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The D-amino acid content of foodstuffs (A Review)

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Abstract. D-amino acids occurring in dietary proteins originate as a consequence of technological intervention while basic materials are being prepared for consumption. Foodstuffs are the most significant sources of D-amino acids, as in the process of cooking or during the various processing procedures used in the food industry dietary proteins undergo racemisation to a greater or lesser degree. Food stores are now selling increasing quantities of foods (such as breakfast cereals, baked potatoes, liquid and powdered infant foods, meat substitutes and other supplements) which in some cases contain substantial quantities of D-amino acids, which in turn possess characteristics harmful with respect to digestion and health. Alkali treatment catalyses the racemisation of optically active amino acids. The degree of racemisation undergone varies from protein to protein, but the relative order of the degree of racemisation of the individual amino acids within proteins shows a high level

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of similarity. The principal factors influencing racemisation are the pH of the medium, heat treatment, the duration of the application of alkaline treatment and the structure of the respective amino acids. D-amino acids formed in the course of treatment with alkalis or heat give rise to a deterioration in quality and reduce the extent to which food thus treated can be used safely. The presence of D-amino acids in proteins leads to a decrease in digestibility and the availability of the other amino acids. This results in a reduction in the quantities of the L-enantiomers of the essential amino acids, as the peptide bonds cannot split in the normal way. Some D-amino acids can exert an isomer-toxic effect and have the capacity to give rise to changes in the biological effect of lysinoalanine. On the other hand, certain D-amino acids may also be of benefit (e.g. in pain relief), and proteins containing D-amino acids of lower digestibility can be used in, for example, diets designed for weight loss.

1 Introduction

Foods contain large quantities of non-natural substances of external origin which influence their digestibility to a considerable degree [44]. An example is the D-stereoisomer amino acids, which are formed from common L-stereoisomer amino acids, either in the course of the production process or as a consequence of changes in the microbiological quality of the foodstuff. The presence of these D-stereoisomer amino acids results in a substantial reduction in the digestibility of dietary protein and the availability of the transformed amino acid. However, despite the fact that D-amino acids in foods are considered undesirable, some hold the opinion that in certain cases D-amino acids can nevertheless be beneficial to the human organism.

As in many other aspects, Pasteur [79] also accomplished pioneering work in this field. He demonstrated that aspartic acid derived from vetch is optically active (chiral), whereas that produced by the heating of ammonium fumarate does not exhibit optical activity. It was subsequently realised that the proteins occurring in the living organism are constructed exclusively from L-amino acids, despite the fact that D- and L-stereoisomers (enantiomers) possess the same chemical and physical properties, with the exception of the rotation of the plane of polarised light. The two stereoisomers rotate this plane in different directions. The stereospecific synthesis of proteins in the living organism [92] could not initially be explained, as a result of which this sphere of issues occupied scientists for almost a century [4].

As methods developed for the separation and determination of amino acid enantiomers have been perfected it has been found that, contrary to previous belief, D-amino acids occur in a great variety of organisms. For example, bacterial cell wall peptidoglycans contain D-aspartic acid, D-glutamic acid and D-alanine [3, 21, 83]; in some marine worms and invertebrates the cellular fluid contains D-amino acids as a main component [20, 36, 40, 74]; in certain marine shellfish quantities of D-amino acids can exceed 1% [41, 82]; and higher plants also contain D-amino acids [84]. Metabolically stable proteins in mammals of longer life span contain major quantities of D-aspartic acid derived from racemisation [1]: the D-aspartic acid concentration of the white matter of the human brain amounts to 3%, the clarified basic protein of the spinal cord to 10% [45, 72]. Clarke [19] verified that aspartic acid racemises in vivo in human tissues, but due to rapid metabolism does not accumulate in measurable quantities.

The chiral amino acids can be transformed into racemic mixtures, the reaction mechanism of this transformation process necessitating the splitting off of the hydrogen of the α-position carbon atom and the formation of the structure of the planar carbanion. The degree of racemisation occurring depends on whether the amino acid occurs free or in bound form in the peptide chain, and is naturally chiefly dependent on temperature and pH, and also on the nature of the R group occurring in the amino acid [2]. On examination of the racemisation of free amino acids Bada [2] and Steinberg et al. [89] established that at 100 °C and at pH between 7 and 8 the half-life of racemisation (i.e., the time taken for the D/L ratio to reach 0.33) for serine is 3 days, for aspartic acid 30 days, for alanine 120 days, and for isoleucine 300 days. Liardon and Lederman [68] reported that at pH 9 and at 83 °C for casein the half-life of racemisation for the above four amino acids, respectively, is as follows: 16 hours, 19 hours, 11 days and 57 days; Friedman and Liardon [49] gave these respective values for soya protein at 75 °C in 0.1 M sodium hydroxide as 9 minutes, 20 minutes, 5 hours and 25 hours. As can be seen from these collected data, in different conditions the respective amino acids show racemisation times of different duration, but the order of the degree of racemisation among the amino acids remains to a certain extent unchanged. The racemisation of serine, cystine and threonine results not only in the corresponding D-enantiomer, but also in an amino acid not constituting one of the components of proteins. For example, in the inter-carbanion state serine can readily lose its OH group in the formation of dehydroalanine. Reaction of dehydroalanine with the ϵ-amino group of lysine results in lysinoalanine [47, 70, 73], an amino acid of which the alanine part is racemic while the lysine part is optically active. In dietary proteins this reaction can result in cross-linking, leading to a reduction in protein digestibility [18, 50]; the lysinoalanine content of the resultant foodstuff also bears toxic effect [62].

From the aspect of nutrition the racemisation of essential amino acids is of the greatest significance. The digestibility and metabolism of the D-enantiomers of the essential amino acids have been studied for some considerable time. It is evident from the work of Neuberger [76] and Berg [7], both of whom summarised earlier studies, that in mammals the D-enantiomers of essential amino acids are utilised to very low degrees, in some cases act as growth inhibitors, and are for the most part excreted in the urine. The following authors have corroborated the results obtained in earlier research [48, 51, 64, 88].

The half-life of racemisation for the essential amino acids has only recently been subjected to investigation. At pH between 7 and 8 Bada [2] measured the half-life of racemisation at 100 °C for isoleucine, leucine and valine at 300 days, and for phenylalanine and tyrosine at 50 days. Working under the same conditions Engel and Hare [39] determined the half-life of racemisation for lysine at 40 days, while Liardon and Lederman [68] measured the half-life of racemisation at pH 9 and 83 °C at 40 days for tryptophan, 20 days for threonine and 2 days for cysteine. Boehm and Bada [10] obtained a value of 30 days for the half-life of racemisation for methionine at 100 °C at pH between 7 and 8. It appears from the empirical data that cysteine is particularly susceptible to racemisation, while the amino acids with aliphatic side-chains are the most stable in this respect. For most of the essential amino acids the half-life of racemisation is longer than that for aspartic acid.

Food proteins exposed to alkali treatment processes or to lengthy heat treatment contain considerable concentrations of amino acids derived from racemisation. Dakin [34] was the first to demonstrate that the digestibility of proteins decreases on exposure to heat or strong alkalis. It is now evident that this reduction in digestibility is related to the formation of lysinoalanine and the racemisation arising [13, 18, 50, 52, 60, 70].

2 D-amino acids of dietary origin

Despite the fact that some insects, worms and marine invertebrates contain substantial quantities of D-amino acids, since such organisms do not constitute main components of the human diet these quantities are insignificant, and their importance can therefore be disregarded. However, in communities in which marine shellfish represent an important source of food account should be taken of D-amino acids consumed in large quantities, not only with respect to nutrition, but also from the toxicological aspect [41], as, for example, quantities of D-amino acids occurring in marine shellfish can exceed 1%. According to Preston [82] in marine molluscs quantities of D-amino acids can vary between 0.11 and 1.6 mM related to body tissue of 70% water content.

The majority of food treatment procedures, performed for the purposes of improving flavour, consistency or non-perishability, and including cooking and baking, involve heat treatment, and in some cases alkaline conditions are also applied. Racemisation induced by such intervention gives rise to D-amino acids in proteins. Fuse et al. [52], Jenkins et al. [63], Liardon and Hurrel [67] and Masters and Friedman [73] demonstrated that considerable quantities of D-amino acids are to be found in some commercially available foodstuffs which have been subjected to the effect of technological processes. Lysinoalanine is present almost universally in food substances [70]. In addition, synthetically manufactured products such as aspartame dipeptide are particularly susceptible to racemisation [9]. Investigations performed by the authors indicate that 10 to 40% of the amino acid content of feather meal produced by means of alkaline hydrolysis undergoes racemisation, the degree of this being dependent on the production parameters [33].

3 Natural basic materials

Milk, meat and the various types of grain, which do not contain substantial quantities of D-amino acids, are often exposed, in the course of preparation for consumption, to conditions which may give rise to racemisation. Milk and dairy products serve as examples of how the composition of natural substances can change [72]. Although untreated (i.e., raw) milk is available in some food stores, most dairy products are first pasteurised (involving heating for 30 minutes at 68–72 °C) or ultrapasteurised (involving heating for 15 seconds at 135–145 °C). They are subsequently subjected to homogenisation and condensation, until a particular product such as milk for commercial consumption, yoghurt or cheese derived from the various milk protein fractions is finally obtained. The latter two dairy products are fermented by means of bacteria, this process also constituting a source of D-amino acids. (The concentration of D-amino acids is hereafter given in accordance with the following: % D-amino acid = $(D/D{+}L)\times 100$).

Payan et al. [80] studied changes occurring by the effect of milk treatment by measuring D-aspartic acid concentration. They found that untreated raw milk contains the lowest levels of D-aspartic acid (1.48%), but that quantities rise with increasing extent of treatment involved in processing (acidophilus milk: 2.05%, fat-free milk powder: 2.15%, kefir: 2.44%, evaporated milk: 2.49%, yoghurt: 3.12%, milk-based infant formula: 4.95%). Thus, products whose production requires heating may have a D-aspartic acid content as high as 5%. The highest ratio of D-aspartic acid is found in infant formulae, which are subjected to technological intervention procedures such as powder drying or heat sterilisation.

On studying the effect of heat treatment and bacteria on the free and protein-bound D-amino acid content of milk Gandolfi et al. [53] established that the free D-amino acid content of raw milk does not increase by the effect of pasteurisation, ultrapasteurisation or sterilisation. The above authors determined free D-alanine content of between 3 and 8%, D-aspartic acid content of between 2 and 5%, and D-glutamic acid content of between 2 and 4% for the milk samples examined. They also ascertained that the free D-amino acid content of raw milk samples increases substantially during storage at 4 °C; therefore, they recommend that D-alanine content be used for the purposes of monitoring bacterial contamination of milk. The D-amino acid content detected in milk protein is attributed to racemisation occurring during protein hydrolysis.

The past few years have seen the development of a number of methods for the determination of the proportion ascribable to microbial origin of the nitrogen-containing matter. Some of these methods were published by Csapó and Henics [21] and Csapó et al. [23, 26]. Recently, in examining the D-amino acid content of foodstuffs, particularly that of milk and dairy products, the attention of the authors has been turned to whether D-glutamic acid (D-Glu) and D-aspartic acid (D-Asp) can be detected in quantities similar to those determined for D-Ala, principally in products associated with bacterial activity. Based on their investigation the authors recommend that the above two D-amino acids be included alongside DAPA as markers for bacterial protein.

Palla et al. [77] determined the free D-aspartic acid content of milk powder at 4–5 and its D-alanine content at 8–12%. With respect to yoghurt, free D-alanine content was measured by the above authors at 64–68%, free D-aspartic acid content at 20–32%, and free D-glutamic acid content at 53–56%. For mature cheese content values for the same D-amino acids of 20–45%, 8–35% and 5–22% respectively were obtained. The free D-phenylalanine content of mature cheese was found to be between 2 and 13%, D-leucine also being detected in minimal quantities in mature cheese. The D-aspartic acid content of roast coffee proved to be 23–38%, its D-glutamic acid content 32–41%, and its D-phenylalanine content 9–12%. On the basis of the measurements recorded the above authors draw attention to the fact that it is not foodstuffs subjected to lengthy heat treatment which contain substantial quantities of D-amino acids, but rather those which have undergone a process of bacterial fermentation.

On examination of free D-amino acids in milk, fermented milk, fresh cheese and curd cheese Bruckner and Hausch [11] established that considerable quantities of D-amino acids occur both in raw milk and in fermented dairy products manufactured from it. The empirical data obtained by the above authors are presented in *Table 1.*

Table 1: Free amino acid content of milk and fermented milk products[1] (mg/100 g)

Amino acid	**Raw/Pasteurised milk**	**Kefir**	**Yoghurt**	**Curdled milk**	**Fresh cheese**	**Harz cheese**
D-Ala	0.003 – 0.012	0.31	1.35	0.46	1.07	2.48
D-Asx[3]	0.017 – 0.038	0.35	0.31	0.25	0.38	0.37
D-Glx[3]	0.070 – 0.190	0.50	1.09	0.58	0.75	2.13
D-Val	-	0.03	-	0.04	0.09	-
D-Leu	-	0.11	-	0.15	0.16	-
D-Lys	-	0.09	-	0.13	0.44	1.49
D-allo-Ile[2]	-	0.07	-	0.02	-	0.27
D-Ser	-	0.02	-	-	-	-
D-Pro	-	-	-	-	-	2.18
Free amino acids (mg/100 g)	3.29 - 10.3	26.2	28.4	36.8	39.2	159
Free D-amino acids (mg/100 g)	0.09 - 0.24	1.48	2.75	1.63	2.89	8.92

[1] % D=(D/D+L)×100

[2] % D-allo-Ile=D-allo-Ile/(D-allo-Ile+L-allo-Ile+D-Ile+L-Ile)

[3] Asx=Asp+Asn, calculated as aspartic acid; Glx=Glu+Gln, calculated as glutamic acid

It may be ascertained from the data given in the above table that yoghurt and cheese contain substantial quantities of D-alanine (1.35–2.48 mg/100 g), D-aspartic acid (0.31–0.37 mg/100 g) and D-glutamic acid (1.09–2.13 mg/100 g), while the quantities of D-lysine (1.49 mg/100 g) and D-proline (2.18 mg/100 g) present may also be considerable. In addition, trace quantities of D-valine, D-leucine, D-allo-isoleucine and D-serine were also detected in fermented dairy products by the above authors. On analysis of the origin of D-amino acids they established that the occurrence of these can, for the most part, be traced back to microbiological intervention, or to microbial contamination in the case of raw or pasteurised samples, or possibly to the unintentional addition to the composite milk of milk derived from cows with subclinical mastitis.

Csapó et al. [31] used ion exchange column chromatography to determine the total free amino acid content of mature Ardrahan Irish and Camembert cheese (in both cases samples being taken from the half centimetre thick external crust and from the inner part), and that of Danish blue, Emmental, Gouda, Mozzarella, Parmesan and Cheddar cheeses produced by various procedures; the free D-aspartic acid (D-Asp), D-glutamic acid (D-Glu) and D-alanine (D-Ala) content of the same cheeses was determined by means of high performance liquid chromatography. Of the free D-amino acids *(Table 2)* average concentrations of 58 μmol/100 g (30.3%) for D-Asp, 117 μmol/100 g (15.8%) for D-Glu and 276 μmol/100 g (37.2%) for D-Ala were determined in the various cheeses. The figures in brackets give the proportions of these D-amino acids in terms of percentage of total free amino acids. The quantities of the respective D-amino acids showed substantial differences between the individual cheeses: the percentage composition of D-amino acids represented by D-Asp varied between 13.9 and 46.3%, by D-Glu, between 12.9 and 26.6%, and by D-Ala, between 16.1 and 48.1%. Apart from the above three D-amino acids only trace quantities of the other D-amino acids were detected in the cheeses, with values on the limit of detectability. Higher D-amino acid content was determined in those Cheddar cheeses for which lactobacilli are also used in the manufacturing process.

In seeking to ascertain what gives rise to the D-amino acid content of commercially available milk Csapó et al. [27, 28, 29] determined the free D-amino acid content of the first jets of milk produced by healthy cows, that of the composite milk excluding the first milk jets, and that of milk samples corresponding to the various grades applied in the mastitis test *(Tables 3 and 4)*. It was ascertained that both the first jets of milk produced and milk from diseased udders contain substantial quantities of D-Asp, D-Glu, D-Ala and D-allo-Ile. In addition to the above amino acids, D-Ser, D-Pro, D-Val, D-Leu and D-Lys were also detected in the milk derived from mastitis-affected udders. The quantities and ratios of D-amino acids determined in milk derived from diseased udders were observed to increase in accordance with the grades designated in the mastitis test. The study verifies that the D-amino acid content of commercially available milk might be attributable to the first jets of milk produced or to milk originating from cows with subclinical mastitis.

Table 2: Principal[1] D-amino acid content of the various cheeses (μmol/100 g)

Cheese	D-amino acid					
	D-Asp	D-Asp %	D-Glu	D-Glu %	D-Ala	D-Ala %
Mature Ardrahan Irish: external crust	74	27.2	173	13.1	433	27.1
Mature Ardrahan Irish: inner part	70	23.2	235	14.4	393	28.2
Camembert: external crust	42	13.9	122	12.9	334	18.0
Camembert: inner part	36	14.0	176	14.8	259	16.1
Danish blue	89	31.1	149	20.2	212	42.4
Emmental	42	26.8	195	26.6	405	45.6
Gouda	61	28.5	244	22.7	462	38.4
Mozzarella	5.2	28.9	9.6	24.0	52	33.3
Parmesan	57	20.8	72	10.6	752	37.3
Commercial Cheddar	74	46.3	45	14.1	96	45.3
Cheddar: expt. 1	74	43.5	62	12.5	153	46.3
Cheddar: expt. 2	89	41.4	65	12.4	165	48.1
Cheddar: expt. 3	59	45.4	53	12.5	161	47.9
Cheddar: expt. 4	41	33.4	42	10.9	125	46.1

[1] % D=(D/D+L)×100 [2] All the D-amino acids were analysed, but apart from a few exceptions, the other D-amino acids proved to be present in very low concentrations, determination of these being unreliable.

Table 3: Free D-amino acid content of milk from healthy and mastitis-affected cows[1]

D-amino acid	**Groups examined, on the basis of the mastitis test**				
(mg/100 cm^3)	**neg. (5)**	+(5)	++(5)	+++(5)	++++(5)
D-Asp	0.021	0.17	0.23	0.32	0.32
D-Ser	-	-	0.02	0.04	0.04
D-Glu	0.053	0.74	0.99	1.48	1.53
D-Pro	-	-	0.04	0.09	0.10
D-Ala	0.043	-	1.13	2.32	2.41
D-Val	-	0.48	0.09	0.09	0.12
D-allo-Ile	-	0.08	0.10	0.12	0.15
D-Leu	-	0.08	0.12	0.17	0.17
D-Lys	-	0.06	0.27	0.36	0.37
Total free		0.11			
D-amino acids	0.117	1.72	2.99	4.99	5.21

[1] Mean for the milk of 5 cows.

Table 4: Free D-amino acid content of milk from healthy and mastitis-affected cows in terms of percentage of total free amino acids[1]

D-amino acid	**Groups examined, on the basis of the mastitis test**				
%	**neg. (5)**	+(5)	++(5)	+++(5)	++++(5)
D-Asp	17.5	19.0	20.1	23.0	22.0
D-Ser	-	-	6.5	8.2	7.7
D-Glu	6.2	29.2	31.0	32.0	32.5
D-Pro	-	-	9.5	11.5	11.2
D-Ala	12.6	22.0	33.0	47.5	48.9
D-Val	-	11.3	12.2	11.1	13.0
D-allo-Ile	-	27.6	28.6	29.6	34.1
D-Leu	-	28.6	31.6	24.6	25.8
D-Lys	-	22.4	32.5	32.0	32.5

[1] Mean for the milk of 5 cows.

4 Foodstuffs subjected to various technological procedures

Modern food industry technology applies a diverse range of procedures for the purpose of modifying the characteristics of proteins in order to improve flavour, consistency and non-perishability. Treatment with heat or alkalis is used preferentially for the manufacture of products possessing particular characteristics, form and function. For example, soya protein is treated with alkalis and heat for the purposes of obtaining, through extrusion, a product of fibrous structure suitable for consumption as a meat substitute. Alkali treatment is also applied in order to obtain flaked maize and tortillas from maize protein. *Table 5* gives the D-amino acid content of various alkali-treated foodstuffs in comparison with untreated controls.

Table 5: D-amino acid content of various foodstuffs (%)[1]

Treated product	**Amino acids**					
(Untreated control; ref.)	**Asp**	**Ala**	**Phe**	**Leu**	**Val**	**Met**
Toast[2]	10.5	2.8	2.4	2.7	1.1	1.7
Bread, [13]	5.6	2.4	2.3	3.2	0.9	2.3
Extruded soya bean meal	7.6	2.2	2.4	2.7	0.8	-
Soya bean meal, [13]	4.4	2.5	2.8	1.4	1.0	-
Soya protein[3]	27.7	9.9	19.7	3.1	1.0	18.2
Untreated, [49]	0.5	0.2	0.5	0.2	0.03	0.3
Zein[4]	40.2	17.6	31.3	5.0	2.9	19.5
Not heat treated, [63]	3.4	0.7	2.2	0.7	0.4	0.9
Hamburger[5]	5.5	2.8	2.7	3.2	1.5	2.9
Raw meat, [13]	6.2	3.2	2.8	3.1	1.6	2.4
Chicken muscle[6]	22.4	0.5	0.4	0.1	-	-
Raw chicken, [67]	2.9	-	-	-	-	-
Bacon, 180 °C[7]	10.7	2.4	3.1	3.1	1.6	-
Not heat treated, [52]	2.4	-	1.8	3.3	0.7	-
Casein, 230 °C[7]	31.0	12.0	-	7.0	4.4	-
Not heat treated, [58, 59]	3.1	1.5	-	-	-	-

[1] D-amino acids % = (D/D+L)×100

[2] The white bread was heated for 1 minute 45 seconds, only its surface having been analysed.

[3] 3 hours, 65 °C, 0.1 N NaOH.

[4] 4 hours, 85 °C, 0.2 N NaOH.

[5] The hamburger was fried on both sides for 4 minutes. The temperature of the pan was 250 °C. Only the surface was analysed.

[6] Heating at 121 °C for 4 hours.

[7] Heated for 20 minutes.

Heat treatment or combined heat and alkali treatment in every case gave rise to D-amino acids in measurable quantities. The highest D-aspartic acid content (31%) was determined in the casein heated to 230 °C for 20 minutes. Comparison of the racemised amino acids reveals that the highest degree of racemisation occurred in aspartic acid. Certain amino acids not included in the table, such as serine and cysteine, probably racemise more rapidly than aspartic acid. It may be stated in general that the essential amino acids do not racemise rapidly unless exposed to high temperature. However, it may also be the case with the essential amino acids that a combination of high temperature and alkali treatment is accompanied by a substantial degree of racemisation.

Authors of other studies have also reported on the high D-amino acid content of treated foods. On examination of the D-Asp content of a number of commercially available foods Masters and Friedman [73] established very high ratios of this D-amino acid in textured soya protein (9%), bacon (13%) and non-milk fat (17%). Finley [43] determined substantial quantities of D-Asp in savoury crackers made from wheat flour (9.5%), wheat cake (11.9%), Mexican pancake (11.6%) and corn cake (15.4%). The data for the fried hamburger indicate that racemisation occurs to only an insignificant degree in that particular food in the course of the frying process. The high ratios of D-amino acids detected in the toasted white bread, the cooked bacon and the chicken meat demonstrate that in some foods substantial degrees of racemisation can arise in the process of cooking, baking or frying.

On examining the effect on food proteins of microwave treatment Lubec et al. [69] fairly recently ascertained that by the effect of microwave treatment of 10 minutes duration the cis-3 and cis-4 hydroxyproline content of all three infant foods examined increased, and only microwave-treated formulae contained D-proline in detectable quantities. The concentration of the cis isomer was found to be 1–2 mg per litre. The above authors point out that if the cis isomer is incorporated into a protein instead of the trans isomer, structural, functional and immunological changes can result.

5 Manufactured foods and artificially produced peptides

This category includes every type of food subjected to substantial levels of technological treatment, or synthetically produced (e.g. aspartame). In some liquid foods the protein is combined with carbohydrate, in the process of which the protein may undergo considerable change. Antibiotic peptides may contain substantial quantities of D-amino acids [8, 87], as may some drugs used in chemotherapy [15]; the residues of these may subsequently result in significant D-amino acid content of foodstuffs produced. On evaluation of data in the literature it may be ascertained that synthetic products contain considerably higher levels of amino acids than natural basic materials, the former being the main sources of the D-amino acid content of foods. Liquid food formulae based on soya protein, actually purchased from health food stores, has been found to contain 13% D-aspartic acid, this being a substantially higher level than that determined in soya-based infant formulae. Finley [43] reported that food products formulated to induce weight loss which had been subjected to alkali treatment proved to contain 50% D-serine, 37% D-aspartic acid and 26% D-phenylalanine; these high quantities of D-amino acids might pose a risk if consumed as the sole source of dietary protein. Such extreme cases are relatively rare, but it should nevertheless be noted that in foodstuffs subjected to lengthy alkali or heat treatment processes a high proportion of the amino acids present may undergo racemisation.

On studying racemisation in aspartame sweetener Boehm and Bada [9] reported that both aspartic acid and glutamic acid racemised rapidly at neutral pH at 100 °C. Racemisation occurs when the sweetener is transformed into a cyclical dipeptide, these being highly susceptible to racemisation. The importance of awareness of this lies in the fact that if sweetener is added to food before, for example, cooking, a high degree of racemisation may result.

6 D-amino acid metabolism

The points outlined above provide clear evidence that D-amino acids can occur in substantial quantities in foods. What is the fate of these unnatural stereoisomers? Since the publication of the pioneering work of Krebs [65] it has been generally known that mammals possess specific enzymes for the purposes of a D-amino acid metabolism. D-amino acids metabolise primarily in the series of reactions of D-amino acid oxidase, in the formation of α-ketoacids [6, 7, 14, 66, 76]. These α-ketoacids may subsequently undergo stereospecific transamination, which results in the L-enantiomer of the original amino acid, which in turn enters the usual metabolic process, or alternatively is broken down directly in another reaction, e.g. by means of oxidative decarboxylation. The transformation of D-amino acids into α-ketoacids takes place principally in the kidneys; thus, dietary D-amino acids first have to diffuse across the membranes to enable them to metabolise via this route. However, the transport mechanisms are stereo-selective and discriminative with respect to D-amino acids [54, 86].

The respective amino acids are oxidised to different degrees by D-amino acid oxidase. The D-enantiomer of aspartic acid, the amino acid which according to investigations is the most susceptible to racemisation, is a highly unsuitable substrate for D-amino acid oxidase. Despite this, Dixon and Kenworthy [38] reported that there occurs in mammals D-amino acid oxidase specific to D-aspartic acid; however, there is none for the other amino acids. Essential amino acids such as lysine and threonine racemise more rapidly than alanine, and are also very unsuitable substrates for D-amino acid oxidase. On the other hand, proline, which does not racemise to a significant degree in the preparation of food products, is the best possible substrate for this enzyme [67]. Thus, it appears that there is no relation between susceptibility to racemisation and readiness to react with D-amino acid oxidase. It can therefore be asserted that the D-amino acid oxidase system in mammals is not sufficiently developed to be capable of meeting the challenge posed by racemised amino acids of food origin. Krebs [65, 66] was still in doubt as to the biological function of D-amino acid oxidase; however, the view now generally held is that D-amino acid oxidase detoxifies D-amino acids which happen to be present or which arrive via bacterial protein [5]. This is corroborated by the fact that rats reared in a germ-free environment show far lower levels of D-amino acid oxidase activity than those reared in a normal environment. Despite this, D-glutamic acid constituting a component part of peptidoglycan occurring in bacterial cell walls is the least suitable substrate for D-amino acid oxidase, undergoing only very slow oxidation with D-aspartic acid oxidase [38]. Although D-amino acid oxidase enzymes enable mammals to metabolise D-amino acids, this route is inefficient and obviously overburdened, since when racemic amino acids enter the organism a high proportion of D-amino acids is excreted in the urine [7, 76]. Free D-amino acids can also be transformed by means of racemases into racemic mixtures or the corresponding L-amino acids. However, since racemases occur primarily in bacteria, this is not a route for the metabolism of D-amino acids in mammals. It is now known that amino acid transaminases also occur only in bacteria.

The main sources of D-amino acids in human foodstuffs are industrially produced proteins. Before D-amino acids present in these proteins are able to metabolise in the series of reactions of D-amino acid oxidase they must be liberated by means of the metabolic enzymes. The first stage of the digestion of dietary proteins results in free amino acids and peptides consisting of small numbers of amino acids [5, 55]; the peptides are subsequently further hydrolysed by peptidases [81, 85]. It is quite evident that peptides containing D-amino acids resist enzyme hydrolysis in the digestive process. Studies involving synthetic peptides indicate that D-aspartic acid [75] and D-methionine [78] are not liberated from the peptide bonds in the course of enzyme hydrolysis, even if all the other adjacent amino acids are L-enantiomers. A number of published works have reported that amino acids racemised to a large extent by the effect of heat and alkali treatment resist proteolytic hydrolysis. On studying the relation between phenylalanine racemisation and protein digestibility Chung et al. [18] established that as the degree of racemisation increases digestibility decreases rapidly. As phenylalanine racemises more slowly than aspartic acid, serine or cysteine, it is evident that proteins containing significant quantities of racemised amino acids are only partially broken down in the course of proteolysis.

The products of the proteolytic hydrolysis of proteins contain racemised amino acids and peptides of low molecular weight containing D-amino acids. Di- and tripeptides diffuse across the membranes, while peptides consisting of larger numbers of amino acids are simply excreted in the faeces. Di- and tripeptides containing D-amino acids are not particularly suitable substrates for D-amino acid oxidase [14, 66]. Under in vitro conditions at pH 7 dipeptides rapidly undergo cyclic transformation into cyclic peptides (diketopiperazine) [89]. Tripeptides are hydrolysed rapidly by non-enzyme processes in vitro in the course of internal ammonolysis, which results in cyclic dipeptides and a free C-terminal amino acid [90]. Cyclic dipeptides are highly susceptible to in vivo racemisation [57, 89]. Thus, if the hydrolytic process were also to occur in vivo, this would lead to the formation of other D-amino acids. However, studies of the metabolism of D-amino acids have not, as yet, devoted attention to the presence of diketopiperazine.

7 D-amino acid digestion

There is not yet sufficient knowledge of the effect on the human organism of long-term consumption of proteins containing racemised amino acids. Masters and Friedman [73] pointed out that no authors have performed specific investigations on the effect exerted by racemised amino acids on the human organism, i.e., how racemisation affects digestibility and the availability of amino acids.

Harmful effects of D-amino acids: The utilisation of D-amino acids bound in proteins depends on whether such D-amino acids are liberated from the L–D, D–L and D–D bonds, and whether the liberated D-amino acids can be transformed efficiently into L-amino acids. At the beginning of the century Dakin and Dudley [35] were the first to observe that in dogs a large proportion of alkali-treated casein was excreted, undigested, in the faeces. Other researchers subsequently determined the digestibility of alkali-treated and non-treated protein. In each case the treated samples exhibited reduced digestibility, attributed primarily to racemisation and/or to the formation of lysinoalanine. On studying racemisation of the amino acids in alkali-treated proteins Hayashi and Kameda [61] reported that even a minor degree of racemisation provokes a major decrease in the digestion of such proteins. The above authors ascribed this reduced digestibility to the fact that racemised amino acids are not substrates for proteases, and also exert an effect on the capacity for liberation of adjacent non-racemised amino acids. Thus, the racemisation of some amino acids may also give rise to substantial loss with respect to the neighbouring essential amino acids, thus reducing the proteolytic digestibility of the protein.

Friedman et al. [50] examined the effect of treatment temperature, duration and pH on the digestibility of alkali-treated casein, trypsin and chymotrypsin. The above authors observed that a decrease in the digestibility of aspartic acid and phenylalanine was accompanied by an increase in lysinoalanine crosslinking and racemisation. Bunjapamai et al. [13] were the first to succeed in distinguishing the effects on in vitro digestibility of racemisation and crosslinking. The main conclusion of this research was that reduced digestibility was caused primarily by racemisation. According to Schwass et al. [86] one D-amino acid is sufficient to render a peptide unfit for transport. These authors stated that racemisation is the one process which alone leads to a reduction in in vitro digestibility and in the in vivo assimilation of enzyme-digested protein.

One highly important issue is whether D-amino acids present in foodstuffs are toxic. It may be established from the outset that the various D- and L-amino acids are equally toxic, as verified by their LD_{50} values [56]. D-proline may be an exception to this, higher lethality in chickens having been ascertained for this enantiomer than for L-proline [17]. It has already been stated that D-proline is the best possible substrate for D-amino acid oxidase. Masters and Friedman [73] reported that some D-amino acids exert their toxic effect over a protracted period. The investigations performed by the above authors indicated that D-serine, lysinoalanine and the various alkali-treated proteins present in foodstuffs provoke pathological changes in the kidneys of rats. Free lysinoalanine is much more highly nephrotoxic than that bound in peptide bonds; it follows from this that the nephrotoxic effect of bound lysinoalanine within alkali-treated proteins will be substantially weaker [47]. According to Degroot et al. [37] rats are particularly sensitive to the nephrotoxic effect of alkali-treated proteins and lysinoalanine, and it is evident from the studies performed by these authors that different species of animal show different degrees of sensitivity in this respect.

Lysinoalanine is an in vitro inhibitor to carboxypeptidases and aminopeptidases, as is D-alanine occurring in alkali-treated proteins [51, 62]. The inhibitory effect of lysinoalanine is manifest in its forming of a complex with the enzyme metal ion involved in the enzyme reaction [62]. The question of whether lysinoalanine and D-amino acids of dietary origin are inhibitors to metabolic enzymes has not yet been investigated; nor are there available at present data on the effect on inhibition of treatment of longer duration.

Beneficial effects of D-amino acids: Reduced digestibility of dietary proteins due to D-amino acids may in certain cases prove advantageous with respect to nutrition, on condition that the substances remaining after proteolytic digestion are not toxic. Racemised proteins may be consumed for a period of a few days in diets designed for weight loss, and in consequence of the very low digestibility of these proteins considerable loss of weight may be anticipated within a short period. It has been demonstrated [16] that D-phenylalanine and D-leucine exert an analgesic effect, due to which they are applied in cases of persistent pain [12]. This analgesic effect is based on the inhibition of carboxypeptidase A and similar enzymes playing a role in the breakdown of opioid pentapeptide in the brain and spinal cord [12]. Friedman et al. [51] reported that the lysinoalanine and D-amino acid content of alkali-treated food proteins also inhibits carboxypeptidase A. The above research findings allow the conclusion to be drawn that the presence of racemic amino acids in food proteins may be beneficial in the relief of pain.

It has been known for some considerable time that D-amino acid sequences are present in most antibiotic peptides. It is therefore conceivable that in the proteolytic breakdown of racemised dietary proteins peptides with antibiotic properties may be formed.

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