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Physicochemical and biochemical parameters in milk of Serbian breastfeeding women

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Background/aim: This study was undertaken to determine the changes and relationships between some important milk constituents as well as physical, rheological, and biochemical parameters of milk obtained from Serbian breastfeeding mothers.
Materials and methods: Physicochemical and biochemical parameters and the concentrations of vitamins, uric acid, and minerals were determined during the three periods of lactation covering colostrum, transitional, and mature milk collected from 67 mothers who had a term-pregnancy.
Results: Large interindividual variations regarding many parameters were found between mothers at the same period of lactation, but the average values were mostly in the expected and recommended ranges. For some parameters, our values are quite different in relation to the milk of women from other countries or data reported by other authors.
Conclusion: Differences in vitamin and mineral contents and physicochemical and rheological characteristics of milk obtained by Serbian breastfeeding mothers compared to that of mothers from other parts of the world have been found. This paper presents the measured data of some physical parameters of human milk about which there is little information in the literature.

Key words: Human milk, vitamins, minerals, surface tension, viscosity

1. Introduction

Human milk is a complex biological fluid that contains various compounds that protect the newborn child. Endogenous antioxidants and vitamins (α-tocopherol, uric acid, vitamin C, vitamins of the B group) are essential for immunity and development of infants. For that reason, it is important to examine the contents of these components in human milk. Physical properties and parameters also indicate breast milk quality. It is known that proteins, fats, and minerals in cow milk significantly affect milk pH, natural acidity, viscosity, surface tension, refractive index, and electrical conductivity influencing fluid milk rheology. Thus, electrical conductivity of the milk depends on the soluble salt fraction, nonelectrolytes, and fat content, while surface tension is influenced by proteins, phospholipids, mono- and diglycerides, and salts of free fatty acids. The latter are formed as a result of lipolysis; therefore, surface tension could be an important parameter of the excess of the milk lipase. The refractive index also depends on total solids and casein content, while natural acidity is influenced by the total acid content (1–3). Although rheological characteristics of cow milk and dairy products have been extensively studied, documentations of these properties of human milk have been scarce. There are limited data about physical and rheological parameters of breast milk even in the most important books in the field.

Human milk composition is dynamic and varies within a diet, over periods of lactation, and between mothers and different populations. It has also been demonstrated that it is influenced by race, religion, and dietary habits; therefore, it largely varies with geographic, urban, and rural regions (2,4). Consequently, in recent years there has been an increasing interest in the estimation of breast milk quality in different geographic regions taking into account the modern lifestyle and dietary habits of the people. In order to investigate regional variations in human milk composition, the aim of this study was to provide information on vitamin and mineral contents and physicochemical and rheological characteristics of milk obtained by breastfeeding mothers in an urban region of Serbia. Breast milk of Serbian mothers was also compared to that of mothers from other parts of the world.

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2. Materials and methods

2.1. Reagents

Analytical grade standards of ascorbic acid and uric acid (Merck, Darmstadt, Germany), (±)α-tocopherol, thiamine, and riboflavin (Sigma-Aldrich, St. Louis, MO, USA) were used for the preparation of stock and working standard solutions of vitamins. Bovine serum albumin was purchased from Serva Feinbiochemica (Serva Feinbiochemica, Heidelberg, Germany). Folin–Ciocalteu phenol reagent (2 M) was obtained from Merck. Ammonium acetate, potassium carbonate, potassium ferricyanide, potassium hydroxide, and sodium hydroxide of analytical grade were purchased from Sigma-Aldrich. Hydrochloric acid, perchloric acid, orthophosphoric acid, methanol, and acetonitrile of HPLC grade were supplied by Merck.

2.2. Instrumentation

The Agilent Technologies 1200 Series apparatus (Agilent Technologies, Santa Clara, CA, USA) with DAD and FLD detectors was used for HPLC analysis. Separation and analysis were performed by using an analytical HPLC columns as follows: α-tocopherol on Restek Ultra IBD C18 (4.6 × 150 mm, 5.0 μm) (Restek, Bellefonte, PA, USA), ascorbic and uric acid on Zorbax Eclipse Plus C8 (3.0 × 150 mm, 3.5 μm) (Agilent Technologies), thiamine and riboflavin on Zorbax Eclipse Plus C18 Solvent Saver Plus column (3.0 mm × 150 mm, 3.5 μm) (Agilent Technologies).

An automatic biochemical analyzer, Beckman Coulter AU 680 (Beckman Coulter, Tokyo, Japan) with Beckman Coulter System reagents, was used for the analysis of minerals, triglycerides, cholesterol, and lipase. An Evolution 60 UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used for proteins and fat determination. The electrical conductivity was measured by a Hanna Instruments EC 215 conductivity meter (Hanna Instruments USA, Smithfield, RI, USA). For the measurement of pH, a CONSORT C830 multiparameter analyzer (Consort bvba, Turnhout, Belgium) was used. The refractive index was measured by using Abbe's refractometer and viscosity by using a capillary flow-type Ostwald U-tube viscometer.

2.3. Sample collection

Fresh milk samples were being collected from 67 healthy donors during 10 months. All the women were from the urban area, well-nourished, nonsmokers, and nonvegetarian. They were between 30 and 44 years old and had a term-pregnancy. None of them used vitamin supplements. The obtained milks from every woman were grouped into three categories based on the postpartum dates: colostrum (1–7 days), transitional (8–14 days), and mature (after 15 days) milk. All the samples were kept in a freezer at –20 °C. The procedure was approved by the Ethics Committee of the Faculty of Medicine, University of Niš, in accordance with the requirements of the TR 31060 project.

2.4. Sample preparation and HPLC analysis of vitamins and uric acid

The optimizations of the procedures for sample preparation and the analysis of α-tocopherol, riboflavin, and thiamine are described in full detail in our previous papers (5–7). The same procedures were applied to the breast milk except that all the samples were first diluted with deionized water by the volume ratio of 1:3. For the ascorbic acid and uric acid determination, modified procedures according to the literature data (8) were applied.

2.5. Determination of minerals

The analysis of the concentrations of minerals in human milk was performed on the automatic biochemical analyzer Beckman Coulter AU 680 using standardized laboratory methods. The concentrations of magnesium and calcium were measured using photometric color testing with xylidyl-blue and Arsenazo III, respectively. Sodium, potassium, and chlorides were determined by ion selective method.

2.6. Assay of total protein content, total lipid content, triglycerides, cholesterol, and lipase

The total protein content was measured by the Lowry colorimetric method (9). The total lipid content was determined by applying the Forcato et al. spectrophotometric method by measuring absorbance of the samples at 208 nm (10). Triglycerides, cholesterol, and lipase were determined by use of a Beckman Coulter AU 680 automatic biochemical analyzer. Lipase activity was expressed in U/L of milk.

2.7. Determination of physical properties

The milk samples were analyzed for pH, titratable acidity, electrical conductivity, refractive index, viscosity, and surface tension. All measurements were performed on undiluted samples at room temperature. The coefficient of viscosity was determined by the flow time of the milk and distilled water through the viscometer at 25 °C (3). The coefficient of surface tension was determined by using a stalagmometer by the drop number method. Titratable acidity (TA) and pH of milk samples were determined at the time of sampling (11). Titratable acidity was determined by titration of 9.0 mL of human milk sample with 0.1 N sodium hydroxide with a potentiometric titrator until pH 8.4. The titratable acidity is expressed as % of lactic acid and is calculated according to the following equation:

$$\% \text{ lactic acid} = (Vg \times 100 \times 0.009) / Vm, \quad (1)$$

where Vg is the volume of NaOH solution added (mL), 0.009 is the equivalent of lactic acid normality, and Vm is the volume of milk used for titration (mL) (11).

2.8. Statistical analysis

All the samples were analyzed at least in triplicate. The results are presented as the mean value ± standard deviation and were processed using SPSS 18.0 commercial software. The statistical analysis was performed using Student's t-test, ANOVA testing, and correlation analysis. The differences between means were considered significant at $P < 0.05$.

3. Results

3.1. Biochemical parameters

The contents of total proteins, lipids, albumin, lipase, triglycerides, and cholesterol in the examined breast milk samples are presented in Table 1. The concentrations of total proteins ($P < 0.001$), albumin ($P < 0.001$), triglycerides ($P < 0.05$), and cholesterol ($P < 0.001$) remarkably decreased throughout the lactation. In contrast to triglycerides and cholesterol levels, total lipids content ($P < 0.001$) and the lipase activity ($P < 0.05$) significantly increased during the period of lactation.

3.2. Vitamins and uric acid

Thiamine and riboflavin levels in all of the analyzed transitional and mature milk samples were statistically higher than in colostrum ($P < 0.001$). Additionally, the concentrations of riboflavin were twice as high as the concentrations of thiamine in all three stages of lactation. Ascorbic acid concentrations in all of the analyzed mature milks were statistically higher than in colostrum ($P < 0.001$). On the other hand, colostrum was characterized by a significantly higher amount of α-tocopherol, which was noticeably greater compared to transitional and mature milks ($P < 0.001$). The obtained levels of uric acid in mature milk were significantly lower ($P < 0.05$) than in colostrum and transitional milks (Table 2).

3.3. Macrominerals

The mean concentrations of the major elements were higher in colostrum than in transitional and mature milks (Table 3). Thus, sodium, potassium, and chlorides decreased significantly during the lactation period ($P < 0.05$), while the average concentrations of calcium remained relatively constant. On the other hand, the mean concentrations of magnesium showed lactation stage-specific differences, whereas its concentration in transitional milk was slightly lower than in colostrum or mature milk ($P < 0.05$).

3.4. Titratable acidity and physical characteristics

Table 4 shows physical parameters of the examined human milk samples. During lactation, a statistically significant decrease was observed for electrical conductivity, viscosity, and titratable acidity ($P < 0.001$). The measured values of the surface tension of collected breast milk samples remained relatively constant for the period of 2 weeks postpartum, with a tendency of slight decrease in mature milks ($P < 0.05$).

4. Discussion

The results about total proteins, lipids, albumin, triglycerides, cholesterol, and lipase confirm the fact that the composition of breast milk considerably changes during the lactation period as a result of both physiological and external factors. The total lipid content in examined transitional and mature milk samples was similar to those reported for Japanese women (12) and corresponds to the expected values (13), while for colostrum these values were lower than in milk from Japanese mothers (10.8 g/L versus 26.8 g/L). The triglycerides content in our study was considerably lower than in most western populations (33–44 mmol/L) (14), while cholesterol levels were in the expected ranges for all three lactation stages and correspond with those given by Kamelska et al. (15). The variations of protein concentrations with a lactation period from transitional to mature milk were different compared to other reports. These values were lower than those reported by Emmett and Rogers (16), Sabahelkier

Table 1. Biochemical parameters of the examined samples of human milk.

Biochemical parameter	Colostrum (Mean ± SD)	Transitional milk (Mean ± SD)	Mature milk (Mean ± SD)
Total proteins (g/L)	21.5 ± 2.1	9.8 ± 3.3 [b]	5.7 ± 2.5 [d,e]
Total lipids (g/L)	10.8 ± 3.5	25.2 ± 2.4 [b]	35.2 ± 1.9 [d,e]
Albumin (g/L)	7.7 ± 1.7	4.2 ± 1.5 [a]	3.1 ± 0.8 [d,e]
Triglycerides (mmol/L)	15.80 ± 1.37	11.08 ± 0.78 [a]	7.70 ± 1.13 [c,e]
Cholesterol (mmol/L)	1.00 ± 0.38	0.25 ± 0.13 [b]	0.10 ± 0.04 [d,f]
Lipase activity (U/L)	1561 ± 190	1880 ± 150 [a]	2341 ± 215 [c,e]

a: $P < 0.05$ colostrum vs. transitional milk, b: $P < 0.001$ colostrum vs. transitional milk, c: $P < 0.05$ colostrum vs. mature milk, d: $P < 0.001$ colostrum vs. mature milk, e: $P < 0.05$ transitional vs. mature milk, f: $P < 0.001$ transitional vs. mature milk.

Table 2. Vitamin and uric acid content of the examined human milk samples.

Compound (μg/mL)	Colostrum (Mean ± SD)	Transitional milk (Mean ± SD)	Mature milk (Mean ± SD)
Thiamine	0.051 ± 0.010	0.094 ± 0.012 [a]	0.248 ± 0.050 [c,e]
Riboflavin	0.113 ± 0.043	0.228 ± 0.035 [b]	0.453 ± 0.050 [c,d]
Ascorbic acid	12.0 ± 3.5	22.6 ± 3.7 [a]	35.4 ± 5.2 [c,d]
α-Tocopherol	12.8 ± 1.5	3.7 ± 1.4 [b]	1.1 ± 0.8 [c,e]
Uric acid	4.6 ± 0.6	3.0 ± 0.7 [a]	1.1 ± 0.4 [c,e]

a: $P < 0.05$ colostrum vs. transitional milk, b: $P < 0.001$ colostrum vs. transitional milk, c: $P < 0.001$ colostrum vs. mature milk, d: $P < 0.05$ transitional vs. mature milk, e: $P < 0.001$ transitional vs. mature milk.

Table 3. Average mineral content of the examined human milk samples.

Element (μg/mL)	Colostrum (Mean ± SD)	Transitional milk (Mean ± SD)	Mature milk (Mean ± SD)
Na	529 ± 20	299 ± 22 [b]	217 ± 25 [d]
K	764 ± 42	567 ± 15 [a]	543 ± 10 [c]
Mg	49.3 ± 10.4	34.7 ± 2.8 [a]	38.9 ± 1.6 [c]
Ca	282 ± 13	275 ± 12	264 ± 14
Cl	1030 ± 61	745.5 ± 18.7 [a]	684 ± 17 [c]

a: $P < 0.05$ colostrum vs. transitional milk, b: $P < 0.001$ colostrum vs. transitional milk, c: $P < 0.05$ colostrum vs. mature milk, d: $P < 0.001$ colostrum vs. mature milk.

Table 4. Physicochemical parameters of the examined human milk samples.

Parameter	Colostrum (Mean ± SD)	Transitional milk (Mean ± SD)	Mature milk (Mean ± SD)
Electrical conductivity (mS/cm)	1.89 ± 0.15	1.55 ± 0.10 [a]	1.33 ± 0.10 [c]
Refractive index	1.3510 ± 0.0013	1.3494 ± 0.0010	(1.3482–1.3490) ± 0.0005
Dynamic viscosity ($Pa\ s \times 10^{-3}$, centipoises)	2.3988 ± 0.0280	1.4442 ± 0.0301 [b]	1.3579 ± 0.0620 [d]
Surface tension (10^{-3} N/m)	47.7314 ± 1.1752	47.6617 ± 0.9881	39.3813 ± 3.8448 [c,e]
Titratable acidity of the fresh milk (% of lactic acid)	0.07 ± 0.004	0.05 ± 0.005	0.02 ± 0.008 [c,e]
pH	6.99 ± 0.03	7.07 ± 0.03	7.24 ± 0.07 [c]

a: $P < 0.05$ colostrum vs. transitional milk, b: $P < 0.001$ colostrum vs. transitional milk, c: $P < 0.05$ colostrum vs. mature milk, d: $P < 0.001$ colostrum vs. mature milk, e: $P < 0.05$ transitional vs. mature milk.

et al. (17), Shamsia (18), Yuen et al. (14), and Guo and Hendricks (13), while the protein content in colostrum was similar to those of other reports.

The variations in concentrations of vitamins and uric acid in milk in different periods of lactation also followed the expected values. The thiamine content in our mature

milk samples was significantly higher than in lactating women of Inner Mongolia of China (0.063 μg/mL) (19), Egyptian mothers (0.13 μg/mL) (18), Caucasian women from the United States (0.037 μg/mL) (20), Spanish women (0.066 μg/mL) (20), and the average value of 0.15 μg/mL reported by Guo and Hendricks (13). The concentration of riboflavin in mature milk was also higher than in milk from Chinese women from Inner Mongolia (0.137 μg/mL) and slightly higher compared to the average value of 0.37 μg/mL (13). The obtained results for thiamine and riboflavin in milk of the women in our study were within the acceptable and recommended limits found in most of the literature (13,16). These values of B vitamins in mature milk are in the range of adequate intake (AI) of 0.2 μg/mL for thiamin and 0.4 μg/mL for riboflavin for infants aged 0–6 months (21), which indicates good maternal intake of B vitamins during lactation. The obtained concentrations of ascorbic acid were found to be slightly below the values reported in most papers, especially for colostrum and transitional milk (13), and also below AI values of 50 μg/mL. The level of ascorbic acid in mature milk was similar to that reported by Shamsia for Egyptian women (18). The variation in concentrations of α-tocopherol is in contrast to the findings of Shi et al. (19), who reported no variations in α-tocopherol during lactation, but is in agreement with the values reported by Emmett and Rogers (16). The average α-tocopherol content in our colostrum and mature milk was lower compared to German women (22 μg/mL and 5.7 μg/mL, respectively) and in Sudan (9.84 μg/mL of α-tocopherol in mature milk), but slightly higher than in milk from French women (1.14 μg/mL of α+γ-tocopherol in mature milk) (20). The α-tocopherol concentrations in our transitional and mature milk were lower than the AI values for infants from 0 to 6 months (5 μg/mL). The concentrations of uric acid are consistent with the results of Atkinson et al. (22). The contents of ascorbic and uric acid (both water-soluble endogenous antioxidants) inversely vary during the nursing period.

The mineral content of human milk is influenced by the nutritional status of a mother (13); therefore, large interindividual variations were registered. The values for calcium, magnesium, and potassium are in the recommended ranges of AI for infants from 0 to 6 months (256 μg/mL, 38.5 μg/mL, and 515 μg/mL respectively), while sodium and chlorides levels were significantly higher (AI is 155 μg/mL for sodium and 230 μg/mL for chlorides (21)), which indicates higher salt intake.

The electrical conductivity of the examined milk samples from Serbian women was within the range given in the literature (2) and that reported by Kermack (150 to 320×10^{-5} ohm^{-1} cm^{-1} for milk in mothers with adequate lactation, i.e. 1.5–3.2 mS/cm) (23). The results obtained in our work indicate that dynamic viscosity declines during lactation, which corresponds to the total protein decrease. Contrary, a negative correlation between lipid content and viscosity was observed (r = –0.73). Furthermore, we found a slight decrease of the surface tension during lactation, which could be related to a total protein decrease and a lipid content increase. For the refractive index (RI) a statistically significant difference between lactation periods was not noticed. Our results are similar to the findings of other authors, who reported that human milk has a higher RI than cow milk (24). In addition, the RI values of our samples showed a very slight decline during lactation and approached the RI for cow milk.

Titratable acidity (TA) and pH are a measure of the milk's natural acidity. Based on the experimental data, a positive correlation between TA and total proteins (r = 0.84) and a negative correlation between pH and total proteins (r = –0.68) were found. Accordingly, lower pH and higher TA values for colostrum can be explained due to its high protein content. The pH values of fresh mature and transitional milk obtained from our volunteers were higher than those reported by Neville and Jensen (pH of mature milk: 6.57–6.85) (2), Shamsia (average pH: 6.89 for the milk of Egyptian women) (18), and Yamawaki et al. (pH is from 6.6 to 6.4 for the milk of Japanese mothers) (12). On the other hand, our values were similar to those given by Hibberd (pH 7.0–7.2) (25) and those for Sudan as reported by Sabahelkier et al. (pH 7.2) (17). The values of pH for colostrum are similar to those for Japanese women (pH 6.9) (12), but they are different from those reported by Hibberd et al. (pH 7.4–7.6) (25). The titratable acidity of fresh mature and transitional milk was lower than that reported by Shamsia (average TA of 0.072% lactic acid for Egyptian women) (18) and Sabahelkier et al. (TA of 0.13% lactic acid for Sudan) (17), which indicates a lower total acid content.

In conclusion, data about vitamin concentrations, particularly those with antioxidative and structural roles, as well as physicochemical parameters are important for the estimation of breast milk quality. The results about vitamins, minerals, proteins, and lipids content as well as physical parameters of milk of the women in this study are mostly in agreement with the average values found in the literature. However, the content of some components in milk from Serbian women was different from the values obtained in other parts of the world. The paper also deals with important physical and rheological characteristics of human milk, about which few papers have been published so far. The obtained results suggest that physical and rheological parameters could be useful for the estimation of the general quality of breast milk.

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