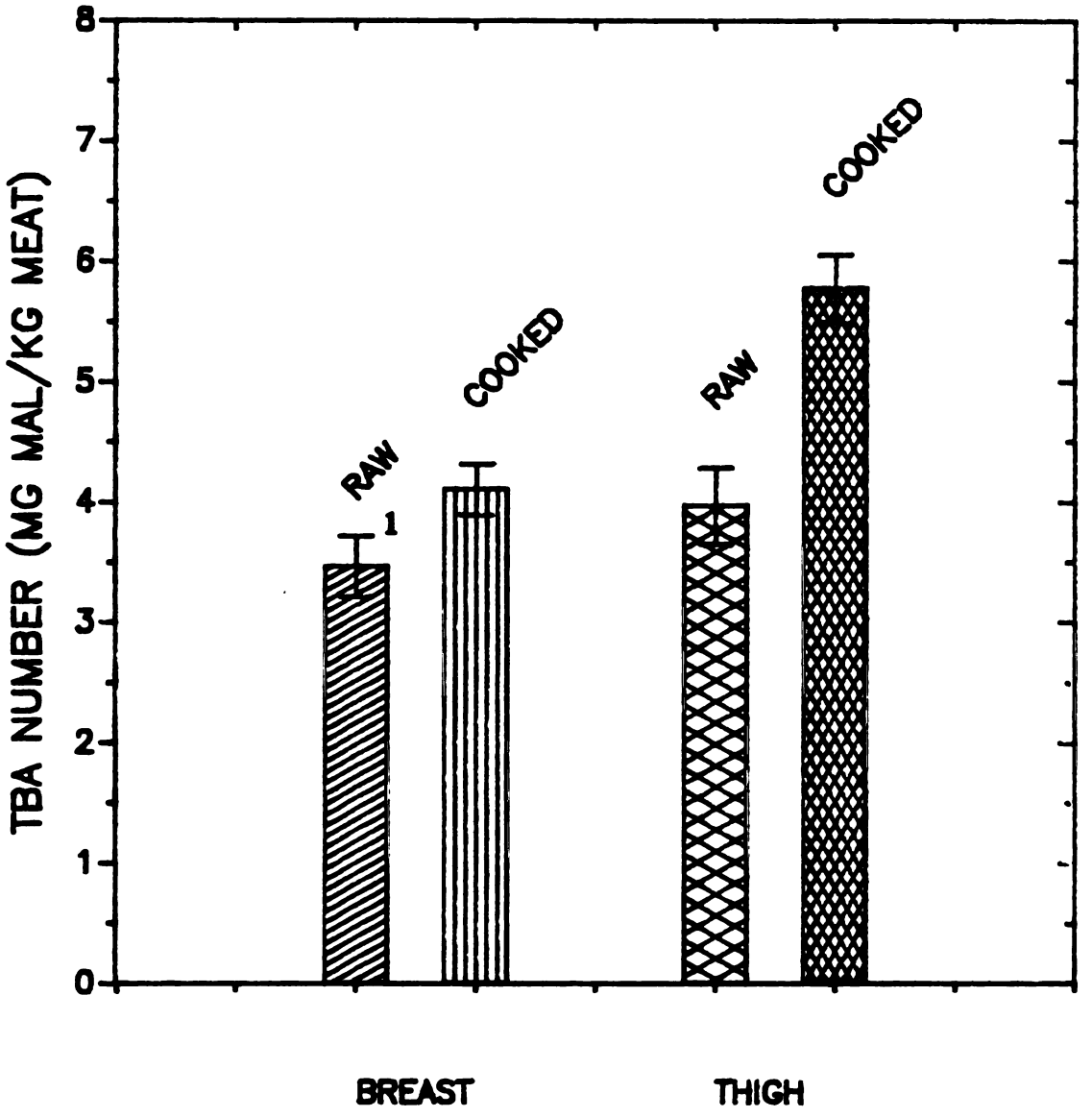



Figure 10. Effect of cooking and turkey meat type on TBA values.

[1]Bars represent standard errors.

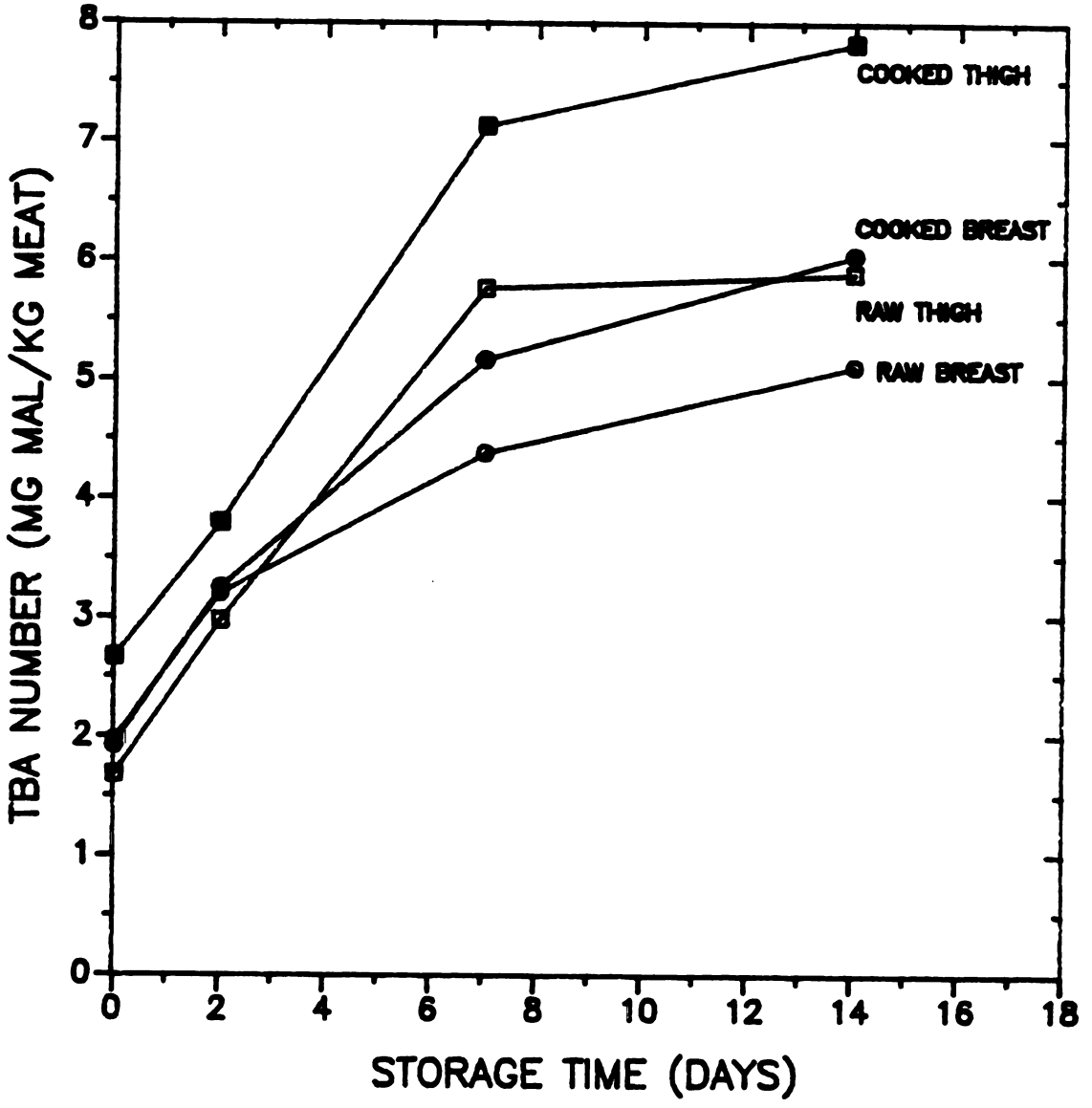



Figure 11. Effect of cooking, meat type and storage on TBA values of refrigerated turkey meat.

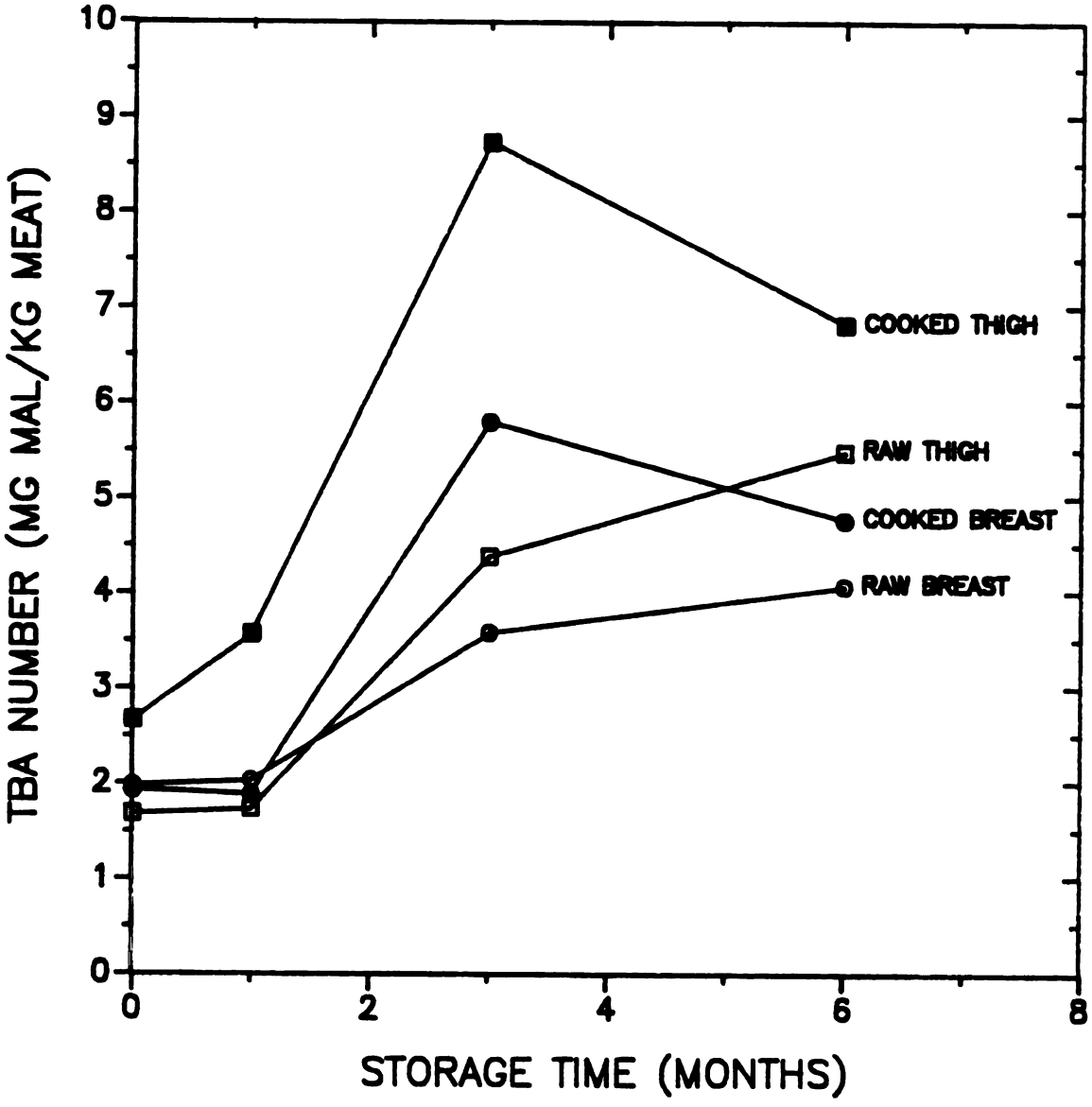



Figure 12. Effect of cooking, meat type and storage on TBA values of frozen turkey meat.

**Treatments**

Lipid oxidation in foods can be catalyzed by certain divalent cations, even when present in trace amounts. Metal cations may come from packaging materials, processing equipment or be present in added ingredients such as salt, spices or flavorings.

In general, TBA results indicated that the relative prooxidant effect of divalent cations and salts on turkey meat were in the following order: $Fe^{3+}$> $Cu^{2+}$> rock salt (RS)> fine flake salt (FFS) > control (CNT) (Figure 13). A significant difference ($P<0.05$) was detected among treatments. These findings agree with Younathan and Watts (1959) that ferric heme was the active catalyst of lipid oxidation in muscle system. However, Brown et al. (1963) and Hirano and Olcott (1971) reported that heme with iron in either the ferrous or ferric states were not different in promoting lipid oxidation. The most important mechanism involved in heme catalyzed lipid oxidation has been considered to be the catalytic decomposition of hydroperoxides to generate free radicals. In contrast, Sato and Hegarty (1971), Love and Pearson (1974) and Igene et al. (1979) concluded that nonheme iron has a stronger prooxidant effect than heme iron. Heme compounds were reported to have little influence on the development of off-flavors or TBA reactive materials in meat. Ferrous iron has been

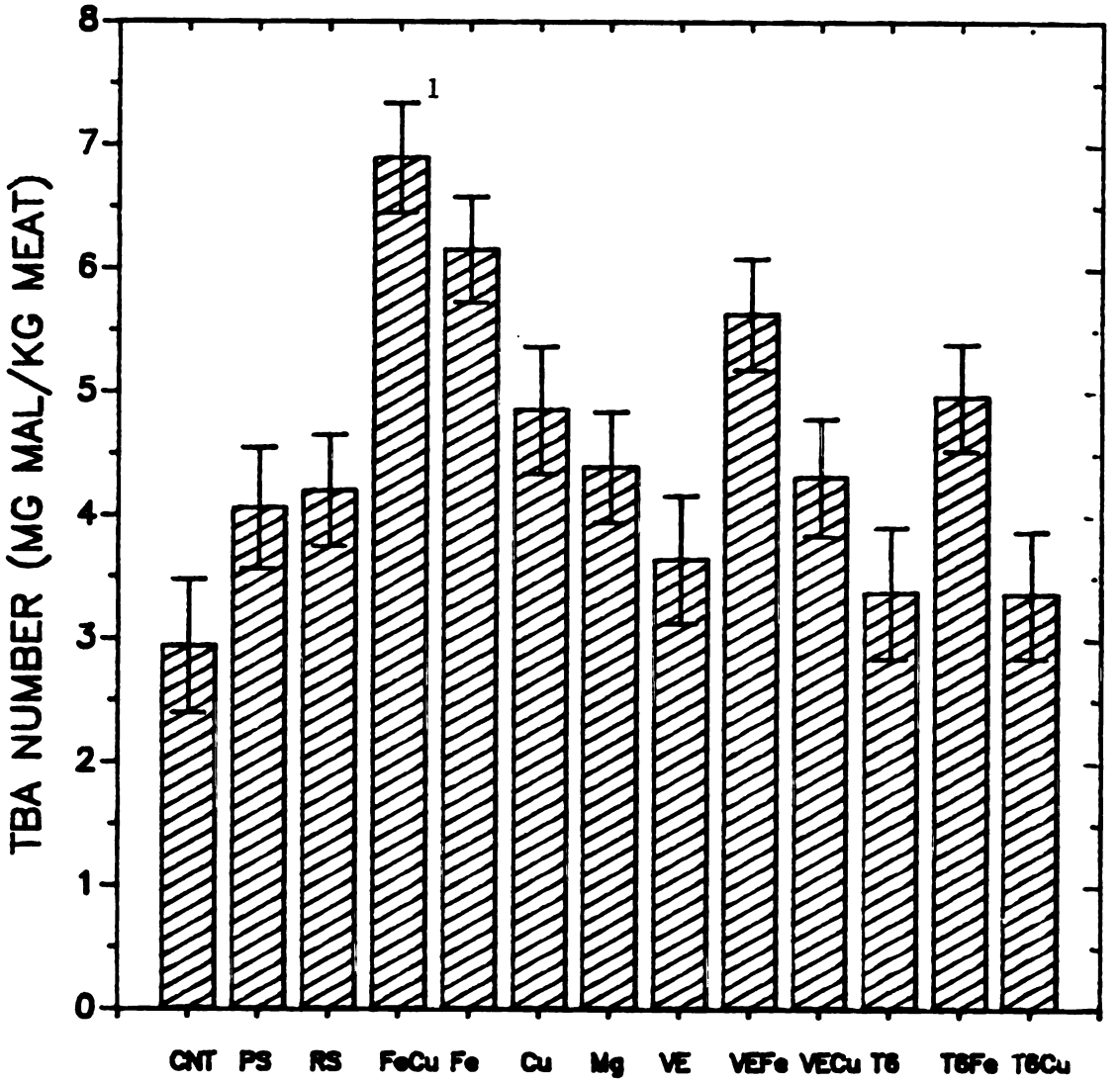



Figure 13. Effect of treatments on TBA values of turkey meat.

control (CNT); pure salt (PS); rock salt (RS); ferrous + cupric ions (FeCu); ferric ions (Fe); cupric ions (Cu); magnesium ions (Mg); vit.E, ascorbyl palmitate, citric acid (VE); tenox 6 (T6)

[1]Bars represent standard errors.

reported to have greater prooxidant activity than ferric iron (Smith and Dunkley, 1962; Brown et al., 1963; Wills, 1965; O'Brien, 1969 and Bidlack and Tappel, 1972). However it has been reported by Govindarajan (1973) that catalysis by nonheme iron appeared to be independent of the ionic state of the metal, although the type of model system used and the concentration of iron or a balance between ferrous or ferric iron affect results (Sato and Hegarty, 1971).

The effect of cooking on the release of nonheme iron was investigated in both turkey breast and thigh meat in order to determine the effect of this catalyst on warmed-over flavor development (Table 11). The presence of fine flake salt (2%) in meat samples had no effect on the release of nonheme iron ($P<0.05$). Breast meat samples which had 50 micrograms/g added iron, were found by atomic absorption to have 41.30 $\pm$ 1.82 and 50.01 $\pm$ 2.13 micrograms/g total iron in the raw and cooked meats, respectively. The corresponding values for thigh meat containing 50 micrograms/g added iron were 56.07 $\pm$ 1.96 and 53.8 $\pm$ 2.17 micrograms/g total iron for the raw and cooked meats, respectively.

The nonheme iron content of raw and cooked turkey breast meat was very large when compared to total iron content (93 to 95% nonheme iron). The values for turkey

Table 11. Total iron, nonheme iron and percentage of nonheme iron in turkey meat.[1]

| Meat type | Total iron (microgram/g) | Nonheme iron (microgram/g) | Nonheme iron (percent) |
|---|---|---|---|
| raw breast | 5.99±Ø.38 | 5.6Ø±Ø.23 | 93.79±5.92 |
| cooked breast | 5.93±Ø.29 | 5.63±Ø.25 | 95.Ø2±1.82 |
| rawthigh | 15.13±Ø.82 | 13.78±1.47 | 91.Ø9±8.25 |
| cooked thigh | 15.13±Ø.59 | 14.19±Ø.67 | 93.86±5.48 |

[1]Values represent means of 6 replicates

nonheme iron are in the range reported for other specie as pork, lamb and beef of 5.2, 7.Ø and 9.8 micrograms/g nonheme, respectively (Schricker et al., 1982). A similar proportion of nonheme iron (91 to 94%) was found forthigh meat although the total and nonheme iron content in turkey thigh meat were about three times greater than breast.

The percent of nonheme iron in turkey meat is much higher than that reported in other species. This may be explained by the low myoglobin content of poultry meat. Yamauchi (1972) showed that chicken breast contained Ø.263 mg myoglobin/g meat compared to Ø.642, 3.169 and 4.3Ø7 mg/g in pork, mutton and beef, respectively. Percent of nonheme iron in cooked and raw turkey meat was not significantly different ($P<Ø.Ø5$). The method of Schricker and Miller (1982) used for this analysis may be not sensitive enough

to detect the changes in nonheme iron as it is present at low concentrations in turkey meat. The method of Igene et al. (1985) was used in this investigation and found to have almost the same or lower sensitivity. In conclusion, the nonheme iron level is very high (about 93%) in turkey meat. It could be one of the major factors which contribute to the lower stability to lipid oxidation in turkey meat, especially thigh meat which has about 3 times more total iron than the breast meat. However the nonheme iron is 20.9 micrograms/g in heated fresh beef as reported by Schricker et al. (1982) and yet beef is one of the more stable meats to lipid oxidation. Thus in a complex meat system there is no one single factor which controls meat stability. Polyunsaturated fatty acids and the ratio of reduced to oxidized forms of iron might be major factors.

Copper acted as a prooxidant and produced TBA values close to that produced by iron in turkey meat (Figure 13, Page 71). Results agree with the data reported by Tichivangana and Morrissey (1985) which indicated that copper catalyzed oxidation followed a pattern similar to that for iron catalysis, but copper was slightly less effective.

Results of copper analysis are presented in Table 12. Breast meat with 50 micrograms/g copper added analyzed by atomic absorption was found to contain a lower level. the results for thigh meat were similar except there was

greater total copper, particularly in the cooked samples. In meat without added copper the level in thigh was 2.5 to 3 times greater than in breast.

Table 12. Copper concentration in turkey meat[1]

| Meat type | Copper (microgram/g) | Added copper (50 micrograms/g) |
|---|---|---|
| raw breast | 0.81±0.35 | 38.42±1.21 |
| cooked breast | 1.09±0.43 | 37.03±1.53 |
| raw thigh | 3.06±0.37 | 39.17±1.30 |
| cooked thigh | 2.49±0.39 | 47.28±1.18 |

[1]Values represent means of 6 replicates

The difference between the copper content in breast and thigh might be one of the factors which make the thigh meat more labile to lipid oxidation as monitored by the TBA method. Castell and Spears (1968) reported that small amounts of copper ions play an important part in the development of oxidation defects in fat-containing food products.

Cupric salts, in very special cases, were reported to have inhibitory effects in lipid oxidation and this was attributed to chain termination (Ingold, 1962). Many aspects of lipid oxidation need to be investigated in order to fully comprehend the prooxidant and inhibitory role

played by copper ions. These might include oxidation of cell components other than lipids, the role of phosphorous-containing compounds, especially nucleotides, PH effects and the effect of high temperature.

Fine flake salt had a significant prooxidant effect ($P<0.05$) in these studies. The literature spanning oxidative deterioration in foods leaves conflicting conclusions insofar as the role of salts is concerned. The prooxidant activity of salt appears to vary with conditions. Under certain conditions salt has been reported to have inhibitory or antioxidant effect (Tarladgis et al., 1960 and Zipser et al., 1964).

The effect of two antioxidants was studied in order to determine the most effective one in turkey meat. Tenox 6 was more effective in controlling the prooxidant effect of both iron and copper. The use of Tenox 6 resulted in significantly lower TBA numbers ($P<0.01$) when mixed with the meat containing salt and added iron and copper. Tenox 6 was the more effective antioxidant in thigh meat containing pure salt (PS) than was the case for breast meat with PS (Figure 5). The antioxidant which contained vitamin E, ascorbyl palmitate and citric acid produced a slight inhibition of the prooxidant activity of iron and copper,but the effect was not significant compared to the treatments which contained iron or copper. Dugan (1976) indicated

that the most effective antioxidants in food systems function by interrupting the free radical chain mechanism, which include BHA, BHT and tocopherol. This group consists mainly of phenolic compounds capable of donating a labile hydrogen. Tenox 6 may have exhibited more antioxidant effect as it contained 10% BHA, 10% BHT, 6% PG and 6% citric acid. It has been reported by Kraybill et al. (1949) and Marion and Forsythe (1964) that BHA was very valuable in delaying autoxidation in foods. BHA with BHT and citric acid provide good processing stability and help stabilize many processed foods (Dugan, 1976). This antioxidant control is achieved as a result of the synergistic interaction of antioxidants.

Shown in Figure 14 is the effect of treatments and refrigerated storage on lipid oxidation of raw and cooked turkey breast meat. In raw breast the TBA number was about 2.5 to 4.25 on 0-day analysis for treatments which have Fe, Cu, VEFe, T6Fe and VECu. The treatments containing T6Cu, FFS, VE, T6 and CNT have a TBA number below 1. This indicates that lipid oxidation started immediately after mixing of treatments with the ground meat for the first group of treatments which have strong prooxidant effect. In the cooked breasts TBA values start between 1.25 and 3.2 and tend to cluster together throughout storage. The effect of treatments andfrozen storage on lipid oxidation of raw and

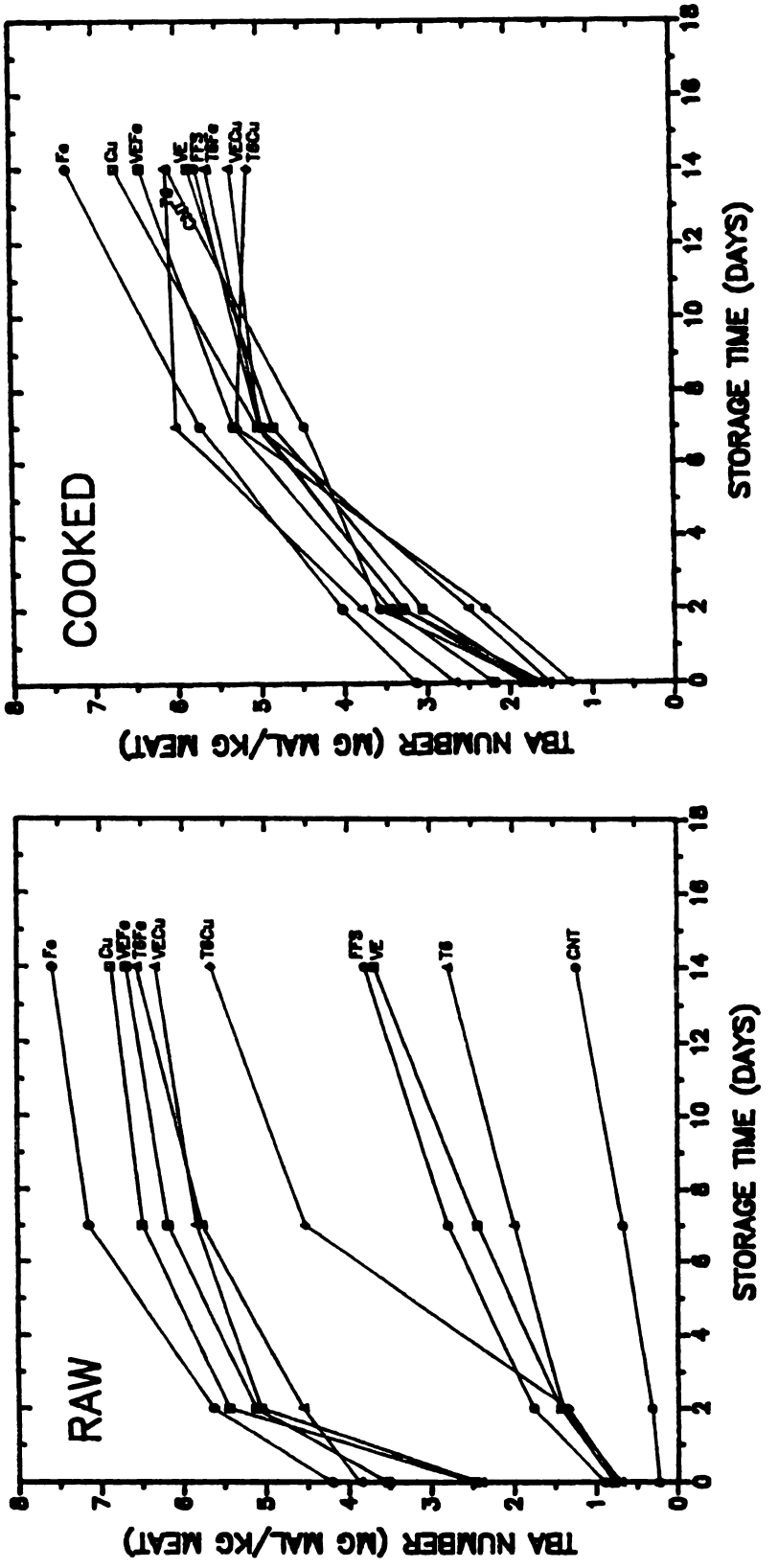



Figure 14. Effect of storage, salt, metal ions and antioxidants on TBA values of refrigerated turkey breast.

cooked turkey breast is Shown in Figure 15. Raw frozen turkey breast with added iron tended to have higher TBA values throughout storage than was the case with other treatments. In the cooked frozen breast the effect of treatment is less since TBA values are more similar. The maximum TBA in all the cooked, breast treatments was reached after 3 months of frozen storage vs. 14 days of refrigerated storage. The decline seen in the sixth month of frozen storage could be explained by the fact that malonaldehyde is not a final product of lipid oxidation and it is labile for further decomposition with prolonged storage.

Figures 16 and 17 show the effect of treatments and storage conditions on lipid oxidation of raw and cooked turkey thigh. The general pattern is similar to the corresponding one of the breast meat, but in the thigh the range of TBA values is higher.

Tenox 6 was a good antioxidant for both raw and cooked turkey meat containing copper and iron, but it was more effective in raw meat (Figure 18 and 13, page 71). Tenox 6 had better antioxidant control on fine flake salt in the raw meat, while the vitamin E, ascorbyl palmitate and citric acid combination had only slight antioxidant control on samples which have Fe, Cu or FFS, whether in the raw or cooked turkey meat (Figure 18).

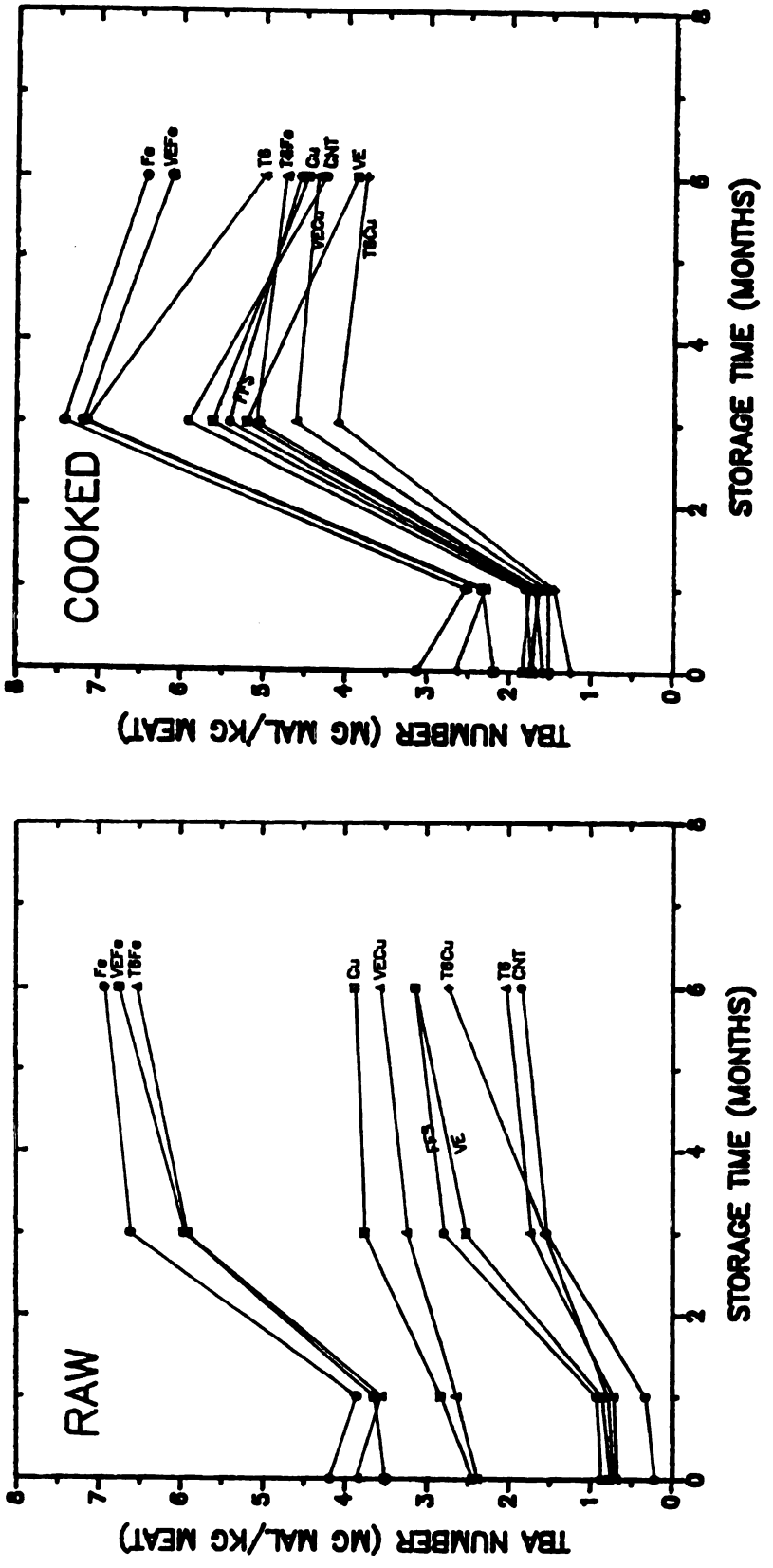



Figure 15. Effect of storage, salt, metal ions and antioxidants on TBA values of frozen turkey breast.

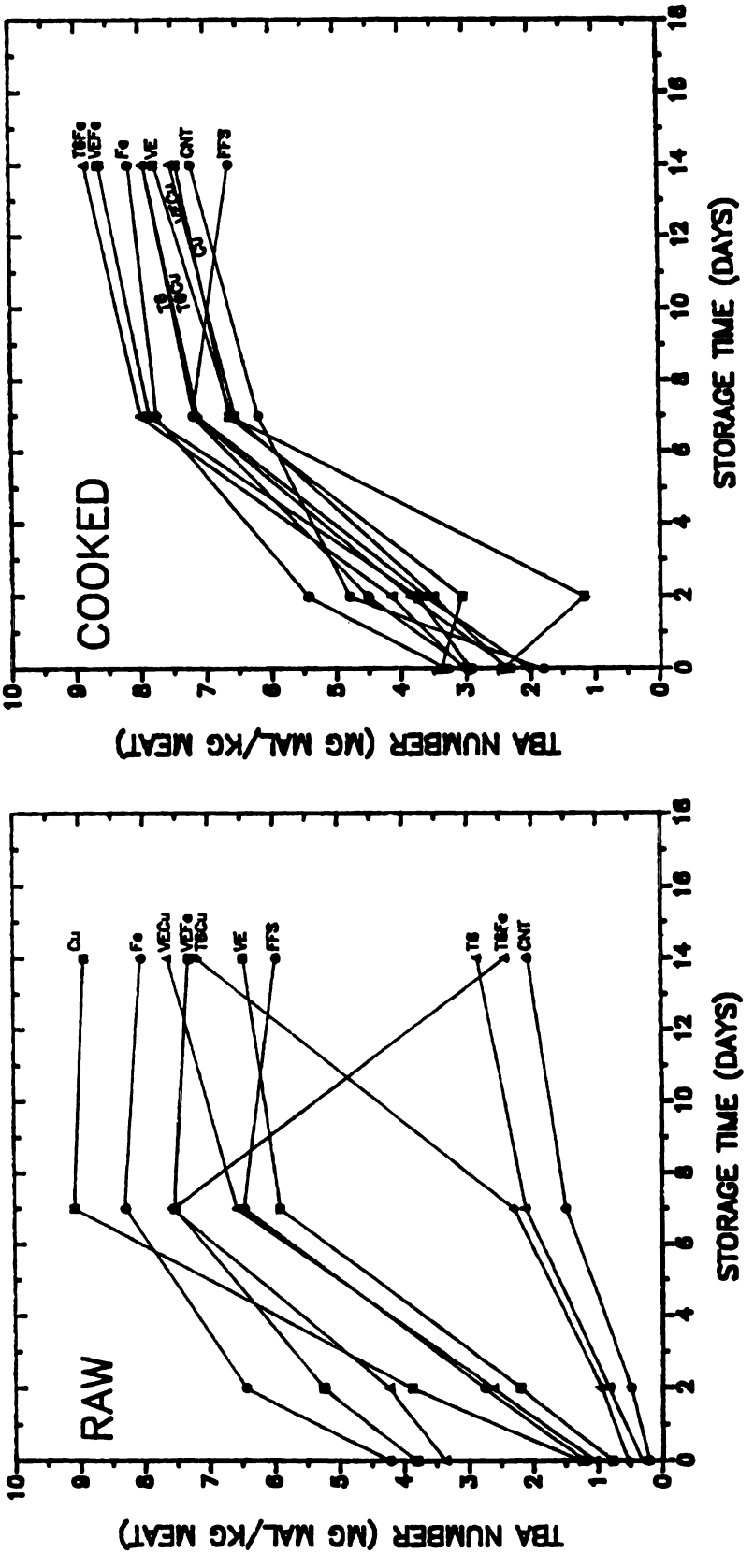



Figure 16. Effect of storage, salt, metal ions and antioxidants on TBA values of refrigerated turkey thigh.

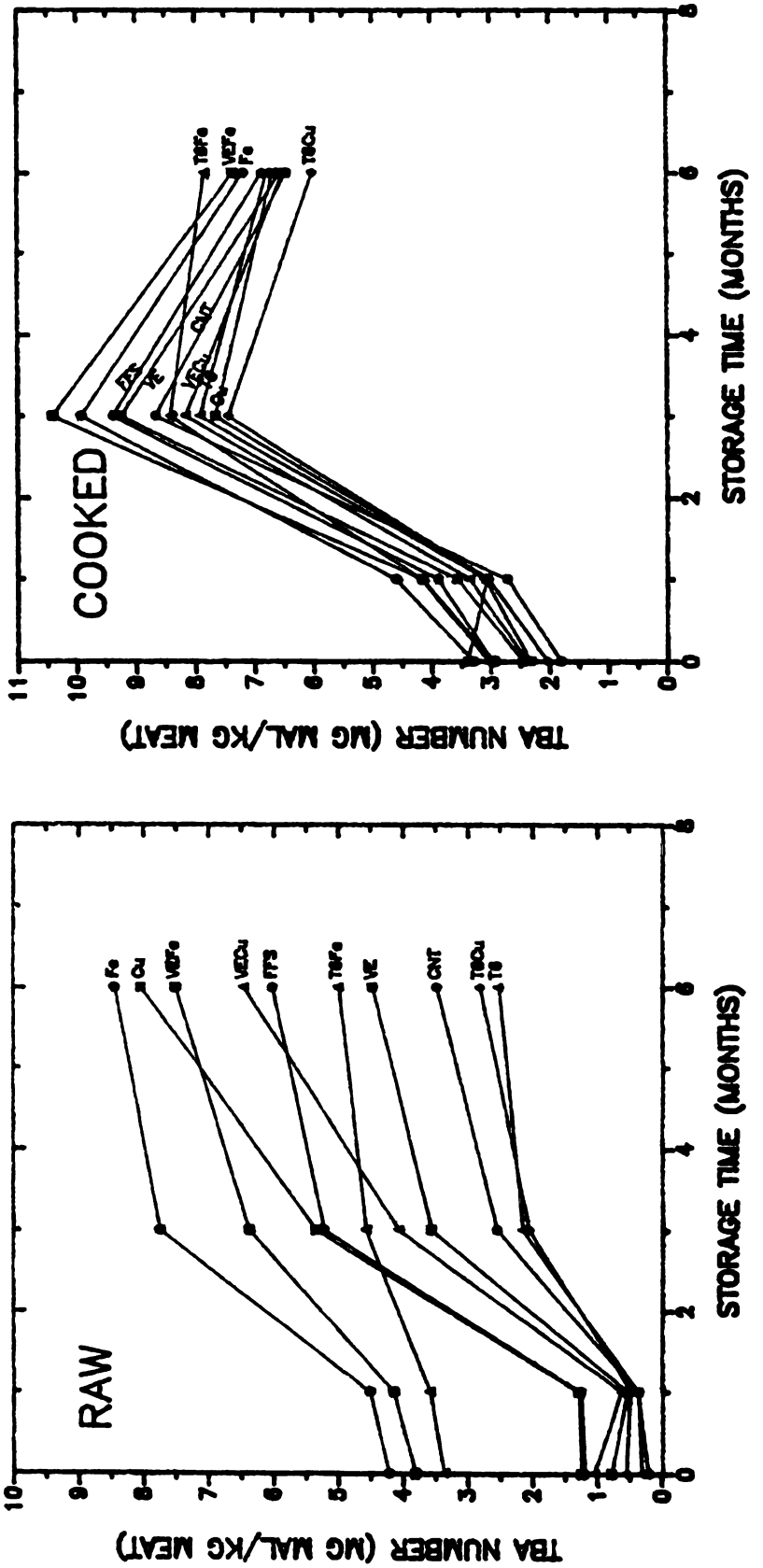



Figure 17. Effect of storage, salt, metal ions and antioxidants on TBA values of frozen turkey thigh.

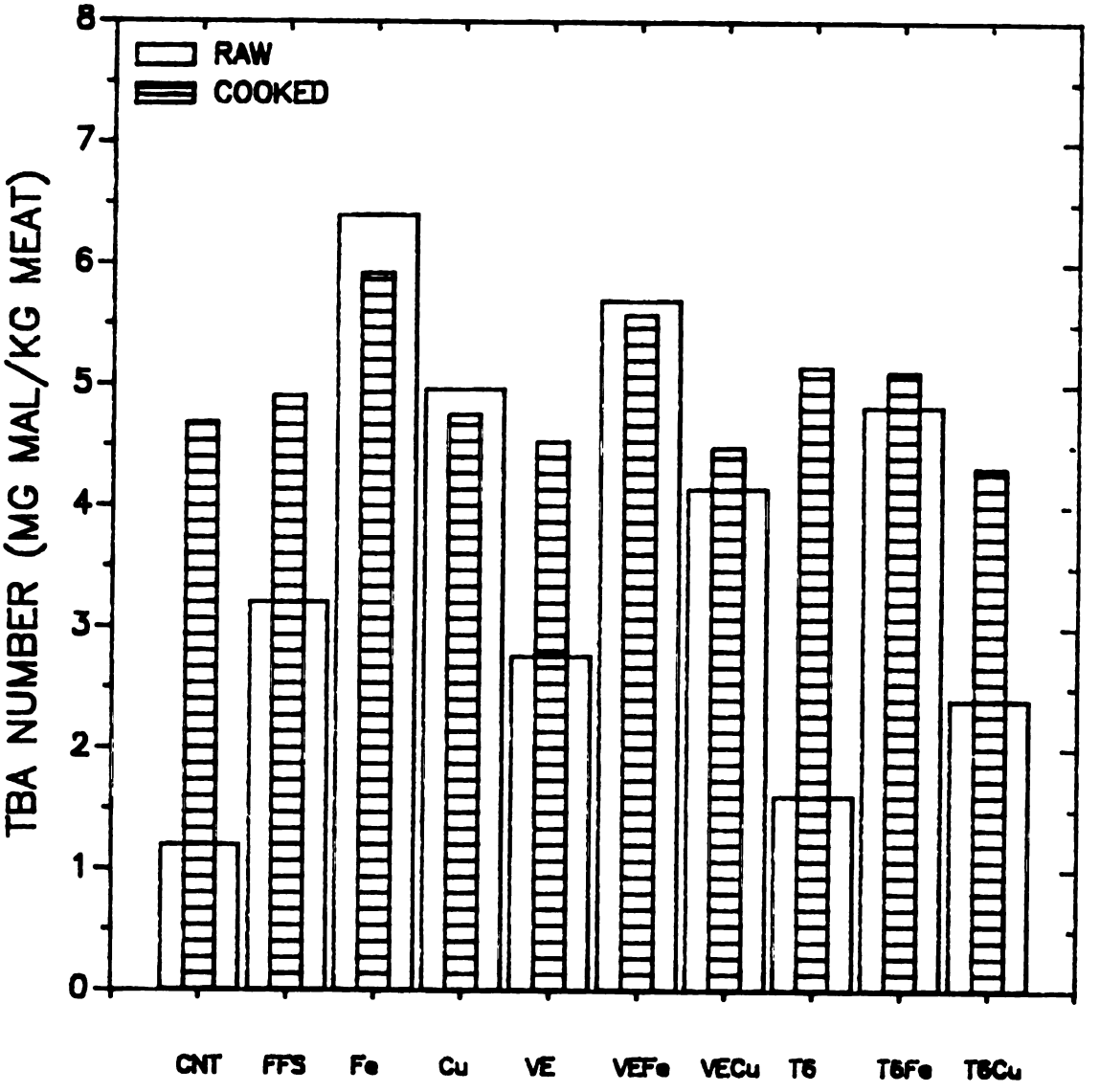



Figure 18. Effect of treatments and cooking on TBA values of turkey meat.

control (CNT); fine flake salt (FFS); ferric ions (Fe); cupric ions (Cu); vit. E, ascorbyl palmitate, citric acid (VE); tenox 6 (T6)

## Lipid Content of Fresh Turkey Breast and Thigh Meat

The composition of lipids extracted from turkey meat are presented in Table 13. The proportion of phospholipids in the total fat was higher in breast than in thigh meat (Table 13), but the total amount of phospholipids was higher in thigh meat, 0.66% vs. 0.25% of meat weight.

Table 13. Lipid content of fresh turkey breast and thigh meat

| Lipids | Meat type | |
|---|---|---|
| | Breast | Thigh |
| Total lipids[1] | 0.75 ± 0.02 | 6.02 ± 0.06 |
| Phospholipids[2] | 33.47 ± 1.11 | 11.01 ± 2.36 |
| Neutral lipids[2] | 66.53 ± 1.11 | 88.99 ± 2.36 |

[1]Expressed as percentage of total tissues

[2]Expressed as percentage of total fat

These findings are in agreement with Wangen et al. (1971) who concluded that on an absolute basis, turkey thigh had the larger concentration of phospholipids but as a percentage of total lipids, phospholipids comprised a larger percentage in breast lipids. A similar trend was

reported by Marion and Miller (1968) and Pikul et al. (1984) for chicken breast and thigh tissues.

### Effect of Frozen Storage and Cooking on Turkey Lipids Content

The proportion of phospholipids in the raw breast meat during frozen storage is shown in Table 14. A decline in phospholipid percentage during storage became significant ($P<0.05$) after one month of frozen storage.

Table 14. Effect of frozen storage on turkey breast phospholipids (PL) and neutral lipids (NL) percentage of total lipids[1,2]

| Lipids | Cooking | Storage time (months) at -25 C | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 3 | 6 | 12 |
| PL | raw | 35.35[a] | 33.61[b] | 32.77[c] | 32.73[c] | 32.86[d] |
| NL | raw | 64.65 | 66.39 | 67.13 | 67.27 | 67.14 |
| PL | cooked | 33.17[a] | 30.41[b] | 30.19[c] | 31.48[d] | 31.09[d] |
| NL | cooked | 66.83 | 69.59 | 69.81 | 68.52 | 68.09 |

[1]Values in the same lipid group and in the same row and bearing the same superscript are not significantly different ($P<0.05$)

[2]Each value represents a mean of 10 determinations

Results also showed that cooking decreased the proportion of phospholipids in total lipids. Level of phospholipids in cooked breast meat were 33.17, 30.41, 30.19, 31.48 and 31.91% of total lipids at 0, 1, 3, 6 and 12 months of frozen storage, respectively. The lowest ratio of phospholipids to neutral lipids in the cooked breast was observed after 3 months of frozen storage. The decrease of the phospholipid proportion with frozen storage and cooking corresponds to the variation in the levels of malonaldehyde as measured by the TBA test (figure 11 and 12 Pages 68 and 69) as well as the changes in fatty acid profile shown later.

The results of this study showing decreased proportion of phospholipids due to frozen storage and cooking are in agreement with the work of Acosta et al. (1966) who reported an apparent decrease in the phospholipid content of turkey tissues frozen for 180 days at -25 C. Davidkova and Khan (1967) and Fishwick (1968) reported a similar trend and concluded that the phospholipid decrease with time of storage at -10 C or below appeared to be largely independent of the actual temperature of storage. They explained the decrease in phospholipid content by the loss of PC and PE. The increased yield of triglyceride after storage was explained by Davidkova and Khan (1967) as resulting from deteriorative biochemical changes which make the

association or binding between triglycerides and proteins less strong, permitting greater extraction.

## Effect of Treatments on Composition of Turkey Lipids

Treatments with pure salt, rock salt, antibiotic and metal cations have minor effects on the proportions of lipids, although some significant effects could be detected (Table 15). Treatments had no detectable effects on lipid

Table 15. Effect of treatments on the percentage of turkey breast lipids

| Treatments | Phospholipids | Neutral lipids |
|---|---|---|
| CNT[2] | 32.48 c[1] | 67.52 cd |
| CMF | 32.79 b | 67.21 e |
| FFS | 32.79 b | 67.21 e |
| NRS | 31.99 e | 67.91 b |
| SRS | 31.07 f | 68.93 a |
| ANT | 32.74 b | 67.26 e |
| $Fe^{2+}Cu^{2+}$ | 32.49 c | 67.51 d |
| $Cu^{2+}$ | 33.05 a | 66.95 f |
| $Fe^{3+}$ | 32.81 b | 67.19 e |
| Mg | 32.26 d | 67.74 bc |

[1]Values in the same lipid groupbearing the same superscript are not significantly different ($P<0.05$)

[2]Definitions are in Table 3, Page 45

oxidation as monitored by changes in fatty acid profile. However, the TBA test did show significant treatment effects (see prior discussion and Figure 13).

## Effect of Storage, Cooking and Treatments on the Composition of Turkey Breast Phospholipids

The proportionate levels of phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), sphingomyelin (SP) and lysophosphatidyl choline (LPC) in fresh turkey breast meat phospholipids were 54.17, 23.89, 14.01 and 7.10%, respectively. These findings are in good agreement with the results reported by Fishwick (1968) and Wangen et al. (1971) in turkey breast phospholipids. Fishwick (1968) found 60.9% PC + phosphatidyl inositol (PI), 29.2% PE, 8.2% SP and 1.7% phosphatidyl serine (PS) while Wangen et al. (1971) reported 51.90% PC, 22.84% PE, 10.42% SP, 9.81% PI + PS and 5% LPC. The levels of PC and PE were found to decrease during frozen storage, while the proportion of SP and to a lesser extent LPC increased (Figure 19). The decline in PC and PE was significant ($P<0.01$) after one month of frozen storage of the breast meat at -25 C, and continued until the twelfth month of storage. The proportion of SP started to increase significantly even before the first month of storage. The increase in LPC was slight but was also significant ($P < 0.01$) after the first

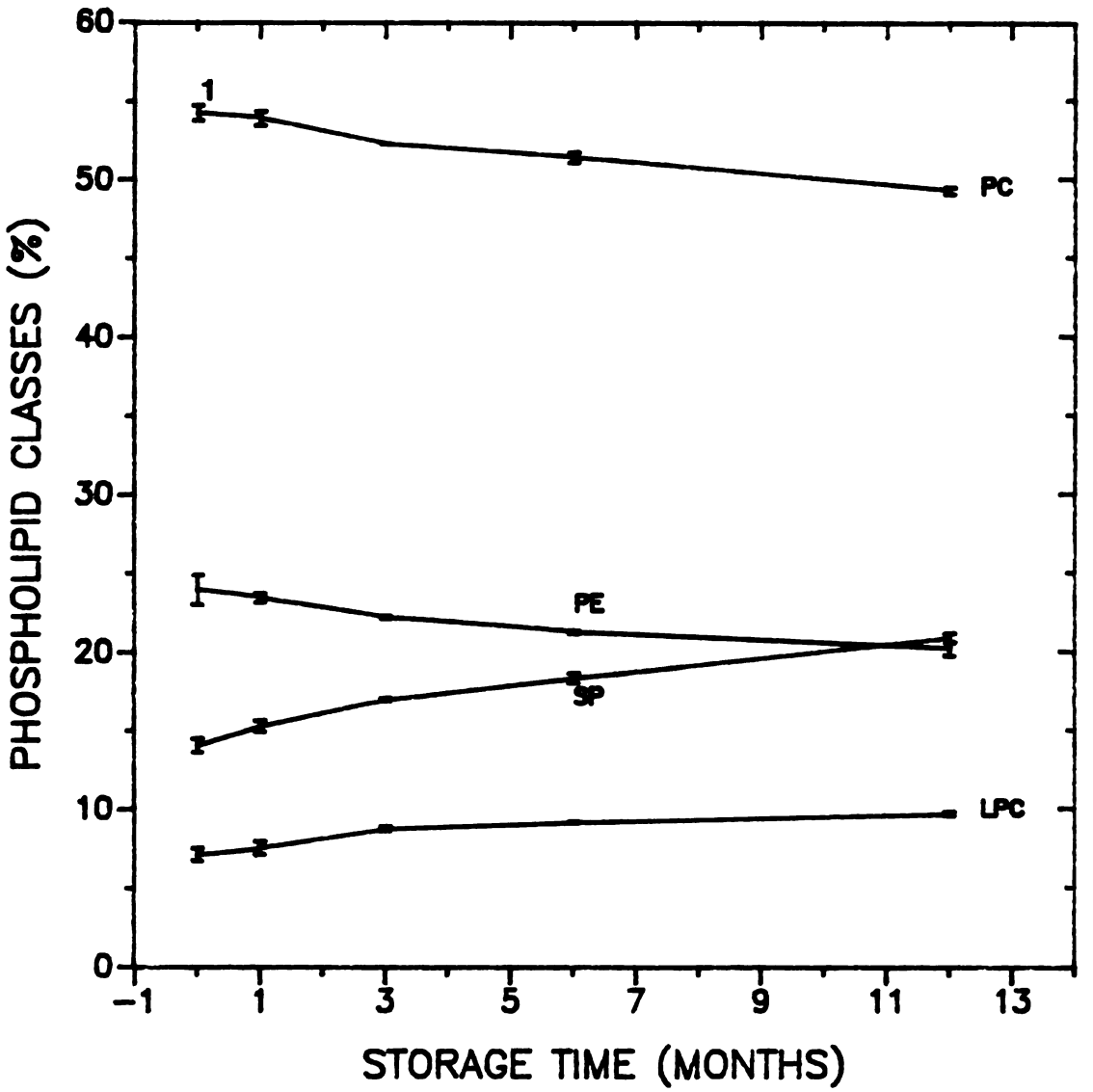



Figure 19. Effect of storage on the distribution of phospholipid classes in turkey breast phospholipids.

phosphatidyl choline (PC); phosphatidyl ethanolamine (PE); sphingomyelin (SP); lysophosphatidyl choline (LPC)

[1] Bars represent standard errors.

month of frozen storage. These changes were explained by Corliss and Dugan (1970) and Tsai and Smith (1971) who reported that PE is the most reactive component in the phospholipids and thus changes in PE may indicate the extent of lipid oxidation. They also reported that PC does not exert a prooxidant effect. However Acosta et al. (1966) reported that PC was more active in the early stages of autoxidation. It is expected that PE is more susceptible to autoxidative degradation than PC (Tsai and Smith, 1971). This is due to the fact that PE contains more highly unsaturated fatty acids than PC (Hornstein et al., 1961; Keller and Kinsella, 1973; Body and Shorland, 1974). On the other hand, the increasing levels of SP and LPC during frozen storage suggest that lipolysis had occurred. Similar findings had been reported by Awad et al. (1968) and Braddock and Dugan (1972) in beef and salmon.

Data in Table 16 show that changes in the proportion of oleate, linoleate, linolenate and arachidonate were not sufficiently consistent to reflect the autoxidative degradation process throughout frozen storage of turkey breast meat. This has been supported by Lea (1953), Hornstein et al. (1961) and Keller and Kinsella (1973). They indicated that the unsaturated fatty acids disappear rapidly at advanced stages of autoxidation. Polyunsaturated fatty acids

Table 16. Effect of frozen storage on the proportion of unsaturated fatty acids in phospholipid [1,2]

| Storage time | Fatty acids | | | |
|---|---|---|---|---|
| | 18:1 | 18:2 | 18:3 | 20:4 |
| 0-Month | 13.38±1.26[a] | 18.62±0.65[a] | 0.18±0.15[a] | 12.08±0.64[ab] |
| 1-Month | 14.15±o.93[a] | 18.64±1.56[a] | 0.18±0.25[a] | 11.82±0.57[ab] |
| 3-Month | 13.19±0.64[ab] | 17.88±0.35[a] | 0.22±0.09[a] | 12.27±0.75[ab] |
| 6-Month | 13.21±0.73[ab] | 18.18±0.59[a] | 0.24±0.70[a] | 12.74±0.93[a] |
| 12-Month | 12.17±0.46[b] | 16.18±0.88[b] | 0.19±0.60[a] | 11.40±1.13[b] |

[1]Each value represents a mean of 20 replicates

[2]Means in the same column bearing the same letter are not significantly different (P<0.01)

(PUFA) appear to be principally responsible for the development of rancidity, therefore both PC and PE could play an important role in the development of rancid flavors in stored meat or meat products because of their high content of PUFAS.

The losses in PE after cooking were greater than in PC (Figure 20), although the decrease in both was significant (P<0.01). Therefore, PE was found to be less stable to cooking than PC. It was indicated by Love and Pearson (1971) and Keller and Kinsella (1973) that the loss of

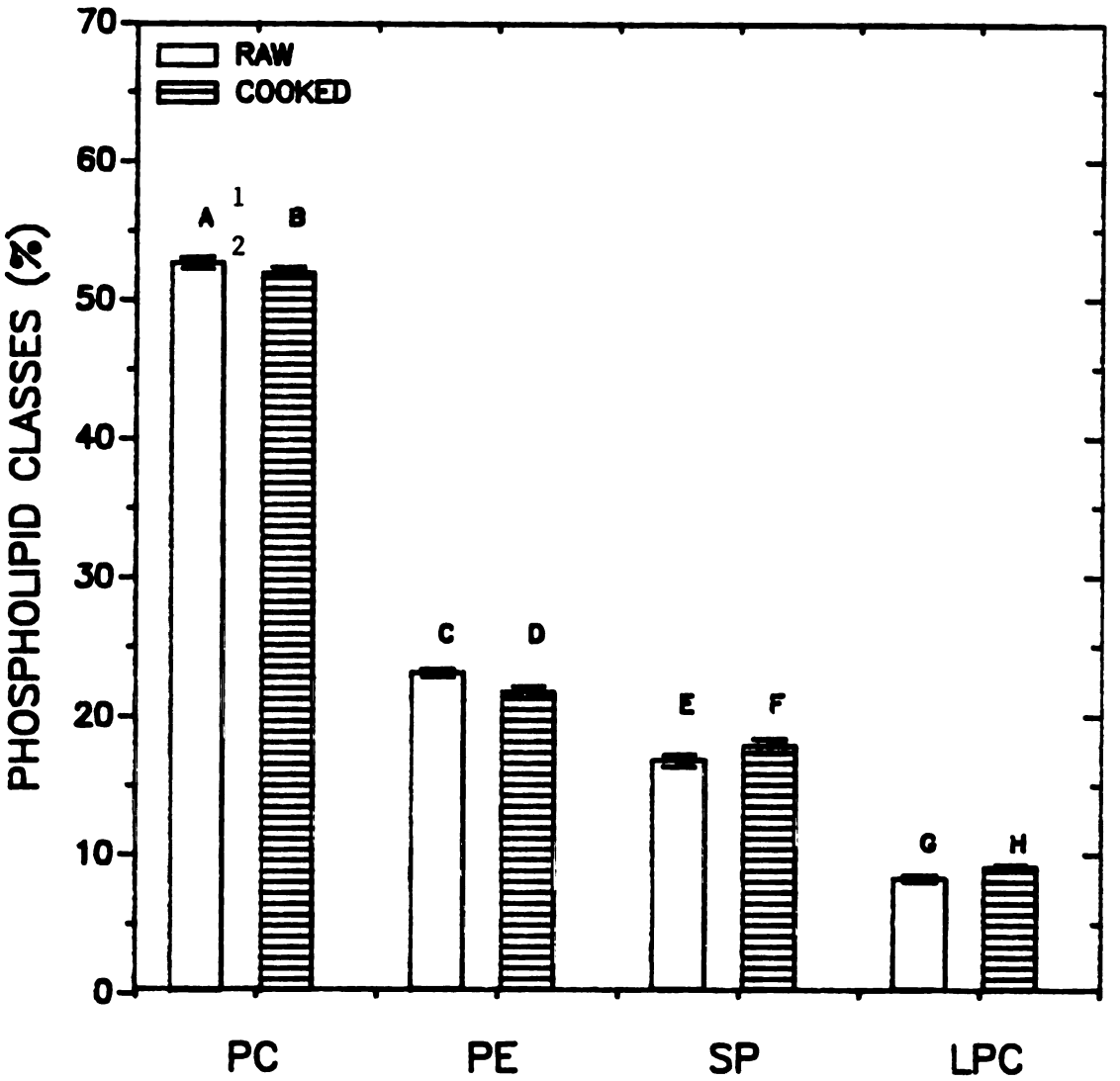



Figure 20. Effect of cooking on the proportion of phospholipid classes in turkey breast phospholipid.

[1]Lipid classes bearing the same letter are not significantly different (P<0.05)

phosphatidyl choline (PC); phosphatidyl ethanolamine (PE); sphingomyelin (SP); lysophosphatidyl choline (LPC)

[2]Bars represent standard errors.

arachidonic acid was consistent with its greater propensity to undergo autoxidation, especially when associated with PE. However, because of the lipolysis, the SP and LPC levels have increased after cooking.

the proportion of PE and PC decreased significantly ($P<0.01$) during frozen storage of turkey breast meat treated with copper, 50 micrograms/g. meat and copper and iron, 1:1 50 micrograms/g meat, (Figure 21). The changes in PC and PE are large over the storage time and appear to be directly related to the development of oxidative deterioration when monitored by the increase of malonaldehyde in samples containing added iron and copper (Figure 13, Page 71). The metal cations act as prooxidants mainly to the polyenoic acids of PE and PC which are extremely reactive and through oxidative degradation produce a number of carbonyl compounds which greatly influence oxidized flavor (Lea, 1957; Younathan and Watts, 1960).

## Changes in Fatty Acid Composition in Phospholipids of Turkey Breast During Frozen Storage and Cooking

The fatty acid composition of phospholipid fraction of raw turkey breast was calculated as a percentage of total fatty acids in the phospholipid fraction. Results are shown in Table 17. The percentage of saturated, mono-, di- and polyenoic fatty acids in the fresh breast at 0-time were

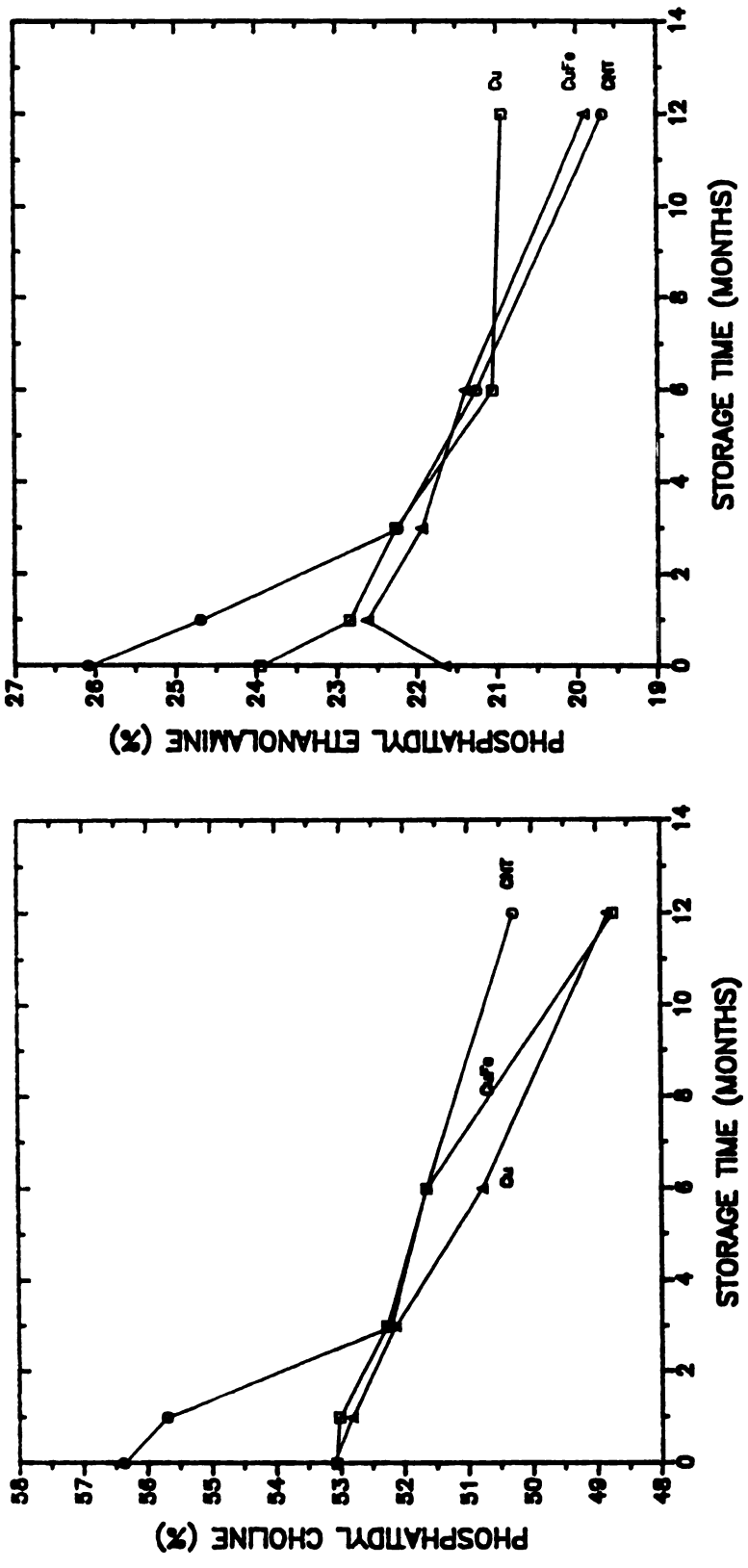



Figure 21. Effect of storage and treatments on the proportion of phospholipid classes in turkey breast phospholipid.

31.35, 12.74, 20.87 and 23.91, respectively (Table 17). These values are in good agreement with the corresponding values 31.90, 12.90, 23.30, and 21.00 reported by Fishwick (1968).

Some changes occurred in the unsaturated fatty acids during frozen storage. The loss in unsaturation could be accounted for by the changes in 18:2, 20:2, 20:3, 20:4, 22:3 and 22:6 fatty acids. The initial levels of dienoic, polyenoic and total unsaturated fatty acids were 20.87, 23.91 and 57.52%, respectively. The proportion of these acids showed a relative decrease at the 12th. month of frozen storage. The reported values for these acids after 12 months storage were 18.90, 21.34 and 52.63%, respectively. Although these observed losses in unsaturated fatty acids would indicate the occurrence of lipid oxidation during frozen storage of raw turkey breast, there were no significant changes for these acids when stored for less than 12 months. These findings lead to the conclusion that fatty acid profile changes are not ideal for monitoring lipid oxidation in stored poultry meat.

Nineteen fatty acids were identified and quantified in the phospholipid fraction from cooked turkey breast meat. The percentage of saturated, mono-, di-, and polyenoic fatty acids in the freshly cooked turkey breast were 37.00, 15.31, 18.83 and 23.66, respectively (Table 18).

Table 17. Changes in fatty acid composition as percent of the phospholipids of raw turkey breast muscle for the indicated frozen storage times [1]

| | Months of storage at -25 C | | | | |
|---|---|---|---|---|---|
| Fatty acid[2] | 0 | 1 | 3 | 6 | 12 |
| 14:0 | 0.31[3] | 0.11 | 0.16 | 0.24 | 0.03 |
| 14:1 | 0.0 | 0.0 | 0.0 | 0.02 | 0.0 |
| 15:0 | 0.0 | 0.07 | 0.0 | 0.02 | 0.0 |
| 16:DMA | 8.45 | 7.41 | 10.70 | 8.38 | 13.03 |
| 16:0 | 14.33 | 18.45 | 13.14 | 14.19 | 14.19 |
| 16:1 | 0.53 | 0.58 | 0.56 | 0.83 | 0.27 |
| 18:DMA | 2.67 | 0.30 | 3.53 | 2.71 | 3.37 |
| 16:2 | 1.63 | 0.0 | 2.19 | 1.61 | 2.05 |
| 18:0 | 16.71 | 17.86 | 15.38 | 16.03 | 16.70 |
| 18:1 | 12.21 | 13.82 | 13.04 | 14.23 | 12.12 |
| 18:2 | 18.99 | 18.62 | 18.00 | 18.68 | 16.74 |
| 18:3 | 0.18 | 0.23 | 0.29 | 0.33 | 0.21 |
| 20:2 | 0.26 | 0.33 | 0.20 | 0.07 | 0.12 |
| 20:3 | 2.01 | 2.43 | 1.41 | 1.71 | 1.59 |
| 20:4 | 12.49 | 11.27 | 12.82 | 12.05 | 12.07 |
| 20:5 | 0.84 | 0.69 | 0.57 | 1.34 | 0.57 |
| 22:3 | 0.22 | 0.25 | 0.07 | 0.06 | 0.08 |
| 22:4 | 3.05 | 3.09 | 3.07 | 2.85 | 2.98 |
| 22:5W6 | 1.36 | 1.19 | 1.27 | 1.20 | 0.94 |
| 22:5W3 | 1.53 | 1.36 | 1.51 | 1.42 | 1.29 |
| 22:6 | 2.24 | 1.94 | 2.08 | 2.02 | 1.61 |
| Sat. | 31.35 | 36.49 | 28.68 | 30.48 | 30.97 |
| Mono | 12.74 | 14.40 | 13.60 | 15.09 | 12.39 |
| Dienoic | 20.87 | 18.95 | 20.40 | 20.36 | 18.90 |
| Polyenoic | 23.91 | 22.45 | 23.09 | 23.01 | 21.34 |
| Unsat. | 57.52 | 55.55 | 57.08 | 58.46 | 52.63 |

[1]Results represent means of 10 determinations

[2]Number of carbon : number of double bonds

[3]Percent of total fatty acids

16:DMA = Dimethyl acetal of hexadecanal

18:DMA = Dimethyl acetal of octadecanal

W = Omega

Table 18. Changes in fatty acid composition as percent of the phospholipids of cooked turkey breast muscle for the indicated frozen storage times [1]

| | Months of storage at -25 C | | | | |
|---|---|---|---|---|---|
| Fatty acid[2] | 0 | 1 | 3 | 6 | 12 |
| 14:0 | 0.23[3] | 0.23 | 0.20 | 0.16 | 0.01 |
| 14:1 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 |
| 15:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:DMA | 4.08 | 9.32 | 8.81 | 9.32 | 14.51 |
| 16:0 | 17.68 | 15.11 | 14.90 | 13.88 | 15.05 |
| 16:1 | 0.69 | 0.41 | 0.35 | 0.27 | 0.20 |
| 18:DMA | 1.14 | 2.73 | 2.68 | 2.75 | 3.60 |
| 16:2 | 0.53 | 1.55 | 1.37 | 1.61 | 2.17 |
| 18:0 | 19.04 | 19.52 | 19.66 | 18.53 | 17.52 |
| 18:1 | 14.54 | 13.37 | 13.33 | 12.21 | 11.91 |
| 18:2 | 18.25 | 17.17 | 17.76 | 17.69 | 15.62 |
| 18:3 | 0.19 | 0.11 | 0.14 | 0.15 | 0.17 |
| 20:2 | 0.04 | 0.04 | 0.11 | 0.04 | 0.09 |
| 20:3 | 2.06 | 1.76 | 1.60 | 1.73 | 1.24 |
| 20:4 | 11.67 | 11.46 | 11.72 | 13.43 | 10.74 |
| 20:5 | 1.13 | 0.59 | 0.52 | 0.54 | 0.58 |
| 22:3 | 0.59 | 0.36 | 0.30 | 0.11 | 0.13 |
| 22:4 | 2.94 | 2.59 | 2.74 | 3.14 | 2.81 |
| 22:5W6 | 1.42 | 1.00 | 0.96 | 1.18 | 0.92 |
| 22:5W3 | 1.59 | 1.18 | 1.16 | 1.40 | 1.23 |
| 22:6 | 2.07 | 1.52 | 1.70 | 1.86 | 1.52 |
| Sat. | 37.00 | 34.86 | 34.75 | 32.57 | 32.58 |
| Mono | 15.31 | 13.78 | 13.68 | 12.48 | 12.11 |
| Dienoic | 18.83 | 18.76 | 19.24 | 19.35 | 17.88 |
| Polyenoic | 23.66 | 20.55 | 20.84 | 23.54 | 19.34 |
| Unsat. | 57.79 | 53.10 | 53.75 | 55.37 | 49.33 |

[1]Results represent means of 10 determinations

[2]Number of carbon : number of double bonds

[3]Percent of total fatty acids

16:DMA = Dimethyl acetal of hexadecanal

18:DMA = Dimethyl acetal of octadecanal

W = Omega

Important changes occurred in these fatty acids during cooking. The saturated and the monoenoic fatty acids increased by 15 and 16.7%, respectively, due to cooking as compared to that in the fresh breast meat values, (Tables 17 and 18) indicating a shift in proportion due to loss, disappearance or failure to detect unsaturated fatty acids.

The effect of cooking on the level of fatty acids in phospholipids, from turkey breast regardless of storage conditions is illustrated in Figures 22 and 23. The proportion of the total unsaturated, di- and tetraenoic fatty acids decreased significantly ($P<0.05$) as a result of cooking. No significant ($P<0.05$) changes were found in mono- and trienoic fatty acids. The level of total saturated fatty acids increased significantly ($P<0.01$). The main change in dienoic acids was due to the autoxidation of linoleate (18:2). Linolenate (18:3) is a polyunsaturated acid which was expected to be more labile to autoxidation, but no significant changes were detected. This might be a result of the extremely low quantities of this acid. However arachidonate (20:4) content was slightly decreased after cooking. The high level of arachidonic acid in phospholipids distinguishes this fraction from neutral lipid.

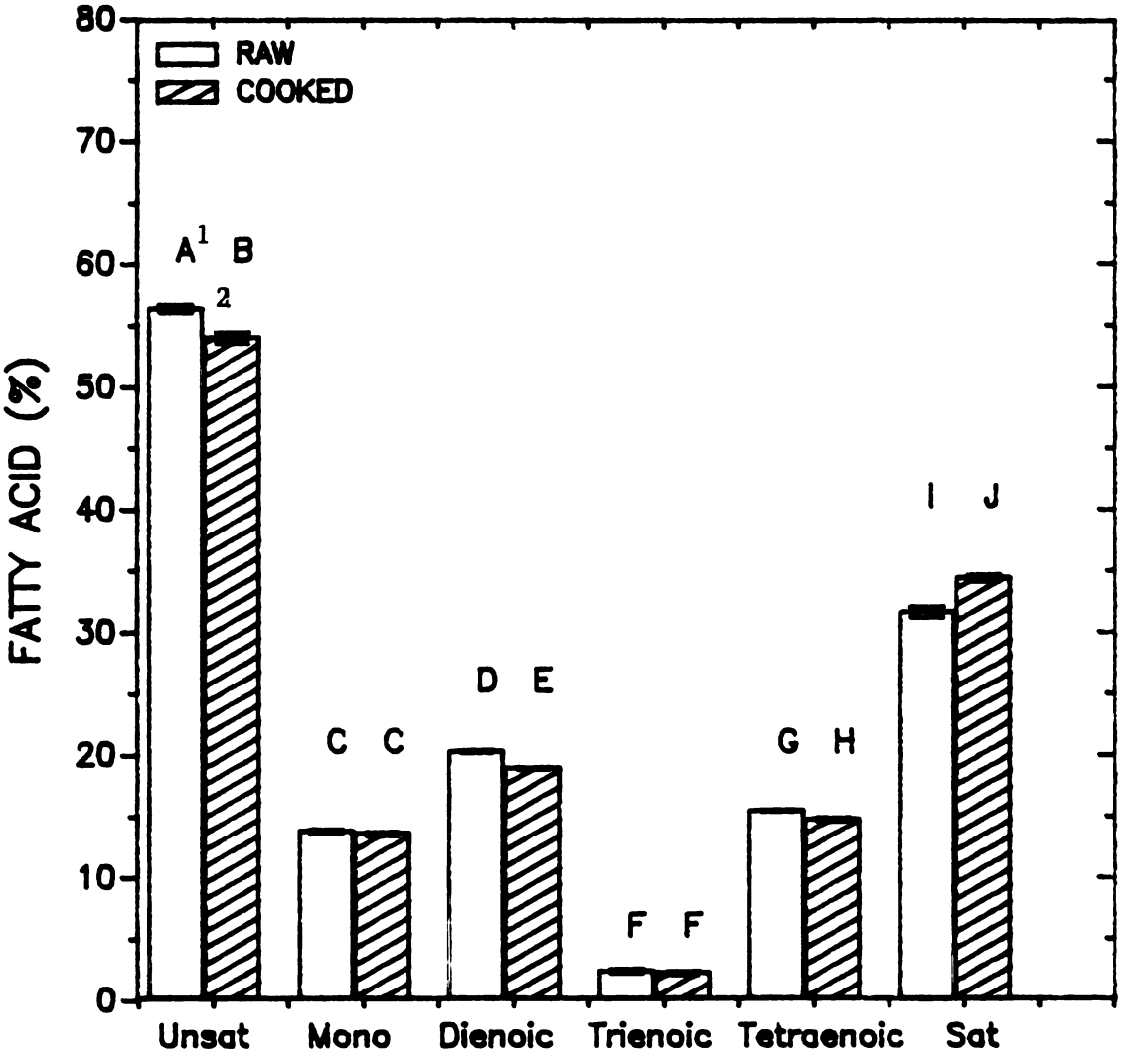



Figure 22. Effect of cooking on the proportion of fatty acids in the phospholipid fraction.

[1]Fatty acid groups bearing the same letter are not significantly different (P<Ø.Ø5)

[2]Bars represent standard errors.

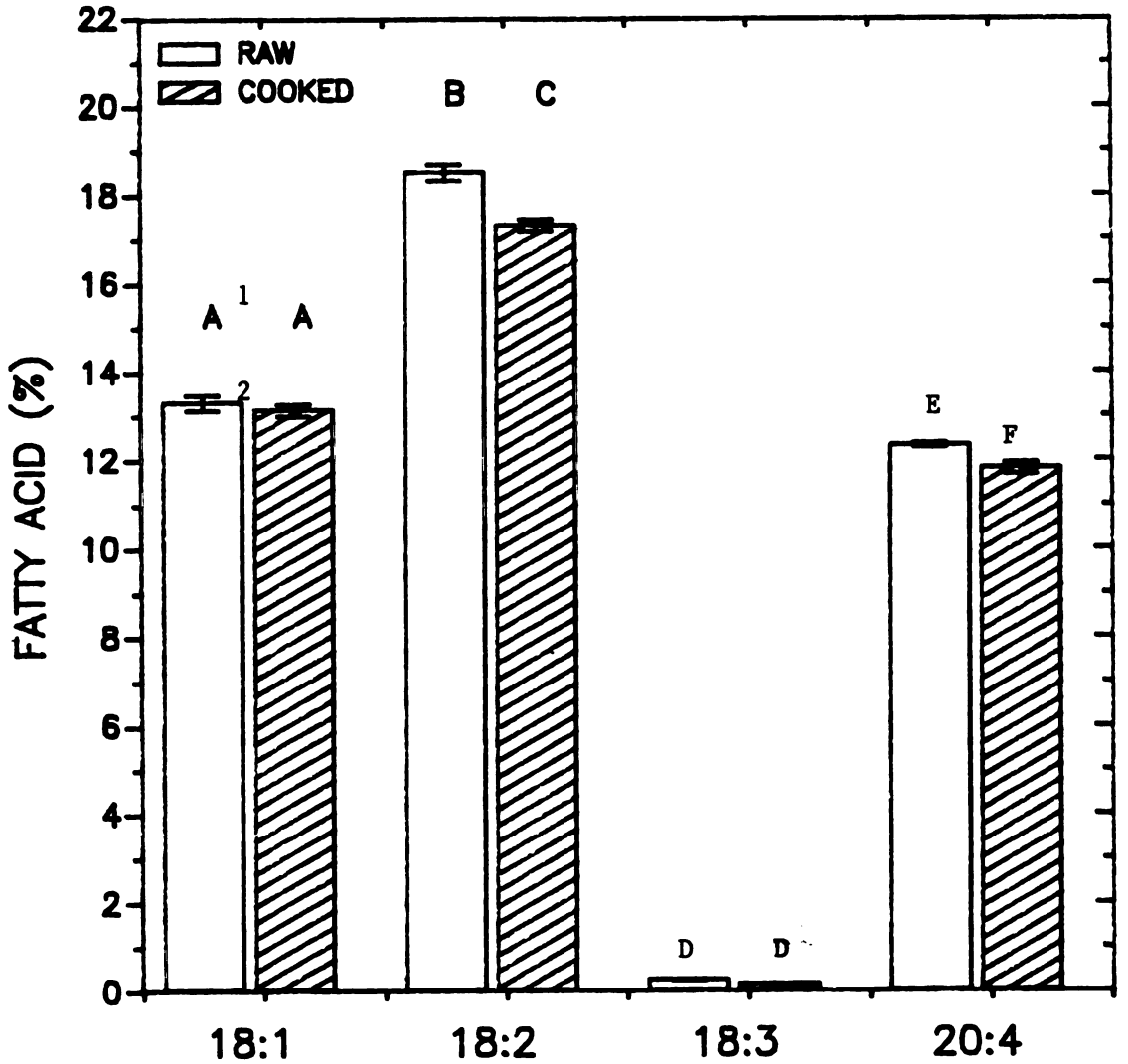



Figure 23. Effect of cooking on the proportion of unsaturated fatty acids in the phospholipid fraction.

[1]Fatty acids bearing the same letter are not significantly different ($P<0.05$)

[2]Bars represent standard errors.

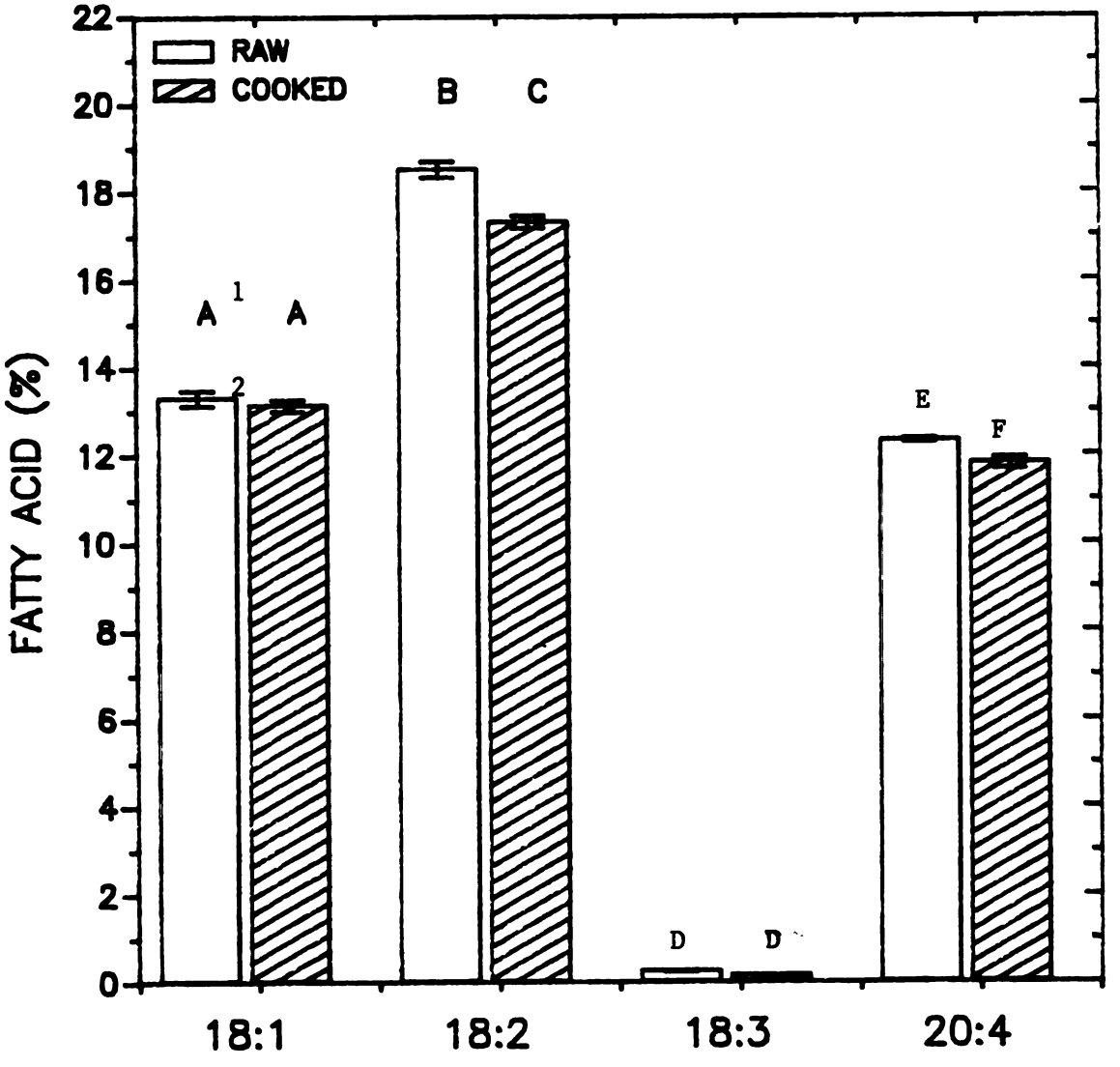



Figure 23. Effect of cooking on the proportion of unsaturated fatty acids in the phospholipid fraction.

[1]Fatty acids bearing the same letter are not significantly different ($P<0.05$)

[2]Bars represent standard errors.

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The decrease in the di- and polyenoic fatty acids is explained by the rapid autoxidation of these acids after cooking. Sato and Hegarty (1971) came to a similar conclusion and suggested that cooking exposes the labile lipid components to catalytic agents. It might also be due to the liberation of heme iron by cooking (Igene et al., 1981). The saturated as well as the monoenoic fatty acids are fairly stable, thus they usually either stay at the same level or increase in proportion to the decrease in polyenoic acids, when measured as percentage of the total composition. However, the results of this work revealed that with prolonged frozen storage the monoenoic acid oxidation was initiated.

Changes in the proportions of fatty acids during frozen storage of the cooked breast meat are similar to those of the raw breast meat. The high susceptibility of cooked meat to autoxidation might be due to the denaturation of the protein which facilitates exposure of iron to unsaturated fatty acids. Eriksson (1975) showed that protein denaturation increased the ability of the heme-containing proteins, peroxidase and catalase to promote lipid oxidation.

## Changes in Fatty Acid Composition Percent in Neutral Lipids of Turkey Breast During Frozen Storage and Cooking

The fatty acid composition from raw and cooked turkey breast muscle was calculated as percentage of the total fatty acids in the neutral lipid fraction. Results are presented in Tables 19 and 20. Twenty different fatty acids were identified and quantified from the neutral lipid fraction. The most prevalent fatty acids had 16 carbon atoms (palmitic and palmitoleic) and 18 carbon atoms (stearic, oleic and linoleic acids). These C:16 and C:18 fatty acids composed 92.5 percent of the total fatty acids in the neutral lipids. Palmitic acid accounted for a major portion of the saturated fatty acids (about 2/3 of that in the fresh raw and the fresh cooked breast muscle). Oleic and linoleic acids accounted for a large percentage of the unsaturated fatty acids (more than 4/5 of that in the fresh raw and in the fresh cooked breast muscle). This distribution of fatty acids in neutral lipids is typical and has been previously reported by Issacks et al. (1964) in triglyceride fraction of adipose tissue of the laying hens and Katz et al. (1966) in neutral lipids from chicken tissues.

Since oxidation depends on the presence of unsaturated fatty acids, an analysis of individual fatty acids was made

Table 19. Changes in fatty acid composition as percent of the neutral lipids of raw turkey breast muscle of the indicated frozen storage times [1]

| | Months of storage at -25 C | | | | |
|---|---|---|---|---|---|
| Fatty acid[2] | 0 | 1 | 3 | 6 | 12 |
| 12:0 | 0.00[3] | 0.00 | 0.05 | 0.06 | 0.07 |
| 14:0 | 1.37 | 1.41 | 1.36 | 1.34 | 1.56 |
| 14:1 | 0.23 | 0.05 | 0.19 | 0.29 | 0.22 |
| 15:0 | 0.14 | 0.04 | 0.09 | 0.18 | 0.16 |
| 16:DMA | 0.35 | 0.19 | 0.44 | 0.18 | 0.15 |
| 16:0 | 21.64 | 21.45 | 22.42 | 21.94 | 22.57 |
| 16:1 | 3.84 | 3.88 | 3.89 | 3.82 | 3.97 |
| 18:DMA | 0.51 | 0.24 | 0.59 | 0.55 | 0.57 |
| 16:2 | 0.41 | 0.19 | 0.38 | 0.46 | 0.43 |
| 18:0 | 8.70 | 8.76 | 9.05 | 8.86 | 9.26 |
| 18:1 | 32.70 | 32.74 | 34.33 | 34.16 | 34.59 |
| 18:2 | 23.19 | 22.27 | 22.41 | 22.62 | 23.33 |
| 18:3 | 1.48 | 1.57 | 1.60 | 1.59 | 1.24 |
| 20:2 | 0.38 | 0.35 | 0.29 | 0.47 | 0.19 |
| 20:3 | 0.38 | 0.35 | 0.22 | 0.42 | 0.14 |
| 20:4 | 2.52 | 1.03 | 0.93 | 1.07 | 0.81 |
| 20:5 | 0.48 | 0.10 | 0.27 | 0.24 | 0.31 |
| 22:3 | 0.11 | 0.04 | 0.10 | 0.08 | 0.0 |
| 22:4 | 0.64 | 0.21 | 0.48 | 0.56 | 0.19 |
| 22:5W6 | 0.33 | 0.15 | 0.21 | 0.28 | 0.02 |
| 22:5W3 | 0.34 | 4.74 | 0.46 | 0.42 | 0.12 |
| 22:6 | 0.24 | 0.20 | 0.26 | 0.44 | 0.09 |
| Sat. | 31.88 | 31.70 | 32.96 | 32.38 | 33.63 |
| Mono | 36.93 | 36.67 | 38.40 | 38.27 | 38.79 |
| Dienoic | 23.97 | 22.81 | 23.08 | 23.54 | 23.95 |
| Polyenoic | 6.52 | 8.39 | 4.51 | 5.08 | 2.92 |
| Unsat. | 67.42 | 67.87 | 66.00 | 66.89 | 65.65 |

[1]Results represent means of 10 determinations

[2]Number of carbon : number of double bonds

[3]Percent of total fatty acids

16:DMA = Dimethyl acetal of hexadecanal

18:DMA = Dimethyl acetal of octadecanal

W = Omega

Table 20. Changes in fatty acid composition as percent of the neutral lipids of cooked turkey breast muscle for the indicated frozen storage times [1]

| | Months of storage at -25 C | | | | |
|---|---|---|---|---|---|
| Fatty acid[2] | 0 | 1 | 3 | 6 | 12 |
| 12:0 | 0.04[3] | 0.00 | 0.00 | 0.00 | 0.00 |
| 14:0 | 1.52 | 1.41 | 1.47 | 11.44 | 1.54 |
| 14:1 | 0.15 | 0.08 | 0.02 | 0.16 | 0.21 |
| 15:0 | 0.09 | 0.06 | 0.00 | 0.04 | 0.18 |
| 16:DMA | 0.38 | 0.03 | 0.00 | 0.04 | 0.08 |
| 16:0 | 22.48 | 22.30 | 22.87 | 22.47 | 23.28 |
| 16:1 | 3.89 | 4.16 | 4.18 | 4.07 | 4.19 |
| 18:DMA | 0.57 | 0.20 | 0.16 | 0.26 | 0.40 |
| 16:2 | 0.42 | 0.18 | 0.10 | 0.22 | 0.30 |
| 18:0 | 9.10 | 8.77 | 8.91 | 8.84 | 9.21 |
| 18:1 | 33.70 | 34.81 | 35.02 | 34.55 | 35.14 |
| 18:2 | 21.89 | 22.22 | 22.03 | 22.92 | 22.76 |
| 18:3 | 1.46 | 1.49 | 1.36 | 1.47 | 1.13 |
| 20:2 | 0.35 | 0.45 | 0.24 | 0.27 | 0.20 |
| 20:3 | 0.58 | 0.41 | 0.22 | 0.21 | 0.12 |
| 20:4 | 1.43 | 1.43 | 1.66 | 1.49 | 0.86 |
| 20:5 | 0.57 | 0.32 | 0.28 | 0.28 | 0.05 |
| 22:3 | 0.16 | 0.28 | 0.14 | 0.14 | 0.01 |
| 22:4 | 0.49 | 0.37 | 0.49 | 0.59 | 0.18 |
| 22:5W6 | 0.25 | 0.21 | 0.46 | 0.19 | 0.01 |
| 22:5W3 | 0.27 | 0.55 | 0.22 | 0.17 | 0.07 |
| 22:6 | 0.22 | 0.28 | 0.17 | 0.17 | 0.03 |
| Sat. | 33.23 | 32.54 | 33.25 | 32.79 | 34.21 |
| Mono | 37.74 | 39.05 | 39.23 | 38.79 | 39.54 |
| Dienoic | 22.65 | 22.85 | 22.37 | 23.41 | 23.26 |
| Polyenoic | 5.43 | 5.33 | 4.99 | 4.71 | 2.41 |
| Unsat. | 65.82 | 67.23 | 66.59 | 66.91 | 65.21 |

[1]Results represent means of 10 determinations

[2]Number of carbon : number of double bonds

[3]Percent of total fatty acids

16:DMA = Dimethyl acetal of hexadecanal

18:DMA = Dimethyl acetal of octadecanal

W = Omega

for neutral lipids in raw and cooked, and fresh and frozen turkey breast muscle. Although neutral lipids have a much less important role in autoxidation of poultry meat because of the lower content of polyunsaturated fatty acids (6.52 and 5.43% in the raw and cooked meat compared to 23.91 and 23.66% in phospholipids of raw and cooked meat) the proportion of polyenoic fatty acids in them consistently decreased during frozen storage with a loss of up to half their proportional amount by 12 months of storage. The greatest difference between the percentage of polyenoic acids in neutral and phospholipids is due to the higher content of arachidonic acid (20:4) in the phospholipid fraction. These results agree with the findings reported by Miller et al. (1962) and Katz et al. (1966).

The effect of cooking on the proportion of fatty acids in neutral lipids is presented in Figures 24 and 25. These graphs show that fatty acids in neutral lipids of turkey breast meat are not greatly altered during cooking. The levels of total unsaturated and saturated fatty acids were not significantly ($P<0.05$) affected by cooking. However as a result of cooking, a slightly greater proportion of monoenoic acids was observed (mostly oleate). The reverse was the case for dienoic acids which amount to about 99% linoleate (18:2).

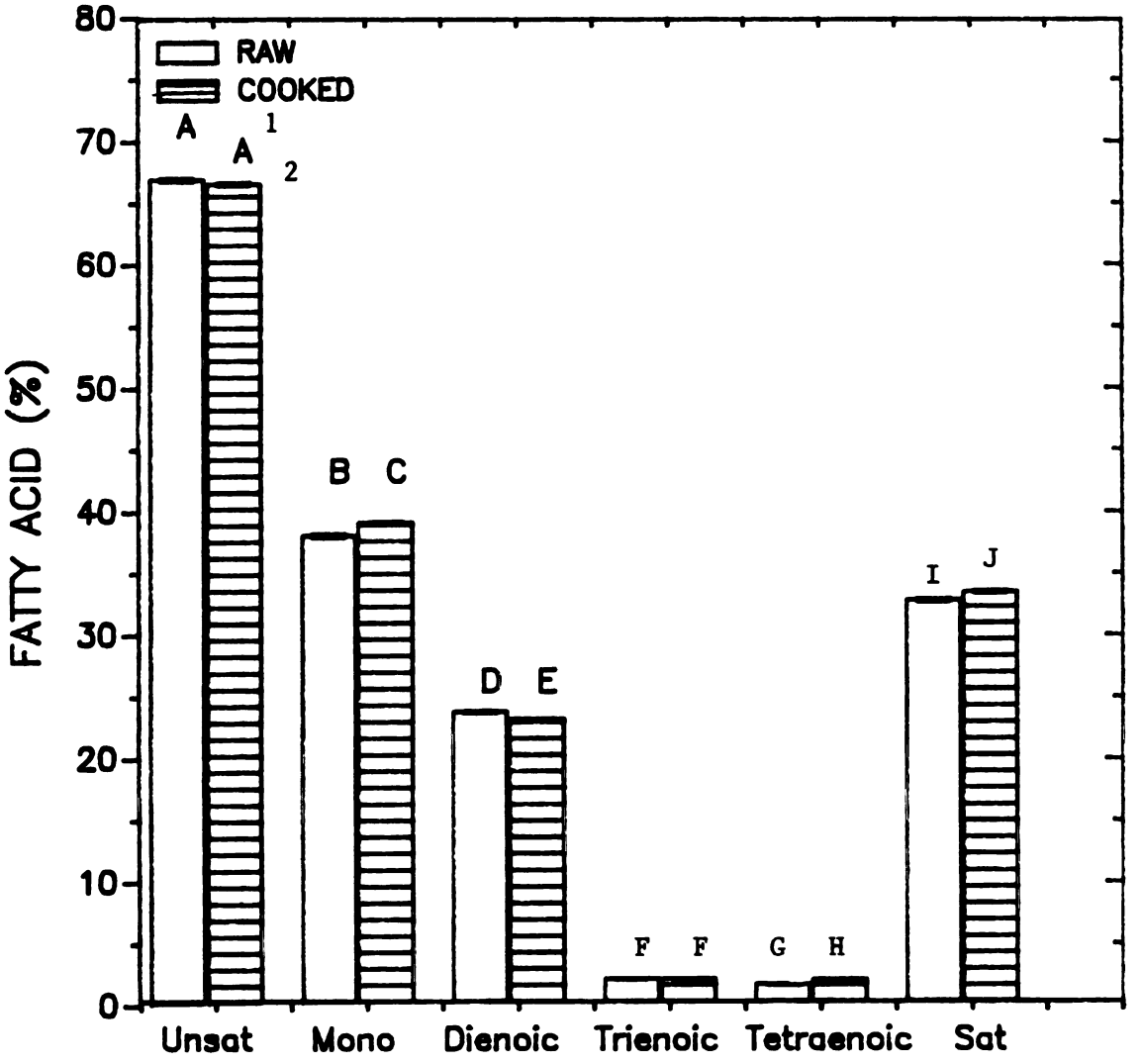


Figure 24. Effect of cooking on the proportion of fatty acids in neutral lipid fraction.

[1]Fatty acid groups bearing the same letter are not significantly different (P<0.05)

[2]Bars represent standard errors.

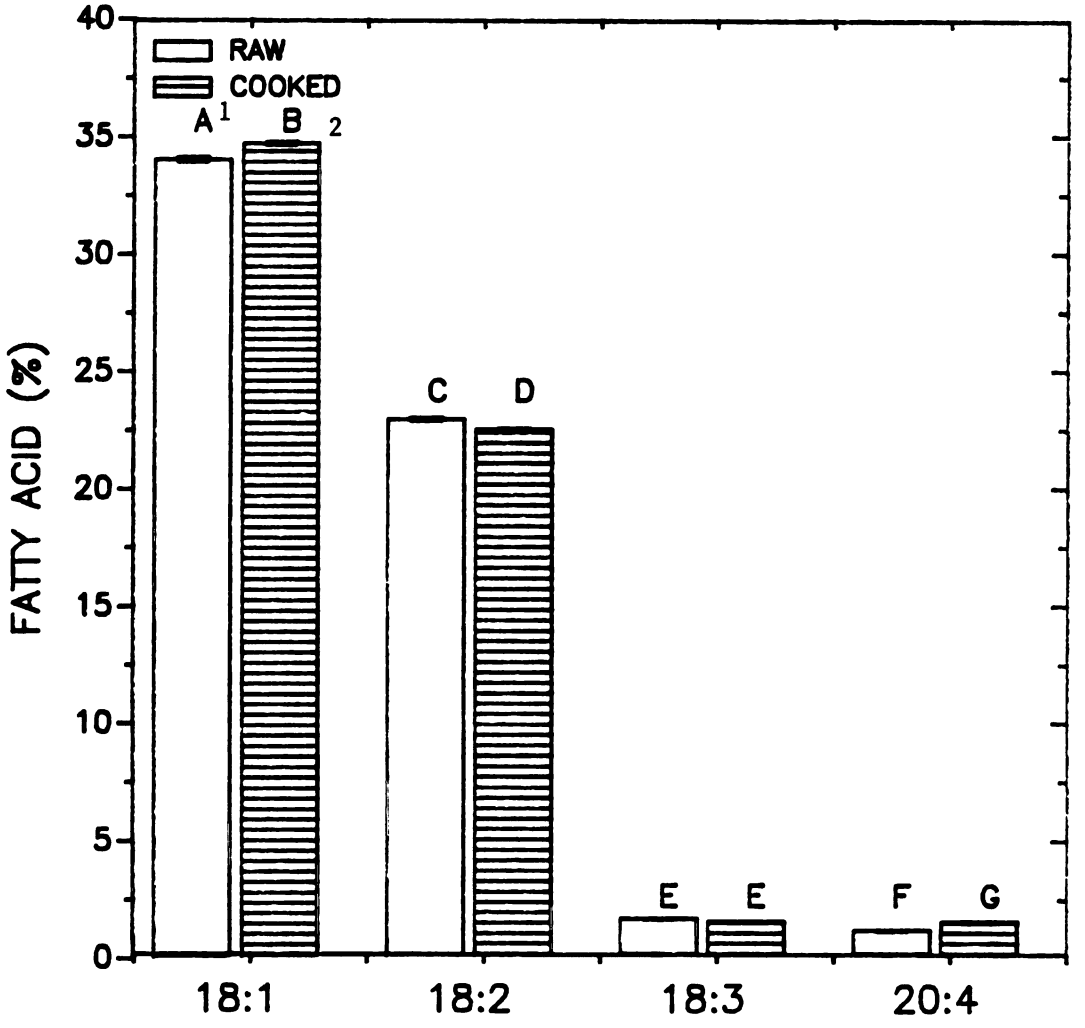


Figure 25. Effect of cooking on the proportion of unsaturated fatty acids in neutral lipid fraction.

[1]Fatty acids bearing the same letter are not significantly different (P<0.05)

[2]Bars represent standard errors.

The results indicate that only minor changes occurred in fatty acid profiles of neutral lipids during frozen storage and cooking of turkey breast meat (Table 19 and 20, Figures 24 and 25). These results are in agreement with the Chang and Watts (1952) and Igene et al. (1981) which showed only slight changes in the fatty acid composition of neutral lipids during storage and cooking.

The effects of frozen storage on the proportion of saturated fatty acids regardless of cooking are illustrated in Figure 26 for both neutral lipids and phospholipids. Significant changes in the unsaturated fatty acids in phospholipids were found during frozen storage of the meat (unlike the neutral lipids). The changes are largely produced by the long term storage (12 mo.) effect. Other changes in unsaturated fatty acids (as shown earlier in Tables 17 and 18) which occurred during the storage intervals of 1, 3, and 6 months are not valuable as indicators of oxidative rancidity.

## Fatty Acid Composition of Fresh Raw Turkey Thigh Lipids

Fatty acid composition of phospholipids and neutral lipid fractions of fresh raw turkey thigh muscle are shown in Table 21. In general the fatty acid profile of thigh meat is similar to that of the breast meat except for some minor differences. The mono- and dienoic acids are higher

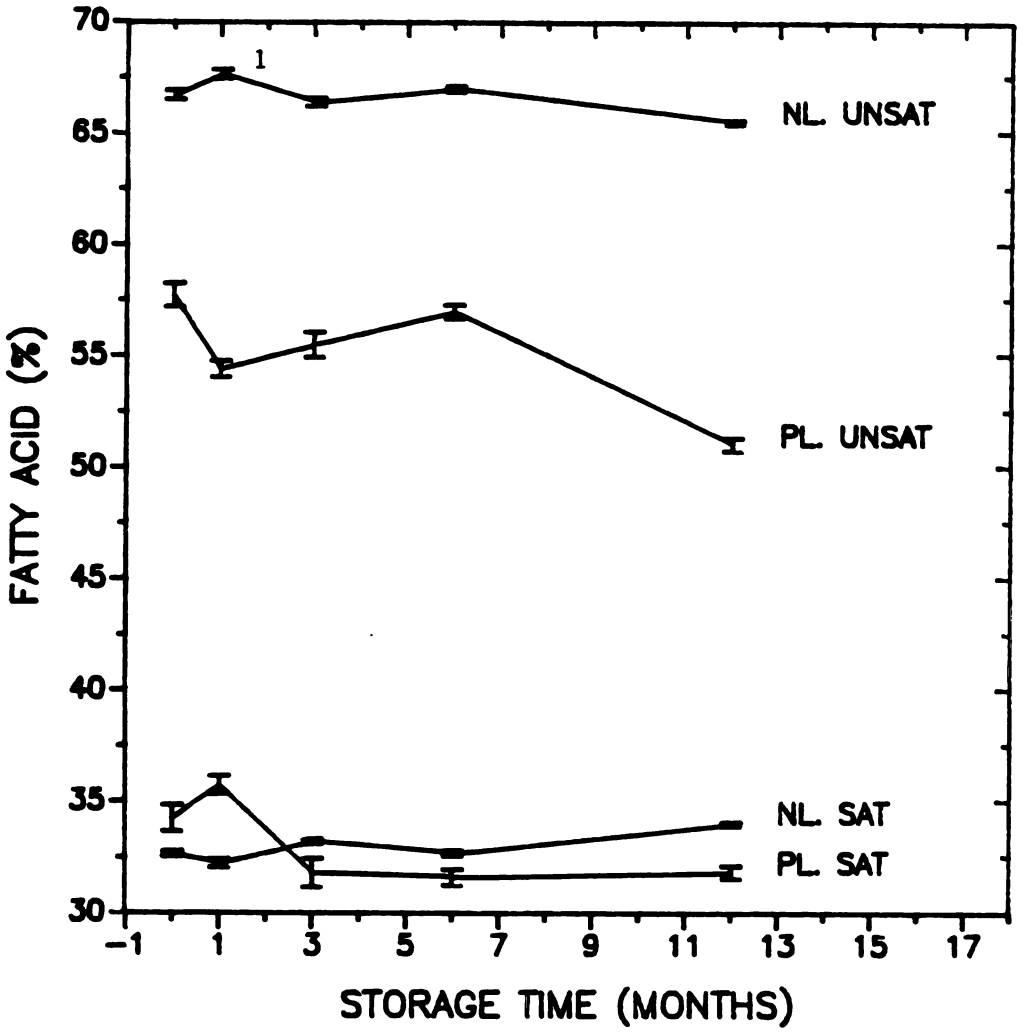


Figure 26. Effect of storage on the composition of saturated and unsaturated fatty acids in neutral and phospholipids.

neutral lipid (NL); phospholipid (PL); saturated (SAT); unsaturated (UNSAT)

[1]Bars represent standard errors.

| | | | |
|---|---|---|---|
| Sat | | | |
| Mon | | | |
| Die | | | |
| Poly | | | |
| Unsa | | | |

[1]Numbe

[2]Value

[3]Percen

[4]Standar

16:DMA =

18:DMA =

ω = Omega

Table 21. Fatty acid profile of fresh turkey thigh lipids

| | Phospholipids | | Neutral lipids | |
|---|---|---|---|---|
| Fatty acid[1] | Mean[2,3] | STD[4] | Mean | STD |
| 12:0 | 0.02 | 0.005 | 0.10 | 0.007 |
| 14:0 | 1.95 | 0.637 | 1.22 | 0.047 |
| 14:1 | 1.22 | 0.686 | 0.25 | 0.031 |
| 15:0 | 0.98 | 0.222 | 0.25 | 0.035 |
| 16:DMA | 4.23 | 1.321 | 0.00 | 0.000 |
| 16:0 | 12.22 | 0.497 | 19.82 | 0.788 |
| 16:1 | 0.93 | 0.156 | 3.92 | 0.902 |
| 18:DMA | 1.18 | 0.511 | 0.42 | 0.105 |
| 16:2 | 0.47 | 0.214 | 0.47 | 0.142 |
| 18:0 | 16.90 | 0.308 | 7.36 | 0.336 |
| 18:1 | 15.09 | 0.914 | 34.15 | 1.229 |
| 18:2 | 23.92 | 0.200 | 26.10 | 0.970 |
| 18:3 | 0.57 | 0.083 | 2.25 | 0.190 |
| 20:2 | 0.22 | 0.041 | 0.47 | 0.084 |
| 20:3 | 0.76 | 0.192 | 0.47 | 0.381 |
| 20:4 | 13.77 | 0.715 | 0.81 | 0.335 |
| 20:5 | 0.46 | 0.248 | 0.76 | 0.583 |
| 22:4 | 2.14 | 0.203 | 0.51 | 0.365 |
| 22:5W6 | 0.98 | 0.154 | 0.36 | 0.227 |
| 22:5W3 | 1.08 | 0.090 | 0.24 | 0.069 |
| 22:6 | 0.89 | 0.078 | 2.65 | 5.175 |
| Sat. | 32.08 | 0.534 | 28.74 | 0.801 |
| Mono | 17.34 | 1.043 | 38.32 | 1.875 |
| Dienoic | 24.61 | 0.364 | 27.04 | 0.996 |
| Polyenoic | 20.65 | 1.668 | 5.48 | 2.331 |
| Unsat. | 62.50 | 1.368 | 70.84 | 0.710 |

[1]Number of carbon : number of double bonds

[2]Values represent means of 5 determinations

[3]Percent of total fatty acids

[4]Standard deviation

16:DMA = Dimethyl acetal of hexadecanal

18:DMA = Dimethyl acetal of octadecanal

W = Omega

in the thigh phospholipids 17.34 and 24.61% vs. 12.74 and 20.87% in the breast phospholipids. This same trend exists in neutral lipids. The mono- and dienoic acids are 38.32 and 27.04%, respectively in thigh meat while the corresponding values for breast meat are 36.93 and 23.97%.

## Identification of Dimethyl Acetal of Hexa- and Octadecanal

Chromatographic results of fatty acids in phospholipids (Tables 17 and 18, Pages 96 and 97) indicated the presence of two unknown compounds. One of them was eluted preceding the methyl ester of hexadecanoic acid and the second was eluted preceding the octadecanoic acid. Methylated fatty acid standards were found to have retention times which did not correspond to those of the unknowns. From the above results and mass spectrophotometric analyses it was confirmed that the compounds were not fatty acid methyl esters and it was suspected that they were plasmalogen aldehyde derivatives or oxidation products. However, the identification and quantification of dimethyl acetals (DMA) of hexa- and octadecanal has not been reported in turkey meat phospholipids. Their identification and proportion in the phospholipid fraction is reported for raw and cooked turkey breast meat throughout 12 months frozen storage.

Dimethyl acetals are formed from methylation of the aldehydes during esterification of the phospholipids. The

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hexa- and octadecanals are long-chain aldehydes released from plasmalogens. Plasmalogens have been found in a variety of animal tissues (Webster, 1960, Rapport and Norton, 1962; Peng and Dugan, 1965 and Neudoerffer and Lea, 1967) These two aldehydes correspond to hexadecanoic and octadecanoic acids and are found as enol ethers.

The gas chromatographic analysis using 10% SP-2330 on 100/120 Supelcoport showed a significantly lower concentration of the 16:DMA in the alkali transesterified sample (6.6%) than in the acid esterified sample (12.2%), but the corresponding values of 18:DMA for the two methods were 2.7 and 2.0%, respectively. However when the saponified portion of phospholipids was extracted and analyzed after being esterified, the result obtained was similar to that reported when the boron trifluoride-methanol method of esterification was used directly.

Gas chromatography-mass spectrophotometric analysis indicated that there were two compounds that are present in substantial amounts in turkey phospholipids and have high molecular weights of 286 and 314 (Figures 3 and 4 Appendix C). These results indicate that the compounds could not be oxidation products. The unknowns produced large mass peaks at 75 m/e and mass units produced were two mass units higher than fragmentation peaks produced by fatty acid methyl esters.

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Friedel and Sharkey (1956) indicated that oxygenated hydrocarbons must contain two oxygen atoms and no unsaturated bonds in order to produce such peaks. The dimethyl acetals produce such peaks. Also the molecular weight of these compounds are the same as those of C:16 and C:18 dimethyl acetals.

Gardner et al. (1972) using infrared spectroscopy reported the possible presence of hexadecanal and octadecanal in chicken meat.

In this work, DMA of hexa- and octadecanal were found to be present in both raw and cooked turkey breast lipids. The quantity of hexa- and octadimethyl acetals in the raw were 8.45 and 2.67%, respectively (Table 17, Page 96). The corresponding values in the freshly cooked breast meat were 4.Ø8 and 1.14% (Table 18, Page 97). These values are in agreement with the results reported by Gardner et al. (1972). They indicated that phospholipids from raw broiler breast contained 8.54% hexadecanal and 2.68% octadecanal, while the cooked muscle contained 8.41% hexadecanal and 1.52% octadecanal. Moerck and Ball (1973) reported a lower level of hexadecanal (2.36%) in bone marrow phospholipids but the octadecanal level was 1.6%.

During 12 months of frozen storage of raw and cooked turkey breast meat the percentage of hexa- and octadecanal increased the hexadecanal by about 21 and 56% in the raw

and cooked samples, respectively. And the octadecanal increased by about 12 and 52% in the raw and cooked samples, respectively. As the aldehyde of these compounds are associated with the membranous structures of the tissues, the prolonged frozen storage and / or cooking might help release them completely because of the deteriorative changes which occur in the meat tissues.

The fresh raw turkey thigh muscle contained lower proportion of both hexa- and octadecanal (4.23 and 1.18%, respectively) compared to fresh raw turkey breast. Gardner et al. (1972) reported in raw chicken thigh meat were 6.00 and 2.38%. The neutral lipids contained traces of these compounds.

## Aerobic Plate Count (APC)

In meat systems microbial growth limits the shelf life and results in important flavor changes in the meat product (Dawson et al., 1975). Although certain lipolytic or fat-splitting microorganisms, such as some species of Pseudomonas, can hydrolyze the fat causing hydrolytic rancidity, the main cause of changes in meat is oxidative rancidity produced by chemical combination of oxygen in the air with the unsaturated fatty acid component of the fat.

Figure 27 demonstrates that the logarithmic growth phase of aerobic organisms for both refrigerated raw breast

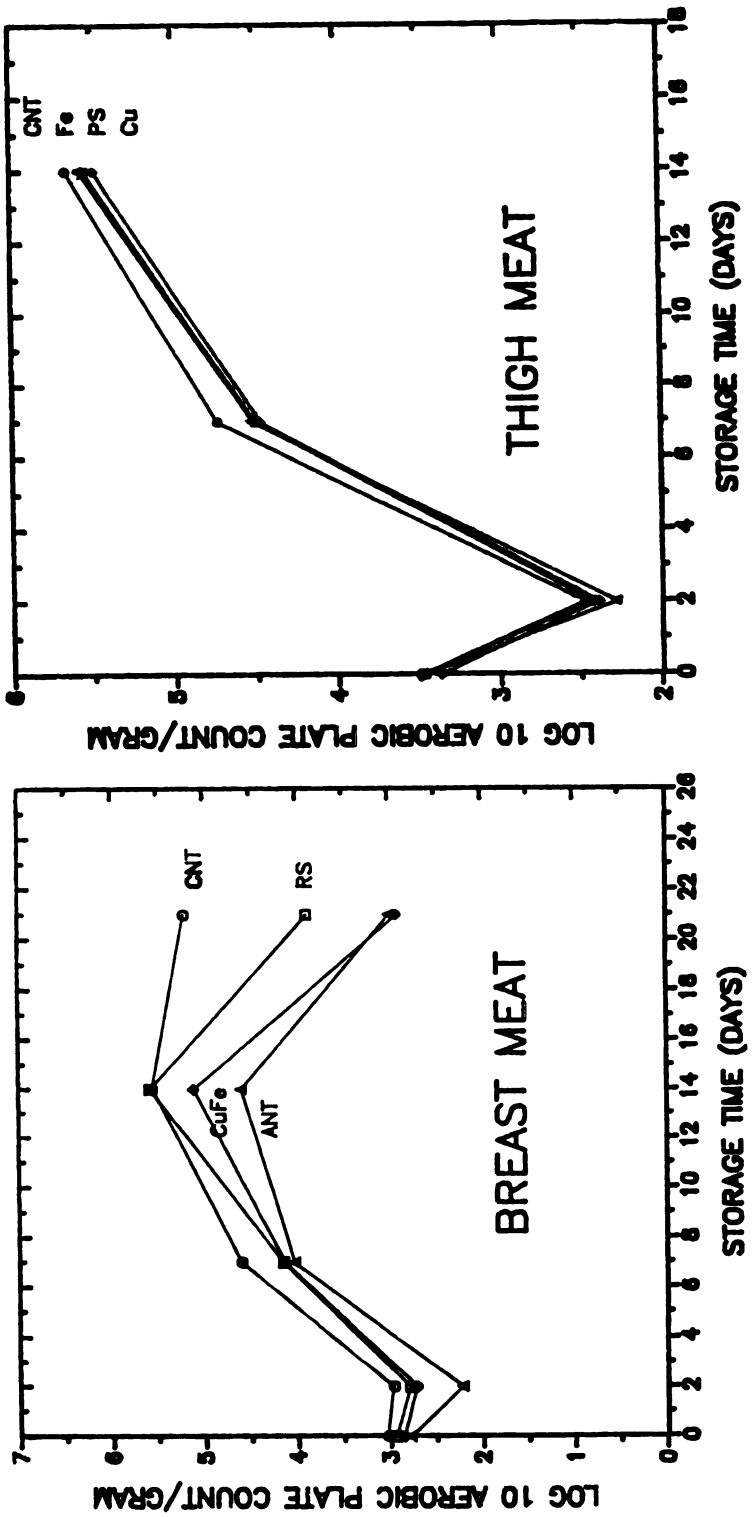



Figure 27.Effect of treatments and storageon aerobic plate count for raw refrigerated turkey meat.

and thigh meats started after a lag phase of 2 days. The shape of the growth curve could be affected by several factors.

The effect of chilling on the microflora in a particular food depends on the temperature characteristics of the organisms as well as the temperature and time of storage. The lag phase which is the period of adjustment increases with decreasing temperature. In a mixed flora of psychrotrophs and mesophiles, low temperature has an important selective action and may affect the composition of the initial contamination or lead to changes in the flora development during processing and storage. For example, chilled beef prepared in a semitropical climate had longer storage life under chilled conditions than did similar beef from cooler areas (ICMSF, 1980a) because the proportion of psychrotrophs is larger in the initial contamination of beef from the cooler areas.

Spoilage is detected when the numbers of the bacteria reaches or surpasses $10^7/cm^2$ of meat surface. The meat surface becomes discolored and odorous, then slime appears and obscures the sheen of the meat surface. This stage of spoilage was detected in raw turkey breast meat refrigerated for 21 days at 4 C, consequently when experiment C was designed the maximum storage time for the refrigerated meat was 14 days.

Figure 28 shows significant (P<0.01) decrease in APC in both raw turkey breast and thigh meats during frozen storage at - 25 C. These results are in agreement with the conclusion of an International Commission on Microbiological Specifications for Foods (ICMSF, 1980b) who stated that freezing reduces the number of the viable microorganisms in raw meat. Some are killed while others are damaged sublethally. At temperatures below - 10 C these sublethally damaged microorganisms die in time, but above this temperature recovery can take place. None of treatments used in breast or in thigh meat have a very significant effect on APC, even the antibiotic which might be used in a concentration not sufficient to suppress the microbial population.

Thermally processed meat should have low APC. Shelf-life is related to the time-temperature combination used for processing, the extent of postcooking contamination, the type of packaging and temperature and time of storage. For instance the APC was < one colony/gram of cooked turkey breast meat for the samples examined immediately after cooking. However the APC started to increase linearly after the second day of refrigerated storage at 4 C (Figure 29). These results agree with the results reported by an International Commission on Microbiological Specifications for Foods (ICMSF, 1980c). The psychrotrophic microorganisms

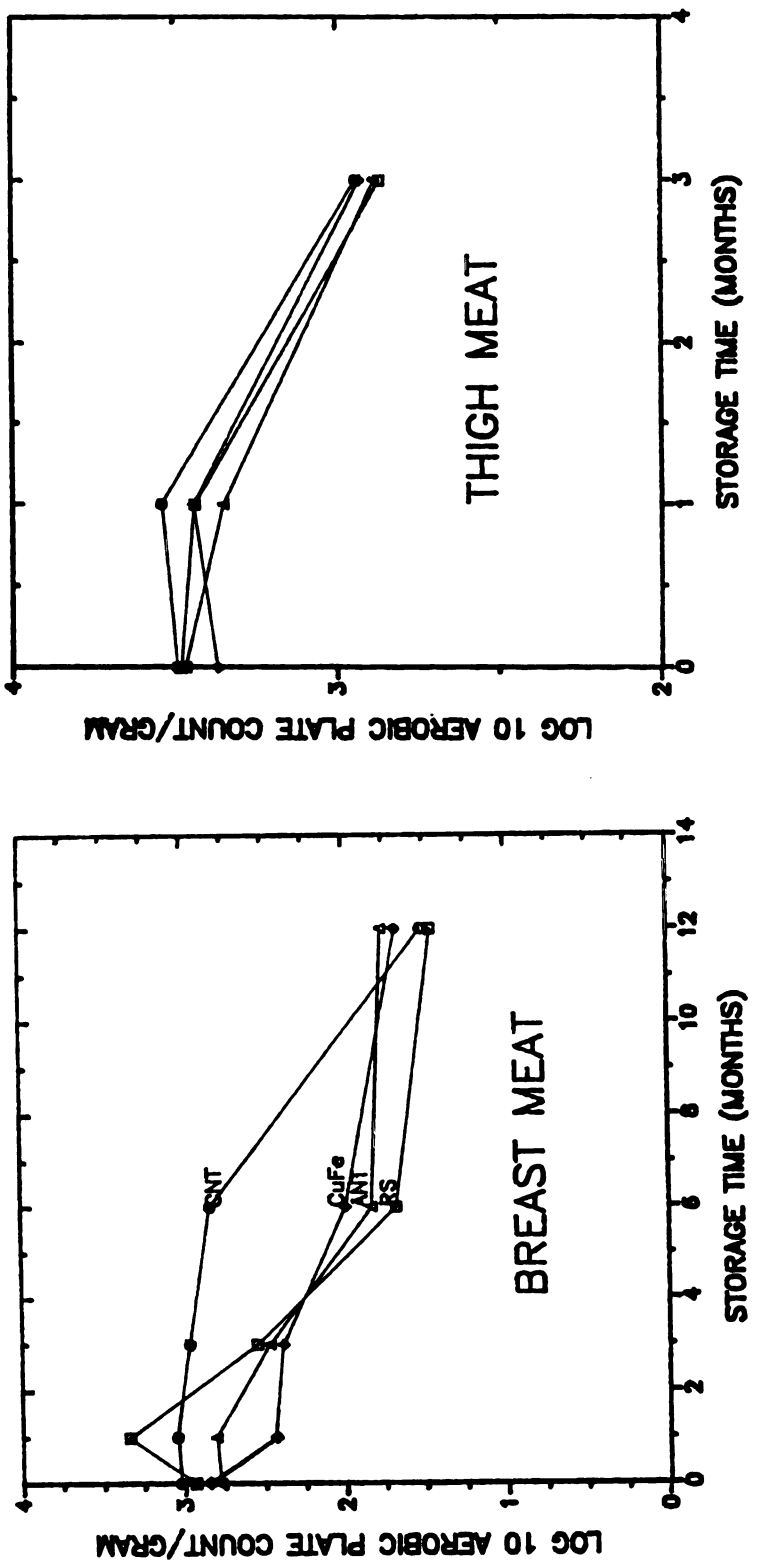



Figure 28. Effectof treatments and storageon aerobic plate count for raw frozen turkey meat.

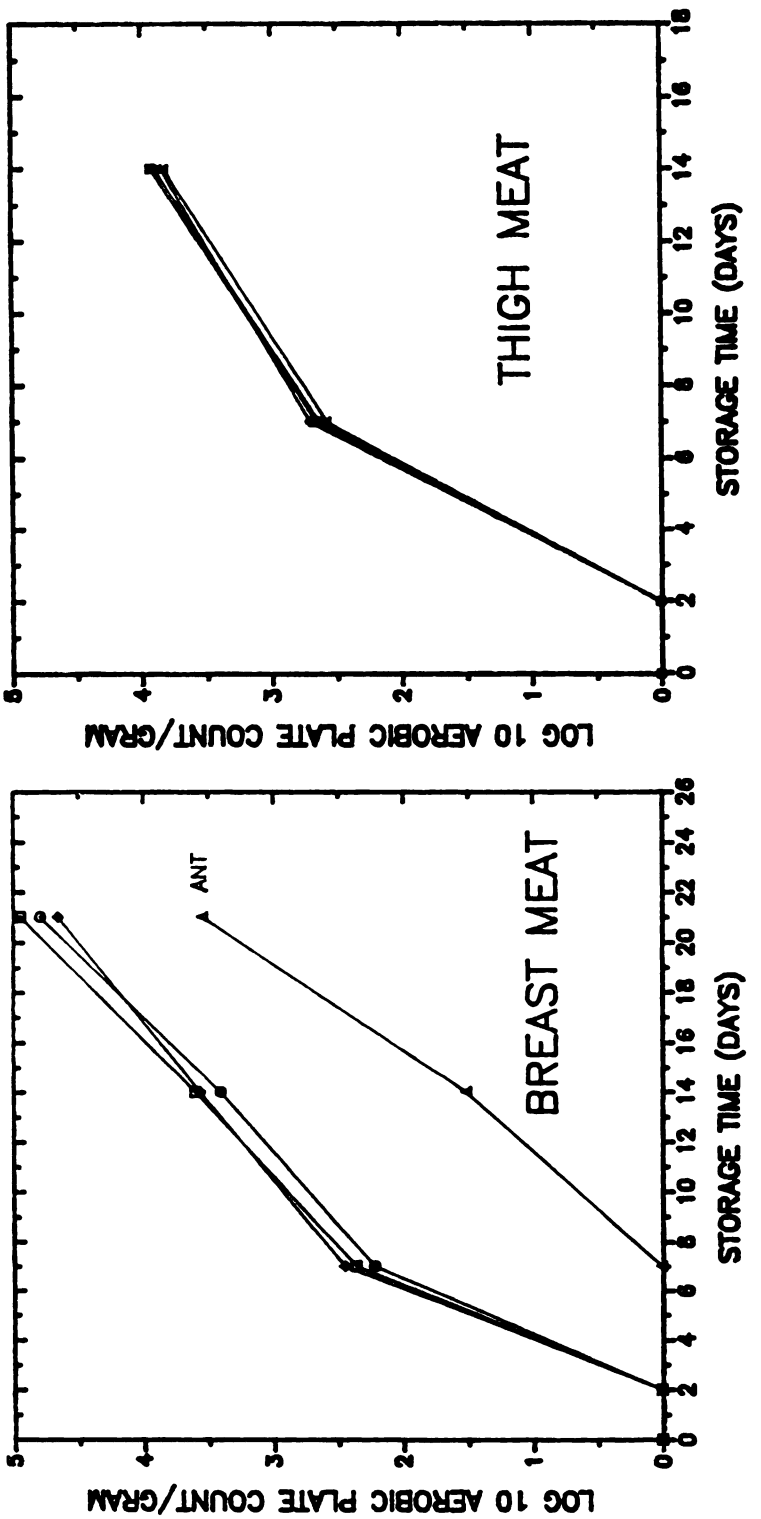



Figure 29. Effect of treatments and storage on aerobic plate count for cooked refrigerated turkey meat.

are capable of rapid growth at temperatures as low as 4 to 6 C.

The APC for the frozen meat samples demonstrated a consistent level during the storage time (Figure 30), but the initial increase detected after the first month of freezing is not due to microbial growth during the freezing, but is likely due to postprocessing contamination during the cutting and packaging.

Moerck and Ball (1979) investigated the influence of various microorganisms on the carbonyl compounds of chicken tissue. Broiler adipose and thigh muscle samples were inoculated with bacteria and yeasts, then stored for three days at 22 C or 7 days at 4 C. Hexane extracted carbonyl compounds were converted to their 2,4- dinitrophenylhydrozone derivatives. Pseudomonas fluorescence and Candida lipolytica produced acetone in both tissue samples. Rhodotorula aurantiaca produced acetone in ground thigh muscle. Pseudomonas fragi and Pseudomonas aeroginosa produced acetone and trace amounts of 2-butanone, 2-hexanone, and 2-octanone in all tissue samples.

Five Pseudomonas species, Achromobacter lipolytica and C. lipolytica decreased the concentration of at least one class of aldehyde in all samples and decreased peroxide level. Trichosporon pullulans and R. aurantiaca decreased

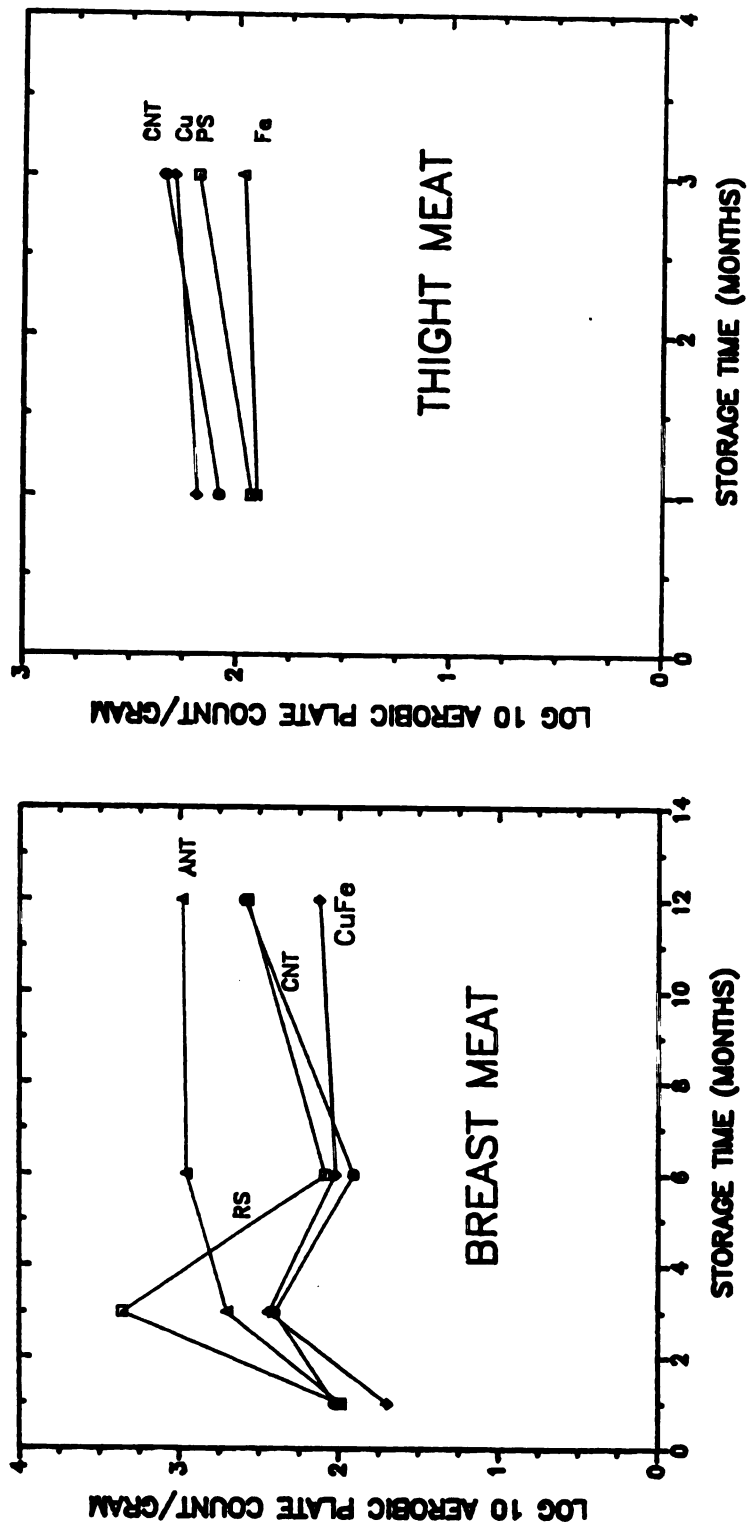



Figure 30. Effectof treatments and storageon aerobic plate count for cooked frozen turkey meat.

the concentration of aldehydes in samples stored for 3 days at 22 C. Dicarbonyl compounds were removed by R. aurantiaca and A. lipolytica. A Micrococcus sp. increased peroxides and aldehydes in stored samples and also rancid adipose tissues. They concluded that it was possible that these microorganisms decreased the carbonyl concentrations by disrupting the normal sequence of autoxidation. Hydroperoxides that were formed during autoxidation may have been decomposed to compounds other than carbonyls, or the formation of hydroperoxides may have been inhibited.

Since the flavor contribution of monocarbonyl compounds is concentration dependent, the microbially induced alterations of monocarbonyl and peroxide concentration could cause a wide range of flavor changes in stored poultry meat.

It is conceivable that manipulation of poultry microflora or their enzymes could bring about desirable changes in warmed-over flavor, but this type of approach needs extensive and thorough investigation.

## SUMMARY AND CONCLUSIONS

Three major experiments were conducted to study the effect of pure salt, rock salt source, an antibiotic, metal ions and antioxidants on the development of oxidative rancidity and warmed-over flavor during refrigerated, and frozen storage of raw and cooked turkey meat.

In the first phase an improved extraction TBA method was developed and correlated with organoleptic evaluation. This method was used throughout the study together with fatty acid profile, proportion of lipid fractions and levels of phospholipid classes for monitoring lipid oxidation in the meat system treatments.

In the second phase a preliminary experiment was designed to study the role of pure salt, rock salt, antibiotic and metal ions on the development of oxidation in a meat model system prepared from ground turkey breast muscle "pectoralis minor".

In the third phase turkey breast and thigh muscles were treated with selected metal ions, fine flake salt and two types of antioxidants in specified design.

The improved extraction TBA method was found to be fast, precise, easier to perform and as accurate as the distillation method. The correlation coefficient between

the two methods was high. When the cooked chicken meat was analyzed after 48 hours of refrigeration, the value of r was 0.93 and when analyzed 2 hours of cooking the value of r was 0.85. The relationship of the two methods with sensory scores was high, r = 0.85 for the extraction and 0.83 for the distillation.

Turkey thigh muscle is more susceptible to lipid oxidation than turkey breast muscle as measured by TBA test ($P<0.001$). The susceptibility of these tissues reached its maximum after seven and fourteen days of refrigeration at 4 C and three and six months of freezing at -25 C. However lipid oxidation developed more in the cooked than in the raw turkey meat ($P<0.001$). The increase in lipid oxidation as represented by TBA values was proportional to the storage time between 0 and 14 days of refrigeration. The TBA value of the cooked meat reached its maximum after 3 months of frozen storage while in the raw state it reached the maximum level after 6 months. The decline in the TBA values after it had reached the maximum level is due to the fact that malonaldehyde is not a final product of lipid oxidation and it is labile for furthe- decomposition with prolonged storage. Statistical analysis of TBA result indicated that the relative prooxidant effects ofdivalent cations and salts on turkey meat were in the following order: ferric ions > cupric ions > rock salt > fine flake

salt > control. However these treatments were found to have no detectable effect on lipid oxidation as monitored by changes in fatty acid profile, per se. while the proportion of phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) was significantly ($P<0.01$) decreased by the effect of iron and copper. The percent of nonheme iron of the total iron in turkey meat was found to be much higher than that reported in other species. This might be due to the lower myoglobin content of poultry meat and it could be one of the major factors which contribute to the lower stability of turkey meat to lipid oxidation, especially thigh meat which has about 3 times more total iron than the breast meat. Also copper content in thigh meat was found to be 3 times that in the breast. Rock salt had a significantlylarger prooxidant effect ($P<0.01$) than pure salts ($P<0.05$) which might be due to the metal impurities.

Tenox 6 was a more effective antioxidant in controlling the prooxidant effect of both iron and copper ($P<0.01$). The other antioxidant which contained vitamin E, ascorbyl palmitate and citric acid as the active ingredients produced no significant control on the prooxidant activity of iron and copper.

The proportion of phospholipids in total fat was higher in breast than in the thigh meat, but the total amount of phospholipids was higher in the thigh meat. There was a

decrease in the proportion of phospholipids with frozen storage and cooking. This decrease corresponds to the variation in the level of malonaldehyde as measured by TBA test. Also, the proportion of the phospholipid classes PE and PC was found to decrease after cooking and during frozen storage ($P<0.01$). However the PE was found to to be more susceptible to autoxidative degradation than PC, this is because the former is much more unsaturated. The loss in the unsaturation could be accounted for by the changes in 18:2, 20:2, 20:3, 20:4, 22:3 and 22:6 fatty acids. The proportion of these acids showed a relative decrease after cooking and after 12 months of frozen storage. But there were no significant changes for these acids throughout the shorter storage periods. These results lead to the conclusion that fatty acid profile changes are not ideal indicators for monitoring lipid oxidation for the stored poultry products. However, only minor changes have occurred in fatty acid profile of neutral lipids during frozen storage and cooking of turkey breast meat.

Dimethyl acetal of hexa- and octadecanal were identified and quantified in phospholipid fraction in raw and cooked turkey breast throughout 12 months frozen storage as well as in the fresh raw thigh meat. The neutral lipids contained traces of these compounds.

By aerobic plate count it was found that spoilage was detected in raw turkey breast meat refrigerated at 4 C after 21 days. The number of viable microorganisms was found to decrease with frozen storage ($P<0.01$).

The results of this study clearly show the sensitivity of the very small quantity of phospholipids in turkey muscle to oxidation and concomitant flavor change. The severe effects of free iron and copper ions along with the presence of salt was also demonstrated.

# APPENDICES

## APPENDICES

### Appendix A

Table 1. Chemical analysis of calcium magnesium free salt (CMF) and fine flake salt[1]

| Chemical | CMF Salt % | Fine Flake Salt % |
|---|---|---|
| NaCl [2] | 99.910 | 99.960 |
| $Na_2SO_4$ | 0.091 | none |
| $CaSO_4$ | 0.000 | 0.038 |
| $CaCl_2$ | 0.000 | 0.001 |
| $MgCl_2$ | 0.000 | 0.003 |
| Ca PPM | 3.000 | 115.000 |
| Mg PPM | 2.000 | 8.000 |
| Cu PPM | 0.090 | 0.080 |
| Fe PPM | 0.250 | 0.300 |

[1]Analyzed by Diamond Crystal Co. St. Clair, Michigan.

[2]On moisture free basis.

Not determined, but typical values are as follows: Ni, 0.08 PPM; Zn, 0.05 PPM; Pb none detected or less known 0.05 PPM.

Table 2. Particle size analysis of calcium magnesium free salt (CMF) and fine flake salt[1]

| On USS | CMF Salt | Fine Flake Salt |
|---|---|---|
| 20 | tr. | 0.0 |
| 30 | 10.0 | tr. |
| 40 | 45.0 | 0.5 |
| 50 | 38.0 | 16.5 |
| 70 | 5.5 | 39.0 |
| 80 | 0.0 | 15.0 |
| 100 | 0.0 | 14.5 |
| Pan | 1.5 | 14.5 |

[1]Analyzed by Diamond Crystal Salt Co. St. Clair, Michigan.

Table 3. Chemical analysis of rock salts for iron and copper content[1]

| Salt type | Iron (PPM) | Copper (PPM) |
|---|---|---|
| Northern rock salt (NRS) | 39.62 | 1.10 |
| Southern rock salt (SRS) | 35.23 | 1.27 |

[1]Analyzed in Department of Animal Science, Michigan State University.

**Appendix B**

Table 1. Triangle test questionnaire for cooked turkey meat.

---

QUESTIONNAIRE FOR TRIANGLE TEST

(Taste for Flavor only)

NAME :_____ DATE :_____

PRODUCT: Turkey Meat

Two of these three samples are identical, the third is different.

1. Please write the code number in the space indicated and check the odd sample.

| Code No. | Check Odd Sample |
|---|---|
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |

2. Indicate the degree of difference between the duplicate samples and the odd sample.

   Slight _____

   Moderate_____

   Much _____

   Extreme _____

3. Acceptability:

   Odd sample more acceptable _____

   Duplicates more acceptable _____

4. Comments:

Table 2. Cooked chicken rating score form.

CHICKEN WARMED-OVER FLAVOR

Name _____

Date _____

Directions: Taste the reference sample first to familiarize your self with fresh chicken flavor. Then taste the coded samples in the order listed below. Mark the degree of warmed-over flavor by placing a vertical line on the horizontal line corresponding to the three digit code.

Evaluate sample for warmed-over flavor only. Expectorate after tasting in the cup provided and rinse mouth with water between samples.

| CODE | NO WARMED-OVER FLAVOR | VERY STRONG WARMED-OVER FLAVOR |
|---|---|---|
| --- — - | I----------I----------I | |
| --- — - | I----------I----------I | |
| --- — - | I----------I----------I | |
| --- — - | I----------I----------I | |
| --- — - | I----------I----------I | |
| --- — - | I----------I----------I | |

## Appendix C

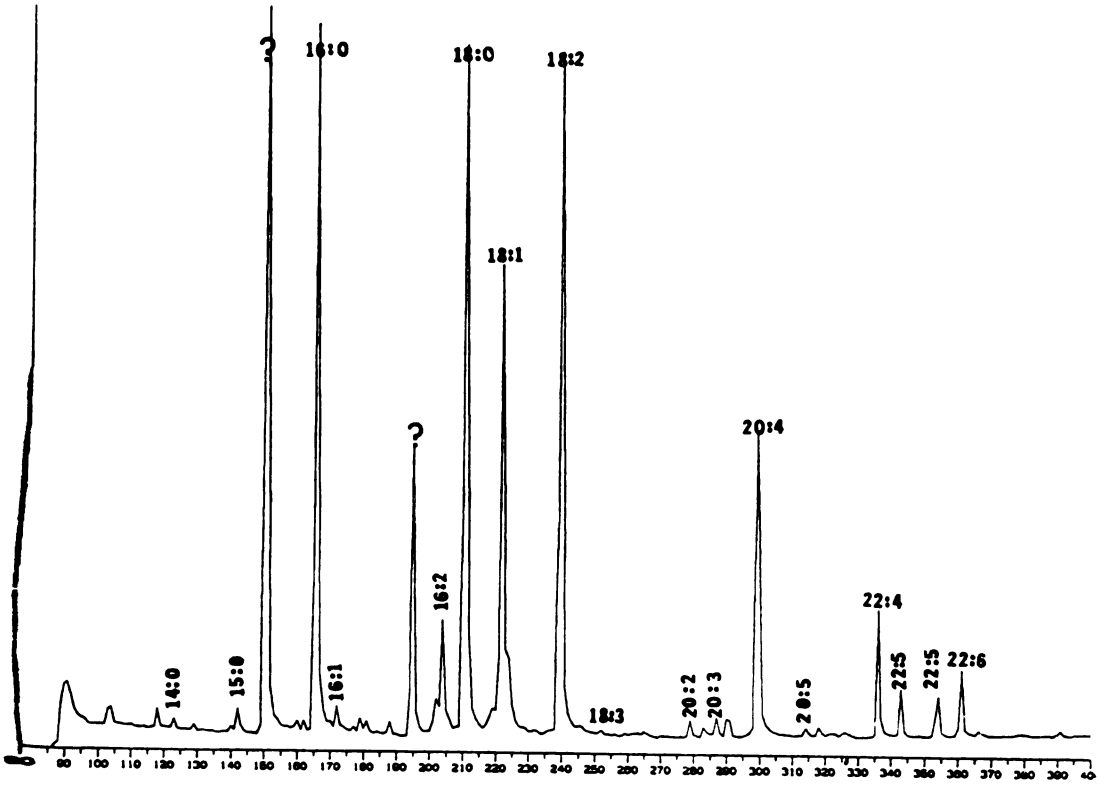



Figure 1. Gas chromatographic spectrum of turkey phospholipid fatty acids.

10% SP-2330 on 100/120 Supelcoport; 30 m x 0.24 mm i.d. capillary column; 0.2 film thickness; helium velocity = 35 cm/sec; column temp. set at 150 C for 5 min. then to 250 C at 10 C/min; inj. temp. = 250 C; det. temp = 300 C.

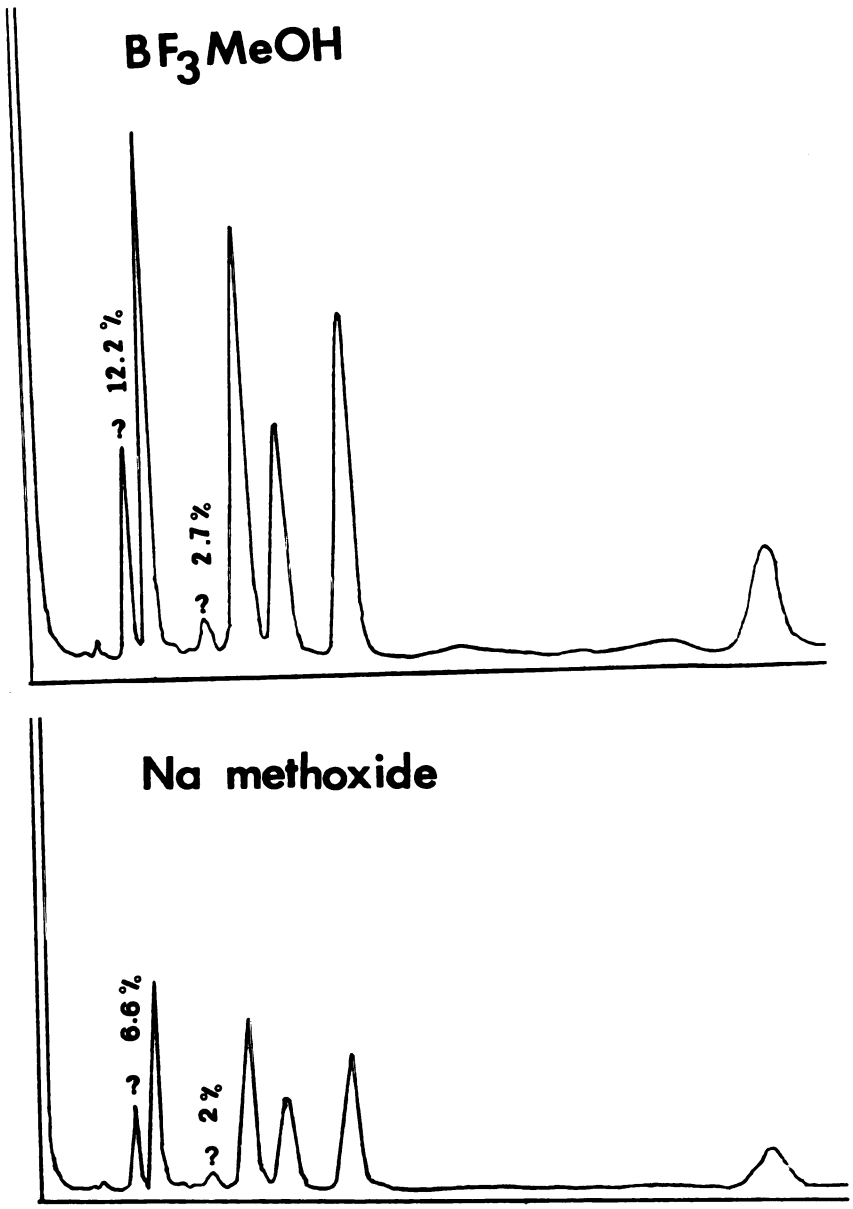



Figure 2. Gas chromatography of turkey phospholipid fatty acids by two methods of methylation.

10% SP-2330 on 100/120 Supelcoport; 2 m x 2 mm i.d. glass column; nitrogen flow rate = 20 ml/min; column temp. set at 150 C for 3 min. then to 220 C at 5 C/min; inj. temp. = 200 C; det. temp. = 300 C.

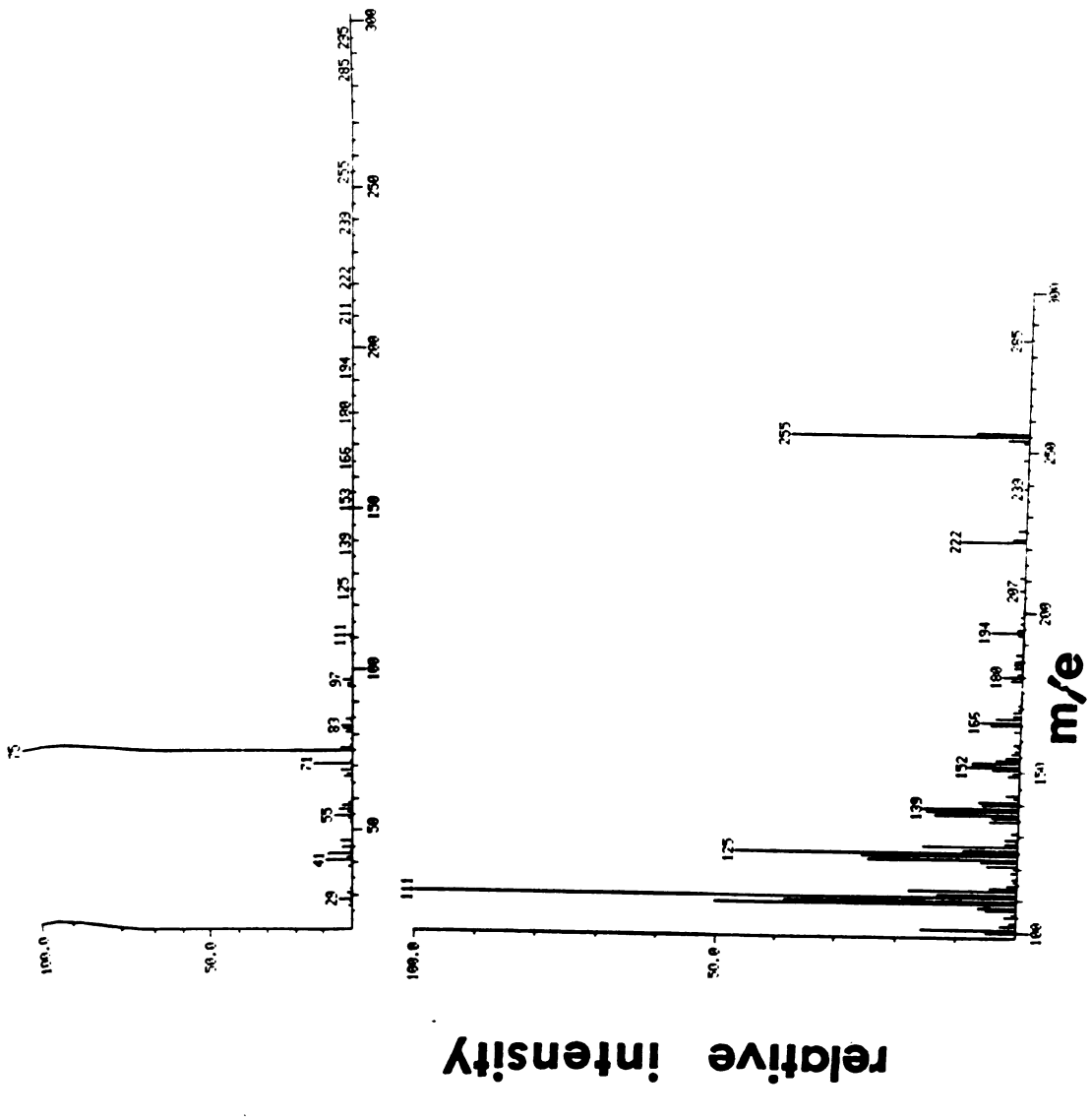



Figure 3. Mass spectrum of dimethyl acetal of hexadecanal.

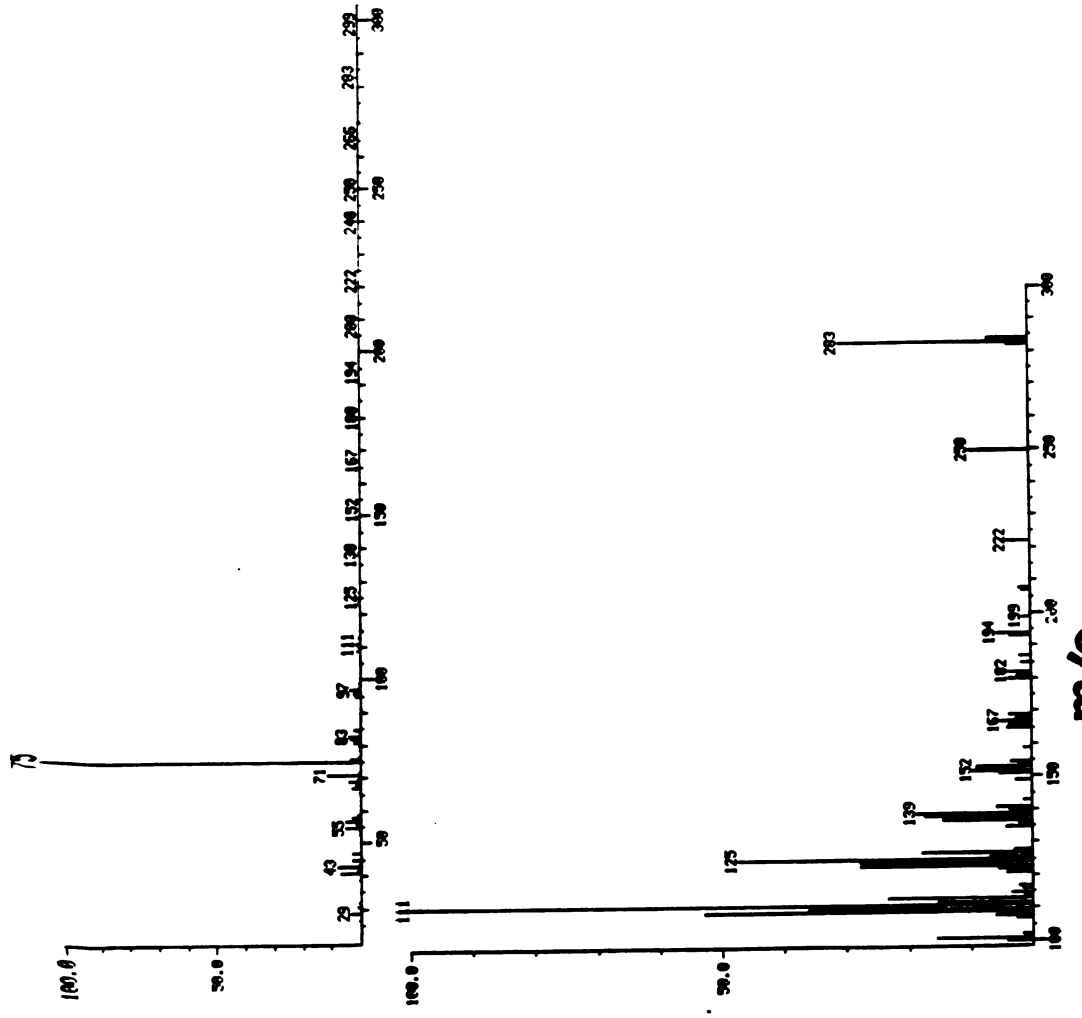
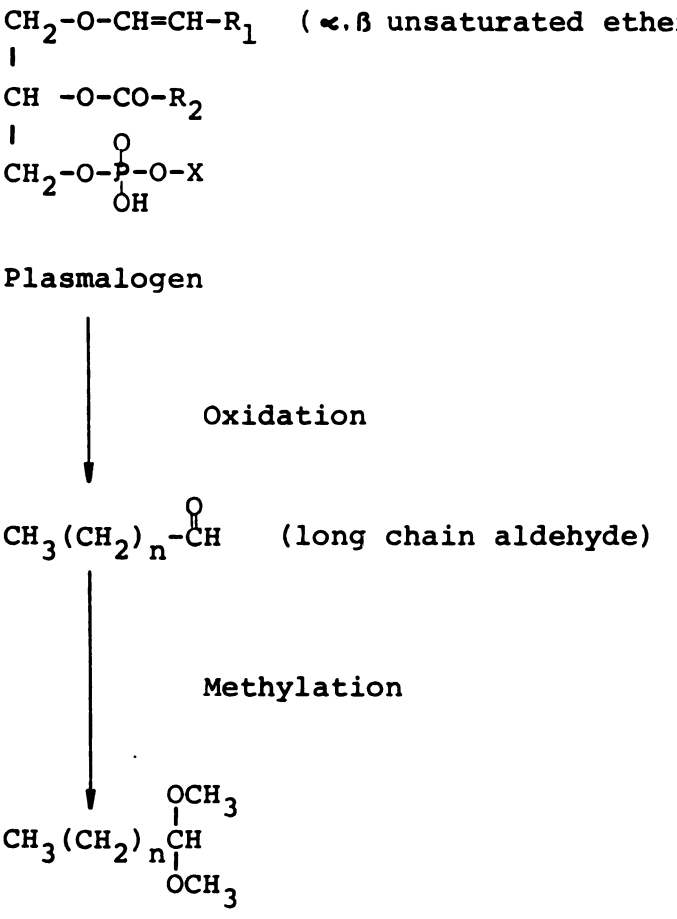



Figure 4. Mass spectrum of dimethyl acetal of octadecanal.

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$R_1$= Fatty aldehyde residue

$R_2$= Fatty acid residue

X = Choline, ethanolamine, or serine residue
(ethanolamine is the most common)

n = 14 in DMA of hexadecanal and 16 in DMA of octadecanal.

Figure 5. Formation of dimethyl acetals from plasmalogens.

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