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Formation of Furan and Methylfuran by Maillard-Type Reactions in Model Systems and Food

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The formation of furan and 2-methylfuran was studied in model systems based on sugars and selected amino acids. Both compounds were preferably formed under roasting conditions in closed systems yielding up to 330 μmol of furan and 260 μmol of 2-methylfuran per mol of precursor. The amounts obtained under pressure cooking conditions were much lower, usually below 20 μmol/mol, except for 2-furaldehyde, which yielded 70−100 μmol/mol of furan. Labeling studies indicated two major formation pathways for both furans: (i) from the intact sugar skeleton and (ii) by recombination of reactive C_2 and/or C_3 fragments. Under roasting conditions in the absence of amino acids, furan was mainly formed from the intact sugar skeleton. Formic and acetic acid were identified as byproducts of sugar degradation, indicating the split off of C_1 and/or C_2 units from hexoses. The presence of alanine, threonine, or serine promoted furan formation by the recombination of C_2 fragments, such as acetaldehyde and glycolaldehyde, which may originate from both sugars and amino acids. In aqueous solution, about half of furan was generated by the recombination of sugar fragments. 2-Methylfuran was preferably formed in the presence of amino acids by aldol-type reactions of C_2 and C_3 fragments with lactaldehyde as a key intermediate, the Strecker aldehyde of threonine. The total furan levels in cooked vegetables were increased by spiking with hexoses. However, in pumpkin puree, only about 20% of furan was formed from sugars, preferably from the intact carbon skeleton.

KEYWORDS: Furan; methylfuran; sugars; amino acids; model systems; labeled precursors; mechanistic study; Maillard reaction; Strecker aldehydes; SPME-GC/MS; CAMOLA

INTRODUCTION

Furan (C_4H_4O, CAS No. 110-00-9) has received considerable attention due to its classification as "possibly carcinogenic to humans" (Group 2B) (*1*) and the relatively high amounts of it in food that had undergone heat treatment, in particular, canned and jarred products (*2*). However, this volatile, food-borne, heat-induced chemical has been known for long time as a food constituent (reviewed in ref *3*). As there is only a limited set of data available on food, which does not represent the average diet, the European Food Safety Agency has called for further quantitative data for furan in food (*4*). In addition, more work is needed on the mechanisms of formation, including precursor composition and heating conditions, to develop concepts for mitigation upon food processing.

Several precursor classes have been suggested to release furan upon thermal treatment, such as ascorbic acid and related compounds, Maillard reaction systems (reducing sugars, specific amino acids), lipids comprising unsaturated fatty acids and the corresponding triglycerides, as well as carotenes and organic acids (*5*−*9*). The variety of precursors is not surprising because furan can be seen as a rather stable reaction product that may be generated from different chemical classes by the degradation and/or recombination of smaller fragments.

So far, mechanistic work has mainly been devoted to ascorbic acid as it had been claimed to be one of the major furan precursors yielding between 0.1 μmol/mol (pressure cooking, γ-irradiation) and about 1 mmol/mol (roasting) of furan. The relatively high furan amounts could be confirmed in model systems, particularly under roasting conditions (*6, 8*). However, under pressure cooking conditions in food, ascorbic acid does not seem to function as a direct precursor but may support furan formation from other precursors such as sugars and/or lipids (*9*). Therefore, our research activities were directed toward other potential furan precursors with a focus on Maillard systems and lipids. This paper deals with furan formation initiated by Maillard-type reactions. The results on furan formation triggered by lipid oxidation will be published elsewhere.

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The aim of this work was to study the formation of furan and its methyl derivative from sugars and specific amino acids in food and model systems simulating food process conditions such as roasting and pressure cooking. Reliable quantitative data were obtained by solid phase microextraction (SPME) in conjunction with gas chromatography/mass spectrometry (GC/MS) using [$^2H_4$]-furan as the internal standard. Mechanistic insight into the degradation steps was achieved by using ^{13}C-labeled precursors.

EXPERIMENTAL PROCEDURES

Materials. The following chemicals are commercially available: L-alanine (ALA, >99.5%), D-arabinose (ARA, 99%), citric acid (monohydrate), disodium hydrogenphosphate (dihydrate, 99%), D-erythrose (ERY, ≥90%), D-fructose (FRU, 98%), furan (stabilized, 99%), d_4-furan (stabilized, 98%), 2-furaldehyde (99%), D-glucose anhydrous (GLU, 98%), 2-methylfuran (MF, 99%), D-phenylalanine (PHE, 98%), L-threonine (THR, 98%), white quartz sand, and silicone oil (oil bath, from −50 to 200 °C) (Sigma-Aldrich, Buchs, Switzerland); L-serine (SER, 99%) and methanol (for analysis) (Merck, Darmstadt, Germany); 3-methylfuran (97%) (Acros Organics, Geel, Belgium); 3-deoxyglucosone (3DG, 95%) (Toronto Research Chemicals, North York, Canada); [U-$^{13}C_6$]-D-glucose, [1,2-$^{13}C_2$]-D-glucose, [U-$^{13}C_6$]-D-fructose, [1-^{13}C]-D-fructose, [U-$^{13}C_3$]-L-alanine, [3-^{13}C]-L-alanine, and [U-$^{13}C_4$]-L-threonine (Cambridge Isotope Laboratories, Andover, MA); and [$^{13}C_1$]-D-arabinose (>98%, Omicron Biochemicals, South Bend, IN).

Sample Preparation. *Roasting Model Systems.* Equimolar amounts of precursors (0.1 mmol each) and sea sand (1 ± 0.05 g) were mixed in a headspace vial (20 mL, clear glass, 75.5 mm × 22.5 mm, 20 mm crimp, rounded bottom, Brechbühler, Geneva, Switzerland) used as a reaction vessel, which was sealed with a crimp cap. The samples were heated at 200 °C for 10 min, simulating roasting conditions. Experiments were carried out in duplicate by using two reaction vessels, both simultaneously immersed into a silicon oil bath. A homogeneous temperature distribution was achieved by magnetic stirring. More experimental details are given in ref *9*.

Aqueous Model Systems. Equimolar amounts of precursors (1 mmol each) were placed in a volumetric flask (10 mL) and dissolved in the buffer solution (pH 4 or pH 7) by stirring. Citric acid-phosphate buffer solutions were used for buffering the aqueous model systems (*10*). An aliquot of the homogeneous solution (1 mL = 0.1 mmol of each precursor) was transferred into the reaction vessel (20 mL headspace vial) and then sealed with a crimp cap. The vials were then homogenized using a Vortex shaker (Vortex Genie 2, Verrerie Carouge, Switzerland) for 30 s. The aqueous samples were heated at 121 °C for 25 min, simulating sterilization conditions. More experimental details are given in ref *9*.

Food Products. Vegetable puree was prepared from pumpkin bought in a local grocery store (Migros) by using a plastic squeezer (Moulinex, Switzerland). The pH values were immediately measured. For spiking experiments, defined amounts of precursors were added into a flask (50 mL) containing the vegetable puree (10 g) and stirred to obtain a homogeneous sample. An aliquot (2 g) was then placed into the reaction vessel (20 mL headspace vial) and sealed. The spiked samples were heated at 123 °C for 22 min, simulating sterilization conditions.

Quantification of Furan and Methylfuran. *Isotope Dilution Assay.* After heat treatment, the samples were cooled in a water bath (~10 °C) for 5 min. The internal standard ($^2H_4$-furan = d_4-furan) was added through the septum with a gastight syringe (10 μL, Sigma-Aldrich), and the vial was vortexed. The added volume of internal standard (ca. 0.8 μg of $^2H_4$-furan/mL of methanol) was adjusted (1−25 μL) in a way that analyte/standard ratios were within the linear range of the calibration curve. The samples were left at room temperature for at least 30 min to achieve equilibrium prior to analysis.

SPME-GC/MS. The analytical method for furan quantification published by Goldmann et al. (*11*) was used after validation with some modifications as recently reported (*9*). In short, sample preparation involved the addition of a deuterated analogue of furan (d_4-furan) to the sample via the septum, extraction from the headspace at 35 °C for 10 min, adsorption onto Carboxen fiber (polydimethylsiloxan, PDMS, film thickness 75 μm, Supelco, Bellefonte, PA), and finally desorption for 2 min. After GC separation, the data were acquired by MS. The chromatographic conditions on a ZebronWAX capillary column (30 m × 0.25 mm, d_f 0.5 μm, Phenomenex, Torrance, CA) have recently been described (*9*).

Quantitative analyses were performed in the selective ion monitoring (SIM) mode and repeated in the full scan mode, whereas the experiments with the labeled precursors were only analyzed in the full scan mode. The parameters of the MS have recently been described (*9*). Quantification was based on MS signals at *m/z* 68 for furan, *m/z* 72 for d_4-furan, and *m/z* 82 for methylfuran. The following qualifiers were used: *m/z* 39 and 69 for furan, *m/z* 42 for d_4-furan, and *m/z* 53 for methylfuran. The quantities of furan and 2-methylfuran were determined relative to the internal standard (d_4-furan). The response factor (R_F) was determined taking into consideration the differences in response of the mass detector between analyte and standard (based on the slope of the calibration curve). For methylfuran, the response factor additionally was influenced by a different recovery rate of analyte and standard during SPME adsorption. A stronger adsorption of the less polar methylfuran explains the relatively low R_F value of 0.36 as compared to the R_F value of 0.97 for furan.

Calculation of Labeling Percentage. The percent labeling distribution of furan and methylfuran was determined by subtracting the naturally occurring percentage of ^{13}C. An additional data treatment was required for methylfuran (*9*), and in contrast to furan, methylfuran generated a $[M - 1]^+$ signal ($[C_5H_5O]^+$, *m/z* 81) through the loss of $H^\cdot$ with a relative intensity of 63% as compared to the molecule ion signal (*m/z* 82). Therefore, the $[M - 1]^+$ signal of methylfuran has to be taken into account when correcting the overall signal intensity to calculate the percent labeling distributions in labeling experiments studying methylfuran formation. The obtained percentages after correction lower than 1% were set to 0% by definition.

Carbon Module Labeling (CAMOLA). Model samples were prepared by mixing equimolar amounts of fully labeled and unlabeled precursors (for the CAMOLA approach, see ref *12*) (i.e., [U-$^{13}C_6$]-glucose and glucose), followed by heat treatment (roasting, aqueous conditions), extraction of furan and methylfuran by SPME as described previously, and GC/MS analysis.

RESULTS AND DISCUSSION

Quantification of Furan and 2-Methylfuran in Model Systems. The formation of furan and 2-methylfuran was studied in model systems simulating roasting and pressure cooking (sterilization) conditions. This study focused on Maillard-type reactions based on sugars, sugar derivatives, and amino acids as putative furan precursors. The amounts of furan and 2-methylfuran were quantified by SPME-GC/MS to evaluate the efficiency of various precursors systems. The results are expressed in micromol of furan or 2-methylfuran per mol of precursor, and the relative standard deviation (RSD) is given in percent. 3-Methylfuran also was detected in various model systems (baseline separated from 2-methylfuran), but the quantities were very low, and therefore, these data are not reported in this paper.

Roasting Conditions. As shown in **Table 1**, the amounts of furan generated from sugars (nos. 1−4) under roasting conditions were in the range of about 70−340 μmol/mol. Arabinose (no. 3) was the most efficient precursor of furan (335 μmol/mol), whereas erythrose was the least efficient (no. 4, 75 μmol/mol). Addition of phenylalanine to glucose resulted in an increase of about 50% (no. 8, 124 μmol/mol), whereas the furan amount decreased by 20% in the binary mixture of fructose and phenylalanine (no. 9, 100 μmol/mol). In contrast, the presence of the amino acids alanine, threonine, and serine (nos. 5−7) all resulted in higher furan amounts, in particular, with glucose.

Table 1. Amounts (μmol/mol) of Furan and 2-Methylfuran Generated in Various Model Systems Containing Sugars, Sugar Derivatives, and Amino Acids

		roasting				pressure cooking (pH 7)				pressure cooking (pH 4)			
no.	model system[a]	furan	RSD	MF	RSD	furan	RSD	MF	RSD	furan	RSD	MF	RSD
1	GLU	79.6	4.3	24.3	4.3	2.05	9.7	0.24	14.4	<0.1		<0.1	
2	FRU	121.5	5.8	23.5	5.1	7.59	0.5	0.91	6.0	0.1	24.4	<0.1	
3	ARA	334.6	0.4	23.7	3.3	17.3	26.1	2.04	30.6	0.42	18.1	<0.1	
4	ERY	74.9	0.7	7.7	6.2	15.7	3.8	3.14	7.1	0.43	10.1	<0.1	
5	GLU + ALA + THR	152.8	4.7	262.3	6.4	8.87	17.7	3.05	26.5	0.62	7.4	<0.1	
6	GLU + ALA + SER	198.5	0.3	207.6	3.7	10.7	8.7	4.65	9.7	1.84	14.2	0.4	8.4
7	FRU + ALA + SER	159.5	11.4	262.9	6.6	9.09	16.4	8.1	22.8	0.53	6.9	0.1	2.6
8	GLU + PHE	123.9	0.9	188.0	5.2	9.11	1.9	11.5	1.3	0.23	30.5	0.1	16.8
9	FRU + PHE	99.6	5.1	158.1	10.3	7.06	18.1	9.0	25.0	0.36	28.8	<0.1	
10	3DG[b]	23.7		15.0		1.9		0.5		1.1		0.1	
11	R5P[b]	69.0		142.1		1.8		3.9		<0.1		0.14	
12	2-furaldehyde	265.0	16.1	1.87	19.4	97.7	0.6	<0.1		70.4	10.8	1.64	6.8

[a] Roasting (200 °C, 10 min) and pressure cooking (121 °C, 25 min). [b] Only one experiment was carried out. RSD = relative standard deviation in percent. Glucose (GLU), fructose (FRU), arabinose (ARA), erythrose (ERY), alanine (ALA), threonine (THR), serine (SER), phenylalanine (PHE), 3-deoxyglucosone (3DG = 3-deoxyhexos-2-ulose), and ribose-5-phosphate (R5P).

However, these values are much lower as compared to those obtained with ascorbic acid under similar reaction conditions (*8, 9*).

The levels of 2-methylfuran generated from pure sugars (nos. 1−4) were significantly lower (8−24 μmol/mol) as compared to furan. However, in Maillard samples (nos. 5−9), and, in particular, in the presence of alanine, the methylfuran amounts (160−260 μmol/mol) were higher as compared to furan. These data support the hypothesis that 2-methylfuran also may be formed by the recombination of C_2 and C_3 fragments originating from sugars and/or amino acids (*8, 9*). The sugar intermediate 3-deoxyglucosone (no. 10) formed low amounts of both furan and 2-methylfuran (15−24 μmol/mol). Ribose-5-phosphate (no. 11) turned out to be more efficient, in particular, with respect to 2-methylfuran (142 μmol/mol). As previously reported (*9*), 2-furaldehyde (no. 12) is an efficient precursor of furan (265 μmol/mol). Overall, the furan yields obtained under dry-heating conditions from sugars and Maillard reaction systems are rather low as compared to other potential precursors such as ascorbic acid and polyunsaturated fatty acids (*5−9*).

Pressure Cooking Conditions. In general, thermal treatment of sugars and/or amino acids under pressure cooking conditions resulted in much lower amounts of furan and 2-methylfuran as compared to the dry-heat treatment. At pH 7, the furan levels were in the range of 2−17 μmol/mol. Pentose sugars, in particular, arabinose (no. 3), tend to generate more furan than hexoses (nos. 1 and 2). However, the presence of an amino acid (nos. 5−9) favors furan formation from glucose (9−11 μmol/mol). 2-Methylfuran was particularly abundant (~10 μmol/mol) at pH 7 in the presence of phenylalanine (nos. 8 and 9). On the other hand, very low amounts of furan (<1 μmol/mol) and 2-methylfuran (<0.1 μmol/mol) were found at pH 4, with the exception of sample 6 (GLU + ALA + SER), resulting in 1.8 and 0.4 μmol/mol, respectively. This is most likely due to the fact that sugar fragmentation and enolization are favored under neutral and alkaline conditions. The sugar intermediate 2-furaldehyde (no. 12) was found to be an efficient precursor of furan under pressure cooking conditions, resulting in 70−100 μmol/mol. 2-Furoic acid also has been shown to be a potential intermediate in furan formation (*8, 9*).

Formation of Furan. The mechanisms of furan formation were investigated using specifically and uniformly ^{13}C-labeled precursors such as sugars and amino acids. The CAMOLA approach (*12*) also has been applied to obtain insight into the basic principles of furan formation (i.e., recombination of fragments as compared to the intact carbon chain). It was observed, indeed, that formation mechanisms depend much on the reaction conditions applied, such as roasting and pressure cooking. Aqueous samples were only heated at pH 7 due to low yields obtained at pH 4 (**Table 1**).

From Sugars under Roasting Conditions. Model mixtures of fully labeled and unlabeled sugars (ratio 1:1) indicated in CAMOLA experiments with both glucose and fructose that furan was formed from the intact sugar only and not by the recombination of fragments. As shown in **Table 2** (nos. 13 and 15), only $[M]^+$ (*m/z* 68) and $[M + 4]^+$ (*m/z* 72) were found, suggesting that unlabeled and labeled furan resulted from unlabeled and labeled glucose or fructose, respectively. As mixed isotopomers at *m/z* 69−71 could not be detected, it can be excluded under these experimental conditions that furan was formed by the recombination of sugar fragments (e.g., $C_1 + C_3$ or $C_2 + C_2$).

In addition, model reactions with single and double ^{13}C-labeled sugars were performed to understand as to which part of the carbohydrate skeleton is incorporated into furan. The experiment with [1,2-$^{13}C_2$]-glucose (no. 14) revealed that furan is mainly formed from the C3−C6 moiety of glucose (62%) and to a lesser extent from the C2−C5 (25%) and C1−C4 moiety (13%). Similar results were obtained with [1-^{13}C]-fructose (no. 16): the C3−C6 and C2−C5 moieties of fructose account for 91% of furan, whereas the C1−C4 moiety contributes only 9%. The data suggest various degradation mechanisms leading to furan by splitting off C_1 and/or C_2 leaving groups. Indeed, formic acid (C_1) and acetic acid (C_2) were identified as byproducts in model system numbers 1 and 2 (data not shown). Labeled sugars formed a mixture of labeled and unlabeled formic and acetic acids. [1,2-$^{13}C_2$]-Glucose (no. 14) gave rise to labeled formic acid and $^{13}C_2$-acetic acid with about 60% yield each, the remaining acids being unlabeled. [1-^{13}C]-Fructose (no. 16) led to labeled formic acid and $^{13}C_1$-acetic acid with 60 and 70% yield, respectively. The labeling pattern suggests that these acids were mainly formed from C-1 and C1−C2 of the sugars, which is consistent with the findings that furan is preferably represented by the C3−C6 sugar moiety.

Several pathways are suggested in **Figure 1** to explain the formation of furan from glucose and fructose, which can be converted into each other via the 1,2-enediol intermediate. The experiment with [1,2-$^{13}C_2$]-glucose is shown in red in **Figure 1**, whereas blue stands for the experiment with [1-^{13}C]-fructose. The first step of the pathway is suggested to be dehydration either at C-3 or C-4 (from the 1,2- and 2,3-endiols, respectively)

Table 2. Percent Labeling Distribution of Furan Generated in Different Model Systems Containing Sugars and Amino Acids

		roasting[a]					pressure cooking (pH 7)[b]				
no.	model system	M	M + 1	M + 2	M + 3	M + 4	M	M + 1	M + 2	M + 3	M + 4
13	GLU + [U-$^{13}C_6$]-GLU (1:1)[c]	52	0	0	0	48	38	0	26	0	36
14	[1,2-$^{13}C_2$]-GLU	62	25	13	0	0	68	12	20	0	0
15	FRU + [U-$^{13}C_6$]-FRU (1:1)[c]	58	0	0	0	42	41	0	19	0	40
16	[1-^{13}C]-FRU	91	9	0	0	0	84	16	0	0	0
17	[1-^{13}C]-ARA	87	13	0	0	0	n.d.	n.d.	n.d.	n.d.	n.d.
18	GLU + [U-$^{13}C_6$]-GLU (1:1) + PHE[c]	50	0	2	0	48	39	0	21	0	40
19	FRU + [U-$^{13}C_6$]-FRU (1:1) + PHE[c]	49	0	2	0	49	45	0	8	0	47
20	GLU + [3-^{13}C]-ALA + THR	67	33	0	0	0	90	10	0	0	0
21	GLU + [U-$^{13}C_3$]-ALA + THR	63	0	37	0	0	93	0	7	0	0
22	GLU + [U-$^{13}C_3$]-ALA + SER	49	0	51	0	0	91	0	9	0	0
23	GLU + [U-$^{13}C_3$]-ALA + [U-$^{13}C_4$]-THR	71	0	29	0	0	86	0	14	0	0
24	GLU + ALA + [U-$^{13}C_4$]-THR	90	0	10	0	0	94	0	6	0	0

[a] Results under dry-heating conditions (200 °C, 10 min). [b] Results from aqueous solutions at pH 7 (121 °C, 25 min). [c] CAMOLA experiment. Glucose (GLU), fructose (FRU), arabinose (ARA), alanine (ALA), threonine (THR), serine (SER), and phenylalanine (PHE). Measured masses: $[M]^+$ (*m*/*z* 68), $[M + 1]^+$ (*m*/*z* 69), $[M + 2]^+$ (*m*/*z* 70), $[M + 3]^+$ (*m*/*z* 71), and $[M + 4]^+$ (*m*/*z* 72). n.d., not determined.

by a β-elimination reaction, resulting in 3-deoxyhexos-2-ulose and 4-deoxy-2,3-diulose. A potential major pathway (A) is suggested to proceed via 4-deoxyhexo-2,3-diulose, which can undergo a second dehydration step, resulting in 4,5-dideoxyhexo-2,3-diulose. The latter compound forms via dehydration and cyclization 2-(1-oxo-2-hydroxyethyl)furan, which can result in furan and glycolic acid by an electrophilic aromatic substitution-type reaction. The latter reaction is analogous to the formation of furan from 2-furaldehyde (*9*). Glycolic acid has been described by Ginz et al. (*13*) as a major degradation product in dry-heated (240 °C, 15 min) glucose and fructose model systems. The formation of 2-(1-oxo-2-hydroxyethyl)furan labeled at the CH_2OH group from [1-^{13}C]glucose has been reported by Tressl et al. (*14*). Furan obtained by pathway A would comprise the C3−C6 moiety of the sugar.

Two minor pathways (B and C), leading to single and double labeled furan from [1,2-$^{13}C_2$]-glucose, may proceed via the 3-deoxyhexos-2-ulose pathway. Similarly to ascorbic acid, hexoses may generate furan from 2-furaldehyde, which is supported by the presence of labeled formic acid released as a sugar degradation product. 2-Furaldehyde is a well-known decomposition product of hexoses (*15*) formed through the 3-deoxyhexos-2-ulose pathway (B) that gives rise to furan comprising the C2−C5 moiety of the sugar. Dry-heating of [1-^{13}C]-glucose was shown to release [^{13}CHO]-labeled 2-furaldehyde as described by Tressl et al. (*14*) in model experiments. Two different mechanisms might operate to transform 2-furaldehyde into furan: (i) electrophilic aromatic substitution with water, forming formic acid as the byproduct and (ii) oxidation to 2-furoic acid followed by decarboxylation. The former mechanism should be preferred under aqueous conditions, whereas the latter might be favored upon dry-heating.

The formation of furan by incorporating the C1−C4 moiety of glucose or fructose (pathway C) could proceed via the β-dicarbonyl route (*16*). The mechanism consists of the transformation of 3-deoxyhexos-2-ulose by a series of enolization steps and dehydration to 2,3-dideoxyhexos-5-ulose, which upon hydrolytic cleavage forms glycolic acid and a C_4 intermediate, the latter yielding furan upon cyclization and dehydration. [1-^{13}C]-Fructose also can form unlabeled furan through 3-deoxyhexos-2-ulose and 4-deoxyhexo-2,3-diulose. The higher production of unlabeled furan (91%) corresponds to the sum of unlabeled and single labeled furan (62% + 25%) from [1,2-$^{13}C_2$]-glucose. It should be mentioned, however, that heating of 3-deoxyhexos-2-ulose (no. 10 in **Table 1**) under roasting conditions showed a lower efficiency in generating furan (−30%) as compared to glucose.

The data show that furan formation from glucose via 4-deoxyhexo-2,3-diulose (62%) is a potential major pathway (A) as compared to that via 3-deoxyhexos-2-ulose (B and C, 38%). Alternatively, and provided that hydrolytic α-dicarbonyl cleavage takes place under dry-heating conditions, furan also might be formed from 1,4-dideoxyhexo-2,3-diulose by α-dicarbonyl cleavage, leading to acetic acid and 2-deoxyerythrose, another direct precursor of furan.

Unlabeled furan from [1-^{13}C]-fructose is formed through 3-deoxyhexos-2-ulose and 4-deoxyhexo-2,3-diulose. Therefore, a direct comparison between the model systems containing 3-deoxyhexos-2-ulose and fructose is not possible. However, fructose showed also a higher potential to generate furan than 3-deoxyhexos-2-ulose (122 and 24 μmol/mol, respectively), thus favoring the major pathway via 4-deoxyhexo-2,3-diulose. In analogy, the formation of furan from pentose sugars may proceed via intermediary 4-deoxypento-2,3-diulose (**Figure 2**, pathway A): hydrolytic β-dicarbonyl cleavage (*17*) may lead to a C_4 intermediate that upon cyclization and dehydration yields furan. Alternatively, also pathway B via 3-deoxypentos-2-ulose and 2-furaldehyde might be considered, in particular, in pentose systems where 2-furaldehyde is one of the major sugar degradation products (*15*). In agreement with the proposed scheme, dry-heat degradation of [$^{13}C_1$]-arabinose (no. 17, **Table 2**) resulted mainly in unlabeled furan with almost 90% yield, thus confirming that a C_1 unit is lost in the reaction cascade that is most likely formic acid corresponding to the C1 carbon of pentose.

From Sugars under Pressure Cooking Conditions. Contrary to dry-heating, both glucose and fructose formed in the CAMOLA experiment (nos. 13 and 15) partially labeled $[M + 2]^+$ furan, apart from unlabeled $[M]^+$ and fully labeled $[M + 4]^+$ furan, in a ratio close to 2:1:2 (**Table 2**). This indicates that sugar fragmentation takes place as another major reaction (i.e., furan is formed in aqueous systems (pH 7) from the intact sugar skeleton (ca. 50−60%) and by sugar fragmentation (ca. 40−50%)). Glucose (52%) tends to fragment more than fructose (38%). Furan formation by fragmentation proceeds via recombination of two reactive C_2 units, such as glycolaldehyde and acetaldehyde (**Figure 3A**). Glycolaldehyde is a well-known degradation product of glucose formed by cleavage between C2 and C3 or C4 and C5 atoms of glucose (*18*). Also, traces of acetaldehyde have been reported as a decomposition product of sugars in wet systems under oxidative conditions (*19*). Aldol-type recombination of these fragments may lead to the intermediate 2-deoxyaldotetrose, which forms furan upon cyclization and dehydration (**Figure 3B**).

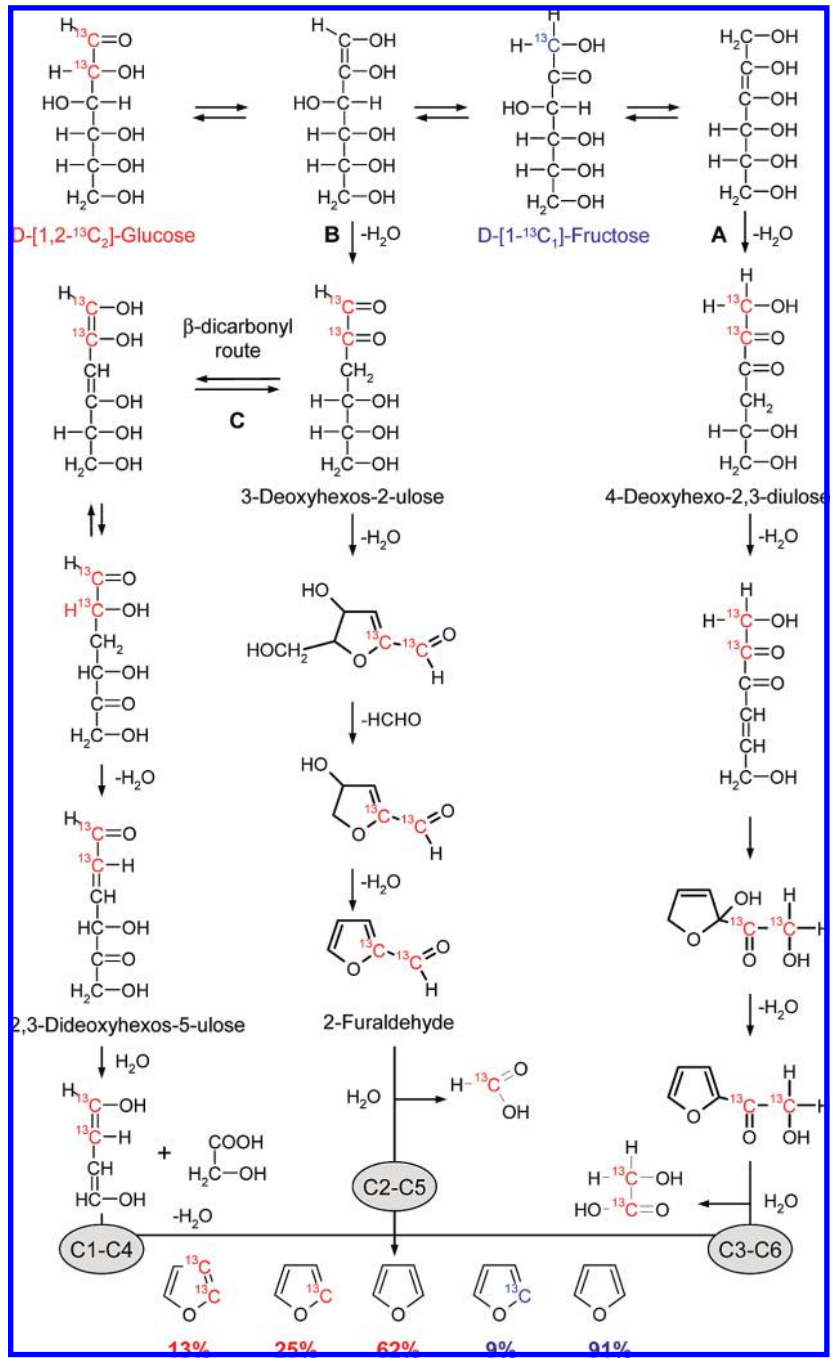

Figure 1. Hypothetical formation pathways of furan from hexose sugars. The labeling positions are marked in red (glucose) or blue (fructose). The percent values for labeled furan correspond to experiments 14 and 16 in **Table 2** obtained under roasting conditions.

Furthermore, double ^{13}C-labeled glucose (no. 14) and single ^{13}C-labeled fructose (no. 16) resulted in the same labeling furan patterns as under dry-heating conditions (**Table 2**), but the percent distributions of the molecule masses were slightly different. The labeling pattern of furan was in agreement with the proposed mechanism (**Figure 4**) as confirmed by the mass spectra (data not shown). The fragment $[^{13}CHO]^+$ (*m/z* 30) was detected in both experiments with [1-^{13}C]-fructose and [1,2-$^{13}C_2$]-glucose.

From Maillard Systems under Roasting Conditions. The possibility of forming furan by the recombination of suitable fragments was further studied in Maillard systems in which sugar fragmentation is promoted by amino acids. CAMOLA experiments in the presence of phenylalanine (nos. 18 and 19) confirmed the findings that furan was to a large extent formed from the intact sugar skeleton. However, traces of double labeled furan $[M + 2]^+$ were also found, which indicates a minor furan formation pathway by the recombination of two C_2 fragments, amounting to about 4% of the total furan formed. This is due to phenylalanine promoting sugar fragmentation.

Model systems were designed to study possible degradation products of amino acids (e.g., Strecker aldehydes) as well. Indeed, one ^{13}C atom was incorporated into furan (33%) when using [3-^{13}C]-alanine (no. 20 in **Table 2**). In addition, [U-$^{13}C_3$]-alanine (nos. 21 and 22) led to double labeled furan (37−51%). The data indicate the C2−C3 moiety of alanine functioning as a reactive C_2 fragment, possibly as the Strecker degradation product acetaldehyde, which may react with the known sugar degradation product glycolaldehyde to form furan (**Figure 4**). Only low threonine levels (10%) were incorporated into furan

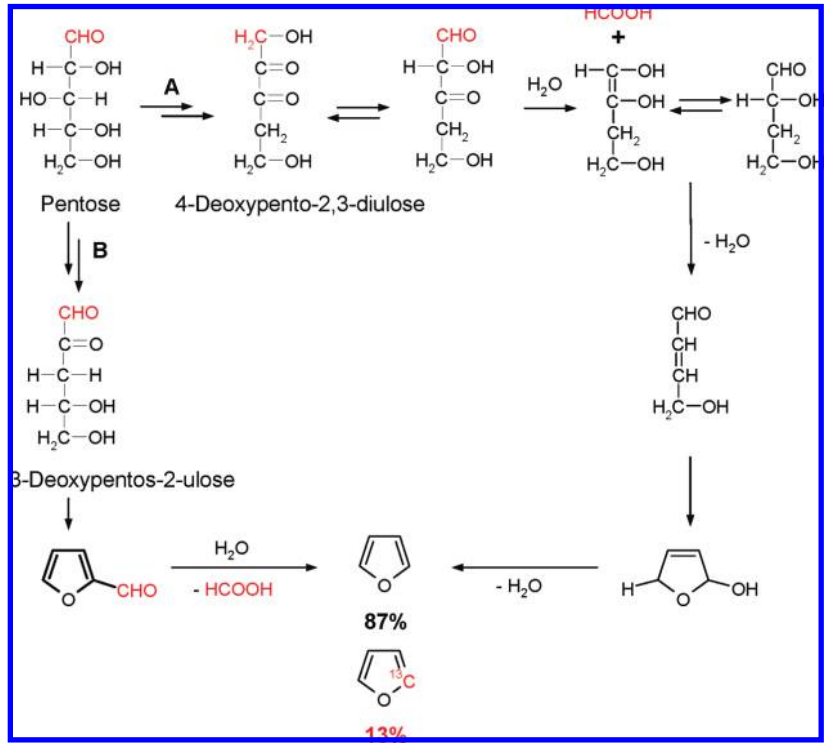

Figure 2. Hypothetical formation pathways of furan from pentose sugars. The percent values for labeled furan correspond to the experiment 17 in **Table 2** performed under roasting conditions.

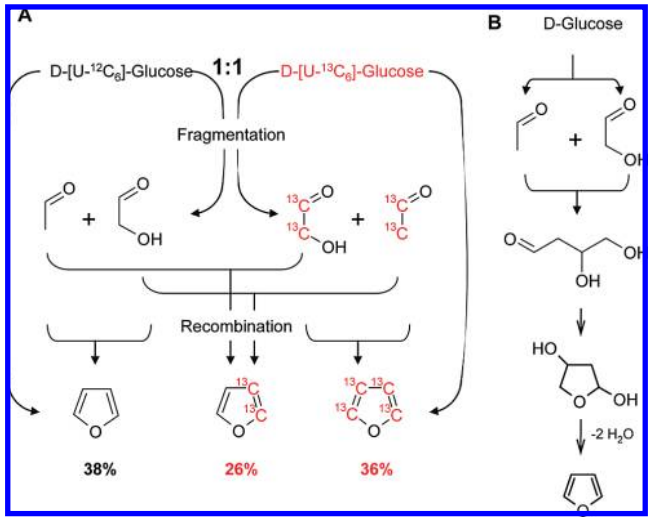

Figure 3. Schematic presentation of the carbohydrate module labeling (CAMOLA) experiment under aqueous conditions at pH 7: (**A**) using equimolar amounts of unlabeled and fully labeled glucose (sample 13 in **Table 2**) and (**B**) scheme leading to furan by recombination of C_2 sugar fragments such as acetaldehyde and glycolaldehyde.

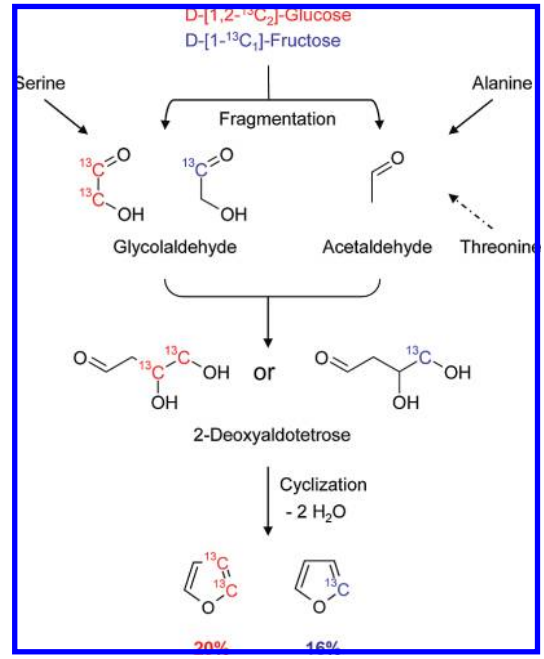

Figure 4. Formation of furan in aqueous Maillard reaction systems containing sugars and specific amino acids. The labeling positions are marked in red (glucose) or blue (fructose). The percent values for labeled furan correspond to experiments 14 and 16 in **Table 2** performed under pressure cooking conditions at pH 7.

(no. 24) as shown by the $[M + 2]^+$ signal, thus indicating that threonine is less efficient in generating acetaldehyde as compared to alanine (no. 21). The acetaldehyde amount seems to be the limiting factor for furan formation by recombination. Even if glycolaldehyde is readily formed from sugars, the addition of serine increases the overall furan level (**Table 1**, no. 7) and promotes furan formation by recombination (**Table 2**, no. 22), resulting in ca. 50% double labeled furan.

From Maillard Systems under Pressure Cooking Conditions. Similarly to pure sugar systems (nos. 13 and 15), CAMOLA experiments suggest two reaction pathways leading to furan in aqueous Maillard systems in the presence of phenylalanine (nos. 18 and 19) at pH 7. The majority is formed from the intact sugar skeleton (ca. 60−80%) with glucose fragmenting more than fructose. The contribution of alanine to the formation of furan under aqueous conditions (nos. 20−22) was only 7−10% as compared to 33−51% observed in the dry system. On the basis of the labeling pattern of precursors, acetaldehyde can

Table 3. Percent Labeling Distribution of 2-Methylfuran Generated in Different Model Systems Containing Sugars and Amino Acids

		roasting[a]						pressure cooking (pH 7)[b]					
no.	model system	M	M + 1	M + 2	M + 3	M + 4	M + 5	M	M + 1	M + 2	M + 3	M + 4	M + 5
17	[1-^{13}C]-ARA	3	97	0	0	0	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18	GLU + [U-$^{13}C_6$]-GLU (1:1) + PHE[c]	49	0	0	0	0	51	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19	FRU + [U-$^{13}C_6$]-FRU (1:1) + PHE[c]	46	0	0	0	0	54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20	GLU + [3-^{13}C]-ALA + THR	41	59	0	0	0	0	93	7	0	0	0	0
21	GLU + [U-$^{13}C_3$]-ALA + THR	49	0	51	0	0	0	93	0	7	0	0	0
22	GLU + [U-$^{13}C_3$]-ALA + SER	100	0	0	0	0	0	100	0	0	0	0	0
23	GLU + [U-$^{13}C_3$]-ALA + [U-$^{13}C_4$]-THR	34	0	0	5	4	57	86	0	0	2	0	12
24	GLU + ALA + [U-$^{13}C_4$]-THR	36	0	3	58	0	3	81	0	2	12	0	5

[a] Results obtained under dry-heating conditions (200 °C, 10 min). [b] Results obtained from aqueous solutions at pH 7 (121 °C, 25 min). [c] CAMOLA experiment. Arabinose (ARA), glucose (GLU), fructose (FRU), alanine (ALA), threonine (THR), serine (SER), and phenylalanine (PHE). Measured masses: $[M]^+$ (*m*/*z* 82), $[M + 1]^+$ (*m*/*z* 83), $[M + 2]^+$ (*m*/*z* 84), $[M + 3]^+$ (*m*/*z* 85), $[M + 4]^+$ (*m*/*z* 86), and $[M + 5]^+$ (*m*/*z* 87). n.d., not determined.

either be 1-^{13}C-labeled from [3-^{13}C]-alanine (no. 20) or fully labeled from [U-$^{13}C_3$]-alanine (nos. 21 and 22). The MS fragmentation pattern was in agreement with the labeling position of 1-^{13}C in furan generated from [3-^{13}C]-alanine (i.e., the fragment $[^{13}CHO]^+$ (*m*/*z* 30) was readily detected indeed (data not shown)).

Slightly more unlabeled furan was formed when replacing threonine by serine (no. 22), as higher amounts of glycolaldehyde (Strecker aldehyde of serine) should be formed in the presence of serine. The experiment with fully labeled threonine (no. 24) resulted in the low incorporation of a C_2 unit (6%), which, however, is not surprising as the Strecker aldehyde of threonine (lactaldehyde) is composed of three C atoms and should rather favor the formation of methylfuran. However, lactaldehyde may partially decompose to acetaldehyde, hence leading to $^{13}C_2$-furan. The differences observed for furan formation in Maillard systems under roasting and aqueous conditions may imply that either alanine is much easier converted to acetaldehyde under roasting conditions as compared to aqueous conditions or that C_2 fragments (possibly glycolaldehyde) are not formed in sufficient quantities from sugars in aqueous systems.

Overall, our data are consistent with the already proposed pathway of furan formation from Maillard reaction model systems (*5*). In particular, we confirm that furan is mainly composed of the C3−C6 moiety of hexoses and that Strecker aldehydes of certain amino acids are incorporated into furan by using for the first time labeled threonine and alanine. In addition, evidence is given for furan formation by two principle pathways: (i) sugar degradation keeping the intact carbon skeleton and (ii) recombination of C_2 fragments that may originate from both sugars and specific amino acids. Key intermediates are (i) 4-deoxy-2,3-diuloses and 2-furaldehyde as well as (ii) acetaldehyde and glycolaldehyde preferably formed from amino acids (e.g., alanine) and sugars, respectively.

Formation of 2-Methylfuran. The formation of 2-methylfuran from sugars was rather marginal (<25 μmol/mol), in particular, in aqueous systems (nos. 1−4, **Table 1**). Maillard reactions systems, however, yielded 2-methylfuran in relatively high amounts (i.e., 160−260 μmol/mol under roasting conditions (nos. 5−9)). Furthermore, pH 7 was more efficient than pH 4 in generating 2-methylfuran. To obtain a more precise insight into formation mechanisms, targeted experiments were performed using labeled sugars and amino acids.

CAMOLA experiments performed under roasting conditions and in the presence of phenylalanine (nos. 18 and 19, **Table 3**) indicated that methylfuran was formed from the intact sugar skeleton without recombination of sugar fragments. In agreement with that, degradation of [$^{13}C_1$]-arabinose (no. 17) almost exclusively led to single labeled methylfuran (97%). Overall, the results obtained in this study for furan and methylfuran (nos. 18 and 19, **Tables 2** and **3**) indicate similar reaction pathways leading to both furans from sugars under roasting conditions. However, more furan than methylfuran was generated in pure sugar systems (nos. 1−4, **Table 1**), while the presence of phenylalanine significantly boosted methylfuran formation (nos. 8 and 9, **Table 1**), which may be due to a higher turnover of sugar degradation. CAMOLA experiments performed in aqueous systems in the presence of phenylalanine indicate that, similarly to furan (nos. 18 and 19, **Table 2**), aqueous systems induced some formation of 2-methylfuran by a recombination of sugar fragments (<40%) with glucose fragmenting more than fructose. However, due to low levels of 2-methylfuran and possible interferences, the exact labeling pattern for 2-methylfuran could not be determined (nos. 18 and 19, **Table 3**).

Specific amino acids such as alanine and threonine contribute significantly to the formation of methylfuran. The $[M + 1]^+$ ion (no. 20, **Table 3**) suggests the incorporation of labeled acetaldehyde from [3-^{13}C]-alanine, which is in agreement with the $[M + 2]^+$ ion (no. 21) in the presence of [U-$^{13}C_3$]-alanine and unlabeled threonine. Interestingly, replacing threonine by serine (no. 22) resulted only in unlabeled 2-methylfuran, thus indicating that no labeled acetaldehyde was incorporated at all. Apparently, the C3 fragment is missing to form 2-methylfuran in the presence of labeled acetaldehyde. The C3 fragment might be lactaldehyde, the Strecker aldehyde of threonine. This also would mean that lactaldehyde is not generated from sugars in sufficient quantities under our experimental conditions. Indeed, little information is found in the literature on the formation of lactaldehyde from sugars that may preferably take place under alkaline conditions (*20*).

The use of fully labeled alanine and threonine (no. 23) resulted mainly in fully labeled 2-methylfuran accounting for about 60%. These data suggest that 2-methylfuran can readily originate from amino acids, provided that they release suitable reactive intermediates. Fully labeled threonine (no. 24) produced mainly $[M + 3]^+$ (ca. 60%). However, the traces of $[M + 2]^+$ and $[M + 5]^+$ suggest several types of reactions leading to 2-methylfuran.

In general, the trends found under pressure cooking conditions (pH 7) were similar to those upon roasting. However, the proportion of unlabeled 2-methylfuran was usually significantly higher in the aqueous samples (nos. 20−24), thus suggesting a higher contribution of the sugar. The major formation pathway leading to 2-methylfuran is summarized in **Figure 5**.

The results obtained suggest that 2-methylfuran can be formed both from sugars and from specific amino acids. In pure sugar systems, it is mainly generated by cyclization and dehydration,

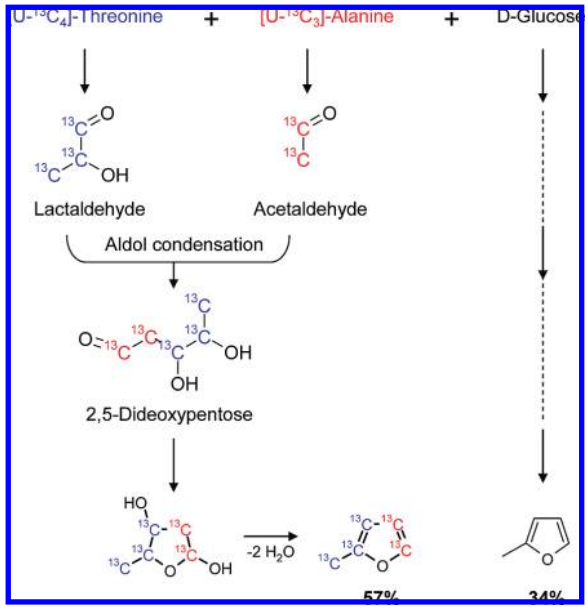

Figure 5. Formation of 2-methylfuran in Maillard reaction systems based on sugars and specific amino acids. The scheme represents the reaction system 23 in **Table 3** performed under roasting conditions.

Table 4. Furan and 2-Methylfuran Content (μg/kg) in Pumpkin Puree Spiked with Different Amounts of Glucose and Fructose

		pressure cooking[a]			
no.	model food[b]	furan	RSD	MF	RSD
25	PP	48.6	3.4	3.8	7.8
26	PP + GLU (2 g/100 g)	64.4	4.2	4.7	13.0
27	PP + GLU (4 g/100 g)	98.8	18.7	6.0	2.2
28	PP + GLU (6 g/100 g)	61.5	10.8	4.9	1.8
29	PP + GLU/FRU (0.78 g/0.62 g/100 g)	124.2	18.7	5.3	17.0

[a] Results obtained under pressure cooking conditions at pH 6.3 (121 °C, 25 min). RSD = relative standard deviation in percent. Pumpkin puree (PP), glucose (GLU), and fructose (FRU). [b] Natural amount of glucose and fructose in PP was 0.78 and 0.62 g/100 g, respectively.

keeping the intact carbon skeleton. In the presence of certain amino acids, it is generated by recombination of the corresponding Strecker aldehydes (e.g., acetaldehyde, lactaldehyde) via aldol-type reactions followed by cyclization and dehydration of the intermediary 2,5-dideoxypentose. In general, the contribution of amino acids to furan and 2-methylfuran is more important under roasting conditions as compared to aqueous systems (**Tables 2** and **3**), thus indicating that dry-heating generates more C_2 and C_3 Strecker aldehydes.

Spiking Experiments in Food Products. Fruits and vegetables contain hexoses such as fructose and glucose. Although these sugars have not shown a high potential to generate furan under aqueous conditions, their relatively high concentrations in fruit and vegetable products could render them still important precursors. Therefore, spiking experiments were performed by adding different amounts of glucose to pumpkin puree. Indeed, the addition of increasing amounts of glucose (2, 4, and 6 g/100 g) to the pumpkin puree resulted in higher furan levels (**Table 4**), which was doubled when adding 4 g/100 g glucose (no. 27). Interestingly, adding 6 g of glucose per 100 g (no. 28) resulted in a relatively lower furan increase (+130%) as compared to the addition of 4 g/100 g (+200%). However, sugars seem to be direct precursors of furan, contrary to ascorbic acid (*9*), even if the yields are low. The fortification of the pumpkin puree with glucose showed almost no increase in the 2-methylfuran content. This is consistent with results of the model systems, where also only trace amounts of 2-methylfuran were generated from glucose.

In further fortification experiments, equivalent amounts glucose (0.78 g) and fructose (0.62 g) were added, as already present naturally, to 100 g of pumpkin puree. The furan content was increased by a factor of 2.5 (no. 29), although the spiked pumpkin puree only contained twice the amount of each hexose. This means that in contrast to glucose, the addition of both glucose and fructose to the model food does not only generate more furan from the hexoses but may also accelerate furan formation from other precursors. The increase of furan could potentially also be caused by the enhancing effect of hexose concentration on furan formation kinetics. As compared to the glucose spiking experiments, more additional furan was generated, although less hexose (sum of glucose and fructose = 1.4 g/100 g) was added. This may be due to the fact that (i) fructose is more efficient in furan formation (**Table 1**) and (ii) fructose and glucose do not follow exactly the same mechanistic pattern. The formation of furan from the intact C_4 carbon skeleton (**Table 2**) is more important in the case of fructose (62%) as compared to glucose (48%).

Furthermore, a CAMOLA experiment was performed in the model food system by adding the naturally occurring amounts of glucose and fructose as uniformly ^{13}C-labeled sugars ([U-$^{13}C_6$]-GLU and [U-$^{13}C_6$]-FRU). To the best of our knowledge, this is the first time that a CAMOLA experiment was applied to food with the aim of investigating formation pathways of target compounds in real food systems. With this fortification, the pumpkin puree contained both sugars as 1:1 mixtures of the unlabeled and fully ^{13}C-labeled isotopomers. The following percent labeling distributions were obtained: for furan 88% $[M]^+$, 3% $[M + 2]^+$, and 9% $[M + 4]^+$ and for 2-methylfuran 100% $[M]^+$, with 0% for all other furan and methylfuran isotopomers. The results indicate that only small quantities of $^{13}C_2$-furan (3%) and $^{13}C_4$-furan (9%) were generated and that there was no incorporation of ^{13}C into 2-methylfuran.

The relative importance of each pathway was calculated based on statistical rules (*12*). As shown in **Figure 6**, 6% (1.5% + 3% + 1.5%, ratio of 1:2:1) of furan was formed by the recombination of two C_2 fragments (e.g., acetaldehyde and glycolaldehyde, see **Figure 4**) and 15% by cyclization of the intact hexose skeleton composed of 7.5% (9% − 1.5%) $^{13}C_4$-furan and 7.5% unlabeled furan. These data confirm that, similarly to aqueous model systems, furan in foods also was formed by (i) cyclization of the intact C_4 moiety of hexoses and (ii) recombination of sugar fragments. In the pumpkin puree, the former pathway was more important (ratio of 7:3). It should be noted, however, that overall, the majority of furan in pumpkin puree (i.e., 79% (100% − (2 × 7.5%) − 6%)) was generated from other precursors than sugars, such as unsaturated lipids, for example.

In conclusion, the formation mechanisms of furan and 2-methylfuran were substantiated using labeled sugar and amino acid precursors. Under pressure cooking conditions, CAMOLA experiments proved that furan and 2-methylfuran formation proceeded to a large extend via recombination of reactive fragments that may originate from both sugars and amino acids. Some key intermediates could be substantiated such as 2-furaldehyde and certain deoxy sugars. The involvement of Strecker aldehydes such as acetaldehyde and lactaldehyde also could be proven by labeling experiments. However, furan formation from sugars and amino acids only represent a minor route. This was

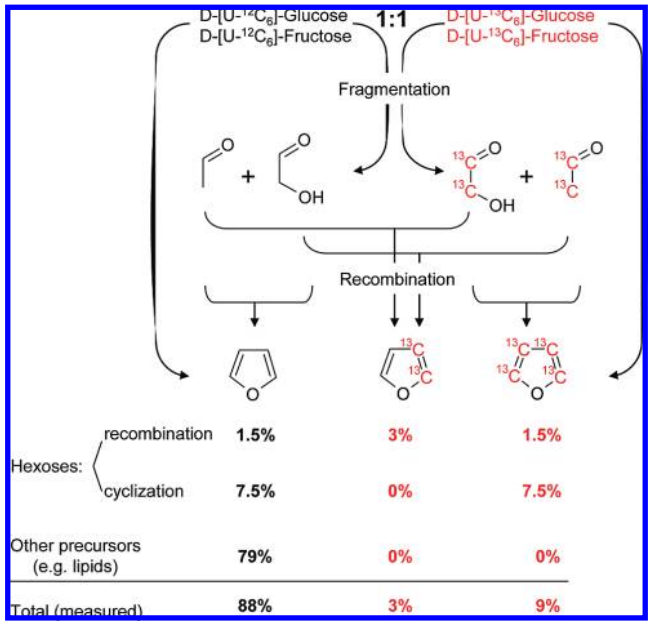

Figure 6. Schematic presentation of carbohydrate module labeling (CAMOLA) experiment performed in pumpkin puree. See text for explanation.

demonstrated by a CAMOLA experiment performed in pumpkin puree, where only about 20% of furan was generated from sugars. Therefore, our next contribution will deal with the formation of furan and 2-methylfuran from polyunsaturated lipids.

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