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Characterization of Iraqi sheep milk fat

Ali Khudhair Jabir Alrikabi
Department of food science-College of agriculture-University of Basrah.

Abstract

Fatty acids have been identified in sheep milk by GC/MS technique and it compared with cow milk. Sheep milk was contained of Odd fatty acids which uncommon in cow milk fat such as cyclo and branch fatty acids. On the other hand, the results showed that the sheep milk fat has been contained of Conjugated fatty acids. Sheep milk was used in manufacturing yogurt and total acidity percentage, total count of lactic acid Bactria and Conjugated linoleic acid (CLA) concentration have been increased during incubation period. Locally manufactured yogurt from sheep milk has been superiority with its antioxidant activity and reducing power and tying ability ferrous ion with yogurt manufactured from cow milk. Yogurt manufactured from sheep milk has ability to retardation corn oil oxidation at different incubation periods.

Introduction

Sheep milk has important in case of nutritional, industrial and therapeutically because it contains varied fatty acids (branch fatty acids and CLA) compare with cow milk (Jooyandeh and Aberoumand, 2010). In addition of sheep milk is suitable for yogurt and cheese processing. These products characterized with featured flavor because of sheep milk containing some rare fatty acids contrary to cow milk which has not these fatty acids (Park et al., 2007).on the other hand, yogurt processing from sheep milk producing great increasing of amino acids quantity because of converting some free fatty acids in milk via presence of starter enzymes (Jandal et al., 1996). So, the present study aimed to identification of fatty acids in Iraqi goat milk fat like Odd fatty acids which has nutritional ,industrial and therapeutically important, also evaluation of CLA and antioxidative activity in yoghurt manufactured from sheep milk.

Materials and Methods

Milk:

Cows' and sheep's milk samples have been produced from agriculture researches station which belong to Agriculture College, Basrah University.

Esterification of fatty acids:

All samples was prepared according to A.O.A.C, 1980 which depended on the esterification of glycerides via its reaction with methyl potassium hydroxide solution which prepared by solvate of 11.2 g of potassium hydroxide into 100 ml. of methanol. Esterification process was conducted by weight of 1 ml of fat sample in tube of 10 ml capacity then heated in bath water till soluble then 5 ml of methyl potassium hydroxide was added and the tube was shake well for 5 minutes then added 5 ml of pure hexane. After that tube content was mixed well till to separate them to two layers. The upper layer contains methyl esters of fatty acids in the hexane while the lower layer contains of saponification items.

Free fatty acids identification by GC-MS technique:

0.5 ml of ester cow and sheep fat was put into injection tube of GC-MS then added to it 1 ml. of pure hexane and shaken well then put in the bearer of GC-MS apparatus.

Determination of conjugated linoleic acid concentration:

A quantity of CLA has been determined by using Spectrophotometer at wave length of 233 nm (Rodriguez et al., 2010). CLA was calculated through the standard curve which prepared according to Zhao et al. (2011) by using different concentrations of standard CLA (Cis9 Tran11) that ranged between (0-12) µ g/ml. souled by hexane and absorbance was measure at length wave of 233 nm.

Calculation of linoleic acid conversion ratio to CLA:

Percentage of linoleic acid conversion ability was calculated according to the way that cited in Nieuwenhove et al.(2007) as illustrated in the following equation:

Processing of yoghurt:

Yoghurt has been manufactured according to the described method by Lee and Lucey (2010). Yoghurt starter is used for processing yoghurt which provided by Chr's-Hansen CO. starter was activated according to provided company. Starter has been activated according to method of provided company. Pollinated milk by this starter is needed 4 hours for coagulating. a single starter from bacteria of L. acidophilus and L. casei has been used after activation by using skimmed milk at temperature of 37 oC/24 hours of incubation. Mixture has been pollinated by 2% (1:1) and incubated at 40 oC temperature. After that, coagulated milk was stored at ± 5 oC temperature. Samples of manufactured yogurt have been exanimated during periods of 1, 2, 4, 6, 8, 10 days.

Antioxidative Activity Determination:

A 20 mg/ g of yoghurt and BHT has been dissolved into 4ml of 95% ethanol then mixed with 4.1 mL of linoleic acid and 8 mL of 0.05M phosphate buffer (pH 7.0) and 3.9 mL of distilled water then kept in containers provided with screw cap at 40 oC/24 hr in the dark. A 0.1 mL of 30% ammonium thiocyanate, added at precisely 3 minute after the addition of 0.1m of 20mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance at 500nm of the resulting red solution was measured (Yakeda et al., 2012). The percent inhibition of linoleic acid peroxidation was calculated as Anti oxidative activity (%inhibition) = (absorbance of the sample)/absorbance of the control)×100.

Measurement of the Reducing Power:

The yoghurt (10, 20, 30, 40 and 50 mg/ mL) or BHT mixed with an equal volume of 0.2M phosphate buffer (pH 6.0) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then an equal volume of 1% trichloroacetic acid was added to the mixture and centrifuged at 6000 rpm for 10 min. The upper layer of the mixture was mixed with distilled water and 0.1% FeCl3 with a ratio of (1:1:2) and measured the absorbance at 700 nm (Yen and Chen, 1995).

Ferrous Ion Chelating Effect:

Reaction mixtures containing 0.1 mg/ mL of the yoghurt, 0.2 mL of 0.5mM ferrous 6, 5, 4, 3, 2, 1 different concentrations (chloride and 0.2 mL of 5 mM ferrozine were incubated at 37 Co for 10 min. A 1.5 mL of deionized water was added to the mixture, the absorbance at 562 nm was measured (Gibbons and Gray, 1998).

Retardation of Corn Oil Auto Oxidation:

0.5 g of corn oil was dissolved in 24 mL of chloroform-methanol mixture (1:2) and 1 mL of the yoghurt was added in various concentrations (2, 4, 6, 8 and 10 mg/ mL). The corn oil was incubated at 45°C and peroxide value was determined periodically (Shantha and Decker, 1994).

Results and Discussion

Identification of Fatty acids by GC/MS technique:

Table 1,Fig 1,2 shows that the fatty acids percentage in sheep and cow milk in order to compare, and which identified by GC-MS. Sheep milk containing many saturated and non saturated fatty acids in forms cis and trans in addition, it containing Odd fatty acids which did not found in cow milk, these fatty acids have a huge industrial important and main roles in presence of characterized flavor of processed cheese from sheep milk as well as it has a nutritional and therapeutically role. Also, some Branch fatty acids have been found which unable their identification by other techniques such as HPLC, but it's identified by GC-MS technique, like Methyl 4,8-dimethylnonanoate, Methyl 10-methyl-undecanoate ،4-methyl- Dodecanoic acid, ،Methyl-13-methyltetradecanoate Hexadecanoic acid, 2-methyl ،14-methylhexadecanoate ،Methyl 8,11,14,17-eicosatetraenoate, but cow milk fat that used to compare did not has these fatty acids.

Also, it can be seen from table 1that saturated fatty acids percentage reached 71.82% and distributed on the fatty acids that contains of C6-C20, while saturated fatty acids gave higher ratio of C10 ، C12 ، C14 ، C16, and C18 which reached to 5.71 ، 5.66 ، 13.79 ، 25.32 ، 10.17 % respectively. these results were convergence to Jooyandeh and Aberoumand(2010) who found that content of sheep milk fat from C14 ، C16, and C18 were 8.3 ، 22.1and 11,6 % respectively. While Niedbalska et al.(2001) found that saturated fatty acids in sheep milk fat (C14 ، C16 and C18) were 4 ، 25.9, and 9.57% respectively.

The result in table 1 also showed that unsaturated fatty acids in sheep milk fat (cis and trans) reached 47.36 %. Fatty acid percentage of C18:1:9c (oleic acid) was higher compared with other unsaturated fatty acids and reached 19.18 %. This acid has many physiological functions such as anticarcinogenic, increasing self body immune and resistance of chronic inflammatory (Darani et al. ,2014).

After that, Ratio of Linolenic acid (C18:2:9,12cis) reached 2.73% which has many interests such as anti database (Almeida et al.,2014), anti cancer (it prevents formation of cancer cells and drawback its growth (Beppu et

al.,2006), also enhancement of nervous, bloodroot ,immunological systems functions and reducing fat content in the human body and improvement of blood cholesterol level (Ponnampalam et al.,2006). C18 :1:11 trans has been reached 1.31% , but cow milk contains 0.03% of this acid. Theses results agreed with who found that content of sheep milk from C18:1:9c , C18:1:9trans, and C18:2:9c,12c reached 23.6, 8.9 and 2.8 % respectively.

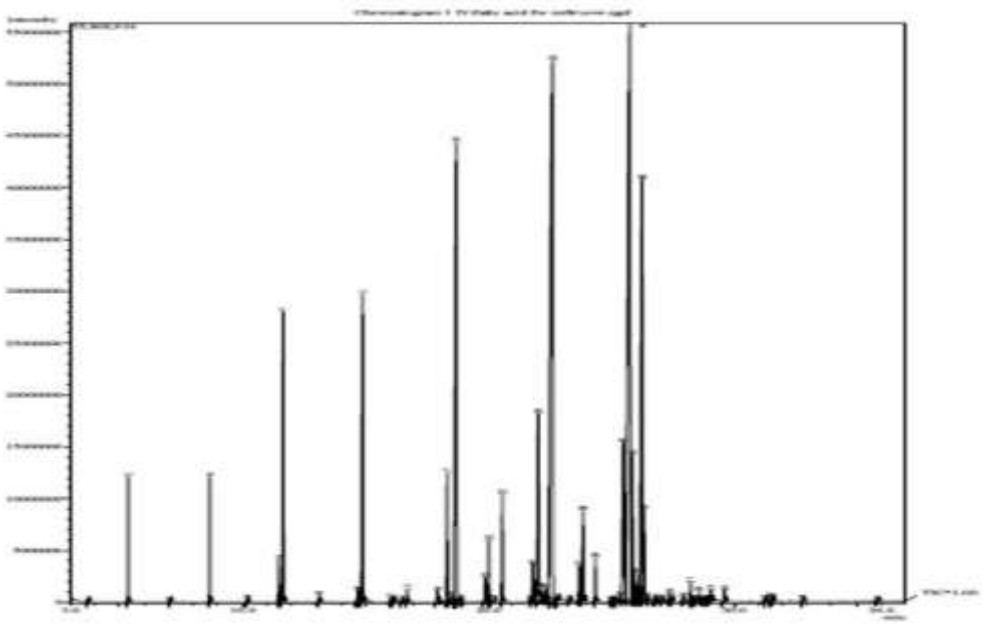

Fig (1) : Identification of Fatty acids in cow milk by GC/MS technique

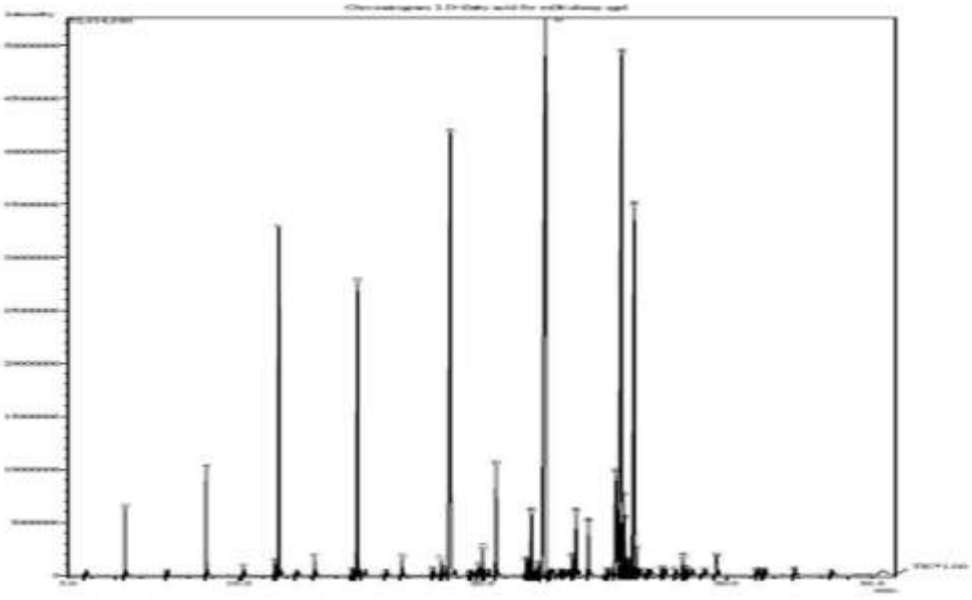

Fig (2) : Identification of Fatty acids in sheep milk by GC/MS technique

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Table (1). fatty acids in the cow and sheep milk that identified by GC-MS technique

Name	Formula	cow%	sheep%
Caproic acid	C6	1.15	0.78
Heptanoic acid	C7	0.02	0.04
Caprylic acid	C8	1.17	1.28
Pelargonic acid	C9	0.04	0.11
4-Decenoic acid	C10 : 1	0.4	0.18
Capric acid	C10	3.13	5.71
Methyl 4,8-dimethylnonanoate	C11 ┤ ┤	-	0.01
Undecanoic acid	C11	0.09	0.26
Methyl 10-methyl-undecanoate	C12 ┤	-	0.04
cis-5-Dodecenoic acid, methyl ester	C12 : 1	0.23	0.09
Lauric acid	C12	4.69	5,66
Dodecanoic acid, 4-methyl	C13 ┤	-	0.02
Tridecanoic acid	C13	-	0.28
Tridecanoic acid, 12-methyl	C14 ┤	0.09	0.12
Methyl 12-methyl-tridecanoate	C14 ┤	0.02	0.07
Methyl Z-11-tetradecenoate	C14 : 1	0.15	0.12
Methyl myristoleate	C14 : 1	1.89	0.26
Myristic acid	C14	11.05	13.79
Methyl 13-methyltetradecanoate	C15 ┤	-	0.16
Pentadecanoic acid	C15	-	0.49
pentadecanoic acid	C15	-	2.12
9-Hexadecenoic acid	C16 : 1	3.9	0.28
11-Hexadecenoic acid	C16 : 1	0.33	0.28
Palmitic acid	C16	21.83	25.32
Hexadecanoic acid, 2-methyl	C17 ┤	-	0.08
14-methylhexadecanoate	C17 ┤	-	0.36
cis-10-Heptadecenoic acid	C17 : 1	-	1.24
l 9-heptadecenoate or 9-17:1	C17 : 1	-	1.05
Margaric acid	C17	-	0.09
gamma.-Linolenic acid	C18 : 3	0.04	0.09
Linoleic acid	C18 : 2	3.98	2.73

6,9-octadecadienoate	C18 : 2	0.19	0.15
Methyl 10-trans,12-cis-octadecadienoate	C18 : 2	1.25	0.71
n-Propyl 9,12-hexadecadienoate	C18 : 2	0.03	2.73
5-Octadecenoic acid	C18 : 1	0.01	0.03
9-Octadecenoic acid	C18 : 1	0.03	1,31
Oleic acid	C18 : 1	24.57	19.18
Oleic acid - cis-13	C18 : 1	0.67	0.25
Oleic acid - cis-7	C18 : 1	0.16	0.03
Stearic acid	C18	11.03	10.17
Methyl 12-cis,10-trans-octadecadienoate	C18 : 2	-	0.71
(E)-9-Octadecenoic acid ethyl ester	C19 : 1	-	0.04
Cyclopropaneoctanoic acid	C19 - ⌂	-	0.15
cis-10-Nonadecenoic acid	C19 : 1	-	0.11
cis-5,8,11-Eicosatrienoic acid	C19 : 3	-	0.48
Methyl 11-eicosenoate	C20 : 1	-	0.15
Methyl 5-eicosenoate	C20 : 1	-	0.04
cis-11,14-Eicosadienoic acid	C20 : 2	-	0.11
Methyl 5,8,11-eicosatrienoate	C20 : 3	0.03	0.48
Arachidonic acid	C20 : 4	0.31	0.54
cis-11-Eicosenoic acid	C20 : 1	-	0.08
Methyl 8,11,14,17-eicosatetraenoate	C20 ┤┤┤┤	-	0.07
5,8,11,14,17- Eicosapentaenoic	C20 : 5	0.05	0.11

As noticed from table 1 that some conjugated fatty acids by 3% have been distributed between 2.9% of C18:2:9c,12c in sheep milk fat, but its ratio in cow milk fat was very little (0.04%). While other conjugated fatty acids was 0.1% Mendoza et al.(2008) found that there is a variation in unsaturated fatty acids ratio in sheep milk fat. Fatty acid of C18:1 has been went one better than the rest fatty acids and its ratio reached 21.1% then C18:2 (3.21%).the results also showed. also, the results showed that the ratio of Odd fatty acids in sheep milk reached 5.61% and distributed between fatty acids C7 ، C9 ، C11 ، C13 ، C15 ، C17 ، C19 by 0.04 ، 0.11، 0.26، 0.28 ، 2.12 ، 0.09 ، 0.04 % respectively. On the other hand these fatty acids were got the better of fatty acids in cow milk. These results were convergent with results of Niedbalska et al.(2001) who noticed that sheep milk contains a different ratios of odd fatty acids such as C13 ، Iso C15 ، anisito C15 ، Iso C17 ، C17 by 0.17 ، 0.34 ،0.47 ، 0.99 ، 0.53 ، 0.30 % respectively, but he did not refer to fatty acids like C7 ، C9 ، C11 ، C19 in his study contrary to the present study which referred to these fatty acids. This because of the differences in milk fat composition as a result to differences in animal variety and its nutrition. Also, the Cyclopropaneoctanoic acid has been identified in sheep milk fat that reached 0.15% contrary to cow milk fat which came down to it completely.

Concentration increase of conjugated linoleic acid in fermented dairy products

Figure 3 illustrates the total acidity percentage in sheep milk that added to it 250 µ g/ml. of sunflower oil and pollinated with local Bactria isolates which cited above by concentration of 3% and incubation temperature

of 37 C for one day. The variation in the total acidity percentage has been determined at periods of 0, 1, 2, 4, 6, 8, 10 days. Bactria isolates showed that a significant effect (P< 0.01) in the total acidity percentage with progress of storage periods. It has been noticed that a simple height in the total acidity at the first hours of storage (0-10) hours, but after 1 day of incubation had noticed a cute height in the total acidity reached 0.78, 0.62% and accompanied it increase in numbers of logarithm lactic acid Bactria was reached 10.5 and 10.4 CFU/ml. for L. acidophilus and L. casei respectively, also the height was continued with progress of cold storage periods and the highest high has been produced at storage time of 10 days and reached 0.85 and 0.74 % and numbers of logarithm Bactria was 8.83 and 8.78 CFU/ml. for previously cited bacteria isolates respectively. the increasing total acidity due to variation salt system especially increasing in phosphate ion, in addition of continuing production lactic acid and other organic acids by local Bactria isolates. This results agreed with Colakoglu and Gursoy (2011) who found that total acidity was increased with progressing storage period to fermented yogurt which ready for drinking by using different isolates from lactic acid Bactria and the maximum was at period of 10 days. Also, Han et al. (2012) found that gradually increasing in the total acidity with progressing storage time and the highest increasing was produced at 6 weeks and reached 1.28%. The differences between periods of 6 to 10 days of storage period were not significant.

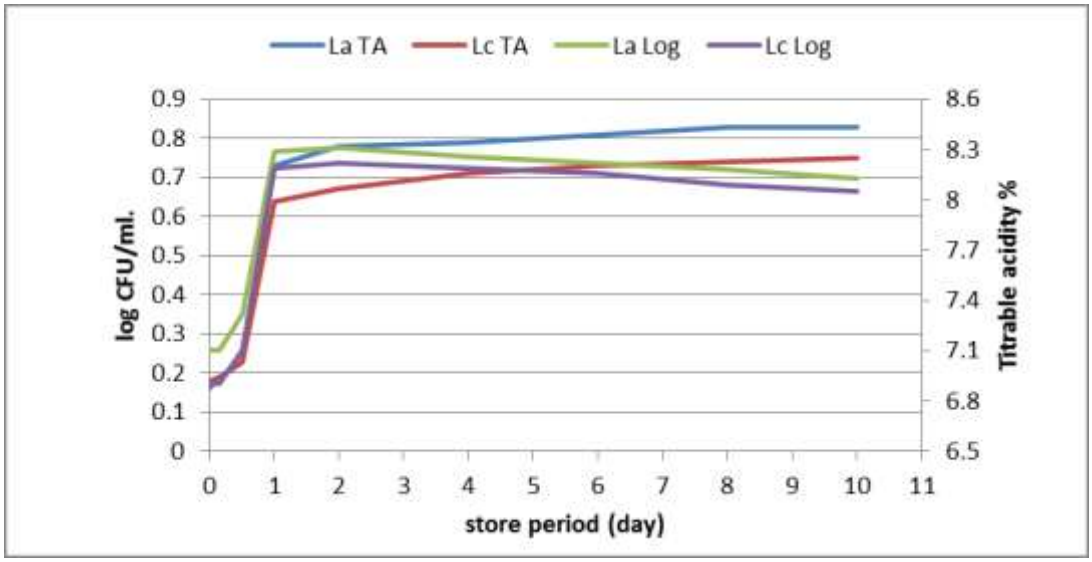

Figure (3): total acidity percentage in the sheep milk that added it 250 mg/ml of sunflower and pollinated by local Bactria isolate and storage time of (0-10) day.

Figure 4 shows concentrated conjugated linoleic acid in sheep milk that added to it 250 µg/ml. of sun flower oil and pollinated by Bactria isolates such as L. acidophilus and L. casei with concentration and incubation of 3% and 37 C/ for 1 day respectively. The variation of concentration of congregated linoleic acid at periods of 0, 1, 2, 4, 6, 8, 10 days was significant (P< 0.01) for all Bactria isolates. The results also showed that there is an increasing concentration of congregated linoleic acid in sheep milk and the period 1 day gave a marked increase by formation congregated fatty acid CLA reached 321.114 and 288.028 µ g/ml. with conversion ratio of 83.489 and 77.616 % and it accompanied increasing of logarithmic Bactria numbers reached 10.5 and 10.4 CFU/ml. to L. acidophilus and L. casei respectively. in addition to period of 2 days produced a slight increase of fatty acid CLA for all these isolates.

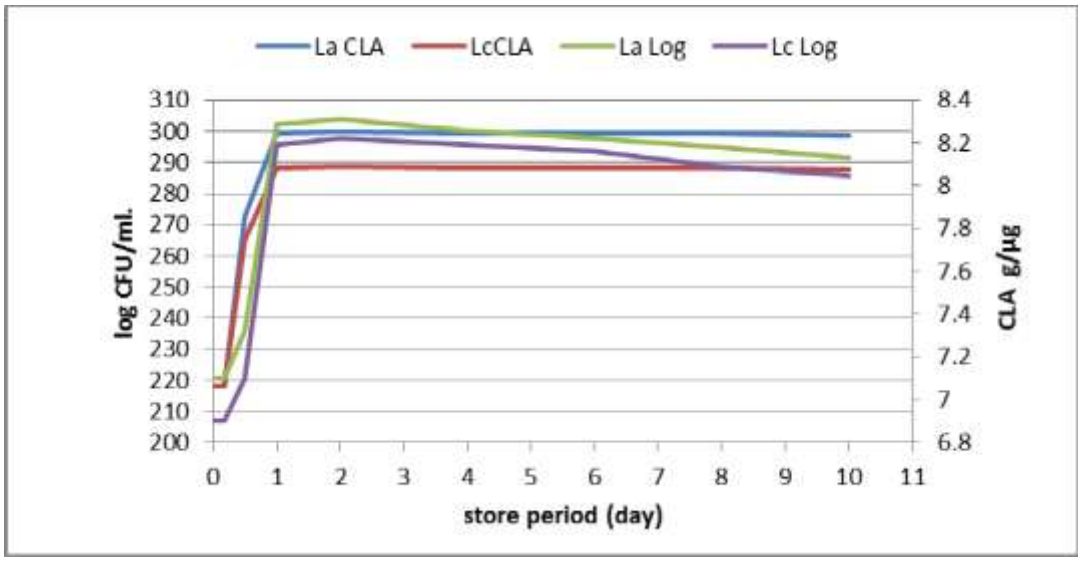

Figure (4): linoleic acid concentration in the sheep milk that added it 250 mg/ml of sunflower and pollinated by local Bactria isolate and storage time of (0-10) day.

The highest of conjugated linolenic acid concentration It may be attributed to increase isomerase activity because most of CLA is produced at the end logarithmic phase and beginning lag phase, also presence of enough quantity of substrate which led to activation of isomerase and optimum temperature for working enzyme. In addition to enough numbers of microorganisms which had a high capacity to produce of enzyme, all these factors led to produce conjugated fatty acid by high concentration during this period. While, gradual reducing produced with progress of storage times because of reducing pH with progress of storage times and it made the media was not fit for working isomerase. These results aged with Van-Nieuwanhove et al. (2007) who found that the highest of concentration of CLA was at 200 µg/ml. **of CLA in milk media.**

Evaluation of antioxidant activity

Table 2 illustrates increasing antioxidant activity of yogurt manufactured from cow and sheep milk with increasing concentration, but the increasing in yogurt of the sheep milk was higher than cow milk because increasing concentration of Conjugated fatty acids which working as antioxidant in the produced yogurt (Liangli,2001; Marinel.2012). As shown in table 2 and 3 that the reducing power of yogurt manufacture from cow milk at all used concentrations was transcend. This means that the antioxidant compounds are mainly answerable for reducing power. Also, it can be seen from tables that BHT activity was higher than yogurt sample because BHT is a compound has high purity.

Table 4 shows that yogurt manufactured from sheep milk has ability to hitch ferrous ion higher than yogurt manufacture from cow milk and compare with EDTA. CLA Forming antioxidant that reacts with free radicals of peroxides then tying ferrous iron and preventing oils oxidation (Ha et al.;1990). Due to these results, the activity of yogurt manufactured from sheep milk to drawback of corn oil oxidation. Ability of yogurt manufactured from sheep milk to drawback with increasing used concentrations and the higher activity was at 2.5 % . the results also showed that retention of oil with its quality characteristics at all concentrations.

Table 2. antioxidant activity for yogurt manufactured from cow and sheep milk (%).

source	Concentration(mg/g)				
	2	4	6	8	10
Sheep yoghurt	38.2	46.7	56.3	58.0	62.3
Cow yoghurt	22.3	32.7	45.5	46.7	49.2
BHT	63.9	75.3	87.7	92.6	98.3

Table 3. ability of reducing power for yogurt manufactured from cow and sheep milk (%).

source	Concentration(mg/g)				
	2	4	6	8	10
Sheep yoghurt	22.6	27.8	34.3	39.7	56.1
Cow yoghurt	11.4	19.4	26.9	29.4	31.7
BHT	56.5	62.7	79.9	88.5	93.2

Table 4. ability of tying ferrous ion for yogurt manufactured from cow and sheep milk (%).

source	Concentration(mg/g)				
	5	10	15	20	25
Sheep yoghurt	36.7	45.5	49.0	56.3	71.3
Cow yoghurt	22.2	27.0	32.2	39.2	41.7
EDTA	65.7	82.4	88.3	92.0	92.2

Table 5. Ability of yogurt manufactured from sheep milk on the retardation of corn oil oxidation by following peroxide values (mg/Kg oil).

Concentration (%)	Store periods (day)					
	0	6	12	18	24	30
0.5	1.5	2.1	4.1	5.1	7.9	9.8
1.0	1.5	2.1	3.6	4.7	6.5	8.7
1.5	1.5	1.9	2.6	3.9	4.6	5.2
2.0	1.5	1.7	2.2	3.2	4.1	4.6
2.5	1.5	1.7	2.2	2.9	3.7	4.2
Control	1.5	3.2	6.7	8.2	10.7	13.1
BHT(0.5)	1.5	1.6	1.9	2.0	2.1	2.3

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