Chromatographia (2020) 83:1539–1551
https://doi.org/10.1007/s10337-020-03958-0

ORIGINAL

Processing of Onion Skin Extracts with Quercetin-Molecularly Imprinted Adsorbents Working at a Wide Range of Water Content

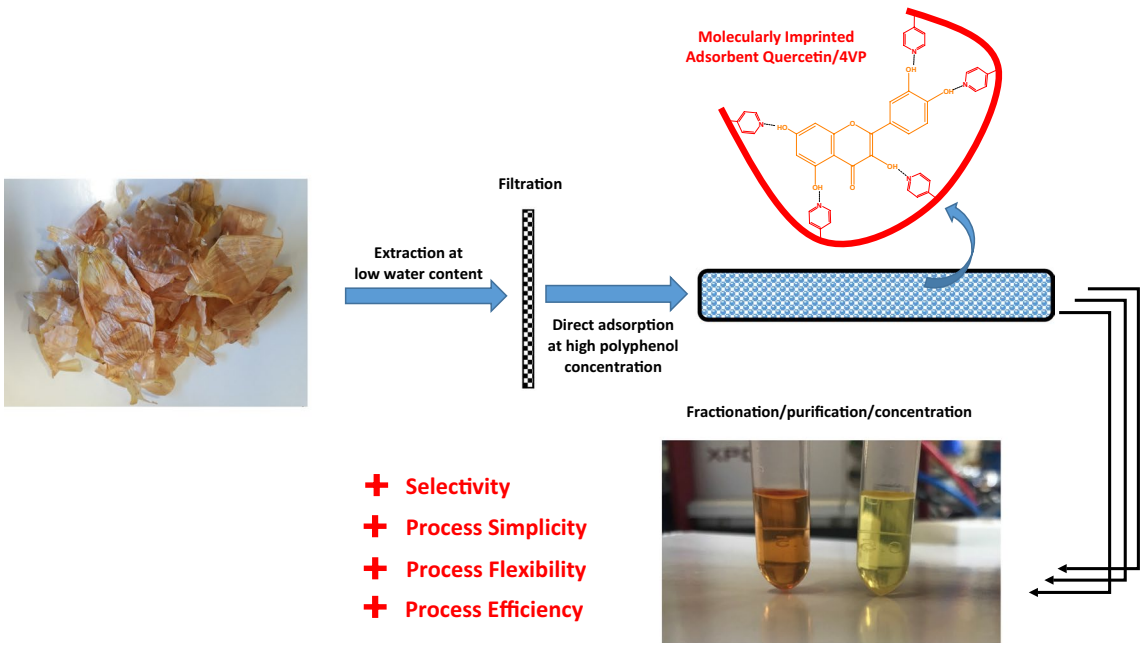
C. P. Gomes[1] · V. Franco[1] · Rolando C. S. Dias[1] · M. R. P. F. N. Costa[2]

Received: 12 December 2019 / Revised: 19 August 2020 / Accepted: 3 September 2020 / Published online: 22 September 2020

Abstract

A simple adsorption process allowing a high retention of polyphenols contained in extracts of onion skin with ethanol/water volume ratio going up to 80/20 is described. We show that the straightforward processing of the extracts is possible, even at low water content, by using quercetin-molecularly imprinted (Q-MIP) adsorbents synthesized with 4-vinylpyridine (4VP) as the functional monomer. The favorable interactions between the pyridyl functional groups of 4VP and the polyphenols, as well as the improved binding site accessibility introduced by molecular imprinting, are at the source for the good performance observed with the Q-MIPs. The usefulness of the Q-MIPs in onion skin polyphenols purification, fractionation and concentration is demonstrated with few sorption/desorption steps and considering sonicated, Soxhlet and supercritical CO_2 extracts. Polyphenol retention of c.a. 88% is possible with Q-MIPs (7% with non-tailored adsorbents) when directly processing ethanol/water 80/20 extracts. Protocatechuic acid and other very hydrophilic molecules (such as simple sugars) were readily removed from the extracts, leaving fractions containing mostly quercetin and quercetin derivatives. Polyphenol recovery higher than 90% (measured with quercetin) and concentration factors up to 34 times were observed with the Q-MIPs.

Graphic abstract



Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10337-020-03958-0) contains supplementary material, which is available to authorized users.

Extended author information available on the last page of the article

Keywords Onion residues · Downstream processing · Polyphenols · Molecular imprinting · Adsorbents

Introduction

Plants synthesize different kinds of polyphenols to control oxidative stress and for protection of external threats such as insect plagues [1–3]. It is commonly accepted that polyphenols show important beneficial effects in human health [4] and therefore, due to their current applications in multiple biotechnological fields and potential future developments, a huge growth for the polyphenols market in the near future is expected [5].

It was observed in several studies that onions, and especially their outer parts, contain appreciable amounts of quercetin (a flavonoid polyphenol belonging to the flavonols class) and also of quercetin derivatives, namely quercetin-4′-glucoside and quercetin-3,4′-diglucoside. Quercetin and its derivatives present strong antioxidant effects with potential impact in the treatment of several diseases, namely diabetes and cancer. These compounds are also prospected as sedative agents. Furthermore, more than 122,000 k tons of onions (dried) are worldwide annually produced, therefore yielding more than 18,000 k tons of onion residues. 4800 k tons of onions are estimated to be annually produced in European Union, leading to more than 700 k tons of onions residues [6]. Using these residues as fertilizers or animal feed is limited due to their inherent composition and susceptibility to microbial spoilage (see [7] and references therein). Therefore, the valorization of onion residues is nowadays an interesting issue, namely in order to avoid the disposal of large amounts of this biomass as it is currently observed. In this context, the recovery of polyphenols from onion residues is an attractive route for biomass valorization by generating high value-added products. Several operations are needed to get bioactive compounds (namely polyphenols) suitable for biotechnological applications from plant residues. Solid–liquid extraction, followed by sorption/desorption processes (to purify and concentrate), are often used. A key issue is the generation of diluted streams with co-extraction of unwanted compounds. In practice, these operations should be performed efficiently and economically to get a feasible industrial application.

Indeed, sorption/desorption processes are commonly carried out to purify, fractionate and concentrate the polyphenols present in plant extracts [8, 9]. Hydrophobic effects are a major mechanism governing the sorption of the bioactive compounds in many adsorbents; hydrogen bonding, ionic interactions or even π interactions can also play a role in the retention/release processes. Often, in order to accomplish a fair amount of polyphenol retention, solvents with high water content must be used. When the valorization of some underutilized effluents in sought, such as in olives debittering, artichoke washing, olive oil or natural juices production, etc., this condition is met because water is mainly used in these processes. However, many plant extracts are produced using low water content solvents in order to increase polyphenols extraction efficiency and concentration (e.g., mixtures containing more than 70% weight ethanol). Thereafter, to perform the subsequent adsorption process in common synthetic resins, preparation steps are needed in order to increase the water content, such as solvents evaporation and re-suspension of dried extracts in aqueous systems. Besides the growth in process complexity and energy consumption, such operations lead to a drop of polyphenols concentration in the liquid because the solubility of many of these molecules is very scarce in aqueous systems. Therefore, the possibility for the direct adsorption of polyphenols present in low water content mixtures deserves some scrutiny.

Molecularly imprinted polymers (MIPs) are synthetic materials akin to artificial antibodies or enzymes as they mimic the "lock and key" principle inherent to antigen/antibody or substrate/enzyme binding. Nowadays, MIPs find special applications in many kinds of separations, solid-phase extraction, as sensors, membranes, catalysis, controlled drug delivery and also as modified adsorbents [10, 11]. This research explores the development of MIPs synthesized with the polyphenol quercetin as the template molecule and 4-vinylpyridine as the functional monomer. The produced MIPs are characterized and applied for the retention and recovery of polyphenols present in onion skin extracts. Samples with high ethanol content, obtained through sonication and Soxhlet or supercritical CO_2 extraction are considered in these studies. The tailored MIP adsorbents are used to handle directly ethanol/water 80/20 v/v onion skin extracts, highlighting the possibility for the design of simple, flexible and efficient downstream processing for polyphenols.

Materials and Methods

Reagents

Quercetin (hydrate, purity 95%) and rutin (purity 97%) were supplied by Acros Organics. Gallic acid (assay 97.5–102.5%, titration), styrene (S, 99% purity), ethylene glycol dimethacrylate (EGDMA, 98% purity), 2,2′-Azobis(2-methylpropionitrile) (AIBN, 98% purity) and Supelite™ DAX-8 (an adsorbent of moderate polarity also referred as polymethylmethacrylate resin) were purchased from Sigma Aldrich. Divinylbenzene (DVB, 80% purity) and 4-vinylpyridine (4VP, 95% purity) were provided by Alfa Aesar. Analytical

reagent grades for acetonitrile (ACN), dimethylformamide (DMF), acetic acid (AcOH), and methanol (MeOH) were bought from Fisher Scientific and for ethanol (EtOH) from PanReac. Millipore water (Milli-Q quality) was used in all the experiments unless otherwise mentioned.

Synthesis of the Quercetin-Molecularly Imprinted Polymers (Q-MIP)

The synthesis of the quercetin-molecularly imprinted polymers (Q-MIP) was performed following procedures similar to those reported in our previous works [12–16]. The precipitation polymerization method was used to produce the adsorbents here addressed, considering ACN/DMF 91/9 v/v or ethanol as solvents. Briefly, quercetin (the selected template polyphenol—T) was mixed with 4-vinylpyridine (the selected functional monomer—FM) and the solvent, up to the formation of a homogeneous solution. The template–functional monomer (T–FM) interaction was then promoted using an ultrasound bath during 30 min. A final solution containing also the required amounts of crosslinker (EGDMA or DVB) and initiator (AIBN) was then prepared and purged with a flow of dry argon for 30 min. The polymerization took place during 24 h in a sealed vessel, using a thermostatic oil bath pre-set to the desired temperature ($T = 60$ °C). The Q-MIP adsorbent particles obtained were cleaned in several centrifugation steps with methanol, methanol/acetic acid and also using Soxhlet extraction in order to remove unreacted chemicals as well as the quercetin polyphenol used as template. Non-imprinted polymers (NIPs) were prepared following the same experimental procedure as for Q-MIPs, but without the presence of the quercetin template. All the kinds of adsorbent particles synthesized were dried in a vacuum oven up to the attainment of a constant product weight.

Characterization of the Adsorbent Particles with FTIR, SEM and BET

The dried synthesized adsorbent particles were characterized through IR spectroscopy with a Perkin Elmer, model Spectrum Two™, instrument. The morphology of the different Q-MIP and NIP particles was obtained by SEM (see Figure S1) at the Materials Center of the University of Porto (CEMUP), using a SEM FEI Quanta 400FEG instrument with the EDS (Energy Dispersive Spectroscopy) system Edax Genesis X4M. The Brunauer–Emmett–Teller (BET) specific surface area and pore volume of the produced adsorbent particles were determined through N_2 adsorption/desorption isotherms at 77 K using a Quantachrome NOVA 4200e adsorption analyzer.

Preparation of Onion Skin Extracts Using Sonication, Soxhlet and Supercritical CO_2 Extraction Techniques

Domestic onion skin residues of the white variety were used in this research. Three different techniques were considered to prepare onion skin extracts, as follows: the sonication was carried out during 1 min, at room temperature, using the ratio 10/100 g/mL of onion skin residues in the hydro-alcoholic mixture EtOH/H_2O 80/20 (v/v). Soxhlet extraction was carried out considering two process cycles and the apparatus loaded with 11.5 g of onion skin residues and 200 mL of EtOH/H_2O 80/20. Supercritical CO_2 extraction was performed at $T = 40$ °C and $P = 160$ bar during 2 h, considering different runs with a mass of onion skin residues in the range 25–30 g and 5–10% of pure ethanol as co-solvent comparatively to CO_2 (800 g of CO_2 were roughly used in these experiments). Before loading in the Q-MIP particles, extracts were filtered through a 0.45-µm nylon filter.

Polyphenols Identification/Quantification Through Gradient HPLC-DAD

A Jasco MD-4010 photo diode array (PDA) detector was used to carry out the HPLC-DAD analyses. Nucleosil® C18 analytical column, $L \times$ I.D. = 15 cm × 4.6 mm and 5 µm particle size, working at room temperature, have been chosen. The flow rate was 1 mL/min and the absorbance changes were monitored at 280 and 360 nm. The mobile phases used for chromatographic analysis were: (A) acetonitrile/water (10/90) at pH = 3 (adjusted with acetic acid) and (B) acetonitrile/water (90/10), also at pH = 3. The linear gradient method was used, starting with 100% of solvent A and ending with 100% of solvent B.

Polyphenols Identification/Quantification Through LC-DAD-ESI–MS^n

These analyses were carried out at the Centro de Investigação de Montanha (CIMO). The chromatographic analysis was performed using a Dionex UltiMate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) system equipped with a diode array detector coupled to an electrospray ionization mass detector (LC-DAD-ESI/MS^n), a quaternary pump, an auto-sampler, a degasser and an automated thermostatic column compartment. Chromatographic separation was carried out with a Waters Spherisorb® S3 ODS2(C18), $L \times$ I.D. = 15 cm × 4.6 mm and 3 µm particle column (Waters, Milford, MA, USA) working at 35 ºC. The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was isocratic 15% B (5 min), 15% B to 20% B (5 min), 20–25% B (10 min), 25–35% B (10 min), 35–50% B (10 min), and

re-equilibration of the column, using a flow rate of 0.5 mL/min.

Solid-Phase Extraction Experiments with Onion Skin Extracts

Solid-phase extraction (SPE) measurements with onion skin extracts were carried out considering procedures similar to those considered in previous works [12–16]. Briefly, packing extraction cartridges containing the selected adsorbents (e.g., 50 mg of dried products) were first conditioned considering several washing steps [12–16]. Cartridges were then loaded with the selected onion skin extract (5 mL) using a constant percolation flow rate (c.a. 0.3 mL/min). Afterwards, washing and elution steps were carried out by percolating the selected solvent (5 mL) through the adsorbents (e.g., washing with water to remove water-soluble compounds and elution with methanol/acetic acid 90/10 v/v to recover polyphenols). The collected fractions correspondent to the diverse loading, washing and elution steps were monitored using batch UV–vis spectrometry and, when required, analyzed by LC-DAD-ESI–MS^n and/or HPLC-DAD. SPE measurements were performed at room temperature.

Sorption/Desorption of Onion Skin Extracts in Continuous Processes

To carry out these experiments, the adsorbent particles were first packed (using a slurry process) in empty HPLC columns (e.g., 250 mg of adsorbent in a column with $L \times$ I.D. = 33 mm × 8 mm). The packed columns were cleaned, conditioned, and afterwards submitted to saturation, recovery and cleaning steps with the selected onion skin extract (e.g., with a flow rate of 1 mL/min). The saturation process was monitored by UV–vis spectrometry analysis of samples collected at prescribed time-instants. Desorption was carried out considering a designed sequence of selected solvents (e.g., starting with water, up to methanol/acetic acid 90/10 v/v at the end) and different fractions were collected during this stage. When required, the recovered fractions were analyzed by LC-DAD-ESI–MS^n and/or HPLC-DAD. Saturation was performed at 25 °C and the recovery at 45 °C. Additionally, in order to prevent the backpressure problem, some saturation/recovery runs were performed with the adsorbents (e.g., 500 mg) in SPE cartridges and using lower flow rates (c.a. 0.3 mL/min). The same analytical procedures considered with the HPLC runs were used with the saturation/recovery experiments performed in SPE cartridges. SPE experiments were carried out at room temperature with some elution steps at 45 °C.

Results and Discussion

Rationale for MIPS Synthesis and Structural Features of the Adsorbents

Table 1 summarizes the conditions used for the synthesis of the quercetin-MIPs considered to target polyphenols present in onion skin extracts. Quercetin was selected as the template (*T*) molecule because it is known to be the major polyphenol in onions. Many other polyphenols in onions are derivatives of quercetin (e.g., quercetin-glycosides, quercetin-diglycosides, quercetin-trimers) and therefore some key structural properties are shared by these compounds. The functional monomer (FM) 4-vinylpyridine (4VP) was mainly used in these Q-MIPs due to its potential favorable interactions with quercetin [12].

Hydrogen bonding of nitrogen in the pyridyl groups of 4VP with the hydroxyl groups of polyphenols, hydrophobic effects involving aromatic units and/or π interactions are plausible interactions predicating the better results observed with 4VP-based MIPs [12]. MIP1 and MIP2 are roughly analogue materials with EGDMA and DVB as crosslinkers (see MIP1/MIP2 comparative assessment in S2). Ester groups of EGDMA are prone to hydrolysis when aggressive application conditions are used (e.g., strong acidic/alkaline conditions), and so a higher chemical resistance is generally observed for DVB-based MIPs, leading to improved reusability and long-term stability [17]. With MIP3, a high value for Y_M (monomers mass fraction) was deliberately used (20%) in order to evaluate its effect on product morphology, since a higher rate of particles coalescence/agglomeration is plausible in these conditions. As discussed elsewhere [12–14], other issues such as the mutual initial solubility of ingredients, polymerization retardation due to the polyphenol template, potential interactions FM/T or intended particles size and rigidity also affect the values selected for Y_{CL} (% mole fraction of crosslinker in monomers mixture), Y_I (mole fraction of initiator comparatively to monomers × 100) and $Y_{FM/T}$ (mole ratio FM/T). Notably, besides the sought molecular imprinting effect (see MIP versus NIP comparison in [12] with measurement of imprinting factors in the order of 1.6), the presence of quercetin in the polymerization system can change the particle morphology (particle nucleation is affected by the polyphenol radical scavenging effect) and the incorporation of the template in the polymer network is also possible (Figure S3).

Because MIPs formation involves a copolymerization, it is important to confirm the incorporation of both the FM and crosslinker in the final network. FTIR was used to obtain the features concerning the chemical composition of the synthesized MIPs and the formation of the expected

functionalized materials was observed (Figure S4). Low values for the specific surface area were observed with the BET measurements for the Q-MIPs synthesized. Values in the range of 2–4 m^2/g were measured for the surface area of these adsorbents and a negligible pore volume was estimated. Much higher values of the surface area (135 m^2/g) and pore volume (0.337 cm^3/g) were measured for DAX8. A low porogenic effect was thus caused in MIPs formation. Despite these differences, Q-MIPs present improved adsorption capabilities for polyphenols as demonstrated below. Functionalization of the surface of the Q-MIP particles and improved mass transfer rates are key factors for these outcomes.

Phenolic Profile of the Onion Skin Extracts

The composition of different parts of the onion (internal bulb, outer skin, top and bottom parts, roots) in diverse varieties (white, yellow and red types) was addressed in previous works, namely concerning structural and non-structural carbohydrates (glucose, fructose, sucrose and low molecular weight fructooligosaccharides), fatty acids, water-soluble/non-soluble fibers, essential oils and polyphenols [7, 18–27]. Concerning the polyphenol composition, the flavonol quercetin and its glucoside derivatives are the major compounds present in onions. A relatively high amount of protocatechuic acid (c.a. 15% in total polyphenol content [27]) is also found in this plant. The antioxidant activity and other biological effects of the flavonols present in onions were also demonstrated in several research works [28–33].

In this work, the polyphenols present in the different onion skin extracts produced (using sonication, Soxhlet and $SCCO_2$ extraction) were identified and quantified through LC-DAD-ESI–MS^n and HPLC-DAD. A simpler and less expensive run is possible with the latter technique, and thus, HPLC-DAD was chosen for most analysis, after getting a satisfactory identification of the polyphenols with LC-DAD-ESI–MS^n. Figure 1 presents the total ion chromatogram (TIC) and photodiode array (PDA) signals observed with the analysis of an onion skin extract through LC-DAD-ESI–MS^n (results for an ethanol/water 80/20 v/v sonicated extract are here presented with additional data in Supplementary Material). Note the different elution times for the same compounds when LC-DAD-ESI–MS^n and HPLC-DAD

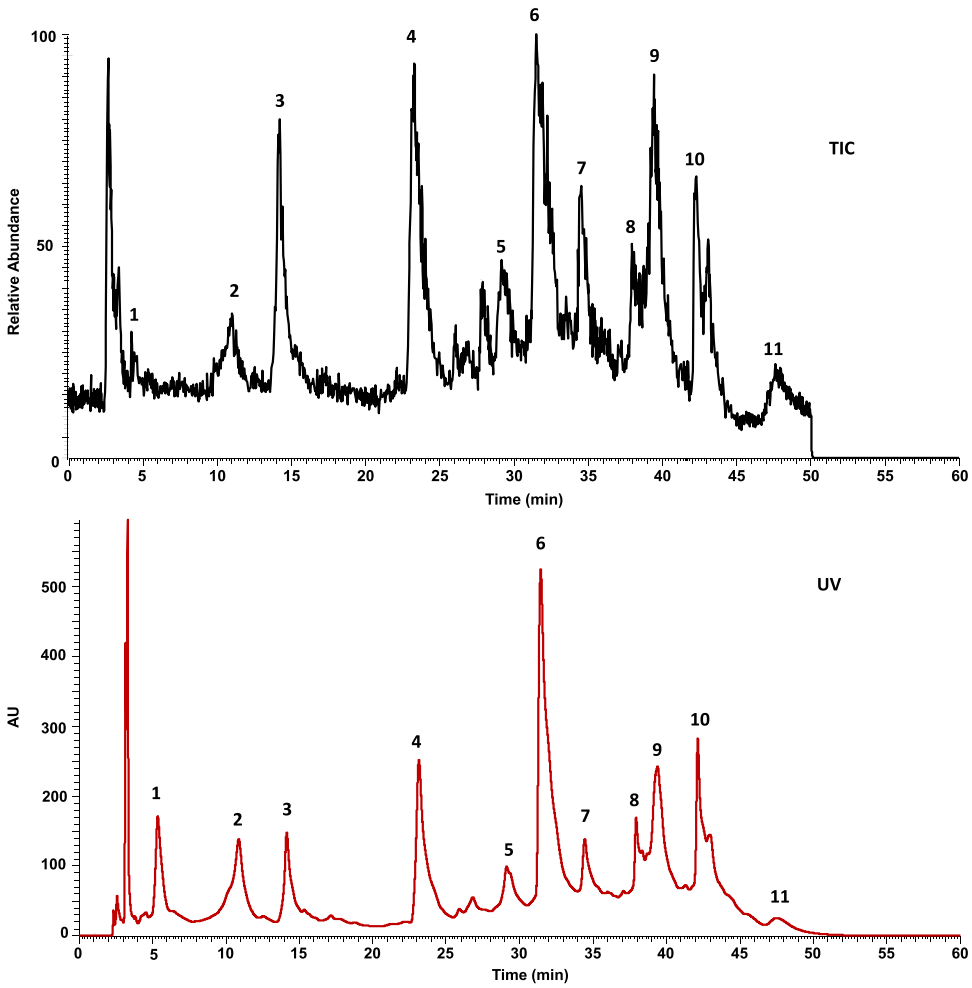


Fig. 1 Total ion chromatogram (TIC) and photodiode array (PDA) signals observed with the LC-DAD-ESI–MS^n analysis of an onion skin extract. Results for the ethanol/water 80/20 sonicated extract are presented here. The PDA UV response at 280 nm was selected for illustration purposes

analysis are compared due to the different running conditions (see the description of both techniques above). These results, in connection with previous works [18, 19], allows to confirm the presence in the extract of protocatechuic acid (peak 1), 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (2), quercetin-diglycoside (3), quercetin-glycoside (4), protocatecoyl quercetin (5), quercetin (6), quercetin dimer-glycosides (peaks 7–9), quercetin dimer (10) and quercetin trimer (11). The chemical structures of these molecules are presented in Fig. 2 and further details concerning these compounds (e.g., occurrence of multiple isomer structures) can be found elsewhere [18, 19].

The yield for the sonicated and Soxhlet extractions, expressed as the total phenol content (TPC) per mass of dry onion skin, was estimated to be 1022 and 1128 mg/100 g, respectively. These values are in a similar range as values reported in other researches, e.g., TPC ~ 1600 mg/100 g for a global extraction yield of 9300 mg/100 g [19], 1361 [29], 785 [28], 1000–2000 [20]. Note, however, that a large variation for onion TPC or individual phenolic molecules is reported (see [19–21, 23–29]), depending on the vegetable variety, the portion used (raw vegetable, tops, bulb, skin), extraction method or quantification technique (e.g., 10.8 mg/100 g [27]). A much lower TPC was observed with our $SCCO_2$, runs (~ 121 mg/100 g), particularly in comparison with related works [19]. This is likely a consequence of the operation conditions used (kind/amount of the CO_2 solvent modifier, continuous/batch run). Optimization of the extraction conditions with $SCCO_2$ is possible [19] but these issues transcend the scope of the present work.

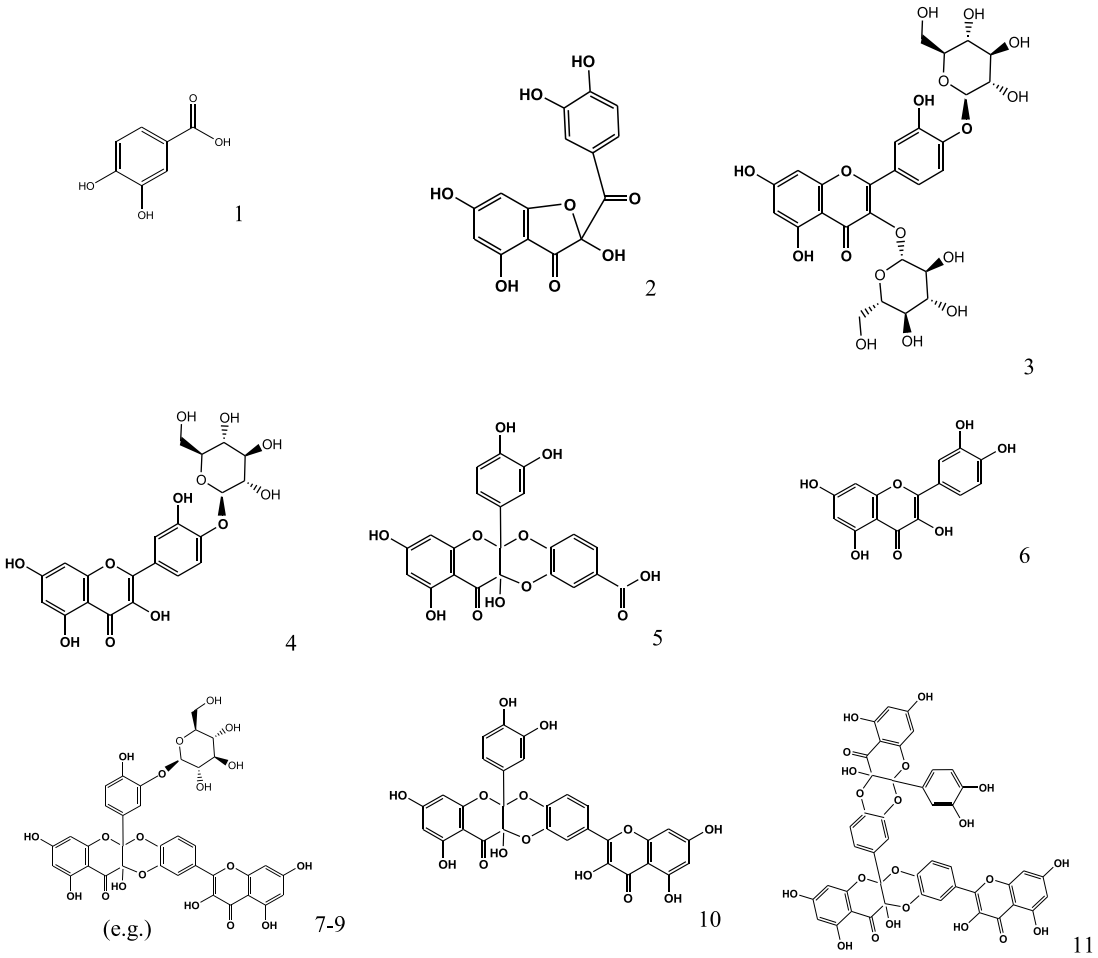


Fig. 2 Structures of some phenolic compounds identified in the extracts of onion skin: (1) protocatechuic acid, (2) 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone, (3) quercetin-diglycoside, (4) quercetin-glycoside, (5) protocatecoyl quercetin, (6) quercetin, (7–9) quercetin dimer-glycosides, (10) quercetin dimer, (11) quercetin trimer. The identification numbers ascribed to the structures are correspondent to the peaks presented in Fig. 1. Further details concerning these compounds, namely the multiple isomer structures, can be found in references [18, 19]

Purification and Fractionation of Onion Skin Polyphenols in Low Water Content Extracts

In recent research works [12, 13] the good performance of tailored adsorbents, namely MIP materials, in the adsorption of polyphenols was demonstrated considering not only standard polyphenol molecules (e.g., polydatin, resveratrol, quercetin, gallic acid or rutin) but also real plant extracts, such as red wine, cork, chestnut shell and olive leaf extracts. The improved polyphenol retention capability of the 4VP MIPs in comparison with other synthetic resins (XAD4, XAD7HP and DAX-8) was highlighted [12]. It was also shown that the efficient adsorption of polyphenols is even possible with the 4VP MIPs when solvents with low water content are considered (e.g., ethanol/water 80/20). Avoiding polyphenols/adsorbent interactions chiefly based on hydrophobic effects is at the source of such outcomes.

In the present work, the improved adsorption capabilities of tailored adsorbents, namely quercetin – MIPs developed with 4VP as FM, are explored for the processing of onion skin extracts. Figure 3 presents a relevant outcome achieved in this context. These results show that a high adsorption capacity (ca. 88%) is observed with MIP1 for onion skin polyphenols in ethanol/water 80/20 while only a residual amount of these compounds (ca. 7%) is adsorbed in the same conditions when using the adsorbent DAX-8. Thus, a simple adsorption process allowing the direct processing of alcoholic extracts is possible when using the developed Q-MIP adsorbents. Working with a wide range of operation conditions (e.g., concerning the composition of the loading solvent) and at high polyphenol concentration are advantages of the Q-MIPs. They can be further exploited to design more flexible and efficient downstream processing for these bioactive compounds (e.g., increasing energy savings). Only by increasing the water content of the extracts it is possible to achieve high polyphenol retention when common synthetic resins are used (hydrophobic effects are promoted). However, water addition induces the precipitation and dilution of many polyphenols of the original alcoholic extracts (as shown below) thus decreasing the efficiency of the global process. Actually, the importance of hydrophobic interactions for polyphenols retention in common synthetic resins has been previously demonstrated considering the XAD4, XAD7HP and DAX-8 absorbents [12].

Having demonstrated the possibility for direct adsorption in the Q-MIPs of onion skin polyphenols contained in ethanol/water 80/20 extracts, the desorption process leading to their purification and fractionation should be addressed. Figure 4 provides results for such an approach, considering a very simple, two-step, desorption process correspondent to washing with EtOH/H_2O 80/20 and elution with MeOH. The washing step with the same solvent used in loading (EtOH/H_2O 80/20) was carried out in this experiment in order to show the release of unbounded or weakly bonded molecules. Protocatechuic acid (1), 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (2), quercetin-diglycoside (3), quercetin-glycoside (4), protocatecoyl quercetin (5) and quercetin (6) are clearly identifiable in this washing fraction. Note that the more hydrophilic molecules, such as protocatechuic acid, were initially weakly adsorbed in the

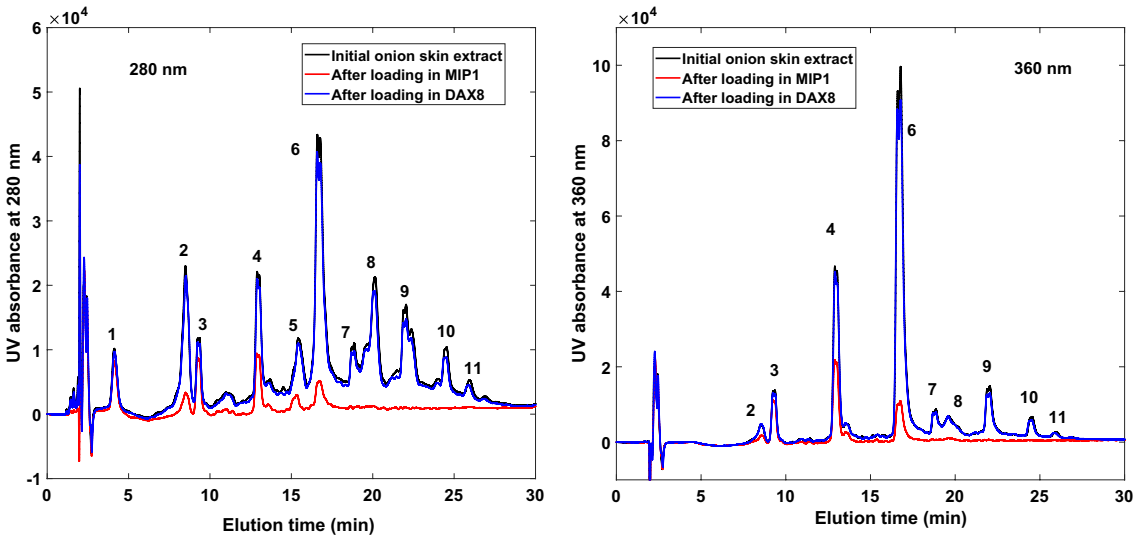


Fig. 3 Comparison of the HPLC-DAD chromatograms for an initial onion skin extract (obtained in ethanol/water 80/20 using sonication technique) and for the correspondent SPE processed solutions, collected at column outlet after extract loading in MIP1 and in DAX-8 (measurements at $\lambda = 280$ and $\lambda = 360$ nm are shown). The identification for the polyphenol peaks presented in the plot is based on the comparison with the chromatograms for standard polyphenols, namely quercetin, and using the data provided by the LC-DAD-ESI–MS^n analysis performed in this research (see Fig. 1 and S6)

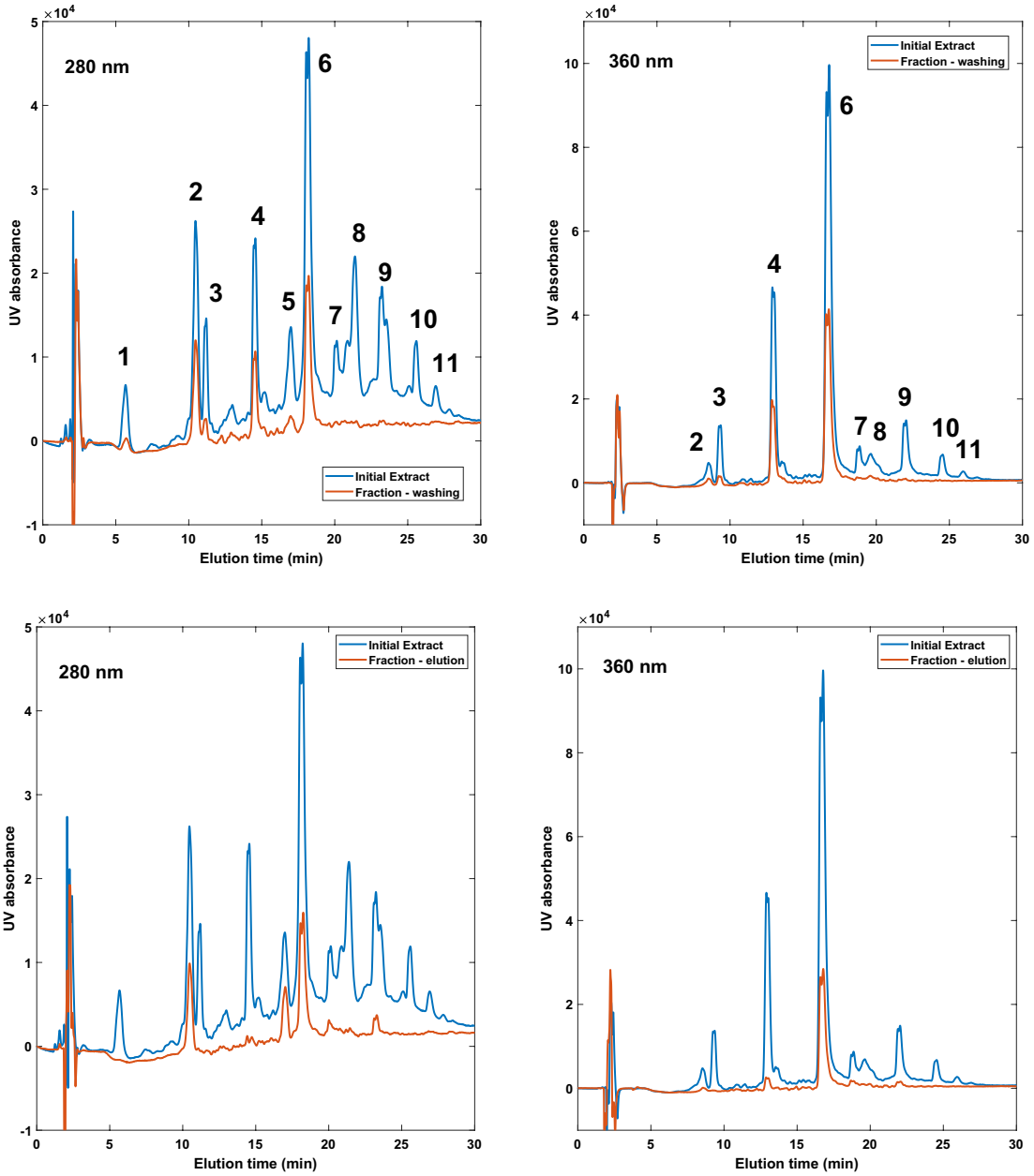


Fig. 4 HPLC-DAD analysis for the fractions collected after the direct SPE adsorption of an onion skin extract (EtOH/H_2O 80/20) in MIP1. The two-step desorption fractions correspondent to washing (with EtOH/H_2O 80/20) and elution (with MeOH) are here presented considering measurements at $\lambda = 280$ nm and $\lambda = 360$ nm

Q-MIP during loading (see Fig. 3) and in this washing step the retained traces are washed out. Thus, a first purification of the extract is possible with this simple procedure by eliminating the more hydrophilic molecules such as protocatechuic acid, simple sugars (e.g., glucose, fructose, sucrose) and other carbohydrates not seen with the UV analysis performed. Furthermore, the elution of quercetin and other low water-soluble polyphenols in the washing step can be avoided if a low alcoholic content solvent is used, as shown below.

In this experiment, the elution step after washing was performed with MeOH and the results obtained are also presented in Fig. 4 (see also quercetin recovery higher than 90% in S2). The fraction collected contains 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (2), protocatecoyl quercetin (5), quercertin (6) and traces of quercetin dimer-glycosides (9). Considering additional steps involving further adsorption/desorption stages with the collected fractions, the individual purification of quercetin and derivatives is possible (see [18] using

XAD-2, chromatography in silica gel and reversed-phase HPLC). However, for many applications, high purification among the individual polyphenols is neither needed nor even advisable. The synergy between different polyphenol molecules seems to be critical for the antioxidant effect and this function can be downplayed with their isolated application.

Figure 5 presents results concerning the use of the Q-MIPs in continuous sorption/desorption processes. The material MIP3 was packed in an empty HPLC column (250 mg in a column with $L \times$ I.D. = 33 mm × 8 mm) and saturated with ethanol/water 80/20 onion skin extracts at $Q = 1$ mL/min, as presented in Fig. 5a for the breakthrough

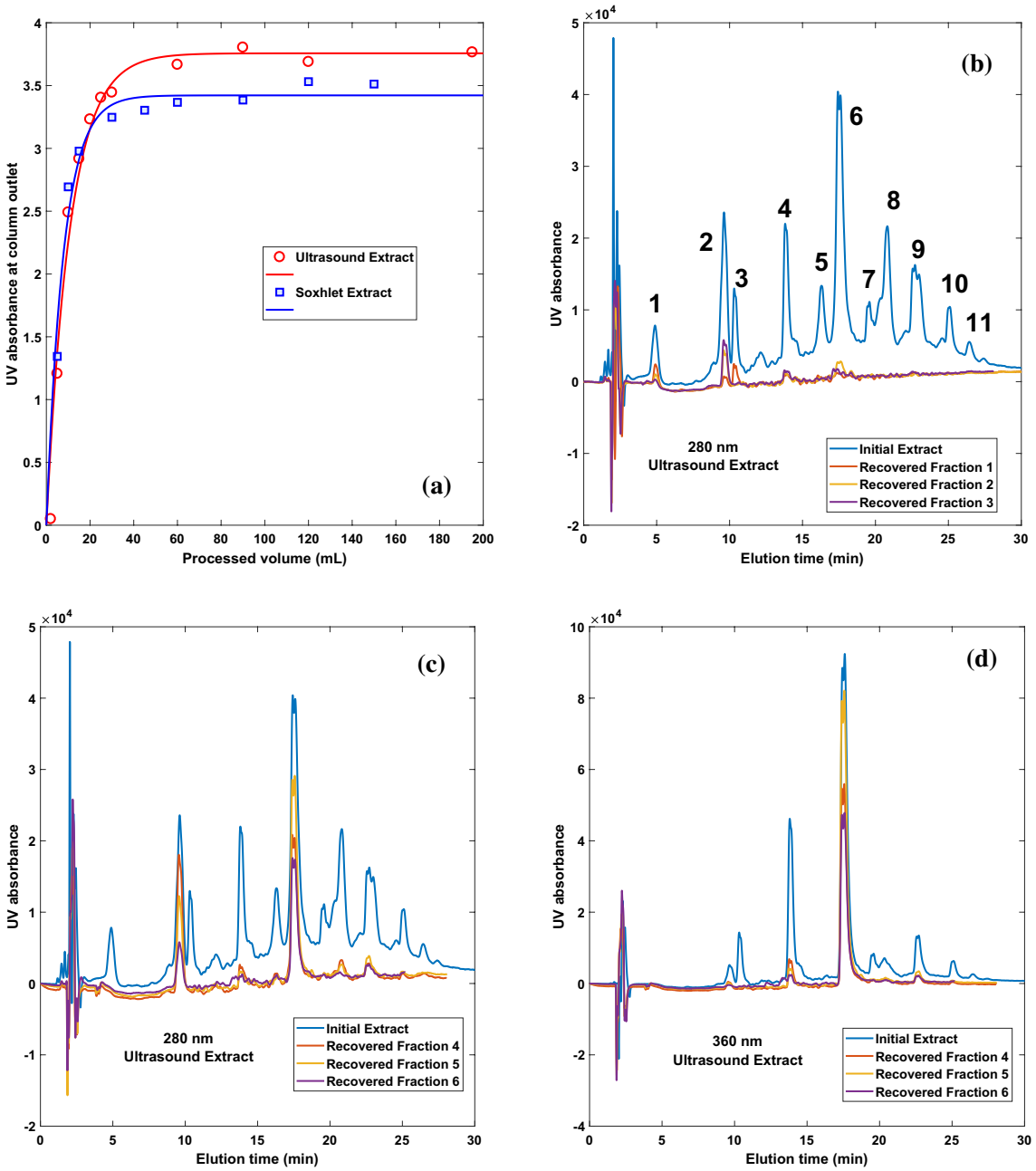


Fig. 5 Adsorption of EtOH/H_2O 80/20 onion skin extracts in MIP3 and subsequent recovery of different fractions. **a** Dynamics of continuous adsorption in a column packed with 250 mg of MIP3 (adsorption of ultrasound and soxhlet extracts are both presented). **b–d** HPLC-DAD analysis for fractions collected with different solvents composed from A = H_2O/MeOH 90/10 at pH = 3 (adjusted with AcOH) and B = MeOH/AcOH 90/10. Fraction 1 A/B = 72/28, Fraction 2 A/B = 46/54, Fraction 3 A/B = 35/65, Fraction 4 A/B = 24/76, Fraction 5 A/B = 8.5/91.5, Fraction 6 A/B = 0/100. Measurements at $\lambda = 280$ are presented in **b** and **c** while measurements at $\lambda = 360$ nm are shown in **d**

curves of sonicated and Soxhlet extracts. Synthesis conditions of MIP3 were changed in comparison with MIP1 to produce particles with higher size leading to continuous processes showing lower backpressure. MIP3 is also able to retain directly the onion skin polyphenols contained in ethanol/water 80/20 onion skin extracts, as shown in Fig. 5a. The purification and fractionation of these compounds is also possible as demonstrated in Fig. 5b–d. Fractionation was performed starting with more hydrophilic solvents up to the use of MeOH/AcOH 90/10 at the end. In fractions 1–3 only traces of protocatechuic acid (1), 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (2), quercetin-diglycoside (3), quercetin-glycoside (4), and quercetin (6) can be identified (Fig. 5b). As showed before, protocatechuic acid is only slightly adsorbed during loading. Other hydrophilic molecules like sugars and diverse carbohydrates are also removed with fractions 1–3 (a first purification of the extract is attained). Fractions 4–6 contain mostly quercetin (6) with 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (2) and traces of quercetin-glycoside (4) and of quercetin dimers/trimers.

Other processing conditions for EtOH/H_2O 80/20 onion skin extracts with the MIPs developed were considered and their good performance for adsorption and subsequent fractionation/concentration of polyphenols was confirmed (S7–S8).

Processing of More Hydrophilic and Diluted Onion Skin Extracts

Many practical systems aiming at the valorization of underutilized streams containing bioactive compounds involve the processing of diluted mixtures with high water content (e.g., olive processing effluents and other cases mentioned above). A pre-separation of weakly water-soluble molecules contained in plant extracts produced with low water content (e.g., fatty acids, some polysaccharides, fibers) can be performed by increasing the water fraction of the mixtures, at the expenses of the precipitation of some compounds of interest (such as polyphenols) and also by causing the operation with more diluted streams. In both cases, hydrophobic effects are potentiated by the presence of water and many different kinds of synthetic resins can be used for purification/fractionation purposes.

It was previously shown that MIP adsorbents can provide high polyphenol retention also with high water content mixtures [12]. Obviously, the selectivity of MIPs decreases substantially when water content is increased. In this section are presented results showing the usefulness of the developed Q-MIPs with more hydrophilic and diluted onion skin extracts. Within this purpose, EtOH/H_2O 50/50 and EtOH/H_2O 20/80 extracts were generated from the original EtOH/H_2O 80/20 extract. The formation of precipitate was visually observed with these operations leading to higher water content extracts. Fatty acids, polysaccharides and fibers become insoluble with these new conditions. The solubility of many polyphenols also decreases in these water-rich conditions (see Figure S9, showing also the ability of MIP1 and MIP2 to retain polyphenols present in the three different EtOH/H_2O mixtures).

The usefulness of the Q-MIPs in these new conditions was assessed with the continuous loading of a Soxhlet EtOH/H_2O 20/80 extract to MIP1 and MIP2 (breakthrough curves and HPLC monitoring of the columns outlet presented in S10-S11). Figure 6 shows the recovery of the polyphenols previously adsorbed in a Q-MIP. After extract loading, fifteen consecutive fractions of 5 mL volume were collected. Fractions 1–3 are correspondent to washing with water, fractions 4–6 to elution with MeOH at room temperature and fractions 7–15 to the elution with MeOH at 45 °C. Figure 6a shows the release of the adsorbed traces of protocatechuic acid (1) caused by the washing with water (sugars and other highly hydrophilic molecules are also desorbed). With the first MeOH fraction (fraction 4) the formation of a precipitate was observed, indicating the presence of polyphenols above the solubility limit. Successive small amounts of this solvent were added to fraction 4 until a clear solution was achieved. The HPLC-DAD analysis of such a solution is presented in Fig. 6b, showing the presence of many polyphenols at a very high concentration comparatively to the initial extract. Quercetin dimers and trimers are at high concentration in this fraction, as well as 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (2) and quercetin-diglycoside (4), particularly in proportion to quercetin (6). Protocatechuic acid (1) still retained in the Q-MIP is eluted in this fraction. Figure 6c compares the phenolic profile correspondent to fractions 6 and 7, showing a high relative content concerning 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (2) and quercetin-diglycoside (4) in fraction 6 and the reverse for quercetin (6) in fraction 7. These results also confirm a higher affinity of the Q-MIP towards quercetin, plausibly introduced by molecular imprinting. Figure 6d shows the huge concentration effect (34 times) achieved through the sorption/desorption process for the quercetin polyphenol and some derivatives.

Conclusions

A high retention of onion skin polyphenols contained in extracts with variable water content (ranging from ethanol/water 80/20 to 20/80 v/v) was shown to be possible with quercetin-molecularly imprinted particles synthesized with 4-vinylpyridine as the functional monomer. Results here presented show that the Q-MIP particles allow working

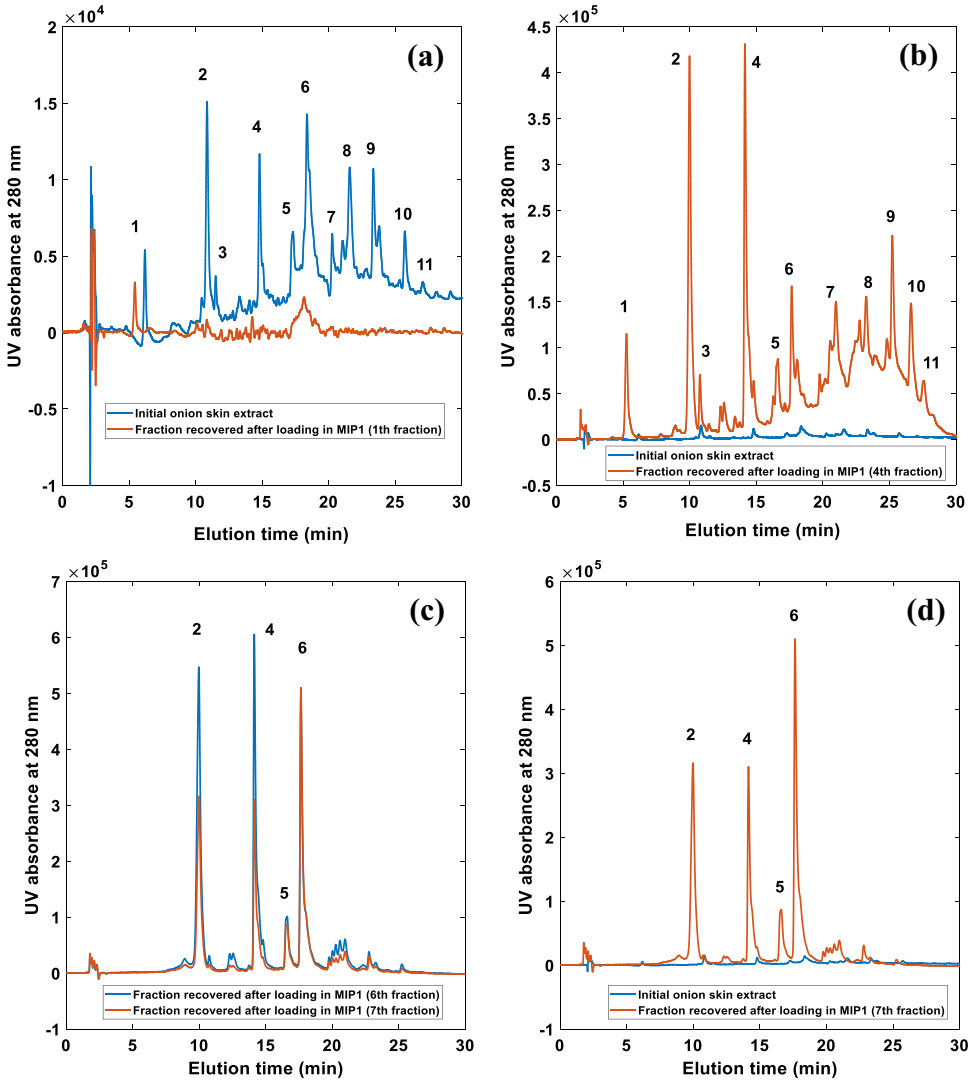


Fig. 6 HPLC-DAD analysis for fractions recovered after loading of an onion skin Soxhlet extract with EtOH/H_2O 20/80 in MIP1 (see S10–S11). **a** Initial extract and fraction 1 (washing with H_2O). **b** Initial extract and fraction 4 (recovered with MeOH at room temperature). **c** Fraction 6 (MeOH at room temperature) and fraction 7 (MeOH at 45 °C). **d** Initial extract and fraction 7

Table 1 Polymerization conditions used in the preparation of different quercetin-MIPs and NIPs

MIP	Functional monomer	Crosslinker	Solvent	Y_M	Y_I	Y_{CL}	$Y_{FM/T}$	Notes
MIP1	4VP	EGDMA	ACN/DMF 91/9	6.4	5.2	49.1	5.0	MIP9 in [12]
MIP2	4VP	DVB	ACN/DMF 91/9	6.4	5.2	49.1	5.0	
MIP3	4VP	EGDMA	ACN/DMF 91/9	20.0	5.0	49.9	20.3	
MIP4	STY	DVB	Ethanol	6.7	1.9	54.2	35.8	MIP11 in [12]

Polymerizations performed at $T = 60$ °C. NIPs were prepared in parallel, following the same experimental procedure as for MIPs, eliminating the presence of the quercetin template (see MIP/NIP performance comparison in [12]).

with a wide range of operation conditions (namely when compared with non-tailored adsorbents) and at a high polyphenol concentration, and thus, a more flexible and efficient downstream processing for these bioactive compounds can be conceived. This work also shows that many new opportunities and future developments are possible with molecular imprinting concerning the development of tailored adsorbents and processes aiming at the valorization of plant residues and biotechnological applications. Extension of the recipes here presented to other polyphenol templates (e.g., for sample preparation in analytical applications), changing of materials functionality through the design of molecular imprinting composition and/or polymerization technique (e.g., reversible deactivated radical polymerization) and the manipulation of particles morphology/surface area are important issues to be addressed in future works.

Acknowledgements This work is a result of project "AIProcMat@N2020—Advanced Industrial Processes and Materials for a Sustainable Northern Region of Portugal 2020", with the reference NORTE-01-0145-FEDER-000006, supported by Norte Portugal Regional Operational Programa (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF) and of Project POCI-01-0145-FEDER-006984—Associate Laboratory LSRE-LCM funded by ERDF through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI)—and by national funds through FCT—Fundação para a Ciência e a Tecnologia.

Compliance with ethical standards

Conflict of interest The authors declare a total absence of conflicts of interest.

References

1. Demidcchik V (2015) Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. Environ Exp Bot 109:212–228
2. Teotia S, Singh D (2014) Oxidative Stress in Plants and Its Management. In: R.K. Gaur and P. Sharma (eds) Approaches to Plant Stress and their Management. Springer India. 10.1007/978-81-322-1620-9_13
3. Séquin M (2017) The Chemistry of Plants and Insects - Plants, Bugs and Molecules, Royal Society of Chemistry, Croydon, United Kingdom **(ISBN: 978-1-78262-448-6)**
4. Watson RR (ed) (2019) Polyphenols in plants: Isolation, Purification and Extract Preparation, 2nd edn. Academic Press, New York **(ISBN: 9780128137680)**
5. Polyphenols Market Size (2019) GRAND VIEW RESEARCH, https://www.grandviewresearch.com/press-release/global-polyphenols-market. Accessed 26 Aug 2019
6. FAOSTAT. Data for Agricultural production (2017) https://www.fao.org/faostat/en/#data/QC. Electronic version acessed in October 2019
7. Kühn S, Wollseifen HR, Galensa R, Schulze-Kaysers N, Kunz B (2014) Sorption of flavonols from onion (*Allium cepa* L.) processing residues on a macroporous acrylic resin. Food Res Int 65:103–108
8. Kammerer DR, Kammerer J, Carle R (2019) Chapter 19—adsorption and ion exchange for the recovery and fractionation of polyphenols: principles and applications. In: Watson RR (ed) Polyphenols in plants, 2nd edn. Academic Press, New York, pp 327–339 **(ISBN:9780128137680)**
9. Pérez-Larrán P, Días-Reinoso B, Moure A, Alonso JL, Domínguez H (2017) Adsorption technologies to recover and concentrate food polyphenols. Curr Opin Food Sci 17:165–172
10. Ye L, Mattiasson B (2015) Molecularly imprinted polymers in biotechnology. Springer, Berlin
11. Whitcombe MJ, Kirsch N, Nicholls IA (2014) Molecular imprinting science and technology: a survey of the literature for the years 2004–2011. J Mol Recognit 27:297–401
12. Gomes CP, Dias RCS, Costa MRPFN (2019) Preparation of molecularly imprinted adsorbents with improved retention capability of polyphenols and their application in continuous separation processes. Chromatographia 82:893–916
13. Gomes CP, Sadoyan G, Dias RCS, Costa MRPFN (2017) Development of molecularly imprinted polymers to target polyphenols present in plant extracts. Processes 5(72):1–24
14. Gomes CP, Dias RCS, Costa MRPFN (2019) Polymer Reaction Engineering Tools to Tailor Smart and Superabsorbent Hydrogels. In: Mondal M. (eds) Cellulose-Based Superabsorbent Hydrogels. Polymers and Polymeric Composites: A Reference Series. Springer Nature, Switzerland AG, https://doi.org/10.1007/978-3-319-77830-3_19
15. Oliveira D, Freitas A, Kadhirvel P, Dias RCS, Costa MRPFN (2016) Development of high performance and facile to pack molecularly imprinted particles for aqueous applications. Biochem Eng J 111:87–99
16. Oliveira D, Gomes CP, Dias RCS, Costa MRPFN (2016) Molecular imprinting of 5-fluorouracil in particles with surface RAFT grafted functional brushes. React Funct Polym 107:35–45
17. Kupai J, Razali M, Buyuktiryaki S, Kecili R, Szekely G (2017) Long-term stability and reusability of molecularly imprinted polymers. Polym Chem 8:666–673
18. Ly TN, Hazama C, Shimoyamada M, Ando A, Kato K, Yamauchi R (2005) Antioxidative compounds from the outer scales of onion. J Agric Food Chem 53:8183–8189
19. Campone L, Celano R, Piccinelli AL, Pagano I, Carabetta S, Di Sanzo R, Russo M, Ibañez E, Cifuentes A, Rastrelli L (2018) Response surface methodology to optimize supercritical carbon dioxide/cosolvent extraction of brown onion skin by-product as source of nutraceutical compounds. Food Chem 269:495–502
20. Khiari Z, Makris DP, Kefalas P (2009) An investigation on the recovery of antioxidant phenolics from onion solid wastes employing water/ethanol-based solvent systems. Food Bioprocess Technol 2:337–343
21. Kiassos E, Mylonaki S, Makris DP, Kefalas P (2009) Implementation of response surface methodology to optimise extraction of onion (*Allium cepa*) solid waste phenolics. Innov Food Sci Emerg Technol 10:246–252
22. Bello MO, Olabanji IO, Abdul-Hammed M, Okunade TD (2013) Characterization of domestic onion wastes and bulb (*Allium cepa* L.): fatty acids and metal contents. Int Food Res J 20:2153–2158
23. Kwak J-H, Seo JM, Kim N-H, Arasu MV, Kim S, Yoon MK, Kim S-J (2017) Variation of quercetin glycoside derivatives in three onion (*Allium cepa* L.) varieties. Saudi J Biol Sci 24:1387–1391
24. Ko EY, Nile SH, Sharma K, Li GH, Park SW (2015) Effect of different exposed lights on quercetin and quercetin glucoside content in onion (*Allium cepa* L.). Saudi J Biol Sci 22:398–403
25. Vazquez-Armenta FJ, Cruz-Valenzuela MR, Ayala-Zavala JF (2016) Onion (Allium cepa) essential oils. In: Preedy VR (ed) Essential oils in food preservation, Flavor and Safety. Academic Press, Cambridge, pp 617–623 **(ISBN: 9780124166417)**
26. Bhagwat S, Haytowitz DB, Holden JM (2014) USDA database for the flavonoid content of selected foods. U.S. Department of Agriculture, Beltsville
27. Lachman J, Proněk D, Hejtmánková A, Dudjak J, Pivec V, Faitová K (2003) Total polyphenol and main flavonoid antioxidants in different onion (*Allium cepa* L.) varieties. Hort Sci (Prague) 30:142–147
28. Nile A, Nile SH, Kim DH, Keum J, Pivec YS, Seok PG, Sharma K (2018) Valorization of onion solid waste and their flavonols for assessment of cytotoxicity, enzyme inhibitory and antioxidant activities. Food Chem Toxicol 119:281–289
29. Sharma K, Ko EY, Assefa AD, Ha S, Nile SH, Lee ET, Park SW (2015) Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. J Food Drug Anal 23:243–252
30. Lesjak M, Beara I, Simin N, Pintać D, Majkić T, Bekvalac K, Orčić D, Mimica-Dukić N (2018) Antioxidant and anti-inflammatory activities of quercetin and its derivatives. J Funct Foods 40:68–75
31. Olayeriju OS, Olaleye MT, Crown OO, Komolafe K, Boligon AA, Athayde ML, Akindahunsi AA (2015) Ethylacetate extract of red onion (*Allium cepa* L.) tunic affects hemodynamic parameters in rats. Food Sci Hum Wellness 4:115–122

32. Lee WS, Yi SM, Yun JW, Jung JH, Kim DH, Kim HJ, Chang S-H, Kim G, Ryu CH, Shin SC, Hong SC, Choi YH, Jung J-M (2014) Polyphenols Isolated from *Allium cepa* L. induces apoptosis by induction of p53 and suppression of Bcl-2 through inhibiting PI3K/Akt signaling pathway in AGS human cancer cells. J Cancer Prev 19:14–21
33. Kawabata K, Mukai R, Ishisaka A (2015) Quercetin and related polyphenols: new insights and implications for their bioactivity and bioavailability. Food Funct 6:1399–1417

Affiliations

C. P. Gomes[1] · V. Franco[1] · Rolando C. S. Dias[1] · M. R. P. F. N. Costa[2]

✉ Rolando C. S. Dias
rdias@ipb.pt

[1] LSRE and Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

[2] LSRE, Faculdade de Engenharia da Universidade Do Porto, Rua Roberto Frias s/n, 4200-465 Porto, Portugal