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# Vital Lipids Contents in Buffalo Butter Oil and its Fractions Prepared by Dry Fractionation

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**Abstracts**

Background and Objective: The fractionation of butter oil to various specific fractions needs recently to multiply its uses and the requirement of its interchangeability. This article is concerning with the distribution of bioactive lipids in buffalo butter- oil and its fractions prepared by the dry fractionation technique. Materials and Methods: Thirty-two samples of Egyptian fresh buffalo milk were collected, divided into 4 composite samples and then converted to butter oil. Each butter oil sample was fractionated to four fractions: three solid fraction treatments at 35°C ($S_{35}$), 25°C ($S_{25}$), 15 °C ($S_{15}$)and liquid fraction at 15°C (L15). Fatty acids profile was assayed included butyric acid (BA), short chain fatty acids (SCFAs), conjugated linoleic acid (CLA), odd & branched chain fatty acids (OBCFAs) and Trans fatty acids by using GC-MS apparatus. Results: Dry fractionation of butter-oil revealed that L15 had a higher content of CLA as well conjugated diene and triene fatty acids, while S25 contained higher content of total OBCFAs as compared to all other fractions. Conclusion: The dry fractionation leads to marked differences in the distribution of bioactive lipids in buffalo butter oil and its fractions. Each fraction could be used for specific purposes in the food industries according to its melting properties and this affect its healthy benefit.

***Key words***: Buffalo milk; butter oil; dry fractionation; bioactive lipids; milk fat; fatty acid; CLA.

## Introduction

From a health point of view, it has been proven that several milk fat groups enhance health benefits either through the synthesis of fatty acids in tissues or the induction of cell pathways. Even though the consuming of short and medium-chain fatty acids offer several health improvements, but many proofs recommended that the polyunsaturated fatty acids (PUFAs) are the essential bioactive lipids as well as conjugated linoleic acid [1, 2]. A large number of various fatty acids had been composed the triglycerides of milk fat. This commands to a heterogeneous composition of triglycerides and a higher melting range, which alters between approximately 40 & 35°C [3, 4]. Butter oil or anhydrous milk fat had been utilized as a source of concentrated-fat for cooking and kitchen necessity. Also, some dairy products such as processed cheese and ice cream were used as an ingredient. Its deficiency of functionality and unsteady of physicochemical properties are considered as a restriction in using it in food manufacturing even though anhydrous milk fat has excellent properties. This is due to the properties of butter oil mainly affected the physical properties of the final dairy product. One of the most important techniques is the modification of milk fat to develop its nutritional and functional properties for extending its use in the food industry through its fractionation [5]. In this regard, chemical methods such as hydrogenation or enzymatic inter-esterification have been applied in modifying milk fat [6, 7]. These established techniques were accepted according to food policies, but they are not common due to the consumer order for edible fats free from any chemical treatments. Physical modification of milk fat can be classified into multiple processes

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that convert the chemical composition of the fat. The former contains fractionation techniques and blending with vegetable oils [8, 9], while the ultimate encompass texturization and cream tempering treatments [10].

The distribution of fatty acids has been affected by the different methods of fractionation on each part, and consequently the effect on their nutritional value [5]. Milk fat can be fractionated by supercritical carbon dioxide extraction [11-14], molecular distillation [15] and by crystallization from the melted fat with or without solvents, [3, 7, 16, 17].

Dry fractionation is one of the methods transacts with the physical technique; comprises two major steps, crystallization and separation. The melting point of triglycerides in milk fats is characterized by a wide range of temperatures from -40 ° C to 40 ° C; so it lets the crystallization out of a series of triglycerides at temperatures below their melting points. When crystallized, the resultant slurry is filtered by vacuum filtration [18, 19] or pressure filtration [20] and filters centrifugation [21]. The advantages of dry fractionation are the absence of organic solvents or process aids, as well as the reasonable cost of treatment and relatively simple equipment, and maintaining the desired flavor of milk fat.

Generally, crystallization from melting is a transition phase from a liquid to a solid state and it can be split up into two steps: nucleation and growth. Before nuclei formation, the mother phase must be supersaturated to provide a thermodynamic force for the crystallization [22]. The super-saturation, which can be achieved by a progressive lowering of the temperature, depends on numerous factors such as specific and latent heat, viscosity, inter-solubility and polymorphism [5]. Once the nuclei have been created, they grow and develop into crystals, which need efficient control to secure suitable heat transfer through the mass. As a matter of fact, both nucleation and growth ordinary happen together while the system is in continuous evolution. Additional alternation in crystals can take place as stable crystals modify their habit and metastable crystals undergo polymorphic transitions [23, 24].

Milk fat fractions had been incorporated in several types of food products. The high melting fractions generally use as shortening in puff pastry, fat bloom inhibitors in chocolate, hard stock for ghee production, cocoa butter replacement , edible films and frozen desserts [25-30]. Other promising applications for low melting fractions include biscuits, short breads, cold spreadable butter, pourable frying oils and improving the re-contestability of milk powder [31, 32].

In fact, numerous researches had been carried out with cow-milk-fat fractionation; however there are few studies that have been announced about buffalo milk fat [13, 14, 33, 34]. Thence, the present study was undertaken to seek the impact of the dry fractionation on the distribution of bioactive lipids in buffalo butter-oil and its various fractions.

## Materials and Methods

### Study area:

Study was carried out in the period of 2016 to 2018 in Dairy Department, National Research Centre, Egypt; and Chemistry Department, Wroclaw University of Environmental & Life Sciences, Poland

### Milk source

Thirty-two samples of buffalo raw milk (the main source of milk in Egypt) were obtained from the Dairy department, Faculty of Agriculture, Cairo University in winter season (November to January). Samples were divided into 4 composite samples. Every group mixed well and kept under freezing conditions till analysis.

### Preparation of butter oil

Butter oil was prepared according to the method mentioned by Amer et al. [18]. Every milk composed sample was skimmed and the obtained cream was churned. The resulted butter was melted at 60°C. Centrifugation on the melted butter was conducted at 4000 rpm for 5 minutes (Sigma 301model, Max speed 6000 rpm and timing range from 0 to 60 minutes), removing the top oil layer, filtering through glass wool and drying the resulting oil over anhydrous sodium sulphate. The oil then re-filtered (under vacuum, Whatman 41 paper) to obtain clear oil (~99.5% fat).

### Fractionation of butter oil

Fractionation of butter oil was prepared by the method according to Van Aken et al. [3]. The obtained butter oil was heated to 70°C for 10 min and then cooled slowly to 35°C using a circulating water bath. The solid fraction ($S_{35}$) was separated from liquid fraction ($L_{35}$) by centrifugation at 5000 rpm for 5 minutes (Refrigerated Sigma 2-16K Centrifuge model,

Power supply 230/50 or 120/60 V/Hz, Maximum rotational speed 15.300 rpm, Maximum capacity 4x100 ml and temperature control up to 60°C). A similar process was used for fractionation of both S35 and $L_{35}$ at 25 and 15ºC respectively, resulting 4 fractions; the solid fraction ($S_{35}$, $S_{25}$ and $S_{15}$) and the liquid fraction ($L_{15}$), respectively (Fig. 1).

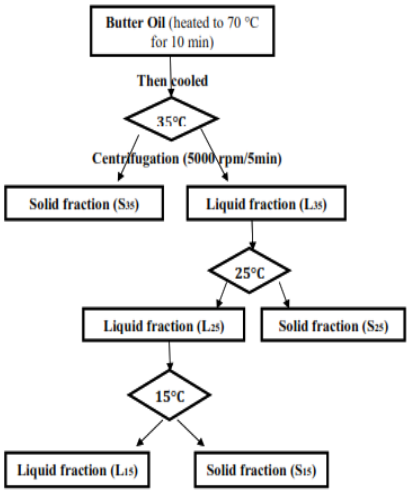


Fig.(1): Schematic diagram of dry fractionation procedure.

**Methods**

1-Determination of total conjugated diene and triene contents:

Conjugated diene acids (CDA) and triene (CTA) were determined according to the modified version of [34] and were calculated using the following equations:

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

CTA (%) = (0.84 x B) / (bc-K0)

Where:

A is the absorbance at 233 nm.

B is the absorbance at 280 nm.

b is the length of cell (cm)

c is gram / liter.

K0 is a constant. (0.07).

2-Extraction of lipids:

The total lipids and phospholipids were extracted according to the methods reported recently by Abd-El-Hamid *et al*. [4].

**3-Determination of fatty acid profile:**

Fatty acids methyl esters were prepared according to the method of Wirasnita et al. [35] and fatty acid profile was assessed using gas chromatography coupled with a mass spectrometer (Shimadzu GC-MS QP 2120, Shimadzu, Kyoto, Japan).

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 Abbas *et al*. [4].

**Statistical Analysis:**

Statistical analysis for obtained data was carried out using analysis of variance (ANOVA) and Duncan tests with the Statistical Analysis System [36]. A probability of $P < 0.05$ was used to establish the statistical significance.

## Results and Discussion

**Conjugated diene (CDA) and triene (CTA) fatty acids**

**Table 1** is conducted conjugated diene and triene fatty acids contents of buffalo butter- oil and its fractions. Significant differences had been detected in their contents between butter-oil and its fractions except solid fractions ($S_{25}$ and $S_{15}$). Results revealed that liquid fraction ($L_{15}$) had gained significantly ($P \leq 0.05$) higher content of conjugated diene and triene than butter-oil and its fractions. The average values of CDA and CTA were 0.9370 and 0.1840% for $L_{15}$, whilst they were 0.8330 and 0.1662% for $S_{15}$, respectively. Values of butter-oil and solid fractions ($S_{25}$ and $S_{35}$) were 0.8680 and 0.1495 % for butter-oil. They were 0.8250 and 0.1670 % for $S_{25}$ as well 0.5770 and 0.1555 % for $S_{35}$, in the same order. These data were come in the same line of as previously reported by researchers [37].

Table 1: Total conjugated diene (CDA) and triene (CTA) fatty acids contents (as % of total fatty acids) in buffalo butter-oil and its fractions

| Samples | CDA (%) | CTA (%) |
|---|---|---|
| Butter oil | 0.8680b ±0.001 | 0.1495d ±0.007 |
| $S_{35}$ | 0.5770g ±0.004 | 0.1555cd ±0.001 |
| $S_{25}$ | 0.8250c ±0.008 | 0.1670c ±0.007 |
| $S_{15}$ | 0.8330c ±0.005 | 0.1662c ±0.007 |
| $L_{15}$ | 0.9370a ±0.007 | 0.1840b ±0.004 |

$S_{35}$= Solid fraction at 35°C; $S_{25}$= solid fraction at 25°C; $S_{15}$= solid fraction at 15°C; $L_{15}$= liquid fraction at 15°C. Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$) . a,b,c ….means significant between rows.

**Butyric acid and short chain fatty acids (SCFAs)**

Contents of butyric acid and short chain fatty acids (SCFAs) of buffalo butter-oil and its fractions were stated at Table 2. All SCFAs contents between butter-

oil and its fractions had significant differences. Buffalo butter-oil and the solid fraction ($S_{25}$) gained higher butyric acid content (1.11 and 1.12%). They were 0.93, 0.84 and 1.06% of total FAs for solid fraction ($S_{35}$), solid fraction ($S_{15}$) or liquid fraction ($L_{15}$), respectively. While Caproic, caprilyic and capric acids were significantly ($P \leq 0.05$) lower in butter-oil and $S_{35}$ as compared to $S_{25}$, $S_{15}$ and $L_{15}$. For butter oil values were 0.32, 0.31 and 2.11%, and they were 0.57, 0.32 and 1.82% of total FAs for $S_{35}$ for solid fractions ($S_{25}$, $S_{15}$) and liquid fraction ($L_{15}$) samples, the corresponding values were 0.77, 0.91 and 2.31% for $S_{25}$ while they were 0.28, 0.58 and 2.22% for $S_{15}$ as well as 0.70, 0.88 and 2.37% of total FAs for $L_{15}$, respectively. The current observations were compatible with those scientists [13, 14] who used supercritical carbon dioxide in fractionation. Solvent (acetone) fractionation and dry fractionation techniques had been employed to fractionate milk fat. They elucidated that butyric acid was the most principle one in milk fat short chain fatty acids [3].

**Table 2: Butyric and short chain fatty acids contents in buffalo butter-oil and its fractions**

| | Samples | | | | |
|---|---|---|---|---|---|
| **Fatty acids (%)** | **Butter oil** | **$S_{35}$** | **$S_{25}$** | **$S_{15}$** | **$L_{15}$** |
| **C4:0 (Butyric)** | $1.11^{c} \pm 0.01$ | $0.93^{e} \pm 0.04$ | $1.12^{c} \pm 0.02$ | $0.84^{f} \pm 0.02$ | $1.06^{d} \pm 0.02$ |
| **C6:0 (Caproic)** | $0.32^{gf} \pm 0.02$ | $0.57^{d} \pm 0.01$ | $0.77^{b} \pm 0.02$ | $0.28^{g} \pm 0.02$ | $0.70^{c} \pm 0.02$ |
| **C8:0 (Caprilyic)** | $0.31^{g} \pm 0.01$ | $0.32^{g} \pm 0.03$ | $0.91^{a} \pm 0.02$ | $0.58^{d} \pm 0.01$ | $0.88^{a} \pm 0.02$ |
| **C10:0 (Capric)** | $2.11^{d} \pm 0.02$ | $1.82^{f} \pm 0.03$ | $2.31^{b} \pm 0.01$ | $2.22^{c} \pm 0.07$ | $2.37^{a} \pm 0.02$ |

$S_{35}$= Solid fraction at 35°C; $S_{25}$= solid fraction at 25°C; $S_{15}$= solid fraction at 15°C; $L_{15}$= liquid fraction at 15°C. Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$). . a,b,c ….means significant between rows.

**Conjugated linoleic acid (CLA) and its isomers**

As shown in Table 3 that CLA (cis-9, trans-11) ratios of L15 were higher (0.91%) than butter-oil, S35, S25 and S15 (0.54, 0.43, 0.80 and 0.84% of total FAs), respectively. It was also obvious that there were no significant ($P > 0.05$) differences in cis-10, cis-12 contents between buffalo butter-oil, S35 and L15 (0.60, 0.67 and 0.63%), while a significant difference among S25 and S15 (0.54 and 0.47% of total FAs) was stated, in order. Regarding to buffalo butter-oil had significantly ($P \leq 0.05$) elevated value in trans-10 cis-12 (0.72%) as compared to S35, S25, S15 and L15 (0.11, 0.70 0.19 and 0.18% of total FAs), in the same order. As for trans-9 trans-11 of solid fractions (S25 and S15) and liquid fraction (L15); they contents were significantly ($P \leq 0.05$) higher (0.34, 0.26 and 0.21%) when differentiated with buffalo butter-oil and S35 (0.07 and 0.03% of total FAs), respectively. As mentioned in previous paper [4] that CLA (cis-9, trans-11) contents in butter were significantly higher than its content in butter milk. While it was no significant difference between cream and butter and their by- products in trans-10, cis-12 and trans-9, trans-11 contents. Recently, Pena-Serna and Restrebd-Benancur [37] mentioned that the content of buffalo ghee was 0.77±0.004 while cow ghee was 1.00±0.003.

**Odd and branched chain fatty acids (OBCFAs)**

Contents of odd and branched chain fatty acids (OBCFAs) of buffalo butter-oil and its fractions were given in Table 4. Visibly, solid fractions ($S_{25}$ and $S_{15}$) and liquid fraction ($L_{15}$) contained higher contents of total OBCFAs than butter-oil and $S_{35}$. Average values were 14.72, 10.66 and 10.41% of total FAs for $S_{25}$, S15 and $L_{15}$, when they were 3.48 and 3.77% of total FAs for butter-oil and $S_{35}$, in the same order. In buffalo butter oil the most paramount ratios for C15:0 and C17:0 (1.04 and 0.65%), as for solid fraction (S35) were C17:0 and C14:0 iso (1.04 and 0.65% of total FAs), respectively. In opposite direction, cis9-C15:1 was the principle content in solid fraction ($S_{25}$) then C15:0, C18:0 anteiso and C17:0 (2.22, 2.16, 2.15 and 1.95%). With regard to solid fraction ($S_{15}$) and liquid fraction ($L_{15}$), it was found that C15:0, C17:0, C14:0 anteiso and C17:0 anteiso were the major values which were 2.34, 2.09, 1.14 and 0.99 % for S15 and 2.26, 2.01, 1.15 and 0.98% of total FAs for L15, in the same order. Data of cow butter-oil for total branched chain fatty acids were 1.73% of total FAs. Also, they pointed that the contented values of C14:0 iso, C15:0 iso, C15:0 anteiso, C16:0 iso and C17:0 anteiso were 0.22, 0.11, 0.54, 0.37 and 0.49% of total FAs, respectively [38].

**Table 3: Conjugated linoleic acid and its isomers contents (as % of total fatty acids) in buffalo butter-oil and its fractions**

| Fatty acids (%) | Samples | | | | |
|---|---|---|---|---|---|
| | Butter oil | $S_{35}$ | $S_{25}$ | $S_{15}$ | $L_{15}$ |
| **Cis9 trans11-C18:2** | $0.54^{d} \pm 0.02$ | $0.43^{e} \pm 0.03$ | $0.80^{b} \pm 0.04$ | $0.84^{b} \pm 0.07$ | $0.91^{a} \pm 0.10$ |
| **Cis10 cis12-C18:2** | $0.60^{a} \pm 0.007$ | $0.67^{a} \pm 0.07$ | $0.54^{c} \pm 0.07$ | $0.47^{d} \pm 0.04$ | $0.63^{a} \pm 0.09$ |
| **Trans10 cis12-C18:2** | $0.72^{a} \pm 0.02$ | $0.11^{d} \pm 0.13$ | $0.70^{a} \pm 0.02$ | $0.19^{c} \pm 0.14$ | $0.18^{c} \pm 0.007$ |
| **Trans9 trans11-C18:2** | $0.07^{c} \pm 0.02$ | $0.03^{c} \pm 0.07$ | $0.34^{a} \pm 0.03$ | $0.26^{b} \pm 0.06$ | $0.21^{b} \pm 0.04$ |

$S_{35}$= Solid fraction at 35°C; $S_{25}$= solid fraction at 25°C; $S_{15}$= solid fraction at 15°C; $L_{15}$= liquid fraction at 15°C. Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$). a,b,c ….means significant between rows.

**Table 4: Odd and branched chain fatty acids contents (as % of total fatty acids) in buffalo butter-oil and its fractions.**

| Fatty acids (%) | Samples | | | | |
|---|---|---|---|---|---|
| | Butter oil | $S_{35}$ | $S_{25}$ | $S_{15}$ | $L_{15}$ |
| **C11:0** | $0.05^{b} \pm 0.001$ | $0.02^{b} \pm 0.01$ | $0.14^{b} \pm 0.02$ | $0.03^{b} \pm 0.007$ | $0.03^{b} \pm 0.007$ |
| **C12:0 iso** | $0.01^{d} \pm 0.001$ | $0.05^{abc} \pm 0.01$ | $0.04^{bcd} \pm 0.02$ | $0.03^{bcd} \pm 0.01$ | $0.03^{bcd} \pm 0.01$ |
| **C12:0 anteiso** | $0.01^{d} \pm 0.001$ | $0.02^{cd} \pm 0.01$ | $0.03^{abcd} \pm 0.0$ | $0.05^{a} \pm 0.007$ | $0.05^{ab} \pm 0.001$ |
| **C13:0** | $0.01^{d} \pm 0.001$ | $0.18^{a} \pm 0.02$ | $0.09^{bc} \pm 0.02$ | $0.10^{b} \pm 0.01$ | $0.11^{b} \pm 0.01$ |
| **C13:0 iso** | $0.03^{cd} \pm 0.001$ | $0.01^{d} \pm 0.007$ | $0.30^{cb} \pm 0.02$ | $0.33^{b} \pm 0.01$ | $0.32^{b} \pm 0.07$ |
| **C14:0 anteiso** | $0.41^{c} \pm 0.01$ | $0.03^{d} \pm 0.007$ | $1.06^{ab} \pm 0.07$ | $1.14^{a} \pm 0.07$ | $1.15^{a} \pm 0.02$ |
| **C14:0 iso** | $0.17^{d} \pm 0.01$ | $0.65^{a} \pm 0.02$ | $0.56^{a} \pm 0.03$ | $0.61^{a} \pm 0.04$ | $0.61^{a} \pm 0.01$ |
| **C15:0** | $1.04^{e} \pm 0.02$ | $0.02^{f} \pm 0.001$ | $2.16^{ab} \pm 0.05$ | $2.34^{a} \pm 0.14$ | $2.26^{ab} \pm 0.04$ |
| **Cis9-C15:1** | $0.01^{b} \pm 0.001$ | $0.08^{b} \pm 0.007$ | $2.22^{a} \pm 0.25$ | $0.03^{b} \pm 0.007$ | $0.02^{b} \pm 0.007$ |
| **Cis11-C15:1** | $0.03^{f} \pm 0.001$ | $0.34^{a} \pm 0.02$ | $0.12^{c} \pm 0.007$ | $0.14^{b} \pm 0.007$ | $0.14^{b} \pm 0.007$ |
| **C18:0 iso** | $0.05^{d} \pm 0.001$ | $0.09^{bc} \pm 0.001$ | $0.20^{a} \pm 0.01$ | $0.19^{a} \pm 0.01$ | $0.19^{a} \pm 0.002$ |
| **C18:0 anteiso** | $0.08^{f} \pm 0.001$ | $0.16^{e} \pm 0.02$ | $2.15^{a} \pm 0.02$ | $0.37^{b} \pm 0.04$ | $0.34^{b} \pm 0.01$ |
| **C16:0 iso** | $0.16^{c} \pm 0.02$ | $0.16^{c} \pm 0.04$ | $0.61^{a} \pm 0.02$ | $0.66^{a} \pm 0.05$ | $0.65^{a} \pm 0.02$ |
| **C17:0** | $0.65^{e} \pm 0.02$ | $1.04^{d} \pm 0.07$ | $1.95^{ab} \pm 0.03$ | $2.09^{a} \pm 0.19$ | $2.01^{a} \pm 0.06$ |
| **Cis9-C17:1** | $0.13^{f} \pm 0.01$ | $0.33^{def} \pm 0.02$ | $1.85^{a} \pm 0.35$ | $0.64^{cd} \pm 0.01$ | $0.63^{cd} \pm 0.01$ |
| **C17:0 iso** | $0.28^{e} \pm 0.007$ | $0.54^{cd} \pm 0.03$ | $0.85^{ab} \pm 0.11$ | $0.92^{a} \pm 0.08$ | $0.89^{ab} \pm 0.02$ |
| **C17:0 anteiso** | $0.36^{d} \pm 0.007$ | $0.04^{e} \pm 0.001$ | $0.95^{a} \pm 0.02$ | $0.99^{a} \pm 0.07$ | $0.98^{a} \pm 0.01$ |
| **Total** | $3.48^{e} \pm 0.02$ | $3.77^{d} \pm 0.04$ | $14.72^{a} \pm 0.14$ | $10.66^{b} \pm 0.17$ | $10.41^{c} \pm 0.11$ |

$S_{35}$= Solid fraction at 35°C; $S_{25}$= solid fraction at 25°C; $S_{15}$= solid fraction at 15°C; $L_{15}$= liquid fraction at 15°C. Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$). a,b,c ….means significant between rows.

### *Trans* fatty acids (TFAs)

On the other way it had been expounded the contents of *Trans* fatty acids of buffalo butter-oil and its fractions in **Table 5.** It was apparent that trans9-C16:1 content had significant ($P \leq 0.05$) value (0.42%) in solid fraction ($S_{35}$) compared to butter-oil, $S_{25}$, $S_{15}$ and $L_{15}$ (0.06, 0.20, 0.21 and 0.23% of total FAs), in order. Nevertheless, trans9-C18:1 gained lower content in all fractions versus to butter-oil. Their values were 4.80, 4.84, 4.85 and 4.85% for $S_{35}$, $S_{25}$, $S_{15}$

and $L_{15}$ against 5.88% of total FAs for butter-oil, respectively. Further, fractions of solid and liquid at 15°C ($S_{15}$ and $L_{15}$) had higher content of trans11-C18:1 (1.57 and 1.63%) than all of butter-oil, $S_{35}$ and $S_{25}$ (1.48, 1.38 and 1.46%), in order. On other side; trans11-C20:1 content was significantly ($P \leq 0.05$) high in $L_{15}$ (0.16%) when differentiated to butter-oil, $S_{35}$, $S_{25}$ and $S_{15}$ (0.02, 0.05, 0.09 and 0.04% of total FAs), in the same order. Same line was found when compared between trans9-C18:1 and trans11-C18:1 contents were 1.73 and 9.65 mg/g fat in soybean oil, while they were 1.92 and 13.12 mg/g fat in low CLA ghee, as well as 1.96 and 16.63 mg/g fat in high CLA ghee, respectively [39].

**Table 5: Trans fatty acids contents (as % of total fatty acids) in buffalo butter-oil and its fractions**

| | Samples | | | | |
|---|---|---|---|---|---|
| | **Butter oil** | **$S_{35}$** | **$S_{25}$** | **$S_{15}$** | **$L_{15}$** |
| **Trans 9-C16:1** | $0.06^{e}$ ±0.001 | $0.42^{a}$ ±0.04 | $0.20^{bc}$ ±0.03 | $0.21^{bc}$ ±0.02 | $0.23^{b}$ ±0.02 |
| **Trans 9-C18:1** | $5.88^{a}$ ±0.07 | $4.80^{abc}$ ±0.04 | $4.84^{abc}$ ±0.57 | $4.85^{abc}$ ±0.14 | $4.85^{abc}$ ±0.34 |
| **Trans 11-C18:1** | $1.48^{bc}$ ±0.10 | $1.38^{cde}$ ±0.02 | $1.46^{bc}$ ±0.06 | $1.57^{ab}$ ±0.02 | $1.63^{a}$ ±0.04 |
| **Trans 11-C20:1** | $0.02^{b}$ ±0.001 | $0.05^{b}$ ±0.001 | $0.09^{ab}$±0.02 | $0.04^{b}$ ±0.007 | $0.16^{a}$ ±0.07 |

$S_{35}$= Solid fraction at 35°C; $S_{25}$= solid fraction at 25°C; $S_{15}$= solid fraction at 15°C; $L_{15}$= liquid fraction at 15°C. Results are presented as means ± standard deviation (SD). Means with the same letter are not significantly different ($P \leq 0.05$). a,b,c ….means significant between rows.

## CONCLUSION

Egyptian buffalo milk is a rich source of bioactive lipids, which considered as good compounds for human health. The differences between the bioactive lipids contents of buffalo butter oil fractions are due to the technological steps (Dry fractionation). Results of the present research indicated that dry fractionation of butter-oil revealed that liquid fraction at 15°C ($L_{15}$) had higher content of CLA as well conjugated diene and triene fatty acids, while solid fraction at 25°C ($S_{25}$) concluded higher ratio of total OBCFAs as compared to all other fractions. Further studies are required to investigate the influence of some bioactive lipid classes on biological activities of human body to employ these characteristics for treating specific patient's conditions. More information about these bioactive lipids advantages must be achieved in multiple researches in near future.

## Conflicts of interest

The authors whose names are listed immediately below certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

## Significant statement

This study confirmed that the technological step (Dry Fractionation) had a clear effective influence on the distribution of all bioactive lipids in buffalo butter oil fractions. The study is concerning with effect of the dry fractionation as a technological step on the distribution of bioactive lipids in buffalo butter- oil and its fractions. The importance of bioactive lipids as healthy natural ingredient must be taken a great consideration in future. Also, it is important to pay attention to the fat of other types of milk such as goats, camels, and sheep.

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