Effect of oxygen availability and pH on the furan concentration formed during thermal preservation of plant-based foods

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Thermally treated fruit- and vegetable-based foods are important contributors to the furan exposure of children and adults. Furan reduction by adding or removing precursors from the product has proven to be challenging, because of major food constituents and interactions involved in the reaction pathways leading to furan formation. Instead of intervening at the precursor level, it might be more feasible to influence these formation pathways by adjusting the matrix properties of the product. As opposed to many previous literature sources, the present study investigated the effects of oxygen availability (normal vs. reduced) and pH (acid vs. low-acid) on the furan formation in a real food system. Different combinations of both matrix properties were prepared in a reconstituted potato purée and subjected to a thermal treatment with a pasteurization or sterilization intensity. Irrespective of addition of the furan precursors ascorbic acid, fructose and fatty acids, a considerable furan reduction was observed for the sterilized purées (F_{121}^{10} = 15 min) with either a reduced oxygen availability (0.1-1.8 mg/L) or at pH 3. The effects of both matrix properties were less pronounced in the pasteurized purées (P_{90}^{10} = 10 min), because of the lower furan concentrations. Even though the mechanisms of furan reduction for both types of matrix properties could not be fully elucidated, the results showed that lowering the oxygen concentration or the pH prior to thermal processing offers a powerful, additional strategy for furan mitigation in thermally treated plant-based foods.

Keywords: furan; precursor; oxygen; pH; thermal pasteurization; thermal sterilization.

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Introduction

Furan (C_4H_4O) is a small organic molecule with high volatility. In 1995, furan was classified as ‘possibly carcinogenic’ to humans after it was proven to be carcinogenic in rats and mice (International Agency for Research on Cancer (IARC) 1995). As a reaction, North American (US Food and Drug Administration (FDA) 2009) and European (European Food Safety Authority (EFSA) 2011) food safety authorities have analyzed a wide range of commercial foods for furan. The highest concentrations are found in coffee products and canned or jarred foods. Shelf-stable fruit- and vegetable-based products (e.g. jarred baby foods, ready-to-eat soups, sauces and juices) are important contributors to the furan exposure of children and adults. Particular concerns exist about the furan exposure of babies and toddlers, because of the sensitivity of these population groups in combination with a high food intake per kg body weight (Lachenmeier et al. 2009; Owczarek-Fendor et al. 2010a). In anticipation of more information on the toxicological effects of furan for humans, actions should be taken to keep the furan levels in all these products as low as can reasonably be achieved by following good practices at all stages of food production and distribution (Council of the European Communities 2009; Joint FAO/WHO Codex Committee on Contaminants in Foods 2011). Modification of the processing conditions for thermal preservation (Fan et al. 2008; Owczarek-Fendor et al. 2010a; Huang et al. 2011; Sevenich et al. 2014; Palmers et al. 2014; Palmers et al. 2015b) or storage (Palmers et al. 2015a; Palmers et al. 2015c) represents an effective way for furan reduction in fruit- and vegetable-based products. However, a considerable furan reduction can sometimes be hampered by microbial safety standards to comply with and/or economic reasons. For such products, mitigation strategies aiming at an intervention in the reaction pathways for furan formation (e.g. by adding or removing substances from the product) might offer an additional approach to reduce the furan concentration without adverse effects on general food safety or quality attributes. In the literature, many possible reaction pathways leading to the formation of furan have been reported, the major precursors being sugars (alone or in combination with amino acids), ascorbic acid and unsaturated fatty acids, followed by amino acids and carotenoids (Locas & Yaylayan 2004; Becalski & Seaman 2005; Fan 2005; Mark et al. 2006; Limacher et al. 2007; Limacher et al. 2008; Owczarek-Fendor et al. 2010a; Owczarek-Fendor et al. 2011; Van Lancker et al. 2011; Huang et al. 2011; Owczarek-Fendor et al. 2012). It is clear that most of these precursors are major constituents of fruit- and vegetable-based foods, thus contributing to appreciated quality attributes such as the nutritional value, rheology, appearance, etc. Instead of intervening at the precursor level, it is therefore more desirable from an application point of view, to influence these pathways by adjusting matrix properties such as the redox condition and the pH of the product.

In the literature, the effect of the redox condition has often been investigated by adding oxidizing or reducing agents (e.g. ferric ions, butylated hydroxytoluene, sulfite, etc.) to model solutions of various precursors (Becalski & Seaman 2005; Mark et al. 2006; Owczarek-Fendor et al. 2010a; Owczarek-Fendor et al. 2011; Mogol & Gokmen 2013), however, with variable results depending on the applied combination of the precursor and redox agentia. Surprisingly, the effect of oxygen on the furan formation has rarely been investigated, even though this approach seems to be one of the most obvious and feasible ways to control the redox condition of the food matrix (Johnson & Decker 2015; Larsen et al. 2015). The effect of pH on the furan formation has been described in the context of ascorbic acid (Fan 2005; Limacher et al. 2007; Fan et al. 2008; Owczarek-Fendor et al. 2010a; Nie et al. 2013a) and sugar degradation (Fan 2005; Limacher et al. 2008; Huang et al. 2011; Owczarek-Fendor et al. 2012; Nie et al. 2013a; Nie et al. 2013b). Like for the redox effect, the pH has variable effects, depending on the precursor. Again, most of the studies are performed with model systems, using phosphate solutions as a buffer for tempering the pH shift during the thermal treatment. However, phosphate ions have recently been shown to enhance furan formation themselves (Fan et al. 2008; Huang et al. 2011). In real food systems, the complexity is further increased, since the pH can have an indirect effect on the furan formation through interactions with proteins, metal ions or even the structure of the matrix. It would therefore be interesting to evaluate the effects of oxygen and pH on the furan formation in real food systems. In this work, potato was selected based on food composition tables (Rijksinstituut voor Volksgezondheid en Milieu 2013), as a vegetable matrix which is naturally low in furan precursors. In addition, potato is a commonly used ingredient of many vegetable-based foods (e.g. jarred baby foods, soups, sauces). By spiking a potato purée with fixed amounts of selected furan precursors (with and without addition of ascorbic acid, fructose or fatty acids) and adjusting the oxygen availability (normal vs. reduced) and the pH (acid vs. low-acid) to targeted levels, a range of well-defined research matrices was obtained, as a compromise between pure model and real systems. The purée formulations were subjected to a thermal treatment with a pasteurization or sterilization intensity and analyzed for furan, to obtain an insight into the effects of precursor composition, pH, oxygen availability and thermal intensity on the furan formation of fruit- and vegetable-based foods. A schematic overview of the experimental approach of the current study is presented in **Figure 1**.

Material and methods

Preparation of the reference and the spiked potato purées

Potatoes (*Solanum tuberosum* 'Challenger') were bought at a local supplier and stored in a cold room at 4 °C until further handling. They were first peeled and then cut into slices of approximately 1 cm thickness, before vacuum-packing in low-density polyethylene bags. To assure that all the changes observed during thermal processing were chemical, the potato slices were blanched at 95 °C for 8 min in a water bath (WBU 45, Memmert, Schwabach, Germany). The blanching conditions were validated using a qualitative and quantitative peroxidase test (Adebooye et al. 2008; Vervoort et al. 2012). After blanching, the plastic bags were immediately cooled in iced water for 10 min, frozen in liquid nitrogen and stored in a freezer at -40 °C. Prior to purée preparation, the potato slices were thawed overnight in a cold room at 4°C. The slices were blended (B-400, BÜCHI, Flawil, Switzerland) with a standardized amount of deionized water to obtain a homogeneous potato purée without addition of furan precursors (further addressed to as 'reference'). The composition of this reference purée was characterized in terms of free sugars, vitamin C and fatty acids (all possible furan precursors) by certified analytical procedures of Eurofins Food Testing Belgium (Brugge, Belgium). The concentration of free sugars (<0.1 g/100 g purée) was found to be below the quantification limit. Also the vitamin C (3.7 mg/100 g purée) and fatty acids (0.5 g/100 g purée, consisting of 7.1% monounsaturated fatty acids, 50.2% polyunsaturated fatty acids and 42.7% saturated fatty acids) concentrations were relatively low, especially when compared with other types of fruit- and vegetable-based foods (Rijksinstituut voor Volksgezondheid en Milieu 2013). To assess whether or not the effects of pH and oxygen availability on furan formation are dependent on the type of precursors in the systems, three additional purée formulations were prepared by spiking the blanched potato slices with fructose (5 g/100 g purée), ascorbic acid (150 mg/100 g purée) or olive oil (3 g/100 g purée). The concentration levels of these precursors were selected as high, but realistic levels for these groups of possible furan precursors in fruit- or vegetable-based products (Rijksinstituut voor Volksgezondheid en Milieu 2013). The different purée formulations were prepared by mixing the blanched potato slices with fixed amounts of precursor solutions. For this, stock solutions of fructose (1 g/ml) (≥99%, AppliChem, Darmstadt, Germany) and ascorbic acid (25 mg/ml) (99%, Acros Organics, Geel, Belgium) were prepared in deionized water, while olive oil (consisting of 70.6% monounsaturated fatty acids, 11.3% polyunsaturated fatty acids and 18.1% saturated fatty acids) (Vandemoortele, Izegem, Belgium) was added as such. Analogous to the reference purée, the mixtures were further diluted with deionized water to obtain a standardized total volume and blended (B-400, BÜCHI, Flawil, Switzerland) to obtain homogeneous purées, ready for pH adjustment or modifying the oxygen availability. In total, four different purée formulations regarding furan precursors were obtained; (i) without additions (reference), (ii) spiked with fructose, (iii) spiked with ascorbic acid and (iv) spiked with olive oil. All potato purées were prepared in duplicate.

Adjusting the pH and oxygen availability of the spiked potato purées

After the purée preparation, the pH and oxygen availability of the different purée formulations were adjusted to the desired levels. The initial pH values of the potato purées ranged between 5.37 (spiked with ascorbic acid) and 6.10 (spiked with fructose), depending on the purée composition. The pH levels of the purée formulations were adjusted to pH 3 and 7 with 1 M HCl (6.3-6.9 ml/100 g purée) or NaOH (1.5-2.3 ml/100 g purée). Opposite to the pH, it is rather difficult to control the oxygen concentration at a controlled, standardized level. Therefore, the purée formulations were manipulated in such a way that oxygen was removed as much as possible ('reduced oxygen availability') or on the contrary, completely allowed ('normal oxygen availability'). For the purées with reduced oxygen availability, the air was removed with a rotary vane vacuum pump (Type RE 2, Vacuubrand, Werdheim, Germany). Next, the sample holders were transferred into a closed environment (Pyramid portable glove bag, Erlab, Val-de-Reuil, France), which was filled with nitrogen to avoid reintroduction of air. In this oxygen-free environment, the samples were flushed with nitrogen for 10 min to continue the removal of dissolved oxygen from the systems. For the purées with normal oxygen availability, no specific measures were taken to avoid oxygen from entering the systems. On the contrary, the purées were intensively stirred for 10 min under normal atmospheric conditions. Due to their inert nature, glass jars (100 ml volume, 95 mm height and 45 mm diameter) were used as sample holders for the thermal treatments. Depending on the targeted oxygen availability, the jars were filled with 85 ± 0.5 g of purée under nitrogen atmosphere or normal atmospheric conditions and then closed with metal lids. The efficiency of the procedures for adjusting the oxygen availability inside the potato purées was validated by non-invasive, optical oxygen measurements (O2xyDot, OxySense, Dallas, Texas) in the purée varieties of the reference, and will be discussed in more detail in the Results and discussion section. In total, four possible combinations of pH and oxygen conditions were obtained for each purée formulation.

Thermal treatments of the potato purées

The purée formulations were subjected to a thermal treatment for obtaining shelf-stable products, with a thermal intensity depending on the pH of the systems. The potato purées at pH 3 were subjected to a severe pasteurization treatment with a process value of P_{90}^{10} = 10 min, the purées at pH 7 were sterilized with a process value of F_{121}^{10} = 15 min. The targeted process values for both treatments were indeed much higher than the theoretical values for microbial safety of the respective types of products, but to avoid product recall and to account for non-uniform impact distributions, process values of this order are frequently applied in food industry. In addition to the pasteurization treatment, the potato purées at pH 3 were also sterilized to investigate the effect of pH on the furan formation. The thermal treatments were performed in a static Steriflow pilot retort (Barriquand, Roanne, France). The glass jars filled with potato purée were loaded into the retort, which was heated (come-up time of 8-10 min) at the processing temperature of 90 (pasteurization) or 121 °C (sterilization). The holding time (±25 min at 90 °C or ±32 min at 121 °C) was calculated in advance to obtain the desired process value in the coldest point of the product. The temperature profiles in the retort and in the coldest point of the product were recorded using type T thermocouples (Ellab, Hilleroed, Denmark) (not shown). After the thermal treatment, the glass jars were immediately transferred to iced water to slow down further chemical reactions. The thermally treated potato purées were emptied in a cold room at 4 °C, frozen in liquid nitrogen and stored in a freezer at -80 °C until analysis.

Quantitation of furan

Quantitation of furan was performed via an isotope dilution assay as described by Palmers et al. (2014), using furan-d_4 as an internal standard. For sample preparation, 2.5 g of the thermally treated purées was weighed into a 10 ml headspace vial with a PTFE/silicone septum seal. The purée was diluted with 2.5 ml of a saturated NaCl solution, 100 µl of furan-d_4 (98%, Sigma-Aldrich, Saint Louis, Missouri) working solution (ca. 0.05 µg/ml in deionized water) and deionized water to obtain a standardized total volume of 6 ml. Furan was extracted by solid phase microextraction (SPME), using a 75 µm carboxen/polydimethylsiloxane fiber (Supelco, Bellefonte, Pennsylvania) which was exposed to the headspace of the samples at 30 °C for 15 min. The analyses were carried out using an Agilent 7890A gas chromatograph and an Agilent 5975C mass spectrometer (Keysight Technologies, Santa Rosa, California), equipped with a HP-PLOT Q column (30 m × 320 µm, 20 µm film thickness, Keysight Technologies, Santa Rosa, California) and using helium as the carrier gas at a constant flow rate of 2 ml/min. Mass spectra were obtained by electron ionization (EI) at 70 eV, in the combined SCAN and SIM mode. The selected ions monitored were *m/z* 68 (quantifier) and 39 (qualifier) for furan and *m/z* 72 (quantifier), 44 and 42 (both qualifier) for furan-d_4. Each sample was analyzed in triplicate. For quantitation, a calibration curve of furan (>99%, Sigma-Aldrich, Saint Louis, Missouri) was prepared in the reference purée without additions, covering the concentration range of 0-50 ng/g purée. The decision limit and the detection capability of the procedure (in accordance with the definitions of European Commission Decision 2002/657/EC) were 1.15 ng/g purée and 1.86 ng/g purée, respectively. Both parameters were established by the calibration curve procedure.

Statistical data-analysis

The statistical data analysis was performed with the SAS statistical software package (SAS Enterprise Guide 4.3, Cary, North Carolina). A mixed model was applied, with fixed factors to estimate the effects of the controllable factors (composition, pH level, oxygen availability, thermal intensity) on the furan concentration of the thermally treated potato purées, and random factors to correct for the correlation between samples that were prepared, thermally treated or analyzed together. Significant differences among the fixed factors were examined using the post-hoc Tukey test at a significance level of 0.05.

Results and discussion

The effects of oxygen availability and pH on the furan concentration of fruit- and vegetable-based foods were investigated in a potato purée without additions (reference) and in potato purées with the addition of the possible furan precursors fructose, ascorbic acid and fatty acids. For each purée formulation, four different combinations of the selected matrix properties were prepared and subjected to a thermal treatment with a pasteurization or sterilization intensity. An overview of the experimental approach of this study is presented in **Figure 1**.

Relative importance of precursors and reaction conditions for furan formation

As can be seen from **Figure 1**, the experimental approach of the present study resulted in a large number of purée formulations (in total 24 combinations consisting of different conditions of precursors, oxygen availability, pH and thermal intensity) to be prepared in duplicate, thermally treated and analyzed for furan. In this section, the relative importance of the selected factors for furan formation in the thermally treated potato purées is investigated. The purée formulations which are illustrative for the effects of oxygen availability and pH, will be discussed in more detail in the following sections. Overall, the furan concentrations of the thermally treated purées varied from not detectable (ND) to 203 ng/g purée. However, the majority of the potato purées had a furan concentration in the lower range, which is indicated by the median and mean concentrations of 18 and 29 ng/g purée, respectively. Even though a comparison with commercially available fruit- and vegetable-based foods is hampered by the variety in matrix properties and the different intensities of the thermal treatment, the furan concentrations of the present study were very similar to results of the reports by the EFSA (e.g. jarred baby foods, with median and mean furan concentrations of 24 and 31-32 ng/g purée, and a maximum value of 233 ng/g purée) (European Food Safety Authority (EFSA) 2011) and by the FDA (only results of individual food products reported) (US Food and Drug Administration (FDA) 2009). For the statistical analysis of the present results, a mixed model regression approach was applied. Given the large number of purée formulations, the potato purées were divided into different groups for the sample preparation (purée preparation and thermal treatment) and furan analysis. The experimental factors (precursor composition, oxygen availability, pH and thermal intensity) are included in the model as fixed main effects, with random effects to capture the possible correlation between the samples that were prepared, thermally treated and/or analyzed together. Interaction effects are omitted from the model, to avoid overfitting of the data and because there is no mechanistic base for most of these effects. The mixed effects model is presented in equation (1):

$$Y_{klm} = \mu + \sum_{i=1}^{4} \beta_{1i} x_{1i} + \sum_{i=1}^{2} \beta_{2i} x_{2i} + \sum_{i=1}^{2} \beta_{3i} x_{3i} + \sum_{i=1}^{2} \beta_{4i} x_{4i} + a_k + b_l + c_m + \varepsilon \qquad (1)$$

where Y_{klm} is the observed furan concentration at the m^{th} repeated observation (m = 1,2,3) from the k^{th} preparation group (k = 1,2,3,4) and the l^{th} analysis group (l = 1,2,…,12). μ represents the intercept, β_{1i}, β_{2i}, β_{3i} and β_{4i} are the regression coefficients of the main effects and x_{1i}, x_{2i}, x_{3i} and x_{4i} are the i^{th} levels of the precursor composition, oxygen availability, pH and thermal intensity, respectively. a_k, b_l and c_m are the random effects of the k^{th} preparation group, the l^{th} analysis group and the m^{th} repetition of furan analysis. The mixed effects model was estimated using generalized least squares for the effects of the experimental factors and restricted maximum likelihood (REML) for the variances of the random effects. The estimates of the regression coefficients are presented in **Table 1**.

Whether or not the selected furan precursors (i.e., fructose, ascorbic acid or fatty acids) were added to the potato purée, had no significant effect on the furan concentration after thermal treatment (individual estimates for β_1 and type III test, results not shown). However, the mixed effects model showed highly significant effects ($p < 0.0001$) for the oxygen availability, pH and thermal intensity. The potato purées with a normal oxygen availability had a clearly higher furan concentration than the purées with a reduced oxygen availability (positive estimate for β_2). Similarly, the potato purées at pH 3 had a significantly lower furan concentration than the purées at pH 7 (negative estimate for β_3), and the pasteurized potato purées had a significantly lower concentration than the sterilized purées (negative estimate for β_4). As mentioned above, the effects of the oxygen availability and pH will be discussed in more detail in the following sections. Nevertheless, it should be noted that the effects of the matrix properties oxygen availability and pH were of a similar importance as the effect of the thermal intensity. The impact of the thermal processing conditions on the extent of furan formation is well-established in the literature. High furan concentrations are also found in food products that are subjected to an intensive thermal treatment for sterilization or roasting purposes (US Food and Drug Administration (FDA) 2009; European Food Safety Authority (EFSA) 2011). However, the potential for decreasing the thermal impact by adjusting the thermal processing conditions, is often limited by microbial safety standards to comply with and/or technological requirements (e.g. the heating rates delivered by the equipment), especially in the case of thermal sterilization of low-acid, conduction-heated foods like vegetable-based products. The results of the present study seemed to demonstrate that adjusting the selected matrix properties prior to the thermal preservation step can be an interesting, additional strategy for furan mitigation in fruit- and vegetable-based foods, next to optimizing the processing conditions themselves. The degradation of the selected furan precursors is strongly influenced by matrix properties such as the oxygen availability and pH. Since the experimental setup of this study was specifically designed for obtaining insight into the effects of both matrix properties on the furan formation, it was not surprising that these effects dominated the effect of adding precursors. To elucidate the specific role of the furan precursors (which can depend on the concentration, conversion efficiency and possible interactions), a specific experimental setup is needed, which was outside the scope of the present study.

Effect of oxygen availability on furan formation in the thermally treated potato purées

To investigate the effect of oxygen on the furan formation in fruit- and vegetable-based food products, two types of purée formulations were included in this study (cf. **Figure 1**). The first type ('normal oxygen availability') was prepared under normal atmospheric conditions, in order to obtain a realistic degree of oxidative degradation reactions during thermal processing. The second type of purées ('reduced oxygen availability') was subjected to vacuum conditions and flushed with nitrogen, to remove the dissolved oxygen as much as possible before the pasteurization or the sterilization treatment. The effectiveness of both procedures for setting the oxygen availability, was checked by measuring the oxygen concentration in the different formulations of the reference purées (i.e., the six combinations of oxygen availability, pH and intensity of the thermal treatment). The results of these measurements showed a clear difference between the oxygen concentrations of the purées with a normal oxygen availability (7.4-7.9 mg/L) and the purées with a reduced oxygen availability (0.1-1.8 mg/L), immediately after the purée preparation. After the pasteurization or the sterilization treatments, the oxygen concentrations of all the reference purées were strongly decreased to maximum levels of 0.3 mg/L. A large part of the oxygen might be expelled from the glass jars because of the thermal expansion of air. The remaining oxygen was consumed in thermal oxidation reactions of various food constituents, such as the possible furan precursors ascorbic acid and fatty acids. As shown by results of the mixed effects model (**Table 1**), this resulted in significantly lower furan concentrations for the purées with a reduced oxygen availability as compared to the purées with a normal oxygen availability. For a better understanding of this effect in specific formulations, the furan concentrations of the pasteurized purées at pH 3 and the sterilized purées at pH 7 are presented in **Figure 2**. Both are interesting reactions conditions from the perspective of food processing, because they closely resemble the pH and corresponding thermal intensity of shelf-stable fruit- and vegetable-based products. From **Figure 2**, it can be seen that the furan concentrations of the pasteurized purées were low (ND-26 ng/g purée) as compared to the concentrations of the sterilized purées (40-114 ng/g purée). The effect of oxygen on the furan formation was more pronounced in the sterilized purées, where a reduced oxygen availability resulted in a considerable decrease in the furan concentrations of the reference purée (60%), as well as in the other purées with addition of ascorbic acid (58%), fructose (35%) or fatty acids (33%). This is a very interesting observation, because it shows the potential for furan reduction by lowering the oxygen concentration of food products with a various composition.

Literature studies directly investigating the effect of oxygen on the furan formation in food or model systems are scarce. Nevertheless, it is generally acknowledged that the furan precursors ascorbic acid and fatty acids are prone to oxidative degradation (Joint FAO/WHO Codex Committee on Contaminants in Foods 2011). Ascorbic acid can also be degraded in a non-oxidative way, but the oxidative pathways (via dehydroascorbic acid and hydrolysis to diketogulonic acid) are faster and dominating in the presence of oxygen (Verbeyst et al. 2013). Under pyrolytic conditions (dry heating to 220 °C) and after replacing air inside the reaction vessel by nitrogen, Mark et al. (2006) have observed a considerable decrease (30%) in the furan amounts from ascorbic acid. This is in accordance with the results of the present study, which seems to confirm the importance of the oxidative degradation pathways for the furan formation from ascorbic acid. Oxygen can also act as an initiator for the thermal oxidation of fatty acids. In studies on model systems, the furan concentration is usually higher as the degree of fatty acid unsaturation is higher and the susceptibility to oxidation increases (e.g. for linoleic and linolenic acid) (Becalski & Seaman 2005; Mark et al. 2006). Discussion is ongoing whether or not unrealistically high degrees of fatty acid oxidation are necessary to form furan in real foods (Owczarek-Fendor et al. 2010b). In the present study, fatty acid oxidation seemed to have only a small contribution to the furan formation. Despite the high, but realistic amounts of polyunsaturated fatty acids in the spiked olive oil (11.3% of the total fatty acids content, or a concentration of ca. 340 mg/100 g purée), a small difference was observed between the furan concentrations of the different purée formulations with addition of fatty acids. Next to the purées discussed above, a strong decrease in the furan concentration was observed for the reference purée (without addition of precursors) and the purée with addition of fructose. Since the latter formulations contained low concentrations of vitamin C (3.7 mg/100 g purée) and fatty acids (0.5 g/100 g purée) (cf. Material and methods), these observations seemed to demonstrate that oxygen has a larger effect on the furan formation in the potato purées than only acting as an initiator for the oxidative degradation reactions. The observed furan reduction might be linked to sugar degradation, which mainly occurs through Maillard reactions under the current conditions (pH 7, high water content). In a very similar research matrix (starch solution at pH 6 with addition of furan precursors), Owczarek-Fendor et al. (2012) have observed a strong synergistic effect on the furan formation between fructose and oxidized soybean oil. Conversely, the addition of ascorbic acid to a fructose-containing solution resulted in a lower than expected furan concentration. Both results indicate that sugar degradation can be affected by changes in the reaction conditions on the redox level, which might be an important part of the explanation for the observed furan reduction in the present study.

Effect of pH on furan formation in the thermally treated potato purées

To investigate the effect of pH on the furan formation in fruit- and vegetable-based foods, purée formulations with different pH levels of 3 and 7 were prepared and subjected to a thermal treatment (cf. **Figure 1**). The selected pH values closely resemble the natural pH of fruit- and vegetable-based food products, respectively. Contrary to most other studies (Fan et al. 2008; Huang et al. 2011; Nie et al. 2013b), the pH of the purée formulations was adjusted using concentrated HCl or NaOH solutions (1 M) instead of using phosphate buffers. This way, the present study utilized the natural buffering capacity of the potato matrix to stabilize the pH during the thermal treatments. Measurements of the pH after the treatments indicated that the pH shift, which was induced by thermal reactions inside the product, was limited to maximally 0.32 pH unit. For a fair comparison of the pH effect, the potato purées at pH 3 and 7 were both subjected to a sterilization treatment (121 °C, $F_0 = 15$ min). For each purée composition, the pH effect was investigated at reduced and normal oxygen availability, even though no interactions were expected between both matrix characteristics. The furan concentrations for the different formulations of the sterilized purées are presented in **Figure 3**. The effect of pH was very similar for the purées with a reduced or a normal oxygen availability. Irrespective of the purée composition (i.e., type of spiked precursor) or the oxygen availability, a pH level of 7 resulted in higher furan concentrations as compared to pH 3. This is again a very interesting observation in the context of furan mitigation, because the observed differences in the furan concentrations of fruit- and vegetable-based foods from monitoring experiments (US Food and Drug Administration (FDA) 2009; European Food Safety Authority (EFSA) 2011) are usually explained by the different thermal intensities of the preservation treatments (e.g. pasteurization vs. sterilization). However, the results of the present study indicate that the lower furan concentrations for fruit-based foods might be partly explained by the acid pH of the products. It should also be noted that the addition of the furan precursors ascorbic acid, fructose or olive oil, did not result in a clear furan increase as compared with the reference purées (see section "Relative importance of precursors and reaction conditions for furan formation"). Possibly, the furan formation from the various precursors was limited by the effects of the thermal intensity, the investigated matrix properties (i.e., oxygen availability and pH) or even other properties (e.g. the amino acid fraction), rather than by the concentrations of the precursors themselves. By spiking the potato purées with high, but realistic concentrations of each precursor, the relative importance of that specific reaction pathway is favored as compared with other pathways. Nevertheless, the observed effect of the investigated matrix property on the furan formation, is always a combination of the effects on all the constituting reaction steps in the potato purée under consideration, as will be explained in the next paragraph.

As mentioned in the Introduction, the effect of pH on furan formation is dependent on several matrix characteristics such as the precursors under consideration and the type of buffer solution. In real food systems, the complexity is further increased, because the pH might have additional, indirect effects on the furan formation through interactions with proteins (unfolding and/or reaction availability) (Cui et al. 2013; Makinen et al. 2016), metal ions (solubility and pro-oxidant properties) (Sorensen et al. 2008) or even the structure of the matrix (Fraeye et al. 2007; Thongkaew et al. 2015). The complexity of the matrix makes it virtually impossible to provide a comprehensive explanation for the effects of pH on the course of a reaction with multiple precursors, such as the furan formation. However, by using a potato purée as a research matrix, the effect of pH on the furan formation could be directly investigated in a commonly used ingredient of various vegetable-based foods. The reduced furan concentration at pH 3 compared to pH 7 was consistently observed for all the different purée compositions included in this study (potato purée with and without addition of ascorbic acid, fructose or olive oil). Given the high starch content of potato purée, it can be hypothesized that the acid pH resulted in a partial hydrolysis of the starch. This could have affected its gelating properties (not characterized), but would also provide a supplementary amount of precursors (i.e., glucose) to the matrices. However, since the reference purées (without spiking additional precursors) showed an increased furan formation at pH 7, the furan increase can most probably be explained by an enhanced degradation of the intrinsic precursors ascorbic acid, sugars and fatty acids at pH 7. Ascorbic acid contains an acidic hydroxyl group with a pK_a value slightly higher than 4 (Davey et al. 2000; Belitz et al. 2009) and is therefore prevalent in the stabile, non-ionized form in the purée formulations at pH 3. At pH 7, however, it exists as a monovalent anion, which is more reactive and prone to oxidative degradation (and furan formation) (see section "Effect of oxygen availability on furan formation in the thermally treated potato purées"). An analogous reasoning can be set up for the monosaccharides fructose and glucose, which are most stable around pH 3-4 (Belitz et al. 2009). Sugars are degraded to furan through Maillard reactions and to a lesser extent also in the absence of amino acids (Locas & Yaylayan 2004; Limacher et al. 2008; Van Lancker et al. 2011). Both reactions are enhanced at pH 7, which has been observed before and is often linked to the favorable conditions for sugar enolization and fragmentation (Limacher et al. 2008; Owczarek-Fendor et al. 2012). Furan formation from fatty acids is a free radical-induced reaction, that is rarely affected by pH. Nevertheless, Fan et al. (2008) and Shen et al. (2015) have observed an optimal furan formation in linoleic and linolenic acid emulsions at pH 6-7 as compared to a lower (pH 3 and 4.55, respectively) or a higher pH (pH 8.68). It is stated that acidic or alkaline model systems can restrain the oxidation of polyunsaturated fatty acids and, therefore, reduce the furan formation (in this case at pH 3). Irrespective of the provided explanation for the pH effect, lowering the pH of the product for furan mitigation should be done with the highest care. As mentioned above, adjusting the pH level can have a large impact on various quality attributes (microbial safety, taste and structure, among others). Reducing the oxygen concentration (see section "Effect of oxygen availability on furan formation in the thermally treated potato purées") might therefore be considered a more feasible approach for furan reduction in shelf-stable, low-acid foods such as jarred baby foods, soups and sauces.

Conclusions

Furan reduction by adding or removing precursors from the product has proven to be challenging, because of major food constituents and interactions involved in the reaction pathways leading to furan formation. Instead of intervening at the precursor level, it might be more feasible from an application point of view, to influence the formation pathways by adjusting the matrix properties of the product. To investigate the effects of the oxygen availability (normal vs. reduced) and the pH (acid vs. low-acid) on furan formation in fruit- and vegetable-based foods, different combinations of both matrix properties were prepared in a potato purée, with and without the addition of the furan precursors ascorbic acid, fructose or fatty acids. Irrespective of the addition of these precursors, a considerable decrease in the furan concentration was observed for the purée formulations with a reduced oxygen availability and the purées at pH 3. In the literature, commercially available, fruit-based food products tend to have a lower furan concentration than vegetable-based foods, which is usually explained by the lower intensity of the thermal treatment for preservation (pasteurization vs. sterilization). Based on the results of this study, the difference in furan concentration can partly be attributed to the acid pH of the fruit-based products. In the context of furan mitigation, priority should be given to the optimization of the thermal preservation process for fruit- and vegetable-based foods (i.e., sterilization of low-acid, conduction-heated foods). If this thermal optimization is hindered by microbial safety standard to comply with or an extended shelf life, lowering the oxygen concentration and/or the pH of the products might offer an additional, powerful strategy for furan mitigation. However, since the mechanisms of furan reduction for both matrix characteristics could not be fully established, the adjustments of the product composition should always be accompanied by a thorough evaluation of the effects on other food safety and quality attributes (e.g. structure, taste, color). Ideally, various mitigation strategies should be brought together in an industrial toolbox for furan reduction, as already done for acrylamide by FoodDrinkEurope (2014). By providing measures at the different steps of the food chain (product formulation, thermal processing and storage), such a toolbox could allow food processors to reduce the furan concentrations of various fruit- and vegetable-based foods, with limited adverse side effects on other important food attributes.

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Tables

Table 1. Estimates of the regression coefficients and significance tests for the mixed effects model describing the furan concentrations in the thermally treated potato purées as a function of precursor composition, oxygen availability, pH and thermal intensity.

Effect	**Value**	**Estimate**	**Standard error**	**DF**	***t* Ratio**	***p* Value**
Intercept (μ)		46.89				
Precursor (β_1)	Reference	4.77	25.76	124	0.19	0.8533
Precursor (β_1)	Fructose	-4.33	9.10	124	-0.48	0.6352
Precursor (β_1)	Ascorbic acid	20.53	12.74	124	1.61	0.1096
Oxygen availability (β_2)	Normal	21.97	3.35	124	6.56	<0.0001
pH level (β_3)	3	-40.39	5.15	124	-7.84	<0.0001
Thermal intensity (β_4)	Pasteurization	-21.97	5.15	124	-4.26	<0.0001

Figure captions

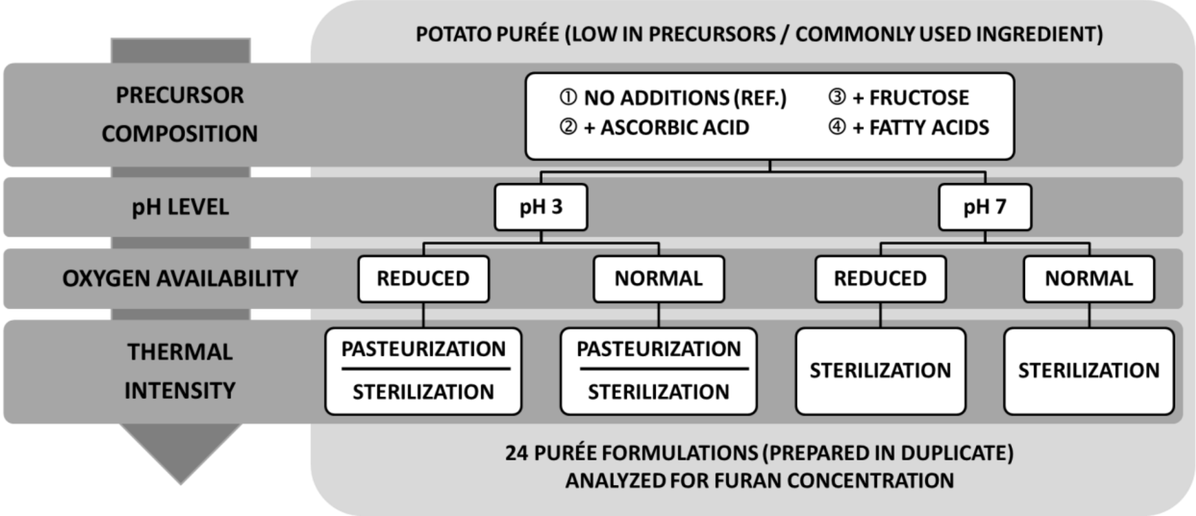

Figure 1. Schematic overview of the experimental approach for purée preparation. All the purée formulations were prepared in duplicate, thermally treated and analyzed for furan.

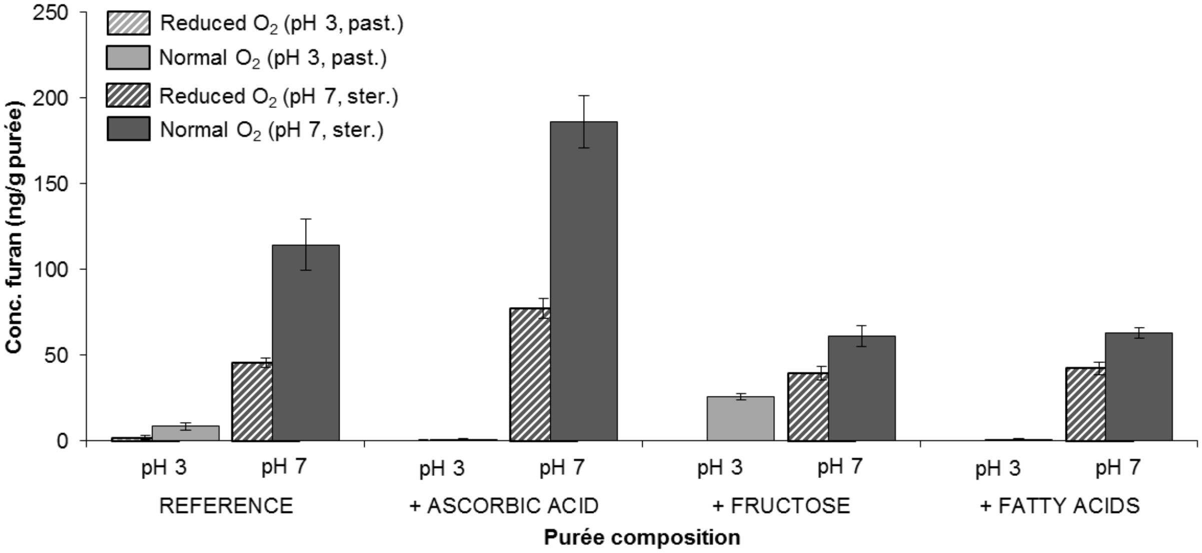

Figure 2. Effect of oxygen availability on the furan concentration of the thermally treated, spiked potato purées (past. = pasteurization, ster. = sterilization).

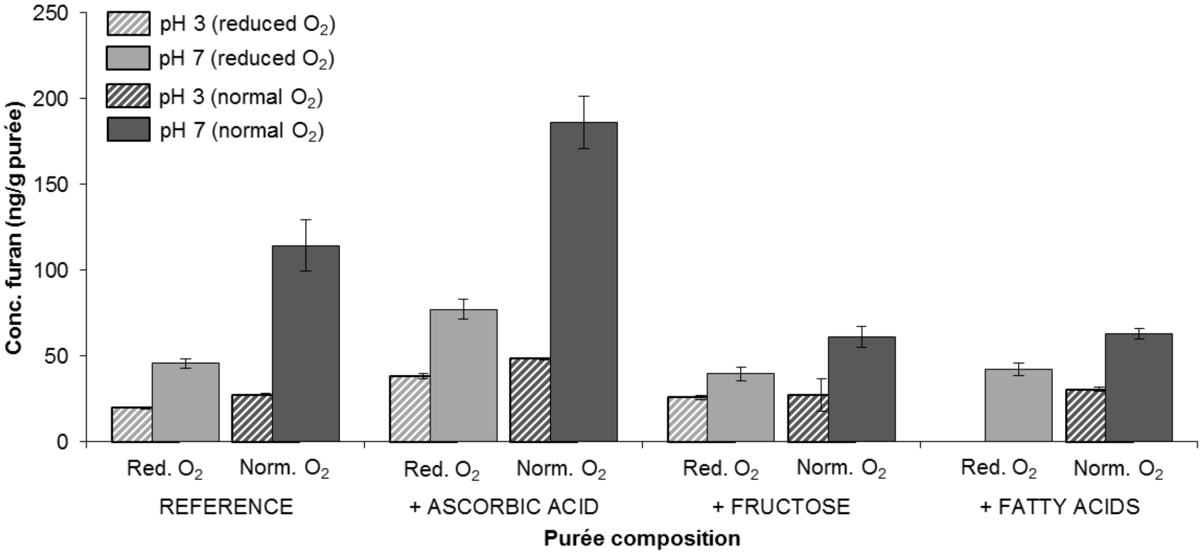

Figure 3. Effect of pH on the furan concentration of the thermally sterilized, spiked potato purées.