JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. **2010**, *58*, 9579–9586 **9579**
DOI:10.1021/jf101671u

Importance of Fat Oxidation in Starch-Based Emulsions in the Generation of the Process Contaminant Furan

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The formation of the possibly carcinogenic process contaminant furan was studied in starch-based emulsions during heat treatments as applied for sterilization. Fresh and oxidized soybean, sunflower, high-oleic sunflower, olive, linseed, and rapeseed oils were compared. Results indicated that both the oil type, in particular, the fatty acid composition, and the oxidation degree of the oil determined the susceptibility of the oils to generate furan upon heating. Thus, oils containing the nutritionally relevant ω-3 unsaturated α-linolenic acid proved to be able to generate significant amounts of furan if the oils were oxidized. No clear relationship between *p*-anisidine values of various oils and the amount of generated furan could be observed. However, in the case of soybean oil, significantly more furan was produced upon an increase in oxidation degree. Surprisingly, furan formation in food-relevant systems containing fresh lipids proved to be a minor route (up to 1.5 ppb furan) compared to a previously studied vitamin C containing model system (up to 13 ppb furan).

KEYWORDS: Furan; simple baby food model system; starch; unsaturated fatty acids; oils; lipid oxidation; 2-butenal; α-linolenic acid; SPME-GC-MS

INTRODUCTION

Furan is a colorless, lipophilic, highly volatile (boiling point of 31 °C), aromatic heterocyclic compound with a low molecular weight of 68. Since 1995, it has been classified as "possibly carcinogenic to humans" in group 2B by the International Agency for Research on Cancer (IARC) (*1*). More comprehensive studies performed in 2004 by the U.S. Food and Drug Administration (FDA) (*2*) have identified furan in a number of foods that had been submitted to a thermal treatment in closed containers, such as cans and jars, with levels ranging from nondetectable to 174 μg/kg. Therefore, the presence of furan in food has become a serious problem, which is still under investigation (*3*−*6*).

Relatively high amounts of furan have been detected in commercial baby foods and infant formulas (*2*, *3*, *7*). The mean furan content in infant formulas was 8 μg/kg, whereas commercial jarred baby food contained an average of 25 μg/kg and a maximum of 210 μg/kg (*3*). Therefore, jarred baby foods are of particular interest as they may form an important part of the diet of many infants, known to be a very susceptible group of consumers (*3*, *8*).

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The common presence of furan in various foodstuffs suggests that there are probably multiple precursors and alternative routes of furan formation rather than a single mechanism. Indeed, literature data suggest that degradation products from carbohydrates, generated in the presence or absence of proteins, from ascorbic acid, and from lipids are all potential precursors. Thus, furan formation from unsaturated fatty acids has been linked to lipid oxidation. Lipid oxidation starts at the allylic position of the double bond of unsaturated fatty acids and results in the deterioration of the quality and acceptability of food products (*9*). The process of lipid oxidation results in the formation of a broad spectrum of compounds, in particular, small volatiles, compounds with keto, epoxy, and hydroxyl groups, and α,β-unsaturated aldehydes exhibiting toxicity and contributing to an off-flavor of the lipid (*10*−*12*). In the literature, polyunsaturated fatty acids are cited as the most important furan precursors, next to ascorbic acid. Becalski and Seaman (*13*) have shown that monounsaturated fatty acids (oleic acid, ω-9-C18:1) did not form furan, whereas in aqueous solutions of linoleic acid (ω-6-C18:2) and α-linolenic acid (ω-3-C18:3), 125 and 625 ppb of furan were generated upon heating during 118 °C for 30 min, respectively. Similar furan levels were also produced when fatty acids were present as triacylglycerols (triglycerides). Thus, the yields for

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Published on Web 08/06/2010

furan generation seemed to be largely dependent upon the degree of fatty acid unsaturation. These findings are in correspondence with those published by Perez-Locas and Yaylayan (*14*), suggesting a crucial role of 4-hydroxy-2-butenal, a typical 4-hydroxy-α,β-unsaturated aldehyde generated during the oxidation of ω-3 fatty acids such as α-linolenic acid, as a potent furan precursor.

In the literature, the mechanism of furan formation and the parameters affecting the formation of this process contaminant have been studied typically in simple model systems under various conditions of heating, such as pressure-cooking (*4*, *13*, *15*) and roasting (*4*), and even under extreme pyrolytic conditions (*14*). Furan formation has been also investigated in real food products, namely, in hazelnuts during roasting (*16*), in pumpkin puree and carrot juice under pressure-cooking conditions (*4*), and in apple and orange juices as a result of ionizing radiation and thermal treatment (*17*). Moreover, furan formation has been reported in green coffee (4 ng/g) during incubation at 40 °C for 30 min (*18*).

In a previous study (*6*), we introduced a research methodology to study furan formation in a more realistic food matrix system, albeit still a model system, consisting of a starch gel. Previously (*6*), we applied this system to elucidate the role of vitamin C as a furan precursor. In the present study, the role of fat oxidation in the generation of furan upon heating of starch-based emulsions is investigated.

MATERIALS AND METHODS

Reagents. Citric acid (monohydrate, 99.5+%) and disodium hydrogen phosphate (dihydrate, 99.5+%) were purchased from Chem-Lab NV (Zedelgem, Belgium). Furan (99+%), D_4-furan (98%), and methyl linolenate, technical (70−80%), were supplied by Sigma-Aldrich (Steinheim, Germany). Methanol (Picograde) was supplied by LGC Promochem (Molsheim, 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). Croton aldehyde (2-butenal) was supplied by Acros Organics (Geel, Belgium). Cold swelling native waxy corn starch was kindly offered by Cargill (Haubourdin, France). DATEM (diacetyl tartaric (acid) ester of monoglyceride) was kindly offered by Palsgaard (Juelsminde, Denmark). Soybean oil (Leiseur brand; 100%), sunflower oil (Vandemoortele brand; 100%), high-oleic sunflower oil (bio, Delhaize brand; 100%), refined olive oil (Bertolli, Unilever brand), rapeseed oil (Vandemoortele brand; 100%), and linseed oil (virgin, Emile Noel 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 blue/PTFE linears were supplied by Grace Davison Discovery Sciences (Lokeren, Belgium). The same vials and septa were used for heating experiments as well as for 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.* Generally, heating experiments were performed with a model food system containing 10% (w/w) of cold swelling waxy corn (WC) starch, 5% (w/w) of oil, and 1% (w/w) of emulsifier DATEM, prepared at pH 6.0 with a 0.56 M phosphate−0.44 M citric acid buffer.

Mixtures of buffer, oil (15%, w/w), and DATEM (3%, w/w) were mixed with an Ultra-Turrax T 25 (IKA, Staufen, Germany), 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 = approximately 200 bar + second stage = 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.

To have similar conditions of sample treatment as for the vitamin C containing system (*6*), typically, 15 g of emulsion/oil-free sample was transferred to a 20 mL headspace vial. The vials were sealed with crimp caps and heated for 20 min at 120 ± 0.5 °C, unless stated otherwise.

The heating experiments were carried out, as described previously by Owczarek-Fendor et al. (*6*) and Mestdagh et al. (*19*), in an oil bath (deep-fryer, Fritel 2505, Belgium), equipped with a temperature sensor (Testo 735-2) and with a stirring mechanism ensuring a homogeneous temperature distribution in the oil bath. Temperatures used throughout heating experiments refer to the temperature of the heating medium. Immediately after heating, samples were cooled to a temperature below 4 °C in an ice−water mixture for a minimum of 30 min. After cooling, the samples were mixed for a minimum of 2 min using a vortex shaker and were placed in a cold room at 4 °C.

These conditions were used throughout all experiments unless otherwise specified.

Influence of Fatty Acid Profile and Oxidative Status of the Oil on Furan Formation. The following fresh oils were used to assess the influence of oil type and fatty acid composition on furan formation: soybean oil, sunflower oil, high-oleic sunflower oil, and olive oil. In addition to fresh oils, also oxidized oils were studied. Therefore, the oils studied were oxidized by incubation of 150 g of oil in 250 mL Erlenmeyer plugged with paper tissue at 60 °C for 6, 9, and 14 days for soybean oil; 3, 6, and 11 days for sunflower oil; 3, 6, 11, and 22 days for high-oleic sunflower oil; and 3, 6, and 22 days for olive oil. Moreover, all of the oils were oxidized at the same time/temperature conditions (11 days at 60 °C). In another series of experiments, fresh rapeseed, linseed, and soybean oils (the latter from a batch different from the one used in the first series of experiments) were also oxidized at 60 °C for 11 days.

In addition, fresh sunflower oil was spiked with methyl linolenate at concentrations of 1, 5, and 10% (w/w). The spiked oils and control sample to which no methyl linolenate was added were oxidized at 60 °C for 11 days.

Fresh and oxidized oils were used to prepare starch-based samples containing oil as described under General Conditions of Sample Preparation. Samples were heated for 20 min at 120 °C and analyzed for furan.

The oxidative status of oils was defined by the *p*-anisidine value (pAV) and the peroxide value (pV). pAV was measured according to American Oils Chemists' Society (AOCS) Official Method Cd 18-90 (*20*) in which the secondary oxidation products are reacted with *p*-anisidine, resulting in the production of colored compounds assessed spectrophotometrically (at 450 nm). pV was determined according to the method of Lea and Wheeler (*21*) by titrimetric measurement of the amount of iodine formed by the reaction of peroxides with iodide ion.

The fatty acid composition of oils was determined according to AOCS Official Method Ce 1b-89 for marine oils (*22*) in which the fatty acid methyl esters were analyzed by gas chromatography (**Table 1**). First, triacylglycerols were saponified with a methanolic NaOH solution. Subsequently, the fatty acids were esterified with boron trifluoride/methanol (BF_3/MeOH) in the presence of NaOH as a derivatization catalyst. Fatty acids were identified on the basis of the GC analysis of reference standards and the retention time.

Influence of the Addition of 2-Butenal to the Oil. (*a*) *Addition of 2-Butenal to Oxidized Sunflower Oil.* Oxidized sunflower oil (incubated for 14 days at 60 °C) was spiked with 2-butenal (freshly distilled prior to application) at the following concentration levels: 100 mg/g of oil and 20, 2, and 0.2 μg/g of oil. The spiked oil samples and a control sample to which no 2-butenal was added were used for emulsion preparation and subsequent heating (20 min at 120 °C) as described above. Samples were analyzed for furan after heating.

(*b*) *Oxidation of Sunflower Oil in the Presence of 2-Butenal.* Fresh sunflower oil was spiked with 2-butenal at concentrations of 2, 0.2, and 0 mg/g of oil. Subsequently, spiked and unspiked samples were incubated at 60 °C for 11 days in closed Erlenmeyers. Afterward, the unspiked control sample was spiked with 2-butenal at concentrations of 2, 0.2, and 0 mg/g of oil. The various spiked oil samples and the oxidized oil sample to which no 2-butenal was added were used for emulsion preparation and subsequent heating (20 min at 120 °C) as described above. Samples were analyzed for furan after heating.

Table 1. Compositional Details of the Main Fatty Acids Present in the Soybean Oil (SBO), Sunflower Oil (SFO), High-Oleic Sunflower Oil (HOSFO), Olive Oil (OO), Rapeseed Oil (RSO), and Linseed Oil (LSO) (Grams per 100 g of Oil)[a]

oil	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
HOSFO	3.71	2.53	84.43	6.26	0.05
OO	12.31	2.93	70.43	10.87	0.68
RSO	4.31	1.51	54.38	18.20	9.09
LSO	4.61	3.47	19.48	16.87	47.01

[a] Mean of duplicate analyses.

Table 2. Furan Formation in Emulsions Containing 5% (w/w) of Fresh or Oxidized Oils, such as Soybean Oil (SBO), Sunflower Oil (SFO), High-Oleic Sunflower Oil (HOSFO), and Olive Oil (OO)[a]

sample (type of oil used)	time of oxidation	pV[b] (mequiv/kg)	pAV[b]	furan[c] (ppb)
oil-free system (starch + DATEM)	–[d]	–	–	1.10 ± 0.30 a
SBO	fresh	0.68 ± 0.03	1.67 ± 0.20	1.04 ± 0.19 a
	6 days	13.90 ± 0.06	1.88 ± 0.17	2.94 ± 0.25 d
	9 days	47.25 ± 0.19	3.59 ± 0.22	5.48 ± 0.72 e
	14 days	130.01 ± 1.73	12.24 ± 0.90	10.51 ± 0.55 g
SFO	fresh	3.45 ± 0.14	4.36 ± 0.03	1.13 ± 0.01 a
	3 days	21.01 ± 2.25	4.95 ± 0.60	1.95 ± 0.08 abcd
	6 days	113.06 ± 0.42	5.74 ± 0.02	2.00 ± 0.10 abcd
	11 days	161.26 ± 2.39	7.30 ± 0.42	2.77 ± 0.19 cd
HOSFO	fresh	4.96 ± 0.07	1.30 ± 0.06	1.08 ± 0.15 a
	3 days	6.78 ± 0.06	1.02 ± 0.01	1.20 ± 0.09 a
	6 days	13.75 ± 0.06	1.75 ± 0.06	1.51 ± 0.06 abc
	11 days	23.35 ± 0.02	1.67 ± 0.08	1.82 ± 0.21 abcd
	22 days	39.44 ± 0.42	1.75 ± 0.04	2.68 ± 0.03 cd
OO	fresh	4.19 ± 0.03	6.01 ± 0.01	1.48 ± 0.00 abc
	3 days	16.45 ± 0.47	5.97 ± 0.01	1.33 ± 0.15 ab
	6 days	26.00 ± 0.17	6.00 ± 0.06	2.57 ± 0.13 bcd
	22 days	49.62 ± 0.36	9.36 ± 0.06	6.86 ± 1.21 f

[a] Heating of the samples was performed at 120 °C for 20 min. [b] Peroxide value (pV) and *p*-anisidine value (pAV); values (mean $\pm$ SD, $n = 3$). [c] Values (mean $\pm$ SD, $n = 3$) with different letters show statistical significance ($\alpha = 0.05$). [d] –, not applicable.

Influence of the Presence of Whey Proteins in the Oil-Containing System. A whey protein (7.5%, w/w)–starch (15%, w/w) suspension was prepared in the previously described phosphate–citric acid buffer (*6*). This suspension was mixed in a 2:1 (w/w) ratio with a soybean oil emulsion prepared as described previously, using soybean oil that had been initially incubated for 17 days at 60 °C. The final mixture was heated (20 min at 120 °C) and analyzed for its furan content as described later.

Furan Analysis. (*a*) *Sample Preparation.* All of the handlings, such as preparations of the stock, standard, and working solutions, preparations of the samples for SPME-GC analysis and the final SPME-GC-MS analysis performed to analyze furan, were described previously by Owczarek-Fendor et al. (*6*). Briefly, approximately 1 g of cooled and mixed sample was transferred to an analytically weighed vial. Immediately, the transferred sample was spiked with 50 μL of D_4-furan working solution in water (approximately 70 pg/μL), and the vial was sealed. The spiked sample was mixed, weighed analytically, and analyzed directly by SPME-GC-MS.

In parallel, an external calibration curve was prepared daily before each analysis by injecting solutions of native furan at exactly known concentrations ranging approximately from 0.7 to 35 ng/mL in the 20 mL headspace vials spiked with a fixed volume (50 μL) of D_4-furan working solution in water (approximately 70 pg/μL).

(*b*) *SPME-GC-MS Analysis.* The method for the quantitative determination of furan used throughout the experiments was based on the method published by Bianchi et al. (*23*). However, it was applied after some modifications as described by Owczarek-Fendor et al. (*6*). In short, the samples were extracted by solid phase microextraction (SPME) followed by GC-MS analysis. The SPME was carried out using an automated CTC Combi-Pal system equipped with a CTC Peltier-Effect Cooler (Interscience, Breda, The Netherlands). The SPME fiber was a CAR-PDMS fiber (75 μm coating phase of carboxen–polydimethylsiloxane) supplied by Supelco (Bornem, Belgium). Extraction of the samples was performed at 4 °C for 26 min after optimization and validation. The GC-MS analyses of the SPME extracts were performed with a Trace GC 2000 (Interscience, Breda, The Netherlands) gas chromatograph coupled to the ion-trap mass spectrometer PolarisQ (Interscience) working at unit mass resolution with an ionization energy of 70 eV and equipped with a Varian CP-PoraBOND Q capillary column (25 m $\times$ 0.32 mm $\times$ 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

Table 3. Influence of Time/Temperature of Heating on Furan Formation in Emulsions Containing 5% of Fresh Soybean Oil (SBO) (pV = 0.68 ± 0.03 mequiv/kg, pAV = 1.67 ± 0.20)

	furan[a] (ppb)	
conditions of heating	system containing SBO	oil-free system (starch + DATEM)
120 °C, 20 min	1.27 ± 0.04 a	1.34 ± 0.02 a
120 °C, 30 min	2.77 ± 0.39 b	2.86 ± 0.03 b
130 °C, 20 min	4.35 ± 0.24 c	4.60 ± 0.19 c
130 °C, 30 min	6.89 ± 0.09 d	6.83 ± 0.12 d

[a] Values (mean $\pm$ SD, $n = 3$) with different letters show statistical significance ($\alpha = 0.05$).

for furan and at m/z 72 for D_4-furan. The following qualifiers were used: m/z 39 for furan and m/z 42 for D_4-furan.

An extensive validation of the analytical method performed in a variety of foodstuffs (data not shown) showed the suitability of the methodology irrespective of the physicochemical structure of the matrix considered.

Analysis of Volatiles from Oxidized Oils. The following oils, soybean, sunflower, high-oleic sunflower, and olive oil, oxidized for 11 days at 60 °C and used for sample preparation and furan analysis, were also submitted to SPME-GC-MS analysis to analyze volatile compounds. First, general flavor volatiles were analyzed with the method described by Jahouach-Rabai et al. (*24*). Briefly, oil samples (approximately 2 g) were placed in 20 mL headspace vials and extracted with an SPME fiber (50/30 μm divinylbenzene−carboxen−polydimethylsiloxane coating, Supelco) during 60 min at 40 °C (after 5 min of incubation) at 250 rpm and desorbed for 2 min at 250 °C in the GC injector. SPME and desorption were performed automatically by means of a multipurpose sampler (MPS-2, Gerstel). The GC-MS analysis of desorbed volatiles was performed with an Agilent 6890 GC Plus coupled to a quadrupole mass spectrometer 5973 MSD (Agilent Technologies, Diegem, Belgium) and equipped with a HP5-MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness). GC analyses were performed using the following conditions: carrier gas, He; flow rate, 1.2 mL/min; injection temperature, 250 °C; oven temperature programmed from 40 to 140 °C at 3 °C/min, then from 140 to 220 °C at 10 °C/min, and held at 220 °C; ionization mode, electron impact at 70 eV.

Table 4. Furan Formation in Emulsions Containing 5% of Oxidized Oils (11 Days at 60 °C), such as Soybean Oil (SBO), Sunflower Oil (SFO), High-Oleic Sunflower Oil (HOSFO), Olive Oil (OO), Rapeseed Oil (RSO), and Linseed Oil (LSO)[a]

type of oil	pV[d] (mequiv/kg)	pAV[d]	furan[e] (ppb)
SBO[b]	136.88 ± 0.03	23.40 ± 0.96	13.25 ± 0.15 a
OO[b]	29.52 ± 0.07	7.39 ± 0.22	3.39 ± 0.07 d
HOSFO[b]	25.75 ± 0.14	1.95 ± 0.31	2.48 ± 0.04 e
SFO[b]	153. 40 ± 1.11	9.86 ± 0.01	1.65 ± 0.05 f
SBO[c]	69.04 ± 0.36	9.31 ± 0.25	5.14 ± 0.19 c
RSO[c]	61.40 ± 0.12	17.90 ± 0.67	10.94 ± 0.44 b
LSO[c]	33.35 ± 1.09	11.32 ± 0.82	10.79 ± 0.14 b

[a] Heating of the samples was performed at 120 °C for 20 min. [b] Oils oxidized in a first series of experiments. [c] Oils oxidized in a second series of experiments; SBO was from a batch of oil different from the one used in the first series of experiments. [d] Peroxide value (pV) and *p*-anisidine value (pAV); values (mean ± SD, n = 3). [e] Values (mean ± SD, n = 3) with different letters show statistical significance (α = 0.05).

Afterward, the same oil samples were also analyzed with the same method as used for furan analysis in order to extract volatiles with a low molecular weight. However, the analysis was performed in the full-scan mode.

In general, volatiles were identified by comparison of mass spectra with mass spectral libraries (Wiley 6th and the NIST Mass Spectral Library-1998), in combination with the retention index. Identification of 2-butenal was additionally confirmed by injection of the authentic reference compound.

Statistical Analysis. The statistical tests were performed using SPSS, v. 16.0. Significance was determined by analysis of variance (ANOVA), followed by Tukey's multiple-comparison test at a significance level of 0.05.

RESULTS AND DISCUSSION

Previous studies with a vitamin C containing starch-based food model system have shown that the applied research methodology was suitable for the repeatable and reproducible generation of furan (*6*). Therefore, on the basis of this experience, lipid-containing

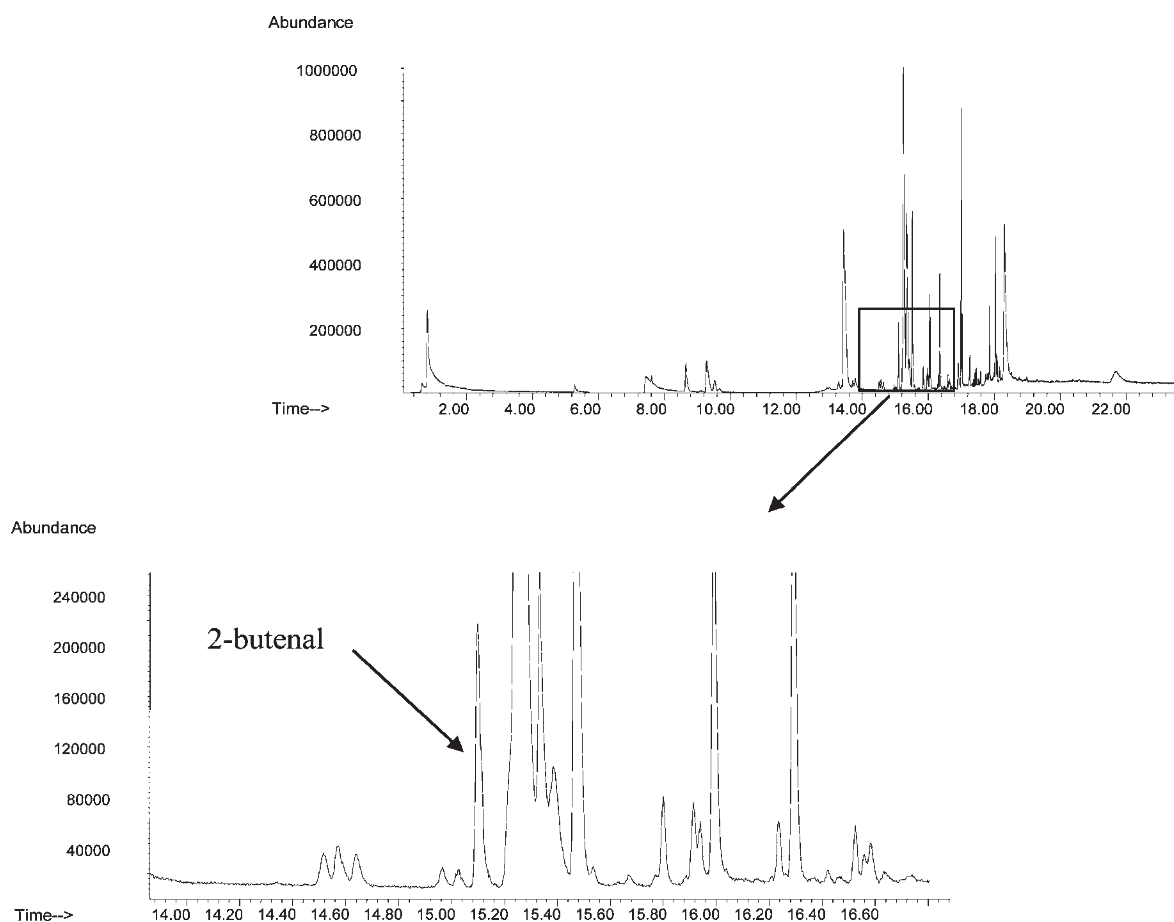

Figure 1. SPME-GC-MS full-scan chromatogram of oxidized soybean oil (pV = 130.0 mequiv/kg and pAV = 12.2) indicating the presence of 2-butenal in this oil.

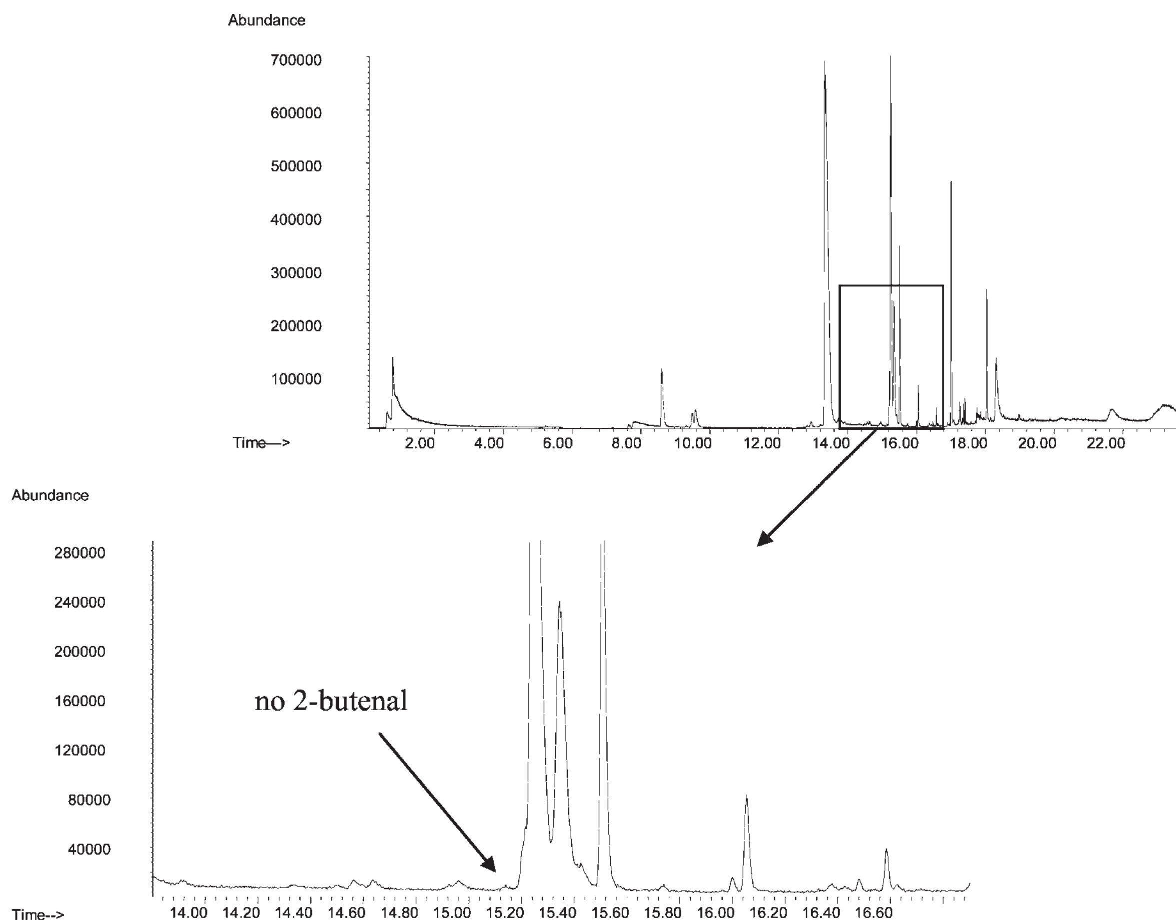

Figure 2. SPME-GC-MS full-scan chromatogram of oxidized sunflower oil (pV = 161.3 mequiv/kg and pAV = 7.3) indicating that 2-butenal was not detectable in this oil.

systems were created to investigate the role of fat oxidation in the generation of furan.

In a first series of experiments, various oils, differing in their fatty acid composition (**Table 1**) were compared for their furan generation potential during heating of starch-based emulsions containing the respective oils. From **Table 2** it can be observed that the generation of furan from fresh oil-based emulsions was very limited, irrespective of the oil used, especially compared with furan generation in the oil-free systems. The latter observation corresponds to our previously (*6*) reported findings that furan is generated in a starch-based gel system in the absence of other furan precursors, indicating furan formation from starch. This is most probably due to a limited starch hydrolysis and formation of small sugar units, which have been reported to generate furan (*25*). Using more intense heat treatments, furan generation in the samples containing soybean oils was, as expected, enhanced, but from the data shown in **Table 3**, it can be concluded that no significant differences were observed between the oil-free and oil-containing systems.

In a second series of experiments, the oils used in the initial experiments were oxidized by incubation at 60 °C for various times, yielding a broad spectrum of (highly) oxidized oils as characterized by their peroxide and *p*-anisidine values, which are indicators for the concentration of, respectively, primary (hydroperoxides) and secondary (carbonyl compounds) oxidation products (**Table 2**). Considering the data for soybean oil-based emulsions, a straightforward relationship between the oxidation status of the oil and the amounts of generated furan upon heating of the soybean oil-containing emulsions could be observed. Significantly higher amounts of furan were generated upon a progressive oil oxidation status. It should be noted, however, that the oxidation status of the least oxidized soybean oil evaluated was already quite high and is not acceptable in practice. It should be stressed as well that in our previous study, dealing with the generation of furan from vitamin C in similar experimental conditions as in the present study, considerably higher amounts of furan were generated (up to 13 ppb) (*6*), compared to the furan levels found in the systems containing fresh and even oxidized oils. Thus, the practical relevance of the contribution of oil oxidation on the generation of furan remains relatively restricted, at least in the model system used in this study.

Considering the other oils under study, it seems that especially in the case of olive oil an increased oxidation status resulted in a clearly more intense furan generation, although less furan was generated compared to the soybean oil-based emulsions. For the other oils considered, only after 11 and 22 days of oxidation a significant increase in the furan content could be observed; however, it should be stressed again that the amounts generated were much lower compared to the soybean oil and also olive oil-based systems. Remarkably, however, considering the oil oxidation parameters of both the soybean and olive oil samples, no clear relationship between the *p*-anisidine value of the respective oils and the amounts of generated furan could be observed. Soybean oil-based emulsions of which the oil had an initial *p*-anisidine value of 3.6 generated 5.5 ppb of furan upon heating, whereas a similar emulsion based on olive oil with a higher *p*-anisidine value of about 6 generated only 1.5−2.6 ppb of furan. This was somewhat surprising, because the *p*-anisidine value is considered to be related to the content of secondary oxidation products, of which some are believed to be furan precursors (*14*). Thus,

it could be concluded that the classical parameters to assess fat oxidation are poor indicators to predict the furan generation potential of various oils, although for one specific oil type, obviously, they remain relevant.

Considering the data with respect to sunflower and high-oleic sunflower oil, it became clear that these oils were nearly unable to generate furan upon heating of emulsions containing these respective oils, even if their oxidation status reached unrealistically high levels. Thus, it seemed that, apart from the oxidation status of the oil, the kind of oil determined the susceptibility of the oil to generate furan by heating of a starch-based oil emulsion. In view of the data published by Becalski and Seaman (*13*), which showed that particularly α-linolenic acid proved to be vulnerable for the generation of furan, it seems logical to conclude from the data observed in **Table 2** and the fatty acid composition of the various oils evaluated (**Table 1**) that particularly oils containing the nutritionally relevant ω-3 α-linolenic acid are more prone to the generation of furan upon heating. This is in agreement with studies with epoxidized and unepoxidized soybean and linseed oil performed by Hasnip et al. (*26*). The authors showed that the epoxidized oils contained furan and that heating epoxidized oils formed more furan in the case of linseed oil as compared to soybean oil due to the higher content of α-linolenic acid.

To allow a better comparison of the various oils with respect to their susceptibility for furan generation, a final experiment in this respect was carried out in which all oils were oxidized under similar circumstances as shown in **Table 4**, prior to emulsification and heating of the resulting starch-based emulsions. In addition, some other α-linolenic acid containing oils (linseed and rapeseed oil) were included as well (**Table 4**). If fresh linseed and rapeseed oils were applied, 2.30 ± 0.23 and 0.20 ± 0.05 ppb of furan were present in the heated emulsions respectively (n = 3). Considering the results for two oxidized soybean oil-based emulsions, it was again confirmed that a high oxidation status resulted in a high furan generation. It should be noted from this perspective that two different batches of soybean oil were used in these series of experiments. Obviously, the oxidation sensitivities of the two different soybean oils were different, giving rise to different oxidation statuses, despite the same incubation conditions, resulting as well in different furan contents of the heated emulsions. From these data, the α-linolenic acid containing oils could be identified as the most vulnerable oils evaluated. Despite the fact that the regular sunflower oil was highly oxidized as well, very limited amounts of furan were generated upon heating of the sunflower oil containing emulsion. The difference between the olive oil and the two sunflower oils proved to be less pronounced in this series of experiments compared to the data shown in **Table 2**. This is in line with the limited content of α-linolenic acid of the former oil and indicates that olive oil is particularly vulnerable for furan generation if it is highly oxidized. Again, it seems unlikely that such extreme circumstances are encountered in practice. In the case of linseed oil-based emulsions, however, a relatively low furan content was found in view of considerably higher α-linolenic acid content compared to rapeseed and soybean oils. Apart from the α-linolenic acid content, however, also the oxidation status of the oil should be considered. As can be noted, the oxidation status of the linseed oil was considerably lower compared to the status of the soybean and rapeseed oils used.

Table 5. Furan Generation in the Systems Containing Oxidized Sunflower Oil (SFO) (pV = 151.65 ± 1.95 mequiv/kg, pAV = 11.23 ± 1.35) Spiked, prior to Emulsion Preparation, with 2-Butenal, Resulting in the Indicated Concentration Levels[a]

2-butenal[b] (ppm)	furan[c] (ppb)
0	2.29 ± 0.05 a
0.01	2.32 ± 0.23 a
0.1	2.10 ± 0.13 a
1	2.22 ± 0.08 a
5000	352.5 ± 53.0 b

[a] Heating of the samples was performed at 120 °C for 20 min. [b] Concentration of 2-butenal in final emulsions. [c] Values (mean ± SD, n = 3) with different letters show statistical significance (α = 0.05).

As shown in **Tables 2** and **3**, both fatty acid composition and oxidation status of the oil seemed to determine the susceptibility of an oil to generate furan upon heating. As the fatty acid composition of the oil not only to some extent determines the oxidation stability of the oil but also influences the types of oxidation products generated, the oils used in this study (soybean, sunflower, high-oleic sunflower, and olive oil) were analyzed using SPME-GC-MS to compare the composition of volatile oxidation products. A first analysis enabled extraction of a broad range of volatiles; however, no specific volatiles could be detected that could be related to furan formation. Nevertheless, the SPME analysis of low molecular weight volatiles resulted in an interesting finding, being the presence of 2-butenal solely in the oxidized soybean and olive oil (among the oils tested), the oils that generated significant amounts of furan. 2-Butenal, a typical oxidation product of α-linolenic acid, has already been identified in thermally oxidized rapeseed oil (*27*) and soybean oil (*28*), in olive oil as a result of UV radiation (*29*), and in linseed oil (*30*). Two examples of chromatograms obtained for oxidized soybean and sunflower oil are presented in **Figures 1** and **2**, respectively. The identity of the 2-butenal peak was based on the mass spectrometric data and on the injection of a pure 2-butenal standard. The content of 2-butenal found in soybean oil was higher than in olive oil, which was similar to the tendency in furan formation. As described by Perez-Locas and Yaylayan (*14*), 2-butenal has been suggested as an intermediate in furan formation via ω-3 fatty acid oxidation. Therefore, the presence of this alkenal in the oil can be an important marker suggesting possible furan formation.

On the basis of these findings, additional experiments with emulsions containing oils spiked with 2-butenal were performed to evaluate if the addition of 2-butenal would enhance furan formation. For this purpose, sunflower oil was chosen, because it was shown in the first part of this study that this oil was unable to generate appreciable amounts of furan even if highly oxidized,

Table 6. Furan Generation in Systems Containing Fresh and Oxidized Sunflower Oil (SFO) Spiked prior to Emulsion Preparation with 2-Butenal and Containing SFO Oxidized in the Presence of 2-Butenal[a]

emulsion with SFO (type of oil)	pV[b] (mequiv/kg)	pAV[b]	furan[c] (ppb)		
			0 ppm[d]	10 ppm[d]	100 ppm[d]
2-butenal added to the fresh oil	2.25 ± 0.05	3.60 ± 0.01	0.89 ± 0.03 a	1.96 ± 0.08 b	8.06 ± 0.31 cd
2-butenal added to oxidized oil (11 days)	110.04 ± 1.40	5.99 ± 0.07	1.94 ± 0.03 b	1.76 ± 0.07 b	8.98 ± 0.55 d
oil oxidized in the presence of 2-butenal (11 days)	–[e]	–[e]	–[f]	7.63 ± 0.05 c	85.68 ± 0.77 e

[a] Heating of the samples was performed at 120 °C for 20 min. [b] Peroxide value (pV) and *p*-anisidine value (pAV); values (mean ± SD, n = 3). [c] Values (mean ± SD, n = 3) with different letters show statistical significance (α = 0.05). [d] Concentration of 2-butenal in final samples. [e] Not analyzed. [f] –, not applicable.

Table 7. Furan Generation in Systems Containing Fresh and Oxidized Sunflower Oil (SFO) (5%, w/w) and SFO Spiked with Methyl Linolenate prior to Oil Oxidation (11 Days at 60 °C)[a]

type of SFO	pV[b] (mequiv/kg)	pAV[b]	furan[c] (ppb)
fresh	4.01 ± 0.00	3.75 ± 0.11	0.06 ± 0.04 a
oxidized	97.55 ± 0.81	9.53 ± 0.06	1.61 ± 0.12 b
+ 1% methyl linolenate, oxidized	103.33 ± 0.80	16.82 ± 0.13	6.17 ± 0.15 c
+ 5% methyl linolenate, oxidized	110.74 ± 0.01	31.25 ± 0.02	17.00 ± 0.44 d
+ 10% methyl linolenate, oxidized	112.38 ± 0.85	51.60 ± 0.31	35.01 ± 0.93 e

[a] Heating of the samples was performed at 120 °C for 20 min. [b] Peroxide value (pV) and *p*-anisidine value (pAV); values (mean ± SD, $n = 3$). [c] Values (mean ± SD, $n = 3$) with different letters show statistical significance ($\alpha = 0.05$).

Table 8. Furan Generation in Systems Containing Fresh and Oxidized Soybean Oils (SBO) (5%, w/w) in the Absence and Presence of 5% of Whey Proteins[a]

emulsion with SBO (type of oil)	pV[b] (mequiv/kg)	pAV[b]	furan[c] (ppb) no proteins	furan[c] (ppb) 5% whey proteins
fresh	1.97 ± 0.03	1.37 ± 0.05	0.88 ± 0.04 a	1.95 ± 0.07 b
5 days oxidized	27.75 ± 0.22	1.93 ± 0.05	3.27 ± 0.23 c	3.04 ± 0.10 c
7 days oxidized	41.57 ± 0.87	4.76 ± 0.20	4.27 ± 0.32 d	3.66 ± 0.22 cd
17 days oxidized	117.32 ± 0.78	13.41 ± 0.11	10.67 ± 0.23 f	7.58 ± 0.44 e
oil-free system	–[d]	–	0.77 ± 0.10 a	1.28 ± 0.04 ab

[a] Heating was performed at 120 °C for 20 min. [b] Peroxide value (pV) and *p*-anisidine value (pAV); values (mean ± SD, $n = 3$). [c] Values (mean ± SD, $n = 3$) with different letters show statistical significance ($\alpha = 0.05$). [d] –, not applicable.

and, moreover, it did not contain detectable amounts of 2-butenal (**Figure 2**). Surprisingly, addition of this 2-alkenal in highly oxidized sunflower oil at concentrations ranging from 0.01 to 1 ppm did not seem to have an impact on the generation of furan (**Table 5**). Only an unrealistically high concentration of 0.5% of 2-butenal in the oil gave rise to the generation of significant levels of furan in the experimental conditions used. In another series of experiments, 2-butenal was added at up to 100 ppm to both fresh and oxidized sunflower oil (**Table 6**). Surprisingly, the amounts of furan generated in the systems containing oils spiked with 2-butenal did not depend on the oxidation status of sunflower oil. Nevertheless, significant amounts of furan were generated if the oils were spiked at an unrealistically high 100 ppm 2-butenal level. In view of these results, it was decided to conduct a series of experiments in which the 2-alkenal was added to fresh sunflower oil that was subsequently submitted to a forced oxidation by incubation of the oil at 60 °C for 14 days. The spiked and oxidized oil was then emulsified, and the starch-based emulsions were subsequently heated. In these series of experiments (**Table 6**) it was shown that much higher amounts of furan were generated as a function of the 2-butenal level, compared to the experiments in which 2-butenal was added to fresh or oxidized oil immediately before emulsion preparation and heating. Moreover, it should be noted that the amounts of furan generated in the presence of a more realistic level of 10 ppm of 2-butenal in the oil were quite comparable with the amounts previously generated in the emulsions based on oxidized soybean oil (**Table 2**). These results clearly show that 2-butenal is not a direct precursor of furan, but necessitates further oxidation, possibly to 4-hydroxy-2-butenal, which as suggested by Perez-Locas and Yaylayan (*14*) is a direct furan precursor. In view of the fact that 2-butenal is a typical oxidation product of all ω-3 unsaturated fatty acids, it can be concluded that foods containing such oils are probably all prone to the generation of furan, especially if no sufficient care is taken with respect to the oxidation status of the lipids. To further corroborate the link between the presence of α-linolenic acid and furan generation, a supplementary experiment was performed in which methyl α-linolenate was added at three concentration levels to sunflower oil, which previously was shown to be a poor furan precursor (**Table 2**). The resulting mixtures were oxidized (11 days at 60 °C) and emulsified as in the previous experiments. The results presented in **Table 7** indicate a perfectly linear ($R^2 = 0.996$) correlation between the furan content and the α-linolenic acid content. These results clearly illustrate the impact of lipid oxidation combined with the presence of α-linolenic acid and, although not experimentally evaluated, with probably other ω-3 polyunsaturated fatty acids as well, on the generation of furan.

In a final series of experiments, the additional presence of whey proteins in the starch-based soybean oil emulsions was evaluated. Apart from lipids, proteins are indeed also major ingredients of, for example, prepared baby foods. The results are summarized in **Table 8**. Although very limited amounts of furan were generated when fresh soybean oil was used for the emulsion preparation, the presence of whey proteins seemed to enhance in a significant way furan formation. This result contrasted with a neutral effect of the proteins when moderately oxidized oils were used. Only in the case when a severely oxidized soybean oil was used for emulsion preparation did proteins inhibit the furan formation during heating of the emulsion. In view of the clear indications that 2-butenal is a key intermediate in the generation of furan from lipids, this inhibitive effect of proteins is no surprise because of the highly reactive nature of these α,β-unsaturated aldehydes toward nucleophilic substances such as proteins. Again, however, it should be noted as well that, on the basis of the findings in the experimental conditions used, it is most likely that, in practice, the impact of the presence of proteins on the generation of furan from lipids will be rather limited, if relevant at all.

In conclusion, it was shown in this study that the role of lipid oxidation in the generation of furan in starch-based emulsions was quite restricted, especially if the results in this paper are compared with those presented in our previous study dealing with vitamin C as the sole dominant furan precursor present (*6*). Only if the oils reach an unrealistically high level of oxidation, relevant levels of furan seem to be generated. In addition, it was shown that the fatty acid composition of the oil seemed to play a crucial role as well, which could be attributed to the formation of the indirect furan intermediate 2-butenal out of the oxidation of ω-3 polyunsaturated fatty acids such as α-linolenic acid. Obviously, food systems are much more complicated than the starch-based model system used in this study. Especially the joint presence of two potent furan precursors such as vitamin C and oxidizing lipids should be considered because of its practical

relevance. The impact of the joint presence of oxidizing lipids together with antioxidants such as vitamin C will be presented elsewhere.

ABBREVIATIONS USED

SBO, soybean oil; SFO, sunflower oil; HOSFO, high-oleic sunflower oil; OO, olive oil; RSO, rapeseed oil; LSO, linseed oil; pV, peroxide value; pAV, *p*-anisidine value; SPME, solid phase microextraction.

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Received for review January 21, 2010. Revised manuscript received July 12, 2010. Accepted July 12, 2010. This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (Contract RT 06/1, FURA). We are indebted to the Research Foundation Flanders (FWO-Vlaanderen) for an Aspirant fellowship to F.V.L. and for a postdoctoral fellowship to A.A.