

# APPLICATION OF CLOUD POINT EXTRACTION USING SURFACTANTS IN THE ISOLATION OF PHYSICAL ANTIOXIDANTS (PHENOLS) FROM OLIVE MILL WASTEWATER

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## SUMMARY

The possibility of the application of Cloud Point Extraction (CPE) procedure on the isolation of natural antioxidants (phenols) from olive mill wastewater (OMW) was determined. The efficiency of the technique was firstly tested on individual phenols and phenol mixtures. The use of a surfactant (Triton X-114) was proven to be, in most of the cases, very efficient for the CPE of individual phenols from their aqueous solutions. The CPE with Triton X-114 achieved a quantitative extraction of individual phenols with recovery values higher than 96%, with one or more successive CPE steps and with a total of 4-6% of surfactant. This procedure was used on OMW (after the removal of fatty substances), and achieved recoveries higher than 60% when 6% of surfactant was used. Since Triton X-114 is a relatively non-toxic reagent and the CPE technique is a simple, fast, low-cost, sensitive and selective procedure, the extracted organic substances from OMW can be applied as natural antioxidants in food technology.

## KEYWORDS:

olive mill wastewater, surfactants, phenol, extraction, cloud point.

## INTRODUCTION

Natural phenolic antioxidants attract a great commercial interest in food technology. Their extraction from plant sources and agricultural wastes (i.e. olive mill wastewater, OMW) with the classic extraction techniques requires extreme high volumes (9-15-fold of the liquid sample) of toxic organic solvents, such as methanol, ethyl acetate and n-propanol [1, 2]. On the contrary, Cloud Point Extraction (CPE) methodology is a clean technology, since it only requires 4-12% surfactant volumes of the liquid sample.

CPE with surfactants as solvents has already found applications in isolation of organic pollutants, such as chlorophenols, heavy metals and PCBs [3]. The use of non-toxic surfactants for removal of organic substances from wastes in temperatures over the cloud point is a known promising alternative, not yet applied for isolation of phenols to be used as physical antioxidants in food technology.

In order to establish simple, fast, low-cost, sensitive, and selectively analytical and preparative methods for extraction, determination and production of physical antioxidants, such as phenolics and tocopherols, suitable for use in dietary applications, the micellar systems (surfactant solutions) can offer a real alternative to organic solvents, for use as non-toxic extractants from liquid and solid samples [4]. Non-ionic surfactants cause no toxicological or dermatological problems, are non-volatile and classified as either relatively non-toxic or harmless reagents [5]. In particular, those without branched aliphatic chains or aromatic moieties are considered to be edible by the U.S FDA [6].

To our knowledge, there is not any application of the CPE using surfactants for the isolation of phenols and other physical antioxidants from plant materials or wastes of plant origin. In this work, the yields of OMW phenols, separated with the aid of a surfactant, were examined.

## MATERIALS AND METHODS

**Materials.** The OMW samples were supplied by an olive mill in Argos city (Greece) and maintained in the refrigerator (6 °C) till use. The phenol standards were purchased from Sigma-Aldrich (Hohenbrunn, Germany) (tyrosol, syringic acid, gallic acid), Fluka (Buchs, Switzerland) (protocatechuic acid, *p*-cumarinic acid, *o*-coumaric acid) and Extrasintese (Genay, France) (oleuropein, luteolin, rutin and apigenin). Triton X-114 was obtained by Sigma Aldrich Hohenbrunn Germany.

**Equipment for CPE.** For the temperature equilibration during CPE, a Konidaris s.a. S/N 70 (Athens, Greece) water-bath was used. The phase separation was carried out with a HERMLE Labortechnik Z 200A (Weningen, Germany) centrifuge.

**Individual and Total Phenols.** Phenols were determined photometrically with a HITACHI U-2000 (Hitachi Ltd., Tokyo, Japan) spectrophotometer by the Folin-Ciocalteu procedure according to Vazquez-Roncero et al. [1], after extraction with ethyl acetate (three successive extraction steps with four-fold solvent volume) and *n*-propanol (two successive extraction steps with two-fold solvent volume), and expressed in ppm (of caffeic acid in the OMW samples) and directly (without extraction) in the aqueous phenol standard samples and mixtures (by means of the Folin-Ciocalteu reagent at 725 nm). The standard phenol solutions (50-500 ppm) used for the CPE experiments were dissolved in a mixture of 5% methanol and 95% water.

**Determination of Water, Fat and Total Phenol Content of OMW.** The water content of the OMW sample was determined gravimetrically by drying at 103 °C. The fat content was determined by triple extraction with hexane and drying at 80 °C. OMW samples used for the CPE experiments contained (%) 84±0.5 of water, 2.3±0.25 of fat and 3.4±0.2 of total phenols (expressed as caffeic acid). In the case of OMW, fat was removed with hexane prior to CPE.

**CPE procedure.** The mixture of sample and surfactant (Triton X-114, concentrations 1-6% by volume) in tapered glass tubes was equilibrated at 55-60 °C for 20 min in the water-bath, and then centrifuged for 5 min at 3,500 rpm. The phases were separated by decanting (1<sup>st</sup> extraction step). The surfactant-rich phase was highly viscous. The volumes of water and surfactant phases were estimated, and recorded for the calculation of phenols' recovery. This CPE procedure was repeated under the same conditions in the water-phase containing the non extracted phenol one (2<sup>nd</sup> extraction step) or two (3<sup>rd</sup> extraction step) times. Every CPE experiment was repeated three times under the same conditions, and thus all the recovery values represent means of three extractions experiments.

**Triton X-114 properties.** The minimum concentration of surfactant required to form micelles is called "critical

micelle concentration", and, in the case of Triton X-114, it is 0.20-0.35 mM and the cloud point temperature is 23-25 °C [4]. The volumes of the surfactant-rich phase ( $V_s$ ) and the aqueous phase ( $V_w$ ) were determined after centrifugation and proved to be dependent on the surfactant concentration at constant temperature. These volumes are necessary for the phenol mass balance, and, thus, the calculation of phenols' recovery (% phenols recovered by the surfactant from the sample). This recovery from the sample is calculated as:

$$\text{Recovery}(\%) = \frac{C_s V_s}{C_o V_o} \times 100 = \frac{C_o V_o - C_w V_w}{C_o V_o} \times 100$$

where:  $C_o$  represents the phenol concentration in the sample-surfactant mixture 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  $V_s$ .

## RESULTS AND DISCUSSION

### One step CPE of individual phenol standards with Triton X-114

The results of the one-step CPE of individual phenolic standards from their 100 ppm aqueous solutions with 4% Triton X-114 are represented in Fig. 1. From the phenol recovery results, it can be concluded that most of the examined individual phenols can be recovered practically quantitative (recovery values >95%), except for gallic acid (recovery 74.2%). Especially in the case of apigenin and luteolin, low surfactant concentrations are sufficient for quantitative removal.

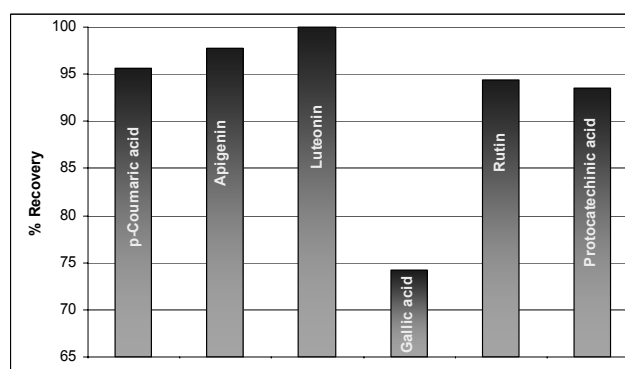


FIGURE 1 - Recoveries (%) by one-step CPE of individual phenolic standards (100 ppm) in aqueous solutions (4% Triton X-114).

The results of the one-step CPE of individual phenol standards in aqueous solutions at three different concentrations with Triton X-114 (1-6%, v/v) addition, are presented in Fig. 2 (results are shown only for protocatechuic acid). Different phenols show individual quantitative, but similar qualitative behavior, in relation to the surfactant

concentration. At higher surfactant levels, all of the phenols tend to a plateau, implying to the necessity of an additional CPE step, in order to achieve quantitative recovery.

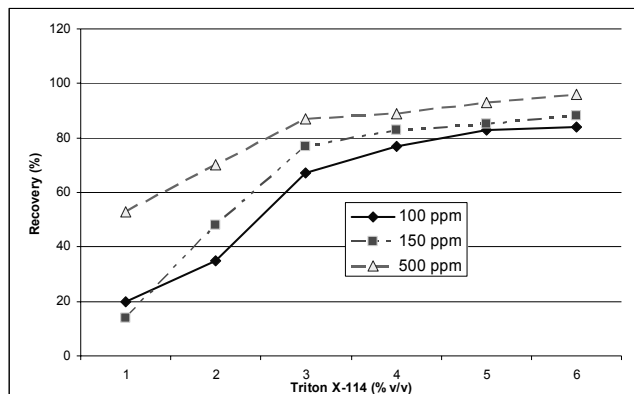


FIGURE 2 - Protocatechuic acid recovery dependence from surfactant concentration at three different phenol concentrations in one-step CPE.

#### One and three step CPE of seven phenols mixture with Triton X-114

The recovery of total phenols by one-step CPE from the phenolic mixture (3 different concentrations 50, 100 and 200 ppm) with 1, 2, 4 and 6% Triton X-114 is represented in Fig. 3. The results showed that the recovery is proportional to surfactant percentage used. As expected, higher percentages of Triton X-114 produced higher recoveries of individual phenols. However, again the recovery remained lower than 60%, implying again the necessity for additional CPE steps.

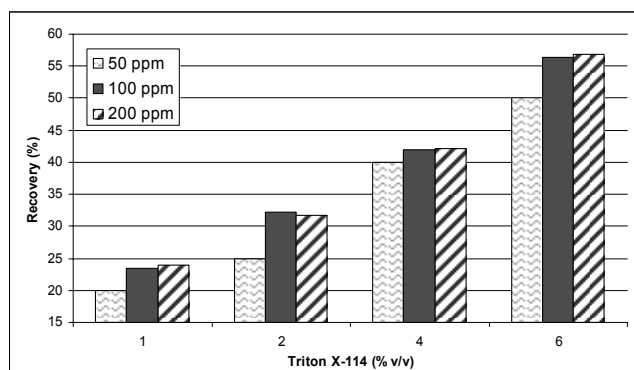


FIGURE 3 - Recovery of total phenols by one-step CPE from phenolics' mixture (3 concentrations 50, 100 and 200 ppm) with 1, 2, 4 and 6% Triton X-114.

In order to test the recovery of phenols using multiple CPE steps, a mixture of seven phenols (*p*-coumaric, gallic, syringic and protocatechuic acid, rutin, luteolin and apigenin; 150 ppm each) was exhibited to 3-step CPE with 2% Triton X-114. The recovery of the phenols from the water phase was estimated and the results are presented in Fig. 4. It is obvious that the total phenols recovery reaches >90%, when a three-step CPE is applied with 2% v/v sur-

factant in each step (in total a surfactant volume of 6% of that of the sample volume is necessary to separate more than 90% of phenols from the aqueous phase). Multiple extraction steps should be applied to reach >90% levels of recovery.

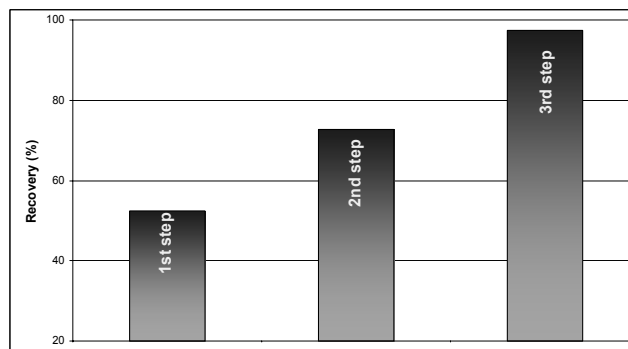


FIGURE 4 - Recovery of seven phenols' mixture in three-step CPE with Triton X-114 (2% each step).

#### Simple and successive CPE of olive mill wastewater (OMW) with Triton X-114.

The results of the total phenol recovery rates with simple and successive CPE of olive mill wastewater (OMW) with surfactant addition (2, 4 and 6% v/v of Triton X-114) are presented in Fig. 5. The multiple steps (1-3) increased the recovery by 117, 76 and 69% in the cases of 2, 4 and 6% (v/v) of Triton X-114, respectively. It seems that multiple steps (even when the lower concentration of surfactant was used) significantly ( $P < 0.05$ ) increased the recovery of phenols from OMW.

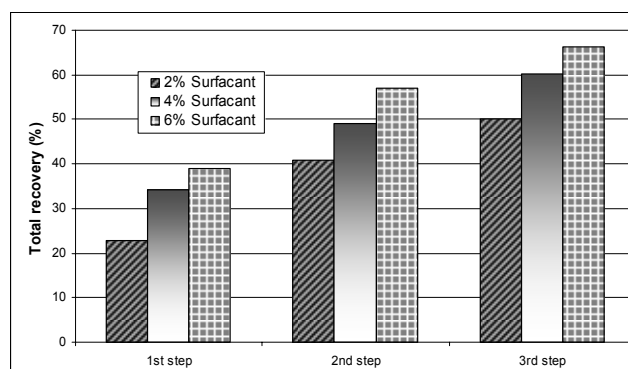


FIGURE 5 - Total phenol recovery with simple and successive CPE of olive mill wastewater (OMW) with various concentrations (2, 4 and 6%, v/v) of Triton X-114.

## CONCLUSION

The cloud point extraction (CPE) with Triton X-114 can successfully be applied in the case of aqueous phenolic solutions and, in the case of absence of fatty substances, phenols could be quantitatively extracted. Individual phenol recovery rates (from the water phase) higher than 96%

can be achieved with one or more successive CPE steps, with a total of 4-6% Triton X-114.

Phenol losses during CPE procedure can be avoided, since the equilibration temperature is lower than 60 °C. In the case of higher phenol concentrations, more surfactant (4-6%) is necessary for total removal. The CPE procedure is a useful tool for pre-concentrations of phenolics, prior to phenol determination by means of photometric identification (with the Folin-Ciocalteu reagent).

The CPE procedure offers an interesting alternative to the liquid-liquid or liquid-solid solvent extraction of phenols, due to its simplicity, low time-, labor- and equipment requirements, and the use of non-toxic extractants. Optimization of CPE conditions (equilibration time, salt addition, pH value) could lead to an even simpler procedure of phenol isolation, making eventually the second extraction step unnecessary. In the case of preparation of physical phenolic antioxidants from wastes of plant origin, the clean CPE technology is a simple economical method, which could be acceptable for dietary applications. After optimization of extraction conditions, the phenols recovery could be increased to values higher than those achieved in this study (about 60%). In relation to classical solvent extraction, CPE requires extractant volume of only 8-12% of sample/waste volume. In the case of OMW, a fat removal step is necessary, prior to CPE, for a quantitative removal, in order to avoid the mixing of Triton X-114 with oil (causes phase separation problems after centrifugation). Important is also the examination of the possibilities to separate the phenols from the surfactant, and to recover the surfactant e.g. by means of pH change or temperature decrease. The use of other surfactants (i.e. Genapol X-080) could lead to higher phenol recovery rates and should be exploited.

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