The Micro-Extraction and Detection of Phenolic Anti-Oxidants from Cereal Products Using MEPS™-GCMS

Introduction

Synthetic phenolic antioxidants are increasingly rejected as acceptable food additives because of their demonstrable or suspected adverse effects on human health. Among the compounds of concern are the butylated hydroxyanisoles (BHA) and butylated hydroxytoluene (BHT), synthetic phenolic antioxidants which are used to stabilize fats and oils at elevated temperatures.

Micro-extraction Packed Sorbent (MEPS™) is a solid-phase technique that allows rapid sample extraction by reducing the volume of sample processed. Because the sorbent device is incorporated directly into a liquid handling syringe, it may also be used with robotic autosamplers for on-line chromatographic analysis. In this example, rice crackers manufactured with sunflower oil that was stabilized with BHA were extracted and analyzed with either water or methanol-water.

Experimental

Plain rice crackers were purchased from a local supermarket. The labelled contents were rice flour (95%), sunflower oil (contains antioxidant 320), salt, sugar, maltodextrin from maize and flavour enhancers.

Method 1: Plain rice crackers (2210 mg) were crushed and suspended in water (20 mL) and then allowed to steep for a further 60 minutes. The gelatinous mass was centrifuged in glass test tubes at 2500 rpm for 10 minutes and the clear liquid pipetted to a new tube for analysis. Recovery of the water was approximately 10% by volume with the remaining volume inseparable from the gelatinous mass.

Method 2: Plain rice crackers (2210 mg) were crushed and suspended in methanol (10 mL) for 30 minutes. The methanol was pipetted to a clean vial and diluted with an equal volume of water.

A C18 MEPS™-BN in a 100 µL syringe was conditioned with methanol (20 µL) and water (20 µL) at 10 µL/sec. The diluted sample (100 – 1500 µL) was loaded to waste in 10 cycles at 10 µL/sec. The sorbent device was incorporated directly into a liquid handling syringe for one-off sample processing. Because the sorbent device is incorporated directly into a liquid handling syringe, it may also be used with robotic autosamplers for on-line chromatographic analysis. The extracted sample volume was 100 µL.

Results and Discussion

Liquid samples were obtained from the rice crackers using two alternative techniques: the first was suspension in water and the second was by extraction into methanol. As the crackers contained both starch (rice flour) and other phenolic compounds, such as coumarin, 4-vinyl-2-methoxyphenol and 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionic acid (BHPP Me). The panel on the right shows the Extracted Ion Chromatogram for m/z 165 for the same analyses.

Extraction of the rice crackers with methanol and subsequent dilution of the methanol with water gave a straightforward method for separating the extracted components from the rice flour base. However, although methanol is a generally poor solvent for aliphatic compounds, it did dissolve a portion of fatty acid (with a portion esterified to methyl esters) and fatty acid glycerides from the crackers. These materials were strongly retained from the diluted sample by the C18-MEPS™ sorbent and eluted from the sorbent by sequential elutions of methanol.

The free acid is a known additive of acrylonitrile-butadiene-styrene polymers of the type used in the packaging and the same compound was detected following extraction of the packaging material with methanol. Methanol is presumably responsible for the in situ esterification of the acid and fatty acids from the sample, either on standing or by the methylolation.

The meat and bone extract of the crackers presents an interesting dynamic for solid-phase extraction methods. The matrix is sufficiently oily that it is co-extracted with the methanol and retained on the C18 sorbent. This material has the potential to act as a competitive sorbent for the desired analytes. The retained oil is also likely to consume much of the sorbent capacity and so decrease the breakthrough volume for extraction (Figure 3) as well as contributing to carryover and incomplete elution of fatty acid methyl esters and monoglycerides.

Conclusion

A simple and rapid MEPS™-GCMS method is presented for the detection of butylated hydroxyanisoles and other phenolic compounds in cereal products such as rice crackers. Sample processing time, including sorbent conditioning and recycle time, was less than 5 minutes for samples of 1 mL in volume. The technique also permitted the detection of phenolic and other compounds that were common to the plastic packaging in which the crackers were presented.