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Sequential molecularly imprinted solid-phase extraction methods for the analysis of resveratrol and other polyphenols

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ABSTRACT

Molecularly imprinted polymers (MIPs) templated with either the phytoalexin, (*E*)-resveratrol, or its structural analog, 3,5-dihydroxy-*N*-(4-hydroxyphenyl)benzamide, have been used in tandem for the sequential extraction of (*E*)-resveratrol from aqueous peanut meal extracts in high purity and in near quantitative yields. Re-processing of the (*E*)-resveratrol-depleted peanut meal extract with the 3,5-dihydroxy-*N*-(4-hydroxyphenyl)benzamide imprinted MIP yielded additional polyphenolic components, identified as A-type procyanidins. Tandem liquid chromatography–electrospray ionization mass spectrometry confirmed the identity and purity of the isolated products. This study documents the advantages of tandem approaches with MIPs for the solid phase extraction and analysis of multiple bioactive compounds present in complex biomass waste streams.

1. Introduction

Consumption of fruits and vegetables rich in phytonutrients is known to promote human health and wellbeing. These phytonutrients include polyphenols and the structurally more complex flavonoids, which are pharmacologically active compounds that have benefits toward combating cardiovascular disease, inflammation and oxidative stress-related disorders [1]. One such polyphenol is (*E*)-resveratrol, **1** (Fig. 1), which is a plant-derived hydroxylated (*E*)-stilbene that is produced in response to fungal disease and UV irradiation [2,3]. (*E*)-Resveratrol is thought to be an early intermediate in metabolic pathways that lead to many more complex bioactive polyphenols and flavonoids [4] that are found in numerous plant species [3].

During manufacture, current food processing practices rarely use all of the available plant tissue and often discard considerable quantities of by-products as waste streams. Many health-beneficial bioactives remain in these high volume wastes, which are frequently disposed as low value stock feed, compost or sent to land fill. The targeted extraction and concentration of these bioactive compounds currently present many challenges because of their low concentrations, albeit large volumes, and structural diversity, which make their separation and extraction both costly and labor intensive. Therefore, the development of simple and robust platform methodologies that enable the convenient, rapid and selective isolation and enrichment of such bioactives will be beneficial to the food and beverage, agriculture and pharmaceutical industries by producing value-add products for possible use as food additives, dietary supplements or as lead compounds in therapeutic drug discovery.

Molecularly imprinted polymers (MIPs) are synthetic materials that are designed to have a molecular memory for a particular target molecule. This memory is imparted to a polymer via two principal approaches, either non-covalent self-assembly or labile covalent assembly, both of which take advantage of specific interactions between the target molecule and functional monomers [5]. Polymerization of the assembled complex in the presence of an excess of cross-linker allows the complementary three-dimensional molecular shape and location of the functional groups of the monomers involved in these interactions to be fixed in spatial terms, whilst subsequent removal of the template molecule, either by a simple extraction procedure or chemical cleavage, affords a cavity that is both spatially and chemically complementary to the template molecule. The resultant highly cross-linked MIPs that are produced are often physically and chemically robust, and are reusable with relatively long shelf life. MIPs have found diverse uses

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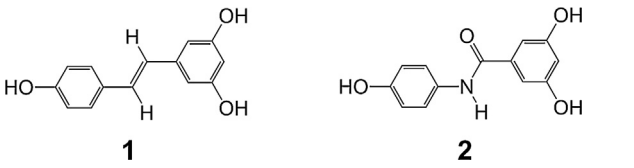
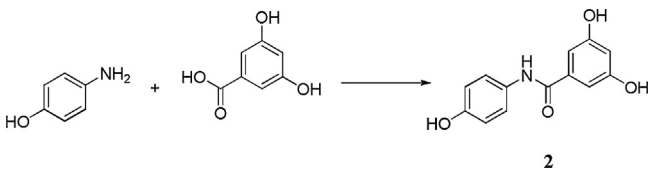


Fig. 1. Structure of the phytoalexin resveratrol, **1**, and the corresponding amide selectophore, **2**.



Scheme 1. *N*-Ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride was added to a solution of 3,5-dihydroxybenzoic acid and 4-aminophenol in DMF to return 3,5-dihydroxy-*N*-(4-hydroxyphenyl) benz-amide (76%) [14].

in the chemical and life sciences, such as applications in chemical and biomimetic sensors, catalytic reactors or chromatographic and solid-phase extraction materials [6–9]. Previously, we have reported the preparation of molecularly imprinted polymers that exhibited a high selectivity toward the polyphenolic compound (*E*)-resveratrol **1** and various structurally related analogs [10–12]. Similarly, resveratrol and related phenolic compounds were determined through the application of a resveratrol-imprinted polymer in a solid phase extraction format in wine and juice beverages [13]. We have also described the preparation of 'selectophore' imprinted polymers, whereby a structural analog of a naturally occurring template, such as (*E*)-resveratrol, can be employed as the molecular template [14], similar to the concept of a pseudo/dummy template [15]. The imprinted polymer so derived contained pseudo-template binding sites that were capable of mimicking the molecular recognition of parent template, albeit with a slightly different structural specificity and selectivity. These differences in specificity and selectivity for the target molecule can be exploited by utilising both the parent template- and selectophore-imprinted polymers in sequence as molecularly imprinted solid-phase extraction (MISPE) materials.

In this paper, we report the sequential application of MISPEs for the enrichment and isolation of (*E*)-resveratrol and other structurally related bioactives, such as the A-type procyanidins, in high purity from a single waste source such as peanut meal. Generic MISPE techniques have previously been used for the extraction and identification of the plant derived bioactives, such as quercetin from red wine samples spiked with this compound [16,17]. More recently, MISPE materials templated with caffeic acid and *p*-hydroxybenzoic acid, respectively, were used for the selective extraction of polyphenols from olive mill waste waters [18]. In these studies, the tandem use of imprinted polymers resulted in the isolation of several compounds, including the two templated compounds (caffeic acid and *p*-hydroxybenzoic acid) and several structurally related compounds such as gallic acid, protocatechuic acid and vanillic acid from the same feedstock. Further, a depletion approach, well established in the proteomic and metabolomic fields of analysis [19,20], based on a composite protein-imprinted macro-porous cryogel for the depletion of human serum albumin has been used for the chromatographic analysis of low abundance proteins in blood plasma [21]. In the context of solid phase extraction approaches, the application of multi-template MISPE methodologies for the efficient chromatographic separation and analysis of naturally occurring bioactive molecules present in complex biomass waste streams thus has considerable potential.

2. Materials and methods

2.1. Compounds

4-Vinylpyridine (4-VP), ethyleneglycol dimethacrylate (EGDMA) and 2,2′-azobis(2-methylpropionitrile) (AIBN), catechin and piceid were obtained from Sigma–Aldrich (Sydney, NSW, Australia). 4-VP and EGDMA were purified immediately prior to use via vacuum distillation and alumina column chromatography, respectively. All solvents were HPLC grade. (*E*)-Resveratrol **1** was synthesized based on adaptation of a method reported by Andrus et al. [27] as described previously in Ref. [10]. The selectophore, a structurally related polyphenolic analog, 3,5-dihydroxy-*N*-(4-hydroxyphenyl) benzamide **2** (Fig. 1) was synthesized via the condensation reaction route summarized in Scheme 1.

2.2. MIP preparation

MIPs and their non-templated counterparts (NIPs) were prepared, and the MIPs evaluated for the presence of any residual template after the template extraction step as previously described [14], using the conditions summarized in Table 1. The MIP_{RES} resin was prepared at 50 °C (18 h) followed by thermal annealing at 60 °C (24 h). The MIP_{AMIDE} resin was produced with a different porogen at 55 °C (40 h). Both procedures produced MIPs with comparable stability and binding performance.

2.3. Reversed-phase chromatographic (RP-HPLC) analysis

RP-HPLC analysis was performed on an Agilent Technologies 1100 LC system (Waldbronn, Germany) consisting of a binary pump with a vacuum degasser, auto-sampler with a 900 μL sample loop, thermostated column compartment and a diode-array detector. Injected samples were analyzed on a double end-capped Zorbax Eclipse XDB-C_{18} column (4.6 × 150 mm, 5 μm particle size) at 40 °C. The mobile phase consisted of 0.1% (v/v) AcOH in H_2O (eluent A) and 0.1% (v/v) AcOH in EtOH/H_2O or MeOH/H_2O (8:2 v/v) (eluent B), applying the following gradient: 0–2 min: 25% B isocratic, 2–6 min: 25–37.5% B, 6–9 min: 37.5% B isocratic, 9–12 min: 37.5–62.5% B, 12–15 min: 62.5% B isocratic, 15–18 min: 62.5–100% B, 18–22 min: 100% B isocratic, 22–25 min: 100–25% B, 25–29 min: 25% B isocratic. A flow rate of 0.5 mL min^{-1} was used. The injection volume was 5 μL whilst analyte detection was achieved with a Agilent Technologies UV–vis diode array detector (80 Hz) set to the absorbance wavelengths of λ = 280, 321 and 370 nm, whilst tandem liquid chromatograph–electrospray ionization mass spectrometry (LC–ESI-MS) analysis was carried out as described previously [10,11] in the positive ion mode with an Agilent 1100 Series LC/MSD-SL ion trap mass spectrometer through an Agilent G1607A orthogonal electrospray interface (Agilent Technologies, Waldbronn, Germany). All system control and data acquisition were conducted with Agilent ChemStation and MSD Trap Control software.

2.4. Preparation of peanut meal extract

Peanut meal (200 g) suspended in EtOH:H_2O (1000 mL, 4:1 v/v) was sonicated for 60 min, after which the mixture was filtered and the solvent evaporated to return the crude extract (17.9 g). The peanut meal extract used in subsequent experiments was prepared by adding this material (10.0 g) to a mixture of EtOH/H_2O (1:1 v/v) and diluting this mixture to a total volume of 500 mL, which was stored at 4 °C until required. Prior to use, the peanut meal extract was brought to room temperature and sonicated to clarify the solution.

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