Tailoring composition and nanostructures in supramolecular solvents: Impact on the extraction efficiency of polyphenols from vegetal biomass

Celia Sánchez-Vallejo[1] Ana Ballesteros-Gómez [1*], Soledad Rubio[1]

[1] *Department of Analytical Chemistry. Institute of Fine Chemistry and Nanochemistry. Universidad de Córdoba, Campus de Rabanales, Edificio Marie Curie (anexo), E-14071 Córdoba, Spain.*

[*] *Corresponding author, email: ana.ballesteros@uco.es, Phone 34 957 218643*

Abstract

Polyphenols are one of the most appreciated antioxidants nowadays. Industry has an enormous interest in their efficient extraction from vegetal biomass, but most of current methods are neither eco-friendly nor cost-effective. Supramolecular solvents (SUPRAS), made up of self-assembled amphiphilic aggregates, have shown a great potential for the extraction of bioactives from biomass as well as for the compliance with many green chemistry criteria. Comparative studies on the extraction capability of different types of SUPRAS are essential for their application-oriented tailoring. In this study, seven SUPRAS made up of reverse hexagonal aggregates and sponge-like structures were synthesized in a variety of hydro-organic mixtures from 1-octanol and 1,2-octanediol, respectively, and their capability for the extraction of polyphenols from raspberries was evaluated. SUPRAS were characterized in terms of formation phase diagrams, composition and structure. Sponge-like SUPRAS of 1,2-octanediol, which had abundant aqueous interconnected channels (35.5-39% w/w), showed excellent solubilisation properties for both the highly polar polyphenol glycoside conjugates and the less polar polyphenols. Optimal values were obtained with SUPRAS of 1,2-octanediol and 1,3-propanediol that provided a total polyphenolic content of 9.57±0.19 mg GAE/g FW sample. This value was up to three times higher than that obtained with ethanolic extracts. Polyphenol glycosides (from quercetin, naringenin, kaempferol, coumaric acid and catechin) were the predominant identified metabolites.

Keywords

Supramolecular solvent; Sponge phase; Liquid chromatography-high resolution mass spectrometry; Polyphenol extraction, raspberries.

1. Introduction

Polyphenols are secondary plant metabolites with antioxidant properties. They are structurally characterised by the presence of one or various six-carbon aromatic rings and two or more phenolic groups. Antioxidants provide beneficial effects to the human health: they help to avoid heart and neurodegenerative diseases and have positive effects on the immune system [1–3].

Thanks to these beneficial properties, polyphenols are interesting for the food, cosmetic and nutraceutical industry [4]. This has led to an increasing interest in the development of cost-effective and greener extraction techniques for these compounds. Polyphenols are present in thousands of plants which contain different profiles of phenolic constituents. Consequently, these techniques should ideally behave as matrix-independent and they should be able to cover a wide polarity range of phenolic compounds. Products, such as cocoa, tea, citrus or berries contain a remarkable concentration of polyphenols [5]. Currently, there are not extraction methods that cover the efficient extraction of a wide polarity range of phenolic compounds in different vegetable matrices.

The most used extraction methods for polyphenols are still based on conventional extraction with organic solvents. Traditional methods such as heat reflux extraction or Soxhlet extraction use high volumes of organic solvents like methanol, ethanol or iso1-propanol, in a ratio up to 1:100 w/v. These techniques also require long times for extraction, which hinders high sample throughput and increases costs [6,7]. Other authors have proposed the use of hydro-organic or organic solvent mixtures to cover the extraction of a wider range of polyphenols [8]. The consumption of organic solvents has been reduced by the application of auxiliary energies (microwaves, ultrasounds, high temperature, pressure, etc.) or they have been replaced with greener solvents [ionic liquids (IL), deep eutectic solvents (DES), biosolvents, and supercritical fluids] [9–15]. Nevertheless, the use of auxiliary energies or of supercritical conditions is costly for process scale-up. IL are also expensive to produce and their constituents are not always eco-friendly. In this sense, the use of DES is a better option. However, in order to achieve efficient extraction of phenolic compounds with these viscous liquids, the application of ultrasound or microwave-assisted energy is usually required, together with relatively high temperatures (40-80 °C), dilution with water to reduce viscosity (5-30% w/w) and long extraction times (up to 90 min) [16–18]. Dispersive liquid-liquid microextraction with

DES has been also proposed for the extraction of polyphenols and phytosterols from oils and milk, respectively. These methods, which were oriented to analytical purposes, required multiple sample preparation steps including an initial liquid-liquid extraction step. [19,20]

Supramolecular solvents (SUPRAS) are nanostructured liquids generated from colloidal solutions of amphiphiles through spontaneous phenomena of self-assembly and coacervation [21]. Their application to the extraction of bioactive compounds from vegetal biomass has been quite testimonial in the last decade [22-27]. However, because of their unique properties for solute solubilisation, their potential in this field is recognized as highly promising [28,29]. Those particular characteristics of SUPRAS that are of interest for the extraction of bioactive compounds include [28-30]; (*i*) the presence of *regions of different polarity* in their nanostructures, where a wide polarity range of substances can be efficiently solubilized through mixed mode mechanisms. (*ii*) The *high concentration of amphiphiles* in the SUPRAS (~0.1-1 g/mL) that results in a large number of binding sites. (*iii*) The *discontinuous character* of SUPRAS, formed by coacervate droplets, that provide a large surface area and, consequently, fast mass transfer. (*iv*) The possibility of *tailoring SUPRAS* nanostructures, composition and properties by judicious selection of amphiphiles and the environment for coacervation. (*v*) The compliance of SUPRAS with many of the criteria set for *green solvents* (e.g. high performance for the intended purpose, synthesis carried out through an energy-saving process that has a high-atom economy, etc.) [33].

So far, the most used amphiphiles for extracting bioactive compounds have been non-ionic surfactants (e.g. surfactants from the Triton X series, alcohols and carboxylic acids). SUPRAS formation was induced by the increase of the temperature of the aqueous colloidal solution [23-26] or by the addition of water (a poor solvent for the amphiphile) to the organic colloidal solution [22,27-29]. In both cases, the coacervation-inducing agent (temperature or water) promoted the growth of the amphiphilic aggregates present in the colloidal solutions by reducing the repulsions among their amphiphile head groups or by decreasing the solvent molecules available for head-group solvation. The extraction efficiency of bioactive compounds (e.g. betaine, saponins, anthraquinones, phenolic compounds, caffeine, carotenoids, etc.) surpassed that of conventional organic solvents. Thus, Ribeiro et al.[23] found that SUPRAS were superior for extraction of saponins from sisal waste (recovery 98.4%) compared with an ethanolic solution (recovery 38.6%). Also

Torres-Valenzuela et al [27] found that SUPRAS extracted ~12-fold and ~19-fold more caffeine than methanol and ethanol, respectively, from coffee cherry pulp.

Both the constituents and the nanostructure of SUPRAS are expected to greatly influence the quantity and the profile of the extracted bioactive compounds. On this basis, this study aims to get an insight and to gain knowledge into those SUPRAS properties driving the extraction of compounds with different polarity, and to lay the foundations to design tailor-made SUPRAS to target specific classes of compounds. For this purpose, seven SUPRAS made up of reverse hexagonal aggregates and sponge-like structures were synthesized in different hydro-organic mixtures from 1-octanol and 1,2-octanediol, respectively, and their capability for the extraction of polyphenols from raspberries was evaluated. The hydro-organic mixtures were made from protic (ethanol, 1-propanol, 1,3-propanediol) and aprotic (tetrahydrofuran) solvents. SUPRAS were characterized in terms of phase diagrams, composition and structure. Extraction efficiencies for the different SUPRAS were evaluated in terms of total polyphenol content and profile. Below, results are discussed on the basis of SUPRAS composition and structure.

2. Experimental

2.1 Reagents

All chemicals were of analytical-reagent grade and employed as supplied. Tetrahydrofuran (THF), ethanol, 1-octanol and acetic acid were purchase from Panreac (Barcelona, Spain). 1-Propanol, gallic acid and Folin-Ciocalteau reagent were supplied by Sigma-Aldrich (Steinheim, Germany) and methanol by Riedel-de Haën (Seelze, Germany). 1,2-octanediol and sodium carbonate were purchase from Aldrich (St Louis, USA) and 1,3-propanediol was supplied by Acros Organics (Geel, Belgium). Ultra-pure water was produced in an Elix® Essential 3 water purification system by Merck Millipore (Madrid, Spain).

2.2 Apparatus

SUPRAS preparation was performed using a vortex for mixing ingredients and a centrifuge for accelerating phase separation. The following devices were used: Vortex mixer equipped with an attachment for different size tubes from Heathrow Scientific

(Vernon Hills, USA), a Mixtasel BLT digitally regulated centrifuge equipped with an angle rotor 16 × 15 mL from JP Selecta (Barcelona, Spain), a Reax Top vortex mixer equipped with an attachment for centrifuge microtubes from Heidolph (Schwabach, Germany) and a MINICEN centrifuge from Ortoalresa (Madrid, Spain).

A Coulometric Karl Fischer titrator with generator electrode without diaphragm from Metrohm (Herisau, Switzerland) and a digital calliper from Medid Precision, S.A. (Barcelona, Spain) were used to measure the water content and volume of the SUPRAS, respectively. The analysis of residual amphiphile in the equilibrium solution after SUPRAS preparation as well as the identification of the phenolic compounds in SUPRAS extracts were done with a Bruker ELUTE UHPLC coupled to a hybrid ion mobility triple quadrupole/TOF (TimsTOF, Q-TOF) equipped with an ESI source operating in positive and negative modes from Bruker Daltonics (Bremen, Germany). The measurement of the total polyphenolic content in SUPRAS extracts was done using a Thermo Spectronic Helios ε spectrophotometer from Labbox (Madrid, Spain).

The characterization of SUPRAS structure was carried out with an EVO LS 15 scanning electron microscope from Zeiss (Oberkochen, Germany)

2.3 Phase diagrams

Phase diagrams for the ternary mixtures of 1-octanol:water:organic solvent and 1,2-octanediol:water:organic solvent were constructed in order to delineate the region for SUPRAS formation. Mixtures of 10 mL of 1-octanol or of 1,2-octanediol (up to 15% w/v) were prepared in hydro-organic solutions (from 95:5 v/v to 20:80 v/v water:organic solvent). Organic solvents were ethanol, 1-propanol, 1,3-propanediol and tetrahydrofuran. The mixtures were vortex-shaken for 5 min to favour the contact between their components and then centrifuged (2,500 rpm, 5 min) to accelerate phase separation. The criterion used to determine the formation of SUPRAS was the formation of two immiscible isotropic liquid phases in the system. The SUPRAS phase stand at the top and the equilibrium solution at the bottom (containing a residual amount of amphiphile near the critical aggregation concentration, water and organic solvent).

2.4 SUPRAS volume and composition

The generated volume of SUPRAS under different synthesis conditions was calculated by measuring its height in a cylindrical tube with a digital calliper and using the equation $V=\pi \cdot r^2 \cdot h$.

The water content in SUPRAS was determined with a coulometric Karl Fischer (KF) titrator. For this purpose, aliquots of 50 µL SUPRAS were weighed and dissolved in 1-2 mL of MeOH. Aliquots of 200 µL of the resulting solutions were injected into the KF titrator. The concentration of amphiphile in SUPRAS was determined gravimetrically after the evaporation of water and the organic solvent. For this purpose, 200 µL aliquots of SUPRAS were weighed, added to an Eppendorf tube and left to evaporate at room temperature for 48 h. The water content of the residue was measured by KF titration to ensure that all the water was evaporated. Finally, the concentration of organic solvent in SUPRAS was calculated by weight difference.

After SUPRAS formation, the residual concentration of amphiphile remaining in the bottom equilibrium solution was measured by LC-APCI (atmospheric pressure chemical ionization) (+)-(high resolution)-MS to monitor acetonitrile adducts of 1-octanol and 1,2-octanediol (see table **S1**) [31]. Haga clic o pulse aquí para escribir texto.Chromatographic separation was carried out on a RESTEK C_{18} column (100 mm x 3.0 mm, 3 µm) preceded by a Phenomenex KJ 0-4282 Security Guard Cartridge Kit precolumn. The mobile phase consisted of (A) water and (B) acetonitrile. The gradient elution program consisted in isocratic conditions for 1 min at 60% (v/v) B and then a linear gradient from 60% to 99% (v/v) of B for 9 min followed by isocratic conditions at 99% (v/v) B for 2.5 min (flow rate 0.2 mL/min). Finally, initial conditions were re-equilibrated for 7 min. The column temperature was set at 35 ºC. Source parameters were as follows: dry heater 200 ºC, dry gas flow 3 $L \cdot min^{-1}$; nebulizer gas pressure 2.5 bar; capillary voltage, 2,500V. Acquisition was done at bbCID (broad band collision induced dissociation) mode (3 Hz, focus on, profile spectra).

2.5 SUPRAS structural characterization

The SUPRAS structure was elucidated by scanning electron microscopy (SEM). The sample preparation was as follow: 10 µL of SUPRAS were fixed with glutaraldehyde and embedded with a 6% (w/v) agarose aqueous solution. The sample was washed three times

with sodium cacodylate and stained with OsO_4 (1%, w/v) for contrast enhancement. Samples were then dehydrated with a graded series of acetone (30, 50, 70, 80, 90, 100 %, v/v) and dried using the critical point drying. Finally, samples were coated with gold and observed under SEM. The accelerating voltage was set at 10 kV.

2.6 SUPRAS extraction of polyphenols in raspberries

The extraction capacity of the different SUPRAS was investigated by extracting polyphenolic metabolites from raspberries (*Rubus idaeus*) that were bought at a local supermarket in Córdoba (Spain) and blended until homogenized. SUPRAS of 1-octanol and 1,2-octanediol were prepared by dissolving 15% w/v of amphiphile in a 15:85 % v/v mixture of organic solvent (ethanol, 1-propanol, 1,3-propanediol or THF) and water (total volume of the synthesis solution 50 mL). Both the SUPRAS and the corresponding equilibrium solutions were separately stored at 4 °C in closed polypropylene bottles until use for at least 1 month.

Aliquots of 200 mg of sample (wet weight) were mixed with 400 µL of SUPRAS and 400 µL of equilibrium solution. The mixture was vortex-shaken (15 min) to favour the extraction and centrifuged (15,000 rpm, 15 min) to accelerate the separation of the SUPRAS phase. SUPRAS extracts were directly analysed with LC-ESI(-/+)-(high resolution)MS/MS for the identification of the main phenolic compounds and by UV spectrophotometry for the determination of the total polyphenolic content by the Folin-Ciocalteu method.

For LC-(ESI)-MS/MS analysis, the stationary phase was the same as that described in section 2.4. The mobile phase consisted of (A) water and (B) methanol both containing 5 mM ammonium formate and 0.01% v/v formic acid for ESI positive mode and 5 mM ammonium acetate for ESI negative mode. The gradient elution program was the same in both polarity modes and consisted in a linear gradient from 4% to 99% v/v of B for 16 min (flow rate 0.2 mL/min) and then isocratic conditions with 99% v/v of B for 3 min (flow rate 0.48 mL/min). Finally, initial conditions were re-equilibrated for 7 min. The column temperature was set at 40 ºC. Source parameters were as follows: dry heater 200 ºC, dry gas flow 3 $L \cdot min^{-1}$; nebulizer gas pressure 2.5 bar; capillary voltage, 3,500V. Acquisition was done with *autoMSMS* mode (10 Hz, focus on, profile spectra). The identification of the metabolites was performed using a library of plant metabolites administered by the equipment vendor and the program MetaboScape (Bruker).

Identification criteria were based on exact mass (≤5 ppm) and isotopic pattern fit (mSigma ≤200).

For the quantification of total polyphenolic content, SUPRAS extracts were diluted 1:30 v/v with MeOH. After that, 50 μL of the diluted sample, 1.5 mL of distilled water, 100 μL of 0.1N Folin Ciocalteau reagent and 300 μL of a 7,5 % w/v sodium carbonate solution were added to 2 mL Eppendorf tubes. After 90 minutes in darkness, the absorbance was measured at 760 nm. Quantitative analysis was conducted using standard solutions of gallic acid, prepared in ultrapure water in the concentration range of 5–1000 mg/L, and subjected to the same procedure than samples.

3. Results and discussion

An array of SUPRAS were synthesized from two amphiphiles (1-octanol and 1,2-octanediol) dissolved in protic (ethanol, 1-propanol, 1,3-propanediol) and aprotic (THF) solvents by addition of water as the coacervation-inducing agent. Water, a poor solvent for these amphiphiles, promoted amphiphile-amphiphile over amphiphile-solvent interactions, which led to aggregate growth and liquid-phase separation [30]. By the synthesis and characterization of these SUPRAS, we aimed to investigate the influence of the polar group of the amphiphile and the coacervation medium on both SUPRAS composition and nanostructure, and how these factors influenced their capability for extraction of polyphenols in plants. This class of bioactive compounds include a wide variety of structures and physicochemical properties, so they were considered excellent candidates for the purpose of this study.

3.1 SUPRAS synthesis

Figures 1 and **2** show the range of concentrations of amphiphile and organic solvent where SUPRAS from 1-octanol and 1,2-octanediol were formed respectively, for each of the ternary mixtures. Above the SUPRAS region, the percentage of water in the ternary mixture was too low to induce the coacervation and below it, the percentage of organic solvent was not enough for amphiphile solubilisation. 1-Octanol did not generate SUPRAS in 1,3-propanediol:water mixtures.

The phase diagrams of 1-octanol were wider and SUPRAS were formed at much lower percentages of water (30-40% v/v) than those from 1,2-octanediol (70-80% v/v). The reason is that adding a second OH- group at the polar head of the amphiphile remarkably

increases its polarity. Water solubility of 1-octanol is 540 mg/L at 25 °C while for 1,2-octanediol it is 3 g/L at 20°C (experimental data obtained from PubChem database). Due to the lower water solubility of 1-octanol in comparison with 1,2-octanediol, it was expected that a lower concentration of coacervation-inducing agent would be required to induce its self-assembly.

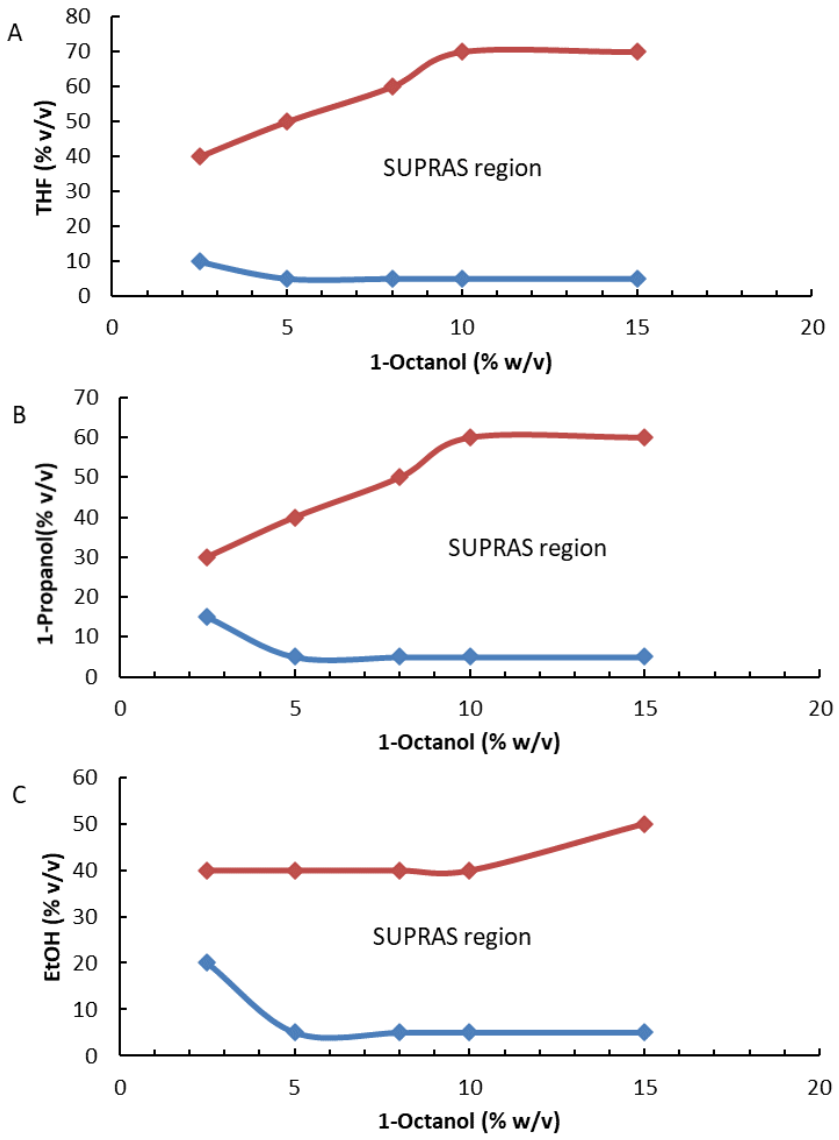


Figure 1. Phase diagrams of 1-octanol in mixtures of water and organic solvents [tetrahydrofuran (THF) (A), 1-propanol (B) and ethanol (C)].

Clear differences were observed between SUPRAS of 1-octanol formed in different organic solvents (Fig. 1). Thus, the solvents with the lowest dielectric constants gave the broadest regions for SUPRAS formation, as it occurred for THF (ε=7.4 at 25 °C),

followed by 1-propanol (ε=20.33 at 25 °C) and ethanol (ε=25.3 at 25 °C). However, this behaviour was different for the more polar 1,2-octanediol-SUPRAS, for which the different solvents generated similar phase diagrams (Fig. 2).

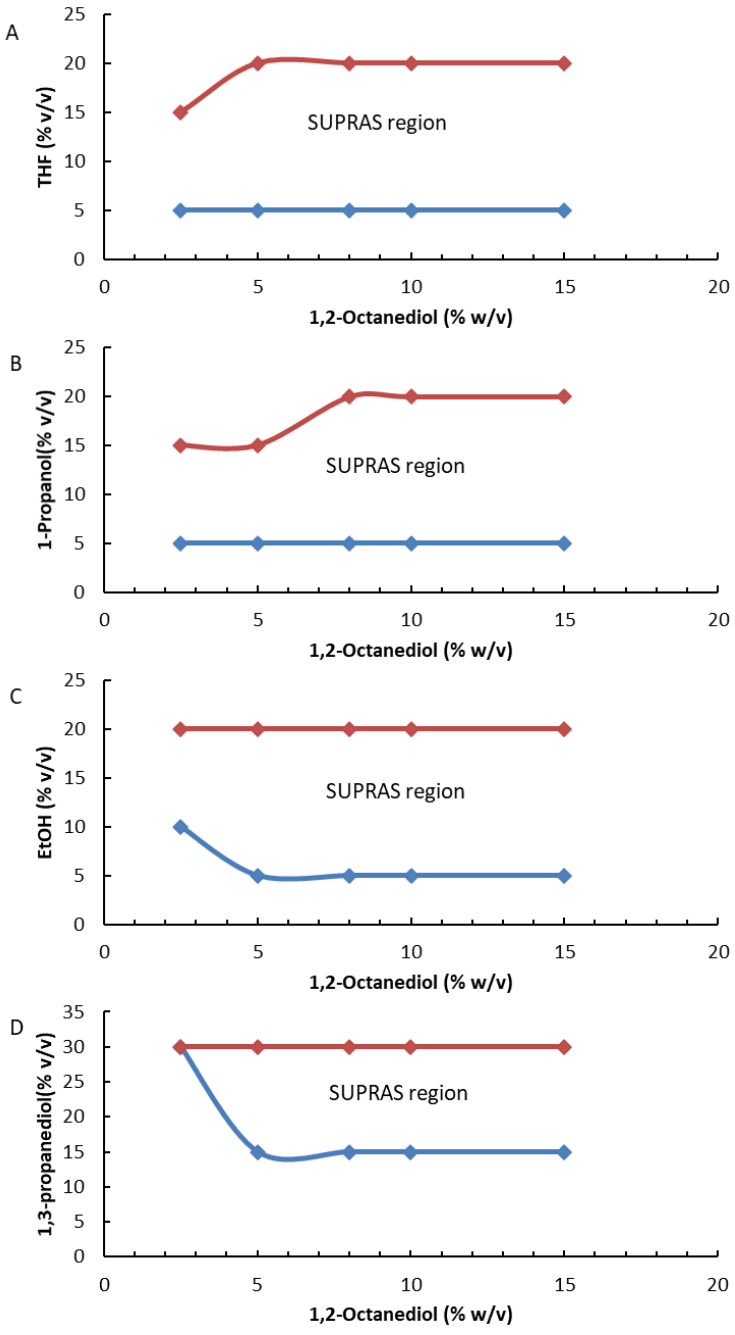


Figure 2. Phase diagrams of 1,2-octanediol in mixtures of water and organic solvents [tetrahydrofuran (THF) (A), 1-propanol (B), ethanol (C) and 1,3-propanediol (D)].

In order to determine the efficiency of the SUPRAS synthesis, the concentration of amphihile remaining in the equilibrium solution after SUPRAS formation was measured by LC-(APCI)-MS (section 2.4). Average values for 1-octanol-based SUPRAS were in the range 1.3-3.1 g/L, 0.08-1.96 g/L and 0.28-0.43 g/L when using THF, 1-propanol or ethanol, respectively. For 1,2-octanediol-based SUPRAS, the concentration of amphiphile in the equilibrium solution was a bit higher, which is in agreement with its higher water solubility and it was in the ranges 8.3-8.6 g/L, 4.9-6.6 g/L, 8.6-8.7 g/L and 10.2-17.2 g/L when using THF, 1-propanol, ethanol or 1,3-propanediol, respectively. Accordingly, the percentage of amphiphile incorporated into the SUPRAS phase after the synthesis was ~90-100 % for both 1-octanol and 1,2-octanediol-SUPRAS under the studied experimental conditions (**Table S2**). Thus, the synthesis of 1-octanol- and 1,2-octanediol-based SUPRAS at room temperature is a high atom-economy process [33], which is a requirement for the preparation of green solvents.

3.2 SUPRAS volume

The volume of SUPRAS generated by coacervation was investigated as a function of the relative proportion of the synthesis ingredients. **Figures S1** and **S2** show the volume of SUPRAS, expressed as µL of SUPRAS per mL of synthesis solution, as a function of the percentage of amphiphile and of organic solvent. As displayed in **Tables S3** and **S4,** it was found that, in all cases, the volume of SUPRAS increased linearly with the percentage of the amphiphile (at a fixed concentration of organic solvent) and exponentially with the organic solvent (at a fixed concentration of the amphiphile)**.** This means that all SUPRAS behaved as environmental-responsive materials since their volume changed with the environment for coacervation (i.e. the organic solvent/water ratio). These trends are in agreement with previously reported SUPRAS [34,35].

Non-linear regression was used to fit a model and propose general equations to predict the volume of SUPRAS as a function of the percentage of amphiphile and organic solvent in the synthesis mixture. **Table 1** shows the predicted equations. The goodness of the fit of these equations are shown in SI (**Figure S3**).

Table 1. Equations to predict the generated volume of SUPRAS as a function of the percentage of amphiphile (%, w/v) and of organic solvent (%, v/v) in the synthesis solution

SUPRAS		Volume equation	R^2
Amphiphile	**Organic solvent**		
1-Octanol	**THF**	y=[(0.207x+13.176)·z]+(6.537x -96.319)	0.9532
1-Octanol	**Ethanol**	y=[(0.502x+6.089)·z]+(-1.554x+29.703)	0.9347
1-Octanol	**1-Propanol**	y=[(0.117x+4.265)·z]+(12.893x-85.864)	0.8922
1,2-Octanediol	**THF**	y=[(0.453x+16.460)·z]+(2.199x-30.981)	0.9893
1,2-Octanediol	**Ethanol**	y=[(0.520x+14.296)·z]+(-0.982x-1.531)	0.9932
1,2-Octanediol	**1-Propanol**	y=[(0.587x+15.944)·z]+(3.276x-35.388)	0.9813
1,2- Octanediol	**1,3-Propanediol**	y=[(0.431x+11.182)·z]+(-2.190x+25.844)	0.9985

y: SUPRAS volume (µL/mL mixture); x: % v/v organic solvent; z: % w/v amphiphile

Under identical synthesis conditions, the volume of SUPRAS from 1,2-octanediol was around 1.4-fold higher than that obtained from 1-octanol, independently of the hydro-organic mixture. This behaviour can be easily inferred from the comparison of the equation coefficients in Tables S3 and S4. The use of different organic solvents for the synthesis of SUPRAS with the same amphiphile did not result in large differences in SUPRAS volumes.

3.3 SUPRAS chemical composition

Tables 2 and **3** show the SUPRAS composition at 15% w/v of amphiphile and different organic solvent:water ratios in the synthesis solution. Regarding 1-octanol-based SUPRAS (Table 2), as the percentage of water (coacervating agent) in the bulk solution decreased, the water and organic solvent contents in SUPRAS increased, and consequently, the amphiphile percentage decreased. Since the volume of SUPRAS also increased accordingly, the amphiphile just become more diluted and solvated in this phase. This behaviour indicated that the chemical composition of the 1-octanol-based SUPRAS was environment responsive and that it could be tailored by just changing the organic solvent:water ratio in the synthesis solution. This is also in agreement with the fact that higher concentration of coacervating agent (water) give rise to more dehydrated polar head groups and, consequently, to more packed SUPRAS phases with less water content, as it happens in salt-induced or temperature-induced SUPRAS [32,35].

The percentage of solvent incorporated into the SUPRAS phase was directly related to its dielectric constant, so that the higher the solvency power of the organic solvent for the nonpolar part of the amphiphile (lower ε), the higher the incorporated value. For example, values of 16.8±0.3, 8.7±0.8 and 3.9±0.5% w/w of organic solvent were measured in 1-octanol-based SUPRAS produced with 10% v/v of THF, 1-propanol and ethanol, respectively. Contrarily, the maximum percentage of water incorporated in 1-octanol-based SUPRAS was inversely related to the dielectric constant of the solvent, so that values of 14.3±0.5, 24.4±0 and 32.9±0.3% w/w of water were obtained for SUPRAS prepared in THF, 1-propanol and ethanol, respectively.

Comparing the composition of 1-octanol (Table 2) and 1,2-octanediol-based SUPRAS (Table 3), the most significant difference was the higher water content of the latter. 1,2-octanediol-SUPRAS contained percentages of water around 35.9 ±0.9, 38±1, 36.6±0.9 and 38.4±0.8 % w/w using THF, 1-propanol, ethanol and 1,3-propanediol, respectively; being this value quite independent of the solubility properties of the organic solvent. The same behaviour was previously reported for 1,2-decanediol-based SUPRAS for which values of water around 30% w/w were measured [34]. The higher water content of diols in comparison with simple alcohols is due to the double head of the amphiphile leading to a higher hydration degree. Furthermore, amphiphilic aggregates made up of double-headed surfactants are expected to be more open. Micellar sizes and aggregation numbers decrease as the number of head groups increase due to electrostatic repulsion and/or steric hindrance, thus favoring the interactions with water molecules [36].

As it occurred for 1-octanol-based SUPRAS, the percentage of amphiphile increased with the increase of the coacervating agent in the bulk solution leading to more packed phases (Table 3). However, differently from 1-octanol-based SUPRAS, the ones synthesized from 1,2-octanediol were less solvated in terms of organic solvent content, which is probably solvating the amphiphile hydrocarbon chains, while the content of the water remained almost constant. Taking into account that the amphiphile and the organic solvent concentration in 1,2-octanediol-based SUPRAS vary with the composition of the bulk solution, we can conclude that they are environmental responsive materials too.

Table 2. 1-Octanol-based SUPRAS composition (at 15% w/v amphiphile)

Synthesis conditions	SUPRAS composition (% w/w)		
THF:H_20 (%,v/v)	1-Octanol	H_2O	THF
5:95	84.4±0.2	3.7±0.1	11.9±0.4
10:90	78.7±0.3	4.52±0.04	16.8±0.3
15:85	68.8±0.7	5.1±0.2	26±0.9
20:80	61.4±0.2	6.00±0.06	32.6±0.3
30:70	47±2	6.9±0.1	46±2
40:60	39.4±0.5	8.6±0.2	52.0±0.3
50:50	31.4±0.8	9.8±0.7	58.8±0.1
60:40	26.2±0.1	11.8±0.3	62±0.4
70:30	21.3±0.8	14.3±0.5	64.4±1.2
1-Propanol:H_20 (%, v/v)	1-Octanol	H_2O	Propanol
5:95	90.3±0.2	6,338±0,002	3.4±0.2
10:90	84±0.7	7.3±0.1	8.7±0.8
15:85	76.1±0.2	9.05±0.03	14.8±0.3
20:80	76±11	9.9±0.4	13.9±0.9
30:70	53.9±0.6	13.7±0.3	32.4±0.3
40:60	41.7±0.4	18.94±0.04	39.3±0.4
50:50	34.4±0.9	24.6±0.3	41±0.6
Ethanol:H_20 (%, v/v)	1-Octanol	H_2O	Ethanol
5:95	93.9±0.5	4.51±0.06	1.6±0.5
10:90	91.2±0.5	4.93±0.02	3.9±0.5
15:85	88.4±0.6	5.7±0.2	5.9±0.4
20:80	84.1±0.1	6.6±0.2	9.2±0.1
30:70	72.8±0.1	9.37±0.03	17.8±0.1
40:60	57.7±0.7	15.7±0.3	27±1
50:50	41.2±0.6	32.9±0.3	25.9±0.9

Table 3. 1,2-octanediol-SUPRAS composition (at 15% w/v amphiphile)

Synthesis conditions	SUPRAS composition (% w/w)		
THF:$H_2$0 (%,v/v)	1,2-octanediol	H_2O	THF
5:95	52.4±0.4	35.4±0.7	12±1
10:90	44±3	36±1	20±4
15:85	38±1	36.4±0.7	25.3±0.7
20:80	32±2	38.6±0.3	29±2
1-Propanol:$H_2$0 (%, v/v)	1.2-octanediol	H_2O	Propanol
5:95	58±2	37.7±0.2	4±2
10:90	50.8±0.1	36±2	13±2
15:85	43.1±0.1	39±1	18±2
20:80	34.1±0.1	43.4±1	22.5±0.9
Ethanol:$H_2$0 (%, v/v)	1.2-octanediol	H_2O	Ethanol
5:95	53.4±0.3	38±1	9±1
10:90	49.7±0.7	36.4±0.3	13.9±0.4
15:85	52.2±0.2	35.8±0.9	12±1
20:80	48±2	36.8±0.4	15±1
1,3-Propanediol:$H_2$0 (%, v/v)	1.2-octanediol	H_2O	Propanediol
15:85	39.6±0.2	37.5±0.2	22.90±0.02
20:80	41.62±3.1	35.5±0.1	23±3
30:70	34.5±0.5	35.0±0.7	30.5±0.2

3.4 SUPRAS nanostructure

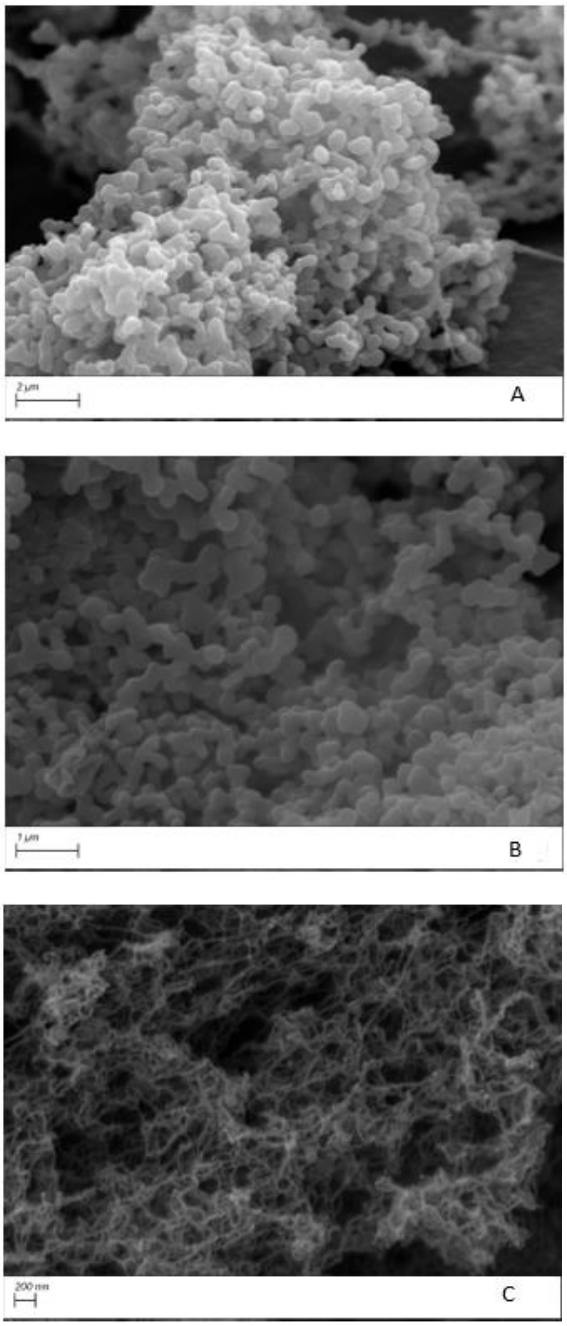
The structure of SUPRAS of alkanols in THF:water media have been reported by Ballesteros-Gómez et al. [35]. These SUPRAS were described as clusters of inverted hexagonal aggregates where the alcohol groups of alkanols surrounded aqueous cavities and the THF solvated their hydrocarbon chains. Figure **S4** shows typical micrographs obtained by optical and cryo-SEM microscopy, along with a schematic of the reported nanostructures. The size of the aqueous cavities can be tailored by controlling the tetrahydrofuran (THF):water ratio in the synthesis mixture. Thus, the lower the water content in the synthesis solution, the larger the aqueous cores in the SUPRAS nanostructures.

Inverted hexagonal aggregates were also supposed to be produced in SUPRAS synthesized from 1-octanol in ethanol-water and 1-propanol-water, here firstly investigated, on the basis of the similar variation of their chemical composition as function of the organic solvent:water ratio in the synthesis solution (Table 2). Thus, the percentage of water in the SUPRAS increased as the water content in the synthesis solution decreased, a behaviour that, so far, has been exclusively found in inverted hexagonal aggregates [33].

Regarding the SUPRAS made up of 1,2-octanediol, all of them here firstly investigated, González-Rubio et al. recently reported that SUPRAS synthesized from 1,2-decanediol in THF:water media gave sponge phases [32]. The different morphology found for alkanols (i.e. hexagonal phases) and 1,2-decanediol (i.e. sponge phases) is a consequence of the very different packing parameter (*g*) of both amphiphiles. The packing parameter depends on both the volume and length of the hydrophobic chain and the effective area per head group [37]. An extra -OH group at the polar head of the amphiphile significantly increases the area of the polar head group, so that the packing parameter for 1-octanol is 1.032 and for 1,2-octanediol is 0.514. These values are commonly used to predict the morphology of the amphiphilic aggregates near the critical aggregation concentration but, so far, they are not useful in predicting the morphology of SUPRAS phases where the concentration of amphiphile is so high as 20-90% w/w (Tables 2 and 3). Nevertheless, without a doubt, the structure of the amphiphile, and so the packing parameter, is a critical factor in driving the resulting SUPRAS nanostructures

The nanostructures of 1,2-octanediol-based SUPRAS were investigated by SEM (section 2.5). **Figure 3** shows representative micrographs obtained for SUPRAS prepared with 15% w/v amphiphile in (A) ethanol:water and (B) THF:water mixtures at a proportion of 15:85 v/v. The same results were obtained for the other hydro-organic mixtures tested (i.e. 1-propanol:water and 1,2-propanediol-water). SEM micrographs showed typical features of a sponge morphology [38,39]; ellipsoidal structures nearly flat and surrounded by a network of curved areas with a smooth appearance (Figure 3A and B). The sponge phase consisted of a random 3D amphiphile bilayer network separated by water pores or interconnected channels (Figure 3C). As mentioned before and under the different synthesis conditions (different organic solvents and organic solvent:water mixtures) the water content of these channels remained constant (average 35.9 ±0.9 %, w/w). These results are in agreement with those recently reported by our group for SUPRAS of 1,2-

418 decanediol in mixtures of THF:water [34]. Consequently, the difference in two carbon
419 atoms of the hydrophobic tail did not influence the SUPRAS structure.



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421

422 **Figure 3.** SEM micrographs of 1,2 octanediol-based SUPRAS synthesized in (A, C)
423 ethanol:water and (B) THF:water. Synthesis conditions: 15% (w/v) of amphiphile and
424 15:85, v/v organic solvent:water.

3.5 Influence of SUPRAS composition and nanostructure on their capability for the extraction of polyphenols from raspberries

The seven SUPRAS synthesized with 1-octanol and 1,2-octanediol were applied to the extraction of polyphenols from raspberries (section 2.6) in order to compare their extraction efficiency for these bioactive compounds. Main extraction techniques and organic solvents used for isolation of polyphenols from red fruits, including raspberries, have been discussed by Hidalgo and Almajano [40]. Among the solvents investigated (e.g. water, acetone, hexane, ethyl acetate, ethanol, methanol, etc.), ethanol was the most efficient for extraction of antioxidants from red fruits [40-42].

Figure 4 shows the total polyphenolic content [mg gallic acid equivalents (GAE)/g fresh weight (FW) sample] for each SUPRAS. Pure ethanol was also employed for comparison as an established media for the extraction of polyphenols. According to bibliography [40] the total polyphenolic content in raspberries by different extraction methods was in the range 2.6-3.7 mg GAE/g FW sample, what is in agreement with the total polyphenolic content obtained in our study by employing ethanol (3.42±0.05 mg GAE/g FW sample).

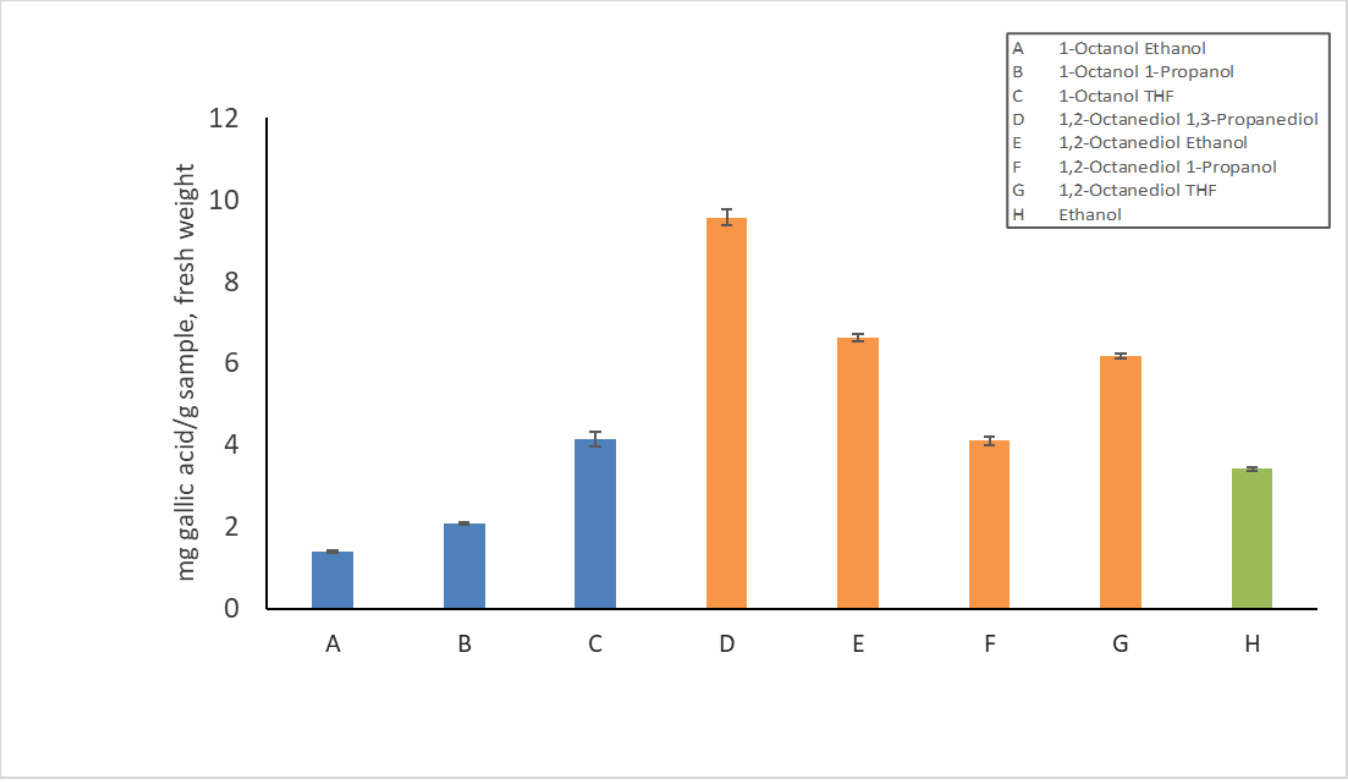

Figure 4. Total polyphenolic content in SUPRAS extracts (15% w/v amphiphile, 15:85 v/v organic solvent:water) and in a conventional ethanol extract from raspberries.

In general, 1-octanol-based SUPRAS extracted polyphenols less efficiently than SUPRAS of 1,2-octanediol, and only those prepared in THF reached similar extraction rates than that of pure ethanol (4.16±0.18 and 3.42±0.05 mg GAE/g FW sample, respectively). The sponge-like SUPRAS were more efficient extraction phases, being also superior to ethanol. The higher water content of these SUPRAS (35.5-39% w/w for 1,2-octanediol-SUPRAS and 4.5-9.1 % w/w for 1-octanol-SUPRAS under the selected synthesis conditions) and their higher expected surface area and more open structure could be the reasoning for this behaviour.

Regarding the influence of the organic solvents in both types of SUPRAS, which were expected to be solvating the hydrocarbon layers of the aggregates, those that were protic were more efficient as their polarity increased (1,3-propanediol>ethanol>1-propanol), while the aprotic THF followed a different trend. Optimal values were obtained with SUPRAS of 1,2-octanediol and 1,3-propanediol that provided a total polyphenolic content of 9.57±0.19 mg GAE/g FW sample. This value was almost three times higher than the one obtained with the ethanol extraction method and than those that were previously reported [40]. These results suggest that the high polarity microenvironments in SUPRAS, along with the strong dispersion interactions provided by the hydrocarbon chains of the amphiphile, give an optimal balance for the efficient solubilisation of polyphenols. It is worth mentioning that the composition of all SUPRAS, except for those with THF, would be readily compatible with cosmetic applications (according to CosIng, *European Commission database for information on cosmetic substances and ingredients*).

In order to find a consistent explanation to the unlike extraction behaviour obtained for the different types of SUPRAS, the phenolic profile was investigated by LC-high resolution (QTOF) MS/MS analysis. The list of identified phenolic compounds and metabolites, along with the exact mass of MS and MS/MS, is shown in **Table S5**. The chemical structure of these compounds is depicted in **Table 4**. Glycosides of phenolic compounds were the predominant metabolites. Berries are rich in sugars, and therefore, polyphenols can easily form glycosides with the sugars present in the matrix. Adding a sugar to the molecule increase the polarity of the parent compound, so that glycosides are always more polar than the molecule from which they derive. Their octanol-water constant, expressed as log, for the identified phenolic compounds (data for glycosides were not found) was from -0.481 to 1.76.

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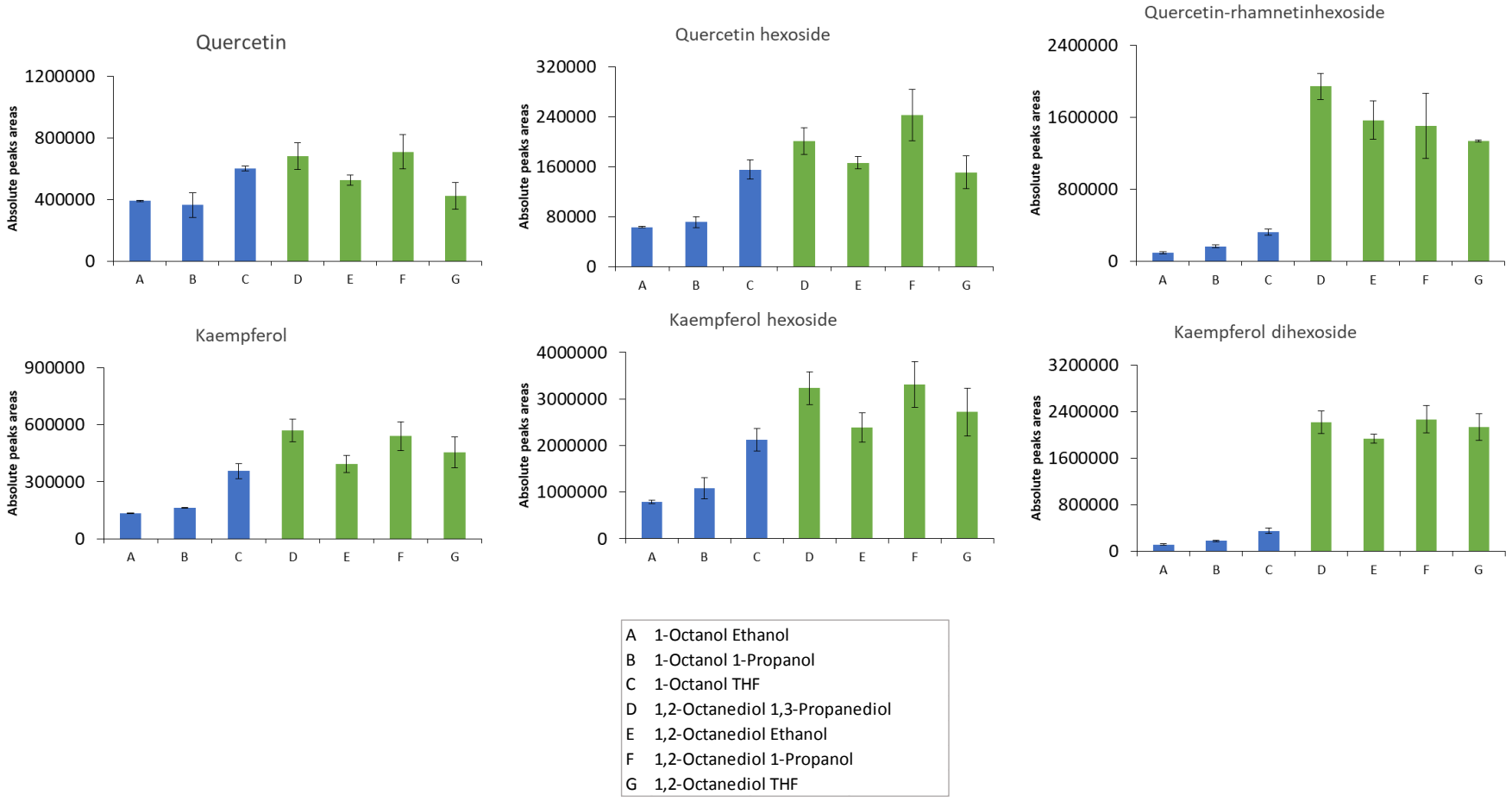


477

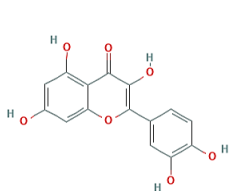
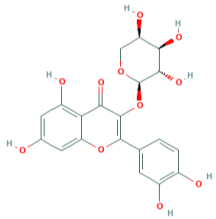
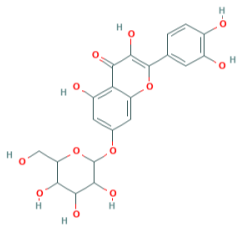
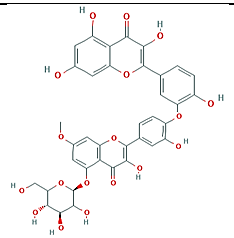
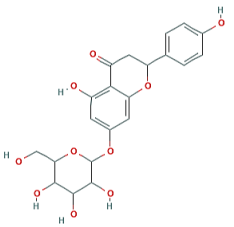
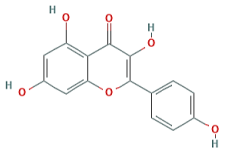
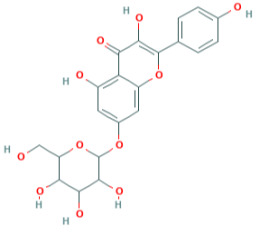
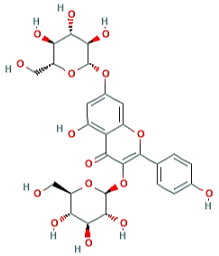
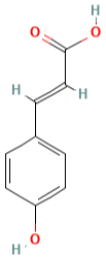
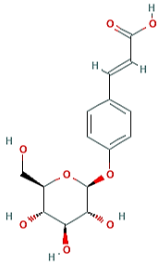
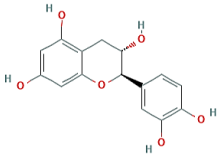
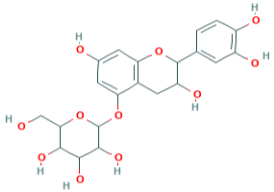
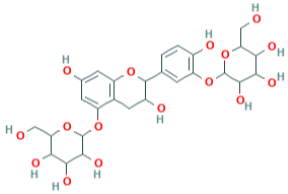
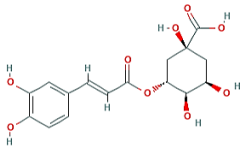
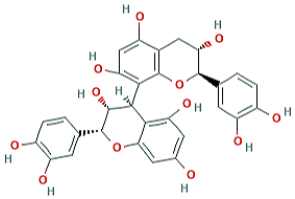
478 **Figure 5**. Extraction of quercetin, kaempferol and their glycosides from raspberries employing different SUPRAS.

Relative extraction efficiencies (expressed as peak absolute areas) for the most abundant phenolic compounds and/or metabolites in SUPRAS extracts are shown in **Figure 5**. The relative extraction efficiencies for other identified compounds are shown in **Figure S5**. For some phenolics, glycosides were the most abundant bioactives (e.g. quercetin rhamnetinhexoside or kaempferol hexoside, Figure 5) while for other ones the predominant form was the parent compound (e.g. catechin or coumaric acid, Figure S5).

By comparison of the relative extraction efficiency of the phenolic compounds and their glycosides, a similar trend was observed in most of cases. As the polarity of the molecule increased due to the addition of increasing sugar units (e.g. quercetin rhamnetinhexoside>quercetin hexoside>quercetin; kaempferol dihexoside> kaempferol hexoside>kaempferol, see Figure 5), the extraction was clearly more efficient by using 1,2-octanediol-based SUPRAS than 1-octanol-based SUPRAS. For example, by using 1,2-octanediol-based SUPRAS, the extraction was more than 2 times higher for kaempferol and kaempferol-hexoside and it was 9 times higher for the most polar kaempferol-dihexoside. These results were in agreement with the measured total phenolic content. We could conclude that SUPRAS of diols greatly improved the extraction efficiency of medium polar and especially of very polar compounds in comparison with SUPRAS based on simple alcohols. The same trend was observed for catechin hexoside and coumaric acid hexoside in comparison with the parent compounds (Fig. S5).

The influence of the different organic solvents for SUPRAS preparation on the extraction efficiency had a less defined pattern for 1,2-octanediol-based SUPRAS compared to 1-octanol-based SUPRAS, in which THF was the most effective solvent. This is probably due to the fact that the water content in SUPRAS is the most influential parameter for the extraction of very polar compounds and this content is independent on the type of organic solvent in diol-based SUPRAS.

505 **Table 4**. Chemical structure of the identified phenolic compounds.

				
Quercertin	Quercertin arabinoside	Quercertine hexoside	Quercertin rhamnetinhexoside	Naringenin hexoside
				
Kaempferol	Kaempferol hexoside	Kaempferol dihexoside	Coumaric acid	Coumaric acid hexoside
				
Catechin	Catechin hexoside	Catechin dihexoside	Chlorogenic acid	Procyanidin dimer

506

4. Conclusions

SUPRAS composition and nanostructure can be easily tailored by judicious selection of the amphiphile and the environmental conditions for coacervation. In this study, we prove that this tailoring is essential for the development of application-oriented SUPRAS with high efficacy for the intended purpose. Thus, by simply doubling the polar group of the amphiphile (i.e. one or two OH groups), SUPRAS with very different nanostructures (sponges from 1,2-octanediol and inverted hexagonal aggregates from 1-octanol) and composition (mainly arising from their distinct water content and consequently the extent of their hydrophilic region) can be obtained, independently of the medium used for coacervation. These differences were found to greatly impact their capability for the extraction of polyphenols from raspberries. Thus, the sponge-like structure of SUPRAS of 1,2-octanediol, that contains abundant aqueous interconnected channels (35.5-39% w/w), solubilised more efficiently a wider polarity range of phenolic compounds. SUPRAS of 1,2-octanediol and 1,3-propanediol provided a total polyphenolic content of 9.57±0.19 mg GAE/g FW sample, a value that was around three times higher than that obtained with pure ethanol. 1-Octanol-based SUPRAS extracted polyphenols less efficiently being the best results obtained with those prepared in THF (4.16±0.18 mg GAE/g FW sample). The LC-MS/MS profile of SUPRAS extracts revealed the presence of quercetin, naringenin, kaempferol, coumaric acid and catechin and their glycosides together with chlorogenic acid and procyanidin.

It is known that the potential of SUPRAS for the extraction of compounds in a wide polarity range is mainly due to the presence of polar and nonpolar microenvironments in their nanostructures, being one of their most outstanding features. This study shows that the broadening of the polar region greatly improves the extraction capability of polar compounds, a factor here firstly investigated. These results highlight the need for more fundamental studies related to the application of SUPRAS in the extraction of bioactive compounds from biomass so that knowledge-based processes can be implemented to take advantage of the opportunities arising from their tailoring.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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