Review

Forty years of furosine – Forty years of using Maillard reaction products as indicators of the nutritional quality of foods

Helmut F. Erbersdobler[1] and Veronika Somoza[2]

[1] Institute of Human Nutrition and Food Science, Kiel, Germany
[2] German Research Centre for Food Chemistry, Garching, Germany

The Maillard reaction products (MRPs) most widely used as markers of the nutritional quality of foods are furosine, N^{ε}-carboxymethyllysine (CML), hydroxymethylfurfural, pyrraline, pentosidine and pronyl-lysine. One of the MRPs identified first was furosine, which was quantified in foods 40 years ago as a chemical indicator of the Amadori compound N^{ε}-fructoselysine. Since then, furosine has gained broad attention by food chemists and biomedical researchers, as its formation upon heat treatment is well characterised. Moreover, it represents the Amadori products from early Maillard reactions in which amino acids react with reducing carbohydrates, resulting in a loss of their availability. This is of importance for the essential amino acid lysine, which is also the limiting amino acid in many proteins. In order to evaluate the nutritional quality of a protein, the concomitant analysis of free – and nutritionally available – lysine and the amount of lysine reacted to form the respective MRP is essential, even for mildly processed foods. The other chemical markers of heat treatment such as CML, pyrraline, pentosidine or pronyl-lysine seem to be useful markers of the advanced stages of Maillard reactions. Compared to the conditions in which furosine is formed, these compounds are generated under more severe conditions of heat treatment. However, the concentrations analysed are significantly lower than those of furosine. Therefore, the nutritional evaluation of a food protein should include not only furosine, but also other chemical markers of heat treatment such as, for example, CML, pyrraline and pentosidine.

Keywords: Amadori products / Furosine / Lysine / Maillard reaction / Other markers of the Maillard reaction

Received: August 24, 2006; revised: February 1, 2007; accepted: February 2, 2007

1 Introduction

The first attempts of protein evaluation were made almost 100 years ago by defining the biological value [1, 2], and 10 years later the term "protein efficiency ratio" (PER) [3] was introduced as a biological marker of protein quality. In addition, amino acid analyses have been used to evaluate the nutritional protein quality for about 60 years [4]. However, soon after the amino acid analysis was applied to heat-treated foods, it became clear that this procedure was not reliable. Mainly amino acids bearing a free amino group such as lysine easily react with reducing sugars during heat treatment. These Maillard reactions result in the formation of compounds in which lysine is no longer nutritionally available, but is quantified as free lysine when the standard procedure of amino acid analysis following a pre-step of acid hydrolysis is applied. Therefore, the determination of available lysine by labelling of the critical e-amino group of lysine with fluoro-dinitrobenzene was proposed [5] and applied worldwide thereafter (*e.g.* [6]).

Further research in this area revealed that available lysine is not the most sensitive chemical marker of graduated heat treatment. Nowadays, a broad variety of Maillard reaction compounds have been identified whose formation depends more closely on the type of heat treatment. Among these, furosine, N^{ε}-carboxymethyllysine (CML), hydroxymethylfurfural (HMF), pyrraline and pentosidine are the most widely used markers for the nutritional evaluation of severely and even mildly heat-treated foods such as ultra-

Correspondence: Professor Helmut Erbersdobler, Institute of Human Nutrition and Food Science, Duesternbrooker Weg 17, 24105 Kiel, Germany
E-mail: helmut@erbersdobler.de
Fax: +49-431-2390276

Abbreviations: CML, N^{ε}-carboxymethyllysine; HMF, hydroxymethylfurfural; **MRPs,** Maillard reaction products; **PER,** protein efficiency ratio

Table 1. Historical review of selected indicators identified as chemical markers of nutritional evaluation of food proteins

1909	[1]	Biological value (humans)
1919	[3]	Protein efficiency ratio (PER)
1924	[2]	Biological value of proteins (laboratory animals)
1946	[4]	Amino acid analysis – chemical score
1955	[5]	FDNB-available lysine
1959	[48]	Hydroxy-methy-furfural (HMF)
1966	[7 – 10]	Furosine
1985/86	[41 – 43][a)]	Carboxy-methyl-lysine (CML)
1980/88	[53, 54][a)]	Pyrraline (ε-pyrrole-lysine)
1989	[56, 57][a)]	Pentosidine
2002	[58]	Pronyl-lysine

a) First detection/identification and first application on foods

high temperature-treated milk or even pasteurised milk. Other markers, which have been identified quite recently, such as pronyl-lysine, are still under investigation. Table 1 gives a historical review of selected chemical markers used for the nutritional evaluation of food proteins.

2 History of several markers and their prevalence in foods

Since its detection 40 years ago [7] furosine has been used as a reliable indicator of thermal damage in foods. Figure 1 shows two of the first chromatograms obtained by ion exchange chromatography with ninhydrin post-column derivatisation of the acid hydrolysate prepared from a mildly and a severely heated dried skim milk, dated 24 March 1966. Only the lower chromatogram displayed in Fig. 1 showed a peak that indicated the presence of furosine, which was simply called "compound x" at that time, as the chemical structure had not been identified yet. The discovery of "compound x" happened accidentally, as the chromatography was run entirely manually with homemade instruments and "compound x" eluted after arginine, the last eluting amino acid. Moreover, at the time we used a rather strong 7.75-M hydrochloric acid for the hydrolysis of the food proteins, a fact that significantly enhances the formation of furosine (see Section 3).

Soon after, structure elucidation revealed that compound x was the derivative of the ε-fructoselysine moiety [8, 9]. Experiments with heated mixtures of lysine and glucose, in which first one and then the other was ^{14}C-labelled, showed that both compounds were involved in the formation of the new compound x [9].

Heyns *et al.* [10] and Finot *et al.* [11] identified the structure of compound x nearly simultaneously as ε-*N*-(2 furoylmethyl)-L-lysine and named it furosine. Almost at the same time, Freimuth and Trübsbach [12] proposed a similar but not fully correct structure.

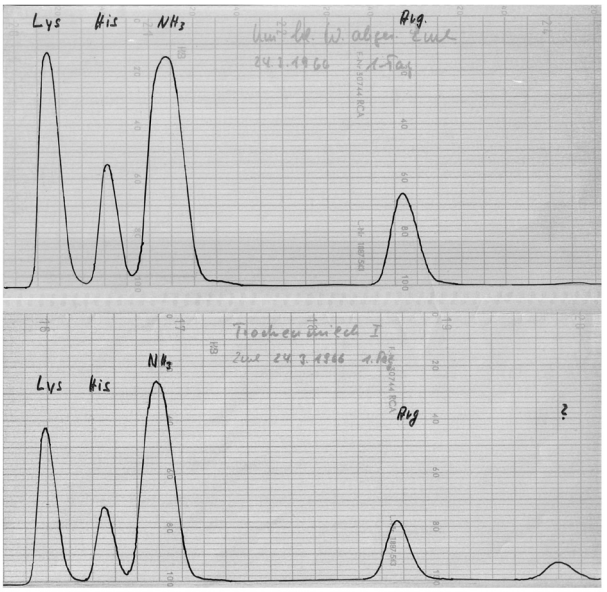

Figure 1. First chromatographs (24 March 1966) with substance x (?).

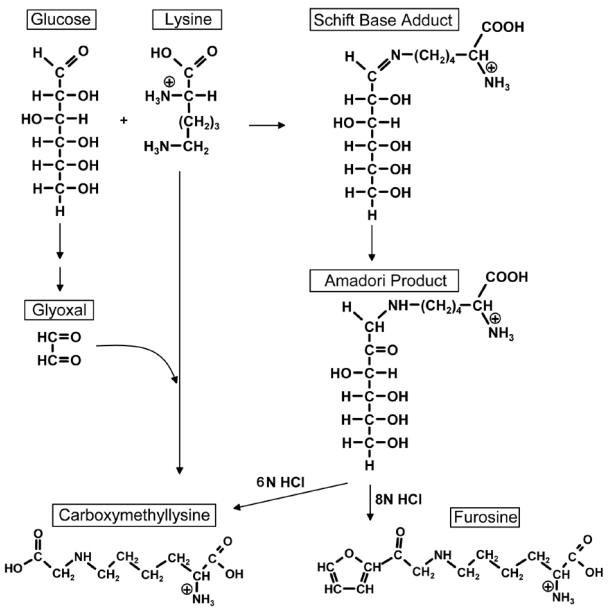

Figure 2. Initial stage of the Maillard reaction with the formation of furosine and N^{ε}-carboxymethyllysine.

The group of Finot *et al.* [13] also identified another Maillard reaction product (MRP) named pyridosine. However, it did not reach the same importance as an indicator of the nutritional value of a heat-treated protein as furosine did. Figure 2 shows the initial stage of the Maillard reaction with the formation of furosine and N^{ε}-carboxymethyllysine.

In the years thereafter, furosine stimulated research in the field of heat damage of proteins, demonstrating the importance of the Amadori products in particular. The use of fur-

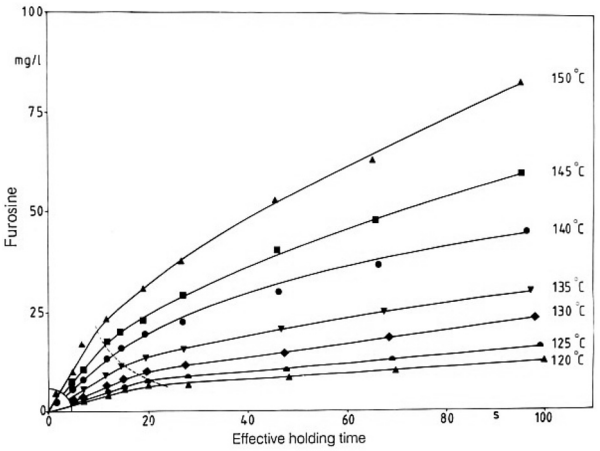

Figure 3. Furosine formation in indirectly UHT-heated milk as a function of temperature [21]. (Reprinted with permission from Th. Mann Publishers)

osine was furthered by analytical improvements starting with the proposals for HPLC techniques by Schleicher *et al.* and Chiang [14, 15] followed by Resmimi *et al.* [16] and others (*e. g.* [17]). Especially after the commercial availability of a pure and stable standard in 1992, the analyses of furosine in heat-treated foods increased substantially and are still applied worldwide. In 1992, Delgado *et al.* [18] proposed a procedure based on ion-pairing HPLC by using sodium-heptanosulphonate and applied it in series of studies. In other approaches, CZE was also used to quantify furosine [19, 20]. Although this method does not seem suitable for the testing of products with low levels of furosine like pasteurised milk and mozzarella cheese [19], its speed and low cost make it attractive for quality control of moderately heat-treated dairy samples [19, 20].

However, the determination of furosine has the disadvantage that this compound is formed from Amadori products with a yield of only 30–40%. On the other hand, this recovery rate is reproducible if consistent analytical conditions are applied. Figure 3 shows an example of furosine values in indirectly ultra-high temperature (UHT)-heated milk as a function of temperature [21]. As can be seen, the furosine content does not increase linearly with increasing heat damage. In more severely heated samples, the Amadori products and their marker furosine decrease again. The reason is the progress of the Maillard reaction, leading to the formation of further intermediates and end products. Table 2 shows an example of a model experiment with a lysine-glucose mixture, heated at 100°C for up to 30 h [22].

Furosine offers the advantage of being a direct marker of lysine reaction products, which are not only of analytical and technological but also of nutritional relevance. It is a representative marker of Amadori products from the early stage of the Maillard reaction that are nutritionally unavailable [23–25]. Table 3 shows results from experiments with

Table 2. Effect of heating time (hours at 100°C) on a 1:1 molar mixture of lysine and glucose in 88 vol.% water on the formation of furosine and CML [22]

Time of heating [h]	mg furosine/g of lysine	mg CML/g of lysine
3	27	2.1
6	40	3.3
9	50	4.5
12	59	5.0
16	68	5.1
20	58	5.4
24	55	6.0
30	45	6.5

Table 3. Effects of lysine supplementation of a severely heat-damaged dried skim milk on weight gains and protein efficiency ratio in young growing rats [26]

Protein source	Dried skim milk	Overheated (scorched) dried skim milk				
Available lysine 1[a)]	6.6	2.2	2.2	2.2	2.2	2.2
Available lysine 2[b)]	8.6	2.7	2.7	2.7	2.7	2.7
Amadori lysine[b)]	0.6	6.2	6.2	6.2	6.2	6.2
Added lysine[c)]	0	0	1.5	2.0	2.5	3.0
g weight gains[d)]	38	4	19	24	34	39
PER[e)]	3.5[c, f)]	0.6[g)]	1.7[h)]	2.0[h)]	2.8[i)]	3.1[i)]

a) Fluorodinitrobenzene as marker [6]
b) By using the furosine method (furosine + lysine analysis, calculation see [28])
c) As lysine mono chloride
d) 2 wk (weanling rats)
e) PER = protein efficiency ratio (gram weight gain/gram protein intake).
f–i) Values with different figures are significantly different ($2\alpha < 0.05$, Wilcoxon test)

rats given heat-damaged dried skim milk without and with the addition of lysine [26]. The supplementation with lysine restored the protein quality. This type of restoration of the nutritional value of a heated casein by lysine addition was first described by Greaves *et al.* in 1938 [27]. However, now it was possible to support the animal trials with modern analytical means. The scorched dried skim milk contained 2.7% available lysine and about 6% lysine bound as Amadori product, both calculated with the furosine method [28]. If the amount of Amadori lysine in the heat-damaged dried skim milk had been nutritionally available, the addition of lysine would not have been effective in restoring the protein quality as shown by an improved weight gain in the animal trial [26].

Furosine analyses were applied to many food items, and especially to dairy products. Together with the lysine content quantified by amino acid analysis, furosine allows the calculation of total, "blocked" (Amadori lysine) and available lysine [28–31]. As Table 4 shows, some foods show high contents of blocked lysine. Furosine contents have been analysed in milk products, cereals, pasta, honey and

Table 4. Levels of furosine, CML and blocked lysine in several heated milks [44]

Products (*n*)	Furosine [mg/kg crude protein]	CML	Blocked lysine [%][a)]
UHT milks (10)	1154	nd	2.7
UHT chocolate drinks (9)	1617	136	3.6
Sterilised milks (6)	3600	12	7.1
Sterilised chocolate drinks (5)	4460	250	9.5
UHT creams (8)	1375	11	2.7
Sterilised creams (6)	3930	522	8.3
Sterilised evaporated milks (13)	9950	430	18
UHT flavoured milks (7)	2694	27	5.1
Dried skim milks (10)	5369	7	11

a) Amadori lysine

many other items to which moderate heat treatment is applied [29, 32–38]. The relevance of furosine is also significant for regulatory uses, for instance, for the production of mozzarella cheese where furosine contents indicate the addition of heat-treated cow's milk to the original product made purely from low temperature-treated buffalo's milk [39].

The most accurate analytical protocol for the direct determination of the main Amadori product, fructoselysine, has been published by Fogliano *et al.* [40]. Herein, a stable isotope dilution assay is described for the quantification of protein-bound *N*-(epsilon)-(1-deoxy-D-fructos-1-yl)-L-lysine (fructoselysine) using a ^{13}C-labelled internal standard after enzymatic hydrolysis.

A marker of the advanced and late Maillard reaction is N^{ε}-carboxymethyllysine (CML), first detected as a marker of glycated proteins in biological material in 1985 and in food proteins in 1986 [41–43]. In more severely heat-treated food items, in which furosine levels have already decreased, CML can provide additional information on the protein damage. However, unlike for furosine, the mechanisms by which CML is formed are not unique, as is shown in Fig. 2. Table 4 shows concentrations of furosine, CML and the calculated amounts of blocked lysine (Amadori lysine) in several milk products [44]. The relation of furosine to CML is not uniform, which also suggests different pathways of CML formation [45–48]. Among the food items analysed, the highest CML contents were found in severely heat-treated, sterilised products.

In dairy products and especially in fruit juices, hydroxymethylfurfural (HMF) [49] is also a common marker resulting from the Maillard condensation reactions. For a long time, HMF determination was insufficiently reproducible between laboratories. Moreover, it is not solely formed in the Maillard reaction but also from the isomerisation and subsequent degradation of sugars. However, several studies on UHT-treated milk demonstrated the usefulness of HMF analyses as a rapid and simple measure of heat damage (*e.g.* [50]). The correlation between HMF and furosine contents proved to be quite good. In our investigations, we obtained correlation coefficients of 0.85 in 190 commercial UHT-treated milks of different origin and of 0.96 in 81 directly UHT-treated model milk samples [51]. Several new HPLC methods produce reliable results [52].

In other papers [53–55] pyrraline (ε-pyrrole-lysine) was shown to increase progressively with heat treatment, indicating that up to 15% of the lysine residues may have been modified. Pyrraline was first reported in dairy and bakery products. Pyrraline appears to be a useful marker of the advanced and late Maillard reaction. Studies by Resmimi and Pellegrino [36] on heat-treated pasta showed that pyrraline is formed significantly later than furosine, but increases linearly under conditions in which furosine decreases. Similar results were obtained by Henle *et al.* [55] with milk products. The concentrations found were about one-fifth of those of furosine. For future studies, a combined evaluation of heated food proteins by furosine and pyrraline analyses would be an interesting approach, as correlation coefficients of both nutritional markers of lysine modification are not available yet.

Pentosidine, another marker of heat treatment, has been quantified in foods as a derivative of arginine, although the range of concentrations was smaller than that of furosine or pyrraline [56, 57].

Another marker of heat-induced lysine damage found among melanoidin structures is pronyl-lysine, recently identified by Lindenmeier *et al.* [58]. Pronyl-lysine was first quantified in bread crust and crumb. Its lysine substitution appears to be quite low. On the other hand, it exhibits strong antioxidative capacities.

3 Characterisation of the quality of markers

First of all, the scope of a marker should be clearly characterised. In the huge field of the Maillard reaction, this means identifying whether the marker will be indicating the early, advanced or late parts of the reaction cascade. Generally, a stable and linear formation with heat treatment is an advantage. The marker should be sensitive on the one hand and should cover a large range of damage on the other. Of course, the marker should be easily detectable and stable during clean-up (*e.g.* hydrolysis) and analysis (chromatography). Table 5 shows the characteristics of the above-mentioned markers. None of the markers is perfect on all counts, and for some of them, such as pronyl-lysine and pentosidine, many criteria are still not known.

With respect to furosine, the recovery rate is reproducible if all conditions of hydrolysis and chromatography are kept stable. The results do not differ greatly among the values obtained between 1972 and 2003, if the molality of the hydrochloric acid used for hydrolysis was above 7.75 [28, 59]. It has also been demonstrated that furosine is stable

Table 5. Characterisation of the quality of the markers

Marker	Fur	HMF	CML	Pyrr	Pent	PronLys
Clear origin	+[a)]	–	–	+	+	+
Stability during clean-up and analysis	(+)	(+)	+	(+)	+	(+)
Linearity	–	–	+	(+)	?	?
Analytical recovery	40%	(+)	90–97	++	?	?
Applied in:	EM[b)]	EAM	ALM	ALM	AM	LM
Range	+++	++	+	++	?	?
Maximal yield%	>30	<10	<10	>10	>10	<10
Biologica relevancel	+++	+	+++	++	+++	+

a) – = not given; + = adequate (+) = partially or under certain conditions adequate; ++ = good, wide or high; +++ = very good, very wide, very high; ? = no or not enough data
b) **E**arly (E), **A**dvanced (A), **L**ate (L) **M**aillard Reaction (M)

Table 6. Recovery (%) of furosine and lysine from Amadori products after hydrolysis with HCl

References	HCl	Furosine	Lysine
Erbersdobler 1970 [29] Mp	7.75	~50	~50
Finot and Mauron 1972 [30] FFL	6.0	29	43
Brandt and Erbersdobler 1972 [28] Mp	7.75	40	50
Bujard and Finot 1978 [31] Mp	6.0	32	40
Krause *et al.* 2003 [59] FFL	8.0	43	43
Krause *et al.* 2003 [59] LL	8.0	50	41
Krause *et al.* 2003 [59] FL	7.4	43	48

FFL = free fructoselysine; FL = fructoselysine, peptide bound; LL = lactuloselysine, peptide bound; Mp = Milk products

Table 7. Yields of "Amadori lysine" (g/16 g of N) in severely heated model proteins

	Initial lysine	Available lysine	%Losses[a)]
Fortified models			
von Wangenheim *et al.* [62]	8.2	4.6	44
Lee [63]	8.8	4.7	47
Faist *et al.* [64]	8.5	4.4	48
Commercial items [34]			
Chocolate and similar items	4–8	2–5	25–71[b)]
Zwieback	2.2	0.5	41[b)]
Condensed milks	8.8	7.2	14[b)]

a) Only as Amadori lysine
b) The items also possibly contained some amounts of other products (*e.g.* lysinoalanine) and totally destroyed lysine, for example, in Zwieback 36%

during ion exchange chromatography with buffers of pH values below ~5 and/or eluting temperatures below ~60°C [60]. Since 1990 [16], most laboratories use HPLC, a method in which furosine has proved to be stable. The most recent data of Krause *et al.* [59] show a differentiation between various Amadori products in relation to the formation of furosine during hydrolysis of the heat-treated proteins. However, not all matrices have been tested. Especially in biological material like faeces and urine, the conversion factors are still questionable [61]. Table 6 shows data for the recovery of furosine procedure as reported in the literature [28–31, 59].

CML and pentosidine are quite stable during hydrolysis, whereas pyrraline has to be analysed following enzymatic hydrolysis [55], and for the determination of pronyl-lysine, a hydrazinolysis using methyl hydrazine must be applied before chromatographic separation techniques [58].

4 Biological significance

All markers discussed here are suitable indicators of protein damage in foods. Furosine, pyrraline and CML represent "blocked" lysine and are not nutritionally available as a lysine source for higher organisms. Furosine is directly derived from Amadori products and hence allows the quantification of "blocked" lysine from this source. Furosine is currently the most specific and important indicator of early Maillard reactions. Together with lysine determination, the calculation of available lysine is possible, at least in all mildly heat-processed foods containing some amount of glucose, lactose, maltose or other saccharides with a reactive glucose moiety.

Table 7 shows examples of very high values of blocked lysine calculated from furosine contents in severely heated model proteins or commercial food items [34, 62–64]. In milk products, up to 70% of lysine was quantified as Amadori lysine, depending on temperature and time of heating [65]. The importance of these findings is demonstrated by the fact that little is known about the quantitative and qualitative aspects of the reaction products of proteins with fructose, *e.g.* in heated fruits or in honey-sweetened and heated items.

5 Future prospects

A look into the main search engines reveals a great number of hits for furosine, although the number of hits for CML, HMF and pentosidine is much higher, presumably because of their predominant use in clinical biochemistry. This indicates the growing interest in MRPs in the fields of food science, nutrition, biochemistry and medicine.

It is not possible within the scope of this paper to discuss extensively the bioactive effects of MRPs, nor the questions pertaining to risk and benefit and nutritional importance. These questions have been discussed elsewhere (*e. g.* [66 – 72].

As can be seen, furosine analysis has many advantages but also some drawbacks which limit its value and application. Some of them can be overcome with a direct determination of fructoselysine after enzymatic hydrolysis of the sample. Initial analytical protocols were established by Henle *et al*. [73]. Now, a stable isotope dilution assay for an accurate quantification of protein-bound *N*(epsilon)-(1-deoxy-D-fructos-1-yl)-L-lysine using a ^{13}C-labelled internal standard seems to be very promising [40].

Due to the links between food science and medical biochemistry regarding the Maillard reaction, it is likely that more markers will be used in both fields in the future [74].

6 References

[1] Thomas, K., Über die biologische Wertigkeit der Stickstoffsubstanzen in verschiedenen Nahrungsmitteln. Beiträge zur Frage nach dem physiologischen Stickstoffminimum, *Arch. Anat. Physiol. Physiol. Abstr.* 1909, 219 – 302.

[2] Mitchell, H. H., A method of determining the biological value of proteins, *J. Biol. Chem.* 1924, *58*, 873 – 903.

[3] Osborne, T. B., Mendel, L. B., Ferry, E. L., A method of expressing numerically the growth promoting value of proteins, *J. Biol. Chem.* 1919, *37*, 223 – 229.

[4] Mitchell, H. H., Block, R. J., Some relationship between amino acid contents of proteins and their nutritive values for the rat, *J. Biol. Chem.* 1946, *163*, 599 – 620.

[5] Carpenter, K. J., The estimation of the available lysine in animal-protein foods, *Biochem. J.* 1960, *77*, 604 – 610.

[6] Erbersdobler, H., Zucker, H., Bestimmung von verfügbarem Lysin in Futtermitteln mit Dinitrofluorbenzol, *Z. Tierphysiol. Tierern. Futtermittelkde.* 1964, *19*, 244 – 255.

[7] Erbersdobler, H. F., Zucker, H., Untersuchungen zum Gehalt an Lysin und verfügbarem Lysin in Trockenmagermilch, *Milchwiss.* 1966, *21*, 564 – 568.

[8] Erbersdobler, H., Bock, G., Untersuchungen zur Identifizierung einer in Hydrolysaten hitzegeschädigter Proteine nachweisbaren ninhydrinpositiven Substanz, *Naturwissenschaften* 1967, *24*, 648.

[9] Brüggemann, J., Erbersdobler, H. F., Fructoselysin als wichtigstes Reaktionsprodukt von Lysin mit Glucose bei Hitzeschädigung von Lebens- und Futtermitteln, *Z. Lebensm. Unters. Forsch.* 1968, *137*, 137 – 143.

[10] Heyns, K., Heukeshoven, J., Brose, K.-H., Der Abbau von Fructose-Aminosäuren zu N-(2-Furoylmethyl)-Aminosäuren. Zwischenprodukte der Bräunungsreaktionen, *Angew. Chem.* 1968, *80*, 627.

[11] Finot, P. A., Bricout, J., Viani, R., Mauron, J., Identification of a new lysine derivative obtained upon acid hydrolysis of heated milk, *Experientia* 1968, *24*, 1097 – 1099.

[12] Freimuth, U., Trübsbach, A., Eine ninhydrinpositive Substanz im Hydrolysat von Proteinen nach Maillard Reaktion, *Die Nahrung* 1968, *12*, 887 – 888.

[13] Finot, P. A., Viani, R., Bricout, J., Mauron, J., Detection and identification of pyridosine, a second derivative obtained upon acid hydrolysis of heated milk, *Experientia* 1969, *25*, 134 – 135.

[14] Schleicher, E., Wieland, O. H., Specific quantitation by HPLC of protein (lysine) bound glucose in human serum albumin and other glycosylated proteins, *J. Clin. Chem. Clin. Biochem.* 1981, *19*, 81 – 87.

[15] Chiang, G. H., A simple and rapid high-performance liquid chromatographic procedure for determination of furosine, a lysine reducing sugar derivative, *J. Agric. Food Chem.* 1983, *31*, 1373 – 1374.

[16] Resmini, P., Pellegrino, L., Batelle, G., Accurate quantification of furosine in milk and dairy products by a direct HPLC method, *Ital. J. Food Sci*. 1990, *3*, 173 – 183.

[17] Clawin-Rädecker, I., Schlimme, E., Bestimmung von Furosin aus pasteutisierter Milch mittels Ionenpaar- Umkehrphasen-Flüssigkeitschromatographie, *Kieler Milchw. Forschber.* 1995, *47*, 169 – 175.

[18] Delgado, T., Corzo, N., Santa-Maria, G., Jimeno, M. L., *et al.*, Determination of furosine in milk samples by ion pair reversed phase liquid chromatography, *Chromatographia* 1992, *33*, 374 – 376.

[19] Tirelli, A., Pellegrino, L., Determination of furosine in dairy products by capillary zone electrophoresis; a comparison with the HPLC method, *Ital. J. Food Sci.* 1995, *7*, 379 – 385.

[20] Delgado-Andrade, C., Rufián-Henares, J. A., Morales, F. J., Fast method to determine furosine in breakfast cereals by capillary zone electrophoresis, *Eur. Food Res. Technol. A*, 2005, *221*, 707 – 711.

[21] Nangpal, A., Reuter, H., Dehn-Müller, B., Erbersdobler, H. F., Formation of furosine during UHT treatment of milk – comparison between direct and indirect heating, *Kieler Milchw. Forschber.* 1990, *42*, 43 – 51.

[22] Hartkopf, J., N-$^{\varepsilon}$carboxymethyl-lysin als Hitzeschädigungsindikator – Modell-untersuchungen zur Bildung in Lebensmitteln, Thesis, University of Kiel, 1993.

[23] Erbersdobler, H. F., Amino acid availability, in: Cole, D. J. A., Boorman, K. N., Buttery, P. J., Lewis, D., *et al.* (Eds.), *Protein Metabolism and Nutrition*, Butterworths, London and Boston 1976, pp. 139 – 157.

[24] Erbersdobler, H. F., The biological significance of carbohydrate-lysine crosslinking during heat treatment of food proteins; in: Friedman, M. (Ed.), *Adv Exp Med Biol: Protein Crosslinking. Nutritional and Medical Consequences*, Plenum Press, New York and London 1977, pp. 367 – 378.

[25] Finot, P.-A., Bujard, E., Mottu, F., Mauron, J., Availability of the true Schiff's bases of lysine. Chemical evaluation of the Schiff's bases between lysine and lactose in milk, in: Friedman, M. (Ed.), *Protein Crosslinking. Nutritional and Medical Consequences*, Plenum Press, New York and London 1977, pp. 343 – 365.

[26] Erbersdobler, H. F., Dümmer, H., Untersuchungen zur analytischen und physiologischen Charakterisierung der Aminosäurenschädigung durch Hitzebehandlung. 3. Untersuchungen an einem überhitzten Milchpulver, *Z. Tierphysiol. Tierern. Futtermittelkde.* 1971, *28*, 224 – 231.

[27] Greaves, E. O., Morgan, A. F., Loveen, M. K., The effects of amino acid supplements and variation in temperature and duration of heating upon the biological value of heated casein, *J. Nutrition* 1938, *6*, 115 – 128.

[28] Brandt, A., Erbersdobler, H. F., Zur Bestimmung von Furosin in Nahrungs- und Futtermitteln, *Landw. Forsch.* 1972, *28/II*, 115 – 119.

[29] Erbersdobler, H. F., Zur Schädigung des Lysins bei der Herstellung und Lagerung von Trockenmilch, *Milchwissenschaft* 1970, *25*, 280 – 284.

[30] Finot, P. A., Mauron, J., Le blocage de la lysine par la réaction de Maillard. II. Propriétés chimiques des derivés *N*-(désoxy-1-D-fructosyl-1) et *N*-(désoxy-1-D-lactulosyl-1) de la lysine, *Helv. Chim. Acta* 1972, *55*, 1153 – 1164.

[31] Bujard, E., Finot, P. A., Measure of available and blocked lysine in industrial milks, *Ann. Nutr. Alim.* 1978, *32*, 291 – 305.

[32] Erbersdobler, H. F., Dehn-Müller, B., Nangpal, A., Reuter, H., Determination of furosine in heated milk as a measure of heat intensity during processing, *J. Dairy. Res.* 1987, *54*, 147 – 151.

[33] Rufián-Henares, J. A., Delgado-Andrade, C., Jiménez-Pérez, S., Morales, F. J., Assessing nutritional quality of milk based sport supplements as determined by furosine, *Food Chem.* 2007, *101*, 573 – 578.

[34] Erbersdobler, H. F., Hupe, A., Determination of lysine damage and calculation of lysine bio-availability in several processed foods, *Z. Ernährungswissenschaft* 1991, *30*, 46 – 49.

[35] Delgado-Andrade, C., Rufián-Henares, J. A., Morales, F. J., Lysine availability is diminished in commercial fibre-enriched breakfast cereals, *Food Chem.* 2007, *100*, 725 – 731.

[36] Resmimi, P., Pelegrino, L., Occurrence of protein-bound lysylpyrrolaldehyde in dried pasta, *Cereal Chem.* 1994, *71*, 254 – 262.

[37] Del Castillo, D. M., Corzo, N., Olano, A., Early stages of Maillard reaction in dehydrated orange juice, *J. Agric. Food Chem*. 1999, *47*, 4388 – 4390.

[38] Sanz, M. L., Del Castillo, D. M., Corzo, N., Olano, A., 2-Furoylmethyl amino acids and hydroxymethylfurfural as indicators of honey quality, *J. Agric. Food Chem.* 2003, *51*, 4278 – 4283.

[39] Commission Regulation (EC) No. 2537/98 of 26 November 1998, *Off. J. Eur. Communities*, L 317, 14 – 18.

[40] Vinale, F., Fogliano, V., Schieberle, P., Hofmann, T., Development of a stable isotope dilution assay for an accurate quantification of protein-bound *N*(epsilon)-(1-deoxy-D-fructos-1-yl)-L-lysine using a (13)C-labeled internal standard, *J. Agric. Food Chem.* 1999, *47*, 5084 – 5092.

[41] Ahmed, M. U., Thorpe, S. R., Baynes, J. W., Identification of N^{ε}-carboxymethyl-lysine a modified amino acid formed by decomposition of fructoselysine in glycated proteins, *Fed. Proc*. 1985, *44*, 1621.

[42] Ahmed, M. U., Thorpe, S. R., Baynes, J. W., Identification of *N*-epsilon-carboxymethyllysine as a degradation product of fructoselysine in glycated protein, *J. Biol. Chem.* 1986, *261*, 4889 – 4894.

Professor Helmut F. Erbersdobler studied Veterinary Medicine in Munich where he later had a position as Associate Professor. From 1980 he was director of the Department of Food Science and Special Human Nutrition at the University of Kiel until he retired in 2003. He is a member of the committee of the German Nutrition Society (6 years as President and 8 years as Vice-President) and is Editor of the Ernährungs-Umschau. His research focused on food processing, protein evaluation and lipid metabolism. Between 1974 and 2004 he won six awards and honors.

[43] Büser, W., Erbersdobler, H. F., Carboxymethyllysine, a new compound of heat damage in milk products, *Milchwiss.* 1986, *41*, 780 – 785.

[44] Erbersdobler, H. F., Drusch, S., Faist, V., Nutritional role of dairy products. Effects of processing on protein quality of milk and milk products, in: Roginski, H., Fuguay, J. W., Fox, P. F. (Eds.), *Encyclopedia of Dairy Sciences*, Academic Press/ Elsevier Science, San Diego 2002, pp. 2137 – 2143.

[45] Zyzak, D. V., Wells-Knecht, K. J., Backledge, J. A., Litchfield, J. E., *et al.*, Pathways of the Maillard reaction in vitro and in vivo, in: Labuza, T. P., Reineccius, G. A. (Eds.), *Maillard Reactions in Chemistry, Food and Health*, Hartnolls Ltd., Bodmin 1994, pp. 274 – 280.

[46] Wells-Knecht, K. J., Zyzak, D. V., Litchfield, J. E., Thorpe, S. R., *et al.*, Mechanism of autoxidative glycosylation: Identification of glyoxal and arabinose as intermediates in the autoxidative modification of proteins by glucose, *Biochemistry* 1995, *34*, 3702 – 3709.

[47] Glomb, A. G., Monnier, V. M., Mechanism of protein modification by glyoxal, glycolaldehyde, reactive intermediates of the Maillard reaction, *J. Biol. Chem.* 1995, *270*, 1117 – 10026.

[48] Kasper, M., Schieberle, P., Labeling studies on the formation pathway of N^{ε}-Carboxymethyllysine in Maillard-type reaction, in: Baynes, J. W., Monnier, V. M., Ames, J. M., Thorpe, S. R. (Eds.), *The Maillard Reaction-Chemistry at the Interface of Nutrition, Aging, and Disease*, New York Academy of Sciences, New York 2005, pp. 59 – 62.

[49] Keeney, M., Basette, R., Detection of intermediate compounds in the early stages of browning reaction in milk products, *J. Dairy Sci.* 1959, *6*, 945 – 960.

[50] Konietzko, M., Reuter, H., Bildung von Gesamt-Hydroxymethylfurfural während der Ultrahocherhitzung von Vollmilch, *Milchwissenschaft* 1986, *41*, 449 – 451.

[51] Erbersdobler, H. F., Müller, B., Dehn-Müller, B., Untersuchungen zur Proteinschädigung in UHT-Milch, *Milchwissenschaft* 1991, *46*, 431 – 434.

[52] Morales, F. J., Romero, C., Jiménez-Pérez, S., Chromatographic determination of bound hydroxymethylfurfural as an index of milk protein glycosylation, *J. Agric. Food Chem.* 1997, *45*, 1570 – 1573.

[53] Nakayama, T., Hayase, F., Kato, H., Formation of epsilon (2-formyl-5-hydroxy-methyl-pyrrol-1-yl)-L-norleucin in the Maillard reaction between D-glucose and L-lysine, *Agric. Biol. Chem.* 1980, *44*, 1201 – 1202.

[54] Chiang, G. H., High performance liquid chromatography determination of pyrrole-lysine in processed food, *J. Agric. Food Chem.* 1988, *36*, 506 – 509.

[55] Henle, T., Schwarzenbolz, U., Walter, A. W., Klostermeyer, H., Protein-bound Maillard compounds in foods: Analytical and technological aspects, in: O'Brien, J., Nursten, H. E., Crabbe, M. J. C., Ames, J. M. (Eds.), *The Maillard Reaction in Food and Medicine*, Royal Society of Chemistry, London 1998, pp. 178 – 183.

[56] Grandhee, S. K., Monnier, V. M., Mechanism of formation of the Maillard protein cross-link pentosidine, *J. Biol. Chem.* 1991, *266*, 11649 – 11653.

[57] Henle, T., Schwarzenbolz, U., Klostermeyer, H., Detection and quantification of pentosidine in foods, *Z. Lebensm. Unters. Forsch.* 1997, *204*, 95 – 98.

[58] Lindenmeier, M., Faist, V., Hofmann, T., Structural and functional characterization of pronyl-lysine, a novel protein modification in bread crust melanoidins showing in vitro antioxidative and phase I/II-enzyme modulating activity, *J. Agric. Food. Chem.* 2002, *50*, 6997 – 7006.

[59] Krause, R., Knoll, K., Henle, T., Studies on the formation of furosine and pyridosine during acid hydrolysis of different Amadori products of lysine, *Eur. Food Res. Technol.* 2003, *216*, 277 – 283.

[60] Hartkopf, J., Erbersdobler, H. F., Stability of furosine during ion-exchange chromatography in comparison with reversed-phase high performance liquid chromatography, *J. Chromatography* 1993, *635*, 151 – 154.

[61] Rérat, A., Calmes, R., Vaissade, P., Finot, P. A., Nutritional and metabolic consequences of the early Maillard reaction of heat treated milk in the pig. Significance for man, *Eur. J. Nutr.* 2002, *41*, 1 – 11.

[62] Wangenheim, B., von, Hänichen, T., Erbersdobler, H. F., Histopathologische Untersuchungen an Rattennieren nach Fütterung hitzegeschädigter Proteine, *Z. Ernährungswissenschaft* 1984, *23*, 219 – 229.

[63] Lee, K.-H., Untersuchungen zur Bilanzierung des Proteins sowie des Fruktoselysins und des Lysinoalanins bei hitzebehandeltem Casein an Ratten mit der Homoarginin-Technik und am Menschen, Thesis, University of Kiel, 1992.

[64] Faist, V., Müller, C., Erbersdobler, H. F., Selective fortification of lysinoalanine, fructoselysine and N^{ε}-carboxymethyllysine in food-based casein model systems, *Nahrung/Food* 2001, *45*, 218 – 221.

[65] Finot, P. A., Deutsch, R., Bujard, E., The extent of the Maillard reaction during the processing of milk, *Prog. Food Nutr. Sci*. 1981, *5*, 345 – 355.

[66] Erbersdobler, H. F., Brandt, A., Scharrer, E., von Wangenheim, B., Transport and metabolism studies with fructose amino acids, *Prog. Food Nutr. Sci*. 1981, *5*, 257 – 263.

[67] Erbersdobler, H. F., Faist, V., Metabolic transit of Amadori products, *Nahrung/Food* 2001, *45*, 177 – 181.

[68] Finot, P. A., Historical perspective of the Maillard reaction in food science, *Ann. N.Y. Acad. Sci*. 2005 *1043*, 1 – 8.

[69] Faist, V., Erbersdobler, H. F., Metabolic transit and in vivo effects of melanoidins and precursor compounds deriving from the Maillard reaction, *Ann. Nutr. Metab.* 2001, *45*, 1 – 12.

[70] Henle, T., Protein-bound advanced glycation endproducts (AGEs) as bioactive amino acid derivatives in foods, *Amino Acids* 2005, *29*, 313 – 322.

[71] Lee, T. C., Kimiagar, M., Pintauro, S. J., Chichester, C. O., Physiological and safety effects of Maillard browning foods, *Prog. Food Nutr. Sci.* 1981, *5*, 1 – 6.

[72] Marko, D., Habermeyer, M., Kemeny, M., Weyland, U., *et al.*, Maillard reaction products modulating the growth of human tumor cells in vitro, *Chem. Res. Toxicol.* 2003, *16*, 48 – 55.

[73] Henle, T., Walter, H., Klostermeyer, H., Evaluation of the extent of the early Maillard-reaction in milk products by direct measurement of the Amadori product lactuloselysine, *Z. Lebensm. Untersuch. Forsch.* 1991, *193*, 119 – 122.

[74] Nursten, H. E., Links between the medical and food science aspects of the Maillard reaction, in: Fogliano, V., Henle, T. (Eds.), *Melanoidins in Food and Health*, COST 919 Proceedings vol. 3., Dresden 2002, pp. 189 – 194.

