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Effect of Freezing and Frozen Storage on Amino Acid Profile and Fatty Acid Pattern in Imported and Local Meat

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ABSTRACT

Due to the effect of cold treatment on the amino acid profile of meat has not sufficiently studied. This study aimed to study the effect of freezing temperature and duration of frozen storage on amino acid profile and fatty acid pattern in imported beef meat, local beef meat, chicken breast and Nile tilapia fish. The effect of freezing on lipid and protein oxidative stability of meat was also measured. The selected meat under research was frozen at different temperature (- 7 ° C) for 1month of local beef, fish and chicken breast meat and (- 18 ° C) for 3 and 6 months of imported meat. The result show the effect of freezing on amino acid as the total amount of essential and nonessential amino acids and amount of each amino acid of meat samples are reduced during the freezing process. However there is no significant difference between the frozen imported meat beef for 3 and 6 months except glycine and glutamic acid were changed from 17.02±0.41 to (14.28±0.66 ; 12.73±0.36) and from (49.24±0.46 to 46.90±0.37 ; 45.05±0.38) respectively. There is a significant effect of frozen storage duration on saturated fatty acids (SFA), unsaturated fatty acids (USFA) and total fatty acids (TFAs). A significant ($P < 0.05$) increase in total volatile nitrogen (TVN) value after freezing for 3 and 6 months in imported beef meat from 14.41±0.21 to 15.50± 0.29 and 18.25±0.27 respectively, also in frozen fish samples from 18.41±0.35 to 21.11±0.47. While there is no significant ($P>0.05$) difference in frozen local beef meat and frozen chicken meat samples. Moreover, there was an increment in the rate of lipid oxidation during frozen storage of the examined samples but within acceptable limit. The present study concluded that cold treatment had a great impact on chemical composition of meat especially amino acids profile and fatty acids pattern regardless the duration of freezing storage.

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1. INTRODUCTION

Freezing is a rapid heat transfer that used for preservation of meat and meat products as it leads to minimal loss of quality during long term storage. Also freezing is one of the easiest, quickest, most versatile and most convenient methods of food preserving. Properly frozen food maintain more of their original colour, flavor, texture and nutrients than foods preserved by other methods (Julie, 2013). Moreover, freezing is used to retard undesirable biochemical reactions in meat but there is some cell disruption and destruction of muscle fiber due to the formation of ice crystals (Sebranek, 1982). To meet the requirements for healthy human diet it is important not only to acquire meat with desirable fatty acids but also to preserve it the best, thus freezing is considered to be an excellent mean aid for maintaining meat quality for long periods and frozen storage has been regarded as useful technological aid (Mateo and Perez, 2004). In many developing countries especially Egypt, meat is widely consumed as a source of protein; it is either eaten cooked or processed into other forms to ovoid associated spoilage (Olaoye et al., 2010; Olaoye and Onilude, 2010). Meat is a nutritious protein rich food which is highly perishable and has a short shelf life unless preservation methods are used (Olaoye and Onilude, 2010). It is an excellent source of high biological value protein and important micro nutrients that are needed for good health throughout life. As the fact that it is either their only source or they have a higher bioavailability of Vit A and Vit B12 occur exclusively in meat and can hardly be compensated for by plant derived pro vitamins (Biesalski, 2005).

Amino acids are a basic building-blocks of proteins, during protein constructions in your cells amino acids join together as long-chains that wrap and twist around each other and give shape to the structure, fundamentally amino acids are joined together by peptide bonds to form the basic structure of protein (Peter, 1998). Amino acids

profile is an important parameter because some amino acids cannot be synthesized in human body and must be obtained from diet (Alina and Ovidu, 2007) thus amino acids play a significant role in determination the nutritional value of meat.

Essential fatty acids are that humans and other animals must ingest because the body requires them for good health but cannot synthesize them (Robert et al., 1980). Omega-3 and omega-6 fatty acids are important in normal functioning of all tissue of body (Linscheer and Vergroesen, 1994; Barnard, 1998) and their deficiencies are responsible for a host of symptoms and disorders including abnormalities in liver and kidney, decreased immune function, depression (Sanders and Emery, 2009), crazy- pavement scaly dermatitis, alopecia and thrombocytopenia (Behram et al., 2004) so, the ideal ratio of omega-6 and omega-3 fatty acid is (5:10 -1) for optimal health benefits (WHO/FAO, 1995).

The health promoting value of meat depends largely on the fat content and fatty acid composition. Also the composition of fatty acids in meat fat affects not only the palatability and dietetic value, but also storageability of meat (Monika et al., 2007).

Broadly our country Egypt depend largely on the imported meat from different countries so in this study we aim to determine the effect of freezing, frozen storage and freezing temperature on the imported and local meat and measure the effect of freezing on lipid and protein oxidative stability of meat.

2. MATERIAL AND METHODS

2.1. Preparation of samples and storage:

This study was done on beef meat both local and imported, chicken breast meat and Nile tilapia fish meat. The five samples of fresh imported meat are of Brazilian origin, were obtained from agriculture outlets, collected from different parts of animal, directly after slaughtering and sent immediately to laboratory analysis. The five samples of local beef meat were collected randomly from different parts of animal and were obtained from local slaughter house immediately after slaughtering and sent immediately to lab analysis. The five samples of fresh Nile tilapia fish meat (*Oreochromis niloticus*) were collected randomly from different parts of fish and were obtained from market in Alexandria and sent immediately to laboratory analysis. The five samples of fresh chicken breast meat were collected randomly from different parts of chicken breasts and were obtained from market in Alexandria immediately after slaughter and sent immediately to laboratory analysis. The five samples of frozen imported beef meat for 3 and 6 months are of Brazilian origin , were obtained from Al- Nagar company in Alexandria and stored in boxes at - 18°c till lab analysis. The preparation of five samples of frozen (local beef meat, chicken breast meat, Nile Tilapia fish) for 1 month by storing fresh samples at 4°c for 4±1 hrs then samples were placed in aluminum foil in the freezer (- 7°c) for 1 month with an identification code. Each sample weight 300 g and was placed in aluminum foil then was transferred in an insulated ice box to the laboratory.

2.2. Sampling:

A total of random cut samples of frozen and fresh meat are divided into nine groups as follow:

Group 1: Fresh imported beef meat.
Group II : Frozen imported beef for 3 months at - 18°C.
Group III : Frozen imported beef for 6 months at - 18°C.
Group IV: Fresh local beef meat.
Group V : Frozen local beef meat for 1 month at - 7°C.
Group VI : Fresh Nile tilapia fish.
Group VII : Frozen Nile tilapia fish for 1 month at - 7°C.
Group VIII : Fresh chicken breast.
Group IX : Frozen chicken breast meat for 1 month at - 7°C .

2.3. Evaluation of amino acids profile by Amino acid analyzer :

The meat sample was defatted using diethy ether and 0.4g was hydrolysed in sealed evacuated Pyrex test tube using 5 ml of 6 N HCL at 110°C for 24hrs.At the end of period, hydrolysate was transferred quantitavely to containers and the HCL was then evaporated to dryness at 50 - 60°C on water bath. Distilled water (5 ml) was added to hydrolysate and then evaporated to dryness to remove the excess of hydrochloric acid and then further addition of distilled water till complete removal the excess of hydrochloric acid and the samples were dried till dry film obtained. The obtained dry film was dissolved in a known volume buffer (0.1 N sodium acetate buffer, PH 2.2) and the solution of sample dilution was filtered through a 0.45 mm membrane filter. The samples were stored frozen in sealed vials till fractionation of amino acids by Amino acid analyzer (LC 3000 Eppendorf).

2.4. Evaluation of fatty acids profile by Gas Chromatography:

2.4.1. Lipid Extraction:

The technique was applied according to method described by Pearson, (1981).

Weigh 2-20 g of the sample into a 250 ml centrifuge bottle, add sufficient water to bring total water present to 16 ml together with 40 ml methanol and 20 ml chloroform. Macerate for 2min; add further 20 ml chloroform and Macerate for 30 sec. Centrifuge the mixture for 10 min at 2000-2500 rpm. Draw off the lower chloroform layer and filter through a course filter paper into a dry weighed flask or beaker. Evaporate the chloroform to dryness.

2.4.2. Methylation of Lipid:

The obtained fatty acid were converted to methyl ester as follow; in a tube weigh 50 mg of Lipid, add 5 ml of methanolic sulphuric acid (1 ml Conc sulphuric acid and 100 ml methanol) and 2 ml of benzene, close the tube well and place it in water bath at 90°c for an hour and half. Cool, add 8 ml water and 5 ml petroleum ether shake strongly and separate out the ethereal layer in a dry tube. Evaporate to dryness. Finally, the methyl ester of fatty acids was performed and the aliquots of this solution were subjected to analysis by GLC (6890 series).

2.4.3. Separation of fatty acid methyl ester:

The fatty acid methyl esters were analyzed by Hewlett Packard gas chromatography (6890 series) equipped with flame ionization detector. The flow rate inside column was1 ml /m. Under these conditions, peak identification was performed by comparison of the relative retention time (RRT) for each peak with those of standard chromatograms. The peak was measured by triangulation and the relative proportions of the individual compound were therefore obtained by determination of the partial areas in relation to the total area.

2.5. Determination of Meat Protein Peroxidation (TVN) :

For determination of total volatile nitrogen the magnesium oxide method was used in which the samples which contain (ammonia, mono-methyl amine, diethyl amine and tri-methyl-amine and other volatile amine). Samples were blended with magnesium oxide and distilled into boric acid. The boric acid was titrated to its original with strong acid (H_2SO_4) at low concentration to determine the amount of the base distilled, which correlated to the total volatile nitrogen as described by AOAC (1990).

10 g of sample was added to the heating flask containing 300 ml distilled water plus 2 g magnesium oxide and anti-bumping granules. In the receiving flask 25 ml of boric acid (2%), a few drops of methyl red indicator was added. The two flasks (heating & receiving) were connected to the evaporator and the water bath was managed. After 25 minutes, distillation was stopped. The content of the receiving flask was transferred to another flask and titrated to the end point by very weak acid 0.05 (H_2SO_4).

The total volatile nitrogen was determined as follows:

$$TVN\ (mg / 100\ mg) = \frac{(V \times N \times 100 \times 14)}{W}$$

V= volume (ml) H_2SO_4 used for sample. N= normality of H_2SO_4. W= weight of sample in grams.

2.6. Determination of Meat Lipid Peroxidation (TBARS) :

TBARS indicate the oxidative changes in muscle foods during frozen storage. The amounts of TBARS in washed, minced meat samples were determined according to method described by Pearson, (1968) and is reviewed by Gray, (1978). The increase in the amount of red pigment formed in the reaction between 2-thiobarbituric acid (TBA) and oxidized lipids as oxidatives rancidity advances has been applied to a wide variety to fatty foods.

Macerate 10 g fatty food with 50 ml water for 2 min and wash into a distillation flask with 47.5 ml water. Add 2.5 ml of 4 M hydrochloric acid to bring the PH to 1.5, followed by 10 min from the time boiling commences. An antifoaming preparation and a few glass beads. Heat the flask by means of an electric mantle so that 50 ml distillate is collected in Pipette 5 ml distillate into a glass-stoppered tube. Add 5 ml TBA reagent (0.2883 g / 100 ml of 90 percent glacial acetic acid), stopper, shake and heat in boiling water for 35 min. Prepare a blank similarly using 5 ml water with 5 ml reagent. Cool the tubes in water for 10 min and measure the absorbance (D) against the blank at 538 nm using 1 cm cells.

TBA no . (as mg malonaldehyde per kg sample) = 7.8 D.

2.6. Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Kirkpatrick. (2013) Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-

Wilk test and D'Agstino test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparison between two independent population were done using independent t-test while more than two population were analyzed F-test (ANOVA) to be used and Post Hoc test (LSD). Significance of the obtained results was judged at the 5% level.

3. RESULTS

3.1. Evaluation of amino acids profile by Amino acid analyzer:

Table 1 and 2 illustrates the total amount of essential and nonessential amino acids and amount of each amino acid of meat samples are reduced during the freezing process. However there is no significant difference between the frozen imported meat beef for 3 and 6 months except glycine and glutamic acid were changed from 17.02±0.41 to (14.28±0.66; 12.73±0.36) and from (49.24±0.46 to 46.90±0.37; 45.05±0.38) respectively. Such changes depend considerably on the structural characteristics of amino acids, their solubility in water and their chemical structure as well as freezing temperature

3.2. Evaluation of fatty acids profile by Gas Chromatography:

The fatty acid composition g / 100 g of total lipid fraction and changes therein during storage period in beef meat either imported or local , fish and chicken meat is summarized in Table 3 and 4 The changes in FA profiles during frozen storage were significant (P <0.05).

3.3 Determination of Meat Protein Peroxidation (TVN) :

Data summarized in Table 5 revealed that there is significant (P >0.05) increase in TVN value after freezing for 3 and 6 months in imported beef meat from 14.41±0.21 to 15.50±0.29 and 18.25± 0.27 respectively, also in frozen fish samples from 18.41±0.35 to 21.11±0.47. While there is no significant (P>0.05) difference in frozen local beef meat and frozen chicken meat samples.

Table 1. Changes of essential and non- essential AAs contents in meat after freezing.

Amino acids (mg / g) protein	Group I	Group II	Group III	Group IV	Group V
Aspartic	15.19±0.27 ^{a}	24.42±0.56 ^{b}	23.41±0.21 ^{b}	29.34±0.31 ^{a}	27.51±0.25 ^{b}
Threonine	11.60±0.30 ^{a}	11.30±0.37 ^{a}	11.22±0.30 ^{a}	12.97±0.39 ^{a}	11.53± 0.25 ^{b}
Serine	10.28±0.32 ^{a}	10.20±0.31 ^{a}	9.42±0.26 ^{a}	10.30±0.25 ^{a}	9.18±0.31 ^{b}
Glutamic	49.24±0.46 ^{a}	46.90±0.37 ^{b}	45.05±0.38 ^{c}	50.70±0.21 ^{a}	45.96±0.43 ^{b}
Proline	14.18±0.72 ^{a}	11.32±0.56 ^{b}	10.98±0.41 ^{b}	13.45±0.33 ^{a}	12.23±0.40 ^{b}
Glycine	17.02±0.41 ^{a}	14.28±0.66 ^{b}	12.73±0.36 ^{c}	14.41±0.28 ^{a}	12.94±0.35 ^{b}
Alanine	15.16±0.30 ^{a}	13.68±0.45 ^{b}	13.54±0.56 ^{b}	16.34±0.48 ^{a}	15.48±0.30 ^{a}
Cysteine	2.34±0.22 ^{a}	1.84±0.21 ^{ab}	1.62±0.14 ^{b}	1.76±0.19 ^{a}	1.43±0.23 ^{a}
Valine	15.70±0.21 ^{a}	14.76±0.42 ^{a}	12.82±0.31 ^{a}	15.70±0.21 ^{a}	14.76± 0.42 ^{a}
Methionine	5.86±0.37 ^{a}	6.71±0.35 ^{a}	7.54±0.45 ^{a}	5.86±0.37 ^{a}	6.71± 0.35 ^{a}
Isoleucine	13.97±0.59 ^{a}	12.35±0.21 ^{a}	12.32±0.32 ^{a}	13.97±0.59 ^{a}	12.35±0.21 ^{b}
Leucine	26.83±0.39 ^{a}	23.20±0.27 ^{b}	23.70±0.28 ^{b}	26.83±0.39 ^{a}	23.20±0.27 ^{b}
Tyrosine	10.64±0.42 ^{a}	9.35±0.27 ^{a}	10.01 ±0.35 ^{a}	10.64±0.42 ^{a}	9.35±0.27 ^{b}
Ph.alanine	11.80±0.38 ^{a}	13.51±0.26 ^{a}	12.0±0.50 ^{a}	11.80±0.38 ^{a}	13.51±0.26 ^{b}
Histidine	15.54±0.25 ^{a}	13.28±0.39 ^{a}	13.18±0.28 ^{ab}	15.54±0.25 ^{a}	13.28±0.39 ^{b}
Lysine	29.21±0.39 ^{a}	25.95±0.58 ^{a}	25.35 ±0.28 ^{a}	29.21±0.39 ^{a}	25.95±0.58 ^{b}
Arginine	21.05±0.34 ^{a}	20.69±0.48 ^{a}	21.26±0.58 ^{a}	21.05±0.34 ^{a}	20.69±0.48 ^{a}
Total essential	142.34±1.13 ^{a}	141.60±0.81 ^{a}	135.79±0.67 ^{b}	146.59±1.16 ^{a}	143.99±0.52 ^{a}
Total non-essential	130.85±0.39 ^{a}	124.08±0.99 ^{b}	121.99±1.06 ^{b}	144.29±0.78 ^{a}	132.08±0.71 ^{b}
Total AAs	273.19±1.18 ^{a}	265.69±1.75 ^{b}	257.78±1.30 ^{c}	290.88±1.62 ^{a}	276.07±0.61 ^{b}

I: Control fresh imported beef, II: frozen imported for 3 months, III: frozen imported for 6 months,
IV: Control local beef meat, V: frozen local beef meat for 1month. ND (not detectable).
*Values are means ± standard errors. Means in a row with a different letter differ significantly.
Values are statistically significant at P < 0.05.
Comparison between group I, II and III was done separate from group IV and V.

Table 2. Changes of essential and non- essential AAs contents in meat after freezing.

Amino acids (mg / g) protein	Group VI	Group VII	Group VIII	Group IX
Aspartic	27.17±0.27 [a]	26.39±0.54 [a]	32.50±0.72 [a]	21.41±0.57 [b]
Threonine	11.61±0.26 [a]	9.87±0.32 [b]	13.68±0.55 [a]	8.63±0.51 [b]
Serine	10.13±0.31 [a]	8.02±0.30 [b]	10.84±0.50 [a]	7.19±0.29 [b]
Glutamic	45.19±0.40 [a]	36.04±0.31 [b]	48.17±0.39 [a]	30.96±0.48 [b]
Proline	9.30±0.29 [a]	9.40±0.19 [a]	12.75±0.29 [a]	8.76±0.28 [b]
Glycine	14.47±0.23 [a]	13.64±0.24 [b]	15.93±0.44 [a]	9.74±0.39 [b]
Alanine	15.47±0.56 [a]	13.63±0.49 [b]	17.73±0.25 [a]	11.64±0.31 [b]
Cysteine	1.62±0.10 [a]	1.72±0.14 [b]	ND	ND
Valine	12.41±0.30 [a]	13.62±0.49 [a]	18.42±0.19 [a]	12.93±0.35 [b]
Methionine	8.57±0.22 [a]	6.50±0.19 [b]	8.74±0.43 [a]	5.61±0.21 [b]
Isoleucine	12.65±0.29 [a]	10.50±0.32 [b]	15.34±0.30 [a]	9.81±0.52 [b]
Leucine	22.53±0.56 [a]	19.70±0.51 [b]	27.38±0.31 [a]	17.67±0.54 [b]
Tyrosine	7.67±0.47 [a]	6.91±0.46 [a]	11.14±0.25 [a]	7.44±0.58 [b]
Ph.alanine	12.40±0.61 [a]	11.39±0.29 [a]	15.10±0.37 [a]	12.71±0.51 [b]
Histidine	11.14±0.53 [a]	9.79±0.28 [a]	22.49±0.53 [a]	16.30±0.53 [b]
Lysine	27.46±0.62 [a]	24.63±0.31 [b]	32.77±0.49 [a]	21.66±0.73 [b]
Arginine	24.52±0.31 [a]	16.23±0.50 [b]	24.52±0.31 [a]	16.01±0.62 [b]
Total essential	141.53±1.15 [a]	123.35±0.44 [b]	162.44±0.38 [a]	105.72±1.26 [b]
Total non-essential	136.44±0.58 [a]	104.66±0.64 [b]	165.05±0.28 [a]	112.76±2.42 [b]
Total AAs	277.97±1.73 [a]	228.01±0.21 [b]	327.50±0.48 [a]	218.48±2.83 [b]

VI: Control fresh fish, VII: Frozen fish for 1 month,
VIII: Control fresh chicken breast, IX: frozen chicken breast for 1 month
ND (not detectable) Values are means ± standard errors.
Means in a row with a different letter differ significantly. Values are statistically significant at $P < 0.05$.
Comparison between group VI and VII was done separate from group VIII and IX

Table 3 . Effect of freezing on fatty acid analysis by Gas Chromatography.

Fatty acids (g /100g) lipid	Group I	Group II	Group III	Group IV	Group V
C4:0 (Butyric)	0.01±0.01	ND	ND	0.0±0.0 [a]	0.0±0.0 [a]
C6:0 (Caproic)	0.02±0.0	ND	ND	0.0±0.0	ND
C8:0 (Caprylic)	0.02±0.0	ND	ND	0.02±0.0	ND
C10:0 (Capric)	0.02±0.0	ND	ND	ND	ND
C12:0 (Lauric)	0.03±0.01 [a]	0.35±0.03 [b]	0.06±0.01 [a]	0.02±0.0 [a]	0.02±0.01 [a]
C13:0 (Tridecyclic)	0.81±0.05 [b]	0.08±0.01 [a]	0.04±0.01 [a]	0.03±0.01 [a]	0.04±0.01 [a]
C14:1 (Myristoleic)	0.74±0.05 [a]	2.76±0.08 [b]	0.07±0.01 [c]	0.27±0.02 [a]	0.15±0.02 [b]
C14:0 (Myristic)	0.29±0.01	1.01±0.01	ND	ND	0.04±0.01
C15:1 (Pentadecanoic)	ND	0.38±0.03	ND	ND	0.02±0.01
C15:0 (Pentadecuclic)	0.05±0.0	ND	ND	ND	ND
C16:1 (Palmitoleic)	0.13±0.01 [a]	0.43±0.03 [b]	0.14±0.03 [a]	0.09±0.01 [a]	0.04±0.01 [b]
C16:0 (Palmitic)	4.51±0.20	ND	ND	1.77±0.08	ND
C17:1 (Heptadecenoid)	0.30±0.04	ND	ND	1.38±0.15[a]	1.35±0.19 [a]
C17:0 (Margaric)	ND	ND	ND	1.90±0.19	ND
C18:3 (Lenolenic)	0.09±0.01	ND	ND	2.27±0.13 [a]	0.06±0.0 [b]
C18:2 (Linoleic)	1.58±0.14 [a]	0.57±0.07 [b]	0.14±0.02 [c]	ND	ND
C18:1 (Oleic)	2.37±0.18	0.15±0.02	ND	ND	ND
C18:0 (Stearic)	5.83±0.28	ND	ND	ND	ND
Saturated FAs	10.85±0.48 [a]	2.16±0.05 [b]	0.10±0.01 [c]	3.74±0.22 [a]	0.10±0.01 [b]
Unsaturated fatty acids	5.11±0.21 [a]	4.14±0.07 [b]	0.58±0.04 [c]	4.01±0.16 [a]	0.26±0.02 [b]
Total fatty acids	15.97±0.62 [a]	6.30±0.07 [b]	0.68±0.04 [c]	7.75±0.27 [a]	0.37±0.03 [b]

I: Control fresh imported beef, II: frozen imported for 3 months, III: frozen imported for 6 months,
IV: Control local beef meat, V: frozen local beef meat for 1month
ND (not detectable). *Values are means ± standard errors.
Means in a row with a different letter differ significantly.
Values are statistically significant at $P < 0.05$.
Comparison between group I, II and III was done separate from group IV and V.

Table 4. Effect of freezing on fatty acid analysis by Gas Chromatography.

Fatty acids (g /100 g) lipid	Group VI	Group VII	Group VIII	Group IX
C4:0 (Butyric)	ND	0.05±0.01	ND	0.09±0.01
C6:0 (Caproic)	ND	ND	0.01±0.0 [a]	0.03±0.01 [b]
C8:0 (Caprylic)	ND	0.01±0.0	ND	0.04±0.01
C10:0 (Capric)	ND	0.03±0.0	0.02±0.01	ND
C12:0 (Lauric)	ND	ND	0.03±0.01	ND
C13:0 (Tridecyclic)	0.14±0.01 [a]	0.12±0.01 [a]	0.05±0.01 [a]	0.18±0.04 [b]
C14:1 (Myristoleic)	0.53±0.04 [a]	0.17±0.01 [b]	0.02±0.01 [a]	0.37±0.03 [b]
C14:0 (Myristic)	0.09±0.01 [a]	0.58±0.03 [b]	ND	0.50±0.04
C15:1 (Pentadecanoic)	0.77±0.06 [a]	0.23±0.03 [b]	0.22±0.02 [a]	0.47±0.03 [b]
C15:0 (Pentadecuclic)	0.54±0.03 [a]	0.34±0.03 [b]	ND	0.22±0.04
C16:1 (Palmitoleic)	ND	0.05±0.01	0.05±0.01	ND
C16:0 (Palmitic)	ND	0.97±0.06	0.59±0.05 [a]	1.18±0.05 [b]
C17:1 (Heptadecenoid)	1.74±0.13	ND	10.06±0.31	ND
C17:0 (Margaric)	0.58±0.06	ND	0.57±0.04	ND
C18:3 (Lenolenic)	0.48±0.03 [a]	0.83±0.06 [b]	10.58±0.29 [a]	0.34±0.02 [b]
C18:2 (Linoleic)	1.35±0.06	ND	7.15±0.57 [a]	0.48±0.03 [b]
C18:1 (Oleic)	1.54±0.01	ND	6.22±0.30	ND
C18:0 (Stearic)	0.06±0.01	ND	0.18±0.01	ND
C21:0 (Heneicosylic)	ND	ND	0.13±0.02	ND
Saturated fatty acids	4.73±0.15 [a]	0.66±0.04 [b]	16.84±0.53 [a]	1.14±0.05 [b]
Unsaturated fatty acids	2.46±0.04 [a]	3.36±0.16 [b]	18.49±0.88 [a]	3.29±0.11 [b]
Total fatty acids	7.19±0.19 [a]	4.02±0.15 [b]	35.33±0.80 [a]	4.43±0.13 [b]

VI: Control fresh fish, VII: Frozen fish for 1 month, VIII: Control fresh chicken breast,
IX: frozen chicken breast for 1 month ND (not detectable). *Values are means ± standard errors.
Means in a row with a different letter differ significantly. Values are statistically significant at $P < 0.05$.
Comparison between group VI and VII was done separate from group VIII and IX

3.4 Determination of Meat Lipid Peroxidation (TBARS) :

Lipid peroxidation was studied with regard to thiobarbituric acid (TBA). The TBA number is a measure of malonaldehyde (MA), a byproduct of lipid oxidation. TBA records revealed an increased rate of lipid oxidation during frozen storage of the examined samples. The significant ($P > 0.05$) increase in the TBA value from 0.19±0.01 to 0.25±0.020; 29±0.01 in imported beef meat frozen for 3 and 6 months respectively ; local meat from 0.29±0.01 to 0.72±0.03; local fish from 0.53±0.04 to 0.72±0.03; local chicken from 0.34±0.03 to 0.59±0.04. Data summarized in Table 6.

4. DISCUSSION

4.1. Effect of freezing on meat amino acids :

The effect of cold treatment on the amino acid composition of meat has not been sufficiently studied. Amino acid profile is an important parameter as some amino acid cannot be synthesized in human body and must be from diet (Alina and Ovidu, 2007) so play a significant role in determination nutritional value of meat (Demby and Cunningham, 1980) as meat has long been known for its nutritive compositions that could explain why it is being consumed by many people worldwide as protein profile of meat consists of AAs that have been described as an excellent due to presence of all essential amino acid required for body, it has been proved that the proteins in meat could not be substituted for plant sources (Olaoye, 2011).

Table 5. Effect of freezing on meat protein peroxidation by total volatile nitrogen (TVN).

Groups	TVN (mg /100 mg)
Group I	14.41±0.21 [a]
Group II	15.50±0.29 [b]
Group III	18.25±0.27[c]
Group IV	15.49±0.33 [a]
Group V	15.60±0.45 [a]
Group VI	18.41±0.35 [a]
Group VII	21.11±0.47 [b]
Group VIII	16.57±0.38 [a]
Group IX	17.07±0.45 [a]

I (control fresh imported beef), II (frozen imported for 3 months) , III (frozen imported for 6 months), IV (control local beef meat) , V (frozen local beef meat for 1 month), VI (control fresh fish), VII (frozen fish for 1 month) , VIII (control fresh chicken), IX (frozen chicken for 1 month) *Values are means ± standard errors. Means in a row with a different letter differ significantly. Values are statistically significant at P <0.05.
Comparison between group I, II and III was done separate from group IV and V. Comparison between group VI and VII was done separate from group VIII and IX.

Table 6. **Effect of freezing on meat lipid peroxidation by thiobarbituric acid (TBA)**.

Groups	**TBARS (mg / kg)**
Group I	0.19±0.01 [a]
Group II	0.25±0.02 [b]
Group III	0.29±0.01 [b]
Group IV	0.34±0.02 [a]
Group V	0.72±0.03 [b]
Group VI	0.53±0.04 [a]
Group VII	0.77±0.02 [b]
Group VIII	0.34±0.03 [a]
Group IX	0.59±0.04 [b]

I (control fresh imported beef), II (frozen imported for 3 months) , III (frozen imported for 6 months), IV (control local beef meat) , V (frozen local beef meat for 1 month), VI (control fresh fish), VII (frozen fish for 1 month) , VIII (control fresh chicken), IX (frozen chicken for 1 month). *Values are means ± standard errors.
Means in a row with a different letter differ significantly. Values are statistically significant at P <0.05.
Comparison between group I, II and III was done separate from group IV and V. Comparison between group VI and VII was done separate from group VIII and IX.

It is obvious from the results obtained in Table (1 and 2) that the total amount of essential and nonessential amino acids and amount of each amino acid of beef meat either imported or local meat, fish and chicken meat are reduced during freezing process regardless of freezing temperature. However such changes depend considerably on freezing temperature and frozen storage .

This agree with Baraneko et al., (2014) who reported the same result of veal meat frozen at temperature of -24°C and -35°C. The result in Table (1 and 2) showed that the chicken breast meat and fish have the highest amount of total essential amino acids compared with other species and has the lowest amount of proline (nonessential amino acid) which decreases nutritive value of the product, so the chicken breast meat and meat have higher nutritional value. This agree with Saad et al., (2013) who reported that chicken breast has high nutritional value than duck meat.

4.2 Effect of freezing on meat fatty acids:

The rate and extent of fat oxidation depend on the degree of fatty acid saturation, oxygen exposure, storage time and temperature (Tomas and Anon, 1990) . This explain what we found in results obtained in Table (3) as C17:1 (Hepta) and C18:3 (w3) were found only in fresh imported beef and disappear in frozen imported beef for 3 and 6 months. Also C18:1 (w9) disappear in frozen imported beef meat for 6 months this explain the great effect of storage time on lipid peroxidation. Changes in lipid contents of chicken breast meat associated with freezing are shown in Table (4) that revealed the disappearance of C16:1, C17:1(Hepta) and C18:1(w9) . This agree with Ayla et al., (2009) who reported that the lipid content of chicken leg and breast meat decreased during frozen storage, due to lipid peroxidation which altered the chemical

composition of meat. Roopma et al., (2012) stated that the decreasing in total lipid content in meat attributed due to lipid oxidation mainly due to losses in triglycerides fraction. This agree with our study as total fatty acids was decreased in all sample species. Alvarez et al., (2009) did not find a significant effect of time of storage on the amount of SFA. This is in contrast to our study as we noticed that there is significant decrease of C12:0 in imported meat stored for 6 months compared to frozen imported for 3 months , moreover the disappearance of C14:0 in frozen imported beef meat for 6 months compared to frozen imported for 3 months , also disappearance of C18:0 and C16:0 in both frozen imported meat, and C16:0 and C17:0 in frozen local beef meat. This is agree with Monika et al., (2007) who reported there was a significant decrease in the level of C16:1 in meat fat of calves and what we observed are in agreement with Popova, (2014) as reported that total SFA decreased through storage , but in contrast to this author as stated that the content of C16:1 of SM muscle of lamb increased at 6th month .

4.3 Effect of freezing on protein peroxidation (TVN):

During frozen storage many reactions occurred between different meat components for example the fish and poultry meat are susceptible to oxidative reactions due to their high concentrations of oxidation catalysts as myoglobin and iorn (Ashgar et al., 1988). This explain what we found as there is significant increase in TVN in frozen fish meat. And this agree with Hassan et al., (2012) who reported that a continuous increasing in the level of total volatile nitrogen with time of storage among fish species. The results in Table 5 showed higher value of TVN in fish and chicken breast meat, this attributed to the higher protein content in chicken breast and fish meat compared with other species. This is agree with Edris et al., (2012) who stated that the TVN value in chicken thigh , breast and drum stick stored at 2-5°C for different periods.

4.4 Effect of freezing on lipid peroxidation (TBA):

Table 6 revealed that the TBA value of frozen imported Brazilian beef meat for 3 and 6 months was 0.25±0.02 and 0.29±0.01 respectively. This not agree with what Hemmat et al.,(2005) stated as they revealed that the mean TBA value of examined (Indian) imported frozen meat was 0.58 ± 0.19, while for (Brazilian) samples was 1.03 ± 0.54. Al-Hamdany, (2009) mentioned that the TBA values in inspected frozen chicken thigh samples were 0.91 – 6.53 mg MDA / kg meat which are markedly different from the current results , and probably the same values obtained by Nam et al. (2000) who recorded TBA values in chicken thigh meat to reach 0.17 mg MDA / kg. According to the limits regulated by (ICOSQC, 1987) who specified the TBA should not exceed more than 5 mg MDA/ kg chicken meat.(Hazhaow et al., 2013) , this in agreement with the current results.

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