D-Amino Acid in Ewe's Milk

The D-Amino Acid content of ewe's milk and certain products of ewe's milk

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Introduction

The foods ingested into the human organism represent the most important source of D-amino acids as food proteins undergo a certain degree of racemisation during cooking as well as during the various processes used in food production. D-amino acids generally diminish product quality and food safety. 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.

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.

Data on cow's milk abound in the literature, but little is published (or at least the findings are inconclusive) on the milk of such small ruminants as ewes, the D-amino acid content of products made from it, and the degree to which heat treatment affects the D-amino acid content of ewe's milk. In the present paper, we wish to report on our findings in this regard.

1. REVIEW OF PREVIOUS STUDIES

As staples, milk and dairy products perfectly illustrate the change in the D/L-amino acid composition of the original raw material. Although raw milk (unheated) is also available to consumers (primarily abroad), most dairy products are first pasteurised by various methods and then homogenised as well as evaporated, curdled etc., thus giving the characteristic features to specific products, such as market milk, yoghurt and various cheeses. These latter two products are fermented with bacteria in a process that also results in the presence of D-amino acids. Much research has been done on the occurrence of D-amino acids in milk and dairy products with the conclusion that they may contain significant amounts of D-aspartic acid, D-alanine and D-glutamic acid. As dairy production involves no alkaline treatment (with the exception of Na caseinate), it can be stated that heat treatment and bacterial activity bring about the growth of D-amino acids in dairy products.

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 (1985) and Steinberg & Bada (1981) 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. According to Liardon and Lederman (1986), in the case of casein, at 83° C with pH 9 the half time of racemisation for these four amino acids is 16 hours, 19 hours, 11 days and 57 days, respectively.

Payan et al. (1985) studied the changes brought about during milk treatment by measuring the concentration of D-aspartic acid. (In what follows, we provide the concentration of each D-amino acid as a percentage of the total: %D-amino acid = (D/D+L)*100.) 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%). The largest percentage of D-aspartic acid can be found in the various kinds of baby formula which undergo such processes as spray drying and sterilization (with heat).

Gandolfi et al. (1992) 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. They found the content of free D-alanine in their milk samples was between 3-8%, the D-aspartic acid content between 2-5% and the D-glutamic acid content between 2-4%. 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 et al. (1989) 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, Bruckner and Hausch (1990) determined that a significant amount of D-amino acid occurs both in raw milk and in fermented dairy products.

Csapó et al. (1995, 1997, 1996-97) studied cow's milk from healthy and mastitic udders as well as the free D-amino acid content of certain cheeses. 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. In addition to these amino acids, they also found D-Ser, D-Pro, D-Val, D-Leu and D-Lys in the milk from the mastitic udders. The amount and proportion of D-amino acids in the milk from the diseased udders grew in line with the mastitest degrees; for example, the D-alanine content was 12.6% for the non-mastitic sample whereas it was 48.9% for the four cross sample, and significant changes were also noted for other amino acids. 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 et al. 1995, 1995-96, 1997).

In studies of 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 _mol/100g (30.3%), D-Glu at 117 _mol/100g (15.8%) and D-Ala at 276 _mol/100g (37.2%). There was a significant difference in the amount of D-amino acids between individual cheeses, and the percentages varied from 13.9-46.3% for D-Asp, 12.9-26.6% for D-Glu, and 16.1-48.1% for D-Ala. The other amino acids could only be detected in trace concentrations and were thus barely noticeable in the cheeses. A larger D-amino acid content was measured in cheddar cheeses which were made using species of Lactobacillus as well.

2. 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 of the Food Industry 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 (Csapó and Einarsson 1993) and by precolumn derivation using chiral reagents o-phthalaldehide/tetra-O-acati-_-D-glycopiranozide (Folestad et al. 1994).

3. FINDINGS

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.

However, the heat sensitivities of the two amino acids appear to diverge. Aspartic acid shows nearly the same D-amino acid content growth at 60 and 70° C whereas the D-amino acid

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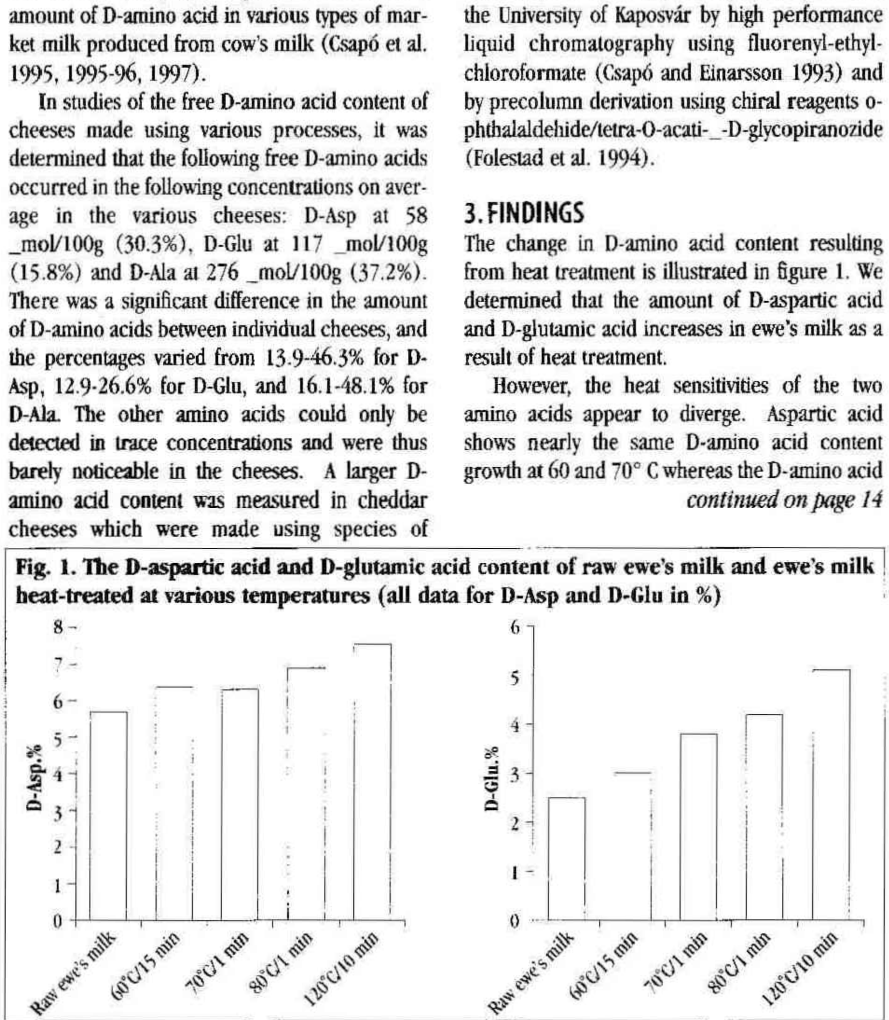
Fig. 1. The D-aspartic acid and D-glutamic acid content of raw ewe's milk and ewe's milk heat-treated at various temperatures (all data for D-Asp and D-Glu in %)



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content clearly grew as of 80° C. The D-glutamic acid content growth, however, was significant and continuous at each successive temperature. In the case of both amino acids, the highest 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.

Based on the data, we can state that heat treatment alone does not bring about a significant 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 certain heat treatments compared to raw milk is demonstrated in chart 1.

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.

Chart 1. The growth rate (%) of D-amino acid content resulting from various heat treatments (Value of raw ewe's milk=100%)

Amino acid	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

Chart 2. The content and percentage of D-amino acid in certain products of ewe's milk

	D-Asp %	D-Glu %	D-Asp/D-Glu
Raw milk	5.92	2.62	2.26
Ewe yoghurt	39.53	27.05	1.46
Yoghurt (cow's milk)	35.14	21.44	1.64
Kashkaval	16.80	13.34	1.26
Cream white cheese	25.53	23.01	1.11
Merino cheese	26.52	23.25	1.14

The data in the chart, 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 appearing in the milk during cold storage have a greater effect on aspartic acid.

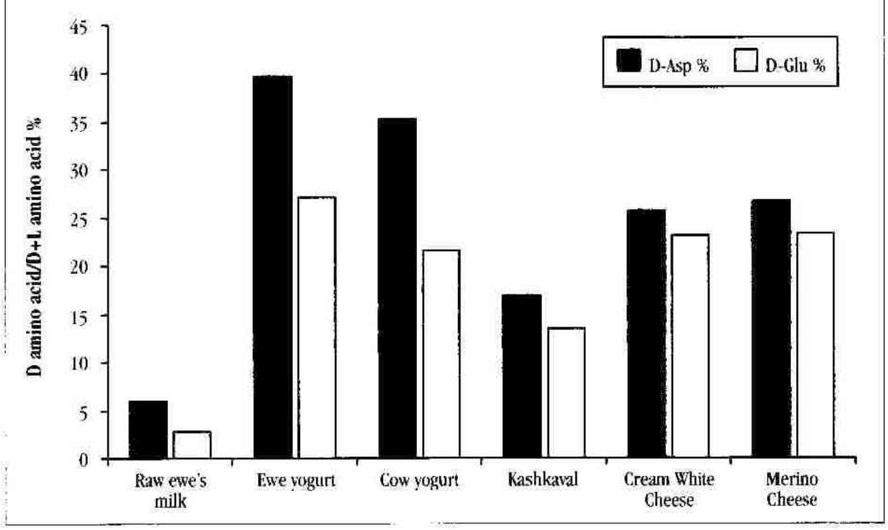
Figure 2 demonstrates the findings of our analyses of D-amino acid content in traditionally aged ewe cheese, fused cheese (Kashkaval), cream white cheese made by ultrafiltration and the fermented product (yoghurt). We can state that all these products contain a significantly higher proportion of D-enantiomers 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.

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, microflora in

Figure 2. The D-aspartic acid and D-glutamic acid content of raw ewe's milk and certain dairy products (all data for D-Asp and D-Glu 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. 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. 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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ABSTRACT

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. Many have studied the presence of D-amino acids in milk and dairy products. However, we have found no research concerning the milk of small ruminants. 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. Al of the investigated products contained significant level of free D-amino acids. The free D-aspartic acid content of the products was 16,9-39,55, while the freed-glutamic acid content was 13,3-27,0% in the percent of total free amino acids. The racemisation of the aspartic acid was higher, than at glutamic acid at every investigated products. The D-amino acid content of sauer-milk products was higher than in the case of different cheeses. ■

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