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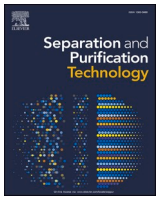

Supramolecular-solvent based extraction of hydroxytyrosol from brines of the processing of table olives

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ABSTRACT

The recovery of bioactive compounds from agri-food waste is a growing sector and in line with the principles of green chemistry. In the last two decades, the development of processes for the extraction and purification of bioactive compounds that are cheaper, faster, simple, efficient, safe and ecological has been investigated. In this study, we develop for the first time a simple liquid–liquid extraction method of hydroxytyrosol from brines of the processing of table olives, an abundant and underutilized waste of the olive industry as alternative to nano-filtration and sorption onto resins. Brines contain a high concentration of phenolic compounds with beneficial health properties, the major compound being hydroxytyrosol. For the extraction of hydroxytyrosol from brines, green supramolecular solvents (SUPRASs) made up of 1,2-hexanediol and water and induced by salts (Na_2SO_4 and sodium citrate) were used, all innocuous and authorized ingredients for use in the cosmetic industry. The extraction process of hydroxytyrosol from brines was optimized (in terms of the amount of 1,2-hexanediol and salt for the synthesis of SUPRAS). The highest concentrations of hydroxytyrosol in the SUPRAS extracts (~0.5–0.9% w/w) were obtained with 4.8% v/v of 1,2-hexanediol and 1 M of Na_2SO_4 for synthesis (equivalent to a SUPRAS:sample ratio of 1:20 v/v in the extraction process). The final extracts were further analyzed by liquid chromatography and high resolution mass spectrometry to identify other compounds of interest.

1. Introduction

Most of the olive production, approximately 90%, is used to obtain olive oil, while the rest, approximately 10%, is used for other production lines, such as the preparation of table olives, which have a growing market. Table olives have been consumed for>2,000 years in the countries of the Mediterranean basin. Spain is the main producing country of table olives, the region of Andalusia representing around 70–80% of the national production [1].

Table olive preparation methods are varied. The main stages in the elaboration are: (a) collection, transport and evaluation, (b) selection, initial classification and washing, (c) alkaline treatment, (d) final washing, (e) placement in brine and fermentation and (f) conditioning, packaging and pasteurization [1]. The purpose of the alkaline treatment is to eliminate the natural bitterness of the fruit (which is due to the presence of the glycoside named oleuropein), to make the skin permeable, to facilitate the seasoning process and to transform the chlorophyll compounds to give the product a yellowish hue. [2]. This process lasts several hours during which the olives are treated with an aqueous solution of sodium hydroxide. Later olives are washed with water to remove the soda and the fermentation stage in brine is carried out. The olives are placed in a 10–12% NaCl brine, adding or not adding sugar, vinegar, other condiments, spices and other authorized substances. [2,3] Finally, and until they are marketed, the olives must be kept in controlled fermenters or drums, inhibiting the proliferation of microorganisms by controlling the pH at acidity values (pH < 4), high salinity (>5% NaCl) or applying heat treatments, such as pasteurization. Once these preparation methods have been carried out, a series of by-products or residues of the olive are obtained, mainly wastewaters. The application of valorization strategies to these residues is scarce nowadays.

Brines from the processing of table olives are hypersaline effluents with a high concentration of organic matter whose discharge requires adequate treatment to avoid risks to health and the environment [4]. In order to improve the profitability of the olive processing chain, it is possible to extract bioactive compounds from these wastewaters, which contain sugars, phenolic compounds, polyalcohols, lipids, and pectins.

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[5] Among the phenolic compounds present in wastewater, phenyl alcohols and, especially, hydroxytyrosol stands out. Hydroxytyrosol is obtained spontaneously by chemical and/or enzymatic hydrolysis of oleuropein. [6,7] It is a compound with antioxidant, antimicrobial, and phytotoxic properties, like other phenolic compounds and it can be used in various topical preparations for anti-aging, anti-inflammatory properties, etc. [8].

Most of the research about brines from table olives has focused on the elucidation of their chemical composition or of its disposal after different chemical, mechanical and microbial treatments to reduce their polluting nature [9]. For the best of our knowledge, the extraction of hydroxityrosol from brines has been only addressed using nanofiltration [10] and amberlite resins [11]. Nanofiltration was proposed to retain 97% of total polyphenols by working at high pressure. Regarding macroreticular polymeric resins, an amount of 5 g of resin was used to treat 40 mL of sample by shaking for 16 h. Desorption of phenols from resins was carried out with 25 mL of ethanol. These two separation techniques are expensive, slow and require larger volumes of organic solvents for elution, pretreatment and sorbent production and regeneration.

Supramolecular solvents (SUPRASs) are discontinuous liquids formed by aggregates of amphiphilic compounds [12–14]. SUPRASs are formed by a two-step self-assembly and coacervation process. First, the formation of a colloidal dispersion occurs in which three-dimensional aggregates in solution (normal or reverse micelles or vesicles) are formed upon reaching a critical aggregation concentration (*cac*). Secondly, and due to the action of a coacervating agent or director of the self-assembly, the repulsion between the aggregates decrease and they assemble and form the nanostructured liquid. The coacervating agent can be a change in pH, temperature, the addition of a salt or the addition of a poor solvent for the amphiphile. The SUPRAS phase emerges from the initial isotropic solution as a new phase that is immiscible with its synthesis medium, which is known as equilibrium solution and contains the amphiphile at its *cac*.

SUPRASs have been proposed as an alternative to the use of conventional organic solvents in extraction and purification processes of very different types of liquid and solid samples thanks to their excellent properties as extractants [12]. They have been successfully used in a wide variety of analytical extraction processes [12,13]. In a lesser extent, they have also been used for the recovery of bioactive compounds of interest for the cosmetic, pharmaceutical or food industry. Thus, SUPRAS have been applied to the extraction of polyphenols from coffee residues and by-products [14–16], from raspberries [17] or from wine sludge [18], carotenoids and polyphenols from algae [19], betaine from beet molasses [20] and saponins from sisal [21].

Advantageous characteristics of SUPRASs include: (a) variety of interactions and presence of microenvironments with different polarity in the aggregates that make them up (hydrogen bonds and ionic interactions in the polar groups, and dispersion interactions in the hydrocarbon chains of the molecules), (b) high concentration of amphiphile (0.1–1 mg/μL) which provides a high extraction efficiency, (c) properties of restricted access materials against macromolecules and other major components of the matrix, (d) simple synthesis through self-assembly processes and (e) less volatile and toxic liquids than conventional organic solvents [12,13].

The use of SUPRASs made up of innocuous and/or sustainable reagents and produced under mild pH or temperature conditions are ideal for complying with the principles of green chemistry and for obtaining extracts that are directly compatible for their application in the cosmetic, food or the pharmaceutical industry. This is the case of the SUPRASs of fatty acids and alcohols in mixtures of water and ethanol, which are authorized components in cosmetics and foods [14–18] and of the recently described SUPRASs of rhamnolipids, a biosurfactant that coacervates in the presence of salts or in a slightly acid medium [22,23].

This study describes the application of a SUPRAS of 1,2-hexanediol formed in salty water (sodium sulfate and sodium citrate) for the recovery of bioactives compounds from fermentation brines from table olive production, hydroxytyrosol being the main target. This type of SUPRAS was recently described to be formed by cubosomic nanostructures with a high water content (36–61%, w/w) and to have a high potential for the simultaneous extraction of both hydrophobic and very polar compounds [24]. Furthermore, 1,2-hexanediol is an authorized compound for cosmetics in the European Union (EC 1223/2009) as well as the tested salts, which facilitates its implementation in the industry. Finally, this method is cheaper, simpler and greener (solvent-free) in comparison with the nanofiltration- and resin-based procedures mentioned above for the extraction of phenols from table olive wastewaters.

2. Experimental section

2.1. Chemicals and reagents

Methanol, ethanol and 1,3-propanediol were supplied by Fisher Scientific (Madrid, Spain). Ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain). 1-Hexanol, 1,2-hexanediol, 3-hydroxytyrosol and the salts sodium sulfate, sodium citrate tribasic dehydrate, sodium carbonate, sodium nitrate and potassium chloride were acquired from Merck (Steinheim, Germany). Nitric acid, formic acid, acetic acid and ethanol were supplied by Panreac Applichem (Steinheim, Germany). The Folin-Ciocalteu phenol reagent was obtained from Scharlau (Barcelona, Spain).

The stock solution of hydroxytyrosol was prepared at a concentration of 500 mg/L in 50/50 v/v methanol:water and stored in an amber glass bottle at −20 °C. The solution was standardized (previous dilution to 10 mg/L with a 94:6 v/v mixture of acidified water with 3% acetic acid and methanol) from the molar extinction coefficient found in the literature of 2.307 M^{-1} cm^{-1} for this solvent mixture at 280 nm in order to correct the concentration due to possible degradation during storage [25].

2.2. Apparatus

The determination of chloride in brines was made by potentiometric titration with an 848 Titrino plus equipment from Metrohm (Herisau, Switzerland) using a standardized $AgNO_3$ solution as titrant.

For sample treatment and SUPRAS formation a vortex shaker and a high-speed mini centrifuge MC15K series LBX (Barcelona, Spain) have been used. To determine the water content in the SUPRAS, a coulometric Karl Fischer titrator from Metrohm (Herisao, Switzerland) was used.

A Thermo Spectronic Helios ε photometer (Labbox, Spain) was used to measure the total polyphenol content in both the brines and the final SUPRAS extracts using the Folin-Ciocalteu method. The absorbance was measured at 760 nm.

The determination of hydroxytyrosol as the major antioxidant in SUPRAS brines and extracts was made using a liquid chromatography system coupled to ultraviolet detection (LC-UV) Spectra System SCM1000, ThermoQuest (San Jose, CA, USA). This consists of an autosampler with a quaternary pump (P4000) and an ultraviolet diode array detector (UV6000LP). A Kromasil C18 (5 μm 150 × 4.6 mm) purchased from Análisis Vínicos (Tomelloso, Spain) was used as an analytical column. The mobile phase used consisted of milli-Q water with 0.2% v/v of formic acid (A) and methanol (B), with a flow of 1 mL/min in gradient (linear gradient from 90% A down to 37% A in 10 min and 5 min for reconditioning the column to the initial gradient composition).

The screening of other polyphenols and bioactive compounds with mass spectral library search was carried out with a Bruker ELUTE UHPLC coupled to a hybrid ion mobility triple quadrupole/TOF (TimsTOF, Q-TOF) equipped with an ESI source operating in negative mode from Bruker Daltonics (Bremen, Germany). Chromatographic separation was carried out on a RESTEK C_{18} column (100 mm × 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) methanol both containing 5 mM ammonium acetate. The gradient elution program

was as follows: 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 °C. Source parameters were as follows: dry heater 200 °C, dry gas flow 3 L·min^{-1}; nebulizer gas pressure 2.5 bar; capillary voltage, 3,500 V. Data acquisition was achieved in auto-MS/MS mode (abundant ions isolation and fragmentation) in order to perform a search on an open access mass spectral library (https://massbank.eu/MassBank/,MassBank_NIST.msp). Identification was carried out on the basis of mass accuracy (<5 ppm), isotopic pattern fit (<50 mSigma) and MS/MS score (score > 800). Values of mSigma are based on the relative mean square of the difference of an experimental mass spectrum from the theoretical isotopic pattern. The lower this value, the more precise the fit. The MS/MS score describes the difference between the measured and the theoretical fragmentation spectrum. The highest the value, the better the fit. Threshold values were set according to the instrument vendor. The data acquisition programs were *Data Analyst* and *Metaboscape* (Bruker Daltonics).

2.3. Brine samples and their characterization

Seven fermentation brine samples were supplied by the company DCCOP located in Monturque (Córdoba, Spain). The samples were from different containers of olives of the *Hojiblanca* variety and they were taken at the end of April 2020 (after containing the olives for six months) and frozen until use. These samples were measured for pH, and the content of NaCl, total polyphenol and hydroxytyrosol.

For the measurement of chloride, 1 mL aliquots of the samples were centrifuged at 10,000 rpm for 15 min to remove suspended solids. A 1:50 v/v dilution was then made with milli-Q water. 10 mL of 6% v/v HNO_3 and 20 mL of an acetic/acetate buffer solution at pH 4.5 were added to aliquots of samples (10 mL). Potentiometric titration was then performed with an automatic buret system using 0.1 M $AgNO_3$ previously standardized against KCl as titrant. The analyses were done in triplicate.

For the measurement of total polyphenols (Folin-Ciocalteu method), 50 μL aliquots of diluted brines (1:10 v/v with a water:methanol 50:50 v/v mixture), blanks (50:50 water:methanol v/v) or gallic acid standards were added to 2 mL Eppendorfs and centrifuged (10,000 rpm 15 min) to remove possible solids. Next, 1.5 mL of distilled water, 100 μL of 0.1 N Folin-Ciocalteu reagent and 300 μL of a 200 g/L sodium carbonate solution were added. After 90 min in the dark at room temperature, the absorbance at 760 nm was measured. Gallic acid standards (50–750 mg/L) were prepared in 50:50 v/v water:methanol from a stock solution thereof prepared in 10 g/L methanol. Gallic acid solutions were stored at −20 °C and in amber bottles until use.

The hydroxytyrosol measurement was performed after diluting the sample ~ 1:40 v/v with water:methanol 80:20 v/v and subsequent centrifugation (10,000 rpm 5 min) to remove suspended solids. The hydroxytyrosol calibration standards were prepared in the range 0.2–30 mg/L in 80:20 v/v water-methanol.

2.4. Formation and characterization of the supramolecular solvent of 1,2-hexanediol in waters and in brines in the presence of salts (sodium sulfate and sodium citrate)

For the formation of SUPRAS from 1,2-hexanediol, the following synthesis solutions were used: 21% v/v of amphiphile and 0.25–1.5 M of Na_2SO_4 or sodium citrate in water or brine (total volume 1.9 mL). The solutions were vortexed for 5 min and centrifuged at 10,000 rpm for 15 min. The SUPRAS volumes (upper phase after centrifugation) were obtained by measuring the height in the cylindrical neck of the centrifuge tubes with a digital caliper and calculating the cylindrical volume occupied by the SUPRAS ($V = \pi r2h$). The water content in the SUPRAS was measured by coulometric Karl Fischer titration by injecting 200 μL aliquots after dilution of the SUPRAS with methanol (1:30, v/v).

2.5. Optimization of the extraction of hydroxytyrosol from brines based on SUPRAS

For the hydroxytyrosol extraction optimization process, 1 mL aliquots of two different brines (brine 1 and 7) was used, to which 1,2-hexanediol was added as amphiphile. SUPRAS formation of 1,2-hexanediol was induced by the addition of salt (Na_2SO_4, sodium citrate). Samples were vortexed for 5 min and ultra-centrifuged (10,000 rpm) for 15 min. After measuring the upper phase (SUPRAS), it was extracted with a syringe and taken to a 25 mL flask to be diluted with water:methanol 80:20 v/v before its analysis by LC-UV to determine the extraction recovery of hydroxytyrosol.

2.6. Characterization of SUPRAS extracts from brines

SUPRAS formed from 0.4 mL of 1,2-hexanediol/mL brine and 1 M Na_2SO_4 (under which we obtained quantitative hydroxytyrosol recovery) were characterized for total polyphenols and profile of bioactive compounds. The extracts were diluted 1:15 v/v with water:methanol 50:50 v/v, vortexed, centrifuged for 15 min at 10,000 rpm to remove possible solids, and 50 μL aliquots were measured to determine the total polyphenol content as described in section 2.3. The diluted extracts were analyzed by liquid chromatography and high resolution mass spectrometry using the instrument and conditions detailed in section 2.2.

3. Results and discussion

3.1. Characterization of fermentation brines of table olives

Table 1 shows the results of the characterization of brine samples. As expected, their pH was slightly acid and was between 3.68 and 4.30. As specified in the Introduction, this acidity is given because after the treatment with NaOH, the olive is washed to remove the soda and it is preserved in the brine, where lactic fermentation takes place, a process that entails a decrease in pH. The salt content in the analyzed samples was between ~ 0.8 and 1 M NaCl (4.6–5.8 % w/v). The pH values and salt content were within the expected range according to the bibliography (pH 3.8–4, 5–6% w/v NaCl). [26].

The hydroxytyrosol content in the brines varied between 1.1 and 1.7 g/L and it was the most abundant phenolic compound in all cases, with a chromatographic peak at 7.80 ± 0.04 min in all chromatograms (λ_{max} 278 nm). As expected, the correlation between the hydroxytyrosol content and total polyphenols content was very high with a Pearson's correlation coefficient of 0.9667. The hydroxytyrosol values were higher than those found in a study that used brines from a wide variety of olives and in which reported values in the ranges of 0.26 ± 0.3 g/L and 0.89 ± 0.2 g/L in the *Gordal* and *Chamomile* varieties, respectively [27]. Likewise, the content of total polyphenols in our study (1.8–3.4 g of gallic acid equivalents/L) was higher than that found in the above mentioned study (0.5–1.4 g of gallic acid/L [27]).

Table 1
Characterization of fermentation brines.

Brine code	pH	NaCl (M)	Total polyphenol content (gallic acid equivalents, g/L)	Hydroxytyrosol (g/L)
1	4.30	0.96 ± 0.08	2.83 ± 0.03	1.53 ± 0.08
2	3.92	0.91 ± 0.06	1.754 ± 0.004	1.1 ± 0.1
3	4.02	0.810 ± 0.007	2.33 ± 0.05	1.3 ± 0.1
4	4.11	0.9 ± 0.1	2.79 ± 0.03	1.6 ± 0.1
5	4.18	0.869 ± 0.003	2.80 ± 0.02	1.6 ± 0.1
6	4.36	0.77 ± 0.02	3.35 ± 0.08	1.7 ± 0.2
7	3.68	0.90 ± 0.02	2.55 ± 0.05	1.4 ± 0.1

3.2. Formation and characterization of the salt-induced 1,2-hexanediol SUPRASs

The formation of SUPRAS from 1,2-hexanediol in the presence of urine containing Na_2SO_4 has been previously described [24]. It is widely accepted that the addition of salt to colloidal systems (in our case normal aggregates of 1,2-hexanediol in water) causes the destruction of the hydration layer of the head groups of the amphiphile [28]. In this way, the effective area per molecule decreases and surfactant monomers can pack more, leading to aggregate growth and liquid phase separation [29]. The salting-out effect decreases the solubility of non-ionic solutes with increasing salt concentration and favors their aggregation. This effect is more significant for the anion than for the cation of the salt, which in our case is sodium [30].

The sign of the Jones-Dole coefficient (B) is a measure of the degree of hydration of the salt ion (positive for kosmotropes or highly hydrated and negative for chaotropes or weakly hydrated) and represents their dehydration strength. The dehydration power and salting-out effect of the anions of the selected salts is greater for citrate (kosmotropic, B: 0.333) than for $SO_4^{=}$ (kosmotropic, B: 0.208) and Cl^- (chaotropic, B: −0.007). Accordingly, the minimum concentration of salt needed for SUPRAS formation was 0.6 M Na_2SO_4 (0.9 mmols) and 0.5 M of tribasic sodium citrate (0.75 mmols) and NaCl (assayed up to 2 M) did not cause coacervation. Below these values, the amphiphile formed an isotropic solution in water.

The formation of SUPRAS with Na_2SO_4 (the optimal coacervation agent finally selected) was also tested by substituting the distilled water with three different brines. In this way, it was intended to see the effect of the presence of the matrix on the formation of SUPRAS and to study the possibility of generating it from the brine salts themselves by adding 1,2-hexanediol. Although the presence of sodium chloride in the sample did not allowed coacervation by itself, it did favor it and phase separation took place at lower Na_2SO_4 concentrations (0.5 M instead of 0.6 M). The generated SUPRASs slightly contained lower water content and they were smaller in volume. The water content and volume of the SUPRASs generated in these experiments ($n = 3$) are shown in Table 2.

As reported before [24], the salt-induced 1,2-hexanediol SUPRASs were characterized by high water content (~25–59%). The SUPRASs formed at higher salt concentrations gave rise to more packed phases with lower water content. In turn, those induced by sodium citrate contained a smaller amount of water and a smaller volume than those induced by sodium sulfate, which is consistent with the greater dehydration power of this anion.

3.3. Optimization of hydroxytyrosol extraction from brines with salt-induced 1,2-hexanediol SUPRASs and potential applicability at the industrial scale

Table 3 shows the extraction efficiency of hydroxytyrosol with SUPRAS formed by 1,2-hexanediol in brines. Different concentrations of Na_2SO_4 (0.5–1.5 M in brine) or sodium citrate (0.5–1.25 M in brine) were used.

The recoveries and concentration factors were quite similar for SUPRASs induced by different concentrations of sodium citrate, while they increased as the concentration of Na_2SO_4 raised between 1 and 1.5 M. The increase in recoveries with increasing salt concentration may be due to the "salting-out" effect that also influences the hydroxytyrosol solute, decreasing its solubility in brine and favoring its partition to the SUPRAS phase. On the other hand, at the same salt concentration, the citrate-induced SUPRAS contained less water (Table 2), which could negatively affect the recovery of a compound as polar as hydroxytyrosol. This could explain the slightly lower extraction efficiencies for this salt at high concentrations despite its greater dehydration power.

The highest recovery values were obtained for 1.5 M Na_2SO_4; recoveries of 61–64% of hydroxytyrosol were obtained in both brines. The concentration factor was 2.0–2.1 under these conditions (or 3.1–3.3 if we assume the removal of water from SUPRAS in a later stage by lyophilization).

The results were compared with the extraction efficiency of SUPRASs formed with 1-hexanol in order to compare the influence of the presence of an additional alcohol group in the amphiphile. The 1-hexanol-based SUPRASs were formed in mixtures of ethanol or isopropanol and water. The SUPRAS of simple alcohols in mixtures of a water miscible organic solvent (tetrahydrofuran, acetone, ethanol, propane, etc.) and water have been described by the research group and used successfully in extraction processes [9]. In this type of SUPRAS, the coacervating agent is water (in this case the brine), which is a poor solvent for the amphiphile, and which, when added to a colloidal dispersion of inverse aggregates in organic solvent, causes their dehydration and aggregation and the formation of the SUPRAS. These SUPRASs were also tested in the presence of 1 M Na_2SO_4 to take into account the "salting-out" effect on the hydroxytyrosol extraction efficiency due to the decrease in its solubility in water and/or in the formation of SUPRAS.

As can be seen in Table 3, the 1-hexanol-based SUPRASs offered very low recoveries (<30 %) at the same percentage of amphiphile as those of 1,2-hexanediol, in fact, they were not significantly different from those obtained by a pure 1-hexanol phase. The use of other alcohols such as isopropanol did not improve the recoveries. The concentrations of water in these SUPRAS, previously investigated by the research group, were between 5 and 10% by weight. This lower water content in the SUPRAS together with the lower polarity of 1-hexanol and the presence of organic solvent (5–10% w/w) gave rise to less polar SUPRASs and lower

Table 2
1,2-hexanediol-based SUPRAS composition under different preparation conditions.

SUPRAS formed with 1,2-Hexanediol/water (Na_2SO_4)			SUPRAS formed with 1,2-Hexanediol/brine (Na_2SO_4)				SUPRAS formed with 1,2-Hexanediol/water (sodium citrate tribasic)		
Na_2SO_4 (M in water)	Water (w/w in SUPRAS)	Volume (µL SUPRAS per µL of 1,2-hexanediol in the preparation mix)	Na_2SO_4 (M)/ brine code	NaCl (M in brine)	Water (w/w in SUPRAS)	Volume (µL SUPRAS per µL of 1,2-hexanediol in preparation mix)	Sodium citrate (M in water)	Water (w/w in SUPRAS)	Volume (µL SUPRAS per µL of 1,2-hexanediol in preparation mix)
0.60	59 ± 4	1.15 ± 0.02	1/1	0.96 ± 0.08	36 ± 2	1.45 ± 0.05	0.5	49.4 ± 0.9	1.79 ± 0.06
0.75	45.7 ± 0.7	1.79 ± 0.02	1/3	0.869 ± 0.003	34.16 ± 0.08	1.4 ± 0.1	0.75	39 ± 1	1.50 ± 0.09
1.00	39 ± 1	1.51 ± 0.01	1/7	0.90 ± 0.02	36 ± 3	1.4 ± 0.1	1	33.29 ± 0.06	1.40 ± 0.05
1.50	36 ± 1	1.52 ± 0.02					1.5[a]	24.92 ± 0.02	1.13 ± 0.04

[a] The salt precipitated in the solution.

Table 3
Extraction efficiency for hydroxytyrosol with SUPRAS of 1,2-hexanediol induced by salts and of 1-hexanol induced by water or with pure 1-hexanol, all of them formed in brines ([a]brine 1, [b]brine 7).

1,2-hexanediol-based SUPRAS				1-hexanol-based SUPRAS or pure 1-hexanol		
Na_2SO_4 (M in brine)	R (%) ± SD [c,d](Concentration factor)	Sodium citrate (M in brine)	R (%) ± SD [c,d](Concentration factor)	Organic solvent	Na_2SO_4 (M in brine)	R (%) ± SD [c,d](Concentration factor)
0.50	[a]50.8 ± 0.1[c](1.4) [d](3.4) [b]43 ± 1.5[c](1.2) [d](2.9)	0.50	[a]54 ± 6[c](1.5) [d](2.9) [b]50 ± 2[c](1.4) [d](2.8)	0 µL (pure 1-hexanol)	0	[a]28 ± 5 [b]25 ± 2
0.75	[a]54 ± 1 (1.8) [d](3.3) [b]53.3 ± 0.5 (1.8) [d](3.3)	0.75	[a]53 ± 3[c](1.8) [d](2.9) [b]51 ± 1[c](1.7) [d](2.8)	200 µL ethanol (14 % v/v)	0	[a]21 ± 3 [b]25 ± 5
1.00	[a]60 ± 1[c](2.1) [d](3.2) [b]59 ± 2[c](2.1) [d](3.2)	1.00	[a]55.4 ± 0.7[c](2.0) [d](2.8) [b]50 ± 4[c](1.8) [d](2.8)	100 µL ethanol (7% v/v)	0	[a]26 ± 8 [b]24 ± 2
1.50	[a]64 ± 2[c](2.1) [d](3.3) [b]61 ± 2[c](2.0) [d](3.1)	1.25	[a]53 ± 4[c](2.3) [d](2.8) [b]52 ± 2[c](2.3) [d](2.8)	200 µL isopropanol (14 % v/v)	0	[a]11 ± 4 [b]not detected
				200 µL ethanol (14 % v/v)	1	[a]44 ± 6 [b]43.6 ± 0.4

SUPRAS formation conditions: 200 µL amphiphile (17 %v/v), 1 mL brine, [c]Calculated according to: Brine volume/SUPRAS volume × Recovery/100; [d]Calculated in the same way as above, taking into account the volume of SUPRAS without water, since this can be removed in a simple lyophilization process.

extraction efficiency for hydroxytyrosol. The presence of salt also improved the recovery in these SUPRASs (~44%) without reaching the values found with 1,2-hexanediol.

Once the greater capacity of the SUPRAS of 1,2-hexanediol for the extraction of hydroxytyrosol was corroborated, the volume of amphiphile was studied by selecting a concentration of salt of 1 M of Na_2SO_4 (recoveries 59–60%). Although the recovery values improved slightly at 1.5 M salt (61–64%), this increase was considered very small in relation to the increase required in the amount of salt (and the concentration factors were similar, 2.0–3.3). The results obtained by modifying the volume of amphiphile used for generating the SUPRAS are shown in Table 4.

Recoveries of hydroxytyrosol above 70% were obtained for brines 1 and 7 using SUPRASs formed from 400 µL of 1,2-hexanediol. These conditions were tested for the other five brines and recoveries varied from 73 ± 2 to 82 ± 3, showing that the extraction method was robust with respect to variations in the sample matrix (e.g. pH, NaCl content, total polyphenols, etc).

The extracts obtained using the different percentages of amphiphile were also characterized in terms of their hydroxytyrosol content. The highest concentrations of hydroxytyrosol in SUPRAS extracts were measured with a concentration of amphiphile of 4.8% v/v for SUPRAS preparation (i.e. 50 µL). Under these conditions, an average value of 6 g/L of hydroxytyrosol or 9.4 g/L (after lyophilization) were obtained. These values are equivalent to ~ 0.5–0.6% w/w of hydroxytyrosol or 0.8–0.9% w/w in SUPRAS extracts, respectively. However, recoveries were around 25% under these conditions. To obtain recoveries above 70%, the amphiphile values required for SUPRAS synthesis were in the range 28–33% v/v of 1,2-hexanediol, which resulted in lower hydroxytyrosol concentrations in SUPRAS due to the larger volume of extractant phase generated (1.59–1.85 g/L of hydroxytyrosol with 28.5% v/v of 1,2-hexanediol for SUPRAS synthesis). So, the optimal SUPRAS treatment conditions will depend on the market demands and the viability of the product.

Likewise, the total polyphenol contents in these SUPRAS extracts were in the range of 3.90 ± 0.06 g of gallic acid/L in brine sample 6 to 2.37 ± 0.07 g of gallic acid/L in brine sample 2, which is in agreement with the initial total polyphenol content of the brines and the achieved concentration factors (Table 1).

Final concentration of hydroxytyrosol in cosmetics were reported in the range 0.0008–0.055 % w/w [31] so that the direct application of our extracts in market products could be possible. As we have mentioned

Table 4
Extraction efficiency for hydroxytyrosol from brines ([a]brine 1, [b]brine 7, 1 M Na_2SO_4) as a function of the volume of 1,2-hexanediol used for SUPRAS formation.

1,2-hexanediol (µL)	Generated SUPRAS volume	R (%) ± SD [c,d](Concentration factor)	Measured concentration of hydroxytyrosol in SUPRAS extracts
25	[a,b]36.25	[a]2.92 ± 0.07[c](1.2) [d](1.9) [b]1.37 ± 0.07[c](0.5) [d](0.8)	[a]1.84 ± 0.08 [d](2.9) [b]0.70 ± 0.04 [d](1.1)
50	[a,b]72.5	[a]25 ± 1[c](3.4) [d](5.3) [b]25.0 ± 0.4[c](3.4) [d](5.3)	[a]6 ± 1 [d](9.4) [b]6 ± 1 [d](9.4)
100	[a,b]145	[a]44.9 ± 0.2[c](3.1) [d](4.8) [b]43.1 ± 0.9[c](3.0) [d](4.6)	[a]5.2 ± 0.3 [d](8.1) [b]4.1 ± 0.4 [d](6.4)
200	[a,b]290	[a]60 ± 1[c](2.1) [d](3.2) [b]59 ± 2[c](2.1) [d](3.2)	[a]3.13 ± 0.01 [d](4.9) [b]2.7 ± 0.1 [d](4.2)
300	[a,b]435	[a]67.2 ± 0.7[c](1.5) [d](2.4) [b]68 ± 1[c](1.5) [d](2.4)	[a]2.4 ± 0.1 [d](3.8) [a]1.956 ± 0.008 [d](3.0)
400	[a,b]580	[a]75.9 ± 0.2[c](1.3) [d](2.0) [b]72 ± 4[c](1.2) [d](1.9)	[a]1.85 ± 0.05 [d](2.9) [b]1.59 ± 0.04 [d](2.5)
500	[a,b]725	[a]76 ± 3[c](1.0) [d](1.6) [b]75 ± 1[c](1.0) [d](1.6)	[a]1.84 ± 0.08 [d](2.9) [b]0.70 ± 0.04 [d](1.1)

SUPRAS formation conditions: 1 mL brine with 1 M Na_2SO_4, [c]Calculated according to: Brine volume/SUPRAS volume × Recovery/100; [d]Calculated taking into account the volume of SUPRAS without water, since this can be removed in a simple lyophilization process.

before, since the ingredients of SUPRAS extracts are all allowed in market formulations, their complete elimination (which would require further purification steps) is not required.

To further implement this methodology at industrial scale and regarding the treatment of the remaining aqueous salty brine after SUPRAS extraction it must be taken into account that the concentration of 1,2-hexanediol in the salty brine would be negligible and near the critical aggregation concentration of the amphiphile, which is usually in the low millimolar range. For further treatment of this wastewater, different mechanical, chemical and biological treatments have been proposed to desalt them and to remove the organic carbon load before discharge [9] that could be used also after SUPRAS treatment.

Finally, for industrial implementation it is worth mentioning that although we employed a centrifugation step to speed up the experiments, phase-separation also occurs spontaneously after leaving the mixture stand still for around 1 h.

3.4. Identification of other compounds of interest in SUPRAS extracts by liquid chromatography and high-resolution mass spectrometry

Although according to the profile observed in LC-UV and the high correlation with the total polyphenol content, it was clear than hydroxytyrosol was the main phenolic compound in brines, the presence of other compounds of interest in the extracts was qualitatively investigated. SUPRAS extracts from brines 1 and 7 obtained under the highest recoveries were directly analyzed with liquid chromatography and high-resolution mass spectrometry (LC-QTOF-MS). Tentative identification was made based on a high resolution MS/MS library from NIST (Table 5). Fatty acids, hydroxyl fatty acids, sugar-based acids and other plant metabolites, as typical compounds present in vegetables, were identified (arachidic acid, behenic acid, threonic acid, 2-hydroxycaproic acid, 1,4-cyclohexanedicarboxylic acid, mannitol and sulfojasmonate). Bacteria fermentation products (3-phenyllactic acid, 4-hydroxyphenyllactic acid, phenylacetic acid and succinic acid) were also detected. The identified phenolic compounds were 3′,4′-dihydroxyphenylglycol, 4-methyl-catechol,catechol, homogentisic acid, homovanillic acid, luteolin, methyl-salicylate, oleuropein and vanillic acid. Catechol and 4-methyl-catechol have been previously identified in olive washing waters by other authors [32], oleuropein and luteolin have been identified in table olives [33], homogentisic, vanillic and homovalinic acids are also common in vegetables. 3,4-Dihydroxyphenylethanol is a common phenolic compounds in olives with important antioxidant activity [34]. Tyrosol was not found, despite being a widely identified compound in other types of olive grove by-products [32,33].

4. Conclusions

This research paper describes the formation of sustainable SUPRASs and their application for the first time to the liquid–liquid extraction of hydroxytyrosol (phenolic compound with high antioxidant activity) from brines, an abundant residue from the processing of table olives that has been scarcely investigated for recovery of antioxidants. Unlike conventional organic solvents, the proposed green SUPRAS is able to extract a very polar bioactive such as hydroxytyrosol (log P –0.7) by liquid–liquid extraction, a very simple technique for residue valorization. Brines of the *Hojiblanca* olive variety were analyzed, which were characterized by an acid pH (3.68–4.30), high salt content (4.6–5.8 % w/v) and total polyphenol content in the range 1.8—3.4 g of gallic acid /L, being the main component hydroxytyrosol (1.1–1.7 g/L). The SUPRASs were made up of non-toxic components that are authorized in the cosmetic industry, specifically 1,2-hexanediol, water and salts (Na_2SO_4 and sodium citrate). The high water content of the SUPRASs together with the salting-out effect allowed the efficient extraction of hydroxytyrosol (a very polar compound, calculated logP −0.7) from brines. Under non-quantitative yields (~25%), maximum concentrations of hydroxytyrosol in the SUPRAS extracts of ~ 0.5–0.9% w/w were obtained using 4.8% v/v of 1,2-hexanediol and 1 M Na_2SO_4 for the synthesis (equivalent to a SUPRAS:sample ratio of 1:20 v/v in the extraction process). The final extracts were also analyzed by LC-QTOF-MS to identify the presence of other compounds of interest, showing a variety of phenolic compounds. The contents by weight of hydroxytyrosol in the final extracts of SUPRAS are viable for commercialization (which could be increased through subsequent purification and preconcentration processes). The potential of table olive brines as a source of phenolic compounds is also highlighted, being a residue that has been less studied than other olive by-products.

CRediT authorship contribution statement

Ana Ballesteros-Gómez: Investigation, Writing – original draft, Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Antonio Serrano-Crespín:** Resources, Writing – review & editing. **Soledad Rubio:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Table 5
Plant metabolites tentatively identified by spectrum library search (LC-QTOF).

Name	Molecular formula	Ions	Mass error (ppm)	Isotopic pattern fit (mSigma)	MS/MS score
1,4-Cyclohexanedicarboxylic acid	$C_8H_{12}O_4$	[M−H]-	0.791	16.2	999.8
2-Isopropylmalic acid	$C_7H_{12}O_5$	[M−H]-	0.68	10.2	967.1
3,4-Dihydroxyphenylglycol	$C_8H_{10}O_4$	[M−H−H_2O]-, [M−H]-	0.941	29.6	939.1
3-phenyllactic acid	$C_9H_{10}O_3$	[M−H]-, [M−H−H2O]-	0.769	11.8	802.4
4-Hydroxyphenyllactic acid	$C_9H_{10}O_4$	[M−H]-	1.697	19	967.7
4-Methylcatechol	$C_7H_8O_2$	[M−H]-, [M−H−H_2O]-	−0.139	2.3	880.2
6-Hydroxycaproic acid	$C_6H_{12}O_3$	[M−H]-	0.332	11.5	995.7
Arachidic acid	$C_{20}H_{40}O_2$	[M−H]-	1.037	11.5	999.5
Behenic acid	$C_{22}H_{44}O_2$	[M−H]-	0.147	49.3	813.3
Cathecol	$C_6H_6O_2$	[M−H]-	0.265	5.7	992.2
D-(-)-Mannitol	$C_6H_{14}O_6$	[M−H]-	0.46	6.2	911.6
Homogentisic acid	$C_8H_8O_4$	[M−H]-	0.44	22.9	858.3
Homovanillic acid	$C_9H_{10}O_4$	[M−H]-	0.415	14.4	845.4
Luteolin	$C_{15}H_{10}O_6$	[M−H]-	0.253	50.0	840.7
Methyl salicylate	$C_8H_8O_3$	[M−H]-	2.075	7.3	917.4
Oleuropein	$C_{25}H_{32}O_{13}$	[M−H]-	0.288	18.6	879.5
Phenylacetic acid	$C_8H_8O_2$	[M−H]-	1.841	20.6	995.5
Succinic acid	$C_4H_6O_4$	[M−H]-	0.568	7	995.9
Sulfo jasmonate	$C_{12}H_{18}O_7S$	[M−H]-	−0.212	26.9	846.6
Threonic acid	$C_4H_8O_5$	[M−H]-	2.336	15.8	957.8
Vanillic acid	$C_8H_8O_4$	[M−H]-	0.604	21.5	809.9

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.

Data availability

Data will be made available on request.

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References

[1] L. Rejano, A. Montaño, F. J. Casado, A. H. Sánchez, A. de Castro. Chapter 1 - Table Olives: Varieties and Variations, Editor(s): V. R. Preedy, R.R. Watson, Olives and Olive Oil in Health and Disease Prevention, Academic Press, 2010, pages 5-15.
[2] A.H. Sánchez Gómez, P. García García, L. Rejano Navarro, Elaboration of table olives, Grasas y Aceites 57 (2006) 86, https://doi.org/10.3989/gya.2006.v57.i1.24.
[3] C. Romero, M. Brenes, P. García-Serrano, A. Montaño, E. Medina, P. García-García, Packing black ripe olives in acid conditions, Food Chem. 337 (2021), 127751, https://doi.org/10.1016/j.foodchem.2020.127751.
[4] P. Gullón, B. Gullón, G. Astray, M. Carpena, M. Fraga-Corral, M.A. Prieto, J. Simal-Gandara, Valorization of by-products from olive oil industry and added-value applications for innovative functional foods, Food Res. Int. 137 (2020), 109683, https://doi.org/10.1016/j.foodres.2020.109683.
[5] A. Roig, M.L. Cayuela, M.A. Sánchez-Monedero, An overview on olive mill wastes and their valorisation methods, Waste Manag. 26 (2006) 960, https://doi.org/10.1016/j.wasman.2005.07.024.
[6] H. Rasouli, M. Hosein Farzaei, R. Khodarahmi, Polyphenols and their benefits: A review, Int. J. of Food Properties. 20 (2017) 1700, https://doi.org/10.1080/10942912.2017.1354017.
[7] A. Bonetti, S. Venturini, A. Ena, C. Faraloni, Innovative method for recovery and valorization of hydroxytyrosol from olive mill wastewaters, Water Sci. Technol. 74 (2016) 73, https://doi.org/10.2166/wst.2016.181.
[8] M. Brenes, A. de Castro, Transformation of oleuropein and its hydrolysis products during Spanish-style green olive processing, J. Sci. Food Agri. 77 (1998) 353, https://doi.org/10.1002/(SICI)1097-0010(199807)77:3<353::AID-JSFA50>3.0.CO;2-G.
[9] A.J. Huertas-Alonso, D.J. Gonzalez-Serrano, M. Hadidi, M. Salgado-Ramos, J. C. Orellana-Palacios, M.P. Sánchez-Verdú, Q. Xia, M.J. Simirgiotis, F.J. Barba, B. N. Dar, A. Moreno, Table Olive Wastewater as a Potential Source of Biophenols for Valorization: A Mini Review, Fermentation 8 (2022) 215, https://doi.org/10.3390/fermentation8050215.
[10] K.Y. Cazares-Carrión, R. Abreu-Naranjo, Optimisation of the nanofiltration process of residual wastewater from table olives using synthetic solutions for the recovery of phenolic compounds, Water Practice & Technol. 16 (2021) 837, https://doi.org/10.2166/wpt.2021.044.
[11] R. Johnson, A.E. Mitchell, Use of Amberlite Macroporous Resins to Reduce Bitterness in Whole Olives for Improved Processing Sustainability, J. Agric. Food Chem. 67 (2019) 1546, https://doi.org/10.1021/acs.jafc.8b06014.
[12] A. Ballesteros-Gomez, M.D. Sicilia, S. Rubio, Supramolecular solvents in the extraction of organic compounds, A review. Anal. Chim. Acta 677 (2010) 108–130, https://doi.org/10.1016/j.aca.2010.07.027.
[13] A. Ballesteros-Gomez, S. Rubio, Environment-Responsive Alkanol-Based Supramolecular Solvents, Characterization and Potential as Restricted Access Property and Mixed-Mode Extractants, Anal. Chem. 8 (2012) 342, https://doi.org/10.1021/ac2026207.
[14] L.S. Torres-Valenzuela, A. Ballesteros-Gómez, S. Rubio, Soledad, Supramolecular solvent extraction of bioactives from coffee cherry pulp, J. Food Eng. 278 (2020) 10993, https://doi.org/10.1016/j.jfoodeng.2020.109933.
[15] L.S. Torres-Valenzuela, A. Ballesteros-Gómez, J. Serna, A. Arango, S. Rubio, Supramolecular solvents for the valorization of coffee wastewater, Environ. Sci. Water Res. Technol. 6 (2020) 757, https://doi.org/10.1039/C9EW01095E.
[16] L.S. Torres-Valenzuela, A. Ballesteros-Gómez, A. Sanin, S. Rubio, Valorization of spent coffee grounds by supramolecular solvent extraction, Sep. Pur. Technol. 228 (2019), 115759, https://doi.org/10.1016/j.seppur.2019.115759.
[17] C. Sánchez-Vallejo, A. Ballesteros-Gómez, S. Rubio, Tailoring composition and nanostructures in supramolecular solvents: Impact on the extraction efficiency of polyphenols from vegetal biomass, Sep. Pur. Technol. 292 (2022), 120991, https://doi.org/10.1016/j.seppur.2022.120991.
[18] M.N. Keddar, A. Ballesteros-Gómez, J.A. Siles, M. Amiali, D. Zerrouki, M.A. Martín, S. Rubio, Efficient extraction of hydrophilic and lipophilic antioxidants from microalgae with supramolecular solvents, Sep. Pur. Technol. 251 (2020) 11732, https://doi.org/10.1016/j.seppur.2020.117327.
[19] A. Chatzilazarou, E. Katsoyannos, O. Gortzi, S. Lalas, Y. Paraskevopoulos, E. Dourtoglou, J. Tsaknis, Removal of polyphenols from wine sludge using cloud point extraction, J. Air Waste Manag. Assoc. 60 (2010) 454, https://doi.org/10.3155/1047-3289.60.4.454.
[20] M. Mohammadzadeh, M. Honarvar, A.R. Zarei, M.M. Akbar Boojar, H. Bakhoda, A new approach for separation and recovery of betaine from beet molasses based on cloud point extraction technique, J. Food Sci. Technol. 55 (2018) 1215, https://doi.org/10.1007/s13197-017-2999-4.
[21] B. Dias Ribeiro, D. Weingart Barreto, M.A. Zarur Coelho, Use of micellar extraction and cloud point preconcentration for valorization of saponins from sisal (Agave sisalana) waste, Food Bioprod. Process. 94 (2015) 601, https://doi.org/10.1016/j.fbp.2014.07.004.
[22] E. Romera-García, A. Ballesteros-Gómez, S. Rubio, Supramolecular biosolvents made up of self-assembled rhamnolipids: synthesis and characterization, Green Chem. 22 (2020) 6115, https://doi.org/10.1039/D0GC02078H.
[23] E. Romera-García, A. Ballesteros-Gómez, S. Rubio, An environmentally stable supramolecular biosolvent: Characterization and study of its potential for the elimination of polar toxic substances in water, J. Clean. Prod. 321 (2021), 128975, https://doi.org/10.1016/j.jclepro.2021.128975.
[24] S. González-Rubio, A. Ballesteros-Gómez, G. Muñoz, S. Rubio, Cubosomic Supramolecular Solvents: Synthesis, Characterization, and Potential for High-Throughput Multiclass Testing of Banned Substances in Urine, Anal. Chem. 94 (2022) 4103–4111, https://doi.org/10.1021/acs.analchem.2c00082.
[25] P. Antolovich, K. Prenzler, D. Robards, D. Ryan, Sample preparation in the determination of phenolic compounds in fruits, Analyst 125 (2000) 989, https://doi.org/10.1039/B000080I.
[26] P. Conte, C. Fadda, A. del Caro, P.P. Urgeghe, A. Piga, Table Olives: An Overview on Effects of Processing on Nutritional and Sensory Quality, Foods 9 (2020) 514, https://doi.org/10.3390/foods9040514.
[27] E. Medina Pradas. Doctoral Thesis. Antimicrobianos polifenólicos y oleosídicos en alimentos derivados de la aceituna. University of Sevilla, 2008. Available at: https://idus.us.es/handle/11441/65021 (access on 15-03-2023).
[28] D.F. Evans, H. Wennerström, The Colloidal Domain: Where Physics, Chemistry, Biology, and Technology Meet, Wiley-VCH, New York, 1999.
[29] N. Vlachy, M. Drechsler, J.M. Verbavatz, D. Touraud, W. Kunz, Role of the surfactant headgroup on the counterion specificity in the micelle-to-vesicle transition through salt addition, J. Colloid Interface Sci. 319 (2008) 542, https://doi.org/10.1016/j.jcis.2007.11.048.
[30] A.M. Hyde, S.L. Zultanski, J.H. Waldm, General Principles and Strategies for Salting-Out Informed by the Hofmeister Series, Org. Process Res. Dev. 21 (2017) 1355, https://doi.org/10.1021/acs.oprd.7b00197.
[31] P. Miralles, A. Chisvert, A. Salvador, Determination of hydroxytyrosol and tyrosol by liquid chromatography for the quality control of cosmetic products based on olive extracts, J. Pharm. Biomed. Anal. 102 (2015) 157, https://doi.org/10.1016/j.jpba.2014.09.016.
[32] S. Jiménez-Herrera, J.M. Ochando-Pulido, A. Martínez-Ferez, Comparison between different liquid-liquid and solid phase methods of extraction prior to the identification of the phenolic fraction present in olive oil washing wastewater from the two-phase olive oil extraction system, Grasas y Aceites 68 (2017) e208.
[33] C. Salis, I.E. Papadakis, M. Hagidimitriou. Identification and quantification of phenolic compounds in fresh and processed table olives of cv. 'Kalamata'. Notulae Botanicae Horti. Agrobotanici. Cluj-Napoca, 49 (2021), 12394. DOI: 10.15835/nbha49212394.
[34] M.D. Ferro, S.A.O. Santos, A.J.D. Silvestre, M.F. Duarte, Chromatographic Separation of Phenolic Compounds from Extra Virgin Olive Oil: Development and Validation of a New Method Based on a Biphenyl HPLC Column, Int. J. Mol. Sci. 20 (2019) 201, https://doi.org/10.3390/ijms20010201.