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Bioactive-Lipids Distribution in Buffalo Fatty Products

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Abstract

Objective: Bioactive lipids play an important role in human health and there is a serious concern about the impact of the technological steps on the distribution of bioactive lipids and phospholipids between the fatty product and their by-products. So, this research is an effort to illustrate the effect of skimming and churning on bioactive lipids distribution. Thirty two individual samples of buffalo raw milk were skimmed to obtain cream and skim milk. Cream was churned to get butter and butter milk.

Methods: Fatty acids profile included butyric acid (BA), short chain fatty acids (SCFAs), conjugated linoleic acid (CLA), odd & branched chain fatty acids (OBCFAs) and trans fatty acids (TFAs) had been assayed by using GC-MS apparatus. Phospholipids (PLs) fractions were determined using 31P-NMR technique.

Results: Data detected that buffalo cream samples had higher content of BA (1.27%) than skim milk (1.07%).
While, butter milk samples contained higher values of BA (1.03%) than butter samples (0.98%). On the other hand, obtained results, manifested that cream sample had higher values of conjugated diene and triene acids (0.7775 and 0.1675%) than skim milk sample (0.7027 and 0.0933%), respectively. At the same time, butter contained higher contents of total OBCFAs than butter milk. Their average values were 10.90% for butter whilst they were 5.53% for butter milk, respectively. With respect to phospholipids, it was found that cream had higher contents of PE phosphatidylethanolamine, SM sphingomyelin and PG than skim milk. Their average values were 37.6, 29.7 and 3.1% versus 31.7, 20.7 and 2.6% of total PLs, in the same order. On contrary, skim milk had higher values of PC Phosphatidylcholine, PS and PI than cream, where their average values were 25.3, 10.3 and 9.4% versus 13.6, 7.2 and 8.8%, of total PLs respectively. It could be observed that butter milk was higher in Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM) as compared to butter (37.3, 32.5 and 18.2% versus 22.4, 29.3 and 17.1% of total PLs) in the same order.

Conclusion: The technological steps lead to remarkble differences in the distribution of bioactive lipids in both cream and butter and their by-products. Each product could be consumed to prevent or treat from specific diseases.

Keywords: Buffalo milk, Bioactive lipids,Phospholipids, Butyric acid,Odd & branched chain fatty acids, Trans fatty acids

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Introduction:

Milk fat not only is a source of energy and fat soluble vitamins, but also typical transport system for plentiful fatty acids associated with improved human health.

Over the past few years, scientific proof has appeared that bioactive compounds which naturally found in milk can lower the risk of a certain diseases **(Martínez- Monteagudo et al., 2015)**. Bioactive lipids which defined as an active specific signaling pathway are participatory in the regulation and maintenance of normal body, functions, and allowing cells to respond appropriately **(Evans and Hutchinson, 2010)**.

Milk bioactive lipids comprise monoglycerides, diglycerides, triglycerides correlated to beneficial fatty acids like short chain, medium chain, conjugated linoleic acid (CLA), and polyunsaturated fatty acids (PUFAs). The minor lipid components such as phospholipids, also carry biological and health promoting activities **(Dhankhar et al., 2016)**.

Butyric acid (BA) which considered one of bioactive lipids; known as butanoic acid. All of ruminant milk types and their dairy products contain Butyric acid. Some authors **Williams et al., (2003)** notified that little concentrations of butyric acid can prohibit growth of human cancer

cell lines. Also, BA plays an important role in controlling cell growth, differentiation and preventing tumor genesis in colon cells **(Dhankhar et al., 2016).**

On another hand, conjugated linoleic acid (CLA) is defined as a group of positional and structural linoleic acid isomers which methylene (CH_2) group in-between the two double bonds doesn't exist. There are four geometric CLA isomers: cis-trans; tans-cis; trans-trans and cis-cis **(Yurawecz et al., 2006)**. The main precursor of CLA is linoleic acid as referenced by **Hur et al., (2017);** thence CLA can be synthesized via conversion of linoleic acid by ruminal bacteria (Propionibacterium) and lactic acid bacteria (Lactobacillus, Lactococcus, and Streptococcus) with bio-hydrogenation. As stated by **Abbas et al., (2014) and Park & Wu (2014);** dairy products are considered the major source of CLA which affected on the reduction of body fat, prevention of cancer & cardiovascular diseases, modulation of immune & inflammatory responses, and improvement of bone health.

Moreover, Milk fat consists of a varied range of odd and branched-chain fatty acids (OBCFAs), with 56 specific isomers and chain lengths varying from 4 to 26 carbon atoms **(Jensen, 2002). In (2006) Vlaeminck et al.**, mentioned that there are three main classes of branch-chain fatty acid in milk fat were even-chain iso acids, odd- chain iso acids, and odd-chain anteiso. Likewise, there are seven major OBCFAs in food products submitted by **Ollberding, (2016)**, which include iso-14:0, iso-15:0, anteiso-15:0, iso-16:0, iso-17:0, anteiso-17:0, and iso-18:0. The healthy benefits of odd and branched fatty acids were proved by insignificant studies.

Wongtangtintharn et al., (2004) outlined that both anteiso and iso branch-chain fatty acids inhibited tumor outgrowth and the highest activity was observed with 16:0 iso. Odd and branched-chain fatty acids prevent fatty acid synthesis of tumor cells through direct effects on fatty acid synthetase and reduce in fatty acid precursor supply.

In another view; phospholipids (PLs) are an important group of biomolecules. They are mainly existed in milk fat globule membranes (MFGM). They play a fundamental role in milk through the emulsification of fat in the aqueous phase. **(Contarini & Povolo, 2013)**. The properties of the raw material and applied technological processes deeply affected in PLs content of dairy products. Any treatment caused a perturbation of the membrane with separation or fractionation of fat globules, such as homogenization or centrifugation, of polar and neutral components of fat influenced PLs composition and distribution in the final product **(Ali et al., 2018)**. Milk fat globule membrane (MFGM) contains approximately 60–70% of total PLs in milk which represented 0.5–1% of the total lipids in milk. Lately, great attempt has been devoted to inspect the possible link between lipid metabolism and various human diseases such as diabetes, stroke, cancer, arthritis, and Alzheimer's **(Gallier et al., 2010; González-Domínguez et al., 2014; Aoyagi et al., 2017)**. A considerable link between phospholipids, gangliosides (a type of glycolipids) and infant cognitive development had been presented by recent infant clinical trials (**Timby et al., 2014 and Liua et al., 2018)**. It was known that PE is a zwitterionic phospholipid mainly located in the internal layer of the MFGM and considered the most unsaturated class but PI is considered an anionic phospholipid mostly found in the internal layer too of the MFGM **(Deeth, 1997)**. The PE concentration influences the phase behavior of PLs mixtures, transforming to a reversed hexagonal phase instead of a lamellar phase at higher concentrations **(Waninge et al., 2003)**. The PC is one of little substances able to penetrate into blood-brain barrier in the body, going directly into the brain cells where it is used for producing acetylcholine (ACh), that may act as a neurotransmitter for memory enhancement **(Smith et al., 2016). Miranda et al., (2008)**, illustrated that exogenous PC was in fact participate in anti-inflammatory signaling networks and exerted an immune-modulatory effect. Moreover, exogenous PC intake could enhance intestinal barrier defense by inhibiting pro-inflammatory cytokine production **(Olson, 2014)**. Sphingomyelin is found in high quantities in the brain and neural tissues, and phosphatidylcholine is the major dietary source of choline (a precursor of acetylcholine synthesis) and also plays a vital role in neuronal membranes **(Ohlsson et al., 2010)**. On the other side, **Boyle et al., 2019** manifested that PL intake improved both of post-stress reaction time performance on an attention-switching task and mid- stress induction energetic arousal. So, they proved that man can increase his cognitive performance advantages dietary when supplementing with bovine milk PLs under conditions of psychosocial stress.

The U.S. Food and Drug Administration, (2003) defined trans fatty acids (TFAs) as "all unsaturated fatty acids that contain one or more isolated double bonds intrans configuration". Multiple researches have implicated that a high consumption of trans fatty acids may be a cause of cardiovascular disease (CVD) and coronary heart disease (CHD). Intake of TFAs should not exceed 1% of total energy as recommended by **World Health Organization, (2003)** to reduce CVD risk. Both of **Mensink et al., (2003) and Mozaffarian et al., (2006)** exhibited that TFAs could raise low-density lipoprotein (LDL) cholesterol concentrations and plasma triglycerides in blood so it might be a cause of atherosclerosis, sudden cardiac death, and other aspects of chronic diseases.

Consequently, the main goal of this research was to elucidate the influence of some technological steps such as skimming and churning on the distribution of the different bioactive-lipids in buffalo cream and butter and their by-products. Also, this research can be utilized nowadays in face of various diseases prevalent, in recommendation to consume a specific type of bioactive lipid to prevent a specific disease.

Materials And Methods:

Materials:

Raw milk

Thirty-two buffalo raw milk samples (each 0.5 kg) were obtained in winter season (November to January) from the Dairy department, Faculty of Agriculture, Cairo University, Giza, Egypt. Every 8 samples of milk were mixed well and give 4 kg, to represent 4 replicates.

Experiments:

1- Cream and skim milk:

Milk samples were skimmed using a milk cream separator (Model 100 L/H 110V USA/Canada Plug) to obtain the cream (~40.0 % fat) and the skimmed milk (~0.5 % fat).

2- Butter and butter milk

A part of the resultant cream was churned by the blender (Heidolph No 50111,

Model RZR1 ; Speed from 35 to 2200 rpm with the hard flipping) for obtaining butter (~80.0 % fat) and separate the butter milk (~1.5 % fat).

Methods:

Extraction of lipids and phospholipids:

The total lipids and phospholipids were extracted according to the method originally described by **Bligh and Dyer, (1959)** as following: Six ml of sample was added to falcon tube 50 ml with 22.5 ml mixture of chloroform and methanol 1:2 (v/v) and vortex well for 2 minutes then 7.5 ml chloroform was added and vortex well for 2 minutes. Followed by add 7.5 ml distilled water and vortex well for 2 minutes.

Centrifugation was carried out at 4000 rpm for 5 minutes to separate the two phases; the upper phase (methanol layer) and the lower phase (chloroform layer) by separation funnel. Then, washing the upper phase and the bottom phase three times to obtain a very clean extraction. Finally, the solvents were removed by rotary evaporator (BUCHI Rotavapor® R110 model, Rotation speed 20 - 280 rpm and con-

trolled temperature ranged from 20-95°C) to obtain the lipid and phospholipid extracts free from solvents.

Determination of total conjugated diene and triene fatty acids contents:

Conjugated diene (CDA) and triene (CTA) fatty acids were determined according to the modified version of the **AOAC, (2012).** CDA and CTA contents were calculated using the following equations:

CDA (%) = (0.84 x A) / (bc-K0) **CTA (%) = (0.84 x A1) / (bc-K0)**

Where: A is the absorbance at 233 nm, b is the length of cell (cm), c is gram per liter A1 is the absorbance at 280 nm K0 is the absorptivity by ester groups (0.07).

Determination of fatty acid profile:

Preparation of fatty acids methyl esters (FAME):

Fatty acids methyl esters were prepared according to the method of **Wirasnita et al., (2013)**; the extracted lipid fraction, about 50 mg, was saponified with 5 ml 0.5 M solution of KOH/ Met OH and subjected to methylation (10 min. at 75°C) in 5 ml 14% (v/v) BF3/ Met OH. Then, water was added to reaction and the methyl esters of fatty acids were extracted with 20 ml of hexane. Fatty acids methyl esters were washed with 10 ml 10% sodium bicarbonate and dried over anhydrous sodium sulphate. The organic phase was evaporated under reduced pressure and stored in - 27°C until chromatographically analysis.

Determination of fatty acids by GC- MS apparatus:

Fatty acid profile was assessed using gas chromatography coupled with a mass spectrometer. The exact structure of iso and anteiso isomers of fatty acids were confirmed also by isolation of M+ ions for branched-chain-fatty acid methyl ester (BCFAME) for fragmentation in EIMS2 mode, according to **Ran-Ressler et al., (2014)**.

Preparation of phospholipid samples

The phospholipids were prepared according to the method mentioned by **Murgia et al., (2003)**; the extracted phospholipid fraction, about 100 mg of sample was extracted with 200 ml of acetone, and the insoluble residue was collected. The residue was then dispersed in 60 ml chloroform/ methanol 2:1 (v/v) and filtered. After filtration, the solution was washed with the same volume of a 0.01M Na4-EDTA- 0.1M NaCl solution. The organic phase was recovered, dried with anhydrous Na2SO4, filtered, and then evaporated at 35°C in a rotary evaporator. One-tenth of dried samples were dissolved in 0.5 ml triethylamine / dimethylformamide /guanidinium hydrochloride (15, 50 ml, and 5 g, respectively) in order to acquire 31P-NMR spectra.

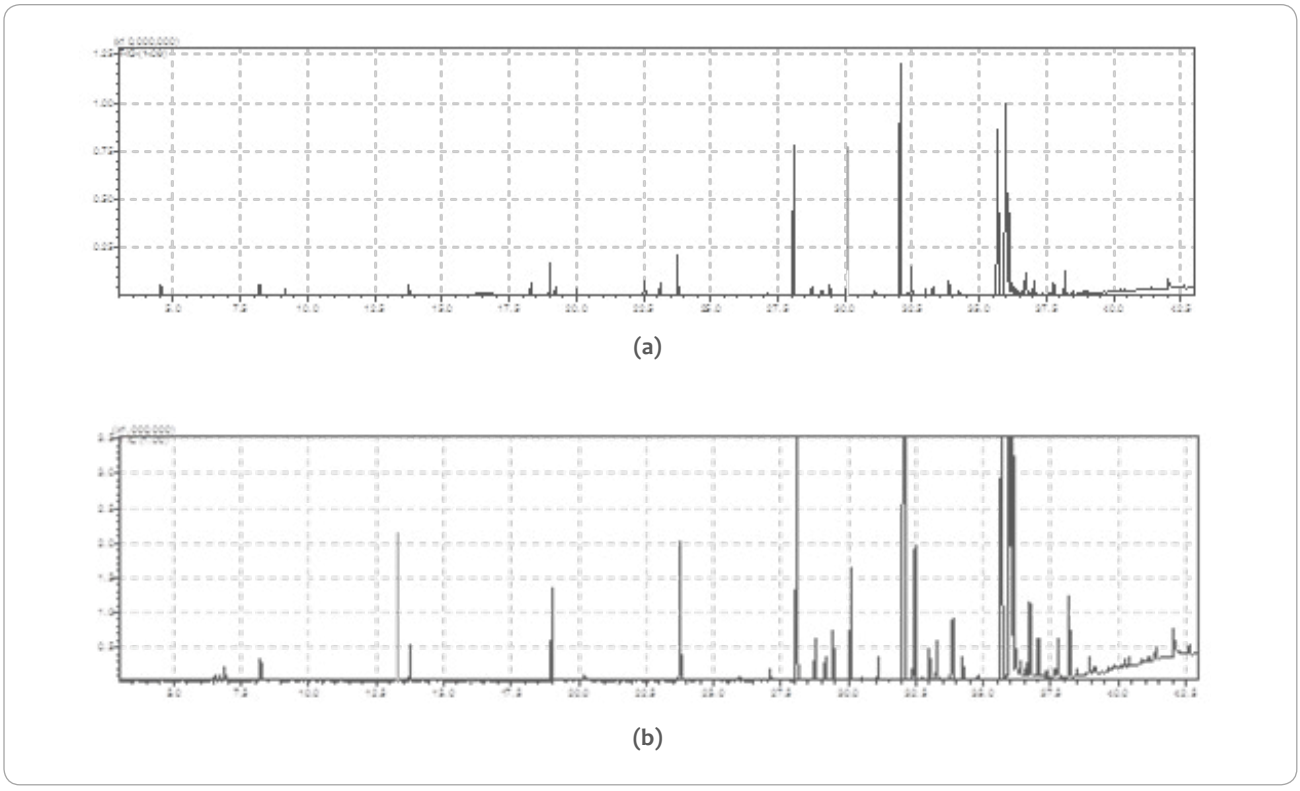
Determination of phospholipid classes by 31P-NMR technique

High-resolution 31P-NMR spectra, acquired at the NMR Service of Istituto di Chimica Biomolecolare del CNR (Pozzuoli, Italy), were obtained at 27°C on a Bruker Avance-400 operating at 161.97 MHz, using an inverse probe fitted with a gradient along the Z-axis. The 1H-decoupled, one-dimensional 31P spectra were obtained using the following conditions: spectral width 200 ppm, delay time 7 s, pulse width of 8.0 µs (60° spin-flip angle), number of scans 3000, number of data points 32 K. Phospholipids samples contained 10% dimetylformamide-d7 for internal lock, and internal phosphatidylcholine was used as reference. Sphingomyelin from chicken egg yolk, rac-1,2- dipalmitoyl-glycero-3-phosphoethanolamine (phosphatidylethanolamine), 1,2-dipalmitoyl-sn-glycero-3-phosphocoline (phosphatideylcholine), 3-sn-phosphstidyl-L-serine from bovine brain (phosphatidylserine), sn-glycerol-3-phosphate (phosphatidylglycerol) and phosphatidylinositol sodium salt from soybean (from Sigma-Aldrich) were used as standards for phospholipids assignments.

Results And Discussion:

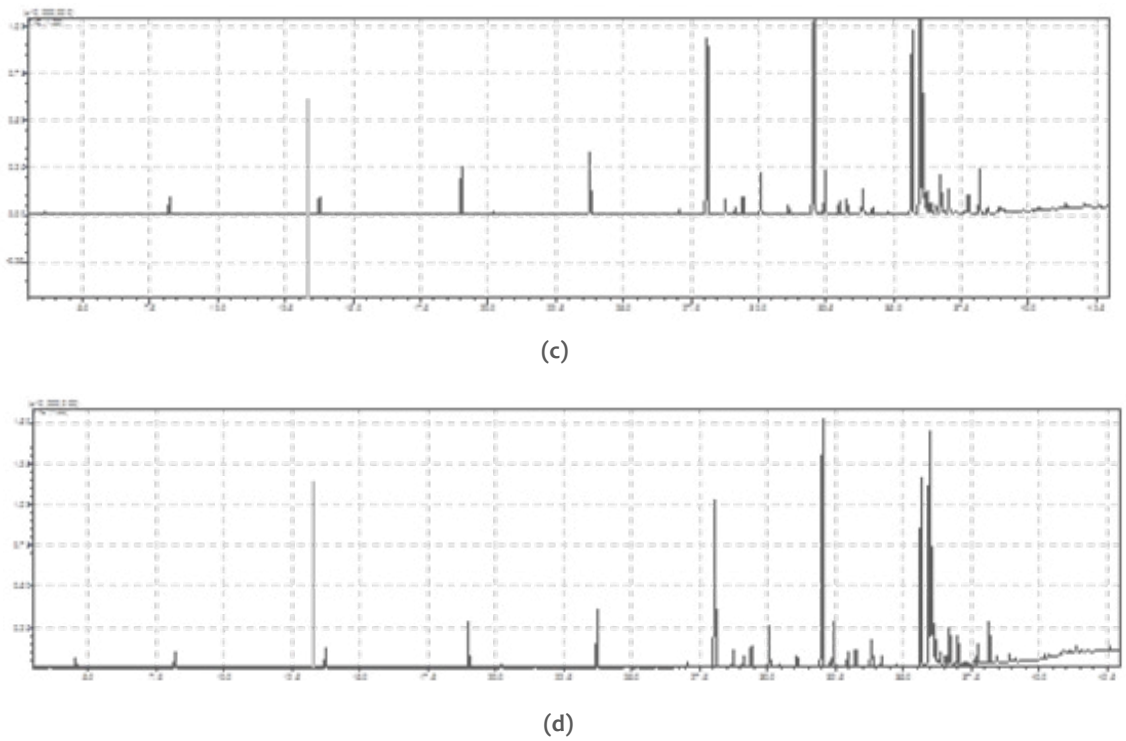
Bioactive lipids in buffalo cream, butter and their by-products:

The distribution of the different bioactive lipids in cream, skim milk, butter and butter milk had been illustrated in the following Fig. 1 (a,b,c,d) ; the results were individually calculated and expressed in separated tables



(a)

(b)



(c)

(d)

Fig. (1): GC/MS chromatograms of buffalo cream, skim milk butter and butter milk.
(a): cream (b): skim milk (c): butter (d): butter milk

Butyric acid (BA) and short chain fatty acids:

Butyric acid and short chain fatty acids (SCFAs) contents of buffalo cream, butter, skim milk and butter milk were presented in Table (1). It was demonstrated that there were significant differences ($p \leq 0.05$) between fatty products and their by- products in all SCFAs contents.

The average values of butyric, caproic, caprilyic and capric acids were 1.27, 0.56, 0.65 and 1.89% for cream, while they were 1.07, 0.75, 0.82 and 2.22% for skim milk, respectively. On the other hand, the corresponding values of butter samples were 0.98, 0.49, 0.51 and 2.31% whilst for butter milk were 1.03, 0.34,0.39 and 1.70% of total FAs, in the same order.

Fatty Acids	Products			
	Cream	Skim milk	Butter	Butter milk
C4:0 (Butyric)	$1.27^{b}\pm0.04$	$1.07^{d}\pm0.02$	$0.98^{de}\pm0.02$	$1.03^{d}\pm0.01$
C6:0 (Caproic)	$0.56^{d}\pm0.02$	$0.75^{cd}\pm0.01$	$0.49^{e}\pm0.02$	$0.34^{f}\pm0.01$
C8:0 (Caprilyic)	$0.65^{c}\pm0.01$	$0.82^{b}\pm0.02$	$0.51^{e}\pm0.01$	$0.39^{f}\pm0.02$
C10:0 (Capric)	$1.89^{c}\pm0.02$	$2.22^{a}\pm0.02$	$2.31^{b}\pm0.01$	$1.70^{b}\pm0.02$

Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$).

Table 1: Butyric and short chain fatty acids contents (as % of total fatty acids) of buffalo cream, butter and their by- products

It was obvious that the highest value of butyric acid was noticed in cream followed by skim milk and finally butter milk and butter. On the same side, **Hassanzadazar et al., (2017)** determined the contents of butyric, caproic, caprilyic and capric acids in cow butter samples. Their average values were 0.69, 0.75, 0.63 and 1.81% of total FAs, respectively.

Total conjugated diene (CDA) and triene (CTA) fatty acids:

Conjugated diene and triene fatty acids contents of buffalo cream, butter and their by-products had been demonstrated in Table (2). Their contents between all samples had great significant differences ($p \leq 0.05$). Buffalo butter had higher conjugated diene acids (CDA) content (0.8580%) than butter milk (0.7290%). As well, buffalo cream contained higher value of CDA (0.7775%) as compared to skim milk (0.7027%). The same trend was realized for conjugated triene acids (CTA); there values were 0.1655 and 0.1577% for butter and butter milk, while they were 0.1675 and 0.0933% for cream and skim milk, respectively. These data were in harmony with the results of **Seçkin et al., (2005)** who displayed that CDAS content of Turkish butter was higher than in Turkish cream (0.79 versus 0.69%), in order.

Products	CDA	CTA
Cream	$0.7775^{d}\pm0.01$	$0.1675^{c}\pm0.003$
Skim milk	$0.7027^{f}\pm0.003$	$0.0933^{e}\pm0.01$
Butter	$0.8580^{b}\pm0.004$	$0.1655^{c}\pm0.007$
Butter milk	$0.7290^{e}\pm0.01$	$0.1577^{cd}\pm0.007$

Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$).

Table (2): Total conjugated diene (CDA) and triene (CTA) fatty acids contents (as % of total fatty acids) in buffalo cream, butter and their by- products

Conjugated linoleic acid (CLA) and its isomers:

Conjugated linoleic acid and its isomers contents in buffalo cream, butter and their by- products were exhibited in Table (3). It could be recognized that CLA (cis-9, trans-11) contents were 0.54 and 0.26% whilst cis-10, cis-12 was 0.50 and 0.65% of total FAs for cream and skim milk, respectively. The identical values of butter samples were 0.74 and 0.44%, while they were 0.17 and 0.42% of total FAs for butter milk, in order. As shown in the same table; non-significant differences ($p \geq 0.05$) were observed between cream and butter and their by- products in trans-10, cis-12 and trans-9, trans-11 contents. Their average values were 0.45 and 0.07% for cream versus
0.47 and 0.07% for skim milk whilst they were 0.41 and 0.08% in butter versus 0.39 and 0.06% of total FAs in butter milk, in the same order.On the same side, Seçkin et al., (2005) mentioned that Turkish cream had higher CLA (cis-9, trans-11) content (5.74 mg/g of fat) when compared to Turkish butter (4.67 mg/g of fat).

Fatty acids	Products			
	Cream	Skim milk	Butter	Butter milk
Cis9 trans11-C18:2	$0.54^{d}\pm0.12$	$0.26^{e}\pm0.21$	$0.74^{a}\pm0.02$	$0.17^{e}\pm0.16$
Cis10 cis12-C18:2	$0.50^{c}\pm0.12$	$0.65^{a}\pm0.16$	$0.44^{d}\pm0.07$	$0.42^{d}\pm0.05$
Trans10 cis12-C18:2	$0.45^{b}\pm0.13$	$0.47^{b}\pm0.29$	$0.41^{b}\pm0.06$	$0.39^{b}\pm0.12$
Trans9 trans11-C18:2	$0.07^{c}\pm0.02$	$0.07^{c}\pm0.01$	$0.08^{c}\pm0.01$	$0.06^{c}\pm0.01$

Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$).

Table (3): Conjugated linoleic acid and its isomers contents (as % of total fatty acids) of buffalo cream, butter and their by- products

Odd and branched chain fatty acids (OBCFAs):

Odd and branched chain fatty acids contents of buffalo cream, butter and their by- products were represented in Table (4). Observably; both buffalo cream and butter and their by- products gained significant differences ($p \leq 0.05$) in total OBCFAs contents. Cream had higher contents (11.43%) of total OBCFAs, than skim milk (7.03%). Also, butter gained higher value of total OBCFAs as compared with butter milk (10.90 versus 5.53% of total FAs). It was also identified that C15:0 was the most abundant in cream followed by C11:0, C13:0 iso and C17:0 (2.15, 1.91, 1.47 and 1.46%), while in skim milk were C15:0, C17:0, C14:0 anteiso and C17:0 anteiso (1.83, 1.49, 0.86 and 0.68 % of total FAs), respectively. On contrary, cis9-C15:1 was the prevelant in butter followed by C15:0, C17:0 and cis9-C17:1 (2.08, 1.97, 1.61 and 1.50%), while in butter milk were C17:0, C15:0, C14:0 anteiso and C17:0 anteiso (1.44, 1.41, 0.58 and 0.55% of total FAs), in the same order.

Cream and butter had higher content of total OBCFAs than skim milk and butter milk. Same line was observed by **Rutkowska et al., (2015)** who pointed that the contents of total OBCFAs in cow cream samples were 4.87 and 4.16% of total FAs for Wielkopolska and Opolskie City, respectively

Fatty Acids	Products			
	Cream	Skim milk	Butter	Butter milk
C11:0	$1.91^{a}\pm0.13$	$0.02^{b}\pm0.01$	$0.04^{b}\pm0.03$	$0.03^{b}\pm0.02$
C12:0 *iso*	$0.03^{bcd}\pm0.01$	$0.02^{bcd}\pm0.01$	$0.06^{ab}\pm0.04$	$0.01^{cd}\pm0.007$
C12:0 *anteiso*	$0.02^{cd}\pm0.01$	$0.04^{abc}\pm0.01$	$0.02^{bcd}\pm0.007$	$0.01^{cd}\pm0.007$
C13:0	$0.04^{d}\pm0.02$	$0.10^{b}\pm0.04$	$0.05^{cd}\pm0.01$	$0.02^{d}\pm0.02$
C13:0 *iso*	$1.47^{a}\pm0.35$	$0.21^{bcd}\pm0.01$	$0.18^{bcd}\pm0.04$	$0.08^{bcd}\pm0.07$
C14:0 *anteiso*	$0.99^{ab}\pm0.07$	$0.86^{b}\pm0.01$	$0.88^{b}\pm0.02$	$0.58^{c}\pm0.24$
C14:0 *iso*	$0.49^{a}\pm0.07$	$0.01^{c}\pm0.01$	$0.41^{b}\pm0.007$	$0.26^{cd}\pm0.12$
C15:0	$2.15^{ab}\pm0.26$	$1.83^{bcd}\pm0.18$	$1.97^{abc}\pm0.07$	$1.41^{cd}\pm0.51$
Cis9-C15:1	$0.03^{b}\pm0.02$	$0.02^{b}\pm0.007$	$2.08^{a}\pm0.09$	$0.01^{b}\pm0.001$
Cis11-C15:1	$0.09^{d}\pm0.007$	$0.08^{d}\pm0.01$	$0.05^{c}\pm0.007$	$0.03^{f}\pm0.001$
C18:0 *iso*	$0.11^{b}\pm0.007$	$0.12^{b}\pm0.01$	$0.12^{b}\pm0.007$	$0.03^{cd}\pm0.03$
C18:0 *anteiso*	$0.25^{c}\pm0.01$	$0.21^{cd}\pm0.007$	$0.24^{cd}\pm0.01$	$0.10^{f}\pm0.03$
C16:0 *iso*	$0.48^{b}\pm0.04$	$0.40^{b}\pm0.07$	$0.40^{b}\pm0.007$	$0.24^{c}\pm0.11$
C17:0	$1.46^{c}\pm0.22$	$1.49^{c}\pm0.03$	$1.61^{bc}\pm0.26$	$1.44^{c}\pm0.29$
Cis9-C17:1	$0.49^{cde}\pm0.04$	$0.39^{def}\pm0.07$	$1.50^{b}\pm0.11$	$0.23^{ef}\pm0.14$
C17:0 *iso*	$0.64^{c}\pm0.09$	$0.55^{cd}\pm0.10$	$0.57^{cd}\pm0.02$	$0.50^{d}\pm0.02$
C17:0 *anteiso*	$0.78^{b}\pm0.11$	$0.68^{bc}\pm0.07$	$0.72^{b}\pm0.12$	$0.55^{c}\pm0.14$
Total	$11.43^{a}\pm0.16$	$7.03^{e}\pm0.11$	$10.90^{b}\pm0.17$	$5.53^{d}\pm0.10$

Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$).

Table (4): Odd and branched chain fatty acids contents (as % of total fatty acids) of buffalo cream, butter and their by- products

Trans fatty acids:

Trans fatty acids (TFAs) contents of buffalo cream, butter and skim milk & butter milk were depicted in Table (5). From the table, it was conducted that skim milk gained higher contents of trans9-C18:1 (4.64%) than cream (3.65%). Moreover, cream contained slight higher value of trans9-C16:1, trans11-C18:1 and trans11- C20:1 than skim milk. Their average values were 0.17, 1.42 and 0.05% for cream, while they were 0.14, 1.28 and 0.04% of total FAs for skim milk, respectively. In the same line, butter contained higher proportion of trans9-C16:1, trans11-C18:1 and trans11-C20:1 than butter milk. Their average values were 0.15, 1.48 and 0.09% for butter, while they were 0.10, 1.27 and 0.08% for butter milk, in order. Furthermore, the average value of trans9-C18:1 was 5.07% in butter milk versus 3.96% of total FAs in butter . Likewise, the results by **Rutkowska et al., (2015)** appeared that contents of total trans-C18:1 in cow cream samples were 2.43 and 3.05% of total FAs whilst trans11-C18:1 content of cow cream was 1.80 and 2.23% of total FAs for Wielkopolska and Opolskie City, in the same order.

Fatty acids	Products			
	Cream	Skim milk	Butter	Butter milk
$Trans_{9}$-C16:1	$0.17^{bcd}\pm0.05$	$0.14^{cd}\pm0.01$	$0.15^{bcd}\pm0.007$	$0.10^{cd}\pm0.02$
$Trans_{9}$-C18:1	$3.65^{cd}\pm0.55$	$4.64^{bc}\pm0.26$	$3.96^{bcd}\pm0.21$	$5.07^{ab}\pm1.23$
$Trans_{11}$-C18:1	$1.42^{c}\pm0.04$	$1.28^{de}\pm0.02$	$1.48^{bc}\pm0.05$	$1.27^{c}\pm0.02$
$Trans_{11}$-C20:1	$0.05^{b}\pm0.04$	$0.04^{b}\pm0.02$	$0.09^{ab}\pm0.02$	$0.08^{b}\pm0.01$

Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$).

Table (5): Trans fatty acids contents (as % of total fatty acids) of buffalo cream, butter and their by- products.

Total polar lipids (PLs) contents:

Table (6) announced total polar lipids content of buffalo cream or butter and skim milk & butter milk. It was obvious that there were significant differences ($p \leq 0.05$) in total PLs contents of cream and butter or their by- products. As it was expected, butter milk had the highest PLs content where its average value was 3.95 g/100g fat because it contains the large amount of the MFGM which considered the main source of phospholipids. As for cream, the content was 0.29 g/100g fat, while it was 0.43 g/100g fat for skim milk and 0.15 g/100g fat for butter, respectively. These differences in distribution of PLs among products are due to the disruption of MFGM and migration of PLs to aqueous phases. The trend was in agreement with **Avalli and Contarini, (2005)** who proved that butter milk had higher contents of total polar lipids (44.85 mg/g of fat) than cream and butter (5.32 and 1.95 mg/g of fat), in order. As well, **Rombaut et al., (2006)** detected that the polar lipids content in skimmed milk were significantly higher than cream. They concluded that the polar lipids are mainly enriched in aqueous phases like skimmed milk, butter milk and butter serum.

Products	Total polar lipids content
Cream	$0.29^{c} \pm 0.007$
Skim milk	$0.43^{b} \pm 0.01$
Butter	$0.15^{d} \pm 0.02$
Butter milk	$3.95^{a} \pm 0.01$

Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$).

Table (6): Total polar lipids contents (g/100g fat) of buffalo cream, butter and their by- products.

Phospholipids classes.

Phospholipids classes content of cream, butter and their by- products were illustrated in Fig. (2). Buffalo cream had higher significant ($p \leq 0.05$) values of phosphatidylethanolamine (PE), sphingomyelin (SM) and phosphatidylglycerol (PG) than skim milk. Their values were 37.6, 29.7 and 3.1% of total PLs for cream versus 31.7, 20.7 and 2.6% for skim milk, respectively. As for skim milk; it contained higher values of phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI) than cream, where their average values were 25.3, 10.3 and 9.4% for skim milk versus 13.6, 7.2 and 8.8% of total PLs for cream, respectively. Further, butter samples had high values of PS, PI and PG than butter milk (16.2, 11.4 and 3.6% versus 9.3, 1.3 and 1.4% of total PLs, respectively. It could be noticed that butter milk was higher in PC, PE and SM as compared to butter (37.3, 32.5 and 18.2% versus 22.4, 29.3 and 17.1% of total PLs), in the same order. So, these results were in compatible with those of **Avalli and Contarini, (2005)** who mentioned that butter milk had higher value of PC (35.5%) and lower value of PI (2.4%) when compared to butter (24.7 and 11.9%) and cream (25.9 and 8.6% of total PLs), respectively. Moreover, cream contained higher value of SM (20.4%) than butter (17.0%) and butter milk (18.5%). However, the findings of this study were not in accordance with the findings of **Rodríguez- Alcalá and Fontecha, (2010)** who stated that the contents of PC, PE, SM, PI and PS in butter milk were 31.0, 30.0, 20.0, 7.0 and 5.0% of total PLs, respectively.

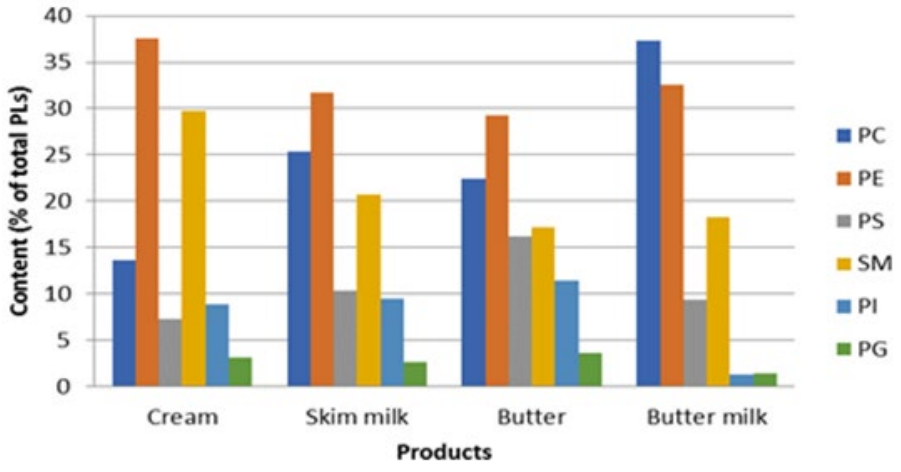


Fig. (2): Phospholipid fractions (as % of total PLs) of buffalo cream, butter and their by- products.
PC= Phosphatidylcholine; PE= phosphatidylethanolamine; PS= phosphatidylserine; SM= sphingomyelin; PI= phosphatidylinositol; PG= phosphatidylglycerol

Conclusion And Recommendations:

Egyptian buffalo milk is a rich source of bioactive lipids which consider as good substances for human health. Data displayed that the highest value of butyric acid was noticed in cream followed by skim milk and finally butter milk and butter. Also, it had been observed that buffalo cream had higher contents of total OBCFAs than skim milk. On contrary, butter milk had lower values of caproic, caprilyic and capric acids than butter. Furthermore, butter milk gained the highest contents of total polar lipids and PC, PE and SM when compared to butter. The technological steps had a clear effective influence on the distribution of all bioactive lipids in milk products.

Thus, it could be recommended that the diet supplementation with butter milk (enriched with phosphatidylcholine) is benefit for memory improvement and as anti- inflammatory agent. Phosphotidycholine produces acetylcholine (ACh) which if it decline by the time aging may be reduce in learning and memory impairment. It also can be adviced that the intake of conjugated linoleic acid-enriched-diet reduced body weight and normalize glucose levels in diabetes patients.

Further studies are required to investigate the influence of total phospholipids and their classes on biological activities of human body to employ these characteristics to treat special patient's conditions. More information about these bioactive lipids advantages must be achieved in multiple researches in the near future**.**

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