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THE D-AMINO ACID CONTENT OF FOODSTUFFS SUBJECTED TO VARIOUS TECHNOLOGICAL PROCEDURES

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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 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.

Keywords: D-amino acids, racemisation, heat treatment, bacterial activity

INTRODUCTION

Foods contain large quantities of non-natural substances of external origin, which influence their digestibility to a considerable degree. 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 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; in some marine worms and invertebrates the cellular fluid contains D-amino acids as a main component; in certain marine shellfish quantities of D-amino acids can exceed 1%; and higher plants also contain D-amino acids. Metabolically stable proteins in mammals of longer life span contain major quantities of D-aspartic acid derived from racemisation: 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%. It was 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. On examination of the racemisation of free amino acids it was established that

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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.
It was 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; it were gave these respective values for soya protein at 75°C in 0.1 M normal 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, 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; the lysinoalanine content of the resultant foodstuff also bears toxic effect.
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 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 half-life of racemisation for the essential amino acids has only recently been subjected to investigation. At pH between 7 and 8 it was 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 it was determined the half-life of racemisation for lysine at 40 days, while others 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. It was 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. It is now evident that the reduction in digestibility is related to the formation of lysinoalanine and the racemisation arising.

D-AMINO ACIDS OF DIETARY ORIGIN

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. It was 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. In addition, synthetically manufactured products such as aspartame dipeptide are particularly susceptible to racemisation. 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.

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. 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)100).
The free D-aspartic acid content of milk powder at 4-5% and its D-alanine content at 8-12% was determined. With respect to yoghurt, free D-alanine content was measured 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 attention is draw 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 it was established that considerable quantities of D-amino acids occur both in raw milk and in fermented dairy products manufactured from it. It may be ascertained that yoghurt and cheese contain substantial quantities of D-alanine (1.35-2.48 mg/100g), D-aspartic acid (0.31-0.37 mg/100g) and D-glutamic acid (1.09-2.13 mg/100g), while the quantities of D-lysine (1.49 mg/100g) and D-proline (2.18 mg/100g) 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.

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.

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 ^{o}C for 20 minutes. Comparison of the racemised amino acids reveals that the highest degree of racemisation occurred in aspartic acid. Certain amino acids, 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 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 very high ratios of this D-amino acid was established in textured soya protein (9%), bacon (13%) and non-milk fat (17%). Substantial quantities of D-Asp was determined 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 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.

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, as may some drugs used in chemotherapy; 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. It was 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 it was 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.

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