CHANGES IN THE D-AMINO ACID CONTENT OF SHEEP MILK RELATED TECHNOLOGIES

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Abstract

We have studied the free D-aspartic acid and free D-glutamic acid content of ewe's milk, heat-treated sheep milk at various temperatures and various products of ewe's milk. Raw ewe's milk didn't contain free D-aspartic and D-glutamic acid in remarkable amount. Whereas, all of the investigated products contained high level of investigated free D-amino acids. The free D-aspartic acid content of the products was 16,9-39,5%, while the free D-glutamic acid content was 13,3-27% in the percent of total free amino acids. The D-amino acid content of fermented milk products was higher than in the case of different cheeses.

***Key words**: free D-aspartic acid, free D-glutamic acid, ewe's milk, dairy products*

INTRODUCTION

Milk and dairy products provide very good examples of the occurrence of D-amino acids in the processing of raw foods. Although a consensus has not been reached on D-amino acids, at present their negative consequences outnumber their positive effects. The presence of D-amino acids in protein reduces digestibility and affects that of other amino acids. Research to date has indicated that it is rather racemisation that is affected, first and foremost, by the pH of the substance, by heat treatment and by alkalisation time as well as by the structure of certain amino acids. The D-amino acid content of the food, we eat is determined by the original D-amino acid content of the raw material, by production methods and by microbiological processes

The D-amino acid content of the food we eat is determined by the original D-amino acid content of the raw material, by production methods and by microbiological processes. Several D-amino acid isomers may have a toxic effect; some may change the biological effect of lisinoalanine 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 dieting (7.)

A number of researchers have analysed the D-amino acid content of milk and various dairy products and concluded that D-amino acid content increases significantly during processing. In their study of the racemisation of free amino acids, Bada (1.) determined that at 100° C with pH between 7 and 8 the half time of racemisation (the period during which the D/L ratio reaches 0.33) for serine is 3 days; they also found that this figure is 30 days for aspartic acid, 120 days for alanine and 300 days for isoleucine.

Payan (12.) studied the changes brought about during milk treatment by measuring the concentration of D-aspartic acid. 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%).

Gandolfi (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 stored at 4° C and thus recommended that the figure for D-alanine content should be used in checking potential bacterial contamination in milk.

Palla (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.

In their study of the free D-amino acids in milk, fermented milk, lactic cheese and quarg, they (2.) determined that a significant amount of D-amino acid occurs both in raw milk and in fermented dairy products.

Csapó et al. (4., 5., 6.) studied cow's milk 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 the milk from the diseased udders grew in line with the mastitest degrees. These studies prove that the first streams during milking and the milk of cows suffering from subclinical mastitis play a significant role in the amount of D-amino acid in various types of market milk produced from cow's milk .

Csapó (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 the 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 measured in Cheddar cheese samples, which were made using species of Lactobacillus as well.

MATERIAL AND METHODS

For our experiments the ewe's milk was heat-treated and the fermented product (yoghurt) was made at the experimental dairy of the College Faculty of Food Engineering at the University of Szeged. We heat-treated the raw ewe's milk at 60, 70, 80, 90 and 120° C. The yoghurt was pasteurised at 75° C and homogenised; it was produced using a Lactobacillus bulgaricus-Streptococcus thermophilus culture.

The 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.).

RESULTS AND DISCUSSION

The ratio of D-aspartic acid and D-glutamic acid was higher in free amino acids, therefore we report only our results related free D-amino acids. The change in D-amino acid content resulting from heat treatment is illustrated in figure 1. We determined that the amount of D-aspartic acid and D-glutamic acid increases in ewe's milk as a result of heat treatment.

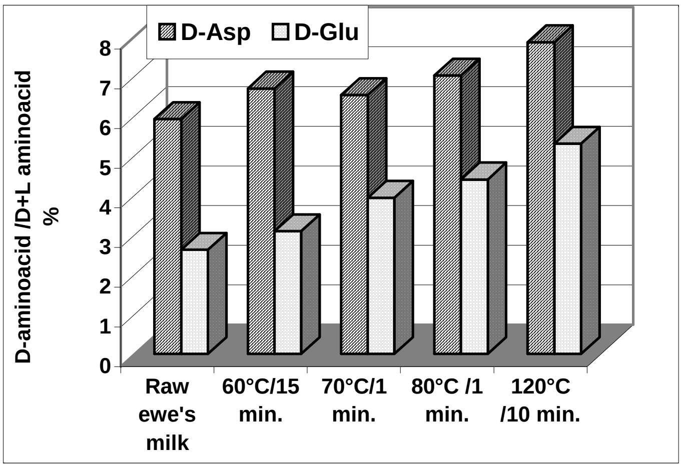

***Fig. 1.** The free D-aspartic acid and D-glutamic acid content of raw ewe's milk and ewe's milk heat-treated at various temperatures (all data for total D-Asp and D-Glu in %)*

However, the heat sensitivities of the two amino acids appear to diverge. Free aspartic acid shows nearly the same D-amino acid content growth at 60 and 70° C whereas the D-amino acid content clearly grew as of 80° C. The free D-glutamic acid content growth, however, was unambiguous and continuous at each successive temperature. In the case of both amino acids, the highest free D-amino acid content resulted from the highest temperature. The divergent heat sensitivities are supported, however, by the fact that in raw milk the existing 3.3% difference in favour of D-aspartic acid decreased to 2.5% after heat treatment at 120° C, 10 min.

Based on the data, we can state that heat treatment alone does not bring about a major change in the D-aspartic acid and D-glutamic acid content of ewe's milk compared to the total given amino acid content (max.: 7.8% D-aspartic acid; 5.3% D-glutamic acid). The effect of investigated heat treatments compared to raw milk is demonstrated in Table 1.

Table 1. *The growth rate (%) of free D-Aspartic acid and Glutamic acid content resulting from various heat treatments (Value of raw ewe's milk=100%)*

	Heat treatment			
Amino acid	**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 brought about 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) effected a 32% increase in D-aspartic acid content while D-glutamic acid content grew by almost 102% (roughly double). Based on the findings of heat treatments at 70 and 80° C, we can state that a temperature increase of 1° C results in an appr. 0.9% increase in D-aspartic acid content and an appr. 1.7% increase in D-glutamic acid content. For glutamic acid, the speed with which D-enantiomers occurred caused by the same degree of temperature increase was double that of aspartic acid.

The data in the table (1.), therefore, prove that when subjected to heat treatment under the same conditions glutamic acid exhibits a greater tendency for racemisation. In this case the 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 microflora prevailing in the udder and/or grown in the milk during cold storage have a greater effect on aspartic acid.

Figure 2 demonstrates the findings of our analyses of D-amino acid content in traditionally aged ewe cheese, fused cheese (Kashkaval), cream white cheese made by ultrafiltration and the fermented product (yoghurt).

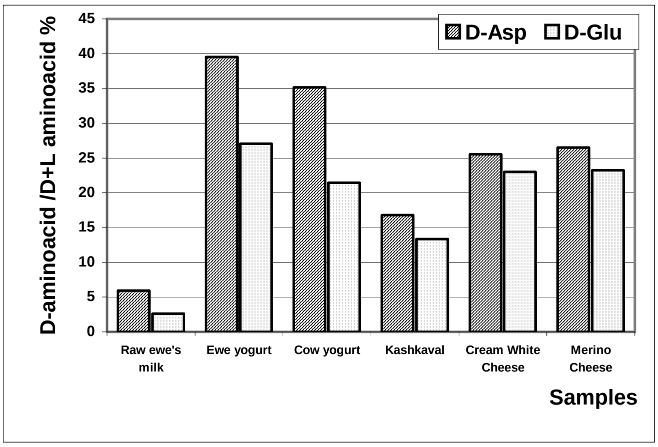

Figure 2. *The free D-aspartic acid and D-glutamic acid content of raw ewe's milk and certain dairy products (all data for total free D-Asp and D-Glu in %)*

We can state that all these products contain a significantly higher proportion of D-enantiomers than raw ewe's milk. Our findings therefore reinforce conclusions in the literature according to which fermentation with cultures greatly increases the D-amino acid content in dairy products. Values and ratio of D-aspartic acid and D-glutamic acid is represented in Table 2.

***Table 2**. The ratio of free D-aspartic acid and free D-glutamic acid in raw ewe's milk and certain products of ewe's milk*

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

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.

The yoghurts contained significantly more D-amino acid than the cheeses. This may be a result of the higher CFU value and the more intensive bacterial activity. Interestingly, the yoghurts representing a pH value of appr. 4.4 exhibited a significantly higher D-Asp/D-Glu ratio than the cheeses (at 1.11-1.26). This ratio is 1.46 for ewe yoghurt and 1.64 for yoghurt made from cow's milk. Also of interest is the fact that the D-Asp/D-Glu ratio is greater for yoghurt made from cow's milk than for ewe yoghurt. However, as the two products were not made under identical conditions we can offer no explanation for this at present.

At the same time, ewe yoghurt has a significantly higher D-amino acid content, which can be explained in part by the fact that the total number of microbes is a great deal higher in ewe's bulk milk than in cow's milk. It may also be concluded that the highest D-Asp/D-Glu ratio is brought about by the natural, or common, micro flora in raw ewe's milk, whereas this value for one of the products made from ewe's milk does not even approach 1.5. In other words, the few cultures commonly used to make dairy products from ewe's milk bring about a D-Asp/D-Glu ratio of between 1.1 and 1.5.

CONCLUSION

Many have studied the presence of D-amino acids in cow's milk and the products of cow's milk. However, we have found no research concerning ewe's milk. We have therefore studied the D-amino acid content of ewe's milk, ewe's milk heat-treated at various temperatures and various products of ewe's milk. According to our findings, raw ewe's milk does not have a high D-aspartic acid (5.92%) and D-glutamic acid (2.62%) content.

Heat treatment brings about no meaningful change in the D-amino acid content of ewe's milk. In the case of the strongest heat treatment D-aspartic acid content increased to 7,85 % and the D—glutamic acid content increased to 5,30 %. In the case of the common pasteurisation of milk we can state that a temperature increase of 1° C results in an appr. 0.9% increase in D-aspartic acid content and an appr. 1.7% increase in D-glutamic acid content.

However, a significant change was detected in the D-amino acid content of every product investigated. The products contained 16.8-39.5% D-aspartic acid and 13.3-27.0% D-glutamic acid. We measured the highest D-amino acid content in those products were made by lactic acid fermentation (yoghurts).

These findings and those of the analyses of the various heat treatments do not enable us to make any generalisations at this point. They call for further study, in particular on temperature and holding time as well as to gain a better understanding of the precise effects of certain cultures and even individual species of bacteria in order to be able to maintain the D-amino acid content at an acceptably low level.

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