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Kurzmitteilungen

Determination of lysine damage and calculation of lysine bio-availability in several processed foods

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Summary: By analyzing lysine and furosine the amount of inactivated lysine in several food systems was determined and the values for available lysine and total lysine were calculated. Considerable heat damage was found in heated cereal products, and in heated milk products, including several formula for children and hospitalized patients. Some products contained more inactivated lysine than available lysine. This may have consequences for the nutrition in low protein consuming populations and leads to errors in predicting the protein quality, e.g., by the recently proposed "Protein Digestibility Corrected Amino Acid Score".

Zusammenfassung: Mit Hilfe der Furosinmethode wurde der Gehalt an inaktiviertem Lysin in verschiedenen Lebensmitteln bestimmt. Der Gehalt an verfügbarem Lysin und Gesamtlysin wurde auf der Basis dieser Werte berechnet. Es zeigte sich, daß in manchen Lebensmitteln eine erhebliche Lysinschädigung zu verzeichnen war, und zwar besonders in erhitzten Backwaren und Cerealien, in Milchprodukten einschließlich mancher Säuglingsnahrung. Dies kann für Bevölkerungsgruppen, die sich proteinarm ernähren, Konsequenzen haben und führt zu einer Fehlbewertung der Proteinqualität auf der Basis sogenannter Amino Acid Scores (z. B. des neuen „Protein Digestibility Corrected Amino Acid Score").

Key words: furosine, fructoselysine, lysine-inactivation; bio-availability of lysine; food processing; Protein Digestibility Corrected Amino Acid Score

Schlüsselwörter: Furosin, Fruktoselysin, Lysin-Inaktivierung, Bioverfügbarkeit von Lysin, Lebensmittelverarbeitung, Protein Digestibility Corrected Amino Acid Score

Introduction

Particularly in processed foods the amino acids determined by amino acid analysis after acid hydrolysis are only partially utilizable by the organism. This is the case mainly in mildly treated proteins where only a small amount of amino acids is destroyed, while the inactivation of several amino acids can be high. The principal reasons for the reduction of the protein quality may be heat treatment, storage, and oxidation. The most

common mechanism in this connection is the so-called Maillard reaction of free amino groups with reducing sugars, which leads to unavailable amino acid sugar reaction products, and in advanced and final stages to a destruction of both the sugar and the amino acid moiety. In heated or stored proteins mainly the free ε-amino group of lysine reacts with glucose, lactose, or maltose, thus forming fructoselysine, lactuloselysine, or maltuloselysine. In this way the lysine moiety becomes unavailable for the higher organisms (inactivated lysine).

A useful indicator for the above-mentioned relative unstable reaction products of lysine (fructoselysine etc.) is furosine, which is formed during acid hydrolysis of the respective proteins. This compound was first detected 25 years ago in our laboratory in Munich (1, 4), and identified by Heyns et al. (7) and Finot et al. (8). Furosine determinations in the meanwhile have been applied in food science and nutrition, in clinical research,

Tab. 1. Contents in lysine, calculated values for inactivated, available lysine, and the percentage of lysine losses in several foods (all values in % of the protein = in g/16 g Nitrogen).

	n	Initial lysine content*	Total lysine**	Inactivated lysine***	Available lysine**	Lysine losses in %	
						De-stroyed[1]	Inacti-vation[1]
Several breads	5	3.2	2.4 ± 1.4	0.3 ± 0.2	2.1	20 %	10 %
Breakfast cereals, processed	7	3.2	2.9 ± 1.1	0.6 ± 0.8	2.3	9 %	19 %
Pasta and other items	20	2.6	2.6 ± 0.6	0.2 ± 0.2	2.4	0 %	8 %
Biscuits and similar items	10	2.6	1.4 ± 0.4	0.5 ± 0.3	0.9	46 %	19 %
Zwieback (hard bread)	2	2.6	1.4–1.6	0.9 ± 1.1	0.5	42 %	38 %
UHT**** milks	27	8.8	8.8 ± 0.4	0.2 ± 0.2	8.6	0 %	2 %
Condensed milks	11	8.8	8.3 ± 0.3	1.2 ± 0.3	7.1	6 %	14 %
Skim milks, spray dried	33	8.8	8.8 ± 0.5	0.6 ± 0.5	8.2	0 %	7 %
Skim milks, roller dried	42	8.8	8.5 ± 0.5	0.9 ± 0.6	7.6	3 %	10 %
Whey, spray dried	33	8.8	8.6 ± 0.4	0.6 ± 0.5	8.0	2 %	7 %
Infant milk formula, dried	44	8.8	8.4 ± 0.6	0.7 ± 0.6	7.7	5 %	8 %
Infant milk formula, sterilized	23	8.8	8.0 ± 0.4	0.9 ± 0.3	7.1	9 %	10 %
Infant mash, processed	5	?	4.3 ± 2.9	0.9 ± 0.3	3.4	?	21 %[2]
Formula diets for tube-feeding, sterilized	19	8.8	8.4 ± 1.1	0.3 ± 0.3	8.1	5 %	3 %
Chocolate and similar items	15	8.0	7.0 ± 0.8	2.0 ± 0.7	5.0	13 %	25 %
Toffee	3	?	3.1–6.2	2.2–4.1	1.3	?	71 %[2]

* suggested lysine content in the raw material

** total lysine = analyzed lysine + 50 % of the inactivated lysine (see text); available lysine = analyzed lysine – 50 % of the inactivated lysine (see text)

*** calculated from the furosine values (see text)

**** UHT = Ultra Heat Treated

[1] % destroyed lysine = (initial lysine content – total lysine) in % of initial lysine content
% lysine inactivation = inactivated lysine in % of initial lysine

[2] inactivated lysine in % of total lysine

and in medical biochemistry. From the furosine values the contents of the lysine-sugar derivatives like fructoselysine can be calculated.

Methods

The food samples were hydrolyzed with 7.8 M hydrochloric acid for 20 h in order to give a high recovery of furosine. Furosine was analyzed on amino acid analyzers using the ion-exchange chromatography technique by a shortened one-column system, increasing in this way the sensitivity and specificity of the detection. Lysine was determined in the same way from diluted hydrolysates, respectively. The details of the analytical procedure are described elsewhere [4]. Since a pure standard of furosine is not available, furosine was calculated from the peak area using the response values for arginine as comparison because arginine is eluted just before furosine and shows a comparable elution behaviour. For the calculation of inactivated lysine from furosine the different molecular weights of furosine and lysine, as well as conversion factors of 2.5 or 3.3 were used. These conversion factors are applied because the recovery of furosine from the fructoselysine moiety is 40 % or 30 % depending on the analytical procedure like the temperature of elution etc. The contents of total and available lysine were calculated from the analyzed lysine, considering that about 50 % of the inactivated lysine moiety are regenerated during the hydrolysis. In food groups of consistent origin also the estimated initial lysine content of the raw material was used for the suggestion of the total destruction of lysine. This appears to be fairly precise in milk products but was obviously only a rough estimate in products like cereal mixtures.

Results and discussion

As Table 1 shows, inactivation represents the main lysine damage in many food systems. Only in visibly browned foods is lysine destruction resulting from advanced Maillard reaction processes predominant. Prior to the use of furosine determinations, lysine inactivation was often only roughly estimated because the routinely used amino acid analysis after acid hydrolysis recovers the main part of the unavailable lysine, and therefore leads to errors in predicting the available lysine content. Now it is clear that some foods like zwieback (hard breads) and other extremely heated items contain more inactivated lysine than available lysine. Some other items like processed breakfest cereals and several milk products contain considerable amounts of inactivated lysine. The losses in available lysine would also have consequences for the recently proposed "Protein Digestibility Corrected Amino Acid Score" (6), giving rise in this way to incorrect opinions about the protein quality of the corresponding food.

This reduction in the bio-availability of lysine can be dangerous, especially in low-protein diets, e.g., for renal patients, because there is no margin of safety to meet the requirements. This will also be important if the food in question is the sole or main source of protein as in infant nutrition. In regions with a shortage in lysine-rich foods, as in developing countries, this may also be of interest. In clinical, and sometimes also in baby nutrition, severe heat-treatment for sterilization is necessary and

very common. The promotion of acceptable products in this field, e.g., by using the milder ultra-heat treatment (UHT) is of great importance.

The lysine sugar reaction products are, as known up to now, not very harmful to man (2). Balance studies with human volunteers have shown (3) that only about 2 % of the ingested fructoselysine were absorbed and excreted in the urine. About 3–6 % were found in the feces. It appears from these results that the main part of the inactivated lysine is decomposed by the bacterial flora in the gut.

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