Egypt. J. Chem. **Vol. 65,** No. SI13B pp. 1389 - 1408 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

MILK BIOACTIVE LIPIDS AS POTENTIAL HEALTHY FRACTIONS: A REVIEW

Hayam Mohamed Abbas [1*]; Jihan Mohamed Kassem [1]; Mohamed Salama [1] and Laila Badawy Abd Elhamid [2]

[1] *Dairy Science Department, Food Industries and Nutrition Research Institute, National Research Centre, 33th El- Bohouth St., Dokki, P.O. 12622, Giza, Egypt.*

[2] *Food Science Department, Faculty of Agriculture, Ain Shams University, Shobra El- Khiema, Egypt.*

Abstract

Fat represents a great component in milk and its products. It is the source of flavor and smooth body of the dairy product. As it well known; fat consists of fatty acids with different structure either short or long chain; saturated and unsaturated fatty acids. Milk fat has a healthy role as it contained bioactive lipids. Bioactive lipids had been defined from several points of view; as bioactive compounds, which are essential and non-essential compounds that occur in nature, they are part of the food chain, and can be shown to have an effect on human health. Besides, these lipids are involved in the regulation and maintenance of normal body, functions, and allowing cells to respond appropriately. The present review dealt with the fractions of bioactive lipids in milk as well as their definitions, their classification, the healthy benefits of each fraction and the factors affect their levels in milk and its products.

Key words: Bioactive lipids, CLA, odd and branched fatty acids, *trans* fatty acids, phospholipids, dairy products.

Introduction

Milk lipids are considered one of the most significant constituent of milk. Their role in the structure, stability, flavor and mouth feel of dairy products or as functional ingredients can't be denied. It is exclusive structure of great ratio of fatty acids of chain length lower than 12-carbon atom; which makes many of its features unique **[1]**.

The milk fat, which is presented as oil-in-water emulsion consists of approximately 97% triglycerides while other milk lipids are diacylglycerol (about 0.5-2%), cholesterol (less than 0.5%), phospholipids (about 1%) and free fatty acids (FFA) about 0.1%. In addition, there are trace amounts of ether lipids, hydrocarbons, fat-soluble-vitamins and flavor compounds **[2]**.

Fatty acids (FAs) of milk fat emerge from two sources: synthesis *de novo* in the mammary glands and the plasma lipids originating from the feed; they differ from each other in their structure. The fatty acids that are synthesized *de novo* are short and medium-chain length acids, from 4:0 to 14:0 and have also some 16:0, whilst the C18 fatty acids and some of 16:0 arise from the plasma lipids. *De novo* fatty acid synthesis accounts about 40% (w/w) of the total fatty acids in milk fat which largely influenced by animal genetics, while lipids of dietary origin gained the rest **[3,4]**.

On the same line, many researches and reviews detected that milk own a rare composition with short, medium chain, polyunsaturated, branched fatty acid and conjugated linoleic acid (CLA) that play principle part in regulating biological activities. This great awareness that milk lipid components accord biological activities besides health beneficial characteristics moreover supported research in the dairy industry for formulating the dairy products with the incorporation of bioactive lipid components **[2]**.

Various factors influenced fatty acid composition of milk fat, such as variation in species, breed, stage of lactation, and feed **[1]**. As well, milk quality depends on a number of factors, even within the same animal species and within the same breed; like animal feeding, rearing systems, and seasonal variability **[5]**. On the other hand, some components choline, ethanolamine, and polyunsaturated fatty acid (PUFA) have promising impacts on the gut

*Corresponding author e-mail: jihankassem997@gmail.com.; (Jihan Mohamed Kassem).
Receive Date: 21 April 2022, Revise Date: 27 May 2022, Accept Date: 05 June 2022
DOI: 10.21608/EJCHEM.2022.135048.5943

mucosa, an inhibitory effect on cholesterol absorption, and an influence on the gut micro-biome and immune function **[6]**.

Thus, this review is a trail to point out the bioactive lipids in milk and milk products. Their definition and therapeutic effects of these fractions and the factors that might be influenced on them are also indicated.

Definitions of Bioactive Lipids

Although the concept of 'bioactive lipids' broadly defined as changes in lipid levels that result in functional consequences has been decades in the making, it has only started to gain traction in the past 20 years, and promises to occupy Center-stage in cell biology research in the twenty-first century. This belated recognition has its roots in earlier preconceived ideas about exclusive roles for lipids in energy metabolism and in membrane structure, which have prevented wider thinking about functional lipids by non-lipidologists. These impediments primarily arise from the many inherent diffi-culties of working with lipids, their enzymes and their targets **[7]**.

Some authors defined the bioactive lipids from several points of view. Generally, **Biesalski et al. [8]** defined them as bioactive compounds, which are essential and non-essential compounds (e.g., vitamins or polyphenols) that occur in nature, they are part of the food chain, and can be shown to have an effect on human health. Also, these lipids are involved in the regulation and maintenance of normal body, functions, and allowing cells to respond appropriately **[9]**.

Classification of Bioactive Lipids in Milk

Bioactive lipids in milk include mono-glycerides, di-glycerides, triglycerides associated with beneficial fatty acids like short & medium chain, conjugated linoleic acid (CLA), and polyunsaturated fatty acids (PUFAs). The minor lipid components like phospholipids and ether lipids also carry biological and health promoting activities **[2]**.

1- Butyric acid, short chain fatty acids

Four carbon atoms, butyric acid (BA) is classified as a saturated short-chain Fatty acid. Its name comes from the Latin word (butyrum) for butter, the material from which BA was discovered. BA occurs in the form of esters in animal fats and plant oils. When butter becomes rancid, BA is released by hydrolysis from its triacylglycerol giving an unpleasant odor. In addition to originate from dietary fat, BA is also produced along with acetic acid and propionic acid in the human gut during the fermentation of dietary fiber by colonic bacteria **[10]**.

Acetic, propionic, and butyric acids are the predominant forms of volatile short chain fatty acids in the gastrointestinal tract. They are produced by fermentation of water-soluble fiber by anaerobic bacteria normally resident in the large bowel **[11]**. The short-chain fatty acids, C4:0 and C6:0, are regarded as present in the milk fat at high level if their proportions are expressed as molar percentages (approximately 10 and 4.5%, respectively). The short (C4:0 to C6:0) and medium-chain fatty acids (C8:0 to C12:0) are unique to milk fat providing flavor or off-flavor depending on their concentrations **[12]**.

2- Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a generic name of a group of positional and structural linoleic acid isomers which don't have methylene (CH2) group in-between the two double bonds. Isomers with double bond in the positions (8 & 10; 9 & 11; 10 & 12 and 11 & 13) every one of them may be in the cis and trans geometrical structures (total of 16 isomers) can be prepared theoretically by the isomerization of linoleic acid. There are four geometric CLA isomers: cis-trans; tans-cis; trans-trans and cis-cis **[13]**.

Song et al. [14] demonstrated that CLA which belongs to a family of geometric and positional isomers of linoleic acid; its double bonds are conjugated in the carbon structure. Recently, **Hur et al. [15]** mentioned that linoleic acid is the main precursor of CLA; thus, CLA can be synthesized via conversion of linoleic acid by ruminal bacteria (Propionibacterium) and lactic acid bacteria (Lactobacillus, Lactococcus, and Streptococcus) with bio-hydrogenation. Accordingly, it is possible to increase the CLA concentrations in milk through feeding animals on a polyunsaturated fatty acid-rich diet, especially linoleic acid.

Therefore, ruminant's milk is characterized and specific with more CLA than Non-ruminants milk. CLA in whole cow milk ranges from 4.5 to 5.5 mg/g of fat **[16]**. The content of CLA in milk and dairy products depends on ruminal production of CLA and trans-11-C18:1 (vaccenic acid) as well as the activity of stearoyl-coenzyme desaturase (SCD) in mammary tissues. Indeed, vaccenic acid

(VA) formed in the rumen can be desaturated by SCD to form rumenic acid (RA), which represents 65 to 97% of total CLA content **[16]**.

Other individual CLA isomers found in ruminant fat represented a very small portion of total CLA and are derived from rumen output. These isomers are found at a low concentration when present, generally they account about 0.5% of the total CLA in ruminant fat **[17]**.

On the other hand, dairy products are considered the master source of CLA **[18]**. Various dairy products which contain different CLA isomers include rumenic acid (C18:2, cis-9 trans-11) ranging from 6 to 16 mg/g of fat. In addition, **Collomb et al. [19]** mentioned that cis-9, trans-11 CLA contents were 51.5 and 53.7 mg/g of total FAs, respectively.

3- Odd and Branched- chain fatty acids

Milk fat consists of a diverse range of odd and branched-chain fatty acids (OBCFAs), with 56 specific isomers being reported, with chain lengths varying from 4 to 26 carbon atoms **[20]**. Odd and branched-chain fatty acids (iso and anteiso) in milk fat largely derive from rumen bacteria, which in turn show large differences in their OBCFAs profile **[21, 22]**.

Vlaeminck et al. [22] classified the major branch-chain fatty acids in milk fat to three main classes; even-chain iso acids, odd-chain iso acids, and odd-chain anteiso. The terms iso and anteiso designate the position of the branch chain in the fatty acid moiety. For iso-methyl fatty acids, the position of the branch chain is located at the penultimate carbon atom; whereas the branch point is lay on the carbon atom number two from the end in anteiso-methyl fatty acids.

The branched chain fatty acid is not routinely recorded in food due to their low concentrations (1–3%) of total lipid content **[23]**. Because their quantitation is sterious by more abundant straight chain saturated fatty acid (SFAs) and monounsaturated fatty acid (MUFAs) peaks in gas chromatography analyses **[24]**. Prior studies noticed that OBCFAs concentrations in the diet derive predominantly from dairy products with small amounts detected in fermented food **[25]**.

Devle et al. [26] mentioned that the contents of odd and branched chain fatty acids may vary in the range of 2.0–3.1% and 1.4–2.4%, respectively. The American diet delivers ~170 and 317 mg BCFAs/d consumed from dairy and beef products, in order. There are seven major OBCFAs in food products, which include iso-14:0, iso-15:0, anteiso-15:0, iso-16:0, iso-17:0, anteiso-17:0, and iso-18:0.

On the other front, **Dingess et al. [27]** concluded that total OBCFAs in milk differed by site, with the highest concentration in Cincinnati City (7.90) followed by Mexico City (6.10) and Shanghai (4.27 mg/100 ml of milk), respectively.

4- Phospholipids

In phospholipids, the sn-1 and sn-2 position of the glycerol backbone are esterified with fatty acids of varying length and degree of saturation. The remaining sn-3 position is esterified with phosphoric acid, which, in turn, is esterified with an alcohol. Depending on the structure of this alcohol, different types and ratios of phospholipids comprise, for example, phosphatidylcholine (PC) 35%, phosphatidylethanolamine (PE) 30%, sphingomyelin (SM) 25%, phosphatidylglycerol (PG), phosphatidylinositol (PI) 5%, or phosphatidylserine (PS) 3%. Depending on the structure of the polar head group and pH of the surrounding medium, PE and PC are zwitter ionic and have a neutral charge at pH 7, whereas PG, PI, and PS are negatively charged at this pH value **[28]**.

Glycerophospholipids and sphingolipids are two principle groups of phospholipids (PLs). Glycerophospholipids are consisting of glycerol, phosphoric acid, fatty acids and a hydroxy compound (e.g., choline, ethanolamine, serine, inositol) whilst, sphingolipids are compounds formed by a long chain base, sphingosine, fatty acids and sugars or phosphoric acid or alcohols **[29]**.

Cerebrosides belong to the simple glycosphingolipids which have a hexose on the C1 atom such as glucosyl and galactosyl ceramide **[30]**. While, gangliosides are complex glycosphingolipids, where the head groups consist of oligosaccharides linked to one or more sialic acids (N-acetyl neuraminic acids). Due to the large head group, the number of different gangliosides is correspondingly large and they are sub-divided by structure into GM, GD and GT **[31]**.

From another view, the milk fat globule membrane (MFGM) contains great attributions of sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine, and some phosphatidylserine, phosphatidylinositol, and glycosphingolipids **[6]**. It is tri-laminar, with a surface-active layer, mainly consisting of proteins, surrounding the intracellular neutral lipids. This inner part is covered by a bilayer

membrane deriving from the secretory cell apical plasma membrane. As it well known; phospholipids are mainly located in the milk fat globule membrane and the outer leaflet of membrane is rich in sphingomyelin and cholesterol **[32, 33, 34]**.

Gallier et al. [35] displayed that MFGM contains approximately 60–70% of total PLs in milk and phospholipids represent 0.5–1% of the total lipids in milk. The majority of milk lipids are sphingolipids and glycerophospholipids, which show polar properties, as they are amphiphilic molecules with hydrophobic fatty acyl chains and a hydrophilic organophosphate (choline, serine, ethanolamine or inositol) head group.

Ribar et al. [36] and **Lopez et al. [37]** mentioned that sphingomyelin is the most amount in milk sphingolipids followed by lactosylceramide (Lac Cer), glucosylceramide (Glu Cer), gangliosides and then less amounts of sphingoid bases. The content of sphingomyelin in milk fat varies between 0.65–1.27 mg/g of fat, with the concentrations in whole milk typically ranging between 26.4–119 mg/100g fat **[38]**.

Further, **Kosmerl et al. [39]** established that there was a meaningful attention recently for the bioactive activities of the MFGM-derived milk PL (MPL), notably sphingomyelin (SM), and numerous benefits to human health. These include their roles in neurodevelopment, gut health, cholesterol absorption, lipid metabolism, and inflammation, deepening the link between dietary lipids and human health.

5- Trans fatty acids

Generally, Trans fatty acids (TFAs) are unsaturated fatty acids with at least a double bond in trans configuration or geometry, i.e., the two hydrogen atoms of the carbons adjacent to the double bond point to opposite directions. The double bonds can be located anywhere along the molecule, so that many positional isomers may exist. The double-bond angle of the trans fatty acids is smaller than the cis isomeric configuration and the acyl chain is more linear, resulting in a more rigid molecule **[40]**.

In 2003; The U.S. Food and Drug Administration **[41]** defined TFAs as "all unsaturated fatty acids that contain one or more isolated double bonds in a trans configuration". It added that trans fatty acids occur naturally in dairy and other animal fats by biological hydrogenation in the rumen, but approximately 80% of dietary TFAs are derived from partially hydrogenated plant oils, with the remainder being supplied by ruminant products. **Goudjil et al. [42]** and **Lock et al. [43]** reported that the major trans isomers in the diet are the trans-C18:1 which found in hydrogenated vegetable oil and ruminant fats, while trans- C18:2 appear only in trace amounts. The most prevalent trans fatty acid in milk is trans11-C18:1.

Factors Affecting the Level and Type of Bioactive Lipids in Milk

There are some factors affecting the level of the bioactive lipids such as:

a- Dietary factors and seasonal variations

It had been observed that synthesis of short chain fatty acids is susceptible to inhibition when increasing dietary levels of certain long-chain fatty acids (LCFAs) in the form of vegetable oils **[13]**. Feeding cows with flaxseed reduced the concentrations of short and medium chain fatty acids and increased the long-chain fatty acids in milk fat **[44]**. **Shingfield et al. [45]** compared between dietary supplementation with fish and sunflower oils in the diet and control one, the results with supplementation diet had clear alterations in milk fatty acid profile. A significant reduction (from 4.29 to 3.01 g/100g of total FAs) in butyric acid content was observed in Holstein-British Friesian animal fed fish and sunflower oils.

Bilby et al. [46] reported that butyric acid content was 2.99, 2.27 and 2.21 g/100 g of total FAs in milk from Holstein cows fed a high-concentrate diet supplemented with fish (2.5%), linseed (5%), whole cottonseed (4.36 %) and sunflower oils (5%), in the same order.

Furthermore, **Glasser et al. [47]** presented that a significant decrease in C4:0 content in milk fat of cows fed linseeds in diet. The butyric acid content in milk fat was reached to 2.58, 2.2 and 2.9 g/100g fat for diet supplemented with linseeds, protected linseeds and linseed oil, respectively, as compared to control (3.3 g/100g fat). **Talpur et al. [48]** studied the seasonal variations in fatty acid composition of milk from ruminants during the four periods of the year. A significant effect of season was observed on the C4:0 content of cow as well as buffalo milk fat. Generally, the concentration of short chain fatty acids (<C14:0) in milk were higher in winter than summer.

However, **Larsen et al. [49]** displayed milk quality as affected by feeding regimens in a country with climatic variation. Milk from Central Sweden differed from milk from southern Sweden that it had a higher content of SCFAs (C4–C14). In addition, it was found that butyric acid content in winter and summer seasons was 4.3 and 4.7 g/100g of total FAs, in order. Where **Bharwade et al. [1]** found that butyric acid content in summer season was higher (2.46 g/100g of total FAs) than winter season (1.50 g/100g of total FAs) in Kankrej Cows, where the green fodder is in summer.

Moreover**, Abbas *et al.* [50]** mentioned that buffalo milk had higher contents of butyric acids in summer and winter season than goat milk whereas the last one had much higher amounts of the rest of short chain fatty acids than buffalo milk. This is attributed to the use of goat milk in blue-veined cheese production.

Concerning CLA, diet is the most significant factor that affecting on CLA content of milk fat, which can be naturally enhanced by fresh pasture feeding **[51]** or through the use of specific dietary formulations including seeds, vegetable oil or fish oil **[52]**. Mustard oil is one of the cheapest fat supplements being used traditionally in India for feeding of lactating animals. It contains about 45% linoleic and 9% linolenic acid content **[53]**. In that study, CLA content in milk was increased 12 fold after their feeding on mustard cake and mustard oil, respectively **[54]**.

Lock and Garnsworthy [55] compared between feeding diets rich with linolenic and linoleic acids and they observed an increase in CLA content of milk when feeding on the first acid. Also, **Kay *et al.* [56]** notified that high CLA content (1.34 and 3.28 g/100g of total FAs) on diet of pasture and supplementation with 150 g of fish oil, in order.

Tyagi *et al.* [57] showed that Indian traditional feeding practices of 13 buffaloes did not influence on CLA content in milk. However, high levels of CLA can be achieved by feeding on a green fodder based ration. It was observed that cows that fed on a high concentrate diet supplemented with linseed, flaxseed, soybean and rich in linoleic acid gain increasing contents of CLA and total conjugated C18:2 fatty acids than supplemented without oils **[58]**.

Dhiman *et al.* [59] reported that cows which feed grazing pasture; give 500% higher CLA content in milk fat (2.21% of total FAs) compared to cows fed a diet containing 50 % conserved forage (hay and silages) and 50% grain (0.38% of total FAs). As well, **Marín *et al.* [60]** clarified that cows feed grazing and low concentrate (1–2 kg/cow/day) give higher levels of CLA and omega-3 (n-3) fatty acids than cows that feed less grazing and greater concentrate (6–8 kg/cow/day).

On the other side, plenty of fresh pastures give the greatest content of CLA, while it decreases throughout the growing season. The reason for this observation remains unclear. The CLA content of buffalo and cow milk was substantially higher during winter season (Feb) when the animals received plenty of greens especially mustard, oat green and berseem. During winter season, the CLA content of buffalo milk was about 50% higher than cow milk. The milk of crossbred cow contained higher levels of CLA than the milk of indigenous Zebu cattle during winter season. The CLA content increased two fold in milk fat from cows grazing on summer pasture compared to total mixed ration (TMR) fed in winter. There was a decrease in CLA content in August followed by an increase in September. The decrease in August was associated with a shortage of grass at this time of year. More grass was available in September due to the use of secondary growths of grass following the ensiling of grass for winter feed **[61]**.

Similarly, **Precht and Molkentin [62]** had observed higher CLA content in European milk in comparison with Nordic countries (0.89 vs. 0.58% of total FAs). This variation can be explained by the different feeding system in winter and summer, as it is well known that pasture feeding (summer) increases the content of CLA in milk fat compared to high concentrate allocations in winter due to the high concentrations of n-3 fatty acids in summer grasses, which are a source of CLA.

Lock and Garnsworthy [61] found that CLA concentration of milk fat varied throughout the year, with the highest values being found in summer months (May, June and July). The CLA content was observed as high as twice in the summer (15 mg/g of fat) than in winter (7.7 mg/g of fat). Besides, **Ledoux *et al.* [63]** reported that summer season have the highest butter CLA contents (0.80 g/100g butter) when compared to winter (0.45 g/100g butter) and spring (0.58g/100g butter). These variations could be due to the animal diet. **Rego *et al.* [64]** mentioned that the CLA (*cis*-9, *trans*-11) content of Holstein cow in spring and winter season was 1.43 and 0.96 g/100g of total FAs, respectively.

Salama *et al.* [65] observed that the buffalo milk was higher than goat milk in conjugated diene and triene fatty acids during winter and summer season. Moreover, it is found that the contents of conjugated fatty acids were higher in winter season when compared to summer season for both types of milk, respectively. These differences attributed to the breed and feeding system that reflects mainly the seasonal variations and their effect on milk composition.

Abbas *et al.* [50] found that total contents of CLA were higher (0.62 and 1.0 %) in goat milk than buffalo milk (0.43 and 0.66 %) during summer and winter season, in the same order.

Increment the forage: concentrate (F: C) ratio in the diet resulted in a higher content of milk odd and branched chain fatty acids (OBCFAs). In addition, excess of *iso* C14:0 and *iso* C15:0 in total OBCFAs were higher than with *anteiso* C15:0, which decreased in some studies. These changes in milk concentrations of *iso*-fatty acids and *anteiso* C15:0 might reflect the differences in the rumen bacterial fermentation induced by variation in the dietary F: C ratio **[66]**.

Supplementation with linoleic acid (C18:2 n-6) and linolenic acid (C18:3 n-3) had small effects on even-chain *iso*- fatty acids. Addendum of fish oil or marine algae minimized these fatty acids to 60% versus the control diet. The odd-chain *iso*-fatty acids increased with an excess degree of unsaturation of the supplemented fatty acids. When fish oil or marine algae were supplemented to the diet, the boost of odd-chain *iso*-fatty acids was mainly due to *iso* C17:0. Compared with the control diet; *iso* C17:0 increased by 95% when fish oil and sunflower oil or marine algae were supplemented **[67, 68]**.

Bernard *et al.* [69] found that the supplementation with linseed oil in dairy goat diets decreased the contents of most *iso* fatty acids in milk fat with a concomitant increase in the proportion of total *trans* C18:1 and total CLA.

Dingess *et al.* [27] studied the influence of diet from different sites on total OBCFAs content in human milk, where the highest concentration was in Cincinnati followed by Mexico City and Shanghai (7.90, 6.10, and 4.27 mg/100 ml of milk), respectively.

On the other side, **Rego *et al.* [64]** studied the impact of seasonal variation on odd and branched chain fatty acid in Azorean dairy herds. They found that the odd- and branched-chain fatty acid were higher in spring, while odd-chain fatty acid (C15:0) were higher in winter.

Abbas *et al.* [50] demonstrated that the seasonal variation is a very important factor of the contents of OBCFAs, where the goat milk in winter contains higher content of OBCFAs than summer. On contrary, buffalo milk contained small amounts in winter when compared to summer season.

As the effective influence of feeding or seasonal variations on fatty acids profile; the minor components such as phospholipids; also have influenced by these factors. **Lopez *et al.* [70]** discovered that a PUFA rich diet produced fat globules with a Foods 2021, 10, 607 7 of 32 larger proportion of phospholipids compared to milk fat derived from a diet with a higher SFA content. This was accredited to the smaller fat globules produced by the PUFA rich diet, resulting in fat globules with a larger surface area/volume ratio, where phospholipids are more abundant due to their presence in the MFGM layer at the surface of the fat globule. The phospholipids derived from the PUFA diet in this study consisted of a larger proportion of long chain and very long chain SFA, reduced C16:0 and increased fractions of C18:0. Research from **Walker *et al.* [71]** recognized higher phospholipid content in milk derived during late lactation than during any other stage, and again, this was linked to a reduced MFG size. In late lactation, there were peak phospholipid contents despite variation between the individual animals sampled. While lactation progressed, a decline in phospholipid and cholesterol concentrations was observed, however, samples for this study were only collected until the 180^{th} day postpartum (mid lactation) **[72]**.

However, supplementation of the diet with 4% soybean oil had no significant effect on sphingomyelin concentrations in milk fat [control diet (1.1 mg/g of fat) vs. supplemented diet (1.3 mg/g of fat)] or in whole milk. Although milk fat composition was altered, the lack of changes in milk fat content and fat globule size likely resulted in the unaltered sphingomyelin concentrations in milk fat between the control cows and those with soybean oil supplemented diets **[71]**. **Graves *et al.* [73]** also mentioned that sphingomyelin

concentration in milk fat was greatest during the summer (1.1 mg/g of fat) and least during the winter (0.95 mg/g of fat); while the concentrations in the spring was intermediate (0.99 mg/g of fat). This confirmed by **Hofi *et al.* [74]** in the past decade that the seasonal variation in the phospholipid content of Egyptian buffalo's milk was higher in summer (29.60 mg/100ml of milk) than in winter (24.69 mg/100ml of milk).

Abbas *et al.* [50] indicated that total polar lipids of buffalo milk in summer and winter season (0.27 and 0.33 g/100g fat) were higher than goat milk (0.20 and 0.26 g/100g fat), respectively.

Trans fatty acids as all fatty acids; highly affect by feeding system and seasonal variation. In an early study had been done by **Chilliard *et al.* [3]** concluded that the supplementation of dairy rations with capsulated vegetable or fish oils increase *trans*11-C18:1 (vaccenic acid) content, where there is a linear relationship between milk CLA and vaccenic acid content.

Lock and Garnsworthy [55] reported that *trans*-C18:1 content was 3.28 g/100g fat when feeding cows on a diet rich in linoleic and linolenic acids, while it was 2.87 g/100g fat when feeding on grass silage (basal diet), respectively. Besides, **Loor *et al.* [17]** mentioned that total *trans*-C18:1 content were 5.0 g/100g of total FAs when feeding cows on a high concentrate diet whilst they were 12.1 g/100g of total FAs when feeding on a high concentrate diet with supplemented at 3% linseed oil, in the same order.

Cívico *et al.* [29] indicated that total *trans*-C18:1 content were 3.61 g/100g of total fatty acid methyl esters (FAME) when feeding goats on a basal diet, while they were 5.72 g/100g of total FAME when feeding on a basal diet supplemented with linseed oil, respectively.

On the other side, numerous studies have shown that *trans* fatty acids contents of milk varies significantly with seasons. **Ledoux *et al.* [63]** mentioned that summer season have the highest butter TFAs contents (2.87 g/100g butter) when compared to winter (1.96 g/100g butter) and spring (2.42 g/100g butter). These variations could be due to the feeding system. Also, **Rego *et al.* [64]** presented that *trans*11-C18:1 and *trans*11 *trans*15- C18:2 contents of Holstein cows in spring were 3.2 and 0.37 g/100g of total FAs, while in winter season were 2.2 and 0.22 g/100g of total FAs, in the same order. **Talpur *et al.* [48]** found that total TFAs contents of buffalo and goat milk samples in summer were 3.39 and 3.15 g/100g fat, while in winter were 2.17 and 2.52 g/100g fat, respectively.

b- Effect of breed and species

Kay *et al.* [75] clarified the fatty acid profile in Holstein and Brown Swiss cows and observed that higher C4:0 content in Brown Swiss (25.5 mg/g of fat) as compared to 23.6 mg/g of fat in Holstein. While, **Ferlay *et al.* [76]** reported that high amount of C4:0 in Montbeliarde cows (4.07 g/ 100g fat) as compared to Tarentaise cows (3.78 g/ 100g fat).

The butyric acid content in milk fat of Pakistani Water Buffalo Kundi was 3.72 and for Nili-Ravi breeds it was 4.20 g/100g fat, respectively **[77]**. Also, **Palladino *et al.* [78]** observed a non-significant difference was observed with 64.5, 60.4 and 61.7 mg BA/g of FAs in Holstein-Friesian, Jersey × Holstein and Jersey breed, in the same order.

On the same side, **Maurice-Van Eijndhoven *et al.* [79]** displayed influence breed on fatty acid profile of milk fat. They noticed that butyric acid content varied between 3.59 - 3.82 g/100g of total FAs for Dutch Friesian, Meuse-Rhine-Yssel, Groningen White Headed and Jersey.

Saroha *et al.* [80] studied the butyric acid content of Indian goat milk and other livestock and they found that short chain fatty acid was 13.51g/100g of total FAME and butyric acid represented 1.34g/100g of total FAME in Indian goat milk. In the same year, **Abbas *et al.* [81]** mentioned that butyric acid in goat milk varied between 2.3-3 g/100g fat, while in cow milk varied between 2.5-6.2 g/100g fat. While, **Teng *et al.* [30]** estimated the fatty acids composition of different milk types. They found that butyric acid content in goat milk was 0.93 g/100 g fat, while in buffalo milk was 1.62 g/100 g fat.

On the same trend, it is a realistic concept that animal breed and species mainly affected all milk composition especially fat content and its individual components. **Lowless *et al.* [82]** compared four breeds of cows i.e. Irish Holstein/ Friesian, Dutch Holstein/ Friesian, Montbeliardes, and Normandes that were grazing pasture. They reported that breed had a clear effect with Montbeliardes, averaging about 13 % greater CLA content in milk fat than the other three breeds.

While, **White *et al.* [83]** made a comparison study on Holstein and Jersey cows that were either fed a TMR confinement or grazing pasture: they found that Holstein cows

had slightly higher milk fat concentrations of CLA (18% greater overall). However, **Kelsey *et al.* [84]** reported that breed accounted for < 0.1% of total variation in CLA concentration in milk fat, when compared CLA content of Brown Swiss and Holstein cows (CLA content 4.1 vs. 4.4 mg /g of fat) fed identical TMR.

Nudda *et al.* [85] mentioned that the range of CLA content in goat milk was 6.4 to 7.9 mg /g of fat, while **Gonzalez *et al.* [86]** had noticed that low CLA proportions (0.48 g/100g fat) in river buffaloes from Argentina. Another trail was conducted by **Kumar [87]** who stated that Murrah buffalo milk contained the highest amount of CLA (15.54 mg/g of fat) as compared to Alpine goat (10.33 mg/g of fat) as well Saanen goat milk (9.32 mg/g of fat).

In India, the CLA content in Kundi was significantly higher (0.80 vs. 0.71g/100g fat) than Nili-Ravi buffaloes. Similar values (0.77 g/100g fat) of *cis*-9, *trans*-11 were represented for Murrah buffaloes fed on green fodder **[87]**.

Sheep and goat milk are richer in CLA than cow's milk. At the same time, the findings found that sheep milk has higher content of CLA than goat milk, the reason was the differences in mRNA of their mammary adipocytes **[88]**. As well, **Teng *et al.* [30]** estimated the fatty acids composition of different milk types and they found that CLA content (*cis*-9, *trans*-11) in goat milk was 0.72 g/100g fat, while in buffalo milk was 0.32 g/100g fat.

The odd and branched chain fatty acids, as all fatty acids; highly affect by breed and species. Little work was conducted with OBCFAs contents; **Devle *et al.* [26]** mentioned that minor amounts of OBCFAs were detected in the ruminant milk such as bovine, caprine, and ovine milk. They found that the values of odd-chain fatty acids (OCFAs) varied between 2.0-3.1% in milk fat from these species, whilst the concentrations of branched-chain fatty acids (BCFAs) were between 1.4–2.4% of total FAs.

Ma *et al.* [28] indicated that there were significant differences in milk OBCFAs composition of different species were observed. In cow, yak, buffalo and Jersey cattle milk, the highest composition of OBCFAs were *iso*-C15:0 and C15:0. In goat milk were C15:0 and *anteiso*-C17:0, while in horse and camel milk were *iso*-C15:0 and *anteiso*-C17:0, in the same order.

On the other hand, total OBCFAs in human milk differed by site, with the highest concentration in Cincinnati followed by Mexico City and Shanghai (7.90, 6.10, and 4.27 mg/100 ml of milk), respectively. The individual concentrations of *iso*-C14:0, *iso*-C16:0, *iso*-C18:0, *anteiso*- C15:0 and *anteiso*-C17:0 also differed between sites. Milk concentrations of *iso*-C14:0, *anteiso*-C15:0 and *iso*-C16:0 were associated with maternal intake of dairy **[27]**.

Cívico *et al.* [29] studied the range of total OBCFAs contents in goat milk fat. They were 2.4–3.0 g/100 g of total FAs whereas **Teng *et al.* [30]** estimated the fatty acids composition of different milk types and they found that OBCFAs content in goat milk was 1.12 g/100g fat, while in buffalo milk was 1.53 g/100g fat.

Regarding phospholipids, in an early study had been done by **Kuchroo and Narayanan [89]** data revealed that buffalo milk had higher PLs content (0.39 g/100g fat) than cow milk (0.36 g/100g fat).

Avalli and Contarini [90] showed that sphingomyelin content of cow milk fat was 0.74 mg/g of fat, and **Rombaut *et al.* [91]** found else contents of 1.27 mg/g of fat. The well-known fact that milk fat globule size (MFGS) is smaller for Holsteins than for Jerseys may be responsible for the greater sphingomyelin contents in Holstein milk fat.

At least part of the breed effect in milk fat globule size is related to milk fat yield; because fat globule size increases with greater milk fat production. The smaller size of the milk fat globule for Holsteins means a greater milk fat globule membrane surface area is present relative to the core lipid volume. Consequently, increase membrane surface, a great content of sphingomyelin is found in Holstein milk fat **[92]**.

Graves *et al.* [**73]** reported that Holstein milk fat contained greater contents of sphingomyelin (1.0 mg/g of fat) than Jersey milk fat (0.84 mg/g of fat). However, sphingomyelin content did not differ significantly in whole milk of Holstein and Jersey cows (0.0350 and 0.0349 mg/g of milk), respectively. While, **Garcia *et al.* [93]** analyzed human milk and found that a lower percentage of phosphatidylethanolamine and a higher percentage of sphingomyelin with respect to the milk of the other species.

Abbas *et al.* [50] found that phospholipids classes content of buffalo and

goat milk in winter season were higher than summer season.

Concerning *trans* fatty acids, animal breed and species mainly affected *trans* fatty acids contents. In an early study by **Alonso *et al.* [94]** who mentioned that total *trans*-C16:1 and *trans*-C18:1 content of goat milk samples were 0.16 and 2.12 g/100g of total FAME, in the same order. While, **Lock and Garnsworthy [55]** found that the contents of *trans*-C18:1 in Friesian cow milk fat varied between 2.87-3.28 g/100g of total FAME.

On the same line, **Loor *et al.* [17]** mentioned that total *trans*-C18:1 content of Holstein cow milk fat was 5.0 g/100g of total FAs when feeding on a high concentrate diet whilst it was 12.1 g/100g of total FAs when feeding on a high concentrate diet with supplemented oil. As determine by **Talpur *et al.* [77]** *trans*11-C18:1 content in milk fat of Pakistani Water Buffalo Kundi and Nili-Ravi breeds. Their contents were 1.7 and 2.7 g/100g of FAME, respectively.

Devle *et al.* [26] notified that TFAs content of cow milk fat was 0.69 g/100g of total FAs, while in goat milk fat was 0.87 g/100g of total FAs as well as in ewe milk fat was 1.0 g/100g of total FAs, in the same order. **Cívico *et al.* [29]** published the range of total *trans*-C18:1 contents of goats fed with different diets; they were 3.61–5.68 g/100 g of total FAME.

Teng *et al.* [30] estimated the fatty acids composition of different milk types. They found that total *trans*-C16:1 and *trans*-C18:1 content of goat milk was 2.36 g/100g fat, while in buffalo milk was 1.16 g/100g fat as well in cow milk was 1.71 g/100g fat.

Abbas *et al.* [50] mentioned that total *trans* fatty acids content of buffalo milk in summer and winter season (4.24 and 5.15 %) were higher than goat milk (4.14 and 5.5 %), respectively. Meanwhile, the same authors concluded that the species of animal as well as seasonal variations possess an important influence on the levels of bioactive lipids as shown in **Table 1**.

Table 1. Bioactive lipids contents (as % of total fatty acids) of buffalo and goat milk samples during summer and winter seasons **[50]**.

Fatty Acids	Summer		Winter	
	Buffalo	Goat	Buffalo	Goat
C4:0	$2.54^{b}\pm0.12$	$2.04^{d}\pm0.04$	$2.97^{a}\pm0.13$	$2.26^{c}\pm0.07$
C6:0	$0.50^{d}\pm0.04$	$1.66^{b}\pm0.02$	$0.77^{c}\pm0.10$	$1.96^{a}\pm0.06$
C8:0	$0.66^{c}\pm0.07$	$1.94^{b}\pm0.01$	$0.70^{c}\pm0.06$	$2.23^{a}\pm0.16$
C10:0	$1.55^{c}\pm0.06$	$6.30^{b}\pm0.01$	$1.70^{c}\pm0.11$	$7.68^{a}\pm0.43$
Cis9 trans11-C18:2	$0.29^{b}\pm0.04$	$0.33^{b}\pm0.07$	$0.43^{a}\pm0.07$	$0.51^{a}\pm0.03$
Cis10 cis12-C18:2	$0.11^{b}\pm0.02$	$0.15^{b}\pm0.01$	$0.16^{a}\pm0.09$	$0.17^{a}\pm0.02$
Trans10 cis12-C18:2	$0.01^{b}\pm0.02$	$0.08^{b}\pm0.07$	$0.04^{b}\pm0.03$	$0.20^{a}\pm0.09$
Trans9 trans11-C18:2	$0.02^{b}\pm0.01$	$0.06^{b}\pm0.03$	$0.03^{b}\pm0.01$	$0.12^{a}\pm0.06$
C11:0	$0.03^{ab}\pm0.01$	$0.05^{a}\pm0.03$	$0.02^{b}\pm0.01$	$0.04^{ab}\pm0.01$
C12:0 *iso*	$0.04^{a}\pm0.008$	$0.03^{a}\pm0.005$	$0.08^{b}\pm0.02$	$0.02^{a}\pm0.07$
C12:0 *anteiso*	$0.06^{b}\pm0.008$	$0.02^{a}\pm0.005$	$0.03^{a}\pm0.008$	$0.05^{ab}\pm0.02$
C13:0	$0.13^{a}\pm0.005$	$0.09^{b}\pm0.03$	$0.07^{c}\pm0.009$	$0.12^{ab}\pm0.01$
C13:0 *iso*	$0.40^{a}\pm0.03$	$0.18^{c}\pm0.01$	$0.23^{cb}\pm0.009$	$0.30^{b}\pm0.05$
C14:0 *anteiso*	$1.30^{a}\pm0.06$	$0.80^{c}\pm0.07$	$0.84^{c}\pm0.08$	$1.14^{b}\pm0.03$
C14:0 *iso*	$0.72^{a}\pm0.04$	$0.54^{c}\pm0.001$	$0.41^{c}\pm0.03$	$0.60^{b}\pm0.05$
C15:0	$2.24^{a}\pm0.02$	$1.25^{c}\pm0.48$	$1.84^{b}\pm0.20$	$2.02^{ab}\pm0.05$
Cis9-C15:1	$0.03^{a}\pm0.01$	$0.04^{a}\pm0.01$	$0.03^{a}\pm0.01$	$0.04^{a}\pm0.01$
Cis11-C15:1	$0.03^{b}\pm0.01$	$0.16^{a}\pm0.003$	$0.06^{b}\pm0.02$	$0.14^{a}\pm0.02$
C18:0 *iso*	$0.16^{a}\pm0.01$	$0.14^{b}\pm0.01$	$0.08^{c}\pm0.01$	$0.13^{b}\pm0.02$
C18:0 *anteiso*	$0.56^{a}\pm0.03$	$0.51^{a}\pm0.02$	$0.23^{c}\pm0.03$	$0.33^{b}\pm0.04$
C16:0 *iso*	$0.78^{a}\pm0.05$	$0.48^{c}\pm0.07$	$0.41^{d}\pm0.03$	$0.64^{b}\pm0.04$
C17:0	$1.79^{a}\pm0.16$	$1.48^{b}\pm0.04$	$1.22^{c}\pm0.11$	$1.87^{a}\pm0.07$
Cis9-C17:1	$0.72^{a}\pm0.04$	$0.65^{ab}\pm0.02$	$0.50^{b}\pm0.15$	$0.60^{ab}\pm0.05$
C17:0 *iso*	$1.03^{b}\pm0.03$	$1.32^{a}\pm0.18$	$0.69^{c}\pm0.06$	$0.92^{b}\pm0.04$
C17:0 *anteiso*	$0.03^{c}\pm0.01$	$0.09^{b}\pm0.04$	$0.05^{cb}\pm0.008$	$1.04^{a}\pm0.05$
$Trans_{9}$-C16:1	$0.36^{b}\pm0.04$	$0.51^{a}\pm0.03$	$0.16^{d}\pm0.01$	$0.22^{c}\pm0.06$
$Trans_{9}$-C18:1	$3.04^{b}\pm0.79$	$2.06^{c}\pm0.07$	$3.83^{a}\pm0.24$	$3.08^{b}\pm0.24$
$Trans_{11}$-C18:1	$0.77^{d}\pm0.04$	$1.48^{c}\pm0.03$	$1.09^{b}\pm0.04$	$2.06^{a}\pm0.03$
$Trans_{11}$-C20:1	$0.07^{a}\pm0.04$	$0.09^{a}\pm0.04$	$0.07^{a}\pm0.01$	$0.14^{a}\pm0.05$

c- Effect of technological steps of dairy products:

The influence of manufacturing conditions or technological steps on the content of fatty acids or CLA and their distribution in milk and its products has been established by little studies.

Shantha *et al.* [95, 96] showed that an increase in processing temperature could slightly raise CLA concentration during the preparation of processed cheese. While the studies of **Luna *et al.* [97]** and **Van Nieuwenhove *et al.* [98]** support that heating at a high temperature does not increase CLA levels in milk fat.

In the dairy products, **Shantha *et al.* [96]** studied the influence of stagnate on the CLA content in butter and cream and they found no change in CLA content as well as yogurt and cheese such as Mozzarella, Gouda and Cheddar. **Gnädig *et al.* [99]** and **Nudda *et al.* [85]** observed no significant effect of cheese processing on the CLA content, including Blue, Edam, Swedish and Swiss cheese types as well as French Emmental and Ricotta cheese made from the corresponding sheep's milk.

Ryhänen *et al.* [100] mentioned that CLA content in milk had been increased by feeding cows a diet with 0.5 kg rapeseed oil per day, and then manufactured butter of this CLA enriched milk. During manufacture of the butter there were no changes in the concentrations of CLA in milk fat. The CLA content in butter was 0.9 to 1.1% of total FAs.

Bisig *et al.* [101] concluded that the CLA content was stable during butter-making out of CLA-enriched milk and no changes in the CLA content during manufacturing or ripening of cheese as well as effect of heating steps showed no changes in CLA content or isomer profiles, with the exception of microwaving, where CLA was decreased by up to 53%.

On the other side, **O'Shea *et al.* [102]** investigated the effect of dry fractionation of bovine milk fat on CLA content in the resulting fractions. They found that the CLA content had been increased from 1.36 to 2.22 g/100 g of total FAME represented 63.2% in the soft fraction compared with the parent fat. While, **Romero *et al.* [103]** found that the fractionation by using supercritical carbon dioxide increase the concentration of CLA by 89% from anhydrous milk fat (AMF).

Phospholipids mainly present in the MFGM, any treatment that produces a disruption of the membrane can affect the distribution and composition of PLs in the final product. Therefore, manufacturing processes and technological steps are the most important factors affecting the content of phospholipids and their distribution in milk and its products **[35]**.

Avalli and Contarini [90] estimated the PLs fractions in cow milk and its products. They found that PLs fractions of milk were 32.3, 9.3, 10.5, 27.3 and 20.5% of total PLs for PE, PI, PS, PC, and SM, respectively. For cream samples, the corresponding values were 42.7, 6.8, 7.2, 14.6 and 28.6% of total PLs, in the same order. Their values for butter samples were 31.0, 11.9, 15.3, 24.7 and 17.1% in sequence. The authors added that buttermilk samples contained 33.5, 2.4, 10.3, 35.5 and 18.3% of total PLs for PE, PI, PS, PC and SM, respectively. They concluded that buttermilk contained the highest amount of total PLs compared with cream or butter, where their values were 4.49 g/100g fat versus 0.54 for cream and 0.20 g/100g fat for butter, in order.

Rombaut *et al.* [104] and **Garczewska-Murzyn *et al.*, [105]** discussed the influence of milk processing such as de-creaming, pasteurization, and churning. They found that the polar lipids in skimmed milk represented more than half of the polar lipids originally present in the raw milk and the polar lipids content in skimmed milk were significantly higher than the polar lipids content of cream. Heat treatment of cream did not significantly affect the polar lipids content. The same authors found that during the churning, there was a preferential flow of polar lipids towards the buttermilk phase: 42.4 % of the cream polar lipids migrated to the buttermilk. Significantly, higher amounts of sphingolipids were found in butter compared with cream and buttermilk. They concluded that the polar lipids are mainly enriched in aqueous phases like skimmed milk, butter milk and butter serum.

Buttermilk and its content of MFGM contain up to 40 (w/w) of phospholipids, where 31% is PC, 30 % is PE, 20 % is SM, 7 % is PI, and 5 % is PS **[105]**. **Gallier *et al.* [35]** mentioned that the milk processing (churning, centrifuging, homogenization, spray drying) affected the profile of milk phospholipids, leading to a loss of sphingomyelin and phosphatidylcholine after centrifugation for cream separation.

On the same trend, **Abd El-Hamid *et al.* [106]** studied the distribution of bioactive lipids in buffalo fatty products as shown in **Table 2**. They reported that buffalo cream contained higher amounts of butyric acid than skim milk. Moreover, buttermilk had higher values of butyric than butter samples. On the other trend, the findings manifested that cream sample had higher values of conjugated diene and triene acids than skim milk, respectively. Meanwhile, butter contained higher amounts of total OBCFAs than buttermilk. Regarding phospholipids, it was found that cream samples contained higher contents of phospholipid classes such as PE, SM and PG than skim milk. On contrary, skim milk had higher values of PC, PS and PI than cream. Moreover, authors observed that buttermilk was higher in PC, PE, and SM as compared to butter samples.

Recently, **Abbas *et al.* [107]** used a new technique (Dry fractionation) to fractionate milk fat. Afterward, authors tracked the distribution of bioactive lipids in buffalo butter oil and its fractions prepared by the new technique as presented in **Table 3**. They found that liquid fraction at 15°C (L15) had a higher content of CLA as well conjugated diene and triene fatty acids, while solid fraction at 25°C (S25) contained higher content of total OBCFAs as compared to the rest fractions. Eventually, authors concluded that dry fractionation led to marked differences in the distribution of bioactive lipids in buffalo butter oil and its fractions. Moreover, each fraction could be used for specific purpose in the food industries according to its melting properties and this affect its healthy benefit.

Table 2. Bioactive lipids contents (as % of total fatty acids) of buffalo cream, butter and their by-products **[106]**.

Bioactive lipids	Products			
	Cream	Skim milk	Butter	Butter milk
C4:0	$1.27^{b}\pm0.04$	$1.07^{d}\pm0.02$	$0.98^{de}\pm0.02$	$1.03^{d}\pm0.01$
C6:0	$0.56^{d}\pm0.02$	$0.75^{cd}\pm0.01$	$0.49^{e}\pm0.02$	$0.34^{f}\pm0.01$
C8:0	$0.65^{c}\pm0.01$	$0.82^{b}\pm0.02$	$0.51^{e}\pm0.01$	$0.39^{f}\pm0.02$
C10:0	$1.89^{e}\pm0.02$	$2.22^{c}\pm0.02$	$2.31^{b}\pm0.01$	$1.70^{h}\pm0.02$
Cis9 trans11-C18:2	$0.54^{d}\pm0.12$	$0.26^{f}\pm0.21$	$0.74^{c}\pm0.02$	$0.17^{g}\pm0.16$
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	$0.07^{c}\pm0.02$	$0.07^{c}\pm0.01$	$0.08^{c}\pm0.01$	$0.06^{c}\pm0.01$
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*	$0.49^{a}\pm0.07$	$0.01^{e}\pm0.01$	$0.41^{b}\pm0.007$	$0.26^{cd}\pm0.12$
C15:0	$2.15^{ab}\pm0.26$	$1.83^{bccd}\pm0.18$	$1.97^{abc}\pm0.07$	$1.41^{ed}\pm0.51$
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	$0.09^{d}\pm0.007$	$0.08^{d}\pm0.01$	$0.05^{e}\pm0.007$	$0.03^{f}\pm0.001$
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*	$0.78^{b}\pm0.11$	$0.68^{bc}\pm0.07$	$0.72^{b}\pm0.12$	$0.55^{c}\pm0.14$
$Trans_{9}$-C16:1	$0.17^{bcd}\pm0.05$	$0.14^{cd}\pm0.01$	$0.15^{bcd}\pm0.007$	$0.10^{ed}\pm0.02$
$Trans_{9}$-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	$1.42^{c}\pm0.04$	$1.28^{de}\pm0.02$	$1.48^{bc}\pm0.05$	$1.27^{e}\pm0.02$
$Trans_{11}$-C20:1	$0.05^{b}\pm0.04$	$0.04^{b}\pm0.02$	$0.09^{ab}\pm0.02$	$0.08^{b}\pm0.01$

Table 3. Bioactive lipids contents (as % of total fatty acids) of buffalo butter-oil and its fractions **[107]**.

Bioactive lipids	Samples				
	Butter oil	S_{35}	S_{25}	S_{15}	L_{15}
C4:0	1.11^{c} ±0.01	0.93^{e} ±0.04	1.12^{c} ±0.02	0.84^{f} ±0.02	1.06^{d} ±0.02
C6:0	0.32^{gf} ±0.02	0.57^{d} ±0.01	0.77^{b} ±0.02	0.28^{g} ±0.02	0.70^{c} ±0.02
C8:0	0.31^{g} ±0.01	0.32^{g} ±0.03	0.91^{a} ±0.02	0.58^{d} ±0.01	0.88^{a} ±0.02
C10:0	2.11^{d} ±0.02	1.82^{f} ±0.03	2.31^{b} ±0.01	2.22^{c} ±0.07	2.37^{a} ±0.02
Cis9 trans11-C18:2	0.54^{d} ±0.02	0.43^{e} ±0.03	0.80^{b} ±0.04	0.84^{b} ±0.07	0.91^{a} ±0.10
Cis10 cis12-C18:2	0.60^{a} ±0.007	0.67^{a} ±0.07	0.54^{c} ±0.07	0.47^{d} ±0.04	0.63^{a} ±0.09
Trans10 cis12-C18:2	0.72^{a} ±0.02	0.11^{d} ±0.13	0.70^{a} ±0.02	0.19^{c} ±0.14	0.18^{c} ±0.007
Trans9 trans11-C18:2	0.07^{c} ±0.02	0.03^{c} ±0.07	0.34^{a} ±0.03	0.26^{b} ±0.06	0.21^{b} ±0.04
C11:0	0.05^{b} ±0.001	0.02^{b} ±0.01	0.14^{b} ±0.02	0.03^{b} ±0.007	0.03^{b} ±0.007
C12:0 iso	0.01^{d} ±0.001	0.05^{abc} ±0.01	0.04^{bcd} ±0.02	0.03^{bcd} ±0.01	0.03^{bcd} ±0.01
C12:0 anteiso	0.01^{d} ±0.001	0.02^{cd} ±0.01	0.03^{abcd} ±0.0	0.05^{a} ±0.007	0.05^{ab} ±0.001
C13:0	0.01^{d} ±0.001	0.18^{a} ±0.02	0.09^{bc} ±0.02	0.10^{b} ±0.01	0.11^{b} ±0.01
C13:0 iso	0.03^{cd} ±0.001	0.01^{d} ±0.007	0.30^{cb} ±0.02	0.33^{b} ±0.01	0.32^{b} ±0.07
C14:0 anteiso	0.41^{c} ±0.01	0.03^{d} ±0.007	1.06^{ab} ±0.07	1.14^{a} ±0.07	1.15^{a} ±0.02
C14:0 iso	0.17^{d} ±0.01	0.65^{a} ±0.02	0.56^{a} ±0.03	0.61^{a} ±0.04	0.61^{a} ±0.01
C15:0	1.04^{e} ±0.02	0.02^{f} ±0.001	2.16^{ab} ±0.05	2.34^{a} ±0.14	2.26^{ab} ±0.04
Cis9-C15:1	0.01^{b} ±0.001	0.08^{b} ±0.007	2.22^{a} ±0.25	0.03^{b} ±0.007	0.02^{b} ±0.007
Cis11-C15:1	0.03^{f} ±0.001	0.34^{a} ±0.02	0.12^{c} ±0.007	0.14^{b} ±0.007	0.14^{b} ±0.007
C18:0 iso	0.05^{d} ±0.001	0.09^{bc} ±0.001	0.20^{a} ±0.01	0.19^{a} ±0.01	0.19^{a}±0.002
C18:0 anteiso	0.08^{f} ±0.001	0.16^{e} ±0.02	2.15^{a} ±0.02	0.37^{b} ±0.04	0.34^{b} ±0.01
C16:0 iso	0.16^{c} ±0.02	0.16^{c} ±0.04	0.61^{a} ±0.02	0.66^{a} ±0.05	0.65^{a} ±0.02
C17:0	0.65^{e} ±0.02	1.04^{d} ±0.07	1.95^{ab} ±0.03	2.09^{a} ±0.19	2.01^{a} ±0.06
Cis9-C17:1	0.13^{f} ±0.01	0.33^{def} ±0.02	1.85^{a} ±0.35	0.64^{cd} ±0.01	0.63^{cd} ±0.01
C17:0 iso	0.28^{e} ±0.007	0.54^{cd} ±0.03	0.85^{ab} ±0.11	0.92^{a} ±0.08	0.89^{ab} ±0.02
C17:0 anteiso	0.36^{d} ±0.007	0.04^{e} ±0.001	0.95^{a} ±0.02	0.99^{a} ±0.07	0.98^{a} ±0.01
Trans9-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
Trans9-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
Trans11-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
Trans11-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.

Health Benefits of Milk Bioactive Lipids

Bioactive lipids have various health benefits related to human health as tumor inhibition, prevent chronic diseases, and enhancing brain functions are represented in **Figure 1**.

Butyric acid as a very short chain fatty acid (C4:0), is one of the most important fatty acids for its potential healthy effect and considered as one of the effective bioactive lipids. Little researches studies were conducted on butyric acid and its estimation **[10]**.

Butyric acid, short chain fatty acids and lauric acid have a positive impact as antiviral, antibacterial, anti-carcinogenic, promote differentiation, inhibit cell growth, induce apoptosis in many human cancer cell lines and prevent the invasion of tumors via inhibitory effects on urokinase **[108**,**109]**. Likewise, **Williams *et al.* [110]** stated that low concentrations of butyric acid can inhibit growth of human cancer cell lines, including prostate and several types of breast and colon cancer. It is believed that butyric acid has the ability to inhibit histone deacetylases, which resulted in histone hyper acetylation and destabilization of 5-chromatin structure that facilitates transcription factor binding and activation of genes associated with cell growth **[2**, **111]**.

Conjugated linoleic acid is considered as anti-obesitic, anti-atherogenic, anti-carcinogenic, and anti-diabetagenic agent as mentioned by **Kilic-Akyilmaz *et al.* [12] , Benjamin and Spener [112]** and **McCrorie *et al.* [113]**. Also, **Park and Wu [114]** observed that CLA can reduce the body fat, prevent of cancer and cardiovascular diseases, modulate of immune and inflammatory responses, and improve of bone health. In addition, CLA was considered to act as an inhibitor of DNA synthesis and consequently can inhibit cancer cells.

Anti-carcinogenic effects have been observed in all cancer types, with doses varying between 55 mg and 3.5 g CLA/day. Milk and its products represent main source of CLA in the human diet, with nearly 70% of the daily requirement, and an average intake of 650 mg/day. That intake value is insufficient for achieving beneficial effects on human health. Various technological alternatives in the field of food technology are exploring ways to produce milk and dairy product rich in CLA. Several studies on human nutrition recorded that daily safe requirement of CLA ranged between 3 and 6 g, although some researches mentioned that administration more than 3.4 g CLA/day has no beneficial action **[115**,**116]**.

Exogenous estrogen administration increases the risk of endometrial hyperplasia and breast cancer, so there is great interest in natural alternatives to estrogen therapy to avoid postmenopausal hazard on women's health. In postmenopausal women, CLA can inhibit the estrogen receptor (ER) signaling in human endometrial and breast cancer cells, acting as an estrogen antagonist through the inhibition of ER alpha (ERα)-mediated responses. **Amaru *et al.* [117]** concluded that CLA effectively reduces breast cancer risk by inhibiting breast tumor initiation, promotion, and progression. Concerning colorectal cancer, CLA intake succeeded in achieving 30% reduction and showed significant role in preventing testicular cancer **[116]**.

On the other trend, little studies dealt with the health benefits of odd and branched fatty acids. Both *anteiso* and *iso* branch-chain fatty acids inhibited tumor outgrowth and the highest activity was observed with 16:0 *iso*, while the inhibitory effects were reduced with an increase or decrease in carbon chain length. Odd and branched-chain fatty acids inhibit fatty acid synthesis of tumor cells through direct effects on fatty acid synthetize and reductions in fatty acid precursor supply **[118]**.

From health points, phospholipids and their digestion products are considered as the most bioactive compounds. Sphingolipids have been implicated as modulators of physiologic and pathophysiologic processes such as inflammatory responses **[119]**. Sphingomyelin can influence cholesterol metabolism and coronary heart disease as well as gangliosides exhibit anti-infection activity and may also protect against mucosal damage **[108]**. In the same side, gangliosides modify the gastrointestinal receptor for microbial toxins, thereby partially preventing some digestive disorders **[120]**. Sphingolipids have an impact on other pathophysiologic processes such as angiogenesis, cell growth, migration, cell death, autophagy, intracellular trafficking, cell adhesion, differentiation, stress, and senescence **[121]**. In addition, potential health benefits may be related to choline, ethanolamine, and polyunsaturated fatty acid (PUFA) content effects in the gut mucosa, an inhibitory effect on cholesterol absorption, and an influence on the gut microbiome and immune function [**6]**. Moreover, phospholipids act as lipoprotein components for transport of fat between gut and liver, as source for acetylcholine (in the case of PC), and as source of (essential) fatty acids and energy **[32]**.

Further, **Dislich and Lichtenthaler [122]** indicated that sphingolipids are associated with age-related diseases and the development of Alzheimer's diseases; sphingo-lipid signalling may play a role in the progressive loss of cell function during the aging process. **Tanaka *et al.* [123]** cleared that sphingomyelin-fortified milk has a positive association with the neurobehavioral development of very low birth weight infants. Lately, **Liu *et al.* [124]** demonstrated that gangliosides have been associated with enhancing spatial learning and affecting brain growth and composition in neonatal piglets.

Growing evidences have confirmed the role of phospholipids in the synthesis and secretion of chylomicrons or very low-density lipoproteins. Additionally, the inclusion of dietary phospholipids can inhibit fatty acids synthesis, and promote fatty acids oxidation to alleviate hepatic lipid deposition. However, dietary phospholipids supplementation did not produce any statistical difference on hepatic lipid content of juvenile Japanese flounder, but significantly promoted hepatic lipid deposition in juvenile stellate sturgeon. Therefore, the impacts of dietary phospholipids on lipid metabolism should be further investigated due to the species-dependent characterization **[125].**

Regarding health impact of TFAs, numerous studies have implicated a high consumption of *trans* fatty acids as risk factors for cardiovascular disease (CVD) and coronary heart disease (CHD). **World Health Organization [126]** recommended that intake of TFAs should not exceed 1% of total energy to reduce CVD risk, without explicitly discriminating between sources of TFAs in the human diet. While, **Mensink *et al.* [127] and Mozaffarian *et al.* [128]** announced that TFAs are known to raise low-density lipoprotein (LDL) cholesterol concentrations and plasma triglycerides in blood. In addition, TFAs are risk factors for atherosclerosis, sudden cardiac death, and other aspects of chronic diseases.

No study has yet found a significant positive relationship between the intake of ruminant-derived TFAs and CHD risk. At the same time, **Elwood *et al.* [129]** mentioned that increased milk consumption is associated with a reduction in CHD risk. **Mozaffarian *et al.* [128]** proposed that reduction in CHD risk with the higher intakes of TFAs from ruminant-derived food. Regarding the substantial risk associated with TFAs from industrial sources, may simply reflect either the lower intake of ruminant TFAs in the human diet or an isomer-specific bioactivity or due to the activity of other compounds in milk and dairy products that negate or mitigate against any adverse effects of TFAs.

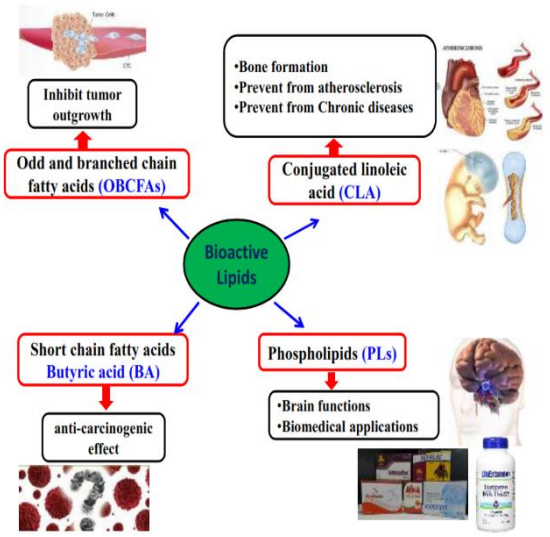

Figure 1. Some health benefits of bioactive lipids in milk and its products

Conclusion

Milk and its products are rich sources of bioactive lipids that consider as good compounds for human health. There are many factors that influence the differences between the bioactive lipids contents of milk such as the species, feeding system and seasonal variations. The outcome of this review is indicated that winter samples contained higher contents of the mentioned-bioactive lipids than summer season, where the green fodder is in winter. Meanwhile, the technological steps had a clear influence on the distribution of all bioactive lipids in milk products, especially CLA and phospholipids. Phospholipids are the most abundant in aqueous phase such as buttermilk and skim milk compared to cream and butter.

Significant Statement

This review confirmed that the feeding system, seasonal variations, and technological processes had a clear influence on the distribution of all bioactive lipids in milk. Consequently, the importance of bioactive lipids as healthy natural ingredients must be taken a great consideration in the future. Meanwhile, it is important to pay attention to the fat of other types of milk such as human, camels, and horses.

References

1. **Bharwade, M.; Balakrishnan, S.; Chaudhary, N. and Jain, A. K.** Fatty Acid Profile and Physico-Chemical Characteristics of Milk Lipids of Kankrej Cow. International Journal of Current Microbiology and Applied Sciences, **(2017)**, 6(8): 3035-3047.
2. **Dhankhar, J.; Sharma, R. and Indumathi, K. P.** Bioactive lipids in milk. International Food Research Journal, **(2016)**, 23(6): 2326-2334.
3. **Chilliard, Y.; Ferlay, A.; Mansbridge, R. M. and Doreau, M.** Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Annales de Zootechnie, **(2000)**, 49(3): 181-205.
4. **Palmquist, D. L.** Milk fat: Origin of fatty acids and influence of nutritional factors thereon. In Advanced Dairy Chemistry Volume 2 Lipids. Springer, Boston, MA. **(2006)**, pp. 43-92.
5. **Mollicam, M. P.;Trinchese, G.; Cimmino, F.; Penna, E.; Cavaliere, G.; Tudisco, R.; Musco, N.; Manca, C.; Catapano, A.; Monda, M.; Bergamo, P.; Banni, S.; Infascelli, F.; Lombardi, P. and Crispino, M.** Milk Fatty Acid Profiles in Different Animal Species: Focus on the Potential Effect of Selected PUFAs on Metabolism and Brain Functions. Nutrients, **(2021),** 13, 1111. https://doi.org/10.3390/nu13041111.

6. **Nilsson, A.; Duan, R. and Ohlsson, L.** Digestion and Absorption of Milk Phospholipids in Newborns and Adults. Front. Nutr., **(2021),** 18 August, vol.8. https://doi.org/10.3389/fnut.2021.724006
7. **Hannun, Y. A. and Obeid, L. M.** Principles of bioactive lipid signalling: lessons from sphingolipids. Nature Reviews Molecular Cell Biology, **(2008)**, 9(2): 139-150.
8. **Biesalski, H. K.; Dragsted, L. O.; Elmadfa, I.; Grossklaus, R.; Müller, M.; Schrenk, D. and Weber, P.** Bioactive compounds: definition and assessment of activity. Nutrition, **(2009)**, 25(11-12): 1202-1205.
9. **Evans, J. F. and Hutchinson, J. H.** Seeing the future of bioactive lipid drug targets. Nature Chemical Biology, **(2010),** 6(7): 476 - 479.
10. **He, B., & Moreau, R.** Lipid-regulating properties of butyric acid and 4-phenylbutyric acid: Molecular mechanisms and therapeutic applications. Pharmacological research, **(2019),** 144, 116-131.
11. **Bergman, E. N.** Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological Reviews, **(1990)**, 70(2): 567-590.
12. **Kilic-Akyilmaz, M.; Ozer, B.; Bulat, T. and Topcu, A.** Effect of heat treatment on micronutrients, fatty acids and some bioactive components of milk. International Dairy Journal, **(2022),** 126 (2022) 105231. https://doi.org/10.1016/j.idairyj.2021.105231.
13. **Yurawecz, M. P.; Mossoba, M. M.; Kramer, J. K. G.; Pariza, M. W. and Nelson, G. J.** Advances in CLA Research, Vol. 3. American Oil Chemists Society, Champaign, IL. **(2006)**, pp.45-93.
14. **Song, K.; Song, I. B.; Gu, H. J.; Na, J. Y.; Kim, S.; Yang, H. S. and Kwon, J.** Anti-diabetic effect of fermented milk containing conjugated linoleic acid on type II diabetes mellitus. Korean Journal for Food Science of Animal Resources, **(2016)**, 36(2): 170-177.
15. **Hur, S. J.; Kim, H. S.; Bahk, Y. Y. and Park, Y.** Overview of conjugated linoleic acid formation and accumulation in animal products. Livestock Science, **(2017)**, 195: 105-111.
16. **Chilliard, Y.; Ferlay, A. and Doreau, M.** Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livestock Production Science, **(2001)**, 70(1): 31-48.
17. **Loor, J. J.; Ferlay, A.; Ollier, A.; Ueda, K.; Doreau, M. and Chilliard, Y.** Concentrate Diets and Polyunsaturated Oils Alter *Trans* and Conjugated Isomers in Bovine Rumen, Blood, and Milk. Journal of Dairy Science, **(2005)**, 88(11): 3986–3999.
18. **Mustafa, A. F.; Chouinard, P.Y. and Christensen D.A.** Effects of feeding micronized flaxseed on yield and composition of milk from Holstein cows. Journal of Science and Food Agriculture, **(2003)**, 83: 920–926.
19. **Collomb, M.; Schmid, A.; Sieber, R.; Wechsler, D. and Ryhänen, E. L.** Conjugated linoleic acids in milk fat: Variation and physiological effects. International Dairy Journal, **(2006)**, 16(11): 1347-1361.
20. **Benjamin, S., Prakasan, P., Sreedharan, S., Andre-Denis, G. Wright, A.G. and Spener, F.** Pros and cons of CLA consumption: An insight from clinical evidences. Nutr. Metab., **(2015)**, 12(4): 1-20.
21. **Jensen, R. G.** The composition of bovine milk lipids: January 1995 to December 2000. Journal of Dairy Science, **(2002)**, 85(2): 295-350.
22. **Vlaeminck, B.; Fievez, V.; Cabrita, A. R. J.; Fonseca, A. J. M. and Dewhurst, R. J.** Factors affecting odd-and branched-chain fatty acids in milk: A review. Animal Feed Science and Technology, **(2006)**, 131(3-4): 389-417.
23. **Fievez, V.; Colman, E.; Castro-Montoya, J. M.; Stefanov, I. and Vlaeminck, B.** Milk odd- and branched-chain fatty acids as biomarkers of rumen function-An update. Animal Feed Science and Technology, **(2012)**, 172(1-2): 51-65.
24. **Hauff, S. and Vetter, W.** Quantification of branched chain fatty acids in polar and neutral lipids of cheese and fish samples. Journal of Agricultural and Food Chemistry, **(2009)**, 58(2): 707-712.
25. **Ran-Ressler, R. R.; Glahn, R. P.; Bae, S. and Brenna, J. T.** Branched-chain fatty acids in the neonatal gut and estimated dietary intake in infancy and adulthood. In: The Importance of Immunonutrition, **Makrides, M.; Ochoa, J.B. and Szajewska, H.** (Eds.), KARGER, Panama. **(2013)**, pp.133-143.
26. **Devle, H.; Vetti, I.; Naess-Andresen, C. F.; Rukke, E.O.; Vegarud, G. and Ekeberg, D.** A comparative study of fatty acid profiles in ruminant and non-ruminant milk. European Journal of Lipid Science and Technology, **(2012)**, 114(9): 1036–1043.
27. **Dingess, K. A.; Valentine, C. J.; Ollberding, N. J.; Davidson, B. S.; Woo, J. G.; Summer, S. and McMahon, R. J.** Branched-chain fatty acid composition of human milk and the impact of maternal diet: the Global Exploration of Human Milk (GEHM) Study. The American Journal of Clinical Nutrition, **(2016)**, 105(1): 177-184.
28. **Ma, L.; Bu, D.; Chen, J. and Wang, J.** Comparison of odd and branched chain fatty acid profiles of cow, yak, buffalo, jerssy cattle, goat, camel and horse milk fat. Joint Animal Meeting, **(2014)**, Kansas City, Missouri. pp.512.

29. **Cívico, A.; Núñez Sánchez, N.; Gómez-Cortés, P.; de la Fuente, M. A.; Peña Blanco, F.; Juárez, M. and Martínez Marín, A. L.** Odd-and branched-chain fatty acids in goat milk as indicators of the diet composition. Italian Journal of Animal Science, **(2017)**, 16(1): 68-74.
30. **Teng, F.; Wang, P.; Yang, L.; Ma, Y. and Day, L.** Quantification of Fatty Acids in Human, Cow, Buffalo, Goat, Yak, and Camel Milk Using an Improved One-Step GC-FID Method. Food Analytical Methods, **2017,** 10(8): 2881-2891.
31. **Ollberding, N. J.; Woo, J. G.; Brenna, T. and Morrow, A. L.** Branched-chain fatty acid composition of human milk and the impact of maternal diet: the Global Exploration of Human Milkre (GEHM). American Journal of Clinical Nutrition, **2016,** 1: 1-8.
32. **Drescher, S. and Hoogevest, P.** The Phospholipid Research Center: Current Research in Phospholipids and Their Use in Drug Delivery. Pharmaceutics, **2020,** 12, 1235. doi:10.3390/pharmaceutics12121235.
33. **Beare-Rogers, J. L.; Dieffenbacher, A. and Holm, J. V.** Lexicon of lipid nutrition (IUPAC Technical Report). Pure and Applied Chemistry, **2001,** 73(4): 685-744.
34. **Sugawara, T.; Zaima, N.; Yamamoto, A.; Sakai, S.; Noguchi, R. and Hirata, T.** Isolation of sphingoid bases of sea cucumber cerebrosides and their cytotoxicity against human colon cancer cells. Bioscience, Biotechnology, and Biochemistry, **2006,** 70(12): 2906-2912.
35. **Gallier, S.; Gragson, D.; Cabral, C.; Jiménez-Flores, R. and Everett, D. W.** Composition and fatty acid distribution of bovine milk phospholipids from processed milk products. Journal of Agricultural and Food Chemistry, **2010,** 58(19): 10503-10511.
36. **Ribar, S.; Karmelić, I. and Mesarić, M.** Sphingoid bases in dairy products. Food Research International, **2007,** 40(7): 848-854.
37. **Lopez, C.; Madec, M. N. and Jimenez-Flores, R.** Lipid rafts in the bovine milk fat globule membrane revealed by the lateral segregation of phospholipids and heterogeneous distribution of glycoproteins. Food Chemistry, **2010,** 120(1): 22-33.
38. **Potočki, S. and Mesarić, M.** Gangliosides in milk. Journal for Dairy Production and Processing Improvement, **2016,** 66(3): 178-186.
39. **Kosmerl, E.; García-Cano, I.; Rocha-Mendoza, D. and Jiménez-Flores, R.** Characterization of milk and soy phospholipid liposomes and inflammation in 3T3-L1 adipocytes. JDS Communications, **2021**; 2: 238–242. https: / / doi .org/ 10 .3168/ jdsc .2020 -0054.
40. **Larque, E.; Zamora, S. and Gil, A.** Dietary *trans* fatty acids in early life: a review. Early Human Development, **2001,** 65(2): S31-S41.
41. **U.S. Food and Drug Administration** Questions and answers about *trans* fat nutrition labeling; **(2003)**. www.cfsan.fda.gov/~dms/qatrans2.html.2003.
42. **Goudjil, H.; Fontecha, J.; Luna, P.; de la Fuente, M. A.; Alonso, L. and Juarez, M.** Quantitative characterization of unsaturated and *trans* fatty acids in ewe's milk fat. Lait, **2004,** 84: 473–482.
43. **Lock, A. L.; Parodi, P. W. and Bauman, D. E.** The biology of *trans* fatty acids: Implications for human health and the dairy industry. Australian Journal of Dairy Technology, **2005,** 60: 134–142.
44. **Chichlowski, M.W.; Schroeder, J.W.; Park, C.S.; Keller, W.L. and Schimek, D. E.** Altering the Fatty Acids in Milk Fat by Including Canola Seed in Dairy Cattle Diets. Journal of Dairy Science, **2005,** 88(9): 3084–3094.
45. **Shingfield, K.J.; Reynolds, C.K.; Hervas, G.; Griinari, J.M.; Grandison, A.J. and Beever, D.E.** Examination of the Persistency of Milk Fatty Acid Composition Responses to Fish Oil and Sunflower Oil in the Diet of Dairy Cows. Journal of Dairy Science, **2006,** 89: 714–732.
46. **Bilby, T.R.; Jenkins, T.; Staples, C.R. and Thatcher, W.W** Pregnancy, Bovine Somatotropin, and Dietary n-3 Fatty Acids in Lactating Dairy Cows: III. Fatty Acid Distribution. Journal of Dairy Science, **2006,** 89(9): 3386–3399.
47. **Glasser, F.; Ferlay, A. and Chilliard, Y.** Oilseed Lipid Supplements and Fatty Acid Composition of Cow Milk: A Meta-Analysis. Journal of Dairy Science, **2008,** 91: 4687–4703.
48. **Talpur, F.N.; Bhanger, M.I.; Khooharo, A.A. and Memon, G.Z.** Seasonal variation in fatty acid composition of milk from ruminants reared under the traditional feeding system of Sindh, Pakistan. Livestock Science, **2008,** 118: 166-172.
49. **Larsen, M.K.; Nielson, J.H.; Buttler, G.; Leifert, C.; Slots, T.; Kristiansen, G.H. and Gustafsson, A.H.** Milk quality as affected by feeding regimens in a country with climatic variation. Journal of Dairy Science, **2010,** 93(7): 2863-2873.
50. **Hayam, M. Abbas; Laila, B. Abd El-Hamid; Abd El-Hamid, A. Asker; Jihan, M. Kassem and Mohamed, I. Salama**. Bioactive Lipids and Phospholipids Classes of Buffalo and Goat Milk Affected by Seasonal Variations. American Journal of Food Science and Nutrition, **2019,** 1(3): 1:13.
51. **Lock, A. L. and Bauman, D.E.** Modifying milk fat composition of dairy cows to enhance fatty

acids beneficial to human health. Lipids, **2004,** 39(12): 1197–1206.

52. **Collomb, M.; Sollberger, H.; Butikofer, U.; Sieber, R.; Stoll, W. and Schaeren, W.** Impact of a basal diet of hay and fodder beet supplemented with rapeseed, linseed and sunflower seed on the fatty acid composition of milk fat. International Dairy Journal, **2004,** 14(6): 549–559.
53. **Tyagi, A.K.; Dhiman, T.R.; Kaur, H.; Singhal, K.K.; Kanwajia, S.K. and Kewalramani, N.** CLA content of milk and milk products as influenced by dietary fat sources in buffaloes. Indian Journal of Animal Science, **2006,** 76(9): 742-746.
54. **Kathirvelan, C.** Influence of mustard oil cake feeding on CLA content of buffalo milk and its anticarcinogenic potency. Indian Journal of Animal Nutrition, **2007,** 24(4), 237-240.
55. **Lock, A. L. and Garnsworthy, P. C.** Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk. Animal Science, **2002,** 74(1): 163-176.
56. **Kay, J.K.; Mackle, T.R.; Auldist, M.J.; Thomson, N.A. and Bauman, D.E.** Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. Journal of Dairy Science, **2004,** 87(2): 236–378.
57. **Tyagi, A.K.; Dhiman, T.R.; Kaur, H.; Singhal, K.K.; Kanwajia, S.K. and Kewalramani, N.** Enhancement of the CLA content of buffalo milk and milk products through green fodder feeding. Animal Feed Science and Technology, **2007,** 133: 351-358.
58. **Bu, D. P.; Wang, J. Q.; Dhiman, T. R. and Liu, S. J.** Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. Journal of Dairy Science, **2007,** 90(2): 998-1007.
59. **Dhiman, T.R.; Nam, S.H. and Ure, A.L.** Factors affecting conjugated linoleic acid content in milk and meat. Critical Reviews in Food Science and Nutrition, **2009,** 45(6): 463–482.
60. **Marín, M. P.; Meléndez, P. G.; Aranda, P. and Ríos, C.** Conjugated linoleic acid content and fatty acids profile of milk from grazing dairy cows in southern Chile fed varying amounts of concentrate. Journal of Applied Animal Research, **2018,** 46(1): 150-154.
61. **Lock, A. L. and Garnsworthy, P. C.** Seasonal variation in milk conjugated linoleic acid and Δ9-desaturase activity in dairy cows. Livestock Production Science, **2003,** 79(1): 47-59.
62. **Precht, D. and Molkentin, J.** Frequency distribution of conjugated linoleic acid and *trans* fatty acids contents in Europeans bovine milk fats. Milchwissenschaft, **2000,** 55: 687– 691.
63. **Ledoux, M.; Chardigny, J. M.; Darbois, M.; Soustre, Y.; Sébédio, J. L. and Laloux, L.** Fatty acid composition of French butters, with special emphasis on conjugated linoleic acid (CLA) isomers. Journal of Food Composition and Analysis, **2005,** 18(5): 409-425.
64. **Rego, O. A.; Rosa, H. J.; Regalo, S. M.; Alves, S. P.; Alfaia, C. M.; Prates, J. A. and Bessa, R. J.** Seasonal changes of CLA isomers and other fatty acids of milk fat from grazing dairy herds in the Azores. Journal of the Science of Food and Agriculture, **2008,** 88(10): 1855-1859.
65. **Salama, M. I.; Laila, B. Abd Elhamid; Hayam, M. Abbas; Jihan, M. Kassem and Asker, A. A.** Conjugated Fatty Acids as Bioactive Compounds of Buffalo and Goat Milk. Journal of Biological Chemistry and Environmental Sciences, **2018,** 13(2): 31-42.
66. **Shingfield, K. J.; Reynolds, C. K.; Lupoli, B.; Toivonen, V.; Yurawecz, M. P.; Delmonte, P. and Beever, D. E.** Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower oil and fish oil. Animal Science, **2005,** 80(2): 225-238.
67. **Singh, A. P.; Avramis, C. A.; Kramer, J. K. and Marangoni, A. G.** Algal meal supplementation of the cows' diet alters the physical properties of milk fat. Journal of Dairy Research, **2004,** 71(1): 66-73.
68. **Jones, E. L.; Shingfield, K. J.; Kohen, C.; Jones, A. K.; Lupoli, B.; Grandison, A. S. and Yaqoob, P.** Chemical, physical, and sensory properties of dairy products enriched with conjugated linoleic acid. Journal of Dairy Science, **2005,** 88(8): 2923-2937.
69. **Bernard, L.; Shingfield, K.J.; Rouel, J.; Ferlay, A. and Chilliard, Y.** Effect of plant oils in the diet on performance and milk fatty acid composition in goats fed diets based on grass hay or maize silage. British Journal of Nutrition, **2009,** 101(2): 213–224.
70. **Lopez, C.; Briard-Bion, V.; Menard, O.; Rousseau, F.; Pradel, P. and Besle, J. M.** Phospholipid, sphingolipid, and fatty acid compositions of the milk fat globule membrane are modified by diet. Journal of Agricultural and Food Chemistry, **2008,** 56(13): 5226-5236.
71. **Walker, G.; Wijesundera, C.; Dunshea, F.; Doyle, P.** Seasonal and stage of lactation effects on milk fat composition in northern Victoria. Anim. Prod. Sci., **2013,** 53, 560–572.
72. **Timlin, M.; Tobin, J. T.;Brodkorb, A.; Murphy, E. G.; Dillon, P.; Hennessy, D.; O'Donovan, M.; Pierce, K. M. and O'Callaghan, T. F.** The Impact of Seasonality in Pasture-Based Production Systems on Milk Composition and Functionality. Foods, **2021,** 10, 607. https://doi.org/10.3390/foods10030607.

73. **Graves, E. L. F.; Beaulieu, A. D. and Drackley, J. K.** Factors affecting the concentration of sphingomyelin in bovine milk. Journal of Dairy Science, **2007,** 90(2): 706-715.
74. **Hofi, A. A.; Hamzawi, L.F. and Mahran, G.A.** Studies on buffalo milk fat globule membrane effect of stage of lactation. Egyptian Journal of Dairy Science, **1977,** 5: 235–240.
75. **Kay, J.K.; Roche, J.R.; Kolver, E.S.; Thomson, N.A. and Baumgard, L.H.** A comparision between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and fatty acid profiles in dairy cows. Journal of Dairy Research, **2005,** 72(3): 322–332.
76. **Ferlay, A.; Martin, B.; Pradel, P.H.; Coulon, J.B. and Chilliard, Y.** Fatty acid composition and milk lipolytic system in Tarentaise and Montbeliarde cow breeds. Journal of Dairy Science, **2006,** 89: 4026-4041.
77. **Talpur, F.N.; Memon N.N. and Bhanger, M.I.** Comparison of Fatty Acid and Cholesterol Content of Pakistani Water Buffalo Breeds. Pakistan Journal of Analytical Environment Chemistry, **2007,** 8: 15-20.
78. **Palladino, R.A.; Buckley, F.; Prendiville, R.; Murphy, J.J.; Callan, J. and Kenny, D.A.** A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. Journal of Dairy Science, **2010,** 93: 2176–2184.
79. **Maurice-Van Eijndhoven, M.H.T.; Hiemstra, S.J. and Calus, M.P.S.** Short communication: milk fat composition of 4 cattle breeds in the Netherlands. Journal of Dairy Science, **2011,** 94(2): 1021 -1025.
80. **Saroha, V.; Kumar, D.; Sharma, A.; Jayakumar, S.; Tyagi, A. K.; Nagda, R. K. and Dixit, S. P.** Quantitative analysis of fatty acid in Indian goat milk and its comparison with other livestock. Journal of Livestock Science, **2014,** 5: 1-8.
81. **Abbas, H.M.; Hassan, F.A.; El-Gawad, M.A.M.A. and Enab, A. K.** Physicochemical characteristics of goat's milk. Life Science Journal, **2014a,** 11(1): 307-317.
82. **Lowless, F.; Murphy, J.; Harrington, D.; Devery, R. and Stanton, C.** Advances in CLA research. Journal of Dairy Science, **1999,** 81: 12-20.
83. **White, S.L.; Bertrand, J.A.; Wade, M.R.; Washburn, S.P.; Green Jr, J.T. and Jenkins, T.C.** Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. Journal of Dairy Science, **2001,** 84: 2295–2301.
84. **Kelsey, J.A.; Corl, B.A.; Collier, R.J. and Bauman, D.E.** The effect of breed, parity, and stage of lactating on conjugated linoleic acid (CLA) in milk fat from dairy cows. Journal of Dairy Science, **2003,** 86(8): 2588–2589.
85. **Nudda, A.; McGuire, M.A.; Battacone, G. and Pulina, G.** Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and Ricotta. Journal of Dairy Science, **2005,** 88: 1311–1319.
86. **Gonzalez, S.; Van Nieuwenhove, C.; Perez-Chaia, A. and DeRuiz Holgado, A.P.** Conjugated linoleic acid in buffalo (Bubalus bubalis) milk from Argentina. Milchwissenschaft, **2004,** 59: (9-10): 506-508.
87. **Kumar, J.S.** The effect of Breed, Parity, Stage of lactation and processing of milk on CLA in Dairy products. M.Sc. Thesis, National Dairy Research Institute, Karnal, India. **2004.**
88. **Kondyli, E.; Svarnas, C.; Samelis, J. and Katsiari, M. C.** Chemical composition and microbiological quality of ewe and goat milk of native Greek breeds. Small Ruminant Research, **2012,** 103(2): 194-199.
89. **Kuchroo, T.K. and Narayanan, K.M.** Effect of sequence of milking on the distribution of fat globule and phospholipids composition of milk. Indian Journal of Dairy Science, **1977,** 30(3): 225-228.
90. **Avalli, A. and Contarini, G.** Determination of phospholipids in dairy products by SPE/HPLC/ELSD. Journal of Chromatography A, **2005,** 1071(1-2): 185–190.
91. **Rombaut, R.; Camp, J.V. and Dewettinck, K.** Analysis of phospho and sphingolipids in dairy products by a new HPLC method. Journal of Dairy Science, **2005,** 88(2): 482-488.
92. **Wiking, L.; Stagsted, J.; Bjorck, L. and Nielsen, J.H.** Milk fat globule size is affected by fat production in dairy cows. International Dairy Journal, **2004,** 14: 909-913.
93. **Garcia, C.; Lutz, N.W.; Confort-Gouny, S.; Cozzone, P.J.; Armand, M. and Bernard, M.** Phospholipid fingerprints of milk from different mammalians determined by ^{31}PNMR: Towards specific interest in human health. Food Chemistry, **2012,** 135: 1777–1783.
94. **Alonso, L.; Fontecha, J.; Lozada, L.; Fraga, M.J. and Juarez, M.** Fatty acid composition of caprine milk: major, branched chain, and *trans* fatty acids. Journal of Dairy Science, **1999,** 82(5): 878–884.
95. **Shantha, N.C.; Decker, E.A. and Ustunol, Z.** Conjugated linoleic acid concentration in processed cheese. Journal of the American Oil Chemists' Society, **1992,** 69(5): 425–428.
96. **Shantha, N.C.; Ram, L.N.; O'Leary, J.; Hicks, C.L. and Decker, E.A.** Conjugated linoleic acid concentrations in dairy products as affected by

processing and storage, Journal of Food Science, **1995,** 60: 695–697.

97. **Luna, P.; Martin-Diana, A. B.; Alonso, L.; Fontecha, J.; de la Fuente, M. A.; Requena, T. and Juarez, M.** Effects of milk fat replacement by PUFA enriched fats on n-3 fatty acids, conjugated dienes and volatile compounds of fermented milks. European Journal of Lipid Science and Technology, **2004,** 106(7): 417–423.
98. **Van Nieuwenhove, C.; Gonzalez, S.; PerezChaia, A. and Holgado, A.P.D.** Conjugated linoleic acid in buffalo (Bubalus bubalis) milk from Argentina. Milchwissenschaft, **2004,** 59: 506–508.
99. **Gnädig, S.; Chamba, J.F.; Perreard, E.; Chappaz, S.; Chardigny, J.M.; Rickert, R.; Steinhart, H. and Sébédio, J.L.** Influence of manufacturing conditions on the conjugated linoleic acid content and the isomer composition in ripened French Emmental cheese, Journal of Dairy Research, **2004,** 71(3): 367–371.
100. **Ryhänen, E.L.; Tallavaara, K.; Griinari, J.M.; Jaakkola, S.; Mantere-Alhonen, S. and Shingfield, K.J.** Production of conjugated linoleic acid enriched milk and dairy products from cows receiving grass silage supplemented with a cereal-based concentrate containing rapeseed oil. International Dairy Journal, **2005,** 15: 207–217.
101. **Bisig, W.; Eberhard, P.; Collomb, M. and Rehberger, B.** Influence of processing on the fatty acid composition and the content of conjugated linoleic acid in organic and conventional dairy products- a review. Le Lait, **2007,** 87(1): 1-19.
102. **O'Shea, M.; Devery, R.; Lawless, F.; Keogh, K. and Stanton, C.** Enrichment of the conjugated linoleic acid content of bovine milk fat by dry fractionation. International Dairy Journal, **2000,** 10(4): 289–294.
103. **Romero, P.; Rizvi, S.S.H.; Kelly, M.L. and Bauman, D.E.** Short communication: Concentration of conjugated linoleic acid from milk fat with a continuous supercritical fluid processing system. Journal of Dairy Science, **2000,** 83: 20–22.
104. **Rombaut, R.; Camp, J.V. and Dewettinck, K.** Phospho- and sphingolipid distribution during processing of milk, butter and whey. International Journal of Food Science and Technology, **2006,** 41(4): 435–443.
105. **Garczewska-Murzyn, A.; Smoczyn´ ski, M.; Kotowska, N. and Kiełczewska, K.** Effect of buttermilk and skimmed milk powder on the properties of low-fat yoghurt. J Food Sci. Technol., **2021**, https://doi.org/10.1007/s13197-021-05227-w.
106. **Laila, B. Abd El-Hamid; Hayam, M. Abbas; Abd El-Hamid, A. Asker; Jihan, M. Kassem and Mohamed, I. Salama.** Bioactive Lipids Distribution in Buffalo Fatty Products. International Journal of Nutritional Science and Food Technology, **2019,** 5(6): 34:42.
107. **Hayam, M. Abbas; Laila, B. Abd El-Hamid; Jihan, M. Kassem and Mohamed, I. Salama.** Vital Lipids Contents in Buffalo Butter Oil and its Fractions Prepared by Dry Fractionation. Egyptian Journal of Chemistry, **2021,** 64(2): 949-956.
108. **Rodríguez-Alcalá, L. M. and Fontecha, J.** Major lipid classes separation of buttermilk, and cows, goats and ewes milk by high performance liquid chromatography with an evaporative light scattering detector focused on the phospholipid fraction. Journal of Chromatography A, **2010,** 1217(18): 3063-3066.
109. **Parodi, P. W.** Cow's milk components with anti-cancer potential. Australian Journal of Dairy Technology, **2001,** 56(2): 65-68.
110. **Williams, E.A.; Coxhead, J.M. and Mathers, J.C.** Anti-cancer effects of butyrate: use of micro-array technology to investigate mechanisms. Proceedings of the Nutrition Society, **2003,** 62: 107–115.
111. **Parodi, P. W.** Nutritional significance of milk lipids. In: Advanced Dairy Chemistry 2: Lipids, **Fox, P.F. and McSweeney, P. L. H.** (Eds.), 3rd edition, Springer, New York. **2006,** pp. 601 –639.
112. **Benjamin, S. and Spener, F.** Conjugated linoleic acids as functional food: an insight into their health benefits. Nutrition and Metabolism, **2009,** 6(1): 36.
113. **McCrorie, T. A.; Keaveney, E. M.; Wallace, J. M.; Binns, N. and Livingstone, M. B. E.** Human health effects of conjugated linoleic acid from milk and supplements. Nutrition Research Reviews, **2011,** 24(2): 206-227.
114. **Park, Y. W. and Wu, Y.** Health benefits of conjugated fatty Acids. In: Conjugated Linoleic Acids and Conjugated Vegetable Oils, **Sels, B. and Philippaerts, A.** (Eds.), The Royal Society of Chemistry, UK. **2014,** pp. 94-116.
115. **Dachev, M.; Bryndová, J.; Jakubek, M.; ˇek Mouˇcka, Z. and Urban, M.** The Effects of Conjugated Linoleic Acids on Cancer. *Processes*, **2021,** 9(3), 454; https://doi.org/10.3390/pr9030454.
116. **Abd El-Gawad, A. M.; Abo El-Hassan, D. G.; Aboul-Enein, A. M.; Abdelgayed, S. S.; Aly, S. A.; Esmat, G.; Mostafa, A. A.; Bakr, M. H.; Ali, R. A. and Ayoub, M. A.** Anticancer activity of milk fat rich in conjugated linoleic acid against Ehrlich ascites carcinoma cells in female Swiss albino mice. Veterinary World, **2021,** 14(3): 696-708. doi: www.doi.org/10.14202/vetworld.2021.696-708.
117. **Amaru, D.L., Biondo, P.D. and Field, C.J.** The role of conjugated linoleic acid in breast cancer

growth and development. Open Nutraceuticals J., **2010,** 3(1): 30-46.

118. **Wongtangtintharn, S.; Oku, H.; Iwasaki, H. and Toda, T.** Effect of branched-chain fatty acids on fatty acid biosynthesis of human breast cancer cells. Journal of Nutritional Science and Vitaminology, **2004,** 50(2): 137-143.
119. **El Alwani, M.; Wu, B. X.; Obeid, L. M. and Hannun, Y. A.** Bioactive sphingolipids in the modulation of the inflammatory response. Pharmacology and Therapeutics, **2006,** 112(1): 171-183.
120. **Haug, A.; Høstmark, A. T. and Harstad, O. M.** Bovine milk in human nutrition– a review. Lipids in Health and Disease, **2007,** 6(1): 25-41.
121. **Nikolova-Karakashian, M. N. and Rozenova, K. A.** Ceramide in stress response. In: Sphingolipids as Signaling and Regulatory Molecules, **Chalfant, C. and Poeta, M. D.** (Eds.), Springer, New York, NY. **2010,** pp. 86-108.
122. **Dislich, B. and Lichtenthaler, S. F.** The membrane-bound aspartyl protease BACE1: molecular and functional properties in Alzheimer's disease and beyond. Frontiers in physiology, **2012,** 3(8).
123. **Tanaka, K.; Hosozawa, M.; Kudo, N.; Yoshikawa, N.; Hisata, K.; Shoji, H. and Shimizu, T.** The pilot study: sphingomyelin-fortified milk has a positive association with the neurobehavioural development of very low birth weight infants during infancy, randomized control trial. Brain and Development, **2013,** 35(1): 45-52.
124. **Liu, H.; Radlowski, E. C.; Conrad, M. S.; Li, Y.; Dilger, R. N. and Johnson, R. W.** Early Supplementation of Phospholipids and Gangliosides Affects Brain and Cognitive Development in Neonatal Piglets–3. The Journal of Nutrition, **2014,** 144(12): 1903-1909.
125. **Huang, Y.; Xu, J.; Sheng, Z.;Xie, R.;Zhang, H.; Chen, N. and Li, S.** Effects of dietary phospholipids on growth performance, fatty acid composition, and expression of lipid metabolism related genes of juvenile hybrid grouper (Epinephelus fuscoguttatus ♀ × E. lancolatus ♂). Aquaculture Reports, **2022,** vol. 22; 100993. https://doi.org/10.1016/j.aqrep.2021.100993.
126. **World Health Organization**. Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series, **2003,** No. 916; www.who.int/dietphysicalactivity/publications/trs916.
127. **Mensink, R. P.; Zock, P. L.; Kester, A. D. and Katan, M. B.** Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. American Journal of Clinical Nutrition, **2003,**77: 1146–1155.
128. **Mozaffarian, D.; Katan, M. B.; Ascherio, A.; Stampfer, M. J. and Willett, W. C.** *Trans* fatty acids and cardiovascular disease. New England Journal of Medicine, **2006,** 354: 1601–1613.
129. **Elwood, P.; Hughes, J. and Fehly, A.** Milk, heart disease and obesity: An examination of the evidence. British Journal of Cardiology, **2005,** 12: 283–290.