



Composition of the mother's milk I. Protein contents, amino acid composition, biological value. A review

J. Csapó^{1,2}

email: csapo.janos@ke.hu

Sz. Salamon¹

email:

salamonszidonia@sapientia.siculorum.ro

¹Sapientia–Hungarian University of Transylvania,
Csíkszereda Campus, RO-530104, Libertatii 1., Miercurea-Ciuc

²University of Kaposvár,
Faculty of Animal Science,
Guba S. u. 40, H-7400 Kaposvár, Hungary

Abstract. The authors have analysed protein contents, protein fractions, free amino acid and total amino acid contents of the mother's colostrum and mother's milk in comparison with the newest publications. They have established that there was no united picture of the different effects on protein contents of the mother's milk. Protein contents of the colostrum of well-nourished mothers were found 6.0%, whereas those of underfed ones 4.5%. Some argue that there is a significant positive relationship between protein contents of food and daily protein intake, as well as protein contents of the mother's milk. Some researchers were found the protein contents of the underfed mothers' milk to be lower, while others found no difference in true protein contents of the milk of underfed and appropriately fed mothers (0.8–1.0%), and more could not

Key words and phrases: mother's milk, protein content, amino acid composition, biological value, NPN-content

evidence a difference in mothers of different nationality. Completed the mother's nutriment with protein, in a part of the experiments protein contents of the mother's milk increased, whereas others have reported reducing protein contents when completing with protein.

Concerning the protein fractions, casein contents of the colostrum were measured to be 3.8 on average; while those of the mature milk 5.7 g/dm³; which values were for the β -casein 2.6 and 4.4 g/dm³, and for the κ -casein 1.2 and 1.3 g/dm³. α -lactalbumin contents were found to be 3.62 and 3.26 g/dm³; lactoferrin contents 3.5 and 1.9; serumalbumin contents 0.39 and 0.41; immunoglobulin A contents 2.0 and 1.0; immunoglobulin M 0.12 and 0.20; and immunoglobulin G 0.34 and 0.05 g/dm³ in the colostrum and the mature milk, respectively.

NPN contents of the mother's milk were measured to be significantly higher (25% in total protein %) than those of the cow's milk (5%), with main component being the urea and free amino acids. It has been established that the total free amino acid provide only 2% of the requirements of a newborn baby, in nutritional respect it is an important fraction as it is easily utilizable for the synthesis of the nerve tissue and the neurotransmitters. Free amino acids are very important for the afterbirth development; especially taurine, serine, glutamic acid and glutamine, which give a considerable portion of the total free amino acids. Taurine was found to be essential for the development of the newborn, as taurine production from cysteinesulfonic acid is rather restricted due the limited activity of the cysteinesulfonic acid decarboxylase enzyme. Taurine takes part in the conjugation of the bilious acids and has a significant role in the formation of the retinal receptors. Serine has an important role in the casein synthesis, as well as it is a precursor of neuroactive substances, and a component of the biosynthesis of phospholipids. High concentration of glutamic acid in the milk can be useful, as glutamic acid has a key position in the amino acid metabolism, and converted into α -ketoglutaric acid it can enter the tricarboxylic acid cycle. By analysis the amino acid composition of the mother's milk protein it was established that around 20% of it is glutamic acid, whereas it contains in the smallest amount histidine, cysteine and methionine. Proportion of the essential amino acids is around 42%, which abundantly cover the requirements of the newborns. Some have established a relation between essential amino acid contents of the milk protein and the essential amino acid contents of the food, while others deny the existence of such a relation. Cysteine/methionine ratio of the mother's milk is higher than that of cow's milk, the amount of phenylalanine and tyrosine is lower, due to the higher proportion of the whey proteins.

1 Non-protein nitrogen content

According to Emmett and Rogers [9] non-protein nitrogen content (NPN) of the mother's milk represents around 25% of the total nitrogen, including urea, uric acid, creatinine, free amino acids, amino alcohols, peptides, hormones, nucleic acids and nucleotides. Their significance is not fully clarified, but some of them contribute to the development of the new-born. The non-protein nitrogen content derives mostly from the blood of the mother and during the lactation shows no considerable change. Various beneficial effects are attributed to non-protein nitrogen, such as e.g. the epidermic growth factor.

Carratu and et al. [4] examined nitrogen-containing components, NPN content of mother's milk in milk samples collected from 195 healthy mothers from different parts of Italy. The mothers fed their newborn babies exclusively with mother's milk for one month. The milk samples were collected at the age of one month of the babies, and previously the mothers were taught how to carry out a correct sampling. The samples were taken during the second and third feeding by hand, the drawn approx. 10 cm³ sample was collected into sterile polypropylene pots and stored at -20 °C until the analyses. Average age of mothers taking part in this experiment was 31 years, their average weight 1 month after the birth was 63 kg, average body mass of the babies were 3360 g, who consumed on the average 673 cm³ milk per day after their birth. NPN content (non-protein content) was 341 mg/dm³ (extreme values: 158 and 635 mg/dm³). NPN represented around 15% of the total nitrogen. It was established that the NPN content of the mother's milk varies between wide limits. The great individual difference can be explained by the fact that this fraction is a heterogeneous mixture of nitrogen-containing substances. Several NPN components of the mother's milk are metabolism products which enter directly from the mother's plasma into the milk gland.

According to Agostini et al. [1, 2] NPN content of the mother's milk including peptides, urea, uric acid, ammonia, creatinine, creatine, nucleic acids, carnithine, amino acids and other components, cannot be regarded as of same value as milk protein nitrogen. NPN content of the mother's milk is significantly higher than that of the cow's milk where this is only 5% of the total nitrogen, while for the mother's milk this ratio can reach even 30 mg/100 cm³.

According to examinations of Räihä [18] the mother's milk contains NPN in a relatively high concentration, up to 25% of the total nitrogen. Main component of this fraction is urea with a concentration of up to 25 mg/100 cm³, as well as creatine (3.7 mg/100 cm³), creatinine (3.5 mg/100 cm³), glucose amine (4.7 mg/100 cm³) and the free amino acids.

According to Picciano [17] NPN content of the mother's milk represents approx. 20–25% of the total nitrogen and remains relatively constant during lactation. It contains around 200 fractions, most important of them are the free amino acids, carnithine, taurine, nucleic acids, nucleotides and polyamines. It appears that some of them, e.g. taurine, purine and pyrimidine bases are essential for the newborn baby.

1.1 Urea content

According to Wu et al. [21] urea content of the Taiwanese mothers is around 30–35 mg/100 cm³. Harzer et al. [11] investigated the change of the urea content of the mother's milk in the early phase of lactation. They took from 10 mothers altogether 78 milk samples (20 cm³) in the first five weeks of lactation on the days 1, 3, 5, 8, 15, 22, 29 and 36, and refrigerated them immediately at –30 °C. No significant differences were found between the colostrum (525 µg/100 cm³), transitional milk and ripe milk (510 µg/100 cm³) in the urea content.

2 Protein content and protein fractions

Wu et al. [21] determined raw protein content of Taiwanese mothers. 264 milk samples were taken from 240 healthy mothers in different phases of lactation, and the samples were arranged in groups as per geographical position. It was experienced that crude protein content of the colostrum decreased very quickly from 2.51% to 1.25% in ripe milk.

Emmett and Rogers [9] examined protein content of the mother's milk in the colostrum, transitional milk and the ripe mother's milk, also taking into consideration the physical condition of the mother. It was established that the protein content of the colostrum (2.0%) is substantially higher than in the transitional milk (1.5%) or in ripe milk (1.3%).

Marína et al. [14] examined the composition of milk of mothers living La Plata in Argentina and established that protein content of milk was not affected by the nutrition of the mother as protein content of the milk of normal, overweight and fat mothers was 9.7; 9.1 and 9.1 g/dm³. Agostini et al. [1, 2] examining the protein content of mother's milk collected milk samples from 16 mothers on the day 4 of the lactation (colostrum), and then in its first and third month. Protein content was determined by the Kjeldahl method. The protein content decreased during the lactation from 1.93 µmol/dm³ to 1.07 µmol/dm³.

Khatir Sam et al. [12] examined protein content of milk of Sudanese mothers. The mothers used a hand-pump for the sampling with the use of which they could take around 100 cm^3 milk per person. Nitrogen content was determined by neutron activation analysis and x-ray spectrometry, respectively. Dry matter content of the milk was measured to be 10.4%, protein content was measured to be 1.23%.

Bener et al. [3] determined milk composition of 26 suckling mothers, starving during the month of Ramadan. The mothers were aged between 20 and 38 years, they were on the average 150 to 160 cm tall and weighed 60 to 70 kg. 35% of them lived in a normal house, while 65% in a villa. The samples were taken between 9.00–11.30 in the morning during the Ramadan (December 9 and January 6); the mothers were both in physically and psychically in a good condition to take the fasting during the Ramadan. None of them smoked and took any medicine during and after the experiment. Each mother broke the fasting after sunset and ate at least once more before sunrise. During the Ramadan protein content of the mothers' milk was 1.62%, after the Ramadan 1.65%, total dry matter 11.50 and 11.30%, fatfree dry matter between 6.69 and 6.70%. No significant difference in the composition could be found between the milk samples taken during and after the Ramadan. The examinations proved that the fasting has no considerable effect on the composition of mother's milk, hence on nutriment supply of the nursed baby.

Yamawaki et al. [22] examined protein content of milk of mothers from different areas of Japan. The milk samples were collected in summer between July and September, and in winter between December and March, respectively, from around 4000 women in different date of lactation (day 1 to day 365). From each mother around 50 cm^3 milk was collected in the part of the day between two nursing, the samples were stored in a deep-freez until the analysis. Along with the sampling, data were collected on smoking habits, vitamin supply, birth-weight of the newborn and that on which of the breasts the sample was taken. The samples were arranged into four groups based on the obtained information: Group A (3170 sample): mothers aged below 40 years, non-smokers, who took vitamin supplementation, and with babies with birth-weight of 2.5 kg or more. Group B (630 sample): age of women and birth-weight of the babies were the same like in group A, but the mothers smoked regularly, took vitamin-supplementation, and received drug treatment during lactation. Group C (30 sample): the only difference from group A was that the mothers were older than 40 years. Group D (200 sample): except birth-weight of the babies (less than 2.5 kg) this group was identical with group A. Mothers of group A were divided into 17 further sub-groups according to season and

lactation state as well as regions of Japan. Protein content was measured to be 1.84% between day 1 and day 5 of the lactation, 1.90% between day 6 and day 10, 1.66% between day 11 and day 20, and 1.25% between day 21 and day 89. The conclusion can be drawn that the protein content decreases significantly during the lactation.

Carratu et al. [4] examined protein content of mother's milk in milk samples collected from 195 healthy mothers from different parts of Italy. The mothers fed their babies exclusively with mother's milk during one month. Protein content and protein fractions were measured by the traditional Kjeldahl method to be on the average 1.26%, which means actually the true protein content as from total nitrogen content the NPN content was deducted, and the remainder was multiplied by 6.25. Lowest measured value was 0.71%, while the highest one 2.10%. It was established that the protein content of the mother's milk remained at a relatively constant value during lactation.

Saarela et al. [19] examined the protein content of mother's milk in the course of the first six months of lactation. Milk samples were taken from 53 mothers who bore their newborns on the average in week 40.2 of the pregnancy; from 36 mothers who gave birth to premature babies in week 31.4 of the pregnancy, from 20 mothers who had a postponed labor. First 30 cm³ of foremilk was taken, then during and at the end of the breast-feeding the same amount of aftermilk was taken, so altogether 483 cm³ of milk samples were analyzed. Out of these samples 253 samples were taken from mothers bearing at normal time, whereas 126 samples from mothers with premature birth, and further 52 foremilk and 52 aftermilk samples were analyzed from mothers bearing at normal time. Protein content of milk of mothers bearing at normal time was 1.98% in week 1 after the childbirth, which decreased at the end of the first month to 1.45%, while at the end of the sixth month to 1.14%. In the same periods protein content of milk of mothers with premature birth was 2.01; 1.51 and 1.13%. Protein content of foremilk of mothers bearing at normal time was measured to be 1.68% in week 1 after the childbirth, which decreased at the end of the first month to 1.33%, while at the end of the sixth month to 1.08%. For the same mothers the result of the aftermilk analysis was 1.64; 1.36 and 1.08%. It was established that there was no significant difference in the protein content of milk between mothers bearing at normal time and with premature birth, respectively, in the first six months of lactation, and no difference was found between the foremilk and aftermilk of mothers bearing at normal time.

Chavalittamrong et al. [5] examined protein content of milk of 153 Thai mothers in the period of between day 0 and day 270 of lactation. The milk

samples were taken 3 hours after nursing. The protein content decreased from 1.56% measured in the first week to 0.6 to 0.7% till day 180 to 207 of lactation. It was established that the protein content was not affected by the age and social status of the mother, these factors had namely influence only on the milk volume.

Manso et al. [13] examining the protein content of mother's milk by capillary electrophoresis during lactation, collected milk samples from five mothers subsequent to the sucking as well as between week 1 and week 18 of lactation. Protein content of the fatfree mother's milk was measured by the Kjeldahl method to be 0.94% which is less than one-third of the cow's milk. Protein content decreased from 1.46% measured in the first week of lactation to 0.66% until week 18 of the lactation, which means a decrease of 0.045% per week.

Murakami et al. [16] examined the protein composition of the mother's colostrum and ripe milk by two-dimensional electrophoresis. Completed their method with gel electrophoresis isoelectric focusing as well as dodecyl sulfate gel electrophoresis, around 400 fractions were detected in case of both the colostrum and the ripe milk. Out of these, the amino acid sequence of 22 main proteins was determined. No essential difference was found regarding the protein fractions between the colostrum and the ripe milk during the lactation.

Emmett and Rogers [9] examined the protein fractions of the mother's milk in the colostrum, the transitional milk and the ripe mother's milk, respectively, taking into consideration the physical condition of the mother. It was established that a considerable part of the colostrum is immunoglobulin A that with high probability does not absorb from the intestines, therefore its nutritional role is negligible, it has an important role in defeating the infections of the digestive organs, however. The mother's milk contain many proteins that are synthesized in the milk gland, such as lactoferrin, α -lactalbumin and casein that are milk-specific proteins. The other part of the proteins comes from the blood, their most typical representative is the serum-albumin. The amount of proteins forming in the milk gland decreases rapidly on the first day of the lactation, whereas concentration of the proteins deriving from the blood changes only to a small extent. Two-third of the proteins of the mother's milk belongs to the group of whey proteins, therefore the amino acid composition of the mother's milk differs substantially from that of the cow's milk, where the proportion of casein is much higher than that whey protein. In the mother's milk occur in the highest concentration α -lactalbumin, lactoferrin, immunoglobulin A, out of which lactoferrin – supporting the absorption of iron – can be found considerable amount in the ripe mother's milk, and as it travels undigested

through the intestines, its antimicrobial effect is also significant.

Montagne et al. [15] performed an investigation of the nutritive and immunological value of mother's milk in 780 samples collected from 79 mothers during the first 12 weeks of lactation. They determined α -lactalbumin, β -casein, serum-albumin, lactoferrin and lysozyme content of mother's milk. Based upon the results they described the dynamics of the mother's milk's development, which they divided into six periods. The first early phase is 1 to 4 days after the childbirth, when the colostrum contains mainly immunoglobulins, the next phase is 5 to 8 days after the childbirth when the concentration of the immunoglobulins decrease significantly, while the amount of in nutritive respect important proteins increase. Afterwards, the milk formed between the days 9 to 18 and 19 to 28 has mainly nutritive role, then between the days 28 to 49 and days 50 to 84 after the childbirth the milk is balanced, it is ideal in both immunological and nutritive respect for the growth and development of the babies nursed, and also the immunoglobulins protect them from the infections.

According to examinations of R  ih   [18] protein content milk of the different mammals varies between 1 and 20%. As protein content is utilized for the synthesis of the newborn's body, protein content of the milk has a close relation with the newborn's growing rate. Optimal if the nutriment content of milk meets the requirement of the newborn, or it is higher a little. Total protein content of the cow's milk was measured to be 3.30%, while that of the mother's milk to be 0.89%, with fairly big dispersion.

Picciano [17] measured the protein content of the mother's milk to be 1.58% at the beginning of the lactation, this value decreased slowly to 0.8–0.9% during the lactation. It was established that the immunoglobulins, the lysozyme and lactoferrin contribute to the protecting mechanism of the digestive system, they transport and absorb different vitamins, they are compounds with hormonal activity, or they have other biological effect (insulin, prolactin, various growing factors). It was also established that protein content of the mother's milk is relatively low compared to the other mammals.

Manso et al. [13] examined protein fractions of the mother's milk and their change during the lactation by capillary electrophoresis. It was established that the mother's milk contains α -s1-casein and κ -casein in a very low concentration, while β -casein is the main fraction of the mother's milk. α -lactalbumin represented 30%, while lactoferrin 7% of the total protein. Both protein fractions reached their highest concentration in the colostrum, then their amount decreased to the fourth week of lactation. Lactoferrin represented 10–25% of the total milk protein, immunoglobulin A 6–16%, whereas lysozyme 0.7–7.0%.

Especially in case of lysozyme were obtained big differences between the entities. Casein content of the mother's milk is approx. 4.6 g/dm^3 ; it can be found in the colostrum in the highest concentration, its amount decreases until the second week of lactation, increases again until the first month, then decreases minimally during the lactation. According to the examinations whey protein content is only a little higher than the casein content in the first week of lactation, their ratio was nearly 1. Afterwards the whey protein content decreased logarithmically during first four weeks of the lactation, in the further phase of lactation it was 41% while the casein content 59% of the total protein. It was established that in the colostrum the whey protein/casein ratio decreased from 90/10 to 60/40 in the early phase of the lactation, whereas in the ripe milk it is settled at 50/50. This high casein and relatively low whey protein ratio was obtained because the lactoferrin and the lysozyme were under-valued, the immunoglobulin A was not examined, and in the casein fraction also the γ -caseins were included.

Hartmann et al. [10] studying the development of the milk gland and the regulation of the milk synthesis compared the composition of the mother's milk with that of different domestic animals. It was established that the mother's milk is low in energy and is in its composition with the exception of the lactose that occurs in highest concentration in the mother's milk, extremely poor compared to the other animal species. There are considerable differences also regarding the protein fractions and other components as well that are not utilized as nutrient in the newborn's organism. In the cow's milk the dominant proteins are the α casein (2.5 g/100 cm^3) and β -lactoglobulin (0.3 g/100 cm^3), and the size of the casein micelles is double of that of the mother's milk. In the cow's milk the main immunoglobulin fraction is the immunoglobulin G (0.06 g/100 cm^3), whose concentration is 0.001 g/100 cm^3 in the mother's milk, and the lactoferrin (0.2 g/100 cm^3) as well as lysozyme (0.05 g/100 cm^3) are present in a very low concentration in the mother's milk. Concentration of the α -lactalbumin in the mother's milk is 0.3 while in the cow's milk 0.1 g/100 cm^3 . The cow's milk almost completely lacks the non-nutritive nitrogen-containing components present in considerable amount in the mother's milk.

According to examinations of R  ih   [18] a part of a few proteins is not utilized in the newborn's organism, because e.g. the immunoglobulin is very stable at a low pH and resists the attack of the proteolytic enzymes. Similarly, the role of lactoferrin is doubtful since it is stable in the presence of hydrolytic enzymes, and it can be found in the faeces of babies nursed with mother's milk in immunological active form. Beyond this, lysozyme resists digestion, its nutritional role is therefore doubtful. These three proteins occur in

a relatively high concentration in the mother's milk, their total concentration reaches 3 g/dm^3 , therefore out of the protein content of the mother's milk only around 7 g are utilized per dm^3 . Assumed that a nursed baby gets in the first month of its life around 180 cm^3 milk per body mass kilograms, this amount of milk contains around 1.3 g protein per body mass kilograms a day.

Milk proteins can be categorized as casein and non-casein proteins. The caseins are milk-specific proteins that contain phosphoric acid in ester bond, their proline content is high, they contain no cystine or only in a very low concentration, their solubility in water between pH of 4 and 5 is low. They form with calcium and phosphorus complex micelles. In the mother's milk the casein represents 30% of the total protein, its amount is 2.5 g/dm^3 , while its proportion is around 80% in the cow's milk. Examining by electrophoresis, the human casein is heterogenous, and can be decomposed into the same fractions as the one in the cow's milk. β -casein is the dominant casein fraction in the mother's milk with around half of the concentration as in the cow's milk. Its phenylalanine and methionine content differ substantially from the β -casein of the cow's milk.

After casein is removed, the remaining whey protein is approx. 20% in the cow's milk, while in the mother's milk more than 65% of the total protein. Main whey protein fractions of the mother's milk are α -lactalbumin (17%; 0.17 g/dm^3), lactoferrin (17%; 0.17 g/dm^3), lysozyme (6%; 0.05 g/dm^3), immunoglobulins (20%; 0.20 g/dm^3) and serum-albumin (6%; 0.05 g/dm^3). Beyond these, there are great numbers of other proteins present in very concentration, like enzymes, growing factors and hormones. α -lactalbumin can be found in each milk containing lactose since this protein is β -sub unit of lactose synthetase. The amino acid sequence of human milk α -lactalbumin differs at 32 places from cow's milk α -lactalbumin. Lactoferrin, the whey protein that occur in the second highest concentration in the mother's milk is a milk-specific iron-binding protein containing one polypeptide chain. In the mother's milk the iron saturation level of the protein is 2–4%, which considerably contributes to the absorption of iron present in the small intestine, to the bacteriostatic effect of the milk. The lactoferrin concentration is higher in the milk of iron-deficient women than in that of ones with normal nutrition, therefore lactoferrin protects the newborn from iron-deficiency.

Mother's milk contains lysozyme in a higher concentration than other milks. This polypeptide consisting of 130 amino acids at 49 places the same amino acids like α -lactalbumin which proves that they formed the similar way during the evolution. Lysozyme hydrolyses the glucoside bonds of the cell membrane of the microorganisms, through which it has an antibacterial role in the diges-

tive system. It resists the digestive enzymes, goes through the digestive tract, therefore its nutritional role is limited. The human milk contains 50 mg/dm^3 albumin on the average, that have a role mainly in the nutrition. Nutritional role of the immunoglobulins is doubtful.

2.1 Modification of the milk protein during heat treatment and storage

According to examinations of Rähä [18] most of the proteins denaturates when being exposed to heat, therefore the enzymatic function ceases due to heat treatment. Although the literature is very uncomplete in this respect, it can be established, however, that lysozyme and immunoglobulin A of the mother's milk hardly lose on their biological activity after being heated up to 62.5°C , but above this temperature they lose their activity quickly and completely. Achievement of the specific temperature, cooling time and duration of the treatment also affect utilization of the protein. The enzymes of the mother's milk are very sensitive to heat impact. The lipase loses quickly its activity; at 50°C it loses 50% of its activity in 5 minutes, while the sulphydryl oxidase desactivates completely at 62.5°C in 30 minutes. If heat treated mother's milk is given to a baby the growth of the bacteria is much faster as in case of the raw milk, therefore it is not practical to pasteurize the mother's milk. Freezing down and warming up, respectively, are much less dangerous than the heat treatment, still freezing damages the macrophages and the lymphocytes therefore it reduces the efficiency of the defence in the newborn.

3 Total amino acid and free amino acid content

3.1 Total amino acid content

Yamawaki et al. [22] examined total amino acid composition of milk of women from different areas of Japan. It was established that total amino acid content increases according to the protein content at the beginning between days 6 and 10 of lactation, then decreases between day 11 and day 89. Total amino acid content is between day 1 and day 5 of the lactation 1904 mg/100 cm^3 , which increased between day 6 and day 10 to 2077 mg/100 cm^3 , then it decreased between the days 11–20 to 1527 mg/100 cm^3 , and between the days 21–89 to 1183 mg/100 cm^3 . There was no significant difference in the total amino acid content between the days 1–5 and days 6–10. From the examinations it was concluded that huge differences could be experienced in the amino acid

composition of the mother's milk in the case of the Japanese mothers, therefore no region-dependent differences could be found.

According to Wu et al. [21] the total amino acid content of the milk of the Taiwanese mothers constituted 80–85% of the crude protein (41–48 mg/100 cm³) during the lactation. The ratio of the essential and non-essential amino acids remained constant during the lactation independently of that in connection with the change of the protein fractions the amino acid content decreased considerably.

Chavalittamrong et al. [5] examined amino acid composition of milk of 153 Thai mothers in the period of between day 0 and day 270 of lactation. According to their investigations the amount of the essential and non-essential amino acids were constant during the lactations, and the amino acid composition was not affected by the age and social position of the mother as these factors had influence on the milk amount only. Analyzing the amino acid composition of the Thai mothers' milk it was established that its methionine, valine and tyrosine content was somewhat lower, while its tryptophan and lysine content higher than the values found in the literature.

DeSantiago et al. [8] examined food consumption of mothers living in the countryside regions of Mexico, aged between 19 and 24 years, and the amino acid composition of blood plasma and the mother's milk. Average milk production of the mothers was 770 cm³. The composition of the food consumed was estimated by computer, based on preliminary determination of the composition of local foodstuffs. The amount of the amino acids was measured by automatic amino acid analyzer. The consumed food contained considerable amount of phenylalanine and leucine, its lysine content was rather low, however; consumed daily lysine amount was by 20% less than suggested to nursing mothers. Also the threonine and tryptophan content of the food was higher than the suggested level. Concentration of the total amino acids in the milk was 24.090 mmol/dm³, the amount of the essential amino acids was measured to be 10.222 mmol/dm³ (42%), while that of the non-essential to be 13.867 mmol/dm³ (58%). The mother's milk contained in the biggest amount glutamic acid (3.664 mmol/dm³), followed by proline (2.479 mmol/dm³), leucine (2.283 mmol/dm³) and valine (2.097 mmol/dm³). In the smallest amount histidin (0.409 mmol/dm³), cysteine (0.309 mmol/dm³) and methionine were found in the mother's milk. No significant relation could be found between the foodstuffs consumed and amino acid composition of the plasma, on the other hand there was a positive correlation between the foodstuff and amino acid content of the milk for most of the essential amino acids. It is characteristic of this food low concentration of the essential amino acids, especially that of

lysine, which can be explained by food consumption based on high volume of maize. Low lysine and high leucine content of maize is well-known, by which it is difficult to ensure the required essential amino acid level for breast-feeding mothers. High volume of glutamic acid present in the milk gland contributes to the synthesis of other amino acids in the course of the transamination. Proline is a limiting amino acid in the milk gland as the milk protein is extremely rich in proline. The proved relationship between the food and amino acid composition of the milk sheds light on that the milk protein synthesis taking place in the milk gland is jointly formed by the catabolism of amino acids and transport of the essential amino acids.

According to examinations of R  ih   [18] the amino acid composition of the mother's milk and cow's milk differs from each other due to the different protein fractions. Due to the low cystine content of the casein, the cystine/methionine ratio of the mother's milk is 2:1 that is much higher than that of the cow's milk (1:3). The cystine/methionine ratio in the mother's milk is very similar to that of the vegetable proteins. The other important difference can be experienced in the case of aromatic side chain amino acids phenylalanine and tyrosine that occur in the whey protein in a much lower concentration than in the casein. The mother's milk contains more threonine because threonine content of the whey proteins is higher than that of the casein. Dominant amino acid of the NPN content of milk is glutamic acid ($170 \mu\text{mol}/\text{dm}^3$) and taurine ($30 \mu\text{mol}/\text{dm}^3$), cow's milk lacks almost entirely the latter, while in mother's milk taurine is of the second highest concentration among the amino acids.

Davis et al. [7] compared amino acid composition of mother's milk to that of chimpanzee, gorilla, baboon and rhesus monkeys, cow, goat, sheep, llama, horse, elephant, cat and rat. Amino acids were given both in $\text{g amino acid}/\text{dm}^3$ milk and in $\text{g amino acid}/100 \text{ g total amino acid}$, and free amino acids and amino acids bonded in proteins were jointly evaluated. No data was published for tryptophan that decomposes completely during the acidic hydrolysis, for glutamine as well as asparagine that transformed into glutamic acid and aspartic acid. It was established that both mother's milk and milk of the different primates contain considerably less amino acid than that of the other animal species due the lower protein content.

Highest total amino acid content was measured in the milk of the rat ($86.9 \text{ g}/\text{dm}^3$), followed by milk of the cat ($75.7 \text{ g}/\text{dm}^3$). Total amino acid content of the milk of the sheep ($54.1 \text{ g}/\text{dm}^3$), the elephant ($37.1 \text{ g}/\text{dm}^3$), the pig ($35.0 \text{ g}/\text{dm}^3$), the cow ($33.6 \text{ g}/\text{dm}^3$), the llama ($29.6 \text{ g}/\text{dm}^3$), the goat ($25.7 \text{ g}/\text{dm}^3$) and the horse ($15.8 \text{ g}/\text{dm}^3$) was significantly higher than that of milk of

the chimpanzee (9.2 g/dm^3), the gorilla (11.5 g/dm^3), the baboon (11.5 g/dm^3) or the rhesus monkey (11.6 g/dm^3). Compared to the above animals, the total amino acid content was the lowest in the human milk with 8.5 g/dm^3 . Amino acid concentration of the mother's milk is practically identical with that of the primates. Besides the primates milk of all the animal species with the exception of the horse contain substantially more amino acid than mother's milk.

Examining the individual amino acids it can be established that in the milk of all the animal species glutamic acid was present in the highest concentration, and in the lowest concentration in the human milk (190 mg/g total amino acid), in the milk of llama, rat as well as chimpanzee was in the highest concentration ($220, 221, 221 \text{ mg amino acid/g total amino acid}$). No extreme differences were found between the species. Leucine was present in the highest concentration in the milk of the cat and the primates (cat: 118 ; human milk: 104 ; rat: 92 ; sheep: $90 \text{ mg amino acid/g total amino acid}$); leucine was followed by proline with 10 to 20% of the total amino acid (pig: 117 ; human milk: 95 ; rat: $75 \text{ mg amino acid/g amino acid}$).

In case of each examined species the essential amino acids represented around 40% , and in this respect the species did not differ significantly from each other. Lowest value was measured in the milk of the horse and the pig ($377; 379 \text{ mg amino acid/g total amino acid}$), whereas the highest one in the milk of the llama and the goat ($443; 433 \text{ mg amino acid/g total amino acid}$). Branched side chain amino acids (valine, leucine, isoleucine) represented around 20% of the total amino acids, and occurred in the milk of the primates in significantly higher concentration. The milk of the pig and the horse contained the lowest amount of these amino acids ($175; 178 \text{ mg amino acid/g total amino acid}$), while the human milk the highest one ($209 \text{ mg amino acid/g total amino acid}$).

Rat and cat milk contained the most sulfur amino acid ($50.7; 44.0 \text{ mg amino acid/g total amino acid}$), whereas proteins of the other species did not differ significantly from each other in this respect ($31.4\text{--}38.4 \text{ mg amino acid/g total amino acid}$). Milk of every animals contains more methionine ($17.0\text{--}24.8 \text{ mg amino acid/g total amino acid}$) and less cystine ($10.1\text{--}16.2 \text{ mg amino acid/g total amino acid}$) than the human milk, whose methionine content was measured to be 16.1 , cystine content to be $20.2 \text{ mg amino acid/g total amino acid}$. Comparing the amount of the other amino acids it was established that pig milk contains the most glycine, and cat milk the least (32 and $10 \text{ mg amino acid/g total amino acid}$).

The highest serine and cystine content was measured in the rat milk (85

and 26 mg amino acid/g total amino acid), the lowest one in the llama's milk (41 and 7 mg amino acid/g total amino acid). Arginine was found in the highest concentration in the milk of the cat (64 mg amino acid/g total amino acid) and the mare (60 mg amino acid/g total amino acid), whereas in the lowest concentration in the goat's milk (29 mg amino acid/g total amino acid). Glycine content of the mother's milk was measured to be 22, serine content to be 61, cystine content to be 20 and arginine content to be 36 mg amino acid/g total amino acid.

It was established that in the mother's milk within the total amino acid content glutamic acid was present in the highest concentration (190 mg amino acid/g total amino acid), and methionine was present in the lowest concentration (16 mg amino acid/g total amino acid). Out of the essential amino acids leucine (104 mg amino acid/g total amino acid) and lysine content (71 mg amino acid/g total amino acid) content was the highest, a medium value was obtained for the isoleucine (53 mg amino acid/g total amino acid), the valine (51 mg amino acid/g total amino acid) and for the threonine content (44 mg amino acid/g total amino acid), whereas histidine concentration was the lowest (23 mg amino acid/g total amino acid). The conclusion was drawn that the composition of the mother's milk is extremely similar to that of the primates and almost identical with that of the anthropoid apes.

3.2 Free amino acid content

Yamawaki et al. [22] examined free amino acid composition of milk of women from different areas of Japan. It was established that glutamic acid (10–51 mg/100 cm³), serin (0.72–1.17 mg/100 cm³), glycine (0.37–0.81 mg/100 cm³), alanine (1.30–1.88 mg/100 cm³) and cystine content (0.57–0.77 mg/100 cm³) increase, while phosphoserine (1.83–0.66 mg/100 cm³), taurine (7.00–6.56 mg/100 cm³), proline (0.77–0.24 mg/100 cm³), leucine (0.76–0.40 mg/100 cm³), tyrosine (0.42–0.24 mg/100 cm³), lysine (1.88–0.38 mg/100 cm³) and arginine content (0.86–0.21 mg/100 cm³) decrease, and the amount of aspartic acid (0.76–0.65 mg/100 cm³), threonine (0.90–0.92 mg/100 cm³), methionine (0.18–0.14 mg/100 cm³), isoleucine (0.28–0.16 mg/100 cm³), phenylalanine (0.34–0.29 mg/100 cm³), ornithine (0.12–0.10 mg/100 cm³) and histidine (0.32–0.41 mg/100 cm³) do not change substantially during the lactation. According to their investigations concentration of the free amino acids is affected considerably by the lactation, however, this effect manifest itself in different ways for the individual amino acids.

Carratu et al. [4] examined free amino acid content of milk of mothers

living in different regions of Italy after precolumn derivatization with FMOC (fluorenylmethyl chloroformate) on C18 column by reversed-phase high performance liquid chromatography. The milk samples were collected in the fourth week of the lactation, and total amount of the free amino acids was measured to be 47 mg/dm³. It was established that glutamic acid was present in the highest concentration in the mother's milk (1171 µmol/dm³), followed by serine (333 µmol/dm³), taurine (301 µmol/dm³), glutamine (259 µmol/dm³), as well as alanine (211 µmol/dm³) and aspartic acid (140 µmol/dm³). Concentration of the rest of the amino acids was lower than 130 µmol/dm³. In the lowest concentration methionin (10.4 µmol/dm³), tyrosine (20.1 µmol/dm³), phenylalanine (20.5 µmol/dm³) and arginine (20.9 µmol/dm³) were present in the mother's milk. Within the total free amino acid content proportion of the essential amino acids was 13%, and that of the non-essential amino acids was 87%.

Wu et al. [21]) determined free amino acid content of milk of Taiwanese mothers, that ranged in the one examined region 43–50 mg/100 cm³, and 40–45 mg/100 cm³ in the other one. In the colostrum the main component was the phospho-ethanolamine, while in the ripe milk the glutamic acid.

According to Agostini et al. [2] total free amino acid concentration of the mother's milk decreases significantly during the lactation, whereas the amount of glutamic acid and glutamine increases along with the time elapsed after the childbirth. Biological significance of the free amino acids of the mother's milk is that the free amino acids contributes to the formation of the utilizable nitrogen reserves of the body and to the free amino acid content of the plasma since free amino acids are absorbed more easily than the amino acids bonded in proteins.

Agostini et al. [2] determined free amino acid content of mother's milk and various pulverized and liquid baby food preparations. The amino acids were determined after precolumn derivatization with fluorenylmethyl chloroformate by reversed-phase high performance liquid chromatography, using UV and fluorescence detection. The milk samples were collected from 16 mothers on the fourth day of the lactation (colostrum), then in its first and third month. It was established that in the mother's milk glutamic acid, glutamine and taurine represent jointly more than 50% of the total free amino acids, whereas in the different milk replacing food preparations the amount of the free amino acids is only 10% of that of the mother's milk, mainly taurine and methionine. The amount of glutamic acid and glutamine is much lower in all of the food preparations than in the mother's milk, therefore with the mother's milk significantly more glutamic acid and glutamine enter the organism of the

babies. This fact explains the difference between babies breast-fed and fed with milk replacer. The role of taurine in the nourishing of the babies has not been fully clarified yet, however, due to its nervous system protecting role it is unconditionally necessary that the baby food preparation contain taurine in an appropriate concentration.

According to their examinations the free amino acid content was the lowest in the colostrum ($2204 \mu\text{mol}/\text{dm}^3$), which increased in the first month to $2679 \mu\text{mol}/\text{dm}^3$ then it reached its maximum value in the third month with $3015 \mu\text{mol}/\text{dm}^3$. Concentration of the essential amino acids in the colostrum was $306 \mu\text{mol}/\text{dm}^3$, between the first and third month of the lactation 283 and $297 \mu\text{mol}/\text{dm}^3$. Out of the non-essential amino acids glutamic acid, taurine and serine were present in the highest concentration. In the course of the lactation (colostrum, 1st month, 3rd month) concentration of glutamic acid, glutamin, glycine, and threonine increased (glutamic acid: 461, 1081, $1382 \mu\text{mol}/\text{dm}^3$; glutamine: 0, 182, $614 \mu\text{mol}/\text{dm}^3$; glycine: 67, 95, $117 \mu\text{mol}/\text{dm}^3$; threonine: 68, 82, $99 \mu\text{mol}/\text{dm}^3$), while the amount of aspartic acid, leucine and lysine decreased (aspartic acid: 44, 41, $24 \mu\text{mol}/\text{dm}^3$; leucine: 51, 40, $37 \mu\text{mol}/\text{dm}^3$; lysine: 93, 36, $33 \mu\text{mol}/\text{dm}^3$).

Concentration of prolin and taurine decreases until the first month, then stabilized for the 3rd month (proline: 32, 18, $19 \mu\text{mol}/\text{dm}^3$; taurine: 396, 278, $279 \mu\text{mol}/\text{dm}^3$), at the same time concentration of the serine increased until the first month, then remained at a constant level (19, 142, $143 \mu\text{mol}/\text{dm}^3$). Concentration of valine (66, 68, $64 \mu\text{mol}/\text{dm}^3$), phenylalanine (17, 20, $18 \mu\text{mol}/\text{dm}^3$) and isoleucine (28, 24, $25 \mu\text{mol}/\text{dm}^3$) showed no special change in the examined period of lactation. Concentration of histidine in the colostrum and in the first month was $16 \mu\text{mol}/\text{dm}^3$, which increased up to the third month to $24 \mu\text{mol}/\text{dm}^3$. Alanine and tyrosine content increased until the first month, then decreased nearly down to the value measured in the colostrum. It was established that in the mother's milk glutamic acid and glutamine represented around 50% of the total free amino acids, whereas in the cow's milk their amount is only $300 \mu\text{mol}/\text{dm}^3$, but this is still higher than in the food preparations, which is the result of the treatment of the cow's milk.

A healthy, breast-fed 4 kg-baby sucks nearly 600 cm^3 milk a day, in which amount around 120 mg free glutamic acid and glutamin are present, that means more than 30 mg per body mass kilograms. This high ratio may be explained by the fact that glutamic acid is the source of α -ketoglutaric acid, a neurotransmitter in the brain, and transamination of glutamic acid results in α -ketoglutaric acid that can enter the gluconeogenesis and is the most important energy supplier of the epidermic cells in the intestines. It is thought to

have a role in the formation of the human immune cells, in the formation of the free amino acid content of the plasma, in the growth of the epidermic cells of the small intestine and in the development of the nerve tissue, therefore supplementation of baby food preparation with glutamic acid and glutamine is indispensably necessary.

Harzer et al. [11] examined the change of the free amino acid content of mother's milk in the early phase of lactation. They took from 10 mothers altogether 78 milk samples (20 cm^3) in the first five weeks of lactation (on day 1, 3, 5, 8, 15, 22, 29 and 36), and refrigerated them immediately at -30°C . The amino acid analyses were carried out using an amino acid analyzer and ninhydrine detection. From the colostrum to the ripe milk the amount of the different amino acids changed as follows ($\mu\text{mol}/100\text{ cm}^3$): glutamic acid (36.6–100.6), glutamine (0.9–20.8), alanine (9.5–19.0), glycine (4.6–11.1), cystine (1.1–2.6) and phospho-ethanolamine content (4.2–9.9) increased, whereas serin (12.1–5.8), phosphoserine (7.9–3.6), aspartic acid (5.6–3.0), arginine (7.3–1.0), lysine (4.6–1.7), isoleucine (1.8–0.9), phenylalanine (0.9–0.6), proline (3.7–2.8), methionine (0.7–0.3), tryptophan and β -alanine content (1.2–0.3) decreased during the lactation. The biggest changes took place during the first five days of the lactation, so the differences between the transitional and the ripe milk are negligible. No significant differences were found between the colostrum, the transitional milk and the ripe milk regarding the total free amino acid ($194\text{--}248\text{ }\mu\text{mol}/100\text{ cm}^3$), taurine ($45.0\text{--}41.5\text{ }\mu\text{mol}/100\text{ cm}^3$), threonine ($5.4\text{--}6.0\text{ }\mu\text{mol}/100\text{ cm}^3$), valine ($3.5\text{--}3.8\text{ }\mu\text{mol}/100\text{ cm}^3$), leucine ($2.6\text{--}2.0\text{ }\mu\text{mol}/100\text{ cm}^3$), histidine ($1.9\text{--}2.0\text{ }\mu\text{mol}/100\text{ cm}^3$) and tyrosine ($2.2\text{--}1.8\text{ }\mu\text{mol}/100\text{ cm}^3$).

Sarwar et al. [20] established that free amino acid content of the mother's milk in case of Italian mothers was $3020\pm 810\text{ }\mu\text{mol}/\text{dm}^3$ that was very similar to the results of Agostini et al. [2] who examining free amino acid content of milk of Canadian mothers, measured in the foremilk $3397\text{ }\mu\text{mol}/\text{dm}^3$, and in the normal and the ripe milk $3069\text{ }\mu\text{mol}/\text{dm}^3$ concentration. As the free amino acid content of the cow's milk a value of $1061\text{ }\mu\text{mol}/\text{l}$ was obtained that was much lower than that of the mother's milk.

According to Emmett and Rogers [9] out of the free amino acids of the mother's milk free taurine occurring in a high concentration in the mother's milk, has a special importance. Although this amino acid is not essential for the babies, babies who were born with low body mass have a low ability to produce taurine. It was established that taurine can be found at certain places of the nervous system in a high concentration, experiments with animals also showed that lack of taurine hinders of the development of the nervous system

and the eyes. It has a role also in the fat absorption since for babies born with low body weight the taurine supplementation increased the efficiency of the fat absorption in case of feeding with mother's milk.

Sarwar et al. [20] examined free amino acid composition of transitional as well as ripe milk of human and of different primates by high performance liquid chromatography after derivatization with phenyl-thiocyanate. Small differences were found in the total free amino acid content of foremilk, transitional and ripe milk of the individual species. Within the free amino acid content in the mother's milk glutamic acid content was in the foremilk 1.412 mmol/dm^3 , in the transitional milk 1.339 mmol/dm^3 and in the ripe milk 2.157 mmol/dm^3 . These values for taurine were 0.388; 0.318; 0.331 mmol/dm^3 , whereas for alanine 0.342; 0.316; 0.294 mmol/dm^3 . Glutamic acid can be found in the highest amount in the milk of the chimpanzee (2.528 mmol/dm^3), followed by the milk of gorilla (1.787 mmol/dm^3), the cow (1.349 mmol/dm^3), elephant (1.332 mmol/dm^3), the swine (1.238 mmol/dm^3) and the horse (1.119 mmol/dm^3). In case of the chimpanzee and gorilla the free amino acid present in the second highest amount is alanine (0.349; 0.453 mmol/dm^3), while in case of the swine and horse is glycine (1.204; 0.947 mmol/dm^3). In case of marine mammals taurine was present in the highest concentration ($6.508\text{--}11.901 \text{ mmol/dm}^3$) in the milk, followed by histidine ($1.907\text{--}5.692 \text{ mmol/dm}^3$), proline (1.671 mmol/dm^3) and arginine ($0.268\text{--}0.362 \text{ mmol/dm}^3$). The most free amino acids were contained in the milk of the Antarctic seals (20.862 mmol/dm^3), elephant seals (16.393 mmol/dm^3), Californian seals (14.748 mmol/dm^3) and Australian seals (12.196 mmol/dm^3). Total free amino acid content of the pig was 7.381 mmol/dm^3 . There was no big difference in the free amino acid content of the milk of the mother (2.157 mmol/dm^3), the mare (3.913 mmol/dm^3), the elephant (3.477 mmol/dm^3), the chimpanzee (4.313 mmol/dm^3) and the gorilla (3.879 mmol/dm^3), while free amino acid content of the cow's milk was 1.061 mmol/dm^3 . Milk of every mammal species has a special free amino acid content that reflects the amino acid requirements of the species.

Cubero et al. [6] investigated the relationship between tryptophan content of the mother's milk and sleeping rhythm of the babies. Alkaline hydrolysis was applied and tryptophan was measured by HPLC. It is well-known that the organism synthesizes out of this amino acid the hormone melatonin responsible for the regulation of the sleep, and also well-known that babies nursed with mother's milk are sleeping more peacefully than the ones fed with baby food preparation. In this experiment 16 babies, aged 12 weeks, were compared, half of them was fed with mother's milk and half of them with baby food prepa-

ration. The 24-hour-sleeping rhythm was compared to tryptophan content of the milk, and concentration of 6-sulfatoxy-melatonin excreted in the urine, respectively. It was established that tryptophan content of the mother's milk showed a minimum value ($55\text{--}60\text{ }\mu\text{mol}/\text{dm}^3$) between 8 and 20 o'clock, it increased after 20 o'clock, and reached its maximum between 3 and 4 o'clock in the morning with $75\text{--}80\text{ }\mu\text{mol}/\text{dm}^3$. In the urine of the babies the 6-sulfatoxi-melatonin followed the change of tryptophan content of the mother's milk with a delay of a couple of hours. It reached its maximum at 6 o'clock in the morning with $20\text{ ng}/\text{cm}^3$, while its minimum between 4 and 8 p.m. with $2\text{--}4\text{ ng}/\text{cm}^3$. In case of breast-fed babies a temporal agreement could be found between tryptophan content of the mother's milk and 6-sulfatoxy-melatonin content present in the urine of the babies.

4 Acknowledgements

Authors are grateful to the Sapientia Foundation, Institute of Research Programs for the financial support.

References

- [1] C. Agostini, B. Carratu, C. Boniglia, E. Riva, F. Sanzini, Free amino acid content in standard infant formulas: Comparison with human Milk, *Journal of the American College of Nutrition*, 19, 4 (2000) 434–438.
- [2] C. Agostini, B. Carratu, C. Boniglia, A.M. Lammardo, E. Riva, E. Sanzini, Free glutamine and glutamic acid increase in human milk through a three-month lactation period, *Journal of Pediatric Gastroenterology and Nutrition*, 31, 4 (2000) 508–512.
- [3] A. Bener, S. Galadari, M. Gilett, N. Osman, H. Al-Taneiji, M.H.H. Al-Kuwaiti, M.M.A. Al-Sabosy, Fasting during the holy month of Ramadan does not change the composition of breast milk, *Nutrition Research*, 21, 6 (2001) 859–864.
- [4] B. Carratu, C. Boniglia, F. Scalise, A.M. Ambruzzi, E. Sanzini, Nitrogenous components of human milk: non-protein nitrogen, true protein and free amino acids, *Food Chemistry*, 81, 3 (2003) 357–362.

- [5] B. Chavalittamrong, S. Suanpan, S. Boonvisut, W. Chatranon, S. N. Gershoff, Protein and amino acids of breast milk from Thai mothers, *American Journal of Clinical Nutrition*, 34, 6 (1981) 1126–1130.
- [6] J. Cubero, V. Valero, J. Sánchez, M. Rivero, H. Parvez, A.B. Rodríguez, C. Barriga, The circadian rhythm of tryptophan in breast milk affects the rhythms of 6-sulfatoxymelatonin and sleep in newborn, *Neuroendocrinology Letters*, 26, 6 (2005) 657–661.
- [7] T.A. Davis, H.V. Nguyen, B.R. Garcia, M.L. Fiorotto, E.M. Jackson, D.S. Lewis, D.R. Lee, P.J. Reeds, Amino Acid Composition of Human Milk Is Not Unique, *American Institute of Nutrition*, January (1994) 1126–1132.
- [8] S. DeSantiago, I. Ramirez, A.R. Tovar, N. Ortiz, N. Torres, H. Bourges, Amino Acid Profiles in Diet, Plasma and Human milk in Mexican Rural Lactating Women, *Nutrition Research*, 19, 8 (1999) 1133–1143.
- [9] P.M. Emmett, I.S. Rogers, Properties of human milk and their relationship with maternal nutrition, *Early Human Development*, 49 (1997) S7–S28.
- [10] P.E. Hartmann, R. A. Owens, D. B. Cox, C. Jacqueline, J.C. Kent, Breast development and control of milk synthesis. The United Nations University Press, *Food and Nutrition Bulletin*, 17, 4 (1996) December.
- [11] G. Harzer, V. Franzke, J.G. Bindels, Human milk nonprotein nitrogen components: changing patterns of free amino acids and urea in the course of early lactation, *The American Journal of Clinical Nutrition*, 40 (1984) 303–309.
- [12] S.A. Khatir, M.O. Mustafa, F.A. EL-Khangi, Determination of protein and trace elements in human milk using NAA and XFR techniques, *Journal of Radioanalytical and Nuclear Chemistry*, 231, 1–2 (1998) 21–23.
- [13] M.A. Manso, M. Miguel, F.R. López, Application of capillary zone electrophoresis to the characterisation of the human milk protein profile and its evolution throughout lactation, *Journal of Chromatography*, 1146 (2007) 110–117.
- [14] M.C. Marína, A. Sanjurjob, M.A. Rodrigob, M.J.T. Alaniza, Long-chain polyunsaturated fatty acids in breast milk in La Plata, Argentina: Relationship with maternal nutritional status, *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 73 (2005) 355–360.

-
- [15] P.M. Montagne, M.L. Cuilliere, C.M. Molé, M.C. Béné, G.C. Faure, Dynamics of the Main Immunologically and Nutritionally Available Proteins of Human Milk during Lactation, *Journal of Food Composition and Analysis*, 13, 2 (2000) 127–137.
 - [16] K. Murakami, M. Lagarde, Y. Yuki, Identification of minor proteins of human colostrum and mature milk by two-dimensional electrophoresis, *Electrophoresis*, 19, 14 (1998) 2521–2527.
 - [17] M.F. Picciano, Nutrient composition of Human milk, *Pediatric Clinics of North America*, 48, 1 (2001) February.
 - [18] N.C.R. Räihä, Nutritional Proteins in Milk and the Protein Requirement of Normal infants. Feeding the normal infant. Palm Springs, CA, April 8–11. (1984) 136–141.
 - [19] T. Saarela, J. Kokkonen, M. Koivisto, Macronutrient and energy contents of human milk fractions during the first six months of lactation, *Acta Padiatrica*, 94 (2005) 1176–1181.
 - [20] G. Sarwar, H.G. Botting, T.A. Davis, P. Darling, P.B. Pencharz, Free amino acids in milks of human subjects, other primates and non-primates, *British Journal of Nutrition*, 79 (1998) 129–131.
 - [21] C.T. Wu, C.C. Chuang, B.H. Lau, B. Hwang, M. Sugawara, T. Idota, Crude protein content and amino acid composition in Taiwanese human milk, *Journal of Nutritional Science and Vitaminology*, 46, 5 (2000) 246–251.
 - [22] N. Yamawaki, Kan-no T. Yamada, T. Kojima, T. Kaneko, A. Yonekubo, Macronutrient, mineral and trace element composition of breast milk from Japanese women, *Journal of Trace Elements in Medicine and Biology*, 19, 2–3 (2005) 171–181.

Received: August, 2009