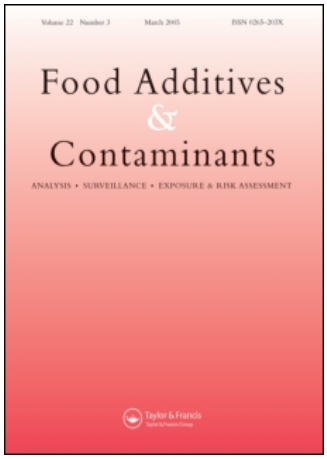


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# Formation of furan and methylfuran from ascorbic acid in model systems and food

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**Abstract**
Previous model studies have suggested ascorbic acid as one of the major sources of furan, a possibly hazardous compound found in thermally processed foods (e.g. canned products, jars). The study showed that about 2 mmol $mol^{-1}$ furan was obtained when dry-heating ascorbic acid, while much lower amounts were formed upon pressure cooking, i.e. 58 μmol $mol^{-1}$ at pH 4 and 3.7 μmol $mol^{-1}$ at pH 7. Model reactions also generated 2-methylfuran (MF). However, the MF levels were generally very low with the exception of the binary mixture ascorbic acid/phenylalanine (1 mmol $mol^{-1}$). Studies with $^{13}$C-labelled ascorbic acid indicated that furan comprises an intact $C_4$ unit, mainly C-3 to C-6, generated by splitting off two $C_1$ units, i.e. $CO_2$ and formic acid. Possible intermediates are 2-deoxyaldoteroses, 2-furoic acid and 2-furaldehyde, which are known as ascorbic acid degradation products. The mechanism of furan formation from ascorbic acid was validated based on the labelling pattern of furan and the identification of $^{13}CO_2$ and $H^{13}COOH$. Furan formation is significantly slowed down in binary mixtures, e.g. the presence of erythrose led to 80% less furan under roasting conditions. This is most likely due to competing reactions in complex systems, thus disfavouring furan formation. The mitigation effect is because furan, contrary to MF, is formed without recombination of ascorbic acid fragments. Therefore, furan levels are definitely much lower in foods than expected from trials with pure ascorbic acid. Consequently, conclusions should be drawn with much caution from model reactions, avoiding extrapolation from oversimplified model systems to food products.

**Keywords:** *Furan, methylfuran, ascorbic acid, model systems, food, labelled precursors, mechanistic study, SPME-GC/MS, isotope dilution assay*

## Introduction

Furan (110-00-9) has received considerable attention due to its classification as 'possibly carcinogen to humans' (Group 2B) by the International Agency for Research on Cancer (IARC) (1994) and the relatively high amounts in food that had undergone heat treatment reported by the US Food and Drug Administration (2004). Furan levels up to about 240 μg $kg^{-1}$ were reported, especially in canned and jarred products (European Food Safety Authority (EFSA) 2004; Reinhard et al. 2004; Becalski et al. 2005). However, furan has been known for long time as food constituent (reviewed by Maga 1979). Current research is mainly devoted to analysis (Becalski and Seaman 2005; Becalski et al. 2005; Goldmann et al. 2005; Senyuva and Goekmen 2005; Bianchi et al. 2006; Nyman et al. 2006; Hasnip et al. 2006), occurrence (EFSA 2004; Reinhard et al. 2004; US Food and Drug Administration 2004; Becalski et al. 2005), and formation (Perez Locas and Yaylayan 2004; Fan 2005; Märk et al. 2006) of this volatile, food-borne process contaminant.

Recently, the formation of furan has been studied in rather simple model systems revealing several precursor classes such as ascorbic acid and related compounds, amino acids and reducing sugars, lipids comprising unsaturated fatty acids and the corresponding triglycerides, but also carotenes and organic acids (Perez Locas and Yaylayan 2004; Becalski and Seaman 2005; Fan 2005; Märk et al. 2006).

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This is not surprising as furan can be seen as a rather stable reaction product that may be generated from different chemical classes by degradation or recombination of reaction fragments. As an example, ascorbic acid may be transformed under non-oxidative pyrolytic conditions to 2-deoxyaldotetrose as key intermediate leading directly to furan (Perez Locas and Yaylayan 2004; Märk et al. 2006). However, no effort has been undertaken so far to elucidate the formation mechanism of furan from ascorbic acid. Thus, the suggested schemes remain of hypothetical nature, in particular when it comes to food systems. Furthermore, mechanistic studies performed in food products are missing, yet many of them are fortified with vitamin C.

Ascorbic acid has been shown as one of the major precursors of furan formed by thermal decomposition (Perez Locas and Yaylayan 2004; Becalski and Seaman 2005; Märk et al. 2006) under pressure cooking (118°C, 30 min), roasting (220°C at 4°C $min^{-1}$ rate, an additional 10 min) or pyrolysis (250°C at 50°C $s^{-1}$ rate, total of 20 s) conditions as well as by $\gamma$-irradiation (Fan 2005) of aqueous samples (5 mg $ml^{-1}$, pH 3–8, 5°C, 0–5 kGy at 0.091 kGy $min^{-1}$). The furan amounts range from 0.1 μmol $mol^{-1}$ (pressure-cooking, $\gamma$-irradiation) to about 1 mmol $mol^{-1}$ (roasting). Interestingly, furan has not been reported in previous studies as a reaction product, neither of ascorbic nor of dehydroascorbic acid decomposed under roasting or cooking conditions, most likely due to inappropriate experimental conditions for analysing furan (Velisek et al. 1976; Vernin et al. 1998).

The aim of the present work was to study the formation of furan and MF from ascorbic acid and related precursors in model systems simulating food preparation conditions such as roasting and pressure cooking. Reliable quantitative data were obtained by solid-phase micro-extraction (SPME) in conjunction with gas chromatography-mass spectrometry (GC/MS) using [$^2H_4$]-furan as the internal standard. Precise mechanistic insight into the degradation steps was achieved by using $^{13}$C-labelled ascorbic acid. In addition, results were validated in food products based on fruits and vegetables by spiking with labelled precursors.

## Materials and methods

### *Materials*

The following chemicals were commercially available: L-ascorbic acid (99%), 2,6-*bis*(1,1-dimethylethyl)-4-methyl-phenol (BHT, 99%), citric acid (monohydrate), disodium hydrogenphosphate (dihydrate, 99%), dehydro-L-ascorbic acid, D-erythrose (90%), furan (stabilized, 99%), $d_4$-furan (stabilized, 98%), 2-furaldehyde (99%), 2-furoic acid (98%), 2-methylfuran (MF, 99%), D-phenylalanine (98%), white quartz sand, silicone oil (oil bath, from −50 to 200°C) (Sigma-Aldrich, Buchs, Switzerland); (+)-D-glucose anhydrous, methanol for analysis, LiChrosolv® water for chromatography (Merck, Darmstadt, Germany); 3-methylfuran (97%) (Acros Organics, Geel, Belgium); L-[1-$^{13}$C]-ascorbic acid, L-[2-$^{13}$C]-ascorbic acid, L-[6-$^{13}$C]-ascorbic acid (Omicron Biochemicals, South Bend, IL, USA). Raw pumpkin puree was obtained from Nestle Weiding (Germany).

### *Sample preparation*

*Roasting model systems.* Equimolar amounts of precursors (0.1 mmol each) and sea sand (1 ± 0.05 g) were mixed into a headspace vial (20 ml, clear glass, 75.5 × 22.5 mm, 20 mm crimp, rounded bottom, Brechbühler, Geneva, Switzerland) used as reaction vessel, which was sealed with a crimp cap. The sea sand was used to achieve a homogeneous distribution of the precursors and to avoid caking. The samples were heated at 200°C for 10 min, which simulates roasting conditions.

Experiments were carried out in duplicate by using two reaction vessels (a 20-ml headspace vial), both simultaneously immersed into a silicon oil bath. A heating/stirring device (IKAMAG® RCT, IKA Labortechnik, Staufen, Germany) heated the silicon oil. The temperature was controlled by a thermostat (IKA-TRON®ETS-D, IKA Labortechnik). A homogenous temperature distribution in the silicon oil bath was achieved by magnetic stirring. This device was used in all heating experiments. For each experimental point, two independent determinations were carried out.

*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 7) by stirring. Citric acid-phosphate buffer solutions were used according to McIlvaine for buffering the aqueous mode systems (Bates 1996). The buffer solutions were prepared by mixing citric acid (0.1 M, component A) and disodium hydrogenphosphate (0.2 M, component B) as follows: 18.15 A + 71.85 B for pH 7, and 61.45 A + 38.55 B for pH 4.

An aliquot of the homogenous solution (1 ml = 0.1 mmol of each precursor) was transferred into the reaction vessel (a 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 of simulating sterilization conditions.

*Food products.* Freshly squeezed fruit and vegetable juices were prepared from navel oranges and organic Swiss carrots bought in a local grocery store (Migros) by using a plastic squeezer and electrical juicer (Vitafruit Type Y36, Moulinex, Switzerland), respectively. The pHs were immediately measured. Aliquots of each juice were prepared for ascorbic acid analysis carried out by a certified laboratory (Regional Laboratory, Nestlé Suisse SA, Orbe, Switzerland) using a validated high-performance liquid chromatography (HPLC) method. For spiking experiments, defined amounts of precursors were added into a flask (50 ml), dissolved in the fruit or vegetable juice (10 g), and stirred to obtain a homogenous sample. An aliquot (2 g) was then placed into the reaction vessel (a 20-ml headspace vial) and sealed. The spiked samples were heated at 123°C for 22 min simulating sterilization conditions in food processing.

*Quantification of furan and MF*

*Isotope dilution assay.* Immediately after the heat treatment the samples were cooled in a water bath (about 10°C) for 5 min to stop the reaction. Then, the internal standard ($^2H_4$-furan) was added through the septum with a gas tight syringe (10 µl, Sigma-Aldrich) and the vial was vortexed. The added volume of internal standard (about 0.8 µg $^2H_4$-furan $ml^{-1}$ methanol) depended on the expected amount of furan formed (1–25 µl). The samples were left at room temperature for at least 30 min to achieve equilibrium before analysis.

*Solid-phase microextraction (SPME) coupled to gas chromatography-mass spectrometry (GC/MS).* The analytical method for furan quantification published by Goldmann et al. (2005) was used with some modifications. This rapid and robust method is based on SPME-GC/MS. Sample preparation involves the addition of the deuterated analogue ($d_4$-furan) to the sample, extraction into the headspace at 35°C for 10 min and finally fibre desorption for 2 min. After GC separation, the data are acquired by MS. The GC-MS used for this study was composed of a Finnigan TRACE GC (ThermoQuest, Milan, Italy), a Finnigan TRACE MS (ThermoQuest), and an Injector MSP2 (Gerstel, Zurich, Switzerland).

The Carboxen™ fibre (polydimethylsiloxan, PDMS, film thickness 75 µm) from Supelco (Bellefonte, PA, USA) was used for analysis. The parameters of the SPME were as follows: Block incubation temperature (35°C), block incubation time (10 min), block incubation agitation (constant 250 rpm), fibre penetration (22 mm), and desorption time (2 min).

Chromatographic separation was performed on a ZebronWAX capillary column (30 m × 0.25 mm, $d_f = 0.5$ µm) (Phenomenex, Torrance, CA, USA). The parameters of the GC were as follows: Carrier gas (helium at 3 ml $min^{-1}$ constant flow), injection mode (split, ratio 1:10), purge flow (26 ml $min^{-1}$), temperature programme (35°C for 3 min, 35 → 150°C at 40°C $min^{-1}$, 150°C for 2 min), and retention time (furan: $2.06 \pm 0.02$ min, MF: $2.61 \pm 0.03$ min).

Quantitative analyses were performed in the single ion-monitoring (SIM) mode and repeated in the full-scan mode, whereas the experiments with the labelled precursors were only analysed in the full-scan mode. The parameters of the MS were as follows: Positive electron impact (EI+) ionization (70 eV), detector voltage (350 V), full scan acquisition ($m/z = 20$–200), SIM acquisition in retention window 1–5 min. Figure 1 shows the full mass spectrum of non-labelled and $^2H_4$-labelled furan. Table I shows the masses monitored for quantification.

*Calculations.* The quantities of furan and MF were determined relative to the internal standard $^2H_4$-furan (=$d_4$-furan). The response factor ($R_F$) was determined taking into consideration the differences in response of the mass detector between analyte and standard. For MF the response factor is additionally influenced by a different recovery rate of analyte and standard during SPME adsorption. A stronger adsorption of the less polar MF explains the relatively low $R_F = 0.36$ compared with 0.97 for furan. The response factor is the slope of the calibration curve. Considering the response factor and the peak areas of analyte and standard, the quantity of furan and MF can be calculated as follows:

$$\text{Furan[mol]} = \frac{d_4\text{-Furan[mol]} - \text{Area}_{\text{Furan,Sample}}}{\text{Area}_{d_4\text{-Furan,Sample}}} \times R_F \quad (1)$$

where $\text{Area}_{\text{Furan,Sample}}$ and $\text{Area}_{d_4\text{-Furan,Sample}}$ are the peak areas of furan and $d_4$-furan, respectively; and $d_4$-furan [mol] specifies the amount of the added internal standard ($d_4$-furan) in mol. The results for MF were calculated in the same manner using the corresponding response factor.

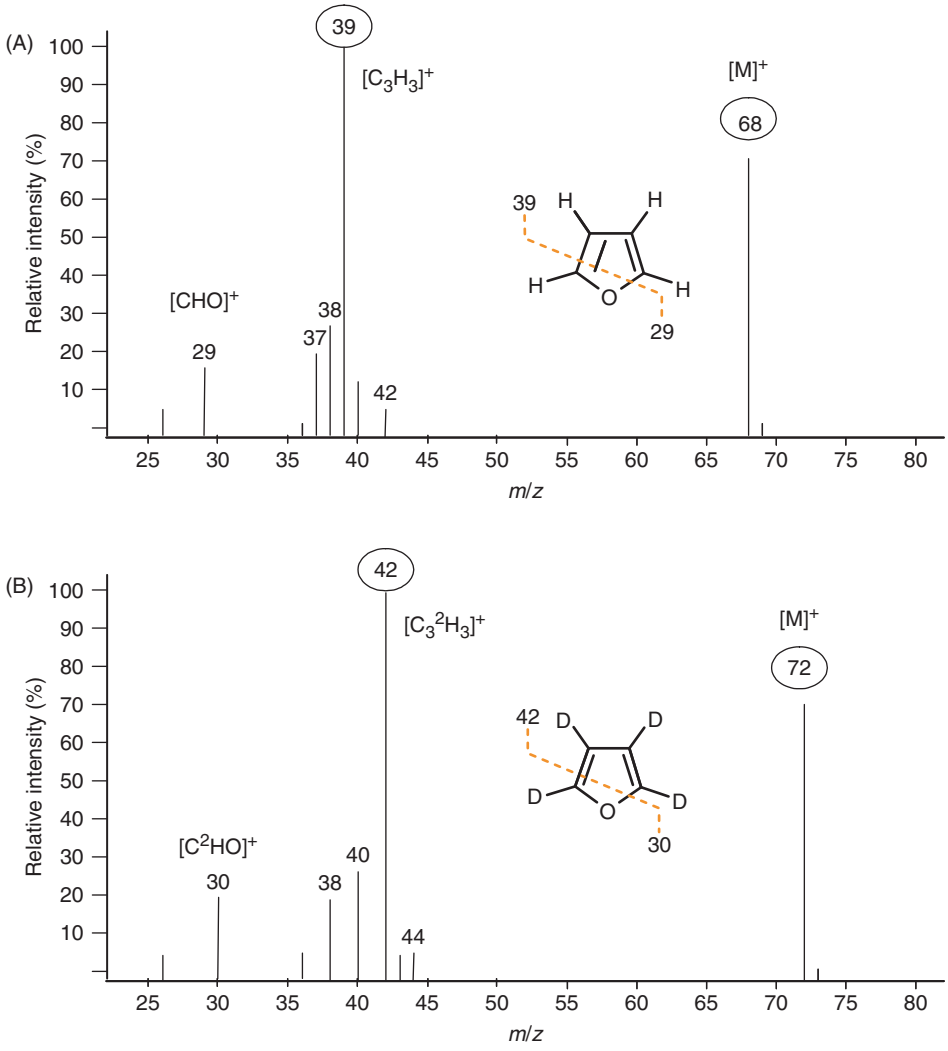


Figure 1. Fragmentation patterns of (A) unlabelled and (B) fully deuterated furan used as the internal standard for quantification.

*Validation of the analytical method*

*Linearity.* Calibration curves were established by adding different amounts of furan and MF (1, 2, 4, 6, 8, 10, 15 and 20 µl of a 0.4 µg ml$^{-1}$ solution in methanol) and a defined amount of $d_4$-furan (5 µl of a 0.4 µg µl$^{-1}$ solution in methanol) through the septum into a sealed headspace vial (20 ml) containing water (1 ml). Furan, MF and $d_4$-furan solutions were pipetted by means of gas-tight syringes (10 or 25 µl; Hamilton Co., Bonaduz, Switzerland). The calibration curves were repeated once per month. Solutions of standards and analytes were kept at 5°C and replaced every 2 weeks. The correlation coefficients were $R^2 > 0.9999$ for furan. The linearity of furan as measured by SPME-GC/MS was evaluated in the range of 0–8.17 µg (0.0, 0.41, 0.82, 1.63, 2.45, 3.27, 4.08, 6.13 and 8.17 µg) as absolute quantity in the headspace vial, which corresponds to 0–1133 µmol mol$^{-1}$ precursor.

Table I. Qualifiers and quantifiers for the quantification of furan and 2-methylfuran.

| Compound | Mass (*m/z*) | Function |
|---|---|---|
| Furan | 68 ($[M]^+$) | Quantifier |
| | 39 ($[C_3H_3]^+$, $[M-CHO]^+$) | Qualifier |
| | 69 ($[M+1]^+$) | Qualifier |
| $[^2H]_4$-Furan | 72 ($[M+4]^+$) | Quantifier |
| | 42 ($[C_3{}^2H_3]^+$) | Qualifier |
| 2-Methylfuran | 82 ($[M]^+$) | Quantifier |
| | 53 ($[C_4H_5]^+$, $[M-CHO]^+$) | Qualifier |

$d_4$-Furan was also used as internal standard for quantifying MF, resulting in calibration curves with $0.9900 < R^2 > 0.9999$.

*Limit of decision (CCα) and detection capability (CCβ).* The following values were determined for

furan (Goldmann et al. 2005): CC$\alpha$ = 25 pg; and CC$\beta$ = 43 pg. These values correspond to the absolute quantity of furan in a 20-ml headspace vial and they are equivalent to 0.034 and 0.086 μg kg$^{-1}$ in the sample, respectively.

*Trueness ($R_F$).* As the response factors ($R_F$) were determined by HS-SPME injections, they also account for differences in recovery during SPME extraction (in addition to the response of the MS signal) between the internal standard $d_4$-furan and furan/MF. An $R_F = 0.97$ was determined for furan; while the recovery factor of MF was $R_F = 0.36$. This is most likely due to a stronger adsorption of the less polar MF on the fibre compared with furan. In addition, the trueness was determined in a dry ascorbic acid model system as well as in several food samples by analysing non-spiked and spiked products with one level of analyte addition. Average recovery values of 98 and 92% were found for furan and MF, respectively, in the ascorbic acid model system as well as in food products (Table II). This shows the high accuracy of the method applied.

*Repeatability.* The repeatability of furan and MF measurements was verified by performing six real repetitions of the heating experiments with ascorbic acid. The results shown in Table III demonstrate the precision of the experimental data, i.e. RSD = 4.5–7.8% for furan and RSD = 20–25% for MF. Most of the experiments were carried out in duplicate, except of a few trials when very expensive chemicals were used such as labelled precursors.

*Leak-proof.* To ensure that no losses of furan and MF occurred from closed vials, 12 vials containing water (1 ml) were spiked with of furan solution (10 μl = 0.0008 μg) through the septum. Six vials were heated at 200°C for 10 min whereas the remaining six samples were left at ambient temperature as control. No losses of furan or MF were observed. Consequently, this simple method was used in all heating experiments.

### *Calculation of labelling percentage*

The per cent labelling distribution of furan and MF was determined by subtracting the naturally occurring percentage of $^{13}C$. An additional data treatment was required for MF. As shown in Figure 2, the highest intensity in the mass spectrum of MF ($C_5H_6O$) was the molecular ion $[M]^+$ signal ($m/z = 82$) followed by the fragment $[C_4H_5]^+$ ($m/z = 53$). Fragments $[C_3H_3]^+$ ($m/z = 39$) and $[CHO]^+$ ($m/z = 29$) were similar to those of furan (Figure 1).

In contrast to furan, MF generated a $[M-1]^+$ signal ($[C_5H_5O]^+$, $m/z = 81$) through the loss of H with a relative intensity of 63% compared with the molecule ion signal ($m/z = 82$). Therefore, the $[M-1]^+$ signal of MF has to be taken into account when correcting the overall signal intensity to calculate the per cent labelling distributions in labelling experiments studying MF formation.

Table II. Trueness of the measurements of furan in model systems and food products.

| Matrix | Spiking (μmol) | Measured (μmol) | Measured without spiking (μmol) | Trueness (%) |
|---|---|---|---|---|
| Ascorbic acid (roasting condition)[a] | 0.1183 | 0.3228 | 0.2047 | 99.8 |
| | | 0.3210 | | 98.3 |
| Pumpkin puree (pressure cooking)[b] | 0.2388 | 0.6512 | 0.4215 | 96.2 |
| | 0.2943 | 0.6938 | | 92.5 |
| Carrot juice (pressure cooking)[b] | 0.2575 | 0.5844 | 0.3155 | 104.4 |
| | 0.2084 | 0.5324 | | 104.1 |

[a]Roasting conditions: ascorbic acid (0.1 mmol) dry-heated at 200°C for 10 min.
[b]Pressure cooking conditions: 121°C for 25 min.

Table III. Repeatability of the heating procedure under various reaction conditions ($n = 6$).[a]

| Condition | Heat treatment | Furan | | 2-Methylfuran | |
|---|---|---|---|---|---|
| | | Average (μmol mol$^{-1}$) | RSD (%) | Average (μmol mol$^{-1}$) | RSD (%) |
| Dry | 200°C, 10 min | 2050.9 | 4.5 | 19.04 | 20.3 |
| Aqueous, pH 7 | 121°C, 25 min | 3.69 | 7.8 | 1.19 | 24.9 |

[a]Ascorbic acid (0.1 mmol) was used as an example.
RSD, relative standard deviation.

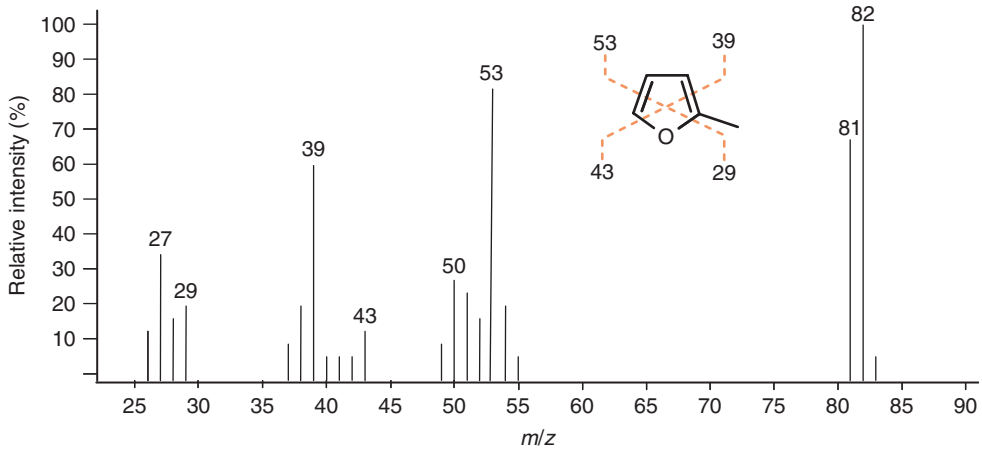


Figure 2. Fragmentation pattern of unlabelled 2-methylfuran.

The obtained percentages after correction lower than 1% were set 0% by definition.

*Identification of carbon dioxide*

As SPME-GC/MS is not suitable for $CO_2$ analysis, an MS-based electronic nose, called SMart Nose® (Smart Nose Ltd, Marin-Epagnier, Switzerland), was employed to prove the presence of carbon dioxide generated from ascorbic acid (Fenaille et al. 2003). This method is based on MS and it allows identification and quantification of both $CO_2$ and $^{13}CO_2$ in the headspace without chromatographic separation. The purge flow was set at 1 min. The headspace volume of 1500 µl was injected manually. The retention window was set between 0 and 50 s. Data were consolidated with the Quadstar™ software (INFICON, New York, NY, USA). The qualitative value of the measured $CO_2$ is given by the integral under the ion current curve.

## Results and discussion

*Quantification of furans in model systems*

The formation of furan and 2-methylfuran (MF) was studied in model systems simulating roasting and pressure cooking (sterilization) conditions. This study focuses on ascorbic acid as one of the putative furan precursors. The effect of additional compounds was also investigated in binary mixtures of ascorbic acid and sugars or amino acids. Some potential intermediates were studied as well to understand better the formation of furan. The amounts of furan and MF were quantified by SPME-GC/MS to evaluate the efficiency of various precursors systems. The results are expressed in µmol furan or MF $mol^{-1}$ of precursor and the relative standard deviation (RSD) is given in per cent. 3-Methylfuran was also 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.

As shown in Table IV, ascorbic acid was the most efficient furan precursor of all model systems studied, generating about 2 mmol $mol^{-1}$ under roasting conditions. However, the transformation yields are still very low (<1 mol%). On the contrary, dehydroascorbic acid formed only trace amounts of furan (40 µmol $mol^{-1} = 2\%$ compared with ascorbic acid), most likely due to the relatively low roasting temperature (200°C) that was below its melting point (228°C). Under pyrolysis conditions at 250, 300 and 350°C, dehydroascorbic acid generated 6, 34 and 56% furan when compared with the yields obtained from ascorbic acid. Furthermore, dehydroascorbic acid can undergo a hemiketal-type ring closure under dry-heating conditions, which prevents furan formation (Perez Locas and Yaylayan 2004).

Addition of a sugar or an amino acid drastically reduced furan formation to an extent of 50–80%. This is rather surprising, as both glucose and erythrose on their own have been reported as furan precursors (Perez Locas and Yaylayan 2004; Becalski and Seaman 2005; Fan 2005; Märk et al. 2006). In particular, erythrose led to an 80% drop of furan, which is similar to the data recently reported (Märk et al. 2006) using slightly different reaction conditions. It seems that furan formation is negatively affected by the presence of additional molecules, which may increase the fragmentation rate or change the redox status of the reaction system. These data confirm the assumption of competing pathways favouring other reactions than furan formation in complex mixtures such as foods, thus leading to lower furan amounts than suggested from simple model systems.

The amounts of MF were rather low compared with furan (Table IV). MF makes less than 1% of the total furans generated from ascorbic acid (sample 1). Binary mixtures tend to increase the MF levels, which is opposite to the furan behaviour. The system ASA/PHE (sample 4) is an exception as almost equal levels of furan and MF were formed, i.e. about 1 mmol $mol^{-1}$ of each. It can be assumed that the formation of MF from ascorbic acid involves fragmentation and recombination steps, unlike the formation of furan. This process can, in particular, be accelerated by amino acids such as phenylalanine, leading to a high total furan level of about 2 mmol $mol^{-1}$ (sum of furan and MF in sample 4), which is equal to the furan amount found in sample 1 (ASA). The amino acid seems to accelerate the fragmentation of ascorbic acid, which reduces furan but favours MF formation, probably by recombination of ascorbic acid fragments.

Pressure cooking conditions resulted in much lower furan amounts as compared with roasting (Table IV). The furan levels generated from ascorbic acid in aqueous media were higher at pH 4 (58 μmol $mol^{-1}$) than at pH 7 (3.7 μmol $mol^{-1}$), which also applied to the binary mixtures (Figure 3). Interestingly, at pH 7 dehydroascorbic acid was more efficient (5.7 μmol $mol^{-1}$) than ascorbic acid (3.7 μmol $mol^{-1}$). A possible explanation is that at pH 7, furan is mainly formed via dehydroascorbic acid as a key intermediate, preferably formed by oxidation favoured at higher pH. The MF amounts were also very low (about 1 μmol $mol^{-1}$). However, the MF portion of total alkylfurans at pH 7 was relatively high (25%). Overall, ascorbic acid is a very minor precursor of furan under food sterilization conditions at pH 7, but might be considered as a potential furan precursor at pH 4. The presence of additional reactants in the model systems led to a decrease of furan, in particular at pH 4.

2-Furaldehyde and 2-furoic acid have been reported as degradation products of ascorbic and dehydroascorbic acid (Velisek et al. 1976; Vernin et al. 1998). This was confirmed in the present study as these intermediates generated relatively high amounts of furan, but only traces of MF. In particular, 2-furoic acid was effective under roasting conditions (1.6 mmol $mol^{-1}$), whereas 2-furaldehyde turned out to be a general precursor of furan in both dry and aqueous model systems (70–260 μmol $mol^{-1}$). Therefore, both compounds should be considered as potential furan precursors: 2-furoic acid mainly in samples containing ascorbic acid, 2-furaldehyde also in sugar or Maillard reaction systems.

*Mechanistic studies on furan formation*

Despite several recently published papers dealing with furan formation, including the use of labelled precursors (Perez Locas and Yaylayan 2004), the mechanisms leading to furan (or MF) are far from being understood. Several possible pathways have been postulated, however, with no or little experimental proof. One objective of the present work was to elucidate further formation mechanisms and substantiate hypotheses by identifying key intermediates and working with labelled precursors. The per cent label distributions were determined by subtracting the naturally occurring percentage of $^{13}C$ for each value.

Mechanistic studies were carried out with L-[1-$^{13}$C]-, L-[2-$^{13}$C]- or L-[6-$^{13}$C]-ascorbic acid under both dry (10 min at 200°C) and aqueous (25 min at 121°C) conditions at pHs 4 and 7. As shown in Table V, there was no incorporation of C-1 and C-2 into furan (samples 1, 2, 5, 6, 9 and 10), whereas L-[6-$^{13}$C]-ascorbic acid (samples 3, 7, 11) only led to mono-labelled furan. These data suggest that furan is exclusively formed from the intact ascorbic acid skeleton, without recombination of fragments that might be generated by decomposition. Furthermore, the labelling pattern was not affected by the type of heating conditions, thus the same percentages were obtained independently of the reaction conditions (dry and aqueous at pHs 4 and 7).

In addition, binary mixtures of equimolar unlabelled ascorbic acid and fully labelled glucose were heated to study whether furan can be generated from fragments formed from ascorbic acid and [U-$^{13}C_6$]-glucose. This set-up can be seen as a modified CAMOLA experiment (Carbon Module Labelling; Schieberle 2005). Under dry-heating conditions, the model system L-ASA/D-[U-$^{13}C_6$]-GLC (sample 4) resulted in 73% unlabelled furan and 27% fully $^{13}C$-labelled furan, indicating that they were formed either from ascorbic acid or from [U-$^{13}C_6$]-glucose. In aqueous systems at pH 7 (sample 8), higher relative amounts were obtained from ascorbic acid (84%) that was even 99% at pH 4 (sample 12). This is in agreement with the observation that furan was preferably generated from ascorbic acid at lower pH (Figure 3). Higher pH, however, accelerates fragmentation reactions of sugars (Ledl and Schleicher 1990) that may favour furan formation from glucose.

As heating of ascorbic acid and labelled glucose did not result in $^{13}C_1$-furan ($m/z = 69$), $^{13}C_2$-furan ($m/z = 70$) or $^{13}C_3$-furan ($m/z = 71$), furan is not generated by the recombination of fragments of glucose and ascorbic acid. However, it cannot be fully excluded from these experiments that furan is

Table IV. Amounts of furan and 2-methylfuran (MF) generated in various model systems containing ascorbic acid (ASA), dehydroascorbic acid (DASA), erythrose (ERY), glucose (GLC), and phenylalanine (PHE).

| | | Roasting[a] | | | | Pressure cooking (pH 7)[a] | | | | Pressure cooking (pH 4)[a] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number | Model system | Furan (μmol mol$^{-1}$) | RSD (%) | MF (μmol mol$^{-1}$) | RSD (%) | Furan (μmol mol$^{-1}$) | RSD (%) | MF (μmol mol$^{-1}$) | RSD (%) | Furan (μmol mol$^{-1}$) | RSD (%) | MF (μmol mol$^{-1}$) | RSD (%) |
| 1 | ASA | 2051 | 4.5 | 19.0 | 20.3 | 3.69 | 7.8 | 1.19 | 24.9 | 57.5 | 26.9 | 0.65 | 33.8 |
| 2 | ASA + ERY | 385 | 5.0 | 17.5 | 12.8 | 4.30 | 4.5 | 1.91 | 23.6 | 27.7 | 25.1 | 0.62 | 35.0 |
| 3 | ASA + GLC | 791 | 16.3 | 184 | 6.0 | 2.77 | 18.6 | 1.52 | 30.4 | 22.8 | 27.0 | 0.58 | 27.4 |
| 4 | ASA + PHE | 990 | 8.4 | 1016 | 5.1 | 0.66 | 15.0 | 0.13 | 18.3 | 17.1 | 4.8 | 0.51 | 3.4 |
| 5 | DASA | 40.2 | 0.3 | 0.67 | 1.1 | 5.71 | 28.0 | 4.43 | 22.1 | 18.9 | 25.6 | 1.06 | 0.3 |
| 6 | 2-Furoic acid | 1656 | 6.9 | 3.21 | 34.8 | 31.4 | 9.7 | 0.10 | 0.9 | 326 | 7.3 | 0.17 | 22.5 |
| 7 | 2-Furaldehyde | 265 | 16.1 | 1.87 | 19.4 | 97.7 | 0.6 | 0.08 | 2.3 | 70.4 | 10.8 | 1.64 | 6.8 |

[a]Roasting (200°C, 10 min), pressure cooking (121°C, 25 min).
RSD, relative standard deviation.

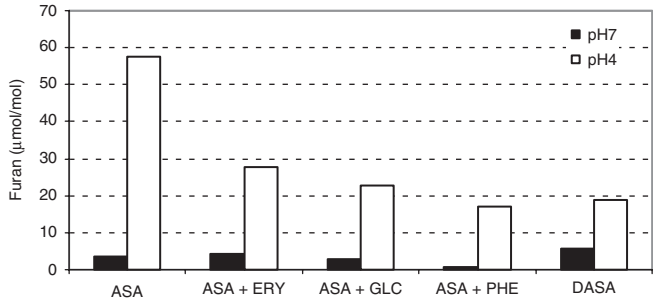


Figure 3. Furan contents (µmol mol$^{-1}$) obtained in aqueous model systems heated at pHs 4 and 7 (25 min, 121°C). ASA, ascorbic acid; ERY, erythrose; GLC, glucose; PHE, phenylalanine; DASA, dehydroascorbic acid.

Table V. Per cent labelling distribution of furan generated from different ascorbic acid (ASA) and glucose (GLC) isotopomers.

| Number | Model system | $[M]^+$ (*m/z* 68) | $[M+1]^+$ (*m/z* 69) | $[M+2]^+$ (*m/z* 70) | $[M+3]^+$ (*m/z* 71) | $[M+4]^+$ (*m/z* 72) |
|---|---|---|---|---|---|---|
| 1, 5, 9 | L-[1-$^{13}$C]-ASA[a,b,c] | 100 | 0 | 0 | 0 | 0 |
| 2, 6, 10 | L-[2-$^{13}$C]-ASA[a,b,c] | 100 | 0 | 0 | 0 | 0 |
| 3, 7, 11 | L-[6-$^{13}$C]-ASA[a,b,c] | 0 | 100 | 0 | 0 | 0 |
| 4 | L-ASA + D-[U-$^{13}C_6$]-GLC (1:1)[a] | 73 | 0 | 0 | 0 | 27 |
| 8 | L-ASA + D-[U-$^{13}C_6$]-GLC (1:1)[b] | 84 | 0 | 0 | 0 | 16 |
| 12 | L-ASA + D-[U-$^{13}C_6$]-GLC (1:1)[c] | 99 | 0 | 0 | 0 | 1 |

[a]Results from dry-heating systems (200°C, 10 min).
[b]Results from aqueous solutions at pH 7 (121°C, 25 min).
[c]Results from aqueous solutions at pH 4 (121°C, 25 min).

also formed by recombination of ascorbic acid fragments (derived from C-3 to C-6). To answer this question, fully labelled ascorbic acid would be necessary, which was commercially not available, to perform a CAMOLA experiment. On the other hand, the absence of $^{13}C_2$-furan (*m/z* = 70) in the labelled ascorbic acid model systems indicates that furan is most likely not formed by recombination of fragments.

Based on experimental results mentioned above, a general formation mechanism of furan from ascorbic acid is proposed that should be valid under both roasting and pressure cooking conditions (Figure 4). Furan formation from ascorbic acid is suggested to start with ring opening followed by $\beta$-elimination of water at C-4. 4-Deoxyascorbic acid can undergo decarboxylation at the C-1 position to yield 3-deoxypentosulose, considering that ß-oxo acids easily decarboxylate. However, it may also be possible that decarboxylation takes place before dehydration.

Dehydration and decarboxylation have already been proposed as the initial steps in furan formation from ascorbic acid (Perez Locas and Yaylayan 2004). The present paper provides unequivocal evidence for the decarboxylation step through monitoring the $CO_2$ by SmartNose. As shown in Table VI, more labelled $^{13}CO_2$ was found in the model system based on L-[1-$^{13}$C]-ascorbic acid as indicated by the ratio 1:1.8 of unlabelled and labelled $CO_2$. On the other side, unlabelled $CO_2$ dominated in the presence of L-[2-$^{13}$C]- and L-[6-$^{13}$C]-ascorbic acid. However, traces of $^{13}CO_2$ were also found, suggesting that decarboxylation may partially also take place at C-2 (via 2-furoic acid as intermediate) and C-6.

Pathway A postulates the degradation of 3-deoxypentosulose to 2-deoxyaldotetrose by an $\alpha$-dicarbonyl cleavage (Perez Locas and Yaylayan 2004), which may alternatively be generated directly from 4-deoxyascorbic acid. The $\alpha$-dicarbonyl cleavage step remains hypothetical, despite the fact that it is frequently used in mechanistic schemes. It has recently been shown that in aqueous systems (pH 6–8, 90–120°C), deoxyosones fragment via a hydrolytic $\beta$-dicarbonyl cleavage and/or an oxidative $\alpha$-dicarbonyl cleavage (Davidek et al. 2006a, 2006b). The fragmentation mechanism under roasting conditions, however, has not yet been studied in detail.

The corresponding counterparts are formic acid and oxalic acid, respectively. Both have been reported as degradation products of ascorbic acid (Kennedy et al. 1989; Shephard et al. 1999). In this study, formic acid could indeed be identified as a typical decomposition product of ascorbic acid (Figure 5A). The highest percentage of labelled formic acid (32%) was found in the experiment with L-[2-$^{13}$C]-ascorbic acid (Table VII). However, unlabelled formic acid was also detected in this sample (Figure 5B). This demonstrates that formic

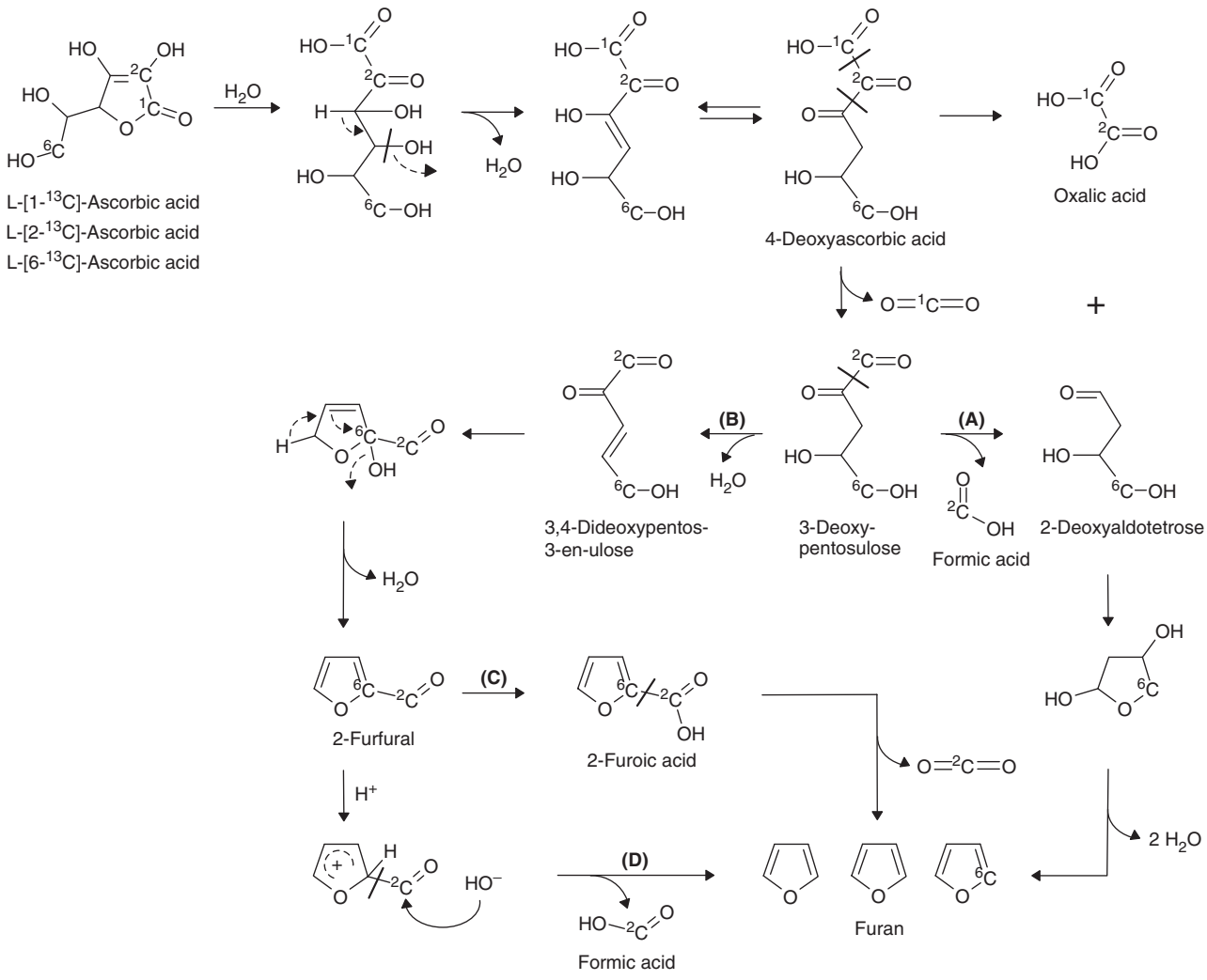


Figure 4. Mechanism proposed for the formation of furan from the intact ascorbic acid skeleton. Experiments were performed using ascorbic acid isotopomers labelled at C-1, C-2-, or C-6. The labelled carbons are marked in the scheme and the same numbering is kept throughout this schematic presentation.

acid is mainly derived from C-2 of ascorbic acid (Figure 4), but also other C-atoms can be transformed into formic acid.

Cyclization and subsequent dehydration of 2-deoxyaldotetrose, which is composed of the C3-C4-C5-C6 carbon atoms of ascorbic acid, gives rise to parent furan in the course of pathway A. Alternatively, 3-deoxypentosulose may dehydrate at C-4 (= C-5 of ascorbic acid) via pathway B leading to 2-furaldehyde by cyclization and additional dehydration. Further oxidation leads to 2-furoic acid (pathway C), which has been shown to be a fairly good precursor of furan (Table IV) with concomitant release of $CO_2$. Furan may also be generated directly from 2-furaldehyde by an electrophilic aromatic substitution-type reaction via pathway (D) with formic acid as by-product. Indeed, formic acid could unequivocally be identified (data not shown) in the model system containing 2-furaldehyde as precursor (sample 7 in Table IV).

Table VI. Formation of $CO_2$ and $^{13}CO_2$ from ascorbic acid (ASA) isotopomers in model systems under roasting conditions (10 min, 200°C)[a].

| Model system | $CO_2$ (ion current) | $^{13}CO_2$ (ion current) | $CO_2/^{13}CO_2$ |
|---|---|---|---|
| L-[1-$^{13}$C]-ASA | $2.869 \times 10^{-10}$ | $5.145 \times 10^{-10}$ | 1:1.79 |
| L-[2-$^{13}$C]-ASA | $18.330 \times 10^{-10}$ | $4.611 \times 10^{-10}$ | 1:0.25 |
| L-[6-$^{13}$C]-ASA | $18.450 \times 10^{-10}$ | $3.026 \times 10^{-10}$ | 1:0.16 |

[a]Natural $CO_2$ content of the air was not subtracted and, therefore, the carbon dioxide ion current only came partly from the decomposition of ascorbic acid.

The pathway via 2,3-diketogulonic acid and aldotetroses, as proposed by Perez Locas and Yaylayan (2004), is less probable because of the low furan yields generated from erythrose (Märk et al. 2006).

Overall, furan comprises an intact $C_4$ unit of ascorbic acid (mainly C-3 to C-6) generated by

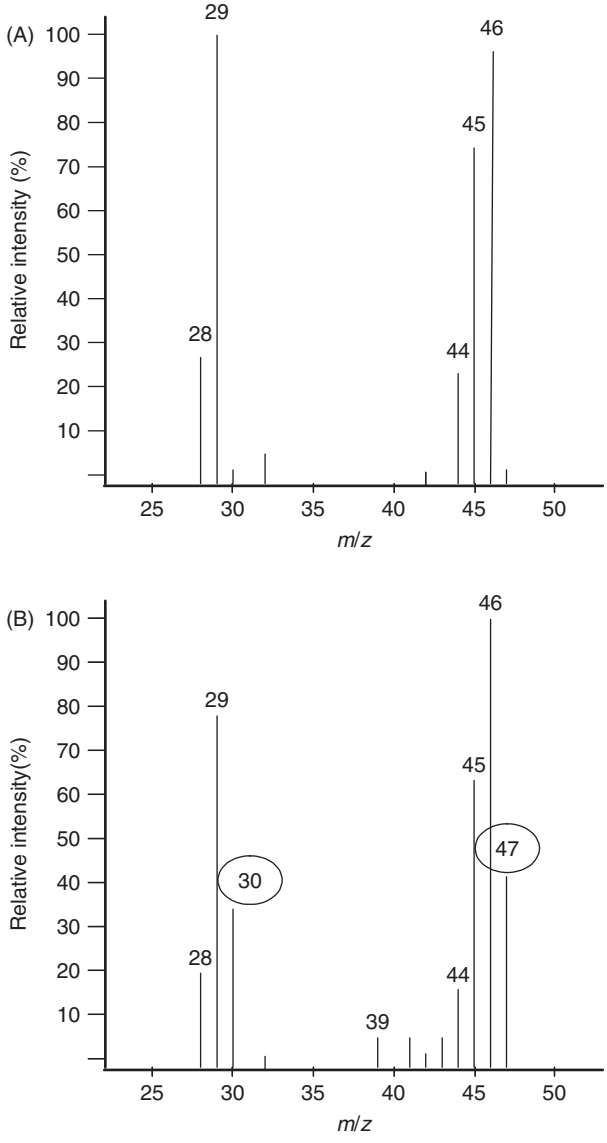


Figure 5. Mass spectra of (A) unlabelled and (B) $^{13}$C-labelled formic acid obtained from model systems containing unlabelled ascorbic acid and L-[2-$^{13}$C]-ascorbic acid, respectively.

liberating two $C_1$ units, i.e. $CO_2$ and formic acid, via various possible intermediates such as 2-deoxyaldoteroses, 2-furoic acid and 2-furaldehyde, all known as ascorbic acid degradation products. Referring to the quantitative results (Table IV), pathway A via 2-deoxyaldoteroses and B/C via 2-furoic acid seem to be the major routes yielding furan upon dry heating (Figure 5). The labelling pattern of furan is well in line with the proposed formation mechanisms from ascorbic acid.

*Mechanistic studies on methylfuran formation*

MF was included in this study for mechanistic reasons as it behaves differently from furan (Märk et al. 2006). Previous data indicated that MF, contrary to furan, is preferably formed by recombination of fragments obtained from various precursors such as sugars, amino acids and ascorbic acid. The results summarized in Table VIII confirm that MF is at least partially formed by fragmentation of ascorbic acid, i.e. 8% unlabelled MF was found in the [2-$^{13}$C]-ASA system upon roasting (sample 2), which can only be formed by recombination of fragments. Similarly, the binary mixture ASA/[U-$^{13}C_6$]-GLC resulted in 6% unlabelled MF (sample 4), indicating more MF to be formed by glucose fragmentation. However, the $C_1$ atom seems not be incorporated into MF as only unlabelled MF was obtained from [1-$^{13}$C]-ASA under both dry-heating and pressure cooking conditions (samples 1, 5 and 9).

Table VII. Formic acid generated by thermal decomposition of ascorbic acid (ASA) isotopomers.

| Model system | HCOOH ($m/z = 46$) | H$^{13}$COOH ($m/z = 47$) |
|---|---|---|
| Ascorbic acid | 100 | 0 |
| L-[1-$^{13}$C]-ASA | 80 | 20 |
| L-[2-$^{13}$C]-ASA | 68 | 32 |
| L-[6-$^{13}$C]-ASA | 84 | 16 |

Values are percentages.

Interestingly, remarkable differences were found for [2-$^{13}$C]-ASA in aqueous systems, i.e. the percentage of MF obtained by recombination raised from 8% (roasting, sample 2) to 33% (pH 4, sample 10) and 75% (pH 7, sample 6), knowing that fragmentation is usually favoured in aqueous solution and with increasing pH (Ledl and Schleicher 1990). In agreement with that, ASA/[U-$^{13}C_6$]-GLC at pH 7 (sample 8) indicated with 16% for $[M+2]^+$ the recombination of a $C_3$ unit of ASA and a $C_2$ unit of the sugar. The data obtained with [6-$^{13}$C]-ASA (samples 7 and 11) are more difficult to interpret. Assuming decarboxylation as a major reaction taking place, MF formation by fragmentation is indicated by 36% of unlabelled MF found in sample 7 at pH 7. However, it should be mentioned that, overall, MF formation from ascorbic acid is definitely a minor reaction pathway.

Unfortunately, the MS signals of MF were rather weak and sufficient intensities were only obtained in the roasting experiments. While [1-$^{13}$C]-ASA resulted only in unlabelled fragments (Table VIII), [2-$^{13}$C]-ASA showed *m/z* 54 and *m/z* 40, but no *m/z* 30, thus indicating $^{13}$C-labelling in position C-3 or C-4. The higher per cent labelling at *m/z* 54 compared with *m/z* 40 also indicated (about 37%) labelling in position C-1 or C-2. On the contrary, [6-$^{13}$C]-ASA led to *m/z* 54 and *m/z* 30, but no *m/z* 40, which points to $^{13}$C-labelling in position C-5. Based on these limited data, it would be very hypothetical to suggest a formation mechanism of MF from ascorbic acid.

Table VIII. Per cent labelling distribution of 2-methylfuran (MF) generated from different labelled ascorbic acid (ASA) and glucose (GLC) isotopomers.

| Number | Model system | $[M]^+$ (*m/z* 82) | $[M+1]^+$ (*m/z* 83) | $[M+2]^+$ (*m/z* 84) | $[M+3]^+$ (*m/z* 85) | $[M+4]^+$ (*m/z* 86) | $[M+5]^+$ (*m/z* 87) |
|---|---|---|---|---|---|---|---|
| 1 | L-[1-$^{13}$C]-ASA[a,d] | 100 | 0 | 0 | 0 | 0 | 0 |
| 2 | L-[2-$^{13}$C]-ASA[a,e] | 8 | 92 | 0 | 0 | 0 | 0 |
| 3 | L-[6-$^{13}$C]-ASA[a,f] | 5 | 95 | 0 | 0 | 0 | 0 |
| 4 | L-ASA + D-[U-$^{13}C_6$]-GLC (1:1)[a] | 6 | 0 | 0 | 0 | 0 | 94 |
| 5 | L-[1-$^{13}$C]-ASA[b] | 100 | 0 | 0 | 0 | 0 | 0 |
| 6 | L-[2-$^{13}$C]-ASA[b] | 75 | 25 | 0 | 0 | 0 | 0 |
| 7 | L-[6-$^{13}$C]-ASA[b] | 36 | 64 | 0 | 0 | 0 | 0 |
| 8 | L-ASA + D-[U-$^{13}C_6$]-GLC (1:1)[b] | 68 | 0 | 16 | 0 | 0 | 16 |
| 9 | L-[1-$^{13}$C]-ASA[c] | 100 | 0 | 0 | 0 | 0 | 0 |
| 10 | L-[2-$^{13}$C]-ASA[c] | 33 | 67 | 0 | 0 | 0 | 0 |
| 11 | L-[6-$^{13}$C]-ASA[c] | 6 | 86 | 8 | 0 | 0 | 0 |
| 12 | L-ASA + D-[U-$^{13}C_6$]-GLC (1:1)[c] | 68 | 0 | 0 | 0 | 0 | 32 |

[a]Results from dry heating (200°C, 10 min).
[b]Results from aqueous solutions at pH 7 (121°C, 25 min).
[c]Results from aqueous solutions at pH 4 (121°C, 25 min).
[d]Labelling pattern of MF generated from L-[1-$^{13}$C]-ASA: *m/z* 53 (100%), *m/z* 39 (100%), *m/z* 29 (100%).
[e]Labelling pattern of MF generated from L-[2-$^{13}$C]-ASA: *m/z* 53 (16%), *m/z* 54 (84%), *m/z* 39 (53%), *m/z* 40 (47%), *m/z* 29 (100%).
[f]Labelling pattern of MF generated from L-[6-$^{13}$C]-ASA: *m/z* 53 (94%), *m/z* 54 (6%), *m/z* 39 (100%), *m/z* 29 (17%), *m/z* 30 (83%).

*Spiking experiments in food products*

The study of model systems was extended to food products containing fruits and vegetables heated under pressure cooking conditions, thus simulating the sterilization process (123°C, 22 min). Two types of products were used, i.e. vegetable puree (pumpkin) and vegetable and fruit juice (carrot and orange). Spiking experiments were conducted with unlabelled and [6-$^{13}$C]-labelled ascorbic acid to evaluate its role as furan precursor in real food environment and estimate the validity of the reaction mechanisms proposed above.

The concentrations of ascorbic acid in the model products ranged from less than 0.1 mg/100 g to about 50 mg/100 g. The amounts added before the heat treatment were 56–58 mg/100 g. As shown in Table IX, the highest increase in furan formation (124%) was observed in the pumpkin vegetable puree caused by the addition of 57.1 mg/100 g ascorbic acid followed by the carrot juice (38%) to which 55.8 mg/100 g ascorbic acid was added. Interestingly, the addition of 58.1 mg ascorbic acid to 100 g orange juice resulted in less total furan levels (−16%). These results clearly miss a direct correlation between the concentration of ascorbic acid present in the samples and the furan amounts generated.

According to the formation pathway of furan from ascorbic acid, C-6 of ascorbic acid is always incorporated into furan (Figure 4). Surprisingly, the [1-$^{13}$C]-furan content was very low (<4%) in all food samples spiked with [6-$^{13}$C]-ascorbic acid (Table IX). For example, labelled furan was hardly detectable in the pumpkin sample though about 124% more furan was found after spiking of 57.1 mg/100 g ascorbic acid. This clearly demonstrates that, under the experimental conditions chosen, ascorbic acid is only a minor precursor of furan in the food products studied. The data also suggest that other compounds must function as precursors, the transformation of which into furan is accelerated in the presence of ascorbic acid.

One explanation for the increase in furan contents in pumpkin puree and carrot juices could be that ascorbic acid acts as a pro-oxidant in such high concentrations rather than as a direct furan precursor. Especially in the pumpkin puree in which the oxidative degradation of lipids is of importance, a pro-oxidative ingredient such as ascorbic acid at high concentrations may increase furan formation. This points to lipids as potential furan precursors that have, indeed, been suggested in previous studies (Perez Locas and Yaylayan 2004, Becalski and Seaman 2005, Märk et al. 2006). Another explanation for such a trend could be the pH decrease from 6.26 to 5.73 after addition of the ascorbic acid, which favours lipid oxidation leading to higher furan levels. In carrot juice, the addition of ascorbic acid probably also accelerated the oxidative degradation of lipids. However, only 4% of the 38% additionally generated furan originated from the added [6-$^{13}$C]-ascorbic acid. Similarly, only 3% of the total furan formed in orange juice spiked with [6-$^{13}$C]-ascorbic acid were incorporated. Moreover, the total furan level decreased, which is difficult to explain based on the data available so far. It seems that the addition of ascorbic acid to food products

Table IX. Spiking experiments with ascorbic acid (ASA) and [6-$^{13}$C]-ASA in food products.

| Product | Vegetable puree (pumpkin) | Vegetable juice (carrot) | Fruit juice (orange) |
|---|---|---|---|
| Natural ASA concentration (mg kg$^{-1}$) | <0.1 | 7.4 | 48.3 |
| Total ASA content after spiking (mg kg$^{-1}$) | 57.1 | 63.2 | 106.4 |
| Added ASA content (%)[a] | 100 | 88 | 55 |
| Furan content without spiking (μg kg$^{-1}$) | 48.6 ± 1.6 | 30.2 ± 5.8 | 20.7 ± 0.4 |
| Furan content with [6-$^{13}$C]-ASA spiking (μg kg$^{-1}$) | 108.9 ± 13.5 | 41.7 ± 0.3 | 17.8 ± 0.2 |
| Deviation in furan level (%)[b] | 124 | 38 | −16 |
| Unlabelled furan content (spiked sample) (%)[c] | 100 | 96 | 97 |
| [1-$^{13}$C]-furan content (spiked sample) (%)[c] | 0 | 4 | 3 |
| pH before spiking | 6.26 | 6.47 | 3.90 |
| pH after spiking | 5.76 | 6.30 | 3.81 |

[a]Added ASA content (%) relative to total ASA amount.
[b]+, Additional formation of furan; −, overall loss of furan.
[c]Spiked amount of [6-$^{13}$C]-ASA in mg kg$^{-1}$: 53.57 (pumpkin vegetable), 54.56 (carrot juice), 56.47 (orange juice).

before thermal treatment, which contain other furan precursors, leads to higher furan levels. Assuming that these furan precursors belong to the chemical class of polyunsaturated lipids (e.g. PUFAs), it is not recommended to fortify such food with vitamin C before thermal treatment. This may in particular be important for canned and jarred food products.

In conclusion, SPME-GC/MS analysis of furan turned out to be a reliable and convenient method 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 roasting conditions. Furan formation was quite sensitive towards changes of the reaction conditions and precursor compositions indicating the complexity of the reaction pathways. Furan amounts were reduced to a great extent by favouring competing reactions and/or intervening at the redox system level. Therefore, the furan levels are definitely much lower in more complex systems such as foods 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 methylfuran from various types of precursors under food processing conditions in order to reduce their formation. Our work on furan formation from sugars, Maillard systems, and lipids, including PUFAs and carotenes, will be published elsewhere.

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