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Mechanistic Insights into Furan Formation in Maillard Model Systems

Fien Van Lancker,[†] An Adams,[†] Agnieszka Owczarek-Fendor,[†,‡] Bruno De Meulenaer,[‡] and Norbert De Kimpe*,[†]

[†]Department of Sustainable Organic Chemistry and Technology and [‡]nutriFOODchem unit, Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium

Furan has recently received considerable attention as a possibly carcinogenic compound occurring in thermally processed foods. Although several food constituents have been identified as furan precursors, multiple formation pathways remain unclear. Therefore, the mechanisms of furan formation in Maillard model systems were studied by means of the carbon module labeling (CAMOLA) technique. Under both roasting and pressure-cooking conditions, furan was formed from glucose via the intact skeleton, and its formation pathways from glucose alone were not amino acid-dependent. However, some amino acids, especially alanine and serine, did influence the furan production by providing an additional formation pathway. Furthermore, most amino acids enhanced the furan production from glucose. Roasting conditions produced 25−100 times higher amounts of furan as compared to pressure-cooking conditions. Surprisingly, in the alanine/glucose model systems, the relative importance of furan production from glucose alone and from the combination of a glucose-derived and an alanine-derived fragment changed completely over a limited time course of 60 min.

KEYWORDS: Furan; Maillard reaction; model systems; CAMOLA; SPME-GC-MS; alanine

INTRODUCTION

Chemical food safety has become an area of concern throughout the past decades. With regard to this topic, the presence of furan in food products, among other contaminants, has become the subject of many studies. Although this compound has been known for a long time as a food constituent (*1*), it received little attention until the report of its possible carcinogenic properties in 1995 (*2*). Since then, it has been classified as "possibly carcinogenic to humans" (group 2B) by the International Agency for Research on Cancer (IARC) (*2*).

It is known that furan is formed as a contaminant during heating processes used for the manufacturing of foods (*1*). Therefore, it is not surprising that high amounts of furan were detected in foods which were subjected to very strong heat treatments during their preparation, such as coffee (*3*). In addition, elevated furan levels were also found in foods that were heat-processed in cans or jars, such as baby foods, as compared to foods that were heat-processed in an open system (*3*−*5*). This difference is due to the high volatility of furan (boiling point = 31 °C; 760 mmHg), which causes the evaporation of furan in an open system. Besides the heating processes during manufacturing, it has been shown that consumer cooking also affects the amounts of furan in food products (*4*, *6*). As expected from its low boiling point, furan concentrations significantly decrease when a food product is reheated at moderate temperatures, especially in a saucepan or when it is stirred during reheating. However, it has been reported that volatilization of furan during food handling and food preparation strongly depends on the food matrix (*7*). Especially lipophilic compounds, such as oils, caused significant retention of furan.

Several precursors of furan have been identified, such as ascorbic acid, sugars, amino acids, Maillard reaction systems, unsaturated fatty acids, and carotenoids (*8*−*15*). Various studies have reported that the highest levels of furan are formed from the degradation of ascorbic acid (*8*, *10*, *11*, *15*). However, because the amounts of ascorbic acid in foods are relatively low as compared to sugars, amino acids, and unsaturated fatty acids, it can be expected that the latter food constituents are more important for the production of furan in foods. In addition to the identification of the precursors, other studies focused on the reaction mechanisms leading to the formation of furan from these precursors (*8*, *12*, *13*). For instance, Limacher et al. (*12*) studied the formation mechanisms of furan from ascorbic acid in model systems. These authors found that high amounts of furan were produced from ascorbic acid alone, whereas binary mixtures (with carbohydrates or amino acids) lowered the furan production. With regard to the mechanistic pathways, it was found that ascorbic acid degradation yielded furan exclusively from its intact skeleton. Furan was mainly formed from C-3 to C-6, generated by splitting off two C_1 units. In addition to ascorbic acid, two studies focused on the mechanisms of furan formation from carbohydrates, amino acids, and Maillard reaction systems (*8*, *13*).

*Author to whom correspondence should be addressed (e-mail Norbert.DeKimpe@UGent.be; phone 00 32 9 264 59 51; fax 00 32 9 264 62 43).

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Both studies showed that, under dry reaction conditions, furan formation from hexoses mainly proceeds via the intact sugar skeleton. Especially the C-3−C-6 fragment generated furan. However, according to Limacher et al. (*13*), fragmentation and recombination of the sugar skeleton also yields furan production under pressure-cooking conditions. In addition, Perez Locas and Yaylayan (*8*) showed that furan can be formed from serine or cysteine in the absence of carbohydrates.

However, many reaction steps and influencing factors remain unclear. For instance, the influence of different amino acids on the mechanism of furan formation from sugars has not been studied yet. However, amino acid dependency has been shown, for instance, for the formation of 2-acetylfuran (*16*). Therefore, the objective of this study was to investigate the amino acid dependency of the formation pathways of furan from glucose. These formation pathways were studied in model systems simulating both roasting and pressure-cooking conditions, by means of the carbon module labeling (CAMOLA) technique (*17*, *18*). In addition, the amounts of furan, produced during these reactions, were determined to study whether certain amino acids decrease or enhance the formation of furan from glucose. In another series of experiments, the progress of the different formation pathways of furan from glucose/alanine model systems was studied as a function of time, under both roasting and pressure-cooking conditions.

MATERIALS AND METHODS

Reagents. Furan (≥99%), glucose (99.5%), glycine (99%), threonine (98%), and purified sea sand (50−70 mesh) were purchased from Sigma-Aldrich (Bornem, Belgium). Phenylalanine (98.5%), cysteine (97%), and serine (99%) were purchased from Janssen Chimica (Geel, Belgium). d_4-furan (99%), alanine (99%), lysine monohydrate (99%), arginine (>98%), and proline (>99%) were purchased from Acros Organics (Geel, Belgium). U-$^{13}C_6$-glucose (99%) was purchased from Cambridge Isotope Laboratories (Andover, MA). Methanol (>99.99%) was of analytical grade.

CAMOLA Experiments. *Preparation of the Samples*. For roasting conditions, 0.25 mmol of $^{12}C_6$-glucose, 0.25 mmol of $^{13}C_6$-glucose, and 1 g of purified sea sand, with or without 0.5 mmol of amino acid, were mixed in a 20 mL headspace vial (Gerstel, Mülheim a/d Ruhr, Germany), used as a reaction vessel, which was sealed with a magnetic crimp cap. The samples were heated at 180 °C for 20 min in a stirred oil bath, simulating roasting conditions.

For pressure-cooking conditions, 0.25 mmol of $^{12}C_6$-glucose and 0.25 mmol of $^{13}C_6$-glucose, with or without 0.5 mmol of amino acid, were weighed in a 1 mL volumetric flask. In the case of model systems without amino acid and with glycine, alanine, proline, phenylalanine, cysteine, serine, or threonine, water was added to obtain the final volume of 1 mL. The pH of the resulting mixture was 6. In the case of lysine and arginine, HCl (2 N) was added until pH 6 was reached and, afterward, water was added to obtain the final volume of 1 mL. The reaction mixtures (1 mL) were transferred in a 20 mL headspace vial, which was sealed with a magnetic crimp cap. The samples were heated at 121 °C for 25 min in a stirred oil bath, simulating pressure-cooking conditions.

All experiments were carried out in triplicate by using three reaction vessels, simultaneously immersed into the oil bath. In the case of $^{12}C_6$-glucose, the results were corrected for natural ^{13}C.

Analysis of the Samples. The samples were analyzed by means of solid phase microextraction (SPME) coupled with GC-MS. SPME experiments were carried out using a 75 μm carboxen−polydimethylsiloxane (CAR-PDMS) fiber (Supelco, Bornem, Belgium). The fiber was exposed to the headspace for 25 min at 35 °C. Desorption was carried out at 300 °C for 5 min. SPME and desorption were performed automatically by means of an MPS-2 autosampler (Gerstel).

GC-MS analyses of the SPME extracts were performed with an Agilent 6890 GC Plus coupled to a quadrupole mass spectrometer 5973 MSD (Agilent Technologies, Diegem, Belgium), equipped with a Varian CP-PoraBOND Q capillary column (25 m length × 0.32 mm i.d.; coating 5 μm film thickness). Working conditions were as follows: injector, 300 °C; transfer line to MSD, 250 °C; carrier gas (He), 1.2 mL min^{-1}; SPME desorption in a CIS-4 PTV injector (Gerstel) in splitless mode; ionization, EI 70 eV. The oven temperature was programmed from 50 to 260 at 8 °C min^{-1} and held for 7 min. Analyses were performed in selective ion monitoring (SIM) mode. MS signals at m/z 68, 69, 70, 71, and 72 were recorded. Corrections were applied for the contribution of natural ^{13}C.

Quantification Experiments. For the quantification experiments, preparation of the samples was performed following a similar procedure as for the CAMOLA experiments. However, in these quantification experiments, no labeled glucose was used. For pressure-cooking conditions, 2.5 mmol of each precursor was dissolved in water to obtain the final volume of 5 mL. Exactly 1 mL of reaction mixture was transferred in a 20 mL headspace vial afterward.

Preparation of the Standards. Stock solutions of furan and d_4-furan were prepared by adding 10 μL of (d_4-)furan via a gastight syringe through the septum of a weighed 10 or 20 mL headspace vial containing 10 or 20 mL of methanol, for roasting and pressure-cooking conditions, respectively. The mixture was then weighed again to determine the exact concentration of furan. Stock solutions were stored at 4 °C for no longer than 5 days. Working solutions were prepared daily by using the same procedure as for the preparation of the stock solutions. For d_4-furan, 200 or 100 μL of stock solution was added to a 10 or 20 mL headspace vial containing 10 or 20 mL of water, using the same procedure, for roasting and pressure-cooking conditions, respectively. For native furan, two working solutions were prepared by adding 20 or 200 μL of stock solution to a 10 mL headspace vial for roasting conditions and 10 or 100 μL of stock solution to a 20 mL headspace vial for pressure-cooking conditions.

For the determination of the furan concentration, calibration standards were prepared daily by injecting aqueous working solutions (20, 50, or 250 μL of the first working solution or 100 or 250 μL of the second working solution) of furan and a fixed volume (200 or 100 μL, for roasting and pressure-cooking conditions, respectively) of d_4-furan into a 20 mL headspace vial containing 1 mL of water by means of a gastight syringe. This yielded exactly known furan solutions. Calibration standards were kept in ice during preparation and were closed immediately afterward.

Spiking of the Samples. All samples were kept in ice before and during spiking, to minimize loss of furan. The headspace vial was opened, spiked with 50 μL of d_4-furan working solution in water, using a gastight syringe, and then sealed again. All samples were vortexed for at least 20 s prior to analysis to ensure homogenization and were analyzed in triplicate.

Analysis of the Samples. SPME experiments and GC-MS analyses were performed as described for the CAMOLA experiments. Analyses were also performed in SIM mode, but quantification was based on MS signals at m/z 68 for furan and at m/z 72 for d_4-furan. The following qualifiers were used: m/z 39 for furan and m/z 42 for d_4-furan.

Statistical Analysis. Differences in furan concentrations were statistically evaluated by means of standard deviations (n = 3) and Student's t test (α = 0.05).

RESULTS AND DISCUSSION

It has been shown that amino acids can influence the formation pathways of certain degradation products of sugars (*16*). Therefore, it was decided to evaluate the amino acid dependency of the formation pathways of furan from glucose. To gain insight into the different formation mechanisms, the CAMOLA technique was used. CAMOLA is a powerful technique to elucidate different pathways for a specific compound and to evaluate the relative importance of each pathway (*17*). Equimolar amounts of unlabeled and fully labeled U-$^{13}C_6$-glucose were mixed with or without an amino acid, and the different isotopomers of furan were analyzed by GC-MS. As illustrated in **Figure 1**, in theory, furan can be formed from glucose either via the intact sugar skeleton or via fragmentation and recombination. In the case when the glucose skeleton is kept intact during furan formation, only U-$^{12}C_4$ and U-$^{13}C_4$ furan should be obtained. On the other hand, if fragmentation and recombination occur, partially labeled isotopomers will be observed (*18*). The addition of amino acids to the mixture of unlabeled and labeled glucose complicates the interpretation of the

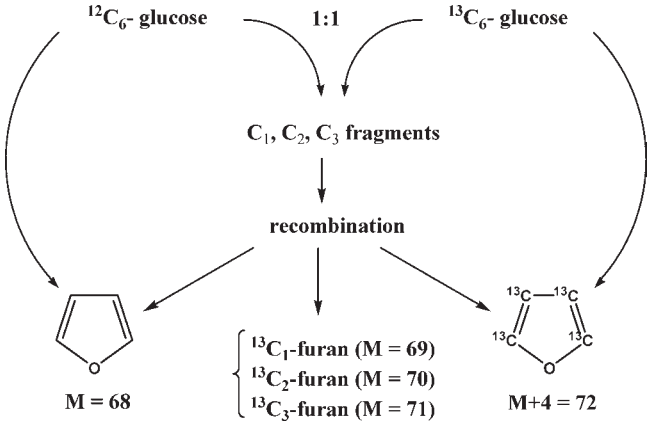

Figure 1. Formation pathways of the different isotopomers of furan during the CAMOLA experiments.

Table 1. Percent Labeling Distribution of Furan Generated in Model Systems Containing Glucose (Equimolar Amounts of $^{12}C_6$-Glucose and $^{13}C_6$-Glucose) and Different Amino Acids under Roasting Conditions (20 min at 180 °C)[a]

	% M	% (M + 1)	% (M + 2)	% (M + 3)	% (M + 4)
glucose	47.5 ± 2.0	0.0 ± 0.3	0.1 ± 0.0	2.8 ± 0.3	47.6 ± 1.9
glucose + Gly	46.3 ± 1.6	0.6 ± 0.1	0.5 ± 0.2	3.6 ± 0.2	47.0 ± 1.5
glucose + Ala	48.8 ± 0.7	1.1 ± 0.1	13.2 ± 0.7	2.6 ± 0.2	32.2 ± 1.3
glucose + Phe	47.8 ± 1.2	0.3 ± 0.3	0.2 ± 0.0	2.9 ± 0.4	46.7 ± 0.9
glucose + Arg	48.1 ± 0.9	1.1 ± 0.0	1.3 ± 0.1	3.5 ± 0.1	43.9 ± 0.8
glucose + Pro	48.4 ± 1.1	0.2 ± 0.1	0.8 ± 0.0	2.8 ± 0.2	45.7 ± 0.9
glucose + Lys	52.8 ± 2.2	0.4 ± 0.1	0.4 ± 0.1	2.7 ± 0.2	41.3 ± 1.9
glucose + Cys	46.6 ± 0.4	0.2 ± 0.0	0.9 ± 0.1	2.9 ± 0.0	47.3 ± 0.4
glucose + Ser	55.9 ± 2.9	0.4 ± 0.2	2.5 ± 0.0	3.3 ± 0.2	39.6 ± 1.1
glucose + Thr	48.2 ± 1.3	0.2 ± 0.2	2.1 ± 0.1	3.0 ± 0.1	44.4 ± 1.6

[a] Values given are averages of triplicate measurements ± standard deviation.

obtained results to some extent, because degradation products of amino acids can also be incorporated into the furan skeleton. However, it is clear that when an amino acid, which is not labeled, is incorporated in the furan skeleton, the partitioning over the different isotopomers of furan becomes asymmetric [% M > % (M+4)]. When the amino acid is not incorporated, the partitioning over the different isotopomers remains symmetric [% M = % (M + 4)], as in case of model systems without amino acids.

It was decided to perform the experiments simulating pressure-cooking conditions without buffer, because it has been shown that the anionic species of the buffer can exert a severe catalytic effect. This has been extensively pointed out for the phosphate ion (*19*, *20*). Preliminary model reactions indeed showed a clear effect of several buffers tested (phosphate and BisTris) on the formation of furan (data not shown). The influence of phosphate on the formation of furan was also shown by Fan et al. (*15*). In the unbuffered systems, the pH remained higher than 5 during heating at 121 °C for 25 min.

Furan Formation in Maillard Reaction Systems. *Roasting Conditions*. The results obtained from the CAMOLA experiments under dry-roasting conditions are depicted in **Table 1**. Corrections were applied for the contribution of natural ^{13}C. As can be seen from the labeling pattern of the experiment without amino acids, glucose-derived furan was almost exclusively formed via the intact sugar skeleton. In addition, because equimolar amounts of labeled and unlabeled glucose were used, a symmetric distribution over the different isotopomers is expected. Therefore, it is assumed that the presence of (M + 3) is caused by impurities of the ^{13}C-labeled glucose. Consequently, formation of furan from glucose via fragmentation and recombination does not take place at all. Similar results were obtained by Limacher et al. (*13*). These authors also used labeled precursors to investigate the formation of furan from carbohydrates and Maillard model systems and reported that both glucose and fructose yielded furan only via the intact sugar skeleton. However, addition of phenylalanine resulted in traces of the (M + 2) isotopomer, indicating a minor furan formation pathway by the recombination of two C_2 sugar fragments. This was not confirmed in this study.

From **Table 1**, it can be seen that alanine and serine are incorporated the most into the furan skeleton. For alanine, the sum of the percentages of (M + 2) and (M + 4) was as good as equal to the percentage of M. This means that alanine provided only one C_2 fragment. This is in accordance with the general assumption that alanine degrades into acetaldehyde, which subsequently condenses with glycolaldehyde derived from glucose to form furan (*8*). About 26% [% (M + 2) × 2] of the total amount of furan was formed via this pathway. The remaining amount of furan resulted from the intact sugar skeleton.

For serine, the percentage of M was much higher than the sum of the percentages of (M + 2) and (M + 4), which implies that serine provided two C_2 fragments for the production of furan, most probably acetaldehyde and glycolaldehyde. About 5% of the total amount of furan was formed from the condensation of glucose-derived glycolaldehyde and serine-derived acetaldehyde, whereas about 14% was formed from the condensation of acetaldehyde and glycolaldehyde, both derived from serine. This implies that serine produced more glycolaldehyde than glucose. Production of furan from serine in the absence of other reactive species has already been demonstrated by Perez Locas and Yaylayan (*8*). These authors also found that in the model systems where excess serine was reacted with glucose (molar ratio 3:1), no furan was formed from the interaction of a glucose and a serine fragment. However, in an excess of glucose, 5% of the total amount of furan was formed from a glucose and a serine C_2 fragment, whereas 15% was formed from the condensation of acetaldehyde and glycolaldehyde, both derived from serine. These percentages are almost the same as in this study, where equimolar amounts of serine and glucose were used.

Furthermore, because for arginine, lysine, and threonine the percentage of M is higher than the percentage of (M + 4), it can be concluded that these amino acids were also incorporated into furan, but to a lower extent. Although cysteine has also been reported to produce furan upon thermal treatment in the absence of any other reactive species (*8*), this was not confirmed in this study.

With regard to the amounts of furan produced during roasting, it can be seen from **Table 2** that most amino acids enhanced the formation of furan from glucose, although they were often not or only to a low extent incorporated into the furan skeleton. This is in accordance with the findings of Perez Locas and Yaylayan (*8*) and Limacher et al. (*13*) and could be expected, because it is known that amino acids enhance the degradation of sugars (*21*). However, the production of furan from Maillard reaction systems in the study of Perez Locas and Yaylayan (*8*) was somewhat different as in this study. Perez Locas and Yaylayan (*8*) reported that the highest amounts of furan were produced in the glucose/cysteine model system, followed by glucose/serine and glucose/alanine, whereas glucose/glycine and glucose/threonine produced the least furan. The major difference between the latter and this study is the high furan production from the glucose/cysteine model system. It is assumed that the differing reaction conditions (pyrolysis at 250 °C) cause this difference, because these authors also reported the production of furan from cysteine in the absence of any other reactive species, which was not confirmed in this study.

As can be seen from **Table 2**, two amino acids, namely, proline and lysine, did not enhance the formation of furan from glucose.

Table 2. Amounts of Furan Generated in Various Model Systems Containing Glucose and Amino Acids, under Roasting (20 min at 180 °C) and Pressure-Cooking (25 min at 121 °C) Conditions[a]

	furan (μmol/mol of glucose)	
	roasting	pressure cooking
glucose	15.1 ± 1.4 a	below limit of quantification[b]
glucose + Gly	47.3 ± 3.9 bc	1.15 ± 0.09 a
glucose + Ala	73.2 ± 3.6 d	0.75 ± 0.07 b
glucose + Phe	34.5 ± 0.4 e	/[c]
glucose + Arg	43.0 ± 1.6 c	0.87 ± 0.12 b
glucose + Pro	13.3 ± 1.1 a	0.22 ± 0.04 c
glucose + Lys	6.4 ± 0.5 f	0.98 ± 0.14 abd
glucose + Cys	47.4 ± 0.6 b	below limit of quantification
glucose + Ser	63.8 ± 4.6 g	1.38 ± 0.05 ae
glucose + Thr	39.3 ± 0.8 h	1.57 ± 0.16 de

[a] Values given are averages of triplicate measurements ± standard deviation. Significant differences ($P < 0.05$) between samples are represented by different letters (a−h) within each column. [b] Limit of quantification = 0.1 μmol/mol glucose. [c] Not performed, due to the low solubility of phenylalanine.

Table 3. Percent Labeling Distribution of Furan Generated in Model Systems Containing Glucose (Equimolar Amounts of $^{12}C_6$-Glucose and $^{13}C_6$-Glucose) and Different Amino Acids under Pressure-Cooking Conditions (25 min at 121 °C)[a]

	% M	% (M + 1)	% (M + 2)	% (M + 3)	% (M + 4)
glucose	below limit of quantification[b]				
glucose + Gly	46.6 ± 0.9	0.3 ± 0.0	0.5 ± 0.0	3.5 ± 0.0	46.9 ± 0.9
glucose + Ala	47.7 ± 0.5	0.4 ± 0.0	4.7 ± 0.7	2.9 ± 0.0	42.1 ± 0.1
glucose + Phe	47.6 ± 0.5	0.3 ± 0.0	0.4 ± 0.0	3.0 ± 0.0	46.6 ± 0.6
glucose + Arg	45.9 ± 1.0	1.3 ± 0.3	0.5 ± 0.0	3.8 ± 0.0	46.5 ± 1.3
glucose + Pro	45.3 ± 1.3	0.3 ± 0.1	1.2 ± 0.7	3.2 ± 0.0	47.9 ± 2.1
glucose + Lys	47.9 ± 0.7	0.3 ± 0.0	0.5 ± 0.0	3.0 ± 0.0	46.2 ± 0.7
glucose + Cys	below limit of quantification				
glucose + Ser	51.5 ± 3.0	0.3 ± 0.2	0.9 ± 0.3	2.8 ± 0.1	42.9 ± 2.5
glucose + Thr	46.1 ± 0.5	0.3 ± 0.0	0.6 ± 0.1	3.0 ± 0.0	48.1 ± 0.5

[a] Values given are averages of triplicate measurements ± standard deviation. [b] Limit of quantification =0.1 μmol/mol glucose.

Proline was found to have no influence on the amount of furan, whereas in the presence of lysine less furan was formed. The lower reactivity of proline is probably due to its secondary amino group. For lysine, it is assumed that the lower furan production is due to the occurrence of more reactive side reactions, most probably of the highly reactive ε-amino group, because it has been suggested that lower furan production can be caused by competing reaction pathways (*11*). As can be seen in **Table 2**, the highest amounts of furan were produced in the model systems containing alanine or serine. This corresponds with the CAMOLA experiments, which showed that these amino acids were incorporated the most into the furan skeleton.

Pressure-Cooking Conditions. The results obtained from the CAMOLA experiments under pressure-cooking conditions are depicted in **Table 3**. Although the amount of furan produced in the amino acid-free model system was below the limit of quantification, it can be assumed from the model systems containing amino acids that, also under pressure-cooking conditions, furan was solely formed via the intact glucose skeleton and not via fragmentation and recombination. This is in contrast to the observations of Limacher et al. (*13*). These authors reported that under pressure-cooking conditions, about half of the furan formed from glucose originated from glucose fragments. These differences can be due to two different reasons. First, these experiments were performed in citrate−phosphate buffers, which are known to influence the formation of furan from different precursors (*15*). Besides the amounts of furan produced, apparently also its formation mechanisms can be highly influenced by the buffer type as well. This is not surprising, because the addition of citrate and/or phosphate ions induces the formation of better leaving groups and thus modifies the reaction pathways (*22*). Second, in this study, the experiments were performed at pH 6, whereas Limacher et al. used pH 7. It is known that between pH 6 and 7, fragmentation increases as the 2,3-enolization pathway dominates.

Furthermore, from **Table 3**, it can also be seen that also under pressure-cooking conditions, alanine and serine are clearly incorporated. However, the percentages of incorporation are lower as compared to roasting conditions. Under pressure-cooking conditions, about 10% of furan formed involved alanine incorporation, whereas this was about 26% for roasting conditions. For serine, about 2% of the total amount of furan was formed from the condensation of glucose-derived glycolaldehyde and serine-derived acetaldehyde and about 7% from the condensation of acetaldehyde and glycolaldehyde, both derived from serine under pressure-cooking conditions, as compared to 5 and 14%, respectively, under roasting conditions. These results show that both glucose degradation and amino acid incorporation are lower under pressure-cooking conditions. No other amino acids were found to be incorporated under pressure-cooking conditions.

With regard to the amounts of furan that were produced during pressure cooking, it can be seen from **Table 2** that also under these conditions most amino acids enhanced the formation of furan from glucose. However, the enhancing properties were somewhat different from those under roasting conditions. The highest amount of furan was produced in the model system containing threonine, followed by serine and glycine. Under these conditions, lysine did not decrease the amounts of furan produced from glucose; probably the adjustment of the pH to about 6 lowered the nucleophilic properties of the ε-NH_2 group. The fact that cysteine did not enhance the production of furan can be due to the formation of hydrogen sulfide, which is released from cysteine upon heating (*21*) and subsequently reacts with the reactive carbonyl groups formed from carbohydrate degradation. Apparently, this reaction becomes more important under pressure-cooking conditions.

Progress of Furan Formation in Glucose/Alanine Model Systems. In another series of experiments, the progress of the different pathways of furan formation from glucose/alanine model systems was studied as a function of time (2−60 min), under both dry (180 °C) and aqueous reaction conditions (121 °C). The model system glucose/alanine was chosen because the previous experiments showed that alanine was incorporated the most into the furan skeleton and high amounts of furan were formed from these model systems.

Roasting Conditions. As can be seen from **Table 4**, the relative abundance of the different isotopomers of furan varied over time, which implies that the importance of the different formation pathways of furan also changed. In the beginning of the reaction, high amounts of (M + 2) were formed. Therefore, it can be concluded that, in this first stage, condensation of alanine-derived acetaldehyde and glucose-derived glycolaldehyde was the main pathway for furan production. As the reaction proceeded, more and more furan was formed from the intact sugar skeleton, and after 20 min, an equilibrium was reached between the two mechanisms of furan formation. From the results presented in **Table 4**, the relative importance of the different formation pathways can be calculated. In these calculations, it is assumed that furan formation from fragmentation and recombination of glucose fragments does not take place, which has been demonstrated from the previous experiments. The results obtained from these calculations are depicted in **Figure 2**. It can be seen that, in the beginning of the reaction, about 80% of the total amount of

Table 4. Progress of the Percent Labeling Distribution of Furan Generated in Dry Model Systems Containing Glucose (Equimolar Amounts of $^{12}C_6$-Glucose and $^{13}C_6$-Glucose) and Alanine during Heating at 180 °C[a]

time (min)	% M	% (M + 1)	% (M + 2)	% (M + 3)	% (M + 4)
2	47.4 ± 2.1	1.4 ± 0.1	39.6 ± 0.4	1.6 ± 0.0	7.9 ± 1.8
5	48.0 ± 2.5	1.0 ± 0.2	26.1 ± 0.7	1.9 ± 0.3	20.9 ± 1.3
10	47.6 ± 0.3	0.8 ± 0.4	20.0 ± 2.1	1.9 ± 0.3	27.7 ± 1.7
20	47.0 ± 2.3	0.7 ± 0.4	15.6 ± 1.9	2.4 ± 0.4	32.3 ± 0.3
30	47.1 ± 1.4	0.9 ± 0.2	14.0 ± 3.1	2.5 ± 0.2	33.4 ± 2.1
60	45.3 ± 0.1	1.3 ± 0.1	17.3 ± 0.7	2.8 ± 0.2	31.3 ± 0.7

[a] Values given are averages of triplicate measurements ± standard deviation.

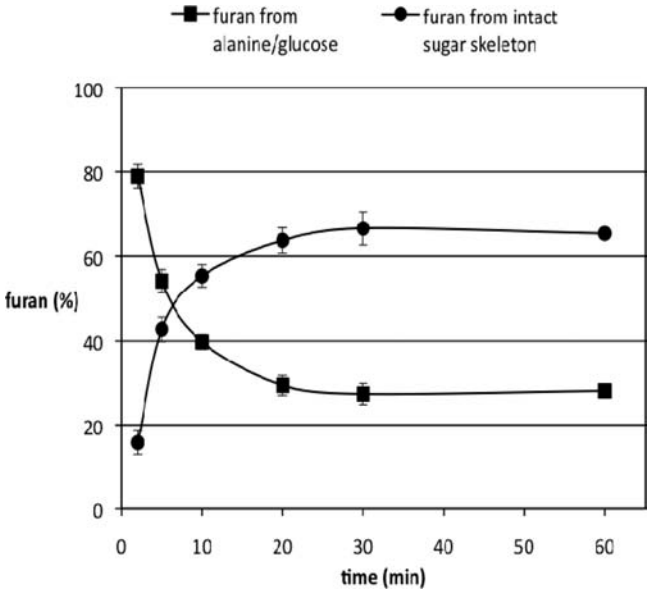

Figure 2. Progress of furan production via alanine/glucose fragments and via the intact sugar skeleton in dry glucose/alanine model systems (180 °C). Error bars represent standard deviations (n = 3).

furan was formed via alanine-derived acetaldehyde and glucose-derived glycolaldehyde, whereas about 16% was formed via the intact sugar skeleton. When equilibrium between both formation pathways was reached (after 20 min), 28 and 66% of the total amounts of furan were produced via the combination of alanine and glucose fragments and via the intact sugar skeleton, respectively.

The evolution of the quantitative production of furan in time from glucose/alanine model systems is depicted in **Figure 3** ("total furan production"). It can be seen that already after 2 min of heating, relatively high amounts of furan were produced under dry-roasting conditions (8 μmol/mol of glucose after 2 min). After 20 min, furan production slowed, resulting in a furan production of 88 μmol/mol of glucose after 60 min. The absolute amounts of furan produced via alanine/glucose fragments and via the intact sugar skeleton under dry-roasting conditions were calculated and are also depicted in **Figure 3**. All curves followed a logarithmic fit. It can be seen that relatively high amounts of furan were formed from alanine and glucose already after 2 min of heating, whereas as the reaction proceeded, low additional amounts of furan were formed via this pathway. These results indicate that, under dry-roasting conditions, alanine is converted into acetaldehyde very quickly. In addition, glycolaldehyde must be formed very quickly from glucose. However, these reactive fragments seem to be consumed quite rapidly. In addition, it seems that cyclization of the sugar skeleton, resulting in the formation of furan from the intact sugar skeleton, requires somewhat more time for initiation, but already after 5 min of reaction, the amounts of furan and the

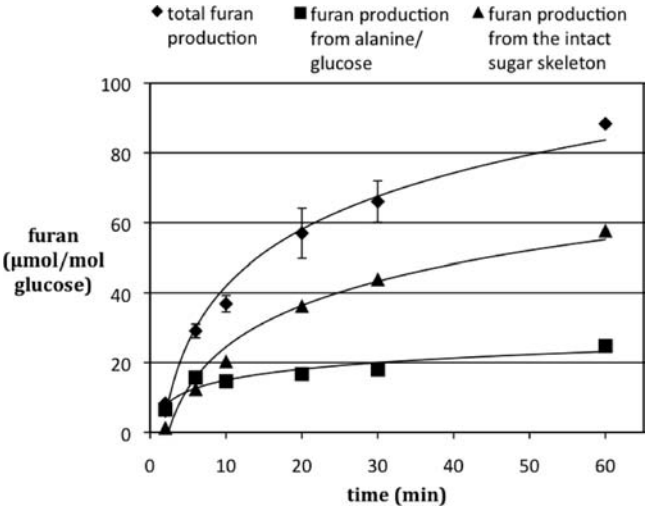

Figure 3. Furan production in dry glucose/alanine model systems (180 °C) with logarithmic fit. Error bars represent standard deviations (n = 3).

Table 5. Progress of the Percent Labeling Distribution of Furan Generated in Aqueous Model Systems Containing Glucose (Equimolar Amounts of $^{12}C_6$-Glucose and $^{13}C_6$-Glucose) and Alanine during Heating at 121 °C[a]

time (min)	% M	% (M + 1)	% (M + 2)	% (M + 3)	% (M + 4)
2	below limit of quantification[b]				
5	below limit of quantification				
10	46.6 ± 4.9	1.0 ± 0.4	0.9 ± 0.1	3.4 ± 0.9	46.1 ± 4.2
20	47.7 ± 0.1	0.4 ± 0.0	3.5 ± 0.1	2.9 ± 0.0	43.3 ± 0.3
25	47.7 ± 0.5	0.4 ± 0.0	4.7 ± 0.7	2.9 ± 0.0	42.1 ± 0.1
30	47.5 ± 0.6	0.6 ± 0.0	7.0 ± 0.5	2.9 ± 0.0	40.0 ± 0.0
60	47.0 ± 0.6	0.6 ± 0.0	7.1 ± 0.2	3.0 ± 0.0	40.3 ± 0.3

[a] Values given are averages of triplicate measurements ± standard deviation. [b] Limit of quantification = 0.1 μmol/mol glucose.

reaction rate from glucose alone were higher than from the combination of alanine and glucose fragments.

Pressure-Cooking Conditions. Also under pressure-cooking conditions, the relative abundance of the different isotopomers of furan changed over time (**Table 5**). However, the progress of furan production was not the same as under dry-roasting conditions. Whereas furan was initially produced mainly from alanine/glucose fragments under dry-roasting conditions, cyclization of the intact sugar skeleton was from the beginning the major route for furan formation under pressure-cooking conditions. Only after 20 min did acetaldehyde from alanine start to be incorporated, and equilibrium between the two sources of furan was reached after 30 min. Also, the relative importance of the different formation pathways was calculated and is depicted in **Figure 4**. It can be seen that, in the beginning of the reaction, about 92% of the total amount of furan was formed via the intact sugar skeleton, whereas only 2% was formed via alanine/glucose fragments. When equilibrium between both formation pathways was reached (after 30 min), 14 and 80% of the total amounts of furan were produced via alanine/glucose fragments and via the intact sugar skeleton, respectively. These results demonstrate that furan formation via alanine/glucose fragments was less important under pressure-cooking conditions as compared to dry-roasting conditions, as was discussed before. However, it must be kept in mind that under pressure-cooking conditions not only the temperature but also the pH plays an important role when it comes to degradation and competing reactions. Therefore, it is possible that in food systems with higher pH values, which favor the 2,3-enolization pathway and thus fragmentation, furan formation via alanine/glucose fragments becomes more important.

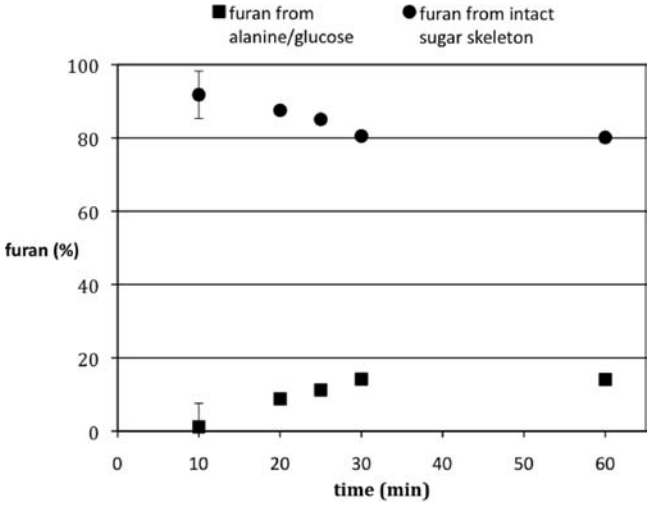

Figure 4. Progress of furan production via alanine/glucose fragments and via the intact sugar skeleton in aqueous glucose/alanine model systems (121 °C). Error bars represent standard deviations ($n = 3$).

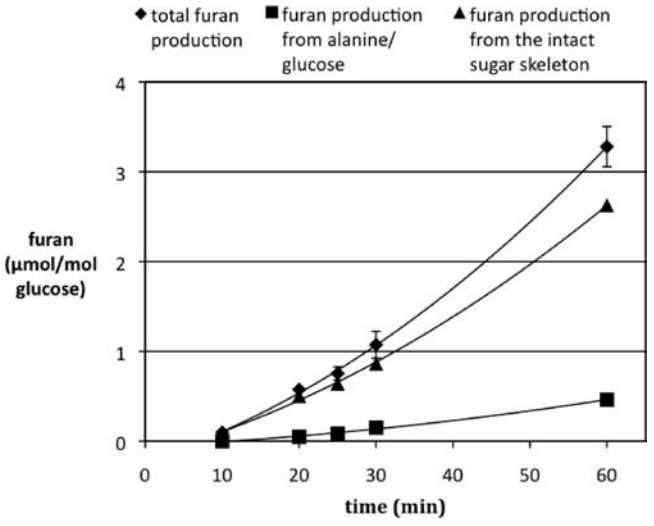

Figure 5. Furan production in aqueous glucose/alanine model systems (121 °C) with polynomial fit. Error bars represent standard deviations ($n = 3$).

The progress of quantitative furan production in aqueous glucose/alanine model systems is depicted in **Figure 5**. It can be seen that under pressure-cooking conditions not only the relative importance of the mechanisms of furan formation but also the kinetics of its formation were completely different from furan formation under dry-roasting conditions. Under aqueous conditions, furan production started much more slowly. Only after 10 min was furan detected. The rate of furan production increased over time, whereas under dry-roasting conditions a plateau was reached. During the complete reaction period monitored (60 min), the amounts of furan produced under pressure-cooking conditions remained much lower as compared to dry-roasting conditions. Naturally, furan production cannot increase indefinitely. Due to depletion of the reaction products, furan production will also slow under pressure-cooking conditions. Monitoring the reaction for a longer period of time is needed to study the kinetics of furan formation more in detail, but it is likely that furan formation under pressure-cooking conditions follows a sigmoid curve (S-curve).

In addition, the absolute furan production via the combination of alanine and glucose fragments and via the intact sugar skeleton was calculated (**Figure 5**). It can be seen that the rate of furan production via both pathways increased with time. This is in contrast to roasting conditions, when the rate of furan production decreased after a certain time. Two hypotheses can be postulated for these observations. First, alanine might be converted to acetaldehyde much more easily under roasting conditions and, second, the C_2 fragments (most probably glycolaldehyde) are not formed in sufficient quantities from sugars under pressure-cooking conditions. Both hypotheses have also been suggested by Limacher et al. (*13*) to explain their findings on furan production in Maillard reaction systems. The high amounts of furan produced during pyrolysis of the model system containing glycolaldehyde and alanine in the study of Perez Locas and Yaylayan (*8*) definitely confirm the first hypothesis. However, both hypotheses may be operative.

In this study, the formation mechanisms of furan were investigated in Maillard reaction systems of amino acids and labeled glucose. The CAMOLA experiments showed that under both dry-roasting (180 °C) and pressure-cooking conditions (121 °C, pH 6, without buffer), glucose-derived furan was formed from the intact sugar skeleton and not from fragmentation and recombination mechanisms. The presence of amino acids did not influence the formation pathways of furan from glucose alone. However, some amino acids (especially alanine and serine) did influence furan production by providing an additional formation pathway. Also, most amino acids enhanced the production of furan from glucose, even if they were not incorporated into the furan skeleton. Furthermore, dry-roasting conditions produced 25−100 times higher amounts of furan as compared to pressure-cooking conditions. This major difference has also been reported by Limacher et al. (*13*). With regard to the progress of furan production in glucose/alanine model systems, it was demonstrated that both the mechanism and kinetics of furan formation as well as its quantitative production were different under dry-roasting conditions as compared to pressure-cooking conditions. Under dry-roasting conditions, furan formation via recombination of alanine-derived acetaldehyde and glucose-derived glycolaldehyde was much more important than under pressure-cooking conditions, especially when relatively short heating periods were considered.

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