Journal of Peptide Science
J. Peptide Sci. 2006; **12**: 291–296
Published online 23 September 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.722

JOURNAL OF PEPTIDE SCIENCE

Glycation of lysine-containing dipeptides

CARMELA MENNELLA, MARIANNA VISCIANO, AURORA NAPOLITANO, MARIA DOLORES DEL CASTILLO and VINCENZO FOGLIANO*

Dipartimento di Scienza degli Alimenti, Università di Napoli ''Federico II'' Parco Gussone, Ed. 84-80055 Portici (NA), Italy

Received 18 May 2005; *Revised 25 July 2005*; *Accepted 28 July 2005*

Abstract: Protein glycation through Maillard reaction (MR) is a fundamental reaction both in foods and in the human body. The first step of the reaction is the formation of Amadori product (AP) that is converted into intermediate and advanced MR products during reaction development. Although the MR is not an enzymatic reaction, a certain degree of specificity in the glycation site has been observed. In the present study, we have monitored the glycation of different lysine-containing dipeptides to evaluate the influence on the NH_2 reactivity of the neighboring amino acid.

Lysine dipeptides were reacted with glucose, galactose, lactose and maltose. The formation and identification of glycated compounds were monitored by mass spectrometry (MALDI-TOF and ESI-MS/MS) and by HPLC of their Fmoc derivatives. MS/MS analysis showed that the glucose APs formed on dipeptides have a characteristic fragmentation pattern: the fragment at $[M - 84]^+$ due to the formation of pyrylium and furylium ion is mainly present in the monoglucosylated form, while the $[M - 162]^+$ and the $[M - 324]^+$ are more evident in the fragmentation pattern of the diglucosylated forms.

The nature of the vicinal amino acids strongly affects lysine reactivity towards the different carbohydrates: the presence of hydrophobic residues such as Ile, Leu, Phe strongly increases lysine reactivity. Contrasting results were obtained with basic residues. The Lys-Arg dipeptide was among the most reactive while the Lys-Lys was not. Copyright © 2005 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Maillard reaction; protein glycation; site specificity; amino acid reactivity; Amadori products

INTRODUCTION

Maillard reaction (MR) between amino groups and sugars is responsible for the formation of color and flavor compounds in many foods. The first step of the reaction is the formation of a Schiff base that, through rearrangements, gives the Amadori product (AP). The formation of APs in reaction systems constituted by one amino acid and one sugar has been widely investigated and the different reactivity of each amino acid has been assessed [1–3].

Free amino acids are present only in some vegetables and fruits; therefore, most of the APs, both in foods and *in vivo*, are formed on the side chain of lysine residues present in the proteins [4]. Many studies have been carried out to quantify APs in model proteins and in foods [5–8]; however, little is known about the factors influencing the reactivity of the different free NH_2 groups present in the proteins.

In the last twenty years, studies by different authors have reported that nonenzymatic glycation is not a nonspecific reaction and that the glycation site specificity is driven by different factors.

A study on hemoglobin [9] proposed that the site specificity of the nonenzymatic glycation is determined by the ability of its microenvironment to isomerize the protein-bound aldose to a protein-bound ketose. The effect of the buffer system on the rate of glycation and site specificity of the free amino groups has previously been described by others [10–13]. Wu and coworkers [14] reported that water activity also influenced the glycation specificity.

Iberg and Fluckiger [15] observed that *in vivo* glycosylation of human serum albumin mainly occurs on lysine residues that are close to another amino group. These authors hypothesized that an acid–base catalysis of the Amadori rearrangement is promoted by near-by amino groups, causing site specificity. Recently, Brock and coworkers [16] confirmed this finding in a study using MS/MS to quantify the extent of carboxymethyl-lysine (CML). Venkatraman *et al.* [17], using stereochemically defined peptide scaffolds, studied the near-neighbor effects on the reactivity of the amino group on the side chains. They found that aspartic acid is an efficient catalytic residue in the Amadori arrangement. The results emphasize the structural determinants of Schiff base and AP formation in the final accumulation of glycated peptides.

This glycosylation specificity has been also verified by Fogliano and coworkers [18] on β-lactoglobulin (β-LG). The authors have shown that Lys_{100}, which, if followed by another Lys in position 101, is preferentially glycated in ultra heat-treated (UHT) milk. In this case, it was found that surface accessibility of the residues is also a factor determining its susceptibility to glycation [18]. On the other hand, other authors found that there is little or no specificity in the glycation site of β-LG,

*Correspondence to: V. Fogliano, Dipartimento di Scienza degli Alimenti, Università di Napoli "Federico II" Parco Gussone, Ed. 84-80055 Portici (NA), Italy; e-mail: fogliano@unina.it

particularly when severe heating conditions are used [19].

Brock *et al.* [16] demonstrated by ESI/LC/MS that carboxymethylation, a consequence of glycation, is a site-specific modification of RNase effected by neighboring amino acids and bound ligands, such as phosphorylated compounds.

From a mechanistic point of view, little information is available concerning the protein-bound APs and the catalyst effect of the neighboring amino acid on their formation. MS techniques have become very useful for analyzing the structure and characterizing posttranslational modifications of glycated proteins and peptides. In the present study, we have evaluated by MALDI-TOF and ESI-MS/MS the formation of lysine-containing dipeptides with reducing sugars (glucose, galactose, lactose and maltose) possessing different degrees of reactivity. The aim of this research was to look at the influence of the *C*-terminal amino acids on the glycation of lysine, employing a simple model system.

MATERIALS AND METHODS

Materials and Equipments

HPLC-grade solvents (methanol, water and acetonitrile) were purchased from Merck (Germany) and from Carlo Erba (Italy) and filtered through disposable 0.45 μm filters purchased from Chemtek (Italy). Peptides were supplied by Bachem (Switzerland). Reagents were from Sigma (USA), if not otherwise specified.

MS/MS analysis was performed by an API 3000 triple-quadrupole mass spectrometer (Applied Biosystem, Toronto, Canada) and a MALDI-TOF mass spectrometer (Kratos II, Shimadzu, Japan). The chromatographic analysis was carried out by HPLC configured with LC-10AD pumps, SLC10A system control and a diode array UV–vis detector (Shimadzu Japan).

Glycation Reaction of Dipeptides

The synthesis of glycated peptides was performed by dissolving the peptide and one of the four carbohydrates (glucose, galactose, lactose and maltose), at a molar ratio 1 : 4 in anhydrous methanol prepared by distillation of a suspension of $LiAlH_4$ in methanol in vacuum. The total final concentration in all cases was 0.18 mmol ml^{-1}.

The mixture was heated in a water bath at 64 °C in stoppered Pyrex vials. Samples were collected after 15, 30, 60 and 120 min, and the formation of the APs was monitored by mass spectrometry.

Analytical Determinations

ESI-MS/MS. The flow injection analysis was performed using a 5 μl loop and the acquisition was carried out by multiple reaction monitoring (MRM) in the positive ion mode in the range of 150–1000 amu using an electrospray source. The instrument was calibrated with ionic adducts of ammonium poly(propylene glycol) (PPG). Data were processed with Analyst 1.4 software (Sciex, Canada).

Deionized water (0. 1% formic acid) with acetonitrile (50 : 50) at 1 ml min^{-1} was used as carrier liquid to obtain better ionization of the precursor ion. The performance of the system was optimized by evaluating the effect of sample injection parameters on the ion intensity in the mass spectrum. The parameters were optimized for each compound analyzed. The voltage and the declustering potential applied were 5500 and 30 V respectively. The collision energy (CE) and collision cell-exit potential (CXP) were optimized for each transition ion. Finally, the collision-induced dissociation was performed using nitrogen as the collision gas in the collision cell.

MALDI-TOF. The instrument was equipped with a nitrogen laser emitting at 337 nm with a pulse width of 3 ns. Positive ions were recorded in linear mode using insulin as the external standard for calibration. A saturated solution (10 mg ml^{-1}) of α-cyan-4-hydroxycinnamic acid was dissolved in acetonitrile/H_2O (with TFA 0.1%) (2 : 3 v/v), and ions were accelerated through an acceleration voltage of 25 kV. Samples were dissolved in deionized water at a concentration of 10 mg ml^{-1}. Slides were prepared using a five-step method. At each step, 0.6 μl was pipetted on to sample plates, and the slides were placed in a fan oven for ∼30 s to evaporate the solvent. Briefly, matrix, sample, matrix, H_2O/TFA 0.1% and finally matrix were layered onto the slides. Kompact 1.2 software was used for data analysis.

HPLC of Fmoc Derivatives. Estimation of the yield of the glycation was carried out by HPLC of the Fmoc derivatives of primary and secondary free amino groups present in the reaction mixtures. Derivatization was performed as reported by Gartenmann and Kochhar [20]. Briefly, 10 μl of reaction mixtures containing APs was dissolved in 500 μl of borate buffer (0.1 M, pH 10.4), mixed with 500 μl of Fmoc 5 mM followed by extraction of the Fmoc in excess with 2 ml of hexane/ethyl acetate (80/20 v/v). Aqueous fraction (20 μl) containing Fmoc derivatives was employed to perform the HPLC analysis. The column was equilibrated in 50% phase A (H_2O, TFA 0.05%) and 50% phase B (CH_3CN, TFA 0.05%). Separation of Fmoc derivatives was performed on a Luna 5 μm, C18, 4.6 mm × 25 cm column (Phenomenex) at a flow rate of 0.8 ml min^{-1}. Gradient elution of Fmoc derivatives was as follows: time 0–5 min, A 50%, B 50%; time 25 min, A 0%, B 100%; time 30 min, A 0%, B 100%; time 33 min, A 50%, B 50%; time 35 min, A 50%, B 50%. Peaks were detected by a diode array at 265 nm followed by ionization by ESI source as previously described. Data were processed by CLASS VP 5.3 software. The peak corresponding to free peptide $(Fmoc)_2$-Lys-X was identified by ESI-MS and quantified by determination of its area. Decrease in the area of the peak was used as an index of peptide consumption by means of the MR.

RESULTS AND DISCUSSION

The ten Lys-X type dipeptides used in this study were reacted for different times with four carbohydrates and the formation of the APs from the different reaction mixtures was evaluated. The first indication of the MR development in the reaction mixtures was

noted by visual inspection and by measurement of the absorbance values at 360 nm. Under the studied conditions, rate of formation of colored compounds was slow. Development of pale yellow color took around 2 h of reaction time. Values of absorbance were not greater than 0.91 for one of the most reactive dipeptides, namely Lys-Phe heated for 2 h.

ESI-MS/MS and MALDI-TOF proved to be particularly useful to monitor the peptide modification during the glycation reaction. Results obtained by MALDI-TOF using Lys-Leu dipeptide are shown as examples in Figure 1, while in Figure 2 the fragmentation pattern obtained by ESI-MS/MS on the glucose APs formed on the same dipeptide (Lys-Leu) is shown.

In Table 1, the most abundant fragments obtained from APs formed on dipeptides upon incubation with glucose are reported. Data show that the APs formed on the different dipeptides possess a common and characteristic fragmentation pattern always mainly constituted by the ions $[M - 162]^+$ and the $[M - 324]^+$ (due to the loss of one or two molecules of glucose, respectively). The fragmentation pattern is also in good agreement with that reported by Horvat and Jakas [21] and by Yeboah and Yaylayan [22], taking into account the different ion mode (negative vs positive) used in our study. The most intense ions that are detected in the MS spectra corresponding to Amadori compounds, particularly when only one APs is present on the peptide, arise from losses of water molecules, resulting in the simultaneous formation of pyrylium $[M - 84]^+$ and furylium $[M - 84]^+$ ions. On the other hand, the immonium ions $[M - 54]^+$ were detected only in APs on Lys-Lys. When two APs were present on the peptide, besides the loss of one and two glucose moieties, the $[M - 120]^+$ ion was also

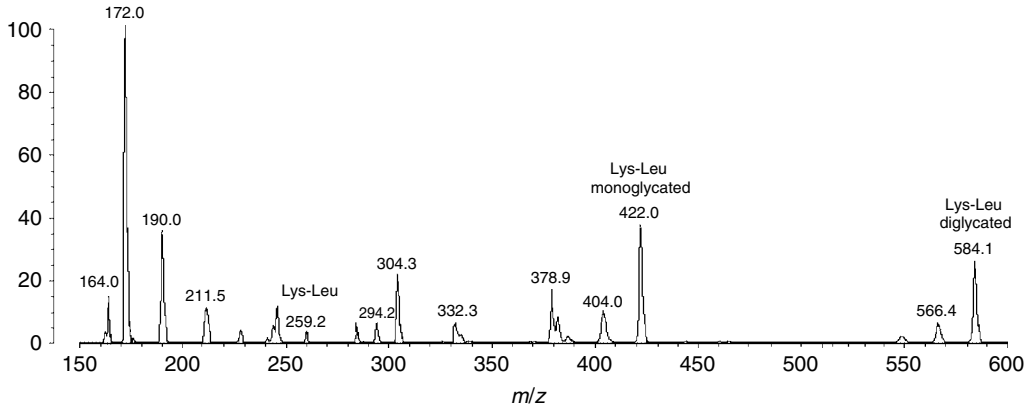

Figure 1 MALDI-TOF mass spectrum of the reaction mixture containing Lys-Leu and glucose after 30 min of heating at 64 °C.

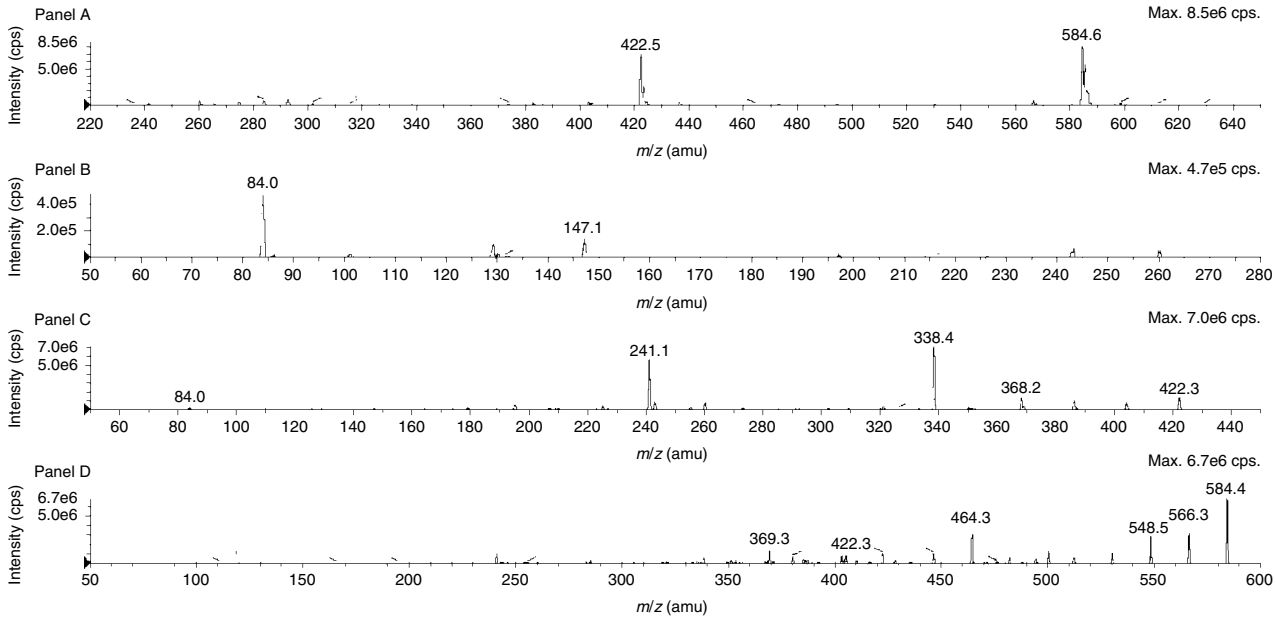

Figure 2 ESI-MS/MS spectrum in MRM mode of the APs formed from Lys-Leu dipeptide with glucose: Panel A: total ion count; Panel B: spectrum of the fragment at 259 $[M - 364]^+$; Panel C: spectrum of the fragment at 422 $[M - 162]^+$; Panel D: spectrum of the molecular ion at 584 $[MH]^+$.

Table 1 ESI-MS/MS fragment ions obtained from reaction mixtures of Lys-X type dipeptides and glucose. Data of monoglycated peptides (only one AP on the peptide) are reported in the grey cells

Dipeptide	Compound	$[MH]^+$ m/z	MS/MS ions	Loss	Fragment lost	CE	CXP
Lys-Arg	Monoglycated	465	429	36	$-2H_2O$	28	13
			345	120	$-5H_2O-CHOH$	24	11
	Diglycated	627	507	120	$-5H_2O-CHOH$	32	9
			609	18	$-H_2O$	32	16
Lys-Ile	Monoglycated	422	338	84	$-3H_2O-CHOH$	28	10
			242	180	$-C_6H_{12}O_6$	31	7
	Diglycated	584	464	120	$-5H_2O-CHOH$	30	12
			368	216	$-2H_2O-C_6H_{12}O_6$	39	11
Lys-Ser	Monoglycated	395	311	84	$-3H_2O-CHOH$	20	9
			215	180	$-C_6H_{12}O_6$	31	10
	Diglycated	557	521	36	$-2H_2O$	31	12
			395	162	$-C_6H_{10}O_5$	32	9
Lys-Leu	Monoglycated	422	338	84	$-3H_2O-CHOH$	32	10
			386	36	$-2H_2O$	26	12
	Diglycated	584	548	36	$-2H_2O$	30	17
			464	120	$-5H_2O-CHOH$	30	16
Lys-Phe	Monoglycated	456	372	84	$-3H_2O-CHOH$	27	11
			276	180	$-C_6H_{12}O_6$	30	8
	Diglycated	618	498	120	$-5H_2O-CHOH$	30	10
			582	36	$-2H_2O$	13	31
Lys-Lys	Monoglycated	437	275	162	$-C_6H_{10}O_5$	26	10
			383	54	$-3H_2O$	22	9
	Diglycated	599	419	180	$-C_6H_{12}O_6$	12	12
			437	162	$-C_6H_{10}O_5$	27	13
	Triglycated	761	743	18	$-H_2O$	32	22
			599	162	$-C_6H_{10}O_5$	28	20
Lys-Val	Monoglycated	407	323	84	$-3H_2O-CHOH$	28	7
			227	180	$-C_6H_{12}O_6$	31	6
	Diglycated	570	450	120	$-5H_2O-CHOH$	31	14
			354	216	$-2H_2O-C_6H_{12}O_6$	39	14
Lys-Gly	Monoglycated	366	282	84	$-3H_2O-CHOH$	26	8
			186	180	$-C_6H_{12}O_6$	31	10
	Diglycated	527	347	180	$-C_6H_{12}O_6$	12	10
			203	324	$-2C_6H_{10}O_5$	15	5
Lys-Tyr	Monoglycated	472	388	84	$-3H_2O-CHOH$	29	12
			292	180	$-C_6H_{12}O_6$	33	8
	Diglycated	633	513	120	$-5H_2O-CHOH$	31	16
			417	216	$-2H_2O-C_6H_{12}O_6$	40	13
Lys-Ala	Monoglycated	380	296	84	$-3H_2O-CHOH$	25	8
			200	180	$-C_6H_{12}O_6$	28	5
	Diglycated	542	362	180	$-C_6H_{12}O_6$	15	11
Ac-Lys-NHMe	Monoglycated	364	202	162	$-C_6H_{10}O_5$	21	17
			328	36	$-2H_2O$	19	10

often present, likely due to the simultaneous formation of pyrylium and furylium combined with the loss of the other two water molecules.

The formation of APs was estimated by HPLC analysis of the Fmoc derivatives. Samples taken at 0, 30, 60 and 120 min of reaction were analyzed. Peaks corresponding to Fmoc derivatives of parent peptides were identified by MS.

Results show that free dipeptide formed the $(Fmoc)_2$-Lys-X derivatives, which have longer retention time than their glycated forms due to their differences of polarity. The area of these peaks was determined and employed to calculate the percentage of reduction of the parent peptide content during the glycation. As expected, a progressive decrease of the parent peptide over time has been observed for all the reaction mixtures under study, but the kinetics of

this decrease was very different among the various peptides. The percentages of residual peptide at different reaction times were calculated and taken as a direct index of reactivity of the peptides in the presence of the different carbohydrates. Table 2 shows the level of glycation obtained in the reaction mixtures constituted by dipeptides and reducing sugars heated at 64 °C for 30 min. The results agree with those previously published indicating that monosaccharides are more reactive than disaccharides [23,24], the order of reactivity was found to be as follows: glucose = galactose > maltose > lactose. The rank of reactivity of the different dipeptides with the four sugars under study was similar. Lys-Arg, Lys-Ile and Lys-Phe became more reactive than other peptides under glycation conditions employing both mono- and disaccharides.

Since it is known that *N*-α-acetylated lysine is more reactive than lysine by means of the MR [25], we have selected the Ac-Lys-NHMe as representative of the reactivity of the ε-NH_2 side chain alone where there is no influence of the neighboring amino acid. In Figure 3, the time-course of the reaction of all dipeptides with glucose is shown. The data demonstrate that the majority of the dipeptides are more reactive than Ac-Lys-NHMe.

Table 2 The percentage of unglycated peptides after 30 min of incubation

	Glucose	Galactose	Maltose	Lactose
Lys-Gly	88.1	90.3	98.0	99.0
Lys-Arg	10.2	11.3	22.0	28.0
Lys-Val	28.5	28.2	53.2	98.5
Lys-Leu	7.0	7.1	35.9	74.6
Lys-Ile	10.6	10.2	32.0	61.6
Lys-Lys	73.6	73.5	75.7	88.2
Lys-Phe	12.6	44.1	48.1	77.6
Lys-Tyr	30.8	36.2	98.5	99.2
Lys-Ser	26.2	30.5	56.1	65.7
Ac-Lys-NHMe	59.4	33.2	96.3	90.2
Lys-Ala	82.7	85.4	97.3	96.3

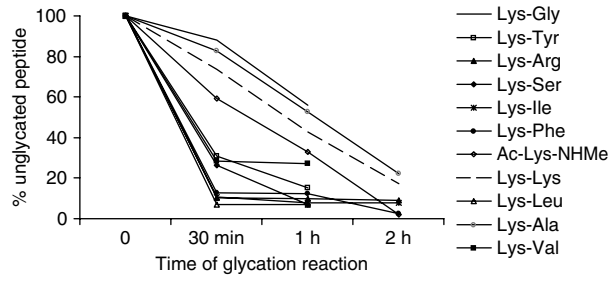

Figure 3 Time-course reaction of the mixtures constituted by lysine containing dipeptides and glucose: (———) Lys-Gly; (□) Lys-Tyr; (▲) Lys-Arg; (♦) Lys-Ser; (⁎) Lys-Ile; (●) Lys-Phe; (◊) Ac-Lys-NHMe; (- - - -) Lys-Lys; (Δ) Lys-Leu; (○) Lys-Ala; (■) Lys-Val.

Peptides constituted by small and uncharged amino acids, Gly and Ala, have shown the lowest reactivity. The presence of hydrophobic residues such as Ile, Leu and Phe strongly increased lysine reactivity while residues of intermediate polarity, such as Val, and those having a hydroxyl moiety, such as Ser and Tyr, also gave good reactivity. Contrasting results were obtained with basic residues. The Lys-Arg dipeptide is among the most reactive while Lys-Lys is as reactive as the Ac-Lys-NHMe.

Investigations on nonenzymatic protein glycation led to results not very different from those described here for simplified models. Indeed, some studies have indicated Lys and Arg residues located near hydrophobic amino acids as specific sites of glycation [26]; while in others, charged amino acids seem to promote the glycation of vicinal basic residues [17,19, 27].

CONCLUSIONS

The study of the reaction between carbohydrates and lysine-containing dipeptides has shown that the reactivity is greatly affected by the neighboring amino acids. These results support all proteins studies [17,28–30] showing the site specificity of the MR with a preferential glycation on selected Lys and Arg residues. However, data suggest that results obtained with dipeptides are mainly dependent on the polarity of the peptides, while in proteins the factors affecting the pK_a of the amino group play a pivotal role in determining the rate of formation of Schiff base adducts together with the proximity of groups capable of proton abstraction. Further studies employing longer peptides and containing acid amino acids are needed in order to give insight on the influence of the neighboring peptide on the reactivity and on the specificity of glycation of free lysine amino groups.

REFERENCES

1. Yaylayan VA, Huyghues-Despointes A. Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. *Crit. Rev. Food Sci. Nutr.* 1994; **34**: 321–369.
2. Martins SIFS, Marcelis ATM, van Boekel MA. Kinetic modelling of Amadori *N*-(1-deoxy-d-fructos-1-yl)-glycine degradation pathways. Part I–reaction mechanism. *Carbohydr. Res.* 2003; **338**: 1651–1663.
3. Hofmann T, Schieberle P. Formation of aroma-active Strecker-aldehydes by a direct oxidative degradation of Amadori compounds. *J. Agric. Food Chem.* 2000; **48**: 4301–4305.
4. Friedman N. Food browning and its prevention: an overview. *J. Agric. Food Chem.* 1996; **44**: 631–653.
5. Vinale F, Monti SM, Panunzi B, Fogliano V. Convenient synthesis of lactuloselysine and its use for LC-MS analysis in milk-like model systems. *J. Agric. Food Chem.* 1999; **47**: 4700–4706.

6. Sanz ML, del Castillo MD, Corzo N, Olano A. Formation of Amadori compounds in dehydrated fruits. *J. Agric. Food Chem.* 2001; **49**: 5228–5231.
7. Del Castillo MD, Corzo N, Olano A. Early stages of Maillard reaction in dehydrated orange juice. *J. Agric. Food Chem.* 1999; **47**: 4388–4390.
8. Sanz ML, del Castillo MD, Corzo N, Olano A. Presence of 2-furoylmethil derivates in hydrolisates of processed tomato products. *J. Agric. Food Chem.* 2000; **48**: 468–471.
9. Nacharaju P, Acharya AS. Amadori rearrangement potential of haemoglobin at its glycation sites is dependent on the three-dimensional structure of protein. *Biochemistry* 1992; **31**: 12 673–12 679.
10. Watkins NG, Neglia-Fisher CI, Dyer DG, Thorpe SR, Baynes JW. Effect of phosphate on the kinetics and specificity of glycation of protein. *J. Biochem. Mol. Biol. Biophys.* 1987; **262**: 7207–7212.
11. Potman RP, van Wijk ThA. Mechanistic studies of the Maillard reaction with emphasis on phosphate-mediated catalysis. In *Thermal Generation of Aromas*, ACS Symposium Series 409, Parliament TH, McGorrin RJ, Ho C-T (eds). American Chemical Society: Washington, 1989; 182–195.
12. Bell LN. Maillard reaction as influenced by buffer type and concentration. *Food Chem.* 1997; **59**: 143–147.
13. Rizzi GP. Role of phosphate and carboxylate ions in Maillard browning. *J. Agric. Food Chem.* 2004; **52**: 953–957.
14. Wu H, Govidarajan S, Smith T, Rosen JD, Ho CT. Glucose-lysozime in a restricted water environment. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, Finot PA, Aeschbacher HU, Hurrel RF, Liardon R (eds). Birkhauser Verlag: Basel, 1990; 85–90.
15. Iberg N, Fluckiger R. Nonenzymatic glycosylation of albumin in vivo. Identification of multiple glycosylated sites. *J. Biochem. Mol. Biol. Biophys.* 1986; **261**: 13 542–13 545.
16. Brock JW, Hinton DJ, Cotham WE, Metz TO, Thorpe SR, Baynes JW, Ames JM. Proteomic analysis of the site specificity of glycation and carboxymethylation of ribonuclease. *J. Proteome Res.* 2003; **2**: 506–513.
17. Venkatraman J, Aggarwal K, Balaram P. Helical peptide models for protein glycation: proximity effects in catalysis of the Amadori rearrangement. *Chem. Biol.* 2001; **7**: 611–625.
18. Fogliano V, Monti SM, Visconti A, Randazzo G, Facchiano AM, Colonna G, Ritieni A. Identification of a beta-lactoglobulin lactosylation site. *Biochim. Biophys. Acta* 1998; **1388**: 295–304.
19. Leonil J, Molle D, Fauquant J, Maubois JL, Pearce RJ, Bouhallab S. Characterization by ionization mass spectrometry of lactosyl beta-lactoglobulin conjugates formed during heat treatment of milk and whey and identification of one lactose-binding site. *J. Dairy Sci.* 1997; **80**: 2270–2281.
20. Gartenmann K, Kochhar S. Short-chain peptide analysis by high-performance liquid chromatography coupled to electrospray ionization mass spectrometer after derivatization with 9-fluorenylmethyl chloroformate. *J. Agric. Food Chem.* 1999; **47**: 5068–5071.
21. Horvat S, Jakas A. Peptide and amino acid glycation: new insight into the Maillard reaction. *J. Pept. Sci.* 2004; **10**: 119–137.
22. Yeboah FK, Yaylayan VA. Analysis of glycated proteins by mass spectrometric techniques: qualitative and quantitative aspects. *Nahrung/Food* 2001; **45**: 164–171.
23. Ledl F, Severin T. Nonenzymatic browning reaction of pentoses and amines. *Z. Lebensm.-Unters. Forsch.* 1978; **167**: 410–413.
24. Leong LP, Wedzicha BL. A critical appraisal of the kinetic model for the Maillard browning of glucose with glycine. *Food Chem.* 2002; **68**: 21–28.
25. Monti SM, Borrelli RC, Ritieni A, Fogliano V. A comparison of color formation and Maillard reaction products of a lactose-lysine and lactose-N (alpha)-acetyl lysine model system. *J. Agric. Food Chem.* 2000; **48**: 1041–1046.
26. Lapolla A, Fedele D, Reitano R, Aricò NC, Seraglia R, Traldi P, Marotta E, Tonani R. Enzymatic digestion and mass spectrometry in the study of advanced glycation end products/peptides. *J. Am. Soc. Mass Spectrom.* 2004; **15**: 496–509.
27. Fenaille F, Morgan F, Parisod V, Tabet JC, Guy PA. Solid-state glycation of beta-lactoglobulin by lactose and galactose: localization of the modified amino acids using mass spectrometric techniques. *J. Mass Spectrom.* 2004;; **39**: 16–28.
28. Kislinger T, Humeny A, Seeber S, Becker CM, Pischetsrieder M. Qualitative determination of early Maillard-product by MALDI-TOF mass spectrometry peptide mapping. *Eur. Food Res. Technol.* 2002; **215**: 65–71.
29. Baldwin JS, Lee L, Leung TK, Muruganandam A, Mutus B. Identification of the site of non-enzymatic glycation of glutathione peroxidase: rationalization of the glycation-related catalytic alterations on the basis of three-dimensional protein structure. *Biochim. Biophys. Acta* 1995; **1247**: 60–64.
30. Shuvaev VV, Fujii J, Kawasaki Y, Itoh H, Hamaoka R, Barbier A. Glycation of apolipoprotein E impairs its binding to heparin: identification of the major glycation site. *Biochim. Biophys. Acta* 1999; **1454**: 296–308.