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The effect of microwave pasteurization on the composition of milk

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Abstract. Free amino acid and total amino acid content, water soluble vitamin content (vitamin C, B_1, B_2, B_6 and B_{12}) and utilizable lysine, lysinoalanine and hydroxymethyl furfurol content of milk were examined after the samples were pasteurized using different heat treatment.

They analyzed the effect of microwave treatment on the amino acids, free amino acid content and biological value compared to the conventional heat treatment technology. In was established that the two applied heat treatments caused practically no change in the amino acid composition of the milk protein neither in case of the essential nor in the case of the non-essential amino acids. The total free amino acid content of the raw milk (20.67 mg/100 g milk) reduced in the milk pasteurized in the traditional way to 8.02, whereas in the microwave pasteurized milk to 8.96 mg amino acid/100 g milk.

Key words and phrases: milk composition, mild pasteurization, microvawe pasteurization

They established, that after mild pasteurization, vitamin C content of milk hardly changed while during microwave pasteurization it decreased to less than its third value. Comparing the composition of raw milk with that of milk pasteurized using the two heat treatment methods, there was no important differences between the four vitamins B. Raw milk and milk pasteurized traditionally and by microwave contained hydroxymethyl furfurol not even in traces, and lysinoalanine content remained for all the three samples below the quantification limit. The utilisable lysine content of the raw milk and the two pasteurized milks was almost the same.

1 Introduction

Prior to the human consumption with the exception of some special dairy products milk has to be pasteurized in order to kill the pathogenic microbes. Pasteurization should be carried out by minimizing the effect on the composition and organoleptic properties of the raw milk. Beside the conventional pasteurization procedures recently the microwave treatment has been used.

In the food industrial practice one of the application fields of the microwave technique is the enhancement of the microbiological safety of the products by pasteurization. According to Pozar [8], during the microwave processes mainly the frequency of 2450 MHz, and in some cases the frequency of 915 MHz have been used. The microwave pasteurization is a promising method as based on the experiences the foodstuffs are damaged less than during the conventional heat treatment, due to the short treatment and radiation time [4, 9, 10, 11]. Sieber et al. [9] when treated milk with microwaves could not detect health-damaging effects. Özilgen and Özilgen [7] examined the kinetics of killing of *Escherichia Coli* by heat during microwave pasteurization, and found that the microwave treatment was applicable at lower temperatures both for pasteurization and sterilization. One of the greatest advantages of the method over the conventional heat treatment is that the product can be treated also when it is already in a closed packaging and by this the product can be stored for a significantly longer time even without added preservatives.

The aim of our research was to investigate if there is a difference between the conventional and microwave pasteurization regarding their effect on the composition of milk.

2 Material and methods

2.1 Milk samples examined

The examined raw milk was obtained from a dairy company in Hargita county, Romania. The normal (mildly) pasteurized milk was obtained by a heat treatment at 72 °C for 40 sec. In case of microwave pasteurized milk samples the milk was pre-heated to 63 °C in a plate heat-exchanger, and was heated to 68 °C by treatment with microwave of 2.45 GHz frequency and was kept at this temperature for 40 sec. The experimental pasteurization equipment consisted of three ALASCA household microwave ovens that were connected into cascade with the ovens placed above each other. Inside of each oven a glass spiral was placed horizontally, that exited the instrument on the rear side. The spiral had an inner diameter of 18 cm, the glass tube had an ID of 20 mm. The three spiral tubes were connected into cascade by flexible tubes. The microwave pasteurization equipment had a capacity of 200 dm^3/h. The raw milk had a fat content of 3.65%, fat free dry matter content of 8.77%, density of 1.028 g/dm^3, total CFU of 2,600,000/cm^3, and *E. coli* number of 1,000/cm^3. After microwave pasteurization at 68 °C total CFU reduced to 1,200/cm^3, no *E. coli* was found, while all the other parameters remained unchanged. The experiments were repeated three times, and the three milk samples from each experiment were analysed.

2.2 Pretreatment and analysis of the samples

Pretreatment of the samples. After sampling the milk samples were cooled down to –25 °C immediately. Sample preparation and analysis were carried out at the Department of Food Science of the Sapientia Hungarian University of Science of Transylvania, Csíkszereda Campus.

The samples were centrifuged at 8,000 g for 15 min in order to remove the cellular elements, then the milk was defatted. Subsequently, to 5 cm^3 of the sample 5 cm^3 of 50% trichloroacetic acid was added and left standing for 20 min. The precipitate was centrifuged for 10 min at 10,000 g [1]. The supernatant was separated, and its vitamin, free amino acid and free D-amino acid content were determined.

Determination of the amino acid composition of milk samples. The total amino acid content was determined after protein hydrolysis [5]. The amino acid analysis was carried out using an INGOS AAA400 amino acid analyzer, on an Ostion Lg ANB cation-exchange resin (35 cm×0.37 cm sized

glass column). Absorbance of the ninhydrin derivatives of the amino acids was measured at the wavelengths of 440 and 570 nm.

During the determination of the free amino acid and free D-amino acid content, derivatization and the analysis were performed using a Varian Pro Star HPLC apparatus. For data collection and evaluation Varian Star 6.0 software was used.

From the free amino acids cyclic derivatives were formed with o-phthaldialdehyde (OPA) and 2-mercaptoethanol [2]. Separation was carried out on a 150×4 mm Supelcosil-C18 column using a two-component (methanol and sodium acetate buffer) gradient system.

From the amino acid enantiomers diastereomer pairs were formed with o-phthaldialdehyde (OPA) and 1-thio-β-D-glucose tetraacetate (TATG) [3]. The separation was carried out on a 125×4 mm Superspher-C8 analytical column using a three-component (methanol, phosphate buffer and acetonitrile) gradient system.

Flow rate was 1 cm^3/min in both cases. The derivatives were detected using a fluorescent detector (excitation wavelength: 325 nm, emission wavelength: 420 nm).

Determination of the vitamin content of milk samples. Separation of vitamins C and B was carried out by reversed-phase HPLC on a Supercosil (C18) column (150×4 mm id). The HPLC system consisted of 2 Varian ProStar 210 pumps, a Varian ProStar 320 UV detector and a Varian ProStar 363 fluorescent detector. Flow rate was 0.8 cm^3/min.

Composition of the mobile phase: For determination of vitamins B: 50:50% mixture of methanol and phosphate buffer. For determination of vitamin C: 10:90% mixture of acetonitrile and acetic acid (0.4%).

Isocratic analysis was performed with a 20 µl injection volume and detectation at 254 nm. Before injection samples were filtrated on a 0.20 µm Millipore (Millipore, Milford, MA, USA) filter. The results were recorded and evaluated with a Varian ProStar 6.0. software.

HMF was also determined by HPLC, using the Varian Pro Star HPLC apparatus, on a Supelcosil C18 column (150×4.6 mm id) and the Pro Star 320 UV-VIS detector. A two-component eluent consisting of acetonitrile and water (5:95, v/v) was used. Flow rate was 1 cm^3/min. HMF was detected at 284 nm.

Utilizable lysine content was determined after derivatization with 2,4-dinitro-1-fluorobenzene (DNFB). Dinitrophenyl-ϵ -amino-lysine (DNP lysine) was determined using amino acid analyzer (INGOS AAA). Lysinoalanine was deter-

mined by the INGOS AAA amino acid analyzer, under conditions similar to those of the normal amino acid analysis. Lysinoalanine elutes after tyrosine and phenylalanine, and before the basic amino acids.

3 Results

3.1 Total amino acid content of milk samples

Amino acid content of the raw milk, the conventionally pasteurized milk (normal milk) and microwave pasteurized milk (MW milk) is shown in *Table 1.*

Table 1: Amino acid content of milk samples heat treated by different manner (n=5)

Amino acid	**Milk samples**					
	Raw milk		**Milk pasteurized**			
			by mild heat treatment		**by microwave heat treatment**	
	g AA/ 100 g milk	g AA/ 100 g protein	g AA/ 100 g milk	g AA/ 100 g protein	g AA/ 100 g milk	g AA/ 100 g protein
Asp	0.216	6.8±0.28	0.207	6.9±0.35	0.212	6.8±0.37
Thr	0.124	3.9±0.32	0.118	3.9±0.29	0.123	4.0±0.36
Ser	0.165	5.2±0.26	0.158	5.3±0.25	0.159	5.1±0.30
Glu	0.694	21.8±0.22	0.650	21.6±0.28	0.669	21. 6±0.24
Pro	0.376	11.8±0.31	0.343	11.4±0.36	0.356	11.5±0.33
Gly	0.058	1.8±0.29	0.055	1.8±0.32	0.058	1.9±0.26
Ala	0.101	3.2±0.36	0.098	3.3±0.31	0.100	3.2±0.34
Cys	0.021	0.7±0.20	0.022	0.7±0.27	0.023	0.7±0.24
Val	0.185	5.8±0.25	0.173	5.8±0.32	0.180	5.8±0.28
Met	0.097	3.0±0.30	0.090	3.0±0.21	0.090	2.9±0.26
Ile	0.154	4.8±0.23	0.146	4.9±0.33	0.140	4.5±0.28
Leu	0.284	8.9±0.28	0.265	9.0±0.24	0.276	8.9±0.31
Tyr	0.130	4.1±0.19	0.127	4.2±0.25	0.132	4.3±0.26
Phe	0.140	4.4±0.22	0.135	4.5±0.28	0.139	4.5±0.31
His	0.086	2.7±0.25	0.079	2.6±0.21	0.080	2.6±0.25
Lys	0.232	7.3±0.30	0.223	7.4±0.24	0.236	7.6±0.28
Arg	0.073	2.3±0.31	0.070	2.3±0.25	0.078	2.5±0.22

After a statistical analysis of the results no significant difference could be established between the amino acid content of the raw milk and the milk pasteurized in the two manners ($P \leq 0.05$). Thus, it can be concluded that

the two kinds of heat treatment practically did not cause any change in the amino acid content of the milk regarding the essential and non-essential amino acids. Change in the ammonia content was also minimal: it was 0.047% for the conventionally pasteurized milk and 0.048% for the microwave pasteurized milk, which was practically identical with the ammonia content of the control.

In *Table 1* the results can be seen also in g amino acid/100 g protein unit which shows the proportion of the individual amino acids in percentage of the milk protein and gives information on the quality of the milk protein. As the amino acid composition expressed in g amino acid/100 g sample hardly changed due to the different treatments, and the total amount of amino acids was nearly the crude protein content for all the three samples, therefore no difference was found in the amino acid composition of the protein between the three milk samples. It can be concluded that the heat treatment we applied did not affect the amino acid content of the milk (g amino acid/100 g sample), and did not influence the amino acid composition of the protein (g amino acid/100 g protein) and the biological value of the protein. Calculating the biological value of milk protein according to Morup and Olesen [6] 81.2 was obtained for the control milk sample, 80.9 for the conventionally pasteurized milk, while 80.8 for the microwave pasteurized milk. These results show that the heat treatment we applied had no effect at all on the biological value of the milk protein.

3.2 Free and free D-amino acid content of milk samples

Table 2 shows the free amino acid content of raw milk and the pasteurized milks in mg amino acid/100 g milk, as well as percentage of free amino acids.

Looking at the free amino acids different conclusions are obtained than in case of the total amino acid content. Total free amino acid content of the raw milk was measured to be 20.67 mg/100 g milk. This value reduced to 8.02 mg amino acid/100 g milk for the traditionally pasteurized milk and to 8.96 mg amino acid/100 g milk for the microwave pasteurized milk, respectively. Among the amino acids the amount of phenylalanine, histidine, leucine, lysine, methionine, valine, aspartic acid, glutamic acid, proline and tyrosine decreased substantially and these differences could be also confirmed statistically. Though some differences were detected in case of the means of isoleucine, threonine, alanine, arginine, cystine, glycine and serine these differences did not proved to be significant.

The amount of phenylalanine, histidine, leucine, lysine, methionine, valine, aspartic acid, proline and tyrosine decreased considerably, while that

of isoleucine, threonine, alanine, arginine, cystine decreased to a less extent, while some increase was obtained for glycine and serine.

Table 2: Free amino acid content of differently treated milk samples (n=5)

Amino acid	Milk samples					
	Raw milk		Milk pasteurized by mild heat treatment		Milk pasteurized by microwave heat treatment	
	mg AA/ 100 g milk	%	mg AA/ 100 g milk	%	mg AA/ 100 g milk	%
Asp	$1.66^{a}\pm0.324$	8.0	$0.41^{b}\pm0.182$	5.1	$0.46^{b}\pm0.277$	5.1
Thr	$0.14^{a}\pm0.113$	0.7	$0.09^{a}\pm0.125$	1.1	$0.07^{a}\pm0.110$	0.8
Ser	$0.07^{a}\pm0.042$	0.3	$0.16^{a}\pm0.037$	2.0	$0.08^{a}\pm0.025$	0.9
Glu	$7.07^{a}\pm0.456$	34.2	$4.75^{b}\pm0.315$	59.2	$5.15^{b}\pm0.422$	57.4
Pro	$4.23^{a}\pm0.337$	20.5	$0.14^{b}\pm0.091$	1.7	$0.23^{b}\pm0.113$	2.6
Gly	$0.33^{a}\pm0.234$	1.6	$0.74^{a}\pm0.421$	9.2	$1.01^{a}\pm0.248$	11.3
Ala	$0.50^{a}\pm0.200$	2.4	$0.28^{a}\pm0.112$	3.5	$0.37^{a}\pm0.154$	4.1
Cys	$0.06^{a}\pm0.032$	0.3	$0.01^{a}\pm0.014$	0.1	$0.02^{a}\pm0.018$	0.2
Val	$1.04^{a}\pm0.066$	5.0	$0.14^{b}\pm0.009$	1.7	$0.18^{b}\pm0.011$	2.0
Met	$0.27^{a}\pm0.098$	1.3	$0.01^{b}\pm0.008$	0.1	$0.03^{b}\pm0.016$	0.3
Ile	$0.14^{a}\pm0.075$	0.7	$0.05^{a}\pm0.030$	0.6	$0.04^{a}\pm0.024$	0.4
Leu	$0.54^{a}\pm0.188$	2.6	$0.04^{b}\pm0.022$	0.5	$0.06^{b}\pm0.019$	0.7
Tyr	$1.40^{a}\pm0.323$	6.8	$0.07^{b}\pm0.034$	0.9	$0.08^{b}\pm0.038$	0.9
Phe	$1.03^{a}\pm0.218$	5.0	$0.08^{b}\pm0.028$	1.0	$0.08^{b}\pm0.021$	0.9
His	$0.45^{a}\pm0.107$	2.2	$0.14^{b}\pm0.083$	1.7	$0.17^{b}\pm0.075$	1.9
Lys	$0.76^{a}\pm0.026$	3.7	$0.20^{b}\pm0.012$	2.5	$0.20^{b}\pm0.017$	2.2
Arg	$0.10^{a}\pm0.051$	0.5	$0.10^{a}\pm0.042$	1.2	$0.10^{a}\pm0.063$	1.1

$^{a\,b}$ Averages in one row with common superscript do not differ ($P \leq 0.05$)

In case of the non-essential amino acids the biggest decrease was experienced for proline and aspartic acid, at the same time in case of glutamic acid which is present in the highest amount, the change is relatively negligible. Summarized, it can be said that the essential amino acid content of the raw milk with the exception of arginine decreases considerably, and also the non-essential amino acids – except glycine and serine – decrease due to the heat treatment.

As the raw milk sample was immediately frozen down along with the heat-treated samples, it could be excluded that the higher free amino acid content was due to the souring of the raw milk, the growth of lactobacilli. It can be assumed that this huge decrease in the amount of free amino acids is due to the technological intervention only. There are two possibilities regarding the changes occuring during the heat treatment. As free amino acids are significantly more reactive than those bound in the peptide chain, it is possible that they reacted with milk sugar during the heat treatment resulting in Maillard reaction products. It is also supported by the fact that there was a decrease of 4 to 5% in the utilizable lysine content due to the heat treatment. This minimal decrease can be a result of the transformation of the free lysine and not of lysine bound in protein. For the rest of the amino acids there is no experimental evidence supporting this theory.

The other possibility may be that the whey proteins coagulated during the heat treatment could absorb the free amino acids on their surface so strongly that we could not remove them from the surface in the course of the determination. In fact, this latter possibility would be the most useful for the practice since no free amino acids would remain in the whey but they would enhance the biological value of the dairy product by being bound on the surface of the protein.

In the course of the examination of the free D-amino acids we could detect D-aspartic acid, D-glutamic acid and D-alanine in the milk samples. Amino acids other than these ones could not be detected at the sensitivity level of our HPLC system. The amount of D-amino acids practically did not change due to the different heat treatments. The amount of D-Asp varied from 0.016 mg/100 g milk in milk pasteurized conventionally to 0.017 mg/100 g milk, to 0.018 mg/100 g milk in the microwave pateurized milk, D-Glu changed from 0.053 mg/100 g milk to 0.052 and 0.054 mg/100 g milk, whereas D-Ala from 0.043 mg/100 g to 0.049 and 0.046 mg/100 g milk. Thus, it can be concluded that during the pasteurization with the applied temperature and time combinations did not change the D-amino acid content of the raw milk. In this respect between the two heat treatment procedures cannot be distinguished.

3.3 Vitamin B and C content of milk

We chose these vitamins because both vitamin C and vitamins B are very sensitive to the technological interventions, especielly on heat treatment. Ascorbic acid and vitamin B content of the raw milk and the differently heat treated milk are shown in the *Table 3.*

As it can be seen vitamin C content hardly changed due to mild pasteurization, however, it reduced to less than its one-third. This is very surprising because the microwave pasteurization was carried out at a lower temperature (68 °C) than the conventional (72 °C) therefore it appears that in the microwave pasteurization not only the temperature but also the energy of the microwave could play a role in the deterioration of the vitamin C content.

Table 3: Vitamin C and B content of raw milk, and milk samples after mild and microwave pasteurization (n=5)

Vitamin contenet mg/dm^3	**Milk samples**		
	Raw milk	**Milk pasteurized by milh heat treatment**	**Milk pasteurized by microwave**
Vitamin C	$22.71^{a}\pm1.273$	$22.11^{a}\pm1.106$	$6.25^{b}\pm0.825$
Vitamin B_1	$0.39^{a}\pm0.083$	$0.27^{a}\pm0.075$	$0.26^{a}\pm0.062$
Vitamin B_2	$1.81^{a}\pm0.324$	$1.63^{a}\pm0.193$	$1.65^{a}\pm0.215$
Vitamin B_6	$0.52^{a}\pm0.153$	$0.48^{a}\pm0.172$	$0.46^{a}\pm0.144$
Vitamin B_{12}	$0.004^{a}\pm0.0021$	$0.004^{a}\pm0.0014$	$0.003^{a}\pm0.0012$

^{ab} Averages in one row with common superscript do not differ p≤0,05.

Out of vitamins B we measured, vitamin B_1 is the most heat sensitive, while the other three have a higher resistance against heat impact. Accordingly, vitamin B_1 content decreased by approx. 33.3% due to both pasteurization procedures. In case of vitamins B_2, B_6 and B_{12} this decrease was around 10-11%, however, it did not prove to be a significant difference.

3.4 Hydroxymethyl furfurol content of milk

The quality of conventionally and microwave pasteurized milk is determined by measuring the hydroxymethyl furfurol (HMF) content. HMF is always present when foods with high protein and sugar content are heat-treated. *Table 4* shows HMF content of the differently heat treated milk samples, sweetened condensed milk and milk powder in μg HMF/100 g sample.

Based upon the results it can be said that the raw milk, the conventionally pasteurized and microwave pasteurized milk did not contain HMF even in traces, thus, in this respect the two pasteurization procedures are equal. In

Table 4: HMF (hydroxymethyl furfurol) content of milk sample with different heat treatment, sweetened condensed milk and milk powder (μg HMF/100 g sample)

Sample		HMF content μg HMF/100 g sample
Raw milk		-
Milk pasteurized by mild heat treatment		-
Milk pasteurized by microwave		-
Sweetened condensed milk	Mean	127
Milk powder	Mean	684

order to check the suitability of the analytical method, we determined the HMF content of a commercially obtainable sweetened condensed milk and a milk powder in three repetitions.

It was established that the sweetened condensed milk contained on the average 127 μg HMF/100 g, while the milk powder contained on the average 684 μg HMF/100 g sample. Comparing these values, it was found that the milk powder contains more HMF than the condensed milk. The reason for this is that milk powder is produced at a higher temperature than the condensed milk, and the formation of the products of the Maillard reaction accelerates at a higher temperature. In the milk powder 600–700 μg HMF/100 g was found.

3.5 Utilizable lysine and lysinoalanine content of milk

Parallelly with the analysis of the HMF content of the milk samples we examined the utilizable lysine and the lysinoalanine content of the milk samples heat-treated differently. The results are shown in *Table 5.*

No lysinoalanine could be detected above the level of the sensitivity of the measurement either in the raw milk or the two heat-treated milk samples. This means that neither threonine (perhaps serine) that is very sensitive to heat treatment, nor cysteine and cystine that are sensitive to heat treatment and oxidation, decomposed considerably, as these two amino acids are the main precursors of lysinoalanine.

Utilizable lysine content of the raw milk was measured to be 0.229%, that of the conventionally pasteurized milk to be 0.217%, whereas that of microwave pasteurized milk to be 0.219%, this is not a significant difference ($P \leq 0.05$).

Table 5: Utilisable lysine and lysinoalanine content of milk samples with different heat treatment (n=5)

Component examined	Milk sample		
	Raw milk	**Milk pasteurized by mild heat treatment**	**Milk pasteurized by microwave**
Utilisable lysine content, %	0.229±0.075	0.217±0.093	0.219±0.104
Lysinoalanine content, mg/dm^3	<5	<5	<5

It can be concluded that during the heat treatment we applied the ϵ-amino group of lysine which is very sensitive to the reducing sugars and heat did not convert to such an extent that could influence its biological utilizability. The around 4–5% difference in the utilizable lysine content indicates that some Maillard reaction product formed.

From our examinations it can be concluded that the two heat treatment methods can be considered as equal in this respect, and neither of them decreased substantially the utilizability of lysine, one of the most important essential amino acids, and neither of them resulted in a considerable lysinoalanine content.

4 Summary

In the course of the experiment we examined milk samples pasteurized using different heat treatment procedures at a dairy company in Hargita County, Romania. We analyzed the effect of the microwave treatment on the components, and compared the data with those obtained in case of the traditional heat treatment.

The two heat treatments applied practically did not cause any change in the amino acid composition of the milk protein regarding. For the free amino acids a considerable difference was obtained between the raw milk and the milk samples heat-treated differently, but between the two heat treatment methods cannot be distinguished in the respect of free amino acids.

Due to mild pasteurization vitamin C content of the milk hardly changed,

while during the microwave pasteurization it reduced to less than its one-third, compared to the raw milk. In case of vitamin B_1 it was established that a loss of 30–40% can be expected, while for the other three vitamins B this decrease was only around 10%.

During the examinations we could not detect hydroxymethyl furfurol and lysinoalanine in any of the milk samples, and we could not establish a significant decrease in the utilizable lysine content due to the pasteurization procedures.

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The effect of thermic treatment conditions on the amino acid composition of soybean and maize

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Abstract. Protein is one of the most valuable components of the fodder, therefore there is a concern preserving of these components in an available form during the different treatments wherein heating is applied. The aim of the research was to investigate the influence of toasting of fullfat soybean and air-drying of corn on the amino acid profile of the products. Dried corn was sampled at two Hungarian drying plants. The corn was dried with an industrial Bábolna B1-15 type gravitational drying tower.

The influence of toasting on two sorts of fullfat soybean products (hydrothermic soy coarse and "natural" hydrothermic soy) was investigated. Soybeans were heat processed with an industrial KAHL HR-1600

Key words and phrases: thermic treatment, soybean, corn, toasting, air-drying, amino acids

type hydrothermic reactor after cracking. The amino acid analysis of samples was carried out with amino acid analyzer, moreover amino acid enantiomers were quantified with a LaChrom type MERCK-Hitachi high performance liquid chromatograph.

The D-amino acid content of the samples before and after drying did not differ significantly, not even at the applied highest temperatures (100 °C drying air temperature and 40–45 °C kernel temperature).

Pressurized steam cooking of fullfat soy did not result in significant increase of the amount of D-enantiomers or the decrease of the concentration of the L-amino acids ($P>0.05$), while the trypsin inhibitor activity (TIA) was reduced to the required level (TIA<1.5 mg/g) and the results of the urease test (ΔpH<0.2) also verified the adequate intensity of the heat treatment.

1 Introduction

Processing steps can increase considerably the amount of D-amino acids, when they include heat treatment, alkaline treatment or fermentation [5, 6, 7]. Processes with heat treatment accelerate racemization, since higher temperatures result in increasing reaction rate of the racemization according to the Arrhenius equation. In case of a given production process a concrete experiment is required in order to decide whether the degree of the conversion into D-amino acids is considerable.

When feeding a crude soybean bean without heat treatment the fodder utilization can be reduced even by 20 to 30% in the case of the monogastric species and young ruminants, as the soya contains considerable amount of antinutritive substances (eg. protease inhibitors, lectins). Some of these loss their activity when high temperature is applied, so there is a chance to lessen the unfavourable effect. There are several thermal procedures to heat treat the soybean: extrusion, puffing-up, as well as toasting, when the soybean is cooked in the presence of water steam at 115–120 °C for 15–40 min [1]. Any of these treatments are used, the working parameters (temperature, moisture content, particle size, other physical effects) should be chosen so that the level of antinutritive substances is appropriately reduced without deterioration of the nutritive value of the protein [9].

In Hungary, among cereals maize is used in the highest amount for foddering purpose. Maize needs to be dried in order to be stored safely. Drying of corn fodders is carried out by blowing-through of high temperature air, and in most of the agricultural plants so-called drying towers are used [4]. Inappropriate drying (overdrying) decreases the digestibility of the feeding stuff

proteins. Deterioration of the corn fodder during the drying can be avoided if the temperature of the drying air does not exceed 80–130 °C, and so the temperature of the seeds does not rise above 60–80 °C. If still overdrying occurs, digestibility of the proteins decreases, utilizability of amino acids reduces. To the highest degree the utilizability of lysine reduces which is especially harmful as to the nutritive value of maize, as in the zein the lysine is the primary limiting amino acid.

The effect of the above thermic treatments on the amino acid content has already been reported in a number of publications, however, according to our knowledge it has not been measured yet in what ratio the amino acids bound in proteins convert into D-amino acids during toasting of the soybean and drying of maize.

2 Material and methods

2.1 Conditions of the drying of maize and toasting of soybeans

The dried maize samples were taken partly at the drying plant of Agria Mezőgazdasági és Szolgáltató Szövetkezet (Agria Agricultural and Servicing Co-operative) in Szentgáloskér, partly at the drying plant of Kapostáj Mezőgazdasági Termelőszövetkezet (Kapostáj Agricultural Co-operative) in Zimány. Drying of the crops was performed at both sites with a Bábolna B1-15 dryer that is commonly used in Hungary. This equipment belongs to the group of the gravitational tower dryers, where the drying (and cooling, respectively) air touches indirectly the particles of the crop. On the border of the drying and the cooling zone there are sensors installed measuring the moisture content of the seeds, the corn temperature and the temperature of the drying air on both sides of the hot air channel (T_1, T_2) at a certaine height. Parameters read off from the displays during the drying and data measured in the collected samples at the Analytical Laboratory are shown in *Table 1.*

During the sampling, sample was taken from a given lot before drying, then from the dried maize continuously until the given lot exited the drier.

The hydrothermal treatment was carried out at the feeding stuff plant of Bóly Rt. (Bóly-Állomáspuszta) in Törökdomb. The whole soybeans stored in silos were carried through two rolling mills after cleaning, where the seeds were cracked into 9 to 12 pieces, then the material entered the toaster (KAHL HR-1600 hydrotermic reactor).

Table 1: Data of plant drying of maize

Conditions of drying		Place of sampling		
		Szentgáloskér	**Zimány (1)**	**Zimány (2)**
Values measured at drying				
Average temperature of the drying air (°C)				
	T_1	100	78	45
	T_2	100	83	39
Moisture content above the cooling zone (%)		20–21	13–14	12–13
Corn temperature (°C)		40–45	39–40	32–33
Values measured in the laboratory				
Moisture content before drying (%)		16.6	17.1	16.6
Moisture content after drying (%)		13.1	12.5	13.1

This autoclave with a mixing part cooks the soya in pressurized steam with an average residence time of 30 min at 120 °C. Due to the wet heat treatment most of the anti-digestive trypsin inhibitors present in the soybean becomes inactivated. During production in the first case the heat treatment is followed by milling with a hammer mill, and the product obtained is sold as "hydrothermal soya grits". In the case of the second product this last milling is omitted, and the soybean remains in cracked form, this is called "natural hydrothermic soya".

2.2 Chemical examinations

The chemical analysis of the samples were carried out at the Department of Chemistry and Biochemistry, University of Kaposvár, Faculty of Animal Science. Moisture content of the samples was determined according to the standard MSZ ISO 1442, and trypsin inhibitor activity of the soya products was determined according to the standard EN ISO 14902. In order to determine the amino acid enantiomers, after hydrolysis of the protein (6 M hydrochloric acid, 24 h, 105±1 °C) diastereomer pairs were formed from the D- and L-amino acids with o-phthaldialdehyde (OPA) and 1-thio-β-D-glucose tetraacetate (TATG) according to the method of Einarsson et al. [3], as well as Csapó et al. [2]. The analyses were performed using a MERCK-Hitachi LaChrom high-performance liquid chromatograph. The derivatives were separated on a Superspher 60 RP-8e column (125 mm×4 mm i.d.). Fluorescent signals of the diastereomers were measured (λ_{ex}: 325 nm, λ_{em}: 420 nm). The statistical evaluation was performed with SPSS for Windows 10.0 (1999) software.

3 Results

3.1 The effect of toasting on the D-amino acid content of the fullfat soya

L- and D-amino acid content of the untreated soybean as well as hydrothermally treated fullfat soybean products is shown in *Table 2.*

Table 2: Amino acid content (g/100 g dry matter) (n=5) and trypsin inhibitor activity (TIA) (n=3) of non-heat treated soya and toasted fullfat soya products

Amino acid g/100 g dry matter	Untreated soybean	Hydrothermal soya grits	Natural hydrothermic soya
L-Asp	3.90±0.10	4.20±0.70	4.10±0.20
D-Asp	0.55±0.04	0.62±0.11	0.65±0.04
L-Glu	6.60±0.20	7.00±1.30	7.10±0.50
D-Glu	0.11±0.03	0.12±0.02	0.12±0.01
L-Ser	1.68±0.05	1.76±0.36	1.78±0.13
D-Ser	0.13±0.01	0.13±0.03	0.13±0.01
L-Val	1.70±0.05	1.78±0.31	1.78±0.14
L-Met	0.25±0.04	0.26±0.04	0.28±0.03
L-Phe	1.81±0.07	1.89±0.37	1.92±0.13
D-Phe	0.08±0.02	0.08±0.01	0.09±0.01
L-Leu	2.70±0.06	2.79±0.59	2.79±0.26
D-Leu	0.27±0.01	0.26±0.06	0.26±0.03
L-Lys	1.56±0.14	1.62±0.43	1.60±0.12
D-Lys	0.03±0.01	0.03±0.01	0.04±0.01
TIA (mg/g)	17.2^{b}±0.5	1.1^{a}±0.2	1.2^{a}±0.3

Mean values without marks being in the same row do not differ (P<0.05).
^{ab} There is no difference between mean values marked with the same letters and being in the same row.

Because of the difference in the water content of the samples the analysis results are given on dry matter basis. D-amino acids found in the untreated control sample were formed probably during chemical preparation of the heat treated samples due to acidic hydrolysis of the proteins [8]. For the time being, according to our knowledge there is no chemical method available, using which this phenomenon could be excluded. If the mean values of D-amino acid content of samples taken before and after the heat treatment differ considerably,

this difference can be regarded as the increase of D-amino acids due to the heat treatment [10].

Practically no detectable change occured in the amount of either D- or L-amino acids due to cooking in the pressurized steam, whereas the activity of trypsin inhibitors reduced appropriately in both of the soya products due to the heat treatment *(Table 2).*

3.2 The effect of drying of the maize on the D-amino acid content

The amount of the examined D- and L-amino acids contained in the maize before and after the heat treatment can be found in *Table 3.*

Table 3: Amount of the examined L-and D-amino acids in the maize before and after drying (g/100g dry matter) (n=5)

Amino acid	Place of sampling					
	Szentgáloskér		Zimány (1)		Zimány (2)	
	Drying		Drying		Drying	
	before	after	before	after	before	after
L-Asp	0.66±0.05	0.68±0.03	0.64±0.04	0.65±0.01	0.70±0.04	070±0.03
D-Asp	0.079±0.006	0.081±0.004	0.077±0.005	0.079±0.002	0.095±0.005	0.093±0.006
L-Glu	1.70±0.10	1.74±0.08	1.86±0.11	1.85±0.07	1.81±0.13	1.89±0.10
D-Glu	0.026±0.002	0.027±0.001	0.030±0.003	0.030±0.001	0.031±0.002	0.032±0.002
L-Ser	0.48±0.03	0.49±0.02	0.50±0.03	0.49±0.03	0.49±0.03	0.49±0.02
D-Ser	0.040±0.008	0.043±0.009	0.034±0.01	0.039±0.01	0.068±0.008	0.065±0.007
L-Val	0.49±0.03	0.49±0.02	0.47±0.03	0.046±0.03	0.50±0.03	0.50±0.02
L-Met	0.04±0.005	0.04±0.003	0.04*±0.006	0.03*±0.002	0.04±0.005	0.05±0.01
L-Phe	0.44±0.03	0.45±0.02	0.49±0.03	0.48±0.02	0.47±0.03	0.49±0.02
D-Phe	0.26±0.01	0.23±0.02	0.17±0.02	0.16±0.04	0.21±0.02	0.21±0.02
L-Leu	1.11±0.05	1.11±0.04	1.28±0.08	1.23±0.09	1.14±0.08	1.22±0.06
D-Leu	0.10±0.03	0.11±0.008	0.15±0.01	0.14±0.02	0.16±0.01	0.16±0.008
L-Lys	0.30±0.05	0.32±0.02	0.30±0.03	0.27±0.06	0.29±0.04	0.29±0.02

Remark: Mean values marked with asterisk differ significantly ($P<0.05$).

In the samples taken before and after drying the amount of the examined D-amino acids did not differ significantly even in the case of drying at the highest temperature (100 °C air temperature, 40–45 °C corn temperature, sampling in Szentgáloskér), however, it should be kept in mind that what we measured was an average value which has importance for the practice. Layers lying closer to the surface of the seeds were exposed to a heat impact of higher degree than the inner parts, and also inside of the dryer there can be inhomogeneous

temperature distribution, so there can be centers with higher and lower heat burden.

In the amount of the measured L-amino acids there was no difference between the control and the heat-treated groups with one exception, in the second sampling (Zimány (1)) L-methionine content of the dried maize was a little lower than that of the untreated sample.

4 Summary

It can be said that the aim of the examined thermal procedures was accomplished without significant increase in the amount of D-amino acids. During toasting of the soybean the trypsin inhibitors were appropriately inactivated, and at the same time the L-amino acid content did not change. The moisture content decreased to the required level during the drying of maize without a considerable loss of amino acids.

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

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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 completting 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/dm^3; which values were for the β-casein 2.6 and 4.4 g/dm^3, and for the κ-casein 1.2 and 1.3 g/dm^3. α-lactalbumin contents were found to be 3.62 and 3.26 g/dm^3; 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/dm^3 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 significants 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 alpha-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 existance 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 cm^3 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 cm^3 milk per day after their birth. NPN content (non-protein content) was 341 mg/dm^3 (extreme values: 158 and 635 mg/dm^3). 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 cm^3.

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 cm^3, as well as creatine (3.7 mg/100 cm^3), creatinine (3.5 mg/100 cm^3), glucose amine (4.7 mg/100 cm^3) 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 cm^3. 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 cm^3) 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 cm^3), transitional milk and ripe milk (510 µg/100 cm^3) 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/dm^3. 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/dm^3 to 1.07 µmol/dm^3.

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 form 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 $30\,\mathrm{cm}^3$ of foremilk was taken, then during and at the end of the breast-feeding the same amount of aftermilk was taken, so altogether $483\,\mathrm{cm}^3$ 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 Räihä [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 αcasein (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 is 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 Räihä [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 Räihä [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 sulfhydryl 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 effienciency 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 cm^3) 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 cm^3. 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/dm^3, the amount of the essential amino acids was measured to be 10.222 mmol/dm^3 (42%), while that of the non-essential to be 13.867 mmol/dm^3 (58%). The mother's milk contained in the biggest amount glutamic acid (3.664 mmol/dm^3), followed by proline (2.479 mmol/dm^3), leucine (2.283 mmol/dm^3) and valine (2.097 mmol/dm^3). In the smallest amount histidin (0.409 mmol/dm^3), cysteine (0.309 mmol/dm^3) 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 extremly rich in proline. The proved relationship between the food and amino acid composition of the milk sheds light on that 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 Räihä [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 vegatable 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 μmol/dm^3) and taurine (30 μmol/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 g amino acid/dm^3 milk and in g amino acid/100 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 g/dm^3), followed by milk of the cat (75.7 g/dm^3). Total amino acid content of the milk of the sheep (54.1 g/dm^3), the elephant (37.1 g/dm^3), the pig (35.0 g/dm^3), the cow (33.6 g/dm^3), the llama (29.6 g/dm^3), the goat (25.7 g/dm^3) and the horse (15.8 g/dm^3) was significantly higher 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 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 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 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 mg amino acid/g total amino acid), whereas the highest one in the milk of the llama and the goat (443; 433 mg amino acid/g total amino acid). Branched side chain amino acids (valine, leucine, isoleucine) represented around 20% of the total amino acids, and occured 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 mg amino acid/g total amino acid), while the human milk the highest one (209 mg amino acid/g total amino acid).

Rat and cat milk contained the most sulfur amino acid (50.7; 44.0 mg amino acid/g total amino acid), whereas proteins of the other species did not differ significantly from each other in this respect (31.4–38.4 mg amino acid/g total amino acid). Milk of every animals contains more methionine (17.0–24.8 mg amino acid/g total amino acid) and less cystine (10.1–16.2 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 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 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 cm^3), serin (0.72–1.17 mg/100 cm^3), glycine (0.37–0.81 mg/100 cm^3), alanine (1.30–1.88 mg/100 cm^3) and cystine content (0.57–0.77 mg/100 cm^3) increase, while phosphoserine (1.83–0.66 mg/100 cm^3), taurine (7.00–6.56 mg/100 cm^3), proline (0.77–0.24 mg/100 cm^3), leucine (0.76–0.40 mg/100 cm^3), tyrosine (0.42–0.24 mg/100 cm^3), lysine (1.88–0.38 mg/100 cm^3) and arginine content (0.86–0.21 mg/100 cm^3) decrease, and the amount of aspartic acid (0.76–0.65 mg/100 cm^3), threonine (0.90–0.92 mg/100 cm^3), methionine (0.18–0.14 mg/100 cm^3), isoleucine (0.28–0.16 mg/100 cm^3), phenylalanine (0.34–0.29 mg/100 cm^3), ornithine (0.12–0.10 mg/100 cm^3) and histidine (0.32–0.41 mg/100 cm^3) 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/dm^3. It was established that glutamic acid was present in the highest concentration in the mother's milk (1171 µmol/dm^3), followed by serine (333 µmol/dm^3), taurine (301 µmol/dm^3), glutamine (259 µmol/dm^3), as well as alanine (211 µmol/dm^3) and aspartic acid (140 µmol/dm^3). Concentration of the rest of the amino acids was lower than 130 µmol/dm^3. In the lowest concentration methionin (10.4 µmol/dm^3), tyrosine (20.1 µmol/dm^3), phenylalanine (20.5 µmol/dm^3) and arginine (20.9 µmol/dm^3) 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 cm^3, and 40–45 mg/100 cm^3 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 μmol/dm^3), which increased in the first month to 2679 μmol/dm^3 then it reached its maximum value in the third month with 3015 μmol/dm^3. Concentration of the essential amino acids in the colostrum was 306 μmol/dm^3, between the first and third month of the lactation 283 and 297 μmol/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 μmol/dm^3; glutamine: 0, 182, 614 μmol/dm^3; glycine: 67, 95, 117 μmol/dm^3; threonine: 68, 82, 99 μmol/dm^3), while the amount of aspartic acid, leucine and lysine decreased (aspartic acid: 44, 41, 24 μmol/dm^3; leucine: 51, 40, 37 μmol/dm^3; lysine: 93, 36, 33 μmol/dm^3).

Concentration of prolin and taurine decreases until the first month, then stabilized for the 3rd month (proline: 32, 18, 19 μmol/dm^3; taurine: 396, 278, 279 μmol/dm^3), at the same time concentration of the serine increased until the first month, then remained at a constant level (19, 142, 143 μmol/dm^3). Concentration of valine (66, 68, 64 μmol/dm^3), phenylalanine (17, 20, 18 μmol/dm^3) and isoleucine (28, 24, 25 μmol/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 μmol/dm^3, which increased up to the third month to 24 μmol/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 μmol/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 (μmol/100 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 (194–248 μmol/100 cm^3), taurine (45.0–41.5 μmol/100 cm^3), threonine (5.4–6.0 μmol/100 cm^3), valine (3.5–3.8 μmol/100 cm^3), leucine (2.6–2.0 μmol/100 cm^3), histidine (1.9–2.0 μmol/100 cm^3) and tyrosine (2.2–1.8 μmol/100 cm^3).

Sarwar et al. [20] established that free amino acid content of the mother's milk in case of Italian mothers was 3020±810 μmol/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 μmol/dm^3, and in the normal and the ripe milk 3069 μmol/dm^3 concentration. As the free amino acid content of the cow's milk a value of 1061 μmol/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 glutamica 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 (6.508–11.901 mmol/dm^3) in the milk, followed by histidine (1.907–5.692 mmol/dm^3), proline (1.671 mmol/dm^3) and arginine (0.268–0.362 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 melatonine 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-melatonine excreted in the urine, respectively. It was established that tryptophan content of the mother's milk showed a minimum value (55–60 μmol/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 75–80 μmol/dm^3. In the urine of the babies the 6-sulfatoxi-melatonine 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 ng/cm^3, while its minimum between 4 and 8 p.m. with 2–4 ng/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-melatonine content present in the urine of the babies.

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Composition of the mother's milk II. Fat contents, fatty acid composition. A review

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Abstract. The authors have analysed fat contents and fatty acid composition of the mother's colostrum and mother's milk in comparison with the newest publications. They have established that the average fat contents of the mother's milk were 3.6–4.0%, and increased during lactation. Among the different ethnic groups there was found no significant difference in the fat contents of the mother's milk, although fat contents of the remainder and milk of corpulent mothers were found high (4–8%).

Saturated fatty acids contribute in 45–55% to the energy value of mother's milk; their amount is 38–41% in the milk fat, which does not change during lactation. With increasing butter consumption palmitic acid and stearic acid contents of the mother's milk increase, and also fatty acid composition of other nutriment is considerably affects the saturated fatty acid contents. Out of the monounsaturated fatty acids oleic acid represents 3–42%, whereas elaidic acid represents 11–12% of milk

Key words and phrases: mother's milk, fat content, fatty acid composition, cholesterol

fat. Multiple unsaturated fatty acid contents of mother's milk are substantially affected by the food composition, and also the significant differences between the ethnic groups can be attributed to differences in the nutrition. During the lactation the amount of the n-3 fatty acids reduces while that of the n-6 fatty acids increase. The geographical and nutritional differences have effect especially on the concentration of long-chain multiple unsaturated fatty acids. Out of the essential fatty acids linolenic acid contents of the mother's milk are between 12–13%, in extreme cases can even reach 20.3%, whereas concentration of the other essential fatty acids is around 0.1–1.5%. No difference was found between the well and badly fed mothers, and the differences between the ethnic groups can also be attributed to the nutritional conditions. During the lactation concentration of both linoleic acid and linolenic acid increases, whereas concentration of arachidonic acid and docosahexaenoic acid decreases. Trans fatty acids form 0.2–17% of milk fat on average. Some believe that consumption of hydrogenated vegetable oils (margarines) does not have any effect on the concentration of trans fatty acids, while others think that it does. Similarly, conjugated linoleic acid contents of milk fat are influenced by the nutriment, and are considerably increased by consumption of alpine butter.

1 Fat content

Yamawaki et al. [54] examined fat 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 on different dates 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 nursings, the samples were stored in a deep-freeze until the analysis. The fat content increased during the lactation; it was significantly higher between days 11–89 of the lactation (3.75–3.90%) than between days 1–10 of the lactation (2.68–2.77%). Along with the sampling, data were collected on smoking habits, vitamin supplementation, birth-weight of the newborn and on whether the sample was taken from the left or right breast. 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 also other 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. Between the formed groups no significant difference was found regarding fat content of the milk.

Rocquelin et al. [41, 42] measured fat content of milk of Congolese mothers in month 5 the lactation to be 2.87%. Fat content of the milk was in a negative relationship with the body mass index. In summary, it was established that the fat content of the milk of the Congolese women depends on the nutrition of the mother.

Saarela et al. [43] examined fat content of the milk during the first six months of the lactation. It was established that the fat content of the aftermilk (5.86%) could be more than double of that of the foremilk, since fat content of the milk increases considerably during the breast-feeding. Total energy content was by 105–126 kJ/100 cm^3 higher in the ripe milk than in the foremilk. It was established that fat and energy content of the mother's milk did not change significantly between the first week and month 6 of the lactation.

Clark et al. [8] examined the total lipid content of milk taken from 10 mothers in week 2, 6, 12, and 16 of the lactation and experienced that it increased significantly from 3.9% measured in week 2 to 5.2% measured in week 16. Glew et al. [16] measured fat content of milk of North-Nigerian fulani nomadic tribes to be 3.05%, that of townmothers to be 3.63%, the difference was not significant between the two groups, however.

According to Bertschi et al. [3] fat content of milk of German mothers consuming butter of different amounts varied between 3.3–3.4%. Marín et al. [30] examined fat content of milk of overweight, normal weight and fat mothers living in La Plata in Argentina and established that fat content of milk of overweight mothers was significantly higher compared to the normal weight and fat mothers (98.1 g/dm^3; 69.2 g/dm^3; 71.5 g/dm^3).

Finley et al. [13] found no difference in milk fat content of vegetarian, semi-vegetarian and non-vegetarian mothers between the three groups. In case of mothers who consumed less than 35 g animal fat a day, fatty acid composition of the milk fat depended significantly on the animal fat intake. For mothers, who consumed more than 35 g animal fat a day, fat content of the mother's milk was in a close positive relationship with the C10:0, C12:0 and C18:3 fatty acids, and in a negative relationship with the palmitic and stearic acid content. Based on the results it was concluded that both for palmitic and stearic acid there was a maximal amount that could be taken by the organism from the blood into the milk gland.

Picciano [38] examining the milk fat content of the mother's milk established that this was the most varied component of the milk, influenced by many

factors. Nourisment of the baby also affects it, as fat content of the mother's milk increases considerably during the breast-feeding. It is influenced by the food of the mother, if it is of low fat content, the endogenous synthesis of the middle-chain fatty acids (C6–C10) increases. Nourisment level of the mother, overweight during the pregnancy can have a connection with the increased milk fat content. Lipids are the most important energy-supplying compounds of the milk, being present in 97–98% as triglycerides in the mother's milk.

Minda et al. [33] collected milk samples from 18 mothers living in Pécs, who gave birth to healthy baby, on the days 1, 2, 3, 4, 5, 6, 7 and then day 14 and 28 after the childbirth. The milk samples were taken between 8 and 10 a.m., and delivered stored at 4–8 °C into the laboratory. The mothers were 29.4±4 years old, and bore in the week 39.1±1.6 of the pregnancy. Surveying the nutrition of the mothers, three of them were completely vegetarian (did not consume even fish), four of them consumed at least once, and 11 mothers consumed in one to three cases fish a week. Fat content of the milk samples varied between 4–8 g/100 cm^3 determined gravimetrically.

Al-Tamer and Mahmood [1] examined composition of milk of mothers with premature and with normal-time childbirth in Iraq. Colostrum and blood samples were taken from mothers bearing in week 39.2 and 32.7, aged 20–40 years. It was established that fat content in the colostrum of mothers with premature childbirth was significantly lower than in the normal colostrum.

Koletzko et al. [24] recommend milk of healthy and well-fed mothers for the nourisment of babies in the first six months of their life. It was established that the fat content of the mother's milk is the main source of energy, contributing in 40–55% to the total energy input. The lipids can be found in the fat micelles with a diameter of 3–5 μm, and containing mainly triglycerides inside. Main components of the fat micelle membrane are the phospholipids and the cholesterol. Lipids of the mother's milk contain the fat-soluble vitamins and the polyunsaturated fatty acids (PUFA) including linoleic acid (LA, 18:2n6) and α-linolenic acid (ALA, 18:3n3). Various PUFAs are proved to have specific biological functions. Linoleic acid e.g. is a component of the skin ceramides that have an important role in formation of the epidermic water barrier. LA and ALA are the precursors of the PUFAs with 20 and 22 carbon atoms. N-6 and n-3 fatty acids take place in such enzyme reactions in which long-chain unsaturated fatty acids (LC-PUFA) are formed, like di-homo-γ-linolenic acid and docosahexaenoic acid (DHA). The LC-PUFAs are important in the formation of the membrane structure, and accumulate perinathalic in tissues rich in membranes like nervous tissue and retina.

2 Lipids in mother's milk

According to Koletzko et al. [24] the mother's milk contains approx. 3.8–3.9% fat, but this value varies between wide limits. The fat in the milk can be found in fat micelles that are formed in the alveolar cells of the milk gland. The fat micelle has a hydrophobic nucleus that is rich in triglycerides and also contains cholesterol ester and vitamin A esters. The surface of the fat micelles contains amphipathic phospholipids as well as proteins, cholesterol and the enzymes. Due to its amphipathic surface the fat micelle is stable in the aqueous medium, and can form the oil in the water emulsion. Major part of the membrane is the apical plasma and membrane of the Golgi apparatus, which distend along with the fat from the cells of the milk gland. The fat micelles have a diameter of 1–10 μm, thus total surface of the micelles in 100 cm^3 milk is 4.5 m^2. To the surface of the fat micelles various lipases are bonded, which contribute to the efficient digestion of the triglycerides. With increasing fat content of the milk during the first four weeks of the lactation also the size of the fat micelles is increasing, resulting in the decrease of the phospholipids and the cholesterol in the membrane. The alveolar cells of the milk gland form the milk fat that can be stimulated by suckling or dosage of prolactin forming in the hypophysis. Major part of the milk fat comes from the foods consumed by the mother, the rest derives from the reserves of the mother's body. A part of the milk fat is synthesized locally in the milk gland from glucose, resulting in mainly C10–C14 fatty acids. The amount of the synthesized middle-chain fatty acids increases in the milk fat when the mother's food is of low fat and high carbohydrate content. There is no significant difference in the lipid composition between mothers with premature and normal time childbirth.

3 Fatty acid composition

3.1 Saturated fatty acids

Shores et al. [48] examined the relationships between copper and middle-chain saturated fatty acids in the milk of 33 Fulani women. Age, height, body mass and number of children of the mothers were recorded. After sample collection the samples were stored at –20 °C until the analyses. A copper content of 399 μg/dm^3 was obtained, capric acid content was measured to be 0.28, lauric acid content to be 9.10 and miristic acid content to be 12.5% in the relative weight% of the total fatty acids. The three middle-chain fatty acids represented altogether 21.5% of the total fatty acids. Significant relationship

was found between the copper content and the three fatty acids, as well as the amount of the total middle-chain fatty acids. According to the authors the relationship between copper and the middle-chain fatty acids can be explained by the fact that either in the milk gland a copper-containing enzyme is necessary for the synthesis of the C10–C14 fatty acids or the middle-chain fatty acids are capable of bonding copper in a special way.

Minda et al. [33] by the examination of saturated fatty acid composition of milk of mothers living in Pécs established that out of the saturated fatty acids palmitic acid reduced during the lactation (25.73–22.33%), whereas the amount of miristic acid and stearic acid showed no substantial change in the first three months of the lactation (C14:0 5.16–6.25%; C18:0 6.96–7.04%).

Marangoni et al. [29] examined fatty acid composition of milk of ten Italian mothers from milk samples taken on the first day of the lactation, then in the month 1, 3, 6, 9 and 12 of the lactation. In the course of the lactation in the total amount of the saturated fatty acids no significant change could be found, their amount varied between 38–41%.

Sala-Vila et al. [44] examined saturated fatty acid composition of the colostrum (day 1–5), transitional milk (day 6–15) and ripe milk (day 15–30) of 66 mothers of Granada bearing at normal time. The milk samples were collected between the second and fourth week of the lactation using hand milk pump. After the collection the samples were immediately frozen down to –80 °C, and stored until the analyses. Determining fatty acid content of the phospholipids it was established that palmitic acid (23.38–24.32%) increased significantly from the colostrum to the ripe milk, while the colostrum contained more stearic acid (24.00–23.49%) than the ripe milk. The amount of the saturated fatty acids (C8:0–C24:0) was measured to be significantly higher in the transitional (56.18%) and the ripe milk (56.89%) than in the colostrum (57.96%).

According to López-López et al. [27] in the human colostrum palmitic acid (19.64–19.9%) and stearic acid (5.24–5.30%) are present in big amount. More than 1% was measured for the lauric acid and miristic acid, whereas the rest of the saturated fatty acids was present in a concentration of below 1% in the milk fat of the mother's milk.

Fidler et al. [11] examined fatty acid composition of the colostrum of mothers living in Slovenia, in town and in country environment. Colostrum samples were taken from 41 Slovenian mothers, out of which 27 lived in town and 14 in the country, 3 days after the childbirth. Determining the fatty acid composition by capillary gas chromatography it was experienced that there was no significant difference in the fatty acid composition of the colostrum's

fat between the townmothers and the mothers from the country. Average saturated fatty acid content of the mothers milk was 37.68%.

Bahrami and Rahimi [2] examined the saturated fatty acid composition of milk of 52 healthy, breast-feeding, West-Iranian mothers aged between 19–39 years, who gave birth to their baby in week 37–45. The milk samples were immediately frozen down to –40 °C, and kept at this temperature until the analysis. The fatty acid composition was determined after derivatization using high-performance liquid chromatography. It was established that out of the saturated fatty acids the middle-chain fatty acids (C6:0–C18:0) represented the main fraction with 37.3%. Schmeits et al. [45] measured the amount of C10:0–C14:0 fatty acids in the milk of 48 mothers living in the countryside territory of Nepal to be 25%.

According to Xiang et al. [52] saturated fatty acid concentration of milk of 41 mothers living in country environment of North China ranged between 34.66–36.59%. Precht and Molkentin [40] established that saturated fatty acid content of milk of 40 German mothers was as follows: C12:0 3.12%, C14:0 6.43%, C16:0 25.28% and C18:0 7.41%. Bitman et al. [4] analyzing the fatty acid composition of the mother's milk established that the amount of the middle-chain fatty acids (C12:0, C14:0, C16:0) in the colostrum of mothers with very premature and premature (23%) childbirth was considerably lower than in the normal colostrum (35%). Glew et al. [16] analyzing the amount of C6:0–C14:0 middle-chain fatty acids established that this was practically the same for both the townmothers (25.2%) and the country mothers (26.6%).

Fidler and Koletzko [10] summarized the results of 15 studies dealing with fatty acid composition of colostrum of mothers from the world's 16 regions. Out of the studies 11 derived from Europe, one from Central America, one from the Caribic, one from Australia and one from Asia. It was established that the amount of the saturated fatty acids was similar in the South-European countries (Spain, France, Slovenia). Colostrum of mothers living in St. Lucia contained more saturated fatty acids due to the foods of high carbohydrate and low fat content. In the colostrum of Australian mothers the amount of the saturated fatty acids was 43.8%.

Bertschi et al. [3] examined the effect of Alpenbutter consumption of German mothers on the fatty acid composition of the fatty acid composition. The butter originated from Graubünden (Switzerland), from 2100 m altitude; the 2 kg packings were stored at –20 °C until they were utilized. The milk samples were taken on day 1, 5, 10, 15 and 20 of the experiment between 8–11 a.m. and the fatty acid composition of the milk was determined by gas chromatography. It was established that due to the alpenbutter supplementation the

proportion of the palmitic acid and stearic acid increased in the milk, as well as total amount of the saturated fatty acids.

Silva et al. [49] determining saturated fatty acid composition of ripe milk of Brazil mothers collected 80 milk samples from 18 healthy donors between week 4–13 of the lactation, who were breast-feeding their baby until week 37–42 after the childbirth. Sampling was done by hand; 10 cm^3 of milk was taken each time that was immediately frozen down to –20 °C and stored until the analyses. Each mother was questioned based on a questionnaire about her nutritional habits, especially regarding the saturated and unsaturated fats, trans fatty acids and carbohydrate consumption (beef, pork, chicken, fish, cakes, rice, flour, margarine, butter, vegetable fats, animal fats, milk, dairy products, vegetables, fruits). On the basis of the questionnaire it was experienced that during the sampling the mothers consumed weekly in most cases rice, cakes, vegetable fats, chicken, fruits and vegetables (7 to 8 times a week), and only in a very few cases fish, butter and animal fat (2 to 3 times a week). The analysis of the fat was carried out in the form of fatty acid methyl esters using a gas chromatograph with a flame ionization detector. Size of the column was 50 m, 0.25 mm, helium as carrier gas with a flow rate of 1 cm^3/min. It was established that out of the saturated fatty acids palmitic acid was present in the highest concentration (17.3%) representing 43.5% of the total saturated fatty acids, followed by miristic acid (7.02%), lauric acid (6.88%) and stearic acid (5.43%). It was concluded that the fatty acid composition of the food affects considerably the fatty acid composition of the mother's milk.

Hayat et al. [20] examined saturated fatty acid composition of milk of 19 healthy Kuwaiti mothers aged between 20–30 years, and parallelly with this based upon a questionnaire the foodstuffs consumed by the individual mothers was surveyed, that proved to be very rich in fats and proteins. The samples were taken using automatic pumps between week 6 and 14 after the childbirth, in the morning. Subsequent to the sampling the samples were placed immediately into ice and examined shortly after the delivery. Fatty acids present in the samples were converted into methyl ester, the examinations were performed by gas chromatography using flame ionization detector and capillary column (50 m long and 0.25 mm internal diameter). According to the examinations composition of the milk was considerably influenced by food consumption of the mothers. It was established that 42.6% of the total fatty acid content was represented by the saturated fatty acids, ouf of them in biggest amount occurred palmitic acid (50.8%), stearic acid (6.5%), miristic acid (6.4%), and lauric acid (6.0%).

Marín et al. [30] examining saturated fatty acid composition of milk of

mothers living in La Plata in Argentina analyzed the relationship between the fatty acid composition and the composition of the foods consumed by the mother. The milk samples were collected between month 1–3 of the lactation manually in the third minute of the breast-feeding. The samples were immediately frozen down to –70 °C and stored until the analyses. The mothers were 16–39 years old. The entire fat amount was extracted using the Folch method and converted into fatty acid methyl esters. The methyl esters were analyzed by gas-liquid chromatography. Capillary column of 30 m length and with 0.25 mm internal diameter, 0.25 μm film thickness and a flame ionization detector was used. In the course of the examinations no difference was found in the amount of the individual saturated fatty acids between the obese and fat mothers despite that the concentration of the C10–C14 fatty acids was the lowest in the case of obese mothers. Within the saturated fatty acids in all three groups palmitic acid was present in the highest concentration (20.58–21.19%). Within the total fatty acid content the amount of the saturated fatty acids was 42.85%. No considerable difference was established in the saturated fatty acid content of the milk of the Argentinian, the Japanese and the Chinese mothers.

Rocquelin et al. [41, 42] examined saturated fatty acid composition of milk of Congolese mothers, with special respect to the nourishment level of the mother and to the satisfaction of the fatty acid requirements of the newborn. In the milk of Congolese women being in the fifth month of the lactation the amount of C8:0–C14:0 fatty acids (25.97%) was relatively high. The fatty acid composition was brought into connection with the consumption of foods with high carbohydrate content, that promote the biosynthesis of the C8:0–C14:0 fatty acids. In summary it was established that the fat content of the milk of Congolese women and the fatty acid composition of the fat depended considerably on the nutrition of the mother.

Xiang et al. [53] examining saturated fatty acid content of milk of Chinese and Swedish mothers analyzed the effect of food on the composition of milk. The Chinese mothers consumed mainly rice, steamed bean, noodles, Chinese cabbage and pork. Food of Swedish mothers consisted of bread, potato, paste, milk, sour milk and cheese. The Chinese consumed more carbohydrates (17% of the energy), less protein (4% of the energy) and fat (12% of the energy) than the Swedish. Fat content of the foods of the Chinese derived from soya oil and pork, whereas that of the Swedish mainly from cheese. It was established that due to the above nutrition the total saturated fatty acid content of the milk of Chinese mothers, with the exception of the arachidic acid (C20:0) and behenic acid (C22:0) was significantly lower (13.39 g/day) than in case of the Swedish

mothers (41.3 g/day). Within this, in the C12:0–C18:0 range the fatty acid content was significantly in case of the Chinese than in case of the Swedish; in the C20:0–C24:0 range an opposite result was obtained, however.

Serra et al. [46] determined fatty acid composition of milk of 20 Italian mothers nourishing according to appetite. The milk samples were taken on day 1, 4, 7, 14 21 and 28 after the childbirth. In the ripe milk the amount of the saturated fatty acids was 45.50%. It was established that fatty acid composition of milk fat of Italian mothers was similar to that of mothers living in the South European countries, which is presumably due to the similar nutrition habits. Laryea et al. [25] examining the fatty acid composition of milk fat of well-nourished Sudanese mother established that the amount of the saturated fatty acids represented 46% of the total fatty acid content.

According to Koletzko et al. [24] major part of the lipids of the mother's milk is composed of the saturated fatty acids. In the vegetable oils the saturated fatty acids take their place more or less randomly in the triglyceride molecule, in the mother's milk, however, the saturated palmitic acid is bonded in the sn-2 position. As the lipolithic enzymes cleave the bonds in the sn-1 and sn-3 positions more effectively, palmitic acid present in the mother's milk can be found mainly in monoglyceride form, whose absorption is greater than that of the free palmitic acid. Due to its the higher polarity, the absorption of the monoglyceride is easier. Most of the palmitic acid absorbes in sn-2 position, keeping its structure after the absorption even in the small intestines. Clinical experiments with baby food preparations confirmed that the palmitic acid absorbes better from the sn-2 position, than it is randomly linked to the glycerine.

According to Picciano [38] saturated fatty acid composition (C12:0–C18:0) of the mother's milk is affected by many factors. These fatty acids contribute in 45-55% to the energy content of the mother's milk.

3.2 Unsaturated fatty acids

Monounsaturated fatty acids Jahreis et al. [22] analyzed the composition of cow's, goat's, sheep's, sow's, mare's and mother's milk. Comparing the monounsaturated fatty acid content measured for the individual species it was established that in case of non-ruminants its concentration was the highest in the sow's milk (51.8±5.8%), followed by the human milk (33.2±2.9%) and the mare's milk (20.7±1.2%). In the case of the ruminants no considerable difference was found within the individual species regarding the monounsaturated fatty acids with concentration ranging between 21.8–23.2%.

According to Xiang et al. [52] monounsaturated fatty acid content of milk of 41 mothers living in country environment of North China in the range C14:1–C24:1 is between 38.0–39.32%. Silva et al. [49] determining the unsaturated fatty acid content of ripe milk of Brazil mothers established that they represented 59.5% of the total fatty acids, out of which 27.6% was the monounsaturated fatty acids. Hayat et al. [20] measured the monounsaturated fatty acid composition of milk of 19 healthy Kuwaiti mothers, aged between 20–30 years, within the total fatty acid content to be 37.3%.

Sala-Vila et al. [44] examined fatty acid composition of the colostrum (day 1–5), transitional milk (day 6–15) and ripe milk (day 15–30) of 66 mothers of Granada bearing at normal time. Determining the fatty acid content of the phospholipids it was established that the amount of the monounsaturated C18:1n9 fatty acids increased significantly from the colostrum to the ripe milk (13.39–14.00%). In the transitional and the ripe milk the amount of the monounsaturated fatty acids was significantly lower (16.60%) than in the colostrum (17.91%), however.

According to López-López et al. [27] in the human colostrum in the highest amount the C18 oleic acid isomers containing one unsaturated bond were present (41.58–42.04%). An amount above 1% was measured also in the case of the palmitoleic acid. Fidler et al. [11] examined fatty acid composition of the colostrum of Slovenian mothers living in town and in country environment. The only difference was in the oleic acid content, which was 36.85% for the mothers living in the country, whereas 34.94% for the townmothers. Total amount of the monounsaturated fatty acids was 40.49%.

Serra et al. [47] determined fatty acid composition of milk of 20 Italian mothers nourishing according to appetite. It was established that with the advance of the lactation the amount of the monounsaturated fatty acids decreased significantly, which was 42.69% in the ripe milk. According to Laryea et al. [25] monounsaturated fatty acid content of milk of well-nourished Sudanese mothers represented 33% of the total fatty acids.

Bahrami and Rahimi [2] examining the fatty acid composition of the milk of 52 healthy West-Iranian mothers established that the monounsaturated oleic acid represented 30.9% while elaidic acid 11.3% of the milk's total fatty acids. According to the examinations of Minda et al. [33] the monounsaturated fatty acid content of the milk of mothers living in Pécs showed some decrease between day 1 and day 28 after the childbirth (36.79–35.75%). Marangoni et al. [28] measured monounsaturated fatty acid content of ten Italian mothers to be between 45-41%.

Marín et al. [30] measured the monounsaturated fatty acid content of milk of

mothers living in La Plata in Argentina to be 34.8%, within which proportion of the 20:1n9 was significantly higher in case of the fat mothers (0.19%) than in the case of normal mothers (0.08%). Total amount of the monounsaturated fatty acids in case of the fat mothers was significantly less compared to the normal and overnourished mothers (33.7%, 36.97%, 33.9%).

Scopesi et al. [46] studied the effect of monounsaturated fatty acid content of the food on the fatty acid composition of the mother's milk in the first month of the lactation in case of 34 breast-feeding mothers. The food's composition was determined six times on the day before the sampling. Milk sample taken on the first day after the childbirth was considered as colostrum, sample taken on days 4–6 as transitional milk, while sample taken on the days 14, 21 and 28 as ripe milk. It was established that the monounsaturated fatty acid content of the mother's food had a significant effect on the composition of the transitional milk.

Xiang et al. [53] examining monounsaturated fatty acid content of milk of Chinese and Swedish mothers analyzed the effect of food on the composition of milk. The Chinese mothers consumed mainly rice, steamed bean, noodles, Chinese cabbage and pork. Food of Swedish mothers consisted of bread, potato, paste, milk, sour milk and cheese. In case of the Chinese mothers the concentration of the total monounsaturated fatty acids was 39.32%, whereas in the milk of the Swedish mothers was 45.15%. Within this in the C14:1–C18:1 range a lower value was obtained for the Chinese mothers, while in the C20:1–C24:1 range the result was opposite.

Polyunsaturated fatty acids Jahreis et al. [22] analyzed the composition of cow's, goat's, sheep's, sow's, mare's and mother's milk. Comparing the polyunsaturated fatty acid content measured for the individual species it was established that its concentration was the highest in the mare's milk (36.8±3.2%), followed by the sow's milk and the human milk (12.5–12.4%). In the case of the ruminants no considerable difference was found within the individual species regarding the polyunsaturated fatty acids with (2.42–4.05%).

Laryea et al. [25] examining the polyunsaturated fatty acid content of milk fat of well-nourished Sudanese mothers established that they represented 21% of the total fatty acids. Xiang et al. [52] determined long-chain polyunsaturated fatty acid composition of milk of 41 mothers living in country environment of North China and measured to be between 25.38–26.00%.

Serra et al. [47] determining the polyunsaturated fatty acid composition of milk of 20 Italian mothers nourishing according to appetite established that with the advance of the lactation out of the long-chain unsaturated fatty

acids the amount of the ω6 and ω3 fatty acids decreased significantly. In the ripe milk the amount of the total unsaturated fatty acids was 54.5%, out of which the polyunsaturated fatty acids represented 11.82%, and within the polyunsaturated fatty acids the long-chain fatty acids represented 1.27%.

Bitman et al. [4] examined the C18:3, C20:3 and C20:4 fatty acids of the mother's milk in case of mothers with very premature (5.6%), premature childbirth (6.2%) and mothers bearing at normal time (1.8%). Colostrum of mothers bearing at normal time contained significantly less out of these fatty acids compared to mothers with premature childbirth.

Fidler et al. [11] examining the fatty acid composition of the colostrum of mothers living in town and country environment in Slovenia did not find significant difference in the amount of the polyunsaturated fatty acids (21.82%). Ratio of the polyunsaturated and saturated fatty acids was 0.58, whereas the n-6/n-3 ratio was 8.0.

Silva et al. [49] examining the polyunsaturated fatty acid composition of the ripe milk of Brazil mothers established that they represented 23.4% of the total fatty acids. The amount of the n-6 long-chain polyunsaturated fatty acids was 1.56%, the C18:2n6/C18:3n3 ratio was 15.35.

Marín et al. [30] examined the long-chain polyunsaturated fatty acid composition of milk of mothers living in La Plata in Argentina and analyzed the relationship between the fatty acid composition and the composition of the foods consumed by the mother. It was established that in case of fat mothers the amount of the polyunsaturated fatty acids increased significantly and also the C18:2n6/total n-6 ratio was significantly higher compared to the ones with normal body weight (0.96%; 0.89%). No significant difference was found between the groups regarding the n-3 fatty acids, but the ratio between the n-6 and n-3 fatty acids was significantly higher for the obese mothers. Comparing the polyunsaturated fatty acid content of the milk of the Argentin, American, Japanese and Chinese mothers it was experienced that the milk of the Argentin mothers contained more C18:2n6 and C18:3n3 fatty acids than that of the rest of the mothers.

Olafsdottir et al. [36] examined the ratio of the polyunsaturated fatty acids in the milk of mothers living in Iceland, who consumed traditionally fish and fish liver oil. From 77 mothers milk samples were taken at 24 hour intervals and analyzing by gas chromatography the fatty acid composition it was established that in the milk of mothers who consumed fish liver oil the polyunsaturated fatty acids were present in a significantly higher concentration. The milk fat contained more docosahexaenoic acid (0.54%) that the milk of the control group (0.30%), similarly, also the amount of eicosapentaenoic (0.16%)

and docosahexaenoic acid (0.22%) was significantly higher than in the control group, where the concentration of these two fatty acids were 0.07 and 0.17%. It was also established that the proportion of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid reach higher value without the amount of other important fatty acids having been decreased. It appears that the fish liver oil is a very important source of fat in respect of nutrition of both the mother and the newborn.

Scopesi et al. [46] studied the effect of polyunsaturated fatty acid content of the food on the fatty acid composition of the mother's milk in the first month of the lactation in case of 34 breast-feeding mothers. The food's composition was determined six times on the day before the sampling. Milk sample taken on the first day after the childbirth was considered as colostrum, sample taken on days 4–6 as transitional milk, while sample taken on the days 14, 21 and 28 as ripe milk. It was established that the polyunsaturated fatty acid content of the mother's food affected only the composition of the ripe milk.

Xiang et al. [53] examining long-chain polyunsaturated fatty acid content of milk of Chinese and Swedish mothers analyzed the effect of food on the composition of milk. The Chinese mothers consumed mainly rice, steamed bean, noodles, Chinese cabbage and pork. Food of Swedish mothers consisted of bread, potato, paste, milk, sour milk and cheese. Total amount of the polyunsaturated fatty acids was higher in case of the Chinese mothers (15.92 g/day) compared to that of the Swedish ones (11.71 g/day). Within this proportion of adrechinic acid (C22:4n6), as well as of the total n-6 and n-3 polyunsaturated fatty acids were significantly higher for the Chinese than for the Swedish. Eicosadienoic acid and clupanodonic acid were, however, significantly higher in case of the Swedish. It was established that due to the nutrition milk of Chinese mothers contained significantly more polyunsaturated fatty acids (26.02%) than that of the Swedish ones (14.14%). With the exception of γ-linolenic acid and docosapentaenoic acid the n-6 polyunsaturated fatty acids were present in the milk of the Chinese mothers in a significantly higher concentration. With the exception of the eicosatrienoic acid the n-3 polyunsaturated fatty acids occured in the milk of the Chinese mothers in a significantly lower concentration.

Hayat et al. [20] examining polyunsaturated fatty acid composition of milk of 19 healthy Kuwaiti mothers aged between 20–30 years established that the amount of the long-chain polyunsaturated fatty acids depends on the composition of the food. In the milk of mothers who consumed bigger amount of fish the fatty acids C22:6n3 and C20:5n3 were present in a significantly higher amount. It was established that 62.7% of the total fatty acid content was

represented by the polyunsaturated fatty acids.

Wijga et al. [51] examined the relationship between the composition of the mother's milk and allergic diseases in case of allergic and non-allergic women. It was established that in case of the children of allergic mothers the amount of n-3 long-chain polyunsaturated fatty acids and the n-3/n-6 ration could be brought into connection with the developed asthma and eczema, no data were found, however, relating to whether fatty acid composition of the milk fat could make one susceptible to these diseases.

Lauritzen et al. [26] compared milk fat composition of non-atopic and atopic Danish mothers. It was established that milk fat of atopic mothers contained in significantly bigger amount C22:5n6 and in lower concentration C20:5n3 fatty acid, at the same time no difference was found between the two groups regarding the rest of the polyunsaturated fatty acids of the mother's milk, relationship was found, however, between the polyunsaturated fatty acid composition of the milk and similar parameters of the food.

Sousa et al. [50] examined fatty acid composition of colostrum, transitional milk and ripe milk of 9 Chilean mothers who bore in week 31 and week 34 of the pregnancy, in the first month after the childbirth. The first milk sample was taken on day 2 and day 3 after the childbirth, the second one between day 15 and day 18. The mothers derived from south-eastern part of Santiago, belonged mainly to low social class, and were in a bad social position. Energy content of their foods came in 20% from fat, in 68% from carbohydrates and in 12% from protein, and also the fatty acid composition of the foods consumed by the mothers was similar. Out of the polyunsaturated fatty acids the n-6's represented 88%, the n-3's 12%, the n-6/n-3 ratio was 7.5:1. Docosahexaenoic acid and eicosapentaenoic acid content of the mother's milk was higher than that of mothers living in the Western industrialized countries.

Patin et al. [37] examined the effect of the sardine consumption on the n-3 fatty acid content of the ripe mother's milk. It is well-known that sardine contains many n-3 polyunsaturated fatty acids. At the beginning of the experiment milk samples were taken from 31 breast-feeding mothers on day 15 and day 30, and the fatty acid composition was determined by gas chromatography. The mothers were divided into two groups regarding sardine consumption. The first group contained mothers who consumed sardine within 3 days prior to the sampling, while to the second group belonged mothers who consumed sardine four days before the sampling. It was established that the n-3 fatty acids of the mother's milk increased considerably at all the three sampling time in the case of the first group, the growth for the second group was only a small one, however. Comparing the n-3 fatty acids of the samples taken at the same

point of time of the two groups, in case of eicosapentaenoic acid for the sample taken on day 30 of the lactation (Group 1: 0.17; Group 2: 0.06%); whereas in case of docosapentaenoic acid at the beginning of the experiment (Group 1: 0.16; Group 2: 0.25%) could be found a significant difference. The amount of the n-3 and n-6 fatty acids showed a significant positive relationship on day 15 and day 30. It was established that fish consumption of the breast-feeding mothers (minimally 100 g sardinia 2–3 times a week) can considerably increase the amount of the n-3 fatty acids in the mother's milk.

According to Picciano [38] the short pregnancy can increase the amount of the long-chain polyunsaturated fatty acids. It is also influenced by the mother's food, as if it is of low fat content, endogenous synthesis of the middle-chain fatty acids (C6–C10) increases, and also the nourishment level of the mother influences it.

Sala-Vila et al. [44] examined polyunsaturated fatty acid composition of the colostrum (day 1–5), transitional milk (day 6–15) and ripe milk (day 15–30) of 66 mothers of Granada bearing at normal time. Determining polyunsaturated fatty acid content of the phospholipids it was established that the amount of the C20:5n3 (0.34–0.81) and C22:2n6 (0.44–0.55) fatty acids increased significantly from the colostrum to the ripe milk. At the same time the colostrum contained more C20:3n-6 (0.62–0.60%), C22:4n6 (0.27–0.06%) and C22:5n3 (0.83–0.65%) fatty acids than the ripe milk. In summary, the amount of the total n-3 polyunsaturated fatty acids decreased from the colostrum to the ripe milk (2.69–2.45%), while the amount of the n-6 fatty acids increased (5.10–5.26%) in this period. It was established that the milk of the mothers of Granada did not differ from the average fatty acid composition of milk of mothers examined in other parts of the world.

Al-Tamer and Mahmood [1] examined composition of milk of mothers with premature and with normal-time childbirth in Iraq. Colostrum and blood samples were taken from mothers bearing in weeks 39.2 and 32.7, whose age varied between 20–40 years. In the milk of mothers bearing at full time the amount of the C20:5n3 and C22:6n3 fatty acids was significantly higher, and some increase could be experienced in the n-3/n-6 ratio. It was established that the C22:6n3 and the n-3/n-6 ratio was lower in the colostrum of the mothers with premature birth than in the normal colostrum. The colostrum's fatty acid composition is considerably influenced by the serum's lipid composition. The difference in the middle-chain fatty acids in the serum and the colostrum explains that these are formed in the milk gland.

Serra et al. [47] determining the fatty acid composition of milk of 20 Italian mothers nourishing according to appetite established that with the advance

of the lactation the amount of the monounsaturated fatty acids decreased significantly, which was in the ripe milk 42.69%.

According to Minda et al. [33] polyunsaturated n-6 fatty acid content of milk of mothers living in Pécs decreased significantly, while major part of the n-3 fatty acids did not show any substantial change during the lactation.

Marangoni et al. [29] could not find a significant difference in the polyunsaturated fatty acid amount (16–16%) of milk of Italian mothers in months 1, 3, 6 and 12 of the lactation.

According to Koletzko et al. [24] the different LC-PUFAs occur in considerable amount in the mother's milk (LC-PUFA = long-chain polyunsaturated fatty acid). Analyzing the fatty acid composition of the ripe milk from industrial countries total n-6 LC-PUFA ranged between 0.83–1.40%, while the total n-3 LC-PUFA between 0.27–0.48% as percentage of the total fatty acids. The fatty acid composition in the studies are extremely similar to each other, irrespective of whether milk of mothers living in Europe or in Africa was examined. The LC-PUFA content appears to be almost entirely independent of life circumstances and the nutrition, irrespective of that they differ considerably between the individual groups. Main PUFAs of the mother's milk are the C20:4n6, C20:3n6 and C20:2n6 all belonging to the n-6 group, as well as docosahexaenoic acid (C22:6n3) and docosapentaenoic acid (C22:5n3) that belong to the n-3 group. The LC-PUFA content decreases in the first month of the lactation which does not mean, however, that the newborns get less such kind of fatty acid as the total fatty acid content considerably increases during the lactation and the amount of the total PUFA (polyunsaturated fatty acid) excreted with the milk remains relatively constant. It is thought that the high LC-PUFA content of the colostrum can be useful for the newborn as the consumed milk amount is little, the PUFA requirement of the newborns on the other hand because of the rapid growth, is high. Amount of some n-6 PUFA like C20:3n6 and C22:5n6 decreases during the lactation. This can be explained by the fact that the milk production during the lactation empties the LC-PUFA reserves of the body, which can serve as source of the milk fat. Comparing the milk composition of mothers bearing early and at normal time it was reported that the colostrum of mothers bearing early contained more LC-PUFA than that of the ones bearing at time. The LC-PUFA decreased in the first month of lactation in the milk of both the mothers bearing early and at time. Feeding with mother's milk has a great advantage for the babies both born prematurely and at time as its high LC-PUFA content satisfies the requirements of the newborn in the first weeks of its life. After the first month of the lactation no further decrease in the LC-PUFA was observed in

the milk of mothers bearing early, but for those who bore at time the decrease continued.

Fidler and Koletzko [10] summarized the results of 15 studies dealing with fatty acid composition of colostrum of mothers from the world's 16 regions. Out of the studies 11 derived from Europe, one from Central America, one from the Caribic, one from Australia and one from Asia. It was established that the amount of the polyunsaturated fatty acids was similar in the South-European countries (Spain, France, Slovenia). Colostrum of mothers living in St. Lucia due to the foods of high carbohydrate and low fat content contained less oleic acid, the rich fish consumption increased the proportion of the n-3 long-chain polyunsaturated fatty acids. In the colostrum of the Australian mothers is the lowest the amount of the polyunsaturated fatty acids, while eicosapentaenoic acid and total n-3 long-chain polyunsaturated fatty acid content of their milk (0.6%, 0.4%, 1.4%) is higher than in case of the European mothers. It was established that the fatty acid composition of the mother's colostrum was affected considerably by the geographical and nutritional differences.

3.3 Essential fatty acids

According to examinations of Marangoni et al. [29] out of the essential fatty acids of milk of Italian mothers in month 1, 3, 6, 9 and 12 of the lactation only the concentration of arachidonic acid show a bigger decrease (1.0–0.5%); the amount of linoleic acid (11.9–12.9%), α-linolenic acid (0.6–0.9%) and docosahexaenoic acid (0.5–0.3%) did not change in the course of the lactation.

According to Glew et al. [16] the differences in two essential fatty acid content (linoleic acid and α-linolenic acid) found in the milk of the Fulani nomadic tribes and townmothers were not significant. Average amount of α-linolenic acid (0.77–0.80%) did not differ in the two populations; the amount of linoleic acid varied between 6.97–7.83% both for the Fulani and townmothers, and was lower than in case of the non-African populations or than in case of the ones living in South part of Niger. The amount of arachidonic acid was significantly higher for the countrymothers (0.62%) than for the townmothers (0.48%).

Silva et al. [49] examining essential fatty acid composition of ripe milk of Brazil mothers established that the major part of the polyunsaturated fatty acids is composed of the essential linoleic acid and α-linolenic acid in a concentration of 20.3% and 1.43%. Concentration of arachidonic acid is 0.53%, that of docosahexaenoic acid is 0.14%, which perfectly corresponds to the needs of the newborn. It was established that milk fat of mothers living in the region

of Viccosa of Brazil contained high amount of linoleic acid and α-linolenic acid in connection with the high, polyunsaturated fatty acid containing oil content of the food. It is also established that the fatty acid composition of the food considerably influences fatty acid composition of the mother's milk.

Hayat et al. [20] examined essential fatty acid composition of milk of 19 healthy Kuwaiti mothers, aged between 20–30 years. According to their investigations composition of milk is considerably influenced by the mothers' food consumption. It was established that the concentration of linoleic acid and linolenic acid depends significantly on the food's composition. Comparing linoleic acid content of milk of mothers with different nationality it was experienced that the milk of the Spanish mothers contained 12.02%, that of German mothers 10.8% and that of Australian mothers 11.0% linoleic acid. Concentration of the α-linolenic acid (n-3) ranged in case of the Arab mothers between 0.3–2.4%, whereas for the German mothers between 0.8–1.2%. It was concluded that the milk composition of adequately nourished mothers satisfied well the requirements ot the newborn.

Knox et al. [23] analyzing the fatty acid composition of milk of 89 Nigerian (Kanuri) mothers wanted to know what relationship between the nutritional level and the amount of the essential fatty acids present in the milk there is. The milk samples were taken in week 1–64 of the lactation using sampling by hand, then cooled down to –20 °C, and delivered at 2–4 weeks into the laboratory for analysis. For determination of the fatty acid composition for the transesterification a chloroform-methanol 2:1 mixture and boron trifluoride in methanol were used, then the analysis was carried out using a gas chromatograph with a flame ionization detector. By arranging the mothers into groups based on the body mass index it was established that in the well-nourished group the ratio of the n-3 and n-6 fatty acids with the exception of the linolenic acid and docosahexaenoic acid did not differ significantly from that of the less well-nourished groups. It was also established that in case of underfed mothers a different mechanism helped the transport of the essential fatty acids into the milk fat than in case of the well-nourished mothers.

Rocquelin et al. [41] examined essential fatty acid composition of milk of Congolese mothers, with special respect to the nourishment level of the mother and to the satisfaction of the essential fatty acid requirements of the newborn. In the milk of Congolese women being in the fifth month of the lactation the amount of the polyunsaturated fatty acids was relatively high, especially that of the n-3 fatty acids (2.39%) consisted mainly of C18:3 and C22:6 fatty acids. The fatty acid composition was brought into connection with the consumption of foods with high carbohydrate content, of fresh-water and salt-water

fishes, vegetable oils, vegetables and high fat content fruits (nuts, avocado), which foods are traditional in Congo. In summary it was established that the fat content of the milk of the Congolese women and the fatty acid composition of the fat depends considerably on the nutrition of the mother, and that the 5-months-old Congolese babies presumably did not receive the n-6 and n-3 essential fatty acids in the amount satisfying their requirements with the mother's milk.

Marín et al. [30] measured linoleic acid content of milk of overweight, fat and normal weight mothers living in La Plata in Argentina to be 6.61; 19.12 and 22.71%. Comparing the polyunsaturated fatty acid content of milk of Argentin, American, Japanese and Chinese mothers it was experienced that the milk of Argentin mothers contained more C18:2n6 and C18:3n3 fatty acids than that of the ones living at other places.

Schmeits et al. [45] determined the fatty acid composition of the milk of 48 mothers living in the countryside territory of Nepal 2–4 weeks after the childbirth. It was established that the linoleic acid concentration of the mother's milk was very low (7.91%), while that of the α-linolenic acid was relatively high (1.93%), arachidonic acid was measured to be 0.35%, docosahexaenoic acid to be 0.21% in the percentage of the total fatty acids. 6.8% of the phospholipids was represented by the two essential fatty acids, and 23% belonged to the C10:0–C14:0 ranged. Arachidonic acid is present in 0.57%, whereas docosahexaenoic acid in 0.78% in the phospholipids. It was concluded that the low linoleic acid content of the milk of Nepalese mothers was in connection with the low linoleic content of the food.

Boylan et al. [5] examined milk composition of mothers with low income in Texas state of the USA in an environment where the fish consumption was very rare. The milk samples were taken from 22 mothers, the same way, between day 8 and day 11 of the lactation, and all the foodstuffs the mothers consumed 24 hours before the sampling were recorded. 19 mothers had never eaten fish in oil, the rest consumed once a year. Another characteristic feature of the nutrition was that the mothers consumed few fruits and vegetables, and also their milk consumption was very low. By the examination of the fatty acid composition of the mother's milk it was established that the docosahexaenoic acid content of the milk fat was extremely low, 0.08% compared to 0.2–0.4% found in the literature. Linoleic acid, α-linolenic acid and other fatty acid content was similar to the literature values. It was established that for mother of Texas who consumed little vegetables, fruits, milk and fish, the docosahexanoic acid content of the milk fat was low.

Glew et al. [17] examining the essential fatty acid composition of milk of

Nepalese mothers looked for a relationship between the essential fatty acid composition of the serum phospholipids and the melting point. From 36 mothers aged between 15–32 years milk and serum samples were taken and the fatty acid composition of the phopholipid fraction was determined. α-linolenic acid content of the milk's lipids was measured to be 1.84%, arachidonic acid content to be 0.43%, docosahexaenoic acid content to be 0.23%, linoleic acid content to be 9.05%. In case of the serum phospholipids and the milk fat a positive relationship was established between the arachidonic acid and α-linolenic acid content. It was established that the arachidonic acid and α-linolenic acid content of the blood of breast-feeding mothers considerably influenced the fatty acid composition of the milk fat.

Xiang et al. [52] determined long-chain essential fatty acid composition of milk of 41 mothers living in country environment of North China. Concentration of linoleic acid (21.47–22.69%) and α-linolenic acid (1.19–1.29%) was found to be very high in the mother's milk. The linoleic acid/αlinolenic acid ratio was measured to be 21.6, which was substantially higher than that reported in case of other countries. Concentration of arachidonic acid (0.51–0.63%) and docosahexaenoic acid (0.18–0.33%) was low, and both were in a positive relationship with the weight of the baby in month 3. The arachidonic acid/docosahexaenoic acid ratio was much higher (2.8) than that was found in case of the vegetarian mothers. As the concentration of arachidonic acid and especially that of docosahexaenoic acid decreased considerably during the lactation, after the third-fourth month of the lactation a docosahexaenoic acid deficiency is expected in case of the breast-fed Chinese country babies. The authors find further studies necessary in order to clarify whether it is necessary to supplement the mother's food with arachidonic acid and docosahexaenoic acid e.g. by adding of fish oil, so that the mother's milk composition becomes optimal for the satisfaction of the babies needs.

According to Sousa et al. [50] docosahexaenoic acid content of milk of Chilean mothers is significantly influenced by the local diet.

Fidler et al. [10] examined the transfer of the increased docosahexaenoic acid content of the food into the milk. As the docosahexaenoic acid is extremely important for the growth of the babies and its conversion from the mother's food into the mother's milk was not circumstantially investigated therefore docosahexaenoic acid labelled with ^{13}C isotope was added to the food and its transfer into the mother's milk was analyzed. Out of 10 breast-feeding mothers 5 received docosahexaenoic acid labelled with ^{13}C isotope, and 5 received placebo. The docosahexaenoic acid labelled with the carbon isotope was administered in one single dose on day 14 of the experiment to the mothers,

then the samples were collected 48 hours afterwards. The docosahexaenoic acid labelled with the isotope was determined by gas chromatography and mass spectrometer measuring the isotope ratio, the fatty acid composition of the milk fat was determined by gas-liquid chromatography. At the beginning of the experiment docosahexaenoic acid content of the mother's milk did not differ significantly for the group received placebo (0.29%) and the group received the supplementation (0.28%), however, two weeks after the docosahexaenoic acid supplementation its concentration increased almost to the double for the group received the supplementation. From the experiment it was concluded that the docosahexaenoic acid supplementation in the mother's food considerably increased its concentration in the mother's milk.

Hibbeln [21] examined docosahexaenoic acid content of the mother's milk due to consumption of various foods of marine origin. It was established that foodstuff of marine origin increased somewhat the docosahexaenoic acid content of the mother's milk. Both consumption of foods of marine origin and docosahexaenoic acid content of the mother's milk were brought into connection with afterbirth depression of the mothers.

Brenna et al. [6] examined long-chain fatty acid composition of milk of 106 mothers. Docosahexaenoic acid content was measured on the average to be 0.23–0.32% (extreme values 0.06 and 1.4%), arachidonic acid content to be 0.13–0.47% (extreme values 0.24–1.0%). A significant relationship was established between the change of the docosahexaenoic acid and that of arachidonic acid.

Xiang et al. [53] examining the essential fatty acid content of milk of Chinese and Swedish mothers analyzed the effect of food on the composition of milk. The Chinese mothers consumed mainly rice, steamed bean, noodles, Chinese cabbage and pork. Food of Swedish mothers consisted of bread, potato, paste, milk, sour milk and cheese. The linoleic acid intake of the Chinese mothers was 14.06 g/day, that of the Swedish mothers 9.91 g/day. The linoleic acid/α-linolenic acid ratio was significantly higher for the Chinese than for the Swedish. It was established that the linoleic acid concentration in the milk of the Chinese mothers was significantly higher (22.69%) than that of the Swedish mothers (10.93%); whereas concentration of α-linolenic acid was in the milk of the Chinese mothers lower (1.19%) than in that of the Swedish mothers (1.60%). It was also established that the linoleic acid/α-linolenic acid ratio was much higher in the milk of the Chinese mothers (22.97) than in that of the Swedish ones (7.50) and also the arachidonic acid/docosahexaenoic acid ratio (3.14; 1.56) was higher. Docosahexaenoic acid content of the food had a positive effect on the milk composition of both the Chinese and Swedish

mothers.

Sala-Vila et al. [44] examined the fatty acid composition of the colostrum (day 1–5), transitional milk (day 6–15) and ripe milk (day 15–30) of 66 mothers of Granada bearing at normal time. Determining the essential fatty acid content of the phospholipids it was established that the amount of C18:2n6 (16.16–18.57%), C18:3n3 (0.17–0.27%) and C20:4n6 (3.66–3.95%) fatty acids increased significantly from the colostrum to the ripe milk. The colostrum contained on the other hand more C22:6n3 (1.53–0.97%) fatty acid than the ripe milk. It was established that the milk of mothers of Granada did not differ significantly from the average fatty acid composition of milk of mothers examined in other parts of the world, it contained only less arachidonic acid (0.2%) than e.g. the milk of the German mothers (1.0%).

López-López et al. [27] compared two direct methods for determination of the fatty acid composition of mother's milk. Fatty acids converted into methyl esters were determined by capillary gas chromatography in human colostrum. According to the first method the fatty acids were transesterified using acetyl chloride, while according to the second method a mixture of boron trifluoride and methanol. No difference was found between the traditional and the elaborated direct method, both of the methods gave the same result. In case of docosahexaenoic acid the results were 112.3 and 114.6 $\mu g/100\,cm^3$, while in case of arachidonic acid 195.3 and 194.7 $\mu g/100\,cm^3$. Advantage of the boron trifluorid-methanol method is that it is much quicker, can be performed more safely and gives a better yield. In the human colostrum the concentration of the linoleic acid was measured to be between 18.25–18.38%.

Fidler et al. [11] examined essential fatty acid composition of the colostrum of mothers living in Slovenia in town and in country environment, which was in case of linoleic acid (18:2n6) 15.26%, for α-linolenic acid (18:3n3) 0.91%, for docosahexaenoic acid (22:6n3) 0.43%, and for arachidonic acid (20:4n6) 1.03%.

Serra et al. [46] examining essential fatty acid composition of milk of Italian mothers nourishing according to appetite established that with the advance of the lactation reduced significantly the amount of arachidonic acid and docosahexaenoic acid. The amount of linoleic acid in the ripe milk was 9.79%, while that of α-linolenic acid 0.36%.

According to Laryea et al. [25] the essential linolenic acid content (18.28%) of milk fat of Sudanese mother is similar to that of mothers living in the developed countries, on the other hand concentration of the C22:6n3 fatty acid was very low in the milk fat. Bahrami and Rahimi [2] examining essential fatty acid content of milk of West Iranian mothers established that linoleic acid

represented 13.8%, linolenic acid 1.1% and arachidonic acid 1.4% of the total fatty acids of the milk.

According to Koletzko et al. [24] arachidonic acid ranged in most of the studies between 0.4–0.6%, docosahexaenoic acid between 0.2–0.4%. It was established that the different researches did not find significant difference in the linoleic acid and α-linolenic acid content of the mother's milk irrespective of the mothers' living place. The essential linoleic acid and α-linolenic acid content of the mother's milk increases with the ripening of the milk, at the same time arachidonic acid decreases by 38%, docosahexaenoic acid by 50% in the first month of the lactation. The DHA content decreases by approx. 20% between week 6 and week 16 of the lactation, after that it does not change until week 30 of the lactation.

Fidler and Koletzko [10] summarized the results of 15 studies dealing with fatty acid composition of colostrum of mothers from the world's 16 regions. Out of the studies 11 derived from Europe, one from Central America, one from the Caribic, one from Australia and one from Asia. It was established that the amount of the essential fatty acids was similar in the South European countries (Spain, France, Slovenia). In case of mothers living in St. Lucia the rich fish consumption increased the amount of docosahexaenoic acid. Comparing the concentration of docosahexaenoic acid for mother living in different regions it can be established that this was the lowest (0.1–0.2%) in the colostrum of the Italian and German mothers, followed by the Spanish, Slovenian, Swedish, French, Panamian, Chinese and Australian (0.4–0.7%), and it was the highest in the colostrum of mother living in St. Lucia (1.0–1.1%). In two French studies [18, 31] it was described that during a two-year period the fatty acid composition of the colostrum did not change practically, two German studies [14, 19], however, reported that during 14 years docosahexaenoic acid and arachidonic acid content of the colostrum increased. According to a Spanish study of Pita et al. [39] covering 13 years α-linolenic acid content of the colostrum decreased during the experiment. According to Gibson and Knebone [15] in the colostrum of the Australian mothers was the lowest the amount of linoleic acid (7.8%) and α-linolenic acid. In contrast with the above docosahexaenoic acid content (0.6%) of the milk of Australian mothers was higher than that of the European mothers. It was concluded that the fatty acid composition of the mother's colostrum was affected considerably by the geographical and nutritional differences.

Patin et al. [37] examined the effect of the sardine consumption on the essential fatty acid content of the ripe mother's milk. The mothers were divided into two groups regarding sardine consumption. The first group contained

mothers who consumed sardine within 3 days prior to the sampling, while to the second group belonged mothers who consumed sardine four days before the sampling. It was established that there was a significant difference in case of docosahexaenoic acid at all the three sampling time (day 1, day 15, day 30) within the groups (Group 1: 0.35%, 0.61%, 0.67%, Group 2: 0.44%, 0.45%, 0.41%). In case of the n-6 fatty acids a significant difference could be established between the two groups only in the linoleic acid concentration at all the three sampling time (Group 1: 21.48%, 22.59%, 24.11%, Group 2: 21.05%, 19.63%, 20.88%). In case of arachidonic acid and linolenic acid there were only minor differences between the two groups. Examining during the lactation the change of the concentration of the n-6 fatty acids it was established that in case of both groups only the concentration of linoleic acid increased, while concentration of linolenic acid and arachidonic acid decreased.

According to Picciano [38] absorption of the essential fatty acids of the mother's milk in the point of view of the newborn is important not only because of the energy supply but because they contribute to the formation of retina and the nervous tissue. Mother's milk is a rich source of linoleic acid (8–17%), α-linolenic acid (0.5–1%) as well as long-chain fatty acids, arachidonic acid (0.5–0.7%) and docosahexaenoic acid (0.2–0.5%).

Minda et al. [33] examined essential fatty acid content of milk of mothers living in Pécs, on the days 1, 2, 3, 4, 5, 6, 7 and then on day 14 and day 28 after the childbirth. In the course of the examinations it was established that out of the essential fatty acids the amount of linoleic acid decreased until day 4 of the lactation (15.00–13.46%), then rose significantly until day 28 of the lactation (15.12–17.24%). The amount of arachidonic acid (1.09–0.41%) decreases significantly, that of α-linolenic acid increases (0.49–0.67%), while the other fatty acids do not show a considerably change during days 1–28 after the childbirth. According to the authors docosahexaenoic acid content of milk of Hungarian mothers is lower than that of many other population.

3.4 Trans fatty acid

Glew et al. [16] examined trans fatty acid content of milk of North Nigerian Fulani nomadic tribes and townmothers. From 41 Fulani breast-feeding mothers and 41 townmothers who were all in a good health condition, around 15 ml milk sample was taken between 8 and 10 a.m., applying a 4–8 minute-hand milking. The fatty acids were transesterified (0.5 M NaOH in methanol, 14% boron trifluoride) and the fatty acid methyl esters were determined by a gas chromatographic method. Main target of the experiment was to compare milk

fat composition of country and townmothers. The Fulani consumed mainly dairy products deriving only from cows, trans fatty acid content of which was lower than that of the townmothers whose diet contained only a few dairy product. It was surprising that the belief that different nutrition results in different milk fat fatty acid composition was proved to be untrue, as regarding the trans fatty acids there was no difference between the two groups of the mothers. Trans fatty acid content in the milk of the Fulani mothers was 0.22%, in the milk of townmothers 0.34%. The t11-C18:1 vaccenic acid represented more than 85% of the trans C18:1 fatty acids in both groups, the rumenic acid (c9, t11-C18:2) isomer, however, represented approx. 40% of the conjugated linoleic acids in both populations. Average amount of the trans fatty acids in both Nigerian populations (0.22–0.32%) was 7–10 times smaller than that of the French mothers or of the ones living in other developed countries.

Jahreis et al. [22] analyzing the trans vaccenic acid content of cow's, goat's, sheep's, sow's, mare's and mother's milk established that its concentration changed in the milk of ruminants seasonally. According to Silva et al. [49] trans fatty acid content of ripe milk of Brazil mothers is only 2.3% which is very low compared to mother's milk samples examined in other parts of the world.

Hayat et al. [20] examined trans fatty acid content of milk of 19 healthy Kuwaiti mothers aged between 20–30 years. Relating to trans fatty acids C14:1t, C16:1t, C17:1t, C18:1t, C20:1t, and C18:2t in case of the Kuwaiti mothers altogether 2.8% was obtained. Comparing with the trans fatty acid content of milk of Canadian and German mothers (7.2%, 4.4%) it can be established that trans fatty acid content of the milk of the Kuwaiti mothers is the lowest, which is attributed to regional difference.

Wijga et al. [51] examined the composition of the mother's milk and the relationship with allergic diseases in case of allergic and non-allergic women. It was established that in case of the children of allergic mothers the amount of trans fatty acids in the mother's milk could be brought into connection with the allergic symptoms, whereas in case of children of non-allergic mothers no such relationship could be found.

Chen et al. [7] examined trans fatty acid isomers of milk of Canadian mothers. Milk samples were collected from 98 mothers 3–4 weeks after the childbirth, which were daily averages. The fatty acids were converted into methyl esters, and the analyses were carried out by gas liquid chromatography using capillary column. Average concentration of the trans fatty acids was found to be 7.19%, where the lower limit was 0.10, the upper limit 17.15%. Out of the 198 samples 25 contained more than 10% trans fatty acid and 13 samples

contained less than 4%. All the trans isomers of linoleic acid were present in the milk fat in 0.89%, with C18:2 c9,t13 in the highest concentration, followed by c9,t12 and c12,t9. On the basis of the results the average daily trans fatty acid consumption of the Canadian breast-feeding mothers was estimated to be 10.6 g per person, but there were ones who consumed even 20.3 g trans isomer. The proportion of the C18:1 trans isomers in the mother's milk was different than in the cow's milk, and was extremely similar to the partially hydrogenated soya and sunflower oil, which supported the hypothesis that the main sources of the trans fatty acids are the partially hydrogenated vegetable oils.

Precht and Molkentin [40] examined linoleic acid, linolenic acid, oleic acid and trans fatty acid content of milk of 40 German mothers. The amount of C18:1 t11 fatty acid was measured to be 2.4%. In the milk the t4 (0.02%), t5 (0.02%), t6–8 (0.21%), t9 (0.37%), t10 (0.32%), t11 (0.68%), t12 (0.23%), t13 (0.15%), t14 (0.18%), t15 (0.09%) and t16 (0.14%) fatty acid isomers containing one unsaturated bond were identified with vaccenic acid as the dominating one. Also the tC14:1 (0.08%) and tC16:1 (0.15%) fatty acids were identified. Further 6 cis and trans linoleic acid isomers were identified with total amount of 1.07%, and also four trans α-linolenic acid isomers with total concentration of 0.11%. In summary, milk fat of milk of German mothers contained 3.81% trans fatty acid where the extreme values were 2.38 and 6.03%. A direct relationship was established between the trans 18:1 fatty acids of the foodstuffs and composition of the lipids of mother's milk. The amount of the fatty acids was as follows: C12:0 3.12%; C14:0 6.43%; C16:0 25.28%; C18:0 7.41%, C18:1 (total) 33.67%; C18:2 (total) 10.63% and α-C18:3 0.87%.

Bahrami and Rahimi [2] comparing fatty acid composition of milk of West Iranian mothers to that of European and American mothers established that the milk of Iranian mothers contained much middle-chain fatty acids and trans fatty acids which can be explained by the low animal protein and animal fat consumption, the high carbohydrate consumption and by the consumption of partially hydrogenated vegetable oils containing much trans fatty acids. They recommend that the Iranian mothers should consume less trans fatty acid containing foods in order to prevent the harmful effect of the trans fatty acids. According to Minda et al. [33] trans fatty acid content of milk of mothers living in Pécs did not change during the lactation.

3.5 Conjugated linoleic acids

Glew et al. [16] examining conjugated linoleic acid (CLA) content of milk of Fulani nomadic tribes and townmothers compared milk fat composition of Fulani country and townmothers. The Fulani consumed mainly dairy products deriving only from cows, CLA content of which was higher than that of the townmothers whose diet contained only a few dairy product. It was surprising that even due to the different nutrition there was no difference regarding the CLA between the two groups of the mothers. The CLA content was in the milk of the Fulani mothers 0.16%, while in the milk of the townmothers 0.14%.

Mosley et al. [35] studied the synthesis of c9,t11-CLA from vaccenic acid in lactating mothers. Four mothers took part in the experiment on the average on day 247 after the childbirth, who were breast-feeding at least six times a day and consumed foods ordered according to the experiment. Subsequent to starvation in the night they received 25 mg/body mass kilogram trans11-vaccenic acid, and sample was taken in 0, 2, 4, 6, 8, 12, 18, 24 and 48 hours after the vaccenic acid consumption. 8 hours after the consumption the milk's average vaccenic acid content was 3.1%, and it reached its maximum in the hour 18 with 7.6%. c9,t11-CLA content of the milk of the ones consumed a diet supplemented by vaccenic acid reached its maximum in the hour 18 with 0.4%, supporting the hypothesis that from the vaccenic acid δ-9-desaturase enzyme formed conjugated linoleic acid. It was established that around 10% of the c9,t11-CLA content of the milk was formed in the milk gland from vaccenic acid.

Bertschi et al. [3] examined the effect of Alpenbutter consumption of German mothers on the CLA content of the mother's milk with special respect to the CLA isomers. They started to collect the milk samples in the Zurich University Hospital from 20 healthy mothers 2–4 days after the childbirth. The mothers were divided into groups based on the food consumption. Group 1 contained mothers who had a normal diet until day 20 after the childbirth, and received between day 1–10 a food supplementation of 40 g margarine/day, containing 24 g fat and 16 g water, and which was given to the food in four equal portions. Between day 11–20 the food was supplemented with extra 30 g alpine butter per day in three portions, containing 25.7 g fat and 4.3 g water, and 2.09 g conjugated linoleic acid in 100 g, which corresponds to around 0.5 g conjugated linoleic acid intake a day. The mothers recorded their food consumption, during which they did not consume alpine butter and beef. Group 2 contained mothers with normal nutrition, for which the daily estimated milk, dairy product and meat consumption. The difference between Group 2 and

Group 1 was that mothers of Group 2 received between day 1–10 30 g/day margarine, between day 11–20 40 g/day alpine butter in four equal portions. The butter originated from Graubünden (Switzerland), from 2100 m altitude; the 2 kg packings were stored at –20 °C until they were utilized. The milk samples were taken on day 1, 5, 10, 15 and 20 of the experiment between 8–11 a.m. CLA content was determined by high-performance liquid chromatography. It was established that due to the alpine butter supplementation the CLA content of the mother's milk increased. The amount of the c9,t11-CLA isomer increased by 49.7%, and a significant increase could be experienced for the t9,t11, t7,c9, t11,c13 and t8,c10 isomers. The remaining nine isomers did not show any change, their concentration was below 5 mg/100 g fat. From the experiments it was concluded that the mother can consciously influence the CLA content of their milk by alpine butter consumption.

McGuire et al. [32] examined CLA content of the mother's milk and the milk replacing food preparations, during which they took milk samples from 14 mothers 3–10 days after the childbirth. The analyses were done using gas chromatograph. Each milk sample contained the c9,t11 CLA isomer in a measurable concentration ranging 2.23–5.43 mg/g with an average and dispersion of 3.64±0.93 mg/g fat. The c9,t11 isomer was 83–100% of the total CLA amount, and out of 14 sample in case of 8 sample only this isomer could be identified. No relationship was found calculating either in fat or milk basis in the CLA content of the mother's milk according to the time elapsed after the childbirth. It was established that the total CLA content of the mother's milk including also the c9,t11 isomer was considerably higher than that of the milk replacing food preparations.

Jahreis et al. [22] recognizing the anti-cancer effect of the c9,t11 CLA isomer analyzed the composition of cow's, goat's, sheep's, sow's, mare's and mother's milk. After the sampling the milks were immediately frozen down to –18 °C. To the esterification a chloroform:methanol 2:1 mixture, to the methylation potassium methylate was used, the analyses were carried out gas chromatographically. It was established that in milk of each species the c9,t11 CLA isomer occured in the majority, with an amount ranging between 0.07–1.35% depending on species in the relative weight percent of the fatty acid methyl esters. As the micro flora of the rumen influences the isomerization of linoleic acid, also the foddering and seasonal effects were taken into consideration during the examinations. The CLA content in the milk of each ruminant changed seasonally (1.28% in July; 0.54% in March). Out of the ruminants, the sheep's milk was the richest in CLA (1.1%), followed by the cow (1.0%) and goat (0.64%). Out of the non-ruminants the mare's milk contained the

least (0.09%) CLA, while that of the sow's 0.2%. The mother's milk contained significantly more conjugated linoleic acid (0.42%) than that of the monogastric domestic animals. In the CLA content of the mother's milk a difference was found between the milk consuming and non-consuming mothers. Comparing the CLA content measured in the individual species it was established that the mother's milk is between the milk of non-ruminants (mare, sow) and that of the ruminants (goat, cow, sheep).

Marangoni et al. [28] examining the essential fatty acid composition of the mother's milk and mother's plasma during the first and third month of the lactation obtained a significant relationship in case of linoleic acid and α-linolenic acid between the composition of the milk and the plasma. In case of the arachidonic acid and docosahexaenoic acid a significant relationship was obtained once, in month 3. It was concluded that in case of linoleic acid and α-linolenic acid there was a close relationship between the composition of the plasma and that of the milk.

Precht and Molkentin [40] examining CLA content of milk of 40 German mothers measured the amount of the main CLA isomer (c9,t11) to be 0.4%.

4 Cholesterol

Clark et al. [8] took milk sample from 10 mothers in the week 2, 6, 12, and 16 of the lactation and determined its total cholesterol and free cholesterol content. The average cholesterol and free cholesterol content of the milk was measured to be 10.3 and 8.3 mg/100 cm^3, which values did not change significantly in the time elapsed after the childbirth.

According to Koletzko et al. [24] the mother's milk has high cholesterol content (10–20 mg/100 cm^3, 250–500 mg/100 g fat). Cholesterol is 90.1% of the total sterols, followed by desmosterol 8.6%, whereas the amount of the phytosterols is negligible. The mother's food appears not to influence the milk's cholesterol content. Children fed with mother's milk receive around 25 mg cholesterol per body mass kilograms a day, this is around 4 mg for the adults. Plasma cholesterol level in breast-fed babies is higher than that of babies fed with milk replacing food preparations, no relationship was found, however, in the plasma lipid level after the breast-feeding depending on whether the baby was breast-fed or fed with food preparation.

According to Picciano [38] cholesterol content of the mother's milk is low at the beginning of the lactation, and increases gradually during the lactation.

Bitman et al. [4] examined the cholesterol content of the mother's milk

2–3 days after the childbirth, as well as on the day 7, 21 and 42 of the lactation. Milk was taken from 18 mothers with very premature childbirth (in week 26–30 of the lactation), from 28 mothers with premature childbirth (in week 31–36 of the lactation) and from 6 mothers bearing at normal time (week 37–47). Colostrum of mothers bearing at normal time contained significantly less out of these fatty acids compared to mothers with premature childbirth. To the transesterification of the fatty acids a chloroform-methanol mixture was used and their amount was analyzed by thin-layer and gas chromatography. Fatty acid composition of the cholesterol esters was determined by gas liquid chromatography after the cholesterol esters had been isolated by preparative thin-layer chromatography. During the lactation concentration of the total cholesterol and cholesterol esters decreased from 5 mg/dm^3 measured in the colostrum to 1 mg/dm^3 in the ripe milk. Fatty acid composition of the cholesterol esters was similar in all the three groups after the childbirth. As weight% proportion of the fatty acids esterified by cholesterol the following was obtained: C10:0, 0.7%; C12:0, 2.6%; C14:0, 2.3%; C16:0, 11.4%; C16:1, 5.0%; C18:0, 8.8%; C18:1, 32.9%; C18:2, 30.6%; C18:3, 1.7%; C20:3, 0.9% and C20:4, 1.8%. Proportion of the unsaturated fatty acids in the cholesterol fatty acid esters was 73%, which is considerably higher than in the milk triglycerides. The biggest difference was obtained in the linoleic acid content, which was 30.6% in the cholesterol esters and 13% in the milk fat. From the results it was concluded that in the fatty acid composition of the cholesterol esters the unsaturated fatty acids dominated and that the fatty acid composition of the cholesterol esters considerably differed from the fatty acid composition of the milk fat.

Clark and Hundrieser [9] compared the cholesterol esters of the mother's milk to the total lipid content. By the analysis of 25 milk samples for total lipid, total cholesterol and free cholesterol, the cholesterol esters and triglycerides were isolated and fatty acid composition of each fraction was determined. Total cholesterol content of the mother's milk was 13.5 mg/dm^3, which was in a significant positive relationship with the total fat content of the milk. Average of the free cholesterol was 10.9 mg/dm^3 which similarly was in a significant positive relationship with the total fat content. With increasing total fat content the fatty acid composition of the cholesterol esters shifted to the saturated fatty acids. The biggest changes were experienced in case of linoleic acid and arachidonic acid that were in negative relationship with the total lipid content. Fatty acid content of the triglycerides exhibited no relationship with the total fatty acid content. From the results it was concluded that the fatty acid composition of the cholesterol esters and that of the triglycerides

differed considerably from each other.

5 Phospholipids

Clark et al. [8] examined the phospholipid content of milk taken from 10 mothers in the week 2, 6, 12, and 16 of the lactation and measured its average amount to be 3.9 mg/100 cm^3, which remained unchanged in the examined period. According to Picciano [38] phospholipid content of mother's milk is lower in the early phase of the lactation, it increases in the course of the lactation, however. According to Koletzko et al. [24] phospholipid concentration of the milk is 25 mg/100 cm^3 and 0.6 g/100 g lipid, respectively. Within the phospholipids phosphatidyl choline represented 28.4%, phosphatidyl ethanolamine 27.7%, phosphatidyl serine 8.8%, phosphatidyl inositol 6.1% and sphingomyelin 37.5%. Phospholipids have emulsion forming feature, they contribute to the stability of the milk fat micelles. Sphingo- and glykolipids as well as ganglioside contribute to the protective mechanism of the body by absorbing bacterium toxins.

6 Triglycerides

Morera et al. [34] examined triglycerides of the ripe mother's milk and fatty acid composition of the triglycerides in the milk of 40 mothers. They tried to ensure that the milk samples derived from circumstances as various as possible, therefore they took samples from different mothers, on different days, at different hours of the day, from the right and left breast by turns, before and after the baby was breast-fed and before and after the mother ate. The triglycerides were determined by high-performance liquid chromatography and linked mass spectrometer, the fatty acid composition was determined by gas chromatography. Analyzing the obtained results by various statistical methods it was established that the triglyceride content of the mother's milk and fatty acid composition of the triglycerides are relatively stable, showed a minimal variability despite subsequent to the statistical analysis they could be arranged into different groups. Between the concentration of the triglycerides and fatty acid composition of the fat in some cases significant relationship could be found. It was established that the properties of the triglycerides in the mother's milk were considerably influenced by external factors like nutrition, nourishment level, length of the lactation and part of the day. Despite this, some triglycerides can be considered as marker of the ripe mother's milk,

as these, their concentration were not affected by the extremely different sampling conditions. According to Picciano [38] in the mother's milk the lipids are the most important energy supplying compounds, occuring in 97–98% as triglycerides. Fatty acid content of the triglycerides is around 88%.

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Composition of the mother's milk III. Macro and micro element contents. A review

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Abstract. The authors have analysed macro and micro element contents of the mother's colostrum and mother's milk in comparison with the newest publications. Calcium contents of the mother's milk varied in most of the studies between 84 and 462, while phosphorus contents varied from 17 and 278 mg/dm^3. The amount of both calcium and phosphorus increased during lactation, but none of these elements was affected by calcium and phosphorus level of the serum, the vitamin supply, age and smoking. Average magnesium concentration of the mother's milk is 30 mg/dm^3, which is not affected by age, vitamin D supply, lactation and diabetes, and even the magnesium supply increases the first day only the magnesium contents of the milk. Sodium contents of the colostrum decreases from 400 mg/dm^3 to 150 mg/dm^3, potassium contents from 600–700 mg/dm^3 to 400–550 mg/dm^3, chloride contents from 600–800 mg/dm^3 to 400–500 mg/dm^3 in the mature milk.

Some of the micro elements occur bonded in protein in the milk, which increase the efficiency of the absorption. Iron contents of mother's

Key words and phrases: mother's milk, macroelements, microelements

milk were found in extreme cases between 0.04–1.92 mg/dm^3, on average 0.40 mg/dm^3, which is not affected by the environment, the mother's nutriment, the iron intake and the contraceptive preparations. Its absorption from the mother's milk is extraordinarily favourable, therefore even a low iron contents are sufficient to satisfy the needs of the babies. Copper contents of the mother's milk vary between 0.03–219 mg/dm^3, on average 0.350 mg/dm^3. The effect of lactation on the copper contents is controversial, and it appears that the copper contents are not influenced by either the nutriment or the copper intake. Its major part if bonded to protein, therefore its absorption is very favourable. Zinc contents of the mother's milk were measured to be between 0.15–5.41 mg/dm^3, it is difficult to specify an average value due to variations of order of magnitude. Similarly, there are extreme value obtained for the manganese contents 0.8–21.5 µg/dm^3, which can be explained by the different manganese intake of the mothers, or by extreme manganese burden of the environment.

Out of the other micro elements, the authors analyze the chromium, nickel, cobalt, molybdenum, selenium, iodine and silicon contents of the mother's milk, while among toxic trace elements cadmium, lead and mercury contents. Amount of latter ones in the mother's milk is affected by smoking, the polluted urban air, exhaust gas of the motor vehicles, the polluted environment and by the number of amalgam fillings. Cadmium contents of the mother's milk were measured to be between 0.07–3.8 µg/dm^3, but in an extremely polluted urban environment it reached even the value of 24.6 µg/dm^3. Even more extreme values were measured for the lead, as its concentration ranged from a couple of tenth to 350 µg/dm^3. Lead contents were increased mainly be the polluted urban air, however, its amount decreased after the unleaded fuels have been widely used. Mercury contents of the mother's milk were affected mainly be number of amalgam fillings and the surface of the fillings. Its amount varied from 0.10 to 6.86 µg/dm^3.

1 Macro elements

1.1 Calcium, phosphorus

Khatir Sam et al. [58] measured calcium content of Sudanese mothers to be 388 mg/dm^3. Bocca et al. [11] determined calcium content of 60 mothers from different areas of Italy, aged between 19 and 40 years, by high-performance liquid chromatography and inductively coupled plasma emission spectrophotometry, and measured it to be 306 µg/cm^3. Based on the measurements a close positive correlation was found between the calcium and magnesium con-

tent.

Dorea [29] analyzing calcium and phosphorus content of mother's milk examined between 1950 and 1999 – based on the works of 169 authors – established that the calcium content varied between 84–462 mg/dm^3 (on the average 252 mg/dm^3), while phosphorus content between 17–278 mg/dm^3 (average value 143 mg/dm^3). Average calcium/phosphorus ratio was 1.7, with extreme values 0.8 and 6.0. In this study the effect of childbirth at young age, duration of the pregnancy, state of underfeeding of the mother, physical burden, various metabolic deseases, different nationality, lactation state, time of separation, milk amount, sampling techniques, environmental differences, social-cultural differences, smoking habits, nutrition, calcium and vitamin D supplementation and contraceptives taken for a long time, on the mother's milk, was specially treated. It was concluded that with the exception of youth motherhood and some metabolic deseases no environmental or other factor did have an effect on the calcium and phosphorus content of the mother's milk.

Shores et al. [86] examined calcium, copper, magnesium, manganese, phosphorus and zinc content as well as middle-chain fatty acid content (capric, lauric and miristic acid) of milk of 33 Fulani mothers, and recorded age, height, body mass of the mothers and number of children. After sampling the samples were stored at –20 °C until the analyses. Calcium content was measured to be 263 mg/dm^3, while phosphorus content to be 165 mg/dm^3. No relationship could be established between middle-chain fatty acids and calcium content.

According to Emmett and Rogers [34] calcium content of mothers of different nationality increases during the lactation from 28 mg/dm^3 in the colostrum to 34 mg/dm^3 in the ripe milk, while phosphorus content from 14 mg/dm^3 to 16 mg/dm^3. Hunt et al. [52] examined calcium content of the mother's milk during first four weeks of the lactation and established that it reduced from 7.01 μmol/dm^3 to 6.68 μmol/dm^3.

Picciano [77] examined calcium and phosphorus content of the mother's milk and experienced that these components are independent of the serum concentration, although Greer et al. [46] established a weak positive relationship between the mother's calcium-intake and calcium content of the milk. In the course of the lactation phosporus content of the mother's milk decreased from 147 mg/dm^3 in the third week of lactation to 107 mg/dm^3 till week 26 of lactation; calcium content decreased from 259 mg/dm^3 to 248 mg/dm^3. In his opinion nutrition of the mother does not affect concentration of these elements.

Yamawaki et al. [97] examined macro and micro element composition of milk of mothers from different areas of Japan by an inductively coupled plasma emission spectrophotomer. The milk samples were collected from around 4000

women in different date of lactation (day 1 to day 365), 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 nursings, the samples were stored in a deep-freeze until the analysis. Along with the sampling, data were collected on smoking habits, vitamin supply, birth-weight of the newborn and on breast position (left or right). 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. Between the formed groups no significant difference was found regarding calcium and phosphorus content of the mother's milk. It was established that concentration of phosphorus and calcium in the milk is higher in the winter months than in the summer (phosphorus: 14.6–15.3 mg/100 cm^3; calcium: 23.7–26.2 mg/100 cm^3), the lactation does not affect the concentration of these components, however.

Honda et al. [49] examining calcium and phosphorus content of the mother's milk, drew 68 mothers, aged between 19 and 38 years, into the experiment, who lived in the non-industrial region of Japan. More than 70% of them was a housewife, and they did not differ significantly from each other in nourishment and state of health. Comparing calcium and phosphorus content of the milk according to the age the mothers, it was established that calcium content of milk of mothers older than 35 years was higher than that of mothers below 35 years (344.4 mg/dm^3, 326.4 mg/dm^3), in the phosphorus content there was no significant difference (191.6 and 188.6 mg/dm^3) between the two groups. No significant difference could be established in case of both elements, either for mothers with the first childbirth or for those with several childbirths (calcium: 327.8; 330.4 mg/dm^3, phosphorus: 183.9; 194.5 mg/dm^3).

1.2 Magnesium

Shores et al. [86] measured magnesium content of milk of Fulani mothers to be 31.2 mg/dm^3. No relationship could be established between middle-chain fatty acids of milk and magnesium content. Butte et al. [12] established signif-

icant difference in the magnesium content of the precolostrum and colostrum, while others could report no such a significant difference. The high magnesium content in the precolostrum is connected presumably with the fact that magnesium is necessary for the formation of the bone's minerals. As ash content of the mother's milk decreases continuously during the lactation, similar changes should occur also in the magnesium content. However, according to Carias et al. [14] as well as Tanzer and Sunel [90] magnesium content of the mother's milk increases slightly in the first six months of lactation. According to Emmett and Rogers [34] magnesium content of milk of mothers with different nationality does not change with the lactation, it is 30 mg/dm^3 on the average. According to Picciano [77] magnesium content of the mother's milk increases from 29.0 mg/dm^3 measured in week 3 of lactation to 33.0 mg/dm^3 till week 26 of the lactation.

According to Hunt et al. [52] magnesium content of the mother's milk increases during the first four weeks of lactation from 1.18 mmol/dm^3 to 1.36 mmol/dm^3, according to Atkinson et al. [6] and Itriago et al. [55] it decreases, whereas according to Allen et al. [2] and Carrion et al. [15] it shows no substantial change in the first phase of the lactation. In the further part of lactation – in connection with the change of the ash content – a minimal change occurs in the magnesium content.

Yamawaki et al. [97] measured the average magnesium content of milk of mothers from different areas of Japan to be 2.7 mg/100 cm^3. It was established that during lactation the magnesium content decreased from 3.2 to 2.5 mg/100 cm^3, but does not change significantly seasonally (2.6–2.7 mg/100 cm^3).

Dorea [31] in a comparative study examining the magnesium content of the precolostrum, the transitional milk and the ripe milk, came to the conclusion that the colostrum and the precolostrum contribute considerably to the satisfaction of the needs of the children born prematurely. Magnesium content of the precolostrum does not differ significantly from that of the ripe milk due to the great variations in the mean values.

According to Fransson and Lönnerdal [42], major part of the magnesium content of the mother's milk is attached to the protein fractions with lower molecular weight and proteins, respectively (53.6%), and only a very small portion of it can be found in the milk fat (1.8%) and fat micelles (0.8%). Magnesium content was measured on the average to be 31 mg/dm^3, with extreme values of 15 and 64 mg/dm^3, and in 75% of the examined cases it did not reach 35 mg/dm^3.

Coni et al. [20] taking magnesium content of the mother's milk 28.0 mg/kg

established that 90% of it is linked to low molecular weight protein fractions.

According to Huang et al. [51] as well as Hua et al. [50] circumstances influencing the mother's metabolism do not affect secretion of magnesium. Magnesium metabolism is affected by changes in the insuline production due to which concentration of the intracellular magnesium increases.

Bitman et al. [10] established that magnesium content of 48.6 mg/dm^3 of milk of diabethic mothers did not change significantly from that of control mothers. Even galactosemie did not affect significantly the milk comparison [38]. During the early pregnancy mineralization of the baby's bones can be disturbed [18] and Lipsman et al. [65] established that magnesium content of teenager mothers is lower than that of older mothers. Bocca et al. [11] measured magnesium content of the mothers milk to be 0.030 μg/cm^3 and established that its concentration in the milk of mothers older than 30 years is higher than in that of younger mothers.

According to Honda et al. [49] magnesium content of milk of Japanese mothers older than 35 years is lower than that of mothers aged below 35 years (32.2 mg/dm^3 and 34.7 mg/dm^3). No significant difference could be found between the milk of mothers with the first childbirth and that of mothers with more childbirth (35.1 and 33.6 mg/dm^3). Number of children [63], state of underfeeding [81] and social position [43] have no significant effect on the composition of the mother's milk. Similarly, loss of magnesium due to physical efforts did not affect magnesium content of milk [37]. Regional differences, the countryside or urban environment did not affect magnesium content of the milk [19, 76] comparing magnesium content of milk of mothers living in various countries (Guatemala, Hungary, Niger, Philippines, Sweden, Zaire) established that there was a substantial difference between the different countries (22.6 and 34.2 mg/dm^3).

According to Karra and Kirksey [56] magnesium content of milk of the American mothers significantly increase in the first three months of lactation despite unchanged magnesium-intake. Magnesium deficiency does not occur either in the developing or in the developed countries, therefore there are no data on the effect of the food's magnesium deficiency on the magnesium content of the milk. Independently of the daily magnesium consumption there was no significant difference in the magnesium content between the milk of the Egyptian (386 mg/day), the American (361–410 mg/day) and the Nepalese mothers (353 mg/day) [57, 70]. The vegetarian lifestyle can affect the magnesium content of the milk, because from the vegatarian foods magnesium is utilized worse [36]. Comparing the magnesium content of milk of vegetarian and non-vegetarian mothers it was experienced that beside the same magne-

sium intake magnesium content of the vegetarian mothers was 27.5 mg/dm^3, whereas that of non-vegatarian mothers 31.1 mg/dm^3. On the other hand, Dagnelie et al. [24] reported that magnesium content of milk of those who consumed macrobiotic diet was lower (31.1 mg/dm^3) than that of pantophage ones (35.8 mg/dm^3). It seem that the components supporting the incorporation of minerals into the bones, e.g. vitamin D, do not influence the magnesium content of the milk. Magnesium content of milk of mothers taking vitamin D was measured to be 21.6–22.8 mg/dm^3, whereas that of mothers not taking vitamin D to be 20.9–25.5 mg/dm^3 [89]. Steroid hormones do not have an influence on the magnesium content of the milk, even though they were taken for a long time before the pregnancy [72].

Some reported magnesium decreasing effect of contraceptives taken during the lactation, while others denied these results, which may be explained by the hormone preparations taken in different doses. Comparing the magnesium content of milk of the group treated with hormones and the control group, it can be established that in case of the control group there was no significant difference according to the lactation, on the contrary, in case of ones taking hormone preparation magnesium content of the milk significantly decreased. After the childbirth, magnesium content of mothers who received a magnesium sulfate therapy was 64 mg/dm^3 24 hours after the start of the treatment, in comparison with the concentration of 47.7 mg/dm^3 of the control group. One day after the treatment, however, there was no difference in the magnesium concentration of either the serum or the colostrum between the two groups [21]. The high-calcium diet did not affect the magnesium status and the absorption of magnesium [95]. For newborns with very low body weight absorption of magnesium was around 86% [6, 66]. According to [66] the increasing concentration of calcium can have a negative effect on the absorption of magnesium, however, several claim the opposite.

1.3 Potassium, sodium, chlorine

According to Emmett and Rogers [34] sodium content of milk of mothers of different nationality decrease during the lactation from 47 mg/dm^3 measured in the colostrum to 15 mg/dm^3 in the ripe milk. Picciano [77] measured sodium content of the foremilk to be 300–400 mg/dm^3, which decreased in the ripe milk to 120–250 mg/dm^3. Honda et al. [49] measured the sodium content in the milk of Japanese mothers older than 35 years to be 371.5 mg/dm^3, while for mothers under 35 years to be 345.9 mg/dm^3. A few difference could be established between the milk of mothers with the first and several childbirths

(373.3 and 327.3 mg/dm^3).

Yamawaki et al. [97] measured the average sodium content of milk of mothers from different areas of Japan to be 13.5 mg/100 cm^3. During the lactation sodium decreased from 32.7 mg/100 cm^3 to 13.9 mg/100 cm^3, its concentration was higher in the summer months than in winter (13.8–13.2 mg/100 ^{3}). According to Picciano [77] potassium content of the mother's milk decreases during the lactation from 600–700 mg/dm^3 to 400–550 mg/dm^3 in the ripe milk.

Honda et al. [49] measured the potassium content in the milk of Japanese mothers older than 35 years to be 678.3 mg/dm^3, while for mothers under 35 years to be 727.8 mg/dm^3. There was a significant difference between the milk of mothers with the first and several childbirths (738.3 and 701.6 mg/dm^3).

Yamawaki et al. [97] measured average potassium content of mother's milk to be 47.0 mg/100 cm^3, which decreased during the lactation from 72.3 mg/100 cm^3 to 46.6 mg/100 cm^3. It was established that its concentration was higher in winter than in the summer (45.5 and 48.5 mg/100 cm^3). Khatir Sam et al. [58] measured chloride content of milk of Sudanese mothers on the average to be 328 mg/100 cm^3, and its potassium content to be 738 mg/dm^3. Picciano [77] measured the chloride content of the mother's milk in the foremilk to be 600–800 mg/dm^3, which value decreased in the ripe milk to 400–450 mg/dm^3. Yamawaki et al. [97] measured the average chloride content of milk of mothers from different areas of Japan to be 35.19 mg/100 cm^3 that was not affected by the lactation, however it was higher in the summer's months than in winter (38.7 and 33.1 mg/100 cm^3).

2 Micro elements

2.1 Connection between micro elements and milk protein fractions

Remy et al. [79] determined macro and micro element content of the human precolostrum by inductively coupled plasma emission technique connected to size-exclusion chromatography and linked mass spectrometer. They analyzed the composition of the precolostrum that was taken from mothers bearing in the week 28 and 32 of the pregnancy in the first month of the lactation. Milk samples taken directly after the childbirth, then on the day 6, 14 and 28 of the lactation were examined. Lead, sulfur, chromium, manganese, iron, cobalt, copper, zink, bromine, selenium, iodine and aluminium content of the milk samples was determined. It was established that the whey protein

fraction of the mother's milk is extremely rich in sulfur, which is due to the high molecular weight sulfur-containing proteins, and low molecular weight substances (glutathione, taurine). Similarly to the sulfur, it was established for all the above macro and micro elements, to what whey protein fraction is attributed their presence.

According to the examinations milk of mothers with premature childbirth differs substantially from the milk replacing preparations in the respect that the different macro and micro elements to what protein fractions are attached. It was also confirmed that the precolostrum is extremely rich in high molecular weight proteins linked to metals, and this is also true to the colostrum and the transitional milk. Amount of the metal-bonding, high molecular weight fractions decreases during the lactation, at the same time the amount of the low molecular weight metal-bonding fractions increases along with the time elapsed after the childbirth. This is very important because absorption of the essential micro elements depends extremely on that in what form they are present in the mother's milk, that is, how the protein modifies utilization of the micro elements. The attention is drawn to that beyond determination of the micro element content it can be also important to know in what form – e.g. linked to proteins – they occur, especially when it is about nourishment of babies born prematurely.

2.2 Iron

Arnaud and Favier [4] examined copper, iron, zinc and manganese content of colostrum and transitional milk of French mothers. From 82 breast-feeding mothers 143 samples were taken; out of the mothers 67 lived in Grenoble, 15 in the neighbourhood. Income, age, number of children, body mass before and at the end of the pregnancy of the mothers were examined. Milk samples were collected between the first and seventh day after the childbirth. During the sampling it was taken care of reducing the contamination with trace elements to the minimum. The sampling was carried out between 9 and 11 a.m.; before the sampling by the hands the breasts were washed with distilled water, then the samples taken were cooled down to –10 °C. Iron concentration of the samples was determined by electrothermal atomic absorption spectrophotometer. It was established that although to the sampling and the storage of the samples special attention was paid, great differences were obtained between the individual samples, for which various physiological and non-physiological factors can be responsible. Trace element concentration of the colostrum is high, and adequately satisfies the micro element needs oft the newborns. Due to the low

milk amount in the first days the newborns require more micro elements in the milk, by which the higher concentration can be explained. It was established that the environment does not have an influence on the iron content of the mother's milk. No relationship was found between age of mother as well as number of children and iron content of the mother's milk. Examining sex and birth weight of the newborn was found no significant relationships. Iron content of samples taken directly after the childbirth decreased from 14 $\mu mol/dm^3$ to 7 $\mu mol/dm^3$ until day 4 of the lactation, then remained at a constant level.

Khatir Sam et al. [58] measured iron content of milk of Sudanese mothers to be 0.56 mg/dm^3. The mothers used hand pump for the sampling, by which they could take around 100 cm^3 milk per person. The examinations were done by neutron activation analysis and X-ray spectrometer.

Dorea and Miazaki [32] examined the effect of contraceptives on the iron and copper content of the mother's milk for 54 Brazilian mothers. The contraceptive tablets contained 0.15 mg levonorgestrel and 0.03 mg ethynyl estradiol each; and active ingredient of the minipill tablets was 0.35 mg norethidron. Examined blood and milk samples were taken before and after of the treatment with contraceptives. Half of the 54 mothers took the hormone preparation and the other half of them was the control group. When evaluating the results social position of the mothers, number of children, duration of the earlier lactation, type of the contraceptive, duration of the application and the age of the mothers were taken into consideration. It was established that on the iron content of the mother's milk the lactation had only a negligible effect, and also the contraceptives did not have a significant influence on the iron content of the mother's milk during first six months of the lactation. Recently, the contraceptives taken in tablet form have been widely used, changing the hormone level of the blood serum, they affect the mineral metabolism [68, 74]. According to Milman et al. [69] and Andrade et al. [3] the hormones have considerable effect on the iron metabolism and reduction of the iron loss due to menstruation. Contraceptives containing oestrogen increased the iron loss the body without influencing its absorption. Despite this, Kirksey et al. [59] established that the contraceptives did not have an influence on the iron content of the milk in the long run.

Al-Awadi and Srikumar [1] examined trace element of milk of 34 Kuwaiti and non-Kuwaiti breast-feeding mothers and established that iron content of the milk of the Kuwaiti mothers was significantly higher (0.43 mg/dm^3) than that of the non-Kuwaiti ones (0.33 mg/dm^3). The milk sample was taken before the nursing in the morning, iron content of the milk was determined by atomic absorption spectrophotomer and it was tried to find a relationship

between the protein and iron content of the milk during the lactation. It was established that the high protein content of the milk is connected with the high concentration of the trace elements, from which it was concluded that the high protein content had a considerable effect on the concentration of the examined elements within this on that of iron and its biological utilization. Bocca et al. [11] measured iron content of milk of Italian mothers to be 0.650 $\mu g/cm^3$ and found a positive relationship between the iron and manganese content.

According to Picciano [77] iron content of the mother's milk in the early phase of the lactation first increases (0.5–1.0 mg/dm^3), then it stabilizes in the ripe milk at around 0.4 mg/dm^3 and it appears that its concentration is not affected by the mother's food. It was reported that the iron content of the mother's milk utilized extremely well, the absorption mechanism has not been clarified yet in every respect, however. Under similar circumstances from the mother's milk iron utilizes five times better than from cow's milk. One-third of the iron content of the mother's milk is attached to the lipid fraction, one-third can be found in the aqueous phase and approx. 10% of it is linked to the casein. The immonologically important iron-binding lactoferrin bonds approx. 20–30% of the iron, and to this is the extreme good utilization of the iron is attributed despite thermal degradation of lactoferrin, but this has no effect on the iron absorption. Also the higher plasma iron concentration of babies fed with mother's milk can be explained by this.

According to Cumming et al. [22]; Fransson and Lönnerdal [41, 43]); Hirai et al. [48] the iron is bonded mainly to the low molecular weight peptides (18–56%), the fat micelles (15–46%) and the lactoferrin (16–40%). Concentration of the main metal transporting proteins decreases with the advance of the lactation, but with the decrease of the lactoferrin the iron concentration of the milk does not change.

According to Dorea [30] iron concentration of the colostrum and the foremilk is significantly higher, and they also claim that the mother's iron reserves do not play a role in the iron content of the mother's milk. Feeley et al. [35] observed significant decrease with the advance of the lactation, on the contrary according to Arnaud et al. [5] there is no significant change in the iron content of the milk during the lactation, and also according to Emmett and Rogers [34], as well as Al-Awaidi the iron content remains at a constant level (0.07 mg/dm^3) with the advance of the lactation. Celada et al. [17] established that the iron content of the mother's milk is independent of the lower iron reserves caused by the increasing number of pregnancy, as well as of the concentration of the serum ferritin and transferrin. No significant difference was found in the iron content of the mother's milk, even then when

the mother was iron defficient, when she consumed too much iron, or she had a normal iron status. Iron supplementation of such an extent, which significantly increased the iron content of the blood, did not affect the iron content of the mother's milk [5, 99]. On the contrary, Fransson [39] reported that iron content of milk of anaemic Indian women was higher that that of mothers with higher haemoglobin level. The iron secretion in the milk gland takes place in a very specific way, and it appears that it absolutely does not depend on that of the other macro and micro elements.

Balogun et al. [7] measured the iron concentration of milk of different mothers to be between 10 and 25 mg/kg. From the animal experiments would be concluded that the mother's iron-intake increases the iron content of the milk, for the human there is no proof for this mechanism, however. It appears that a one-time high iron containing mineral intake does not have an influence on the composition of the mother's milk. Many studies prove that there is no difference in the iron content of the mother's milk when different nutritional habits are compared in the same cultural circle, and also there is no difference between the vegetarians, the non-vegetarians and the different nationalities [36, 78]. In Brasil and the United States, during whether the pregnancy or the lactation the food was completed with iron, this did not affect the iron content of the milk [28, 98].

Zapata et al. [98] also reported that a daily iron supplementation of 40 mg increased the total iron-binding capacity, but did not have a significant effect on the iron content of the mother's milk. It was established that an iron supplementation of 100 and 200 mg a day, respectively, did not influenced the iron content of milk of Nigerian mothers in the last six months of the pregnancy. Today it appears to be clear that the iron reserve of the body does not influence the transfer from the blood serum into the milk. Despite that there is a significant difference in the fat content of the foremilk and the ripe milk, and there is a relationship between the fat and iron content, some claim that the iron content of the foremilk and the ripe milk differs significantly, while others say it does not. Despite that the iron content of the mother's milk varies in extreme cases between 0.04–1.92 mg/dm^3, it appears that in case of breast-fed babies no iron deficiency is expected in the first six months of the lactation. The iron reserve of the newborn's liver balances the lack for the mother's milk [27]. During the first four months the mineral-intake of the babies fed with mother's milk decreases significantly, but as the utilization of the iron from the mother's milk is very high, the feeding with mother's milk completely satisfies the requirements of the growth at this age [13]. Although with advancing lactation the iron intake decreases, it completely satisfies the

needs compared to children fed with baby food preparation.

Krachler et al. [60] measured iron content of transitional and ripe milk of 27 mothers be on the average 380 $\mu g/dm^3$ by a traditional ICP-MS (inductively coupled plasma emission – mass spectrometry) technique.

Santos da Costa et al. [83] examining the micro element content of colostrum of 50 Brazilian mothers from the first day of the lactation till the fourth day, determined the iron, copper and zinc content. By the whole reflexion X-ray fluorescence analysis the iron content of the colostrum was measured to be 1.72 mg/dm^3. The applied analytical technique was proven to be suitable for the determination of the trace element content of the colostrum, as by a simple measurement it makes possible a multi-element analysis and it does not require preliminary concentration of the sample.

Domellöff et al. [26] examined the iron, zinc and copper content of milk of 191 Sweedish and Honduran mothers, as well as the relationship between the mineral status of the mother. The milk samples were collected for nine months, and also the zinc and copper content of the plasma as well as in connection with the iron status the haemoglobin, plasma ferritin, soluble transferrin receptors and zinc porphirin amount were determined. It was established that the iron content of the milk of the Honduran mothers was significantly lower (0.21 mg/dm^3) than that of the Sweedish mothers (0.29 mg/dm^3). The iron content was in a positive relationship with the energy content of the food. It was concluded that the iron content of the milk was not affected by the mineral status of the mother during the period of nine months after the childbirth, therefore it was assumed that iron got in the milk gland by some active transport. Iron content of the milk decreased during the breast-feeding.

Hunt et al. [53] studying the iron content of milk of mothers with premature childbirth and with normal bearing time between the first and twelfth week of the lactation established that in the milk of mothers bearing at normal time the iron content decreased from 355 $\mu g/dm^3$ to 225 $\mu g/dm^3$, while in case of mothers with premature from 406 $\mu g/dm^3$ to 287 $\mu g/dm^3$.

Leotsinidis et al. [62] examined the toxic and essential trace elements of the mother's milk and factors influencing the composition of the mother's milk. Into the experiment 180 Greek mothers were drawn, who gave birth to healthy babies. Based on a questionnaire the food consumption of the mothers was precisely gauged, during which information relating to 22 different foodstuffs was collected. Age, height of the mothers, body mass before and after the pregnancy, smoking habits, marital status, qualification, occupation, number of children and food supplementation consumed during the pregnancy were recorded. Milk samples of 10–20 cm^3 were taken by the hand into polyethylene

pots previously treated with nitric acid and autoclaved at 150 °C for 3 hours, then the samples were stored at –20 °C until the analysis. The iron content was determined by flame atomization atomic absorption spectrophotometer and it was established that this decreased during the lactation. 34% of the mothers taking part in the experiment smoked during the pregnancy, and almost each of them consumed some kind of food supplementation. Between the data relating to the mothers and the iron content of the milk (431 $\mu g/dm^3$) there was no significant relationship. Decrease of the iron content is closely connected with the lower protein and fat content of the transitional and ripe milk, as it is well-known that half of the iron content is bonded to the protein fractions, whereas the other half of it can be found in the fat. Iron content of the mother's food did not influenced the milk composition, from which it can be concluded that the iron is getting in the milk gland by active transport. It was come to the conclusion that although the micro elements of the mother's milk contribute to different extent to the satisfaction of the baby's needs, supplementation of the mother's milk with micro elements is not necessary.

Yamawaki et al. [97] measured the average iron content of milk of mothers from different areas of Japan to be 119 $\mu g/100\,cm^3$. It was established that the iron increases during the lactation from 110 $\mu g/100\,cm^3$ to 180 $\mu g/100\,cm^3$, and that its concentration is higher in the winter's months than in the summer (129; 108 $\mu g/100\,cm^3$).

Shashiraj et al. [85] analyzing the relationship between the iron and lactoferrin concentration of the mother's milk as well as the mother's hemoglobin and iron status established that on the first day after the childbirth the iron content of the mother's milk was between 0.86–0.89 mg/dm^3, which decrease to 0.33–0.34 until week 14 of the lactation, and to 0.26–0.27 mg/dm^3 until month six of the lactation. In the same period the concentration of lactoferrin is 12.02–12.91 g/dm^3; 5.84–6.68 g/dm^3 and 5.85–6.37 g/dm^3.

2.3 Copper

Khatir Sam et al. [58] measured copper content of milk of Sudanese mothers to be 34.8 mg/dm^3. According to Balogun et al. [7] copper concentration of the different mother's milks is between 1.7 and 5.9 mg/kg. Turan et al. [92] determined copper content of colostrum of 30 Turkish middle-class mothers by electrothermal atomic absorption spectrophotometer. For the digestion of the samples and to remove the fat wet ashing was applied. Copper content of the colostrum was measured to be 278 $\mu g/dm^3$.

According to Dorea [30] copper content of the colostrum and the foremilk

is significantly higher, while according to others it completely corresponds to that of the ripe milk. Feeley et al. [35] observed a decrease with advancing lactation in the copper content of the milk. Arnaud and Favier [4] examining copper content of colostrum and transitional milk of French mothers by atomic absorption spectrophotometer established that copper content of samples taken directly after the childbirth was 17 $\mu mol/dm^3$, which decreased on the second day to 13 $\mu mol/dm^3$, while between the fourth and seventh day remained at a constant level. The lactation state had no significant effect on the copper concentration. It was also established that the copper content was significantly affected by the nutrition of the mothers. According to Emmett and Rogers [34] copper content of milk of mothers of different nationality (0.05 mg/dm^3) does not change during the lactation. According to Picciano [77] the copper content of the mother's milk in the early phase of the lactation is 0.5–0.8 mg/dm^3, which stabilizes in the ripe milk at a concentration value of 0.4 mg/dm^3. It appears that the food of the mother does not influence its concentration.

Rossipal and Krachler [80] examined content of 19 trace elements of 79 milk samples taken from 46 healthy mothers between day 1–293 of the lactation. Barium, beryllium, bismuth, cadmium, cobalt, caesium, copper, mercury, lanthanum, lithium, manganese, molybdenum, lead, rubidium, antimony, tin, strontium, thallium and zinc content was determined not only in the course of the lactation but also during the nursing. In the case of the copper content a decrease was reported during the lactation. Concentration of the copper in the colostrum (day 1–3) is 549 $\mu g/dm^3$; in the transitional milk (day 42–60) 241 $\mu g/dm^3$; whereas in the ripe milk (day 97–293) 148 $\mu g/dm^3$. After the suckling the concentration of the copper increased even up to 60%.

Leotsinidis et al. [62] examining the composition of the mother's colostrum, transitional milk and ripe milk established that concentration of the copper increased in the course of the lactation. The average copper concentration was measured to be 368 $\mu g/dm^3$. Between body mass of the mothers and copper content of the milk there was only a little relationship. Although consumption of rice and potato increase the copper content, food of the mother affects it to a small extent.

Yamawaki et al. [97] measured the average copper content of milk of mothers from different areas of Japan to be 35 $\mu g/100\,cm^3$. It was established that the lactation does not influence the copper content of the mother's milk, however, its concentration is higher in the summer months than in the winter (36; 34 $\mu g/100\,cm^3$).

Hunt et al. [53] investigating the copper concentration of milk of mothers

with premature childbirth and with normal bearing time established that it decreases significantly between week 1 and week 12 of the lactation, in the milk of mothers bearing at normal time from 651 μg/dm^3 to 361 μg/dm^3, whereas in case of mothers with premature childbirth from 542 μg/dm^3 to 425 μg/dm^3. According to Salmenpera et al. [82] the copper content of the serum is bonded almost entirely to the celluloplasmin but this does not influence the copper uptake of the milk gland. No increase of the copper content in the mother's milk was found even in such extreme cases where copper content of the blood serum considerably increased due to disease. Although intravenous copper injection increased copper content of the blood serum of the bearing mothers, but did not affect substantially copper concentration of the colostrum [71]. It seems therefore that the mechanism relating to the copper taking place in the milk gland are not affected by the serum copper concentration. The copper secretion in the milk gland takes place in a very specific way and it appears that it does absolutely not depend on that of the rest of the macro and micro elements. Partition of the copper between the different protein fractions of the mother's milk appears to be independent. 15–20% of the copper content of the mother's milk can be found in the fat micelle membrane [67], whereas 20–25% is bonded the copper-containing protein celuloplasmin [64, 96]. Concentration of the major metal transporting proteins reduces with the advance of lactation.

There is no data available regarding whether decrease of the concentration of the copper-containing proteins have an effect on the milk's copper content in the first month of lactation. From the animal experiments would be concluded that the mother's copper-intake increases the copper content of the milk, for the humans there is no proof for this mechanism, however. It appears that a one-time high copper-containing mineral intake does not have an influence on the composition of the mother's milk. Many studies prove that there is no difference in the copper content of the mother's milk when different nutritional habits are compared in the same cultural circle, and also there is no difference even between the vegetarians, the non-vegetarians and the different nationalities [36, 78]. Lipsman et al. [65] reported that Nepalese women consumed foods with significantly higher copper content than the American ones, in the comparison of milk no significant difference could be found, however. Despite this, in the United States milk of the Spanish women contains significantly less copper than that of other nationality. It was established that a copper supplementation of 100 and 200 mg/day in the last six months of the pregnancy did not influence the copper content of the milk of Nigerian mothers. Experiments in Ethiopia and India proved that there can be differences in the milk's copper content between the different ethnical groups [40, 25].

It was also reported that babies of underfed mothers consumed milk with lower copper content, but the experiments show that the copper content of the mother's food does not influence the milk's copper concentration, in fact low or high copper supplementation does not have an effect on it [16]. Oral copper supplementation significantly increases the serum copper level, however, neither the copper concentration of the serum nor the extent of the supplementation have an effect on the copper content of the mother's milk. Today it appears obvious that even the body's copper reserve does influence the transfer from the blood serum into the milk. The oestrogen-containing contraceptives increased the body's copper lost without influencing its absorption. Despite this Kirksey et al. [59] by the examination of the contraceptives in the long run established that they did not affect the copper content of the milk, although they decreased the copper content of the serum in case of mothers, who took contraceptives prior to the pregnancy.

According to Dorea and Miazaki [32] the contraceptives do not affect the copper content of milk of Brazilian mothers in the first six months of lactation. It was established that the copper content in the mother's milk significantly decreased during the lactation. Despite that copper content of the mother's milk varies in extreme cases between 0.03–2.19 mg/dm^3, in case of babies fed with mother's milk in the first 6 months of the lactation no copper deficiency is expected. Copper reserve of the newborn's liver balances the lack for mother's milk [27]. Although with advancing lactation the copper intake decreases, the mother's milk still completely satisfies the requirements of the breast-fed babies in comparison with children fed with baby food preparations.

Bocca et al. [11] measured copper content of milk of Italian mothers to be 0.370 $\mu g/cm^3$ and established that the copper content is significantly higher in case of mothers living in town and there is a close negative relationship between the copper and zinc content. Al-Awadi and Srikumar [1] examined copper content of milk of Kuwaiti and non-Kuwaiti breast-feeding mothers and established that this was significantly higher in case of the Kuwaiti mothers (0.71 mg/dm^3) than in case of the non-Kuwaiti mothers (0.59 mg/dm^3). Irrespective of the mother's nationality the copper content of the milk decreased between month 6 and 12 of the lactation. Santos da Costa et al. [83] measured copper content of the colostrum of Brazilian mothers to be 0.54 mg/dm^3.

Honda et al. [49] measured the copper content of mothers older than 35 years to be 263.0 $\mu g/dm^3$, while that of mothers under 35 years to be 312.6 $\mu g/dm^3$. No significant difference was found in the milk of mothers with the first and several childbirth. According to Domellöff et al. [26] copper content of milk of Honduran mothers is significantly lower (0.12 mg/dm^3) than that of Sweedish

mothers (0.16 mg/dm^3). Between the energy intake and copper content of the mother's plasma no significant relationship was found and it was concluded that during the nine months' period after the childbirth the mother's mineral status did not influence the copper content of the milk.

Shores et al. [86] measured the mother's milk's copper content to be 399 µg/dm^3, and analyzed the capric (0.28); lauric (9.10) and miristic acid content (12.5%) of the milk fat. Significant relationship was found between the copper content and the amount of the three fatty acids. According to the authors the relationship between the copper and the middle-chain fatty acids can be explained by the fact that in the milk gland a copper-containing enzyme is necessary for the synthesis of the C10–C14 fatty acids or the middle-chain fatty acids are capable of bonding the copper in a special way.

Coni et al. [20] analyzed content and absorption of some trace element of milk of 30, healthy mothers living in Torino. During sampling care was taken to avoid contamination of the samples. The ripe milk samples were taken in the second month of the lactation from mothers who were taught the careful and precise sampling. The sampling was done by hand using talkum-free rubber gloves, then the approx. 10 g of mother's milk was stored in polyethylene pots. After appropriate sample preparation the analyses were carried out by quadrupole inductively coupled plasma emission mass spectrometry. A copper content of 552 µg/kg was obtained. Beyond determining the micro elements it was also examined to what protein fractions the substances of interest are bonded in the milk. In order to determine this, the milk protein fractions were divided into five fractions using size-exclusion chromatography. The first fraction contained proteins with molecular weight above 2000 kDa (α-, β-, κ-casein), the second fraction proteins between 2000–500 kDa (immunoglobulins), the third fraction were of 500–100 kDa (human serum albumin, lactoferrin), the fourth fraction contained fractions between 100–2 kDa (α-lactalbumin), and fraction five contained fractions with molecular weight below 2 kDa (proteose-pepton, free amino acids). It was established that copper occur in the first and second, as well as in the fourth and fifth fraction in the same concentration. The final conclusion was that the specific ligands being in the mother's milk, like proteins and enzymes of different molecular weight, were in a close relation with the trace elements, increasing their biological utilization.

2.4 Zinc

Arnaud and Favier [4] examined zinc content of the colostrum and transitional milk of French mothers by flame atomic absorption spectrophotometer and established the it increased from 130 μmol/dm^3 measured on the first day to 180 μmol/dm^3 on the second day, then continuously decreased till the fourth day of the lactation, and on the day 7 became constant at 80–90 μmol/dm^3. The zinc content exhibited a maximal value on day 2.

Emmett and Rogers [34] established that the zinc content of milk of mothers of different nationality showed only a little change during the lactation (0.6–0.3 mg/dm^3). Rossipal and Krachler [80] measured the zinc content of the colostrum to be 4.7 mg/dm^3, that of the transtitional milk to be 0.56 mg/dm^3 and that of the ripe milk to be 0.38 mg/dm^3. According to Picciano [77] the mother's milk contains around 4–12 mg/dm^3 zinc that decreases till month 6 of the lactation to 1.1 mg/dm^3, till month 12 to 0.5 mg/dm^3. It appears that the mother's food does not influence the concentration of these elements. It was also reported that the zinc content of the mother's milk was extremely well utilized in the organism of the newborn.

Yamawaki et al. [97] measured the average zinc content of milk of mothers from different areas of Japan to be relatively high, 145 μg/100 cm^3. It was established that during the lactation the zinc content decreased from 475 μg/100 ml to 177 μg/100 cm^3, and it was higher in the winter's months than in the summer (159; 132 μg/100 cm^3).

Hunt et al. [52] examining the zinc concentration of the mother's milk established that in case of a healthy childbirth it decreased between the first and fourth month of the lactation from 0.04 μmol/dm^3 to 0.02 μmol/dm^3. Hunt et al. [53] in the course of another investigation examining the zinc concentration of milk of mothers having premature childbirth and mothers bearing at normal time established that between week 1 and 12 of the lactation it decreased significantly in the most cases, in the milk of mothers bearing at normal time from 4060 μg/dm^3 to 1190 μg/dm^3, whereas in that of mothers having premature childbirth from 5970 μg/dm^3 to 1270 μg/dm^3.

Frković et al. [45] measured the zinc content of the mothers milk to be 4.98±2.53 mg/dm^3. Comparing age of mothers, environment of the home, smoking habits it was experienced that zinc content of milk of mothers under 25 years was higher that that of mothers above 25 years, however, in case of the other parameter no difference was found. Honda et al. [49] measured the zinc content of milk of mothers older than 35 years to be 5.41 mg/dm^3, while that of mothers under 35 years to be 5.90 mg/dm^3. In case of mothers having the

first childbirth this value was significantly higher (6.27 μg/dm^3) than in case of mothers having more childbirth (5.36 μg/dm^3). Bocca et al. [11] measured the zinc content of milk of Italian mothers to be 2.72 μg/cm^3. Evaluating the results based on the age of the mothers it was established that the zinc content was the higher in the milk of mothers under 30 years.

Khatir Sam et al. [58] measured zinc content of milk of Sudanese mothers to be 1.64 mg/dm^3. According to Al-Awadi and Srikumar [1] the zinc content in the milk of the Kuwaiti mothers is significantly higher (3.2 mg/dm^3) than in the non-Kuwaiti ones (2.4 mg/dm^3). Between month 6 and 12 of the lactation independent of the nationality the zinc content decreased in the mother's milk. Shores et al. [86] measured the zinc content of the mother's milk to be 2.93 mg/dm^3, Turan et al. [92] measured the zinc content of colostrum and milk of Turkish mothers by flame atomic absorption spectrophotometer to be 12.9 mg/dm^3.

Santos da Costa et al. [83] found the zinc content of the colostrum of Brazilian mothers on the average to be 6.97 mg/dm^3 between the first and fourth day of lactation. According to Domellöff et al. [26] zinc content of milk of Sweedish mothers is 0.46 mg/dm^3, that of Honduran mothers is 0.70 mg/dm^3. A negative relationship was found between the energy-intake and the zinc content, and it was also established that the zinc concentration increased during the breast-feeding. It was concluded that the zinc content of the milk during nine months after the childbirth was not influenced by the mother's mineral status.

Leotsinidis et al. [62] determined the zinc content of the mother's milk using flame atomization atomic absorption spectrophotometer and obtained a value of 5010 μg/dm^3. Its amount decreases in the course of the lactation which is in a close connection with the lower protein and fat content of the transitional and ripe milk since it is well-known that zinc is bonded to the protein fractions. Fruits and rice increased the zinc content, while the other foods of the mother did not affect the milk's zinc content. Coni et al. [20] measured the zinc content of the mother's milk to be 3080 μg/kg. It was established that zinc is mainly linked to the low molecular weight protein fractions.

2.5 Manganese

According to Arnaud and Favier [4] manganese content of colostrum and transitional milk of French mothers increases from 120 nmol/dm^3 measured on the first day to 220 nmol/dm^3 on the second day, then continuously decreasing reaches a value of 70–80 nmol/dm^3 on the seventh day. It was established that

the nutrition of the mothers as well as the environment did not have an effect on the manganese content of the mother's milk. Rossipal and Krachler [80] measured the manganese content of the mother's colostrum, transitional milk and ripe milk between day 1–3 of the lactation to be 7.2 $\mu g/dm^3$; between day 42–60 to be 3.9 $\mu g/dm^3$ and between day 97–293 to be 4.0 $\mu g/dm^3$. According to Picciano [77] the manganese content in the first month of the lactation in the ripe milk is on the average 6 $\mu g/dm^3$ that decreases between month 3 and 6 of the lactation to 3 $\mu g/dm^3$.

Yamawaki et al. [97] measured the average manganese content of milk of mothers from different areas of Japan to be 1.1 $\mu g/100\,cm^3$. It was established that in the course of the lactation the manganese content decreased from 1.2 to 0.8 $\mu g/100\,cm^3$ and was higher in the winter's months than in the summer (0.9–1.2 $\mu g/100\,cm^3$).

According to Khatir Sam et al. [58] the manganese content of milk of Sudanese mothers is 14.2 $\mu g/dm^3$ on the average. Krachler et al. [60] measured manganese content of transitional and ripe milk of 27 mothers to be 6.3 $\mu g/dm^3$. Shores et al. [86] measured the concentration of the manganese in the milk of Fulani mothers to be 16 $\mu g/dm^3$. Between the middle-chain fatty acids and the manganese content no relationship could be found.

Al-Awadi and Srikumar [1] examining manganese content of milk of 34 Kuwaiti and non-Kuwaiti breast-feeding mothers established that it decreased in both cases during the lactation. Manganese content of milk of the Kuwaiti mothers was measured to be 6.0, and that of the non-Kuwaiti mothers to be 5.7 $\mu g/dm^3$. According to Turan et al. [92] manganese content of the colostrum of middle-class Turkish mothers is 43.2 $\mu g/dm^3$. Coni et al. [20] measured manganese content of milk of 30 mothers living in Torino to be 16 $\mu g/kg$. It was established that manganese occur in a considerable amount, around 70% in the middle molecular weight protein fraction.

Sharma and Pervez [84] determined manganese content of milk and blood of 120 mothers living in the neighbourhood of a steel factory in Middle India, and compared with milk of mothers who lived far away from this environment. In case of manganese a close relationship was obtained between the concentrations in the blood and the mother's milk. The manganese content was measured to be between 0.8–21.5 $\mu g/dm^3$. Comparing the data of milk of mothers living at the polluted area and that of mothers living far away from that it was established that in case of the manganese accumulation in the blood is four times bigger than in the mother's milk. Manganese content of milk of mothers aged between 20–25 years was 4.6 $\mu g/dm^3$, whereas that of mothers aged 40–45 years was 24.5 $\mu g/dm^3$. In similar age groups in case of mothers

living far away from the industrial areas, in pollution-free environment, the manganese content was between 0.1 μg/dm^3 and 1.5 μg/dm^3. Leotsinidis et al. [62] determined manganese content of the mother's milk by electrothermal atomic absorption spectrophotometer to be 3.58 μg/dm^3. It appears that by consumption of nuts the manganese content of the mother's milk can be influenced.

2.6 Other micro elements

Kumpulainen et al. [61] examined chromium burden of 50 breast-feeding Finnish mothers and chromium content of the mother's milk, respectively. Parallelly with the milk analysis the chromium content of the consumed foodstuffs was measured, which was 31 μg/day on the average (the extreme values were 25–37 μg/day). These values are much lower than that are considered as still tolerable. Chromium concentration of the mother's milk varied in case of higher chromium intake between 0.19–0.69 μg/dm^3, whereas in case of lower chromium intake between 0.24–0.54 μg/dm^3, thus it can be concluded that chromium consumption of the mothers has no effect on the chromium content of the mother's milk. Khatir Sam et al. [58] measured the chromium content in the milk of Sudanes mothers to be 1.11 μg/dm^3.

Krachler et al. [60] determined chromium content of transitional and ripe milk of 27 mothers by a common ICP-MS (inductively coupled plasma emission-mass spectrometer) technique, and measured the average chromium concentration to be 24.3 μg/dm^3. Wappelhorst et al. [94] examined the absorption of chromium from foodstuffs and its transfer into the mother's milk in case of German mothers. The composition of the foods consumed by the mothers was analyzed then milk samples were taken between week 2 and week 8 of the lactation. Destruction of the samples was carried out using a microwave pressure destructor, the listed elements were analyzed by inductively coupled plasma emission and linked mass spectrometer, and the chromium content was measured to be 0.100 μg/kg. Based on the chromium content of the food and the milk was calculated in what portion the chromium are transferred into the milk. The calculated transfer factor was on the average 6.9, which in the case of the individuals significantly differed from each other. These differences were explained by the differences of the mother's milk and the milk production depending on the individuals, as well as by the individual differences relating to the absorption of chromium.

Turan et al. [92] measured the chromium content of the colostrum of 30 Turkish mothers to be 8.6 μg/dm^3, nickel content to be 27.8 μg/dm^3. Ya-

mawaki et al. [97] measured the average chromium content of milk of mothers from different areas of Japan to be 5.9 μg/100 cm^3, selenium content to be 1.7 μg/100 cm^3. It was established that in the course of the lactation the chromium content increased from 1.7 to 5.0 μg/100 cm^3, whereas the selenium content decreased from 2.5 to 1.8 μg/100 cm^3. Selenium content of the Japanese mothers was found relatively high and it was established that it was influenced mainly by selenium intake along with the food. The chromium concentration was found to be higher in the summer's months than in the winter (6.7; 5.1 mg/100 cm^3), the selenium content (1.8; 1.7 μg/100 cm^3) hardly changed seasonally, however. Wappelhorst et al. [94] measured cobalt content of milk of German mothers to be 0.058 μg/kg, molybdenum content to be 0.008 μg/kg.

Krachler et al. [60] measured the average cobalt concentration of the mother's milk to be 0.19 μg/dm^3, nickel content to be 0.79 μg/dm^3, selenium content to be 17 μg/dm^3 and vanadium content to be 0.18 μg/dm^3. According Khatir Sam et al. [58] molybdenum content of the mother's milk was 3.84 μg/dm^3, cobalt content was 1.23 μg/dm^3, and nickel content was 7.8 μg/dm^3. Rossipal and Krachler [80] examining cobalt and molybdenum content of 79 milk samples taken from 46 healthy mothers established that the cobalt increased almost to its double value during the lactation (colostrum day 1–3: 1.35 μg/dm^3; transitional milk day 42–60: 1.64 μg/dm^3; ripe milk day 97–293: 2.96 μg/dm^3); and the concentration of the molybdenum decreased (9.00 μg/dn^3 in the colostrum, 1.02 μg/dm^3 in the transitional milk and 1.56 μg/dm^3 in the ripe milk). During the suckling concentration of the molybdenum increases even by 60%, therefore this has to be taken into consideration when collecting mother's milk samples.

According to Picciano [77] selenium concentration of the mother's milk in connection with some selenium-containing protein fractions, is high at the beginning of the lactation (40 μg/dm^3), while in the ripe milk it varies between 7–33 μg/dm^3 on the average due to differences of the geographical circumstances. Selenium status of the mother influences extremely the selenium content of the milk, that decreases considerably with the advance of the lactation. Selenium content of the milk is in a positive relationship with the plasma selenium concentration of the newborn and with the activity of a selenium-containing enzyme, the glutathione peroxidase. Iodine content of the mother's milk considerably changes due to geographical environment and the mother's iodine intake. In iodine-deficient areas the iodine content of the mother's milk is 15 μg/dm^3, but in case of consumption of appropriate iodine-containing foodstuffs can be by an order of magnitude higher (150 μg/dm^3). Fluorine

content of the ripe milk is between 4–15 $\mu g/dm^3$.

Bermejo-Barrera et al. [9] determined the silicon content in the milk of 13 mothers by electrothermal atomic absorption spectrophotometry. The milk samples were taken that they were not polluted by the environment. Average silicon content of the samples was 112 $\mu g/dm^3$, where the extreme values ranged between 50–440 $\mu g/dm^3$. With the exception of one sample (440 $\mu g/dm^3$), the samples showed values between 50 and 164 $\mu g/dm^3$. Theodorolea et al. [91] determined selenium content of milk of Greek mothers using electrothermal atomic absorption spectrophotometry and chemical modifying. 5–10 cm^3 milk was collected from the mothers by hand-pump in glass vessels, and stored at –18 °C until the analysis. Using the elaborated new method as selenium content of the mother's milk 16.7–42.6 $\mu g/dm^3$ was obtained, on the average 27.4±5.5 $\mu g/dm^3$.

Hunt et al. [53] examined boron concentration of milk of mothers with premature childbirth and mothers bearing at normal time and established that the boron concentration was practically unchanged between week 1 and week 12 of the lactation in case of mothers bearing at time (30 and 28 $\mu g/dm^3$), but considerably changed in case of mothers with premature childbirth (37 and 27 $\mu g/dm^3$). Selenium content decreased in the milk of mothers bearing at normal time from 26.9 $\mu g/dm^3$ to 18.6 $\mu g/dm^3$, in the milk of mothers with premature childbirth from 28.7 $\mu g/dm^3$ to 20.4 $\mu g/dm^3$. Hunt et al. [52] examining the boron concentration of the mother's milk established that in case of a healthy childbirth boron content of the mother's milk between month 1 and 4 decreased from 42 $\mu g/dm^3$ to 35 $\mu g/dm^3$.

3 Poisonous trace elements

3.1 Cadmium

Frković et al. [44] examined cadmium content of milk of mothers living at the North-Adriatic part of Croatia between September and January. Milk samples were collected from 29 mothers out of whom 14 gave birth to the first child, 12 to the second one, and 13 to the third one. The heavy metal content was determined by a graphite cuvette atomic absorption spectrophotometer, with atomizing temperature of 2060 °C. Cadmium content of mother's milk in the neighbourhood of Rijeka varied between 0.45–9.10 $\mu g/dm^3$, with average value of 2.54 $\mu g/dm^3$. Comparing the cadmium content of milk of mothers of different ages, with one and more children, smoking and non-smoking, as well as living in town and in the countryside, no significant difference between the

different groups could be established [75].

According to Rossipal and Krachler [80] the concentration of cadmium in the colostrum is much higher (1.1 μg/dm^3) than in the transitional (0.18 μg/dm^3) or ripe milk (0.24 μg/dm^3). Coni et al. [20] measured cadmium content of the mother's milk to be 0.8 μg/kg. It was established that cadmium occured mainly in the low and the high molecular weight protein fraction. Turan et al. [92] measured cadmium content of the colostrum of Turkish middle-class mothers to be 2.8 μg/dm^3.

Honda et al. [49] examined cadmium content of the mother's milk due to cadmium consumption of different amounts. The cadmium amount entered the organism was established based on a questionnaire and it was taken into consideration whether it was about smoking or non-smoking mothers. Cadmium content of examined mother's milk varied between 0.07–1.23 μg/dm^3, and was not affected by the age of mother and the course of the childbirth. Between cadmium content of the mother's milk and the urine there was a significant relationship. Between cadmium and calcium content of the mother's milk a negative relationship was established.

Sharma and Pervez [84] found no close relationship between the cadmium concentration in the blood and the mother's milk. Cadmium content of the mother's milk varied between 0.1–3.8 μg/dm^3. It was established that cadmium content of milk of older mothers was higher than that of the younger ones. Cadmium content of milk of mother aged between 20–25 years was measured to be 0.6 μg/dm^3, while that of mothers aged between 40–45 year to be 0.3 μg/dm^3. In similar age groups, in case of mothers living far away from the industrial areas, in pollution-free environment, the cadmium content varied between 0.1 μg/dm^3 and 0.3 μg/dm^3.

Ursinyova and Masanova [93] determined cadmium, lead and mercury content of milk of 158 healthy mothers who lived in eight differently polluted areas of the Slovak Republic. The effect of age of mother, family position, tooth fillings, sex and birth mass of the newborn as well as smoking habits in the family on the composition of the mother's milk. The examined mothers were 25.6 years old on the average, had 6.9 filled teeth and gave birth to their child in week 40 of the pregnancy. Average body mass of the newborns was 3.45 kg, 54.4% of them was a boy, 45.6% of them a girl. 22.8% of the mothers smoked before the pregnancy, 3.8% also during the pregnancy, and 42.7% of the fathers smoked. In the average of 158 analyses the cadmium content of the mother's milk was measured to be 0.36 μg/kg. It was established that both the active and passive smoking significantly increased the cadmium content of the milk. Comparing the cadmium content of milk of mothers living in

different parts of the world, outstandingly high value was obtained in the milk of German urban mothers (countryside: 17.3 $\mu g/dm^3$, urban: 24.6 $\mu g/dm^3$).

Leotsinidis et al. [62] examining cadmium content of the mother's milk by electrothermal atomic absorption spectrophotometry established that it was for 11% of the colostrum samples below the detectation limit. Similar ratios were obtained also in the case of the transitional milk. It was established that the decrease of the milk's cadmium content was in a close connection with the lower protein and fat content of the transitional and ripe milk, since it is well-known that major part of the cadmium can be found in the fat. 34% of the mothers taking part in the experiment smoked during the pregnancy, and almost each of them consumed some kind of food supplementation. By the examination of the cadmium content of the colostrum, the transitional milk and ripe milk it was established that this decreased in the course of the lactation. Average cadmium concentration of the mother's milk was 0.130 $\mu g/dm^3$. It was established that milk of smoking mothers contained more cadmium and that consumption of meat of animals (lamb, calf) from clean pasture reduced the amount of the poisonous micro elements, consumption of fresh vegetables and the nuts increased the cadmium content, however.

3.2 Lead

Frković et al. [44] measured lead content of milk of Croatian mothers to be 7.3 $\mu g/dm^3$, which was considerably lower than that of milk made from food preparations. Lead content of the mother's milk varies in the industrialized countries between 5–20 $\mu g/dm^3$, in strongly polluted areas it can be 20times higher, however [88]. According to an investigation in Mexico the average lead content of the mother's milk is 62 $\mu g/dm^3$ with extreme values of 9 and 350 $\mu g/dm^3$ [73]. According to a Sweedish analysis in a polluted environment the lead content of the mother's milk is 0.9 $\mu g/dm^3$, while in a less polluted environment is around 0.5 $\mu g/dm^3$. This very low lead content can be explained by the drastically reduced lead emmission on the one hand, and the spreading of unleaded fuels. In a comparative investigation in 6 countries Palminger et al. [75] measured lead content of the mother's milk to be between 2.0–17.8 $\mu g/dm^3$. Lead content of milk samples measured in the neighbourhood of Rijeka varied between 0.3–44.0 $\mu g/dm^3$. Comparing lead content of milk of mothers aged above and below 25 years, having one and more children, living in Rijeka and in the neighbourhood of Rijeka, smoking and non-smoking, it was established that lead content of milk of mothers below 25 years was higher (10.4 $\mu g/dm^3$) compared to that of mothers older than

25 years (5.7 μg/dm^3), the difference was not significant, however [87]. Lead content of milk of mothers having one child was 5.8 μg/dm^3, whereas that of mothers with more children was 8.7 μg/dm^3, the difference is not significant. No significant difference was found in the lead content of milk of the smoking (5.7 μg/kg) and non-smoking mothers (7.9 μg/kg). The difference was significant only between mothers living in Rijeka (10.6 μg/kg) and in the region (4.7 μg/kg). Lead exposition of mothers living in town also appeared in the higher lead content of milk. Turan et al. [92] measured lead content of the colostrum of Turkish middle-class mothers to be 14.6 μg/dm^3.

In their examinations Rossipal and Krachler [80] established that the decrease of lead from the colostrum to the ripe milk was substantially smaller compared to the other trace elements (1.0–0.12 μg/dm^3). Coni et al. [20] measured the lead content of the mother's milk to be 13 μg/kg. It was established that lead was bonded in the same concentration both to high and low molecular weight protein fractions.

Gundacker et al. [47] collected 5–10 cm^3 milk samples from 59 mothers living in Vienna (urban), 47 mothers living in Linz (industrial) and 59 mothers living in Tulln (countryside), aged 29±5 years, and determined the lead content. 60% of the mothers gave birth to her first child. Dwelling place, nutritional habits of the mothers were surveyed, data were collected on the smoking as well as regarding tooth filling and tooth extraction. The milks were lyophilized between –20 °C and –48 °C, then homogenized, and the samples were taken out of this homogenous "milk" then after appropriate preparation the analysis was done by atomic absorption spectrophotometry. Lead content of the mother's milk was measured to be 1.63±1.66 μg/dm^3. It was established that in Austria the lead exposition considerably decreased during the last 20 years. In 1981 lead content of milk was measured to be 50 μg/dm^3, which reduced to 1993 to 36 μg/dm^3. According to their investigation lead content of the mother's milk was affected mainly by the dwelling place (Tulln: 1.22 μg/dm^3, Linz: 2.48 μg/dm^3, Vienna: 1.29 μg/dm^3), consumption of fish (0.80–1.82 μg/dm^3) and corns (1.46–1.71 μg/dm^3), vitamin supplementation (1.78 μg/dm^3) and the smoking. Lead content of milk of smoking mothers was higher (2.40 μg/dm^3) than that of the non-smoking ones (1.57 μg/dm^3). Lead content of milk of mothers weighing less than 60 kg was measured to be 1.81 μg/dm^3. These values for mothers weighing between 60–80 kg was 1.52 μg/dm^3, and for mothers weighing more than 80 kg was 1.36 μg/dm^3. In summary, it was established that the lead concentration was below the critical level in the examined milk samples. Based on the investigations in the year 2002 it is not to be expected that lead content of milk of healthy mothers

influence the health of the breast-fed baby.

Leotsinidis et al. [62] determined lead content of the mother's milk by electrothermal atomic absorption spectrophotometry. The average lead content was measured to be 0.44 $\mu g/dm^3$, which decreased in the course of the lactation. Milk of mothers who lived in urban environment contained more lead. It was also established that its concentration was higher in the milk of mothers living in urban environment as well as in industrialized regions than in that of mothers living in the countryside. It seems that consumption of red meat decreases, while cheeses, especially the Greek feta cheese and the rice increase the mother's milk's lead content. Consumption of meat of animals (lamb, calf) from clean pasture reduced the amount of the poisonous micro elements. Comparing the values obtained for lead with data from the previous years, it was established that it decreased by almost two orders of magnitude in the last years which was due to the widespread unleaded fuels.

Sharma and Pervez [84] compared lead content of milk of mothers living in polluted environment in Middle India, and that of mothers living more distant from this environment. Toxic element content of the blood was found to be significantly higher than that of mother's milk, and in case of lead a close relationship was obtained between concentrations in the blood and the mother's milk. Lead content of milk of mothers aged between 20–25 years was 3.6 $\mu g/dm^3$, whereas that of mothers aged between 40–45 years was 16.7 $\mu g/dm^3$. Lead content of milk of 20–25 years old mothers living far away from the industrial areas in unpolluted environment was 0.1 $\mu g/dm^3$, while that of mothers aged between 40–45 years was 0.7 $\mu g/dm^3$.

Ursinyova and Masanova [93] measured average lead content of milk of 158 healthy Slovak mothers to be 3.4 $\mu g/kg$. Comparing the values for the milk of mothers living in different parts of the world, it can be established that lead content is outstandingly high in the milk of Italian mothers living in town (126.55 $\mu g/dm^3$), followed by lead content of milk of mothers living countryside (46.52 $\mu g/dm^3$), Singaporean (47.7 $\mu g/dm^3$), Austrian (in 1993: 35.8 $\mu g/dm^3$, in 2000: 1.5–1.8 $\mu g/dm^3$), Malaysian, Canadian and mothers living in different parts of China.

3.3 Mercury

Drasch et al. [33] examined mercury content of 70 milk samples taken from 46 German mothers on the first seven days of the lactation in the function of amalgam filling and other factors. Mercury content of nine milk samples was measured on the average to be 0.37 $\mu g/dm^3$, where the extreme values

varied between 0.20–6.86 μg/dm^3, in the case of most of the milk samples between 0.4 and 2.5 μg/dm^3. Mercury content of the mother's milk had a positive relationship with the number of teeth filled with amalgam, as in case of mothers whose teeth were filled not with amalgam, the mercury content was less than 0.2 μg/dm^3, in case of those having 1 to 7 teeth filled with amalgam the mercury content was up to 0.50–0.57 μg/dm^3, and for those with more than 7 teeth filled, the mercury content was 11 μg/dm^3. The frequency of fish consumption also increased the mercury concentration in the milk, whereas age of mothers was not in a significant relationship with it. Comparing the mercury content of the mother's milk it was established that mercury content of colostrum samples taken on the second and third day of the lactation was higher, afterwards the same and lower, respectively, than that of the baby food preparations.

Rossipal and Krachler [80] examining mercury content of colostrum, transitional and ripe milk of 46 healthy mothers on day 1–293 of the lactation, established that it decreased from 2.7 μg/dm^3 measured in the colostrum to 0.52 μg/dm^3 in the transitional milk, then stabilized at this value in the ripe milk.

Gundacker et al. [47] measured mercury content of milk of mothers living at different places of Austria to be 1.59±1.21 μg/dm^3. Mercury content of 9% of the samples exceeded 3.5 μg/dm^3.

According to their investigation mercury content of the mother's milk was affected mainly by the dwelling place (Tulln: 1.07 μg/dm^3, Linz: 1.82 μg/dm^3, Vienna: 2.17 μg/dm^3), consumption of fish (1.54–1.92 μg/dm^3) and corns (0.87–1.85 μg/dm^3), vitamin supplementation (1.96 μg/dm^3) and the smoking. Mercury content of milk of smoking mothers was lower (1.42 μg/dm^3) than that of the non-smoking ones (1.60 μg/dm^3). Mercury content of milk of mothers weighing less than 60 kg was measured to be 2.09 μg/dm^3, that of mothers weighing between 60–80 kg to be 1.38 μg/dm^3, and that of mothers weighing more than 80 kg to be 1.24 μg/dm^3.

Sharma and Pervez [84] measured mercury content of milk of 20–25 years old mothers living far away from industrial areas, in unpolluted environment to be 0.1 μg/dm^3, and that of mothers aged between 40–45 years to be 0.9 μg/dm^3. A close relationship was obtained between concentrations in the blood and mother's milk.

Ursinyova and Masanova [93] measured mercury content of milk of Slovak mothers to be 0.72 μg/kg. It was established that only the amalgam tooth filling caused significantly higher mercury content in the milk, therefore with increasing number of teeth filled with amalgam significantly increased the

mercury content.

Da Costa et al. [23] examined mercury content of milk of 23 Brazilian mothers between day 7 and day 30 of the lactation in the function of whether the mothers had amalgam tooth filling and what surface of the filling had, because mercury used for filling teeth in dental treatments is the primary source of mercury for the humans. Number of teeth filled with amalgam in case of mothers drawn into the examination was 6.87. Average mercury content of the mother's milk varied between 0 and 23.07 μg/kg, with an average value of 5.73 μg/kg. Between number of teeth filled with amalgam and mercury concentration of the mother's milk a significant positive relationship was found.

4 Other poisonous trace elements

Rossipal and Krachler [80] examined content of 19 trace elements of 79 milk samples taken from 46 healthy mothers on days 1–293 of the lactation. Barium, beryllium, bismuth, cadmium, caesium, lanthanum, lithium, rubidium, antimony, tin, strontium, thallium content was determined not only in the course of lacatation, but also during the nursing. It was established that concentration of such toxic elements like thallium (0.13 μg/dm^3, 0.08 μg/dm^3), barium, beryllium, lanthanum, lithium and antimony was considerably higher in the colostrum than in the ripe milk, and it appears that bismuth, caesium and strontium does not change during the lactation. Concentration of barium, caesium, rubidium, strontium decreases the suckling, which can mean even a difference of 60%, that must be taken into consideration when collecting the mother's milk samples.

Krachler et al. [60] determined toxic element of transitional and ripe milk of 27 mothers and of four baby food preparation (silver, aluminium, arsenic, gold, platinum, scandium, titanium) by traditional ICP-MS (inductively coupled plasma emission-mass spectrometer) technique. The average aluminium concentration was measured to be 67 μg/dm^3, arsenic concentration to be 6.7 μg/dm^3 and silver concentration to be 0.41 mg/dm^3 with extreme values between 0.13–42.0 μg/dm^3. Average concentration of gold was 0.29 μg/dm^3 with extreme values between 0.10–2.06 μg/dm^3. These extreme values were explained by the tooth fillings and use of jewelry. Platinum concentration was very low in each sample, remained below the detectation limit of 0.01 μg/dm^3.

Coni et al. [20] analyzed some trace elements of healthy mothers in Torino and their absorption. For the mother's milk samples the following trace element concentrations were obtained: barium 17 μg/kg, bismuth 0.1 μg/kg,

lithium 1 μg/kg, strontium 85 μg/kg, thallium 0.09 μg/kg. Beyond determination of the micro elements it was also examined to what protein fractions the materials of interest were linked in the milk. In order to determine this the milk protein fractions were divided into five parts by size-exclusion chromatography. It was established that bismuth and lithium occured mainly in the low and high molecular weight protein fractions in nearly equal concentration. Barium, strontium and thallium belonging to the group two of the elements are bonded to the low molecular weight fractions.

Wappelhorst et al. [94] determined antimony, cerium, gallium, lanthanum, niobium, ruthenium, silver, thorium, titanium and uranium content of milk of 19 mothers living in Germany and examined the absorption of the foodstuff, the transfer into the mother's milk. Based on micro element content of the foodstuff and the milk it was calculated in what ratio the micro element present in the foodstuff transferred into the milk. The calculated transfer factor for silver was 5.1, for cerium 16.1, for gallium 19.1, for lanthanum 13.8, for niobium 20.7, ruthenium 4.1, for antimony 13.2, for thorium 20.2, for titanium 5.6, and for uranium 21.3. The transfer factors significantly differed from each other in case of the individuals. These differences were explained by the differences in the milk production and in the absorption of the elements both depending on the entities. Average silver content of the mother's milk was measured to be 0.334 μg/kg, cerium content to be 0.030 μg/kg, gallium content to be 0.027 μg/kg, lanthanum content to be 0.043 μg/kg, niobium content to be 0.023 μg/kg, ruthenium content 0.180 μg/kg, antimony content to be 0.041 μg/kg, thorium content to be 0.028 μg/kg, titanium content to be 0.080 μg/kg and uranium content to be 0.022 μg/kg.

Sharma and Pervez [84] did not find a close relationship between the concentration of arsenic in the blood and mother's milk. Arsenic content of milk of mothers aged 20–25 years, living in polluted area was 0.9 μg/dm^3, and that of mothers aged between 40–45 years was 5.2 μg/dm^3. In similar age groups, in case of mothers living far away from industrial areas, in unpolluted environment the arsenic content ranged between 0.1–0.9 μg/dm^3.

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Changes in fatty acid composition and conjugated linoleic acid contents of sour dairy products caused by pure cultures

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Abstract. In this research we have investigated the effect of various pure cultures *(Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus lactis subsp. lactis biovar, Lactobacillus diacetilactis, Lactobacillus acidophilus, Bifidobacterium lactis)* on fatty acid composition of soured dairy products (Sana, yoghurt) manufactured using different technologies, with special regard to conjugated linoleic acid (CLA). It was established that the cultures we used and which are also commonly used in the dairy industry, had only a slight effect on fatty acid composition of milk. Although minimal differences were found in case of the individual fatty acids, however, due to the small differences it can be established that the cultures have no influence on nutritional value of milk fat.

Key words and phrases: fatty acid composition, conjugated linoleic acid, dairy products, pure bacteria cultures, different technologies

1 Introduction

Fatty acid composition of milk fat, especially owing to short-chain fatty acids present in relatively big amount, is ideal for the human organism because triacylglycerols containing short-chain fatty acids can be more easily attacked by the digestive enzymes. Milk fat contains relatively small amount of unsaturated fatty acids, despite this it can contain considerable amount of essential fatty acids needed to satisfy the requirements of the human organism and due to its animal origin it contains also the essential arachidonic acid [1]. Milk fat can contain also conjugated linoleic acids (CLA) in considerable quantity, which have according to the latest researches many useful physiological effects. Among others their antioxidant effect, that is they prevent the membranes from the attacks of free-radicals, was proven, consequently they can have significant role in the anti-cancer fight [2, 3].

Composition of dairy products manufactured by adding pure cultures is determined to the greatest extent by the composition of the raw milk, since the cultures produce rather aroma materials and they affect fatty acid composition only to a smaller extent. As regards CLAs some have experienced that as an effect of pure cultures CLA contents of dairy products increased and adding of linoleic acid resulted in a higher CLA contents [4].

It was also established that CLA contents of dairy products manufactured by fermentation could vary, as certain cultures were capable of producing CLA from linoleic acid during the souring [7]. Some reports that CLA contents of cheeses can increase during maturation, others, however, did not establish such relationship [4]. According to most of the authors CLA contents of dairy products depend mainly on CLA contents of the milk used for the production; technological processes can, however, significantly influence CLA contents of the finished product [5, 6]. Some reports that starter cultures can produce CLA in considerable amount, others, however, could not establish such relation. Since until recent times it did not manage to give a definite answer to the question what effect microorganism had on CLA contents of the product, therefore we have decided to examine fatty acid composition and CLA contents of dairy product manufactured from cattle's milk (Sana, yoghurt). By our investigations we would like to draw attention to the outstanding health-protecting effects of soured dairy products.

2 Material and methods

2.1 Used bacteria and the production of soured dairy products

Lactic acid producing *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus diacetilactis* and *Lactobacillus acidophilus* are used for the production of dairy products manufactured by fermentation, while *Lactobacillus lactis subsp. lactis biovar, Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus* are used for the production of the very popular yoghurt. Due to proteolitic enzymes of *Lactobacillus delbrueckii subsp. bulgaricus* increase the free amino acid contents, especially proline contents of the yoghurt, with a concentration up to 300–500 mg/kg of the latter. Due to the activity of *Streptococcus salivarius subsp. thermophilus* carbamide contents of the yoghurt reduce to 10% of the original value. In the mixed cultures the phyla *Bifidobacterium lactis* and *Lactobacillus* show better growth and souring ratio than each separately what requires a symbiotic fermentation behaviour. Both phyla can be used alone, but they can be employed excellently together with other phyla, as well. In the experiments according to the individual species and mixtures that optimal temperature and duration were applied where the reproduction was the most intensive. For the mesophil species the ideal temperature is between 15–32 °C, for termophil bacteria 45–60 °C.

For the production of soured dairy products a milk supplied to a dairy company in Székelyland was used which was pasteurized at 78 °C for 50 sec. Temperature of the sample No. 1 was set to be 27 °C and a pure culture mix of *Lactobacillus lactis subsp. lactis* and *Lactobacillus lactis subsp. cremoris* was added, subsequently the sample was incubated at 27 °C over 8 hours in a thermostat, then was refrigerated. After incubation the pH was 4.36. For the sample No. 2 the same cultures, temperature and duration were applied, therefore this sample could be regarded as repetition of the sample No 1. After incubation the pH was 4.43. Temperature of sample No. 3 was set to be 27 °C and a pure culture mix of *Lactobacillus lactis subsp. lactis, Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus* was added. The sample was incubated at 27 °C over 7 hours, then refrigerated. After incubation the pH was 4.9. Temperature of sample No. 4 was set to be 28 °C and a pure culture mix of *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris,* and *Lactobacillus lactis subsp. lactis biovarn* was added. The sample was incubated at 28 °C for 7 hours, then refrigerated. After incubation the pH was 4.56. Temperature of sample No. 5 was set to be

28 °C and a pure culture mix of *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus diacetilactis* was added. The sample was incubated at 28 °C for 14 hours, then refrigerated. After incubation the pH was 4.56. Temperature of sample No. 6 was set to be 46 °C and a pure culture mix of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus* was added. The sample was incubated at 46 °C for 6 hours, then refrigerated. After incubation the pH was 4.21. Temperature of sample No. 7 was set to be 46 °C and a pure culture mix of *Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus* and *Streptococcus salivarius subsp. thermophilus* was added. The sample was incubated at 46 °C for 6 hours, then refrigerated. After incubation the pH was 4.3. Temperature of sample No. 8 was set to be 46 °C and a pure culture mix of *Streptococcus thermophilus* and *Bifidobacterium lactis* was added. The sample was incubated at 46 °C for 6 hours, then refrigerated. After incubation the pH was 4.22. Sample No. 9 was the milk pasteurized at 78 °C for 50 sec, which was subsequently refrigerated. Sample No. 10 was the unpasteurized raw milk sample used as a control sample. In order to terminate bacterial activity, after the incubation the samples were immediately cooled down to –25 °C. Continued activity of lipase was not disturbing since during the analysis relative weight% of fatty acids was determined after transesterification, therefore free fatty acids formed due to lipase did not affect the result.

2.2 Determination of fatty acid composition

Sample preparation. A sample quantity containing approx. 0.5–1.0 g fat was destructed with 8–20 cm^3 of hydrochloric acid (37%) for 1 hour on hot water bath. After having cooled down, 7 cm^3 of ethanol was added. Lipids were extracted with 15 cm^3 diethylether and 15 cm^3 benzine (b.p.<60 °C), and the organic layers were combined. From a portion of this solution, containing approx. 150–200 mg fat, the solvents were removed at 80 °C under reduced pressure (a complete evaporation not necessary).

Transesterification. To the residue 4 cm^3 of 0.5 M sodium hydroxide methanol solution was added and boiled until all the fat drops disappeared (approx. 5 min), then 4 cm^3 of 14% boron trifluoride methanol solution was added, boiled for 3 min, finally 4 cm^3 of hexane, dried on water-free sodium sulphate, was added and boiled for 1 min, and the mixture was allowed to cool down. Saturated aqueous sodium chloride solution was added and after having separated the organic layer was collected into a 4 cm^3 vial containing water-free

sodium sulphate and was directly examined by gas chromatography.

Conditions of the gas chromatographic analysis. Instrument: Chrompack CP 9000 gas chromatograph. Column: 100 m×0.25 mm id, CP-Sil 88 (FAME) phase. Detector: FID 270 °C. Injector: splitter, 270 °C. Carrier gas: He, 235 kPa. Temperature program: 140 °C for 10 min; at 10 °C/min up to 235 °C; isotherm for 26 min. Injected volume: 0.5–2 µl.

2.3 Determination of conjugated linoleic acid contents

Lipid extraction. To a milk sample amount containing approx. 0.3 g fat 80 cm^3 of a 3:2 mixture of hexane and isopropanol (referred to as HIP) was added. The sample was dispergated in the liquid using a dispersion apparatus (Ultra-turrax T25 basic, manufactured by IKA) at 9.500 rpm for 2 min. The suspension was filtrated through a membrane filter (MN640W, 90 mm diameter). The filter was washed three times with 10 cm^3 of the HIP mixture and the organic layers were combined. 5 g of water-free sodium sulphate was added and the liquid was shaken up in order to eliminate water. The liquid was decanted and the solvents were removed at 80 °C under reduced pressure. The residue was washed into a 10 cm^3 volumetric flask with hexane.

Methylation. 0.5 cm^3 of the hexane solution obtained in the manner described above was taken into a 4 cm^3 capped vial and 0.5 cm^3 4 M sodium methylate methanol solution was added, it was shaken up and warmed to 50 °C and kept at this temperature for 30 min. Subsequently, 1 cm^3 of hexane and 1 cm^3 of water were added, it was shaken up, and after the layers have separated, 1 cm^3 of the organic layer was placed into a 5 cm^3 volumetric flask, to the aqueous layer 1.2 cm^3 of hexane was added, it was shaken up and 1 cm^3 of the hexanic layer was taken into the volumetric flask. This extraction with hexane was repeated twice more, the last time as far as it was possible the whole hexanic layer was collected, and the volumetric flask was filled up to 5 cm^3 with hexane, and the obtained solution was stored in a screw capped vial refrigerated until the analysis.

Conditions of the gas chromatographic analysis Instrument: Temperature program: column temperature 140 °C for 10 min; at 5 °C/min up to 235 °C; isotherm for 30 min. Injected volume: 2 µl. Other conditions are identical with those of described for determination of fatty acid composition.

3 Results

Short-chain fatty acids (C6–C12) of soured dairy product samples produced by adding various cultures are shown in *Table 1.*

Table 1: Short-chain fatty acid (C6–C12) contents of milk and dairy products

Various cultures	Fatty acid*			
	Caproic acid	Caprylic acid	Capric acid	Lauric acid
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 7 hours	1.10	0.85	1.93	2.29
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 8 hours	1.60	1.30	2.90	3.26
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 27 °C, 7 hours	1.21	0.91	2.08	2.50
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. lactis biovar, 28 °C, 8 hours	1.22	0.96	2.18	2.63
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 28 °C, 14 hours	1.30	1.00	2.26	2.66
Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 46 °C, 6 hours	1.55	1.18	2.68	3.03
Lactobacillus delbrueckii subsp. bulgaricus,Streptococcus salivarius subsp. thermophilus Bifidobacterium lactis, 46 °C, 6 hours	1.27	0.96	2.14	2.50
Bifidobacterium lactis, Streptococcus salivarius subsp. thermophilus 46 °C, 6 hours	1.11	0.90	2.05	2.43
Milk pateurized 78 °C, 50 min	1.40	1.10	2.46	2.90
Unpasteurized milk	1.14	0.92	2.13	2.56

*In relative weight% of fatty acid methyl esters.

Amount of miristic acid, palmitic acid, stearic acid and oleic acid which are the main fatty acid components is shown in *Table 2,* while amount of the essential and semi-essential fatty acids is shown in *Table 3.*

Having evaluated the results it was established that fatty acid composition of pasteurized milk and that of raw milk were practically identical within the limit of error of the measurement, and it can be established also for most of the cultures used, that the microorganisms did not produce any significant effect on the fatty acid composition. Apart from some minor discrepancies the data for each sample are practically identical, and although it is imaginable that carrying out the analyses with higher number of sample, significant differences could be obtained for some fatty acids, these differences would be, however, probably so slight that they would not affect nutritional value of soured dairy products.

Individually evaluating the fatty acids, it can be established that in the range of C6:0 and C15:0 the results practically coincide. For palmitic acid in case of the aroma and carbon dioxide producing cultures *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. lactis biovar* was found minimally deviating value whereas in each other cases the results were almost identical. For C16:1 and C17:0 this sample had the lowest value while in each other cases there was no difference between the samples. In case of stearic acid for the samples produced with the cultures *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus* (28 °C); *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus* (46 °C); *Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium lactis* (46 °C) was found the lowest concentration whereas in the other cases the data practically coincided.

Evaluating all the samples, the biggest differences could be observed in case of elaidic acid where the lowest value was found to be 2.89% in case of the sample produced with *Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus* (46 °C), the highest value was found to be 7.58% for the sample produced with *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris,* and *Lactobacillus lactis subsp. biovar* (28 °C). In case of oleic acid the highest value was measured to be 28.60% for the sample produced with *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus,* while the oleic acid contents were of the lowest value with 23.58% for the sample produced with *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris,* and *Lactobacillus lactis*

subsp. lactis biovar (28 °C).

Table 2: Miristic acid, palmitic acid, stearic acid and oleic acid contents of milk and dairy products

Various cultures	Fatty acid*			
	Myristic acid	**Palmitic acid**	**Stearic acid**	**Oleic acid**
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 7 hours	9.35	27.31	12.85	25.64
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 8 hours	11.33	26.66	10.93	25.93
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 27 °C, 7 hours	9.81	27.53	12.48	23.59
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. lactis biovar, 28 °C, 8 hours	10.21	27.85	11.97	24.45
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 28 °C, 14 hours	10.63	29.13	10.67	28.58
Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 46 °C, 6 hours	10.91	27.97	11.15	25.75
Lactobacillus delbrueckii subsp. bulgaricus,Streptococcus salivarius subsp. thermophilus Bifidobacterium lactis, 46 °C, 6 hours	9.60	28.00	12.32	24.50
Bifidobacterium lactis, Streptococcus salivarius subsp. thermophilus 46 °C, 6 hours	9.53	27.11	13.17	24.13
Milk pateurized 78 °C, 50 min	10.88	27.73	11.50	24.30
Unpasteurized milk	10.07	27.70	12.50	25.02

*In relative weight% of fatty acid methyl esters.

Table 3: Linoleic acid, linolenic acid and conjugated linoleic acid contents of milk and dairy products

Various cultures	Fatty acid*		
	Linoleic acid	**Linolenic acid**	**Conjugated linoleic acid**
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 7 hours	2.13	1.67	0.49
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 8 hours	2.32	1.46	0.51
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 27 °C, 7 hours	2.10	1.62	0.49
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. lactis biovar, 28 °C, 8 hours	2.10	1.61	0.50
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 28 °C, 14 hours	2.76	1.32	0.47
Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 46 °C, 6 hours	2.20	1.45	0.46
Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus Bifidobacterium lactis, 46 °C, 6 hours	2.01	1.61	0.48
Bifidobacterium lactis, Streptococcus salivarius subsp. thermophilus 46 °C, 6 hours	2.13	1.60	0.51
Milk pateurized 78 °C, 50 min	1.95	1.55	0.46
Unpasteurized milk	2.06	1.67	0.48

*In relative weight% of fatty acid methyl esters.

For all other cultures oleidic acid contents varied from 24.1 to 26.0%. For linoleic acid the sample obtained with *Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus* shows a somewhat deviating value, however, for all other fatty acids there is no substantial difference

in the concentration of the individual fatty acids. Proportion of elaidic acid in raw and pasteurized milk sample ranged between 5.31–5.51%, which barely changed due to the cultures. The highest value was reached with 7.58% for the sample *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus,* 27 °C, 7 h, whereas the lowest value with 2.89% for the sample *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris,* 28 °C, 14 h.

Conjugated linoleic acid contents of raw milk was measured to be 0.48%, that did not change considerably either due to pasteurization or to the cultures used. From our examinations we could establish that CLA contents of raw milk were not lost after treatment with the cultures since soured products contained almost the same amount of CLA as the raw milk.

In summary, it can be said that due to the cultures we used and which are also commonly used in the dairy industry, original fatty acid composition of milk barely changed. Minimal discrepancies could be found for the individual fatty acids between the cultures, but these differences are so slight that it cannot be supposed that they could be supported also statistically by examinations carried out in higher numbers.

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Increase of conjugated linoleic acid content of dairy products by adding sunflower oil

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Abstract. In our experiments we investigated the effect of linoleic acid supplementation on the CLA production of *Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus casei.* We established that a supplementation with 100 µl/100 ml sunflower oil with high linoleic acid content increased CLA content of the sour final product, from 116 to 178 for *Lactobacillus acidophilus,* while for *Lactobacillus plantarum* from 116 to 187 mg/100 g fat (about 40%). Supplementation with amounts higher than 100 µl sunflower oil reduced the CLA content. In the case of *Lactobacillus casei* the the increment percent of CLA was only 20%, and it appears that in the range of 100–1500 µl/100 ml sunflower oil supplementation the amount of linoleic acid does not affect the CLA content.

Key words and phrases: dairy products, sunflower oil, conjugated linoleic acid

1 Introduction

Fatty acid composition of milk fat, especially due to short chain fatty acids present in a relative high amount, is ideal to the human organism as triglycerides containing short chain fatty acids can be attacked more easily by digestive enzymes. Unsaturated fatty acid content of milk fat is relatively low, despite this it can contain considerable amount of the necessary essential fatty acids to satisfy the human needs, and due to its animal origin it also contains the essential arachidonic acid [2]. The milk fat can contain considerable amount of conjugated linoleic acids (CLA) that have many useful physiological effect according to the references. Their antioxidant properties were also proved, protect cell membranes from the attack of free radicals. Due to this feature, they can have a significant biological role [3, 5].

The composition of dairy products produced by addition of bacterial cultures is mainly determined by the composition of the starting milk, since the cultures produce mainly aroma substances, and they have less influence on the fatty acid composition, the technological processes, however, can considerably affect the CLA content of the final product [8, 9]. According to some studies the starter cultures can produces considerable amount of CLA, while others could not establish such a relationship. As until now there has been no unequivocal answer to what effect the microorganisms have on the CLA content on the product, therefore in an earlier research we examined fatty acid composition and CLA content of dairy products produced from cow's milk.

We found several studies in the literature in which the CLA producing capability of different bacterium species was examined [1, 6, 10]. Some researchers reported that the examined bacterium species were able to produce CLA from linoleic acid during the souring [4, 6]. On the basis of the results the pure cultures *Lactobacillus plantarum, Lactobacillus casei,* and *Lactobacillus acidophilus* were found to be the most suitable for increasing the CLA content of dairy products produced by fermentation.

There are relatively few experiments where linoleic acid is added to the milk before the souring in the pure form of a vegetable oil. Ming and Shuting [7] examined the CLA producing capability of *Lactobacillus acidophilus* in milk containing lucerne seed oil (the lucerne seed oil contained approx. 40% linoleic acid). In case of the other two bacterium species no studies were found, therefore our aim was to examine the CLA producing capability of the pure cultures *Lactobacillus plantarum, Lactobacillus casei,* and *Lactobacillus acidophilus* when sunflower oil with high linoleic content was added in various doses.

2 Material and methods

In the course of the examinations the sunflower oil was added before the fermentation. We used a sunflower oil with linoleic acid content of 62.7 relative weight% of fatty acid methyl esters as source of linoleic acid. The starter cultures were obtained from the Corvinus University, Budapest, as aslant agar. The temperature of the cultures was 4 °C and they were covered with paraffin oil. From the bacteria a mother souring mixture was prepared by adding the pure cultures to 50 ml of pasteurized milk, then the mixture was incubed at 38 °C for 24 h. From the mother souring mixture obtained 1.0 ml was used for each samples. For the sample preparation 100 ml of freshly pasteurized, cooled milk with a fat content of 3.2% was used. To the pasteurized milk 1.0 ml the mother souring mixture and 50, 100, 150, 200, 300, 400, 600, 1000 and 1500 µl of sunflower oil was added. Blank samples were also prepared in case of all the three pure cultures. The samples and the blanks were incubed at 38 °C for 24 h, and stored in a deep-freeze until the analysis of the CLA content.

2.1 Lipid extraction

A milk sample amount containing approx. 0.3 g fat was pipetted into a 100 ml beaker, and 80 ml of organic solvent mixture (3:2 mixture of hexane and isopropanol, HIP) was added. The sample was dispergated in the solvent mixture (IKA Ultra-turrax T25 basic dispersion apparatus, 9.500 RPM, 2 min). The emulsion was filtrated on a paper filter (MN640W, 90 mm diameter) into a 250 ml Erlenmeyer flask. The paper filter was washed three times with 10 ml of HIP mixture, the organic layers were combined. 5 g of waterfree sodium sulfate was added and the liquid was shaken up in order to eliminate water. The organic layer was decanted from the salt and evaporated under reduced pressure at 80 °C. The residue was washed with n-hexane into a 10 ml measuring flask (hexane solution).

2.2 Methylation

0.5 ml of the hexane solution was taken into a 4 ml capped vial and 0.5 ml 4 M sodium methylate methanol solution was added, it was shaken up and kept at 50 °C for 30 min. Subsequently, 1 ml of hexane and 1 ml of water were added, it was shaken up, and after the layers have separated, 1 ml of the organic layer was pipetted into a 5 ml volumetric flask, to the aqueous layer 1.2 ml of hexane was added, it was shaken up and 1 ml of the hexanic layer was taken into the volumetric flask. This extraction with hexane was repeated twice more, the

last time as far as it was possible the whole hexanic layer was collected, and the volumetric flask was filled up to 5 ml with hexane, and the obtained solution was stored in a screw capped vial refrigerated until the analysis.

2.3 Conditions of the gas chromatographic analysis

The apparatus was a Chrompack CP 9000 gas chromatograph. The dimension of the column were: 100 m×0.25 mm the stationary phase was CP-Sil 88 (FAME). The detector was a FID at 270 °C, the injector was splitter at 270 °C. The carrier gas was helium at 235 kPa. The column temperature was programmed: 140 °C for 10 min, at 5 °C/min up to 235 °C, isotherm for 30 min. The injected volume was 2 µl. For the preparation of the CLA standards, CLA mix obtained from Sigma was used.

3 Results

Table 1 shows the change in the CLA content of milk with the pure cultures and with increasing volume of sunflower oil.

The c9,t11-CLA content of the raw milk was as a mean value of five measurements 117.92 mg/100 g milk which changed to an average value of 116.43 mg/100 g fat in the pasteurized milk. This, however, does not mean a decrease due to the pasteurization since this decrease is minimal, and it is within the error limit of the measurement. In case of samples with *Lactobacillus acidophilus* the highest c9,t11-CLA content was measured when 100 µl sunflower oil was added (178.37 mg/100 g fat). This maximal value decreased to 141.82 µg/100 g fat when 150 µl sunflower oil was added. The decrease was continued when 200–400 and 600–1500 µl was added from 105.91–112.42 mg/100 g fat, to 90.30–91.30 µg/100 g fat.

Almost the same tendency can be observed in case of *Lactobacillus plantarum* when the CLA content increases to 147.85 mg/100 g fat when 50 µl sunflower oil was added, and to 186.88 mg/100 g fat when 100 µl of sunflower oil was added. After that, the decrease shows a little different tendency in comparison with *Lactobacillus acidophilus* since when 150–1500 µl of sunflower oil was added the CLA content decreased from 148.81 to 117.29 mg/100 g fat. *Lactobacillus casei* exhibits a different tendency than the other two bacteria, it appears that the sunflower oil supplementation in the range of 50–1500 µl does not affect the CLA content. When 50 µl of sunflower oil was added the CLA content increased to 139.11 mg/100 g fat, then it reached its maximum with 142.94 mg/100 g fat when 400 µl of sunflower oil was added.

Table 1: Change of CLA content of milk produced by cutures as a function of added sunflower oil content

Sample		CLA-content mg/100 g fat		
		Lactobacillus acidophilus	***Lactobacillus casei***	***Lactobacillus plantarum***
Raw milk		117.92±0.17^{a}	117.92±0.17^{a}	117.92±0.17^{a}
Pasteurized milk		116.54±0.42^{a}	116.54±0.42^{a}	116.54±0.42^{a}
Amount of sunflower oil (µl/100 ml)	50	140.17±2.25^{b}	139.46±1.62^{b}	147.65±1.59^{b}
	100	178.64±2.32^{c}	135.42±1.37^{b}	188.64±3.39^{c}
	15	179.86±1.37^{c}	135.94±1.85^{b}	148.94±1.99^{b}
	200	110.75±4.03^{a}	141.17±2.62^{b}	129.60±2.48^{d}
	300	111.45±4.28^{a}	138.85±3.30^{b}	128.87±4.38^{d}
	400	102.67±3.85^{a}	142.22±2.59^{b}	120.97±2.09^{a}
	600	90.30±3.10^{d}	141.92±6.42^{b}	117.17±4.41^{a}
	1000	84.05±3.74^{d}	137.28±2.25^{b}	115.32±2.92^{a}
	1500	87.58±2.57^{d}	139.69±7.61^{b}	117.67±2.57^{a}

Even at the addition of 1500 µl of sunflower oil the CLA content was 135.65 mg/100 g fat. Comparing the response of the three lactobacilli to the addition of linoleic acid it can be established that in the case of *Lactobacillus acidophilus* and *Lactobacillus plantarum* when 100 µl/100 ml of sunflower oil was added the amount of CLA increases by 35-40%, while in the case of *Lactobacillus casei* only an increase of 20% was experienced. In the case of this latter bacterium in the 50–1500 µl/100 ml range the amount of CLA remained almost unchanged, whereas in case of *Lactobacillus acidophilus* and *Lactobacillus plantarum* a definite maximum was found at the addition level of 100 µl/100 ml sunflower oil.

Based upon our examinations it can be said that in the case of pure cultures applied in the practice certain caution should be exercised when adding sunflower oil prior to the fermentation since there are pure cultures that are nearly indifferent to the amount of added linoleic acid *(Lactobacillus casei),* and there are others that react with maximal production of CLA upon addition of optimal amount of linoleic acid *(Lactobacillus acidophilus, Lactobacillus plantarum),* and there can be such pure cultures where the addition of linoleic acid can decrease the CLA content of the soured final product. We recom-

mend to make the above trial with each lactic acid bacteria used in the practice in order to obtain the optimal CLA production. In case of the cultures we used the favourable effects reported in the literature, that is, the microbes can convert the added linoleic acid into CLA in 20 to 60% [3, 9, 10], could not be reached , which can be explained by the difference between the bacterium species. It cannot be found in the literature, however, that there can be an optimal linoleic acid intake for each bacterium (in our case in 100 µl sunflower oil /100 ml milk), above which the linoleic acid can act as growth inhibitor, reducing the amount of CLA, in fact, the CLA content can decrease even below the value of the starting milk.

4 Summary

In this research the effect of linoleic acid supplementation on the CLA production of *Lactobacillus acidophilus, Lactobacillus plantarum* and *Lactobacillus casei* was examined. It was established that a supplementation with 100 µl/100 ml sunflower oil with high linoleic acid content increased the CLA content of the sour final product, from 116 mg/100 g fat to 178 for *Lactobacillus acidophilus,* while for *Lactobacillus plantarum* to 187 mg/100 g fat. Supplementation with more than 100 µl sunflower oil reduced the CLA content. In the case of *Lactobacillus casei* the CLA content increment percent is only 20% (from 116 mg/100 g fat to 143 mg/100 g fat), and it appears that in the range of 100–1500 µl/100 ml sunflower oil supplementation the amount of linoleic acid does not affect the CLA content.

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