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Fatty acid compositions of colostrum and mature breast milk in Turkey (Mardin)

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ABSTRACT

In this study, 25–25 milk samples (25 colostral and 25 mature) collected at different lactational stages were used to analyse the fatty acid composition of breast milk. A gas-chromatographic method was used to perform and analyse the transmethylation of total milk lipid extracts. The milk samples contained 20 different fatty acids. Palmitic acid (C16:0), stearic acid (C18:0), myristic acid (C14:0), oleic acid (C18:1n-9), and linoleic acid (LA, C18:2n-6) were the major components of total lipid, phospholipid (PL), and triacylglycerol (TAG) fractions. Colostrum had a lower percentage of polyunsaturated fatty acids (PUFAs), a higher percentage of saturated fatty acids (SFAs), and a lower level of eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) in total lipid than mature milk. Triacylglycerol and PL fractions between colostrum and mature milk samples did not differ statistically.

KEYWORDS

phospholipids, breast milk, chromatography, triacylglycerols

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1. INTRODUCTION

Many nutrients and immunologically active substances included in human milk are necessary for a newborn's healthy development as well as the maturation of their immune systems against a range of illnesses and infections. Around 87–88% of human milk is composed of water, and the solid components are 7% carbohydrates, 1% proteins, and 3.8% lipids. In recent years, there has been a focus on the nutritional and physiological value of PUFAs in infant nutrition, particularly DHA and arachidonic acid (C20:4n-6, AA), which are structural components of the lipid matrix of cellular and subcellular membranes. In addition to having structural characteristics, several PUFAs serve as precursors in the production of eicosanoids, which have crucial regulatory roles. PUFAs are formed through a series of enzymatic desaturation and elongation steps from linoleic (C18:2n-6) and linolenic acids (C18:3n-3; ALA). Dietary intake and endogenous lipid metabolism influence the fatty acid (FA) content of breast milk. Many other factors, such as maternal age, term of delivery, stage of lactation, and gestational diseases, may influence the FA composition of milk (Argov-Argaman et al., 2016). Breast milk fatty acid profile and TAGs content changes have been reported at different stages of lactation (Lubetzky et al., 2012). Fatty acids are the primary constituents of milk fat. These are primarily esterified in the form of TAGs, which account for 98% of milk fat. A smaller proportion of fatty acids are esterified as PLs, which are found in a membrane that surrounds and stabilises the lipidic core of the milk fat globule. Throughout the third trimester of pregnancy and throughout the first two years of life, the most important PUFAs (AA and DHA) are incorporated into the PL membranes of the retina and brain. Approximately 85% of the PUFA in mature human milk is in the form of TAGs, and 15% is in the form of PLs. Human milk contains 20–70% SFA, 23–55% MUFA, 6–36% PUFA, and 0.3–8% long-chain PUFA. SFAs and MUFAs make up the majority of the fatty acids in human milk, followed by PUFAs, particularly omega-3 and omega-6 PUFAs, which have significant biological functions (Innis, 2014). Among the PUFA, C18:2n-6 and C18:3n-3 are essential because they are not synthesised in the human body and serve as precursors to AA, and C22:6n-3 is linked to normal brain development, particularly in childhood (Innis, 2014). The three stages of milk production are colostrum (1–5 days postpartum), transitional milk (6–15 days postpartum), and mature milk (after 15 days). There have been no recent studies in Turkey that focused on the influence of lactation stage on FA composition in the TAG and PL fractions. The purpose of this study is to look at the fatty acid compositions of total lipid, PL, and TAG in colostrum and mature milk.

2. MATERIALS AND METHODS

2.1. Lipid extraction and transmethylation of fatty acids

25–25 milk samples (25 mothers provided colostrum and the same 25 mothers provided mature milk samples later) collected at two lactational phases were used to analyse the fatty acid composition of breast milk. Colostrum was collected 1–5 days after birth, and mature milk was collected 15 days later. After the baby's regular feed, the mother collected the milk and expressed it with a breast pump. Prior to analysis, a 10 mL sample was frozen at −80 °C in a freezer. Thawed breast milk samples were mixed with the extracting solution (methanol-chloroform, 1:2 v/v). The samples' total lipid content was fractionated using the thin-layer

chromatography method. A thin layer of 30 g silica gel and 50 mL pure water was applied to 20 × 20 cm plates for this purpose, and they were dried in an oven at 100 °C for one hour. The samples' total lipid extracts were spotted onto the plates in a single row. TAG and PL were separated using a mobile phase of diethyl ether/petroleum ether/acetic acid (20:80:1 by volume) (Stanley-Samuelson and Dadd, 1983). The lipid fractions were revealed using 2′,7′-dichlorofluorescein. Acidified methanol was used to transmethylate the TAG and PL fractions. Two hours at 85 °C were spent refluxing it. In hexane, the fatty acid methyl esters (FAMEs) were extracted.

2.2. Gas chromatography analysis

Gas chromatography (Shimadzu GC 2010 PLUS) was used to analyse the fatty acid methyl esters using a flame ionisation detector. A BPX70 (70% Cyanopropyl Polysilphenylene-Siloxane) capillary column (30 m × 0.25 mm × 0.25 μm film thickness) was used. Helium was used as the carrier gas, flowing at a rate of 0.5 mL min^{-1}. The compressed air and hydrogen flow rates were 300 and 30 mL min^{-1}, respectively. Following were the temperature profiles: starting temperature of 170 °C (2 min), heating rate of 2 °C min^{-1}, final temperature of 220 °C, injector and detector temperatures of 250 °C. The GC Solution (Version 2.4) computer program was used to identify FAMEs and calculate their concentrations. The standard deviation (SD) was determined in SPSS (16.0) for all analyses, and the data is presented as the mean of the SD for the triplicate results. One-way analysis of variance was used to compare fatty acid percentages. The Tukey HSD test was used to determine differences. The statistics determined that the differences were significant when the data was $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. FA composition of colostrum and mature milk

The fatty acid contents of total lipid, TAG, and PL fractions of colostrum and mature milk are shown in Tables 1 and 2, respectively. While C12:0 and C14:0 were present in similar percentages in the TAG and PL fractions, total lipid contained higher concentrations of both fatty acids in colostrum. C8:0 was found in very small amounts in the total lipid and PL fractions but increased in the TAG fraction in mature milk. C10:0 was detected in both total lipids and fractions in colostrum and mature milk. In total lipid, TAG, and PL fractions, C12:0, C14:0, and C18:0 fatty acids were found in nearly identical ratios in mature milk. C16:0 was found to be the main constituent of the total lipid, TAG, and PL fraction in colostrum and mature milk. It was determined that this fatty acid was lower in the TAG fraction (23.50%) compared to the others in colostrum. This saturated fatty acid was found to be more abundant in the PL fraction (38.94%) of mature milk. Total SFA was found to be 58.11% in total lipid, 36.07% in the TAG fraction, and 53.96% in the PL fraction in colostrum. Total SFA was found in small amounts in the total lipid and TAG fractions, but it was detected at a significant rate (59.44%) in the PL fraction due to the influence of C16:0 in mature milk. In our study, levels of C14:0, C16:0, and C16:1n-7 decreased from colostrum to mature milk in total lipid. C18:1n-9 and C16:0 fatty acids are synthesised *de novo* in breast tissue, other maternal organs, or from the mother's food. As in our study and many other studies (Silva et al., 2005; Mihályi et al., 2015; Zhao et al., 2018;

Table 1. Fatty acid profile of total lipids, triacylglycerols, and phospholipids in 25 colostrum samples (% of total FA)

Fatty acids	Colostrum total	Colostrum TAG	Colostrum PL
C8:0	0.01 ± 0.01[a]	0.03 ± 0.01[b]	0.04 ± 0.01[c]
C10:0	0.91 ± 0.02[a]	0.12 ± 0.03[b]	0.44 ± 0.02[c]
C12:0	6.53 ± 0.98[a]	2.31 ± 0.36[b]	2.31 ± 0.45[b]
C14:0	10.21 ± 0.99[a]	4.79 ± 0.56[b]	5.55 ± 0.34[b]
C15:0	0.02 ± 0.01[a]	0.26 ± 0.06[b]	0.26 ± 0.07[b]
C16:0	31.46 ± 1.90[a]	23.50 ± 1.54[b]	32.89 ± 1.65[a]
C17:0	0.11 ± 0.05[a]	0.25 ± 0.04[b]	0.20 ± 0.08[b]
C18:0	8.83 ± 0.76[a]	4.61 ± 0.56[b]	12.19 ± 0.87[c]
C20:0	0.03 ± 0.01[a]	0.20 ± 0.06[b]	0.08 ± 0.01[c]
∑SFA	58.11 ± 2.33[a]	36.07 ± 1.76[b]	53.96 ± 2.98[a]
C16:1 n-7	3.47 ± 0.55[a]	1.07 ± 0.78[b]	0.85 ± 0.04[b]
C18:1 n-9	28.14 ± 1.22[a]	34.60 ± 1.90[b]	24.38 ± 1.36[a]
C20:1 n-9	0.19 ± 0.01[a]	0.76 ± 0.06[b]	1.10 ± 0.99[c]
∑MUFA	31.80 ± 1.29[a]	36.43 ± 2.35[b]	26.33 ± 2.08[c]
C18:2 n-6	7.79 ± 1.06[a]	23.98 ± 1.89[b]	14.36 ± 1.09[c]
C18:3 n-3	0.20 ± 0.02[a]	0.28 ± 0.01[a]	0.28 ± 0.05[a]
C20:2 n-6	1.01 ± 0.90[a]	1.17 ± 0.07[a]	0.60 ± 0.05[b]
C20:3 n-6	0.33 ± 0.03[a]	0.75 ± 0.06[b]	0.63 ± 0.34[b]
C20:4 n-6	0.32 ± 0.01[a]	0.69 ± 0.06[b]	2.10 ± 0.98[c]
C20:5 n-3	0.11 ± 0.01[a]	0.37 ± 0.05[b]	0.96 ± 0.04[c]
C22:5 n-3	0.10 ± 0.04[a]	0.06 ± 0.01[b]	0.47 ± 0.07[c]
C22:6 n-3	0.13 ± 0.05[a]	0.10 ± 0.01[a]	0.21 ± 0.08[b]
∑PUFA	9.99 ± 1.22[a]	27.40 ± 1.04[b]	19.61 ± 1.23[c]
∑n-3	0.54	0.81	1.92
∑n-6	9.45	26.59	17.69
n-3/n-6	0.05	0.03	0.10

Means are the averages of 3 replicates. Values reported are means ± standard deviation; means followed by different letters in the same line are significantly different ($P < 0.05$) by Tukey's test.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

Yuan et al., 2019; Chen et al., 2020; Floris et al., 2020), C16:0 was the most abundant fatty acid in both mature milk and colostrum among SFAs. Likewise, SFA was found at a high rate, as in other studies (Floris et al., 2020; Zhang et al., 2022). In MUFAs, C18:1n-9 was found to be the predominant fatty acid in colostrum and mature milk. C18:1n-9 levels were similar in the total lipid and TAG fractions but lower in the PL fraction (19.25%) in mature milk. The PL fraction contained the least amount of C16:1n-7, followed by the TAG fraction and total lipid in colostrum. C18:1n-9, an important energy source for the newborn, is the most abundant fatty acid among MUFAs in both mature milk and colostrum. According to some recent studies, the amount of C18:1n-9 was between 31 and 39% (Zhu et al., 2021; Zhang et al., 2022). Despite not being an essential fatty acid, oleic acid is particularly significant because, in addition to its regular roles as a fatty acid, it lowers the melting point of TAGs, which is necessary for the production, transport, and metabolism of milk fat globules. C20:1n-9 increased in colostrum PL

Table 2. Fatty acid profile of total lipids, triacylglycerols and phospholipids in 25 mature milk samples (% of total FA)

Fatty acids	Mature total	Mature TAG	Mature PL
C8:0	0.07 ± 0.01[a]	0.47 ± 0.04[b]	0.08 ± 0.01[a]
C10:0	1.21 ± 0.11[a]	0.69 ± 0.06[b]	0.31 ± 0.03[c]
C12:0	6.70 ± 1.20[a]	4.93 ± 0.99[a]	3.96 ± 0.76[b]
C14:0	6.25 ± 1.95[a]	5.63 ± 0.77[a]	7.35 ± 0.50[a]
C15:0	0.17 ± 0.02[a]	0.20 ± 0.03[b]	0.24 ± 0.01[b]
C16:0	20.92 ± 1.09[a]	22.39 ± 1.50[a]	38.94 ± 1.06[b]
C17:0	0.20 ± 0.02[a]	0.22 ± 0.06[a]	0.25 ± 0.01[a]
C18:0	3.88 ± 0.99[a]	4.86 ± 0.89[b]	7.85 ± 1.24[c]
C20:0	0.08 ± 0.02[a]	0.12 ± 0.07[b]	0.46 ± 0.04[c]
∑SFA	39.48 ± 2.33[a]	39.51 ± 2.56[a]	59.44 ± 2.09[b]
C16:1 n-7	2.65 ± 1.00[a]	1.38 ± 0.56[b]	1.35 ± 0.78[b]
C18:1 n-9	31.78 ± 1.29[a]	34.56 ± 1.20[a]	19.25 ± 1.06[b]
C20:1 n-9	0.25 ± 0.02[a]	0.29 ± 0.06[a]	0.36 ± 0.03[b]
∑MUFA	34.68 ± 2.20[a]	36.23 ± 2.77[a]	20.96 ± 2.31[b]
C18:2 n-6	23.40 ± 1.06[a]	21.72 ± 1.66[a]	15.97 ± 1.54[b]
C18:3 n-3	0.38 ± 0.03[a]	0.25 ± 0.02[b]	0.22 ± 0.06[b]
C20:2 n-6	0.45 ± 0.05[a]	0.57 ± 0.10[b]	0.42 ± 0.04[a]
C20:3 n-6	0.55 ± 0.05[a]	0.61 ± 0.03[a]	0.56 ± 0.01[a]
C20:4 n-6	0.56 ± 0.06[a]	0.50 ± 0.04[a]	1.05 ± 0.88[b]
C20:5 n-3	0.26 ± 0.02[a]	0.35 ± 0.04[b]	0.58 ± 0.05[c]
C22:5 n-3	0.06 ± 0.01[a]	0.05 ± 0.01[a]	0.54 ± 0.11[b]
C22:6 n-3	0.14 ± 0.04[a]	0.11 ± 0.01[a]	0.16 ± 0.06[a]
∑PUFA	25.80 ± 1.26[a]	24.16 ± 1.06[a]	19.50 ± 1.44[b]
∑n-3	0.84	0.76	1.50
∑n-6	24.96	23.40	18.00
n-3/n-6	0.03	0.03	0.08

Means are the averages of 3 replicates. Values reported are means ± standard deviation; means followed by different letters in the same line are significantly different ($P < 0.05$) by Tukey's test.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

as compared to colostrum total. Total MUFA was observed to have low levels in the PL fraction and similar levels in the total lipid and TAG fractions in colostrum. It was found that total MUFA contents were similar in total lipid and TAG fractions but lower in PL fractions in mature milk. Hayat et al. (1999) found MUFA at 37.3% and unsaturated fatty acids at 57.5% in the milk of Kuwaiti women. VanderJagt et al. (2000) found MUFA at 31.0% in the milk of Nigerian women. Vegetable oils, such as soybean oil, sunflower oil, and olive oil, are the primary sources of these fatty acids. In a study in Iraq, it was determined that mature milk contained more fat, and MUFAs did not differ (Al-Tamer and Mahmood, 2004). The content of essential fatty acids in human milk lipids is of special interest because of their eminent physiological significance. The essential fatty acid C18:2n-6 was the most prevalent one in human milk. C18:2n-6 and C18:3n-3 are essential fatty acids that cannot be synthesised by the human body and must therefore be obtained through diet. According to studies, approximately 30% of the

C18:2n-6 in milk is directly transferred from the mother's diet, and the amount of C18:2n-6 in human breast milk is significantly correlated with the amount of C18:2n-6 consumed by the mother. Linoleic acid accounted for approximately 7.79–23.98% of the colostrum and mature fatty acids in the current study. C18:2n-6 had the lowest amount in total lipid, followed by the PL fraction, and the highest in the TAG fraction in colostrum. The content of one of the polyunsaturated fatty acids, C18:2n-6, was shown to be reduced in the PL fraction while remaining the same in the total lipid and TAG fractions in mature milk. The amounts of C18:2n-6 in colostrum and mature milk in the PL fraction were found to be close to each other. In our study, C18:2n-6 content was found to be higher than that obtained in previous studies (Knox et al., 2000; Glew et al., 2001). Among the essential fatty acids, C18:3n-3 was found to be similar to the ratio of Kuwaiti, Chinese, Indian, American, and Dominican mothers (Hayat et al., 1999; Schmeits et al., 1999). PUFAs, such as EPA and DHA, have also been identified as important for brain health. Both fatty acids were found in the highest amounts in PL fraction in colostrum. Low quantities of EPA and DHA were found in the total lipid, TAG, and PL fractions of mature milk. In comparison to the total lipid and TAG fractions, the PL fraction was shown to include more of these two fatty acids in mature milk. In this study, DHA levels in colostrum were found to be higher than in mature milk in the PL fraction. During the third trimester of pregnancy and the first two years after birth, when the brain is rapidly developing, these PUFAs are in high demand by the infant. DHA is one of the main n-3 PUFAs. This fatty acid was determined to be 0.14% in mature milk and 0.13% in colostrum. According to Jensen (1999), DHA ranges from 0% to 2.78%. Harris et al. (1984), de la Presa-Owens et al. (1996), and Fok et al. (2016) found that the amount of DHA in the milk of mothers fed with seafood increased. Arachidonic acid increased progressively from total to PL in colostrum. Levels of C20:4n-6 increased from colostrum to mature milk in total lipid. Arachidonic acid has also received wide attention because it is a precursor of prostaglandins. One of the three major long-chain PUFAs in brain grey matter is AA. Arachidonic acid was around 0.56% in mature milk, and the AA content of mothers in Nigeria (VanderJagt et al., 2000) and Kuwait (Hayat et al., 1999) was similar. Milk from Nepalese, American, and Australian mothers contained lower levels of AA (Schmeits et al., 1999). The amounts of AA were higher in colostrum than in mature milk, as determined by Sala-Vila et al. (2005). Small amounts of C18:3n-3, C20:2n-6, C20:3n-6, and C22:5n-3 were also detected in colostrum and mature milk. As found by Sala-Vila et al. (2005), C20:3n-6 levels were lower in mature milk. Total PUFA was found to be 9.99% of total lipid, 27.40% in the TAG fraction, and 19.61% in the PL fraction in colostrum. The highest n-3/n-6 ratio was also detected in the PL fraction (0.10) in colostrum.

4. CONCLUSIONS

In this study, the TAG and PL contents and compositions of human milk from lactating women were evaluated. In the study, the fatty acids profile in the milk samples taken from 25 mothers changed. It is thought that differences in breast milk may change depending on factors such as the mothers' age, genetic constitution, illnesses, culture, and most importantly, the mother's dietary habits.

Ethical approval: All applicable national guidelines for the care of tested persons were followed.

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