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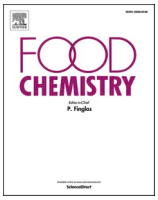

Furfural analysis of aqueous and oily foodstuffs using a single modified paper for combined headspace extraction, derivatization and paper spray mass spectrometry

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ABSTRACT

Furfurals, including 2-furaldehyde, 5-methylfurfural and 5-hydroxymethylfurfural, widely exist in carbohydrate-rich daily foods, and may have toxic effects on humans. Here, a new headspace extraction-paper spray mass spectrometry (HSPS-MS/MS) method was established for furfural detection, in which the extraction and derivatization of volatiles with pre-loaded derivatization agent on paper tips is combined with paper spray mass spectrometry for detection. By this simple and cheap approach, interference of non-volatile matrix compounds is prevented, and the derivatization agent improves electrospray-type ionization efficiency, thus increasing selectivity and sensitivity. The approach was optimized, by investigating positioning during extraction, extraction duration, derivatization agent, addition of internal standard for quantification and finally validated. For this, the developed method was benchmarked against HPLC-UV and could obtain detections limits of 0.32–0.40 μg mL^{-1} for 2-furaldehyde, 5-methylfurfural and 5-hydroxymethylfurfural in olive oil. Moreover, fast screening of free furfurals in soy sauce, coffees and teas was demonstrated with the HSPS-MS/MS method.

1. Introduction

Furfurals including 2-furaldehyde (2-F), 5-methylfurfural (5-MF) and 5-hydroxymethylfurfural (5-HMF), formed by acid-catalyzed degradation of reducing sugars or through Maillard reactions, widely exist in carbohydrate-rich daily foods (Martins et al., 2022; Shen et al., 2022). The Maillard reaction lowers the quality of food products, as it can change the food color, flavor, functional properties and nutritional value (Wu et al., 2021). The resulting furfurals are reported to be cytotoxic, genotoxic and harmful to the eyes, mucous membranes and skin when present above certain limits (Lee et al., 2019; Qiu et al., 2022). In particular, 5-HMF serves as an important indicator for the degree at which the Maillard reaction has occurred, and can be a useful tool for evaluating the freshness and quality of foods (Wu et al., 2009). Therefore, it is important to determine the concentration of furfurals in foods for quality and safety control.

Currently, various analytical techniques for the separation and characterization of furfural compounds in milk powder, vinegar, beer and other foods have been reported, such as high-performance liquid chromatography (HPLC) with ultraviolet detection (UV), HPLC coupled with electrospray ionization mass spectrometry (HPLC-ESI-MS), and gas chromatography coupled with electron ionization mass spectrometry (GC-EI-MS) (Abu-Bakar et al., 2014; Chen et al., 2023; Cui et al., 2020; Du et al., 2018; Giordano et al., 2003; Li et al., 2023; Loi et al., 2011; Wang et al., 2023; Zang et al., 2018). These reported methods all require sample pretreatment or column-based separation, e.g., to increase the extraction efficiency of 5-HMF in *Fructus Corni* (Du et al., 2018). Also, chemical derivatization is applied to increase the ionization efficiency and to enhance the sensitivity in MS (Zhang et al., 2019). GC–MS is the most widely used technology for the high throughput analysis of

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furfurals, due to their volatility and the fact that EI-MS allows for identification based on database matching. It is worth noting that due to the complexity of food samples, sample preparation is typically necessary for GC–MS. Salt-assisted liquid-liquid extraction (LLE) followed by dispersive solid phase extraction, or headspace solid-phase microextraction (HS-SPME) are commonly used, which enrich the furfurals and remove interferences.

A more convenient approach would be direct analysis with mass spectrometry, without extensive sample preparation, or chromatographic separation, which is known as ambient ionization mass spectrometry (AIMS). Such techniques (Bartella et al., 2022; Birse et al., 2022; Jastrzembski et al., 2017; Klampfl & Himmelsbach, 2015; Lara-Ortega et al., 2018; Rajchl et al., 2013) have already been demonstrated in the area of food analysis. For example, Direct Analysis in Real Time (DART) with solid-phase mesh-enhanced sorption from the headspace was applied for trace volatile analysis in grape macerates (Jastrzembski et al., 2017), and DART with high-resolution time-of-flight mass spectrometry (TOF-MS) was used for 5-HMF quantification (Rajchl et al., 2013). Low-Temperature Plasma Mass Spectrometry (LTP-MS) and paper spray mass spectrometry (PS-MS) were compared for direct olive oil analysis (Lara-Ortega et al., 2018). Amongst many AIMS approaches, PS-MS is probably one of the most straightforward and cost-effective, as it only requires a paper tip for generation of electrospray (Liu et al., 2010). The sample is added to a triangular paper substrate, where some analyte separation/extraction from matrixes can take place, and electrospray can be achieved, albeit with limited selectivity (McBride et al., 2019; Talarico et al., 2024). The type of paper used plays an important role for PS-MS, and different types of paper modification have been studied to improve the performance, and importantly the selectivity of PS-MS, including reactive paper spray (Borden et al., 2022; Feranes et al., 2020; Han et al., 2018; Liu et al., 2016; Sarkar et al., 2017). In our previous work, chemical modification of paper substrates was investigated as a means to improve selectivity to specific classes of compounds, such as boronate affinity paper spray for catecholamines, and Ti^{4+}-modified paper spray for phospholipids (Luo, van Beek, Chen, Zuilhof, & Salentijn, 2022; Luo, van Beek, Chen, Zuilhof, & Salentijn, 2023). In both approaches, the interaction between the modified paper and analytes took place at a specific pH, and the analytes could be released by selecting a different pH in the spray solvent. For such non-volatile analytes, direct extraction from a solution is required. However, when the analytes are volatile, such as with furfurals, more selective approaches are typically used, based on that very volatility.

SPME is a highly effective sample-preparation technique that combines sampling, extraction, clean up, and enrichment into a single step (Zhou et al., 2023), with various coatings and shapes of support that define the applicability of the technique (Reyes-Garces et al., 2018). This selective pretreatment method can effectively avoid contamination with high boiling point matrix constituents and has good sensitivity. Moreover, derivatization can be done on the SPME fiber itself to improve sensitivity of compounds and increase ESI-MS ionization efficiency (Zhang et al., 2019).

Considering the volatility of furfurals, and the absorptive characteristics of paper, the objective of this work was to develop a paper device that could exhibit a dual function, as (i) extraction tool instead of a headspace SPME fiber for extraction of volatiles, and (ii) direct spray substrate for MS by ESI-like paper spray. Such headspace paper spray MS (HSPS-MS) would represent a convenient, but also a cheap and environmentally friendly approach. In order to improve the direct ESI-MS ionization efficiency, derivatization was applied for furfural analysis, not only to improve the sensitivity of furfural detection, but also enhance the selectivity and on-paper retention of furfurals over other volatile compounds. The method was thoroughly validated for analysis in olive oil and benchmarked against HPLC-UV.

2. Experimental

2.1. Chemicals and materials

2-Furaldehyde (2-F) and phenylhydrazine were purchased from Titan Scientific Co., Ltd. (Shanghai, China). 5-Methylfurfural (5-MF) and 2-furoic hydrazide were purchased from Bichen Biochemical Technology Co. Ltd. (Shanghai, China). 5-Hydroxymethylfurfural (5-HMF) was purchased from Innochem Scientific Co., Ltd. (Beijing, China). 2-Furaldehyde dimethylhydrazone was purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). 2-Hydrazinopyridine hydrochloride, 2,4-dinitrophenylhydrazine and Girard's Regent P were purchased from MACKLIN Biochemical Technology Co. Ltd. (Shanghai, China). Methanol (MeOH, HPLC-grade) and acetonitrile (ACN, HPLC-grade) were purchased from Innochem Scientific Co., Ltd. (Beijing, China). Chromatography paper was purchased from J&K Scientific Co., Ltd. (Beijing, China). Filters (13 mm * 0.22 μm, Nylon 6) were purchased from BKMAM Biotechnology Co., Ltd. (Changde, China). Water was purified using a Milli-Q purification system (Millipore Corp., Bedford, MA, USA). Olive oil, soy sauce, coffee and tea were purchased from a local supermarket in Changsha (detailed ingredients of food samples are listed in the Supporting Information, SI, Table S1).

2.2. Preparation of blank paper spray tips and headspace paper spray tips

The preparation of blank paper spray tips has been described in previous work (Luo, van Beek, Chen, Zuilhof, & Salentijn, 2022; Luo, van Beek, Chen, Zuilhof, & Salentijn, 2023). Paper triangles were cut with a CUTOK DC craft cutting plotter (Hefei CNC Equipment Co. Hefei, China). The cut tips (base width = 9 mm, height = 13.2 mm) were washed with methanol twice, dried overnight in a fume hood, and stored in a clean plastic bottle.

2-Hydrazinopyridine was used as derivatization agent. 100 μg mL^{-1} 2-hydrazinopyridine solution in MeOH was prepared, and 20.0 μL of this solution were dropped on each triangular paper; these were dried and used in the next steps. These tips are from here on referred to as headspace paper spray (HSPS) tips.

2.3. Instrumental setups

Paper Spray-Tandem Mass Spectrometry (PS-MS/MS) PS-MS/MS analyses were performed by connecting a custom-made paper spray setup (see SI, Fig. S1), also described in previous work (Luo, van Beek, Chen, Zuilhof, & Salentijn, 2022; Luo, van Beek, Chen, Zuilhof, & Salentijn, 2023), with an LCMS 8040 triple quadrupole mass spectrometer (Shimadzu Corp., Japan) with unit resolution. The setup was placed in front of the mass spectrometer and the paper tips were positioned ~5 mm from the orifice. HSPS tips were placed above the sample solution in a closed vial (see also Section 2.4) for furfural extraction and afterwards, clamped with an alligator clip. Then spray solvent (20.0 μL) was dropped on the tip and spray voltage (+ 4.0 kV) was applied in positive mode to generate spray for ~1 min. Full scan mass spectra were acquired (m/z 50–300) under the following conditions: 250 °C desolvation temperature; 300 °C heating block temperature. Multiple reaction monitoring (MRM) conditions were optimized by Labsolutions software, and used for the analysis of the three furfural derivatives and internal standard (see SI, Table S2 for MRM settings, and Figs. S2 & S3 for chemical structures of the furfural derivatives and internal standard). Signals were averaged over the entire duration of the spray.

HPLC-MS/MS HPLC-MS/MS analyses were performed using an LCMS 8050 triple quadrupole mass spectrometer (Shimadzu Corp., Japan). The MS/MS conditions were same as PS-MS/MS mentioned before, except for: nebulizing gas at 3.0 L min^{-1}, drying gas and heating gas at 10.0 L min^{-1}, and interface temperature at 250 °C. A C18 column (5 μm, 250 × 4.6 mm, GL Sciences Inc., Japan) was employed for the separation of furfurals, The mobile phase consisted of (A) water and (B) methanol.

The injection volume was 10 μL. The gradient elution conditions (with a constant flow of 1 mL min^{-1} with high pressure gradient) were as follows: linear increase from 40% to 85% B in 9 min; 85% B until 11 min, then linear decrease of B to 40% in 1 min; 40% B until 20 min (Liu, 2022).

HPLC-UV HPLC-UV analyses were performed using an LCMS 8050 with ultraviolet detector (288 nm, Shimadzu Corp., Japan). The column, mobile phase and gradient elution conditions are the same as for the HPLC-MS/MS method.

2.4. Optimization of HSPS tips

The HSPS-MS method was optimized with respect to the type and amount of derivatization agent, addition of internal standard, position, duration and temperature of extraction. The final protocol used is listed here: HSPS tips were placed above 1000 μL of standard solution, after 5 h of extraction/derivatization at room temperature (25 °C), HSPS tips were analyzed by PS-MS/MS. Twenty μL of MeOH with 2-furaldehyde dimethylhydrazone (IS, 1.0 μg mL^{-1}) was loaded by micropipette on the centre of the paper tip surface as spray solvent. Eq. 1 was used for calculation of analyte concentration.

$$\textit{Analyte concentration}\ (\mu g/mL) = \frac{\left(\text{peak area of derivatives}/\text{peak area of IS}\right) - b}{a} \quad (1)$$

In which, a is the slope and b the intercept of the calibration curve. This protocol is used for all experiments, unless otherwise specified.

Derivatization agent HSPS tips were loaded in the center by micropipette with 20.0 μL of 100 μg mL^{-1} solution of different derivatization agents: phenylhydrazine, 2-furoic hydrazide, 2-hydrazinopyridine, 2,4-dinitrophenylhydrazine, and Girard's Reagent P (SI, Fig. S4). Different HSPS tips were placed above 1000 μL mL of 5 μg mL^{-1} 5-HMF in MeOH. After 5 h of extraction/derivatization, HSPS tips were analyzed by PS-MS/MS.

Addition of internal standard Internal standard (IS, 2-furaldehyde dimethylhydrazone) was added in the spray solvent to perform quantitative analysis with the HSPS tips. Six standard solutions (mixture of 0, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10 μg mL^{-1} 2-F, 5-MF, 5-HMF each in MeOH) were prepared to construct a calibration curve.

Extraction height HSPS tips were placed above 1000 μL of 5 μg mL^{-1} 2-F, 5-MF, 5-HMF in MeOH at four different heights with respect to the solvent level for 5 h (1–5 cm, SI, Fig. S5). Afterwards, PS-MS/MS was performed as described above, with 20.0 μL of MeOH with 5 μg mL^{-1} IS. All further extractions were carried out with an extraction height of approximately 1.2 cm above the solvent from position 1.

Amount of derivatization agent HSPS tips were loaded with 20.0 μL of solutions with different concentrations of 2-hydrazinopyridine (50, 100, 250, 500, 1000 μg mL^{-1}). After drying, these tips were placed above 1000 μL of 5 μg mL^{-1} 2-F, 5-MF, 5-HMF in MeOH at position 1 (SI, Fig. S5) for 5 h. Afterwards, PS-MS/MS was performed as described above. Twenty μL of MeOH with 5 μg mL^{-1} IS was used as spray solvent. The same experiments were performed with a blank MeOH solution (as comparison). A loading concentration of 100 μg mL^{-1} 2-hydrazinopyridine was used in subsequent experiments.

Extraction time HSPS tips were placed above 1000 μL of 5 μg mL^{-1} 2-F, 5-MF, 5-HMF in MeOH at position 1. After extraction/derivatization for various durations (1, 3, 5, 8, 10 h), the treated papers were analyzed by PS-MS/MS. Twenty μL of MeOH with 5 μg mL^{-1} IS was used as spray solvent. The same experiments were performed for different concentrations of mixtures of furfurals as comparison. (0, 0.1, 1.0 μg mL^{-1} 2-F, 5-MF, 5-HMF in MeOH).

Extraction temperature HSPS tips were positioned above 1000 μL of 5 μg mL^{-1} 2-F, 5-MF, 5-HMF in MeOH at position 1 for 5 h, at temperatures of 25, 30, 40, 50, 60 and 70 °C. The temperature was controlled by a water bath with thermostat. Afterwards, they were analyzed by PS-MS/MS. Twenty μL of MeOH with 5 μg mL^{-1} IS was used as spray solvent. Further experiments were conducted with extraction at 25 °C.

2.5. Analytical performance of HSPS-MS/MS

Method validation Calibration curves of standard solutions (six standard solutions of 0, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10 μg mL^{-1} 2-F, 5-MF, 5-HMF in MeOH) were measured in triplicate and constructed by adding IS to the spray solvent. Next, calibration curves were constructed for the mixtures of furfurals in olive oil, with different spiked furfural concentrations (0, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10 μg mL^{-1} 2-F, 5-MF, 5-HMF each in a mixture); every sample was measured in triplicate. The HSPS tips were placed above these samples for 5 h at position 1. Afterwards, they were analyzed by PS-MS/MS directly. The ratio between analyte (derivatives) and IS signals was used to construct a calibration curve.

Limit of detection (LOD) and quantification (LOQ) were estimated from the standard deviation of 10 replicates at the lowest spiking concentration (S_a) and the slope of the calibration curve (b): LOD = $3S_a/b$ and LOQ = $10S_a/b$.

Method comparison Five olive oil samples with different spiked concentrations in different oils were analyzed on three different days. Each sample condition was replicated six times. Experimental details for HSPS-MS/MS and HPLC-UV method validation are shown in SI, Table S3.

Accuracy was calculated as the relative deviation (%) of the calculated mean value from the actual concentration. Precision was expressed as the relative standard deviation (RSD %).

Repeatability and intermediate precision were calculated from 3 olive oil samples with the same spiked concentrations in the same oil, measured on three different days. One-way ANOVA (SPSS Statistics software) was used for statistical evaluation. Repeatability is obtained by taking the square root of the within-group mean square term, which represents the within-group variance, and relating this to the total mean. Intermediate precision is calculated by combining the within- and between-group variance components, and relating this to the total mean (Magnusson & Örnemark, 2014).

Robustness was evaluated with 3 different olive oil samples with the same spiked concentration, and statistically evaluated by one-way ANOVA (SPSS Statistics software).

The HPLC-MS/MS and HPLC-UV method were used as benchmark. Prior to injection, olive oil samples were pretreated by liquid-liquid extraction (Sun et al., 2020). The sample (1000 μL) was mixed with ACN (sample: ACN 1:1 *v*/v), and after approximately 3 min of mixing, centrifuged for 3 min at 9000 rpm, and the supernatant was taken. After repeating three times, the supernatant was combined and filtered (13 mm * 0.22 μm, Nylon 6 filter). 1000 μL filtered supernatant was taken for further analysis.

2.6. Foodstuff analysis by HSPS-MS/MS

Furfural quantitative analysis in olive oil Eight olive oil samples including four fresh (unheated) samples and four heated samples were

prepared. Fresh samples were bought from a local supermarket, and heated samples were obtained after fresh samples had been heated for 10 min at 120 °C. Then, these samples were analyzed directly by the developed HSPS-MS/MS workflow. HPLC-UV was used as benchmark.

Furfural qualitative analysis in liquid foods Nine different foods (3 soy sauce, 3 coffees and 3 teas) were bought from a local supermarket and then 1000 μL was taken directly and put in a vial without pretreatment. The samples were then analyzed directly by the developed HSPS-MS/MS method.

3. Results and discussion

3.1. Optimization of the headspace paper spray - MS/MS method

In this work, paper spray tips were used for headspace SPME, rather than coated fibers, for the extraction of furfurals. The non-polar properties of furfurals limit their ionization efficiency, while the volatility limits their prolonged residence on a solid substrate. In order to solve both issues, on-paper derivatization is applied. The derivatized furfurals will be easily protonated due to the presence of an amino group. Moreover, they will also have increased boiling points, due to the increase in molecular weight. In other words, after settling on the paper, and reacting, it would be more difficult to return to the gas phase, thus they are trapped on the paper. Based on this principle, a paper-based headspace extraction method coupled to paper spray mass spectrometry was developed.

Derivatization agent Different derivatization agents were loaded on HSPS tips and compared. Five compounds with a hydrazine group were used for the combined furfural extraction and derivatization (SI, Fig. S4). The 2-hydrazinopyridine derivative gave a good MS signal by our HSPS method, while the others did not (SI, Fig. S6). This is due to the fact that the 2-hydrazinopyridine derivative is more easily protonated, and thus this compound was selected for the subsequent experiments (Fig. 1). As high selectivity is achieved by (i) using a derivatization that is specific for aldehydes, and (ii) the use of MRM, no chromatographic separation was used in this study. Nonetheless, to prove that a chromatographic step is indeed unnecessary, a careful evaluation of potential matrix effects was carried out (see below).

To demonstrate the effect of derivatization, the paper with reactant (2-hydrazinopyridine) was compared to traditional PS-MS tips (Fig. 2). With the HSPS paper, a clear 5-HMF-derivative signal (*m/z*: 218.10, Fig. 2A) was obtained, while no corresponding MS signal was obtained with a blank paper tip (Fig. 2B). Also, a 5-HMF standard solution was analyzed by PS-MS directly without headspace extraction, and the ionization was also limited (*m/z*: 127.05, Fig. 2C), demonstrating the need for on-paper derivatization. Several steps in the analytical procedure for furfural extraction and derivatization were optimized. Below, the optimization procedures, including internal standard addition, positioning during extraction, derivatization agent, extraction time, and extraction temperature are described.

Addition of internal standard We hypothesized that the quantitative analysis of the furfurals would benefit from the choice of an appropriate internal standard (IS). 2-Furaldehyde dimethylhydrazone was chosen for this purpose, as it has a similar structure as the furfural derivatives (SI, Fig. S3), cannot react with derivatization agent or furfurals and exhibits a good response in PS-MS. The IS was added via the spray solvent. It could be shown that there is a good linearity over the range of 0.1–10 μg mL^{-1} of furfurals in methanol (SI, Fig. S7), with correlation coefficients (*r*) between 0.995 and 0.998. Accuracy and precision of this method in methanol were evaluated based on three samples with different concentrations and found to be acceptable (SI, Table S4, accuracy, −15.5 - +13.8; precision, 3.1% - 10.2%).

Extraction height During headspace analysis, samples are generally placed in sealed vials with a relatively large volume of air above the samples. Once the sample is introduced into the vial and the vial is sealed, an equilibrium will establish between the liquid phase (sample) and the gas phase (air). When the HSPS paper (or during SPME, a fiber) is introduced in the headspace, its position might affect its ability to extract furfurals. Therefore, the position of the HSPS tip was evaluated. As shown in the SI, Fig. S8, there is no significant difference for 2-F at different positions ($p > 0.05$), but 5-MF and 5-HMF show small but significant differences between the different positions. Even though position has some influence on the extraction efficiency, the variation (85%–128%, except for 5-HMF in position 2) is limited. Moreover, using a standardized inset for positioning the tip in a reproducible position, would prevent most variation. The limited variation is not unexpected, as diffusion occurs rapidly in the gas phase and thus little variation was expected in the small volume of the closed sample container (Sghaier

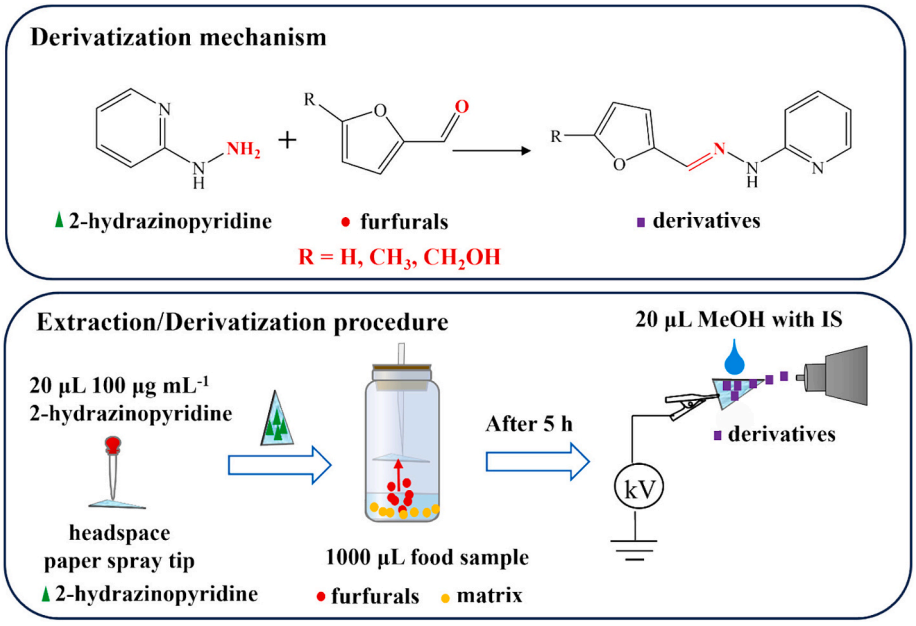

Fig. 1. (A) Overview of the derivatization reaction. (B) Schematic diagram of the HSPS-MS/MS procedure. 2-Hydrazinopyridine was dropped on a paper tip and dried. Furfurals entered the headspace from the food sample and were derivatized on the paper. Finally, the paper was analyzed by PS-MS/MS.

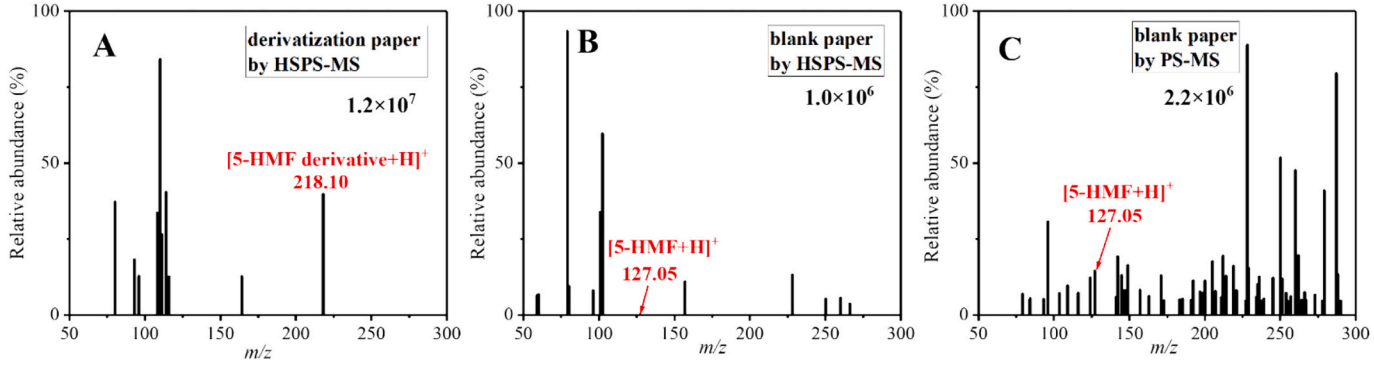

Fig. 2. Mass spectrum of 5 μg mL^{-1} 5-hydroxymethylfurfural (5-HMF) in MeOH analyzed by placing an (A) HSPS tip or (B) blank paper tip ~1.2 cm above the solution for 5 h and subsequently performing PS-MS, or (C) by performing direct PS-MS with blank paper by depositing the solution on the tip. *m/z* 218.10 is [5-HMF derivative + H]$^+$; *m/z* 127.05 is [5-HMF + H]$^+$. MRM was used to confirm identity.

et al., 2016).

Amount of derivatization agent Next, the amount of derivatization agent was optimized. 2-Hydrazinopyridine was dropped on triangular shaped paper as derivatization agent in different quantities. With an increase in the amount of derivatization agent amount, the extraction efficiency significantly increased for a 5 μg mL^{-1} furfural solution (Fig. 3). Compared with 50 μg mL^{-1} derivatization agent, the use of 100 μg mL^{-1} results in significantly more signal ($p < 0.05$) for the three furfurals. While further increases up to 1000 μg mL^{-1} generally leads to higher signal, more background signal is observed as well with a 0 μg mL^{-1} furfural solution in these cases. Taking 5-HMF as example, compared with ESI-MS/MS, a stronger background signal from a blank is observed in PS-MS/MS mode (SI, Fig. S9), which is typical for PS-MS approaches. At a concentration of 100 μg mL^{-1} of reagent, the best ratio between signal/background was observed, and this was used for all next steps. Then, the amount of derivatization agent left after extraction was determined (SI, Fig. S10). When the derivatization agent was used at a concentration of 50 μg mL^{-1}, after the extraction/derivatization procedure, only a small amount of derivatization agent could be detected, and it is thus expected that it has been almost completely consumed. In other words, 1 μg derivatization agent was used up for furfurals trapping. On the other hand, when the derivatization agent was deposited at concentrations at or above 100 μg mL^{-1}, there would be a surplus available for derivatization of all furfurals. Similar experiments were conducted for samples with higher concentrations of furfurals, to ensure that the amount of reagent loaded on the tips would not be depleted. When the concentration of furfurals increased, the required quantity of derivatization agent also increased (SI, Fig. S11), but when the loading concentration of 100 μg mL^{-1} was used, samples containing the three furfurals at concentrations up to 20 μg mL^{-1} would not entirely deplete the available reagent. 2 μg derivatization agent was used up for 20 μg mL^{-1}furfurals trapping. Therefore, this loading concentration was chosen for subsequent steps.

Extraction time Next, the extraction time was optimized. Furfurals in MeOH were extracted with HSPS tips and the extraction time was varied, as were the concentration levels of three furfurals (Fig. 4). Especially when analyzing low concentrations (1.0 μg mL^{-1}), a longer extraction time was needed to obtain sufficient signal for the product ions. Also, the signal intensity shows that 5-HMF is the most difficult to move into the gas phase. 5-HMF has the largest molecular weight and an additional polar group, resulting in a higher boiling point (291 °C at 760 mmHg), compared with 2-F (162 °C at 760 mmHg) and 5-MF (187 °C at 760 mmHg); experimental boiling points are obtained from Chemspider database. For the 5 μg mL^{-1} furfural sample solution, there is no significant difference ($p > 0.05$) between 1 h and 3 h for 2-F and 5-MF, and all three analytes show increased extraction from 3 h to 5 h ($p < 0.05$). Longer extraction times did not result in a further increase for 5-MF and 5-HMF ($p > 0.05$). Thus, an extraction time of 5 h was selected for the final protocol. When 0 μg mL^{-1} solutions were analyzed after different durations of the extraction, the previously reported background signal is stable over time. Therefore, this can be corrected for by calibration.

Extraction temperature Finally, the influence of the temperature during the extraction process was assessed, which in previous experiments was done at room temperature. As is shown in the SI, Fig. S12, an increased temperature can obviously promote the extraction efficiency, as it speeds up the evaporation of the furfurals, especially when the temperature is >50 °C, there is a significant increase in extraction efficiency. However, considering that the final application is detection of furfurals in foods, room temperature is more suitable for the final protocol, as it will not affect the foods itself. However, if faster analysis is needed, and the higher temperature would not affect the matrix or analyte formation/stability, then the application of a higher temperature for extraction might be considered. The final protocol is shown in Fig. 1.

3.2. Analytical performance of HSPS-MS/MS

Method validation As described above, there is good linearity for the calibration curves of a furfural standard solution in MeOH by HSPS-MS/MS. Here, matrix-matched calibration curves were constructed for a mixture of 2-F, 5-MF, 5-HMF in olive oil, as the solvent/sample type could affect the extraction by the developed method. HPLC-MS/MS and HPLC-UV were used as comparison (example of a chromatogram is shown in the SI, Fig. S13). The results of the HSPS-MS/MS method (SI, Fig. S14) show that analysis in this oil indeed affects the performance of the method (see Table 1), and there is an obvious matrix effect in olive oil (SI, Table S5), and thus matrix-matched calibration curves are necessary for quantitative analysis. LODs of 0.32–0.40 μg mL^{-1} (SI, Table S6) in olive oil can still be achieved. 2-F and 5-MF are not allowed in some foods, such as milk and honey (National Standard of the People's Republic of China, 2014). However, 2-F, 5-MF or 5-HMF are frequently detected in foods, such as crude palm oil, in which the detected concentration is higher than 0.4 μg mL^{-1} (Loi et al., 2011). Therefore, the achieved LOD of HSPS-MS/MS method is appropriate for fast screening of food quality. Also, precision, accuracy, repeatability, intermediate precision and robustness were evaluated by the analysis of 5 samples with different spiked concentrations, on different days, and in 3 different olive oil matrixes (SI, Table S3). The results are shown in Table 2, demonstrating precision (4.2% - 16.7%) and accuracy (−5.9% - +13.5%) are acceptable for 5 spiked samples in 3 different olive oils and analyzed on 3 different days; repeatability (9.2–12.3%) and intermediate precision (11.2–14.0%) are also acceptable for 1 sample across 3 different days according to a within-group and between-group comparison; no significant difference ($p > 0.05$) for 3 samples measured across 3 different days with the same spiked concentrations was found.

Method comparison HPLC-MS/MS and HPLC-UV methods were used as comparison for furfurals analysis without derivatization. The results

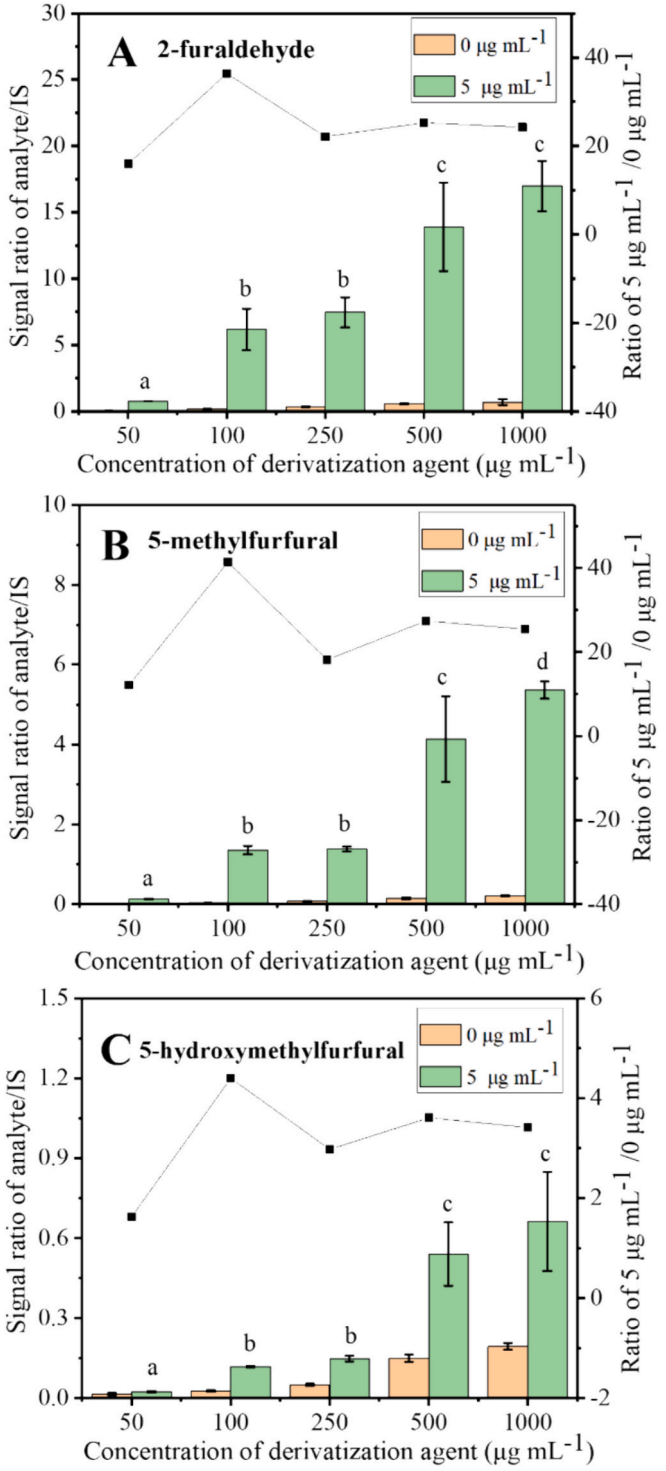

Fig. 3. Signal ratio of analyte/IS at different concentrations of derivatization agent for 5 μg mL^{-1} furfural mixture sample or blank sample. Derivatization paper tips with 50, 100, 250, 500, 1000 μg mL^{-1} 2-hydrazinopyridine were prepared. After extraction/derivatization for 5 h, the treated papers were analyzed by HSPS-MS/MS: (A) 2-F derivative (*m/z* 188.10 → 94.10); (B) 5-MF derivative (*m/z* 202.10 → 94.10); (C) 5-HMF derivative (*m/z* 218.10 → 94.10). Internal standard (*m/z* 139.15 → 44.3) was added in the spray solvent. Datapoints (5 μg mL^{-1}) that do not share a letter (a-d) are significantly different determined by one-way ANOVA ($p < 0.05$).

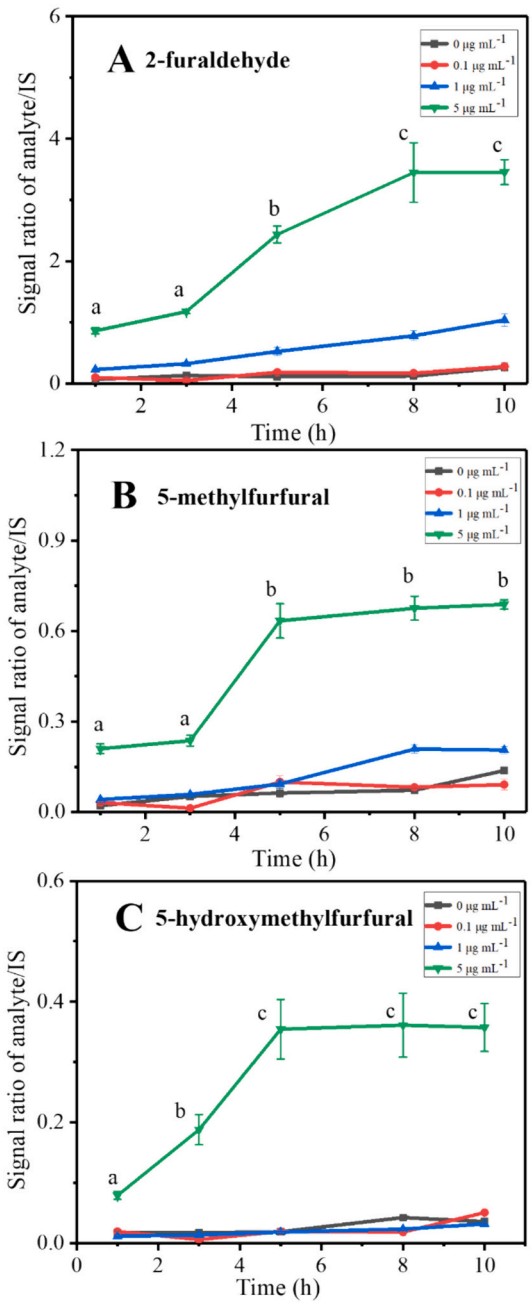

Fig. 4. Signal ratio of analyte/IS with different extraction times for different concentrations of mixed furfural samples. 0, 0.1, 1, 5 μg mL^{-1} furfural mixture was prepared. After extraction/derivatization for 1, 3, 5, 8, 10 h, the treated papers were analyzed by PS-MS/MS. Datapoints (5 μg mL^{-1}) that do not share a letter (a, b, c) are significantly different determined by one-way ANOVA ($p < 0.05$).

(SI, Figs. S15-S17) show that the LOD for the HPLC-MS/MS method is poorer than for the HPLC-UV method (SI, Tables S7 & S8), especially for 2-F, which is likely caused by its non-polar character and thus low ionization efficiency. This again shows the advantage of combining extraction and derivatization on a single substrate, such as in the developed HSPS-MS/MS method. While the HPLC-MS/MS could thus also be further improved by first derivatizing the analytes, here HPLC-UV was chosen as benchmark method. Also, HPLC-UV was used to determine 2-F, 5-MF, and 5-HMF in blank olive oil, which all were not present at or above the LOD of the HPLC-UV method. An overview of results of these calibration curves by HSPS-MS/MS and HPLC-UV is

Table 1
Analytical performance of three methods for furfurals analysis.

Method	Furfurals	Linear equation	Correlation coefficients (r)	Linear range (μg mL^{-1})	LOD/LOQ (μg mL^{-1})
HSPS-MS/MS for the determination of furfurals in fresh olive oil	2-F	$y = 0.048x + 0.080$	0.991	0–10	0.40/1.33
	5-MF	$y = 0.017x + 0.160$	0.999	0–10	0.39/1.30
	5-HMF	$y = 0.014x + 0.099$	0.971	0–10	0.32/1.07
HPLC-UV for the determination of furfurals in fresh olive oil	2-F	$y = 1.4E^4x + 0.9E^4$	0.999	0–10	0.13/0.43
	5-MF	$y = 1.8E^4x + 0.4E^3$	0.993	0–10	0.05/0.16
	5-HMF	$y = 2.8E^3x + 0.6E^2$	0.991	0–10	0.08/0.27
HPLC-MS/MS for the determination of furfurals in fresh olive oil	2-F	$y = 1.3E^4x + 1.1E^3$	0.999	0–10	1.92/6.40
	5-MF	$y = 3.7E^4x + 2.9E^2$	0.997	0–10	0.09/0.30
	5-HMF	$y = 5.5E^5x + 3.6E^4$	0.999	0–10	0.11/0.36

shown in Table 1.

According to the validation results in Table 2, as expected, HPLC has the superior performance, yet needs a column-based separation and re-equilibration of 20 min after each analysis. Moreover, while the UV detector is sensitive enough, it lacks the specificity of mass spectrometry, and MS/MS without derivatization is not sensitive enough. The developed HSPS-MS/MS can employ MS/MS as detector while achieving the derivatization and extraction at the same time. A detailed comparison between HSPS-MS/MS and HPLC-UV methods with different parameters is shown in Table 3. HSPS-MS/MS requires a long pretreatment time compared to HPLC-UV, but the steps for HSPS-MS/MS are easy, and without need for column-based separation. While there is no obvious advantage for HSPS-MS/MS when performing one single analysis (mainly due to time commitment), the time per sample dramatically decreases when 76 samples are analyzed in parallel (as done in this work for validation experiments).

3.3. Foodstuff analysis by HSPS-MS/MS

The validated quantitative method was then applied to determine the concentrations of free furfurals in different brands of olive oil (fresh/unheated and heated samples) to evaluate its applicability. HPLC-UV

Table 3
Comparison of HSPS-MS/MS and HPLC-UV method for different parameters.

Parameter	HSPS-MS/MS	HPLC-UV
Pretreatment time	5 h	20 min
Equipment or materials needed for pretreatment	glass bottle, paper, needle	vial, stirrer, centrifuge, filter
Steps for pretreatment	1	4
Equipment analytical time	30 s	20 min
Solvent for one analysis	20 μL	21 mL
Time for one sample (including pretreatment)	~ 5 h	~ 40 min
Time for fifty samples (including pretreatment)	~ 6 h	~ 17.5 h

Table 2
Precision, accuracy, repeatability, intermediate precision and robustness of HSPS-MS/MS compared to HPLC-UV in olive oil samples for the analysis of furfurals.

Analyzing day	Olive oil	Analyte	Spiked Concentration (μg mL^{-1})	HSPS-MS/MS		HPLC-UV	
				Precision (RSD, %)	Accuracy (%)	Precision (RSD, %)	Accuracy (%)
First day	Olive oil 1	2-F	0.5	16.7	+5.0	6.2	+2.5
		5-MF	2.5	17.2	+13.5	5.0	−0.1
		5-HMF	1.5	10.5	+3.2	10.1	−3.8
Second day	Olive oil 2	2-F	0.5	15.7	+12.9	5.3	+2.7
		5-MF	2.5	6.9	+5.3	5.6	+5.9
		5-HMF	1.5	5.8	+2.8	2.6	−2.0
Third day	Olive oil 3	2-F	0.5	8.1	−5.9	7.5	−4.4
		5-MF	2.5	7.9	+7.8	5.0	+3.4
		5-HMF	1.5	15.6	−0.3	3.4	−3.3
First day	Olive oil 1	2-F	0	N. D.	N. D.	N. D.	N. D.
		5-MF	1.5	7.9	+8.9	2.8	+1.9
		5-HMF	4	13.5	+6.1	4.5	+2.1
First day	Olive oil 1	2-F	2.5	13.1	+13.5	3.2	+2.3
		5-MF	0.5	17.5	+10.9	4.7	−1.1
		5-HMF	3	11.5	−2.1	7.1	−3.1
Second day	Olive oil 1	2-F	2.5	7.4	+1.8	4.7	−0.5
		5-MF	0.5	5.9	−1.0	6.0	−1.2
		5-HMF	3	7.0	+3.2	3.2	1.5
Third day	Olive oil 1	2-F	2.5	4.2	+2.1	4.1	+2.4
		5-MF	0.5	7.1	−2.4	7.2	−2.8
		5-HMF	3	11.9	+9.0	4.6	+0.3
Repeatability (RSD, 100%)		2-F	/	9.3		4.1	
		5-MF		12.3		6.4	
		5-HMF		10.4		5.2	
Intermediate precision (RSD, 100%)		2-F	/	11.2		4.2	
		5-MF		14.0		6.9	
		5-HMF		11.3		5.3	
p value for different olive oil matrix		2-F	/	0.072		0.123	
		5-MF		0.486		0.183	
		5-HMF		0.795		0.877	

N. D.: Not detected.

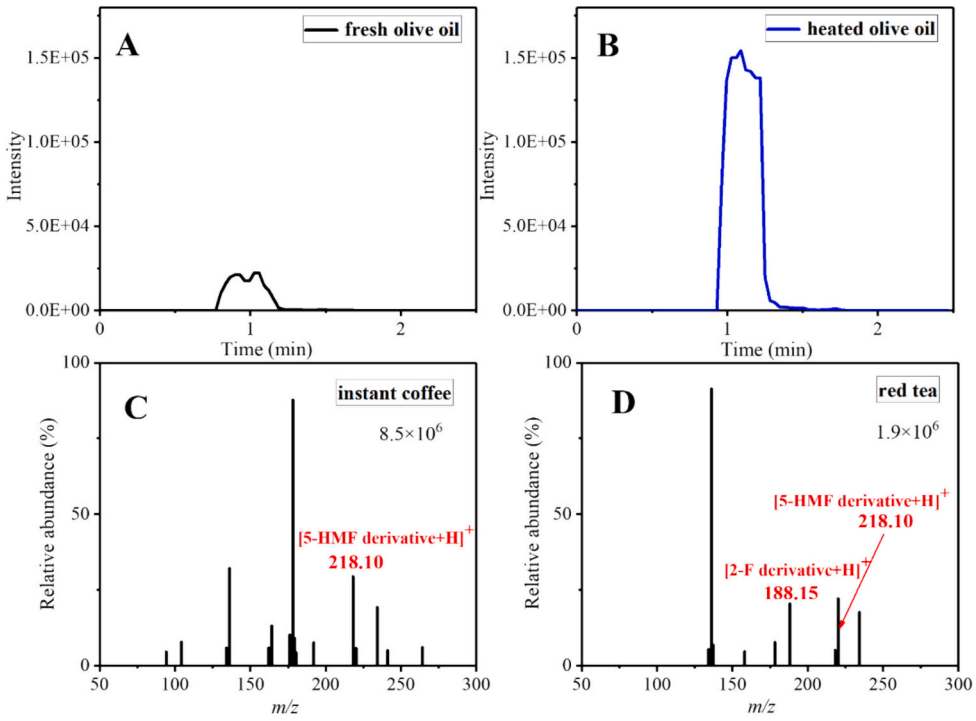

Fig. 5. Extracted ion chronogram of 5-hydroxymethylfurfural (5-HMF) in MRM mode (m/z 218.10 → 94.10) by HSPS-MS/MS for fresh olive oil (A) and heated olive oil (B), and mass spectrum of instant coffee (C) and red tea (D) by HSPS-MS/MS. m/z 218.10 is [5-HMF derivative + H]$^+$; m/z 188.15 is [2-F derivative + H]$^+$. MRM was used to confirm identity.

was used for comparison. As summarized in the SI, Table S9, 5-HMF was detected in all the investigated heated olive oil samples and no furfurals were detected in fresh olive oil samples by both methods. The extracted ion chronogram of 5-HMF in MRM mode for fresh and heated olive oil is shown in Fig. 5A & 5B. In fresh olive oil, no signal above the LOD could be observed for 5-HMF. On the other hand, for heated olive oil, there is an obvious increase in 5-HMF above the LOD. Mass spectra of fresh and heated olive oil are shown in the SI, Fig. S18.

The developed HSPS-MS/MS was also used for the fast screening of free furfurals in some other kinds of foods to demonstrate its versatility. Different brands of soy sauce, coffee and tea (3 products for each sample type; 9 samples in total) were bought in a local supermarket. These foodstuffs are all liquid and could be directly analyzed by the developed HSPS-MS method. As is shown in Fig. 5C & 5D, 5-HMF could be detected in one instant coffee, and both 2-F and 5-HMF have been detected in one red tea. Mass spectra of the other samples without detected furfurals are shown in the SI, Fig. S19. While such screening is only qualitative, as appropriate calibration will need to be developed for each matrix, it shows the broader applicability of this method.

4. Conclusions

A new combined paper-based headspace extraction-paper spray mass spectrometry method was established for furfural detection. It is based on the extraction and derivatization of volatiles with a pre-loaded derivatization agent, 2-hydrazinopyridine, on paper tips. We demonstrated the selectivity of this approach, since the extraction removes the interference of non-volatile matrix compounds, while the derivatization improves the detectability by MS and on-paper retention of the furfurals, simultaneously increasing both the selectivity and sensitivity. This developed method can be used for furfural enrichment from foods, with e.g. LODs of 0.32–0.40 μg mL^{-1} for 2-furaldehyde, 5-methylfurfural and 5-hydroxymethylfurfural in olive oil. This method is easy to use, with short analysis times, since foods can be analyzed directly without complicated pretreatment. Even though one extraction is optimized for a long duration of 5 h per sample, many food samples can be extracted simultaneously, which is quite convenient and faster compared to HPLC when analyzing e.g. fifty or more samples. Results show that an increased temperature can promote the extraction efficiency, and thus reduce extraction time, which might be optimized for specific applications to increase the throughput. The method has potential for the application to analysis of different kinds of foods and applied for food quality control, as preliminarily demonstrated for soy sauce, and instant tea and coffee. Also, the methodology might be translatable to the analysis of other volatile compounds, if appropriate derivatization chemistry can be applied.

CRediT authorship contribution statement

Wei Luo: Writing – original draft, Methodology, Investigation. **Yu Qin:** Methodology. **Teris A. van Beek:** Writing – review & editing. **Bo Chen:** Writing – review & editing, Supervision, Funding acquisition. **Han Zuilhof:** Writing – review & editing, Supervision, Funding acquisition. **Gert IJ. Salentijn:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.140481.

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