Multivariate optimization of headspace trap for furan and furfural simultaneous determination in sponge cake

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ABSTRACT

Furan, a possibly carcinogenic compound to humans, and furfural, a naturally occurring volatile contributing to aroma, can be both found in thermally treated foods. These process-induced compounds, formed by close reaction pathways, play an important role as markers of food safety and quality. A method capable of simultaneously quantifying both molecules is thus highly relevant for developing mitigation strategies and preserving the sensory properties of food at the same time. We have developed a unique reliable and sensitive headspace trap (HS trap) extraction method coupled to GC-MS for the simultaneous quantification of furan and furfural in a solid processed food (sponge cake). HS Trap extraction has been optimized using an optimal design of experiments (O-DOE) approach, considering four instrumental and two sample preparation variables, as well as a blocking factor identified during preliminary assays. Multicriteria and multiple response optimization was performed based on a desirability function, yielding the following conditions: thermostatting temperature, 65 °C; thermostatting time, 15 min; number of pressurization cycles, 4; dry purge time, 0.9 min; water / sample amount ratio (dry basis), 16; and total amount (water + sample amount, dry basis), 10 g. The performances of the optimized method were also assessed: repeatability (RSD: ≤3.3% for furan and ≤2.6% for furfural), intermediate precision (RSD: 4.0% for furan and 4.3% for furfural), linearity (R^2: 0.9957 for furan and 0.9996 for furfural), LOD (0.50 ng_{furan} $g_{sample\ dry\ basis}^{-1}$ and 10.2 $ng_{furfural}$ $g_{sample\ dry\ basis}^{-1}$), LOQ (0.99 ng_{furan} $g_{sample\ dry\ basis}^{-1}$ and 41.1 $ng_{furfural}$ $g_{sample\ dry\ basis}^{-1}$). Matrix effect was observed mainly for furan. Finally, the optimized method was applied to other sponge cakes with different matrix characteristics and levels of analytes.

1. Introduction

Furan, possibly carcinogenic to humans [1], has been widely studied in food for over a decade, especially after the findings of its formation in heat-processed products [2]. It is currently considered a health concern by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) given the calculated margins of exposure in its latest risk assessment [3]. Among furanic compounds present in foods, furfural is also of particular interest. It is a naturally occurring volatile in many food items such as fruits, meat products and beverages [4,5]. In addition, furfural is used as a flavoring agent in food contributing almond, baked potatoes, bready, burnt and spicy notes [6]. Furan and furfural are present at trace levels in foods and can both be formed in thermally treated foods by close reaction pathways like caramelization and Maillard reaction. Furthermore, furfural can also be a precursor of furan under certain conditions [7]. While furan is important for food safety, furfural can be considered as a marker of aroma. Hence, these furanic compounds are complementary for assessing food quality and their simultaneous analysis is of great interest in improving the quality of thermally processed foods, i.e. mitigating furan formation while preserving food aroma attributes.

Headspace techniques are frequently applied to analyze furanic compounds in food, among which static headspace (HS) and headspace solid phase-microextraction (HS-SPME) are the most common. HS-SPME methods are recognized as being more sensitive than static HS [7], but they require fiber pre-selection as an additional step. *In situ* formation of furan has been reported both in HS-SPME [8] and HS techniques [9], related to elevated desorption and thermostatting temperatures, respectively. The headspace trap (HS trap) extraction

technique could be a suitable alternative, more reliable and less time-consuming than HS SPME and possibly avoiding artifact formation, along with higher sensitivity than HS [10]. In HS trap, vials are first heated at a fixed temperature for a constant time in order to reach equilibrium between the sample and its headspace. Afterwards, vials are pressurized and volatile analytes are released into a solid-phase trap by differential pressure, focusing in the sorbent during a given number of pressurization cycles. A dry purge can then be set in order to eliminate moisture; finally the trap is thermally desorbed and analytes are injected into the gas chromatograph [11,12]. HS trap extraction has been efficiently applied for the analysis of volatile organic compounds [13], spirits [14], apple juice [15], hop [16], cheese-related bacteria aroma compounds [17] and more recently for furan quantitation in bread crust [10]. Until now, very few studies on the determination of both furan and furfural have been carried out, namely, in coffee by HS-SPME [18] and headspace liquid phase microextraction (HS-LPME) [19], and deep-fried fish by HS-SPME [20]. Indeed, analytical challenges may arise since both compounds are often found at trace levels, for which optimization is all-important, and they exhibit different properties such as volatility and polarity. Actually, simultaneous determination may result in conflicting experimental conditions when individually optimizing each response [21]. Therefore, a multiple response optimization approach is more suitable.

Moreover, their extraction from the food matrix might be critical for their detection and consequently for their quantitation. Several factors might influence extraction, such as instrumental and sample preparation variables. A univariate approach for optimization, that is varying one factor at a time, does not take into account the possible interactions between or among variables and can give misleading results. A more reasonable alternative consists in applying a multivariate approach, that is varying several factors at a time, which can be performed by using a chemometric tool: the design of experiments (DOE) [22]. In fact, the one-variable-at-a time approach provides local information while the statistical design of experiments results in global knowledge, since it reveals significant factors and interactions [23]. Among the different types of experimental designs, the optimal design of experiments (O-DOE) has been receiving increasing attention due to its flexibility and the possibility to use several optimality criteria. For example, it can handle irregular experimental regions or other constraints [24]. In addition, DOE can be used along with response surface methodology (RSM) for optimization purposes [25].

This paper focuses on the multivariate optimization (using an O-DOE approach) and validation of a reliable and sensitive HS trap extraction method for the simultaneous determination of furan and furfural in sponge cake by GC-MS. Sponge cake has been chosen as an interesting food application for two reasons: it is a solid food matrix, made-up with several ingredients such as sugars, fatty matters and starch which lead to a complex structural and chemical composition and it is prone to contain both furanic compounds at different levels, depending on the thermal conditions applied [26–28].

2. Materials and methods

2.1. Chemicals and standards

Furan (purity: 99%), d4-furan (purity: 98%) and furfural (purity: 99%) were supplied by Sigma-Aldrich, while d4-furfural (purity: 99.7%) was obtained from CIL Cluzeau, CDN Isotopes (Pointe-Claire, Canada). Methanol (purity: 99.9%) was purchased from Carlo Erba (Val-de-Reuil, France). Ultrapure water (> 5 MΩ.cm) was produced using a Millipore Elix 3 System from Millipore SAS (Molsheim, France).

2.2. Standard solutions

Stock and standard solutions were prepared in the same room at 18 °C, while HS samples were prepared in a separate room to avoid any cross-contamination from the surrounding air. Stock solutions of d4-furan, d4-furfural, furan and furfural in methanol were prepared separately at a concentration of 2.5 g L^{-1}, put into hermetic amber glass bottles and stored at −20 °C for up to one month. For isotope dilution assays, a standard solution of d4-furan at a concentration of 25 ng µL^{-1} was prepared daily by diluting the stock solution in ultrapure water. A mixed standard solution containing 0.5 and 15 ng µL^{-1} of d4-furan and d4-furfural, respectively, was prepared daily by diluting the standard solution of d4-furan and the stock solution of d4-furfural in ultrapure water. For the standard addition method, successive dilutions of furan and furfural stock solutions in ultrapure water were made in order to obtain working solutions containing both furan and furfural at increasing concentrations according to the validation ranges (0–18.8 ng$_{furan}$ g$_{water}$$^{-1}$ and 0–563 ng$_{furfural}$ g$_{water}$$^{-1}$). All working solutions were kept at 4 °C until use. All glassware was previously baked out at 55 °C for at least 24 h to avoid any contamination prior to the analysis.

2.3. Sponge cake preparation

Sponge cake formulation and preparation were adapted from Fehaili et al. [26]: 45% pasteurized whole egg (Ovipac, Ovoteam, France), 25% sucrose (Tereos, France), 1% NaCl (La Baleine, France), 25% flour (type 55; Winny, Verdeeld door, Belgium), 4% non hydrogenated palm oil (Palva, Celys, France). Flour, palm oil and whole eggs (all stored at −20 °C) were thawed at room temperature, 4 °C and water bath at 50 °C, respectively. Sponge cake batters (500 *g*) were then prepared by mixing and beating whole eggs, sucrose and salt using a kitchen appliance (Kenwood Chef, KM300, UK) for 10 min at maximum speed. Flour was gently added during 1.5 min at minimum speed and the whole was beaten at the same speed for 0.5 min. Palm oil was gently added during 15 s at minimum speed and the batter beaten for 1 min at the same speed. Then, 20 g of batter were poured into 21 aluminum molds (8.0 cm x 4.5 cm x 3.5 cm) and baked at 170 °C for 25 min in a tailor-made instrumented oven (Bongard, Wolfisheim, France) designed to ensure thermal homogeneity [26]. Immediately after baking, the 21 sponge cakes were put into hermetically sealed glass jars and frozen at −20 °C. Frozen sponge cakes were ground in batches by means of a Grindomix GM200 knife mill, equipped with a stainless steel bowl and titan knifes (Retsch GmbH, Haan, Germany). Each batch was ground using the following conditions: 3000 rpm for 10 s, 6000 rpm for 20 s and 3000 rpm for 10 s. All batches were then mixed together at 2000 rpm for 5 s and composite samples were stored at −20 °C until analysis.

Additional samples were produced for testing the applicability of the optimized analytical method. Three new samples were prepared according to the same making procedure and formulation except for palm oil addition and baking temperature: 1) formula without palm oil baked at 170 °C; 2) formula containing palm oil baked at 200 °C and 3) formula without palm oil baked at 200 °C. These samples were designed to offer different matrix properties, as well as levels of process-induced compounds, such as furan and furfural.

2.4. HS trap/GC-MS procedure

Furan and furfural analyses were carried out simultaneously by means of a TurboMatrix Headspace Sampler HS 40 Trap (Perkin Elmer, Llantrisant, UK) equipped with an Air monitoring trap containing a two-sorbent bed, Carbotrap and Carbosieve SIII (Perkin Elmer) and a Trace GC Ultra gas chromatography system coupled to an ISQ single quadrupole mass spectrometer (Thermo Scientific, Rodano, Italy).

2.4.1. HS sample preparation

All HS vials were previously baked out in a muffle furnace at 350 °C