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Furan Formation from Lipids in Starch-Based Model Systems, As Influenced by Interactions with Antioxidants and Proteins

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ABSTRACT: The formation of furan upon sterilization of a lipid-containing starch gel was investigated in the presence of various antioxidants, namely, α-tocopherol, β-carotene, and ascorbic acid, with and without proteins. Results indicated that α-tocopherol did not significantly influence furan formation from oxidized lipids. β-Carotene, suggested previously to be a furan precursor itself, did influence the generation of furan in a concentration-dependent manner, although to a limited extent. Surprisingly, the presence of lipids seemed to limit the furan generation from β-carotene. Interestingly, the addition of ascorbic acid to the emulsions containing soybean or sunflower oils considerably enhanced the formation of furan from these oils. This was also the case when fresh oils were applied, shown previously to be nearly unable to generate furan. This observation can be explained by an intensified ascorbic acid degradation stimulated by the presence of lipids.

KEYWORDS: furan, starch-based model system, lipid oxidation, β-carotene, α-tocopherol, ascorbic acid

INTRODUCTION

Since furan, a small heterocyclic organic compound, has been classified as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC),[1] its presence in foods has acquired significant attention. However, furan has been known for a long time as a food constituent.[2] Because of its high volatility (boiling point of 31 °C), substantially hindering its analysis, it was only recently that more comprehensive studies, reporting furan in a number of food products, have been performed.[3] Its presence in a broad range of foods is related to its multiple routes of formation, involving mainly thermal degradation of food constituents, such as vitamin C and its derivatives, carbohydrates (in the presence and absence of amino acids or proteins), polyunsaturated fatty acids, and carotenoids.[4,5]

Many studies have been performed to investigate furan formation from different precursors mainly in simple model systems under pressure-cooking conditions,[6−9] under roasting conditions,[7,9] at high temperatures of pyrolysis,[4] and moreover, as a result of ionizing radiation.[8] Investigation of furan formation has also been carried out in real food samples, such as pumpkin puree and carrot juice under pressure-cooking conditions,[7,9] in apple juice and orange juice as a result of ionizing radiation and thermal treatment,[10] and in hazelnuts under roasting conditions.[11] Moreover, it has also been reported that furan can be formed at lower temperatures; in particular, 4 parts per billion (ppb) of furan was found after incubation of a green coffee extract at 40 °C for 30 min.[12]

Recently, a more realistic starch-based model food system, similar to baby food, has been created to study furan formation from vitamin C[13] and lipids[14] under pressure-cooking conditions. As a further elaboration of the latter study, in the present paper, the interactions between antioxidants, proteins, and lipids and their influence on furan formation are investigated.

Yaylayan[15] hypothesized that furan originates from 2-butenal, a direct oxidation product of ω-3 unsaturated fatty acids, involving further oxidation to 4-hydroxy-2-butenal, cyclization of 4-hydroxy-2-(*Z*)-butenal, and subsequent dehydration. This scheme is elaborated in Figure 1. In view of this proposed mechanism, antioxidants can potentially inhibit furan formation from lipids, because they are well-known scavengers of free radicals formed during the lipid oxidation processes.

Thus, it has been shown in the literature that antioxidants, such as tocopherol acetate and butyl hydroxyanisole (BHA), reduced furan generation from polyunsaturated fatty acids (PUFAs) up to 70% under pressure-cooking conditions.[6] On the contrary, another study,[16] investigating the influence of antioxidants under roasting conditions, showed an increase in furan formation from linoleic acid upon the addition of butylated hydroxytoluene (BHT) and only a slight decrease in furan formation from α-linolenic acid in the presence of α-tocopherol.

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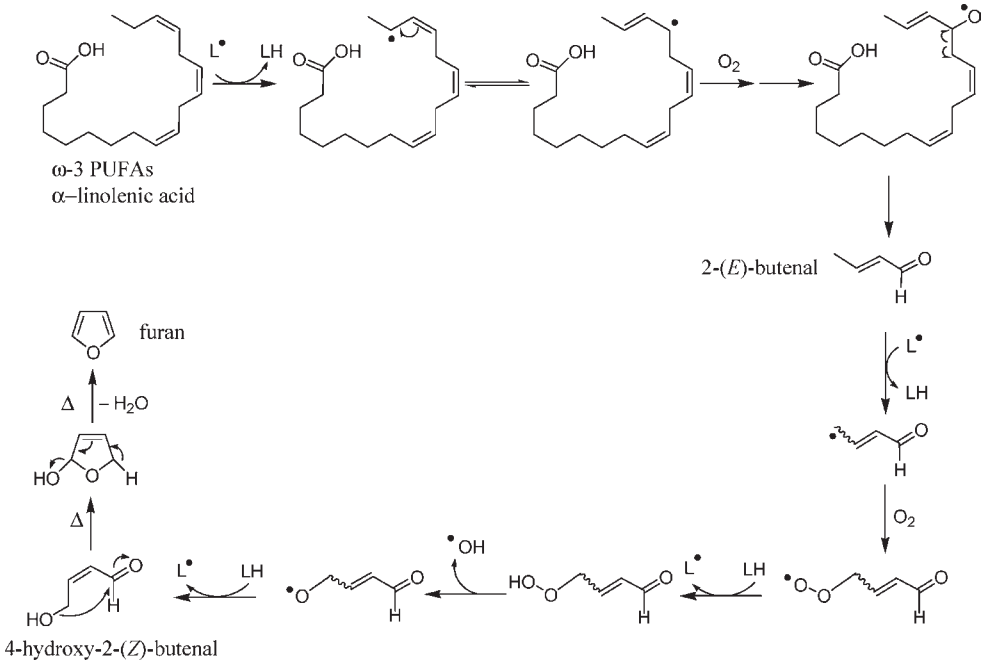

Figure 1. Proposed pathway of ω-3 fatty acid oxidation leading to furan (on the basis of ref 15, with modifications).

In contrast to these antioxidants, β-carotene and other carotenoids have been suggested as furan precursors by Becalski and Seaman.[6] Because no experimental details were provided in this study and no further studies have been performed in this regard, the role of carotenoids with respect to furan formation remains unclear.

Concerning ascorbic acid, another compound having antioxidative properties, it should be stressed that it is considered as the most important precursor of furan, especially in simple model systems studied under dry heating conditions.[7,16] However, studies with binary mixtures of ascorbic acid and amino acids, sugars, or lipids, have indicated that significantly less furan was formed in these complex model systems. Under roasting conditions, a mixture containing equimolar amounts of ascorbic acid and α-linolenic acid generated 46% less furan than ascorbic acid alone.[16] On the other hand, studies performed by Limacher et al.[7] with food samples (pumpkin puree and carrot juice) showed that the addition of ascorbic acid before thermal treatment led to higher furan levels, especially in the pumpkin puree.

To investigate the interactions of lipids with antioxidants and their influence on furan formation upon pressure cooking of the previously described starch-based lipid-containing system,[14] α-tocopherol, β-carotene, and ascorbic acid were incorporated in such a system in the presence and absence of proteins.

MATERIALS AND METHODS

Reagents. Citric acid (monohydrate, 99.5+%), disodium hydrogen phosphate (dihydrate, 99.5+%), L-(+)-ascorbic acid (99+%), methanol (p.a.), ethanol (p.a.), and ethylenediaminetetraacetic acid (EDTA) were purchased from Chem-Lab NV (Zedelgem, Belgium). Furan (99+%), d_4-furan (98%), fresh β-carotene [type II, synthetic, ≥95% (HPLC)], and orthophenylenediamine dihydrochloride were supplied by Sigma-Aldrich (Steinheim, Germany). Methanol (Picograde) was supplied by LGC Promochem (Molsheim, France). Hexane (HPLC-grade) and methanol (HPLC-grade) were purchased from VWR (Fontenay Sous Bois, France). Milli-Q water was prepared using a Millipore system (Brussels, Belgium). Whey protein isolate (Lacprodan DI-9224) was provided by Acatris Food Belgium (Londerzeel, Belgium). Fresh α-tocopherol was kindly offered by DSM Nutritional Products NV (Deinze, Belgium). Cold swelling native waxy corn starch (WC starch) and diacetyl tartaric (acid) ester of monoglyceride (DATEM) were kindly offered by Cargill (Haubourdin, France) and Palsgaard (Juelsminde, Denmark), respectively. Soybean oil (SBO) (Leiseur brand; 100%) and sunflower oil (SFO) (Vandermoortele brand; 100%) were obtained from local supermarkets.

Materials. Headspace (HS) vials for CTC PAL, 20 mL, clear glass, DIN-crimp neck, and magnetic crimp cap (gold) with silicone/polytetrafluoroethylene (PTFE) septa were supplied by Grace Davison Discovery Sciences (Lokeren, Belgium). The same vials and septa were used for heating experiments as well as solid-phase microextraction (SPME) analysis. The thermometer (Testo 735-2) connected with a temperature probe was supplied by Testo (Ternat, Belgium). The dispersing instrument Ultra-Turrax (T 25 digital) was supplied by IKA (Staufen, Germany).

Sample Preparation. *General Conditions of Sample Preparation and Emulsion Preparation.* General sample preparation and heating conditions were similar as in our previous research.[13,14] Generally, heating experiments were performed with a model food system containing 10% (w/w) WC starch, 5% (w/w) oil (soybean oil or sunflower oil), and 1% (w/w) emulsifier DATEM, prepared at pH 6.0 or 4.0 with 0.56 M phosphate/0.44 M citric acid buffer.

The final samples were prepared in two steps. First, buffer, oil (15%, w/w), and DATEM (3%, w/w) were mixed with an Ultra-Turrax T 25, and subsequently, this pre-emulsion was homogenized by means of an APV Lab 2000 two-stage homogenizer (APV, An SPX Brand, Erpe-Mere, Belgium) at a total pressure of approximately 250 bar (first stage at approximately 200 bar + second stage at 50 bar). In parallel, a 15% (w/w) starch suspension was prepared in the same buffer by slow addition of the starch powder to a buffer solution in the appropriate ratio, while mixing intensively. Subsequently, the resulting starch suspension was mixed with the oil-in-water emulsion at a 2:1 (w/w) ratio.

Oil-free samples containing 1% (w/w) DATEM and 10% (w/w) WC starch were prepared as well. For this purpose, DATEM was first dispersed in a phosphate/citric acid buffer and starch powder was added to the resulting mixture in an appropriate ratio as described above.

Table 1. Compositional Details of the Main Fatty Acids Present in the Used SBO and SFO (g/100 g of Oil)[a]

oils	palmitic acid (C16:0)	stearic acid (C18:0)	oleic acid (C18:1)	linoleic acid (C18:2)	linolenic acid (C18:3)
SBO	10.60	3.82	24.91	52.27	6.38
SFO	6.09	3.89	26.22	61.75	0.16

[a] Mean of duplicate analysis.

Afterwards, similar to our previous studies,[13,14] 15 g of emulsion/oil-free sample was transferred to a 20 mL HS vial, which was sealed with a crimp cap and heated in an oil bath (deep-fryer, Fritel 2505, Belgium) for 20 min at 120 ± 0.5 °C. With regard to the amount of heated sample, it was considered, on the basis of the experiments with the vitamin-C-containing system,[13] to use higher sample amounts (always 15 g) during heating in a 20 mL vial. Moreover, in such a way, more realistic HS volumes as compared to jarred foods, e.g., baby foods, were obtained.

Immediately after heating, samples were cooled in an ice−water mixture for a minimum of 30 min, mixed using a vortex shaker, and placed in a cold room at 4 °C.

Those conditions were used throughout all experiments unless otherwise specified.

Oxidation of Oils. Fresh and oxidized soybean oil and sunflower oil were used. Typically, oxidized oils were obtained by incubation of approximately 150 g of oil in a 250 mL Erlenmeyer plugged with paper tissue, at 60 °C for several days, as specified for each experiment. The fatty acid composition of the fresh oils was assessed according to the American Oil Chemists' Society (AOCS) Official Method Ce 1b-89 for marine oils,[17] by means of gas chromatography analysis of the fatty acid methyl esters, as described in our previous research[14] (Table 1). The oxidative status of the oils used was evaluated by determining the *p*-anisidine value (pAV) according to the AOCS Official Method Cd 18-90[18] and the peroxide value (pV) according to the method by Lea and Wheeler.[19] Moreover, the furan content in the fresh and oxidized oils used for the emulsion preparation was determined as described later under Furan Analysis.

Influence of α-Tocopherol. Fresh and oxidized (14 days at 60 °C) soybean oils were first analyzed for tocopherols by normal-phase high-performance liquid chromatography (HPLC)/fluorescence according to the AOCS Official Method Ce 8-89. For this purpose, approximately 1 g of oil was transferred to a volumetric flask of 25 mL and then dissolved in hexane (HPLC-grade, VWR). Subsequently, a subsample was filtered through a 0.45 μm HPLC filter (13 mm, nylon, syringe filters, Alltech Associates, Lokeren, Belgium) into a 1.5 mL glass vial and analyzed by means of HPLC, as described in more detail by Vandekinderen et al.[20]

The thermally oxidized soybean oil was spiked with α-tocopherol previously dissolved in ethanol, at the following concentration levels: 115 and 230 ppm. Fresh and oxidized soybean oils with and without the addition of α-tocopherol were used to prepare starch-based emulsions at pH 6.0 as described before. Samples were heated for 20 min at 120 °C as described above and were analyzed for furan.

Influence of β-Carotene. *Addition of β-Carotene to the Fresh and Oxidized Soybean Oil.* In this experiment, furan formation was investigated in the lipid system containing β-carotene at three different concentrations: 3.5, 7, and 14 mg/100 g of final sample. For this purpose, β-carotene was first dissolved in fresh and oxidized (8 days at 60 °C) soybean oils at the following concentrations: 70, 140, and 280 mg/100 g of oil. The oils containing β-carotene and the control oil samples to which no β-carotene was added were used for emulsion preparation at pH 6.0 and subsequent heating (120 °C, 20 min) as described above and were analyzed for furan.

Addition of β-Carotene to the System without Oil. This experiment was performed with oil-free starch-based samples (10%, w/w) to which β-carotene was added. For this purpose, β-carotene was first dissolved in ethanol at the following concentrations: 0.875, 1.75, and 3.5 mg/mL and then added to the starch gel (10.42%, w/w; pH 6.0) containing DATEM (1.04%, w/w) at a 1:24 (v/w) ratio. The resulting samples were heated (120 °C, 20 min) as described before and analyzed for furan.

Oxidation of Oils in the Presence of β-Carotene. β-Carotene was dissolved first in fresh soybean oil at two different concentrations: 70 and 140 mg/100 g of oil. Oils with and without β-carotene were thermally oxidized for 5, 13, and 17 days at 60 °C and were used afterward for the preparation of the emulsion at pH 6.0 and subsequent heating.

Degradation of β-carotene in oil, because of thermal incubation, was assessed by spectrophotometric measurement (450 nm) of an oil−β-carotene sample dissolved in hexane.

Influence of Vitamin C. Fresh and oxidized soybean oil and sunflower oil (incubated at 60 °C for 17 and 14 days, respectively) were used to prepare emulsions at pH 4.0 as described before. Successively, 17.35% (w/w) starch gel was prepared with a 0.56 M phosphate/0.44 M citric acid buffer at pH 4.0. The same buffer was used to prepare ascorbic acid solutions (pH 4.0) at a concentration of 1 and 5 mg of ascorbic acid/mL. Subsequently, the resulting emulsions, starch gel, and ascorbic acid solutions were mixed at a 10:17:3 (w/w/v) ratio by means of a vortex shaker. The obtained samples were subjected to heat treatment and analyzed for furan. From a nutritional point of view, the vitamin C content is considered as a sum of ascorbic acid and its oxidized form dehydroascorbic acid. However, because ascorbic acid is present the most in food and is a more efficient furan precursor,[13] this was the starting product used in the experiments.

Influence of Vitamin C and Proteins. This experiment was performed with a model system at pH 4.1 and containing 10% (w/w) WC starch, 5% (w/w) oil, 1% (w/w) DATEM, 5% (w/w) whey proteins, and 0.5 mg of ascorbic acid/g of sample. Similar to the previous experiment, fresh and oxidized soybean oil and sunflower oil were used to prepare emulsions with a phosphate/citric acid buffer at pH 4.0. Successively, the same buffer was used to prepare a mixture of whey proteins (7.7%, w/w) and starch 15.4% (w/w), as well as an ascorbic acid solution at a concentration of 30 mg/mL. The obtained emulsions, starch−protein mixture, and ascorbic acid solution were mixed at a 20:39:1 (w/w/v) ratio and additionally homogenized by means of an Ultra-Turrax (1 min, 1000 rpm). The resulting samples were heated and analyzed for furan.

Vitamin C Analysis. Emulsions containing vitamin C (0.5 mg of ascorbic acid/g) and fresh or oxidized (60 °C for 10 days) soybean oil (5%, w/w) were analyzed for vitamin C (the sum of ascorbic acid and dehydroascorbic acid) before and after heating (120 °C for 20 min), according to the procedure described by Dodson et al.[21] and Vandekinderen et al.,[20] with some modifications. All glasswork was protected from sunlight during the analysis. Approximately 2 g of starch-based sample, not heated as well as heated, was transferred to a 25 mL volumetric flask, diluted with an extraction buffer [0.1 M citric acid + 1.7 mM EDTA in methanol/water (1:19, v/v)] and shaken. After filtration over a folded filter, 5 mL of the filtrate was brought in a tube containing 100 mg of acid-washed active charcoal to convert the present ascorbic acid into dehydroascorbic acid. The tube was shaken vigorously for 30 s and centrifuged for 11 min at 6000*g*. Again, the supernatant was filtered, and 500 μL of the filtrate was transferred to a 1.5 mL Eppendorf tube, to which 500 μL of mobile phase (methanol/water, 55:45, v/v; pH 2.3) was added. The Eppendorf tube was shaken and centrifuged for 10 min at 10 000 rpm. Subsequently, 400 μL of supernatant was transferred to another Eppendorf tube, to which 500 μL of mobile phase and 100 μL

Table 2. Furan Formation in Buffered (pH 6.0) Starch-Based [10% (w/w) WC Starch] Emulsions Containing 5% (w/w) Fresh or Oxidized (14 Days at 60 °C) SBO to Which α-Tocopherol and/or β-Carotene Was Added[a]

sample composition						
	oxidative status of oil			α-tocopherol in oil (ppm)		
	pV[b] (mequiv/kg)	pAV[b]	β-carotene (mg/100 g of sample)	present	added	furan[c] (ppb)
fresh	1.88 ± 0.02 a	1.84 ± 0.05 a	—[d]	130	0	0.75 ± 0.11 a
			—	15	0	8.37 ± 0.21 b
			—	15	115	8.52 ± 0.42 b
			—	15	230	8.49 ± 0.43 b
oxidized	89.01 ± 0.58 b	12.13 ± 0.10 b	3.5	15	0	8.94 ± 0.28 b
			3.5	15	115	8.89 ± 0.42 b
			3.5	15	230	8.81 ± 0.03 b

[a] Samples were heated at 120 °C for 20 min. [b] Values (mean ± SD; $n = 3$) with different letters show statistical significance within pV or pAV. [c] Values (mean ± SD; $n = 3$) with different letters show statistical significance ($\alpha = 0.05$) within furan concentrations. [d] Not applicable.

of a 2.5 mg/mL orthophenylenediamine solution (OPD) in the mobile phase was added. External calibration was performed as described by Vandekinderen et al.[20] After 60 min of incubation in the dark, the obtained fluorophore [3-(1,2-dihydroxyethyl)furo[3,4-*b*]quinoxaline-1-one] was determined by means of HPLC with fluorescence detection using a LiChrosorb RP18 column (250 mm × 4.6 mm inner diameter × 1.4 in., 10 μm, 60 Å, Varian, CA).

Furan Analysis. Furan was determined by means of SPME coupled with gas chromatography−mass spectrometry (SPME−GC−MS) based on a method published by Bianchi et al.,[22] however applied after some modifications as described previously.[13]

Briefly, the quantitative analysis was based on an isotope dilution assay using d_4-furan. Sample preparation involved transfer of a cooled and mixed sample (approximately 1 g) to another analytically weighed 20 mL HS vial, spiking with 50 μL of d_4-furan working solution in water (approximately 70 pg/μL), sealing, mixing, and weighing.

In parallel, a calibration curve was prepared daily by injecting solutions of native furan at exactly known concentrations, ranging from 0.7 to 35 ng/mL in 20 mL HS vials, containing 50 μL of d_4-furan working solution in water (approximately 70 pg/μL).

The SPME was carried out as described by Owczarek-Fendor[13] using an automated CTC Combi-Pal system equipped with a CTC Peltier-effect cooler (Interscience, Breda, The Netherlands). Samples were exposed to a CAR-PDMS fiber (75 μm coating phase of carboxen−polydimethylsiloxane) supplied by Supelco (Bornem, Belgium) for 26 min at 4 °C. These conditions were applied after optimization and validation. The GC−MS analyses of the SPME extracts were performed with a Trace GC 2000 (Interscience) gas chromatograph coupled to the ion-trap mass spectrometer PolarisQ (Interscience) working at unit mass resolution with ionization energy of 70 eV and equipped with a Varian CP-PoraBOND Q capillary column (25 m × 0.32 mm × 5 μm). Analyses were performed in selective ion monitoring (SIM) mode, and the limit of detection (LOD) was 0.18 ng/g. Quantification was based on MS signals at m/z 68 for furan and m/z 72 for d_4-furan. The following qualifiers were used: m/z 39 for furan and m/z 42 for d_4-furan.

Statistical Analysis. The experimental data were analyzed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test at a significance level of 0.05 using the statistical analysis software SPSS, version 16.0.

RESULTS AND DISCUSSION

Similar to our previous study,[14] dealing with furan formation in lipid-containing systems, fresh and oxidized oils were used to investigate the interactions between lipids, antioxidants, and proteins and their influence on furan formation. Vegetable oils were applied because they are a source of unsaturated fatty acids known to generate furan, while saturated fatty acids do not form furan.[6]

The method for furan quantification involves a sample transferring step, which, as presented in our previous study,[13] results in a limited loss of furan. Therefore, it should be realized that furan concentrations reported are underestimations of the actual amounts of furan produced. Taking into account this disadvantage, the same repeatable and reproducible methodology was applied throughout all of our studies,[13,14] enabling a comparison of the results obtained for different furan precursors under different conditions.

At first, the furan content of fresh and oxidized (11 days at 60 °C) soybean oil and sunflower oil was determined immediately after oxidation (without heating). Furan in fresh and oxidized sunflower oil and in fresh soybean oil was below the limit of quantification (LOQ = 0.18 ppb), while in oxidized soybean oil, furan concentrations up to 5 ppb were found. Taking into account, however, the 20-fold dilution of oil in the final starch-based samples (5%, w/w) and the preparation in an open system, it can be assumed that the furan content in the final samples before heating is negligible.

In a first series of experiments, the influence of the addition of α-tocopherol on the furan formation from oxidized soybean oil was evaluated. Because the original α-tocopherol content in the oil was largely depleted by the oxidation procedure (Table 2), this antioxidant was added after oxidation to restore the original concentration in the fresh oil. In addition, a 2-fold higher amount was added. Furthermore, it should be mentioned that the oxidized soybean oil also contained γ-tocopherol (±835 ppm) and δ-tocopherol (±360 ppm) and that these levels were comparable to those present in the fresh oil. Similarly, Player et al.[23] also observed a higher stability of γ- and δ-tocopherol than α-tocopherol in soybean oil after 24 days of incubation at 50 °C and a complete degradation of α-tocopherol after 16 days of storage in the same conditions. Generally, as reported by Jung and Mean,[24] the higher the antioxidant activity of tocopherols, the lower their stability in vegetable oil.

As can be observed from Table 2, no significant effect of α-tocopherol addition on furan generation upon heating could be noticed at any of the concentration levels evaluated. The data

Table 3. Furan Formation in Buffered (pH 6.0) Starch-Based [10% (w/w) WC Starch] Emulsions Containing 5% (w/w) Fresh or Oxidized (8 Days at 60 °C) SBO to Which β-Carotene Was Added[a]

sample composition					
	oxidative status of oil		β-carotene		
	pV[b] (mequiv/kg)	pAV[b]	in oil (mg/100 g of oil)	in sample (mg/100 g of sample)	furan[c] (ppb)
fresh	1.98 ± 0.35 a	2.14 ± 0.04 a	0	0	0.94 ± 0.01 a
			70	3.5	1.18 ± 0.04 a
			140	7	1.67 ± 0.09 b
			280	14	2.39 ± 0.04 c
oxidized	77.20 ± 1.18 b	14.67 ± 0.17 b	0	0	6.68 ± 0.09 d
			70	3.5	6.97 ± 0.13 de
			140	7	7.49 ± 0.41 e
			280	14	8.20 ± 0.13 f

[a] Samples were heated at 120 °C for 20 min. [b] Values (mean ± SD; $n = 3$) with different letters show statistical significance within pV or pAV. [c] Values (mean ± SD; $n = 3$) with different letters show statistical significance ($\alpha = 0.05$) within furan concentrations.

Table 4. Furan Formation in Buffered (pH 6.0) Starch-Based [10% (w/w) WC Starch] Systems Containing β-Carotene[a]

β-carotene (mg/100 g of sample)	furan[b] (ppb)
3.5	1.45 ± 0.01 a
7	2.34 ± 0.05 b
14	4.19 ± 0.09 c

[a] Samples were heated at 120 °C for 20 min. [b] Values (mean ± SD; $n = 3$) with different letters show statistical significance ($\alpha = 0.05$).

shown in Table 2 further confirmed the results of our previous study dealing with the role of lipid oxidation on furan formation,[14] being that fresh soybean oil did not give rise to the formation of significant amounts of furan upon heating. In the proposed reaction mechanism (Figure 1), furan is produced from 2-butenal, a direct oxidation product of ω-3 unsaturated fatty acids, such as α-linolenic acid. Therefore, it can be suggested, on the basis of the data presented in Table 2, that the oxidized intermediate, 4-hydroxy-2-butenal, was already present in sufficient quantities in the oxidized oil, prior to the start of the final heating process. As a consequence, any potential radical scavenging effect of α-tocopherol during the final heating step does not result in a decreased furan formation.

In parallel, the combined effect of α-tocopherol and β-carotene addition was evaluated as well. Although β-carotene is a radical scavenging antioxidant as well,[25] no significant effects on the furan formation were observed from the combined addition of β-carotene and α-tocopherol (Table 2). At higher concentrations (and without the addition of α-tocopherol), however, β-carotene proved to enhance furan formation significantly in a concentration-dependent manner, in both fresh and oxidized soybean oil, albeit in a limited manner (Table 3). These data seem to support the previous reports suggesting that β-carotene as such is a furan precursor itself.[6] To corroborate this hypothesis, furan formation was studied in a lipid-free starch gel system, to which only β-carotene was added (Table 4). Again, furan was produced upon heating these gel systems, in which, apart from starch, β-carotene was the only potential furan source. When the data in Table 4 are compared to those in Table 3, interestingly, it could be observed that the presence of fresh lipids seems to decrease the formation of furan from β-carotene.

To further investigate the impact of β-carotene on the formation of furan upon heating, the β-carotene−soybean oil mixtures were oxidized by incubation at 60 °C, prior to emulsion preparation and heating (Table 5). Considering the data for the β-carotene-containing systems oxidized for the same time, the increase in furan formation upon an increase in the β-carotene concentration was found to be significant only in the case of mixtures incubated for 17 days. Similarly, a higher degree of oil oxidation resulted in a significantly increased furan formation, which corresponds to our previous results.[14] In general, when the data for the emulsions containing β-carotene and prepared with oxidized soybean oil (Table 3) are compared to those of the emulsions made with oil incubated with β-carotene for 13 and 17 days (Table 5), a slight increase in furan formation in the latter experiments can be observed. However, because of the difference in oxidative status of the oils, no discerning conclusion can be made. It should be stressed that, because in the latter experiment, β-carotene was co-oxidized together with the oil upon incubation at 60 °C, its content decreased in comparison to the former experiment, despite the same initial concentration in the oil. β-Carotene degradation was also visually detectable because of the less intense yellowish color of these oxidized mixtures. Taking this into account, it was expected that heating the emulsions prepared with β-carotene and oxidized together with the oil would affect furan formation, as compared to emulsions to which β-carotene was added after oxidation. However, the obtained results seem to suggest that fresh β-carotene and its intermediate oxidation products, within some level, seem to have a comparable efficiency in furan formation.

Because our previous study showed that ascorbic acid was a very potent furan precursor, especially in the presence of starch,[13] the combined effect of lipids and ascorbic acid was investigated as well (Table 6). Two types of oil were considered: soybean oil and sunflower oil. These experiments confirmed the previously observed trend,[14] namely, that soybean oil was prone to the formation of furan upon oxidation, while sunflower oil was not, even if it was highly oxidized. Previously,[14] it was explained that these results could be attributed to the fact that soybean oil, in contrast to sunflower oil, contains α-linolenic acid (Table 1). Studies performed with other α-linolenic-acid-rich vegetable oils (linseed and rapeseed oils) and sunflower oil supplemented with

Table 5. Furan Formation in Buffered (pH 6.0) Starch-Based [10% (w/w) WC Starch] Emulsions Containing 5% (w/w) SBO Oxidized in the Absence or Presence of β-Carotene at 60 °C[a]

sample composition					
	β-carotene		oxidative status of oil		
oil oxidation time (day)	initial concentration (mg/100 g of oil)	after oxidation (mg/100 g of sample)	pV[b] (mequiv/kg)	pAV[b]	furan[c] (ppb)
5	0	0	32.22 ± 0.64 a	3.83 ± 0.08 a	3.89 ± 0.51 a
	70	2.9	38.35 ± 0.83 b	3.86 ± 0.59 a	4.52 ± 0.18 a
	140	5.8	36.60 ± 0.45 b	4.27 ± 0.97 a	5.19 ± 0.82 ab
13	0	0	68.90 ± 1.08 c	9.53 ± 0.29 b	6.00 ± 0.25 b
	70	1.9	77.67 ± 1.36 d	9.92 ± 0.95 bc	7.90 ± 0.40 c
	140	4.2	69.27 ± 0.07 c	9.44 ± 1.29 bc	8.57 ± 0.55 c
17	0	0	91.30 ± 1.17 e	12.50 ± 0.73 cd	7.46 ± 0.13 c
	70	1.5	97.28 ± 0.65 f	13.08 ± 0.45 d	10.00 ± 0.49 d
	140	2.8	93.99 ± 1.91 ef	13.52 ± 0.04 d	12.06 ± 0.45 f

[a] Samples were heated at 120 °C for 20 min. [b] pV and pAV of oils oxidized in the presence of β-carotene; values (mean ± SD; $n = 3$) with different letters show statistical significance within pV or pAV. [c] Values (mean ± SD; $n = 3$) with different letters show statistical significance ($\alpha = 0.05$) within furan concentrations.

Table 6. Furan Formation in Buffered (pH 4.0) Starch-Based [10% (w/w) WC Starch] Emulsions Containing No Oil, Fresh or Oxidized (17 Days at 60 °C) SBO (5%, w/w), or Fresh or Oxidized (14 Days at 60 °C) SFO (5%, w/w) in Combination with AA[a]

sample composition				
	oxidative status of oil			
type of oil	pV[b] (mequiv/kg)	pAV[b]	AA (mg/g sample)	furan[c] (ppb)
SBO oil fresh	2.04 ± 0.05 a	1.55 ± 0.11 a	0	0.44 ± 0.01 a
			0.1	11.79 ± 0.41 f
			0.5	14.29 ± 0.67 g
SBO oxidized	119.93 ± 3.01 c	15.47 ± 0.11 d	0	8.75 ± 0.20 e
			0.1	21.11 ± 1.26 i
			0.5	30.58 ± 3.31 j
SFO fresh	4.05 ± 0.05 b	4.06 ± 0.01 b	0	0.51 ± 0.01 a
			0.1	7.23 ± 0.32 de
			0.5	8.02 ± 0.08 e
SFO oxidized	162.00 ± 3.78 d	7.10 ± 0.03 c	0	2.54 ± 0.22 b
			0.1	12.38 ± 0.55 f
			0.5	19.08 ± 1.75 h
no oil	–[d]	–	0	0.67 ± 0.10 a
			0.1	4.37 ± 0.17 c
			0.5	6.38 ± 0.17 d

[a] Samples were heated at 120 °C for 20 min. [b] Values (mean ± SD; $n = 3$) with different letters show statistical significance within pV or pAV. [c] Values (mean ± SD; $n = 3$) with different letters show statistical significance ($\alpha = 0.05$) within furan concentrations. [d] Not applicable.

methyl linolenate confirmed these findings.[14] Also, the emulsions containing vitamin C and soybean oil were more prone to generate furan upon heating as compared to those prepared with sunflower oil. Remarkably, the amounts of furan formed in the model system containing soybean oil or sunflower oil and ascorbic acid were significantly higher than the sum of the amounts of furan formed in the corresponding lipid- and ascorbic acid-free systems. When comparing the furan generation in the soybean oil-containing system without and with 0.5 mg/g of ascorbic acid, it could be hypothesized that, in the latter system, 21.9 ppb (=30.6 − 8.7) of furan was generated from ascorbic acid, supposing a constant furan generation out of the oxidized oil. This value, however, is considerably higher compared to the furan generated in the fresh soybean oil-containing system, in which 0.5 mg/g of ascorbic acid was present. Similarly, it could be hypothesized that, in the oxidized soybean oil system containing

Table 7. Vitamin C Concentrations before and after Heating of Starch-Based [10% (w/w) WC Starch] Emulsions Containing No Oil or Fresh or Oxidized (10 Days at 60 °C) SBO (5%, w/w)[a]

			vitamin C[c] (mg/g of sample)	
	pV[b] (mequiv/kg)	pAV[b]	before heating	after heating
no oil	–[d]	–	0.47 ± 0.00 d	0.37 ± 0.01 c
SBO fresh	2.99 ± 0.05 a	3.64 ± 0.00 a	0.48 ± 0.00 d	0.32 ± 0.01 b
SBO oxidized	75.87 ± 1.61 b	10.97 ± 0.05 b	0.49 ± 0.00 d	0.26 ± 0.00 a

[a] Samples were heated at 120 °C for 20 min. [b] Values (mean ± SD; $n = 3$) with different letters show statistical significance within pV or pAV. [c] Sum of AA and dehydroascorbic acid analyzed by HPLC; the initial concentration of vitamin C added to the samples was 0.5 mg of AA/g. Values (mean ± SD; $n = 3$) with different letters show statistical significance ($\alpha = 0.05$) within vitamin C concentrations. [d] Not applicable.

Table 8. Furan Formation in Buffered (pH 4.1) Starch-Based [10% (w/w) WC Starch] Emulsions Containing 5% (w/w) Fresh or Oxidized (17 Days at 60 °C) SBO or Fresh or Oxidized (12 Days at 60 °C) SFO in Combination with AA (0.5 mg of AA/g of Sample) and Proteins (5%, w/w)[a]

sample composition				
	oxidative status of oil			
type of oil	pV[b] (mequiv/kg)	pAV[b]	additives	furan[c] (ppb)
SBO fresh	2.05 ± 0.08 a	1.42 ± 0.11 a	–[d]	0.49 ± 0.01 a
			AA	10.95 ± 0.87 de
			AA + proteins	12.11 ± 0.31 e
SBO oxidized	117.59 ± 0.41 c	15.50 ± 1.51 d	–	8.95 ± 0.46 cd
			AA	25.71 ± 0.44 h
			proteins	15.24 ± 0.15 f
			AA + proteins	22.22 ± 1.54 g
SFO fresh	2.23 ± 0.06 a	3.66 ± 0.01 b	–	0.46 ± 0.01 a
			AA	10.93 ± 0.48 d
			AA + proteins	11.77 ± 0.65 de
SFO oxidized	110.04 ± 1.39 b	5.99 ± 0.06 c	–	1.47 ± 0.00 b
			AA	12.38 ± 0.11 e
			proteins	1.90 ± 0.02 b
			AA + proteins	10.44 ± 0.57 d
no oil			AA	8.10 ± 0.12 c

[a] Samples were heated at 120 °C for 20 min. [b] Values (mean ± SD; $n = 3$) with different letters show statistical significance within pV or pAV. [c] Values (mean ± SD; $n = 3$) with different letters show statistical significance ($\alpha = 0.05$) within furan concentrations. [d] Not applicable.

0.5 mg/g of ascorbic acid, 15.7 ppb (=30.6 − 14.9) of furan was generated from oxidized fat, supposing a constant furan generation out of ascorbic acid. Again, this value is considerably higher than the amount of furan in the system containing oxidized soybean oil, but no ascorbic acid was present. Thus, it can be concluded that a synergistic effect between ascorbic acid and lipids occurred. With regard to the data for the systems containing oxidized sunflower oil and ascorbic acid, furan formation was considerably enhanced in an ascorbic acid concentration-dependent manner, although ascorbic acid-free emulsions were almost not prone to furan formation upon heating. It seems that the presence of lipids, even if they are fresh and thus unlikely to contain the supposed furan precursor 4-hydroxy-2-butenal, enhances furan generation from ascorbic acid and/or vice versa. To clarify these findings, the degradation of ascorbic acid after heating at 120 °C for 20 min was studied in the absence and presence of fresh and oxidized soybean oil (Table 7). These results indicate that ascorbic acid degradation is enhanced in the presence of soybean oil, in particular oxidized soybean oil. Thus, it can be assumed that the presence of lipids increases ascorbic acid degradation and, thus, furan formation from ascorbic acid. Remarkably as well, these results contrast totally with the merely inhibitive effect of lipids on the furan formation out of β-carotene.

The obtained results (Table 6) are contrasting with those reported earlier by Märk et al.[16] These authors studied furan formation under dry roasting conditions and observed a decrease in the generation of furan by combining α-linoleic acid and

ascorbic acid, as compared to the system containing only ascorbic acid. Similarly, other binary systems of ascorbic acid with amino acids and sugars proved to be less efficient to generate furan compared to the pure ascorbic acid system. Obviously, care should be taken in comparing such studies, especially in view of the fact that, in our study, no dry roasting conditions were applied and, moreover, a more realistic and more complex starch-based model system was used.

On the other hand, studies performed by Limacher et al.[7] with food samples (pumpkin puree and carrot juice) spiked with ascorbic acid before thermal treatment led to higher furan levels, especially in the pumpkin puree. Limacher et al.[7] suggested that ascorbic acid acted as a pro-oxidant rather than a direct furan precursor in the conditions tested. However, on the basis of our results, showing that even highly oxidized sunflower oil almost did not generate furan upon sterilization (see ref 14 and Table 6) and that ascorbic acid degradation was enhanced in the presence of both fresh and oxidized soybean oil (Table 7), it can be hypothesized that lipid oxidation products enhanced furan formation from ascorbic acid. A catalytic influence by, for example, a change in the pH seems less likely because of the strong buffer used to prepare the model system. On the basis of those results, it can be concluded that the combination of lipids and vitamin C could be one of the most relevant routes of furan formation in foods, for example, in mixed baby foods (e.g., salmon−spinach based).

As a further elaboration of the latter experiments, the impact of the presence of proteins in these more complex model systems was evaluated (Table 8). Similar to our previous studies,[13,14] whey proteins were used because of their good solubility at pH 4 and also because they are typical proteins applied in baby foods. Considering the combination of lipids and proteins, experiments were only performed using oxidized oil, because it had been shown that fresh oils are not able to generate significant amounts of furan.[14] In the case of sunflower oil, no significant effect on the limited amounts of furan generated was observed upon the addition of proteins. For the soybean oil-based system, however, furan formation nearly doubled upon the addition of whey proteins. Thus, it seems that proteins enhanced furan formation from oxidized lipids containing polyunsaturated ω-3 fatty acids, such as α-linolenic acid. This observation was quite surprising because, in view of their nucleophilic character, it was supposed that proteins would react with the reactive 2-alkenals produced during lipid oxidation and, thus, partially deplete the available pool of furan precursors. Moreover, this was in contrast with our previous study,[14] showing almost no effect of proteins on furan formation from oxidized oils at pH 6. Probably, the acidic conditions applied in the present experimental conditions decreased the nucleophilic character of the protein side chains. Another explanation could be, however, a Maillard-type reaction between proteins and carbonyl compounds derived from oxidized oils,[26] possibly leading to furan, among other compounds.

However, if proteins were added to systems containing both ascorbic acid and lipids, a different trend was observed. For the fresh soybean oil- or sunflower oil-containing systems, although no significant difference was found upon the addition of proteins, a slight increase in furan formation can be observed. However, for the oxidized oil-containing system, the presence of proteins resulted in a small but significant reduction of furan formation compared to the protein-free system. Thus, our results seem to indicate that proteins can play a dual role in the generation of furan, depending upon the main furan precursor present.

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ABBREVIATIONS USED

WC, starch, waxy corn starch; SBO, soybean oil; SFO, sunflower oil; pV, peroxide value; pAV, *p*-anisidine value; AA, ascorbic acid; SPME, solid-phase microextraction.

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