Vet.Med.J.,Giza. Vol.52, No.3. (2004) : 415-428.

BIOCHEMICAL DIFFERENTIATION BETWEEN FRESH AND THAWED RED BUFFALO MEAT.

SAMIA EL ZEINI* and B.G.A. FAHMY**

*Food hygiene department and **Biochemistry, nutritional disease and toxicology department, Animal health institute, Dokki, Cairo, Egypt.

Received: 21.4.2004.
Accepted: 23.6.2004.

SUMMARY

Ten kilograms of gluteal muscle of native buffalo was divided into 10 equal parts. Each kilogram was subdivided into 4 parts each of 250 grams. The First part was examined as fresh meat, the other three groups, were kept in deep freezer (-20°C) in plastic package for three successive weeks. After each week the samples were allowed to thaw at room temperature (25-28°C) for 2.5 hours, then examined and refrozen. The samples were examined for three successive weeks. PH, moisture, ash, total protein, sodium, potassium, iron, ALT, AST, total protein of thawed meat and drip were measured and polyacrylamide electrophoresis was carried out during study. Humidity and pH as well as ash and total protein percentages were decreased after thawing for three weeks. Sodium and iron were increased but potassium was decreased. ALT and AST were also decreased, total protein for both soluble protein and drip was decreased. Protein bands profile demonstrates characteristic changes in meat and its drip.

INTRODUCTION

The application of freezing for the preservation of food has been practiced for several years to maintain its quality during storage, distribution and marketing. Although meat freezing is generally conceded to cause tissue damage and some quality loss, it remains the method of preference for long-term storage (Lind et al, 1971; El-Badawi and Hamm, 1976; Varnam and Sutherland, 1998 and Lyon et al, 2000). Temperature

fluctuation and/or abuse generally during transportation, storage and/or consumption are the main problems. Those problems are direct contributors to the biochemical and physicochemical changes of the muscle structure (Benjakul et al, 1997 and Benjakul and Bauer, 2001). In low industrial facilities problems come out (like electricity cut off which cause storage temperature fluctuation, shipping problems and/or the frozen meat is not stored at correct temperature throughout its storage life) (Varnam and Sutherland, 1998).

The changes associated with frozen storage are due to the osmotic removal of water, denaturation of protein and mechanical damage that lead to release of enzymes and other components (Considine and Considine, 1982; Restle et al., 1996 and 2002).

Frozen meat which is thawed out, yield on abundant supply of extracts, forming an excellent medium for bacterial growth that enhanced the physical and chemical changes in meat leading to lowering its keeping quality and market ability. Bacteriological evaluation of fresh and thawed meat has been conducted by many authors (Berry et al., 1984 a & b and Chang et al., 2003), while chemical evaluation is still not well documented. Fresh meat may be substituted with frozen-thawed meat. Consumers can not differentiate easily between fresh and frozen-thawed meat. Therefore, the purpose of the present investigation is to determine the effect of multiple freeze-thaw cycle on biochemical and physicochemical changes in the buffloe red meat muscle and there after to differentiate between fresh and thawed frozen meat.

MATERIAL AND METHODS

A total of 10 kilograms of fresh lean buffloe meat from gluteal region from 10 bufflow were obtained from cairo abbatoare (EL-basatine area, cairo) and divided into 10 equal parts. All samples were free of fat. Each kilogram of lean meat was subdivided into 4-equal parts. The First part was directly examind, while the other three parts were packaged in polyethylene bags, sealed and subjected to freezing (-20°C). At the end of the first week, the frozen samples in the sealed packages were then placed at room temperature (25 ± 3°C) for 2.5 hours till complete thawing. One of the three samples was subjected to investigations while the other two samples were re-frozen for another weak. At the end of the second weak frozen samples were thawed again and one of them was examined while the third sample was re-frozen, thawed and examined at the end of the third weak under the previous conditions.

Meat samples (fresh and frozen-thawed) were analyzed for the following physicochemical parameters; meat (color and texture) and juice (color, quality and quantity). Meat and juice color were estimated by naked eye. Texture was tested by touch and pressure. Moisture and protein contents

were determined according to standard methods of AOAC, (1990). pH was determined using pH meter(ph mater E512 Metrohom herisaa) according to ISO, (1974). Two grams of meat were ashen in hot-air oven at 550°C for 4-6 hours. Ash content was determined using the method of Aurand, (1987). Sodium, potassium, and total iron were determined in ash of fresh and thawed meat according to Trinder, (1951); Sunderman and Sunderman, (1958) and Georgy (1980), respectively.

Two grams of meat were chopped into small pieces with 15 ml normal saline (0.09%) and immediately centrifuged at 5000 r.p.m. for 4min. at 4°C to obtain meat exudates. Exudates from the meat samples were collected using Pasteur pipette and its volume was measured and used for determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity using the method of Rietman and Frankle, (1957).

The frozen meat was left for 2.5 hours until complete thawing in their plastic bags to drip. For determination of polyacrylamide fractions of protein (tissue-drip), total protein was determined by Lowery method, (Lowery et al, 1951). 10µl of drip was used for determinarion of total protein and protein fractions in the same manner as fresh and thawed meat assay.

10µl sample was used using SDS-polyacrylamide electrophoresis to determine protein fractions in fresh and thawed meat. Protein fractions using poly-acrylamide (8% acrylamide) gel electrophoresis, (Laemmli, 1970) using standard molecular weight protein (BioRad, USA). The gels were scanned using Image densitometer scanner (G-700), (BioRad, USA). The determination of protein molecular weight was carried out by using computer software (Gel pro-analyzer, pro-plus, version 3, Media Siber-Ntics, USA).

The statistical analysis was carried out using both t-test and one way ANOVA using the formula tabulated in Steel and Torrie, (1980).

RESULTS

After two weeks freezing, the color of the outer surface of thawed meat was changed to bright red with flabby texture. While after three weeks, the color appeared more paler and the texture was more flabby. Results of chemical analysis of fresh and thawed meat are recorded in Tables 1, 2, 3, 4 and 5. The humidity decreased by about 2.47 % in the thawed meat after the third freeze-thaw cycle compared with fresh meat. The pH increased by about 15.46% after the third freeze-thaw cycle. Total protein significantly ($P < 0.001$) decreased after the second and the third weak of freeze-thaw cycle as compared with fresh meat. The ash content significantly ($P < 0.001$) decreased in thawed meat especially after first freeze-thaw cycle. However its level returned to approach the original value of fresh

meat after the third freeze-thawed cycle.

The Iron content was significantly decreased in thawed meat especially after first thawing ($P<0.01$), but increased after the second and third freeze-thawing cycle ($P<0.001$).There was significant ($P<0.001$) increase in sodium content in ash of freeze-thaw meat compared with fresh meat. Potassium level was significantly ($P<0.001$) decreased after the first weak, however its level retuned toward the fresh value after the second and third freeze-thaw.

Both ALT and AST enzymes activities was decreased ($P<0.001$) as the freeze-thaw cycles increased. After three cycles of freeze-thawing, the activities of ALT decreased by 1.5 fold, while the activities of AST decreased after the first and the third freezing and thawing cycle by 17 fold and 16 folds respectively (Table 1). The drip volume increased sharply 3-fold in the freeze-thawed meat. The total protein of drip was decreased after two and three freezing by 15% and 14% respectively as compared to the first week.

SDS-poly acrylamide slap gel electrophoresis for meat and drip proteins are shown in Tables 2, 3, 4 and 5 and Figer 1. Fresh meat fractionated into 7, 4, 1 bands of high(272-97 kD), medium (88-58 kD) and low (56-36 kD) molecular weight, respectively. After first freez-thawing the number of all band was increased specially the medium and low molecular weight proteins(table 2).

Table 3 demonstrated that protein fraction of molecular weights of 272.52-154.2, 110.93-97, 80.01, 66-58.09, 51.13 KD was found in both fresh and freez-thawed meat. Protein fraction of molecular weights 116, 88.5, 72.67, 56.47, 47.97, 36.13 and 45 KD was not found in fresh meat. The Protein fraction of 116 and 56.47 KD apeared in the samples after the 1st and 2nd freeze-thaw week, but disappeared after the 3rd week. The band of molecular weight of 88.5 and 47.97 KD appeared after the 1st and 3rd week of freeze-thawing. The band of molecular weight of 72.67 appeared in meat after all freeze- thaw cycles. The band of molecular weight of 45 appeared in meat after 2nd and 3rd week of freeze ñthaw cycle. The band of molecular weight of 36.13 appeared in meat the 1st week only and disappeared later on.

The number of protein bands of fractionated protein of drip of thawed meat was recorded in table 4. The number of bands of high, medium and low molecular weight was higher in the third week of freeze-thawing cycles as compared to the 1st and 2nd week.

Table 5 showed that protein fraction of molecular weight of 116, 76, 51.13, and 45 KD was not found in drip after the first week. The Protein fraction of 116 and 76 KD apeared after the 3rd freeze-thaw week. Table 5 demonstrated that there was 8 protein bands present in freeze-thaw drip. One protein band (272.25KD) was present

in 100% of freeze-thaw muscle drip. One band (116KD) was present in third freeze-thaw drip (50% of samples). One protein band (205KD) was present in first and third freeze-thaw drip. The rest of the protein bands (154.21, 110.93, 106.08, 101.44 and 97) did not show any significant differences between the three freeze-thaw cycles.

Table 1: Physical and chemical parameters of fresh and thawed buffloe meat (Mean ±SD, n = 10).

Parameters	Fresh meat	Thawed meat		
		1st week	2nd week	3rd week
1.Muscle: Humidity(%)	74.63±0.55	75.14±0.08	73.67±1.48	72.78±0.19***
pH	5.56±0.09	5.20±0.12**	6.30±0.06***	6.42±0.18***
Total protein (gm/100gm)	17.92±0.44	17.12±0.74	12.77±0.08***	12.02±0.09***
Ash (%)	1.34±0.04	0.34±0.12***	1.31±0.02	1.21±0.10
Iron (mg/100g)	3.92±0.10	2.78±0.68**	7.14±0.84***	4.92±0.51**
Sodium (mg/100g)	464.62±5.06	512.18±3.3***	526.12±5.2***	514.20±7.3***
Potassium (mg/100gm)	14.98±0.54	8.00±0.84***	13.42±0.46***	12.50±0.48***
ALT (GPT) (IU/g)	162.83±2.96	163.05±3.27	115.94±5.4***	127.83±2.28***
AST(GOT) (IU/g)	193.28±2.15	11.67±1.41***	11.28±0.84***	13.11±1.58***
2.Drip:dripVolume (ml/250g)	1.53±0.35	5.42±0.65***	4.05±0.33***	3.55±0.47***
Total protein (gm/ 100dL)		9.52±0.25	8.33±0.24***	8.64±0.24***

** Significant at P< 0.01; *** Significant at P< P0.001

Table 2: Distribution of protein bands in buffloe meat:

Mol. weight	Fresh Meat	Thawed meat		
		1st week	2nd week	3rd week
272-97 kD	7	8	8	7
88-58 kD	4	6	5	6
56-36 kD	1	4	3	3
Total	12	18	16	16

Table 3: SDS-electrophoritic pattern of protein fractions of fresh and freeze-thaw buffloe meat.

Protein bands (Mol wt (Kd)	Fresh Meat		Thawed meat						Ap.	Pr.	Tr.
			1st week		2nd week		3rd week				
	%	Conc	%	Conc	%	Conc	%	Conc			
272.52	10.15 ± 0.51	1.29 ± 0.07	8.47 ± 0.44	0.91± 0.06***	6.62 ± 0.46	0.68± 0.05***	9.16 ± 0.05	0.89± 0.05***	F& T	100 T	D
205	4.78 ± 0.24	0.61 ± 0.03	3.74 ± 0.26	0.40± 0.03***	5.86 ± 0.29	0.5 9± 0.04	2.48 ± 0.15	0.24± 0.01***	F& T	67T	D
154.21	11.57 ±0.81	1.47 ± 0.09	5.95 ± 0.27	0.64± 0.04***	4.56± 0.32	0.47± .03***	10.93± 0.55	1.06± .07***	F& T	66T	D
116			4.50 ± 0.32	0.48 ± 0.04	7.2 ± 0.50	0.74 ± 0.05			T Only	50A	A
110.93	8.7 ± 0.52	1.11 ± 0.05	2.80 ± 0.20	0.30± 0.02***	9.15 ± 0.46	0.93± 0.06**	7.1 ± 0.43	0.89 ±0.06**	F& T	50T	V
106.08	6.5 ±0.46	0.83 ±0.05	6.4 ±0.38	0.69 ±0.04	3.12± 0.22***	0.32± 0.02***	6.65 ±0.04	0.65± 0.04***	F& T	50T	V
101.44	7.45 ±0.2	0.95 ±0.07	6.7 ±0.36	0.72± 0.04***	3.67 ±0.18	0.38± 0.02***	7.25 ±0.44	0.70± 0.04***	F& T	50T	V
97	7.89 ±0.55	1.00 ±0.05	3.7 ±0.26	0.40± 0.03***	7.3 ±0.37	0.75± 0.05***	6.65 ±0.46	0.65± 0.06***	F& T	50T	V
88.50			6.75 ± 0.35	0.73 ± 0.05			7.4 ±0.37	0.72 ±0.04	T only	100T	I
80.01	6.91 ± 0.36	0.88 ± 0.04	3.63 ±0.18	0.39± 0.02***	6.35 ± 0.32	0.65± 0.04***	5.9±0.41	0.57± 0.04***	F& T	50T	I
72.67			2.86 ±0.20	0.31 ±0.02	4.37 ±0.32	0.45 ±0.01	2.25 ±0.14	0.22 ±0.01	T Only	50A	V
66	9.4 ± 0.66	1.19 ± 0.07	6.4 ± 0.45	0.69±0.0 5***	8.51± 0.43	0.87± 0.05***	8.54± 0.43	0.83± 0.09***	F&T	84T	I
61.92	4.58 ± 0.27	0.58 ± 0.03	3.50 ± 0.21	0.38± 0.02***	2.99 ± 0.21	0.31± 0.02***	2.4 ± 0.17	0.23± 0.02***	F&T	50T	D
58.09	9.57 ± 0.48	1.22 ± 0.06	5.85 ± 0.41	0.63± 0.04***	7.45 ± 0.37	0.76± 0.05***	8.77 ± 0.44	0.85± 0.05***	F&T	67T	I
56.47			6.35 ± 0.45	0.68 ± 0.05	5.00 ± 0.40	0.51 ± 0.05			T only	50A	D
51.13	12.50 ± 0.63	1.58 ± 0.08	10.8 ± 0.54	1.16± 0.07***	12.12 ± 0.61	1.24± 0.07***	3.73 ± 0.18	0.36± 0.02***	F&T	85T	D
47.97			6.07 ±_0.49	0.65 ± 0.05			3.7 ±_0.29	0.36 ±_0.03	T Only	50A	D
45					5.75 ±_0.46	0.59 ±0.05	7.1 ± 0.57	0.69 ±0.06	T Onl	50A	I
36.13			5.55 ±_0.44	0.60 ±_0.05					T-On	50A	A
Total	100	12.70 ± 0.54	100	10.76± 0.25***	100	10.21± 0.48***	100	9.71± 0.48***			

N =10, Mean ± SD, *** significant at P< 0.001, KD: K-Dalton, Tr. = Trend ,the trend of the increase or decrease of protein bands level directions, Ap. = Appearance, Appearance of the protein band either in fresh or/and freeze-thaw sample from total samples, Pr. = Presence , the presence of protein band in the sample from the total samples, F&T = appearance of protein band in both fresh and freeze-thaw samples, T only = appearance of protein band in freeze-thaw sample only, 100T = presence of protein band in 100% of total samples, 50A = presence of protein band in 50% of freeze-thaw samples. D = decreased trend of the protein band, I = increase in trend of the protein band, V = variable trend of protein band, C = constant trend of protein band

Table 4.: Distribution of protein bands in buffleo meat drip.

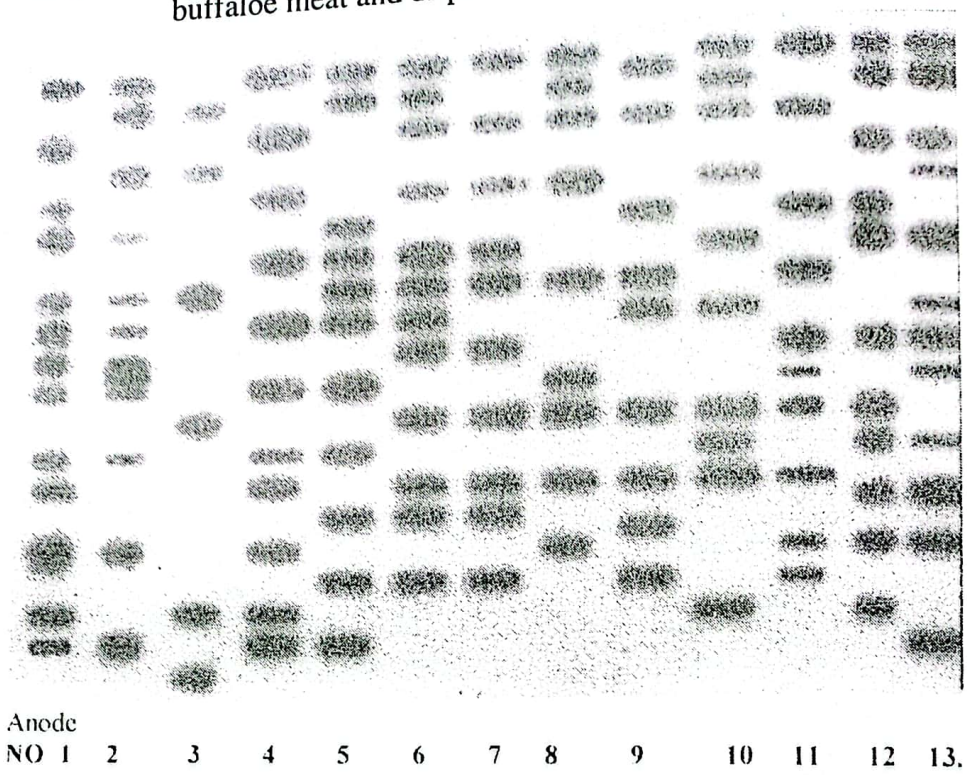
	Thawed meat		
(kD)	1st week	2nd week	3rd week
High (272-97)	7	6	8
Medium(88-58)	6	5	7
Low (56-36)	4	3	4
Total	17	14	19

Table 5.: SDS-electrophoritic pattern of protein fractions of the drip of freeze-thaw buffleo meat.

Protein bands (Mol wt (Kd)	Thawed meat						Ap.	Pr.	Tr.
	1st week		2nd week		3rd week				
	%	Conc	%	Conc	%	Conc			
272.52	7.85± 0.39	0.75± 0.03	8.09±0 .41	0.67±0.03*	6.19±0.28	0.54±0.06***	3 T	100T	D
205	3.64±0 .14	0.35± 0.02			5.57±0.22	0.48± 0.01***	1+ 3	50T	I
154.21	6.1± 0.24	0.58± 0.03	14.15±0.57	1.17± 0.06	4.09± 0.18	0.35± 0.02	3 T	66.T	V
116					7.15± 0.36	0.62± 0.03	3 T	50A	A
110.93	5.9± 0.27	0.56± 0.02	8.42± 0.42	0.70± 0.06***	6.65± 0.27	0.57± 0.03	3 T.	66T	I
106.08	3.54± 0.16	0.34± 0.02	3.7± 0.15	0.31± 0.01	3.52± 0.14	0.30± 0.03	3 T.	50T	N
101.44	5.66± 0.28	0.54± 0.02	5.9± 0.4	0.49± 0.03	4.49± 0.18	0.39±0.01***	3 T.	68T	N
97	5.74± 0.26	0.55± 0.02	2.96± 0.15	0.25± 0.01***	5.36± 0.27	0.46±0.02***	3 T.	85T	V
88.50	3.62± 0.16	0.34± 0.02	7.77± 0.39	0.65± 0.03***	5.75± 0.29	0.49±0.04***	3 T.	84T	I
80.01	6.1± 0.31	0.58± 0.03	1.64± 0.08	0.14± 0.04***	6.74±0.31	0.58± 0.04	3	68T^	V
76					4.34± 0.22	0.37± 0.02	3rd	50A	A
72.67	7.5± 0.34	0.71± 0.05	8.11± 0.41	0.67± 0.03	3.27± 0.17	0.28±0.01***	3 T.	68T	D
66	6.6± 0.33	0.63± 0.03			6.65± 0.33	0.57± 0.03	1+3	34T	C
61.92	6.1± 0.31	0.58± 0.03	5.52± 0.25	0.46± 0.02***	3.54± 0.14	0.31± 0.01***	3 T	68T	D
58.09	3.61± 0.16	0.34± 0.02	7.04± 0.35	0.59± 0.04***	3.85± 0.19	0.33± 0.02	3 T	67T	V
54.50	9.74± 0.39	0.93± 0.04			3.97± 0.16	0.34± 0.02***	1+3 T	100A	D
51.13			13.17±0.53	1.09± 0.06	6.1± 0.24	0.53± 0.03***	2+3 T.	100A	D
47.97	12.22±0.65	1.16± 0.05			6.69± 0.30	0.58± 0.03***	1+3	100A	D
45			7.47± 0.37	0.62± 0.03			2nd T	50A	D
36.13	6.10± 0.31	0.58± 0.04	6.08± 0.30	0.51± 0.02	6.10±0.35	0.53± 0.02	3T	68T	N
Total	100	9.52± 0.48	100	8.33± 0.43***	100	8.64± 0.43***			

N =10, Mean ± SD, *** significant at P< 0.001, KD: K-Dalton, Tr. = Trend ,the trend of the increase or decrease of protein bands level directions, Ap. = Appearance, Appearance of the protein band either in fresh or/and freeze-thaw sample from total samples, Pr. = Presence , the presence of protein band in the sample from the total samples, F&T = appearance of protein band in both fresh and freeze-thaw samples, T only = appearance of protein band in freeze-thaw sample only, 100T = presence of protein band in 100% of total samples, 50A = presence of protein band in 50% of freeze-thaw samples, D = decreased trend of the protein band, I = increase in trend of the protein band, V = variable trend of protein band, C = constant trend of protein band.

Fig.(1): Gel scanned (Protein bands) of standard, fresh and thawed buffaloe meat and drip.



1. No.3: standard molecular weight protein.
2. No 7,9: protein fraction of fresh buffaloe meat.
3. No.8,9,10,11,12,13: protein of fresh fractions of freeze-thawed buffalo meat.
4. No.1,2,4,5,6: protein fractions of freeze-thawed buffalo drip.

DISCUSSION

Freezing is one of the most important methods used for food preservation particularly during transportation for the purpose of export. Thawing is the main problem in freezing process. Thawing is usually associated with changes in physical and chemical meat quality. Jakobsson and Bengstsson, (1973) observed that thawing causes change in meat color , from fresh one to Hunter redness. Moreover slow thawing of fast frozen meat leads to growth of a large ice crystal associated with leakage through cell membrane and collection of water (drip) in the extracellular space (Varnam and Sutherland, 1998 and McGeehin et al., 2001). The present data is in agreement with those of Insausti et al, (2001) who found maxiumum water loss reaching to 2.64% after 10 days of freezing storage. The values of pH were between 5.3 and 5.6 in fresh beef meat. In the present study pH was increased by about 15.46% after the third freeze-thaw cycle. This increase in pH may be attributed to changes caused by the autolytic enzymes and avilability of microorganismes activity to denaturate protein and provide more amonia nitrogen.

Total protein was decreased by 32.92% after second freeze-thaw cycle as compared to fresh meat. Such data indicate that protein content of the muscle is adversly affected by freezing and thawing even after the first freez-thaw cycle. The decreased protein content was suggested to be due to an increase in protein denaturation after thawing (El-Zeini et.al., 2002). The decrease in the ash content of the first freeze-thaw cycle may be attributed to the large volume of drip loss (Abeni & Bergoglio, 2001) .

In the currnt experiment, the Iron content was significantly decreased in thawed meat especially after first thawing ($P<0.01$). It has been suggested that short term freezing decreased heme iron (Decker and Welch, 1990). However its level was increased after the second and third freeze-thawing cycle ($P<0.001$). This pattern is quite similar to that of ash percent. The increase in iron may be to the heme break down and consequently increase in the non-heme iron. This result was in agreement with that of Gomez and Roberts, (1992) who reported that the decrease in heme iron was inversely related to non-heme iron content.

The sodium ion is an important extra cellular anion and help in control acids base balance and fluid balance in the body and in translation of electrical stimulus (Soliman and Moty, 1985). In the present study, there was significant increase in sodium content in ash in thawed meat compared with fresh meat ($P<0.001$). The increase in the sodium content of ash may due to changes in muscle membrane permeability and loss of water during thawing. The increase in sodium concentration correlates with the observed decrease in humidity. On the other hand, Firstenberg-Eden et al., (1980) observed that freezing and storage for 14 days had effect on ions concentration of muscle cells. The decrease in potassium ion level is due to loss of muscle membrane permeability due to successive freezing and thawing. Another factor, that drip withdraw off potassium content of the muscle, assist this observation that drip volume was high after first freeze-thaw cycle then decreased in the successive freeze and thaw. After three cycles of freeze-thawing, the activities of ALT decreased by 1.5 fold, while that of AST decreased after the first and the third freezing and thawing cycle by 17 fold and 16 fold respectively. This indicates the AST is more sensitive to freezing-thawing than ALT. The decrease in ALT and AST levels in freeze-thaw meat may due to denaturation of enzyme (Cao et al.,2003). Freeze-thaw processing potentially disrupts muscle cells, leading to the release of enzymes from mitochondria into sacroplasma (Sair et al, 1999).

There was decrease in drip volume reached from 354.24 to 264.70 and 232.03 % after first, second and third freezing thawing cycle respectively. Such reductions may be attributed to, depletion of water due to recrystallization process, and or to loss of drip in samples. The results showed a

decrease of the total protein content in drip due to repeat freeze and thawing. Our present data is in accordance with data published by Siddaiah et al., (2001). The decrease in total protein may be coincided with increase in the number of fragmented protein bands appeared after PAGE electrophoresis in the same study or due to increase non-protein nitrogen content.

Using SDS-poly acrylamide slap gel electrophoresis, Twelve protein bands were present in fresh meat (63.15% of total bands). Nineteen protein bands were present in freeze thaw meat (100% of total). This indicated that the more freezing and thawing, the more fragmentation of the muscle proteins. This is probably due to the changes in pH which increase the activity of proteases and accelerate proteolysis. Results reported here are in agreement with those of Ertbjerg et al.,(1999) who reported that the proteolytic degradation pattern of myofibrillar proteins during storage differed from that of fresh samples,

In table (3), The appearance of seven protein bands in freeze-thaw meat only (36.84% of total) (116, 88.10, 72.67, 56.47, 47.97,45 and 36.13 KD) is due to protein fragmentation as a result of repeated freezing and thawing cycle.

Two protein bands (205 and 154.21 KD) were present in 66% of the total samples. Other protein bands (110.93, 106.08, 101.44 and 97 KD) were present in 50% of the total samples. It can be concluded therefore, that the first three protein bands in this region (272.52, 205 and 154.21 KD) could be used to differentiate between fresh and freeze-thaw meat. The disappearance of the first three bands (272.52, 205 and 154.21 KD) together could indicated whatever this freeze-thaw meat is in the first, second or third freeze-thaw cycles. Varnam and Sutherland, (1998) mentioned that dissociation and denaturation of the myosin head appears.

Differentiation between fresh and freeze-thaw samples can be obtained by the presence of one protein band (72.67 KD (5.26%)) in meat after three successive freeze-thaw cycles.

The appearance of protein bands with low molecular weight (36.13, 47.97 KD) after first freeze-thaw cycle, the appearance of medium protein band (45 KD) after second freezing-thawing cycles and appearance of protein bands (88.10 and 47.97 KD) after the third freeze-thaw cycle can be used as indicator to differentiate between frozen-thawed and fresh meat.

The protein fractions of the drip were represented in table 4. The first observation is the increase in the number of protein bands in the drip even with decrease in the volume of the drip. Twenty protein bands are present in meat drip of freeze-thaw meat (protein bands standard ranged between 257-36 KD) (molecular weight).

Result reported here indicated two important points first: most of protein bands decrease in its concentration or percentage with increasing the freeze-thaw cycle number. Second, the number of bands increased with increase freeze and thaw cycle number. In parallel with this conclusion, in the SDS-PAGE electrophoresis, Ertbjerg et al., (1999) observed that increased ($P<0.001$) degradation of a 39-kDa band in exercise-treated porcine longissmus muscle. SDS-electrophoresis was not only used to differenate between freeze and thaw beef meat, but also between meats from different species, Cota-Rivas and Vallejo-Cordoba (1997) and Vallejo-Cordoba and Cota-Rivas,(1998). Protein denaturation continues during storage, which tends to enhance the effect of initial freezing (Nagaraj et al., 2001). The increase in the autolytic defgramntion products was observed at frozen beef meat. No difference in degradation productes were obtained among samples subjected to different freeze-thaw cycles ($P>0.05$). However, a slight non-significant increase in degradation products was found in three freeze-thaw samples. Suggesting that a higher release of proteolytic enzymes possibly occurred, due to freeze-thaw process. From the above results, we can depend on the first three protein bands in this region (272.52, 205 and 154.21 KD) to determine if the sample came from either fresh or freeze-thaw meat. This is because of two reasons; the first, presence of the first band in 100% of meat sample and the second and third protein bands in 67% of the total samples. The second reason is the trend of these bands which is constant but it decreased in freeze-thaw meat compared with fresh meat. The first protein bands (272.52 KD) was among the protein bands with high molecular weight, we can depend upon in determination of the difference between both fresh meat and freeze-thaw meat. The decrease of the first three bands (272.52, 205 and 154.21 KD) together were indicative either this freeze-thaw meat in the first, second or third freeze-thaw cycles.

There was increase in band number especially after third freeze-thaw cycle. Except one band (72.67) was present in the three freeze-thaw cycle. After the first freeze-thaw cycle all 4 bands were present (88.50, 56.47, 47.97 and 36.13KD), after second freeze-thaw cycle 2 bands were present (56.47 and 45KD), after third freeze-thaw cycle 3 bands (88.50, also present 47.97 and 45KD). This means that with increasing the number of freeze-thaw cycle, increase the number of protein bands with low molecular weight.

The present results indicated that determination of the number of freeze-thaw cycle can be determined from both medium and low molecular protein bands.

It can be conclouded that the outer surface become more pale and the amount of exudate (drip) increase by thawing and the texture more flabbey. ALT (GPT) and AST(GOT) amount decreased in thawed meat and AST(GOT) is more

sensetive parameter for detection and defferentiation between fresh and thawed meat. In fresh buffloe meat, twelve bands were indicated and with thawed meat it increased up to 18, 16 and 16 by the third week of repeated frozen and thawing. The drip obtained from thawed buffloe meat were denoted by 17 bands after first week increased to 19 bands by third one indicated the increase freeezing and thawing cylcle, the drips had more number of protein bands examined by elelctrophorisis. It can be concluded that by the physical and biochemical deep investigation we can confirm the basis of the meat coming from the buffaloe species whatever it was freshly slaughter or kept for three weeks of freezing and thawing either continuous or interrupted.

REFERENCES

Abeni, F. and Bergoglio, G. (2001):Characterization of different strains of broiler chicken by carcass measurement, chemical and physical parameters on breast muscle. Meat Sci., 57 (2): 133-137.

AOAC. (1990): Associations of official analytical chemists, official Methods of analysis, 15th ed.. USA, WshingtonDC.

Aurand,L.W.;Woods, E.A. and Wells, M.R.(1987): Food composition and analysis. P48. An Avi Book, Pub. Van Nostrand Reinhold company, New York.

Benjakul, S. Seymour, T.; Morrissey, M. and An, H. (1997):Physicochemical changes in Pacific ehiting muscle proteins during iced storage. J. Food Sci.,62: 729-733.

Benjakul, S., and Bauer,, F.(2001):Biochemical and physicochemical changes in cat fish (Silurus glanis Linne) muscle as influenced by different Freeze-thaw cycles. Food chemistry, 72:207-217.

Berry, B.W.; Leddy, K. and Rothenberg, C.A.(1984a): Survival and growth of Staphylococcus aureus on tempreutureñabused beef livers. J. Food. Prot., 47 (4): 260-262.

Berry, B.W.; Leddy, K. and Rothenberg, C.A.(1984b): Study of the sarcoplasmic and myofibrillary proteins of sublimation dried meat by the method of high-volt electrophorisis in starch gel. Vor. Pitan., 3-4 (2): 71-80.

Cao E, Chen Y.; Cui Z. and Foster PR.(2003): Effect of freezing and thawing rates on denaturation of proteins in aqueous solutions. Biotechnol Bioeng., 82(6):684-90.

Chang, V.P.; Mills, E.W. and Cutter, C.N. (2003): Comparison of recovery methods for freeze-injured Listeria monocytogenes, Salmonella Typhimurium and Campylobacter coli in cell suspenstion and associated pork surface. J. Food Prot., 66,5 :798-803.

Considine, D.M. and Considine, D.G. (1982) : Food and food production encyclopedia. pp 772-774. Van Nostrand Reinhold Co., N.York.

Cota-Rivas M, Vallejo-Cordoba BV. (1997): Capillary electrophoresis for meat species differentiation. J. Capillary Electrophor., 4(4):195-9.

Decker, E.A. and Welch, B. (1990): Role of ferritin as lipid oxidation catalyst in muscle food. J. Agri. And Food chemistry, 38 :674-677.

El-Badawi, A.A. and Hamm, R.(1976): Influence of temperature and rate of freezing of bovine muscle on the subcellular distribution of some mitochondirial enzyme. Z. Lebensum. Unters. Forsch, 24(1): 217-226.

El-Zeini, samia,Hala,H. Wehab and Iman M.Nasr (2002):Biochemical and bacteriological evaluation of disagriable broiler carcasses. J.Egypt. Vet. Med. Assoc. 62,(6B):163-178.

Ertbjerg, P.; Henckel, P.; Karlsson, A.; Larsen, L.M. and Moller, A.J. (1999): Comparison of recovery methods for freeze-injured Listeria monocytogenes, Salmonella Typhimurium and Campylobacter coli in cell suspenstion and associated pork surface. J. Food Prot., 66(5) :798-803

Firstenberg-Eden R, Rowley DB, Shattuck GE. (1980): Factors affecting inactivation of Moraxella-Acinetobacter cells in an irradiation process. Appl Environ Microbiol., 40(3):480-485.

Georgy, M.A. (1980);Application of some microanalytical methods for the determination of some elements in their biological reservoir and their influeence on the heals of Egyptian farm animal., PhD thesis,(physiology) Faculty of science ,Cairo university.

Gomez, J. and Roberts, G.L. (1992): Processing and frozen storage effect on the iron content of cod and mackerel. J. Food Sci., 57(2) :1339-1339.

Insausti, K.; Beriain, M.J.; Purroy, A.; Albirti,P. and Alzueta, M.(2001): Shelf life of beef from local Spanish cattle breeds stored under modified atmosphere. Meat Sci., 57 (3): 273-281.

ISO (1974): International organization for standardization P2917-2974. meat and meat products : measurement of pH.

Jakobsson, B. and Bengstsson, N. (1973): Freezing of raw beef :influence of Aging freezing rate and cooking methods on quality and yield. J.Food.Sci., 38 : 560-563.

Laemmli, U. K. (1970): Protein fractions using polyacrylamid gel electrophorisis. Nature 227: 680-685.

Lind, L. M.; Harrison, L.D. and Krop, H. D.(1971): Freezing and thawing rates of lamb chops: effects on platability and related characteristics. J. Food.Sci., 36 (3): 60-63.

Lowery, O.H.; Roserbrough, L.D.;Farr,A.L. and Randall, R.J.(1951): Protein measurment with folin reagent. J. Biol. Chem., 193:256-275.

Lyon, B.G.; Bery, B.W.; Soderberg, D. and Clinch, N. (2000): Visual color and doneness indicators and the incidence of premature brown color in beef patties cooked to four end point temperature.J. Food. Prot.,63 (10): 1389-1398.

McGeehin, B.; Sheridan, J. and Butler, F.(2001): Factors affecting the pH decline in lamb after slaughter. Meat Sci., 58 (1): 79-84.

Nagaraj, N.S.; Anilakumar, K.R. and Santhanam, K. (2001): Effect of temperature on myofibril fragmation during post-mortem storage of muscle. J. Food. Sci. and Technology. Mysore, 38 (1):52-53.

Reitman, S. and Frankle, S. (1957): Calormitric determination of S-GOT and S-GPT activity. Am. J. clin. Path 28: 56-60.

Restle, J.; Faturi, C.; Bernardes, R. C.; Alves, F.; Menezes, K. and de Souza, F.C. (2002): Effect of breed and heterosis on the physical composition of carcases and meat qualitative charactaistic of freed lot finished cull cows. Revist. Brasileira. De Zootecnia, 31 (3):1378-1387.

Restle, J.; Keplin, L. A.; Vaz, F. N.; Muller, L.; Silveira, K. and Nunes, F.C. (1996): Quality of meat from confined charolais steers, slaughtered at different body weights. Ciencia. Rural., 26(3): 463-466.

Sair, A.I.; Booren, A.M.; Berry, B.W. and Smith, D.M. (1999): Residual triose phosphate isomerase activity and color measurements to determine adequate cooking of ground beef patties. J. Food Prot., 62(2) :156-161.

Siddaiah, D.; Reddy, G.V.; Raju, C.V. and Chandrasekhar, T.(2001): Changes in the lipid, protein and kamaboko forming ability of silver carp mince during frozen storage. Food. Research Int., 34 (1): 47-53.

Soliman, M. K. and Moty, A.B. (1985): Veterinary clinical and laboratory diagnosis. pp197. Scientiefic book center, Cairo.

Steel, R. G. and Torrie, K. (1980): Principles and procedures of statistics.(2nd ed.) McGraw Hill. New York.

Sunderman, F.W.,Jr and Sunderman, F.W.(1958): Determinations of potassium in small amount of tissue. Am. J. Clin. Pathol.,29 :95-99.

Trinder,P.(1951): Rapid test of sodium determinations Analyst, 76:596.

Vallejo-Cordoba B, Cota-Rivas M.(1998) Meat species identification by linear discriminant analysis of capillary electrophoresis protein profiles. J Capillary Electrophor. 5(5-6):171-175.

Varnam, H.A. and Sutherland, J.P. (1998). Meat and meat products (Technology, chemistry and microbiology). Pp.358-367 Chapman & Hill LTD. London.