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ORIGINAL PAPER

Recovery of Natural Antioxidants from Olive Mill Wastewater Using Genapol-X080

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Abstract The possibility of applying cloud point extraction (CPE) using Genapol X-080 as surfactant for the separation of phenolic compounds from olive mill wastewater was examined. The ability of the surfactant to recover individual and mixtures of polyphenols and tocopherols in various concentrations from aqueous solutions was tested before its application for the recovery of phenols from olive mill wastewater. Many of the examined individual polyphenols were recovered at high percentage. Especially, in the case of Luteolin, low surfactant concentrations were sufficient for quantitative removal. The recovery appeared proportional to the surfactant concentration. The complete recovery of tocopherols was also possible. Total phenol recovery by simple and successive CPE of olive mill wastewater with various concentrations (2, 5 and 20%, v/v) of Genapol X-080 was up to 89.5%.

Keywords Cloud point extraction · Genapol X-080 · Olive mill wastewater · Polyphenols · Surfactants · Tocopherols

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Introduction

Polyphenols from plant sources are interesting natural antioxidants, used to enhance the food properties, for nutritional purposes and for preservation [1, 2]. Though, the extraction of phenolic compounds from plant materials and wastes is a complicated and costly process as it requires large quantities of toxic and flammable organic solvents [3, 4]. Liquid–solid phase-, solid phase-, supercritical fluid-, accelerated pressurized- and microwave-assisted extraction techniques are not satisfactory for analytical purposes or for industrial production of phenolic antioxidants for dietary applications [5, 6]. Specifically, solid phase extraction leads to lower phenolics recovery, while supercritical fluid extraction using liquid CO_2 requires expensive, high pressure equipment [5].

Surfactants are amphiphilic molecules, with a polar, hydrophilic head and a lipophilic tail consisting of a hydrocarbon linear or branched aromatic or non-aromatic chain, which aggregate in aqueous solutions to form micelles. As indicated by Katsoyannos et al. [7], micellar systems using non-toxic surfactants (non-ionic, without branched aliphatic chains or aromatic moieties) are appropriate for the isolation of natural antioxidants (phenols), which then can be used in dietary applications. Today, liquid–liquid phase separations of non-ionic or zwitterionic surfactant micelles (i.e., cloud point extraction-CPE) are employed, while the use of charged surfactant species is still limited [8]. Common surfactants used are Triton (X-100 and X-114), PEG-6000, Ponde-7.5, Ponde-10, C_8-Lecithin, Brij (−30, −56 and −97) and many others [5, 9]. The anionic surfactants sodium dodecylsulfate, sodium dodecylbenzenesulfonic acid and sodium dodecanesulfonic acid can also form two phases in the presence of high concentrations of HCl [10]. To our

knowledge no analytical application of the cloud point phenomenon has been described for cationic surfactants.

Aqueous solutions of most non-ionic surfactant micelles become turbid after heating to a temperature known as the cloud point. Upon heating above the cloud point temperature (CPT), a micellar solution of a non-ionic surfactant is separated and two phases are formed. One of them is surfactant-rich (i.e. viscous surfactant phase) and the other one aqueous (i.e. water phase), containing a surfactant concentration close to the critical micelle concentration (CMC) at that temperature. When surfactant molecules concentration increases above CMC, surfactant monomers spontaneously accumulate to form colloidal-sized clusters (micelles) [8]. Depending on the specific surfactant and solution conditions, micelles can adopt a variety of shapes, ranging from roughly spherical to ellipsoidal. The mechanism by which separation occurs is not yet clear [10]. Some authors have proposed that it would be due to an increase in the micellar aggregation number (an increase in micellar size) when temperature is increased [11, 12]. Lindman and Wennerstroem [12] have suggested that the phase separation mechanism would be caused by a change in micellar interactions, which are repulsive at low temperature but predominantly attractive at high temperatures. Nilsson et al. [13] have explained the cloud point phenomenon on the basis of the dehydration process that occurs in the external layer of micelles of non-ionic surfactants when temperature is increased. The dielectric constant of water decreases on increasing temperature, rendering it a poorer solvent for the hydrophilic portion of the surfactant molecule [8].

The micellization progress including the percentage of encapsulation and the factors influencing it are still the subject of some debate. The CMC parameters, aggregation number (the number of surfactant molecules per micelle), and more subtle structural aspects (degree of surface roughness, or irregularity, size and size distribution, surface tension, etc.) depending on the microstructure of the non-ionic surfactant [5].

It has been shown that phase separations result from the competition between entropy (which favors miscibility of micelles in water) and enthalpy (which favors separation), and so the clouding and phase-separation procedure is reversible. Re-establishment of the initial solution conditions drives the micelles to merge with the aqueous phase, re-producing a homogeneous system [8].

The optimization of the CPE conditions requires the variation of factors such as equilibration time (over the CPT), pH, salt addition, concentration and nature of the surfactant. CMC vary in the range 0.01–45 mM. CPT of 10–93 °C in most cases, also vary with extreme values of 5 and 120 °C [6]. Surfactant can be separated from the analyte by change of the temperature, pH, ultrafiltration or dilution with water, acetonitrile or methanol [9, 14].

Quina and Hinze [9] reviewed the applications of the surfactant-mediated CPE and their potential. Examples of CPE applications are purification of proteins, pre-concentration of several environmental toxic organic compounds, isolation of enzymes, vancomycin, avidin, PCBs, PAHs, DDT, Vitamins K1 and K2, fungicides, pesticides, aromatic amines, fulvic and humic acids. [9, 10, 15], chlorophenols [11] and nitro-phenols from aqueous solutions [14].

The organic matter of olive mill wastewater (OMW) consists of polysaccharides, sugars, polyphenols, polyalcohols, proteins, organic acids and oil and contain considerable amounts of suspended solids [16]. OMW, which is mainly produced in the Mediterranean area, represents a serious environmental problem because of its large volume and high pollutant load, characterized by the presence of biodegradable as well as recalcitrant and biostatic compounds [17]. Despite the existing laws, OMW is frequently dumped, untreated, either on the soil or into watercourses. This causes problems of phytotoxicity (due to polyphenols), destruction of cultures, bad odors, proliferation of insects, contamination of underground water, increase in salinity and reduction in the permeability of the soil, which decreases the degree of aeration [18].

In previous work, we presented preliminary results concerning the CPE application of a surfactant (Triton X-114) on the isolation of polyphenols from OMW [7]. Polyphenols were recovered up to 60%. The aim of the present work was to exam Genapol X-080 (oligoethylene glycol monoalkyl ether) [6], as the surfactant in CPE to separate polyphenolic compounds and tocopherols from OMW. The recovery values were also determined. Genapol X-080 was chosen for the following reasons: (a) Minimal absorption in the range near 280 nm (the most common wavelength for general detection of phenolics by HPLC) [1], and (b) the lack of the need to remove fat prior to CPE since the isotridecyl group of Genapol X-080 renders the surfactant less oil soluble and therefore the adequate phase separation after centrifugation is much easier. Additionally, Genapol X-080 has been previously applied to extract vitamins A and E from human serum and whole blood [19]. The same surfactant was also successfully applied to preconcentrate nitro- and chloro-phenols from seawater prior to their HPLC-UV detection [6].

Experimental Procedures

Materials

The OMW samples were supplied by an olive mill in the city of Argos (Greece) and maintained at 6 °C until use. Tyrosol, syringic acid, α-tocopherol, and δ-tocopherol were

purchased from Sigma-Aldrich (Hohenbrunn, Germany). Methanol, acetonitrile, Folin-Ciocalteu reagent and Genapol X-080 were purchased from Merck (Darmstadt, Germany) while protocatechuic acid, *p*-coumaric acid, *o*-coumaric acid, gallic acid and rutin were obtained from Fluka (Buchs, Switzerland) and oleuropein, luteonin and epicatechin from Extrasyntese (Genay, France).

Equipment for CPE

For the temperature equilibration (20 min at 55 °C) during the CPE, a Konidaris S.A. S/N 70 (Athens, Greece) water bath was used. The phase separation was carried out with a HERMLE Labortechnick Z 200A (Weningen, Germany) centrifuge.

Individual and Total Polyphenols Recovery from Aqueous Solution

Phenols were photometrically determined with a HITACHI U-2000 (Hitachi Ltd., Tokyo, Japan) spectrophotometer by the Folin–Ciocalteu procedure according to Vazquez Roncero et al. [20]. Extraction with ethyl acetate (3 × 12 ml) and *n*-propanol (2 × 6 ml) and directly (without extraction) in the aqueous standard polyphenolic samples and polyphenolic mixtures by means of the Folin–Ciocalteu reagent at 725 nm. The standard phenol solutions (50–300 ppm) used for the CPE experiments were dissolved in a mixture of 5% methanol and 95%water and extracted with one step CPE using Genapol X-080 at a concentration of 0.2, 0.5, 1.5, 2.5 and 5.0 (% v/v).

Determination of Water, Fat and Total Phenols Content of OMW

The OMW was removed from the fridge and left to stand for 2 h in order to reach ambient temperature. Then, OMW was centrifuged for 10 min at 4,000 rpm (4,110 *g*) using a Sorvall General-purpose RC-3 Automatic Refrigerated Centrifuge (Ivan Sorvall Inc., Newtown, CT, USA) to remove the solids before the CPE experiments. The water content of the OMW sample was determined by drying at 103 °C for 24 h and then calculating the water loss by differences in weight of the wet and dry samples. For the determination of the fat content, 10 g of wastes was extracted by *n*-hexane (3 × 10 ml) in a separating funnel. Then, the hexane layers were combined and the solvent was evaporated at 80 °C. The remaining oil was weighed and its percentage in OMW was calculated.

Determination of the Tocopherol Composition and Recovery

The method used was adapted from Tsaknis et al. [21] using a Waters 600E HPLC pump (Millipore Corp., Waters Chromatography Division, Milford, USA) fitted with a Waters μ-Porasil, 125 Å, 10 μm, 3.9 × 300 mm column and a Waters 486 UV-Vis tunable absorbance detector set at 295 nm. The recovery of tocopherols using CPE was evaluated. Individual tocopherols, alpha and delta at concentrations of 150 and 60 ppm, respectively, and their mixtures, were subjected to a one or two step CPE with 2, 5 and 20% Genapol X-080.

CPE Procedure

The method is schematically represented in Fig. 1. Phenolic solutions and OMW samples were adjusted to pH value of 2.5–3.5 with 2N sulfuric acid prior to CPE. NaCl (5%) was also added to the sample in order to facilitate the phase separation process by increasing the density of the bulk aqueous phase [6, 11]. Additionally, NaCl reduces the cloud point temperature [5]. The procedure was carried out according to Katsoyannos et al. [7]. A mixture of the sample (30 ml), salts and surfactant (Genapol X-080) in concentrations of 2, 5 or 20% by volume was vigorously agitated for 1 min followed by equilibration at 55°C for about 20 min. Changes in surfactant concentration also changes the time required for CPE. The time ranged from 15 to 25 min until CPE takes place depending on surfactant concentration (increase of surfactant also increases time needed for CPE). The mixture was centrifuged (5 min at 3,500 rpm, 3,595*g*) and the phases were separated by decanting (first extraction step). The surfactant-rich phase was highly viscous. The volumes of the water and surfactant phases were recorded after centrifugation, and used for the calculation of the polyphenols or tocopherols recovery. After decanting, the non-extracted phenols contained in the water-phase were extracted using the same procedure once (second extraction step) or twice (third extraction step). Every CPE experiment was repeated three times under the same conditions thus all of the recovery values represent mean values of three extraction experiments.

The minimum critical concentration required for Genapol-X080 to form micelles (CMC) was 0.05–0.35 mM, and the cloud point temperature 25–42°C [9]. The phenol recovery by the surfactant from the sample was calculated as [4]:

$$\text{Recovery } (\%) = \frac{CsVs}{CoVo} \times 100 = \frac{CoVo - CwVw}{CoVo} \times 100$$

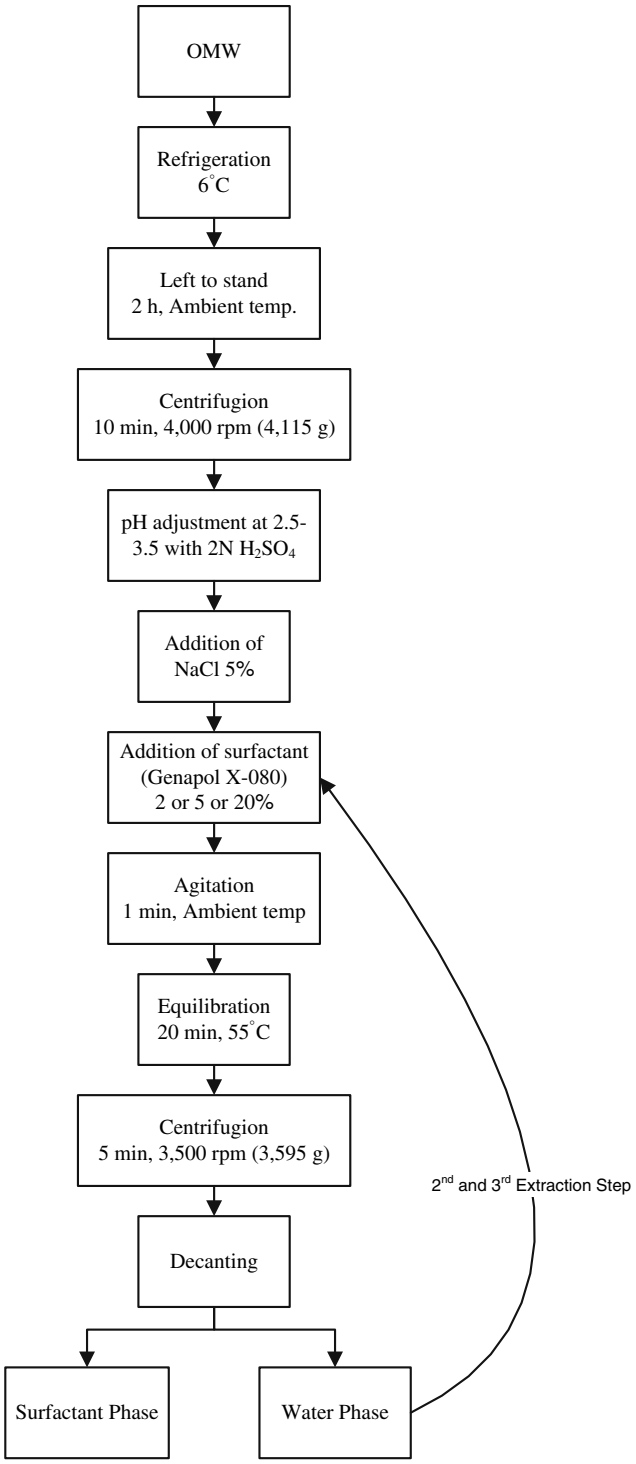

Fig. 1 Schematic representation of the CPE procedure

where, *C*o represents the phenol concentration in the initial sample of volume *V*o, *C*w represents the phenol concentration in the water-phase of volume *V*w and *C*s represents the phenol concentration in the surfactant-phase of volume *V*s.

Statistical analysis

Results are displayed as means of three determinations and standard deviation (SD) (in parenthesis), of three simultaneous assays in all methods. Statistical significance (at $P < 0.05$) of the differences between mean values was assessed by ANOVA test using SPSS (SPSS Inc., Chicago, USA) software.

Results and Discussion

The possibility of applying CPE with Genapol X-080 for separation of polyphenolic compounds and tocopherols was determined. The ability of the surfactant to recover individual and mixtures of polyphenols and tocopherols in various concentrations was tested before its application for the recovery of phenols from OMW.

The volumes of the surfactant-rich phase (*V*s) and the aqueous phase (*V*w) were determined after centrifugation, since they were necessary for the phenol mass balance and thus for the calculation of the phenols recovery (% phenols recovered from the sample by the surfactant) [7]. The results showed that these volumes were dependent on the surfactant concentration at constant temperature (Fig. 2).

The concentration factor $Fc = Cs/Co$ and the *V*s/*V*w ratio are important factors for the yield of the separation. For maximum extraction yield, the antioxidant recovery and pre-concentration should reach maximum values. Thus, *F*c should achieve maximum values, while the *V*s/*V*w-ratio minimal values, which means *V*s should be minimum and *C*s maximum. As surfactant concentration increases, recovery values increase but the *V*s/*V*w decreases rapidly (from 3.3 to 0.07) and consequently an undesirable decrease of the concentration factor occurs. According to Quina and Hinze [9], the phase volume ratio *V*s/*V*w following the phase separation step can be very low, on the order of 0.007– 0.04. Consequently, any desired analyte that is bound to the micellar aggregate can be separated and extracted into the small volume element of the surfactant-rich phase in a manner akin to a conventional liquid–liquid extraction step. However, since the volume of surfactant-rich phase must be manageable, a compromise must be reached so that the surfactant concentration will allow a high phase ratio and a manageable surfactant-rich phase [5].

The *V*s/*V*w ratio increases with increased temperature. Paleologos et al. [8] reported that temperature seems to play a role in enhancing preconcentration efficiency and enhancement factors. They also reported that applying elevated temperatures leads to dehydration of the micelle, increasing the phase–volume ratio and thus the recovery enhancement by a factor as great as 3. Therefore, elevated

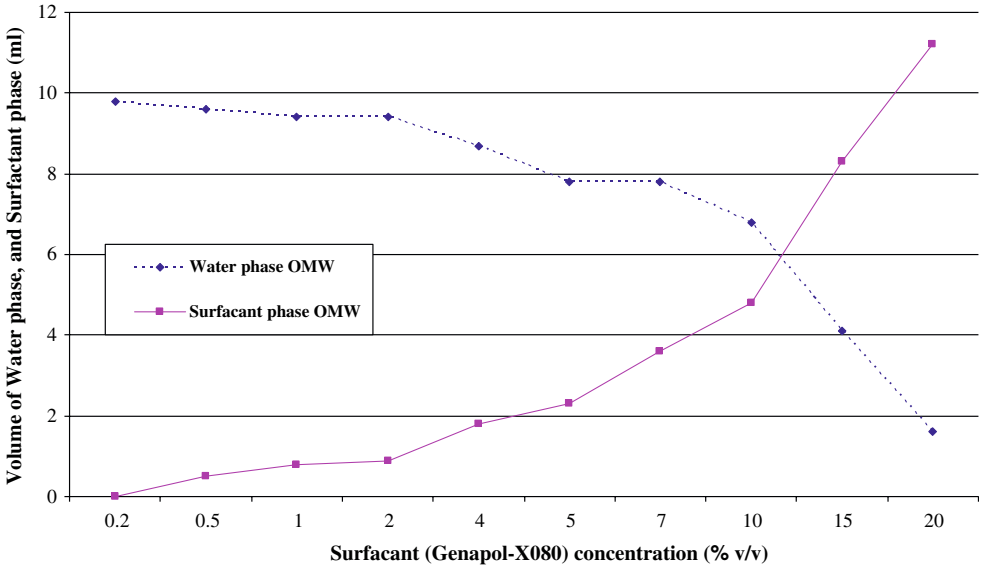

Fig. 2 The relationship between the measured volume of water and surfactant phases after centrifugal separation and surfactant concentration (% v/v-Genapol X-080)

temperatures during the CPE procedure are expected to be the most important condition within the limits of 60 °C for phenols. Thus, CPE temperature should be considered as a relevant criterion of high priority during optimization and scale-up.

One Step CPE of Individual and Mixed Polyphenol Standards with Genapol X-080

The results of the single step CPE of individual polyphenol standards (aqueous solutions with 5% methanol) at two or three different concentrations with Genapol-X080 in the surfactant concentration range from 0.2 to 5% (v/v) are presented in Table 1. It is obvious that many of the examined individual polyphenols can be recovered at high percentage. Especially, in the case of Luteonin, low surfactant concentrations are sufficient for quantitative separation. The recovery of the various polyphenolic compounds appeared proportional to the surfactant concentration. However, the percentage of recovery of each individual polyphenol was dependent on its physicochemical characteristics.

In the case of higher phenol concentration, higher surfactant concentration was necessary for a total phenol removal (Table 1). In previous work [7], at higher surfactant (Triton X-114) concentrations, the phenol recoveries tended to a plateau, implying the need for an additional CPE step in order for a quantitative recovery to be achieved. In present work, as indicated in Table 1, this plateau was only observed for a few phenols (gallic acid 100 and 300 ppm, *p*-coumaric acid 300 ppm, Luteonin 50 and 100 ppm). As indicated by Mahugo Santana et al. [6], the recoveries increase as the concentration of surfactant increases, although the changes are not the same for all compounds.

The recovery of phenols by one step CPE from polyphenol mixture (each at a concentration of 50 ppm) with 2, 5 and 20% Genapol X-080 is presented in Table 2. The results showed that the recovery was proportional to the percentage of surfactant used. As expected, higher percentage of Genapol X-080 produced significantly ($P < 0.05$) higher recoveries of individual phenols (Table 2).

One and Two Step CPE of Individual Tocopherols and Tocopherol Mixture with Genapol X-080

The results (Table 3) showed that the recovery of tocopherols was quantitative even when only 5% of the surfactant was used. So, there was no need to apply higher surfactant concentrations since the recovery was not significantly ($P > 0.05$) higher between 5 and 20% Genapol X-080. Furthermore, higher surfactant concentrations are undesirable, as they lead to lower *Fc*- and higher *Vs*/*Vw* values. When two-step CPE was used, complete (up to 100%) recovery of tocopherols was possible.

Simple and Successive CPE of OMW with Genapol X-080

Before the use of OMW samples for CPE experiments, water, fat and total phenols content was determined. OMW samples contained (%) 87.3 ± 0.4 of water, 1.7 ± 0.2 of fat, 10.8 ± 0.2 of total solids and 0.36 ± 0.1 of total

Table 1 Individual phenol recovery (%) by one step CPE with 0.2–5% (v/v) Genapol X-080 at different phenol concentrations

Individual compound	Concentration (ppm)	% (v/v) surfactant					P^*
		0.2	0.5	1.5	2.5	5.0	
Gallic acid	50	39.9 (1.2)[a]	55.9 (1.0)	69.4 (1.6)	73.8 (1.8)	83.8 (1.9)	*
	100	83.0 (2.1)	85.5 (1.1)	87.6 (1.5)	90.2 (1.0)	92.1 (1.9)	**
	300	17.3 (0.9)	21.8 (1.0)	24.7 (1.1)	43.6 (1.4)	56.4 (1.5)	**
Protocatechuic acid	50	17.6 (1.3)	25.7 (0.6)	41.8 (0.9)	52.4 (1.1)	58.1 (1.0)	*
	100	42.2 (1.5)	47.6 (1.2)	56.7 (1.6)	60.2 (1.3)	69.8 (1.0)	*
	200	29.2 (1.8)	41.1 (1.6)	53.0 (0.6)	68.3 (1.7)	77.8 (1.4)	*
Oleuropein	50	43.1 (0.5)	72.2 (1.9)	83.7 (2.2)	90.1 (2.6)	97.7 (2.0)	*
	300	20.7 (0.2)	46.3 (0.8)	59.2 (0.7)	68.3 (1.1)	77.3 (1.1)	*
o-Coumaric acid	50	29.1 (0.2)	43.8 (0.8)	58.4 (0.9)	78.6 (1.6)	93.9 (1.6)	*
	100	35.5 (1.4)	47.6 (1.7)	56.3 (1.4)	72.4 (1.5)	84.2 (1.2)	*
	300	51.6 (1.8)	61.6 (1.5)	68.5 (1.6)	72.2 (1.0)	77.9 (1.1)	*
p-Coumaric acid	100	50.7 (1.3)	59.4 (1.1)	82.2 (2.1)	87.2 (2.3)	91.9 (1.9)	*
	300	47.7 (1.7)	64.1 (1.8)	81.7 (1.9)	83.6 (1.6)	85.9 (1.5)	* Except for 1.5–5.0
Rutin	50	41.4 (1.2)	60.1 (1.3)	83.5 (1.0)	93.0 (1.9)	97.4 (1.9)	*
	100	46.6 (2.0)	66.6 (2.1)	74.2 (1.6)	82.3 (1.5)	86.6 (1.9)	*
	300	35.7 (0.6)	53.3 (0.8)	76.2 (1.3)	81.8 (1.5)	87.1 (2.0)	*
Epicatechin	50	18.2 (0.1)	44.6 (0.7)	46.6 (1.0)	62.8 (1.7)	77.8 (1.4)	*
	100	30.6 (1.0)	53.0 (1.2)	76.0 (1.3)	85.2 (1.8)	98.5 (1.4)	*
	300	34.4 (1.0)	37.5 (1.1)	65.2 (2.0)	71.2 (1.2)	75.1 (1.0)	*
Luteonin	50	93.4 (2.1)	96.7 (2.3)	97.5 (2.0)	97.1 (2.4)	–	**
	100	64.9 (1.6)	67.4 (1.5)	68.4 (1.8)	83.8 (1.9)	–	* Except for 2.5
Syringic acid	50	21.9 (0.5)	42.3 (0.4)	62.8 (1.1)	82.7 (1.5)	90.0 (1.4)	*
	100	17.3 (1.0)	30.1 (1.6)	52.1 (1.4)	64.5 (1.8)	86.3 (1.7)	*
	300	34.9 (1.2)	39.0 (1.8)	55.1 (2.0)	66.0 (2.2)	89.6 (2.3)	*
Tyrosol	50	20.3 (1.0)	26.6 (1.1)	31.2 (1.8)	36.7 (1.9)	63.3 (2.0)	*
	100	29.0 (1.0)	34.5 (1.4)	43.9 (1.5)	58.5 (1.9)	69.8 (1.7)	*

* P Indicates significant difference ($P < 0.05$) of recoveries among different surfactant concentrations

** Indicates no significant difference ($P < 0.05$)

[a] Values are means of three determinations. Standard deviation is given in parenthesis

Table 2 Phenol recovery (%), from a mixture of ten phenols (50 ppm each), by one step CPE with 2–20% (v/v) Genapol X-080 at the same temperature (55 °C)

% (v/v) Surfactant	Phenol recovery (%)
2	65.1 (1.0)[a]
5	77.3 (1.1)
20	94.9 (1.8)

[a] Values are means of three determinations. Standard deviation is given in parenthesis

phenols (expressed as gallic acid). The pH value was 5.5 ± 0.1.

The results of the total phenol recovery with simple and successive CPE of OMW with various concentrations (2, 5 and 20%, v/v) of Genapol X-080 are presented in Table 4. The multiple steps (1–3) increased the recovery by about 42, 28 and 18% in the case of 2, 5 and 20% (v/v) of surfactant, respectively. The multiple steps (even when the lower concentration of surfactant was used) significantly ($P < 0.05$) increased the recovery of phenols from OMW.

As indicated by the results, CPE with Genapol X-080 can successfully be applied in the case of aqueous solutions of polyphenolic compounds and a quantitative extraction of polyphenols can be achieved. In most cases, individual phenol recovery values (from the water phase) higher than 40% can be achieved with one CPE step with a low surfactant concentration (1.5%). Phenol losses during the CPE procedure are avoided, since the equilibration temperature is lower than 60 °C. The CPE procedure appears to be a useful tool for pre-concentration of phenolic compounds prior to phenol determination by means of photometrical identification (with the Folin-Ciocalteu Reagent). Additionally, CPE could be used to remove phenolics prior to utilization of OMW (e.g. fermentation by microorganisms

Table 3 Tocopherol recovery (%), from an α- and δ-tocopherol mixture (150 and 60 ppm, respectively), by one or two step CPE with 2–20% (v/v) Genapol X-080

One step	% (v/v) Surfactant			P^*
	2	5	20	
α-Tocopherol	69.6 (1.3)[a]	96.7 (2.1)	98.7 (2.3)	* Only between 2 and 5%
δ-Tocopherol	64.3 (1.1)	96.2 (1.9)	98.4 (2.4)	* Only between 2 and 5%
Total tocopherol	67.1 (0.9)	96.5 (2.0)	98.7 (2.0)	* Only between 2 and 5%
Two step	2	5	20	
α-Tocopherol	–	100.0 (2.8)	–	–
δ-Tocopherol	–	99.4 (2.0)	–	–
Total tocopherol	–	99.7 (1.9)	–	–

* Indicates significant difference ($P < 0.05$) of recoveries among different surfactant concentrations

[a] Values are means of three determinations. Standard deviation is given in parenthesis

Table 4 Phenol recovery (%) by two or three step CPE with 2–20% (v/v) Genapol X-080 from olive mill waste water

No of steps	% (v/v) Surfactant			P^*
	2	5	20	
1	63.2 (1.1)[a]	68.2 (0.5)	74.8 (2.0)	*
2	73.6 (2.1)	77.1 (0.9)	81.6 (2.3)	*
3	89.5 (1.1)	87.1 (0.6)	88.3 (0.7)	* Only between 2 and 5%

* P indicates significant difference ($P < 0.05$) of recoveries among different surfactant concentrations

[a] Values are means of three determinations. Standard deviation is given in parenthesis

that can not resist the high phenol content of OMW). The use of Genapol X-080 improved the recovery of phenols from OMW to higher values (up to 89.5%) than with the surfactants (Triton X-114) previously reported (up to 66%) [7]. An additional advantage of Genapol X-080 in relation to Triton X-114 is the minimal absorption in the range near 280 nm (the most common wavelength for general detection of phenolics by HPLC) [1]. Furthermore, the fat removal step prior to CPE is not needed in order to avoid the mixing of the surfactant with oil (a fact that causes problems with the phase separation after centrifugation). The polar-head group of Genapol X-080 is similar to that of Triton X-114 but the hydrophobic chains are different. Genapol X-080 has an aliphatic carbon chain with a branched methyl group (isotridecyl group), while Triton X-114 contains an octylphenol group [22, 23]. This isotridecyl group of Genapol X-080 renders the surfactant less oil soluble and therefore the adequate phase separation after centrifugation is much easier.

The CPE procedure offers an interesting alternative to the liquid–liquid or liquid solid solvent extraction of phenols due to its simplicity, fewer time-, labor- and equipment-requirements and the use of non-toxic extractants [7]. CPE, as a clean technology, could be a simple and economical solution for the preparation of natural phenolic antioxidants, acceptable for dietary applications.

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