THE FREE D-ASPARTIC ACID AND D-GLUTAMIC ACID CONTENT OF SHEEP MILK AND SHEEP MILK PRODUCTS

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ABSTRACT

The role of D-amino acids of foods in the human health is a strongly discussed topic and usually, data came from the investigation of cow milk. We have studied the free D-aspartic acid and free D-glutamic acid content of sheep milk, heat-treated sheep milk at various temperatures and various products of sheep milk. Raw sheep milk didn't contain free D-aspartic and D-glutamic acid in remarkable amount and ratio 5.92% free D-aspartic acid; 2.62% free D-glutamic acid). Our heat-treatments didn't cause major change in the free D-aspartic and D-glutamic acid content (max.: 7.8% free D-aspartic acid; 5.3% free D-glutamic acid in total free aspartic and glutamic acid). Contrary, all of the investigated products contained high level of free D-amino acids. The free D-aspartic acid and free D-glutamic acid content of the products were 16,9-39,5%,and 13,3-27% in the percent of total free amino acids. The racemization of aspartic acid was higher, than that of glutamic acid in every product. The D-amino acid content of fermented milk products was higher than in different cheeses.

Keywords: free D-aspartic acid, free D-glutamic acid, sheep milk, dairy products

1. INTRODUCTION

Free D-amino acid content of different foodstuffs are determined basically by the original free D-amino acid content of the raw material, by production methods and by microbiological processes. Several D-amino acid enantiomers may have toxic effect; some may change the biological effect of lisino-alanine as well. On the other hand, certain D-amino acids may be useful (e.g. in pain relief), and proteins containing D-amino acids with reduced digestibility may be used, e.g. in special diets [7]. We learn more and more about the racemization of different peptides and researchers can use modern method to determine the D-amino acids [3, 11, 12, 13, 14].

A number of researchers have analysed the D-amino acid content of milk and various dairy products concluding that D-amino acid content increases significantly during the processing of raw milk. Ref. 1. determined at 100° C, at 7-8 pH , that the half-life of racemisation (time needs to reach 0.33 D/L ratio) for serine is 3 days, 30 days for aspartic acid, 120 days for alanine and 300 days for isoleucine.

Ref. [1] studied the changes of racemization of D-aspartic acid during milk treatments (Hereinafter, we give the concentration of D-amino acids as a percentage of the total - same - free amino acids: D-amino acid (%) = (D/D+L)x100. Raw milk contained the smallest amount of D-aspartic acid (1.48%). However, this amount increased in direct proportion to the number of treatments (acidophilus milk: 2.05%; low fat milk powder: 2.15%; kefir: 2.44%; evaporated milk: 2.49%; yoghurt: 3.12%; milk-based baby formula: 4.95%).

Ref. [9] analysed the effects of heat treatment and bacteria on the content of free D-amino acid in milk and D-amino acid bonded in protein. They determined that the free D-amino acid content did not grow in raw milk under the effects of pasteurisation, ultra-high pasteurisation or sterilization. In contrast, they discovered that the free D-amino acid content of the raw milk samples grew significantly when it was stored at 4° C and thus recommended that the figure for D-alanine content should be used in checking bacterial contamination in milk.

Ref. [11] found the free D-aspartic acid content of milk powder to be between 4-5% and that of D-alanine to be between 8-12%. They measured the D-alanine content of yoghurt at 64-68%, D-aspartic acid at 20-32% and free D-glutamic acid at 53-56%. These values in aged cheese were between 20-45%, 8-35% and 5-22%, respectively. They measured the free D-phenylalanine content of aged cheese as being between 2-13% and even managed to demonstrate the presence of a minimal amount of D-leucine in the aged cheese. Based on their figures, they point out that it is not those foods that are subjected to long periods of heat treatment which contain large amounts of D-amino acids but rather those that undergo microbiological fermentation.

Studying the free D-amino acids in milk, fermented milk, lactic cheese and quarg, [2] determined that significant amount of D-amino acid can be explored both in raw milk and in fermented dairy products.
Ref. [4, 5, 6] studied samples from healthy and mastitic udders. They determined that during milking both samples from the initial streams of milk and those from the diseased udders contained large amounts of D-Asp, D-Glu, D-Ala and D-Ile. The amount and proportion of D-amino acids in milk from the diseased udders grew in line with the Masti-test degrees. These studies prove that the first streams during milking and the milk from cows suffering in subclinical mastitis play a significant role in the amount of D-amino acids in various types of market milk produced from cow's milk.
Ref. [7] investigated the free D-amino acid content of cheeses made using various processes, it was determined that the following free D-amino acids occurred in the following concentrations on average in various cheeses: D-Asp at 58 μmol/100g (30.3%), D-Glu at 117 μmol/100g (15.8%) and D-Ala at 276 μmol/100g (37.2%). A larger D-amino acid content was determined in Cheddar cheese samples, which were made using Lactobacillus species as well.

2. MATERIAL AND METHODS

In our experiments sheep milk samples after different heat-treatments and yoghurt samples were investigated produced in Dairy Lab of Fac. of Engineering University of Szeged. We heated raw sheep milk at 60, 70, 80, 90 and 120° C. Yoghurt was pasteurised at 75° C and then homogenised; and Yo-fast 88 starter (Ch. Hansens, Danmark) was used for fermentation at 45°C.
D-amino acid content of the freeze-dried samples was determined at the Institute of Chemistry of the Faculty of Veterinary Science at the University of Kaposvár by high performance liquid chromatography using fluorenyl-ethyl-chloroformate [3] and by precolumn derivation using chiral reagents o-phthalaldehide/tetra-O-2,3,4,6-tetra-O-acetyl-thio-β-D-glucopyranose [8]. The results are given as free D-amino acids in the percentage of all free amino acids.

3. RESULTS AND DISCUSSION

The ratio of D-aspartic acid and D-glutamic acid were remarkable in samples, therefore we report about results considering these two amino acids.
We found higher amount of D-aspartic acid and D-glutamic acid in heat-treated sheep milk samples compared to raw milk. The changes are illustrated in Fig. 1.

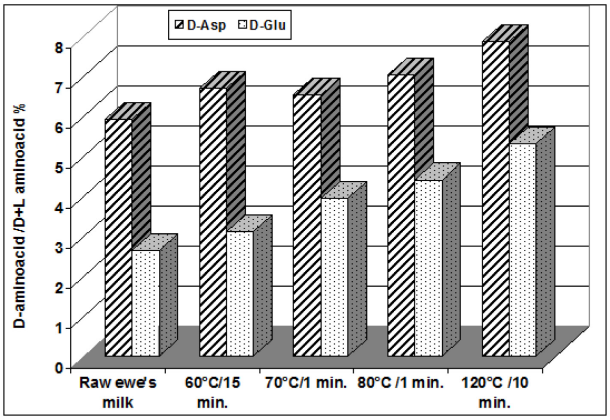

Figure 1. The free D-aspartic acid and D-glutamic acid content of raw sheep milk and sheep milk heat-treated at various temperatures (all data for total D-Asp and D-Glu in %)

However, the heat sensitivities of investigated amino acids seem to be different. Free aspartic acid shows nearly the same increase of D-enantiomers at 60 and 70°C, whereas bigger difference was explored at 80°C. Free D-glutamic acid increase was unambiguous and continuous at each successive temperature. In the case of both amino acids, the highest free D-amino acid content came from samples of highest temperature. The idea of divergent heat sensitivities are suggested, and it is confirmed with the decreased difference between D-aspartic acid and D-glutamic acid (from 3.3% to 2.5% at 120°C, 10 min.).

We can state that the heat treatment (alone) did not result major changes in the free D-aspartic acid and free

D-glutamic acid content of sheep milk compared to the total given free amino acid content (max.: 7.8% D-aspartic acid; 5.3% D-glutamic acid). The effect of investigated heat treatments is demonstrated in Tab. 1. Values are expressed as a proportion of data from raw milk.

Table 1. The growth rate (%) of free D-Aspartic acid and free D-Glutamic acid content resulted by various heat treatments (Value of raw sheep milk=100%)

Amino acids	**Heat treatment** 60° C 15 min.	70° C 1 min.	80° C 1 min.	120° C 10 min.
D-aspartic acid	113.0	110.2	119.0	132.6
D-glutamic acid	117.8	149.9	167.5	201.9

Heat treatment at 60° C for 15 minutes resulted roughly the same change for the two amino acids, but glutamic acid growth was comparatively greater at 70° C. Heat treatment at 120° C (sterilization) resulted 32% increase in D-aspartic acid content while D-glutamic acid content grew by almost 102% (roughly double). Based on the findings came from heat treatments at 70 and 80° C, we can state that temperature increase of 1° C results in an approx. 0.9% increase in D-aspartic acid content and an approx. 1.7% increase in D-glutamic acid content. At same temperature, the speed rate of the racemization of free glutamic acid, was double than that of aspartic acid. Glutamic acid exhibits a greater predisposition for racemisation; D-enantiomer occurs more rapidly and in larger amounts than in the case of aspartic acid. The higher D-aspartic acid content of raw milk suggests, however, that the micro flora prevailing in the udder and/or grown in the milk during cold storage have a greater effect on the condition of aspartic acid.

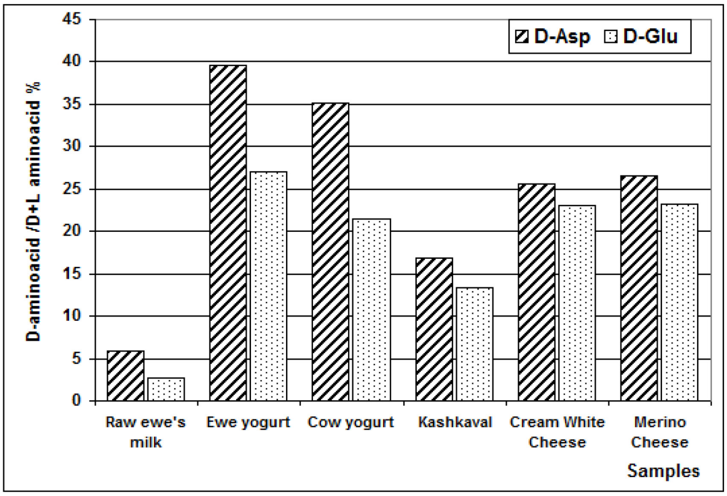

Figure 2. The free D-aspartic acid and D-glutamic acid content of raw sheep milk and certain dairy products (all data for total free D-Asp and D-Glu in %)

Fig. 2 demonstrates our results came from the investigations raw sheep milk and different product made from sheep milk (Pasta filata type cheese as Kashkaval, semi hard cheese as Merino, Cream white cheese made from ultrafiltered milk and yogurts). We can state that all these products contain significantly higher proportion of D-enantiomers than raw sheep milk.

Our findings reinforce the conclusions in the literature suggested that the fermentation of milk with dairy cultures greatly increases free D-amino acid content in dairy products. Values and ratio of D-aspartic acid and D-glutamic acid is represented in Tab. 2.
Of the two amino acids, a higher D-aspartic acid content and a lower D-glutamic acid content was found in all the dairy products. D-amino acid content was roughly the same for traditionally aged cheese (merino)

and acid rennet cream white cheese. The lower values for Kashkaval cheese may have resulted from the heat effect of soaking in warm brine as well as the lower water activity of the cheese.

Table 2. The ratio of free D-aspartic acid and free D-glutamic acid in raw sheep milk and certain products of sheep milk

	D-Asp/D-Glu
Raw sheep milk	2.26
Yoghurt from sheep milk	1.46
Yoghurt (from cow's milk)	1.64
Kashkaval cheese	1.26
Cream white cheese	1.11
Merino cheese	1.14

The yoghurts contained significantly more D-amino acid than cheeses. This may be a result of the higher CFU value and the more intensive bacterial activity. Interestingly, yoghurts representing a pH value of approx. 4.4 exhibited a significantly higher D-Asp/D-Glu ratio than that of cheeses (at 1.11-1.26). This ratio is 1.46 for sheep yoghurt and 1.64 for yoghurt made from cow's milk. Furthermore, the D-Asp/D-Glu ratio was higher in yoghurt made from cow's milk than in yoghurt made from sheep milk. However, we cannot serve correct explanation for this result at present because of the different production conditions (industrial cow's milk yogurt was measured).

At the same time, sheep yoghurt represented a significantly higher D-amino acid content, which can be explained partly by the fact that the total bacteria count is a great deal higher in sheep bulk milk than in cow's milk. Another consequence also can be concluded; likely, the natural, original micro flora of raw sheep milk determines the D-Asp/D-Glu ratio of raw milk. In the other words, at normal condition, the racemization of aspartic acid caused by usual micro flora is deeper, whereas the ratio of D-Asp/D-Glu in products made from sheep milk shows a markedly lower value: 1.11-1.64. It seems likely, that this ratio is determined by many factors as the original micro flora of milk, the processes in the technology and the effect of different starters.

4. CONCLUSION

Many studies proved the presence of D-amino acids in cow's milk and the products made from cow's milk. However, we have not found research concerning sheep milk. Therefore, we have studied the D-amino acid content of sheep milk, sheep milk samples were heat-treated at various temperatures and various products made from sheep milk. According to our findings, raw sheep milk does not have high free D-aspartic acid (5.92%) and free D-glutamic acid (2.62%) content.

Heat treatment did not result remarkable change in the investigated D-amino acid content of sheep milk. In the case of the strongest heat treatment, free D-aspartic acid content increased to 7,85 % and the free D-glutamic acid content reached 5,30 %. In the case of the common pasteurisation of milk, we can state that a temperature increase of 1° C results approx. 0.9% increase of free D-aspartic acid content and approx. 1.7% increase of free D-glutamic acid content.

However, remarkable change of free D-amino acid content was detected in every product samples. The products contained 16.8-39.5% free D-aspartic acid and 13.3-27.0% free D-glutamic acid. We determined the highest free D-amino acid contents in products were made by lactic acid fermentation (yoghurts).

These findings and those of the analyses of samples from the various heat treatments do not enable us to generalise at this point. They call for further study, in particular, to investigate the effect of heating temperature and holding time as well as to gain a better understanding of the precise effects of certain cultures and even individual bacteria species, to ensure that we can keep D-amino acid content of milk products at an acceptably low level.

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