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Quantitation of Furan and Methylfuran Formed in Different Precursor Systems by Proton Transfer Reaction Mass Spectrometry

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Furan has recently received attention as a possibly hazardous compound occurring in certain thermally processed foods. Previous model studies have revealed three main precursor systems producing furan upon thermal treatment, i.e., ascorbic acid, Maillard precursors, and polyunsaturated lipids. We employed proton transfer reaction mass spectrometry (PTR-MS) as an on-line monitoring technique to study furan formation. Unambiguous identification and quantitation in the headspace was achieved by PTR-MS/gas chromatography−mass spectrometry coupling. Ascorbic acid showed the highest potential to generate furan, followed by glyceryl trilinolenate. Some of the reaction samples generated methylfuran as well, such as Maillard systems containing alanine and threonine as well as lipids based on linolenic acid. The furan yields from ascorbic acid were lowered in an oxygen-free atmosphere (30%) or in the presence of reducing agents (e.g., sulfite, 60%), indicating the important role of oxidation steps in the furan formation pathway. Furthermore, already simple binary mixtures of ascorbic acid and amino acids, sugars, or lipids reduced furan by 50−95%. These data suggest that more complex reaction systems result in much lower furan amounts as compared to the individual precursors, most likely due to competing reaction pathways.

KEYWORDS: Furan; methylfuran; ascorbic acid; lipid oxidation; linoleic acid; linolenic acid; Maillard reaction; PTR-MS

INTRODUCTION

The U.S. Food and Drug Administration (FDA) has recently reported on the occurrence of furan (110-00-9) in certain food products that are subject to thermal treatment (*1*). Since the FDA announcement, considerable research has been devoted to the analysis and formation of this volatile food-borne contaminant. Furan has received attention due to its classification as "possibly carcinogenic to humans" (group 2B) by the International Agency for Research on Cancer (*2*). Furan levels up to about 240 μg/kg were reported in food, especially in canned and jarred products (*3*−*5*). However, furan has been known for a long time as a food constituent (*6*), for example, in caramel, coffee, bread, canned meat, and heated soy protein (*7*−*11*). In most thermally treated products, the parent furan was accompanied by a series of alkylated analogues, in particular 2-substituted alkylfurans such as 2-methyl- and 2-ethylfuran (*6*).

The formation of furan and its derivatives has early been associated with thermal treatment of Maillard reaction precursors (*6*) or lipids (*12*, *13*). Pyrolysis of carbohydrates at extreme temperatures of up to 300 °C resulted in the formation of furan, 2-methylfuran (MF), and further alkylated derivatives (*14*−*16*). Unfortunately, in many cases, single sugars were reacted at very high temperatures (300 °C), which do not represent food-processing conditions. On the other hand, furan formation from sugars is favored under more drastic reaction conditions (≥300 °C) (*17*), where radical-mediated cracking processes occur. Not only hexoses but also pentoses, tetroses (erythrose), and polysaccharides generated furan and alkylated derivatives, while glyceraldehyde (triose) was less efficient (*17*). Recently, alkylated furan derivatives have been identified in samples obtained by thermal degradation (250 °C, 10 min) of melanoidins prepared from bread crust, coffee, and tomato (*18*).

There are only a few reports on the formation of furan under food-processing conditions. A lactose−casein browning system kept in the dry state at 75% relative humidity resulted in a brown cake after 8 days of storage, which contained furan and 2-MF among 40 other volatile reaction products identified in the dichloromethane extract (*19*). Several alkylated furans have also been identified in complex Maillard reaction systems leading to baked potato aroma under microwave irradiation and conventional oven-heating conditions (*20*) as well as in glucose/

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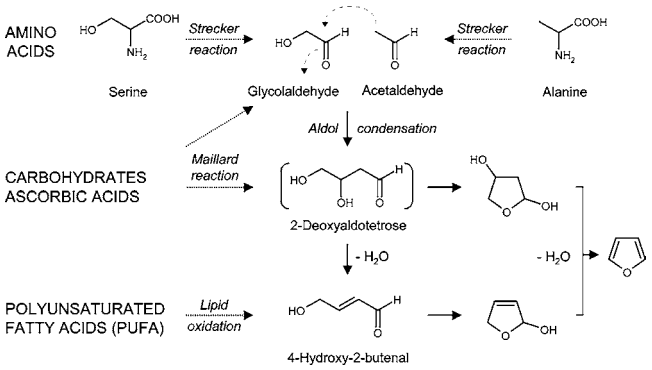

Figure 1. Hypothetical formation pathways based on Maillard precursors, ascorbic acid, and/or polyunsaturated lipids leading to furan by thermally induced reactions (according to ref *22*, with some modifications). Dotted lines represent a series of reactions.

glycine model systems under extrusion conditions (*21*). Thus, these data indicate that furan and 2-MF can, indeed, also be formed under rather mild reaction conditions characteristic for food processing.

Recently, the formation of furan has been studied in simple model systems revealing three main precursor classes, i.e., (i) ascorbic acid and related compounds, (ii) Maillard type systems containing amino acids and reducing sugars, and (iii) lipid oxidation of unsaturated fatty acids or triglycerides (*22*, *23*). Model studies using ^{13}C-labeled glucose and/or serine have shown acetaldehyde and glycolaldehyde as key fragments, which upon aldol condensation, cyclization, and dehydration result in furan (**Figure 1**) (*22*). The key intermediates acetaldehyde and glycolaldehyde may originate from sugars and/or defined amino acids. Alternatively, ascorbic acid may be transformed under nonoxidative pyrolytic conditions to 2-deoxyaldotetrose as a key intermediate that can either lead directly to furan or, alternatively, via 4-hydroxy-2-butenal, which is a well-known lipid degradation product (**Figure 1**).

Because of the volatility of furan and its alkyl derivatives, the most frequently used analytical methods were pyrolysis gas chromatography−mass spectrometry (GC-MS) and headspace GC-MS. Recent quantitative results have been obtained by isotope dilution assay based on deuterated furan used as an internal standard and headspace GC-MS as sampling and detection methods (*5*, *24*). We have recently reported on the time-resolved on-line analysis of acrylamide by proton transfer reaction mass spectrometry (PTR-MS), which does not require any chromatographic separation (*25*). Coupling of PTR-MS with GC-MS has been shown as a powerful analytical tool for unequivocal identification of volatile organic compounds (VOCs) representing a particular mass trace (*26*), which also allows direct and absolute quantitation of VOCs released from complex mixtures (*27*).

The present study was performed to obtain additional and more precise information about the various precursor systems and reaction parameters leading to furan and its methyl analogue with the aim of reducing its formation. PTR-MS was used as a convenient analytical technique allowing on-line monitoring of furans released into the headspace.

EXPERIMENTAL PROCEDURES

Materials. The following chemicals were commercially available: L-ascorbic acid (>99.0%), D-erythrose (≥90.0%), L-alanine (>99.5%), L-serine (>99.5%), L-threonine (>99.5%), glycine (>99.0%), 2,6-bis-(1,1-dimethylethyl)-4-methyl-phenol (BHT, purum), sodium sulfite anhydrous (>98.0%), linoleic acid (~99.0%), linolenic acid (~99.0%), glyceryl trilinoleate (trilinolein, ≥98.0%), glyceryl trilinolenate (trilinolenin, ≥98.0%), (±)-α-tocopherol (≥98.0%), and furan (stabilized) (Fluka, Buchs, Switzerland); dehydro-L-ascorbic acid, 2-MF (99%), and glycolaldehyde dimer (Aldrich, Buchs, Switzerland); (+)-D-glucose anhydrous (Merck, Darmstadt, Germany); ferric(III)-sulfate anhydrous (Riedel-de Haën, Hanover, Germany); and sea sand (Amtech-Chimie SA, Lausanne, Switzerland). Tenax TA traps (Tenax TA 60−80 mesh, 15 gm) were from Phase Separation Ltd. (Deeside, United Kingdom), and the permeation tubes (Dynacal permeation device, type: STD) were from VICI Metronics (Schenkon, Switzerland).

Sample Preparation. Equimolar amounts of precursors (0.35 mmol each) were mixed in the dry state and homogenized in the reaction vessel. Serine, alanine, threonine, BHT, and sodium sulfite were ground prior to use. The quantity of materials of antioxidants amounted to 1.2% (w/w) of the chemical precursor. The amounts of ferric sulfate added (Fe^{3+} ions) accounted for not more than 1% (w/w) of the total mass. In some of the experiments (see text), approximately 1 g of sea sand was added and entirely mixed with the precursors. The samples were distributed inside the reaction vessel with the highest possible surface contact. For each experimental point, two independent determinations each with duplicate injections were carried out.

Experimental Setup. The detailed description of the release setup used and the instrumentation applied has recently been described for the analysis of acrylamide (*24*). The reaction vessel (100 mL) was placed inside the oven and heated to 220 °C with a heating rate of 4 °C/min. The purge flow, consisting of dry air/nitrogen, was maintained at 600 sccm (= standard cubic centimeters per minute), and the dilution flow was maintained at 5190 sccm. Only 25 sccm was introduced into the PTR-MS drift tube, while the remaining volume was released through an exhaust line. The on-line PTR-MS was used in a parallel operation mode; that is, (i) within the scan mode, it monitored the masses from *m*/*z* 21 to *m*/*z* 220 with a dwell time of 0.1 s per mass, and (ii) within the selected ion count mode, the masses of major interest were recorded with a prolonged dwell time of 2 s.

A new coupling device between GC-MS and PTR-MS was used (*25*, *26*) for further investigations to identify the VOCs. The sample headspace was trapped on Tenax tubes by connecting three traps at subsequent time intervals of 2 min to the exhaust line of the release setup. For the trapping time of 2 min at a trapping flow rate of 50 sccm, the trapping efficiency was found to be between 92 and 96% for furan and 94% for 2-MF within the employed Tenax temperature range of 30−40 °C. The trapped volatiles were thermally desorbed using an automatic thermal desorber (ATD 400, Perkin-Elmer, Boston, MA), cryofocused, and injected into the DB-Wax capillary GC column (60 m × 0.53 mm, 1 μm film thickness) (J&W Scientific, Folsom, CA). The column was kept at 20 °C for 1 min, heated to 220 °C with a 4 °C/min ramp, and kept at this temperature for another 10 min. The column outlet was split and led simultaneously into the electron impact (EI)-MS and PTR-MS to analyze the temporally separated compounds. Identification was achieved by comparing (i) the EI fragmentation pattern of the analyte with that found in the Wiley database (*28*) and (ii) its retention time with that of the injected reference compound. The GC-PTR-MS spectrum allowed determination of the relative contributions of different compounds with identical masses to the on-line PTR-MS signal (*26*).

Quantitation by PTR-MS. For absolute headspace quantitation, the relevant parameters of the PTR-MS and the proton transfer reaction rate constants of the compounds of interest are required (*27*). The concentration of VOCs can be calculated without any calibration provided that the known parameters are as follows: reaction chamber pressure and temperature, reaction time, primary ion signal intensity, signal intensity of the ion trace, the mass discrimination of the quadrupole, and the reaction rate constant. Most of the required parameters can be obtained directly during the measurement, except the reaction rate constant. This constant can be calculated based on theoretical models or measured by using a calibration gas. A full description of the quantitation by PTR-MS has been given at the 1st PTR-MS symposium (*27*). Another well-established method is using a selected ion flow drift tube (*29*), where the intensity of the primary ions is preselected and measured before they undergo reactions with the compounds of interest inside the flow drift tube. The reaction rate

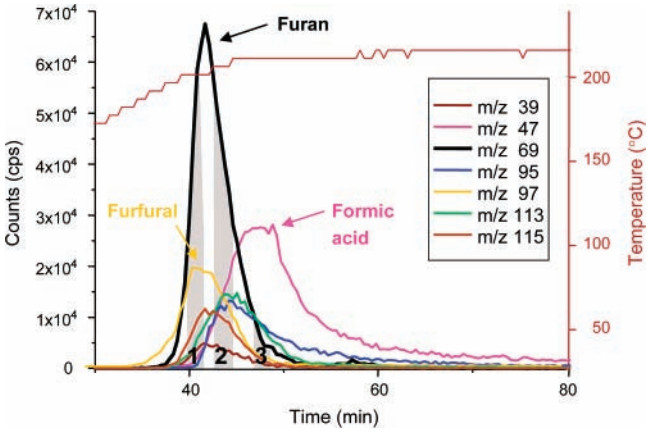

Figure 2. PTR-MS traces of selected ions obtained by heating ascorbic acid. Some of these traces were identified by coupling with GC-MS after trapping the headspace on three Tenax tubes as indicated by the gray zones numbered 1, 2, and 3 (see also text).

constants can then be calculated directly through the ratio of the signal intensities of the primary ions and the product ions.

In this study, an alternative technique was applied using a Dynacalibrator (Dual chamber model 500, VICI Metronics) in combination with PTR-MS to measure the reaction rate constants of furan and 2-MF. A permeation tube containing the compound of interest was installed inside a temperature-controlled chamber, which was purged with nitrogen (190 sccm) and connected to the PTR-MS inlet system. The permeation tube was calibrated through its weight loss during several days. The release of a well-defined amount of furan and MF, respectively, was measured on-line by PTR-MS. Thereafter, the reaction rate constants could be determined by comparing the integrated signal intensities of the ion traces at *m*/*z* 69 and *m*/*z* 83 with the released amounts of the corresponding compounds.

RESULTS AND DISCUSSION

On-line Monitoring of Furans. The PTR-MS procedure for analyzing furan and MF was developed using reaction systems based on ascorbic acid and linolenic acid, respectively. A large number of VOCs was produced upon thermal treatment of these furan precursors. **Figure 2** shows selected mass traces obtained by heating ascorbic acid. The major signals were represented by the ions at *m*/*z* 69, 47, and 97, followed by many other ion traces. The ions at *m*/*z* 69 and *m*/*z* 47 may correspond to furan and formic acid, respectively. However, it is well-known that one ion trace may represent various compounds (*26*). Therefore, the recently described PTR-MS/GC-MS coupling was employed to elucidate the composition of various ion traces with particular interest in *m*/*z* 69. Aliquots of the headspace were trapped on three Tenax cartridges (**Figure 2**) and analyzed off-line by GC-MS as described for the analysis of acrylamide (*25*). In analogy, the conditions for analyzing MF (mixture of 2- and 3-isomers) by PTR-MS were optimized using the ion at *m*/*z* 83 formed upon heating linolenic acid (data not shown).

As shown in **Figure 3A**, thermal decomposition of ascorbic acid led, indeed, to many VOCs. The compound with the retention time at t_R = 8.9 min was identified as furan by comparing its EI mass spectrum with that of the reference compound. Furthermore, only one volatile constituent was found by GC-MS with the mass at *m*/*z* 68 (**Figure 3B**), thus confirming that this mass trace was homogeneous, solely represented by furan, and not contaminated by further ions originating from other VOCs. All reaction systems were characterized by the combined PTR-MS/GC-MS method to define the purity of the ion traces at *m*/*z* 69 for furan and at *m*/*z* 83 for 2-/3-MF.

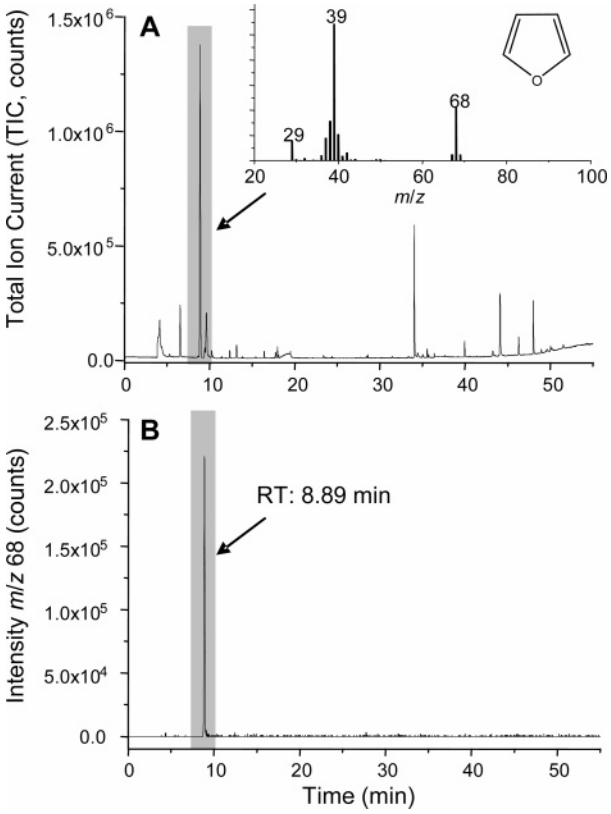

Figure 3. (**A**) GC total ion count signal of a Tenax trap obtained from the ascorbic acid experiment showing the fragmentation pattern of furan. (**B**) Only one volatile compound was found with *m*/*z* 68 eluting at 8.89 min, which was identical to the injected reference compound furan.

Ascorbic acid was formed in the narrow temperature range of 180−210 °C (T_{max} = 195 °C), which lasted for about 10 min (**Figure 4A**). Interestingly, the furan formation profile was different in lipid reaction systems based on linolenic acid, in which furan was generated and released at lower temperatures (110−220 °C, T_{max} = 180 °C) and on a broader time scale of about 70 min (**Figure 4B**). While the ion trace at *m*/*z* 69 was homogeneous in the ascorbic acid sample (94−100% purity), it only partially represented furan in the lipid-based systems with a purity of about 10−25%, determined by Tenax trapping and GC-MS analysis. As the ion trace composition was changing with time, different percentage contributions during the respective time windows had to be considered for analysis.

Maillard reaction systems resulted in quite complex peak profiles and, in general, relatively low-intensity furan signals, which complicated data interpretation. Again, coupling of PTR-MS with GC-MS turned out to be an essential and indispensable tool to obtain reliable results on furan and MF by analyzing the individual Tenax traps. Furan formation started at about 150 °C and continued to be formed during 150 min (T_{max} = 220 °C). The purity of the furan and MF ion traces was in the range of 45−65% (data not shown).

Quantitative Monitoring of Furans. The PTR-MS signals (cps) of furan and MF were converted into concentrations (μg of released quantity of furan and MF per μmol of precursor) using the PTR rate constants of 2.28×10^{-9} and 2.22×10^{-9} cm^3/s, respectively, determined by experimental measurements. Direct quantitation of furan was possible in the ascorbic acid samples due to high purity of the signal at *m*/*z* 69 representing 100% furan in almost all traps obtained from model systems containing only ascorbic acid as the furan precursor. In all other reaction systems, the relative composition of the ion trace at

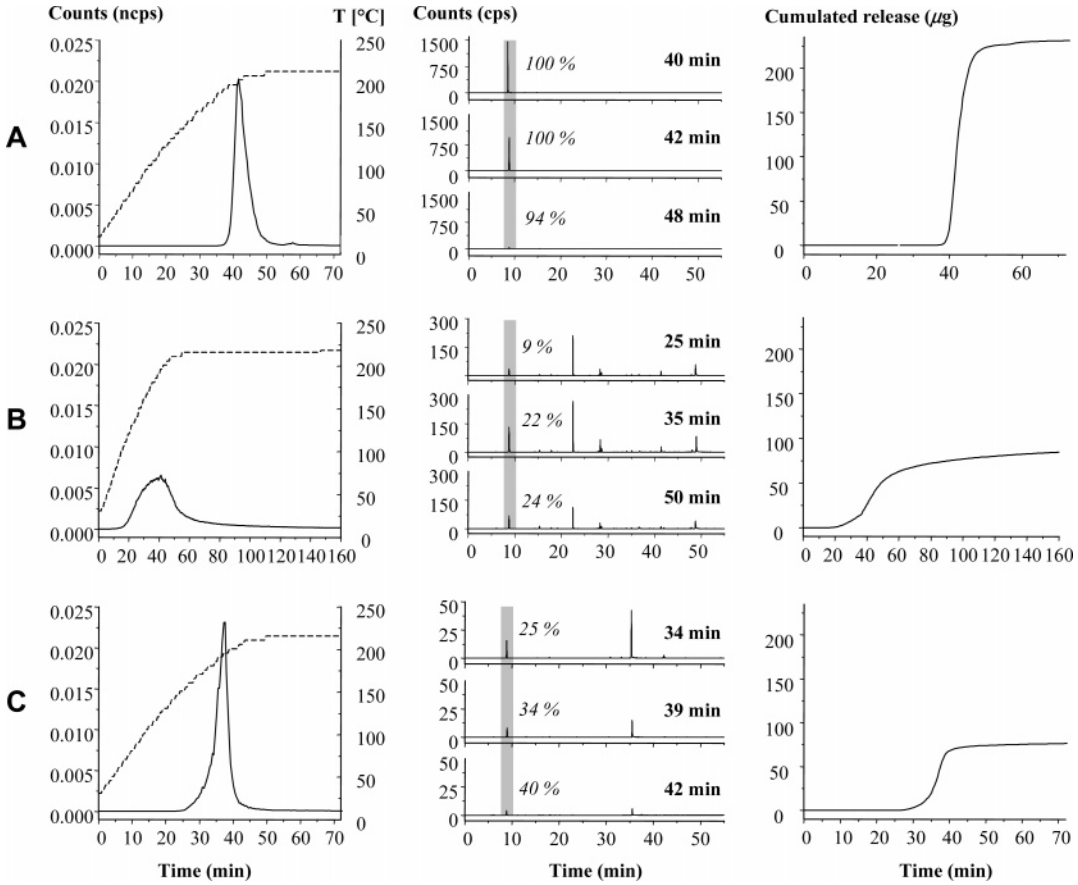

Figure 4. Release curves (*m*/*z* 69) obtained by on-line PTR-MS in normalized counts per seconds (ncps) toward primary ions (1st column), PTR-MS signal (*m*/*z* 69) of the trapped volatiles after GC separation in counts per seconds (cps) with the highlighted peaks corresponding to furan (2nd column), and the total amount of furan in μg released into the headspace (3rd column). All plots vs time in minutes: (**A**) ascorbic acid, (**B**) linolenic acid, and (**C**) ascorbic acid in the presence of sodium sulfite (1.2%, w/w).

m/*z* 69 (or at *m*/*z* 83 for MF) was determined by GC-MS, which was used to correct the PTR-MS signal intensities accordingly prior to the calculation of concentrations. Therefore, the data obtained for lipids and Maillard samples are of a semiquantitative nature. Nevertheless, the cumulated data obtained allow a fair comparison of the trends observed in the various reaction systems. It should also be noted that, in general, PTR-MS data only indicate the concentration in the headspace but not in the actual sample. However, as recently confirmed for acrylamide (*30*), PTR-MS data, indeed, reflect well the trends in the formation of VOCs as compared to those obtained by liquid extraction.

The amounts of furan detected in the headspace of the different precursor systems are summarized in **Table 1**. In general, ascorbic acid (sample 1) showed the highest potential to form furan, resulting in about 10 mmol furan per mol ascorbic acid. In contrast, dehydroascorbic acid (sample 2) showed low efficiency in generating furan, most likely due to cyclization to the corresponding hemiketal that is favored under dry conditions (*22*). Perez Locas and Yaylayan (*22*) also observed a similar trend using dry heating conditions, although less drastic as compared to our results. On the other side, Becalski and Seaman found about 10 times higher furan amounts formed from dehydroascorbic acid under aqueous pressure-cooking conditions (*23*). Interestingly, some articles dealing with thermal degradation of ascorbic or dehydroascorbic acid did not report furan at all as a volatile constituent (*31*, *32*). On the other hand, the authors found 2-furoic acid, which may release furan by decarboxylation (*23*).

Table 1. Formation of Furan and MF from Various Reaction Systems Based on Ascorbic Acids, Maillard Type Precursors, and Lipids[a]

	model system	furan (μmol/mol)	VC[b] (%)	MF[c] (μmol/mol)	VC[b] (%)
1	ascorbic acid	9950	7.8	ND	
2	dehydroascorbic acid	270	3.9	ND	
3	erythrose	1674	8.6	ND	
4	glucose + alanine + threonine	749	11.3	797	1.4
5	linoleic acid (C18:2; 9,12)	681	26.2	ND	
6	trilinolein	1727	21.5	ND	
7	linolenic acid (C18:3; 9,12,15)	3270	0.9	391	8.2
8	trilinolenin	4747	23.2	995	57

[a] Quantitative data refer to concentrations in the headspace. [b] Variation coefficient. [c] MF representing the sum of the 2- and 3-isomers (ND, not detected).

The aldotetrose erythrose (sample 3) produced significantly (6-fold) lower amounts of furan as compared to ascorbic acid, which is in agreement with literature data (*22*), despite the fact that it is supposed to be an intermediate in the formation pathway from ascorbic acid (**Figure 5B**). Apparently, the aldotetrose formed as an intermediate in sample 1 can easily be reduced by ascorbic acid to generate furan, which is not the case in sample 3 due to the lack of reducing species. It may also be, however, that the formation of furan via the intermediate 2-deoxy-aldotetrose (**Figures 1** and **5E**) is more efficient than via the corresponding C_4-aldose sugars.

Sample 4 composed of glucose, alanine, and threonine resulted in equal amounts of furan and MF. The formation of furan is shown in **Figure 1**: Acetaldehyde (Strecker aldehyde

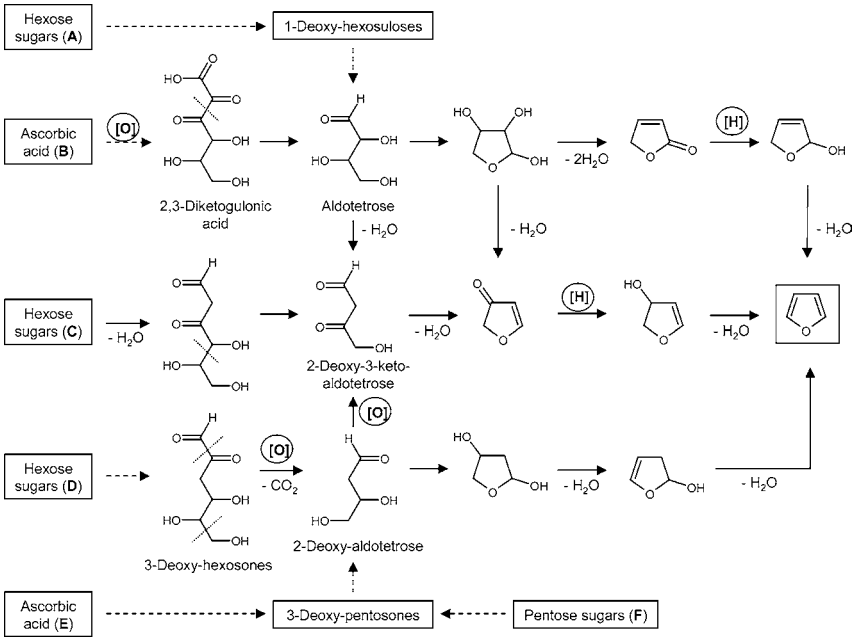

Figure 5. Schematic formation of furan from pentose and hexose sugars as well as ascorbic acid (according to ref *22*) by competing pathways involving oxidation ([O]) and reduction ([H]) steps.

of alanine) and glycolaldehyde, a well-known sugar degradation product (*33*), combine to 2-deoxyaldotetrose, which upon cyclization and dehydration gives rise to furan. The formation of 2-MF (**Figure 6**) can be explained by aldol condensation of the corresponding Strecker aldehydes, i.e., acetaldehyde and lactaldehyde, followed by cyclization to the hemiacetal and dehydration reactions. The formation of Strecker aldehydes was promoted in the presence of glucose, which resulted in sufficient amounts of α-dicarbonyls to perform the Strecker type degradation reaction of amino acids. Thus, depending on the composition of the reaction system, various furan derivatives can be expected upon heat treatment of Maillard precursors. Contrary to literature data (*22*), binary mixtures of glycolaldehyde and serine or alanine led only to traces of furan under the reaction conditions used in this study (data not shown), which is most likely due to the lack of α-dicarbonyls required for initiating the Strecker type degradation reaction.

In general, furan can also be formed from hexose sugars in the presence of any amino acid (**Figure 5**). Key intermediates have been suggested as follows (*22*): 1-deoxyhexoluses leading to aldotetroses (**A**), 2-deoxy-3-keto-aldotatrose (**C**), and 3-deoxy-osones yielding 2-deoxy-aldotetrose (**D**), which can also be formed from 3-deoxy-pentosones (**E**, **F**). These intermediates give rise to five-membered cyclic compounds, leading to furan by reduction and dehydration. As shown in **Figure 5**, the redox system plays a pivotal role in furan formation, as many transformations involve oxidation and/or reduction steps. Therefore, not only the concentration of the potential precursors is important but the whole composition of the reaction or food system.

Samples 5−8 containing lipids as precursors resulted in fairly high furan concentrations in the headspace (**Table 1**). In particular, polyunsaturated lipids having three double bonds (C18:3), such as linolenic acid and the corresponding triglyceride, were prone to generating furan (3.3−4.8 mmol/mol). Linoleic acid and trilinoleate, containing two double bonds (C18:2), formed less furan (0.7−1.7 mmol/mol). Becalski and Seaman (*23*) have also observed this phenomenon. As compared to the fatty acids, the corresponding triglycerides generated 1.5−2.5-fold higher levels of furan due to the 3-fold higher amount of

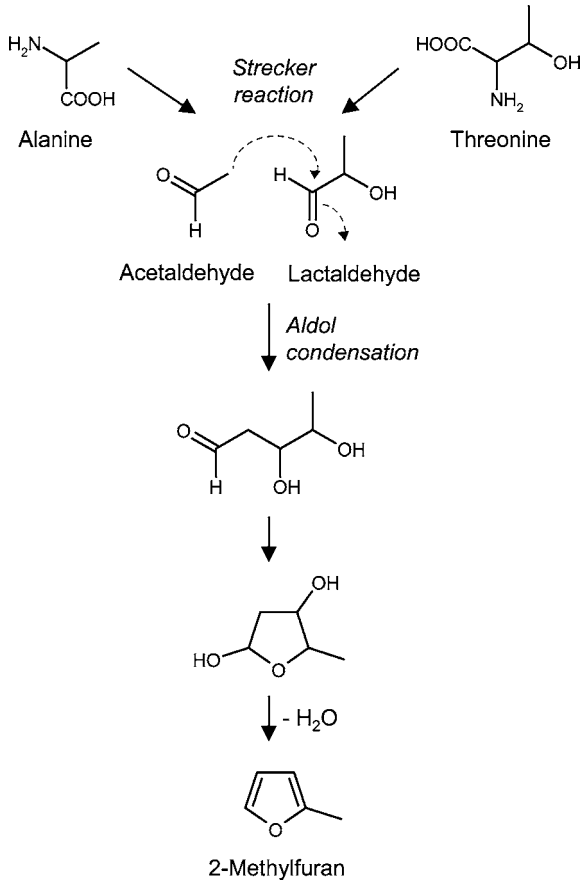

Figure 6. Schematic formation of 2-MF via Maillard type reactions using suitable precursors, such as reducing sugars and specific amino acids, i.e., alanine and threonine, forming Strecker aldehydes as key intermediates.

fatty acids. The higher furan levels observed in triple unsaturated lipids (linolenic acid, trilinolenin) are most likely due to the fact that they oxidize easier than double unsaturated lipids (linoleic acid, trilinolein). Interestingly, triple unsaturated lipids also generated MF, in contrast to double unsaturated lipids,

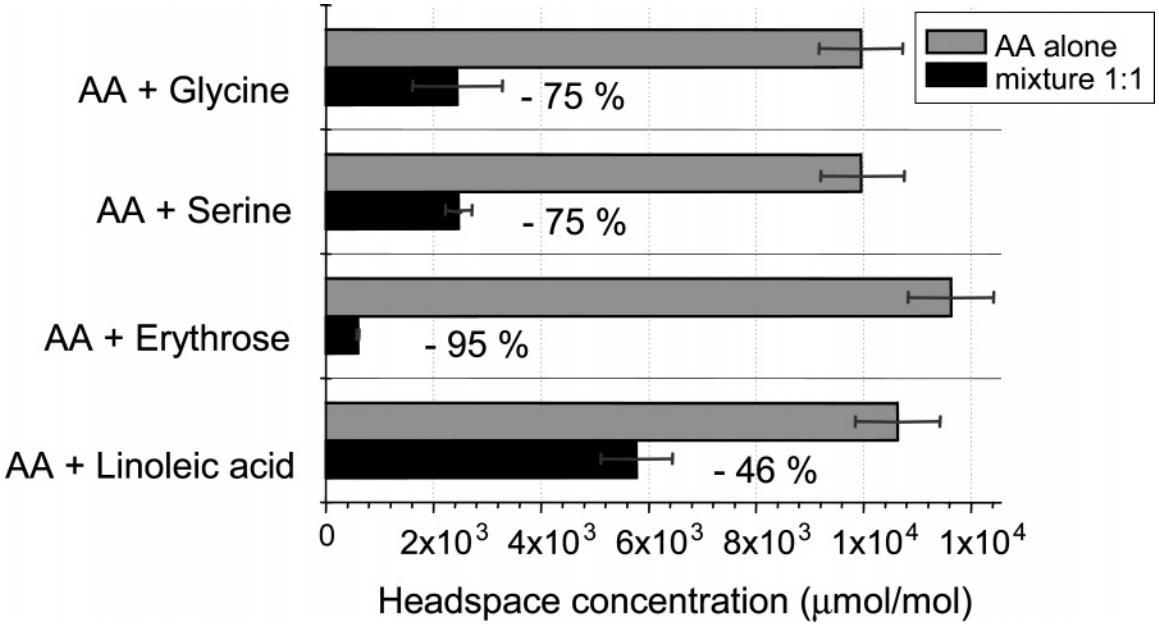

Figure 7. Comparison of furan formation from ascorbic acid (AA) alone (gray bars) and binary mixtures, i.e., in the presence of equimolar amounts of additional compounds (black bars).

which is reported for the first time in this paper. About 10−20% of the total amount can be attributed to methylated furan derivatives (**Table 1**). An exact determination of the substitution pattern was not performed in this study. Literature data, however, indicate 2-MF to largely dominate over 3-MF (*6*). Recently, β-carotene has been reported as a precursor of 2- and 3-MF (*23*).

It should be mentioned that the quantitative results obtained by PTR-MS only reflect the concentration of furan in the headspace, not in the sample, even though the measurements were always carried out until the release curve completely went back to base level. To check if some furan might still be trapped inside the reaction matrix, defined experiments were repeated with the admixture of sea sand to enhance the surface contact of the sample. Indeed, for linoleic acid, the observed furan level was somewhat higher (∼1.1 mmol/mol). However, this effect was less pronounced in the ascorbic acid and Maillard samples, which is most likely due to production of CO_2 during the heating process. The resulting foamy structure probably facilitated evaporation of volatile compounds, in particular those with low boiling points such as furan and MF.

Overall, our data obtained under dry roasting conditions indicate ascorbic acid as the major precursor of furan, followed by glyceryl trilinolenate. However, the transformation yields determined by PTR-MS are very low, i.e., ≤1 mol %. Under pressure-cooking conditions (118 °C, 30 min), Becalski and Seaman (*23*) found the highest amounts of furan when heating glyceryl trilinolenate (∼570 μg/kg) or linolenic acid (∼620 μg/kg), in particular in the presence of catalytic amounts of ferric ions (up to about 1000 μg/kg).

Effect of Additives. The formation of furan and MF was further investigated in the presence of various additives, such as ferric ions and antioxidants. These compounds are known to affect lipid oxidation, which should also influence the formation of furans. The presence of ferric ions significantly increased the furan concentration in samples containing lipids with two double bonds, i.e., linoleic acid (+79%, sample 9) and glyceryl trilinoleate (+29%, sample 10) (**Table 2**). Surprisingly, triple unsaturated lipids produced lower levels (up to −60%) of furan and MF in the presence of ferric ions (samples 11 and 12).

Table 2. Formation of Furan and MF in Various Model Systems as Affected by Selected Additives[a]

	model system	furan (μmol/mol)	VC[b] (%)	MF[c] (μmol/mol)	VC[b] (%)	deviation[d] (%)
9	linoleic acid + Fe^{3+}	1216	14.5	ND		+79
10	trilinolein + Fe^{3+}	2222	19.0	ND		+29
11	linolenic acid + Fe^{3+}	1736	16.6	1207	35.4	−20
12	trilinolenin + Fe^{3+}	1666	16.8	448	25.6	−63
13	linoleic acid + BHT	1736	3.6	ND		+155
14	linolenic acid + α-tocopherol	2615	19.1	422	25.9	−17
15	ascorbic acid + N_2 purge	6915	7.8	ND		−31
16	ascorbic acid + Na-sulfite	4255	21.4	ND		−57

[a] Quantitative data refer to concentrations in the headspace. [b] Variation coefficient. [c] MF representing the sum of the 2- and 3-isomers (ND, not detected). [d] As compared to the original amounts of the sum of furan and MF. Additives used as follows: ferric (Fe^{3+}) ions, $Fe_2(SO_4)_3$ (1.2%, w/w); Na-sulfite, sodium sulfite, Na_2SO_3 (1.2%, w/w); (±)-α-tocopherol, 3,4-dihydro-2,5,7,8-tetramethyl-2-[4,8,12-trimethyltridecyl]-2*H*-1-benzopyran-6-ol (1.2%); and BHT, 2,6-bis(1,1-dimethylethyl)-4-methylphenol (1.2%, w/w).

Becalski and Seaman (*23*) have observed similar effects under pressure-cooking conditions for glyceryl trilinolenate (−20%) but not for linolenic acid (+60%). The formation of MF was favored in the linolenic acid system (samples 7 and 11, +300%), while a reduction of −55% was found with trilinolenin (samples 8 and 12).

Mitigation experiments were performed in model systems to reduce the amounts of furan by intervening at certain steps of the formation pathways. The initial stage of the reaction cascade in lipid oxidation and ascorbic acid or sugar degradation involve oxidation steps, whereas the actual furan formation often requires a reduction step (**Figure 5**). Therefore, addition of components with antioxidative properties or just removing oxygen was evaluated in selected model systems to reduce furan formation.

Surprisingly, linoleic acid showed an unexpected increase of +155% in the presence of the antioxidant BHT (sample 13). Furthermore, this amount exceeded by +40% that of sample 9 containing ferric ions. On the other hand, linolenic acid produced only a slightly lower amount of furan and MF (−20%) in the presence of the antioxidant α-tocopherol (**Table 2**, sample 14),

which was similar to that obtained with ferric ions (sample 11). On the basis of the hypothesis shown in **Figure 1**, only oxidative steps are involved in the formation of 4-hydroxy-2-butenal from polyunsaturated fatty acids (PUFAs), followed by cyclization and dehydration to yield furan. Therefore, antioxidants were expected to reduce furan formation. Indeed, recent investigations have shown under pressure-cooking conditions furan reductions of up to 70% from PUFAs in the presence of commercially used antioxidants (tocopherol acetate, BHA), unfortunately without providing experimental details (*23*). However, antioxidants are also known to develop prooxidative properties at higher concentrations. These findings support the assumption that the formation pathways of furan and MF from lipids are far from being understood. Different mechanisms may exist requiring distinct conditions. Additional studies are in progress to explain the different tendencies in furan formation found for double and triple unsaturated lipids, which will be published elsewhere.

Replacing air by nitrogen resulted in a remarkable decrease of furan (−30%) from ascorbic acid (**Table 2**, sample 15). This could even be improved by the addition of sodium sulfite leading to a major furan reduction of −60% (sample 16). This may be explained by the multifunctional properties of sulfite, acting as an antioxidant (radical scavenger) and nucleophile by reacting with the carbonyl groups of ascorbic acid and its further intermediates. The influence of sodium sulfite is also clearly visible in **Figure 4** (**A** vs. **C**). The on-line *m*/*z* 69 release curves showed a very similar shape and area, but the composition of the signal was different in the presence of sulfite (2nd column), thus leading to reduced amounts of furan released into the headspace (3rd column). Interestingly, the volatile composition changed in the presence of sulfite and resulted in additional compounds releasing ions at *m*/*z* 69. This is most likely due to the reaction of ascorbic acid with sulfite leading to new chemical structures as compared to pure ascorbic acid (sample 1).

Binary Mixtures. The data obtained so far indicate ascorbic acid as the major precursor of furan. In food systems, however, ascorbic acid is usually accompanied by many other compounds such as sugars, amino acids, lipids, etc. Therefore, in another series of experiments, we have investigated binary model mixtures composed of equimolar amounts of ascorbic acid and additional compounds, some of which are furan precursors on their own. The results plotted in **Figure 7** show a significant reduction of furan (−75%) in the presence of the amino acids glycine or serine. These data indicate that amino acids, in general, might reduce the level of furan to approximately one-quarter. In the presence of erythrose, the furan reduction was even more pronounced and close to −95%, despite the fact that each of the compounds may function as a furan precursor. The mitigation in the ascorbic acid/linoleic acid system can partly be explained by the reduced oxygen supply for ascorbic acid embedded in the lipid phase. Overall, it seems that in all of these samples competitive reaction mechanisms take place leading to a partial suppression of the furan formation. As a consequence, ascorbic acid loses much of its furan-generating potential in the presence of additional food constituents. In addition, the pH seems to play an important role in furan formation from sugars and ascorbic acid under pressure-cooking conditions, which is favored at pH 3 as compared to pH 7, as recently reported by Fan (*34*).

In conclusion, the combination of PTR-MS and GC-MS revealed to be a powerful and convenient experimental setup to obtain qualitative and quantitative results on furan and MF generated from specific precursors upon thermal treatment. Significant amounts of furan and MF were detected in the headspace of model systems under dry roasting conditions. Furan formation was quite sensitive toward changes of the reaction conditions and precursor compositions indicating the complexity of the reaction pathways. Furan amounts were reduced to a great extent by favoring competing reactions and/or intervening at the redox system level. Therefore, the furan levels are possibly much lower in more complex systems than one would expect from the data obtained with pure ascorbic acid. Consequently, conclusions should be drawn with much caution, avoiding data extrapolation from oversimplified model systems based on single precursors to complex food products. Additional work may help to understand the formation mechanisms of furan and MF from various types of precursors under food-processing conditions in order to reduce their formation.

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LITERATURE CITED

(1) U.S. Food and Drug Administration (May 7, 2004; updated June 7, 2004), http://www.cfsan.fda.gov/~dms/furandat.html.

(2) International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; IARC: Lyon, France, 1995; Vol. 63, pp 3194−3407.

(3) European Food Safety Authority. Report on the scientific panel on contaminants in the food chain on provisional findings on furan in food. *EFSA J.* **2004**, *137*, 1−20.

(4) Reinhard, H.; Sager, F.; Zimmermann, H.; Zoller, O. Furan in foods on the Swiss market−Method and results. *Mitt. Lebensmittelunters. Hyg.* **2004**, *95*, 532−535.

(5) Becalski, A.; Forsyth, D.; Casey, V.; Lau, B. P.-Y.; Pepper, K.; Seaman, S. Development and validation of a headspace method for determination of furan in food. *Food Addit. Contam.* **2005**, *22*, 535−540.

(6) Maga, J. A. Furans in foods. *CRC Crit. Rev. Food Sci. Nutr.* **1979**, *11*, 355−400.

(7) Sugisawa, H. Thermal degradation of sugars. II. Volatile decomposition products of glucose caramel. *J. Food Sci.* **1966**, *31*, 381−385.

(8) Stoffelsma, J.; Sipma, G.; Kettenes, D. K.; Pypker, J. New volatile components of roasted coffee. *J. Agric. Food Chem.* **1968**, *16*, 1000−1004.

(9) Mulders, E. J.; Maarse, H.; Weurman, C. Odor of white bread. I. Analysis of volatile constituents in the vapor and aqueous extracts. *Z. Lebensm.-Unters. Forsch.* **1972**, *150*, 68−74.

(10) Persson, T.; von Sydow, E. Aroma of canned beef. Gas-chromatographic and mass-spectrometric analysis of the volatiles. *J. Food Sci.* **1973**, *38*, 377−385.

(11) Qvist, I. H.; von Sydow, E. C. F. Unconventional proteins as aroma precursors. Chemical analysis of the volatile compounds in heated soy, casein, and fish protein model systems. *J. Agric. Food Chem.* **1974**, *22*, 1077−1084.

(12) Frankel, E. N.; Neff, W. E.; Selke, E.; Brooks, D. D. Thermal and metal-catalyzed decomposition of methyl linolenate hydroperoxides. *Lipids* **1987**, *22*, 322−327.

(13) Mottram, D. S. Meat. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Marcel Dekker: New York, 1991; Vol. 44, Food Science and Technology, pp 107−177.

(14) Greenwood, C. T.; Knox, J. H.; Milne, E. Analysis of the thermal decomposition products of carbohydrates by gas chromatography. *Chem. Ind. (London)* **1961**, 1878−1879.

(15) Walter, R. H.; Fagerson, I. S. Volatile compounds from heated glucose. *J. Food Sci.* **1968**, *33*, 294−297.

(16) Heyns, K.; Stute, R.; Paulsen, H. Browning reaction and fragmentation of carbohydrates. I. Volatile products from the thermal degradation of D-glucose. *Carbohydr. Res.* **1966**, *2*, 132−149 (in German; *Chem Abstr.* **1966**, *65*, 38725).

(17) Heyns, K.; Klier, M. Browning reactions and fragmentation of carbohydrates. IV. Comparison of the volatile degradation products upon pyrolysis of mono-, oligo- and polysaccharides. *Carbohydr. Res.* **1968**, *6*, 436−448 (in German; *Chem Abstr.* **1968**, *69*, 10648).

(18) Adams, A.; Borrelli, R. C.; Fogliano, V.; De Kimpe, N. Thermal degradation studies of food melanoidins. *J. Agric. Food Chem.* **2005**, *53*, 4136−4142.

(19) Ferretti, A.; Flanagan, V. P.; Ruth, J. M. Nonenzymatic browning in a lactose-casein model system. *J. Agric. Food Chem.* **1970**, *18*, 13−18.

(20) Yaylayan, V. A.; Forage, N. G.; Mandeville, S. Microwave and thermally induced Maillard reactions. In *Thermally Generated Flavors*; Parliament, T. H., Morello, M. J., McGorrin, R. J., Eds.; American Chemical Society: Washington, DC, 1994; pp 449−456.

(21) Ames, J. M.; Guy, R. C. E.; Kipping, G. J. Effect of pH, temperature, and moisture on the formation of volatile compounds in glycine/glucose model systems. *J. Agric. Food Chem.* **2001**, *49*, 4315−4323.

(22) Perez Locas, C.; Yaylayan, V. A. Origin and mechanistic pathways of formation of the parent furan—A food toxicant. *J. Agric. Food Chem.* **2004**, *42*, 6830−6836.

(23) Becalski, A.; Seaman, S. Furan precursors in food: A model study and development of a simple headspace method for determination of furan. *J. AOAC Int.* **2005**, *88*, 102−106.

(24) Goldmann, T.; Perisset, A.; Scanlan, F.; Stadler, R. H. Rapid determination of furan in heated foodstuffs by isotope dilution solid-phase microextraction-gas chromatography−mass spectrometry (SPME-GC-MS). *Analyst* **2005**, *130*, 878−883.

(25) Pollien, P.; Lindinger, C.; Yeretzian, C.; Blank, I. Proton-transfer reaction mass spectrometry, a tool for on-line monitoring of acrylamide in food and Maillard systems. *Anal. Chem.* **2003**, *75*, 5488−5494.

(26) Lindinger, C.; Pollien, Ph.; Ali, S.; Yeretzian, C.; Blank, I.; Märk, T. Unambiguous identification of volatile organic compounds by proton-transfer-reaction mass-spectrometry (PTR-MS) coupled with GC-MS. *Anal. Chem.* **2005**, *77*, 4117−4124.

(27) Pollien, Ph.; Lindinger, C.; Ali, S.; Yeretzian, C. Absolute quantification of headspace volatiles by PTR-MS. 1st International Conference on PTR-MS and its Applications, Igls/Innsbruck, Austria, January 18−23, 2003; pp 153−156, http://www.uibk.ac.at/c/c7/c722/Tagungen/PTRMS03/proceedings_2nd_ed.pdf.

(28) *Wiley Registry of Mass Spectral Data*, 7th ed.; McLafferty, F., Ed.; Database, with NIST Spectral Data; Wiley: Chichester, 2003.

(29) McFarland, M.; Albritton, A. L.; Fehsenfeld, F. C.; Ferguson, E. E.; Schmeltekopf, A. L. *J. Chem. Phys.* **1973**, *59*, 6620−6628.

(30) Robert, F.; Vuataz, G.; Pollien, P.; Saucy, F.; Alonso, M.-I.; Bauwens, I.; Blank, I. Acrylamide formation from asparagine under low-moisture Maillard reaction conditions. 1. Physical and chemical aspects in crystalline model systems. *J. Agric. Food Chem.* **2004**, *52*, 6837−6842.

(31) Vernin, G.; Chakib, S.; Rogacheva, S. M.; Obretenov, T. D.; Parkanyi, C. Thermal decomposition of ascorbic acid. *Carbohydr. Res.* **1998**, *305*, 1−15.

(32) Velisek, J.; Davidek, J.; Kubelka, V.; Zelinkova, Z.; Pokorny, J. Volatile degradation products of L-dehydroascorbic acid. *Z. Lebensm.-Unters. Forsch.* **1976**, *162*, 285−290.

(33) Ledl, F.; Schleicher, E. New aspects of the Maillard reaction in foods and in the human body. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 565−594.

(34) Fan, X. Formation of furan from carbohydrates and ascorbic acid following exposure to ionizing radiation and thermal processing. *J. Agric. Food Chem.* **2005**, *53*, 7826−7831.

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