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

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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 hydroxyphenols such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) and bisphenol A. Simple methods for the removal of food matrices are necessary for the detection of these compounds in regulatory compliance programmes.

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 used with robotic autosamplers for on-line chromatographic analysis. In this example, rice crackers manufactured with sunflower oil that was stabilized with BHA (Figure 1) were crushed and extracted with either water or methanol-water.

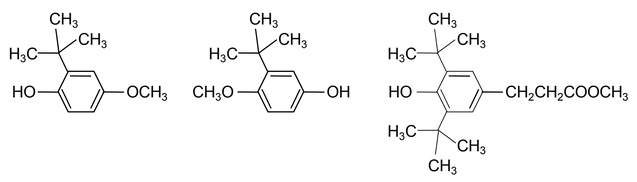

Figure 1: Butylated hydroxyanisole (BHA) can be either 2-butyl-4-methoxyphenol or 3-butyl-4-methoxyphenol. Some samples also showed the presence of methyl 3-(3,5-ditert-butyl-4-hydroxyphenyl) propionate (BHPP Me).

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 (627, 631). Packaging consisted of an ABS tray and unidentified outer plastic wrapper.

Method 1: Plain rice crackers (2210 mg) were crushed and suspended in water (20 mL), sonicated for 10 minutes 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 (2230 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™ BIN on 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 was washed with water (20 µL) and dried with air (3 x 80 µL) at 80 µL/sec. The cartridge was eluted with methanol (20 µL) and the fraction analyzed directly. Processing time was less than 3 minutes for a 200 µL sample.

Gas Chromatography Mass Spectrometry was performed on a 6890GC-5973N MSD (Agilent Technologies, CA, USA) equipped with an ETP electron multiplier (SGE Analytical Science, VIC, Australia) and a BPX5 column (30 m x 0.25 mm i.d., 0.25 µm film thickness, SGE). Injections of 2 µL were splitless at a temperature of 200 °C. The purge flow was 50 mL/min with a nominal inlet pressure of 92 kPa. The oven temperature was programmed from 50 °C (hold for 2 min) to 300 °C (hold for 3 min) at 20 °C/min. The carrier gas was helium at a flow rate of 1.5 mL/min in constant flow mode. EI mass specta were collected over the range 50-550 Da at 2 scan/sec. The transfer line temperature was 280 °C, the quadrapole was 150 °C and the source was 230 °C. Chromatographic data was acquired and processed using ChemStation software (Version 100 D.02.00.275, Agilent Technologies).

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 sunflower oil, both techniques yielded fractions that were complicated by matrix coextractants.

By avoiding a long predigestion step to breakdown the polysaccharide content, the aqueous extract was free from a high concentration of low molecular weight sugars but also gave a low yield of aqueous material for further processing. Not surprisingly, the aqueous fraction gave a very clean extract when prepared using a C18 MEPS™ in combination with a water wash to elute the weakly bound fraction (Figure 2). BHT and other phenolic components were readily detected with some components attributable to the cracker and others probable contaminants from the packaging. Carryover following a single wash of the sorbent with one bed volume of methanol was less than 5 % for BHA. Carryover was effectively eliminated by five methanol washes of 2 bed volumes each (20 µL) to waste.

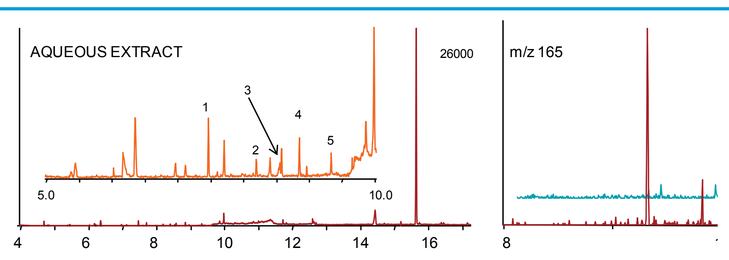

Figure 2: The Total Ion Chromatogram (right) and expanded TIC (inset) for a C18-MEPS™ fraction obtained from the aqueous preparation of rice crackers. Phenolic components are coumarin (1), 4-vinyl-2-methoxyphenol (2), 4-hydroxybenzaldehyde (3), vanillin (4) and BHA (5). The panel on the right shows the Extracted Ion Chromatogram for BHA for the first methanol elution and carryover into the third elution with methanol.

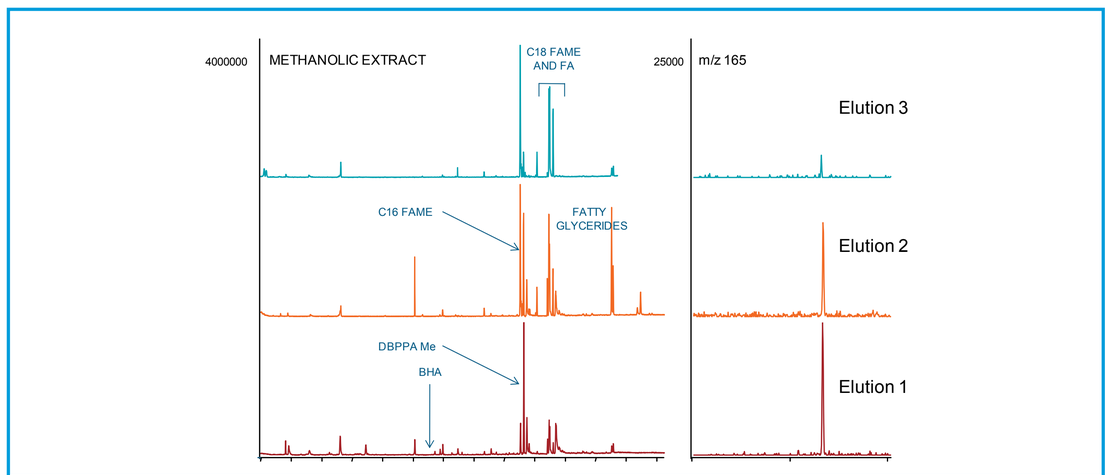

Figure 3 : The Total Ion Chromatogram (left) for the sequential elution of three methanol fractions for a C18-MEPS™ fraction obtained from the methanolic preparation of rice crackers. The right panel shows the Extracted Ion Chromatogram for BHA (m/z 165) for the same analyses.

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 glyceride 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 (Figure 3). BHA was also recovered by this technique and showed low carryover beyond the third fraction of methanol. Also detected on the basis of its abundant peak and distinctive mass spectrum was another butylated phenolic; the methyl ester of 3-(3,5-di-t-butyl-4-hydroxyphenyl)propanoic acid (BHPP Me) (Figure 4).

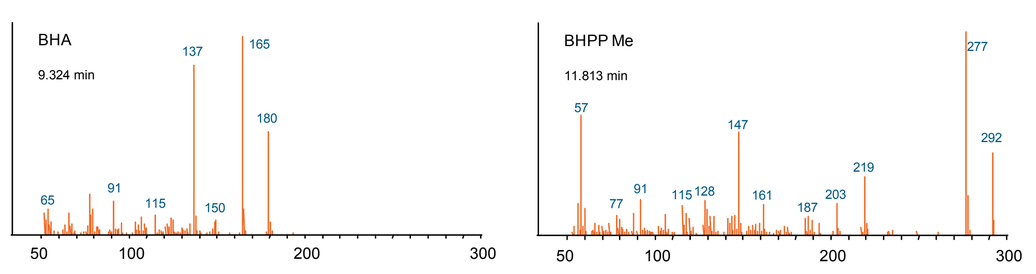

Figure 4: The EI mass spectra for BHA and methyl 3-(3,5-ditert-butyl-4-hydroxyphenyl) propionate (BHPP Me).

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 methylation.

The methanol extraction 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 5) as well as contributing to carryover (incomplete elution) into a second volume of eluting solvent.

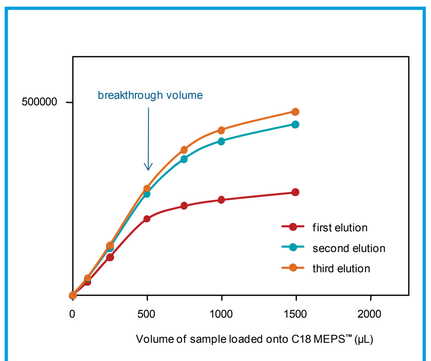

Figure 5: Peak area for BHA eluted from C18 MEPS™ with three sequential fractions of methanol when loaded with different volumes of sample solution.

While methanol is competitive as an elution solvent for BHA and related compounds, it is not a good solvent for oily material and so the fatty acid methyl esters and monoglycerides are incompletely eluted. Recycling of the sorbent is completed by elution with a good solvent for fatty esters and monoglycerides such as isopropanol, dichloromethane or mixtures thereof to ensure stripping of the strongly retained fraction.

Conclusion

A simple and rapid MEPS™ – GCMS method is presented for the detection of butylated hydroxyanisole 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.

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