Relative importance and interactions of furan precursors in sterilized, vegetable-based food systems

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Mitigation strategies aiming at an intervention in the reaction pathways for furan formation (e.g. by adjusting precursor concentrations) might offer an additional route for furan reduction in sterilized, vegetable-based foods, without adverse effects on other food safety or quality attributes. As a first step towards product reformulation, the aim of the present study was to determine the relative importance and interactions of possible furan precursors in these types of foods. Based on an I-optimal experimental design, potato purée (naturally low in furan precursors) was spiked with known amounts of sugars, ascorbic acid, olive oil and β-carotene, and subjected to a thermal sterilization. Significant correlations were observed between furan concentrations after thermal treatment and starting concentrations of ascorbic acid and monosaccharides (i.e., fructose and glucose). Ascorbic acid had a clear furan reducing effect as an antioxidant, by protecting (polyunsaturated) fatty acids against oxidative degradation. Fructose and glucose were the main precursors, which can most probably be attributed to their high, but realistic concentrations in the product. The contributions of fatty acids and β-carotene were strongly dependent on redox interactions with other food constituents. In the same potato purées, only low concentrations (0-2 ng/g purée) of 2-methylfuran were detected, indicating that the direct importance of the spiked food constituents as a precursor for methylfuran formation was rather small. Based on the results of this study, reducing the amount of monosaccharides or adjusting the redox conditions of the matrix are suggested as two possible approaches for furan mitigation on the product side.

Keywords: furan; methylfuran; precursor; thermal sterilization; optimal experimental design.
Introduction

Furan (C₄H₄O) 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). A recent risk evaluation by the Joint FAO/WHO Expert Committee on Food Additives (2011) has indicated a human health concern for furan. Consequently, actions should be taken to minimize exposure to an acceptable level. Sterilized, vegetable-based foods (jarred baby foods, ready-to-eat soups, sauces, etc.) are important contributors to the furan exposure of children and adults (US Food and Drug Administration (FDA) 2009; European Food Safety Authority (EFSA) 2011). In their previous work, the authors of the present study have investigated the potential of furan mitigation measures in vegetable-based foods, by modifying the processing conditions for thermal preservation or storage. In this context, innovative high-pressure high-temperature (HPHT) processing presented itself as an interesting alternative for conventional thermal sterilization (Palmers et al. 2014). Following a HPHT treatment, the furan concentrations of eleven individual vegetable purées (e.g. broccoli, carrot, potato) decreased to levels close to the analytical limits (1-2 ng/g purée). Similar results were observed by Sevenich et al. (2014) in vegetable-based baby food systems. In both studies, the decrease in furan concentration could be attributed to the shorter processing times as compared with conventional retort treatments, which resulted from the application of compression heat and the corresponding increase in the heating and cooling rates for the purées (Palmers et al. 2015b). Sevenich et al. (2015) even showed that it is possible to scale-up this technology to a pilot scale, whilst keeping the same reduction of furan concentrations. The effect of storage was investigated for a similar range of vegetable purées as described above (Palmers et al. 2015a). After 5 months of storage at temperature-abuse conditions of 35 °C, the majority of the sterilized vegetable purées showed a considerable increase in furan concentration. This furan formation during storage could be reduced by storing the vegetable purées at a refrigerated temperature of 4 °C, at which the furan concentrations remained approximately constant over the same period of 5 months. The importance of the storage temperature
for controlling furan formation during storage was confirmed by means of a kinetic experiment (Palmers et al. 2015c), using pasteurized fruit juices as a research matrix.

Shelf-stable, vegetable-based foods are particularly susceptible to furan formation (Jestoi et al. 2009; Mesias-Garcia et al. 2010), because of favorable conditions on both the product (natural mixtures of all known furan precursors) and the processing side (intensive heat treatment for preservation). Even though recent literature has indicated the potential of furan mitigation measures by changing the processing conditions of thermal preservation and storage, a substantial furan reduction might not always be feasible, because of microbial safety standards to comply with and because of economic reasons. For such products, mitigation strategies aiming at an intervention in the reaction pathways for furan formation (e.g. by changing the product composition) 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 ways 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). However, the majority of these studies applied simple model systems (consisting of only a single or few constituents) to investigate the role of the different precursors for furan formation. Moreover, these systems are often subjected to extreme processing conditions (e.g. pyrolysis, roasting), which are not frequently applied in food industry, especially for thermal preservation of vegetable-based foods. As a first step towards product optimization, the aim of the present study was, therefore, to determine the relative importance and interactions of the furan precursors in realistic, vegetable-based food systems. A potato purée (naturally low in furan precursors) was spiked with known amounts of fructose, glucose, sucrose, ascorbic acid, olive oil and β-carotene. Based on an I-optimal experimental design, 40 different purée formulations were prepared for thermal sterilization and analysis for furan. A mixed model regression was applied to detect significant correlations between the furan concentrations after thermal
treatment and the precursor concentrations in the starting material. As recently proposed by Becalski et al. (2010) and adopted in the recommendations of the Joint FAO/WHO Expert Committee on Food Additives (2011), also the concentrations of 2- and 3-methylfuran were monitored. Both alkyolated derivatives of furan might be of toxicological interest, since animal studies have shown that they can be metabolically activated in a similar way as furan (Gill et al. 2014a; Gill et al. 2014b).

Material and methods

Selection of the research matrix

As a first step in setting up the experimental design for the present experiment, an appropriate research matrix had to be selected. Based on the available food composition tables (Rijksinstituut voor Volksgezondheid en Milieu 2013), potato was selected as a vegetable matrix which is naturally low in furan precursors. In addition, potato is a commonly used ingredient of many vegetable-based foods. By spiking a potato purée with fixed amounts of selected furan precursors, strictly defined and controlled matrix compositions were obtained, as a compromise between a pure model and real systems. 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. To verify whether the selected potatoes were actually low in furan precursors, the blanched potatoes were characterized in terms of free sugars, vitamin C, fatty acids and total carotenoids concentrations. For this characterization, a homogenous potato purée was prepared by blending (B-400, BUCHI, Flawil, Switzerland) the blanched potato slices with a standardized amount of deionized water. The analyses of free sugars and fatty acids concentrations were performed by Eurofins
Food Testing Belgium (Brugge, Belgium). The vitamin C concentration was measured by means of HPLC and UV detection according to the procedure by Verbeyst et al. (2013), and the concentration of total carotenoids was determined spectrophotometrically as described by Knockaert et al. (2011). The concentrations of free sugars (<0.1 g/100 g purée) and fatty acids (<0.2 g/100 g purée) were found to be below the respective quantification limits. Also the concentrations of vitamin C (4.4 ± 0.2 mg/100 g purée) and total carotenoids (44 ± 10 µg/100 g purée) were very low, especially when compared with the selected concentration levels for spiking (cf. “Preparation of the spiked potato purées”). In other words, the results confirmed that the selected potatoes were low in furan precursors, and therefore, could be considered as a suitable matrix for setting up the I-optimal experimental design for the present study. The characterization of the blanched potato purée was further completed with determinations of the pH (5.95 ± 0.01), starch content (10.9 g/100 g purée) and dry matter content (14.22 ± 0.21 g/100 g purée). Even though the latter factors might affect the furan formation in vegetable-based foods, they were considered to be fixed matrix properties over the entire experimental range and will therefore not further be discussed.

**Preparation of the spiked potato purées**

Sugars, vitamin C, fatty acids and carotenoids were selected as different groups of possible furan precursors to be added to the potato purée. Fructose (≥99%, AppliChem, Darmstadt, Germany), glucose (≥99.5%, Sigma-Aldrich, Bornem, Belgium) and sucrose (≥99%, AppliChem, Darmstadt, Germany) were added as different types of sugars, which are most abundant in fruit and vegetables. Vitamin C was added in the form of ascorbic acid (99%, Acros Organics, Geel, Belgium), carotenoids in the form of β-carotene (≥97%, Sigma-Aldrich, Bornem, Belgium). Olive oil (fatty acid composition of 10.6% C16:0, 1.0% C16:1, 3.7% C18:0, 78.5% C18:1, 5.0% C18:2, 0.6% C18:3, 0.4% C20:0, and 0.2% C20:1) (Vandemoortele, Izegem, Belgium) was added as a source of (unsaturated) fatty acids. Given the complexity of amino acids as possible furan precursors (diverse and wide range of concentrations), the amino acid composition of the selected potato purée was considered as a fixed property. In order to be
able to estimate main, quadratic (the curvature) and interaction effects of the selected furan precursors, each precursor had to be spiked in at least three different concentration levels. The highest concentration was selected based on food composition tables (Rijksinstituut voor Volksgezondheid en Milieu 2013), representing a relevant maximum concentration for vegetables and vegetable-based products. The intermediate level was exactly half of this maximum concentration. At the lowest concentration level, no additional precursors were added to the potato purée. The precursor levels were included in the experimental design as coded (standardized) variables, which are presented together with the selected concentration levels in Table 1. The different purée formulations (cf. “Experimental design”) were prepared by mixing the blanched potato slices with fixed amounts of precursor solutions. Therefore, stock solutions of fructose (1 g/ml), glucose (0.5 g/ml), sucrose (1 g/ml) and ascorbic acid (25 mg/ml) were prepared in deionized water. Olive oil was added as such (fatty acids were spiked without β-carotene), or as a mixture with β-carotene (1667, 3333 or 6667 µg/g olive oil, depending on the concentration levels of both precursors that had to be spiked). The concentrations of all these stock solutions were optimized taking into account the maximum solubility of the precursors and the targeted value of the concentration levels. The mixtures of the potato slices and added solutions were further diluted with deionized water to obtain a standardized total volume, and blended (B-400, BUCHI, Flawil, Switzerland) to obtain a homogenous purée.

**Experimental design**

Since each of the selected furan precursors could be spiked at three different concentration levels, it was not feasible to prepare all possible purée formulations ($3^6 = 729$ possible combinations). Instead, an I-optimal experimental design consisting of 40 different purée formulations was generated with the help of the JMP statistical software (JMP Pro 11, Cary, North Carolina), assuming a full second-order response surface model in the six precursor concentrations. The optimal design approach is a flexible approach that takes into account practical constraints when performing an experiment, such as constraints on combinations of concentrations and the need to split an experiment over several days.
The design utilized here is I-optimal, which means that it minimizes the average prediction variance over the entire set of test combinations under consideration, and thereby guarantees precise predictions (Goos & Jones 2011). Due to its lipophilicity, β-carotene could only be spiked in combination with the olive oil (however in different concentration ratios). This constraint on the concentrations of olive oil and β-carotene was taken into account by the I-optimal design approach. In addition, two blocking factors were included in the experimental design and analysis, to divide the 40 purée formulations into two blocks of 20 purées for the preparation of the samples (purée preparation and thermal treatment) and into ten blocks of 4 purées for furan analysis. The optimal experimental design approach ensures that any systematic differences due to the time of preparation and/or the time of the furan analysis impact the estimation of the precursors’ effects as little as possible. The I-optimal experimental design is presented in Table 2.

**Thermal sterilization**

The spiked potato purées were subjected to a thermal sterilization in a static Steriflow pilot retort (Barriquand, Roanne, France). Due to their inert nature, glass jars (100 ml volume, 95 mm height and 45 mm diameter) were used as sample holders. The jars were filled with 85 ± 0.5 g of potato purée and then closed with metal lids. Next, they were loaded into the retort and sterilized at a processing temperature of 121 °C. The holding time (±32 min) was calculated in advance to obtain an process value of \( F_{121.1 \degree C}^{10 \degree C} \) \( (F_0) = 15 \text{ min} \) in the coldest point of the product. The targeted process value was indeed much higher than the theoretical value for microbial safety of low-acid, shelf-stable foods (‘botulinum cook’ or \( F_0 = 2.5-3 \text{ min} \)), but to avoid product recall and to account for non-uniform impact distributions, \( F_0 \)-values of this order are frequently applied in food industry. The temperature profiles in the retort and the product were recorded using type T thermocouples (Ellab, Hilleroed, Denmark) (results not shown). After sterilization, 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 and methylfuran

Quantitation of furan, 2- and 3-methylfuran was performed by means of an isotope dilution assay as described by Palmers et al. (2014), using furan-d₄ as an internal standard. For sample preparation, 2.5 g of the thermally treated potato purées was weighed into an 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-d₄ (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 and derivatives were 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 HP-PLOT Q column (30 m × 320 µm, 20 µm film thickness, Keysight Technologies, Santa Rosa, California) using helium as the carrier gas at a constant flow rate of 2 ml/min. Mass spectra were obtained by electron ionisation (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-d₄. For quantitation, a calibration curve of furan (>99%, Sigma-Aldrich, Saint Louis, Missouri) was prepared in the blanched potato purée, covering the concentration range of 0-50 ng/g purée. According to our own findings (results not shown), the behavior of 2- and 3-methylfuran towards the internal standard furan-d₄ was not significantly different from the behavior of furan. Therefore, the same calibration curve was used to estimate the concentrations of both methylfurans. For these compounds, the selected ions were m/z 82 (quantifier), 81 and 53 (qualifiers). The decision limit and the detection capability of the procedure were 1.15 ng/g purée and 1.86 ng/g purée, respectively. Each sample was analyzed in triplicate.
**Statistical data-analysis**

The statistical data analysis was performed using the JMP statistical software (JMP Pro 11, Cary, North Carolina). A response surface model was fitted to the data using linear mixed model regression, with fixed effects (main, quadratic and second-order interaction effects) for the controllable factors and random effects to capture the possible correlation between samples coming from the same purée preparation block, the same analysis block and the same purée formulation (repetition of analysis). A significance level of 0.05 was used for selecting significant effects. The response surface modeling approach is discussed in more detail in the section “Response surface model analysis of the furan concentrations in the thermally treated purées” of the results and discussion.

**Results and discussion**

Based on the I-optimal experimental design (cf. “Material and methods”), potato purée (naturally low in furan precursors) was spiked with known amounts of mono- and disaccharides (fructose, glucose and sucrose), ascorbic acid, olive oil (source of fatty acids) and β-carotene. This way, 40 different mixtures of possible furan precursors were prepared to closely resemble the compositions of jarred baby foods and similar vegetable-based foods. After thermal treatment with the aim of sterilization (121 °C, $F_0 = 15$ min), the potato purées were analyzed for furan and the alkylated derivatives 2- and 3-methylfuran. The discussion of the results starts with a visual interpretation of the furan concentrations, followed by regression response surface modeling of the concentrations, an explanation of the significant effects and the relevance of the response surface in the context of furan mitigation. Some general trends in the methylfuran concentrations of the thermally treated potato purées are discussed afterwards.

**Visual interpretation of the furan concentrations in the thermally treated purées**

The furan concentrations of the thermally treated purées and their corresponding standard deviations are represented in Fig. 1, together with the decision limit (CC$_\alpha$, 1.15 ng/g purée) and the detection capability (CC$_\beta$, 1.86 ng/g purée) of the analytical procedure. All potato purées contained detectable
amounts of furan, with concentrations varying from 4 to 36 ng/g purée. With a mean concentration of 17 ng/g purée, the furan concentrations of the present study were in the same range as the concentrations reported in the literature for commercially available, vegetable-based products (mean concentrations of 31-32, 8-11 and 23-24 ng/g purée for baby foods, sauces and soups, respectively) (European Food Safety Authority (EFSA) 2011). In order to detect possible trends in the susceptibility of the potato purées to furan formation, the purées are shown in increasing order of concentration in Fig. 1. The composition of the purée formulations with the highest and the lowest furan concentrations were investigated in more detail. Combinations 20, 35, 36, 37 and 38 had the highest furan concentrations. These combinations were characterized by relatively high sugar concentrations (10-12.5 g/100 g purée) and low amounts of ascorbic acid (no ascorbic acid was added for combinations 20, 35, 36 and 37). All these potato purées contained olive oil (1.5-3 g/100 g purée) as a source of (poly-)unsaturated fatty acids, in most cases accompanied by the presence of β-carotene (only combination 36 did not involve addition of β-carotene). Combinations 1, 4, 16, 23 and 40 had the lowest furan concentrations. These combinations had low sugar concentrations (2.5-7.5 g/100 g purée) and a high concentration of ascorbic acid (150 mg/100 g purée for combinations 4, 16, 23 and 40). These combinations contained varying amounts of olive oil and β-carotene. When comparing the purée compositions of the combinations with the highest and the lowest furan concentrations, it seemed that the sugar and ascorbic acid concentrations were the most important factors affecting the furan formation in the potato purées. High sugar concentrations without ascorbic acid seemed to lead to high furan concentrations, regardless of the concentrations of olive oil and β-carotene. Moreover, the purée formulations showed a continuous range of furan concentrations, starting from the lowest concentrations for combinations 1, 4, 16, 23 and 40, and ending with the highest values for combinations 20, 35, 36, 37 and 38. This way, the wide spread of furan concentrations showed that the experimental design led to a broad variety in purée compositions. At the same time, it demonstrated the potential for furan reduction in vegetable-based foods by changing the precursor concentrations in the product. In order to identify statistically significant associations,
based on the 40 different purée compositions and the observed furan concentrations, there is a need for a sound statistical analysis that takes into account the correlation between purées that were prepared, thermally treated and/or analyzed together. Therefore, the dataset resulting from the I-optimal experimental design was re-analyzed using a mixed model regression approach (cf. “Response surface model analysis of the furan concentrations in the thermally treated purées”).

Response surface model analysis of the furan concentrations in the thermally treated purées

Due to the size of the experiment, it was not feasible to perform the sample preparation steps (purée preparation and thermal treatment) and the furan analysis for all the purée formulations in the same time frame. Therefore, the I-optimal experimental design included two blocking factors to assign the 40 different purée formulations to (i) two preparation blocks and (ii) ten groups for furan analysis. The analysis was performed in triplicate, so that each purée formulation gave rise to three measurements of the furan concentration. Mixed model regression was a suitable approach for statistically analyzing these furan concentrations (cf. “Visual interpretation of the furan concentrations in the thermally treated purées”), because it allowed to account for the mutual dependencies between the concentrations due to the preparation and analysis blocks. To describe the effects of the controllable, fixed factors on the furan concentrations, a full second-order response surface model was used. Since there are six controllable experimental factors in the experiment (concentrations of fructose, glucose, sucrose, ascorbic acid, fatty acids and β-carotene), this model involved 27 fixed effects for 40 observations: 6 main effects, 6 quadratic effects and 15 second-order interactions. For modeling purposes, the concentration levels of the furan precursors were converted into coded (standardized) variables (cf. Table 1). The response surface model equation, including the fixed and random effects, is represented in equation (1):

\[ Y_{kln} = \mu + \sum_{i=1}^{6} \beta_i x_{iklm} + \sum_{i=1}^{6} \beta_i x_{iklm}^2 + \sum_{i<j=1}^{6} \beta_{ij} x_{iklm} x_{jklm} + a_k + b_l + c_m + \epsilon_{kln} \tag{1} \]
where \( Y_{klmn} \) was the observed furan concentration at the \( n \)th repeated observation \((n = 1,2,3)\) from the \( m \)th purée formulation \((m = 1,2,3,4)\), the \( k \)th preparation block \((k = 1,2)\) and the \( l \)th analysis block \((l = 1,2,\ldots,10)\). \( \mu \) represented the intercept, \( x_{ijklm} \) and \( x_{jklm} \) were the coded concentration levels of the \( i \)th and \( j \)th controllable experimental factors, the regression coefficients \( \beta_i \), \( \beta_{ii} \) and \( \beta_{ij} \) were the the main effects, the quadratic effects and the interaction effects, respectively, and \( a_k \), \( b_l \) and \( c_m \) were the random effects of the \( k \)th preparation block, the \( l \)th analysis block and the \( m \)th purée preparation. The model was estimated using generalized least squares for the experimental factors’ effects, in combination with restricted maximum likelihood (REML) for the variances of the random effects. A stepwise backward elimination procedure was used (where insignificant terms were dropped one by one) to obtain the final reduced model presented in Table 3. The adjusted coefficient of determination value \((R^2_{\text{adj}}, 0.9855)\) and the root mean square error (RMSE, 1.228) both indicated a good fit of the selected model, which is confirmed by the parity plot and a plot of the residuals in Fig. 2.

The reduced response surface model (Table 3) involves an intercept, five main effects and three interaction terms describing the empirical relationship between the furan concentrations of the thermally treated purées and the concentration levels of the experimental factors in the starting material. The intercept can be considered as a reference value for the furan concentrations, and the estimated value (16.84 ng/g purée) was close to the mean concentration of the tested purées. The strongest main effects were observed for fructose \((p = 0.0007)\) and ascorbic acid \((p < 0.0001)\), followed by glucose \((p = 0.0519)\), whose significance was close to the postulated significance level \((\alpha = 0.05)\). Fructose and glucose have positive main effects, as opposed to ascorbic acid, which has a negative main effect on the furan concentration. The main effects of fatty acids \((p = 0.2011)\) and \( \beta \)-carotene \((p = 0.2617)\) were not significant, but they were retained in the model because they are included in significant interaction terms. This was not the case for sucrose, which did not appear in any statistically significant term. Sucrose was therefore completely removed from the model. These observations were in agreement with the visual observations described in the section “Visual interpretation of the furan concentrations in the
thermally treated purées”. All quadratic terms of the selected furan precursors were insignificant and therefore removed from the model. This implies that the association of every individual precursor with the furan concentration can be described by a linear relationship, for given levels of the remaining precursors. Three interaction terms had significant explanatory value for the furan concentrations of the purée formulations. Important positive correlations were observed when β-carotene was combined with fructose ($p = 0.0246$) or glucose ($p = 0.0013$). Through its interaction with monosaccharides, β-carotene has a larger impact on furan formation than as a direct furan precursor. Furthermore, both interactions of β-carotene contribute to the importance of monosaccharides for furan formation in vegetable-based systems. Finally, a strongly negative interaction ($p = 0.0085$) was observed for fatty acids and ascorbic acid, implying that combining both compounds in the food matrix reduced the furan concentrations of the thermally treated potato purées.

It should be noted that the described relationships between precursors and furan concentration were only empirical, which means that strictly speaking, there was no proof for a causal relationship between both types of compounds in the present study. Nevertheless, by setting-up a well-balanced experimental design (with a sufficiently large sample size and distribution, randomizing the experimental runs with each block and controlling all factors which are not explicitly included in the experiment), using strictly defined but realistic research systems such as the purée formulations of the present study, the potential of response surface methodology to obtain insight into the complex relationship between furan and its precursors, was exploited to the largest possible extent. In the next section, an attempt is made to integrate the observed main effects and interaction effects and to provide an overall explanation for the reaction pathways leading to furan formation in the spiked potato purées.

**Interpretation of the response surface describing furan as a function of purée composition**

The reduced response surface model (Table 3) describing the relationship between the purée composition (i.e., the precursor concentrations) and the furan concentrations of the thermally treated purées, involved nine terms (intercept, main effects and interaction terms) and five of the six original
Because most of these factors appear in a significant interaction term, the interpretation of the relative importance of the precursors for furan formation is not trivial. To visualize the interaction effects and study the joint impact of pairs of experimental factors, three-dimensional response surface plots, representing the furan concentration as a function of two experimental factors (given fixed concentrations for the other factors) are used. With the help of these response surface plots, the results of the present study can be interpreted graphically and compared to the available literature in order to obtain an increased understanding of the furan formation in the present purée formulations and possibly also other types of vegetable-based foods.

Sugars are often named as one of the most important furan precursors in foods (Yaylayan 2006; Crews & Castle 2007; Bolger et al. 2008). Three types of sugars were spiked in the present study (i.e., fructose, glucose and sucrose). Significant effects on the furan concentration were only observed for fructose and glucose. Based on just the main effects, it would be tempting to state that fructose was the most efficient precursor for furan formation among the tested sugars. However, the larger interaction effect for glucose with β-carotene means that the role of glucose is of a similar importance as that of fructose. The response surface plot in Fig. 3A shows the combined effect of the fructose and glucose concentrations on the furan concentrations of the thermally treated potato purées, given fixed, average concentrations of ascorbic acid (80 mg/100 g purée), fatty acids (1.5 g/100 g purée) and β-carotene (5000 µg/g purée). This plot clearly shows that, all other concentrations being equal (ceteris paribus), the furan concentrations originating from both precursors were additive, with a slightly faster furan formation for fructose. This observation is in agreement with the literature, where fructose is usually reported to be the most efficient precursor among the hexose sugars, before glucose and sucrose (Fan 2005; Limacher et al. 2008; Owczarek-Fendor et al. 2012). In food systems with a high water content and low-acid pH, like the purée formulations of the present study, Maillard reactions are the dominating pathways leading to furan formation from sugars (Belitz et al. 2009), although sugars can also degrade to furan in the absence of amino acids (Yaylayan 2006). The inactivity of sucrose as a furan precursor can most probably be explained by the same low-acid pH of the purée formulations (pH 5.95), at which
Sucrose hydrolysis (to generate fructose and glucose) is expected to be small (Owczarek-Fendor et al. 2012).

The interactions between fructose or glucose and β-carotene were more challenging to interpret. In the literature, little information can be found on possible interactions between the degradation reactions of sugars and carotenoids. However, some Maillard reaction products (e.g. Amadori compounds) have been described to promote lipid oxidation (Zamora & Hidalgo 2008). In a similar way, reaction products of the fructose and glucose degradation might have promoted the oxidative degradation of β-carotene, resulting in higher furan concentrations than when both precursors were not combined in the same starting material. The response surface plot in Fig. 3B shows the combined effect of the fructose and β-carotene concentrations on the furan concentrations of the thermally treated potato purées, given fixed, average concentrations of glucose (2.5 g/100 g purée), fatty acids (1.5 g/100 g purée) and β-carotene (5000 µg/100 g purée). In addition to the increased furan formation when fructose and β-carotene are combined in the matrix, this plot indicates that β-carotene reduced the furan formation in the presence of low monosaccharide concentrations in the potato purées (no additional fructose added). This effect is probably due to the antioxidant properties of β-carotene, which can partly protect other precursors such as fatty acids against oxidative degradation to furan. In the presence of high monosaccharide concentrations, β-carotene had a positive impact on the furan formation. The opposite effects of β-carotene at low and high monosaccharide concentrations explain why the main effect of β-carotene was small and insignificant. An identical response surface plot for the glucose and β-carotene concentrations (graph not shown) confirmed that the monosaccharides fructose and glucose had a very similar effect on the furan concentrations of the thermally treated potato purées.

Ascorbic acid appears twice in the reduced response surface model: it has a significant main effect and a significant interaction term with fatty acids. Since both effects are negatively related to the furan concentration, ascorbic acid had a clear reducing effect on the furan formation in the thermally treated
potato purées. This is an interesting observation, because, in the literature, ascorbic acid is generally presented as an (important) precursor for furan formation (Crews & Castle 2007). In order to investigate the joint effect of ascorbic acid and fatty acids concentrations on the furan concentrations of the thermally treated potato purées, the response surface plot for these two furan precursors is shown in Fig. 4, given fixed, average concentrations of fructose (2.5 g/100 purée), glucose (2.5 g/100 g purée) and β-carotene (5000 µg/100 g purée). The significance of the interaction effect implies that the effect of ascorbic acid on the furan formation in the potato purées was dependent on the fatty acids concentration. At low concentrations of fatty acids in the starting material, ascorbic acid had little or no effect on the furan concentration after thermal treatment. However, when fatty acids were spiked at the highest concentration level, ascorbic acid had a clear reducing effect as an antioxidant, possibly protecting fatty acids against oxidative degradation to furan. Various authors (Locas & Yaylayan 2004; Mark et al. 2006; Limacher et al. 2007; Mogol & Gokmen 2013) state that ascorbic acid can degrade to furan in an oxidative or a non-oxidative manner, the oxidative reaction pathways being faster and prevailing in the presence of oxygen (Esteve et al. 1999; Rojas & Gerschenson 2001; Oey et al. 2006). Moreover, various aldohexose compounds originating from the ascorbic acid degradation are well-known intermediate products of the sugar degradation to furan (Locas & Yaylayan 2004; Mark et al. 2006; Limacher et al. 2007). This shows that the role of ascorbic acid for furan formation is complex, because it strongly depends on the presence of other compounds (e.g. fatty acids, sugars) and on the specific reaction conditions (e.g. oxygen, pH) in the food system under consideration. As observed for β-carotene, ascorbic acid can act as a furan precursor itself, but at the same time, it seems to protect other precursors against oxidative degradation to furan.

The response surface plot in Fig. 4 also shows the furan concentration as a function of the fatty acids concentration, when no ascorbic acid was added to the purées. Remarkably, in that case, there was a positive relation between the concentration of fatty acids in the starting material and the furan concentration of the thermally treated potato purées. In other words, when the ascorbic acid level was low, degradation of fatty acids contributed substantially to the furan concentration. When the ascorbic
acid level was high, large concentrations of fatty acids led to a reduction of the furan formation. Based on the literature, the relative importance of fatty acids for furan formation remains unclear. Furan formation from the fat fraction of the food is mostly attributed to the oxidative degradation of polyunsaturated fatty acids, mainly linoleic acid (C18:2) and linolenic acid (C18:3) (Becalski & Seaman 2005; Mark et al. 2006). The olive oil which was used to spike the potato purées of the present study, contained both linoleic acid (5% in the olive oil, or 0.15 g/100 g purée) and linolenic acid (0.6% in oil, 0.018 g/100 g purée), in low, but realistic concentration levels (cf. “Material and methods”). No specific measures were taken to control the oxygen concentration of the present purées. Moreover, some literature sources state that unrealistically high degrees of oxidation have to be reached in order to form furan (Owczarek-Fendor et al. 2010b; Owczarek-Fendor et al. 2012). Nevertheless, the results of the present study seemed to suggest that fatty acids could have an important contribution to the furan formation of vegetable-based foods, and that the redox condition of the matrix (i.e., antioxidant concentration) would be an important factor to control the furan concentration in such a case. As already mentioned above, β-carotene might have similar antioxidant properties as ascorbic acid. However, such properties were only observed when β-carotene was spiked at the highest concentration level (10000 µg/100 g purée), in the presence of low concentrations of monosaccharides (absence of fructose, glucose concentration of 2.5 g/100 g purée). At higher sugar concentrations, the antioxidant properties seemed to be dominated by the positive interaction effects between β-carotene and fructose or glucose.

Optimization of the product composition for furan mitigation

In the previous section, the relative importance of the furan precursors in the thermally treated potato purées was studied by means of a response surface model (Table 3) and response surface plots (Fig. 3 and 4). Based on the response surface model, an optimized product composition for minimal or maximal furan concentrations can be put forward within the experimental framework of this study. The optimized compositions (obtained from the response optimizer in the JMP statistical software) are presented graphically using the prediction profilers in Fig. 5. The minimum and maximum predicted furan
concentrations are 1 and 38 ng/g purée, respectively. These concentrations are in the same range as the experimentally observed minimum and maximum concentrations (4 and 36 ng/g purée, respectively). The predicted minimum corresponds to a product composition consisting of potato purée spiked with large concentrations of ascorbic acid (150 mg/100 g purée), fatty acids (3 g/100 g purée) and β-carotene (10000 µg/100 g purée), and without adding fructose or glucose. The purée composition corresponding to the prediction maximum involves the same concentration levels of fatty acids (3 g/100 g purée) and β-carotene (10000 µg/100 g purée). However, this purée formulation also contains large concentrations of fructose (5 g/100 g purée) and glucose (5 g/100 g purée), without the addition of ascorbic acid. A comparison of the two formulations again indicates that the concentrations of the monosaccharides fructose and glucose, on the one hand, and the ascorbic acid concentration, on the other hand, are the major factors affecting the furan concentration of the thermally treated potato purées.

The relative importance of the different precursors for furan formation is expected to be a function of both the concentration and the conversion efficiency of the degradation reactions to furan. Among the precursors investigated in this study, sugars and (total) fatty acids (g/100 g product) were present in the highest concentrations, followed by ascorbic acid (mg/100 g product) and carotenoids (µg/100 g product). This partly explains why the monosaccharides fructose and glucose were found to be the primary source for furan formation in the thermally treated potato purées. Ascorbic acid, which was present in a lower range of concentrations, still had an important reducing effect on the furan concentration through the redox interaction with the (polyunsaturated) fatty acids of the olive oil. Fatty acids and β-carotene were shown to be of less importance for the furan formation, since they appeared in high concentrations in the optimized product compositions for both the minimum and the maximum furan concentrations. The extent of fatty acid degradation seemed to be limited in the thermally treated purées, which can be explained by the small (but realistic from an application point of view) proportion of polyunsaturated fatty acids in the olive oil, and the presence of antioxidants ascorbic acid and β-carotene. The antioxidant activity of β-carotene could only be observed in the absence of monosaccharides. In the opposite case, they contributed to the furan formation through a significant
redox interaction with fructose and glucose. As mentioned before, sucrose does not appear in the reduced response surface model. It has no significant effects on the furan concentrations of the thermally treated potato purées. Therefore, from the perspective of furan mitigation, sucrose might be the best way of adding sugars to a food product, since its concentration does not seem to affect the furan formation.

The identification of the relative importance and interactions of furan precursors in the purée formulations of the present study is one step closer towards product reformulation for furan reduction in vegetable-based foods. According to the reduced response surface model, reducing the concentrations of the monosaccharides fructose and glucose on the one hand, and increasing the antioxidant capacity of the matrix (e.g. by increasing ascorbic acid concentration) on the other hand, seem to offer two promising approaches for furan mitigation. However, it should be noted that the optimized product compositions were obtained by an extrapolation of the response surface model. For example, the specific purée formulation for minimum furan formation was not part of the experimentally prepared purée formulations, and the corresponding prediction of the furan concentration was lower than the experimentally observed minimum. Moreover, because of the empirical nature of the response surface model, the optimized product compositions cannot be directly translated to the increased complexity of real products. Future work should aim to include the possible effects of other product characteristics, such as the presence of amino acids (contribution to Maillard reactions and direct precursor) or matrix properties (e.g. pH and oxygen concentration). This way, the complexity of the response surface model can be systematically increased, with the final aim of obtaining a comprehensive model describing furan formation in a variety of (fruit- and) vegetable-based food products. One of the major advantages of response surface methodology, is that the model obtained from the present study can serve as a starting point for such type of experiments, to save time and costs.

*Trends in methylfuran concentrations*
In addition to furan, the thermally treated potato purées were analyzed for 2- and 3-methylfuran. Major food constituents such as sugars (alone or in combination with amino acids) (Mark et al. 2006; Limacher et al. 2008), ascorbic acid (Limacher et al. 2007), fatty acids (Mark et al. 2006) and β-carotene (Becalski & Seaman 2005) have also been reported as possible precursors for the formation of both methylfurans. Given the wide range of purée compositions and possible precursors that were included in the present study, the experimental setup provided an excellent opportunity for obtaining insight into the main precursors and reaction pathways leading to methylfuran formation. Remarkably, 2-methylfuran was only detected in very low concentrations, and 3-methylfuran was not detected at all. The concentrations of 2-methylfuran in the potato purées varied between 0 and 2 ng/g purée (Fig. 6). These concentrations were of the same order of magnitude as the analytical limits of the procedure and should therefore be interpreted with care. However, in a previous study by the same authors (Palmers et al. 2014), eleven vegetable purées (without addition of precursors) were thermally treated and analyzed for 2- and 3-methylfuran. About half of the thermally treated purées (bell pepper, broccoli, red carrot, pumpkin and spinach) had a summed methylfuran concentration (9-23 ng/g purée) in the same range as the corresponding furan concentration. The other half of the purées (orange, purple, and yellow carrots, onion, potato and red beet) contained 2- and 3-methylfuran in concentrations close to the detection capability (1.86 ng/g purée). These results showed that in both studies, the potato purée contained low methylfuran concentrations, no matter whether possible precursors were added to the matrix (like in the present study) or not (Palmers et al. 2014). In other words, the selected food constituents (sugars, ascorbic acid, fatty acids and β-carotene) were not a direct precursor for methylfuran formation, as opposed to furan. This was a very interesting observation, because it points to the importance of different matrix characteristics than the selected food constituents as limiting factors in the formation of 2- and 3-methylfuran in the spiked potato purées.

Based on the current literature information, the possible reaction pathways leading to methylfuran formation can be divided into two groups. Sugar degradation and Maillard reactions are considered as
one group, oxidative degradation reactions (i.e., polyunsaturated fatty acids and β-carotene) as the other. 

Ascorbic acid degradation might be considered as a minor route compared with both groups (Limacher et al. 2007). Of course, interactions might exist as well between these reaction pathways leading to methylfuran formation. The suggested reaction pathways indicate that the amino acids composition and/or the redox condition might be two relevant matrix characteristics for methylfuran formation, which were not included in the present study. Moreover, fundamental differences have been observed in the formation mechanisms of furan and both methylfurans. Furan seems to be formed mainly from the intact carbon skeleton, whereas labeling studies indicate that 2- and 3-methylfuran are more often formed through fragmentation and recombination steps (Mark et al. 2006; Limacher et al. 2008). As a result, it is currently very difficult to control the methylfuran concentrations in vegetable-based foods. There is a need for more fundamental insight into the reaction pathways for 2- and 3-methylfuran formation. More specifically, it might be interesting to elucidate the roles of amino acids and the redox condition, in order to be able to understand and control the formation of both methylfurans in vegetable-based and other types of foods.

**Conclusions**

So far, research on furan mitigation in vegetable-based foods has mostly been targeted at the levels of thermal processing for preservation and the final preparation steps prior to consumption. Reducing furan concentrations by product reformulation has proven to be challenging, because of the many desirable nutritional, structural, and other quality-related properties of the precursors. Based on the results of this study, reducing the amount of monosaccharides or adjusting the redox conditions of the matrix (e.g. by increasing antioxidant concentrations and/or reducing the oxygen availability) can be suggested as two possible approaches for controlling the furan formation via measures on the product side. For example, replacing part of the fructose and glucose concentrations in vegetable-based foods by a disaccharide like sucrose might result in a significant decrease in the furan concentration. It should be noted that these results are only valid for low-acid, semi-solid foods like the spiked potato purées of this study. Since the
presented conclusions are obtained from empirical models (without a real mechanistic base), the mitigation approaches should always be implemented by carefully evaluating the product composition (precursor concentrations and matrix properties) and the intensity of the thermal preservation treatment for the product under consideration. Ideally, the experimental attempts should result in the establishment of an industrial toolbox for furan mitigation, such as the one for acrylamide by FoodDrinkEurope. By providing possible furan mitigation measures at the different steps of the food chain (product formulation, thermal processing and storage), such a toolbox might enable food processors to reduce the furan concentrations of various vegetable-based (and possibly other types of) foods, without compromising on other important food safety or quality attributes. In addition to furan, concerns have been postulated on possible adverse health effects associated with the consumption of alkylated derivatives of furan, in particular 2- and 3-methylfuran. However, the spiked potato purées of the present study contained only 2-methylfuran in low concentrations (0-2 ng/g purée). In order to explain the (apparent) contradiction of these results with the higher methylfuran concentrations observed in some commercial vegetable-based foods, there is a need for more fundamental insight in the reaction pathways leading to 2- and 3-methylfuran (e.g. by experiments with labeled precursors).

Acknowledgements

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Huang XS, Duan HY, Barringer SA. 2011. Effects of buffer and temperature on formation of furan, acetic acid and formic acid from carbohydrate model systems. Lwt-Food Science and Technology. 44:1761-1765.


Table 1. Coding and concentration levels of the selected furan precursors.

<table>
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<tr>
<th>Precursor</th>
<th>Symbols</th>
<th>Concentration level*</th>
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</thead>
<tbody>
<tr>
<td>Fructose (g/100 g purée)</td>
<td>$x_1$</td>
<td>$X_1$</td>
</tr>
<tr>
<td>Glucose (g/100 g purée)</td>
<td>$x_2$</td>
<td>$X_2$</td>
</tr>
<tr>
<td>Sucrose (g/100 g purée)</td>
<td>$x_3$</td>
<td>$X_3$</td>
</tr>
<tr>
<td>Ascorbic acid (mg/100 g purée)</td>
<td>$x_4$</td>
<td>$X_4$</td>
</tr>
<tr>
<td>Fatty acids (olive oil) (g/100 g purée)</td>
<td>$x_5$</td>
<td>$X_5$</td>
</tr>
<tr>
<td>β-Carotene (µg/100 g purée)</td>
<td>$x_6$</td>
<td>$X_6$</td>
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</table>

* $x_1 = (X_1 - 2.5)/2.5; x_2 = (X_2 - 2.5)/2.5; x_3 = (X_3 - 2.5)/2.5; x_4 = (X_4 - 75)/75; x_5 = (X_5 - 1.5)/1.5; x_6 = (X_6 - 5000)/5000."

Table 2. I-optimal experimental design, with coded variables indicating the precursor concentrations (cf. Table 1), and the blocking factors to divide the 40 purée formulations into two preparation blocks ($B_1$) and ten analysis blocks ($B_2$).

<table>
<thead>
<tr>
<th>Purée ID</th>
<th>Coded variables</th>
<th>Blocking factors</th>
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<tr>
<td></td>
<td>$x_1$ $x_2$ $x_3$ $x_4$ $x_5$ $x_6$</td>
<td>$B_1$ $B_2$</td>
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<td>1 1</td>
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<td>29</td>
<td>1 1 1 1 -1 -1 2</td>
<td>2 8</td>
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Table 3. Effect estimates and significance tests for the reduced response surface model describing the furan concentrations in the thermally treated potato purées as a function of the coded precursor concentrations.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Standard error</th>
<th>DF</th>
<th>t Ratio</th>
<th>p Value</th>
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<td>Intercept</td>
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<td></td>
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<tr>
<td>Fructose</td>
<td>3.22</td>
<td>0.82</td>
<td>22.58</td>
<td>3.91</td>
<td>0.0007</td>
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<td>Glucose</td>
<td>1.84</td>
<td>0.90</td>
<td>23.88</td>
<td>2.05</td>
<td>0.0519</td>
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<td>Ascorbic acid</td>
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<td>0.93</td>
<td>23.28</td>
<td>-5.01</td>
<td>&lt;0.0001</td>
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<td>Fatty acids</td>
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<td>1.18</td>
<td>26.88</td>
<td>1.31</td>
<td>0.2011</td>
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<td>β-carotene</td>
<td>0.98</td>
<td>0.85</td>
<td>23.44</td>
<td>1.15</td>
<td>0.2617</td>
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<tr>
<td>Fructose * β-carotene</td>
<td>2.29</td>
<td>0.96</td>
<td>24.00</td>
<td>2.40</td>
<td>0.0246</td>
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<tr>
<td>Glucose * β-carotene</td>
<td>3.45</td>
<td>0.93</td>
<td>21.58</td>
<td>3.70</td>
<td>0.0013</td>
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<td>Ascorbic acid * fatty acids</td>
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<td>1.16</td>
<td>21.78</td>
<td>-2.89</td>
<td>0.0085</td>
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**Fig. 1.** Overview of the furan concentrations and their standard deviations for the thermally treated potato purées, prepared according to the I-optimal experimental design presented in Table 2. The decision limit (CCα, 1.15 ng/g purée) and detection capability (CCβ, 1.86 ng/g purée) of the analytical procedure are represented by the dashed and solid line, respectively.

**Fig. 2.** Parity plot (left) and plot of the residuals (right) for the reduced response surface model presented in Table 3. The dashed line in the parity plot represents the mean of the observed furan concentrations.
Fig. 3. Response surface plots showing the combined effect of (A) the fructose and glucose concentrations and (B) the fructose and β-carotene concentrations on the furan concentration of the thermally treated potato purées, based on the response surface model presented in Table 3. Where appropriate, the concentrations of glucose (2.5 g/100 g purée, right), ascorbic acid (80 mg/100 g purée), fatty acids (1.5 g/100 g purée) and β-carotene (5000 µg/100 g purée, left) were fixed at average levels.
Fig. 4. Response surface plot showing the combined effect of the ascorbic acid and fatty acids concentrations on the furan concentration of the thermally treated potato purées, based on the response surface model presented in Table 3. The concentrations of fructose (2.5 g/100 g purée), glucose (2.5 g/100 g purée, right) and β-carotene (5000 µg/100 g purée, left) were fixed at average levels.
**Fig. 5.** Product compositions leading to (A) minimum or (B) maximum furan concentrations in the thermally treated potato purées. The black lines show the change in the furan concentration as a function of the concentration of each precursor, given fixed values for the others (in red). The optimal settings of the precursor concentrations are also indicated by the vertical dotted lines, the horizontal dotted lines indicate the corresponding predicted furan concentration.
Fig. 6. Overview of the 2-methylfuran concentrations and their standard deviations for the thermally treated potato purées, prepared according to the I-optimal experimental design presented in Table 2. The decision limit (CCα, 1.15 ng/g purée) and detection capability (CCβ, 1.86 ng/g purée) of the analytical procedure are represented by the dashed and solid line, respectively.