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COMPOSITION AND STABILITY OF LIPIDS FROM MECHANICALLY PROCESSED POULTRY MEATS (MPPM)

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ABSTRACT

COMPOSITION AND STABILITY OF LIPIDS FROM MECHANICALLY PROCESSED POULTRY MEATS (MPPM)

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Lipids from light and dark mechanically processed chicken and turkey meats (MPCM and MPTM), light and dark hand deboned chicken and turkey meat (HDCM and HDTM), their corresponding bone residues and skin tissues were analyzed for total cholesterol contents, phospholipid contents (total and fractions) and fatty acid distribution profiles.

Small differences were found among fatty acid components of neutral lipids from various tissue samples while phospholipid fatty acid components of MPCM and MPTM resembled more the fatty acids of their corresponding bone or hand deboned meat phospholipids than skin phospholipids. Quantitation and classification of phospholipids indicated that the total phospholipid content and quantity of each phospholipid class in MPPM were most similar to those found in their bone tissues. Total cholesterol contents revealed that cholesterol content of MPPM lipids more closely resemble cholesterol content of skin lipids or bone lipids than muscle lipids. From quantities of each component found in their composite tissues, models for MPCM and MPTM lipids were set up. Based on fat contributed by each tissue, a value of 1:3:6 meat fat: bone fat: skin fat was suggested as a combination for MPCM fat. A model of 1:4:5 meat fat: bone fat: skin fat was hypothesized as a MPTM lipid model

Pantipar P. Jantawat

Storage stability of mechanically processed poultry meats was evaluated in two different studies. The first study involved the effect of inert gases vacuum packed and prefreezing hold time on storage stability of MPPM. MPCM and MPTM were packed along with either N_2 or CO_2 or under vacuum, and frozen at -18°C either immediately after packing or after 72 hrs. holding at 4°C. All treated samples were stored at -18°C up to 4 months. Samples were evaluated for oxidative and hydrolytic rancidity by following the decrease in polyunsaturated fatty acid (C 18:3-22:6), the development of TBA reactive substances and changes in total phospholipids.

Vacuum and N_2 packed samples from every treatment gave significantly lower TBA numbers and higher unsaturation ratios than CO_2 packed samples of corresponding treatments. Vacuum packaging was comparable to N_2 packaging with respect to the development of TBA reactive substances found in most treatments. Advantages for vacuum packaging over N_2 and CO_2 packaging were observed in phospholipid losses found in MPTM samples but these advantages were not significant in MPCM samples. Significantly higher losses of polyunsaturated fatty acids, total phospholipids and increases in TBA numbers were found in samples which were held 72 hrs. at 4°C before freezing.

The second part of the stability study involved the effect of air at various tension levels on storage stability of MPPM and their lipid extract samples. Air pressures equivalent to 0, 5, 15 and 30 in. of Hg. were assigned to MPCM, MPTM and their lipid extract samples. All treated samples were stored up to 3 months at -18°C. Changes in phospholipid unsaturation ratios (C 18:3-22:6 / C 16:0), 2-thiobarbituric acid tests and losses in total phospholipids were

used to follow lipid oxidation reactions. MPCM samples packed at 5 in. of air were found to be comparable to vacuum packed samples with respect to the development of TBA reactive substances and loss of polyunsaturated fatty acids. For MPTM, TBA absorption values of samples packed under vacuum were significantly lower than those found in samples packed at 5, 15, and 30 in. of air after the first month of storage. Significant differences in mean unsaturation ratios were observed between MPCM and MPCM lipid extract samples, but these results were not observed between MPTM and MPTM lipid extract samples. Significant differences between these two types of stored samples within the same meat species however, were found in their TBA absorption values and total phospholipid contents.

To

My parents and my husband

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INTRODUCTION

In recent years, mechanically deboning processes have been used extensively to remove poultry meats from bones. This deboning process has been found to enhance the utilization of poultry meat sources. Chicken necks and backs or frames, turkey racks and spent laying hens which were of low market value, are now being mechanically deboned, and used in emulsified and other processed food products. However, it has been found that mechanical deboning alters the lipid and protein composition of the meat. These changes result in flavor instability and formation of some undesirable functional characteristics of the meat.

The nature, proportion and unsaturation degree of unsaturated fatty acids present in a lipid system or food will indicate the approximate susceptibility of that product toward oxidative deterioration. Generally speaking, the higher the proportion and degree of unsaturation of the fatty acids the more labile the lipid system is to oxidation. The mechanical deboning process was reported to incorporate heme and lipid components from bone and skin into the resulting meat (Froning 1970). Therefore lipids from these sources might also affect flavor quality of the meats and also be responsible for stability problems found in subsequent utilization and storage of the meats.

Cholesterol,which has been identified as a risk factor in

occurrence of premature coronary heart desease has been found to be higher in turkey frankfurters made from mechanically processed meat than those made from the hand processed meat. Thus, the availability of detailed lipid compositional data for both the meat and its components may help to elucidate the source or sources of this sterol and possibly, the way to minimize it.

Many attempts have been made to slow down or prevent oxidative rancidity in mechanically processed meat. In freezing preservation of ground fresh meat, the main consideration in packaging the meat is the exclusion of oxygen. The mechanism of lipid oxidation in fresh meat is complicated due to the presence of heme pigments which are generally accepted as biocatalysts for the meat lipid oxidation. Neil and Hasting (1925) demonstrated rapid conversion of hemoglobin to methemoglobin at intermediate rather than at very high or very low oxygen tension. According to Tarladgis (1961), iron in metmyoglobin or hemoglobin was highly effective in initiating the chain reaction mechanism in the lipid autoxidation process.

Storage of meat in an atmosphere containing inert gases has been found to retard bacterial action as well as oxidative changes. Various fresh meats were found to remain palatable during frozen storage much longer when packed in nitrogen or vacuum packed. However, while lowered oxygen tension accelerates oxidation of hemoproteins to their oxidized forms, lowering of the pH was also found to further accelerate this reaction. Some inert gases such as CO_2 in the presence of water in the meat tissue, might cause lowering of the meat pH. Thus, the study of behavior of oxygen at various levels as well as the comparison of benefits or disadvantages of

some different storage atmospheres might be helpful in improving the meat preservation.

Specific objectives of this study were:

1. To study the composition of mechanically processed poultry meat lipids in comparison with those of the hand deboned meats, skin and their corresponding bone residues.
2. To compare the effect of oxygen at different levels on storage stability of mechanically processed poultry meats.
3. To compare storage stability of mechanically processed meat packaged in the presence of some inert gases with that vacuum packaged.
4. To study the effect of prefreezing hold time on subsequent storage stability of mechanically processed poultry meats.
5. To study and compare the storage stability of meat lipids as they naturally occur, with extracted meat lipids which are freed from other components.

LITERATURE REVIEW

Composition of Meat Lipids

Meat lipids are classified according to their sites of distribution and composition into two major groups. Intermuscular fat is stored as large deposits in adipose tissue or under the skin. This fraction was found to compose mainly of triglycerides. Another group was found intramuscularly. These muscle tissue lipids are an integral part of such cellular structures as muscle cell wall, mitochondria and microsomes. They are separated from the depot fat, are highly unsaturated and many of them are combined with protein (Watts, 1961; Love, 1972; Pearson et al., 1977).

Terroine (1920) classified structural fats as "element constant" while the depot fats were named "element variable." This classification was proposed to differentiate the fact that the element variable can either be drawn up to furnish energy for body processes or deposited when there is an ample supply of food and less energy is required. The constant element on the other hand, remains relatively stable in quantity, to preserve the essential structure of the body.

Composition of Poultry Lipids

Hand Deboned Poultry Tissues

Certain fats, such as those of fish, poultry and pork have been reported to be much more easily oxidized than those from other animals such as beef and lamb (Watts, 1954). These differences are

largely attributed to the total lipid content, phospholipid content and fatty acid compositions of each species. Besides, variations among tissues of the same animal also exist. White chicken meat, the lowest in total lipids, was found to contain almost equal amounts of neutral and phospholipids. Dark meat contained about twice as much of the total lipids. However, lipid of dark meat has been found to have only about half as much of the phospholipid content, when compared to that found in the white meat. Skin and depot tissue fats were reported to contain low quantities of phospholipids (Katz et al., 1966; Acosta et al., 1966; Lee and Dawson, 1973). Moerck and Ball (1973) reported that chicken bone marrow contained a slightly higher percentage of phospholipids than most other tissues.

Although variations were found in phospholipid content of different tissues, it was pointed out that the components of phospholipids, expressed as a percentage of total phospholipids, are somewhat similar in most animal tissues (Pearson et al., 1977). Phosphatidyl choline and phosphatidyl ethanolamine were found to be the predominant components of poultry tissue phospholipids. The lesser components found were sphingomyelin, phosphatidyl serine, phosphatidyl inositol and lysophosphatidyl choline (Peng and Dugan, 1965; Davidkova and Khan, 1967; Wangen et al., 1971 and Lee and Dawson, 1973).

Fatty acid composition of neutral and phospholipids from poultry tissues have been studied by various workers (Peng and Dugan, 1965; Machlin et al., 1962; Katz et al., 1966; Marion et al., 1967 and Lee and Dawson, 1973). Predominant fatty acids of the neutral lipids were palmitic acid, stearic acid, oleic acid and linoleic acid. These fatty acids account for approximately 95% of the total fatty

acids of the neutral lipids. Relative quantities of these major fatty acids were shown to be similar among various types of tissue. Predominant fatty acids of phospholipids were palmitic acid, stearic acid, oleic acid, linoleic acid and arachidonic acid. These fatty acids have been found to comprise approximately 75% of the total fatty acids found in muscle phospholipids. However, the concentration and kind of fatty acids in phospholipids varied among different tissues, and arachidonic acid attributed most to these differences, the quantities of this C 20:4 fatty acid was lower in skin and depot phospholipids than in muscle phospholipids.

Difference in fatty acid composition in total lipid of chicken and turkey skins was reported by Miller et al. (1962). Their studies revealed that the largest difference in fatty acid content between turkey and chicken skin was observed in the eighteen carbon polyunsaturated fatty acids. Turkey skin contained approximately 60% more linoleic and 50% less linolenic acid than did chicken skin.

Very few published data are available on the fatty acids of poultry bone marrow. Seigel and Latimer (1971) stated that chicken tibia bone marrow contains high levels of unsaturated fatty acids. Moerck and Ball (1973) found that approximately 91% of fatty acids in triglyceride fraction of chicken bone marrow lipid were comprised of stearic acid, oleic acid, linoleic acid and palmitic acid, while high levels of polyunsaturated, 20 to 24 carbon fatty acids, were found in the phospholipid fraction.

Mechanically Deboned Poultry Meats

Compositions of mechanically deboned poultry meat have been subjected to extensive studies by various investigators (Goodwin et al., 1968; Froning, 1970; Satterlee et al., 1971; Dimick et al., 1972; Grunden et al., 1972; Froning and Johnson, 1973 and McMahon and Dawson, 1976). Mechanically deboned poultry meat was found to have higher lipid content than the hand deboned meat. The lipid component from bone marrow and skin was claimed to account for the large increase in fat content of mechanically deboned poultry meat. Moerck and Ball (1974) studied lipid oxidation in mechanically deboned chicken meat. They reported that about 1.4% of the total lipid in mechanically deboned chicken meat was phospholipids, while neutral lipids comprised approximately 98.6%. The predominant fatty acids of the triglyceride fraction were palmitic, stearic, oleic and linoleic acids. The phospholipid fraction contained higher levels of 18-carbon saturated and 20:3 to 22:6 carbon polyunsaturated fatty acids than did the triglyceride fraction.

Cholesterol Content of Poultry Tissues

A comprehensive review of available data for cholesterol content of food, including methods for its determination, was recently prepared by Sweeney and Weihrauch (1976).

Mickelberry et al. (1964) demonstrated that dietary fat influenced moisture, fat, cholesterol content and iodine value of both cooked and uncooked broiler tissue. Further investigation by this group of researchers in 1966 indicated that most of the cholesterol in chicken occurred as free cholesterol and that only a small

fraction (2-10%) existed in the form of esters.

For total cholesterol content of raw chicken tissues, the following values were reported in mg per 100 g of the wet tissue: white meat 57-67; dark meat 82-148; skin 109-472. For cooked tissue, the values were: white meat 82-84; dark meat 91-96 and skin 91 mg per 100 g of wet tissue (Mickelberry et al., 1966; Marion and Woodroof, 1965 and Feeley et al., 1972). Nockles (1973) reported the total cholesterol content of certain tissues of hen in mg per 100 g of dry tissues. He obtained the following values: dry thigh muscle 161; dry liver 1040; and dry adipose tissue, 201. Kritchevsky and Tepper (1961) reported 110 mg per 100 g for total cholesterol content in skinless turkey meat. Neudoerffer and Lea (1968) studied the effects of dietary fat on the amount and proportions of the individual lipids in turkey muscles. They found a total cholesterol content of 84 mg per 100 g meat and 116 mg per 100 g meat in breast and dark turkey meat respectively. Hartung et al. (1973) determined total cholesterol content of cooked commercial broad breasted turkey meat. They obtained 68.3 to 94.2 mg per 100 g for roasted white turkey meat and 73.8 - 130 mg per 100 g for roasted dark turkey meat. Standal et al. (1970) found a value of 111 mg per 100 g meat of total cholesterol in smoked turkey. Moerck and Ball (1973), separated chicken bone marrow lipid by using Unisil column chromatography. They reported the free cholesterol content of femur, tibia and ilium-ischium bone to be 1.4, 1.3 and 1.3% of total lipid content respectively. The values for cholesterol esters for these bones were: 0.3% for femur bone; 0.2% for tibia bone and 0.2% for ilium ischium bone.

Only one report was found containing data for cholesterol content in mechanically deboned poultry meat. Values of 2.5 g cholesterol and 0.5 g cholesterol esters per 100 g of total lipids were found in mechanically deboned chicken meat by Moerck and Ball (1974).

Meat Lipid Oxidation

Lipid Autoxidation

Mechanism of lipid autoxidation has been reviewed by many authors including: Dugan, 1961; Lundberg, 1962; Shultz et al., 1962; Labuza, 1971; Sherwin, 1972 and Sato and Herring, 1973. The generally accepted autoxidative mechanisms involve a three step series of reactions, as shown in the following scheme (Dugan, 1971):

Initiation:

$$RH + \text{initiator} \rightarrow \text{free radical } R^{\circ}$$

Propagation:

$$R^{\circ} + O_2 \rightarrow ROO^{\circ}$$

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

Termination:

$$R^{\circ} + R^{\circ} \rightarrow \text{inert products}$$

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Initiation step involves the formation of a free radical species. Under sufficient energetic applications, with certain kinds of energy such as light, heat, enzyme, metal and some radiation particles, the labile hydrogen atoms at the double bonds of unsaturated fatty acids can be abstracted from their sites.

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free radical formed with molecular oxygen to form a peroxy radical (ROO°). A new free radical is then formed as a result of the reaction between the peroxy radical and a nonoxidized unsaturated fatty molecule (RH).

Formation of non free radical products was considered to be the termination of the chain reaction.

Oxidation of Tissue Lipids

Tissue lipid oxidation has been found to contribute markedly to the undesirable flavor changes which occur in stored meat. A significant quantity of phospholipids have been reported in muscle lipids (Katz et al., 1966; Peng, 1965 and Acosta et al., 1966). Because of their contents of unsaturated fatty acids, these phospholipids were found to be very susceptible to oxidative rancidity (Watts, 1954; Younathan & Watts, 1959; El-Gharbawi and Dugan, 1965; Love and Pearson, 1971 and Lee and Dawson, 1973). These protein bound lipids have been found to autoxidize much more readily than the free glyceride fat. According to Chipault and Hawkins (1971), autoxidation of meat lipid occurs in two stages. The protein bound lipids oxidize first without an induction period, their initial rate of oxidation then decreases as time progresses. After a period of lower oxygen absorption, the free glyceride fats begin to autoxidize in the autocatalytic manner characteristic of autoxidation in isolated glyceride fat.

Meat Lipid Prooxidants

Heme pigments have been generally recognized as one of the most important prooxidants for meat lipid oxidation (Watts and Peng,

1947; Zipser and Watts, 1961; Love and Pearson, 1971 and Lee et al., 1975). Banks (1944) and Tappel (1953) proposed that preformed linoleate peroxide was necessary for hematin catalysis. According to Tappel (1962), hematin catalysis involves the formation of lipid-peroxide-hematin compounds, and their subsequent decomposition into free radicals could have a propagation effect on the chain reaction step in the autoxidation process mechanism. Tarladgis (1961) hypothesized the mechanism of heme catalyzed lipid oxidation. He postulated that iron in metmyoglobin was a paramagnetic substance which was in a high spin state. This highly energetic iron could cause the formation of the first free radical of the chain reaction in the autoxidation process.

The ratio of hemoprotein to unsaturated fatty acids in muscle tissue has been reported to have a certain influence on the extent of the catalytic activity of the pigments. Kendrick and Watts (1969) studied the acceleration and inhibition of lipid oxidation by heme compounds. They summarized that, for a maximum catalytic activity, the ratio of quantity of the pigments and unsaturated fatty acid of the system must exist at an optimum value. According to Hirano and Olcott (1971), the rate of oxidation of linoleate solutions was catalyzed by low concentrations of heme and heme-proteins and inhibited by higher concentrations. Lee et al. (1975) found that the ratio of relative concentrations of polyunsaturated fatty acid to hemoprotein in mechanically deboned chicken meat was in the range where heme catalized oxidation would occur at near the maximum rate. He thus pointed out that this could be one reason why this deboned meat is very susceptible to oxidative rancidity spoilage during

utilization and storage.

Non heme iron has also been reported to have an important role in accelerating the oxidation of muscle lipids. Heavy metals (Cu, Fe, Ni, etc.) have been reported to increase the rate of oxidative deterioration in food lipids (Smith and Dunkley, 1962; Ingold, 1962). Wills (1966) pointed out that the peroxidation of fat in tissue mitochondria and microsomal fractions was easily induced by iron. Other investigators also reported similar results (Liu and Watts, 1970; Love, 1972; Uri, 1961 and Heaton and Uri, 1961).

Other factors affecting lipid oxidation in meat are light, temperature and salts (Watts, 1954 and Dugan, 1961). Sherwin (1968) mentioned enzymes, moisture, heat, light, other oxidized fats and acids as prooxidants of tissue and other lipid oxidative rancidity.

Stability of Poultry Meats

Storage Stability of Ground Poultry Meats

Sato and Hegarty (1971) stated that any process that causes a disruption in the muscle membrane system could cause exposure of lipid components to oxygen and other prooxidant substances. As a result, an acceleration in the development of lipid oxidation could easily occur. Marion and Forsythe (1964) studied oxidation of lipids in raw ground turkey tissue held at 4°C for 1 week, and reported a rapid increase in TBA numbers in both white and dark meat. Keskinel et al. (1964) found that a raw ground turkey meat which was held at 5°C up to three weeks showed a rapid increase in TBA numbers. Dhillon and Maurer (1975b) reported high initial TBA numbers for ground hand deboned turkey meat. Dawson and Schierholz (1976) studied the

development of lipid oxidation in turkey meat products which were either roasted whole or boned, ground and broiled as patties. They found that TBA numbers were highest from ground cooked patties held 7 days at 3°C, followed respectively by ground raw patties held 7 days, freshly roasted meat and freshly ground patties. They then concluded that stability of turkey meat was influenced by cooking, grinding and storage, and the combination resulted in maximum lipid oxidation. Dawson et al. (1975) further reported that the high TBA numbers found in turkey patties prepared from ground thigh meat and held at 3°C up to 10 days, could have been effectively controlled by treating with a commercial antioxidant mixture, containing butylated hydroxyanisole, propyl gallate and citric acid.

Storage Stability of Mechanically Deboned Poultry Meats

Composition and storage stability of mechanically deboned poultry meat have been the subject of numerous studies since 1970. Froning (1976) in his recent review on composition, functional property and stability of mechanically deboned poultry meat stated that the mechanical deboning process may cause considerable cellular disruption, protein denaturation and increase lipid and heme oxidation in the resulting meat. Besides, oxygen could oftentimes be mixed into the deboned meat during the extrusion process.

Various workers have found that the machine deboning process incorporates lipid from skin and bone into the resulting meat. Thus, lower protein and higher fat contents than that found in the hand deboned meat have been observed in various sources of mechanically deboned poultry meat (Goodwin et al., 1968; Froning, 1970; Froning

et al., 1971; Satterlee et al., 1971; Dimick et al., 1972; Grunden et al., 1972; Froning and Johnson, 1973 and McMahon and Dawson, 1976).

Besides lipids, workers have reported substantial quantities of heme components in mechanically deboned poultry meat. Froning and Johnson (1973) reported a threefold increase in the total heme pigments in mechanically deboned fowl meat as compared to those of the hand deboned meat from the same sources. Cunningham and Mugler (1973) and Lee et al. (1975) reported similar results.

Considering all of the mentioned factors, it's obvious that lipid oxidation must be considered as one of the most serious problems involved in storage and utilization of mechanically deboned poultry meat.

Maxon and Marion (1970) reported that both lipid oxidation and hydrolytic deterioration occurred in lipids of mechanically deboned turkey meat. They found that besides a linear response of TBA number with storage time, total phospholipids decreased during the 7 days storage at 4°C and in the freezer at -20°C. Schnell et al. (1971) demonstrated that particle size of mechanically deboned chicken influenced its TBA numbers. According to these workers, smaller particle sizes resulted in larger TBA numbers. Dimick et al. (1972) used carbonyl contents and organoleptic tests to evaluate the quality of mechanically deboned poultry meat. They reported that the quality of mechanically deboned poultry meat could be maintained up to 6 days at 3°C. They also observed that no differences in storage stability were noted where poultry parts were deboned immediately or held 5 days at 3-4°C prior to the deboning. Johnson et al. (1974), however, noted a significant flavor loss in mechanically deboned

turkey meat after 12-14 weeks of frozen storage. Dhillon and Maurer (1975a) studied storage stability of comminuted meat including mechanically deboned chicken meat, centrifuged mechanically deboned chicken meat, hand deboned chicken meat, mechanically deboned turkey meat, centrifuged mechanically deboned turkey meat and ground beef, at -25°C for 6 months. They observed that highest initial TBA numbers were found in mechanically deboned chicken meat, mechanically deboned turkey meat and centrifuged mechanically deboned turkey meat. Of all samples studied, they found mechanically deboned chicken meat to be affected the most by frozen storage.

Storage stability of products containing mechanically deboned poultry meat was also studied by many researchers. Froning et al. (1971) incorporated 15% mechanically deboned turkey meat either fresh or after 90 days frozen storage into red meat frankfurters. They reported that the TBA number indicated that frankfurters containing 15% fresh mechanically deboned turkey meat were comparable to all red meat franks in flavor stability. However, after 90 days frozen storage mechanically deboned turkey meat products were significantly inferior. Cunningham and Mugler (1973) reported that the excellent qualities of mechanically deboned chicken meat weiners could be maintained through 6 months storage at -30°C. Dhillon and Maurer (1975a) formulated summer sausages with 50% mechanically deboned chicken meat and 50% ground beef, 50% mechanically deboned turkey meat and 50% ground beef and 100% ground beef (control). After storage for 6 months at -25°C, quality measurements by TBA numbers and sensory evaluations indicated that there was only a slight decline in quality. Summer sausages containing mechanically deboned chicken

meat showed the greatest quality loss. However, the products were well accepted and there was no comment on any flavor differences. Uebersax et al. (1978a) used various concentrations of mechanically deboned turkey meat in turkey meat loaves. They reported an increase in TBA numbers with increase of mechanically deboned turkey meat substitution for both raw and precooked foil wrapped or vacuum sealed loaves.

Mechanism of lipid oxidation and interaction of heme and lipid components in mechanically deboned meat have also been studied. Moerck and Ball (1974) reported that considerable autoxidation deterioration occurred in the highly unsaturated phospholipid fatty acids of mechanically deboned chicken meat, whereas the triglyceride fraction failed to exhibit an apparent oxidation after the meat was stored at 4°C for 15 days. They also found a high correlation between the phospholipid fatty acid oxidation and TBA numbers of the meat. Lee et al. (1975) destroyed hemoprotiens in a mechanically deboned chicken meat homogenate by treatment with H_2O_2. They reported that the catalytic function was decreased to less than 10% of the original activity. They thus concluded that hemoproteins were the predominant biocatalyst of lipid oxidation in mechanically deboned chicken meat.

Various efforts have been made to alleviate the stability problems found in mechanically deboned poultry meats. Froning and Johnson (1973) centrifuged mechanically deboned fowl meat at 20,000 rpm for 15 minutes in a refrigerated Sorval centrifuge (5°C). They reported that centrifugation significantly increased the protein content and significantly decreased the fat content of the end products.

Centrifugation in addition, significantly reduced changes in TBA numbers of mechanically deboned fowl meats.

Various additives have been utilized to maintain storage stability of mechanically deboned poultry meat. Butylated hydroxy anisole, commercial mixture of antioxidants containing 20% butylated hydroxyanisole, 6% propyl gallate, and 4% citric acid in propylene glycol and food grade phosphates have been found to be effective in maintaining flavor stability of mechanically deboned poultry meat (MacNeil et al., 1973; Moerck and Ball, 1974 and Froning, 1973).

MacNeil et al. (1973) found a rosemary spice extract to be effective in maintaining lower TBA numbers in simulated mechanically deboned poultry meat, after the meat was stored at 3°C for 11 days. Uebersax et al. (1978b) reported that invivo tocopherol supplementation through diet and subcutaneous injections were effective in lowering TBA numbers for mechanically deboned turkey meat and mechanically deboned turkey meat substituted loaves.

Effects of Diets on Poultry Fat Composition and Stability

The lipid contents of the tissue greatly reflect the rearing conditions and diet of the birds. Likewise, the fatty acid content of poultry reflects the fatty acid content of dietary fat (Marion and Woodroof, 1963; Isaacks et al., 1964; Neudoerffer and Lea, 1968; Edward et al., 1973; and Porter and Britton, 1974).

Kummerow et al. (1948) studied the effect of variations in diet on the characteristics of fat extracted from 18 different groups of immature turkeys. They observed that when compared with control birds, the fat extracted from birds which had been supplemented with

linseed oil was least stable, and those from birds which had been supplemented with choline chloride or ethanolamine hydrochloride was most stable.

Klose et al. (1952) reported that turkeys on low fat diet was roughly estimated to contain 28% total saturated fatty acids and 68% octadecenoic acids. With further studies on quality and stability of turkey as a function of diet, Klose et al. (1953) postulated that the tendency of fat to deteriorate in frozen storage may be predicted in part from either the induction period or fatty acid composition of the carcass fat.

Marion and Woodroof (1963, 1966) studied the effects of diet on fatty acid composition and stability of chicken broiler carcasses. They reported that the fatty acid composition of breast, thigh and skin responded to the dietary fats by tending to assume the fatty acid composition of the fat in the diet. Feeding of different fats resulted in an increased carcass deposition of the major fatty acids present in each fat. Regarding stability, diets containing coconut oil or beef tallow produced carcasses with lower TBA numbers than diets containing high protein or menhaden oil. Marion et al. (1967), in a study on the effects of dietary fat and protein on lipid composition and oxidation in chicken meat, obtained good correlation coefficients between TBA numbers and level of each lipid component.

Bartov and Bornstein (1977) determined the effect of graded increments of α-tocopherol acetate, in diets containing various fat supplements at different concentrations on stability of abdominal fat and meat of broilers. They observed that diets containing α-tocopherol acetate significantly improved stability of abdominal fat

and meat in broilers having relatively saturated carcass fat, whereas its beneficial effect was rather limited as the degree of unsaturation of carcass fat increased.

Porter and Britton (1974) observed that feeding chickens with full-fat soybeans resulted in changes of fatty acid composition of their fat. The fat was found to be soft, oily and more susceptible to oxidative rancidity.

Effects of Species on Composition and Stability of Poultry Fats

No significant variation in major components and fatty acid composition of poultry fat were reported among fat samples from different species of poultry (Mecchi et al., 1952; Miller et al., 1962; Marion and Woodroof, 1965; Marion et al., 1970 and Wangen et al., 1971). Pereira (1975), studied fatty acid composition of processed fat from chicken, turkey and duck, and reported that differences in fatty acid composition due to species difference did exist, but that these differences were expected to be less marked than differences imparted by dietary fat changes within the same species.

Stability variations among species of poultry fat, however, have been reported by many investigators. In the field of poultry products, turkeys are known to be more susceptible to rancidification than chickens, and, due to seasonal production, are generally stored for longer time periods (Klose et al., 1952). Nutter et al. (1943), studied the chemical composition of depot fats in chickens and turkeys, and reported that turkey fat has a greater tendency toward rancidification than chicken fat. They attributed this phenomenon to the lower content of natural antioxidant in turkey fat. Criddle

and Morgan (1951) reported results in which turkeys on zero and low tocopherol diets were judged rancid after 3 months of frozen storage and those receiving high tocopherol diet did not become rancid until after 9 months storage. Mecchi et al. (1952) studied the lipid composition of chicken and turkey carcass fat in an attempt to correlate composition with rancidity development. They concluded that fatty acid composition differences in chicken and turkey fats were not sufficient to explain the greater tendency of turkey fat toward rancidification. Baker (1958) compared rate of rancidity development in chicken, duck and turkey fats. They reported that both hydrolytic and oxidative rancidity were greater for turkey and duck fats and lowest for chicken fat. Mecchi et al. (1956 a and b) determined tocopherol content in chicken and turkey fats, and reported about 5 times more tocopherol in chicken fat than in turkey fat. They then, postulated that the difference in quantity of this natural antioxidant might be responsible for the difference in stability of turkey and chicken fats. Pickett et al. (1967) demonstrated a reduction in rancidity development in male turkey carcasses, during 2 months of storage, as a result of an injection of 600 I.U. of tocopherol. Jacobson and Koehler (1970) in studies on rancidity development during short time storage of cooked chicken and turkey meat, however, reported only a slight difference in TBA numbers and sensory scores between these two types of meat.

A somewhat contrary result was found in mechanically deboned poultry meats. Dhillon and Maurer (1975 a and b) studied storage stability of mechanically deboned turkey meat and mechanically deboned chicken meat, at -25°C for 6 months. Their chicken meat

samples were affected more than meat from turkey, during frozen storage, as indicated by TBA increase and taste panel results. When these 2 types of meat were formulated separately at 50% level with ground beef in summer sausages, those sausages containing chicken meat showed greater quality loss. They speculated that this difference may have been particularly due to higher fat-water combination in mechanically deboned chicken meat which was composed of just backs and necks.

Extraction of Tissue Lipids

For quantitative extraction of tissue lipids, a polar solvent, usually in combination with a non polar solvent is necessary. Nelson (1975) stated that covalently bound lipids must be subjected to a hydrolysis procedure before they can be extracted with organic solvents of any polarity. The polar solvent is able to disrupt the hydrogen bonding of protein bound lipids, thus allowing free access of non polar solvent to the lipids. Folch et al. (1957) used 2:1 chloroform-methanol mixture to extract tissue lipids and aqueous solutions for their purifications. Ostrander and Dugan (1961), evaluated 4 lipid extraction methods and proposed a modification of the method by Bligh and Dyer (1959), using methanol-chloroform-water as the extracting solvent, to be most satisfactory in extraction of meat lipids. Radin (1969) also reported that chloroform-methanol appeared to be a good solvent combination for extracting wet tissue. Sheppard et al. (1974) compared 8 lipid extraction methods by analyzing 8 different food samples. Based on the recovery of total lipid, fatty acid distribution and triglyceride recovery, they found 2 methods to be satisfactory: a 4N HCl digestion followed by ethyl

ether extraction and another one, a 2:1 chloroform: methanol extraction. In their recent study, Hubbard et al. (1977) used 7 extraction methods to compare the efficiency of total lipid extraction from samples of 8 different food products. They reported that, on the basis of total lipid recovered and amounts of fatty acids and sterols present, the chloroform methanol method described by Folch et al. (1957) was selected as the most effective method.

Separation of Phospholipids from Neutral Lipids and Fractionation of Phospholipids

Silicic acid column chromatography has been used by many workers to separate polar lipids present in animal tissues (Hanahan et al., 1957; O'Brien and Benson, 1964; Katz et al., 1966; Peng and Dugan, 1965 and Nelson, 1975). This separation method was found to be useful in separation of a sample into major lipid classes such as neutral lipids, glycosphingolipids and phospholipids.

Rapid growth of thin layer chomatography (TLC) as a tool for lipid separation has occurred in the past decade. It has advantages over the silicic acid column separation method with respect to resolution, speed of separation, and generally simpler procedures (Nelson, 1975). Silicic acid column chromotagraphy, however, was found to be useful as an adjunct to TLC. It was reported to be a superior technique in preparing lipid samples for further analysis by TLC (Nelson, 1975).

Two dimentional TLC on silica gel plate was found to be the most convenient and reliable method, currently available for separation of a complex mixture of phospholipids. Skipski et al. (1962) separated phospholipids and cerebrosides on silica gel G plate using

a mixture of chloroform, methanol, acetic acid and water as development solvents. Parker and Peterson (1965) found a special washed silica gel-H plate and solvent mixture containing chloroform, methanol, acetic acid and water to be a rapid and accurate micro method for fractionation of phospholipids prior to their quantitative determinations. Rouser et al. (1966) used a two dimensional TLC with solvent pairs: chloroform-methanol-water and n-butanol-acetic acid-water; and chloroform-methanol-28% aqueous ammonia followed by chloroform-acetone-methanol-acetic acid-water to improve separation of phospholipids prior to analysis of their phosphorus contents. Nelson (1975) concluded that TLC is a precise, accurate and reproducible method for fractionation of complex phospholipids.

Many methods including gravimetric, colorimetric and titrimetric have been used in phosphorus analyses. Among these, colorimetric methods are usually preferred for their adaptability to the micro-method and for their simplicity. Colorimetric methods described by Morrison (1964), Parker and Peterson (1965) and Rouser et al. (1966) are among those that are generally accepted and used currently.

Gas Liquid Chromatography (GLC)

Methylation of Fatty Acids

Purposes of fatty acid esterification prior to GLC analysis are to prepare volatile esters from relatively non volatile compounds and to reduce the polarity of the compounds. Two techniques are commonly used in fatty acid derivatization: direct interesterification reaction and liberation and isolation of fatty acids from lipids by saponification, acid hydrolysis or enzymatic hydrolysis and

subsequent esterification of the liberated fatty acids

Choices of reagents and methods should depend on the type of compounds one wishes to analyze (Anon, undated). For fatty acids containing eight or more carbons the BF_3 - MeOH or BCl_3 - MeOH reagents will give good results. For low molecular weight fatty acids BF_3 - BuOH reagent is preferable. For fatty acids, triglycerides phospholipids etc., which are difficult to esterify, the saponification-esterification procedure will do the job. Commonly used fatty acid esterification methods at present, include those described by Morrison and Smith, 1964; McGinnis and Dugan, 1965 and Metcalfe et al., 1966.

Gas Liquid Chromatographic Analysis of Fatty Acid Methyl Esters

A gas liquid chromatographic technique was introduced in 1952 by James and Martin, as a method for carboxylic acid separation. Since then, it has been developed and used widely as a reliable tool in many analytical fields, including lipids.

Choice of liquid phase in GLC analyses of lipids depends on composition of sample. For an efficient, normal separation, liquid phase should be similar in chemical structure to component of mixture. Orr and Callen (1958) recommended polyester type liquid phase to shorten analysis time and to provide a good resolution in separation of esters of common and polyunsaturated fatty acids. Of these types, adipate and succinate polyesters of diethylene glycol are most widely used. Kuksis (1965) recommended various silicone polymers, of which the most popular has been SE-30, for a high temperature separation of natural triglycerides.

Mehlenbacker (1960) pointed out that elution characteristics of individual compounds in GLC analysis depends on type and amount of liquid phase, temperature, gas flow rate, and type of compound analyzed, while resolution of chromatographic peaks was related to column efficiency and solvent efficiency. Seino et al. (1973) studied influence of operating conditions on determination of fatty acid methyl esters. They found that sample size and flow rate of carrier gas had significant influences on the analytical values.

Identification of various peaks on fatty acid GLC chromatogram can be done in many ways. Retention volume or time which is characteristic of the samples and liquid phase can be used to identify samples. James (1960) introduced an identification method in which retention times of standard fatty acids were plotted versus their chain length on semilogarithmic graph paper. Since a plot of the log of the retention times is proportional to some increasing property of the homologous series, identification of members of each homologous series can thus be obtained. This method is advantageous in that only 2 or 3 compounds are needed to establish the slope of the line and thus can be used to identify other members of the same series.

Evaluation of Lipid Oxidations

Numerous methods are available for evaluation of lipid oxidation, however, none of them has been found to be ideal. The choice of method used, thus, will depend normally, on factors such as: types of products to be analyzed, nature of problems and equipment availability.

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available for determination of lipid oxidation can be classified into 4 groups: 1) methods based on lipid compositions; 2) methods based on absorption of oxygen; 3) methods based on the intermediate formation of lipid peroxides and 4) methods based on the measurement of one or more of the final reaction products or classes of products resulting from peroxide decompositions.

In most lipid composition studies, fatty acid analyses will be done. Quantity of unsaturated fatty acids as well as their degrees of unsaturation in a lipid system, in general, will indicate the approximate susceptibility of that system toward oxidative deteriorations. Decreases in degree of fatty acid unsaturation as storage time of fat increases have been observed. And, in some cases this reduction can be positively correlated with other methods used to measure the progress of lipid oxidation (Lee and Dawson, 1973 and Moerck and Ball, 1974).

Measurement of products formed subsequent to decomposition of peroxides are also used to measure oxidative rancidity in animal tissues. Sinhuber et al. (1958) reported that condensation products of one molecule of malonaldehyde with 2 molecules of 2-thiobarbituric acid (TBA) reagent was responsible for the red color developed in rancid salmon oil. Tarladgis et al. (1960, 1964) reported that free malonaldehyde was produced during the oxidative breakdown of unsaturated fatty acids from food products. According to these researchers, malonaldehyde itself did not contribute to the rancid odor in food. However, good correlations between TBA test and rancid flavor development have been reported by many workers (Younathan and Watts, 1959; Jacobson and Koehler, 1970; Webb et al.,

1972 and Johnson et al., 1974). Another method which is generally accepted, in this group is carbonyl-test (Henick et al., 1954; Dugan, 1955 and Lea and Swoboda, 1958). An advantage of using TBA test to determine rancidity development in animal tissues is that the test can be applied directly to the products without prior extraction of lipid.

Cholesterol Analyses

Steps necessary to determine the cholesterol content of products are extraction of lipids, separation of cholesterol from lipids and interfering materials and detection and measurement of isolated cholesterol (Sweeney and Weihrauch, 1976).

In general, any lipid extraction method that will satisfactorily extract lipids from muscle tissue should be suitable for extraction of tissue cholesterol. Sweeny and Weihrauch (1976) stated that a mixture of polar and non polar solvents gave better cholesterol extraction from dairy products or from any other food products in which part of the cholesterol may be bound to a lipoprotein or some other substance in food. Hubbard et al. (1977) reported that the method described by Folch et al. (1957) was the most effective method among 7 other methods they evaluated, in extraction of cholesterol from some food products.

Separation of cholesterol from lipid and interferring materials can be done in various ways. Digitonin was used by many workers to precipitate free cholesterol from lipid extracts (Sperry and Brand, 1943; Hunter et al., 1945; Sperry, 1963 and Tu et al., 1967). Kabara and McLaughlin (1961) and Edwards et al. (1964) reported that

tomatine was more specific than digitonin for cholesterol precipitation. Various chromatographic methods have been used to separate cholesterol. Moerck and Ball (1973, 1974) used a mixture of different polar solvents to elute cholesterol and cholesterol esters from Unisil columns. Thin layer chromatography (TLC) has been used by many investigators to separate cholesterol and cholesterol esters (Skipski et al., 1968; Thorpe et al., 1969; Tattrie, 1972 and Teichman et al., 1974). The most commonly used adsorbent in TLC isolation of cholesterol is silica gel G. Common solvents used for developing the chromatograms include: ethyl ether -petroleum ether, hexane-ethyl ether-acetic acid, chloroform-methanol and chloroform-methanol-water. Gas liquid chromatography has been recently used widely for both sterol separation and determination. According to Sweeney and Weihrauch (1976), it has greater specificity and more accuracy for cholesterol analysis than colorimetric methods, especially when the sample being assayed contains sterols and other interfering materials.

MATERIALS AND METHODS

Materials

Meat Samples

All meat samples were obtained from a commercial poultry processing plant in Michigan. Mechanically processed meats were processed through a Behive mechanical deboning machine (Model AU968MF, Behive Machinery Co., Sandy, Utah).

Composition Study - Light hand deboned poultry meat (HDPM) was processed from breast meat while dark hand deboned meat was obtained from thigh and drumstick meats. Light and dark skins were obtained from their corresponding light and dark meat pieces. Light mechanically processed poultry meat (MPPM) was processed from hand boned breast racks whereas the dark MPPM was from back racks and portion of necks. Bone samples, both light and dark, were separated by the deboning machine from either white or dark machine processed meats. Chicken meat and products were obtained from freshly dressed (not frozen) adult hens (fowl), and further processing was accomplished as soon as major muscles were removed from carcasses for use in other products. Turkey meat and products were obtained from previously processed and frozen birds. The original source and length of storage of these turkeys is unknown. Turkeys were thawed in cold water prior to hand boning major muscle and machine deboning of remaining bony portions. All samples were packed in cryovac bags. The closed

bags were then packed along with dry ice and ice in an insulated box, and transferred to the Food Science Laboratory.

Stability Study - Both mechanically processed chicken and turkey meats were processed from whole carcasses. Meat samples were packaged in 18 kg corrugated board boxes, lined with plastic sheets. Temperature of meat at time of receiving at laboratory ranged from 4-7.5°C.

Materials for Chromatographic Analyses

Stainless steel columns (0.32 cm x 1.83 m) packed with 10% Diethylene glycol succinate-phosphoric acid (DEGS-PS), on 80-100 mesh supelcoport, were obtained from Supelco Inc. The columns were conditioned by temperature programming from 50°C to 190°C at 2°C/min., for 48 hrs. The column flow rate during the conditioning period was 20 ml/min.

For sterol analysis, a silane treated glass column (0.635 cm x 1.83 m) packed with 3% OV-17 on 100-200 mesh gas-chrome Q, was obtained from Applied Science Laboratories Inc. The column was conditioned prior to use by programming from 50°C to 300°C, at 2°C/min. for 48 hrs. Column flow rate during conditioning was 80 ml/min.

Silica gel G and H plates were purchased from Applied Science Laboratories Inc. or from Supelco Inc. All plates were activated at 105°C for 1 hour before use.

Silicic acid (100 mesh) was obtained from Mallinkrodt Co. Inc. Preparation of the column was as follows:

Silicic acid was washed several times with deionized distilled water to remove the fines. Water was removed from washed acid

by passing the slurry through Büchner funnel fitted with # 1 filter paper. The precipitate was then rinsed on the filter, twice, with anhydrous methanol.

Washed silicic acid was activated at 105°C for 24 hours. A 25 g sample was then dispersed in chloroform and poured into a 1.5 x 30 cm glass column fitted with a sintered glass disc and Teflon stopper at its lower end. Silicic acid was allowed to settle and chloroform was drained from the column by applying a slight suction. A 1 cm layer of granular anhydrous sodium sulfate was then placed on top of the acid column. The column was rinsed several times with chloroform prior to use.

Reference Standards

Standard cholesterol (99 + % pure) and 5α-Cholestane (99 + % pure) was obtained from Applied Science Laboratories, Inc.

Mixture of fatty acid methyl esters were obtained from Supelco Inc. and Applied Science Laboratories Inc.

Standard phosphorus solutions were made from dried potassium dihydrogen phosphate primary standard (Fisher Scientific Company).

Phospholipid standard mixtures were obtained from Supelco Inc.

Solvents

Analytical reagent grade chloroform and methanol were freshly redistilled prior to each use to remove any contaminating peroxides. When the redistilled chloroform required storage for more than 1 day, a 0.25% methanol by volume was added to prevent its decomposition. All other solvents, unless otherwise specified, were a

'Distilled in Glass' high purity solvent grade obtained from Burdick and Jackson Laboratories. All solvents were stored at 3°C.

Glassware

All glassware which might come in contact with sterol and fatty acid methyl esters was subjected to a special treatment. First the glassware was washed through a normal laboratory washing procedure. After drying, they were soaked in concentrated sulfuric acid overnight. After soaking, they were rinsed copiously with water and distilled water. Before use, all surfaces which would come in contact with the sample were rinsed twice with either chloroform or a 2:1 chloroform methanol solution.

All screw caps used in sterol and fatty acid analyses were cleaned by using a sonic type cleaner. After cleaning, they were rinsed with distilled water and a 2:1 chloroform methanol solution. Teflon pads which were soaked and rinsed well with chloroform were used as liners for all screw caps.

Silane treated glass centrifuge tubes and their stoppers used to derivatize cholesterol were prepared and cleaned according to AOAC (1976) method.

Glassware used for phosphorus determinations was cleaned by rinsing with water. After each use, they were soaked with conc. sulfuric acid overnight. After soaking, they were rinsed well with distilled water, followed with deionized distilled water and were dried prior to use.

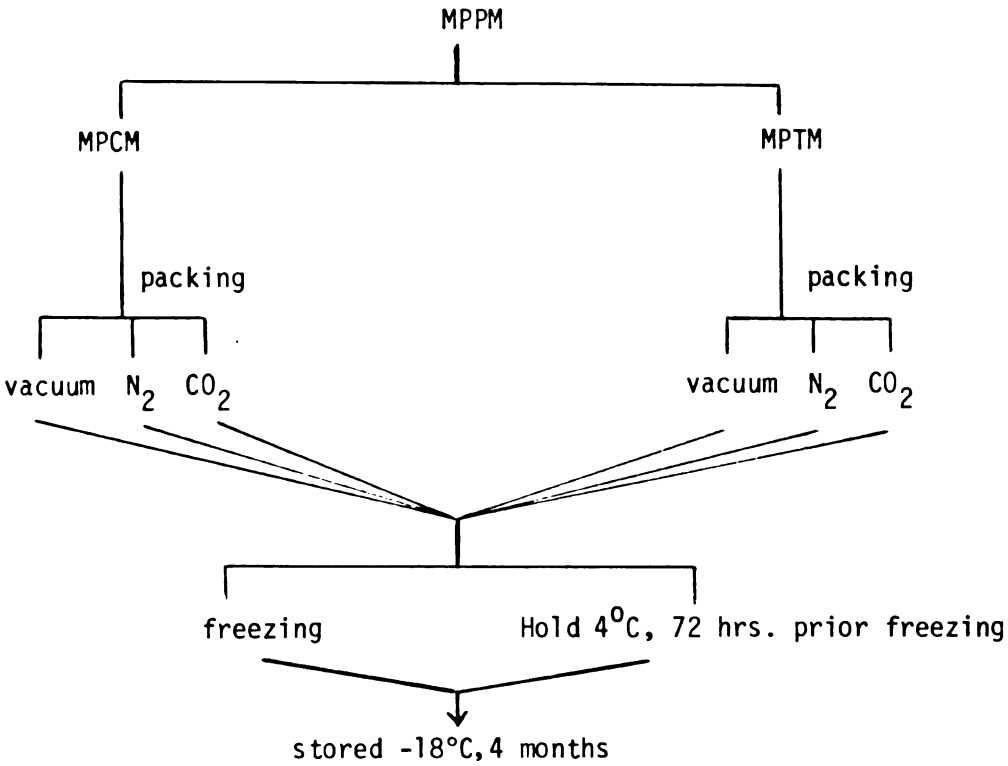
Preparation of Meat Samples

Composition Study

Hand deboned meats, skins and bone residues were ground through a Hobart meat grinder, fitted with a 3 mm hole plate. All ground tissues including mechanically processed meats were hand mixed, to assure their homogeneity, then vacuum packed at about 150 g level, in # 13 IKD plastic bags and stored at -18°C.

Inert Gas Study

Mechanically processed chicken and turkey meats were individually mixed at medium speed in a Hobart mixer (Model K-5A), under N_2, for 2 minutes. Mixing was performed to obtain homogeneity within each batch of meat. After mixing, the meat was packed in 100 g portions in 170 x 100 mm^2 # 13-IKD plastic bags. One third of the total packages were then vacuum sealed by using a Kenfield vacuum packaging sealer. The rest of the packages were then flushed with either N_2 or CO_2 gas and sealed so that a constant and appropriate volume of either of the gases was confined within each bag. After sealing, half of the packages from each treatment were randomly selected and stored in a walk in type freezer at -18°C. The remaining packages were transferred to a home style refrigerator and held there for 72 hours. Temperature variations during this holding period ranged from 2.5-5°C. At the end of the holding period, all packages were transferred to the freezer and stored at -18°C, for 4 months. Treatment diagram is illustrated as follows:

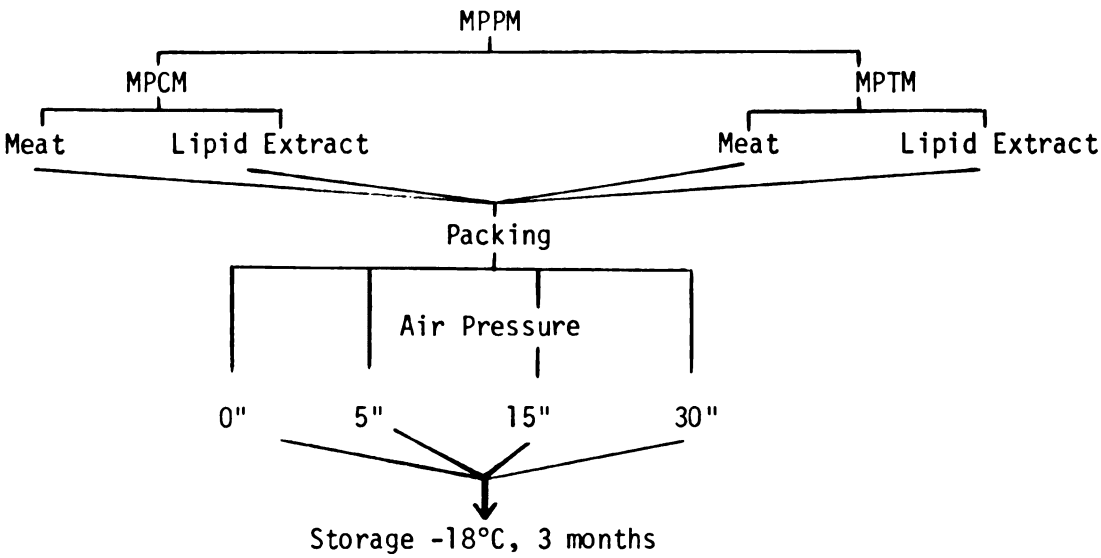


At the end of each specified interval, samples were randomly selected for analyses. All samples were partially thawed at room temperature for 1 hour, prior to analyses.

Air Tension Study

All meat samples were mixed at a medium speed, on a Hobart mixer (Model K-5A), under N_2 for 2 minutes. After mixing, each meat item was vacuum packed in a # 13 IKD plastic bag in such a manner that they were uniformly spread in a 2.54 cm thickness layer within each bag. After sealing, they were held in the freezer at -18°C overnight. Frozen meats were then cut into 5.08 x 5.08 x 2.54 cm^3 by using a Hobart meat saw (Model 5212). Each meat block was wrapped with a piece of low density polyethylene sheet and arranged into a prepared storage chamber. Lipid extracts from 100 g of meat, stored in a 30 x

35 mm wide mouth bottle type glass vials, were arranged within the same storage chamber. A low density polyethylene wrap was selected to help minimize moisture loss from the meat surface and to minimize the interfering effect of the transportation of microclimatic O_2 into the meat block during storage. After the chamber was closed, an assigned quantity of air pressure was created within each chamber. The chamber was first evacuated by using a Duo-Seal vacuum pump (model 1400). After complete evacuation, a known quantity of air was injected back into the chamber, and the chamber was sealed. A U-type vacuum guage was used to assure the appropriate quantity of air within each chamber. The closed chamber was then placed in a cryovac bag and the bag was sealed with a Tipper Clipper bag sealer (model 72105). Storage chambers were stored in the freezer at -18°C for 3 months. At one month intervals, the products in one chamber from each treatment were randomly selected for chemical analyses. Treatment diagram for this study is as follows:



Extraction of Total Lipids

The method described by Folch et al. (1957) was used to extract total lipids from all samples. A weighed quantity of ground tissue containing at least 0.5 g of fat was homogenized at high speed in a Vortex homogenizer with a volume of 2:1 chloroform:methanol (about 20 times the sample weight) for 2 minutes. The extracting solvent and tissue residue were then transferred to Büchner funnel fitted with # 1 Whatman filter paper and the filtrate transferred to a 500 ml separatory funnel. The residual cake and filter paper were re-extracted for 1 minute with an additional solvent system of about 5 times the sample weight. The extracting solvent was filtered and collected in the separatory funnel. The crude extract was washed with 0.2 its volume with a 0.74% potassium chloride solution and allowed to stand at -18°C overnight to facilitate its separation. The chloroform layer was collected by passing the solution through a glass funnel containing about 20 g of anhydrous sodium sulfate into a glass stoppered round bottom flask. The water layer was washed 2 more times with 20 ml portions of chloroform, and the solvent layers were combined. Solvent was evaporated at 20°C by using a rotary vacuum evaporator (Rinco Instrument Co.). After drying, traces of chloroform were further evaporated under a N_2 stream. The total lipid was stored in a vacuum desicator at -18°C when not used immediately.

Separation of Phospholipids from Neutral Lipids

Composition Study - Column chromatography was used to separate phospholipids from neutral lipids in this study.

A 0.25 g sample of lipid in chloroform, was added to the column.

Elution of each fraction was performed under a gentle pressure. A stream of nitrogen was used to flush the sample from the top of the column throughout the elution period. The sample was eluted from the column at a rate of 2 drops per second. A 15 ml portion of solvent was used for each elution until the total volume of solvent was 20 times the lipid sample weight. Neutral lipids were eluted with chloroform while methanol was used as eluant for phospholipids. Purity of each separating fraction was checked by thin layer chromatography. A 0.25 mm thickness silica gel G plate was used. A solvent system consisting of petroleum ether, ethyl ether and acetic acid (90:10:1 by volume) was used to check purity of phospholipids. Purity of neutral lipids was confirmed by using a mixture of chloroform methanol-water (65:25:4 by volume) as the developing solvent.

The solvent was evaporated from each lipid fraction on a Rinco rotary vacuum evaporator. Last traces of solvent were removed under a N_2 stream.

Phospholipids from each separation were quantitated by adding accurately 5 ml of 2:1 chloroform methanol into the concentrated residue. After thoroughly shaking, a 1 ml aliquot was taken and dried in a forced air oven at 105°C to a constant weight. The remaining solution was stored under N_2 in a 4 ml Teflon lined screw cap vial at -18°C until use.

Stability study - Thin layer chromatography was used to separate phospholipids from neutral lipids for both stability studies. A 0.50 mm thick layer silica gel G plate was used for this separation. Fat samples dissolved in chloroform were applied under N_2 along the bottom of the plate in several spots, 0.5 cm apart. A 10 µl

microsyringe was used for application of the sample. The plate was developed in a saturated chamber, by using chloroform as a developing solvent. After each development, the lipid bands were visualized by spraying with 0.5% iodine in methanol solution. Iodine was allowed to sublimate from the plate under a N_2 stream, prior to the removal of lipids from the plate. Phospholipids, were then scraped from the bottom of the plate and transferred into a 20 ml screw cap test tube with the aid of several 2 ml portions of 2:1 chloroform methanol solvent. The remaining neutral lipids were then scraped into another tube. Diethyl ether was used as an eluting solvent for the neutral lipid. After elutions, solvents were evaporated from the sample under N_2 stream, and the samples were subjected to methylation for GLC analysis.

Methylation of Lipids

Methylation of lipids was performed according to the method described by Morrison and Smith (1964).

Solvent was evaporated from lipid sample under N_2. One ml of 14% boron trifluoride in methanol was added under N_2 to the polar lipid residue in a 10 x 125 mm test tube. The tube was sealed with Teflon-lined screw caps and heated for 10 minutes in a boiling water bath. The non polar lipid residue was treated in the same manner except that 0.2 ml of benzene was added to the sample prior to heating and the heating time was 30 minutes. After heating, samples in test tubes were cooled to room temperature and the methyl esters were extracted. A 3.0 ml portion of petroleum ether and 1.5 ml of distilled water were added to the sample in the tube and the tubes were

shaken briefly on a test tube shaker and let stand for 2 minutes. The upper petroleum ether layer was removed. Re-extraction was performed with an additional 1.5 ml of petroleum ether. The methyl ester solution was stored under N_2 at -18°C in a 4 ml Teflon lined screw cap. Fatty acid methyl ester solutions were concentrated under N_2 prior to injection into the GLC.

Gas Chromatographic Analyses of Fatty Acid Composition of Polar and Non Polar Lipids

Gas chromatographic analyses were performed by using an F&M model 810 dual column gas chromatograph, equipped with a flame ionization detector. Helium was used as the carrier gas at a flow rate of 30 ml per minute. The hydrogen flame was fed with 35 ml per minute hydrogen and 350 ml per minute compressed air. Temperature was programmed from 150 to 190°C at a rate of 4°C per minute. Temperatures of detectors and injection ports were maintained at 255°C and 250°C respectively. A sample of 2.0 µl was injected for each analysis.

Fatty acid methyl esters were identified by comparing their relative retention times (relative to methyl palmitate), using a plot of logarithm of the relative retention time versus the number of carbon atoms. After peak identification, the percentage of each fatty acid ester was calculated by dividing the area of each individual peak by the total area of all peaks.

Classification of Phospholipids

Phospholipid solutions obtained from silicic acid column separation were applied on 0.5 mm, thick layer silica gel G plates,

along with standard materials. The plates were developed in a chamber saturated with a mixture of solvents containing 65:25:8:4 chloroform: methanol:acetic acid: water (by volume). After each development, spots were visualized with iodine vapor and located. Identifications were made by comparing the Rf values of the unknown spots with those of standards. Various quantities of spots were then scraped directly into 30 ml Kjeldahl flasks. Adjacent areas of blank silica gel corresponding in size to the areas containing phospholipids were also scraped into digestion flasks to serve as blank determinations for the analysis (Skipski et al., 1962).

Quantitation of Total Phospholipids

Separation of phospholipid from neutral lipid was done by the thin layer chromatographic method previously described under the title "Separation of Phospholipids from Neutral Lipids." After all phospholipid spots were visualized and located, they were scraped into a 30 ml Kjeldahl digestion flask. Blank samples were obtained in the same manner as those described for phospholipid fractionation.

Phosphorus Determination

The total phospholipids and their fractions were quantitated by analyzing the phospholipid phosphorus using the method described by Rouser et al. (1966).

Samples in Kjeldahl flasks were digested with 0.9 ml of 72% perchloric acid, using a low heat setting on an electrically heated digestion rack (Laboratory Construction Co., Model A). The flasks were shaken occasionally during the digesting period. After 30 minutes, each sample was allowed to cool and the flask side was

rinsed with 5 ml of deionized distilled water. One ml of 2.5% ammonium molybdate was added to the sample. After swirling, 1 ml of freshly prepared 10% ascorbic acid was added and the content of the flask was mixed and transferred to a 10 ml centrifuge tube. The flask was rinsed with 2 ml deionized distilled water and the rinsings were combined. The tubes were then heated in a boiling water bath for 5 minutes. After cooling, the absorbance of the supernatant solution was read at 820 nm using Bausch & Lomb Spectronic-20. Water was used to adjust the zero reading and a mean value from several blank determinations was subtracted from the sample reading. Phosphorus content of each sample was determined directly from a standard curve.

Phosphorus Standard Curve

A solution containing 0.1 mg of phosphorus per ml, was made by diluting 0.4393 g of dried potassium dihydrogen phosphate primary standard to one liter with deionized distilled water. Various amounts of standard solution to contain 2-10 µg of phosphorus were pipetted into Kjeldahl flasks, and further treated in the same manner as those for each food sample. Concentrations of phosphorus in micrograms were plotted against their absorbances.

2-Thiobarbituric Acid (TBA) Test

A 10 g portion of mechanically processed poultry meat was homogenized with 50 ml of distilled water, at medium speed for 2 minutes, in Virtis homogenizer. The resulting mixture was then quantitatively transferred, with the aid of 47.5 ml distilled water, into a 500 ml distilling flask. Two and one-half ml of 4N HCl were added

to lower the pH to about 1.5. A few glass beads were added and the mixture sprayed with Dow Corning antifoam. After thorough mixing, the flask was connected to the distilling apparatus. The distilling unit was composed of a 30.5 cm long distilling column connected to the condensor with a bending shoulder, and a 50 ml graduated cylinder served as a receiver. Distillation was continued at a rate in which 50 ml of the distillate was collected within 10-15 min., subsequent to boiling.

Two aliquot portions of the distillate were pipetted and transferred to the reacting tubes. Accurately, 5 ml of 0.02 M 2-Thiobarbituric acid in 95% redistilled glacial acetic acid were added and the tubes were capped. After thoroughly mixing and heating in a boiling water bath for 35 min., they were cooled in cold water for 10 min. The absorbance was determined at 532 nm against a reagent blank in which 5 ml of distilled water was used in place of the distillate.

The TBA number was calculated by multiplying the mean absorbance by 7.8, distillation constant, (Tarladgis et al., 1960) and reported as mg TBA reactive substance per 1000 g of meat.

TBA Absorption Values

A quantity of lipid extract equivalent to 10 g of mechanically processed poultry meat (2.0 g for turkey lipid extract and 2.3 g for chicken lipid extract) was weighed into 500 ml distilling flask. Fifty ml of distilled water were added and the flask was stoppered and shaken for 2 min. on a test tube shaker. An additional 47.5 ml distilled water were used to rinse the flask stopper and then combined

with the contents of the flask. Further treatments were done in the same manner as those for the tissue determination. TBA numbers of lipid extract were reported as TBA absorption values.

Cholesterol Analyses

Total Cholesterol

Cholesterol analysis was done according to the method described in AOAC (1976).

Extraction of Tissue Lipids

Tissue lipids were extracted and purified by using the method described by Folch et al. (1957).

Saponification and Extraction of Nonsaponifiable Fraction

An accurate volume of chloroform-lipid extract to contain 250-500 mg of fat was filtered through a glass funnel containing a pledget of glass wool and about 25 g anhydrous sodium sulfate. After several rinsings with chloroform, the solvent was evaporated to dryness on a Rinco rotary vacuum evaporator at 20°C. The residue was dissolved in 70 ml of petroleum ether and filtered through Whatman no. 1 filter paper containing about 20 g anhydrous sodium sulfate into a 300 ml glass stoppered Erlenmayer flask. The round bottom flask was rinsed with several 10 ml portions of petroleum ether and the rinsings combined. Petroleum ether was then evaporated to dryness on a vacuum rotary evaporator at 25°C.

The fat residue was saponified with 8 ml conc. KOH solution (60 g KOH in 40 ml water), and 40 ml of reagent alcohol (a mixture of ethyl alcohol: methyl alcohol: isopropyl alcohol, 90:5:5) on a

magnetic stirrer hot plate. The sample was gently stirred by means of a magnetic stirrer bar throughout the saponification process. A 1 meter long glass column was attached to the reacting flask during saponification to prevent an excessive loss of the solvent. At the end of the digestion, 60 ml of reagent alcohol were introduced into the flask through the column. After cooling, 100 ml of benzene (accurately measured) were added to the sample and the flask was shaken vigorously for 30 sec. The flask contents were then transferred into a 500 ml separatory funnel and the benzene layer was separated by shaking with 200 ml of 1 N KOH solution. After the aqueous layer was discarded, the benzene layer was washed with 40 ml of 0.5 N KOH solution, followed by 4 x 40 ml portions of distilled water. The washed benzene solution was then filtered through Whatman # 4 filter paper containing about 15 g of anhydrous sodium sulfate into a glass stoppered flask. An additional 20 g of anhydrous sodium sulfate were added into the flask. After vigorously shaking, the flask was allowed to stand for 15 minutes. A 50 ml aliquot portion of benzene was then pipetted into a 100 ml round bottom ground-glass-jointed-flask and evaporated to dryness at 30°C on a rotary vacuum evaporator. After drying, 3 ml of acetone were added and the mixture was re-evaporated to dryness. The residue was then dissolved in 3 ml dimethylformamide.

Derivatization

An accurate 1.0 ml portion of the extracted nonsaponifiable solution in dimethylformamide solution was derivatized in a silane treated 15 ml centrifuge tube. Each sample solution was shaken vigorously on

a test tube mixer with 0.2 ml hexamethyldisilazane and 0.1 ml trimethylchlorosilane. After standing 15 min., 1 ml of 5 α-cholestane internal standard solution (0.2 mg per ml 5 α-cholestane in n-heptane) and 10 ml of distilled water were added into the tube, and the tube was shaken for 1 min. Duplicate 2 μl heptane layers were injected into the GLC equipment.

Cholesterol Standard Curve

A cholesterol standard solution in dimethylformamide was made to contain 2.0 mg of cholesterol per one ml of the solution. Further dilution provided a concentration range from 0.05 to 1.25 mg/ml. A 0.2 mg per 1 ml solution of 5 α-cholestane in n-heptane was also prepared. Derivatization of cholesterol standard solutions was then performed on a 1 ml aliquot of each standard dilution, in the same manner as that described for sample derivatization. Duplicate 2 μl of heptane layer of each dilution was injected into the GLC equipment. Peak area ratio at each concentration, calculated by dividing cholesterol peak area by 5 α-cholestane peak area, was then plotted against standard cholesterol concentrations.

Free Cholesterol

TLC separation of total lipids was performed on a 0.5 mm thick layer silica gel H plate. A solvent system containing 90:10:1 hexane-ethyl ether-acetic acid was used. The plate was developed in a chamber saturated with solvent. A 0.5% iodine in anhydrous methanol was used as visualizing agent. Separated free cholesterol was identified by comparing its Rf value with that of pure cholesterol standard. A row of cholesterol spots was then scraped into a test tube with the

aid of 5 ml chloroform. Additional 3 x 5 ml portions of chloroform were used to elute the samples out of the silica gel powder. The filtered solvent was then evaporated under N_2 stream at 45°C. The dried residue were dissolved in 1 ml of dimethylformamide and subjected to derivatization.

Standard curves for free cholesterol were prepared by spotting known quantities of cholesterol standard solutions on silica gel H plates. The plates were then developed with the same kind of solvent and procedure as those described for the samples. Further treatments followed the same manner as those for the standard until completion of derivatization. Duplicate 2 µl of standard materials and sample were injected in to the GLC equipment.

Gas Liquid Chromatographic Analysis of Cholesterol

Analysis of cholesterol was accomplished by using an F & M model 810 dual column gas chromatograph, equipped with a flame ionization detector. Nitrogen was used as the carrier gas at a flow rate of 35 ml per min. A 35 ml per min. hydrogen and 350 ml per min. of compressed air were used to feed the hydrogen flame. A temperature programming operation was performed from 240 to 275°C at a rate of 6°C per min. The detector and injection port temperatures were kept constant at 300 and 275°C respectively. The emerging peaks were identified by comparing their retention times with those of standards. Peak areas were determined for both sample and internal standard. The relative area ratio of sample to internal standard was then used to determine cholesterol concentration directly from a standard curve.

Statistical Analyses

Statistical analyses were performed by using a Michigan State University computer program identified as Analysis of Variance Program on MSU STAT System Package and run on a Control Data Corporation (CDC) 6500 computer at MSU computer laboratory.

RESULTS AND DISCUSSION

Part I. Composition Studies

Lipids from light and dark mechanically processed poultry meats (MPPM), their corresponding hand deboned meats (HDPM), bone residues and skin tissues were analyzed for total cholesterol contents, phospholipid distributions and fatty acid compositions. Attempts were made to correlate components of MPPM lipids with those found in HDPM, bone residues and/or skin tissue lipids. The purpose of this study was to determine the source or sources of fats which entered into MPPM during the machine deboning processes.

Fatty Acids

Fatty acid distribution profiles for composite tissue samples including neutral and phospholipids from light and dark chicken and turkey tissues are shown in Table 1 and 2 respectively. Examples of chromatograms for neutral and phospholipid fatty acids from gas liquid chromatographic analyses are shown in appendices A and B.

Fatty Acids from Neutral Lipids

Ten different fatty acids were identified and quantified from neutral lipids of dark and light chicken and turkey tissues. The most prevalent fatty acids found in this fraction for all types of tissues examined were the 16-carbon atom fatty acids (palmitic and palmitoleic acids) and 18 carbon atom fatty acids (stearic acid,

Table 1a. Fatty acids of the neutral lipids from chicken tissues.[a]

Fatty Acid [b,c]	Source of Fatty Acids							
	MPM[d]		HDM[e]		Bone Residue		Skin	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Chicken Fatty Acids of Neutral Lipids								
12:0	1.61 ± 0.14	1.23 ± 0.07	1.62 ± 0.14	1.64 ± 0.07	0.90 ± 0.14	1.27 ± 0.06	1.18 ± 0.14	1.34 ± 0.16
14:0	2.25 ± 0.25	2.48 ± 0.12	2.85 ± 0.22	2.77 ± 0.20	2.50 ± 0.13	2.00 ± 0.10	1.96 ± 0.17	2.06 ± 0.16
16:0	23.31 ± 0.22	20.67 ± 0.24	21.35 ± 0.49	17.42 ± 0.19	21.99 ± 0.28	23.33 ± 0.18	25.41 ± 0.35	20.97 ± 0.72
16:1	5.70 ± 0.13	7.40 ± 0.22	6.84 ± 0.15	10.10 ± 0.14	6.18 ± 0.19	6.45 ± 0.25	5.77 ± 0.15	6.88 ± 0.47
18:0	7.46 ± 0.26	8.35 ± 0.15	10.35 ± 0.43	11.48 ± 0.23	9.06 ± 0.42	6.91 ± 0.24	6.21 ± 0.25	8.02 ± 0.41
18:1	34.92 ± 0.29	32.68 ± 0.07	32.68 ± 0.27	28.54 ± 0.51	31.73 ± 0.16	36.39 ± 0.05	35.46 ± 0.50	32.65 ± 0.89
18:2	23.23 ± 0.09	25.40 ± 0.10	22.42 ± 0.27	25.51 ± 0.31	26.22 ± 0.37	22.36 ± 0.28	22.45 ± 0.44	25.80 ± 0.72
20:0	-	-	-	-	-	-	-	-
18:3	1.53 ± 0.14	1.74 ± 0.06	1.94 ± 0.13	2.51 ± 0.21	1.35 ± 0.20	1.26 ± 0.11	1.54 ± 0.05	2.26 ± 0.14
20:3	-	-	-	-	-	-	-	-
20:4	t	t	t	t	t	t	-	-
Total Saturation	34.63	32.73	36.17	33.31	34.45	33.51	34.76	32.39
Total Unsaturation	65.38	67.22	63.87	66.66	65.48	66.46	65.22	67.59
Unsaturation Ratio	1.89	2.05	1.77	2.00	1.90	1.98	1.88	2.09
Monoene	40.62	40.08	39.52	38.64	37.91	42.48	41.23	39.53
Diene	23.23	25.40	22.42	25.51	26.22	22.36	22.45	25.80
Triene	1.53	1.74	1.94	2.51	1.35	1.26	1.54	2.26

[a] Calculated as percentage of total fatty acids in neutral lipids.

[b] Number of carbons: number of double bonds.

[c] Mean and standard deviations from 4 determinations.

[d] Mechanically processed meat.

[e] Hand deboned meat.

Table 1b. Fatty acids of the neutral lipids from turkey tissues.[a]

Fatty Acid [b,c]	Source of Fatty Acids							
	MPM[d]		HDM[e]		Bone Residue		Skin	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Turkey Fatty Acids of Neutral Lipids								
12:0	0.66 ± 0.03	0.70 ± 0.06	1.42 ± 0.10	1.42 ± 0.09	0.96 ± 0.03	0.56 ± 0.02	1.46 ± 0.21	1.53 ± 0.35
14:0	4.33 ± 0.13	3.36 ± 0.20	3.15 ± 0.18	3.15 ± 0.38	3.01 ± 0.57	2.57 ± 0.19	3.10 ± 0.09	3.06 ± 0.27
16:0	21.60 ± 0.26	19.58 ± 0.15	19.69 ± 0.56	20.37 ± 0.50	23.34 ± 0.72	21.24 ± 0.56	24.11 ± 0.27	19.06 ± 0.76
16:1	6.37 ± 0.26	4.41 ± 0.18	4.67 ± 0.17	4.39 ± 0.48	6.27 ± 0.17	5.42 ± 0.22	4.28 ± 0.44	6.35 ± 0.20
18:0	11.05 ± 0.17	10.46 ± 0.11	12.35 ± 0.29	11.32 ± 0.63	9.56 ± 0.42	11.00 ± 0.36	10.56 ± 0.46	10.10 ± 0.36
18:1	30.00 ± 0.45	33.33 ± 0.18	28.77 ± 0.62	29.50 ± 0.47	32.93 ± 0.52	30.68 ± 0.29	30.57 ± 0.54	30.29 ± 0.33
18:2	22.78 ± 0.18	25.75 ± 0.14	27.77 ± 0.33	27.71 ± 0.67	22.54 ± 0.24	27.06 ± 0.50	24.08 ± 0.24	27.16 ± 0.29
20:0	0.34 ± 0.05	-	-	-	-	-	-	-
18:3	1.86 ± 0.20	1.56 ± 0.10	2.19 ± 0.06	2.10 ± 0.26	1.40 ± 0.27	1.56 ± 0.35	1.84 ± 0.16	2.39 ± 0.34
20:3	-	-	-	-	-	-	-	-
20:4	t	t	-	t	t	t	-	-
Total Saturation	37.64	34.10	36.61	36.20	36.81	35.35	39.38	33.74
Total Unsaturation	61.01	65.05	63.40	63.70	63.14	64.71	60.61	66.18
Unsaturation Ratio	1.62	1.91	1.73	1.76	1.72	1.83	1.54	1.96
Monoene	36.37	37.74	33.44	33.89	39.20	36.10	34.85	36.64
Diene	22.78	25.75	27.77	27.71	22.54	27.06	24.08	27.16
Triene	1.86	1.56	2.19	2.10	1.40	1.56	1.84	2.39

[a]Calculated as percentage of total fatty acids in neutral lipids.

[b]Number of carbons: number of double bonds.

[c]Mean and standard deviations from 4 determinations.

[d]Mechanically processed meat.

[e]Hand deboned meat.

oleic acid and linoleic acid). These C_{16} and C_{18} carbon fatty acids comprised approximately 94-96% of the total fatty acids found in the neutral lipids. Among these fatty acids, palmitic acid accounted for the majority of saturated fatty acids while oleic and linoleic acids are the major unsaturated fatty acids found in this fraction. These results are typical for both chicken and turkey lipids and for all tissues examined. Marion and Woodroof (1966), Katz et al. (1966), and Moerck and Ball (1974) reported similar results in the non polar lipid fatty acids of chicken breast muscles, thigh muscles, skins and mechanically processed chicken meats.

The most obvious difference in neutral lipid fatty acids of light and dark tissues was the variation in quantities of total saturated and unsaturated molecules. Of all tissue studied, neutral lipids from light tissues were more saturated than those found in the dark tissues. These results are indicated by the magnitudes of unsaturated to saturated fatty acid ratios. Obvious differences in unsaturation ratios were shown in lipids from light and dark skins and from mechanically processed meats. In most of the hand deboned and bone tissue lipids, however, these differences were not as apparent as those found in other types of tissue pairs.

In all tissues studied, neutral lipids from turkeys contained lower quantities of unsaturated fatty acids than chicken lipids. However, since dietary histories and ages of the two species are not known, no attempts were made to evaluate these differences. The main reason for this omission is that large variability of fatty acid proportions could also be imparted by these factors as well as by the real species variations.

There was little variation in the unsaturation ratio of different neutral lipids from various tissue samples. This result is true for both chicken and turkey tissues. It is evident from the data that the unsaturation ratio patterns of light and dark MPCM and MPTM closely resemble those of their corresponding light and dark skin lipids. However, with these small differences among all types of tissue of each species, it can not be concluded with confidence that neutral lipid fatty acid distributions from MPPM most closely resemble those found in skin tissues. Thus, at this point, it will be most reasonable to conclude that the similarities in relative amounts of fatty acids found in neutral lipids from four types of tissue of each meat species are of major significance. These similarities have also been reported by Katz et al. (1966), Lee and Dawson (1973) and Moerck and Ball (1974).

Fatty Acids from Phospholipids

Quantitative evaluation of phospholipid fatty acids of all tissue items showed that palmitic, (C 16:0), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2) and arachidonic acids (C 20:4) are the predominant fatty acids in this fraction (Table 2). These acids accounted for approximately 80 percent of the total fatty acids found in phospholipids. The total polyunsaturated fatty acids ranged from 30 to 50% and arachidonic acid accounted about 30-35% of these polyunsaturated fatty acids. The phospholipid fractions for all tissue samples also contained high levels of polyunsaturated 20 to 24 carbon fatty acids. These characteristics are typical for phospholipid fatty acid distributions and have been previously reported

Table 2a. Fatty acids of the phospholipids from chicken tissues[a]

Fatty Acid [b,c]	MPCM		HDCM		Bone Residue		Skin	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
14:0	0.57 ± 0.07	0.76 ± 0.13	0.60 ± 0.04	0.30 ± 0.05	0.61 ± 0.12	0.71 ± 0.06	0.40 ± 0.08	0.46 ± 0.05
Unknown 1	2.59 ± 0.08	1.61 ± 0.10	2.91 ± 0.09	2.23 ± 0.14	0.31 ± 0.13	1.24 ± 0.19	1.83 ± 0.17	0.17 ± 0.03
16:0	16.04 ± 0.33	16.02 ± 0.21	16.60 ± 0.46	15.61 ± 0.09	17.31 ± 0.25	14.89 ± 0.18	23.11 ± 0.33	21.43 ± 0.20
16:1	1.64 ± 0.08	1.56 ± 0.16	1.86 ± 0.14	1.66 ± 0.13	1.78 ± 0.08	2.08 ± 0.16	1.69 ± 0.04	0.77 ± 0.02
17:0	0.44 ± 0.02	0.27 ± 0.03	0.28 ± 0.01	0.34 ± 0.02	0.24 ± 0.10	0.28 ± 0.06	1.12 ± 0.25	0.58 ± 0.08
Unknown 2	1.20 ± 0.10	0.65 ± 0.11	1.65 ± 0.06	1.11 ± 0.18	t	t	t	t
18:0	14.56 ± 0.26	15.02 ± 0.16	11.13 ± 0.25	14.63 ± 0.13	16.93 ± 0.12	14.81 ± 0.49	19.50 ± 0.51	17.27 ± 0.22
18:1	16.77 ± 0.39	17.44 ± 0.66	14.50 ± 0.39	17.13 ± 0.12	18.00 ± 0.06	16.99 ± 0.13	20.04 ± 0.14	18.69 ± 0.35
18:2	15.84 ± 0.21	17.35 ± 0.26	16.11 ± 0.23	18.76 ± 0.09	15.87 ± 0.17	15.48 ± 0.39	14.91 ± 0.18	16.38 ± 0.08
18:3	0.55 ± 0.04	0.94 ± 0.09	1.04 ± 0.11	0.70 ± 0.10	0.52 ± 0.06	0.88 ± 0.11	0.35 ± 0.07	0.50 ± 0.12
20:0	1.15 ± 0.16	0.43 ± 0.02	0.23 ± 0.02	0.29 ± 0.04	0.27 ± 0.12	0.30 ± 0.04	0.23 ± 0.04	0.58 ± 0.12
20:2	0.54 ± 0.06	0.57 ± 0.09	1.05 ± 0.18	0.25 ± 0.10	0.34 ± 0.09	0.46 ± 0.07	0.20 ± 0.03	0.33 ± 0.09
20:3	2.63 ± 0.12	1.86 ± 0.11	2.95 ± 0.05	1.05 ± 0.08	2.05 ± 0.05	2.80 ± 0.28	2.02 ± 0.33	2.99 ± 0.11
20:4	14.71 ± 0.37	15.99 ± 0.27	14.50 ± 0.31	15.66 ± 0.12	16.02 ± 0.07	18.50 ± 0.20	9.77 ± 0.49	11.29 ± 0.30
20:5	t	t	t	t	t	t	t	t
22:4	5.19 ± 0.15	5.17 ± 0.12	6.43 ± 0.17	5.21 ± 0.18	4.65 ± 0.32	5.84 ± 0.20	2.49 ± 0.25	4.21 ± 0.19
22:5	1.77 ± 0.20	1.29 ± 0.04	2.60 ± 0.11	1.64 ± 0.14	0.93 ± 0.02	0.88 ± 0.06	0.46 ± 0.06	1.05 ± 0.23
22:6	2.44 ± 0.08	1.70 ± 0.13	3.46 ± 0.21	1.90 ± 0.07	1.86 ± 0.10	1.64 ± 0.10	0.83 ± 0.06	1.28 ± 0.10
24:0	t	t	t	t	t	t	t	0.81 ± 0.13
24:2	1.39 ± 0.10	1.35 ± 0.02	2.05 ± 0.19	1.56 ± 0.05	1.68 ± 0.08	1.28 ± 0.08	0.56 ± 0.08	1.01 ± 0.07
Total Saturation	32.76	32.5	28.84	31.17	35.36	30.99	44.36	41.13
Total Unsaturation	63.47	65.22	66.55	65.52	63.70	66.83	53.32	58.5
Unsaturation Ratio	1.94	2.00	2.31	2.10	1.80	2.16	1.20	1.42
Monoene	18.41	19.00	16.36	18.79	19.78	19.07	21.73	19.46
Diene	17.77	19.27	19.21	20.57	17.89	17.22	15.67	17.72
Triene	3.18	2.80	3.99	1.75	2.57	3.68	2.37	3.49
Tetraene	19.90	21.16	20.93	20.87	20.67	24.34	12.26	15.50
Pentaene	1.77	1.29	2.60	1.64	0.93	0.88	0.46	1.05
Hexaene	2.44	1.70	3.46	1.90	1.86	1.64	0.83	1.28

[a]Calculated as percentage of total fatty acids in phospholipids.

[b]Number of carbons: Number of double bonds.

[c]Means and standard deviations from 4 determinations.

Table 2b. Fatty acids of the phospholipids from turkey tissues[a]

Fatty Acid [b,c]	MPTM		HDTM		Bone Residue		Skin	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
14:0	0.43 ± 0.07	0.34 ± 0.03	0.46 ± 0.01	0.36 ± 0.04	0.49 ± 0.02	0.51 ± 0.04	0.54 ± 0.06	0.87 ± 0.20
Unknown 1	1.92 ± 0.19	1.95 ± 0.24	2.53 ± 0.24	3.34 ± 0.03	1.49 ± 0.14	1.45 ± 0.04	0.97 ± 0.07	0.18 ± 0.02
16:0	17.45 ± 0.91	16.59 ± 0.29	15.99 ± 0.09	16.29 ± 0.09	17.02 ± 0.17	15.28 ± 0.39	22.80 ± 0.06	21.78 ± 0.58
16:1	0.84 ± 0.04	1.07 ± 0.02	0.64 ± 0.15	0.62 ± 0.04	1.20 ± 0.01	1.39 ± 0.01	0.79 ± 0.17	0.91 ± 0.21
17:0	0.34 ± 0.01	0.41 ± 0.06	0.54 ± 0.03	0.48 ± 0.04	0.45 ± 0.06	0.54 ± 0.12	0.94 ± 0.15	0.55 ± 0.15
Unknown 2	1.52 ± 0.54	1.53 ± 0.05	1.41 ± 0.07	1.43 ± 0.14	1.49 ± 0.24	1.87 ± 0.45	t	t
18:0	16.01 ± 0.74	15.13 ± 0.07	14.24 ± 0.15	15.12 ± 0.11	15.58 ± 0.11	14.47 ± 0.28	21.37 ± 0.09	22.24 ± 0.52
18:1	16.81 ± 0.54	16.86 ± 0.33	14.90 ± 0.23	16.05 ± 0.09	17.65 ± 0.01	16.16 ± 0.19	20.11 ± 0.13	18.40 ± 0.60
18:2	16.35 ± 0.09	16.42 ± 0.62	17.40 ± 0.25	18.72 ± 0.31	15.93 ± 0.02	15.44 ± 0.22	15.35 ± 0.24	14.67 ± 0.47
18:3	0.70 ± 0.08	0.84 ± 0.06	0.35 ± 0.13	0.40 ± 0.01	0.48 ± 0.11	0.54 ± 0.01	0.52 ± 0.16	0.78 ± 0.10
20:0	0.37 ± 0.06	0.44 ± 0.06	0.37 ± 0.02	0.24 ± 0.06	0.51 ± 0.07	0.51 ± 0.06	0.34 ± 0.16	0.60 ± 0.11
20:2	0.27 ± 0.05	0.32 ± 0.09	0.13 ± 0.007	t	1.38 ± 0.25	1.23 ± 0.24	0.17 ± 0.02	0.30 ± 0.02
20:3	1.08 ± 0.07	1.03 ± 0.05	0.88 ± 0.06	0.48 ± 0.06	0.98 ± 0.10	0.63 ± 0.03	1.56 ± 0.30	2.00 ± 0.21
20:4	15.25 ± 0.58	17.55 ± 0.55	16.44 ± 0.75	17.13 ± 0.15	15.46 ± 0.18	18.42 ± 0.35	9.85 ± 0.16	10.98 ± 0.09
20:5	t	t	0.57 ± 0.14	0.51 ± 0.10	t	t	t	0.26 ± 0.001
22:4	4.33 ± 0.27	4.81 ± 0.10	4.22 ± 0.15	3.38 ± 0.07	4.75 ± 0.02	6.38 ± 0.39	2.62 ± 0.15	2.29 ± 0.23
22:5	1.98 ± 0.25	1.44 ± 0.01	2.74 ± 0.03	1.57 ± 0.07	1.85 ± 0.13	1.54 ± 0.12	0.41 ± 0.11	0.73 ± 0.24
22:6	3.22 ± 0112	2.45 ± 0.28	4.80 ± 0.06	3.22 ± 0.04	1.89 ± 0.08	1.66 ± 0.12	1.10 ± 0.15	1.27 ± 0.15
24:0	t	t	t	t	t	t	t	0.80 ± 0.03
24:2	1.13 ± 0.08	0.85 ± 0.15	1.42 ± 0.06	0.64 ± 0.06	1.12 ± 0.03	1.52 ± 0.19	t	0.43 ± 0.03
Total Saturation	34.60	32.90	31.53	32.49	34.05	31.31	45.99	46.84
Total Unsaturation	61.96	63.64	64.49	62.72	62.69	64.91	52.48	53.02
Unsaturation Ratio	1.79	1.93	2.05	1.93	1.84	2.07	1.14	1.13
Monoene	17.65	17.93	15.54	16.67	18.85	17.55	20.9	19.31
Diene	17.75	17.59	18.95	19.36	18.43	18.19	15.52	15.40
Triene	1.78	1.87	1.23	0.88	1.46	1.17	2.08	2.78
Tetraene	19.58	22.36	20.66	20.51	20.21	24.80	12.47	13.27
Pentaene	1.98	1.44	2.74	1.57	1.85	1.54	0.41	0.73
Hexaene	3.22	2.45	4.80	3.22	1.89	1.66	1.10	1.27

[a]Calculated as percentage of total fatty acids in phospholipids.

[b]Number of carbons: Number of double bonds.

[c]Means and standard deviations from 4 determinations.

by various workers (Marion and Woodroof, 1966; Katz et al., 1966; Lee and Dawson, 1973 and Moerck and Ball, 1974).

There were no apparent variations in the fatty acid compositions of light and dark tissue phospholipids. Slightly higher quantities of arachidonic acids and slightly lower quantities of palmitic acids were found in almost all the phospholipids from dark tissues when compared to those from the light ones. Most of the dark tissue phospholipids also had slightly more unsaturated molecules than their corresponding phospholipids from light tissues. The only exceptions are phospholipids of hand deboned meat and of turkey skin, where these are nearly equal. In general, the relative distribution of fatty acids in phospholipids of these two types of tissue are almost identical. These results are true for all types of tissue items examined.

The greatest difference between phospholipid and neutral lipid fractions were the high levels of C20 to 24 polyunsaturated fatty acids and low levels of oleic acid and linoleic acids in phospholipids. These results corroborate previous findings by Marion and Woodroof (1966), Katz et al. (1966), and Lee and Dawson (1973). Moerck and Ball (1974) explained that, since arachidonic acids found in poultry meat were synthesized from linoleic acids, the phospholipid fractions which were higher in arachidonic acids thus, were lower in linoleic acid than the triglyceride fractions.

The most obvious difference among phospholipids from different types of tissue are the quantities of arachidonic acids and the levels of the total polyunsaturated 20 to 24 carbon fatty acids. The percentage of arachidonic acid is lowest in phospholipid of skin tissues. This result is true for both chicken and turkey. Unusually

high levels of arachidonic acid were found in phospholipids from bone residues of dark meat of both chicken and turkey. Dietary fat might be responsible for this result. As mentioned in the literature review section, many workers have found that dietary fats affected composition of poultry body fats. Thus, a high percentage of unsaturated molecules might be expected in fat from birds fed with diets containing high levels of unsaturated fats.

The C20 to 24 polyunsaturated fatty acids were lowest in skin tissues, highest in hand deboned tissues and comparable between machine processed meats and bone tissues. The low percentages of polyunsaturated C20 to 24 fatty acid in skin tissues accounted for the high levels of saturated fatty acids found in this tissue. As is evident from the table, highest levels of palmitic and stearic acids were found in skin phospholipids of both chicken and turkey.

Unlike tetraenes, the quantities of pentaenes and hexaenes were highest in hand deboned tissue phospholipids, instead of bone or machine processed tissues. These results, although not expected, can be reasonably explained. Both machine processed meats and bone samples were ground and subjected to high tension treatments during the deboning process. According to Holman (1954), the increase in each double bond in a polyunsaturated fatty acid could increase the rate of autoxidation by a factor of 2. Thus, during the processing period, followed by subsequent handling and storing, pentaenes and hexaenes which are most vulnerable to oxidative attack might undergo oxidation. As a result, lower levels of these acids were then quantitated at time of analyses.

When comparing the unsaturation ratios of all types of tissues,

it is obvious that, except for skin tissue, there are small differences among phospholipid fatty acids of MPPM, HDPM and bone tissues. These results, as well as relative quantity of individual major fatty acids, found in each tissue, suggest that fatty acid distributions in phospholipid fractions of MPPM resemble more those of the hand deboned meats or bone tissues than skins.

Classifications and Quantitations of Phospholipids

Total phospholipid phosphorus compounds including their classifications and quantities are shown in Table 3.

Light hand deboned chicken and turkey lipids contained the highest amount of total phospholipids, and the smallest values were found in skins from dark meat from both species. For all tissues investigated, lipids from light tissues contained a higher proportion of phospholipids than those of the dark ones. This result corroborates those findings by Katz et al. (1966).

Five classes of phospholipids were identified and quantified from chicken and turkey tissues. Phosphatidylcholine, phosphatidylethanolamine, sphingolipid and phosphatidylserine are predominant components of muscle and bone phospholipids and comprise approximately 90% of total phospholipids. Traces of lysophosphatidylcholine were also found in these tissue phospholipids. Skin phospholipids, on the other hand, was found to be richer in lysophosphatidylcholine and only traces of phosphatidylserine were detected in skin phospholipids of both chicken and turkey. Davidkova and Khan (1967) and Lee and Dawson (1973) reported similar findings for chicken muscle and skin phospholipids. The most prevalent of the phospholipids found in all types of tissue was phosphatidylcholine. This component accounted for

Table 3. Total phospholipids and phospholipid classes from chicken and turkey tissues

Phospholipid Class	MPM[b]		HDM[c]		Bone		Skin	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Chicken	Phospholipid Phosphorus (mg/g fat)							
Total phospholipids[a]	2.18 ± 0.05	0.98 ± 0.02	13.95 ± 0.65	6.12 ± 0.20	2.23 ± 0.01	1.13 ± 0.08	0.83 ± 0.01	0.42 ± 0.006
Lysophosphatidylcholine	t	t	t	t	t	t	0.02	0.02
Spingomyelin	0.22	0.12	0.82	0.32	0.22	0.08	0.09	0.10
Phosphatidylcholine	1.10	0.50	7.09	2.73	1.20	0.63	0.43	0.17
Phosphatidylserine	0.08	0.02	0.31	0.14	0.07	0.02	t	t
Phosphatidylethanolamine	0.59	0.35	4.45	2.39	0.52	0.32	0.23	0.09
Turkey								
Total phospholipids[a]	1.88 ± 0.03	1.13 ± 0.06	8.56 ± 0.40	4.24 ± 0.05	1.82 ± 0.02	1.69 ± 0.03	0.60 ± 0.02	0.36 ± 0.003
Lysophosphatidylcholine	t	t	t	t	t	t	0.09	0.03
Sphingomyelin	0.20	0.12	0.60	0.23	0.15	0.14	0.09	0.05
Phosphatidylcholin	0.94	0.51	5.22	2.35	0.80	0.82	0.26	0.17
Phosphatidylserine	0.11	0.05	0.18	0.18	0.08	0.09	t	t
Phosphatidylethanolamine	0.44	0.30	1.85	1.01	0.59	0.52	0.17	0.08

[a]Means and standard deviations from 4 determinations.

[b]Mechanically processed meats.

[c]Hand deboned meats.

from 40 percent (dark chicken skin) to 60 percent (light HDCM) of the total phospholipid contents. The next major component found in almost all the tissues is phosphatidylethanolamine. The only exception is the phospholipids of skin from dark meat of chicken where the quantities of its sphingolipid and phosphatidylethanolamine are almost equal. Light and dark tissue phospholipids within and among bird species showed similar trends for both compositions and relative quantities of the major phospholipid classes.

It is evident from table 3, that the total phospholipid contents of both MPCM and MPTM were intermediate between those of bone and skin tissue or most resemble those from bone tissue, when compared among the three. Quantitation of phospholipid classes also support these results. Except for sphingolipids from dark MPCM, the quantities of phosphatidylcholine, phosphatidylethanolamine and sphingolipid of both MPCM and MPTM were found to be somewhat similar to those of bone tissues rather than the hand deboned or skin tissues.

Cholesterol

Total cholesterol values for all tissues are given in Table 4. An example of a chromatogram for total cholesterol is given in Appendix C. Mean values are given in mg per 100 g fat for tissue correlation purposes and in mg per g tissues to compare with previously reported results by other researchers.

Total cholesterol contents for light HDCM, dark HDCM, light chicken skin and dark chicken skin are 42.8, 70.4, 75.03, and 91.01 mg/100 g tissues respectively. These values were found to be lower than those reported by Mickelberry et al. (1964, 1966) who reported

Table 4. Total cholesterol[a] content of chicken and turkey tissues

Meat Type	MPM[b]		HDM[c]		Bone		Skin	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Chicken								
mg cholesterol/g fat	5.40 ± 0.14	5.23 ± 0.14	17.90 ± 0.42	11.50 ± 0.57	13.50 ± 0.14	10.10 ± 0.29	2.03 ± 0.03	2.30 ± 0.03
mg cholesterol/100 g tissue	73.50 ± 2.12	110.00 ± 4.07	42.80 ± 1.82	70.40 ± 2.26	101.25 ± 1.76	147.00 ± 4.22	75.03 ± 1.24	99.01 ± 1.19
Turkey								
mg cholesterol/g fat	7.65 ± 0.07	5.75 ± 0.21	16.40 ± 0.28	11.75 ± 0.35	12.70 ± 0.28	8.85 ± 0.07	1.38 ± 0.02	1.79 ± 0.04
mg cholesterol/100 g tissue	80.72 ± 0.99	114.38 ± 4.41	41.83 ± 2.04	55.76 ± 0.84	75.50 ± 0.71	98.33 ± 0.78	53.00 ± 0.77	72.03 ± 1.60

[a]Means and standard deviations from 4 determinations.

[b]Mechanically processed meat.

[c]Hand deboned meat.

the values of 57.7, 82.6 and 109 mg/100 g for chicken breast, thigh and skin tissues. Sweeney and Weihrauch (1976) however, clearly indicated that dietary fats and age of the bird influence cholesterol content of the bird tissues. In addition to these biological factors, methods for determinations also affect final cholesterol values. Thus, large variations can be expected from different laboratories. These explanations are also true for turkey tissues. Cholesterol contents of turkey tissue found in this study were: 41.8, 55.8, 53 and 72 mg/100 g for light meat, dark meat, light skin and dark skin while those obtained by Neudoerffer and Lea (1968) were: 84 mg/100 g for breast meat and 116 mg/100 g for dark meat.

Light tissue lipids from both turkey and chicken contained higher levels of cholesterol than dark tissue lipids, when reported as mg cholesterol per g fat. However, when the values were calculated on tissue weight basis, dark tissues contained higher levels of cholesterol than their corresponding light ones. Differences in total lipid contents between light and dark tissues are responsible for these results. As shown in Appendix D, dark tissue, in general had about twice as much lipid as did white tissues.

From data shown in Table 4, it is apparent that sources of cholesterol in MPPM come from their three component tissue cholesterols. Cholesterol contents (mg/g fat) of both MPCM and MPTM are lower than those found in their corresponding HDM and bone tissues but higher than those of skins. For MPCM, the values obtained are closer to those of skin rather than hand deboned meat or bone tissues. Thus from this result alone it seems appropriate to conclude that a greater amount of fat from skin enters into MPCM during the

machine deboning processes. For turkey, cholesterol content per g of MPTM fats more closely resembled those from bone fats when compared among the three types of tissue. So, at this point, the conclusion is that bone lipid cholesterol appears to have a significant contribution to cholesterol content of MPTM.

Free cholesterol contents of MPCM and MPTM are reported in Table 5. An example of a chromatogram for free cholesterol is given in Appendix E. It is apparent that most of the cholesterol in both chicken and turkey tissues are in the form of free cholesterol. This finding coroborates the work of Mickelberry et al. (1964) and Moerck and Ball (1974). For MPCM only about 6-8% of their total cholesterol was in the form of cholesterol esters. Higher levels of ester forms are found in MPTM. For this particular meat species, approximately 12-13% of the total cholesterol was cholesterol esters in their tissue lipids.

Correlations

An attempt was made to correlate quantities of cholesterol, phospholipid and fatty acid distribution profile found in each type of mechanically processed meat with those found in their composite tissues. Various proportions of meat lipid:bone lipid:skin lipid which were likely to be representative models for MPPM lipids were set up. Calculations were made in accordance with each set of the hypothesized proportion to obtain the final quantities of cholesterol, phospholipids (total and fractions) and fatty acid compositions in mechanically processed meat lipids. It was found that a model of meat fat:bone fat:skin fat of 1:3:6 seemed to be most appropriate

Table 5. Free cholesterol[a] content of mechanically processed chicken and turkey meats

Meat Type	Total Cholesterol		Free Cholesterol	
	Light	Dark	Light	Dark
MPCM				
mg cholesterol/g fat	5.40 ± 0.14	5.23 ± 0.14	5.05 ± 0.24	4.79 ± 0.20
mg cholesterol/100 g tissue	73.50 ± 2.12	110.00 ± 4.07	68.53 ± 3.95	105.00 ± 4.20
MPTM				
mg cholesterol/g fat	7.65 ± 0.07	5.75 ± 0.21	6.68 ± 0.12	5.07 ± 0.09
mg cholesterol/100 g tissue	80.72 ± 0.99	114.38 ± 4.41	70.79 ± 1.42	102.67 ± 2.17

[a]Means and standard deviation from 4 determinations.

for MPCM fats based on fat contributed by each tissue. For turkey, a slightly different proportion was concluded. A value of 1:4:5 meat fat:bone fat:skin fat was hypothesized as MPTM lipid model. Comparisons of calculated and analyzed total cholesterol and total phospholipid contents of lipids from both meat species are shown in Table 6.

Table 6. Comparisons of analyzed values and calculated values of total cholesterol and total phospholipid contents from mechanically processed chicken and turkey meats

Meat Types	Total Cholesterol (mg/g fat)		Total Phospholipids (mg/g fat)	
	Calculated Values	Analyzed Values	Calculated Values	Analyzed Values
MPCM				
Light	5.56	5.40	2.55	2.18
Dark	5.40	5.23	1.20	0.98
MPTM				
	7.40	7.65	1.88	1.87
Light	5.60	5.75	1.28	1.13
Dark				

From previous findings and discussions, conclusions were drawn as follows:

1) There was little difference among the fatty acid components of neutral lipid from various tissue samples.
2) Phospholipid fatty acid components of MPPM resembles the fatty acids of bone or hand deboned meat phospholipids more than those from skin phospholipids.

3) Total phospholipid contents and quantity of each phospholipid class in MPPM are most similar to those from bone tissues.

4) Cholesterol contents of MPPM resembles the cholesterol contents of skin tissues or bone tissues more closely than that from muscle tissues.

In contrast to the hypothesized models, the above conclusion indicated that, except for neutral lipid fatty acids, bone tissue lipids seem to have a predominant effect on MPPM lipid composition. However, as evident from data shown in Table 6, total cholesterol and total phospholipid contents of both MPCM and MPTM lipids are in good agreement with their corresponding hypothesized values. For phospholipid classes and phospholipid fatty acids of MPCM and MPTM, although the actual findings seem to be contrary to the hypothesized models, they are explainable. Although it was hypothesized that 50-60% of fats in MPPM are from skin fats, skin fats contain the lowest levels of phospholipids. Thus, even if 5-6 times as much skin fat as meat fat did enter into the machine processed meat fats, the proportion of skin phospholipids in the resulting MPPM phospholipids were still lower than those from either the hand deboned meat or bone tissue. As a result, the MPPM phospholipid fatty acids and their phospholipid classes resemble most the bone and hand deboned tissue phospholipids or bone phospholipids instead of skin phospholipids.

According to the suggested models fatty acid composition of MPPM neutral lipids should resemble most those found in their corresponding skin neutral lipids. However, as already mentioned in the

previous discussion little differences were shown among neutral lipid fatty acids from various tissues within each bird species. Thus, specific conclusions can not be drawn with confidence for this particular lipid component.

Part II. Stability of MPPM

A. Effects of Inert Gas and Vacuum Packaging on Storage Stability of Mechanically Processed Poultry Meats

Mechanically processed chicken and turkey meats (MPCM and MPTM) were individually mixed under inert atmospheres, packed along with either N_2 or CO_2 gases or under vacuum and frozen at -18°C, immediately after packaging or after holding for 72 hrs. at 4°C. Samples were stored up to 4 months at -18°C. For each evaluation period, frozen samples were randomly selected, partially thawed at room temperature and prepared for chemical evaluations. This experiment was designed to study storage stability of mechanically processed poultry meat (MPPM), under different types of microenvironmental atmospheres. Along with the above factor, the effect of prefreezing hold time on subsequent storage stability of MPPM was observed. The specific purpose of this study was to evaluate the influences of packaging methods and storage conditions on oxidative rancidity development in MPPM, in order to develop practical approaches to aleviate stability problems.

Changes in Fatty Acid Composition

Mean values for fatty acid unsaturation ratios of both MPCM and MPTM are presented in Table 7. Statistical analyses of these data are shown in Tables 7and 11. Fatty acid distribution profiles for MPCM and MPTM phospholipids before and after 4 months storage at -18°C are shown in Appendices F and G.

Earlier studies by many researchers revealed that the neutral lipid fraction of various kinds of meat including poultry, oxidized very slowly compared to phospholipids (El Gharbawi and Dugan, 1963;

Table 7. Mean unsaturation ratios[1] and their Tukey separations[2] for MPCM and MPTM packed under CO_2 or N_2 or vacuum and stored at -18°C for 4 months.

Meat Type	Treatment	Storage time (mo.)			
		0	2	3	4
		Unsaturation Ratio			
MPCM	Immediate freezing				
	CO_2	2.15^{a}	1.68^{a}	0.88^{a}	0.73^{a}
	N_2	2.05^{ab}	1.69^{a}	0.84^{a}	0.69^{b}
	Vacuum	2.18^{a}	2.06^{b}	1.04^{b}	1.18^{b}
	72 hrs. prefreezing hold time				
	CO_2	1.86^{a}	1.25^{ba}	0.91^{a}	0.52^{ba}
	N_2	1.78^{a}	1.41^{b}	0.74^{a}	0.75^{b}
	Vacuum	1.80^{a}	1.63^{b}	0.92^{a}	0.93^{b}
MPTM	Immediate freezing				
	CO_2	1.53^{a}	1.05^{a}	0.80^{a}	0.54^{a}
	N_2	1.56^{a}	1.18^{b}	0.81^{a}	0.85^{b}
	Vacuum	1.60^{a}	1.14^{a}	0.95^{b}	0.93^{b}
	72 hrs. prefreezing hold time				
	CO_2	1.56^{a}	0.91^{a}	0.87^{a}	0.37^{a}
	N_2	1.56^{a}	0.97^{ab}	0.88^{a}	0.74^{b}
	Vacuum	1.60^{a}	0.82^{a}	0.65^{b}	0.71^{b}

[1]Mean of 2 replicates, expressed as polyunsaturated, C 18:3 to 22:6/palmitic acid.

[2]Comparison among packaging treatments of each storage interval and freezing treatment. Like letters among treatments within a column denote no significant difference (P = 0.05).

Keskinel et al., 1964; Lee and Dawson, 1973 and Moerck and Ball, 1974). Special attention is thus spent only on the changes in fatty acid composition of phospholipid fractions. It was evident from the fatty acid composition of meat from all treatments after storage that hexaenoic, pentaenoics, tetraenoics and trienoics were the major substrates of oxidative deterioration in both species of meat. Hence, the progress of autoxidation of polyunsaturated 18:3 to 22:6 carbon fatty acids were selected as the indices to follow autoxidative deterioration of MPPM phospholipids. Using palmitic acid as the stable component, the unsaturation ratios were calculated from total percentage area of polyunsaturated 18:3 to 22:6 carbon fatty acids over percentage area of palmitic acid. Changes in the unsaturation ratios for both MPCM and MPTM are graphically presented in Figures 1 and 2.

Three way analysis of variance for this particular test showed a significant treatment effect for all 3 main factors, both in MPCM and MPTM. Significant interactions were also observed in MPCM between freezing-storage and gases-storage and for all types of 2 and 3 way interactions for MPTM.

Total lipid content of mechanically processed meat samples for this study were: 17.29% of wet tissue for MPCM and 16.98% of wet tissue of MPTM. It is apparent from the data that unsaturation ratio for all samples decreased as storage time increased. For MPCM, there appeared to be an induction period between 0 and 2 months of storage since the unsaturation ratios of most samples declined slowly during this time. This induction period was then followed by an accelerated rate of autoxidation which continued to about 3 months, and slowed after the third month. These autoxidative trends were

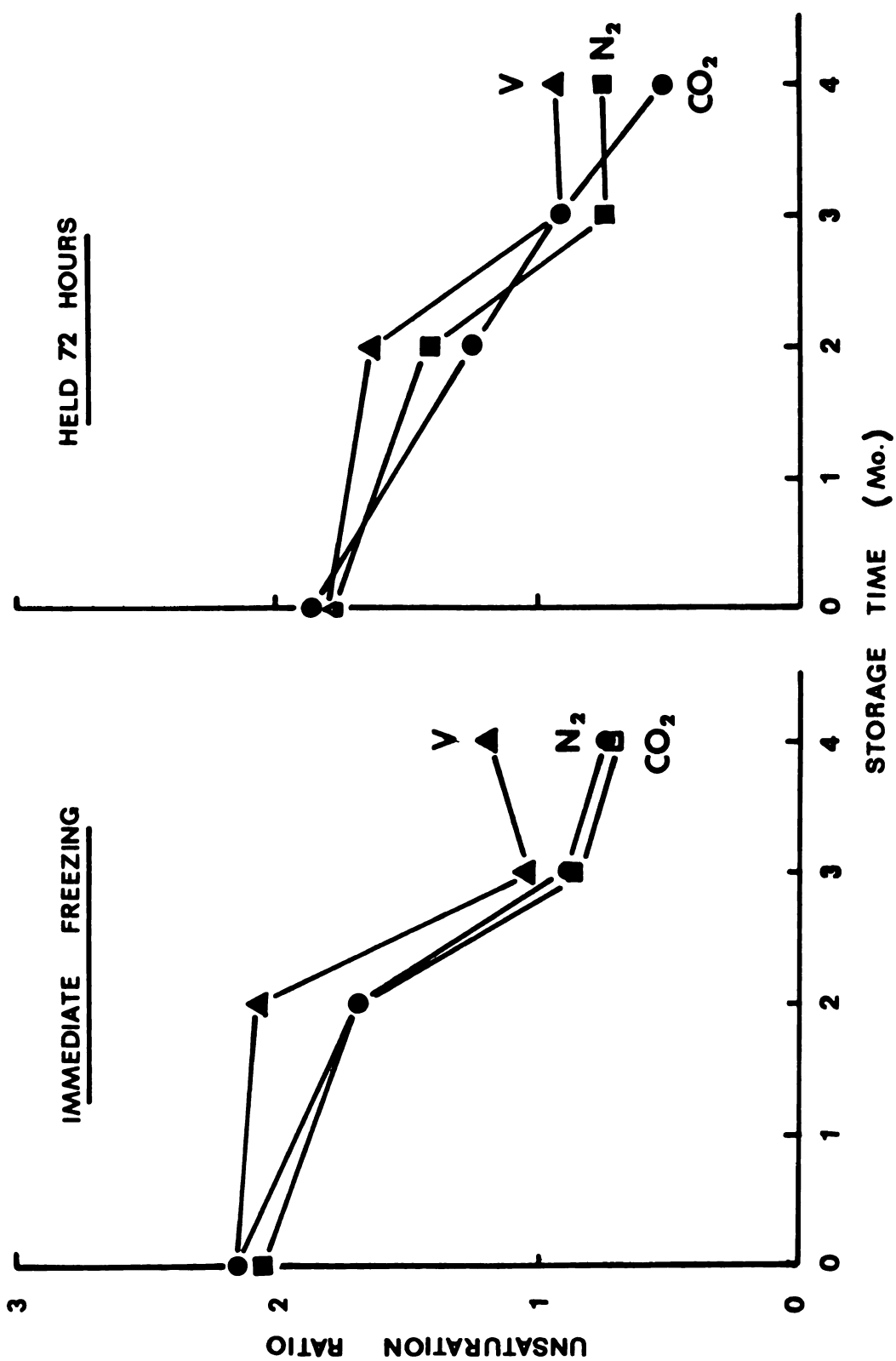


Figure 1. Oxidation of C 18:3 to 22:6 polyunsaturated fatty acids in phospholipids of MPCM packed under N_2 or CO_2 or vacuum and stored at -18°C, either immediately after treatments or after 72 hrs. holding at 4°C.

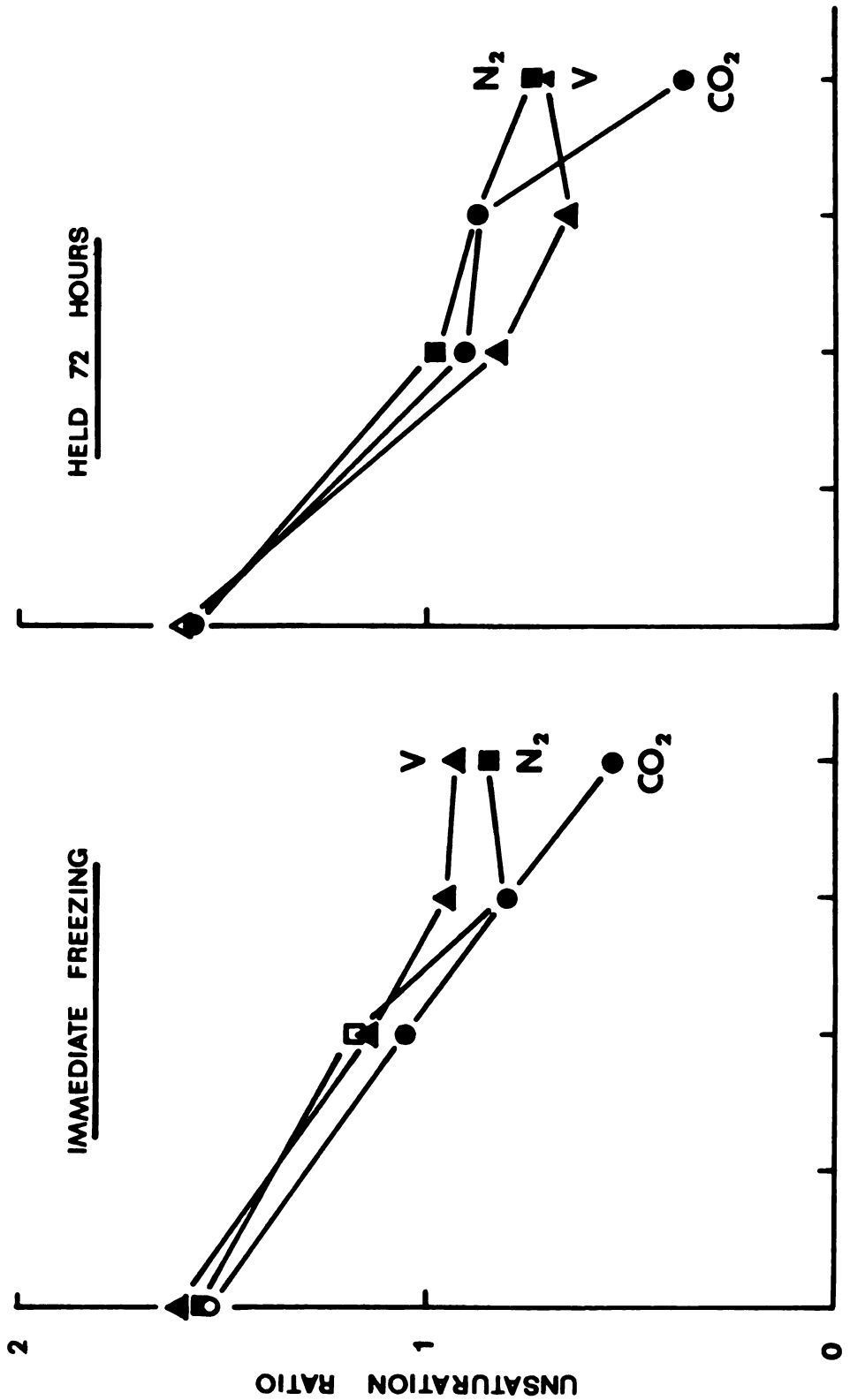


Figure 2. Oxidation of C 18:3 to 22:6 polyunsaturated fatty acids in phospholipids of MPTM packed under CO_2 or N_2 or vacuum and stored at -18°C, either immediately after treatments or after 72 hrs. holding at 4°C.

not observed in MDTM phosphospholipids. No apparent induction periods for the autoxidation which resulted in the decrease in unsaturation ratios were found in MDTM samples. Decreases in unsaturation ratios were rather steady for most of the storage period. A slightly lower decreasing rate was observed in some treatments, during later storage periods.

Samples packed under vacuum had higher numbers of unsaturated molecules (C 18:3 - C 22:6) in their phospholipids when compared with those found in samples packed in N_2 or CO_2, at the end of storage period. The only exception was the vacuum packed MDTM samples which were held 72 hrs at 4°C before freezing. For these samples, unsaturation ratios were almost equal to those of the samples packed under N_2. These observations are supported by statistical analyses. Tukey multiple comparisons of the means for MDCM samples indicate that at the 5% level, the average mean values for vacuum treated sample was different from the average means of the other two treatments, at the fourth month of storage. These results are true for both freezing treatment groups of MDCM. For MDTM the advantage of vacuum storage was shown only in the samples which were frozen without delay. In most of the samples, CO_2 packaging was found to be the least advantageous when compared among three types of packing, at the fourth month of storage.

Visual observations of the meat packages during frozen storage revealed water losses from the surface of meat samples which were stored under N_2 or CO_2. Gas packed products in general, have some space left between surface of the meat and the film, whereas in vacuum packed products, the surface of the meat adhered thoroughly

to the surface of the packages. Losses of water from the surface of meat packed under N_2 or CO_2 could occur as a result of exchanges in microclimatic water vapor between meat and voids between surface of meat and packaging film. Correlation of desiccation or "freezer burn" with oxidative rancidity and discoloration of meat has been observed earlier by Ramsbottom (1947) and Steinberg et al. (1949). Watts (1954) stated that a lowering of the meat pH can occur as a result of CO_2 packing. Oxidation of reduced myoglobin and hemoglobin pigments to their oxidized form could be accelerated by this acid pH. The ferric heme pigments have been reported to be active biocatalysts in initiating lipid oxidation in meats (Tarladgis, 1961 and Watts, 1961). This could explain why vacuum packed samples are better than those packed in N_2 and CO_2 with respect to oxidation of unsaturated fatty acid molecules, and why the unsaturation ratio of phospholipids from samples packed under CO_2 are the lowest among the three, at the end of the storage period.

Holding meat samples at 4°C for 72 hrs. prior to freezing resulted in a significant increase in the development of lipid oxidation. As evident from the data (Table 1) and statistical analyses, unsaturation ratios of both MPCM and MPTM phospholipids from most treatments are lower in samples which had a delaying time between processing and freezing (followed by frozen storage), when compared with another group which was frozen immediately after packaging. As previously pointed out in Part I of this study, autoxidation of highly unsaturated fatty acids found in the phospholipid fraction of MPPM might have already been initiated by the machine deboning process. Subsequent holding of the meat below freezing temperature hence,

could result in a favorable condition for further autoxidative deterioration of these fatty acids. Vacuum packaging or packaging of the meat under inert atmosphere during these holding periods might partially minimize some of the oxidation reactions. However, considering other types of prooxidants which are naturally present in the meat as well as atmospheric O_2 which was incorporated into the meat tissues during the deboning process, it is obvious that oxidative degradation of polyunsaturated fatty acids of meat samples stored under vacuum or inert gasses was not eliminated completely.

2-Thiobarbituric Acid (TBA) Tests

In order to determine if the changes in polyunsaturated fatty acids were attributable to oxidation, TBA tests were used for numerically assessing the degree of lipid oxidation in MPPM samples during frozen storage. The mean TBA numbers are reported in Table 8, and statistical analyses are summarized in Tables 8 and 11. Figure 3 and 4 show graphic presentation of the mean TBA numbers for MDCM and MDTM respectively.

Analyses of variance showed significant effects for all of the main treatments. Significant interactions were also found in freezing-storage and packing-storage of MDCM and for all 2 and 3 way interactions of MDTM.

Small differences in TBA numbers were found among variously treated MPCM samples between 0 and 2 months of storage. Significant increases in TBA numbers of these samples were evident at the end of 4 months of storage. As previously shown in the changes in unsaturation ratios, there appeared to be induction periods of about 2 months

Table 8. Mean TBA numbers[1] and their Tukey separations[2] for MPCM and MPTM packed under CO_2 or N_2 or vacuum and stored at -18°C for 4 months.

Meat Type	Treatment	Storage time (mo.)			
		0	2	3	4
		TBA numbers			
MPCM	Immediate freezing				
	CO_2	0.86^{a}	1.18^{a}	4.40^{a}	4.24^{a}
	N_2	1.02^{a}	0.83^{a}	1.58^{b}	1.81^{b}
	Vacuum	0.85^{a}	0.92^{a}	1.57^{b}	1.24^{b}
	72 hrs. prefreezing hold time				
	CO_2	0.83^{a}	2.47^{a}	4.63^{a}	5.09^{a}
	N_2	1.23^{a}	1.32^{b}	1.47^{b}	2.33^{b}
	Vacuum	1.23^{a}	1.20^{b}	1.71^{b}	2.19^{b}
MPTM	Immediate freezing				
	CO_2	4.08^{a}	6.95^{a}	8.55^{a}	9.23^{a}
	N_2	3.97^{a}	6.05^{b}	7.43^{ba}	5.85^{b}
	Vacuum	3.68^{a}	5.63^{b}	6.56^{b}	6.39^{b}
	72 hrs. prefreezing hold time				
	CO_2	5.37^{a}	8.21^{a}	9.58^{a}	7.68^{a}
	N_2	4.60^{ab}	6.39^{ba}	6.47^{ba}	8.54^{b}
	Vacuum	4.26^{b}	5.27^{b}	4.44^{b}	7.45^{a}

[1]Mean of 2 replicates within 8 determinations expressed as mg. TBA reactive substance per 1000 g meat.

[2]Comparison among packaging treatments of each storage interval and freezing treatment. Like letters among treatments within a column denote no significant difference (P = 0.05).

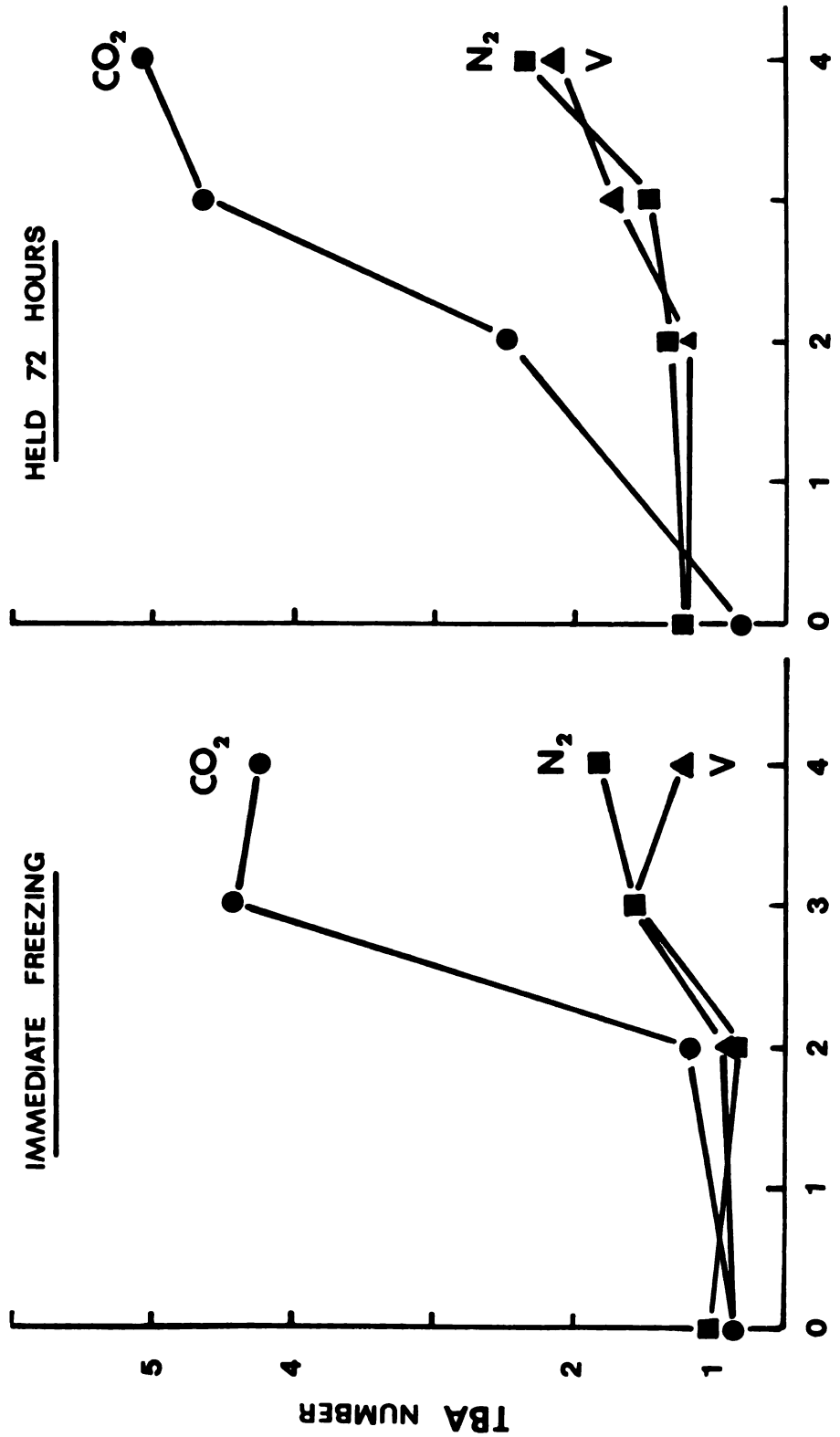


Figure 3. TBA numbers of MPCM packed under CO_2 or N_2 or vacuum and stored at -18°C, either immediately after treatments or after 72 hrs. holding at 4°C.

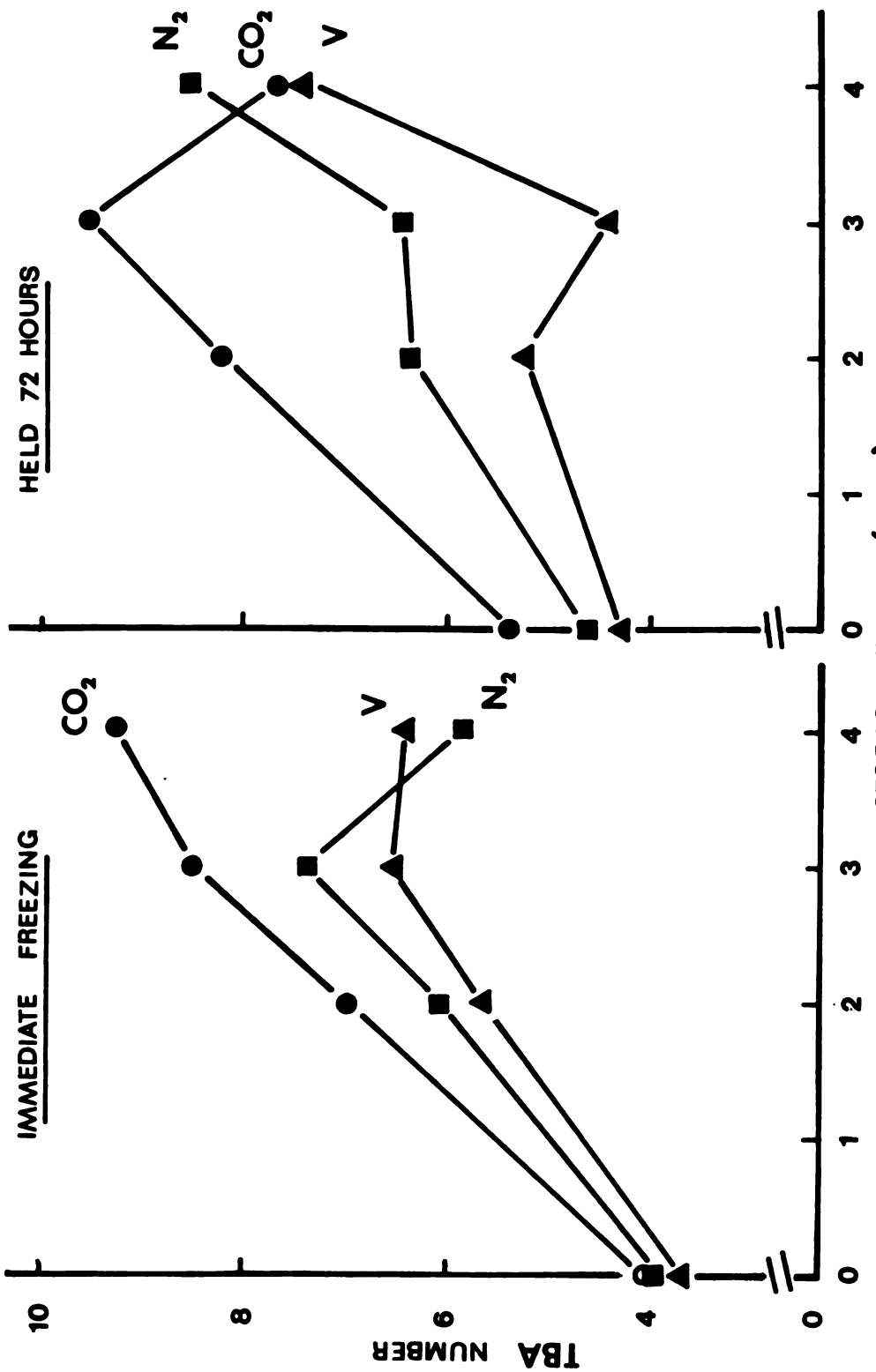


Figure 4. TBA numbers of MPTM packed under CO_2 or N_2 or vacuum and stored at -18°C, either immediately after treatments or after 72 hrs. holding at 4°C.

during which the rate of autoxidation was quite slow. High initial TBA numbers were observed in all treatments of MPTM. Unlike MPCM lipids, there was no obvious induction period of autoxidation shown in any treatment of MDTM. Rapid increases in TBA numbers were found in most of the treatments during freezing and subsequent storage. At the end of 4 months storage, significant effects of frozen storage on TBA changes were shown in all treatments of MPTM.

Tukey comparisons of mean TBA numbers for MPCM revealed that samples stored under CO_2 develop significantly higher TBA numbers than those stored under vacuum or in N_2, at the end of the third and fourth month of storage. This significant difference was shown as early as during the second month of storage for MPCM samples which were held at 4°C for 72 hrs. before freezing. However, there was no significant advantage of vacuum packing over N_2 packing at the end of storage for this particular meat. TBA numbers of MPTM stored under CO_2 markedly exceeded those of the meat samples stored under vacuum or N_2. These results were especially obvious for MPTM which was held at refrigerator temperature before freezing. Significant differences between TBA numbers of samples packed under CO_2 and those stored under vacuum and N_2 were observed as early as at the end of the second month of storage. The benefit of vacuum packaging over N_2 and CO_2 packaging was shown in both groups of MPTM samples at the end of the third month. However, these advantages were not significant at the end of the storage period.

For samples which were frozen with and without delay, a highly significant difference in TBA numbers was observed between these two groups. In both MPCM and MPTM, mean TBA numbers of all samples

frozen immediately after packing were lower than those found in samples which were held 72 hrs. at 4°C before freezing. This result was obviously shown in MPCM at the end of 4 months of storage, and at the third month for MPTM.

Correlation of Unsaturation Ratios and TBA Numbers

In order to determine if a relationship existed between phospholipid fatty acid oxidation and TBA numbers, the mean unsaturation ratios were correlated with the mean TBA numbers across storage time. The results are presented in Table 11.

High correlation coefficients were found for most samples packed under CO_2. The only exception is that of MPTM which was held 72 hrs. at 4°C before freezing, where low correlation ($r = 0.61$) was observed. All samples packed under N_2 showed good correlation coefficients between oxidation of polyunsaturated (18:3 - 22:6) fatty acid in their phospholipids and the development of TBA reactive substances ($r = 0.82 - 0.92$), during frozen storage. The only vacuum packed sample which failed to show a good correlation between these 2 tests was vacuum packed MPTM samples which were held 72 hrs. at 4°C before freezing ($r = 0.48$).

Low correlations were, in general, found between those groups of samples in which either the TBA numbers or unsaturation ratio fluctuated highly during frozen storage. For example, CO_2 packed MPTM sample which was held 72 hrs. before freezing storage developed TBA numbers as high as 9.58 mg TBA reactive substance per 1000 g of meat at the third month of storage and then dropped to 7.68 mg TBA reactive substance per 1000 g of meat at the end of the fourth

Table 9. Correlation coefficients between unsaturation ratios and TBA numbers

Sample Treatment	Correlation Coefficient
MPCM	
Immediate freezing	
CO_2	-0.97
N_2	-0.92
Vacuum	-0.95
72 hrs. prefreezing hold time	
CO_2	-0.97
N_2	-0.82
Vacuum	-0.89
MPTM	
Immediate freezing	
CO_2	-0.98
N_2	-0.87
Vacuum	-0.99
72 hrs. prefreezing hold time	
CO_2	-0.61
N_2	-0.91
Vacuum	-0.48

month.

Although there were few treatments which failed to exhibit high correlation coefficients between the two tests, correlation coefficients obtained from the majority of the treated MPTM and MPCM samples led to the conclusion that there is a relationship between oxidation of polyunsaturated fatty acids found in phospholipid fraction of MPCM and MPTM and the developments of TBA reactive substances in the meat sample during frozen storage. These relationships have been previously reported in lipids from MPCM, by Moerck and Ball (1974).

Total Phospholipid Phosphorus

The mean total phospholipid phosphorus values for MPCM and MPTM samples are presented in Table 10, and are graphically illustrated in Figures 5 and 6. Statistical analyses of these data are shown in Tables 10 and 11.

As evident from the data and graphic presentation, a considerable decrease in the total phospholipid contents were apparent in samples from all treatments as frozen storage time of the meat progressed. Analyses of variance also indicated significant effects of storage on changes in phospholipids from both MPCM and MPTM samples. A slight decline in the phospholipid contents of samples from all treatments was evident during the first 2 months of storage, then a high loss was shown in samples from most treatments between 2 and 3 months to 4 months of storage.

At the end of the storage period, MPCM samples packed under N_2 or CO_2 showed slightly higher phospholipid losses than those samples packed under vacuum. This trend was observed in both freezing groups

Table 10. Mean total phospholipid phosphorus[1] and their Tukey separations[2] for MPCM and MPTM packed under CO_2 or N_2 or vacuum and stored at -18°C for 4 months.

Meat Type	Treatment	Storage Time (mo.)			
		0	2	3	4
		Phospholipid P (mg/g fat)			
MPCM	Immediate freezing				
	CO_2	1.23	1.07	0.87	0.53
	N_2	1.13	1.05	0.79	0.56
	Vacuum	1.13	0.96	0.73	0.65
	72 hrs. prefreezing hold time				
	CO_2	1.01	1.00	0.77	0.43
	N_2	0.99	0.96	1.06	0.49
	Vacuum	0.97	1.07	0.90	0.96
MPTM	Immediate freezing				
	CO_2	1.08^{a}	0.90^{a}	0.68^{a}	0.30^{a}
	N_2	1.10^{a}	1.05^{b}	0.87^{ba}	0.43^{ba}
	Vacuum	1.12^{a}	0.99^{b}	0.95^{b}	0.86^{b}
	72 hrs prefreezing hold time				
	CO_2	0.94^{a}	1.08^{a}	0.53^{a}	0.19^{a}
	N_2	0.94^{a}	0.89^{b}	0.70^{b}	0.36^{ba}
	Vacuum	1.00^{a}	0.94^{b}	0.74^{b}	0.52^{b}

[1]Mean of 2 replicates with 4 determinations.

[2]Comparison among packaging treatments at each storage interval and freezing treatments. Like letters amont treatments within a column denote no significant difference (P = 0.05).

Table 11. Analyses of variance of the unsaturation ratios, TBA numbers and phospholipid phosphorus

Source of Variation	d.f.	Mean Square		
		Unsaturation Ratio	TBA Number	Phospholipid Phosphorus
MPCM				
Storage	3	0.91[a]	10.04[a]	0.48[a]
Freezing	1	0.67[a]	2.46[a]	0.002
Packing	2	0.26[a]	13.59[a]	0.02
Freezing-storage	3	0.07[a]	0.44[a]	0.05[a]
Freezing-packing	2	0.02	0.15	0.06[a]
Storage-packing	6	0.05[a]	2.43[a]	0.06[a]
Freezing-storage-packing	6	0.01	0.11	0.03[b]
MPTM				
Storage	3	1.84[a]	98.60[a]	1.64[a]
Freezing	1	0.16[a]	5.01[a]	0.40[a]
Packing	2	0.05[a]	65.40[a]	0.22[a]
Freezing-storage	3	0.03[a]	5.87[a]	0.04[a]
Freezing-packing	2	0.03[a]	3.60[a]	0.03[a]
Storage-packing	6	0.04[a]	5.74[a]	0.11[a]
Freezing-storage-packing	6	0.01[a]	9.45[a]	0.02[a]

[a]Significant at 0.01% level.

[b]Significant at 0.05% level.

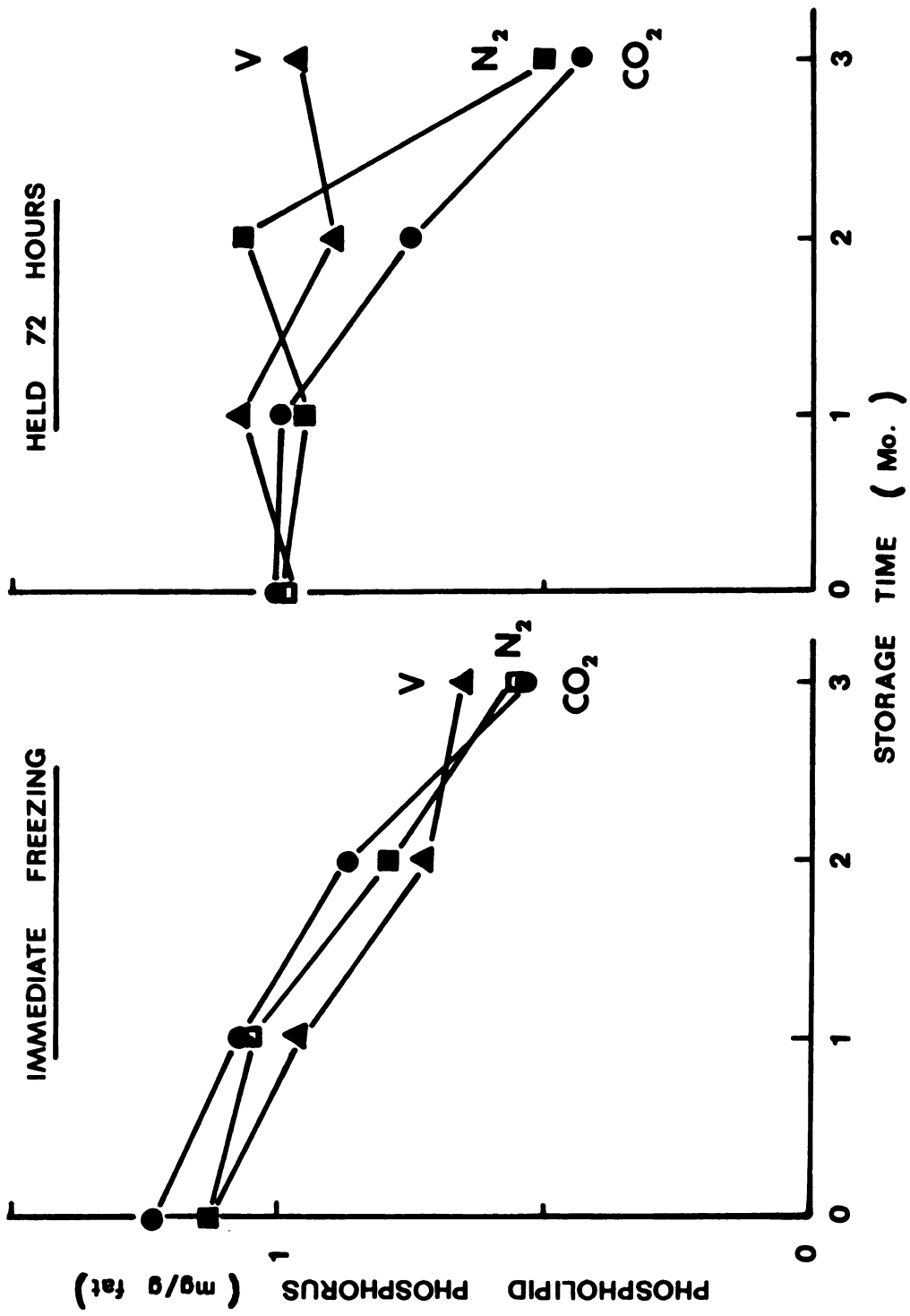


Figure 5. Total phospholipid phosphorus of MPCM packed under CO_2 or N_2 or vacuum and stored at -18°C, either immediately after treatments or after 72 hrs. holding at 4°C.

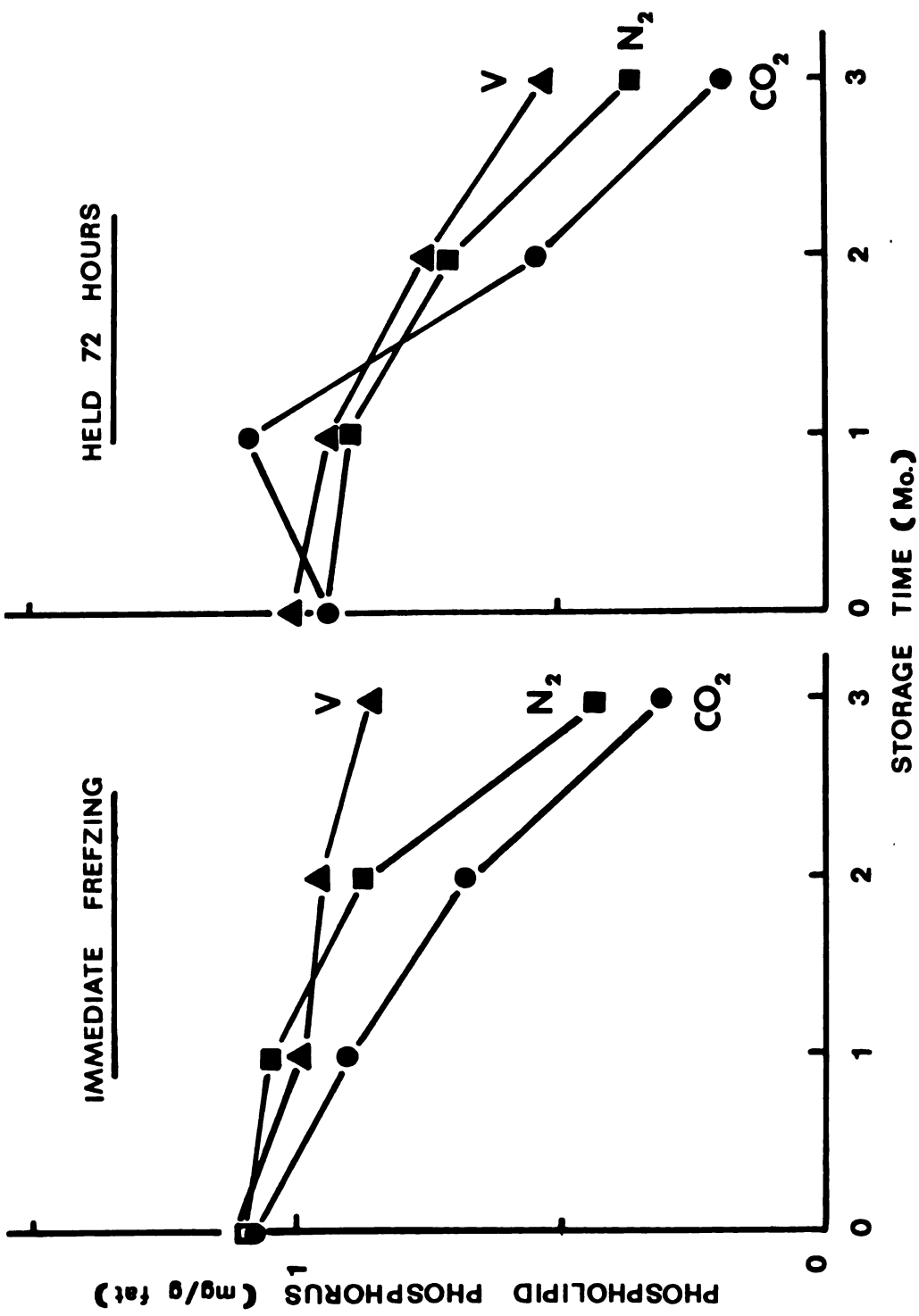


Figure 6. Total phospholipid phosphorus of MPTM packed under CO_2 or N_2 or vacuum and stored at -18°C, either immediately after treatments or after 72 hrs. holding at 4°C.

of MPCM. However these differences are not statistically significant. Unlike MPCM, significant differences among three types of packings were shown in total phospholipid contents of MPTM samples. Losses in phospholipid content were lowest in samples packed under vacuum and highest in those packed along with CO_2. These results are true for both groups of samples which were frozen with and without delay.

The effect of holding the meat at refrigerator temperatures before freezing on subsequent loss of total phospholipids are not significantly shown in MPCM. However, highly significant effects of this treatment were apparent in MPTM. Significant interactions between freezing-storage and freezing-packing were observed in phospholipid phosphorus from both types of meat.

Lipolysis of phospholipids during frozen storage has been observed in meat from various animals. McMurray and Magee (1972) explained that the enzyme phospholipase which occurred in mammalian tissue can cause a release of fatty acids from phospholipids. Formation of free fatty acids and water soluble decomposed phospholipids were reported to occur as a result of the hydrolysis of phospholipids (Awad et al., 1968 and Keller and King, 1973). Other reactions which might also involve and cause breakdown of phospholipids include lipid oxidation, lipid protein copolymerization and lipid browning reactions.

All meat samples were allowed to thaw for only one hour at room temperature prior to each analysis. This short thawing period was selected so that the meat would be soft enough for convenient preparation for each chemical analysis. With this treatment, a negligible amount of drip loss was found in the meat packages at each thawing. Thus, losses of total phospholipids from both MPCM and MPTM upon

frozen storage apparently were not from drip loss. Besides, all of the variously treated samples were frozen and thawed under similar conditions. Hence, loss of phospholipids may be attributed as a result of storage rather than freezing and thawing per se. Davidkova and Khan (1967) and Awad et al. (1968) reported decreases in phospholipid concentration with formation of free fatty acids, during frozen storage of chicken muscle and cod muscle. They explained that the drop in phospholipid concentration could be accounted for by the enzymatic hydrolysis of phospholipids.

Significant differences in phospholipid losses among various treatments of MPTM indicated that enzymatic hydrolysis may not be the only cause for phospholipid degradation noted in this meat type. Close agreements between TBA numbers and phospholipid content of MPTM samples indicated that a breakdown of phospholipids occurred as a result of oxidative degradation in addition to hydrolytic degradation. There was no significant effect of packing and freezing on total phospholipid changes at the end of 4 months storage for MPCM. This result led to the conclusion that the drop in phospholipid concentration in this meat during frozen storage may be mainly accounted for by the enzymatic hydrolysis reaction. The oxidation reaction has only a minor influence on phospholipid losses found in MPCM. This result seems reasonable since oxidative deterioration of MPTM samples, as indicated by changes of TBA numbers and unsaturation ratio, exceeded by far those of MPCM samples.

B. Effects of Air at Various Levels on Storage Stability of Mechanically Processed Poultry Meats

In this study lipid oxidation in MPCM and MPTM during frozen storage, was followed over a wide range of air levels. Air pressures of 0, 5, 15 and 30 in. of Hg were assigned to MPPM and MPPM lipid extract samples, and the samples were stored at -18°C up to 3 months. At each test period, the samples were thawed at room temperature for 1 hr. and prepared for chemical evaluations. Changes in quantities of polyunsaturated fatty acids (C 18:3 - C 22:6) and 2-thiobarbituric acid tests were used for numerically assessing the degrees of lipid oxidation. Degradation of phospholipids as a result of frozen storage of the meat and their lipid extracts under these air tensions was also quantitated. This experiment was designed to determine maximum limit of air which may exist in the meat packages without causing development of oxidative rancidity in the meat samples. Along with air, storage stability of meat lipids in absence of all other meat components was also studied.

Changes in Fatty Acid Compositions

The mean phospholipid unsa uration ratios (C 18:3 - 22:6/ C 16:0) are reported in Table 12. Their statistical analyses are presented in Table 12 and 15. Fatty acid compositions of MPCM and MPTM phospholipids before and after 3 months storage are presented in Appendices H and I. Changes in the unsaturation ratios from samples from all treatments of MPCM and MPTM are graphically shown in Figures 7 and 8.

Significant differences in unsaturation ratios were found in MPCM as a result of storage, air tension, and extraction of meat

Table 12. Mean unsaturation ratios[1] and their Tukey separations[2] for MPCM, MPTM and their lipid extract samples, packed at 0, 5, 15 and 30 in. of air and stored at -18°C for 3 months.

Meat Type	Treatment	Storage Time (mo.)		
		0	2	3
		Unsaturation Ratio		
MPCM	Stored as meat			
	0" air pressure	1.92^{a}	1.42^{ba}	0.92^{a}
	5" air pressure	1.92^{a}	0.65^{b}	0.83^{a}
	15" air pressure	1.92^{a}	0.86^{ba}	0.38^{b}
	30" air pressure	1.92^{a}	0.53^{b}	0.31^{b}
	Stored as lipid extract			
	0" air pressure	1.79^{a}	1.06^{a}	0.97^{a}
	5" air pressure	1.79^{a}	1.07^{a}	0.82^{b}
	15" air pressure	1.79^{a}	0.81^{b}	0.62^{b}
	30" air pressure	1.79^{a}	0.66^{b}	0.53^{b}
MPTM	Stored as meat			
	0" air pressure	1.43^{a}	0.70^{a}	0.68^{a}
	5" air pressure	1.43^{a}	1.00^{a}	0.58^{b}
	15" air pressure	1.43^{a}	0.60^{a}	0.26^{b}
	30" air pressure	1.43^{a}	0.59^{a}	0.31^{b}
	Stored as lipid extract			
	0" air pressure	1.42^{a}	0.69^{a}	0.80^{a}
	5" air pressure	1.42^{a}	0.57^{a}	0.71^{ba}
	15" air pressure	1.42^{a}	0.49^{b}	0.56^{b}
	30" air pressure	1.42^{a}	0.49^{b}	0.37^{b}

[1]Mean of 2 replicates, expressed as polyunsaturated C 18:3 - 22:6 fatty acids/palmitic acids.

[2]Comparison among packaging treatments of each storage interval and extraction treatment. Like letters among treatments within a column denote no significant difference (P = 0.05).

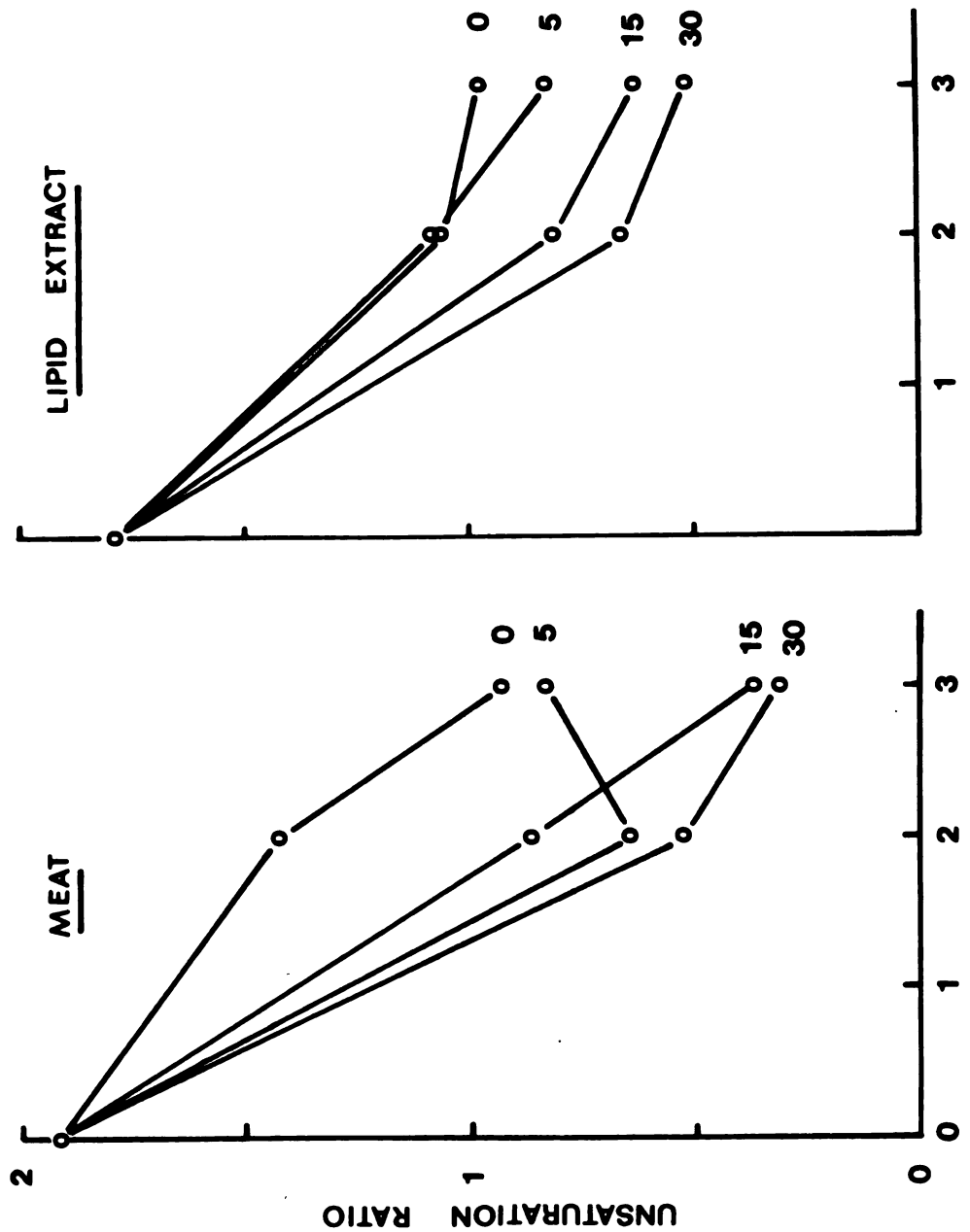


Figure 7. Unsaturation ratios of MPCM and MPCM lipid extract samples packed at 0, 5, 15 and 30 in. of air and stored up to 3 months at -18°C.

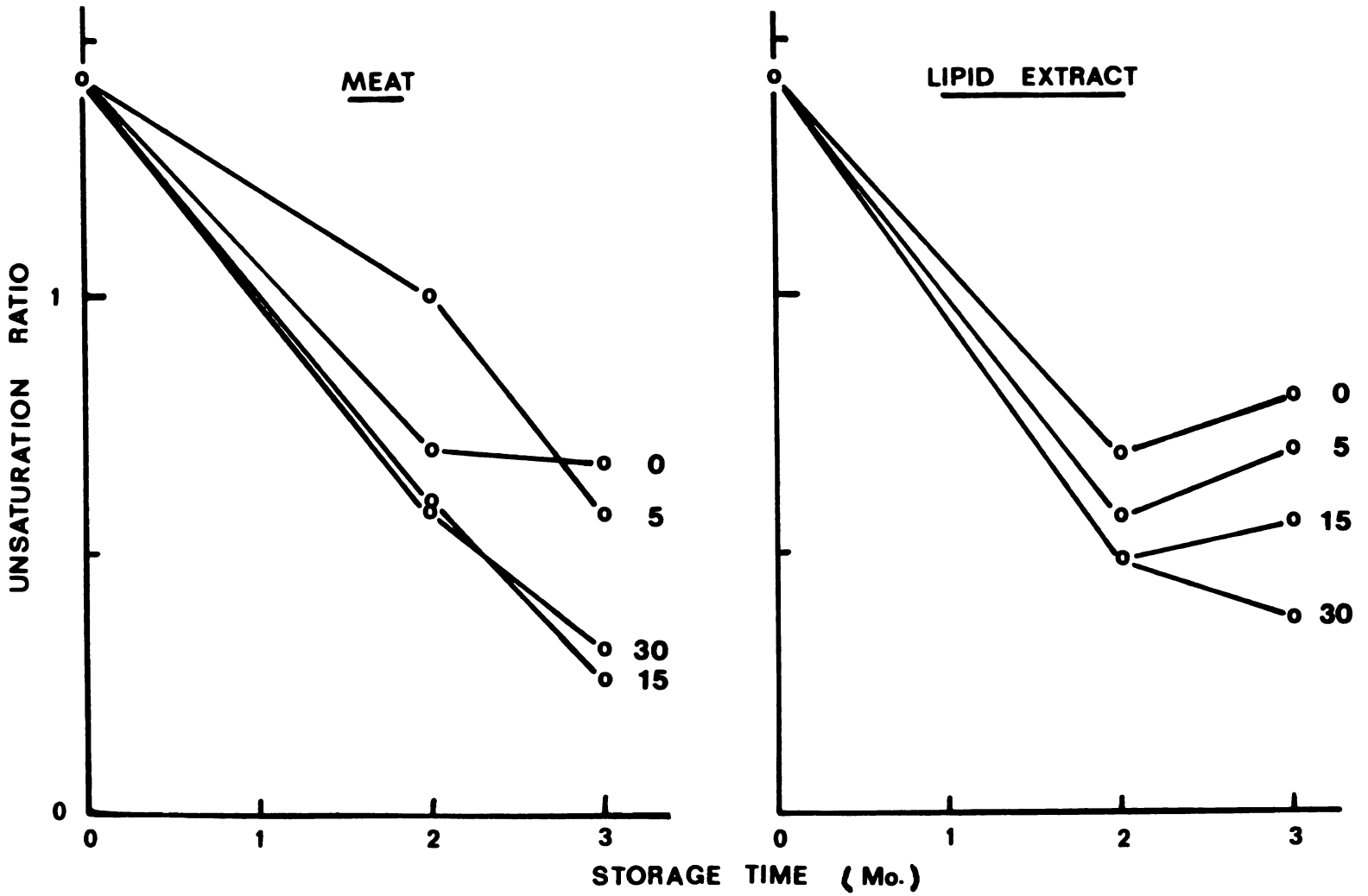


Figure 8. Unsaturation ratios of MPTM and MPTM lipid extract samples packed at 0, 5, 15 and 30 in. of air and stored up to 3 months at -18°C.

lipids. There was no significant effect of lipid extraction on changes in unsaturation ratio of MPTM. Significane of 2 and 3 way interactions for all treatment combinations were found in both MPCM and MPTM phospholipid unsaturation ratios.

It is evident from the data and statistical analyses that a marked decrease in unsaturation ratios was found in samples from all treatments at the end of 3 months of storage. No apparent induction period nor any specific or typical trend of lipid autoxidation were observed in treatments examined. The short storage period (3 months) or the missing data (no test at 1 month interval) might have affected these results.

Significant differences in unsaturation ratios were found among means of different air level treated samples. For MPCM, at the end of 3 months storage, the quantities of unsaturated molecules (C 18:3 - 22:6) left in the phospholipids were comparable between samples stored at 0 and at 5 in. of air and between those stored at 15 and 30 in. of air. There was a significant difference in the mean values of unsaturation ratios between these 2 pairs of samples. Similar results were observed in MPCM lipid extracts and in MPTM samples. Similar results, as previously mentioned for these 3 groups of samples was observed in MPTM lipid extract. The only exception was that there were significant differences between unsaturation ratios of samples stored at 15 and 30 in. of air for MPTM lipid extract samples.

When MPCM samples were stored as lipid extracts, significantly higher quantities of polyunsaturated fatty acids (C 18:3 - 22:6) were found in their phospholipids when compared to those found in their corresponding samples which were stored as meats. Higher unsaturation

ratios were also observed in MPTM lipid extract samples. However, these differences were not statistically significant.

From the results obtained, it appears that 5 in. of air was comparable to vacuum storage with respect to oxidation of polyunsaturated fatty acids in MPPM phospholipids. Non-significant differences in the mean unsaturation ratios found between samples stored at 15 and 30 in. of air also led to the conclusion that air at 15 in. had the same effect as air at 30 in. on oxidation of MPPM phospholipid fatty acids.

Lipid extract samples were expected to develop autoxidative deterioration more slowly and to a lesser degree than their corresponding meat samples upon frozen storage. Extraction of muscle lipids with chloroform-methanol and purification of the crude extracts with aqueous solution should provide meat fats which were free from most other water soluble components including the meat pigments and non-heme iron which have been reported to be present as trace components in MPPM. Thus, the autoxidation reactions which occur in isolated lipids should be entirely different from those which occur when these lipids are in the meat samples. On the other hand, when lipids are in the meat tissues, some of them might bind with proteins and be present in the form of lipoprotein complexes. Protein and water in meat tissues together might partially protect the lipids from being reached by O_2 molecules which surround the meat blocks. Lipid extract samples, in contrast, were exposed directly to O_2 molecules. This factor might have some compensation effect on losses of prooxidant substances found in lipid extract samples. As a result, a rapid development of oxidation was also found in the lipid extract samples. Hence, at the end of 3

months storage, there were no significant differences in fatty acid oxidation of MPTM and MPTM lipid extract samples, or only low level significant differences in mean unsaturation ratios found between MPCM and MPCM lipid extract samples.

2-Thiobarbituric Acid (TBA) Tests

TBA absorption values obtained for variously treated MPCM and MPTM samples are presented in Table 13 and graphically shown in Figures 9 and 10. Analysis of variance and Tukey mean separations for these data are presented in Tables 13 and 15, respectively.

Analysis of variance for TBA absorption values showed significant effects for storage, air tensions and lipid extraction treatments for both MPCM and MPTM. Significance of all 2 and 3 way interactions was also observed in TBA absorption values from these 2 types of meats.

TBA absorption values of all samples increased as a result of frozen storage. The induction period for the development of TBA reactive substances seems to occur between 0 to 2 months of storage for MPCM samples stored at 0 and 5 in. of air. MPCM samples stored at 15 and 30 in. of air showed a rapid increase in TBA absorption values between 0 and 1 to 2 months of storage and then slowed down. Similar trends of this rise and decline were observed in MPCM lipid extract samples. The TBA absorption values, however, behaved differently for MPTM samples. Rapid increases were observed in all treatments of MPTM early in the storage. There was no apparent oxidation induction period in any treatment of MPPM. After a 2 month frozen storage period, TBA absorption values of MPTM samples increased to approximately 1.5 to 1.6 nm and then, with subsequent storage periods, some of these values dropped.
These increases and declines in TBA absorption values however, were

Table 13. Mean TBA absorption values[1] and their Tukey separations[2] for MPCM, MPTM and their lipid extract samples packed at 0, 5, 15 and 30 in. of air and stored at -18°C for 3 months.

Meat Type	Treatment	Storage Time (mo.)			
		0	1	2	3
		TBA Absorption Value (nm)			
MPCM	Stored as meat				
	0" air pressure	0.089[a]	0.072[a]	0.111[a]	0.235[a]
	5" air pressure	0.089[a]	0.070[a]	0.173[a]	0.304[a]
	15" air pressure	0.089[a]	0.076[a]	0.762[ba]	0.885[ba]
	30" air pressure	0.089[a]	0.454[b]	1.106[b]	1.173[b]
	Stored as lipid extract				
	0" air pressure	0.059[a]	0.116[a]	0.185[a]	0.293[a]
	5" air pressure	0.059[a]	0.132[a]	0.421[ba]	0.454[ba]
	15" air pressure	0.059[a]	0.270[ba]	0.700[b]	0.632[b]
	30" air pressure	0.059[a]	0.444[b]	0.727[b]	0.625[b]
MPTM	Stored as meat				
	0" air pressure	0.426[a]	0.600[a]	1.000[a]	1.150[a]
	5" air pressure	0.426[a]	0.819[ba]	1.258[b]	1.318[b]
	15" air pressure	0.426[a]	1.168[bc]	1.571[b]	1.398[b]
	30" air pressure	0.426[a]	1.335[b]	1.492[b]	1.383[b]
	Stored as lipid extract				
	0" air pressure	0.365[a]	0.330[a]	0.214[a]	0.400[a]
	5" air pressure	0.365[a]	0.394[a]	0.300[a]	0.460[a]
	15" air pressure	0.365[a]	0.485[a]	0.600[ba]	0.710[ba]
	30" air pressure	0.365[a]	0.723[b]	0.780[b]	0.939[b]

[1]Mean of 2 replicates with 6 determinations.

[2]Comparison among packaging treatments of each storage interval and extraction treatment. Like letters among treatments within a column denote no significant difference (P = 0.05).

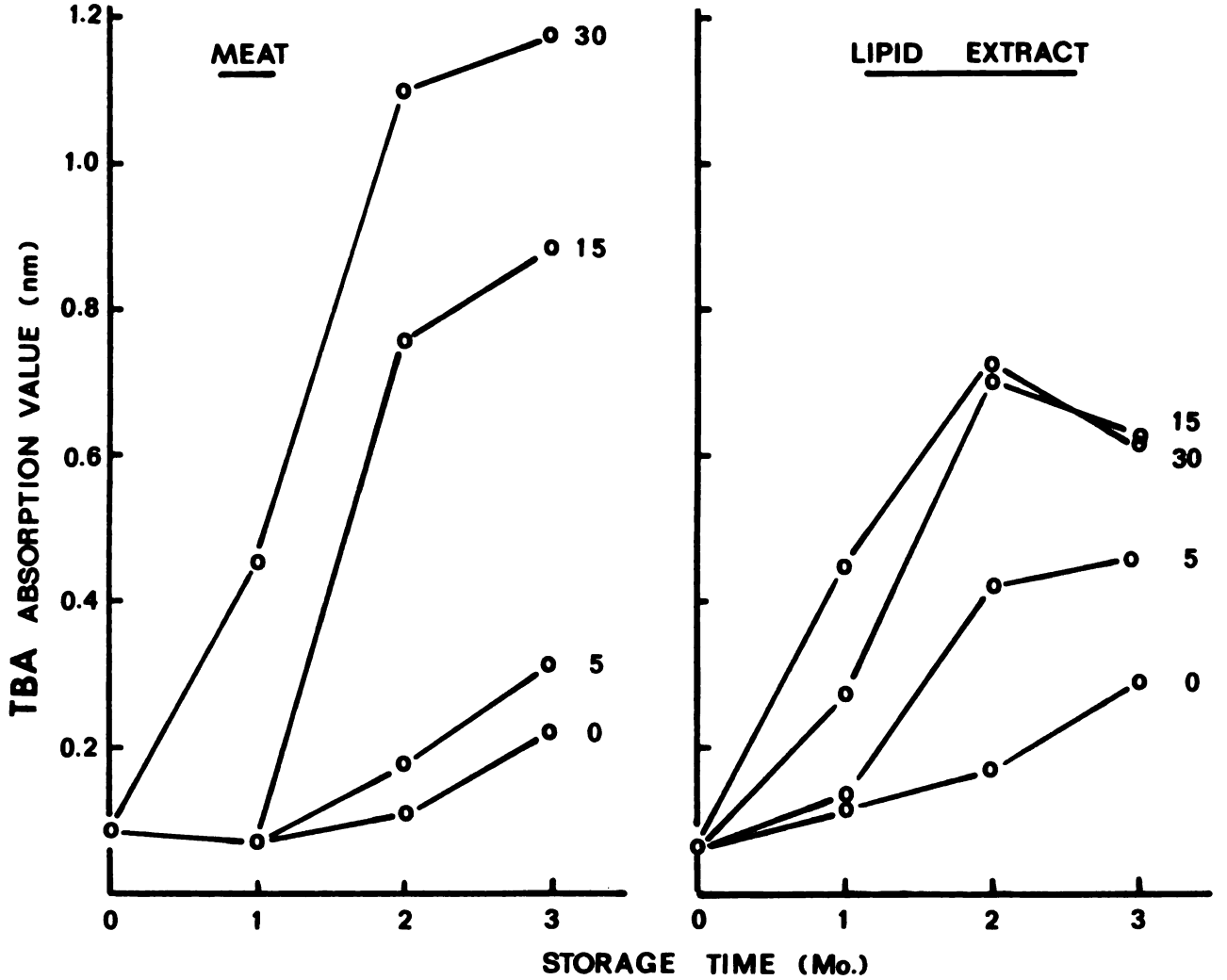


Figure 9. TBA absorption values of MPCM and MPCM lipid extract samples, packed at 0, 5, 15 and 30 in. of air and stored up to 3 months at -18°C.

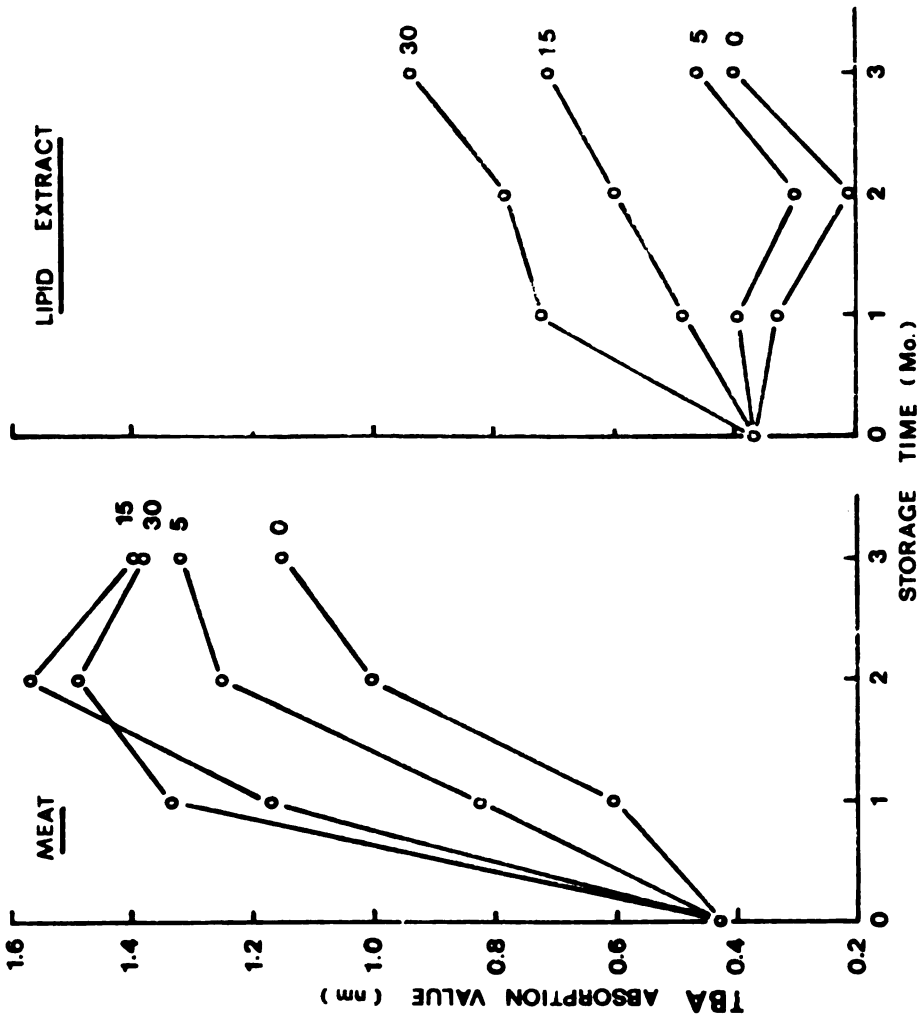


Figure 10. TBA absorption values of MPTM and MPTM lipid extract samples, packed at 0, 5, 15 and 30 in. of air and stored up to 3 months at -18°C.

not observed in MPTM lipid extract samples. A rather steady increase in TBA absorption values was observed for samples from most treatments within this group.

Significant differences in TBA absorption values were found among different air level treatments for both MPCM and MPTM samples. Tukey mean separations indicated that at the end of the first month, there were no significant differences in TBA absorption values between MPCM samples stored at 0, 5 and 15 in. of air. At the end of the second month, however, significant differences in mean TBA absorption values were found between samples stored at 0, 5 in. of air and those stored at 15 and 30 in. of air. This difference was still observed at the end of the 3 months of storage period. MPCM lipid extract samples developed significant differences in TBA absorption values between samples stored under vacuum and those stored at 5, 15 and 30 in. of air after the second month of storage. For MPTM samples, significant differences in TBA absorption values between samples stored under vacuum and those stored at 5, 15 and 30 in. of air was observed, after the first month of storage. At the end of 3 months, these results were still observed among various treatments within this group. MPTM lipid extract samples showed significant differences in TBA absorption values between samples stored at 0, 5 and 13, 30 in. of air after 3 months storage.

The results obtained from TBA tests indicate that air at 5 in. of Hg had the same effect as air at 0 in. or vacuum storage with respect to development of TBA reactive substances in MPCM for a 3 month storage period. For MPTM samples, 5 in. of air in their packages resulted in differences in TBA absorption values from those stored

under vacuum after the first month of storage.

Significant differeuces in TBA absorption values were observed between MPPM and MPPM lipid extract samples, after 3 months storage. These results were not in good agreement with results obtained from changes in phospholipid unsaturation ratios. One factor which should be mentioned here, is the difference in response to TBA test which might occur between these two types of products. TBA analyses were applied directly to meat samples without extraction of the meat lipids. Handling of meat at elevated temperatures during analyses and heating of the meat during the distillation processes might result in a favorable condition for catalytic action of natural prooxidant substances found in the meat, on meat lipid oxidation. Different forms of samples thus might respond differently to this particular testing method. This difference might also contribute to the highly significant difference in TBA absorption values found between these two treatment groups.

Total Phospholipid Phosphorus

Mean total phospholipid phosphorus for all MPCM and MPTM samples evaluated over a 3 month period are presented in Table 14 and their statistical analyses are summarized in Tables 14 and 15. Figures 11 and 12 show graphic presentation of the mean total phospholipids for MPCM and MPTM, respectively.

It is apparent from statistical analyses that phospholipid losses found in both chicken and turkey were influenced by storage as well as air tensions and extraction of the meat lipids. All three factors caused highly significant mean value differences in all types of samples examined. Significant interactions, both 2 and 3

Table 14. Mean total phospholipid phosphorus[1] and their Tukey separations[2] for MPCM, MPTM and their lipid extract samples, packed at 0, 5, 15 and 30 in. of air and stored at -18°C for 3 months.

Meat Type	Treatment	Storage Time (mo.)			
		0	1	2	3
		Phospholipid P (mg/g fat)			
MPCM	Stored as meat				
	0" air pressure	1.10^{a}	0.94^{a}	0.77^{a}	0.70^{a}
	5" air pressure	1.10^{a}	0.77^{ba}	0.97^{ba}	0.90^{ba}
	15" air pressure	1.10^{a}	0.99^{aba}	0.68^{aba}	0.71^{aba}
	30" air pressure	1.10^{a}	1.03^{aba}	0.67^{aba}	0.33^{bbb}
	Stored as lipid extract				
	0" air pressure	1.02^{a}	0.96^{a}	1.11^{a}	0.99^{a}
	5" air pressure	1.02^{a}	1.25^{b}	0.81^{b}	0.88^{a}
	15" air pressure	1.02^{a}	0.87^{a}	0.91^{b}	0.83^{a}
	30" air pressure	1.02^{a}	0.99^{a}	0.79^{b}	0.97^{a}
MPTM	Stored as meat				
	0" air pressure	1.01^{a}	0.95^{a}	0.90^{a}	0.79^{a}
	5" air pressure	1.01^{a}	0.83^{aa}	0.96^{aa}	0.61^{aa}
	15" air pressure	1.01^{a}	0.82^{aaa}	0.71^{aba}	0.56^{baa}
	30" air pressure	1.01^{a}	0.72^{baa}	0.64^{bba}	0.28^{bbb}
	Stored as lipid extract				
	0" air pressure	0.99^{a}	0.92^{a}	0.92^{a}	0.81^{a}
	5" air pressure	0.99^{a}	1.00^{a}	0.71^{aa}	0.86^{a}
	15" air pressure	0.99^{a}	0.98^{a}	0.68^{baa}	0.76^{a}
	30" air pressure	0.99^{a}	0.98^{a}	0.52^{baa}	0.67^{a}

[1]Mean of 2 replicates with 4 determinations.

[2]Comparison among packaging treatments of each storage interval and extraction treatment. Like letters among treatments within a column and a row (vertically) denote no significant difference (P = 0.05).

Table 15. Analyses of variance of the unsaturation ratios, TBA absorption values and total phospholipid phosphorus

Source of Variation	Unsaturation Ratio		TBA Number		Phospholipid Phosphorus	
	d.f.	Mean Square	d.f.	Mean Square	d.f.	Mean Square
MPCM						
Storage	2	6.88[a]	3	0.95[a]	3	0.50[a]
Extraction	1	0.02[b]	1	0.01[a]	1	0.32[a]
Air	3	0.34[a]	3	0.65[a]	3	0.07[a]
Extraction-storage	2	0.05[a]	3	0.03[a]	3	0.15[a]
Extraction-air	3	0.04[a]	3	0.09[a]	3	0.03[b]
Storage-air	6	0.09[a]	9	0.11[a]	9	0.03[a]
Extraction-storage-air	6	0.05[b]	9	0.03[a]	9	0.13[a]
MPTM						
Storage	2	3.82[a]	3	1.04[a]	3	0.36[a]
Extraction	1	0.001	1	4.43[a]	1	0.06[a]
Air	3	0.12[a]	3	0.44[a]	3	0.06[a]
Extraction-storage	2	0.10[a]	3	0.48[a]	3	0.08[a]
Extraction-air	3	0.02[b]	3	0.02[b]	3	0.01
Storage-air	6	0.04[a]	9	0.05[a]	9	0.02[b]
Extraction-storage-air	6	0.01[b]	9	0.02[a]	9	0.01

[a]Significant at 0.01% level.

[b]Significant at 0.05% level.

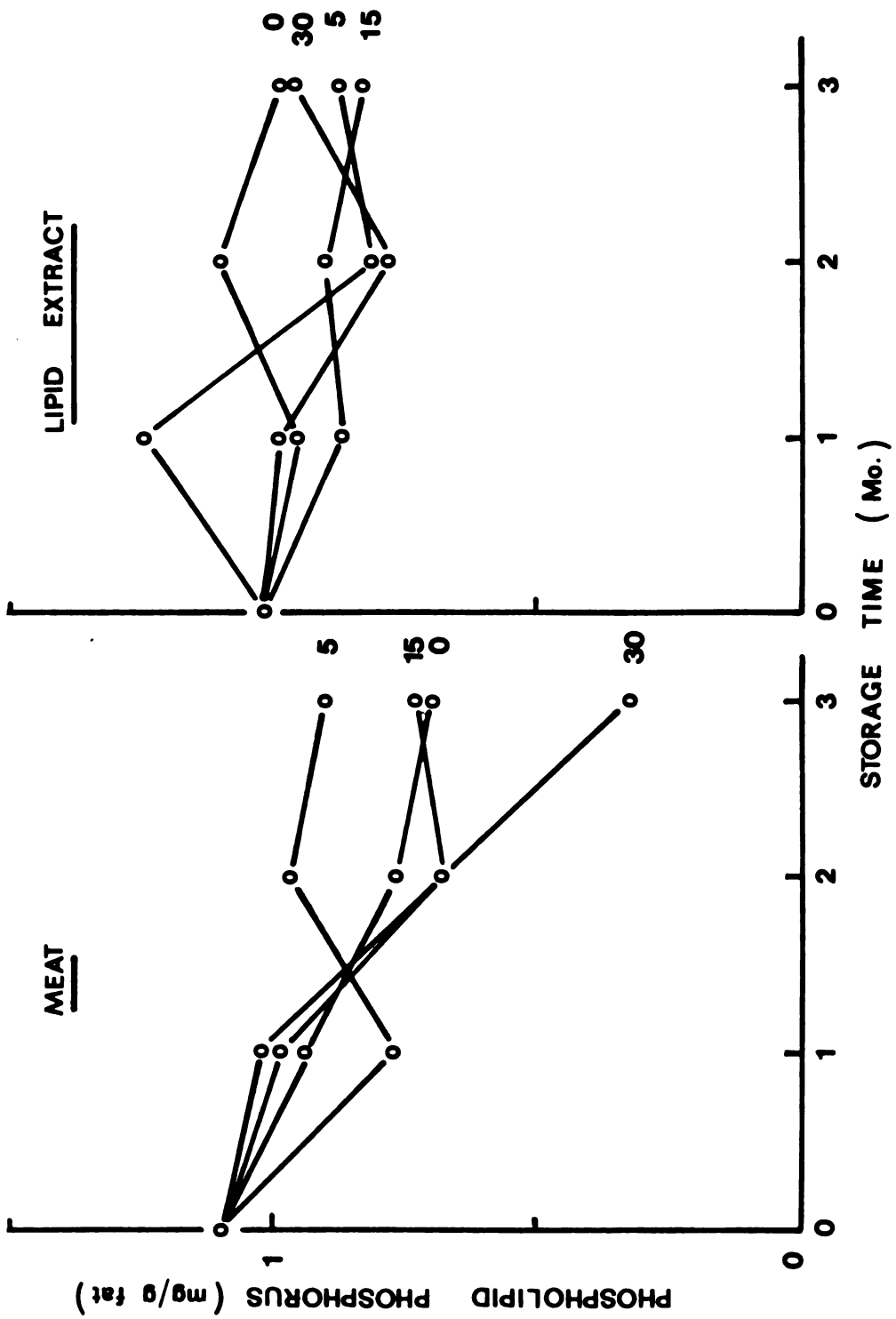


Figure 11. Total phospholipid phosphorus of MPCM and MPCM lipid extract samples, packed at 0, 5, 15 and 30 in. of air and stored up to 3 months at -18°C.

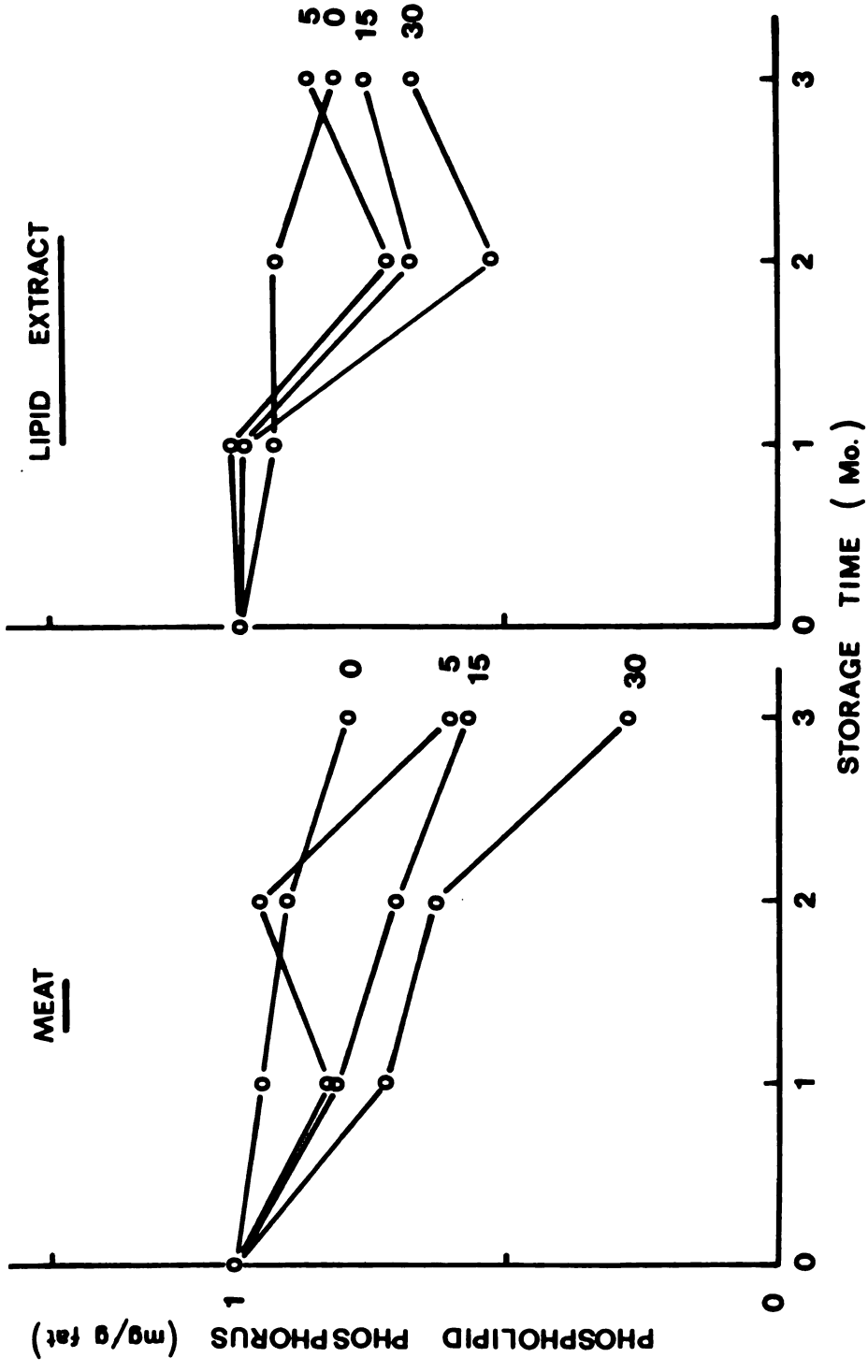


Figure 12. Total phospholipid phosphorus of MPTM and MPTM lipid extract samples, packed at 0, 5, 15 and 30 in. of air and stored up to 3 months at -18°C.

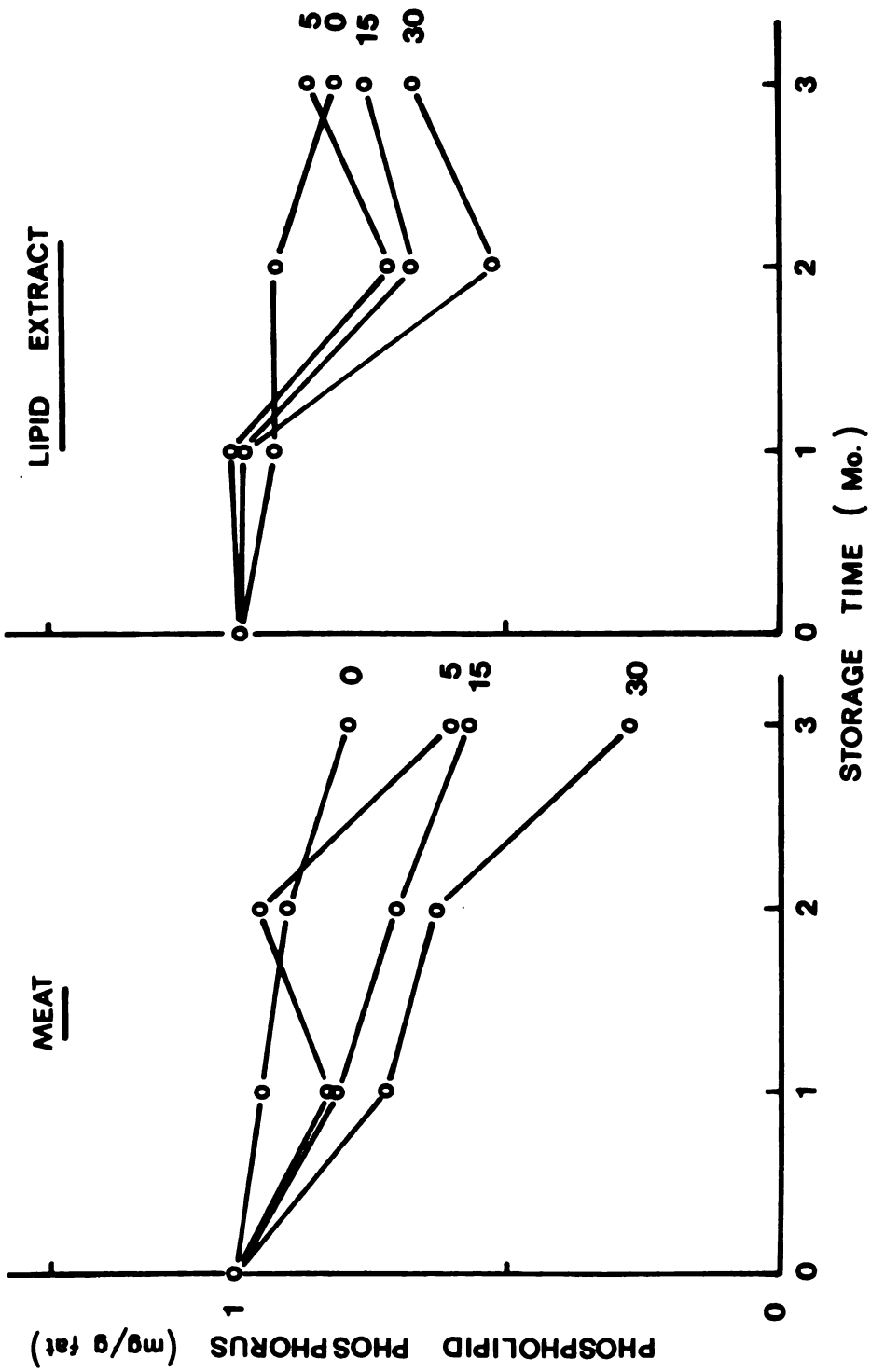


Figure 12. Total phospholipid phosphorus of MPTM and MPTM lipid extract samples, packed at 0, 5, 15 and 30 in. of air and stored up to 3 months at -18°C.

ways, were observed in MPCM samples. Significant interactions in MPTM however, were found only between extraction-storage and air-storage.

Storage of meats and their lipid extract samples at freezer temperature (-18°C), resulted in significant losses of total phospholipids. Considerable decreases in total phospholipid contents were observed in most samples as the frozen storage time progressed. No typical or common trend was observed for these decreases. Different samples seemed to behave differently with respect to degradation of phospholipids during the 3 months storage period.

A marked difference in total phospholipid content was evident between samples stored as meats and as lipid extracts, at the end of the third month of storage. For all treatments from these 2 groups, samples stored as meat exhibited substantially lower phospholipid contents than those samples stored as lipid extracts. This result was true for both chicken and turkey.

At the end of 3 months of storage, MPCM samples stored at 30 in. of air showed the highest loss in total phospholipids. There was a significant difference in mean phospholipid content between samples stored at 5 and 15 and 30 in. of air. Vacuum packed samples, however failed to show a significant difference in total phospholipid loss when compared with samples packed at 15 in. of air. This result led to the conclusion that 5 in of air tension was by far the best in retarding loss of MPCM phospholipids. PHospholipid contents in MPCM samples packed at 0 and 5 in. of air were comparable at the end of the 3 months storage, while samples stored at 30 in. of air showed a significantly lower level of phospholipids among the 4 treatments within this group. No significant differences were found

among total phospholipid contents of MPCM lipid extract and MPTM lipid extract samples packed at various air levels, at the end of 3 months of storage. Differences among these 4 air level treatments were found in both MPCM lipid extract and MPTM lipid extract samples at the end of 2 months storage, however they did not show up at the end of the storage period.

The decline in phospholipid concentration in meat samples during frozen storage can be accounted for by the enzymatic hydrolysis of phospholipids. The enzyme phospholipase which occurs in mammalian tissues can cause release of fatty acids from phosphoglycerides (McMurray and Magee, 1972). Other reactions which might involve losses of phospholipids during storage of the meats are lipid oxidation, lipid protein copolymorization and lipid browning reaction. Phospholipid break down during frozen storage of meats may result in rancidity and browning of the meats (Caldwell et al., 1960 and Greene, 1971).

Samples stored as meat showed significantly greater phospholipid losses than those stored as fat extracts after 3 months storage. This result was observed in both chicken and turkey. Conceivably, the phospholipid decreases noted in lipid extract samples might occur as a result of reactions other than enzymatic reactions. Processes used in extraction of muscle lipid out of the tissues could exclude the tissue enzymes as well as other components. Thus, phospholipid decreases noted in these samples may have been mainly caused by lipid oxidation reactions while combinations of reactions were responsible for the degradation of phospholipids found in samples which were stored as meats.

SUMMARY AND CONCLUSIONS

Composition and storage stability of mechanically processed chicken and turkey meats (MPCM and MPTM) were evaluated in consecutive studies. In the composition study, lipids from light and dark MPCM, MPTM and their corresponding composite tissue lipids were analyzed for total cholesterol contents, phospholipid phosphorus (totals and fractions) and fatty acid composition. Attempts were made to correlate components of MPCM and MPTM lipids with those found in lipids from their composite tissues. From the analyses of data the following conclusions have been reached:

1) There was little difference among the fatty acid components of neutral lipid from various tissue samples.
2) Phospholipid fatty acid components of MPPM resemble more the fatty acids of bone or hand deboned meat phospholipids than skin phospholipids.
3) Total phospholipid content and quantity of each phospholipid classes in MPPM are most similar to those of bone tissues.
4) Cholesterol contents of MPPM lipids more closely resemble cholesterol content of skin tissue lipids or bone tissue lipids than muscle lipids. Based on total cholesterol contents, total phospholipid contents and phospholipid fatty acid components found in lipids from composite tissues

from each bird species, a model of 1:3:6 meat fat: bone fat: skin fat was proposed for mechanically processed chicken fat and a value of 1:4:5 meat fat: bone fat: skin fat was suggested for mechanically processed turkey fat.

Stability studies were divided into 2 parts. The effects of inert gases (N_2 and CO_2) and vacuum packing on storage stability of MPCM and MPTM were evaluated. In this study, MPCM and MPTM were packed along with either N_2 or CO_2 gas or vacuum packed and frozen at -18°C, immediately after packing or after a 72 hrs. holding period at 4°C. Changes in phospholipid unsaturation ratio (C 18:3 - 22:6/C 16:0), 2-thiobarbituric acid tests and changes in total phospholipid contents were used to follow the development of lipid oxidation and hydrolytic reactions. Analyses of data obtained from this experiment led to the following conclusions:

1) Vacuum and N_2 packaging treatments resulted in significantly lower in losses of polyunsaturated fatty acids and developments of TBA reactive substances found in meat samples from most treatments at the end of 3 months storage.
2) Vacuum packaging treatments were comparable to N_2 treatments in the development of TBA reactive substances found in samples from most treatments. However, for the decrease in unsaturation ratios, this result was observed only in MPTM samples.
3) There were no significant differences in total phospholipid phosphorus in MPCM samples packed under CO_2, N_2 vacuum. The advantage of vacuum packed over N_2 and CO_2 packed, however, was observed in total phospholipid losses found

in MPTM samples.

4) TBA numbers, loss of phospholipid polyunsaturated fatty acids and degradation of phospholipids were significantly higher in MPCM and MPTM samples which were held 72 hrs. at 4°C prior to frozen storage, when compared with those products which were frozen immediately after packaging.

The second part of stability studies involved the effect of air at various tension levels on storage stability of MPPM. In this study, air pressures equivalent to 0, 5, 15 and 30 in. of Hg were assigned to MPPM and MPPM lipid extract samples and the samples were stored at -18°C upto 3 months. At each test period, samples were analyzed for changes in phospholipid unsaturation ratio (C 18:3 - 22:6/C 16:0), 2-thiobarbituric acids, and total phospholipid contents. From analyses of data obtained, conclusions can be drawn as follows:

1) A partial vacuum (air at 5 in.) was comparable to vacuum packaging for products stored up to 3 months concerning changes in phospholipid unsaturation ratio and formation of TBA reactive substances in PPCM. For MPTM however, vacuum packaging was significantly better than other treatments in preventing of the development of TBA reactive substances.

2) Vacuum and 5 in. air tension packaging treatments were comparable in prevention of phospholipid degradation reactions, found in MPPM at the end of 3 months storage.

3) Significant differences between MPPM samples stored as meat and as lipid extracts were observed in most treatment

groups and testing methods. The only exception for these was the changes in unsaturation ratio of MPTM samples. In all significant cases, samples stored as meats developed higher and faster lipid oxidation and hydrolytic reactions than their corresponding lipid extract samples.

PROPOSAL FOR FUTURE RESEARCH

1. Compare composition of lipids from intact chicken and turkey bone marrows with those found in their corresponding bone residues, separated by the deboning machines.
2. Verify the hypothesized mechanically processed poultry meat (MPPM) lipid models by preparing model systems for MPPM lipids according to the hypothesized models. Then compare components of real MPPM lipids with those found in their model system.
3. Evaluate effect of pH (both higher and lower than the normal MPPM pH) on rate of lipid oxidation of MPPM.
4. Evaluate the effect of air at tension levels range from 5 to 15 in. of Hg on storage stability of MPPM.

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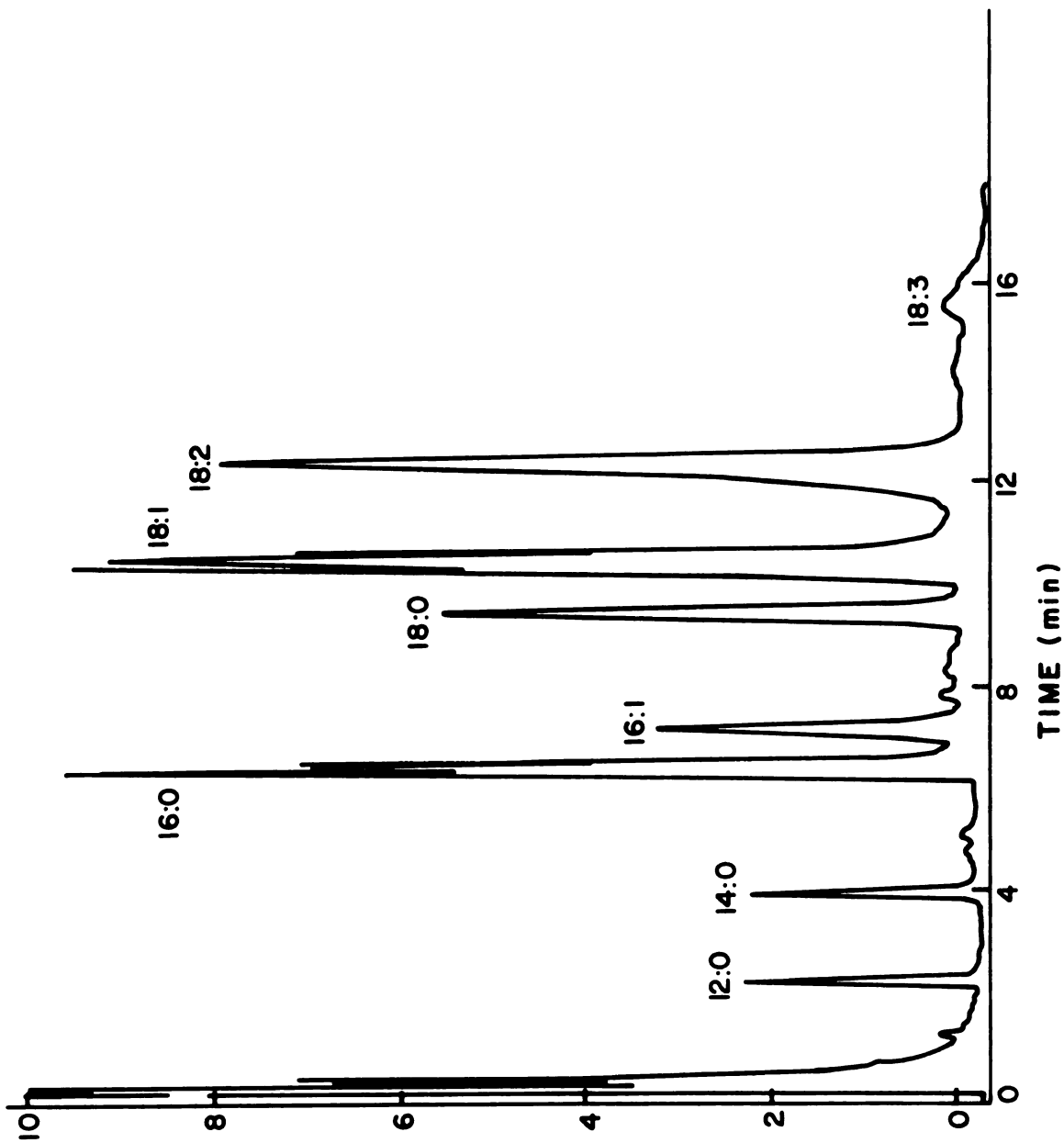
APPENDICES

APPENDIX A

CHROMATOGRAM FOR NEUTRAL LIPID FATTY ACIDS

Appendix A

Chromatogram for neutral lipid fatty acids
(automatic attenuation for highest peaks)

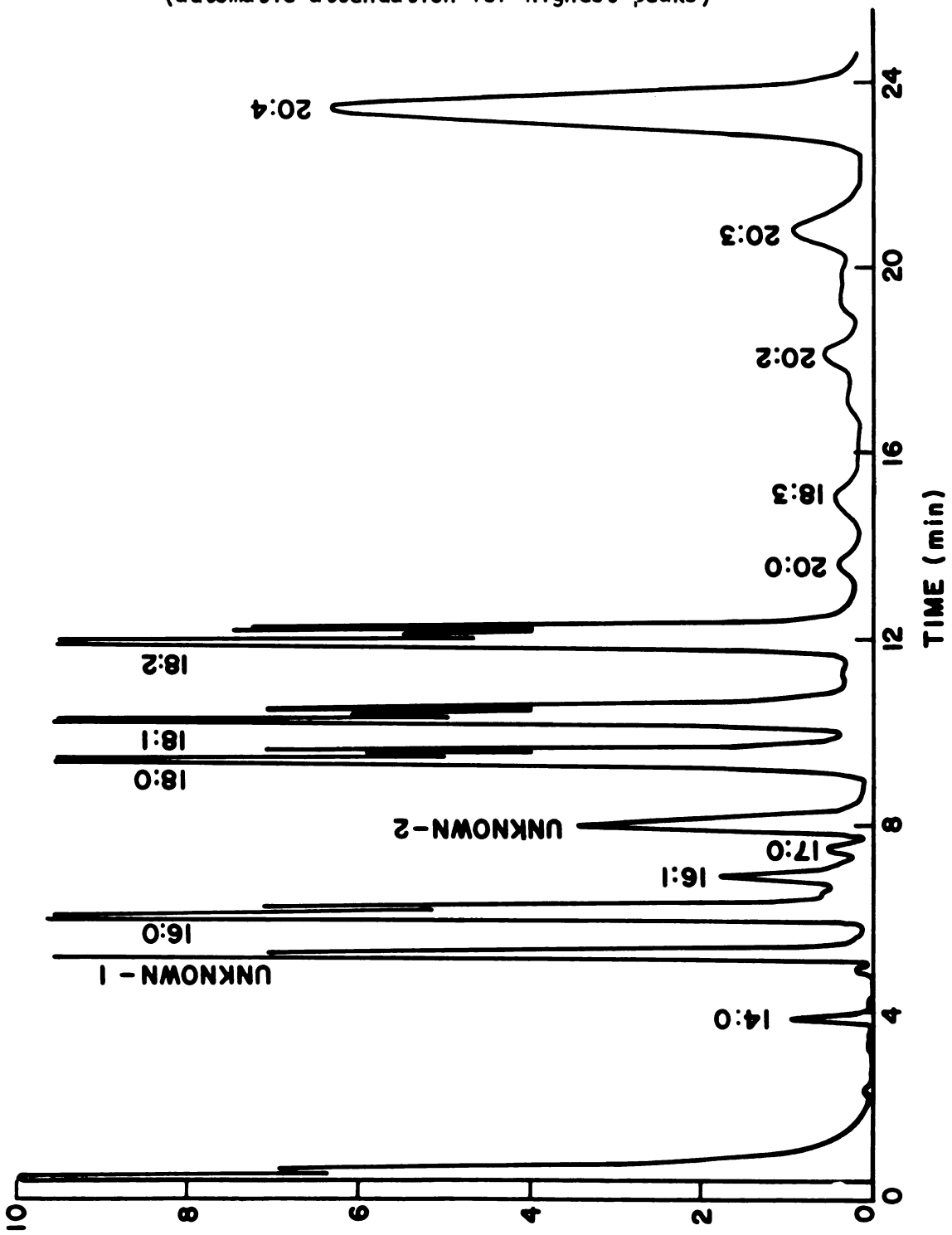


APPENDIX B

CHROMATOGRAM FOR PHOSPHOLIPID FATTY ACIDS

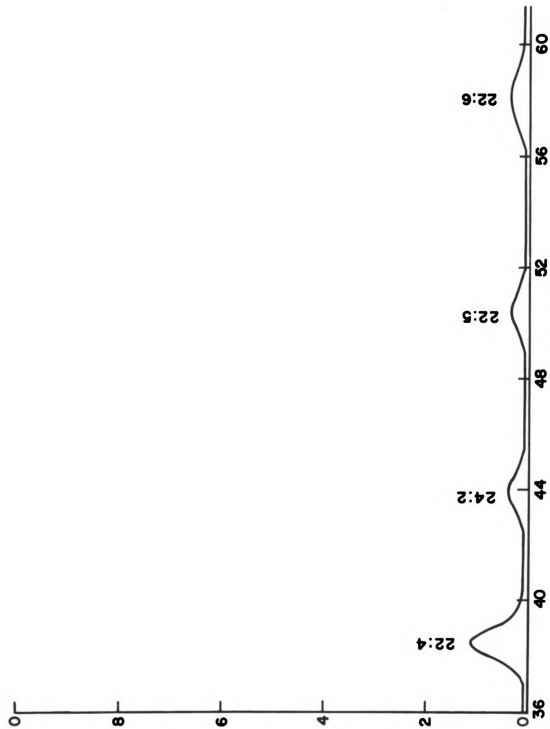
Appendix B

Chromatogram for phospholipid fatty acids
(automatic attenuation for highest peaks)



Appendix B

(continued)

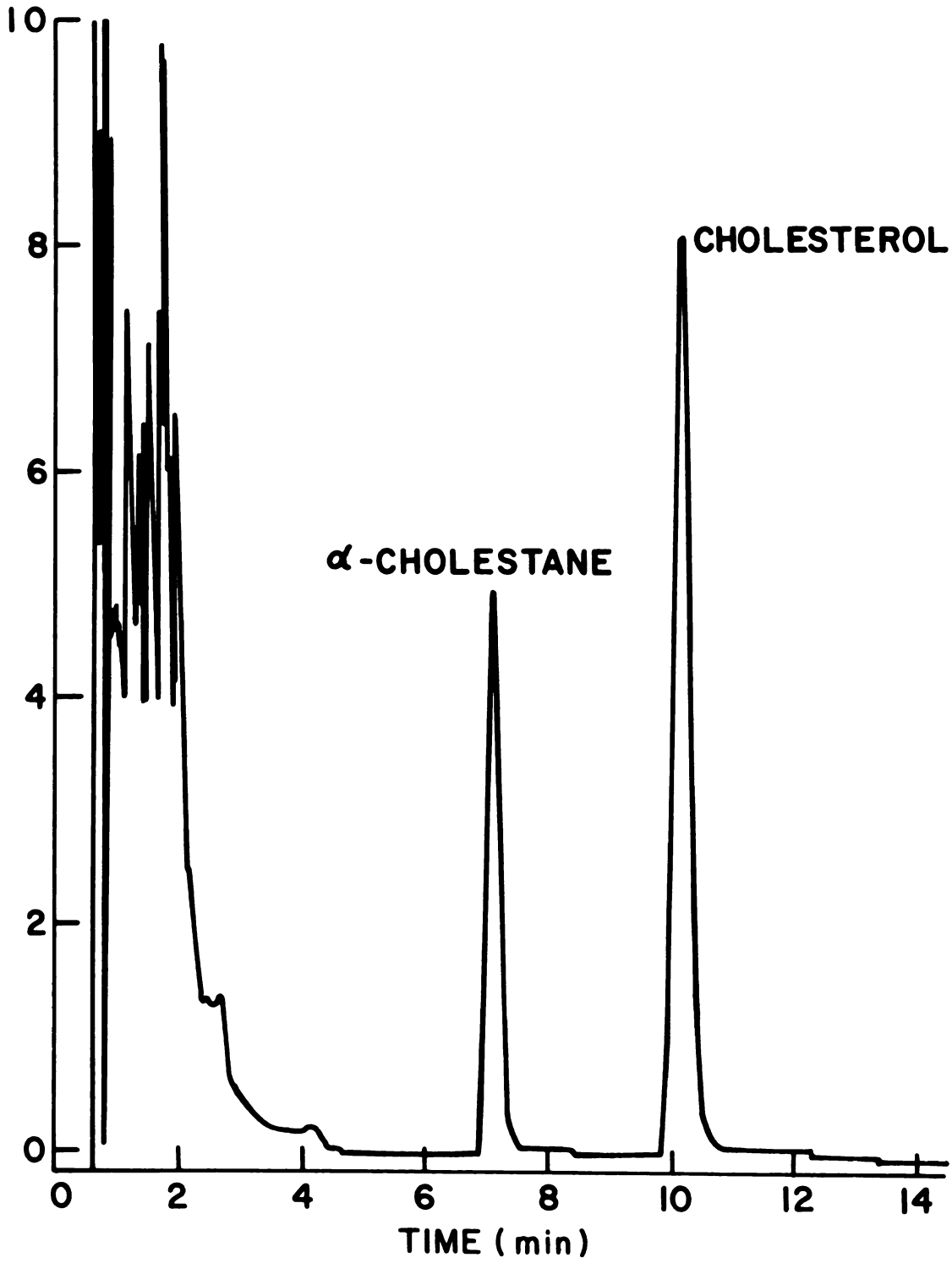


APPENDIX C

CHROMATOGRAM FOR TOTAL CHOLESTEROL

Appendix C

Chromatogram for total cholesterol



APPENDIX D

TOTAL LIPID CONTENTS OF CHICKEN AND TURKEY TISSUES

Appendix D

Total lipid contents of chicken and turkey tissues

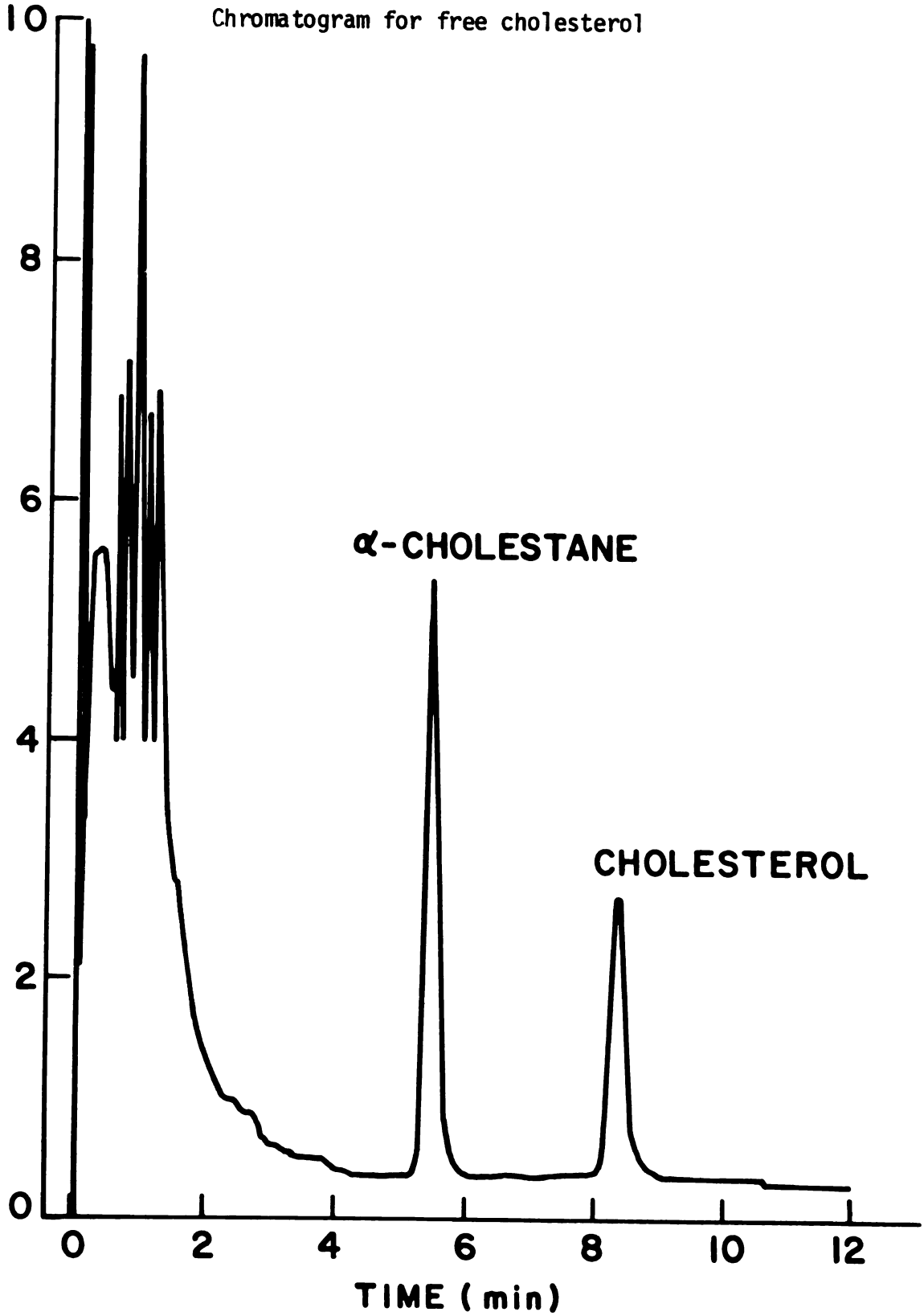
Sample	% Total Lipid	
	Light Tissue	Dark Tissue
Chicken		
MPCM	13.58	21.47
HDCM	2.09	4.14
Bone residue	7.49	14.65
Skin tissue	37.08	42.83
Turkey		
MPTM	10.60	19.79
HDTM	2.54	4.76
Bone residue	5.90	11.46
Skin tissue	38.66	40.27

APPENDIX E

CHROMATOGRAM FOR FREE CHOLESTEROL

Appendix E

Chromatogram for free cholesterol



APPENDIX F

CHANGES IN FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM MPCM PACKED WITH CO_2 OR N_2 OR UNDER VACUUM AND STORED AT -18°C FOR 4 MONTHS

Appendix F. Changes in fatty acid composition of phospholipids from MPCM packed with CO_2 or N_2 or under vacuum and stored at -18°C for 4 months

Storage Time (mo.)	0						2					
Freezing	FI[a]			FH[b]			FI[a]			FH[b]		
Packing	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum
Fatty Acids	Percent (GLC Peak Area)											
14:0	0.54	0.71	1.01	1.05	0.90	0.72	0.49	0.32	0.35	1.01	0.81	0.37
Unknown 1	0.40	0.54	0.42	0.84	0.58	0.38	1.48	1.18	1.15	2.84	2.55	1.12
16:0	13.35	14.30	13.70	14.8	15.02	15.45	15.38	15.18	13.30	16.50	15.10	15.35
16:1	1.08	0.98	0.84	1.31	1.62	1.46	0.45	0.98	0.96	0.99	1.05	0.99
17:0	0.19	0.15	0.20	0.34	0.21	0.35	1.03	0.33	0.61	0.45	0.74	0.49
Unknown 2	t	t	t	-	-	t	0.32	0.23	0.33	0.34	0.35	0.50
18:0	15.14	14.80	15.16	15.89	15.96	15.00	15.52	16.30	15.20	16.67	15.84	17.07
18:1	19.02	18.43	17.42	18.03	18.04	17.81	17.58	19.91	19.17	18.28	19.54	19.74
18:2	18.43	17.65	17.54	16.95	17.54	18.21	19.26	16.05	18.82	19.60	20.11	16.27
18:3	1.22	1.42	0.98	1.32	1.24	1.30	1.03	1.15	1.05	1.13	1.56	1.00
20:0	0.43	0.54	0.43	0.60	0.39	0.25	0.49	0.74	0.76	0.78	0.62	0.71
20:2	0.43	0.35	0.42	0.96	1.05	0.79	0.75	1.03	0.78	0.61	0.87	1.12
20:3	1.91	2.01	1.74	1.98	2.41	2.60	2.38	1.81	2.66	1.80	1.72	1.77
20:4	15.92	16.05	16.58	15.92	14.96	14.86	15.88	14.05	16.91	11.61	12.62	16.25
22:4	5.12	4.89	6.03	4.40	4.66	4.44	3.45	4.33	4.16	4.06	3.23	3.88
22:5	1.82	1.75	1.65	1.22	1.04	1.41	1.49	1.75	1.08	0.59	0.84	1.01
22:6	2.70	3.10	2.89	2.75	2.46	3.16	1.66	2.49	1.56	1.43	1.38	1.11
24:2	2.29	2.33	2.93	1.94	2.02	1.80	1.36	2.11	1.15	1.03	1.06	1.25
Total Saturation	29.65	30.50	30.50	32.08	32.48	31.77	32.91	32.87	30.22	35.41	33.11	33.99
Total Unsaturation	69.94	68.69	69.02	66.78	67.04	67.84	65.29	65.66	68.30	61.13	63.98	64.39
Total C 18:3 - 22:6	28.69	29.22	29.87	27.59	26.77	27.77	25.89	25.58	27.42	20.62	21.35	25.02
Unsaturation ratio	2.15	2.04	2.18	1.86	1.78	1.80	1.68	1.69	2.06	1.25	1.41	1.63

Appendix F. (Continued)

Storage Time (mo.)	3						4					
Freezing	FI[a]			FH[b]			FI[a]			FH[b]		
Packing	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum
Fatty Acids	Percent (GLC Peak Area)											
14:0	0.84	0.51	0.99	0.74	0.47	0.67	1.78	2.15	1.55	1.56	1.86	0.91
Unknown 1	2.50	2.28	3.19	1.54	1.68	2.13	1.55	1.31	1.79	1.66	1.12	1.69
16:0	19.96	19.37	17.85	18.85	20.55	17.89	21.26	19.78	18.00	24.40	22.52	19.42
16:1	2.53	1.56	1.80	2.29	1.53	1.50	0.95	1.26	0.78	0.99	1.08	0.63
17:0	0.51	0.46	0.63	0.71	0.59	0.33	0.96	0.87	0.86	1.01	1.06	0.98
Unknown 2	1.25	0.94	0.50	0.25	0.33	0.23	0.65	0.80	0.19	0.38	1.26	0.34
18:0	18.66	20.37	18.66	18.34	18.88	19.77	19.73	23.15	18.57	21.62	20.27	19.24
18:1	22.23	22.51	21.93	22.62	23.90	23.23	16.46	17.61	18.80	17.47	17.12	19.49
18:2	12.06	14.37	14.34	15.16	14.95	16.18	16.85	16.41	16.20	16.08	14.41	16.81
18:3	0.72	0.67	1.38	1.46	0.94	0.93	1.41	0.56	1.35	1.63	0.47	1.52
20:0	0.55	0.48	0.51	0.77	0.61	0.47	2.19	0.71	0.63	0.73	0.60	1.12
20:2	0.85	0.46	0.60	0.67	0.47	0.87	1.31	1.47	0.68	0.99	1.06	0.71
20:3	1.37	0.92	1.52	1.40	0.92	1.73	1.07	0.95	2.76	2.09	0.84	0.46
20:4	11.54	12.42	12.65	10.23	10.38	11.56	11.13	10.56	14.84	7.65	14.25	13.06
22:4	2.78	1.38	2.34	3.20	1.95	1.41	1.95	1.62	2.30	1.02	1.41	3.00
22:5	0.26	0.29	0.22	0.24	0.40	0.60	t	t	t	-	-	t
22:6	0.84	0.51	0.49	0.52	0.70	0.26	-	t	t	-	-	-
24:2	0.56	0.45	0.40	1.01	0.74	0.24	0.79	0.80	0.67	0.72	0.68	0.63
Total Saturation	40.52	41.19	38.63	39.41	41.10	39.13	45.92	46.66	39.64	49.32	46.31	41.67
Total Unsaturation	55.74	55.54	57.67	58.80	56.88	58.51	51.92	51.24	58.38	48.63	51.32	56.31
Total C 18:3 - 22:6	17.51	16.19	18.60	17.05	15.29	16.49	15.56	13.69	21.25	12.59	16.97	18.04
Unsaturation Ratio	0.88	0.84	1.04	0.91	0.74	0.92	0.73	0.69	1.18	0.52	0.75	0.93

[a]Frozen immediately after treatments.

[b]Frozen after holding at 4°C for 72 hrs.

APPENDIX G

CHANGES IN FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM MPCM PACKED WITH CO_2 OR N_2 OR UNDER VACUUM AND STORED AT -18°C FOR 4 MONTHS

Appendix G. Changes in Fatty Acid Composition of Phospholipids from MPTM, packed with CO_2 or N_2 or under vacuum and stored at -18°C for 4 months

Storage Time (mo.)	0						2					
Freezing	FI[a]			FH[b]			FI[a]			FH[b]		
Packing	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum
Fatty Acids	Percent (GLC Peak Area)											
14:0	0.49	0.56	0.71	0.37	0.29	0.64	0.79	0.36	0.34	0.95	0.81	0.83
Unknown 1	1.92	1.90	1.63	1.77	1.40	1.50	2.82	1.27	1.11	1.17	2.72	1.25
16:0	15.53	16.92	16.58	15.57	16.39	15.34	18.40	17.68	17.31	17.94	18.10	18.17
16:1	0.98	0.84	1.05	0.94	0.84	1.43	1.11	0.68	0.78	1.13	1.00	0.85
17:0	0.44	0.34	0.60	0.33	0.32	0.43	0.52	0.43	0.46	0.71	0.68	0.49
Unknown 2	t	-	-	0.19	-	0.21	0.29	0.32	0.29	0.32	0.33	t
18:0	16.44	15.82	16.49	16.07	16.54	16.51	18.58	19.15	18.50	19.65	18.87	18.86
18:1	17.02	16.71	15.44	17.80	17.44	17.43	20.08	20.78	21.52	20.74	20.86	21.57
18:2	20.19	18.02	19.01	20.51	18.96	19.23	16.08	15.93	17.91	19.34	16.99	19.49
18:3	1.10	1.65	1.76	0.89	1.25	0.89	1.81	1.28	1.51	1.02	1.03	0.83
20:0	0.48	0.65	0.22	0.31	0.33	0.59	0.86	0.71	1.03	0.61	0.63	0.75
20:2	0.41	0.62	0.66	0.62	0.71	0.67	0.91	0.95	0.65	0.61	0.66	1.20
20:3	1.12	1.30	1.46	1.20	1.45	1.68	2.14	1.94	1.99	1.67	0.97	1.46
20:4	14.35	15.90	15.85	14.86	15.58	15.08	10.37	13.56	13.11	9.95	11.44	9.08
22:4	4.41	4.32	4.02	4.16	4.32	4.44	3.66	2.81	1.31	2.48	2.63	2.65
22:5	1.23	1.44	1.46	1.07	1.19	1.02	0.58	0.64	0.79	0.49	0.63	0.85
22:6	2.32	1.98	2.05	2.17	1.76	1.84	0.69	0.70	0.95	0.78	0.81	0.86
24:2	1.55	1.02	0.99	1.34	1.20	1.05	0.34	0.83	0.44	0.44	0.82	0.82
Total Saturation	33.38	34.29	34.60	32.65	33.87	33.51	39.15	38.33	37.64	39.86	39.09	39.10
Total Unsaturation	64.68	63.80	63.75	65.56	64.70	64.76	57.77	60.10	60.96	58.65	57.84	59.66
Total C 18:3 - 22:6	24.53	26.39	26.60	24.35	25.55	24.60	19.25	20.93	19.66	16.39	17.51	14.90
Unsaturation Ratio	1.53	1.56	1.60	1.56	1.56	1.60	1.05	1.18	1.14	0.91	0.97	0.82

Appendix G. (Continued)

Storage Time (mo.)	3						4					
Freezing	FI[a]			FH[b]			FI[a]			FH[b]		
Packing	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum	CO_2	N_2	Vacuum
Fatty Acids	Percent (GLC Peak Area)											
14:0	0.45	0.98	0.76	0.61	0.44	0.39	0.88	0.76	0.56	0.74	0.80	0.62
Unknown 1	1.54	2.56	2.56	1.86	1.46	1.58	3.16	2.44	1.42	2.32	2.70	2.08
16:0	19.79	18.55	18.74	18.47	19.27	18.62	20.10	19.90	20.36	21.74	20.03	21.10
16:1	0.90	1.20	1.20	1.60	2.63	1.22	2.44	1.61	1.26	2.06	1.54	1.36
17:0	0.54	0.52	0.57	0.77	0.87	0.51	1.45	0.45	0.35	1.66	0.63	0.35
Unknown 2	1.05	0.93	0.34	0.41	0.84	0.32	2.16	1.26	0.88	1.30	1.30	0.84
18:0	19.32	16.07	19.74	19.12	18.10	20.83	19.33	22.25	22.63	22.93	22.63	23.47
18:1	21.88	19.43	21.27	23.61	20.90	25.44	23.75	17.38	17.88	23.46	17.82	19.39
18:2	16.56	21.80	14.64	15.10	16.72	16.43	14.40	14.90	13.29	14.42	15.13	13.62
18:3	1.08	1.39	1.21	1.19	1.13	1.32	0.69	0.70	0.57	0.35	0.62	0.91
20:0	0.67	0.85	0.68	0.82	0.69	0.85	0.67	1.16	1.18	0.41	1.22	0.87
20:2	0.90	0.88	0.75	0.68	0.81	1.98	0.51	0.61	0.54	0.74	0.96	0.50
20:3	0.72	1.76	1.22	0.36	1.64	0.53	0.37	0.68	0.78	0.50	0.65	0.62
20:4	10.28	8.05	11.47	10.93	9.67	8.29	7.74	12.90	13.56	5.61	11.15	10.61
22:4	3.47	3.10	1.82	2.99	3.21	1.66	2.02	2.63	4.04	1.55	2.31	2.93
22:5	t	0.41	0.85	0.30	0.31	0.21	t	-	-	-	-	-
22:6	0.26	0.29	1.26	0.27	0.93	t	t	-	-	-	-	-
24:2	0.57	1.23	0.91	0.92	0.38	0.31	0.34	0.37	0.66	0.37	0.50	0.73
Total Saturation	40.77	36.97	40.49	39.79	39.37	40.69	32.43	44.52	45.08	45.82	45.31	46.41
Total Unsaturation	56.62	59.54	56.60	57.95	58.33	57.39	52.26	51.79	52.58	50.55	50.68	50.67
Total C 18:3 - 22:6	15.81	15.00	17.83	16.04	16.89	12.01	10.82	16.92	18.95	8.01	14.73	15.07
Unsaturation Ratio	0.80	0.81	0.95	0.87	0.88	0.65	0.54	0.85	0.93	0.37	0.74	0.71

[a]Frozen immediately after treatments.

[b]Frozen after holding at 4°C for 72 hrs.

APPENDIX H

CHANGES IN FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM MPCM AND THEIR LIPID EXTRACT SAMPLES, PACKED AT 0, 5, 15 AND 30 IN. OF AIR AND STORED UP TO 3 MONTHS AT -18°C

Appendix H. Changes in fatty acid composition of phospholipids from MPCM and their lipid extract samples, packed at 0, 5, 15 and 30 in. of air and stored up to 3 months at -18°C

Storage Time (mo.)	0		2							
Sample	Meat	Lipid Extract	Meat				Lipid Extract			
Packing (in. Hg)			0	5	15	30	0	5	15	30
Fatty Acids	Percent (GLC Peak Area)									
14:0	0.69	0.35	0.69	0.85	0.85	0.53	0.50	0.83	0.96	0.93
Unknown 1	2.55	1.04	1.24	0.94	2.54	1.50	1.03	1.02	2.96	1.92
16:0	14.06	14.67	16.71	21.08	19.71	19.99	18.65	18.32	18.54	19.93
16:1	1.25	1.02	0.74	0.94	1.23	1.18	1.04	2.20	1.55	1.45
17:0	0.40	0.50	0.34	0.47	0.51	0.48	0.54	0.53	0.85	0.60
Unknown 2	1.60	t	0.23	0.28	0.30	0.26	t	0.19	0.29	0.40
18:0	15.09	16.65	17.99	19.96	19.38	21.55	19.27	18.92	18.94	20.72
18:1	16.14	18.64	19.45	22.35	22.41	25.42	22.67	21.26	22.96	24.01
18:2	18.64	18.26	16.90	15.95	14.22	16.50	14.50	14.82	15.24	14.34
18:3	1.03	1.23	1.42	1.18	1.35	1.43	0.64	1.03	1.15	1.13
20:0	0.57	0.70	0.85	0.84	0.71	1.10	0.50	0.77	0.69	0.42
20:2	0.49	0.85	0.77	1.44	0.58	0.87	0.76	0.84	1.20	1.25
20:3	1.67	1.70	1.17	0.80	1.85	0.50	0.84	1.72	0.99	1.18
20:4	16.72	17.40	15.73	10.32	11.13	6.20	14.28	13.21	10.67	9.04
22:4	4.12	3.53	3.66	0.95	2.55	2.49	2.61	3.16	1.96	1.87
22:5	1.28	1.01	0.66	0.27	t	t	0.72	0.28	0.21	-
22:6	2.18	1.33	1.02	0.22	t	t	0.74	0.20	t	-
24:2	1.49	1.16	0.41	1.13	0.68	0.62	0.64	0.69	0.82	0.82
Total Saturation	30.81	32.87	36.58	43.20	41.16	43.65	39.46	39.25	39.98	42.60
Total Unsaturation	65.01	66.13	61.94	55.55	55.32	55.21	59.44	59.53	56.75	55.09
Total C 18:3 - 22:6	27.00	26.20	23.66	13.74	16.88	10.62	19.83	19.60	14.98	13.22
Unsaturation Ratio	1.92	1.79	1.42	0.65	0.86	0.53	1.06	1.07	0.81	0.66

Appendix H. (Continued)

Storage Time (mo.)	3							
Sample	Meat				Lipid Extract			
Packing (in Hg)	0	5	15	30	0	5	15	30
Fatty Acids	Percent (GLC Peak Area)							
14:0	0.69	0.85	1.80	1.95	0.39	0.88	1.02	1.01
Unknown 1	2.26	2.84	2.62	2.24	1.15	2.81	3.37	2.00
16:0	18.71	19.30	21.33	22.11	18.68	19.22	20.23	19.49
16:1	0.74	2.04	2.40	2.15	0.68	1.07	1.21	1.05
17:0	0.34	0.88	0.69	0.88	0.40	0.58	0.78	0.58
Unknown 2	0.23	0.29	0.34	0.63	0.29	0.81	0.29	0.61
18:0	18.99	18.21	21.66	20.76	19.84	19.74	19.12	21.42
18:1	22.01	19.93	24.29	25.09	21.53	22.14	23.00	24.58
18:2	16.91	16.81	14.95	14.80	16.83	15.02	16.53	16.37
18:3	1.42	0.96	1.29	1.63	1.31	1.04	1.10	0.78
20:0	0.85	0.61	0.66	1.09	0.65	0.74	0.65	0.68
20:2	0.77	1.38	1.15	1.27	0.97	0.77	0.84	1.38
20:3	1.17	0.33	0.43	0.83	1.89	0.96	0.74	0.71
20:4	11.73	12.03	5.47	3.82	13.07	11.51	10.02	7.90
22:4	1.66	1.77	0.95	0.63	1.54	1.78	0.67	1.01
22:5	0.56	0.59	-	-	0.22	0.21	-	-
22:6	0.60	0.40	-	-	0.12	0.22	-	-
24:2	0.41	0.78	-	-	0.44	0.48	0.47	0.41
Total Saturation	39.58	39.85	46.14	44.84	39.96	41.16	41.76	43.18
Total Unsaturation	57.92	56.94	50.93	52.31	58.60	55.20	54.58	54.19
Total C 18:3 - 22:6	17.14	16.08	8.14	6.91	18.15	15.72	12.53	10.40
Unsaturation Ratio	0.92	0.83	0.38	0.31	0.97	0.82	0.62	0.53

APPENDIX I

CHANGES IN FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM MPTM AND THEIR LIPID EXTRACT SAMPLES, PACKED AT 0, 5, 15 AND 30 IN. OF AIR AND STORED UP TO 3 MONTHS AT -18°C

Appendix I. Changes in Fatty Acid Composition of Phospholipids from MPTM and their lipid extract samples, packed at 0, 5, 15 and 30 in. of air and stored up to 3 months at -18°C

Storage Time (mo.)	0		2							
Sample	Meat	Lipid Extract	Meat				Lipid Extract			
Packing (in. Hg)			0	5	15	30	0	5	15	30
Fatty Acids	Percent (GLC Peak Area)									
14:0	0.68	1.00	0.55	0.84	1.15	0.50	1.39	1.47	0.99	1.14
Unknown 1	0.80	0.78	0.71	1.83	1.60	1.66	2.43	2.99	2.16	3.11
16:0	15.20	16.23	20.28	18.63	20.39	20.51	19.97	20.50	22.24	20.96
16:1	1.79	0.88	1.52	1.29	1.44	2.17	2.38	1.07	2.49	3.24
17:0	0.38	0.60	0.52	0.39	0.43	0.92	1.48	0.70	0.99	1.46
Unknown 2	1.33	0.26	0.24	0.22	0.26	0.64	0.42	0.42	0.21	-
18:0	17.60	15.97	20.45	19.09	20.93	21.45	19.73	20.06	21.88	21.35
18:1	21.08	19.91	24.61	21.89	24.03	24.16	21.50	22.83	22.91	24.03
18:2	17.03	18.70	15.53	15.68	15.16	14.08	15.01	15.57	13.31	11.60
18:3	0.87	1.26	0.94	0.77	1.03	1.14	1.08	1.22	0.86	0.96
20:0	0.56	0.88	0.77	0.39	0.45	0.59	0.78	0.82	0.93	1.00
20:2	0.79	0.54	0.65	1.06	0.99	0.75	0.86	1.47	0.96	0.61
20:3	1.08	1.68	1.20	1.35	0.45	0.51	1.51	1.89	1.04	0.74
20:4	14.18	15.32	9.58	13.00	9.31	8.84	8.32	6.65	7.45	8.67
22:4	3.80	3.01	1.82	2.35	1.04	1.65	2.40	1.39	1.60	1.20
22:5	0.62	0.89	0.22	0.35	0.42	-	0.19	0.25	-	-
22:6	1.20	0.94	0.18	0.20	-	-	0.20	0.22	-	-
24:2	0.01	1.05	0.29	0.69	0.67	0.41	0.38	0.47	-	-
Total Saturation	34.42	34.68	42.57	39.34	43.35	43.97	43.35	43.55	47.03	45.91
Total Unsaturation	63.45	64.28	56.53	58.63	54.77	53.71	53.83	53.03	50.62	51.05
Total C 18:3 - 22:6	21.75	23.10	14.23	18.71	12.25	12.14	13.70	11.60	10.95	10.37
Unsaturation Ratio	1.43	1.42	0.70	1.00	0.60	0.59	0.69	0.57	0.49	0.49

Appendix I. (Continued)

Storage Time (mo.)	3							
Sample	Meat				Lipid Extract			
Packing (in. Hg)	0	5	15	30	0	5	15	30
Fatty Acids	Percent (GLC Peak Area)							
14:0	0.89	1.86	1.77	1.61	0.51	0.62	1.06	0.42
Unknown 1	2.84	4.02	3.91	3.48	1.82	1.96	2.93	1.82
16:0	20.31	20.00	22.16	21.96	20.06	19.00	21.93	21.91
16:1	0.97	1.06	1.08	2.50	1.53	1.43	1.35	1.28
17:0	0.62	0.54	0.82	1.14	0.49	0.37	0.59	0.46
Unknown 2	0.19	0.23	0.24	1.80	0.86	0.24	1.48	0.37
18:0	20.75	20.60	22.80	21.83	20.28	20.97	19.37	22.63
18:1	24.63	24.30	26.08	25.03	22.38	23.95	23.17	25.43
18:2	13.59	14.14	13.67	13.08	14.73	16.77	15.61	15.99
18:3	1.42	0.60	1.14	1.40	0.67	0.87	1.38	1.24
20:0	0.36	0.41	0.79	0.78	0.49	0.49	0.99	0.81
20:2	0.78	0.63	0.87	0.92	0.57	0.63	0.81	0.65
20:3	1.16	0.23	0.26	0.30	0.95	0.60	0.83	0.51
20:4	9.18	9.34	3.31	3.58	12.47	10.42	8.90	5.58
22:4	1.95	1.22	1.07	0.62	2.44	1.33	0.49	0.68
22:5	-	0.18	-	-	-	-	-	-
22:6	-	-	-	-	-	-	-	-
24:2	0.37	0.52	-	-	0.44	0.33	-	-
Total Saturation	42.93	43.41	48.34	47.32	41.83	41.45	43.94	46.23
Total Unsaturation	54.05	52.32	47.48	47.43	56.18	56.33	52.54	51.56
Total C 18:3 - 22:6	13.71	11.57	5.78	6.82	17.54	13.55	12.23	8.01
Unsaturation Ratio	0.68	0.58	0.26	0.31	0.80	0.71	0.56	0.37