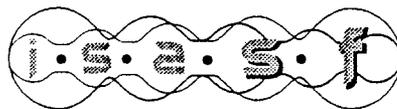


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COMPARISON OF LIQUID MODIFIERS USED WITH SUPERCRITICAL CARBON DIOXIDE AND THEIR EFFECT ON ANALYTE YIELD AND THE AMOUNT OF INTERFERENCES IN THE RESULTANT EXTRACT

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Rolled oats samples spiked with deoxynivalenol (DON) were extracted using ISCO Model 3560 automated supercritical fluid extractor, equipped with dual pumping capability. Methanol, acetonitrile, water and their mixtures were used as static and dynamic modifiers with supercritical carbon dioxide. Initially, the extraction results using just methanol were compared with those using acetonitrile, and mixtures of these two liquids as a modifier. It was found that the identity and concentration of the modifier had a prominent effect on the amount of interferences found in the HPLC analyses (as judged from the total HPLC chromatogram peak area). The highest recovery of DON was obtained with methanol, but mixtures of methanol and acetonitrile gave similar results. Next, liquid modifiers were added directly on to the sample prior SFE, and the SFE was performed dynamically with acetonitrile as a modifier in CO₂. The yield of hexane-soluble fat increased as the amount of static modifier increased, the modifier being acetonitrile, or a mixture of methanol and acetonitrile. In contrast, the amount of added acetonitrile/water (84:16) had no effect on the yield of fat. It was also found that the more water was added as a static modifier, the less fat was extracted. However, the yield of the analyte was found to be higher when adding the 84:16 -mixture of acetonitrile/water, or neat water as static modifiers on to the sample. As a practical compromise, the 84:16-mixture was used as the dynamic modifier. The modifier concentration (5-20 %) in the carbon dioxide affected the analyte yield, which ranged from 40 to over 100 % recovery. However, the effect on fat yield was negligible.

BACKGROUND

In analytical methods for the determination of different mycotoxins in food commodities, the isolation and clean-up procedures needed prior the quantitation are the most tedious steps. Supercritical fluid extraction (SFE) has shown some potential in replacing the liquid organic solvents in the isolation of aflatoxins, fumonisins and trichothecenes [1-5]. In previous studies

we have used methanol as a modifier with supercritical carbon dioxide for the SFE of trichothecene mycotoxins from grains and cereals [6-7]. The use of modifier in carbon dioxide was necessary to obtain a decent recovery of deoxynivalenol (DON), which is a moderately polar non-volatile compound. These relatively harsh SFE conditions resulted in extracts having a number of unidentified compounds. These coextractants interfered with the quantitation of the mycotoxin moieties and clean-up methods were needed to prior HPLC analysis to solve the problem. The aim of this investigation was to evaluate whether other liquid modifiers could replace methanol without affecting the analyte yield; yet simultaneously decrease the amount of interfering compounds in the extracts.

MATERIALS AND METHODS

The rolled oats samples (about 2 g) spiked with about 1 ppm deoxynivalenol (10 μ l, dissolved in methanol) were added into extraction cells (volume 10 ml) between layers of granular anhydrous sodium sulfate. ISCO model 3560 automated extractor with dual pumps was used in this study. Methanol, acetonitrile, water and their mixtures were used as liquid modifier with supercritical carbon dioxide. The modifier was introduced to by adding 0.3-1.4 ml of the liquid directly to the sample in the cell (i.e., static modifier addition), and/or by using the modifier pump (dynamic modification, 5-20 %, v/v). The other SFE conditions were kept constant: the extraction pressure was 5400 psi (37.2 MPa), the temperature 60 $^{\circ}$ C, the flow rate 0.6 ml/min, and the extractives collected into methanol (5 ml). The SFE protocol had a short static extraction step, which facilitated the better interaction of the fluid and the modifier with the sample matrix prior the dynamic extraction. The extracts were defatted using 2 x 2 ml hexane, which yielded the gravimetric amount of fat extracted from the sample (% fat). The collection solvent was evaporated into dryness and the extract was reconstituted into HPLC mobile phase. The amount of the analyte, DON was determined quantitatively and interfering compounds qualitatively from the HPLC-UV chromatograms. The methods used are described in more detail in reference [8].

RESULTS AND DISCUSSION

The average composition (fresh weight basis) of rolled oats is as follows: about 60 % of carbohydrates, 15 % of protein, 7-10 % of fat, and 5 % of dietary fiber. The water content is about 10-15 %. The components which are readily soluble in the fluid are volatiles, some additional, more polar molecules, and fatty constituents. Many of the most volatile compounds are lost during the collection and evaporation steps, because no attempt was made to limit their loss. Still, due to the nonspecific detection system (UV at 222 nm) several unidentified components were found in the HPLC-chromatograms of the extracts. The effects of different modifiers on analyte yield and the amount of fat extracted are shown in Figures 1, 2, and 3. In figure 1 are shown the analyte peak heights obtained by HPLC, when methanol, acetonitrile and their mixtures were used as dynamic modifiers with supercritical carbon dioxide. It was apparent that the nature of the modifier had an effect on the semi-quantitative yield of interferences as judged from the total peak area (not shown here). The amount of

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interferences in the chromatograms seemed to be parallel with the yield of the analyte. The highest yield of DON was obtained with methanol, but mixtures of methanol and acetonitrile also gave similar results. When the dynamic SFE using the modifiers shown in the Figure 1. were performed, the yield of fat did not vary much with the modifier concentration (5-20 %, V/V).

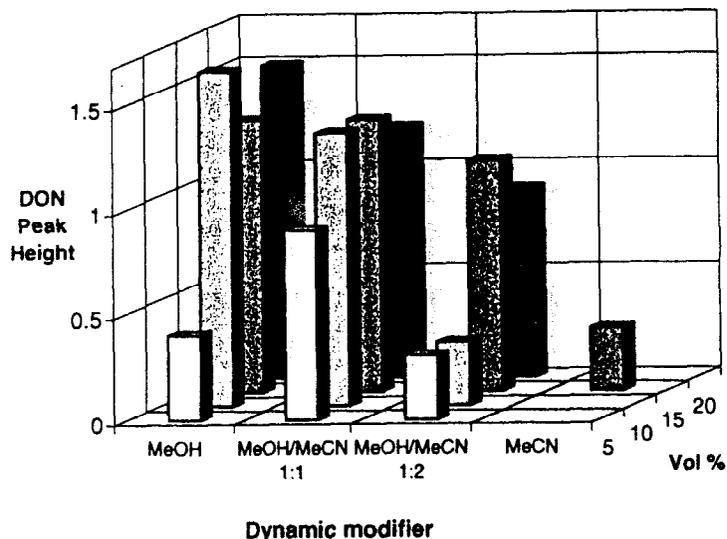
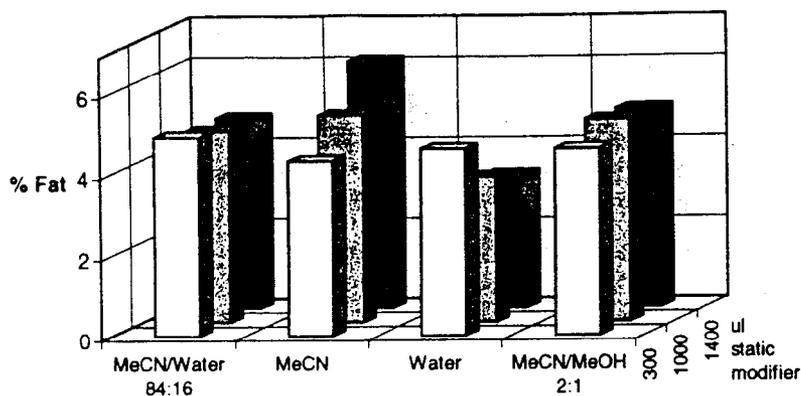


Figure 1:
 The yield of DON when methanol (MeOH), acetonitrile (MeCN) and their mixtures are used as dynamic modifiers (5-20 %, V/V) in supercritical carbon dioxide.

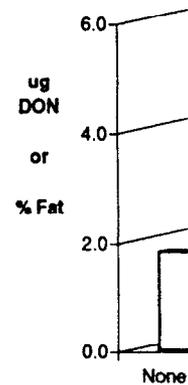
Next liquid modifiers were added directly on to the sample prior SFE (static modifiers), and the SFE was performed using acetonitrile (15 %, V/V) as a dynamic modifier in CO₂. In Figures 2 and 3 are shown the yield of the analyte, and the amount of fat extracted. The yield of hexane-soluble fat depended on the amount of static modifier added, when the static modifier was acetonitrile, or a mixture of methanol and acetonitrile (Figure 2). However, the amount of added acetonitrile/water (84:16), which is usual solvent for the liquid solvent extraction of trichothecenes, did not affect the yield of fat (Figure 2). Also it can be seen in the Figure 2, that the more water was added, the less fat was extracted.



Dynamic extraction: 15 % (v/v) MeCN in CO₂

Figure 2: Comparison of some static modifiers by means of the amount of fat extracted (Fat %).

The yield of the analyte was found to be higher when adding acetonitrile/water (84/16), or neat water as static modifiers on to the sample, whereas the use of methanol, acetonitrile, or their mixture did not affect the yield, when compared with dynamic extraction using 15 % (v/v) acetonitrile in carbon dioxide (marked "None" in Figure 3). The additional water inhibited the SFE of fat from the matrix, when compared with other liquid modifiers. The aliquot of water added corresponds the water content of 25 %, which increases the moisture of the rolled oats sample up to approx. 40 %. The possible reasons for inhibition of the SFE of fat at higher water levels have been discussed previously [9-10]. In contrast, the explanation for the higher extraction yield of the analyte is not so obvious. It has been noted, that the high water content of the sample increases the solubility and/or extractability of the more polar constituents in the SFE processes [11], but the actual mechanisms are not understood.



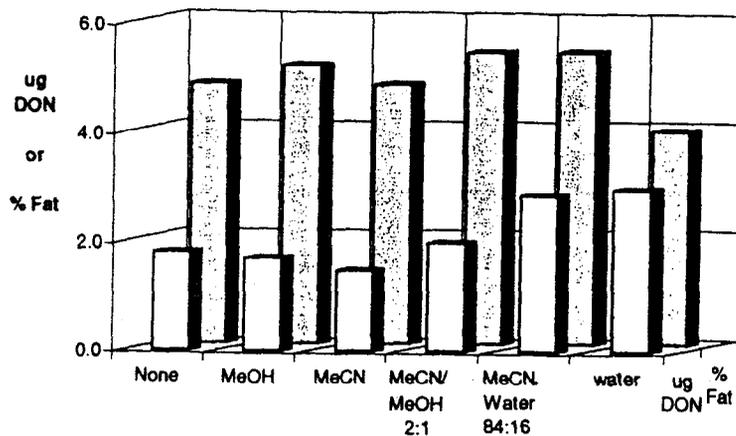
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Figure 3: The yields of the analyte for acetonitrile modified

The moistening of the sample during preparation. For the dynamic modifier. The analyte yield, which is negligible (ranging from naturally contained extraction [8]).

CONCLUSIONS

The present study shows that carefully choosing the static modifiers and the careful selection of the dynamic modifiers are essential for the a



Static modifier volume 500 μ l ; Dynamic SFE: 15 % MeCN in CO_2

Figure 3:

The yields of the analyte and coextracted fat using different static modifiers (500 μ l) with acetonitrile modified supercritical carbon dioxide.

The moistening of the samples prior SFE however would add one more step to sample preparation. For that reason, the acetonitrile/water (84:16)-mixture was chosen as the dynamic modifier. The modifier concentration (5-20 %) in the carbon dioxide affected the analyte yield, which ranged from 40 to over 100 % recovery, but the effect on fat yield was negligible (ranging from 4.3 to 4.6 %). However, when applied to the isolation of the analyte from naturally contaminated wheat and oat grains the SFE was not as efficient as liquid solvent extraction [8].

CONCLUSIONS

The present study shows that sometimes selectivity towards a target analyte can be obtained by carefully choosing the identity, the amount, and the mode of addition of the liquid modifier. The careful selection of the modifier is particularly needed in those cases, where the modifiers are essential for the adequate recovery of the analyte, and the coextractives in the SFE-derived

extracts interfere with the subsequent processes or analytical methods. Still, in each case, the role of modifiers needs to be studied experimentally, since our knowledge on the interactions of the modifier and/or modified fluid with the matrix components (including the analytes) in SFE processes is limited.

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ABSTRACT

A nonrecirculated solvent was used to extract oil from chopped seeds using supercritical fluid extraction. Operating conditions were optimized for maximum oil solubility, the oil-CO₂ was liberated to the atmosphere. Results showed that the solubility was exponential with increasing temperature. At 10% butanol, the solubility was higher. On the other hand, the addition of small quantities of phospholipids (PL) to the solvent increased the solubility. Finally, co-solvents were analyzed.

INTRODUCTION

Previous studies have shown that the extraction of oils from seeds using supercritical fluid extraction is similar or better than that of conventional solubility was typical. It was found that the addition of small quantities of phospholipids (PL) to the solvent increased the selectivity of the extraction.

Modifiers can be used to modify the solvent. Several authors have shown that the addition of phospholipids (PL) to the solvent increases the solubility of the co-solvent and the selectivity of the extraction.