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RESEARCH ARTICLE

Characteristics of Fat Milk Iraqi Buffalo *(Bubalus bubalis)*

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ABSTRACT:
Buffalo milk is the most prevalent milk in the south Iraq. Few studies have focused on the physical and chemical properties of the Iraqi buffalo milk fat, in this study focus on fatty acid of Iraqi buffalo milk and the yogurt produced from it. The fatty acids and its ratio were diagnosed by GC-MS,the results showed that the yogurt made from the starter Y_{480} contained higher amounts of fatty acids compared with the milk used in the industry, alsothe fat of the buffalo milk was characterized by containing important biological fatty acids more than fat of cows milk. Evaluated the total content of conjugated linoleic acid (CLA)of buffalo milk fat compared with cow milk fat treated with linoleic acid isomerase was for different incubation periods. The period of 30minute showed higher CLA for bothtypes. The Antoxidative activity, Chelating offerrous ion, reducing force and hydrogen peroxide were also evaluated. The results showed that buffalo milk fat was superior in all studied properities compared with cow milk fat.

KEYWORDS: Buffalo milk, Fatty acids, Antioxidants, Gas chromatography mass spectrometer GC-MS, Yoghurt.

INTRODUCTION:
Milk is a complex biological fluid secreted by mammals and used in the production of many important nutritional products, the buffalo milk is used in many countries of the world asa commercial source in the manufacturing of dairy products, including India, Pakistan and Egypt, as it has double the amount of fat in the milk of cows which (7.4-8.8%)[1]. Buffalo spread the in the south of Iraq, especially in the areas of the marshes, the product made from which was not enough scientific studies, especially with regard to the nutritional and therapeutical aspect.

Many studies on the milk and its products have shown interest in the nutritional, healthy and on the consumer to contain the milk fat on the fatty acid which called conjugated linoleic acid which is a mixture from geometrical and positional isomersof linoleic acid C18:2, the isomer C9T11 the most effective and presence in foods, thatbelongs to it the many biological benifits including theantioxidant activity, the proportion of this isomer to 90% of the total linoleic acid isomers,CLA exists in many of foods, especially milk being secreted in the paunch of ruminants[2] because of ability of the microbiology whichis settlement in paunchon formation process ofisomers and biohydrogenation in a presence of linoleic acid which works as a substrate to producelinoleic acid isomerase which generates by some of the microbiology in in the paunch of ruminants which converted linoleic acid to conjugated linoleic acid, in addition to the role of an enzyme desaturase which secreted from lactic glands which transfer vaccinic acid(T11 C18:1) to CLA[3].

The buffalo milk under thestudyis having a good content of CLA which having biological importance, especially with regard to the antioxidant activity[4], so the study aimed to describe the content of the buffalo milk fat by

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estimating its content of fatty acids and treatment withlinoleic acid isomerase to enhance the biologicalactivity of milk and assessment the content of CLA and found a relationship between this content and itsbiological activity by measuring the antioxidant activity and their relationship in mechanism this activity.

MATERIALS AND METHODS:

Materials:

Milk cows were obtained from agricultural research station/Faculty of Agriculture, University of Basra, the buffalo milk were obtained from one of the educators in the Qarma area/Basra governorate.

METHODS:

Diagnosing Fatty Acids by GC-MS:

1-Fat Extraction:

Extracted the fat of cows and buffalo milkaccording to the method of[5]by mixing 3 ml of sample with 6 ml of isopropanol and vortex for 1 min.and added 5 ml from pure hexane (99%) with vortex for 1 min., then centrefuging at 2000 rpm/min. for 5 min. in 4 ^{o}C.

2-Esterification:

The esterification was done according to[6] depending on glycerides reaction with methyl potassium hydroxide which prepared by soluble 11.2 gm from potassium hydroxide in 100 ml methanol, weight 1gm of sample in tube capacity of 15 ml and added 5 ml ofmethyl potassium hydroxidewith vortex for 5 min., then added 5 ml from pure hexane, shaked the contents and left until spread two layers, the upper layer contains methyl esters for fatty acid in hexane while lower layer contains saponin materials.

3-GC-MS:

The samples diagnosing by gas chromatography mass spectrophotometry type Shimadzu GC/MS–PQ2010 Ultra in GC-MS lab. at agriculture college, university of Basra according following conditions:

Column type DP-5MS (30 M×0.25 mm i.d., filmthickness 0.25µm), Heleium gas at flow rate 1 ml/sec., injector temperature was 280 ^{o}C, programme of GC oven was justified at pretemperature 100 ^{o}C for 1 min. then rised the temperature to 280 ^{o}C (6degrees per min.) and matching the resulting peaks with 08 NIST library.

Estimation the Total Content of Conjugated Linoleic Acid (CLA):

Estimated CLA according to the method of[7] by incubated 10 gm of cows and buffalo milk fat with 2 mg/gm of linoleic acid isomerase for incubation periods (0-50) min., then mixing 2 gm of cows and bufallo milk fat with 10 ml isopropanol very well by vortex for two min. and added 8 ml of hexane, also vortex for two min. centerfuging at 3000 rpm/min. the upper layer of supernatant was taken and measured the absorbance at 233 nm according to the standard curve of standard CLA that prepared with concentration between (0-400) µg/ml soluble in hexane and measurement the absorbance at same wave length.

Measurement of Antioxidant Activity:

Antioxidant activity was measured according to the method of[8], 15 gm of cows and buffalo milk fat which showing a higher content of CLA was prepared with concentration ranged (10-50) mg/ml centerfuging at 9000 rpm/min. and 1 ml of supernatant mixing with 4 ml ethanol concentration of 95% and 4.1 ml linoleic acid concentration of 2.5% in ethanol and 8 ml of phosphate buffer 50 mM,pH 7 . The mixture was incubated in a temperature of 45 ^{o}C for 24 hours, then added 0.1 ml of this mixture to 9.7 ml of ethanol concentration of 75% and 0.1 ml of ammonium thiocyanates concentration of 30% and added 0.1 ml of ferrous chloride concentration of20 mM prepared in 3.5% hydrochloric acid to a mixture of interaction to form a red colored complex with lipid peroxides resulting from oxidation, the blank sample prepared in the same way above except mixed 1 ml of distilled water instead of the sample, the absorbance of samples and blank were measured at 500 nm, which has a clear maximum absorbancefor complexes of antioxidant compoundswith ferrous ion, an antioxidant activity measured accordance with the following equation:

Antioxidant activity (%)=
100–[(absorbance of sample/absorbance of blank)×100]

Estimation the Ability of Linking Ferrous Ion:

The ability of linking ferrous ion estimated according to the method described by[9], included mixing 0.4 gm of cows and buffalo milk fat which showed the higher content of CLA with concentration ranging between (2-10) mg/ml and 0.4 mlferrous chloride(2 mM) and 0.4 of 8-hydroxy quinolone (5 mM) prepared in ethanol (98%), the mixtureincubation for 10 minutes at room temperature in a dark place, then measured at 562 nm. Also estimated the ability of linking ferrous ion to EDTA and citric acid in the same circumstances for the purpose of comparison, the blank was prepared in the same way, except for the addition of the samples, the ability of linking measured according to the following equation:

Ability of linking ferrous ion(%)=
1–(absorbance of sample/absorbance of blank)×100

The Ability of Capturing of Hydrogen Peroxide:

Estimated the ability of capturing hydrogen peroxide according to the method described by[10]. Included mixing 1gmof cows and buffalo milk fatwith concentration ranging between (2-10) mg/ml with 0.6 ml of hydrogen

peroxide (2 mM), the blank preparedfrom 1ml of phosphate buffer without the addition of samples, as well as the use ascorbic acid andrutein compound to comparison with samples, then leave the mixture for 10 minutes, then measured the absorbance at 230nm, the ability was calculated according to the following equation:

Ability of capturing hydrogen peroxide (%) =(absorbance of sample/absorbance of blank)×100

The Measurement of the Reductionist Force:

Estimated the reductionist force according to the method described by[11]. Bymixing 0.3 gm of cows and buffalo milk fat which showed the higher content of CLA with concentration ranging between (5-25) mg/ml with 0.3 ml of ferricpotassium cyanide (1%) and 0.3 ml of phosphate buffer (2M) at pH 6.6. The mixture was incubated at temperature of 50 ºC for 20 minutes, then added 0.3 ml of chloride acetic acid concentration of 1% andcenterfuging at 6000 rpm/min for 10 min.at 4 ºC. After the separation of the upper layer of the mixture was withdrawn and mixed with 0.12 ml of chloride ferric (0.1%) and added 0.6 ml of distilled water, vortexed the mixture and leave for 10 minutes as the blank was preparedfrom all the previous components except the sample. An industrial anti-oxidant BHT was used under the same conditions and concentration for comparison, then measuring the absorbance at 700nm and the reductionist force was calculated according to the following equation:

Reductionist force (%)=
1–(absorbance of sample/ absorbance of blank)×100

Statistical Analysis:

Using the experiement of two factors by using complete random design (C.R.D.) and the comparison between the averages of the trearments by using the least significantdifference(L.S.D.)at level of $p<0.05$ using the statistical analysis program[12]in the analysis of the data.

RESULTS AND DISSCUSION:

Diagnosing Fatty Acids by GC-MS Technique:

Showing (Fig. 1,2,3) diagnosis the fatty acids in the fat of cows and buffalo milk, as well as yoghurt fat made from thebuffalo milk by using starter Y_{450} respectively. Diagnosing the fatty acids in the milk was to compare the fatty acids in yoghurt to notice the changes in it after processing. The figures show a presence of 46 peak for cows milk fat and 37 peak for buffalo milk fat and the yoghurt made from it, as well as (Tables 1,2,3) explain the percentages for fatty acids in the fat of cows and buffalo milk, as well as yoghurt fat made from the buffalo milk respectively. The results showed presence of saturated fatty acids, unsaturated fatty acids, cyclic fatty acids, methylated fatty acids and branched fatty acids, but there is a clear difference in this percentages. As compare the fatty acids in the fat of cows and buffalo milk, the latter contains fatty acids that having a biological, medicinal, processing and nutritional importance more than cows milk, such as 8-Octadecenoic acid,which is responsible of showing the unique flavor forthe product, as well as contribute to ease the digestion process and provide the body with energy directly, instead of stored in the adipose tissuesand forms a ratio of 18.26% higher than its percentage in cows milk which equal to (14.6%)[13].

The fat of buffalo milk also contains fatty acids in a very low rates but itshaving a biological importance, including the fatty acid 10,13-Octadecadienoic acid with ratio of 1.46% and not exists primarily in the fat of cows milk, which has the capacity to inhibition some cancerous tumors, through its work as antioxidant, as well as its ability to reduce the risk of suffering from atherosclerosis[14]. The fat of buffalo milk contains too the fatty acid Nonadecanoic acid and not exists in the fat of cows milk.

Notice from the table increasing a percentage of the conjugated fatty acid Methyl Cis9 trans11 Octadecadienote from 1.03% in the fat of cows milk to 3.12% in the yoghurtand from 1.43% in the fat of buffalo milk to 5.67% in the yoghurt. Also found that conjugated linoleic acid (CLA) plays an important role in reducing the level of glucose in blood because of decreasing the fatty acids in the liver, fats, and triglycerides and thereby reduce the obesity causing in reducing the risk of diabetes[15].

The long chain unsaturated fatty acids boost the nervous system, while the short chain unsaturated fatty acids having an importance in the growth of neurons, either the medium length chainunsaturated fatty acids are important in a differentiation of neurons, these acidswere diagnosed in the fat of buffalo milk[16].

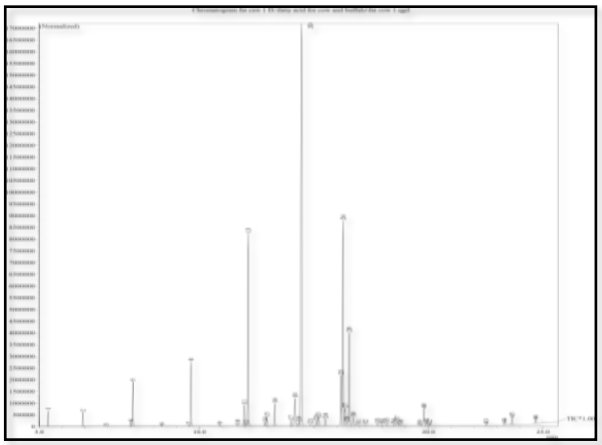

Fig. 1:Chromatogram of the fatty acids for milk fat of Iraqi cow diagnosing by GC-MS

Table 1:Percentage of the fatty acids for milk fat of Iraqi cow diagnosing by GC-MS.

Peak	R.Time	Name	Area%
1	3.392	Hexanoic acid, methyl ester	0.53
2	4.913	Octanoic acid, methyl ester	0.63
4	6.994	4-Decenoic acid, methyl ester	0.22
5	7.096	Decanoic acid, methyl ester	2.37
6	8.350	Undecanoic acid, methyl ester	0.06
7	9.549	cis-5-Dodecenoic acid, methyl ester	0.12
8	9.634	Dodecanoic acid, methyl ester	3.81
9	10.891	Tridecanoic acid, methyl ester	0.13
10	11.667	Methyl 12-methyl-tridecanoate	0.09
11	11.953	Methyl myristoleate	1.35
13	12.132	Methyl tetradecanoate	2.84
15	12.949	Tridecanoic acid, 12-methyl-, methyl ester	0.52
16	13.291	Pentadecanoic acid, methyl ester	1.41
17	14.007	Hexadecanoic acid, methyl ester	0.38
18	14.172	9-Hexadecenoic acid, methyl ester, (Z)-	2.16
19	14.350	11-Hexadecenoic acid, methyl ester	0.37
20	14.468	Hexadecanoic acid, methyl ester	38.03
23	15.191	Hexadecanoic acid, 14-methyl-, methyl ester	0.75
24	15.499	Heptadecanoic acid, methyl ester	0.48
25	16.187	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	3.74
26	16.275	8-Octadecenoic acid, methyl ester	14.60
27	16.366	9-Octadecenoic acid (Z)-, methyl ester	1.89
28	16.433	trans-13-Octadecenoic acid, methyl ester	0.34
29	16.545	Octadecanoic acid, methyl ester	6.46
32	17.245	Methyl 9,10-methylene-octadecanoate	0.08
33	17.782	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.18
34	17.971	Methyl 10,13,16-docosatrienoate	0.13
35	18.187	cis-11-Eicosenoic acid, methyl ester	0.25
36	18.485	Methyl 18-methylnonadecanoate	0.17
39	19.625	cis-7,10,13,16-Docosatetraenoic acid, methyl ester	0.06

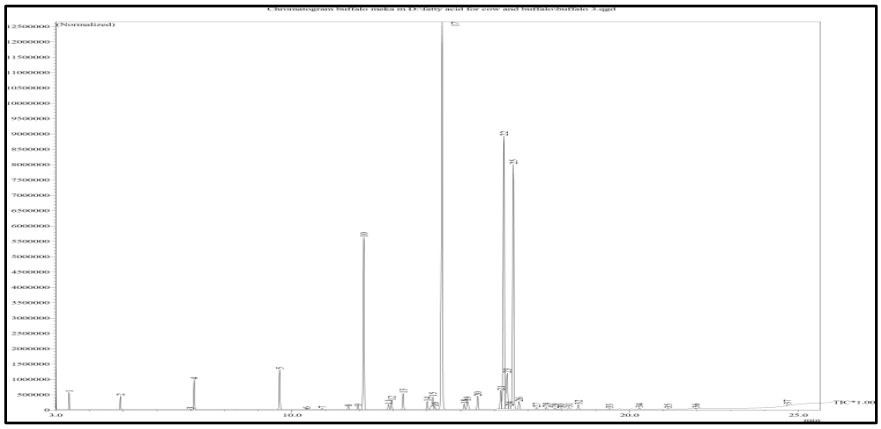

Fig. 2:Chromatogram of the fatty acids for milk fat of Iraqi buffalo diagnosing by G

Table 2:Percentage of the fatty acids for milk fat of Iraqi buffalo diagnosing by GC-MS

Peak	R.Time	Name	Area%
1	3.395	Hexanoic acid, methyl ester	0.66
2	4.916	Octanoic acid, methyl ester	0.56
4	7.099	Decanoic acid, methyl ester	1.59
5	9.634	Dodecanoic acid, methyl ester	2.40
7	10.894	Tridecanoic acid, methyl ester	0.08
8	11.671	Methyl 12-methyl-tridecanoate	0.20
9	11.955	Methyl myristoleate	0.21
10	12.128	Methyl tetradecanoate	11.59
12	12.951	Tridecanoic acid, 12-methyl-, methyl ester	0.58
13	13.293	Pentadecanoic acid, methyl ester	1.05
14	14.008	Hexadecanoic acid, methyl ester	0.54
15	14.174	9-Hexadecenoic acid, methyl ester, (Z)-	1.11
17	14.451	Hexadecanoic acid, methyl ester	30.26

18	15.106	Heptadecanoic acid, methyl ester	0.38
19	15.194	Hexadecanoic acid, 14-methyl-, methyl ester	0.77
20	15.503	Heptadecanoic acid, methyl ester	0.89
21	16.188	10,13-Octadecadienoic acid, methyl ester	1.46
22	16.280	8-Octadecenoic acid, methyl ester	21.51
23	16.375	9-Octadecenoic acid (Z)-, methyl ester	3.19
24	16.450	13-Octadecenoic acid, methyl ester	0.27
25	16.560	Octadecanoic acid, methyl ester	18.26
26	16.729	Methyl 10-trans,12-cis-octadecadienoate	0.52
27	17.247	cis-10-Nonadecenoic acid, methyl ester	0.13
28	17.534	Nonadecanoic acid, methyl ester	0.15
29	17.787	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.09
30	17.977	Methyl 10,13,16-docosatrienoate	0.05
31	18.209	cis-11-Eicosenoic acid, methyl ester	0.17
32	18.488	Methyl 18-methylnonadecanoate	0.36
33	19.406	Heneicosanoic acid, methyl ester	0.06
34	20.292	Methyl 20-methyl-heneicosanoate	0.10
35	21.147	Methyl 20-methyl-docosanoate	0.05
36	21.969	Tetracosanoic acid, methyl ester	0.06

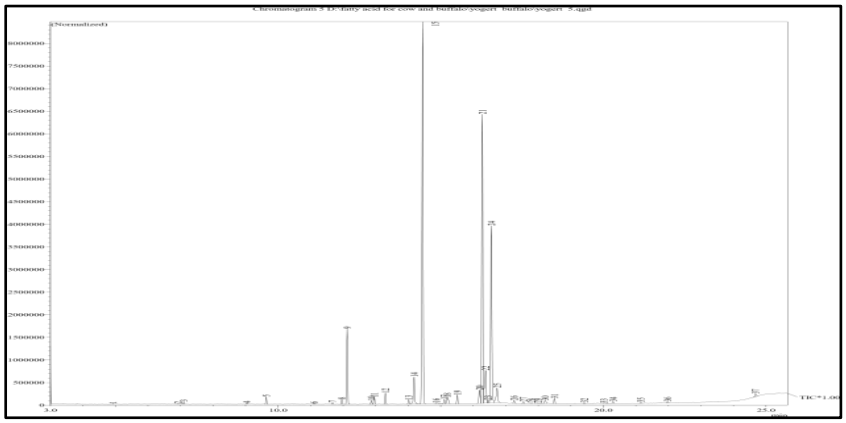

Fig. 3:Chromatogram of yoghurt content from buffalo milk fat diagnosing by GC-MS

Table 3:Percentage of yoghurt content from buffalo milk fat diagnosing by GC-MS

Peak	R.Time	Name	Area%
3	7.102	Decanoic acid, methyl ester	0.17
5	9.636	Dodecanoic acid, methyl ester	0.54
7	11.675	Methyl 12-methyl-tridecanoate	0.12
8	11.960	Methyl myristoleate	0.38
9	12.125	Methyl tetradecanoate	5.84
10	12.868	Pentadecanoic acid, methyl ester	0.30
11	12.958	Tridecanoic acid, 12-methyl-, methyl ester	0.58
12	13.300	Pentadecanoic acid, methyl ester	0.90
14	14.181	9-Hexadecenoic acid, methyl ester, (Z)-	2.55
15	14.448	Hexadecanoic acid, methyl ester	33.64
18	15.200	Hexadecanoic acid, 14-methyl-, methyl ester	0.85
19	15.510	Heptadecanoic acid, methyl ester	0.73
20	16.193	10,13-Octadecadienoic acid, methyl ester	1.38
21	16.280	8-Octadecenoic acid, methyl ester	26.33
22	16.379	9-Octadecenoic acid (Z)-, methyl ester	3.93
23	16.453	Methyl 13-octadecenoate	0.31
24	16.556	Octadecanoic acid, methyl ester	15.95
25	16.734	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	1.93
26	17.254	cis-10-Nonadecenoic acid, methyl ester	0.29
27	17.541	Nonadecanoic acid, methyl ester	0.18
28	17.794	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.05
29	17.986	Methyl 8,11,14-eicosatrienoate	0.06
30	18.210	Methyl 11-eicosenoate	0.36
31	18.497	Methyl 18-methylnonadecanoate	0.47

32	19.414	Heneicosanoic acid, methyl ester	0.10
36	21.973	Tetracosanoic acid, methyl ester	0.13

Total Content of Conjugated Linoleic Acid (CLA):

Explain(Fig. 4) the total content of conjugated linoleic acid (CLA) in cows and buffalo milk, the results show a higher content of CLA was found at a period of incubation of 30 minute in both type of milk, and reached to 270 and 312 µg/gm respectively after 30 minute of incubation, then began to lowering at 40 and 50 minute of incubation, but buffalo milk was the highest content of CLA compare with cows milk.An increasing the content of CLA in the yoghurt may be attribute to a presence oflinoleic acid isomerase which convert linoleic acid to conjugated linoleic acid[17].

The results of the statistical analysis showed superiority the period of 30 minute of incubation significantly in its total content of CLA compare with the rest incubation periods, also the fat of buffalo milk was superior significantly on the fat of cows milk in all periods of incubation at level of $p<0.05$, one of the important factors in increasing the concentration of CLA in buffalo milk was the high proportion of fat in the buffalo milk, as well as the effect of the nutrition of animal[18].

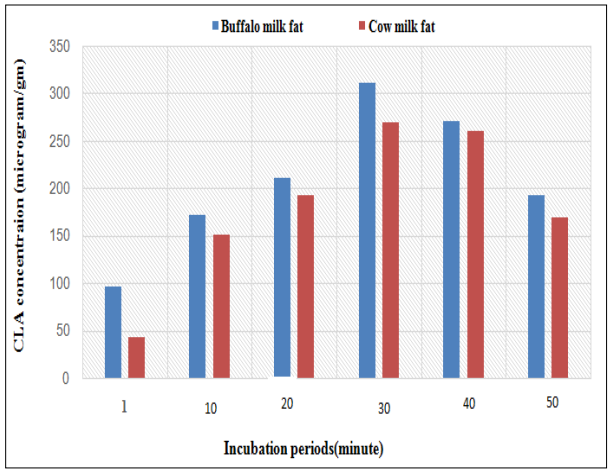

Fig. 4: CLA concentration (µg/gm) in in cow and buffalo milk fat

An Antioxidant Activity in Milk:

The results of the measurement for an antioxidant activity in fat samples of cows and buffalomilk showing in (Fig.5) treated by linoleic acid isomerase with different concentrations, the results showed superiority the fat of buffalo milk significantly on the fat of cows milk at level of $p< 0.05$, but BHT showed higher activity compared with the other samples being its a pure compound in all concentrations, while the higher activity at 40 and 50 µg/gm was found notsignificantly.

An antioxidant activity for fat milk attribute to contain CLA a particular buffalo milk fat as shown in table (4). CLA works on keeping and absorb some of the encouraging elements of oxidation such as Fe ad Zn and others, and sprinkling of free radicals and thereby inhibition the antioxidant activity of fats in the foods[19].

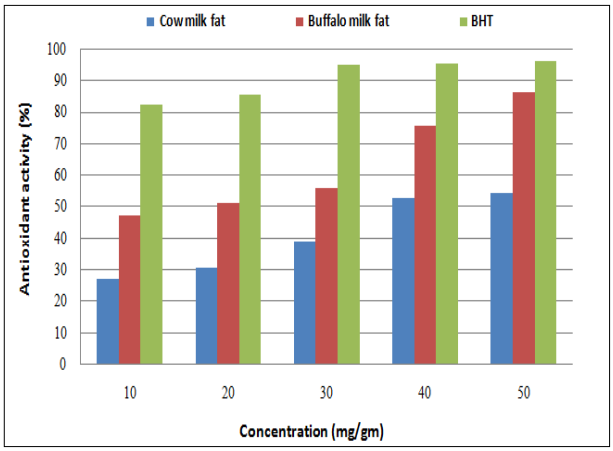

Fig. 5:Antioxidant activity (%) in cow and buffalo milk fat compared with BHT

Abilityof Linking Ferrious Ion:

Showing (Fig. 6) the percentages for abilitythe fat of cows and buffalomilktolinking ferrious ion compared with EDTA, the results showed the superiority of buffalo milk fat with higher ability in linking ferrous ion compared with cows in all concentration, itssusceptibility due to a higher containing of CLA. Statistically found strong positive correlation between the values of antioxidant activity and ability of linking ferrious ion to the value of the correlation coefficient forcows milk fat $R^2 = 0.911$and $R^2 = 0.949$ in buffalo milkfat,that means the antioxidants compounds in the fat of the CLA has the ability to link the metal components causing of fat oxidation especially ferrous ion.

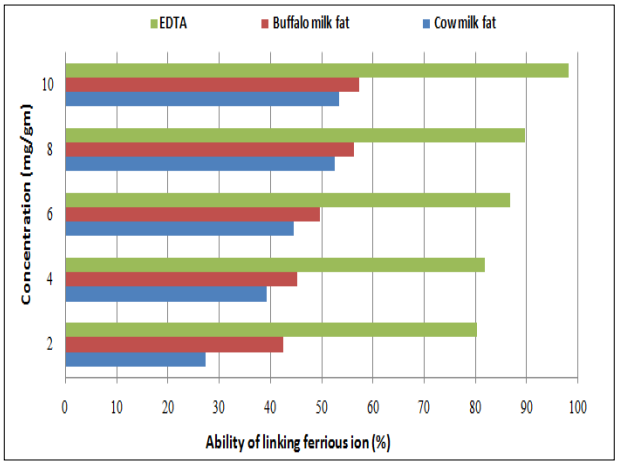

Fig. 6:Ability of linking ferrious ion(%) in cow and buffalo milk fat compared with EDTA

The Ability of Capture Hydrogen Peroxide:

The (Fig. 7) shows the ability of buffalo milk fat of capture hydrogen peroxide to link ferrous ion comparison with ascorbic acid, the results showed the superiority of buffalo milk fat in a higher capture hydrogen peroxidecompared with cows in all concentration. Statistically found strong positive correlation between the values of antioxidant activity and ability ofcapture hydrogen peroxide,the value of the correlation coefficient for cowsmilk fat was R^2 =0.954and R^2=0.976 in buffalo milk fat,that means the antioxidant compounds in the fat of the CLA has the ability to block off the oxygen and prevent a series of free radicals and thus stopping the production of peroxides.

Hydrogen peroxide is one of the types of interactions oxygenwhich attack cell membranes such as fatty membranes which leads to many healthy disorders and thus have a positive impact antioxidants to protect the human body from disorders that occur through interactions oxygen[20].

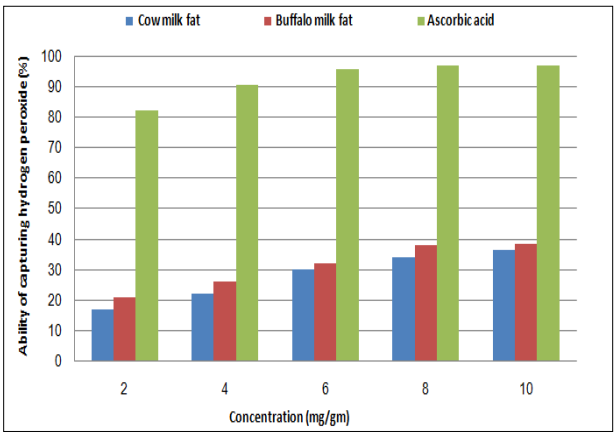

Fig. 7:Ability of capturing hydrogen peroxide (%) in cow and buffalo milk fat

The Measurement of Reductionist Force:

The (Fig. 8) explained thereductionist force for the vital compoundsin milk fat of cows and buffalo in a comparison with BHT, it is clearfy from the results exceedingthe reductionist force with increasing of concentration and superiority of buffalo milk fat in its reductionist force on cows milk fat. It was shown from the results of the statistical analysis presence of asignificant differences between the concentration used and the samples under discussion. The reason for the increase in the force may be due to the ability of the reductionist antioxidant compounds to reduce ferric ion to ferrous ion this resembles the work of the BHT through the reduction of the $Fe^{+3}$to Fe^{+2} (Perenlei *et al.*,2011). Also shown statistically strong positive correlation between the measurement of reductionist force and the values of the antioxidant activity, the value of the correlation coefficient for cows milk fat R^2=0.894and R^2=0.921 in buffalo milk fat, That means theantioxidant compounds in the fat of the CLA has the capacity to reduce ferric ion to ferrous ion.

Reduced the antioxidant compounds ferric ion in complexity potassium ferricyanide $[K_3Fe(CN)_6]$ to ferrous ion in the interaction of the reductionist force therefore, the change of yellow to green or dark blue to be the result of a reductionist force forthe antioxidant compounds or the presence of ferrous ion which features in dark blue color.The test of the reductionist force is a quantitative or semi-quantitative method to estimate the amount of the antioxidant compounds by its few concentration that participate in extinguishing free radicals or their ability to grant hydrogen as well as the number and location of the hydroxyl radicals have a role in the increase of reductionist force and thus increase the activity of reductionist antioxidants[21].

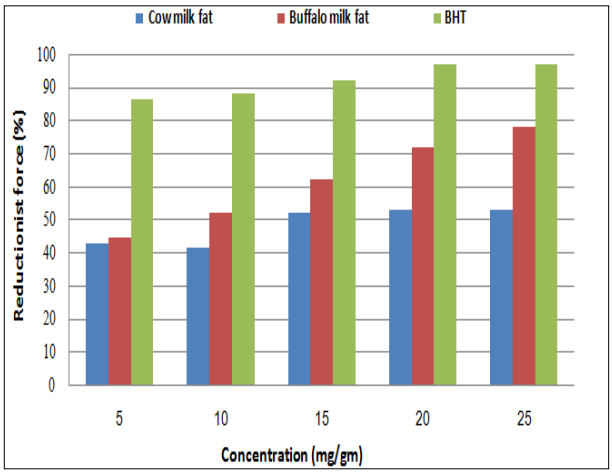

Fig. 8:Reductionist force (%) in cow and buffalo milk fat

REFERENCES:

1. Brijesha, N.; Aparna, H. S. (2017). Buffalo (*Bubalus bubilis*) colostrum and milk fat globule membrane fractions are potent antioxidants. Journal of Applied Biology & Biotechnology Vol. 5 (02), pp. 089-093.
2. Aydin, R. (2005). Conjugated linoleic acid chemical structure , sources and biological properties. J. Turk. Vet. Anim. Sci., 2: 189 – 195.
3. Abu-Gazaleh, A. A. ; Schingoethe, D. J. ; Hippen, R. J. and Whitlock, L. A.(2002). Feeding fish meal and extracted soybean enhanced the conjugated linoleic acid (CLA) content of milk . J. Dairy Sci., 85: 624-631 .
4. Dhiman, T. R.; Nam, S. H. and Ure, A. L. (2005). Factors effecting conjugated linoleic acid content in milk and meat. Critical Review in food science and nutrition, 45 (6): 463 – 482.
5. Rodrigues, L. M.; Braga, T; Malcata, F. X. ; Gomes, A. and Fontecha, J. (2010). Quantitative and qualitative determination of CLA production *Bifidobacterium* and lactic acid bacteria by combining spectrophotometric and Ag^+ - HPLC techniques. Food Chem., 125: 1272 – 1378 .
6. A.O.A.C (1975).Official methods of analysis association of official analytical chemist. 13th , Washington, D. C.,USA .
7. Colakoglu, H. and Gursoy, O. (2011). Effect of lactic adjucted culture on conjugated linoleic acid concentration of yoghurt. J. Food Agric. And Envier. 9(1): 60 – 64 .

8. Gjorgievski, N.; Tomovska, J.; Dimitrovska, G. Makarijoski, B. and Shariati, M. (2016). Determination of the antioxidant activity in yoghurt. J. Hygienic Eng. and design, 15(2): 88 – 92.
9. Gűlcin, I.; Oktay, M.; Kerecz, E. and Kűfrevioĝlu, O.(2003).Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chemistry*, 83:371-382.
10. Türkoğlu, S.; Çelik, S.; Türkoğlu , Đ.; Çakılcıoğlu, U. and Bahsi ,M. (2010). Determination of the antioxidant properties of ethanol and water extracts from different parts of Teucrium parviflorum Schreber. *AfricanJournal of Biotechnology*, 9(40):6797-6805.
11. Cheng, H. (2010). Volatile from compounds in yoghurt. Critical reviews in food science and nutrition, 50: 938 – 950.
12. SPSS (2012). Statistical packages of social sciences. Version (21). USA .
13. Lindmark-Mansson, H. and Akesson, B. (2000). Antioxidative factors in milk. Britsh J. Nutr., 84(1): 103 – 110.
14. Park, Y.W.(2001).Proteolysis and Lipolysis of goat milk cheese. The American Dairy Science Association , Vol. 84 , E. Suppl.
15. Ryder, I. W. ; Porto-CArrero, C. P. ; Song, X. M. ; Cui, L. ; Yu, M. ; Combatsiaris, T. ; Galuska, D. ; Bauman, D. E. ; Barbano, D. M. ; Charron, M. I. ; Zierath, I. R. and Housekenecnt, K. L. (2001). Isomer specific antidiuretic properties of conjugated linoleic acid : Improved glucose tolerance, skeletal muscle insulin action and UCP-2 gene expression . J. Am. Diabetes Assoc.., 50: 1149 – 1157 .
16. Vidu,L. ; Livia, S. and Vasile ,V.(2015) . The content of minerals and fatty acids in buffalo milk. Romanian Biotechnological Letters, Vol. 20, No.1
17. Darani, K. K. ; Reihani, F. S. and Feili, R. (2014). Bio-production of Conjugated Linoleic Acid in Yoghurt by Probiotic Bacteria. J. Int. Biotechnol., 3: 62-68.
18. Peşmen,G.(2016). The conjugated linoleic acid (CLA) contents of various milk and dairy products and affecting factors. International Journal of Innovative Research in Science, Engineering and Technology.Vol.(5) , No.(12).
19. Roberto, W. G.; Gordon, M. H. and Walker, A. F. (2008). Effects of enhanced consumption of fruit and vegetables on plasma antioxidant status and oxidative resistance of LDL in smokers supplemented with fish oil.Eur. J. Clin. Nutr.,57: 1303-1310.
20. Moskovitz, J.; Yim,K.A. and Choke,P.B.(2002). Free radicals and disease .Arch.Biochem.Biophys.,397:354-359.
21. Paul, R. and Ghosh, U. (2011). Effect of thermal on ascorbic acid content pomegranate juice. Indian J. of Biotechnology, 11: 309–313.