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Grau de Química

Treball Final de Grau

Assessment of polymeric resins for the recovery of polyphenols.
Avaluació de resines polimèriques per a la recuperació de polifenols.

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Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

Marie Curie

Als tutors d'aquest treball final de grau, la Dra. Mercè Granados i el Dr. Xavier Saurina, per haver-me guiat i donat suport sempre que ho he necessitat durant la realització d'aquest projecte.

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A tots vosaltres, moltes gràcies.

REPORT

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1. SUMMARY

Dietary polyphenols have received a great attention among food scientists, nutritionists and consumers due to their roles in human health. The main reason for this interest is the antioxidant properties of polyphenols. Research in recent years strongly supports a role for polyphenols in the prevention of cancer and degenerative, cardiovascular and neurodegenerative diseases.

This research is part of a much broader project related to the valorization of different types of wastes from a circular economy perspective. Particularly, this research is related to the recovery of polyphenols from agri-food sector solid wastes such as fruits and vegetables. It focuses on the assessment of polymeric resins with different physicochemical properties by studying the behaviour of a synthetic mixture of representative polyphenols.

In first place, kinetic studies have been carried out with resins with different polymeric structures (PAD610, PAD950, PAD900, MN202, MN270 and Aurix 100) that show fast adsorption processes for most of resins. Moreover, it has been observed that extraction of gallic acid and hydroxytyrosol, which are more polar compounds, is less efficient than the others.

Secondly, there have been carried out studies about the ratio between the amount of resin and volume of solution and about the effect of pH. Those studies have proven that MN202 resin presents the highest retention capacity for all the compounds.

In addition, the adjustment of experimental data of gallic acid to Langmuir isotherm has been checked, showing an excellent fitting.

Lastly, preliminary elution studies have been also conducted and it has been found that after 60 minutes the recovery efficiency of many compounds is around 80%.

Keywords: polyphenols, resins, recovery, agri-food wastes, HPLC.

2. RESUM

Els polifenols de la dieta han rebut una gran atenció entre científics alimentaris, nutricionistes i consumidors degut al seu paper a la salut humana. La raó principal d'aquest interès han sigut les propietats antioxidants dels polifenols. La investigació en els últims anys recolza firmament el paper dels polifenols en la prevenció de càncers i malalties degeneratives, cardiovasculars i neurodegeneratives.

Aquesta recerca forma part d'un projecte molt més ampli relacionat amb la revalorització de diferents tipus de residus des d'un punt de vista d'economia circular. Particularment, està relacionat amb la recuperació de polifenols dels residus sòlids de la industria agroalimentària, com fruites i verdures. Es centra en l'avaluació de resines polimèriques amb diferent propietats fisicoquímiques, estudiant el comportament d'una mescla sintètica de polifenols representatius.

En primer lloc, s'han realitzat estudis cinètics amb resines amb diferents estructures polimèriques (PAD610, PAD950, PAD900, MN202, MN270 i Aurix 100), que mostren processos d'adsorció ràpids per a la majoria de les resines. A més, s'ha observat que l'extracció de l'àcid gàl·lic i l'hidroxitirosol, els compostos més polars, és menys eficient que la resta.

En segon lloc, s'han dut a terme estudis sobre la relació entre la quantitat de resina i el volum de solució i estudis sobre l'efecte del pH. Aquests estudis han demostrat que la resina MN202 presenta la capacitat de retenció més elevada per a tots els compostos.

A més, s'han ajustat les dades experimentals de l'àcid gàl·lic a una isoterma de Langmuir i mostren un ajust excel·lent.

Per últim, s'han dut a terme assajos preliminars d'elució i s'ha trobat que, després de 60 minuts, l'eficiència de recuperació per a la majoria dels compostos està al voltant d'un 80%.

Paraules clau: polifenols, resines, recuperació, residus agroalimentaris, HPLC.

3. INTRODUCTION

A large amount of solid wastes is generated in the agri-food sector (production industries of vegetable juices, olive oil, etc.). These residues, generated by fruits and vegetables processing, may have relatively high levels of natural polyphenols.

At present, it is betting on revalue these wastes from the extraction of polyphenols, which later will have an application as nutraceuticals or in the cosmetic sector.

Dietary polyphenols have received a great attention among food scientists, nutritionists and consumers due to their roles in human health. The main reason for this interest is recognition of their great abundance in our diet and the antioxidant properties of polyphenols. Research in recent years strongly supports a role for polyphenols in the prevention of cancers, degenerative diseases, cardiovascular diseases and neurodegenerative diseases [1].

Normally, and despite the potential value that polyphenols have, agri-food residues are frequently thrown away due to shortage of efficient technologies for their extraction and purification. Therefore, it's important and necessary to find effective methods to recover compounds, such as polyphenols, from these solid wastes.

3.1. POLYPHENOLS

Polyphenols are natural compounds that have one or more phenol structural units. Their structures may vary from a simple phenolic molecule to a high-molecular mass and complex polymer.

"Polyphenol" is a general term for several subgroups of phenolic compounds, however, its use has been somewhat confusing because studies have shown that different subgroups of polyphenols may differ significantly in their properties [1].

These compounds may be subdivided into two major groups, the flavonoid and non-flavonoid compounds [2].

3.1.1. Flavonoids

Flavonoids have a common C6-C3-C6 carbon skeleton which contains two units of phenolic nature (C6). Nowadays, more than 4000 flavonoids have been identified from plant sources [3].

Flavonoids can be further divided into different sub-groups, primarily differing on the basis of oxidation state of central carbon. These sub-groups include flavanones, flavanols, flavonols, isoflavonoids, flavones and anthocyanins. The following table has been adapted from [3] and [4].

Table 1. Main families of flavonoid compounds.

Type of flavonoid	Characteristics	Richest vegetable sources	Main representative compounds
Flavanones	They are generally glycosylated by a disaccharide at position 7.	Citrus fruits, tomatoes and aromatic plants (mint).	Naringenin, hesperetin and eryodictyol.
Flavanols	They exist in both the monomer form (catechins) and the polymer form (proanthocyanidins).	Fruits, red wine, green tea and chocolate.	Catechin and epicatechin
Flavonols	Are the most ubiquitous flavonoids in foods. These compounds are present in glycosylated forms.	Onions, curly lake, leeks, broccoli and blueberries. (red wine and tea)	Quercetin, kaempferol
Isoflavonoids	They are flavonoids with structural similarity to estrogens, but they are not steroids. They are sensitive to heat and are often hydrolysed to glycosides during the industrial processing.	Leguminous plants (soy)	Genistein, daidzein and glycitein
Flavones	These are much less common than flavonols in fruit and vegetables. The polymethoxylated flavones are the most hydrophobic flavonoids.	Parsley, celery and skin of citrus fruit.	Apigenin, luteolin
Anthocyanins	They are pigments dissolved in the vacuolar sap of the epidermal tissues of flowers and fruit, to which they impart a pink, red, blue or purple colour.	Red wine, cereals, fruits and root vegetables (aubergines, cabbage, beans, onion, radishes)	Cyanidin

The structure of one of the main representative polyphenols of each type of flavonoids is shown below in figure 1.

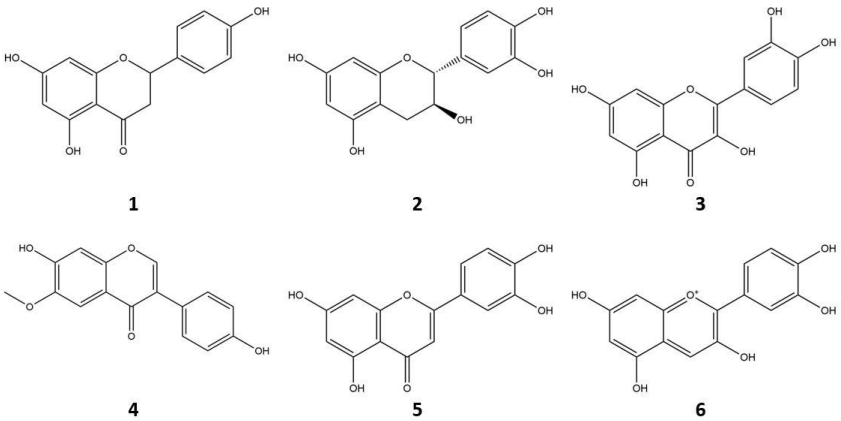


Figure 1. General structures of flavonoids.

(1) Naringenin (flavanones)
(2) Catechin (flavanols)
(3) Quercetin (flavonols)
(4) Glycitein (isoflavonoids)
(5) Luteolin (flavones)
(6) Cyanidin (anthocyanins)

3.1.2. Non-flavonoids

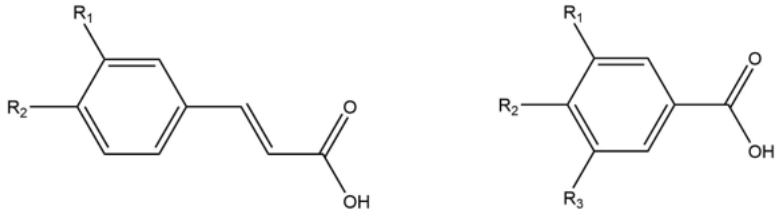
There are three families of non-flavonoids polyphenols: phenolic acids, stilbenes and lignans.

Phenolic acids are key polyphenols in numerous fruits and vegetables like kale, onions, broccoli, tea extracts and grape extracts [3].

We can distinguish two classes of phenolic acids: derivatives of benzoic acid and derivatives of hydroxycinnamic acid, based on C3-C6 and C1-C6 backbones respectively.

Hydroxybenzoic acids (e.g. gallic acid, hydroxybenzoic acid) are rare in the human diet and this is the reason that such compounds are not suggested playing an important role in human health.

Hydroxycinnamic acids (e.g. caffeic acid, sinapic acid) are more common, but they are rarely found in the free form, except in processed food that has undergone freezing, sterilization or fermentation. Instead, they naturally occur as esters with quinic acid or sugars. The general structure of phenolic acids is shown in Figure 2.



R_1=OH Coumaric acid
R_1=R_2=OH Caffeic acid

R_1=R_2=R_3=OH Gallic acid
R_1=R_2=OH Protocatechuic acid

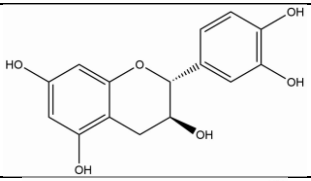
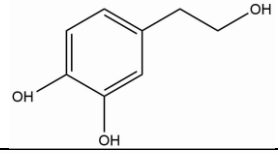
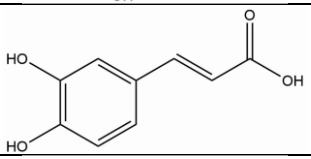
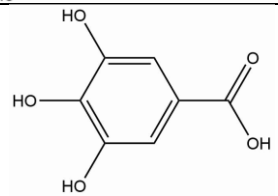
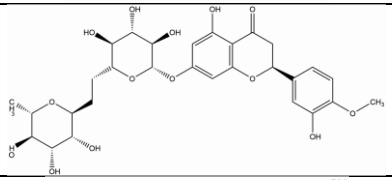
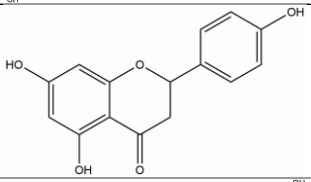
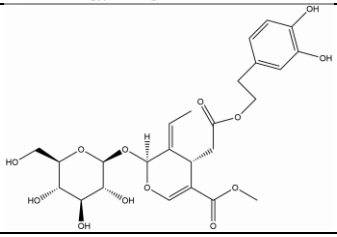
Figure 2. General chemical structure of phenolic acids. Left: hydroxycinnamic acids; right: hydroxybenzoic acids.

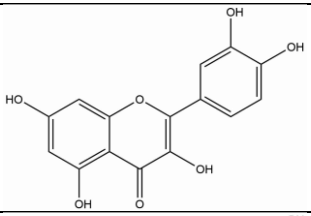
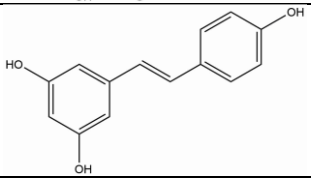
Stilbenes are found in low quantities in the human diet. One of these is resveratrol (see structure in Table 2), which exists in *cis* and *trans* conformations and has been found in low quantities in wine. This compound has been extensively studied and anticarcinogenic effects have been shown during screening of medicinal plants [4].

Lastly, lignans are formed of 2 phenylpropane units. They are found in linseed and other cereals, grains, fruits and vegetables.

This project focuses on the recovery of polyphenols from waste generated in the agri-food sector (companies producing olive oil and wine cellars). The following table shows the representative polyphenols that have been studied, their pK_a, molecular formula and what type of polyphenol are. They have been selected as a model of the most abundant phenolic compounds occurring in the waste samples under study.

Table 2. Representative polyphenols studied.

Name	Molecular formula	Chemical structure	Type of polyphenol	pK_a
Catechin	$C_{15}H_{14}O_6$		Flavonoid (flavanol)	9.0
Hydroxytyrosol	$C_8H_{10}O_3$		Other polyphenols (tyrosol)	9.45
Caffeic acid	$C_9H_8O_4$		Phenolic acid (hydroxycinnamic acid)	4.62
Gallic acid	$C_7H_6O_5$		Phenolic acid (hydroxybenzoic acid)	3.94
Hesperidin	$C_{28}H_{34}O_{15}$		Flavonoid (flavanone)	8.61
Naringenin	$C_{15}H_{12}O_5$		Flavonoid (flavanone)	7.91
Oleuropein	$C_{25}H_{32}O_{13}$		Other polyphenol (tyrosol)	4.94

Quercetin	$C_{15}H_{10}O_7$		Flavonoid (flavonol)	8.45
Resveratrol	$C_{14}H_{12}O_3$		Stilbene	8.99

3.2. RECOVERY OF POLYPHENOLS

Polyphenols can be extracted from solid wastes from agri-food industries using conventional extraction with methanol, ethanol or water, but they also can be extracted using non-conventional techniques. Some examples of these non-conventional techniques are pressurized liquid extraction, microwave assisted extraction and ultrasound assisted extraction [5]. Moreover, all extracts obtained must be purified.

Polymeric adsorbents suppose a new trend in the recovery of polyphenols, and adsorption processes have been identified as a good technology for the recovery of organic compounds from the extracts.

Owing to their chemical stability, high adsorption capacity, limited toxicity, selectivity and ease of regeneration at moderated temperatures, resins are the most studied adsorbents. Ion-exchange resins are proposed for the adsorption of phenolics and natural extracts. Furthermore, non-ionic resins are frequently used [6].

There are a lot of interactions between the adsorbent and the molecules like acid-base interaction, Van der Waals, hydrogen bond, covalent bond, etc. Anyway, a reversible adsorption is desirable if the objective is to recover the solute.

Adsorption is a relatively flexible and simple technology and requires product separation and concentration stages to obtain a phenolic-enriched extract. It is possible to simultaneously recover, purify and concentrate the target compounds in a phenolic rich product. This extract may be used as bioactive product.

3.2.1. Resins

Polymeric resins are spherical synthetic polymers with defined pore structure and high surface area for efficient and selective removal of organic molecules, primarily in aqueous applications [7].

The adsorption efficiency of polymeric adsorbents is affected by the following factors.

- Chemical interactions.
- Molecule size.
- Capacity: more molecules can be adsorbed with a great surface area.
- Solvent: organic molecules can be ionized in polar and protic solvents. It causes an attraction with ion-exchange or hydrophilic groups.

In this project, there have been studied six different resins: PAD900, PAD950, PAD610, MN202, MN270 and Aurix 100.

3.2.1.1. Non-functionalized resins

All the resins mentioned before, except for Aurix 100, are non-functionalized polymer matrices. It means that they do not have any surface modification or ion-exchange ability. We can distinguish three types: aromatic polystyrenic or polydivinylbenzene and aliphatic methacrylate.

In Table 3 is shown what type is each resin and the polymeric structure of the three types.

Aromatic polystyrene adsorbents have strong affinity for hydrophobic molecules such as polyphenols [7]. Aliphatic methacrylic resins display lower hydrophobic behaviour and have good affinity for molecules with aliphatic or semi-aliphatic rings and chains. These adsorbents will develop hydrogen bonding in non-polar conditions due to the interaction of the hydrogens of the carbonyl group from the polyester resin matrix associated with hydroxyls on the target molecules.

These resins are stable in alkaline or acidic solutions and in most organic solvents.

Table 4 (adapted from [7]) contain information about physical characteristics of each non-functionalized resins such as specific surface area, average pore diameter, pore volume and an image of its look.

Table 3. Polymeric structure of the resins used. Table adapted from [7].

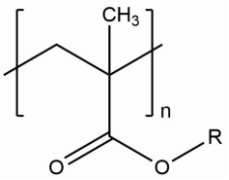
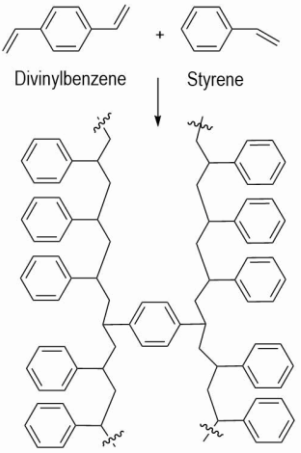
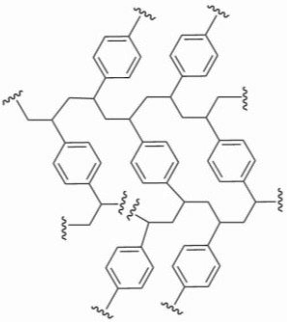
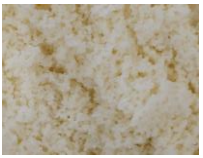
Methacrylic	Polystyrenic	
	Polystyrene cross-linked with divinylbenzene	Polydivinylbenzene with no polystyrene
PAD950, PAD610	MN202, MN270	PAD900
		

Table 4. Physical characteristics and image of non-functionalized resins.

Resin	Specific surface area (m^2/g)	Average pore diameter (Å)	Pore volume (mL/g)	Remarks and applications	Image
MN270	1200	80	0.5	Microporous matrix for small organics / volatile organic compounds removal.	

PAD610	490	300	1.2	A moderately hydrophilic adsorbent used for peptide and vitamin recovery and removal of organic pollutants from ground water.	
PAD950	450	120	0.6		
MN202	950	220	0.3	Extraction of small to large molecules from aqueous solutions such as peptides and proteins.	
PAD900	850	220	1.9		

3.2.1.2. Ion-exchange resins

Aurix 100 is an IE resin used habitually for recovery of gold, but it has also been used for phenol removal from aqueous solutions [8].

Its structure is based on a styrene divinylbenzene based macroreticular resin bead functionalized with guanidine groups (Figure 3). In contact with an aqueous solution, the guanidine group will extract a proton from water at pH's typically less than 11.5 to form a guanidine cation. At pH's above 13, the guanidinium cation gives up its proton to re-form a neutral guanidine group [9].

The appearance of this resin is shown in Figure 4.

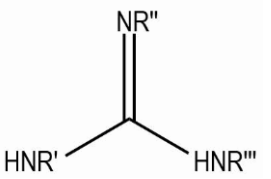


Figure 3. Guanidinium group

Figure 4. Aurix 100 resin beads.

4. OBJECTIVES

This work is part of a much broader research project related to the valorization of different types of wastes from a circular economy perspective. Particularly, it is related to the recovery of polyphenols from agri-food sector solid wastes (fruits and vegetables). It focuses on the assessment of polymeric resins for the recovery of polyphenols to obtain concentrates of interest.

The objectives of the work are:

- To evaluate polymeric resins with different physicochemical properties, studying the behaviour of a synthetic mixture of representative polyphenols, considering the composition of the natural extracts.
- To investigate the effect of the composition of the solvent and the pH on the retention/elution of the different polyphenols, using batch methodology.

5. EXPERIMENTAL SECTION

5.1. STANDARDS AND SOLVENTS

5.1.1. Polyphenols

These compounds have been used to create a synthetic mixture for the analysis of polyphenols in the solutions by high performance liquid chromatography (HPLC).

- Gallic acid, Sigma Aldrich (St. Louis, USA)
- Catechin, TCI (Tokyo, Japan)
- Resveratrol, TCI (Tokyo, Japan)
- Naringenin, Carbosynth limited (Berkshire, England, United Kingdom)
- 2-(3,4-Dihydroxyphenyl) ethyl alcohol, TCI (Tokyo, Japan)
- Quercetin, Merck (Darmstadt, Germany)
- Hesperidin, Glentham (Wiltshire, England, United Kingdom)
- Caffeic acid, Sigma Aldrich (St. Louis, USA)
- Oleuropein, Carbosynth limited (Berkshire, England, United Kingdom)
- Luteolin, Carbosynth limited (Berkshire, England, United Kingdom)

Initial standard solutions with a concentration of 5000 mg mL^{-1} has been prepared in DMSO and stored in the refrigerator to prevent a possible degradation of polyphenols. The working mixture is composed of aqueous solutions of 25 mg mL^{-1} each polyphenol except for oleuropein that is at 100 mg mL^{-1}.

5.1.2. Resins

- PuroSorb PAD950, Purolite Ltd (Llantrisant, Wales, United Kingdom)
- PuroSorb PAD900, Purolite Ltd (Llantrisant, Wales, United Kingdom)
- PuroSorb PAD610, Purolite Ltd (Llantrisant, Wales, United Kingdom)

- Macronet MN202, Purolite Ltd (Llantrisant, Wales, United Kingdom)
- Macronet MN270, Purolite Ltd (Llantrisant, Wales, United Kingdom)
- Aurix 100, BASF (Monheim am Rhein, Germany)

5.1.3. Solvents

- Acetonitrile for UHPLC Supergradient, ACS, Panreac (Castellar del Vallès, Spain)
- Ethanol, Honeywell
- Water purified with a Milli-Q equipment (Merck Millipore)
- Dimethyl sulfoxide, Merck (Darmstradt, Germany)

5.1.4. Other reagents

- Formic acid 98-100% w/w, Merck (Darmstadt, Germany)
- Hydrochloric acid 32% w/w, Merck (Darmstadt, Germany)
- Acetic acid 99-100% w/w, J.T.Baker (Deventer, The Netherlands)
- KH_2PO_4, J.T.Baker (Deventer, The Netherlands)
- H_3BO_3, Merck (Darmstadt, Germany)

5.2. LABORATORY EQUIPMENT AND INSTRUMENTATION

The HPLC system used has been an Agilent Series 1100 HPLC chromatograph by Agilent Technologies with a quaternary pump, a degasser, an automatic injection system and a diode array and fluorescence detectors. All the components are from 1100 series, except for the automatic injector and the degasser, which are from the 1200 series. The chromatographic column used is a Kinetex C18 (100 mm x 4.6 mm, particle size 2.6 μm, 100 Å). The software used for instrumental control and data processing has been the Agilent ChemStation for LC.

The balance that has been used to weigh resins and standards is Sartorius TE214S, which has an error of ±0.1 mg.

The pH of buffer solutions has been measured with a Crison Basic-20 pH-meter.

5.3. METHODOLOGY

5.3.1. Analysis of polyphenols by HPLC

A Kinetex C18 column has been used to perform the chromatographic separation by HPLC. The mobile phase was an aqueous phase (A) of 0.1% formic acid and the organic phase (B) was ACN. The elution gradient for the determination of the composition of the polyphenolic mixtures of standards in the evaluation of absorption and release processes is given in Table 5. The flow rate used has been 1 mL min^{-1} and the injection volume 5 µL. In these conditions, the time of the chromatogram is 50 minutes. Detection has been performed at 280, 310 and 370 nm.

Table 5. Elution gradient program for the determination of polyphenols using HPLC.

Time (min)	Percentage of organic phase (% ACN)
0	5.0
30	20.0
40	45.0
40.2	5.0
50	5.0

In addition, for the assessment of the absorption isotherm of gallic acid, isocratic elution with 6% ACN has been used. Under these circumstances, retention times is 5.5 min.

In Figure 5 the chromatogram of a standard solution containing a mixture of polyphenols at 25 ppm and 100 ppm for oleuropein is shown.

Furthermore, Table 6 shows the retention time and the appropriate wavelength to perform a good quantification of each compound.

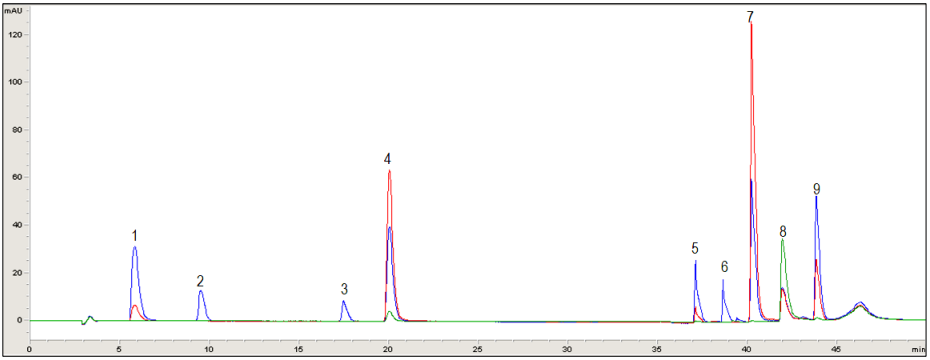


Figure 5. Chromatogram of standard solution containing all polyphenols studied.
■ 280 nm ■ 310 nm ■ 370 nm

Table 6. Retention time of polyphenols.

Peak	Polyphenol	t_R (min)	Wavelength (nm)
1	Gallic acid	5,8	280
2	Hydroxytyrosol	9,5	280
3	Catechin	17,4	280
4	Caffeic acid	20,1	310
5	Hesperidin	37,1	280
6	Oleuropein	38.6	280
7	Resveratrol	40,2	310
8	Quercetin	42,0	370
9	Naringenin	43,8	280

5.3.2. Kinetics

The first extraction experiment to choose the most suitable resins and study the kinetics was performed as follows:

- A solution with a concentration of 100 ppm from oleuropein and 25 ppm from the rest of polyphenols is prepared in mili-Q water.

- 0,2 g of each resin (PAD610, PAD900, PAD950, MN202, MN270 and Aurix100) is weighed in Falcon tubes.
- 10 mL of the mixture of polyphenols are added to each resin.
- The solutions are stirred for 2 hours.
- Aliquots of 250 µL are sampled in the following times: 5 minutes, 15 minutes, 30 minutes, 1 hour and 2 hours.

An image of the rotary agitator used to stir the mixtures with the resins is shown in Figure 6.

Figure 6. Falcon tubes in the rotary agitator.

5.3.3. Study of pH influence on the extraction

The study of pH influence on the resin's extraction has been made with resins PAD610, MN202 and Aurix 100 using several buffer solutions at pH 1, pH 3.5, pH 4.5, pH 5.5, pH 7 and pH 10.

The procedure for the resin's extraction has been the same as the explained in the previous section (5.3.2) but only with 0.1 g of resin and sampling aliquots only at 60 minutes.

5.3.4. Ratio between the resin amount and volume of solution

In order to study how the amount of resin and the volume of sample influence in the extraction efficiency, the ratios shown in Table 7 have been assayed.

Table 7. Quantities of resin and solution used to do the study.

Grams of resin (g)	0.2	0.2	0.2	0.1	0.5	1
Volume of polyphenols mixture (mL)	2	4	10	10	2	2
Ratio	0.01	0.02	0.05	0.1	0.25	0.5

5.3.5. Isotherms

To construct the isotherm of gallic acid, the concentration of these polyphenols in aqueous solution has been modified while the amount of resin remained constant.

For each polyphenol, 2 mL of aqueous solution at each concentration, 0.2 g of resin and one hour of stirring have been used. Concentrations assayed have been 25, 50, 100, 150, 200, 250, 300, 375, 425 and 500 mg mL^{-1}.

5.3.6. Elution experiments

It has been done preliminary assays of elution. It has been reported in the literature an effective elution of polyphenols with ethanol [10] and an ethanol:water solution [11].

Therefore, the preliminary experiments of elution have been performed as follows:

- After adsorption of polyphenols (Fig.7), resins are filtered and then dried by air.
- 0.1 g of each resin, in duplicate, (MN202, PAD610 and Aurix 100) is weighed in a Falcon tube.
- 1 mL of absolute ethanol or 1 mL of 80% ethanol is added to each resin.
- The solutions are stirred for 1 hour.
- Aliquots of 250 µL are sampled and then analysed by HPLC.

Figure 7. Resins before filtration.

6. RESULTS AND DISCUSSION

The extraction of polyphenols in aqueous solution has been studied with different resins, changing parameters like the pH and the ratio between resin amount and volume of solution. In addition, preliminary experiments of elution have been done.

6.1. KINETICS EXPERIMENTS

Initially, there were six resins with four different polymeric structures. Table 3 specifies what type is each resin.

In order to determine which resins are the most suitable, it has been done an extraction of polyphenols from a standard solution with each resin for 120 minutes (section 5.3.2).

The extracts have been analysed by HPLC. It has been integrated the area of each compound at the appropriated wavelength (280, 310 or 370 nm) in order to establish the content of polyphenols in the extract at different times.

In Figure 8 are shown the results obtained for all resins. In each graph is represented how the percentage of extraction of each polyphenol changes with time.

It can be observed that the more optimum extraction is produced with the resin MN202 (D), which is polystyrenic and hydrophobic.

Other polystyrenic resins such as (C) and (E) have different behaviour and less efficient. Resin MN270 has an average pore diameter considerably less than other of the resins used (see Table 4). Therefore, it does not adsorb compounds with a big structure like catechin, oleuropein and hesperidin. However, it is observed a good adsorption for smaller and polar compounds.

Methacrylic resins, (A) and (B) have a similar behaviour between them. They are hydrophobic resins and show an efficient adsorption for less polar compounds like flavonoids, but they are not efficient for polar compounds such as gallic acid or hydroxytyrosol.

Lastly, IE resin (F) has a diverse behaviour depending on the compound. It only adsorbs efficiently some flavonoids such as quercetin and naringenin.

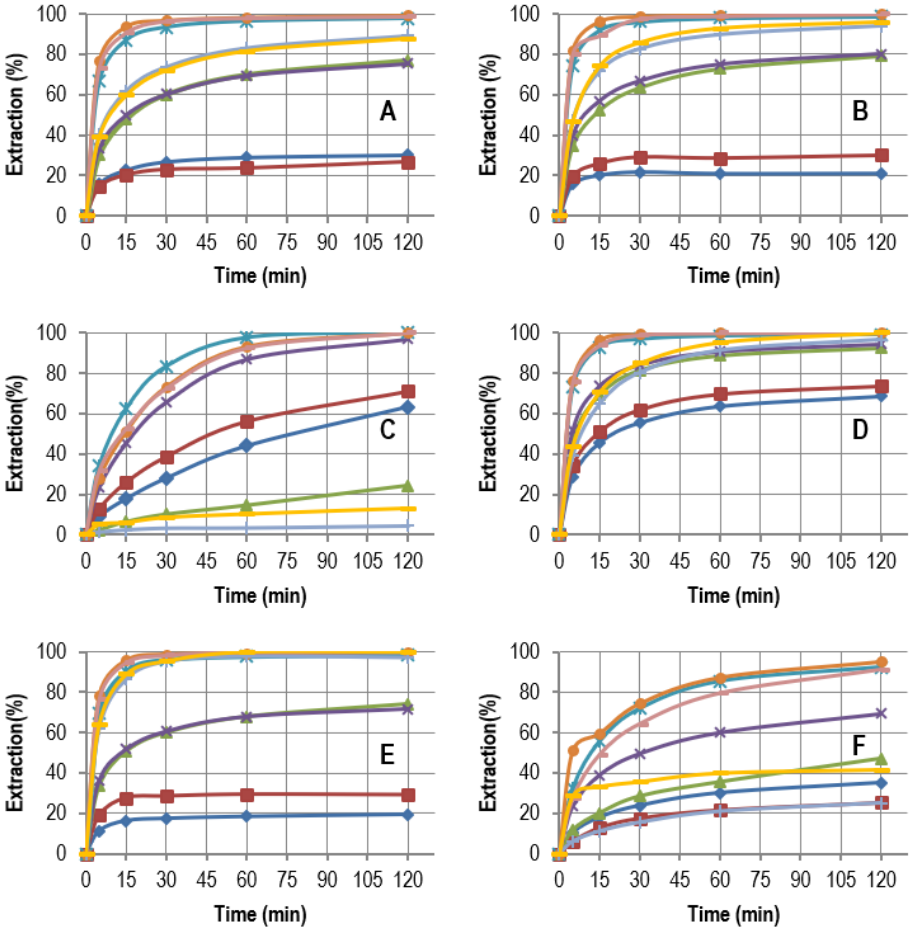


Figure 8. Kinetics experiments with six resins.
Resins assignment: (A) PAD950 (B) PAD610 (C) MN270 (D) MN202 (E) PAD900 (F) Aurix 100
Polyphenols assignment: ♦ gallic acid ■ hydroxytyrosol ▲ catechin × caffeic acid × resveratrol
● quercetin | hesperidin − naringenin – oleuropein

Regarding kinetics, in general all adsorption processes are fast except for MN270 and Aurix 100. With these two resins, it can be observed that the percentage of extraction continues increasing after 60 minutes whereas with the other four resins the percentage doesn't change significantly.

Considering the results obtained, it is decided to perform the following experiments only with three resins, one of each type. Therefore, the selected resins are MN202 which is polystyrenic, PAD610 which is methacrylic and Aurix 100 which is an IE resin.

6.2. EFFECT OF THE RATIO BETWEEN THE RESIN AMOUNT AND VOLUME OF SOLUTION

Six different ratios (see section 5.3.4.) have been used to study their effect in the extraction efficiency. Results are shown in Figure 9, and as it can be observed, percentage of extraction increases as ratio increases.

Resin PAD610 (A) could be a good option to perform the recovery of polyphenols except for the most polar compounds (gallic acid and hydroxytyrosol), as it is needed a big amount of resin to obtain a large percentage of extraction.

Furthermore, it seems that the best resin is MN202 (B) because the percentage of extraction is close to 100% for all these polyphenols also with small ratios, that is with small amounts of resin. This is an important aspect consider in order to reduce the costs in account of the use of resin.

Aurix 100 (C) shows a low percentage of extraction for the major part of these polyphenols at ratios resin:solution below 0.25. However, the recovery of gallic acid is slightly better than with resin PAD610.

In summary, considering the ratio resin mass/volume solution it is proved that resin MN202 is which that present for all the compounds more retention capacity.

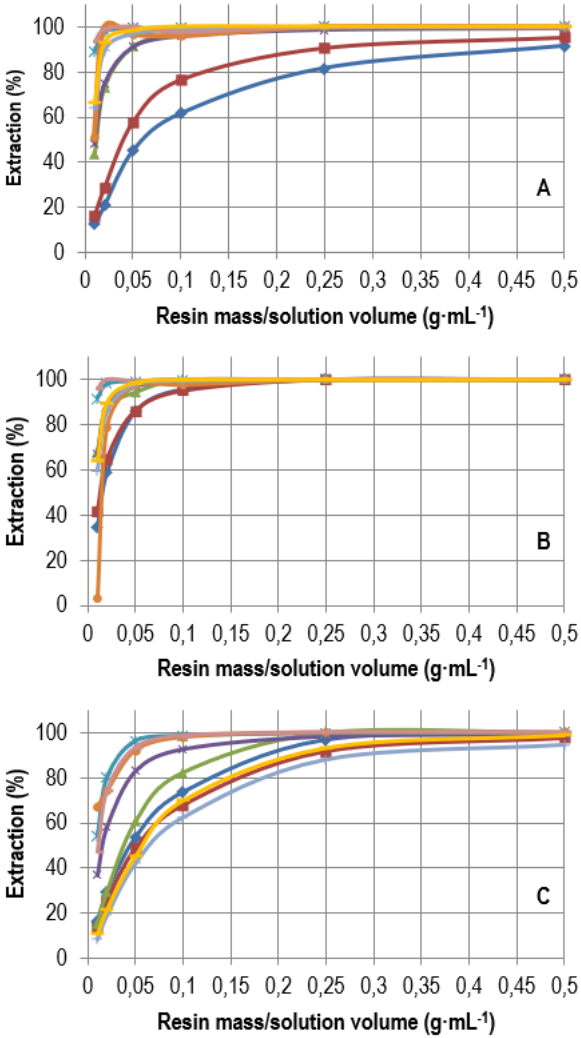


Figure 9. Effect of the ratio resin:solution on the extraction efficiency.
Resins assignment: (A) PAD610 (B) MN202 (C) Aurix 100
Polyphenols assignment: ♦ gallic acid ■ hydroxytyrosol ▲ catechin × caffeic acid × resveratrol ● quercetin | hesperidin − naringenin – oleuropein

6.3. EFFECT OF pH

These experiments have been done getting together 0.1 g of resin and 10 mL of a solution of polyphenols, changing the pH of solution between 1 and 10 and following the procedure described in section 5.3.3.

It has been observed that from pH 6 some compounds (gallic acid, catechin and hydroxytyrosol) experiment degradation. Furthermore, quercetin has not been plotted in this graph because anomalous results have been obtained.

Results are shown in Figure 10. Resins MN202 (B) and PAD610 (A) shows similar trends, although extraction is more elevate with resin MN202. As it was expected, considering the results obtained with another experiments, the most polar compounds (gallic acid and hydroxytyrosol) experiment less retention.

In the case of gallic and caffeic acids it can be observed that from pH 4 a decrease of extraction is produced. This can be attributed to the beginning of predomination of anionic species, because of the dissociation of carboxylic group. There are other polyphenols, such as resveratrol, hesperidin, naringenin and oleuropein, in which the extraction decreases at pH over 7. Is at this moment, when it starts to produce in a relevant way the dissociation of phenol group.

To interpret the behaviour of resin Aurix 100 (C) it is needed to consider that is an IE polymeric resin, with guanidine groups, and it is mainly in cationic form at pH's typically less than 10.

It can be observed that the extraction of polyphenols is less effective than with resins PAD610 and MN202. The compound who presents the best extraction, and without influence of pH in studied interval is resveratrol.

In the case of gallic acid, hydroxytyrosol and caffeic acid it can be observed an increase of extraction from pH 4, because they are in anionic form, but above pH 5, degradation is too much important. Moreover, extraction of oleuropein and hesperidin is lower than the expected.

In summary, it is important to perform these experiments at pH's below 5 in order to avoid polyphenols degradation. In this range, pH does not affect the retention of compounds in resins.

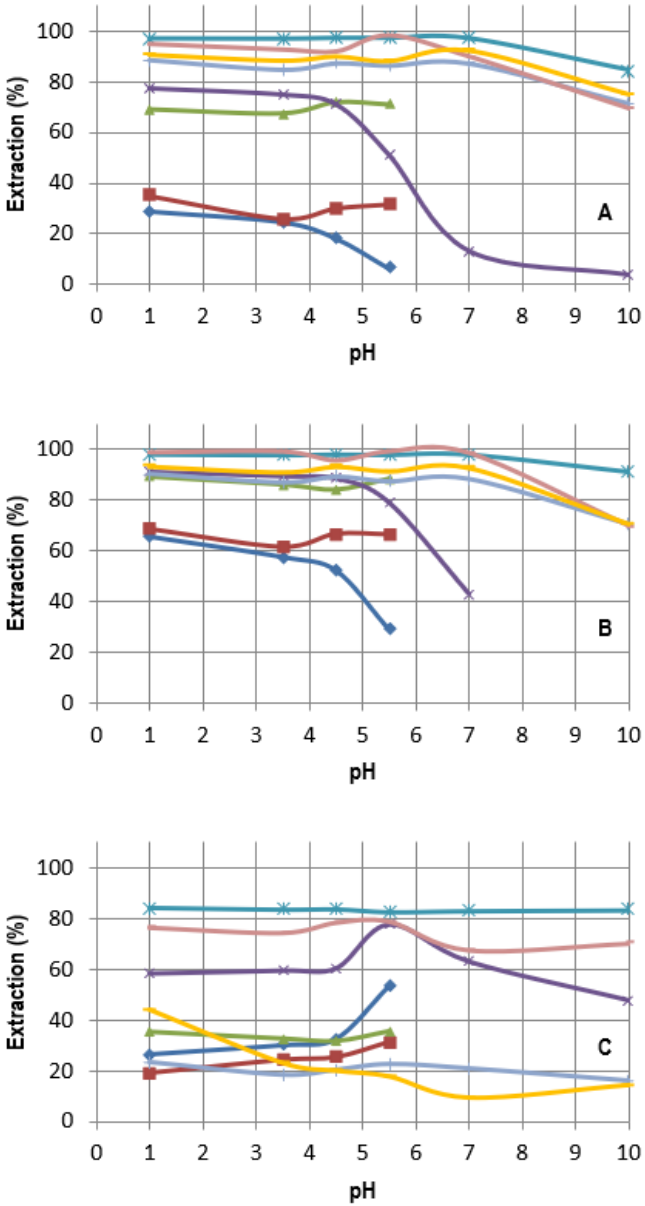


Figure 10. Effect of the pH on the extraction efficiency.
Resins assignment: (A) PAD610 (B) MN202 (C) Aurix 100
Polyphenols assignment: ♦ gallic acid ■ hydroxytyrosol ▲ catechin × caffeic acid × resveratrol
| hesperidin – naringenin – oleuropein

6.4. ADSORPTION ISOTHERM OF GALLIC ACID

The studies done until the moment indicates that MN202 resin seems to provide the best performance, comparing with the other five resins assayed.

Gallic acid has been selected because its solubility in water allows us to evaluate a larger interval of concentrations that with the rest of studied polyphenols. The conditions under which the experiments have been performed are specified in section 5.3.5.

Results are shown in Figure 11.

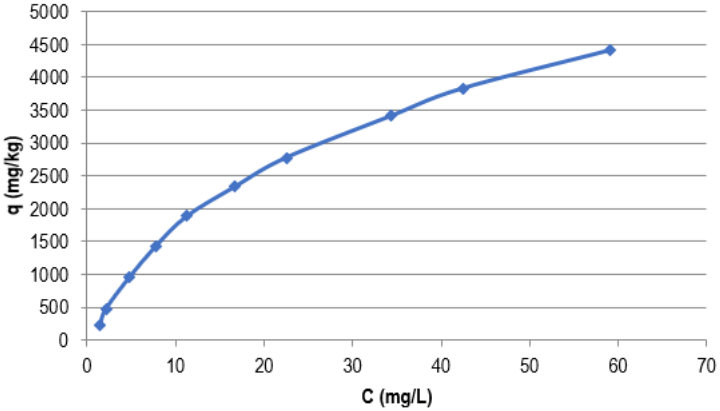


Figure 11. Adsorption isotherm of gallic acid onto MN202 resin.

This isotherm presents a Langmuir behaviour. The Langmuir isotherm basic assumption theory is that adsorption takes place at specific homogeneous sites within the adsorbent. It means that when a phenol molecule occupies a site, no more adsorption can take place at this site [8]. Langmuir isotherm can be expressed by the equation:

$$q = \frac{K_L q_{max} C}{1 + K_L C} \qquad (1)$$

where, q is the total concentration of gallic acid in resin (mg Kg^{-1}), q_{max} is the maximum loading of the resin (mg Kg^{-1}), C is the total concentration of gallic acid in solution (mg L^{-1}), and K_L is the Langmuir adsorption constant (L mg^{-1}).

The isotherms parameters (q_{max} and K_L) have been determined from the linearized form of Eq. (1).

The linearization is given by the following expression:

$$\frac{1}{q} = \frac{1}{q_{max}} + \frac{1}{K_L q_{max}} \cdot \frac{1}{C} \quad (2)$$

The representation of 1/q versus 1/C corresponds to a straight line. Also, the relationship between q_{max} and K_L with the intercept and the slope of the linear function is given as:

$$\text{intercept: } \frac{1}{q_{max}} \quad (3)$$

$$\text{slope: } \frac{1}{K_L q_{max}} \quad (4)$$

The linearized Langmuir isotherm model is shown below (Fig. 12).

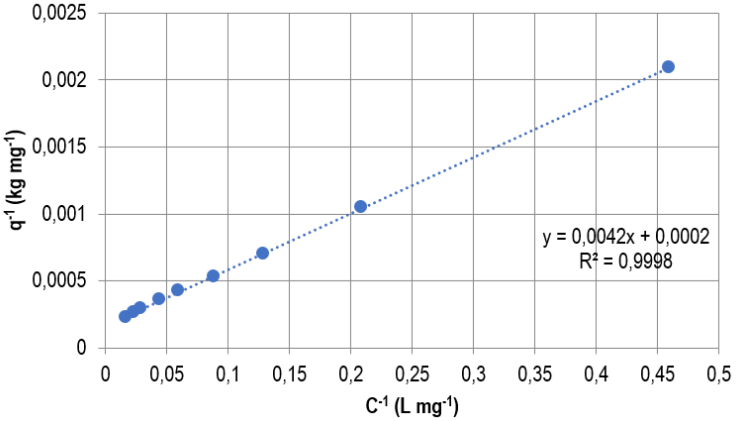


Figure 12. Linearized Langmuir isotherm model for gallic acid onto MN202.

As it can be observed in the graph, this linearization presents an excellent fitting. From the values of slope and intercept it has been obtained a result of $3{,}99 \times 10^{-2}$ L mg^{-1} for K_L and a result of $5{,}99 \times 10^{3}$ mg Kg^{-1} for q_{max}.

6.5. ELUTION EXPERIMENTS

Preliminary assays of elution with ethanol and a with a solution of ethanol:water (80:20, v/v) have been done as is described in section 5.3.6.

Results of elution for three resins (PAD610, MN202 and Aurix 100) are shown in Figure 13.

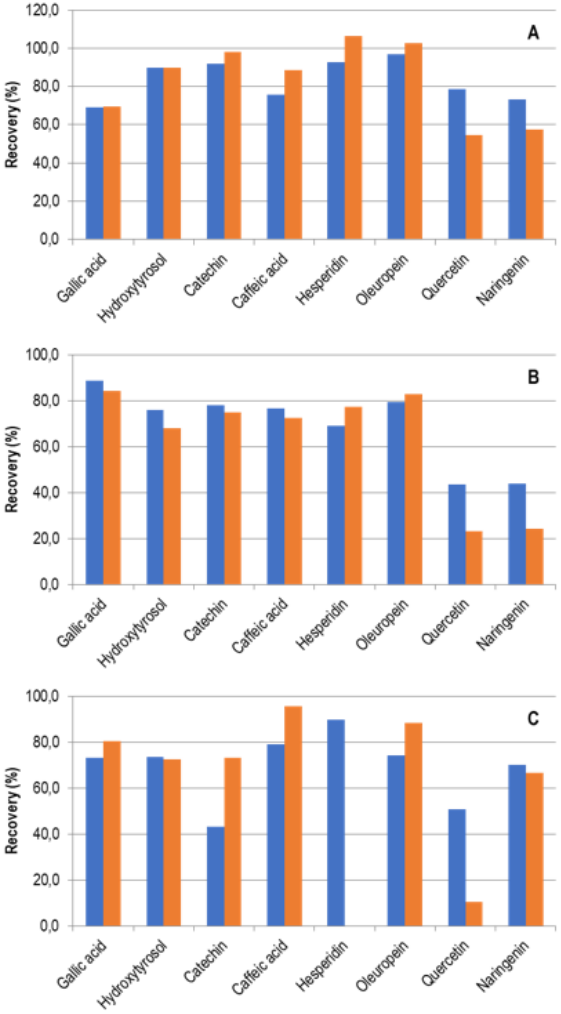


Figure 13. Elution of polyphenols after 60 minutes with ethanol ■ and 80% ethanol ■
Resins assignment: (A) PAD610 (B) MN202 (C) Aurix 100

Resveratrol has not been plotted in these graphs because anomalous results have been obtained.

The best results are obtained for resins PAD610 (A) and MN202 (B), showing a percentage of recovery around 80% for all the compounds, except for quercetin and naringenin. It has not been observed relevant differences between results obtained with both eluents.

Aurix 100 (C), also shows a good recovery for many compounds but its behaviour is much more varied.

7. CONCLUSIONS

Once the experimental work has finished, the most relevant conclusions that are drawn from this study about the recovery of different polyphenols with various polarities are the following:

- After assessing the kinetic extraction of polyphenols with resins with different polymeric structure, methacrylic (PAD950 and PAD610), polystyrene cross-linked with divinylbenzene (MN202 and MN270), polydivinylbenzene (PAD900) and ion-exchange (Aurix 100), it has been verified that, in general those show fast adsorption processes, except for MN270 and Aurix 100. From these results it has been decided to select two non-functionalized resins for their assessment, PAD610 and MN202, and to include an ion-exchange resin, Aurix 100.
- The extraction of most polar polyphenols, such as gallic acid and hydroxytyrosol, is less efficient than the other compounds with all the resins studied.
- Considering the ratio between the amount of resin and volume of solution it has been proved that MN202 resin presents the highest retention capacity for all the compounds. After studying the effect of pH on the retention of polyphenols, it has been checked that it is important to do these experiments at pH's below 5, in order to avoid degradation processes for some polyphenols. In addition, it has been observed that between pH's 1 and 5, there is no effect on the retention.
- In the preliminary studies of elution with ethanol and a solution of ethanol:water (80:20, v/v) it has been observed that there are not relevant differences between the results obtained with both eluents. Resins PAD610 and MN202 show similar results, with a total recovery (extraction and elution) for many compounds around 80%, except for quercetin and naringenin, which is around 40%.

From these results we can conclude that MN202 resin is a good option for future studies of polyphenols recovery from waste extracts generated by fruits and vegetables processing.

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9. ACRONYMS

ACN: acetonitrile

DMSO: dimethyl sulfoxide

HPLC: high performance liquid chromatography

IE: ion-exchange

t_R: retention time