FURANEOL® AND MESIFURAN IN STRAWBERRIES – AN ANALYTICAL CHALLENGE

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Abstract

2,5-Dimethyl-4-hydroxy-3(*2H*)-furanone (Furaneol®) and 2,5-dimethyl-4-methoxy-3(*2H*)-furanone (mesifuran) are very important flavour compounds in various types of foods, as for example in strawberries. Due to the chemical and physical properties, resulting in a high affinity to the strawberry matrix especially of Furaneol®, the analysis is rather complex. It was the aim of this study to compare different extraction methods (i.e. headspace- as well as direct immersion-solid phase microextraction and solid phase extraction) and to develop a sensitive and reproducible analytical method for the quantitative determination of the two compounds. Solid phase extraction after aqueous extraction of the analytes from the strawberry matrix on a polymeric reversed phase matrix turned out to be the method of choice. With this method, Furaneol® as well as mesifuran can be easily extracted from the strawberries with high reproducibility and sufficient sensitivity. Quantitative determination is performed by using gas chromatography on a polar analytical column.

Introduction

2,5-Dimethyl-4-hydroxy-3(*2H*)-furanone (Furaneol®, DMHF) and 2,5-dimethyl-4-methoxy-3(*2H*)-furanone (mesifuran, DMMF) (Figure 1) are very important natural flavour compounds in various fruits. Furaneol® is for example considered to be an impact compound for strawberry flavour.

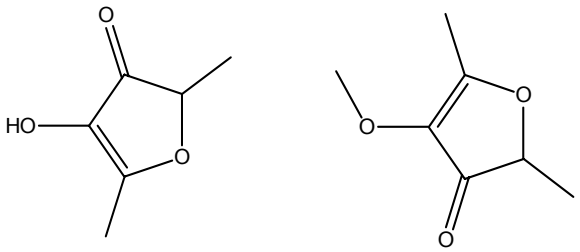

Figure 1. *Chemical structures of Furaneol® and mesifuran.*

Both compounds show very low odour threshold values (thresholds in water $4*10^{-5}$ mg kg^{-1} for Furaneol®; $3*10^{-5}$ mg kg^{-1} for mesifuran). Performing sensory evaluation, Furaneol® is described as caramel, burnt sugar-like and mesifuran as a smell similar to fermented fruit, and earthy-moldy (the descriptors were given by our trained sensory test panel from aqueous solutions of the compounds). For strawberries, very large differences in concentrations have been reported for both compounds (0-37 mg

kg^{-1} for Furaneol® (1, 2, 8) and 0-23 mg kg^{-1} for mesifuran (3, 4, 8)). Reasons for these variations in concentrations may be found for example in different strawberry varieties, or differing ripening stages of the fruits.

Regarding the facts that both compounds are thermally instable and that both show varying stability depending on the pH value (5, 6) in combination with the high affinity of Furaneol® to the strawberry matrix based on its pronounced polarity, the large variations in concentrations reported for strawberries might also be due to parameters used for the extraction and the subsequent analysis, respectively. As a consequence, we aimed to develop a sensitive and highly reproducible analytical method for the quantitative determination of Furaneol® and mesifuran from the strawberry matrix. Three different analytical approaches were chosen: (i) headspace solid phase microextraction (SPME), (ii) direct immersion SPME, (iii) solid phase extraction (SPE). The evaluation of the methods was mainly based on the ability to extract both compounds simultaneously, as well as on parameters like reproducibility, selectivity and sensitivity to the target compounds.

Experimental

Headspace SPME. 0.5 g, 2.0 g or 5.0 g of homogenized strawberries were transferred into a 20 mL headspace vial. For the sample development differing amounts (0.5 g or 2.0 g) of either NaCl or Na_2SO_4 were added. 1,2,3-Trichloropropane (50 µg kg^{-1}) was added as internal standard. The sample was stirred thoroughly using a glass coated magnetic stirrer. After 5 minutes equilibrium time, headspace sampling was performed over a period of 30 min at a sampling temperature of 45°C or 65°C using a DVB/Carboxen™/PDMS fibre (2 cm stable flex). After sampling, the SPME fibre was immediately transferred into the GC-injection port for thermodesorption (injector temperature: 270°C). The fibre was kept in the injector for 10 minutes. The GC separation was performed on an HP5 column (column dimensions 30 m x 0.25 mm x 1.0 µm). The detection was performed by mass spectrometry in the scan mode as well as in the selected ion mode (*m/z* 128 and 85 for Furaneol®, *m/z* 142 and 71 for mesifuran).

Direct immersion SPME. Experiments for the direct immersion SPME were performed from aqueous solutions (sample amount 10 mL) containing about 1 mg L^{-1} per compound. Different SPME fibres (i.e. PEG, PDMS/DVB, PUA, PDMS, PA, Carboxen™/PDMS, DVB/Carboxen™/PDMS) were investigated with respect to their ability to enrich Furaneol® and mesifuran equally reproducibly. The fibres were immersed into the aqueous solution which was stirred at room temperature for 20 min. Afterwards, thermodesorption was performed in the injection port of the GC at 250°C or 270°C (injector temperature was depending on the used fibre material). The influence of NaCl-addition on the yields and the reproducibility was investigated (addition of 4 g NaCl per 10 mL aqueous sample). The GC separation was performed on an HP Innowax (column dimensions 30 m x 0.25 mm x 0.5 µm). The detection was performed via mass spectrometry in the scan mode as well as in the selected ion mode (*m/z* 128 and 85 for Furaneol®, *m/z* 142 and 71 for mesifuran).

Solid phase extraction (SPE). 1.0 g of homogenized strawberries was mixed thoroughly with 50 mL buffer solution (potassium tartrate with tartaric acid; pH ~3.5). Maltol (5 µg absolute) was added to the extract as internal standard. After centrifugation, an aliquot of 10 mL was transferred onto the SPE cartridge (Strata X, polymeric reversed phase, particle size 33 µm, 500 mg/3 mL; Phenomenex). The SPE cartridge was conditioned with 5 mL methanol and 5 mL deionised water prior to

use. The elution of the analytes was performed with acetone (1 mL was discarded, 2 mL were collected). After centrifugation, the eluent was injected directly into the GC. The GC separation was performed on a DB Wax (column dimensions 30 m x 0.32 mm x 0.25 µm), the detection was performed by FID. The identity of the compounds was confirmed by GC-MS analyses.

Results and Discussion

Headspace SPME. Headspace SPME is a well suitable method for the determination of volatile compounds from various food matrices. This was shown several times in literature. In our studies concerning the flavour of strawberries, headspace SPME is also used in order to gain 'aroma profiles' of the investigated fruits. Analyzing these profiles, it turned out that mainly for the determination of Furaneol®, but also for mesifuran, the used parameters did neither yield in sufficient recoveries nor high reproducibilities for the two compounds. In order to enhance the performance of the method, several parameters were modified: (i) the sample amount was varied (0.5 g, 2.0 g, 5.0 g), (ii) different sampling temperatures were chosen (45°C, 65°C), (iii) different types of salt were added in different amounts. The results showed that none of the taken actions could increase the performance of the extraction with regard to the furanoid compounds. While other volatile compounds like various esters or aldehydes can be determined with high reproducibility, the two compounds of interest, especially Furaneol®, could not be determined neither with sufficient yields, nor with high reproducibility using headspace SPME.

Direct immersion SPME. Based on the results obtained from headspace SPME, we concluded that the high affinity of Furaneol® based on its high polarity could be the reason for the bad reproducibility obtained in headspace analysis. As a consequence, we moved to direct immersion SPME, in order to avoid the transfer of Furaneol® and mesifuran from the sample matrix into the headspace. Method development was started with aqueous solutions of Furaneol® and mesifuran. Seven different SPME fibre types were investigated. The only fibre that enriched Furaneol® as well as mesifuran in reasonable amounts was a DVB/Carboxen™/PDMS fibre (2 cm stable flex). The addition of NaCl – in order to increase the extraction yield – led to a drastic decrease in the extraction yield for Furaneol®, whereas the yields for mesifuran increased. In addition, the reproducibilities even from the aqueous solutions were very poor for either compound. Due to those problems, which already arose in the aqueous solution without the presence of any further fruit components, we did not follow this way any further.

Solid phase extraction (SPE). The group of *V. Ferreira* recently showed a quantitative way for the enrichment of Furaneol® from wine using SPE on chemically modified reversed phase material (styrene-DVB) (7). For the extraction of Furaneol® and mesifuran from the strawberry matrix, we modified the procedure that was described in (7). The analytes were extracted out of the strawberries with a tartaric acid buffer at the pH of the strawberry matrix (pH 3.5). Maltol was added to the system as internal standard to control the procedure. After centrifugation, an aliquot of the extract was directly transferred onto the preconditioned SPE cartridge. Amongst various polar solvents that were tested for the elution of the analytes, acetone showed the best performance for this task. This fairly simple method shows high linearity (for Furaneol® as well as for mesifuran from 0.25 to 200 mg kg^{-1} strawberries), high recoveries of the analytes (95-110%) as well as high

reproducibility. The quantitative procedure was fully validated within this concentration range.

Based on these results, we selected the solid phase extraction as the method of choice for the extraction of Furaneol® and mesifuran out of the strawberry matrix. To demonstrate the applicability of the method to the strawberry matrix, Table 1 shows Furaneol® and mesifuran concentrations of different strawberries varieties determined by this method. For future studies, this method will be used to determine the influence of various external parameters on the flavour formation in the strawberry plant and the fruit.

Table 1. *Furaneol® und mesifuran concentrations in different strawberry varieties.*

Strawberry variety	Furaneol® conc. [mg kg^{-1}]	Mesifuran conc. [mg kg^{-1}]
Elsanta	30.7	7.3
Senga Sengana	17.4	5.9
Antea	24.3	4.8
Woodland strawberry (*Fragaria vesca*)	22.4	4.7

Acknowledgement

This work was supported by the Austrian Research Promotion Fund (FFG) and by Grünewald.

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