DOCTORAL (PhD) DISSERTATION THESES

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THE COMPOSITION OF MILK AND DAIRY PRODUCTS PRODUCED IN SZÉKELYLAND, WITH SPECIAL RESPECT TO THE TOTAL GERM NUMBER OF THE MILK RAW MATERIAL

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1. BACKGROUND OF THE RESEARCH, OBJECTIVES

Our foods can contain either due to technological interventions or due to changes in microbiological conditions in the food considerable amount of D-amino acids. The researches showed that D-amino acid content of milk and dairy products can be attributed mainly to microbial activity and the technological intervention can have here only a minor role. It appers to be proven that D-amino acids present in traces in mixed milk of healthy cows are the result of bacterial infection during subclinical mastitis, and they enter the milk as metabolic products of bacteria. We established that D-amino acid content of commercially obtainable milk can be caused by milking of the first milk flows rich in bacteria to the mixed milk on the one hand, and the presence of bacteria causing mastitis, the metabolism product of these bacteria and after death of the D-amino acid content of peptidoglycans being in the cell wall. It was also established that according to the degrees of the Mastitest the quantity of total free and free D-amino acids increased in the milk. It is obvious from our examinations that the free amino acid and free D-amino acid are affected mainly by the microbial condition of the milk raw material.

It is known that D-stereoisomer amino acids are not or not easily utilized by the human organism, their harmful effects were reported in several publications. It is also known that the presence of D-amino acids in the proteins reduces digestibility and in bigger volumes they can act as growth inhibitors. In nutritional scientific respect an important fact is that D-amino acids and peptides containing D-amino acids have a different taste than the corresponding L-stereoisomers.

As in case of countries recently joined the European Union dairy producers are reduced to occassionally produce various dairy products complying with the standards out of milk with germ number of several millions considered to be unsuitable for human consumption in EU countries in the first phase of our experiments we examined total free and free D-amino acid content of milk with various total germ numbers. During this we wanted to establish a relationship between germ number and total free and free D-amino acid content of milk, then we looked for an answer to the question how free amino acid content of milk raw material affects free amino acid composition of dairy products matured for shorter and longer time, manufactured from it.

In Székelyland, more closely also in Hargita county, the occasionally extremely high germ number of milks from the small farms and from a few large-scale agricultural plants is a basic problem for the dairy companies and milk processors. In some periods germ numbers above one million are not seldom, in fact occasionally the total CFU can reach the even 3 million. From such a milk raw material it is very difficult to produce good quality dairy products. Because of the above we targeted to establish how the quality of the raw material, namely, total CFU affects the composition of various dairy products produced by souring, manufactured in Székelyland. As it is widely known that the free amino acids and D-amino acids significantly affect the taste and aroma of the dairy products, we set as our task to examine how free and free D-amino acid content of the milk and the dairy products manufactured from it changes.

In the third phase of our research we examined if it is possible to elaborate such a procedure for the pasteurization of milks with high CFU, that does not require a temperature higher that desired and keeping at this temperature, whereas it kills the mircroorganisms perfectly. Therefore we studied the changes taking place during microwave pasteurization, as recently beside the traditional pasteurization procedures the microwave treatment has been used for the pasteurization of milk. There is not much known about the properties of milk obtained by microwave pasteurization, and about the effect of microwaves on the milk's composition. Therefore we investigated how the microwave treatment affects protein content and free amino acid composition of milk. We focused on the „sensitive" amino acids (Tyr, Lys, Met and Cys) and examined whether there was a considerable change in case of these amino acids compared to the traditional technology.

The natural food raw materials like milk do not contain considerable amount of D-amino acids in crude condition, however, they are frequently exposed in the course of preparation for the consumption − like eg. pasteurization − to such circumstances that can cause racemization. Therefore we examined free amino acid and free D-amino acid content of the raw milk, conventionally pasteurized as well as microwave pasteurized milk. In the course of the examinations we tried to find out whether there was a change in the free amino acid content, any possible damage to the milk protein, whether D-amino acids have formed due to the traditional and microwave heat treatment, as well as to compare the efficiency of the two pasteurization procedures regarding the maintaining the quality of the milk protein.

Subsequently we analyzed how the microwave treatment affected the water soluble vitamin content of the milk. As the most heat sensitive are vitamin C and vitamins B, we tested the microwave method by examining the concentration of vitamin C, B_1, B_2, B_6 and B_{12}, comparing to the traditional pasteurization.

We also set as a task to measure the utilizable lysine content, the concentration of lysinoalanine and hydroxymethyl furfurol (HMF) which is the most commonly detected Maillard reaction product of the milk. The Maillard reaction products contribute to the taste and aroma of the pasteurized milk, but they can also reduce considerably the biological value of the protein mainly by blocking the ε amino group of lysine.

The goals of the dissertation were as follows:

- *Examination of free amino acid and free D-amino acid content of milks of various CFU.*

- *Examination of the effect of milks with various total CFU on the composition of the dairy product.*
    - Changes in the composition of Sana in the function of total CFU of the milk raw material.
    - Changes in the composition of Dalia in the function of total CFU of the milk raw material.
    - Changes in the composition of Telemea and cow's curd in the function of total CFU of the milk raw material.

- *Examination of the composition of milk with high CFU during different heat treatments.*
    - Amino acid composition, biological value
    - Free amino acid and free D-amino acid content
    - Vitamin C and vitamin B content
    - Utilizable lysine content, lysinomethionine and hydroxymethyl furfurol content

2. MATERIALS AND METHODS

2.1. Total CFU as well as composition of the milk and dairy products

2.1.1. Milk samples examined, milk sampling

Milk samples with different CFU and dairy products produced from them were collected in two phases from a dairy company in Székelyland. In the first phase of our experiments in April 2006, we could collect milk samples with total CFU varied between 1,230 and 2,950 thousand. In the second phase of our experiments in November and December 2007, we collected samples with total CFU between 220 and 390 thousand. At the dairy company in this period these milks were used to produce yoghurt, Sana, curds, Telemea and the cheese Dalia. The samples were mixed milks from which the company produced also the consumption milk in addition to the mentioned dairy products. As a control sample milk of total CFU less than 100,000 was used, obtained from the cattle farm of the University of Kaposvár, Faculty of Animal Science, and which was taken from a mixed milk of around 100 Holstein-Friesian cows. After the sampling and determination of the total CFU the milk samples were immediately cooled down to −25 °C and were kept at this temperature until the preparation for chemical analysis.

2.1.2. Determination of the total CFU

For determination of the microbe number direct bacterium counting was applied. The milk sample taken into a sterile test tube was thoroughly mixed and a tenfold dilution was prepared (the diluting solution was a 0.85% sodium chloride solution which was sterilized in autoclave beforehand). 1 cm^3 of the pasteurized milk sample was added to 9 cm^3 of sterile dilutant water, then 1 cm^3 of the thoroughly mixed diluted sample was pipetted onto a sterile Petrifilm plate with a culture medium. The Petrifilm plate was incubed at 37 °C for 24 h, and the developed colonies were directly counted with the use of a colony counter.

2.1.3. Dairy products examined

From the dairy company in Székelyland yoghurt, Sana, curds, Telemea and cheese Dalia were obtained for analysis. The company documentation showed which dairy product from milk of what average total CFU was produced, so the examined products could be sorted individually as per CFU. Out of dairy products examined, the curds, yoghurt, Sana and Telemea are considered for products matured over a short time while the cheese Dalia as product matured over a longer time. The examined dairy products were produced by keeping the Rumanian standards and specifications as well as the hygienic regulations.

2.1.4. Chemical analysis of the samples

2.1.4.1. Sample preparation

In the first phase of our experiments sample preparation and the analytical measurements were carried out at the University of Kaposvár, Faculty of Animal Science, Department of Chemistry and Biochemistry. The milk samples were after defrosting and warming up to 30 °C centrifuged at 8,000 g for 10 min in order to remove the cellular elements, and also defatting of the milk was done. Subsequently, to 50 cm^3 of sample 50 cm^3 of 25% trichloroacetic acid was added, left standing for 20 min, and centrifuged at 10,000 g for 10 min. The pH of the supernatant was adjusted to be 7 with 4 M sodium hydroxide solution for the determination of both free amino acid and free D-amino acid content. The obtained solution was dried-up using a lyophilizer at a plate temperature of 10 °C, then for the determination of free amino acid content the dried-up material (pH=7) was solved in 10 cm^3 of sodium acetate buffer, whereas for the determination of free D-amino acids in 1 cm^3 of bidistilled water. The prepared samples were stored at −25 °C until being analyzed. In case of the analysis of dairy products, they were homogenized with distilled water so that the dry matter content of the obtained mixture similarly to milk is between 12−15%. Subsequently, the completely milk-like homogenized samples were treated as they had been milk samples.

2.1.4.2. Analytical methods, instruments, chemicals

For the determination of free amino acid and free D-amino acid content derivatization and analysis were carried out using a Merck-Hitachi LaChrom HPLC apparatus. Chemicals used for sample preparation, derivatization and analysis were reagents of analytical grade. For the determination of free amino acids precolumn derivatization was carried out with o-phthaldialdehyde (OPA) and 2-mercaptoethanol (MeOH). Separation of free amino acids was carried out on a reversed-phase (LiChrospher 100 Rp-18, 125 × 4 mm, 4 μm) analytical column. The formed derivatives were detected using a fluorescence detector (excitation wavelength: 325 nm, emission wavelength: 420 nm).

For the determination of free D-amino acids from the amino acid enantiomers diastereomer pairs were formed with o-phthaldialdehyde (OPA) and 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopiranoside (TATG). The separation of the enantiomers was carried out on a reversed-phase (Superspher 60 RP-8, 125×4 mm, 4 μm) analytical column. Detection was carried out using a fluorescence detector.

2.2. Comparison of the different pasteurization procedures

2.2.1. Milk samples examined, pasteurization procedures

The examined raw milk was obtained from another dairy company in Hargita county. 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 of a wavelength of 12.2 cm, 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 so that the microwave pasteurization equipment worked with a capacity of 200 l/h. The experiments were repeated three times, and the three milk samples from each experiment were analysed.

2.2.2. Sample preparation and analysis

2.2.2.1. Determination of the amino acid content of the milk samples

After a sample preparation similar to as described in Chapter 2.1.4. the analytical measurement was carried out at the Department of Food Science of the Sapientia Hungarian University of Science of Transylvania, in Csíkszereda. 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 applying the derivatization methods described in Chapter 2.1.4.

During the determination of the total amount of amino acids, the crude protein content was determined by the Kjeldahl method (Hungarian Feeding Stuff Codex (1991) Chapter 6.1.).

After hydrolysis of the proteins the amino acid analysis was carried out using an INGOS AAA400 amino acid analyzer, on an Ostion Lg ANB ion-exchange resin (column: 35 cm x 0,37 cm), with sodium citrate buffers and precolumn derivatization of the amino acids with ninhydrin.

2.2.2.2. Determination of the vitamin content of the milk samples

After appropriate preparation of the samples, the separation of vitamins C and B was carried out on a reversed-phase (150x4 mm id) Supercosil (C18) LC column with a Varian ProStar HPLC, for determination of vitamins B with a 50:50% mixture of methanol and phosphate buffer, for determination of vitamin C using a 10:90% mixture of acetonitrile and acetic acid (0.4%) with isocratic analysis.

2.2.2.3. Determination of the HMF content of the milk samples

HMF was determined using a Varian Pro Star HPLC apparatus, on a Supelcosil LC-C18 reversed-phase analytical column (150x4.6 mm id) and a Pro Star 320 UV-VIS detector. A two-component eluent (a 5:95% mixture of acetonitrile and water) was used. HMF was detected at 284 nm.

2.2.2.4. Determination of the utilizable lysine content

The determination was carried out after a derivatization with 2,4-dinitro-1-fluorobenzene (DNFB) using the INGOS AAA automatic amino acid analyzer. The total lysine content of the sample was determined from the sample not treated with DNFB, the amount of the utilizable lysine was calculated from the difference of the two analyses.

2.2.2.5. Determination of the lysinoalanine content

The determination of the lysinoalanine was carried out as described earlier for the total amino acid determination, using the INGOS AAA amino acid analyzer.

2.3. Statistical analysis

The results were evaluated using SPSS for Windows 17.0 (SPSS Inc., 2009) statistical software. In order to decide whether there is a statistically verifiable difference in the composition of the raw milk, milk samples with different total germ numbers, dairy products produced from them, correlation and regression analysis was carried out. The difference between total amino acid, free amino acid and free D-amino acid content of the differently treated milk samples was examined by two-sample t-test.

3. RESULTS AND THEIR EVALUATION

3.1. The effect of the total germ number on the total free and free D-amino acid content of milk

In the first phase of our experiments we examined the effect of the total germ number on the total free and free D-amino acid content of milk. Ouf of the amino acids we focused on aspartic acid, glutamic acid and alanine since the D-enantiomers of these three amino acids occur in the highest amount in milk. In 2006 the total CFU of milk samples obtained from the dairy company varied between 1.23–2.95 million, while in 2007 between 220–390 thousand.

In the range of total CFU of 100–390 thousand the concentration of both the L and D enantiomers increased somewhat. No important changes happened in the CFU range between 390 thousand and 1.23 million, in fact not even in the CFU range of $1.23 \cdot 10^6$ and $1.53 \cdot 10^6$ where neither the amount of the free L amino acids nor the amount of the free D-amino acids showed essential changes, although both the concentration of the free L and D amino acids and the proportion of the D amino acids increased continuously in the function of the total germ number. This minimal change continued up to the total CFU of $2.20 \cdot 10^6$ where there was an almost explosion-like increase in both the amount of the total free amino acids and the amount of the free D-amino acids, and this increase, and this increase applied also to the proportions of the D-amino acids within the total free amino acids. It appears therefore that up to a CFU of 1.5−1.6 million there are no considerable changes in the free amino acid and free D-amino acid content of the milk. After this period, however, the increase is fast.

Summerized it can be said that for each free amino acid we examined, the concentration of both the free D-amino acids and the L-amino acids increase, but the increase of the D-amino acids is relatively bigger since this ratio increased in case of aspartic acid compared to the control milk up to CFU of $2.95 \cdot 10^6$ this ratio increased from 11.11% to 21.97%, in case of glutamic acid from 5.23% to 25.30%, and in case of alanine from 11.85% to 33.37%.

3.2. The effect of the total germ number of milk on the composition of the dairy products

In the next phase of our researches we examined how the increased amount of the free D- and L-amino acids affected the composition of the dairy products. Knowing the relationship between the total germ number of the milk raw material and the concentration of the D-amino acid it can be assumed that the milk raw material can affect the composition of the dairy product produced from it. In order to prove this hypothesis we examined the composition of 10 Sana produced from milks with different germ numbers, 10 Dalia, 3 Telemea, 2 cow's curd, and 1 yoghurt.

3.2.1. Changes in the composition of Sana in the function of total CFU of the milk raw material

In the total CFU range of 220–390 thousand 6, in the total CFU range of 1.23–2.95 million 4 Sana were analyzed that were produced from milks with total CFU of 1.23; 1.35; 1.53 and 2.95 million. It was established that with increasing total germ number of the milk raw material in case of all the three amino acids the amount of both the D- and L-enantiomer also increases and this increase becomes considerable after total CFU of $1.53 \cdot 10^6$, as the Sana produced from the milk with total CFU of almost 3 million contains the highest amount of L- and D-amino acids. We did not experience essential changes within the individual amino acids regarding the D- and L-ratios. The ratio of the D-glutamic acid is the lowest within the total free amino acids with 24‒25%, followed by D-aspartic acid with 30‒32%, and D-alanine with almost 40%.

3.2.2. Changes in the composition of Dalia in the function of total CFU of the milk raw material

We could analyze in the total CFU range of 220‒390 thousand 6 and in the total CFU range of 1.25‒2.91 million 4 Dalia cheeses. We established that in the case of Dalia the changes are even less than those measured for Sana. For the Dalia cheeses produced from milk with total CFU of 220‒2912 thousand the amount of L-aspartic acid varied between 12.55‒16.75 mg/100 g, the amount of D-aspartic acid between 5.26‒6.32 mg/100 g. The amount of both enantiomers increased somewhat with the increase of the total germ number resulting in a practically unchanged ratio of D-aspartic acid being between 29.42‒30.16%. The amount of L-glutamic acid varied between 38.40‒48.25 mg/100 g in the examined period, while the amount of the D-glutamic acid varied between 11.64‒13.52 mg/100 g with practically unchanged ratio of D-glutamic acid (ranged between 22.66‒24.62%). The L-alanine content of the Dalia cheese produced from the milk of total CFU of 220 thousand was 18.45 mg/100 g, which increased to 27.35 mg/kg with the increase of the total germ number. In the same period the amount of the D-alanine increased from 12.30 mg/100 g to 17.80 mg/100 g with pratically unchanged ratio of D-alanine varying between 39.99‒41.21%.

3.2.3. Changes in the composition of Telemea and cow's curd in the function of total CFU of the milk raw material

In case of Telemea we analyzed products produced from milks with total CFU of 1.32; 1.66 and 2.20 million. In this total CFU range except L-glutamic acid we obtained an increase for each amino acid and enantiomer. However, as the total CFU range was not wide enough, we could not drawn a definite conclusion similar to those for the previous two dairy products from our examinations. In the examined total CFU range the amount of L-aspartic acid varied between 0.86−1.50 and that of D-aspartic acid between 0.39−0.61 mg/100 g, the amount of L-glutamic acid between 3.06−3.49, that of D-glutamic acid between 0.75−0.94 mg/100 g, the amount of L-alanine between 1.69−1.97, and that of D-alanine between 1.07−1.35 mg/100 g. Similarly to the previously examined two materials we found the percentage ratio of D-glutamic acid to be the lowest with 19.73−22.54%, the proportion of D-aspartic acid ranged between 28.99−31.14%, and that of D-alanine between 38.81−40.60%. It appears therefore that in the examined range in case of Telemea there is only a slight relation between the total germ number of the milk raw material and the produced dairy products.

3.2.4. The relationship between the ripening time and D-amino acid content

By the examination of the relationship between the quality of dairy products produced from milk raw materials of different total germ numbers and the total germ number we established that the percentage of the D-amino acids within the total free amino acids was not affected either by the total germ number of the milk raw material or the kind of dairy product. The proportion of D-aspartic acid is around 30% for most of the examined dairy products, although this proportion for Sana and the cow's curd is a little higher, for the Dalia a little lower. The percentage of D-glutamic acid varies between 18–27%, which is higher for Sana than in case of Dalia, the lowest is for Telemea. The proportion of D-alanine is around 40% for each dairy product independently of the germ number of the milk. Within the examined three amino acids the proportion of D-glutamic acid is the lowest, that of D-alanine is the highest, while D-aspartic acid exhibits a medium value nearer to D-glutamic acid.

For fresh dairy products and for those matured for a short time (Sana, Telemea) a relationship can be established between total germ number and D-amino acid content and this relation applies in most cases also to the L-enantiomers. Despite the fact that total germ number has a substantial effect on the concentration of both enantiomers, ratio of the enantiomers is not affected by the total germ number. For those dairy products, however, which are ripened over a longer time (Dalia) and for those where amino acid production capability of microbial cultures significantly exceeds production of microorganisms originally present in the milk raw material no effect of the milk raw material can be expected, thus, free amino acid and free D-amino acid content of the milk products seem to be independent of the composition of the milk raw material.

3.3. Changes in the composition of milk with high total CFU due to different heat treatments

3.3.1. Total amino acid content of the milk samples

The amount of the essential amino acids was measured to be equal independently of whether in case of untreated raw milk or milk heat treated in different way. No difference was found in the cistine content that is sensitive to oxidation, ranging between 0.021 and 0.023%, in the methionine content that varied between 0.090 and 0.097%. Similarly, there was a minimal difference in the to the heat treatment very sensitive threonine (0.118–0.124%) and tyrosine (0.127–0.132%) content. Thy lysine content in the three milk samples varied between 0.223–0.236%. Similar to the essential amino acids no change was experienced also in the case of the non-essential amino acids due to the heat treatment.

From our examinations we can conclude that the two heat treatments we applied practically did not cause any changes in the amino acid content regarding either the essential or the non-essential amino acids.

3.3.2. Amino acid composition and biological value of the protein

As the amino acid composition hardly changed due to the different treatments and the sum of the amino acids nearly gave the crude protein content for all of the three samples, therefore we could not find any differences even in the amino acid composition of the protein between the three milk samples. Calculating the biological value of milk protein according to Morup and Olesen (1976) 81.2 was obtained for the control milk sample, 80.9 for the conventionally pasteurized milk, while 80.8 for the microwave pasteurized milk. The results prove that the applied heat treatment had no effect at all on the biological value of the milk protein.

3.3.3. Free amino acid content of the milk samples

Total free amino acid content of the milk samples. Total free amino acid content of the raw milk was measured to be 20.67 mg/100 g milk, which value reduced to 8.02 mg amino acid/100 g milk for the traditionally pasteurized milk, whereas to 8.96 mg amino acid/100 g milk for the microwave pasteurized milk. 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.

The huge decrease observed in the amount of the free amino acids can be due to the technological intervention only. It is possible that as the free amino acids are significantly more reactive than those bound in the peptide chain, therefore they reacted with the milk sugar during the heat treatment resulting in Maillard reaction products. It is supported by the fact that we experienced a decrease of 4–5% in the utilizable lysine content due to the heat treatment, and we assume that this is the result of the transformation of the free lysine. The other possibility may be that the whey proteins coagulated during the heat treatment could absorb the free amino acids on their surface, and this binding is so strong that we could not remove the free amino acids from the surface in the course of the determination.

Summarized it can be established that we obtained a considerable difference regarding the free amino acids between the raw milk and the milk samples heat treated in different ways, however, we could not distinguish between the two heat treatments as to the free amino acids.

Free D-amino acid content of the milk samples. 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. We established that due to the different heat treatments the amount of the D-amino acids practically did not change. It can be concluded therefore that during the pasteurization with the applied temperature and time combinations did not change the D-amino acid content of the raw milk, and in this respect between the two heat treatment procedures cannot be distinguished.

3.3.4. Vitamin B and C content of milk

Vitamin C content of the raw milk was measured to be 22.71 mg/l which reduced during the normal pasteurization to 22.11 mg/l, and during the microwave pasteurization to 6.25 mg/l, that is, due to mild pasteurization the vitamin C content hardly changed, however, during the microwave pasteurization it reduced to less than its one-third. In the case of vitamin B_1 a decrease of 30–40% can be calculated with, however, for the other three vitamins a reduction of approx. 10% can be expected during the heat treatment. Vitamin B content of milk pasteurized by the two methods can be considered practically as identical. It can be stated therefore, that in the respect of the vitamin B content the normal and the microwave pasteurized milk can be considered as equal, however, during the microwave pasteurization a considerable deterioration of vitamin C must be calculated with.

3.3.5. Hydroxymethyl furfurol content of milk

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 can be regarded as equal.

3.3.6. Utilizable lysine and lysinoalanine content of milk

In the course of the measurement of the lysinoalanine content no lysinoalanine could be detected above the level of the sensitivity of the measurement either in the raw milk or in the two heat-treated milk samples. For all three samples the lysinoalanine content remained below 5 mg/ dm^3 therefore 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% thus during the two kinds of heat treatment we applied an around 4−5% decrease in the utilizable lysine content can be observed, and in this respect the two heat treatment methods can be considered as equal.

4. CONCLUSIONS, SUGGESTIONS

4.1. The effect of the total germ number on the total free and free D-amino acid content of milk

Our experiments proved that even the milk of low total germ number contains in minimal amount D-amino acid, in the first place D-aspartic acid, D-glutamic acid and D-alanine. With the increase of the germ number the concentration of both the L- and D-amino acids increased and the increase is gradual up to the range of 1.5–2.0 millions, then suddenly the amount of both the free amino acids and the free D-amino acids rises and within the amino acids also the proportion of the D-amino acids shows a definite increase. At the highest germ number the amount of the free aspartic acid exceeds 1.8, that of glutamic acid 6.0, and that of alanine 7.2 mg/100 g. In the examined range the proportion of D-aspartic acid increased from 11% to 22%, that of glutamic acid from 5% to 25%, and that of alanine from 12% to 33%. The increase is more significant for the D-amino acids which results in the increase of the ratio of the D-amino acids within the total amino acids.

4.2. The effect of the total germ number of milk on the composition of the dairy products

Both enantiomers of all three amino acids examined (aspartic acid, glutamic acid, alanine) of Sana produced from milk with total CFU of 220–390 thousand increased somewhat in the function of the germ number. The increase took place in the similar manner for both enantiomers, therefore the ratios did not change practically in the examined period. In the range of 1.23–2.95 million CFU in case of all the three amino acids the amount of both the D- and the L-enantiomer, which increase became significant after 1.5 million CFU and the L-amino acid content of Sana produced from a milk of almost three million CFU is 2–3 times higher than that of the product produced from the milk with lower germ number, and the same can be said about the amount of the D-amino acids, therefore in this range no essential change was experienced as to the ratio of D- and L-amino acids. D-glutamic acid represented around 25%, D-aspartic acid 32% and D-alanine around 40% within the total amino acids. We established that up to a CFU of around 500 thousand no considerable increase of the free amino acid content must be reckoned with, however, in the case of a CFU of an order of magnitude of a million the amount of both the L- and D-amino acids can be significantly higher compared to the products made from milk with lower CFU.

Regarding the analysis of the Dalia cheese we established that in the CFU range examined the amount of the L-amino acids hardly showed a change and the same can be said about the amount of the three D-amino acids we examined. Summarized it can be said that in case of the Dalia cheese the changes are quite minimal in the function of the germ number. The proportion of D-glutamic acid ranged between 21.85–24.62%, that of D-aspartic acid between 27.11–29.42%, and that of D-alanine between 39.43–41.85%.

Examining the total free amino acid and free D-amino acid content of Telemea and the curd – except L-glutamic acid – for each amino acid and each enantiomer we obtained an increase but since the total germ number range was not wide enough a definite conclusion could not be drawn on the effect of the germ number.

It was established that for fresh dairy products and for those are matured for a short time (Sana, Telemea) with the increase of the total germ number the amount of both the D- and the L-enantiomers increased, but the ratio of the enantiomers is not affected by the total germ number. For those dairy products, however, which are ripened over a longer time (Dalia) and for those where the amino acid production capability of microbial cultures significantly exceeds the amount produced by the microorganisms originally present in the milk raw material, no effect of the milk raw material can be expected, thus, the free amino acid and free D-amino acid content of these milk products seem to be independent of the composition of the milk raw material.

4.3. Changes in the composition of milk with high total CFU due to different heat treatments

We established that due to the microwave pasteurization the amino acid composition and the from this calculated biological value of the milk protein are practically identical with those of the original raw milk. The total free amino acid content of the raw milk reduces to around 40–45% which can be explained by changes taking place during the pasteurization. According to our hypotheses the free amino acids were either utilized in the Maillard reaction or bonded by the coagulated whey proteins during the heat treatment. Between the heat treatment methods, however, we could not make a difference regarding the free amino acids. We established that the temperature and keeping at the temperature applied during the two kinds of pasteurization did not cause an increase of the D-amino acids.

In the case of the vitamin B_1 a loss of around 30–40% was obtained, and for the other vitamins B around a loss of 10% for both heat treatment processes. We experienced a considerable loss during the microwave pasteurization in the case of vitamin C that in the mildly pasteurized milk hardly changed compared to the raw milk, whereas in the microwave pasteurized milk reduced to around its one-third. Examining the hydroxymethyl furfurol, utilizable lysine and lysinoalanine content of milk we obtained only a minimal difference for both pasteurization procedures compared to the raw milk, and between the two procedures in the respect of these components we could not establish any difference.

5. NEW SCIENTIFIC RESULTS

– We established that with the increase of the germ number of milk the concentration of both the free L- and the free D-amino acids increases. The increase in the range of 50-400 thousand is minimal, then up to 1.5 million total CFU continuously increases, after 1.5–2.0 million CFU the concentration of both the free amino acids and the free D-amino acids increases, and beyond the increase of the total quantity with increasing germ number the proportion of the D-amino acids increases.

– We established that for fresh dairy products and for those matured for a short time with the increase of the total germ number of the milk raw material the amount of both the D- and L-amino acid enantiomers increases, the ratio of the enantiomers is not affected by the total germ number, however. For dairy products ripened over a longer time due to the amino acid production of the applied pure cultures, the total germ number of the milk raw material has no effect on the free amino acid and free D-amino acid content of the dairy products.

– The microwave pasteurization similarly to the traditional pasteurization does not change the amino acid composition and biological value of the milk protein, reduces the free amino acid content of milk, has no effect on the free D-amino acid content of milk, does not cause considerable amount of hydroxymethyl furfurol and lysinoalanine formation, and does not reduce the utilizable lysine content. It decreases by around 10–40% the vitamin B content and results in substantial deterioration of vitamin C compared to the traditional, mildly performed pasteurization.

6. SCIENTIFIC PAPERS AND PRESENTATIONS ON THE SUBJECT OF THE DISSERTATION

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7. SCIENTIFIC PAPERS AND PRESENTATIONS OUT OF THE SUBJECT OF THE DISSERTATION

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