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The Effect of pH on the Formation of Furfural Compounds in the Glucose and Fructose with Amino Acid Enantiomers in Maillard Reaction

– Research Note –

Ji-Sang Kim and Young-Soon Lee†

Department of Food and Nutrition, Kyung Hee University, Seoul 130-170, Korea

Abstract

This study was conducted to investigate the effect of pH on the formation of furfural compounds from glucose and fructose reacting with amino acid enantiomers in the Maillard reaction. Hydroxymethylfurfural (HMF) content was highest at pH 4.0, and decreased with increasing pH. HMF was significantly higher in glucose-based systems than fructose-based systems. Furfuryl alcohol (FFA) and 5-methyl-2-furaldehyde (MF) were not increased with increasing pH, and only small amounts were formed. In addition, 2-furaldehyde (F) was found to increase in the systems, as pH increased. However, the content was small and variable. 2,5-Dimethyl-4-hydroxy-3(*2H*)-furanone (DMHF) was only found in Glc/D-Asn, Glc/L-Lys and Fru/D-Lys system, but the content was not increased with increasing pH. 2-acetylfuran (AF) was higher in Glc (or Fru)/L-Lys and Glc (or Fru)/D-Lys systems at pH 7.0. However, at pH 4.0, the content of AF was higher in the Glc (or Fru)/Gly and Glc (or Fru)/L-Asn systems. Therefore, this study aimed to observe the effect of pH, sugars and amino acid enantiomers on the production of furfural and related compounds by the Maillard reaction. A clear tendency was observed for some classes of compounds to be more easily formed at higher or lower pH. HMF was more readily formed at lower pH, while FFA, F, DMHF and MF were inhibited by acidic conditions. Particularly, compounds like FFA, F and MF were not affected by pH changes. In addition, DMHF and MF were only formed in L-Lys and D-Lys system.

Key words: amino acid enantiomers, furfural compounds, Maillard reaction

INTRODUCTION

The reaction between reducing sugars and amino acids is known as the Maillard reaction or non-enzymic browning reaction (1). The Maillard reaction is a complicated reaction that produces a large number of the so-called Maillard reaction products (MRPs) such as aroma compounds, ultra-violet absorbing intermediates, and dark-brown polymeric compounds named melanoidins (2).

The conversion of free or protein- or peptide-bound physiological L-amino acids into their mirror images (enantiomers) named D-amino acids is of great nutritional and physiological interest (3). The change of chirality in amino acids is commonly referred to as racemization or epimerisation if several chiral centers are involved, although, in the strict sense, racemic amino acids contain equal amounts of D- and L-amino acids (4). The Maillard reaction can also explain the formation of D-amino acids in food. Brückner et al. (4) have recently pointed out that D-amino acids are formed during heating of aqueous solutions of L-amino acids (2.5 mM) together with an excess (278 mM) of saccharides (glucose, fructose, and saccharose) at 100°C for 24～96 h in aqueous solutions of pH 2.5 (AcOH) or pH 7.0 (NaOAc). Thus, the formation of D-amino acids in many foods of plant and animal origin is the result of non-enzymic browning since the presence of amino acids together with saccharides is common. As for the racemization mechanism, it is postulated that the reaction of amino acids with glucose or fructose starts with the reversible formation of Schiff bases. The degree of racemization depends in particular on steric and electronic properties of the amino acid side chains. It should be noted that the early stages of the Maillard reaction proceeds readily under mild conditions (4,5) and do not require alkaline or acidic condition. This new racemization mechanism based on the relatively stable Amadori compounds has been used to explain the generation of free D-amino acid in foods such as dried fruits, concentrated plant juices and fortified wines (6). Recently, experiments in which synthetic Amadori compounds are heated have demonstrated that they are sources of amino acid-enantiomers (7-9). Amino acid racemization, however, is very much dependent on temperature, pH, and presence of catalysts (10). Furthermore, convincing evidence has recently been established that D-amino acids are formed in the course of the Maillard reaction (4,7,8).

†Corresponding author. E-mail: yysllee@khu.ac.kr
Phone: +82-2-961-0881, Fax: +82-2-968-0260

Non-enzymic browning has been considered one of the major causes of quality and colour loss during the processing and storage of food products. Some furanic compounds, one class of heterocyclic compounds that have been reported in a wide variety of food systems (11), were produced from the degradation of sugars by heating. 5-Hydroxymethylfurfural (5-HMF) and furfural are the principal degradation products of the hydrolysis of hexoses and pentoses, respectively (12). Furfurals are intermediary compounds in the formation of pigments (melanoidins) in the most advanced stages of the Maillard reaction (13). The furfural group includes six different compounds: 5-hydroxymethyl-2-furaldehyde (HMF), furfuryl alcohol (FFA), 2-furaldehyde (F), 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone (DMHF), 2-acetylfuran (AF) and 5-methyl-2-furaldehyde (MF). 5-HMF has been correlated with colour changes in fruit juices while furfural is widely accepted as an indicator of flavour changes (14). DMHF, which is an important flavour compound that can be found in various fruits (15,16) and possesses stronger antioxidative activity (17), is also one of the putative degradation products of sugars (18) and can be generated directly from hexoses (19). AF and FFA are also the degradation products of sugars (11,20). F content has been demonstrated in several food matrices; for this reason, the F content is useful as an off-flavour indicator (21-24). In fact, when food is subjected to heat treatment and storage at inappropriate temperatures, different furfural derivatives (HMF, AF, FFA, DMHF, F and MF) can be generated, and these compounds serve as indicators of the extent of the Maillard reaction (25-27). Despite the diverse applications of Maillard reaction in the food industry, no investigations, so far, have tried to identify the furfural components responsible for the characteristic flavours produced. In addition, no reports on the presence of furfural compounds due to different amino acid enantiomers are available. The objective of this study was, therefore, to investigate the effect of pH on the formation of furfural compounds derived from glucose and fructose reacting with amino acid enantiomers in Maillard reaction.

MATERIALS AND METHODS

Chemicals

D-glucose, D-fructose, glycine, L-asparagine, D-asparagine, L-lysine and D-lysine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). HMF (5-hydroxymethyl-2-furaldehyde), FFA (furfuryl alcohol), F (2-furaldehyde), DMHF (2,5-dimethyl-4-hydroxy-3(*2H*)-furanone), AF (2-acetylfuran) and MF (5-methyl-2-furaldehyde) were purchased from Fluka (St. Louis, MO, USA). Sodium carbonate and sodium hydrogen phosphate were purchased from Merck Co. (Darmstadt, Germany). HPLC-grade water was purchased from J. T. Baker (Phillipsburg, NJ, USA). Reagents were of the highest reagent grade quality and used without any further purification.

Preparation of Maillard reaction products (MRPs)

Glucose, fructose and amino acids were dissolved in 100 mL of 0.5 M sodium acetate buffer, pH 4.0, 0.5 M phosphate buffer, pH 7.0 or 0.5 M sodium carbonate buffer, pH 10.0 to obtain a final concentration of 1 M. Twelve model systems were prepared, composed of glucose (Glc), glucose/glycine (Glc/Gly), glucose/L-asparagine (Glc/L-Asn), glucose/D-asparagine (Glc/D-Asn), glucose/L-lysine (Glc/L-Lys), glucose/D-lysine (Glc/D-Lys), fructose (Fru), fructose/glycine (Fru/Gly), fructose/L-asparagine (Fru/L-Asn), fructose/D-asparagine (Fru/D-Asn), fructose/L-lysine (Fru/L-Lys) and fructose/D-lysine (Fru/D-Lys). The reaction mixtures were then distributed among screw-capped glass, Schott tubes (16×160 mm), each containing a minimum of 10 mL. Model solutions, prepared at least in duplicate, were heated without pH control at 100°C for 2 h. The heating was carried out in a silicone oil bath and the proper safety measures were taken. After heating, model solutions were withdrawn and immediately cooled in ice water.

Determination of furfurals compounds

The solutions were heated in a silicone oil bath for 2 h, followed by filtration through a Millex-HN nylon clarification kit with a 0.45 μm pore size (Millipore, Bedford, MA, USA). The filtrate was injected into a high-performance liquid chromatographic system (HP 1100 series, Hewlett Packard, Wilmington, DE, USA) that consisted of a Supelcosil LC-C18 Column (150× 4.6 mm I.D., 5 μm particle size, Supelco, Bellefonte, PA, USA), a Agilent quaternary pump (Hewlett Packard, model G1311A, Wilmington, DE, USA), a variable wavelength detector (Hewlett Packard, model G1314A, Wilmington, DE, USA), a autosampler (Hewlett Packard, model G1313A, Wilmington, DE, USA) and a Chemstation software (Hewlett Packard, Wilmington, DE, USA). The mobile phase was acetonitrile : water in a 5:95 volume ratio with a flow rate of 1.0 mL/min and an injection volume of 1 μL. Detection was in the wavelength gradient at 284 nm for HMF and F, and 280 nm for AF and DMHF, and 215 nm for FFA, and at 293 nm for MF. Furfurals were quantified by interpolation in a calibration curve in the range 0.05～0.5 mg mL^{-1} of HMF, F, AF, DMHF, FFA and MF. Standard HMF, F,

AF, DMHF, FFA and MF (Fluka) were injected into the chromatographic system to identify the corresponding retention time and elution profile in the chromatogram of the browning sample solutions. Triplicate samples were prepared, and each sample was injected 3 times. Each experiment was repeated 4 times.

Statistical analysis

All experimental data were analyzed by analysis of variance (ANOVA) and significance of differences among means from triplicate analysis at ($p<0.05$) were determined by Duncan's multiple range tests using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The results obtained in the determination of furfural compounds are shown in Tables 1, 2 and 3. Numerous furfural compounds can be formed in processed food during thermal processing or storage at inappropriate temperatures (28). HMF is not present in fresh, untreated foods, but it is formed as an intermediate product of the Maillard reaction upon heating at high temperatures (29,30). It is also formed from the degradation of sugars as a result of caramelisation (31). Although the toxicological relevance of HMF is not clear, as in vitro studies on genotoxicity and mutagenicity have given controversial results (32-34), its accumulation is considered an undesirable byproduct of the Maillard reaction. F is another common Maillard product, differing from HMF by the absence of the aldehyde group in 2 positions.

Table 1 shows the content of furfural compounds by amino acids enantiomers at pH 4.0. In glucose-based systems, HMF, FFA, AF and MF were detected. Particularly, HMF and AF were found in all systems, except for the Glc/D-Lys system. The content of HMF (18.13 mM/L) and AF (20.77 mM/L) were highest in the Glc/L-Asn system. The content of HMF and AF in glucose-based systems exhibited the following order: L-Asn> D-Asn ≥ Gly> L-Lys. According to the isomers, HMF and AF content from the L-isomer were higher compared with D-isomer. Moreover, FFA and MF were found in Glc/L-Lys and Glc/D-Lys systems, but only in small quantities. On the other hand, in fructose-based systems, all furfural compounds were detected. HMF and AF were found in all systems, except for the Fru/L-Lys and Fru/D-Lys systems. The content of HMF (8.09 mM/L) and AF (9.26 mM/L) were highest in Fru system. The content of HMF and AF in fructose-based systems exhibited the following order: Fru> Gly> L-Asn> D-Asn. Specially, DMHF was only found in the Fru/D-Lys system. The differences became significant ($p<0.05$) according to amino acids enantiomers. HMF and AF content from the L-isomer were especially higher compared with the D-isomer.

The content of furfural compounds formed in the presence of different amino acids enantiomers at pH 7.0 is shown in Table 2. In glucose-based systems, all furfural compounds were detected. HMF and AF were found in all systems, except for the Glc system. The content of HMF (7.67 mM/L) and AF (8.79 mM/L) were highest in the Glc/D-Lys system. The content of HMF and AF in glucose-based systems exhibited the following order: D-Lys> L-Asn> L-Lys≥ D-Asn> Gly. Moreover, amounts of furfural compounds formed were significantly different according to amino acids enantiomers ($p<0.05$). The contents of HMF and AF in Glc/L-Asn and Glc/D-Lys system were more than their isomer. In addition, FFA and MF were present except in the Glc system, but the

Table 1. Contents of furfural compounds by amino acid enantiomers at pH 4.0[1)]

Samples	pH 4.0					
	HMF	FFA	F	DMHF	AF	MF
Glc	0.51±0.03[2)d3)]	−	−	−	0.58±0.03[d]	−
Glc/Gly	15.18±0.76[b]	−	−	−	17.39±0.87[b]	−
Glc/L-Asn	18.13±0.91[a]	−	−	−	20.77±1.04[a]	−
Glc/D-Asn	16.49±0.82[b]	−	−	−	18.89±0.94[b]	−
Glc/L-Lys	3.51±0.18[c]	0.02±0.00[a]	−	−	4.02±0.20[c]	0.02±0.00[a]
Glc/D-Lys	−	0.01±0.00[b]	−	−	−	0.01±0.00[b]
Fru	8.09±0.40[a]	−	−	−	9.26±0.46[a]	−
Fru/Gly	5.91±0.30[b]	−	−	−	6.77±0.34[b]	−
Fru/L-Asn	2.19±0.11[c]	−	−	−	2.50±0.13[c]	−
Fru/D-Asn	1.91±0.10[d]	−	−	−	2.19±0.11[d]	−
Fru/L-Lys	−	0.01±0.00[a]	0.01±0.00[b]	−	−	0.01±0.00[a]
Fru/D-Lys	−	0.01±0.00[a]	0.04±0.00[a]	0.70±0.19[a]	−	0.01±0.00[a]

[1)]Data expressed as mM/L of furfural compound.
[2)]Values are mean±standard deviation of three experiments.
[3)]Means in a column followed by different superscripts are significantly different at the $p<0.05$ level.

Table 2. Contents of furfural compounds by amino acid enantiomers at pH 7.0[1)]

Samples	pH 7.0					
	HMF	FFA	F	DMHF	AF	MF
Glc	−	−	−	−	−	−
Glc/Gly	3.28±0.16[2)d3)]	0.02±0.00[b]	−	−	3.76±0.19[d]	0.01±0.00[b]
Glc/L-Asn	5.97±0.30[b]	0.01±0.00[c]	−	−	6.84±0.34[b]	0.01±0.00[b]
Glc/D-Asn	4.47±0.22[c]	0.01±0.00[c]	0.01±0.00[a]	0.42±0.02[b]	5.12±0.26[c]	0.01±0.00[b]
Glc/L-Lys	4.59±0.23[c]	0.01±0.00[c]	−	0.71±0.04[a]	5.25±0.26[c]	0.01±0.00[b]
Glc/D-Lys	7.67±0.38[a]	0.03±0.00[a]	0.01±0.00[a]	−	8.79±0.40[a]	0.02±0.00[a]
Fru	0.08±0.00[e]	0.01±0.00[c]	−	−	0.09±0.00[d]	0.01±0.00[a]
Fru/Gly	2.00±0.10[d]	0.03±0.00[a]	−	−	2.29±0.11[c]	0.02±0.00[a]
Fru/L-Asn	2.17±0.11[c]	0.02±0.00[b]	−	−	2.48±0.12[c]	0.02±0.00[a]
Fru/D-Asn	2.65±0.13[b]	0.02±0.00[b]	−	−	3.03±0.15[b]	0.02±0.00[a]
Fru/L-Lys	8.36±0.42[a]	0.02±0.00[b]	0.02±0.00[a]	−	9.57±0.48[a]	0.01±0.00[b]
Fru/D-Lys	8.07±0.40[a]	0.03±0.00[a]	0.01±0.00[b]	−	9.24±0.46[a]	0.02±0.00[a]

[1)]Data expressed as mM/L of furfural compound.
[2)]Values are mean±standard deviation of three experiments.
[3)]Means in a column followed by different superscripts are significantly different at the $p<0.05$ level.

contents were remarkably small. F was only detected in D-isomer system. DMHF was found in Glc/D-Asn (0.42 mM/L) and Glc/L-Lys (0.71 mM/L) systems. On the other hand, in fructose-based systems, all of the furfural compounds were detected, except for DMHF. The content of HMF (8.36 mM/L) and AF (9.57 mM/L) were highest in the Fru/L-Lys system. The content of HMF and AF in fructose-based systems exhibited the following order: L-Lys> D-Lys> D-Asn> L-Asn> Gly> Fru. Moreover, furfural compounds varied according to amino acids enantiomers. The contents of HMF and AF in Fru/D-Asn and Fru/L-Lys system were more than their isomer. In addition, FFA and MF were found in all systems, while F was only detected in Fru/L-Lys and Fru/D-Lys system, but the contents were remarkably small.

The content of furfural compounds by amino acids enantiomers at pH 10.0 is shown in Table 3. In glucose-based systems, FFA, F, DMHF and MF were detected. However, the furfural compounds were not detected in Glc/Gly system. The content of FFA (0.04 mM/L) and MF (0.03 mM/L) were highest in the Glc system, while F was highest in the Glc/D-Lys system, but the content was small. Moreover, DMHF was only found in Glc/D-Asn and Glc/L-Lys systems. According to amino acids enantiomers, the furfural compounds were not significantly different, except for F of Glc/L-Lys and Glc/D-Lys. On the other hand, in fructose-based systems, all of the furfural compounds were detected. Only HMF and AF were found in Fru/Gly systems. The content of FFA and MF were highest in the Fru system, but the content was small. F was found in all systems, except for Fru and Fru/Gly systems. Furthermore, DMHF was only found in Fru/D-Lys systems, but in small quantities.

Table 3. Contents of furfural compounds by amino acid enantiomers at pH 10.0[1)]

Samples	pH 10.0					
	HMF	FFA	F	DMHF	AF	MF
Glc	−	0.04±0.00[a]	0.01±0.00[c]	−	−	0.03±0.00[a]
Glc/Gly	−	−	−	−	−	−
Glc/L-Asn	−	0.01±0.00[b]	0.01±0.00[c]	−	−	0.01±0.00[b]
Glc/D-Asn	−	0.01±0.00[b]	0.01±0.00[c]	0.14±0.01[b]	−	0.01±0.00[b]
Glc/L-Lys	−	0.01±0.00[b]	0.02±0.00[b]	1.06±0.05[a]	−	0.01±0.00[b]
Glc/D-Lys	−	0.01±0.00[b]	0.05±0.00[a]	−	−	0.01±0.00[b]
Fru	−	0.05±0.00[a]	−	−	−	0.04±0.00[a]
Fru/Gly	0.16±0.01[2)a3)]	0.01±0.00[c]	−	−	0.18±0.01[a]	0.01±0.00[c]
Fru/L-Asn	−	0.02±0.00[b]	0.01±0.00[c]	−	−	0.02±0.00[b]
Fru/D-Asn	−	0.02±0.00[b]	0.02±0.00[b]	−	−	0.02±0.00[c]
Fru/L-Lys	−	−	0.80±0.00[a]	−	−	−
Fru/D-Lys	−	0.01±0.00[c]	0.02±0.00[b]	1.06±0.05[a]	−	0.01±0.00[c]

[1)]Data expressed as mM/L of furfural compound.
[2)]Values are mean±standard deviation of three experiments.
[3)]Means in a column followed by different superscripts are significantly different at the $p<0.05$ level.

In summary, the content of HMF was highest at pH 4.0, and the content decreased with increasing pH. HMF was especially higher in glucose-based systems than in fructose-based systems. This higher conversion of glucose into HMF could be due to a glucose-specific reaction pathway involving a 1,2-endiol intermediate in acidic media (31,35-37). FFA and MF were not increased as the pH increased, and furthermore the content was quite small. In addition, F was found in most systems with increasing pH. However, the content was small and variable. Ferrer et al. (38) reported that F contents vary in an irregular way with the storage time and temperature. DMHF was only found in Glc/D-Asn, Glc/L-Lys and Fru/D-Lys systems, but the content was not increased. AF was high in Glc (or Fru)/L-Lys and Glc (or Fru)/D-Lys system at pH 7.0. However, at pH 4.0, the content of AF was higher in Glc (or Fru)/Gly and Glc (or Fru)/L-Asn systems. According to Mottram and Leseigneur (39), concentrations of furans/furfural/furanones (DMHF) dropped markedly as the pH increased in model systems containing ribose and amino acids.

In conclusion, this study observed the effect of pH, sugars and animo acid enantiomers on the different furfural contents in the Maillard reaction. A clear tendency was observed for some classes of compounds to be more readily formed at higher or lower pH. HMF was more readily formed at a lower pH, while FFA, F, DMHF and MF were inhibited by acidic conditions. Particularly, compounds like FFA, F and MF were not affected by pH changes. In addition, DMHF and MF were only formed in L-Lys and D-Lys systems.

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REFERENCES

1. Maillard LC. 1913. Formation de matieres humiques par action de polypeptides sur sucres. *C R Acad Sci* 156: 148-149.
2. Wijewickreme AN, Kitts DD, Durance TD. 1997. Reaction conditions influence the elementary composition and metal chelating affinity of nondialyzable model Maillard reaction products. *J Agric Food Chem* 45: 4577-4583.
3. Friedman M. 1999. Chemistry, nutrition, and microbiology of D-amino acids. *J Agric Food Chem* 47: 3457-3479.
4. Brückner H, Justus J, Kirschbaum J. 2001. Saccharide induced racemization of amino acids in the course of the Maillard reaction. *Amino Acids* 21: 429-433.
5. Ledl F, Schleicher E. 1990. New aspects of the Maillard reaction in foods and in the human body. *Angew Chem Int Ed Engl* 29: 565-594.
6. Pätzold R, Nieto-Rodriguez A, Brückner H. 2003. Chiral gas chromatographic analysis of amino acids in fortified wines. *Chromatographia Suppl* 57: S207-S212.
7. Pätzold R, Brückner H. 2005. Mass spectrometric detection and formation of D-amino acids in processed plant saps, syrups, and fruit juice concentrates. *J Agric Food Chem* 53: 9722-9729.
8. Pätzold R, Brückner H. 2006a. Gas chromatographic detection of D-amino acids in natural and thermally treated bee honeys and studies on the mechanism of their formation as result of the Maillard reaction. *Eur Food Res Tech* 223: 347-354.
9. Pätzold R, Brückner H. 2006b. Gas chromatographic determination and mechanism of formation of D-amino acids occurring in fermented and roasted cocoa beans, cocoa powder, chocolate and other cocoa products. *Amino Acids* 31: 63-72.
10. Bada JL. 1972. Kinetics of racemization of amino acids as a function of pH. *J Am Chem Soc* 94: 1371-1373.
11. Yeo H, Shibamoto T. 1991. Effects of moisture content on the Maillard browning model system upon microwave irradiation. *J Agric Food Chem* 39: 1860-1862.
12. Espinosa-Mansilla A, Salinas F, Berzas-Nevado JJ. 1992. Differential determination of furfural and hydroxymethylfurfural by derivative spectrophotometry. *J AOAC Int* 75: 678-684.
13. Morales FJ, Romero C, Jimenez-Pérez S. 1992. An enhanced liquid chromatography method for 5-hydroxymethylfurfural determination in UHT milk. *Chromatographia* 33: 45-48.
14. Blanco-Gomis D, Gutierrez-Alvarez MD, Sopena-Naredo L, Mangas-Alonso JJ. 1991. High-performance liquid chromatographic determination of furfural and hydroxymethylfurfural in apple juices and concentrates. *Chromatographia* 32: 45-48.
16. Sanz C, Perez AG, Richardson DG. 1994. Simultaneous HPLC determination of 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone and related flavor compounds in strawberries. *J Food Sci* 59: 139-141.
17. Eiserich JP, Macku C, Shibamoto T. 1992. Volatile antioxidants formed from an L-cysteine/D-glucose Maillard model system. *J Agric Food Chem* 40: 1982-1988.
19. Blank I, Fay LB. 1996. Formation of 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(*2H*)-furanone through Maillard reaction based on pentose sugars. *J Agric Food Chem* 44: 531-536.
20. Lee HS, Nagy S. 1988. Relationship of sugar degradation to detrimental changes in citrus juice quality. *Food Technol* 11: 91-97.
21. Mijares RM, Park GL, Nelson DB, Mciver RC. 1986. HPLC analysis of HMF in orange juice. *J Food Sci* 51: 843-844.
22. Tu D, Xue S, Meng C, Espinosa-Mansilla A, De la Pena AM, Lopez FS. 1992. Simultaneous determination of 2-furfuraldehyde and 5-(hydroxymethyl)-2-furfuraldehyde by derivative spectrophotometry. *J Agric Food Chem* 40: 1022-1025.
23. Dinsmore HL, Nagy S. 1974. Improved calorimetric determination for furfural in citrus juices. *J Assoc Off Anal Chem* 57: 332.
24. Marcy JE, Rouseff RL. 1984. High-performance liquid

chromatographic determination of furfural in orange juice. *J Agric Food Chem* 32: 979-981.
25. Guerra-Hernandez E, Garcia-Vilanova B, Montilla-Gomez J. 1992. Determination of hydroxymethylfurfural in baby cereals by high performance liquid chromatography. *J Liq Chromatogr* 15: 2551-2559.
25. Krammer GE, Takeoka GR, Buttery RG. 1994. Isolation and identification of 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone glucoside from tomatoes. *J Agric Food Chem* 42: 1595-1597.
26. Lo Coco F, Ceccon L, Valentini C, Novelli V. 1992. High-performance liquid chromatographic determination of 2-furaldehyde in spirits. *J Chromatogr* 590: 235-240.
27. Albalá-Hurtado S, Veciana-Nogués MT, Mariné-Font A, Vidal-Carou MC. 1998. Changes in furfural compounds during storage of infant milks. *J Agric Food Chem* 46: 2998-3003.
28. Lo-Coco F, Valentini C, Novelli V, Ceccon L. 1994. High performance liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in processed citrus juices. *J Liq Chromatog* 17: 603-617.
28. Naim M, Wainish S, Zehavi U, Peleg H, Rouseff RL, Nagy S. 1993. Inhibition by thiol compounds of off-flavor formation in stored orange juice. 1. Effect of L-cysteine and N-acetyl-cysteine on 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone formation. *J Agric Food Chem* 41: 1355-1358.
29. Berg HE, van Boekel MAJS. 1994. Degradation of lactose during heating of milk. 1. Reaction pathways. *Neth Milk Dairy J* 48: 157-175.
30. Morales FJ, Romero C, Jiménez-Pérez S. 1997. Chromatographic determination of bound hydroxymethylfurfural as an index of milk protein glycosylation. *J Agric Food Chem* 45: 1570-1573.
31. Kroh LW. 1994. Caramelisation in food and beverages. *Food Chem* 51: 373-379.
32. Cuzzoni MT, Stoppini G, Gazzani G, Mazza P. 1988. Influence of water activity and reaction temperature of ribose-lysine and glucoselysine Maillard systems on mutagenicity, absorbance and content of furfurals. *Food Chem Toxicol* 26: 815-822.
33. Janzowski C, Glaab V, Samimi E, Schlatter J, Eisenbrand G. 2000. 5-hydroxymethylfurfural: assessment of mutagenicity, DNA damaging potential and reactivity towards cellular glutathione. *Food Chem Toxicol* 38: 801-809.
34. Lee YC, Shlyankevich M, Jeong HK, Douglas JS, Surh Y. 1995. Bioactivation of 5-hydroxymethyl-2-furaldehyde to an electrophilic and mutagenic allylic sulfuric-acid ester. *Biochem Biophys Res Comm* 209: 996-1002.
35. Antonelli A, Chinnici F, Masino F. 2004. Heat-induced chemical Antonelli, A during the production of traditional balsamic vinegar: a preliminary approach. *Food Chem* 88: 63-68.
36. Hodge JE. 1953. Chemistry of browning reactions in model systems. *J Agric Food Chem* 1: 928-943.
37. Yeboah FK, Alli I, Yaylayan VA. 1999. Reactivities of D-glucose and D-fructose during glycation of bovine serum albumin. *J Agric Food Chem* 47: 3164-3172.
38. Ferrer E, Alegria A, Farre R, Abellán P, Romero F. 2002. High-performance liquid chromatographic determination of furfural compounds in infant formulas－Changes during heat treatment and storage. *J Chromatogr A* 947: 85-95.
39. Mottram DS, Leseigneur A. 1990. The effect of pH on the formation of aroma volatiles in meat-like model systems. In *Flavour Science and Technology.* Bessiere Y, Thomas AF, eds. John Wiley & Sons, Chichester, UK. p 121-124.

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