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# EFFECT OF FREEZING, FROZEN STORAGE AND COOKING ON THE CHEMICAL CHANGES AND QUALITY CHARACTERISTICS OF LAMB MEAT. II. FATTY ACIDS, PHOSPHOLIPIDS, CHOLESTEROL AND CHOLESTEROL ESTERS.

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## Introduction

Lipids comprise about 11 to 30% of the carcass weight and 15 to 20% of the live weight (Olcott 1964). Intramuscular fat of muscle, like that of other metabolically active tissues, has a considerable content of phospholipids and unsaponifiable constituents, such as cholesterol. The predominant fatty acids in meat tissues are saturated palmetic and stearic acids and unsaturated oleic acid. Zhgun et al., (1976).

Freezing and frozen storage can produce profound effects on the structural and chemical properties of muscle foods, including changes in muscle fibers, lipids and proteins, all of which have the potential for significantly influencing the quality attribute of meat and meat products, Miller et al (1980).

Although various workers have noted that tissue lipids were stable during frozen storage (Keskinel et al., 1964 and Witte et al., 1970), breakdown of triglycerides and phospholipids by lipases and autoxidation has been observed in frozen muscle tissue held at various temperatures and for different storage times Keller and Kinsella, 1973).

Lamb is considered one of the meat sources in Egypt, as it represents about 16% of the total annual slaughtered animals. Flavor, odor, physical and chemical characteristics, and cooking properties are determined factors in the acceptance of fresh and frozen lamb.

The method of cooking (type of heat) recommended for specific cuts of meats has been based on the classification of cuts as tender or less tender, according to the relative quantity of connective tissue and muscle fibers in the muscles. Usually moist heat is recommended for less tender cuts, whereas, dry heat is recommended for tender cuts, Freger et al., (1972).

Limited work has been reported on the effect of cooking after frozen storage on the lipid fraction of lamb. Therefore, this investigation was conducted to study the effect of freezing and cooking methods on the alterations on lipid fractions i.e., fatty acids, phospholipids, cholesterol and choelsterol esters in lamb.

## Materials and Methods

Materials: See Part I.

### Analytical Methods:

Lipid extraction was started approximately 6 hours after the meat samples were cooked. The total lipids were extracted from 5 gm. of homogenized samples by the method of Floch et al.,(1957). The total lipids were then further fractionated to neutral and polar lipids by the technique of Mabrouk et al (1969), using(2.5x50cm) glass column containing silica gel Bio-Sil BH, 100-200 mesh (Calibiochem KIB, Los Angelos Calif.). In the following sequence, neutral lipids with $CHCl_3$, glycolipids with acetone and phospholipids with $CH_3OH$, were eluted from the column.

Neutral lipids were further fractionated by the method of Mangold(1961) using the TLC technique into Free Cholesterol(F.Ch.), cholesterol ester (Ch.E), triglycerides (TG) and Free fatty acids (FFA). The spots were visualized by iodine vapor and were identified by the RF values of the authentics and marked with a fine needle. The spots containing F.Ch. and Ch. E. were scraped and eluted with 20 ml. ether. The eluates were then evaporated and cholesterol esters were quantitatively determined according to Carr and Drekter(1956).

The phospholipids fraction was purified on a preparative TLC plates using the method of Turkki and Campbell (1967). Then the fraction was eluted by using a mixture of methano water(4:1v/v). The phosphorus content of the phospholipids fraction was determined calorimitrically using ammonium molybdate as described in the A.O.A.C.(1975).

The fatty acids were separated and estrified according to the method of Katz et al (1966), and were then fractionated and identified by the GLC (4 pye unicum 104 Flo.). The GLC was performed under the following conditions: polyethylene glycol adepote(PEGA) column, nitrogen flow rate 50 ml/min. hydrogen flow rate 5 ml/min. and oxygen flow rate 400 ml/min.

## Results and Discussion

Meat lipids are considered very sensitive to freezing and freezing storage, and hence, many alterations in their characteristics might occur. The lipid fractions liable to changes during freezing, storage and subsequent cooking are: fatty acids, the phospholipids, and cholesterol and its esters.

Table: I, shows the gas chromatographic analysis of the methyl esters of the intramuscular fats of fresh, boiled and roasted lamb, for both L.D. and B.F. muscles. The results indicated that oleic, linoleic and palmitic acids were the most predominant fatty acids in the two muscles. However, there was a great variation between the L.D and B.F. muscles in the proportions of the different fatty acids. Longissimus Dorsi muscle contained more unsaturated fatty acids(85%) than B.F muscle (58%). Meanwhile, palmitic and stearic acids concentrations were higher in B.F muscle than in L.D muscle. Besides, L.D muscle contained twice as much linolenic acid (30.43%) as the B.F muscle (16.35%). These results are expected, and may be attributed to factors, such as species, diet, sex, age and muscle type Lawire (1974).

Also, the results indicated that lamb had a unique fatty acid composition. For lamb contained higher percentages of unsaturated fatty acid, than other types of meat, i.e. camel and beef. That might be one major

factor affecting lamb's characteristic flavor.

Results given in table: I and Fig. 1 & 2, also show the effect of boiling on the fatty acids content of both L.D and B.F muscles of lamb. There was a noticeable decrease in the total unsaturated fatty acids and an increase in the saturated ones. The percentage increase reached as high as 60.5%, while the percentage decrease was only 16.63%. The most pronounced decrease was in both oleic and linoleic acids, while the most noticeable increase was in both palmitic and stearic acids. Changes in the fatty acids concentration might be due to the destruction and oxidation of intramuscular lipids components caused by heating. However, oxidation during cooking is an important phenomena in developing the flavor of any meat, Badings,(1960). Meanwhile, Zocchi(1974), reported that during boiling free oleic acid tended to remain within the meat,while palmitic acid distributed itself between the broth and the meat.

When comapring the fatty acids content of roasted lamb to fresh and boiled lamb, roasted lamb(L.D muscle) contained a lower percentage(52.27%) of unsaturated fatty acids. On the contrary, roasted lamb meat from the same muscle L.D contained a higher percentage (47.86%)of saturated fatty acids. However, there was a significant decrease in linolenic acid concentration in the roasted samples, 4.71%(roasted lamb) versus 30.42%(fresh lamb). Such a decrease might be due to the oxidation and destruction of linolenic acid to carbonyl compounds which contributes to the pronounced flavor of roasted meat. Meanwhile,there was an increase in the concentration of other fatty acids such as palmitic and stearic which could be due to the decomposition of intramuscular fat components and the release of such fatty acids. Similar changes occurred in the meat samples of B.F muscle, accompanied by a noticeable decrease in linolenic acid concentration and a marked increase in the palmitic concentration.

Table: II & III indicate the effect of freezing storage, up to three months, on the fatty acids content of raw, boiled and roasted lamb samples from both L.D and B.F muscles. It could be observed that the total percentage of the unsaturated fatty acids decreased after freezing storage at -20$^{0}$C. The percentage decrease reached 21.29% in L.D muscle and only 9.24% in B.F muscle after one month of storage. However, this decrease was pronounce in the oleic acid concentration. On the other hand, there was an increase in the concentration of saturated fatty acids which reached as high as 64.82% in L.D and only 13.44% in B.F muscle. The changes in the fatty acid content, during freezing of lamb meat, might be due to the oxidation of some unsaturated fatty acids and the hydrolysis of some fat components(glycerides and phospholipids)through the effect of lipases. This effect produces several active chemical intermediates, free radicals, hydroperoxides and carbohyl compounds,Lea(1962). As the time of frozen storage increased i.e.after 2 and 3 months, the same trend of changes in fatty acids seemed to continue without significant difference between the two muscles (Table II & III). This indicates that lamb lipids continue to deteriorate during frozen storage of lamb meat. However, this continuous deterioration would affect the overall acceptability of the meat.

The effect of different cooking methods on the fatty acids composition, after frozen storage,are tabulated in the same tables(II & III) and are graphically represented in Figs. 1 & 2. From the results given ,the changes in the fatty acid composition, particularly between the saturated and unsaturated fatty acid ratios, was more pronounce in the lamb samples prepared by the boiling rather than the roasting procedure. Also, the results indicated that the changes in L.D muscle were much more drastic than those in B.F muscle. However, this might be due to the original difference in structure between the two muscles. In addition, a sharp decrease was evidenced in the unsaturated fatty acids, after one month of storage. Afterwards the unsaturated fatty acids increased gradually with the advancement of time of storage. However, the saturated fatty acids showed some changes, but in the other direction. This could be due to the effect the freezing process has on the lipids fraction of the meat. However, the increase in the unsaturated fatty acids might be due to the increase in the enzymic hydrolysis i.e. lipases with the promotion of autoxidation of the glyceride moitey which leads to the release of more free fatty acids.

Table I: Fatty acids content of Longissimus Dorsi and Biceps Femoris muscles of intramuscular lipids of fresh, boiled and roasted lamb meat.

| Fatty acids Percentage | Longissimus Dorsi | | | Biceps Femoris | | |
|---|---|---|---|---|---|---|
| | Fresh | Boiled | Roasted | Fresh | Boiled | Roasted |
| Unsaturated | | | | | | |
| Tetradecanoic | 0.02 | 0.50 | 3.57 | 0.27 | 0.10 | 0.27 |
| Palmitoleic | 1.43 | 0.54 | 0.43 | 0.29 | 0.41 | 0.59 |
| Heptadecanoic | 0.71 | 0.54 | 0.43 | 0.80 | 0.38 | 0.32 |
| Oleic | 48.61 | 30.17 | 37.14 | 34.73 | 41.71 | 37.04 |
| Linoleic | 3.81 | 2.69 | 5.79 | 5.61 | 4.05 | 4.30 |
| Linolenic | 30.42 | 36.24 | 4.71 | 16.35 | 1.69 | 1.34 |
| Total | 85.00 | 70.86 | 52.07 | 58.05 | 48.34 | 43.86 |
| Saturated | | | | | | |
| Myristic | 1.59 | 1.29 | 2.93 | 2.64 | 2.13 | 2.74 |
| Pentadecenoic | 0.64 | 0.86 | 0.36 | 0.02 | 0.22 | 0.27 |
| Palmitic | 8.42 | 15.30 | 27.30 | 25.89 | 28.38 | 33.33 |
| Heptadecenoic | 0.40 | 0.43 | 0.50 | 0.53 | 0.63 | 0.69 |
| Stearic | 0.48 | 11.31 | 16.71 | 11.86 | 20.32 | 19.09 |
| Total | 11.53 | 29.19 | 47.86 | 40.94 | 51.68 | 56.12 |

Table II: Effect of frozen storage at (-20$^{0}$C) and cooking method on the fatty acids content of lamb meat(Longissimus Dorsi muscle)

| Fatty acids percentage | 1 month | | | 2 months | | | 3 months | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fresh | Boil. | Roas. | Fresh | Boil. | Roas. | Fresh | Boil. | Roas. |
| Unsaturated | | | | | | | | | |
| Tetradecanoic | 0.29 | 0.26 | 0.16 | 0.15 | 0.30 | 0.10 | 0.17 | 0.40 | 1.13 |
| Palmitoleic | 0.14 | 0.45 | 0.32 | 0.75 | 0.33 | 0.46 | 1.08 | 0.22 | 0.69 |
| Heptadecanoic | 0.29 | 1.48 | 0.79 | 0.12 | 0.79 | 0.80 | 0.08 | 0.26 | 0.32 |
| Oleic | 32.37 | 32.91 | 30.02 | 33.70 | 27.17 | 35.93 | 33.87 | 11.71 | 40.83 |
| Linoleic | 1.73 | 5.19 | 2.82 | 1.70 | 3.17 | 2.93 | 1.69 | 0.44 | 2.53 |
| Linolenic | 32.08 | 0.22 | 28.05 | 34.03 | 47.32 | 20.64 | 36.69 | 77.07 | 16.12 |
| Total | 66.90 | 40.51 | 62.16 | 70.45 | 79.08 | 60.86 | 73.58 | 90.10 | 62.62 |

Boil. = Boiled. Roas. = Roasted

Phospholipids content of both L.D and B.F muscles of fresh, frozen stored and cooked lamb meat was determined to follow any changes that might take place in that important fraction of lipids. The results indicated that lamb meat in general, contained more phospholipids than that of any other kinds of meat, i.e. buffalo, beef or camel. Meanwhile,L.D

Table II (Cont.)

| Fatty acids percentage | 1 month | | | 2 months | | | 3 months | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fresh | Boil. | Roas. | Fresh | Boil. | Roas. | Fresh | Boil. | Roas. |
| Saturated: | | | | | | | | | |
| Myristic | 0.72 | 1.99 | 0.80 | 0.73 | 9.72 | 0.83 | 0.89 | 9.24 | 1.13 |
| Pentadecenoic | 0.87 | 2.18 | 1.28 | 0.72 | 1.25 | 3.20 | 0.41 | 1.21 | 2.27 |
| Palmitic | 17.05 | 35.12 | 19.43 | 16.39 | 10.40 | 20.54 | 14.73 | 4.69 | 22.37 |
| Heptadecenoic | 0.72 | 0.96 | 0.53 | 0.66 | 1.27 | 1.52 | 0.32 | 0.16 | 0.50 |
| Stearic | 13.87 | 19.24 | 15.81 | 11.07 | 7.28 | 14.22 | 10.11 | 3.55 | 17.10 |
| Total | 32.78 | 59.49 | 37.85 | 29.55 | 20.92 | 39.30 | 26.41 | 9.84 | 38.37 |

Table III: Effect of frozen storage at (-20°C)and cooking method on the fatty acids content of lamb meat (Biceps Femoris muscle)

| Fatty acids percentage | 1 month | | | 2 months | | | 3 months | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fresh | Boil. | Roas. | Fresh | Boil. | Roas. | Fresh | Boil. | Roas. |
| Unsaturated | | | | | | | | | |
| Tetradecanoic | 0.00 | 0.09 | 0.25 | 0.15 | 0.30 | 0.11 | 0.32 | 0.09 | 0.87 |
| Palmitoleic | 0.53 | 0.33 | 0.46 | 0.98 | 0.32 | 0.42 | 0.22 | 2.39 | 0.06 |
| Heptadecanoic | 1.14 | 0.98 | 0.88 | 0.75 | 1.00 | 1.45 | 0.62 | 0.32 | 0.30 |
| Oleic | 46.01 | 29.91 | 40.17 | 39.01 | 32.10 | 35.94 | 33.70 | 56.56 | 27.89 |
| Linoleic | 4.25 | 2.46 | 3.83 | 4.22 | 5.10 | 2.88 | 4.61 | 4.06 | 1.00 |
| Linolenic | 0.76 | 23.04 | 16.00 | 11.29 | 32.32 | 20.62 | 17.20 | 6.10 | 42.96 |
| Total | 52.69 | 56.81 | 61.59 | 56.40 | 71.15 | 61.02 | 56.67 | 69.43 | 73.08 |
| Saturated | | | | | | | | | |
| Myristic | 1.21 | 1.35 | 1.46 | 1.87 | 1.90 | 1.92 | 0.94 | 1.83 | 0.76 |
| Pentadecenoic | 3.72 | 1.67 | 0.83 | 2.32 | 2.50 | 3.02 | 0.01 | 0.10 | 2.09 |
| Palmitic | 21.83 | 25.08 | 26.25 | 20.96 | 10.40 | 6.60 | 30.89 | 18.18 | 11.83 |
| Heptadecenoic | 1.06 | 0.46 | 0.58 | 0.89 | 2.99 | 1.79 | 0.50 | 0.23 | 0.52 |
| Stearic | 19.48 | 14.63 | 9.29 | 16.66 | 10.96 | 24.98 | 10.99 | 9.76 | 11.74 |
| Total | 47.30 | 43.19 | 28.41 | 42.70 | 28.75 | 38.30 | 43.33 | 30.16 | 26.94 |

Boil.= Boiled Roas. = Roasted.

muscle contained more phospholipids than B.F muscle i.e.2.73mg/100g and 1.57 mg/100 g respectively. However, this is expected as different total intramuscular lipids showed great variation among different muscles and different meats and hence the other fat fractions. Hofmann et al.,(1974).

During frozen storage of lamb meat, there was a clear decrease in the phospholipids concentration in both muscles under investigation i.e. L.D and B.F. The percentage decrease reached its maximum, 86.45% in L.D and 92.35% in B.F after 3 months of storage (Figs.3 and 4). Fresh uncooked L.D muscle contained 2.73 mg phospholipids/100 g of lipids. It decreased to 1.86 mg after 2 months of storage and to 0.37 mg after 3 months of storage. Comparatively, the B.F muscle had an original content of 1.5 mg/100 g lipids and it decreased to 0.63 mg after 2 months of storage and 0.12 mg after 3 months storage.

The mechanism of phospholipids loss or disappearance during frozen storage is not clear. It is likely that several complicated reactions may be involved. These might include oxidation, lipid "browning" reactions, lipid-protein co-polymirzation reactions and lipolysis or enzymatic degradation, Lea and Dawson (1973).

It is also evident from the results that the two methods of cooking used(i.e.boiling & roasting)had a different effect on the phospholipids concentrations,and that the different muscles reacted differently to the effect of the method of cooking.The use of moist heat,i.e.boiling,for one hour induced a clear decrease in the concentration of the phospholipids.However, the loss was more pronounced in B.F muscle than in L.D. The percentage loss reached as high as 18% and 44.58% in both muscles respectively.This decrease might be due to the release of fats to the broth or to more destruction whcih might have occurred in the phospholipids,due to the effect of moist heat.

On the other hand,the use of dry heat(at 170°C for one hour)for cooking lamb induced an increase in the phospholipids concentration,as it increased slightly after roasting.The obtained results are in agreement with those reported by Campbell and Turkki(1967), as they indicated in increase in the phospholipids content of beef and pork muscles after roasting at 172°C.However, the explanation of that increase was not quite clear.

By comparing the phospholipids concentration of cooked lamb meat samples at zero time with those cooked after frozen storage for 1,2 and 3 months intervals,a general decreasing trend was observed. The decrease of the phospholipids concentration in all treatments was mainly due to the freezing process. The method of cooking does not seem to interefere with that trend. However, the percentage loss was more obvious in bioled than roasted samples. Meanwhile, the two different muscles, i.e.L.D and B.F reacted similarly (Figs. 3 and 4).

Cholesterol in animal muscles is usually encountered in both the free and esterfied forms. Table:IV represents the cholesterol and the cholesterol ester content of intramuscular fat of L.D and B.F. muscles of fresh, boiled and roasted lamb meats during frozen storage. The results indicated that fresh lamb meat of L.D muscle contained more cholesterol & cholesterol ester than that of B.F muscle. This increase in cholesterol and cholesterol ester in L.D muscle might be due to the increasing amount of intramuscular fat. Longissimus Dorsi muscle has been rated among the most marbled muscles in the caracass, in all kinds of meat,Griswold (1955).

On the other hand,the obtained results indicated a higher concentration of both cholesterol and cholesterol esters in lamb meat as compared with other kinds of meat i.e.,camel,beef,pork and buffalo(Tu et al.,1967).

Cooking of fresh lamb meat either by dry heat or by moist heat caused a clear decrease in the amount of cholesterol and cholesterol ester contents. Such a decrease was more obvious in case of L.D muscle than B.F muscle. Besides it could be noticed from Table IV that more than 60% of the cholesterol content was lost during boiling of L.D samples,while the loss during boiling of B.F muscle was only about 45%.Such a decrease might be due to either the release of some cholesterol and cheolstero esters to the broth through the effect of heating and/or the degradition of cholesterol by auxidation,Awad et al.,(1968).The same results also indicated that the decrease in cholesterol and cholesterol ester of roasted samples followed the same trend.

During frozen storage of lamb meat at -20°C the cholesterol content tended to increase especially after one month of storage. This increase was pronounced in both L.D and B.F muscles.However, as the frozen storage time progress,cholesterol also continued to increase,but at a lower rate.The increase in the amount of cholesterol during frozen storage might be due to the effect of lipases,leading to the hydrolysis of the intramuscular fat. Table :IV shows that raw lamb meat of L.D muscle frozen for one month contained more cholesterol than that of B.F muscles from the same animal.Meanwhile,no clear differences were observed in the amount of cholesterol esters between the muscles i.e.L.D and B.F. During the second and third months of storage the same results were observed. However,this was expected since fresh L.D muscle contained more cholesterol than B.F muscle.

Cooking lamb after frozen storage for different periods induced a more obvious decrease in its cholesterol content regardless of the cooking method. It seems that any type of heat is capable of inducing destruction in the cholesterol fraction. However, roasting had a less pronounced effect on the cholesterol loss as indicated by the results shown in Table:IV.

Table IV: Cholesterol and cholesterol ester contents of intramuscular fat of L.D and B.F muscles of fresh, boiled and roasted lamb meat during frozen storage.

| Treatment | L.D. Muscle | | B.F. Muscle | |
|---|---|---|---|---|
| | Cholesterol* | Cholesterol ester* | Cholesterol* | Cholesterol ester |
| Fresh | 84.05 | 8.10 | 76.00 | 7.30 |
| Boiled | 28.00 | 5.55 | 42.00 | 4.73 |
| Roasted | 30.00 | 4.07 | 39.28 | 4.20 |
| Frozen Storage | | | | |
| one month | | | | |
| Fresh | 91.25 | 6.96 | 83.64 | 6.38 |
| Boiled | 12.75 | 4.15 | 11.46 | 3.17 |
| Roasted | 44.23 | 5.08 | 20.20 | 3.19 |
| Tow months | | | | |
| Fresh | 94.98 | 9.20 | 89.97 | 8.86 |
| Boiled | 16.02 | 4.12 | 13.77 | 2.35 |
| Roasted | 56.17 | 5.13 | 27.80 | 3.95 |
| Three months | | | | |
| Fresh | 98.17 | 9.46 | 93.10 | 8.97 |
| Boiled | 17.80 | 2.97 | 15.55 | 0.98 |
| Roasted | 37.32 | 3.93 | 32.70 | 2.33 |

* Calculated as mg/100 gm. lipids.


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