AQUEOUS MICELLAR TWO-PHASE EXTRACTION OF PHENOLIC COMPOUNDS FROM PRETREATED OLIVE MILL WASTEWATERS

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Abstract :

The olive oil sector is a very important economic activity in the all the Mediterranean countries. This agrochemical industry produces great amounts of organic residual materials, both liquid and solid, depending on the system used to extract the olive oil. Olive mill wastewater (OMW) is the main liquid effluent generated by this industry and constitutes a serious environmental pollution problem. The phenolic content of these effluents is responsible for its phytotoxicity and antimicrobial effects. However, polyphenols are also known as powerful antioxidants with interesting nutritional and pharmaceutical properties. The recovery of these commercially valuable compounds will reduce the environmental impacts and will generate economic benefits. Nonionic surfactants micellar system was applied to concentrate the natural phenolic compounds from olive mill wastewaters. In this study parameters affecting the cloud point extraction (CPE) including surfactants concentrations, pH and equilibration temperature were investigated. Up to 30 % of the phenolic content was extracted from OMW in a one step CPE. The highest yield was obtained when using 7 % of Genapol X-080 at pH 2 and 60°C. The obtained results showed that aqueous two phases extraction which is based on micellar solubilisation is a simple, low cost, and an efficient method for the recovery of phenolic compounds from OMW. It must be regarded as an interesting option for the natural biophenols recovery from the OMW.

Keywords: olive mill wastewater; aqueous two phases; phenolic compounds; non ionic surfactants.

1. INTRODUCTION

Olive oil production is a significant agricultural activity with a great economic importance particularly in the entire Mediterranean basin. The annual worldwide olive oil production has exceeded 3 million tons for 2010-2011 season (IOOC, 2012). However, this agroindustrial activity generates significant volumes of very polluting wastes (50% wastewater, 30% insoluble solids) (Komnitsas and Zaharaki, 2012). Olive mill wastewaters (OMW) are considered a major environmental issue in the main olive-producing countries of the Mediterranean region. The dark color, the bad smelling, the acute toxicity are the main features of the OMW pollution. The toxicity, which is mainly due to the high concentrations of the natural phenolic compounds, explains the biodegradation resistance of these effluents (Ergül et *al*., 2011). Nevertheless, the presence of high concentrations of polyphenols can identifies OMW as a cheap source of natural antioxidants of great interest for pharmaceutical, cosmetics and food applications. Excellent biological properties in terms of antioxidant, free radical scavenging and antimicrobial activities of the biophenols of OMWs have been reported by many studies (Allouche et *al*., 2004; Obied et *al*., 2005; El Abbassi et *al*., 2012). Until now, several methods have been developed aiming the recovery of phenolic compounds from OMW, including solvent extraction (Lesage-Meessen et *al*., 2001), adsorption onto resins (Bertin et *al*., 2011), supercritical fluid extraction (Lafka et *al*., 2011), selective concentration by ultrafiltration (Galanakis et *al*., 2010) and integrated membrane systems (El-Abbassi et *al*., 2009; Garcia-Castello et *al*., 2010). Besides, some of these technologies show significant and inherent disadvantages. High costs of adsorbents or membranes and the use of expensive, toxic and inflammable organic solvent also limited the applications of some of these recuperative processes.

Alternatively, the micellar aqueous two phase extraction method also called cloud point extraction method has become increasingly popular. This extraction method is based on the property of most nonionic surfactants to form micelles in aqueous solutions and to separate into two phases: a surfactant-rich phase with a small volume and a dilute aqueous phase, when heated above a particular temperature, known as the cloud point temperature. When solutes are present in the solution, they partition between the two phases. Interactions occur due to adsorption of the solute on the surface of the micelles or some others sites within micelles. Cloud point extraction (CPE) methodology has been firstly used for preconcentration of trace-metal ions in the form of their hydrophobic complexes (Watanabe, 1982). However, most of these aqueous surfactant two-phase systems have been proposed as concentrations methods before chemical analysis in order to replace toxic solvents (Ballesteros-Gómez et al., 2010). Recently, cloud point extraction, as observed by several authors, is an effective technique for removing dissolved organic contaminants from wastewater and groundwater (Arunagiri et al., 2014, Haddou et al., 2014 ; Pan et *al*., 2010 ; An et *al*., 2010 ; Taechangan et al., 2009, Wang et al., 2009). the cloud point extraction technique has been successfully employed for the preconcentration and recovery of different organic compounds such as phenols, polycyclic aromatic hydrocarbons and polychlorinated compound.

The CPE provides simple, cheap, highly efficient and speedy design of extraction procedures. This technique becomes an alternative to conventional solvent extraction because of high capacity to concentrate a wide variety of analytes with high recoveries and concentration factors.

The aim of this work is the development of a cloud point extraction procedure for the recovery of phenolic compounds from pretreated OMWs. Three non-ionic surfactants Genapol X-080 Tween 80 and Triton X100 were tested and the parameters affecting the cloud point extraction including surfactants concentrations, pH and equilibration temperature were investigated.

2. MATERIAL AND METHODS

2.1 Reagents

Polyethylene glycol monoalkyl ether (GenapolR X-080), Sodium dodecyl sulfate (SDS), Folin Ciocalteu reagent and anhydrous sodium carbonate were purchased from Sigma-Aldrich (Germany). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Merck (France). All chemicals used were of analytical-reagent grade and were prepared using distilled water.

2.2 Sample preparation

OMW samples were obtained from a 3-phase extraction unit from Marrakech (Morocco) during the 2012-2013 season. Ultrafiltration (UF) was carried out at room temperature and under constant trans-membrane pressure (5 bars) in a pilote plant equipped with a ceramic membrane with a molecular weight cut-off of 150 kDa. The permeate stream was collected and stored at 25 ± 2°C until treatment.

Conductivity and pH of OMWs samples were determined by digital calibrated pH and conductivity meter (MultiLab P5 - WTW, Germany). Total solids were determined by weight difference before and after drying samples over night at 105 °C. The chemical oxygen demand (COD) was determined by the dichromate method as described by LaPara et *al.*, 2000. The total phenolic content (TPC) was determined colorimetrically using Folin Ciocalteu method (Catalano et *al.*, 1999). The main physicochemical characteristics of the samples are summarized in Table1.

TABLE 1: **Main physicochemical characteristics of raw and ultrafiltred OMW**

Parameter	**Unit**	**Raw OMW**	**Ultrafiltred OMW**
pH	-	4.50±0.06	4.45±0.14
Electrical Conductivity	mS/cm	7.02±0.54	7.15±0.42
Dry Residue	g/l	119±1.14	86±0.86
Total Phenolic Content	g/l	4.12±0.47	3.85±0.035
Chemical Oxygen Demand	g of O_2/l	156±1.23	107±1.02

Values are the average of three measurements ± standard deviation.

2.3 Micellar cloud point extraction procedure

For the cloud point extraction experiments, an aliquot of 20 ml of ultrafiltred Olive Mill Wastewater (UFOMW) containing different concentrations of nonionic surfactants 1-7 % (w/v) was incubated in a thermostatic bath maintained at temperature above the cloud point. The incubation time was 30 min, until the solution completely separates into two distinct phases. The effects of equilibration temperature and pH were also evaluated. After phase separation, the surfactant-rich phase was eliminated and a sample of the aqueous phase (lower phase) was then removed and analyzed. The phenolic content was determined colorimetrically using Folin Ciocalteu method. The ratio of phases was obtained by measuring the volumes of the respective phases.

2.4. Extraction efficiency estimation

Complete separation of the two phases was obtained after centrifugation of the tubes at 1500 rpm for 5 min. After a careful separation of both phases, the volume of each phase was accurately determined and their ratio calculated.

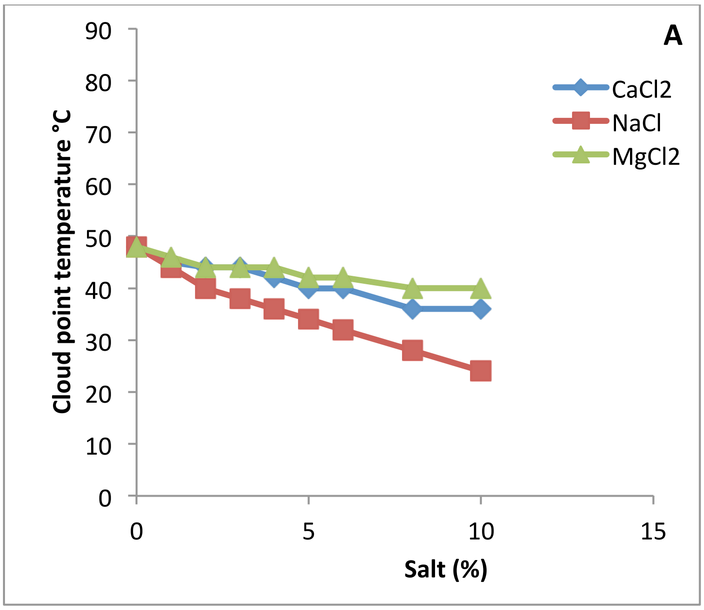
The recovery rate (E) of the phenolic compounds into the coacervate is calculated according to the following equation: $\mathbf{E=100*(1-(C_d/C))}$

Where C_d are equilibrium concentration of the phenolic compounds in diluted phase and C stands for the initial concentration of the phenolic compounds in the UFOMW.

3. RESULTS AND DISCUSSION

3.1 Effect of salts on the cloud point temperature

Micellar extractions efficiency depends on numerous factors such as type and concentration of surfactant, pH of solution, ionic strength and equilibration temperature. Additives play also a key role in tuning the cloud point values of nonionic surfactants and also in the extraction. The addition of salts may facilitate phase separation since it increases the density of the aqueous phase and decrease the cloud point of nonionic surfactants (Mukherjee et *al.*, 2011). In order to achieve an easy phase separation, and preserve the bioactive properties of the natural phenolic compounds, three different salts (NaCl, $CaCl_2$ and $MgCl_2$) were tested for their ability to lower the cloud point temperature of the selected surfactants. 3% of each surfactant was added with different salt concentrations (0 to 10%). The determined cloud point temperatures (CPT) of the Ganapol 080, Triton X-100 and Tween 80 in the UFOMW were respectively 48 °C, 65°C and 78°C. The addition of salts decreased the cloud point of the different surfactants. The CPT of the Genapol was decreased from 48 to 40 °C when 10% $MgCl_2$ or $CaCl_2$ are added to the UFOMW (Fig 1, A). CPT of the Triton X-100 and Tween 80 were also lowered by about 15 °C as a result of the addition of 10% of the $MgCl_2$ or $CaCl_2$ (Fig. 1 B&C). Excepting for the Tween 80, NaCl has lead to more important decreases. Addition of 10% NaCl leads to a CPT decrease of 20 to 30 °C.



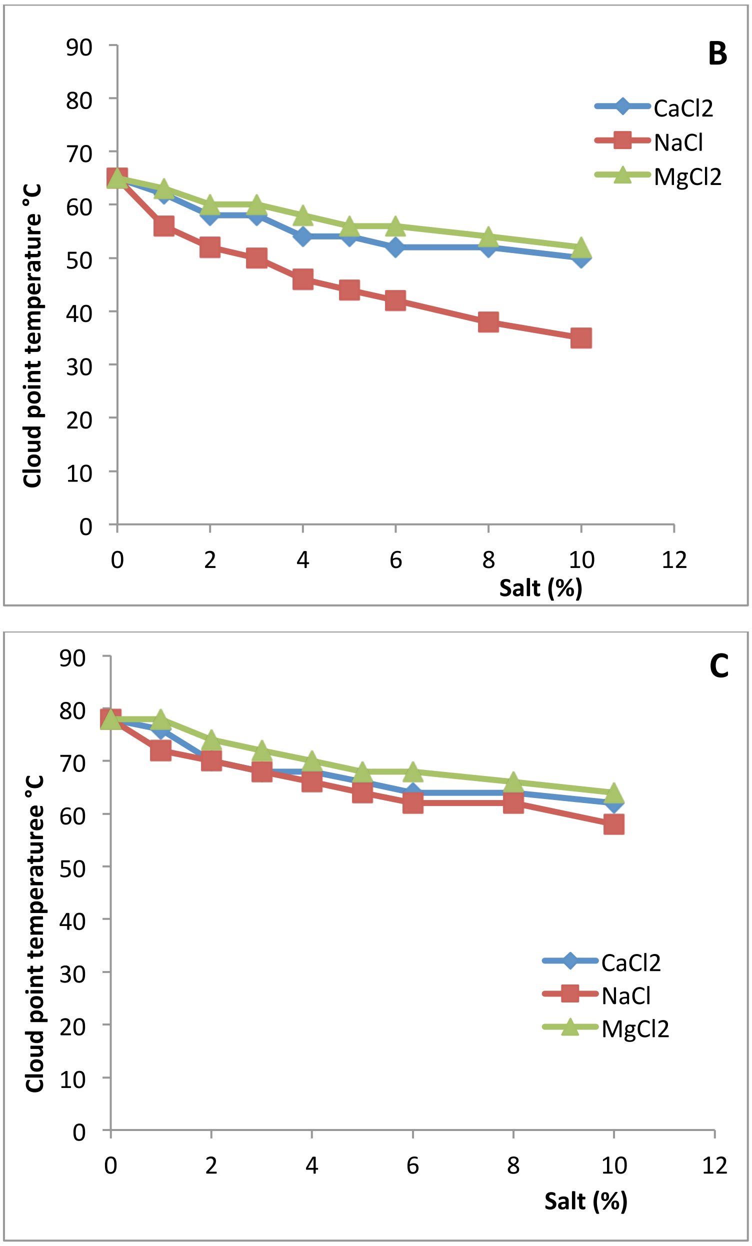
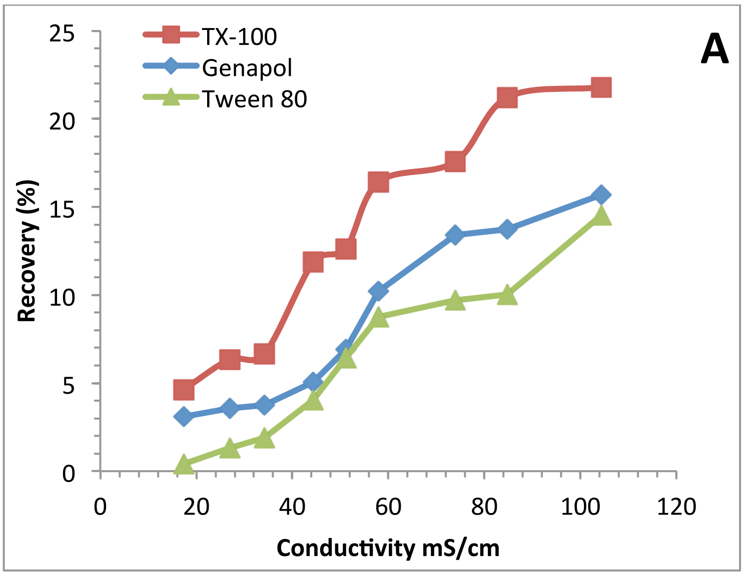


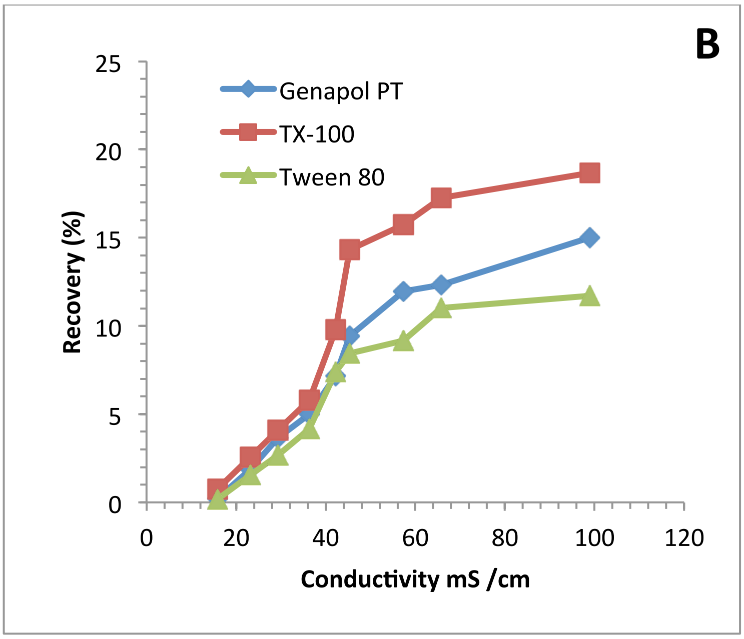
Figure 1: Effect of salts addition on the cloud point temperature of Genapol-080 (**A**), Triton X-100 (**B**) and Tween 80 (**C**) in Ultrafiltred Olive mill wastewaters. *(surfactant concentration 3% w/v ; pH 4,5 ; equilibration duration 30 min)*

3.2 Effect of salts addition on removal of the phenolic compounds

Results depicted in Fig. 2 show that the best extraction efficiencies increased with the increase of the salts concentrations (monitored here with conductivity measuremants). Triton X-100 showed the best removal rates, the highest recovery of the phenolic compounds (22%) was obtained when combining 3 wt % TX100 and $CaCl_2$ to reach 100 mS/cm which, correspond to the addition of approximately 10%. The Tween 80 was found to be the less efficient for the extraction of the phenolic compounds from the UFOMW. At the best Tween has allowed the elimination of 14% of the total phenolic compounds.

Almost similar extraction efficiencies were obtained with the different tested salts for each surfactant. To avoid a secondary pollution, which may be caused by NaCl, 10% $CaCl_2$ (w/v) was adopted for next experiments as the optimum amount to achieve the best extraction efficiency.





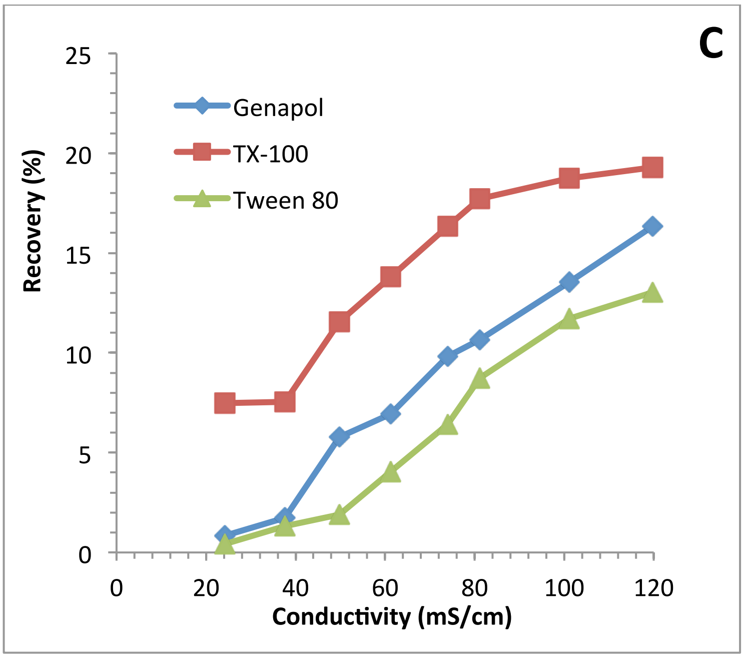


Figure 2: Effect of different Salts $CaCl_2$ (**A**), $MgCl_2$ (**B**) and NaCl (**C**) on the removal of phenolic compounds from UFOMW. *(equilibration temperatures : 40°C, 50°C and 60°C respectively for Genapol 80, TX-100 and Tween 80 ; pH 4,5 ; equilibration duration 30 min)*

3.3 Effect of the surfactant concentration

The effect of the surfactant concentration ranging from 1 - 7% (w/v) was examined and the results are depicted in Figure 3. The extraction efficiency of phenolic compounds increased with the increase of surfactant concentration. Removal of the phenolic compounds increased with the increase of the surfactant concentration. Triton X-100 lead to the best recovery rates followed by Genapol. Maximum recovery rates (38%, 32% and 20%), were obtained when using 7 % (w/v) of Triton X-100, Genapol and Tween 80 respectively.

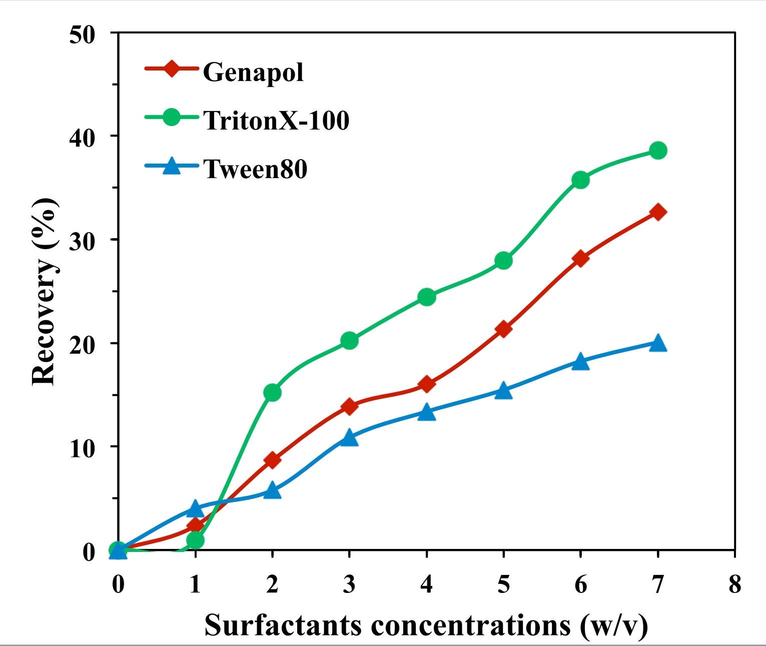


Figure 3: Effect of surfactants concentrations on phenolic compound extraction rates. equilibration temperaturs *(equilibration temperatures : 40°C, 50°C and 60°C respectively for Genapol 80, TX-100 and Tween 80 ; pH 4,5 ; equilibration duration 30 min)*

3.4 Effect of equilibration temperature

Theoretically, CPE methodology is achieved at equilibration temperatures 15 to 20 °C higher than the cloud point temperature of the surfactant (Santalad et *al.,* 2009). According to our previous determinations, the effect of the equilibration temperature was studied over the range 50-80°C for the Triton X-100, 40-70 °C for the Genapol and 60-90°C for the Tween 80, at different surfactant concentration (1-5% (w/v)). Figure 4 shows that the highest extraction efficiency is obtained at 50-60°C for Triton X-100, and for Genapol and 70-80°C for the Tween 80. Generally, the extraction rates were lowered at higher temperatures. The greatest extraction rates (30 %) was obtained at 50 and 60°C when using 5% Triton X-100 and 26% with 5% Genapol. Thus, the optimum equilibration temperatures were selected as 60°C for both Triton X-100 and Genapol and 80°C for the Tween. In aqueous solutions, nonionic surfactants are present in a hydrated state. As temperature increased, the hydrogen bonds between surfactant and water molecules are broken and dehydration occurs leading to a decrease of surfactant-rich phase volume.

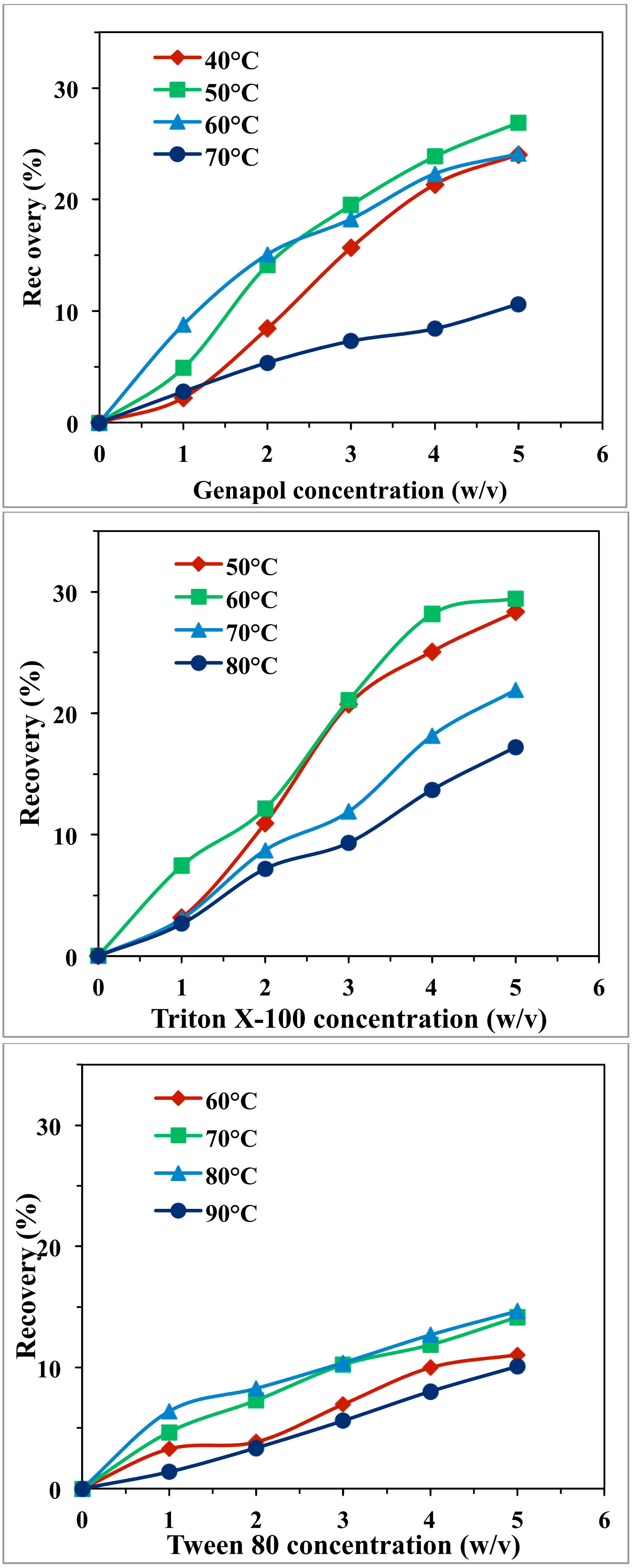
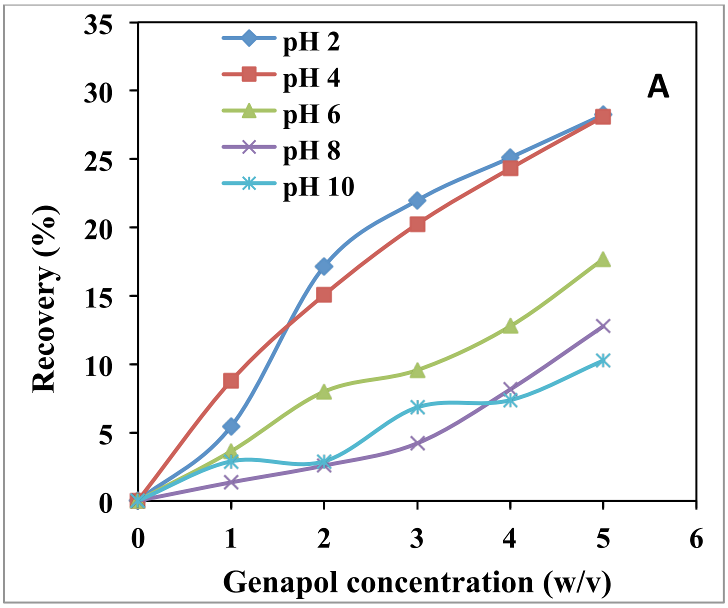


Figure 4: Effect of equilibration temperature on phenol recovery with different nonionic surfactants. (A: Genapol 080 ; B: Triton X-100; C: Tween 80). *(pH 4,5 ; equilibration duration 30 min)*

3.5 Effect of pH

The influence of pH on the partitioning of ionizable solutes is a key parameter to control the extraction efficiency. Our results revealed that the pH greatly influence the extraction of the phenolic compounds (Figure 5). The highest extraction rates were obtained at acidic conditions. pH 2 and 4 has allowed the maximum recovery for all the tested surfactants. Simple phenolic compounds are known to be weakly acidic with pKa values from 2.5 to 4.9. These phenolic acids are greatly dissociated at pH values above 5 while under pH 2.7 they remain mostly undissociated and may present less interaction with water molecules. Because of their predominant undissociated state at acidic pH the simple phenolic compounds are more prone to interact with the more hydrophobic micellar phase.

Other works had reported on the influence of pH on the extraction efficiency of aromatic compounds such as phenol or nitrophenol using micellar two-phase systems (Wei et al. 2008, Haddou et al. 2007). However, the partitioning of solutes in micellar two-phase systems has not been completely elucidated. The influence of different process parameters such as temperature or the influence of electrolytes remains controversial.



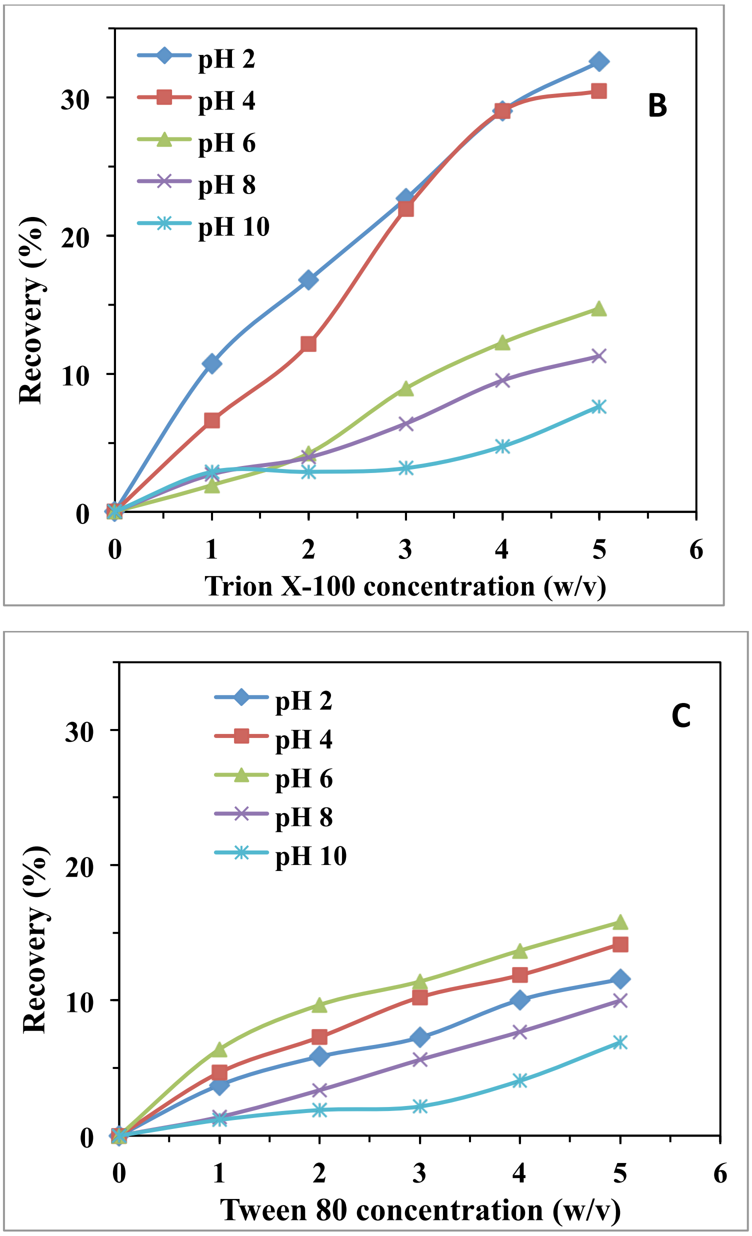


Figure 5: Effect of pH on phenol recovery with different nonionic surfactant concentration. *(equilibration temperatures : 40°C, 50°C and 60°C respectively for Genapol 80, TX-100 and Tween 80; equilibration duration 30 min)*

4. CONCLUSION

The micellar aqueous two-phase extraction has been proven to be an effective method for the extraction of the natural phenolic compounds from ultafiltred OMW using Triton X-100 and Genapol 80. Such removal can be of interest both for the depollution of these agroindustrial wastewater and for the valorization of the natural phenolic compounds as natural bioactive substances. To preserve the bioactive properties of the natural phenolics, the extraction temperature must be as low as possible. The addition of salts decreased the cloud point of the different surfactants. 10% $CaCl_2$ lowered the cloud points of the TX-100 and Tween 80 by 15°C.

The surfactant concentration, the pH, and the equilibration temperature influence the rate of extraction. Acidic pH allowed better recoveries of the phenolic compounds. Up to 30 % of the phenolic content was extracted from ultrafiltred OMW by one step CPE using Triton X-100 or Genapol.

The obtained recoveries can be improved by implying additional CPE steps. So, the CPE of natural phenolics is a promising and interesting alternative to the liquid–liquid extraction of phenolic compounds due to its simplicity, less time, equipment requirements and the use of non-toxic extractants. However, it is presently difficult to fully elucidate the mechanism of the phenolic compounds partitioning in micellar aqueous two-phase systems. Thus, more systematic work is required to understand how the different natural phenolic compounds interact with the non-ionic surfactant micelles and how the different parameters influence these interactions.

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