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Article

Recovery of polyphenols from camu-camu (*Myrciaria dubia* H.B.K. McVaugh) depulping residue by cloud point extraction☆

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ABSTRACT

In this study the potential of cloud point extraction formed by a non-ionic surfactant was used in order to separate polyphenols from industrial residues of camu-camu. The effects of operational conditions of the cloud point extraction (CPE) on the polyphenol recovery and volumetric ratio were investigated. The results showed a maximum recovery of 95.71% that was obtained using 7.0 wt% Triton X-114, native pH (3.25), and 80 wt% polyphenol extract at 30 °C. The use of cloud point extraction was successful to recover the polyphenols from agroindustrial residue since it is a simple as well as of low-cost technique.

1. Introduction

Camu-camu (*Myrciaria dubia* McVaugh) is a reddish fruit of about 2.5 cm in diameter typically found in the Amazon region [1,2]. It belongs to the family of *Myrtaceae* showing itself as a good source of ascorbic acid, phenolic components as well as carotenoids [3–7]. For this reason, it has been processed to produce juices or concentrates to formulation of drugs and food products [6,8,9]. A consequence of this processing is the generation of residues thus causing environmental problems [10–12]. In order to reduce this impact, since up to 40% of fruit mass is due to seeds and shells [9], studies have been carried out in order to add-value to such residues. For instance, recently it has been revealed that in this residue there still are bioactive components [1] that show anti-cancer activity [13].

Extraction systems have been used to recover and to purify bioactive compounds allowing the obtainment of preparations with specific properties as well as leading to improvements in the assays of polyphenols [14]. In the last decades, the aqueous micellar extraction system called cloud point extraction (CPE) has been pointed as an alternative to the traditional liquid–liquid extraction system [15]. The main advantages of the CPE are the following: (a) is simple to operate; (b) reduces extraction time; (c) is low-cost; (d) is environmentally friendly for the biomolecules; and (e) achieves higher concentration factors [16,17]. This method exploits the properties of the surfactants to form biphasic systems when heated in such way that the solute can migrate preferably to the diluted phase (poor-micelle phase) or coacervate phase (rich-micelle phase) [18,19]. Therefore CPE has been successfully used to separate and concentrate organic compounds, mainly natural phenolic components [15,20–27]. Even though there are some reports dealing with the recovery of phenolic components from residues using CPE such as olive mill wastewater (OMW) [15,20,22,24,26], rice straw hydrolysate [21], corn stover hydrolysate [28] and wine sludge [23] to the best of our knowledge there is no report of use of CPE for recovery and concentration of camu-camu (*Myrciaria dubia* McVaugh) depulping residue.

In this study we have exploited the CPE using as surfactant agent the Triton X-114 in order to extract phenolic components of the aqueous extract of camu-camu (*Myrciaria dubia* McVaugh) depulping residue. The behavior of the phases in the micellar system was evaluated by the coexistence curves in the presence of extract and salt. Also, we have investigated the effects of equilibrium temperature, pH, the percentages of surfactant, extract and salt in the yield as well as volumetric ratio of the CPE.

2. Materials and Methods

2.1. Chemical

The fresh residue of camu-camu (*Myrciaria dubia* McVaugh), basically composed of seeds and shells, was kindly supplied by Cupuaçu of Amazon

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Co. (Amazonas, Brazil). Non-ionic surfactant Tert-octylphenoxypoly (ethoxyethanol) (Triton X-114), Folin–Ciocalteu reagent and phenolic standards such as (+)-catechin, eugenol, quercetin, vanillin, ellagic acid, gallic acid, syringic acid and vanillic acid were acquired from Sigma-Aldrich (MO/USA). The chemicals sodium chloride, calcium carbonate, hydrochloric acid, and sodium hydroxide were of analytical grade and were supplied by Synth Co. (São Paulo/Brazil). Deionized water was used in the CPE experiments and was prepared using a MilliQ system.

2.2. Dried camu-camu (Myrciaria dubia McVaugh) and polyphenol extract

In order to preserve the residue properties a drying processing was carried out using a freeze dryer Liobras model L101 (Campinas, Brazil) at −40 °C and under a vacuum pressure of 13 Pa. Next, powder samples (1.0 g) were extracted by deionized water in the proportion of 1:100 (*w*/*v*) for 30 min in an ice bath and thus centrifuged at 2100*g*. All aqueous extracts were prepared immediately before the CPE experiments.

2.3. Coexistence curve diagram

The coexistence curves were determined for the CPE systems using two approaches. Firstly, the presence or absence of sodium chloride was investigated. Secondly, the percentage of extract content was also altered. Therefore, defined mixtures of Triton X-114 (0.5 wt%–10.0 wt %), extract (20 wt%–80 wt%), sodium chloride (0–4.0 wt%) and water were incubated in a thermostatic bath (Tecnal TE-184) with a temperature control of 0.1 °C. The cloud point temperature (CPT) was defined as the temperature in which the first turbidity in the solution was observed according to Albertsson [29], Blankschtein *et al.* [30] and Lopes *et al.* [31]. The experiments were carried out in duplicate and the values of CPT are the average of those obtained in the assays.

2.4. CPE procedure

Firstly, the pH of the polyphenol extract aliquots (3.25) was adjusted to the desired value by adding a solution of 1.0 $mol \cdot L^{-1}$ hydrochloric acid or 5.0 $mol \cdot L^{-1}$ sodium hydroxide. Next, the extract was transferred to conical flasks together with Triton X-114, sodium chloride and water to give a final total mass of 5.0 g. The tubes were shaken and incubated in a thermostatic bath using a temperature higher than the cloud point for 3 h. After phase separation, the phase volume was recorded, the coacervate phase was discarded and the diluted phase was collected and assayed. All CPE experiments were carried out in triplicate and Tukey's test was performed using the software Statistica 7.0 (StatSoft, Microsoft/USA) at a significance level of 5% ($p < 0.05$).

The effects of both the percentage of Triton X-114 (1.0 wt%–10.0 wt%) and incubation temperature (30–60 °C) were observed simultaneously, while a univariate approach was used for the pH (3–10), percentages of extract (10 wt%–90 wt%) and sodium chloride (0–10.0%). The optimum operational condition was chosen by the agreement of these five variables on the recovery of polyphenols.

The polyphenol recovery (Recovery) was calculated according to Eq. (1):

$$\text{Recovery} = \frac{C_C V_C}{C_O V_O} \times 100\% = \frac{C_O V_O - C_D V_D}{C_O V_O} \times 100\% \quad (1)$$

where C_O is the total phenolic concentration in the extract obtained from the camu-camu residue of volume V_O, C_C is the total phenolic concentration in the coacervate phase of volume V_C and C_D is the total phenolic concentration in the diluted phase of volume V_D.

The volumetric ratio of CPE (ϕ_C) was measured by visual observation of the ruler (scale) on the conical tube surface and defined as shown in Eq. (2):

$$\phi_C = \frac{V_C}{V_D} \quad (2)$$

2.5. Determination of total phenolic content and partial characterization

The content of the total phenolic extracts obtained from the camu-camu residue as well as from the diluted phase of the CPE system was determined using a modified method of Folin–Ciocalteu [32]. Sample aliquots of 0.5 ml were reacted with 2.5 ml of Folin reagent (1:10, pre-diluted with MilliQ water) for 2 min, then 2.0 ml of 75 $g \cdot L^{-1}$ sodium carbonate solution was added. Reactional mixture was carried out in a thermal bath at 50 °C for 5 min and absorbances were recorded using a spectrophotometer (ThermoSpectronic Genesys 10UV–Vis) at 760 nm. The high surfactant content in the coacervate phase caused a precipitation in the Folin reagent; this fact hampered the quantification of the phenolics in this phase. Additionally, the effect of the pH as well as the sodium chloride concentration was taken into consideration. A partial characterization was performed, in this case, standard curves were obtained using phenolic standard such as (+)-catechin, eugenol, quercetin, vanillin; ellagic, gallic, syringic and vanillic acids.

The polyphenols existing in the extract as well as in the diluted phase were identified by HPLC using the chromatographic platform Accela (Thermo Scientific, USA); the system has a diode array detector (running from 220 nm to 360 nm) as well as an automatic sample injector and automatic fraction collector. For the experiments a Shim-pack CLC-ODS(M) column (250 mm × 4.6 mm, Shimadzu, Japan) was used. Under a flow rate of 1000 $\mu l \cdot min^{-1}$ and oven temperature of 30 °C, elution was carried out in gradient by the mixture of 1.0 vol% acetic acid (A) and acetonitrile (B) in the following proportions: 100% A as initial condition, 100% A to 70% A at 10 min, 70% A to 30% A at 15 min, and 30% to 0 until 25 min. Finally, elution was maintained only with acetonitrile for a further 5 min. Samples were filtered using a membrane of 0.22 μm pore size and assayed in three replicates.

3. Results and Discussion

3.1. Phase diagram of the coexistence curves

The coexistence curve reflects the limit condition of a monophasic and biphasic region in a phase diagram. Therefore it plays a key role for the CPE operation [33]. As expected, the CPTs in the control coexistence curve (without extract) increased with the addition of Triton X-114, as showed in Fig. 1(a). This behavior was also observed in other coexistence curves with camu-camu residue extract. The presence of extract strongly reduces the CPTs, proportionally to mass percentage of extract. Possibly, the polyphenols existing in the extract interact with the surfactant then reducing their solubility in water. This behavior also was observed in the other phenolic components [15,34,35].

The addition of inorganic salts to the CPE system has been a routine practice aiming the change in the phase behavior. In this case, the salt sodium chloride has been commonly used [17,26,34]. In the present study, it was observed that the presence of this salt affected considerably the cloud point temperature of the CPE system composed of 7 wt% Triton X-114 and 80 wt% extract [Fig. 1(b)]. In this case, the cloud point temperature dropped linearly from 22.1 °C (without salt) to 16.7 °C (4 wt%) with the increase of the percentage of sodium chloride. This behavior can be attributed to the salting-out effect of the surfactant, in which the electrolytes added caused the dehydration of the coacervate phase and then leading to an increase in the interaction among the surfactant molecules [31,34,37].

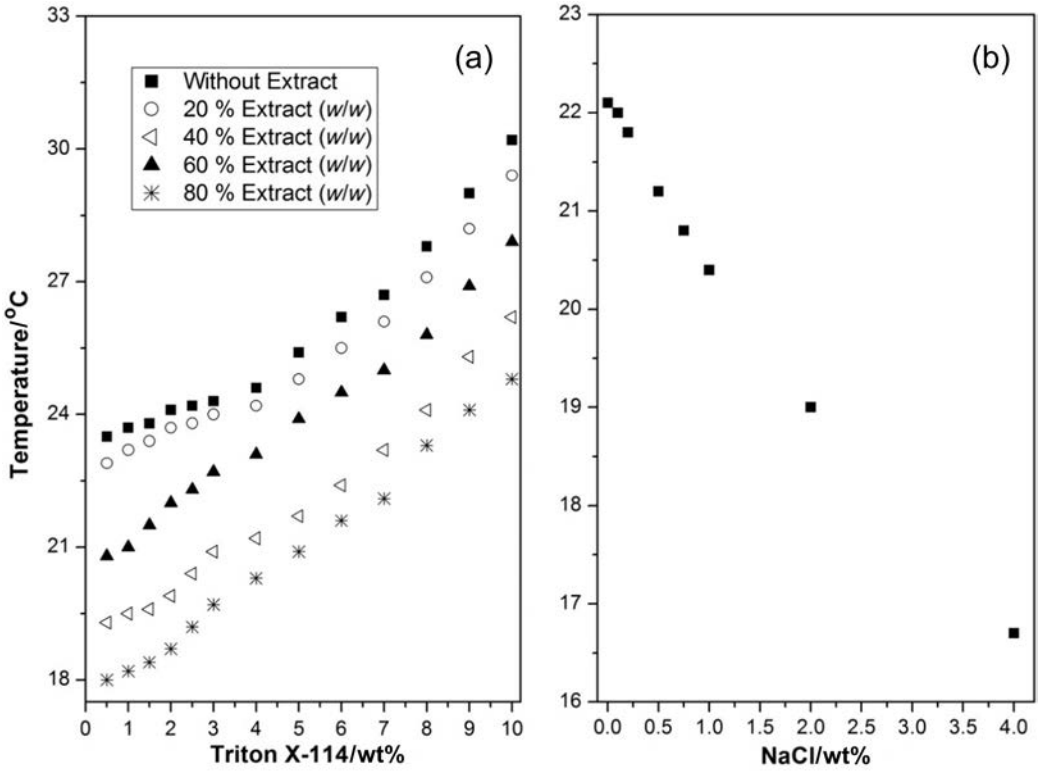

Fig. 1. Cloud point values in function of the Triton X-114 and percentage of extract (a). Influence of the sodium chloride in the cloud point (b).

3.2. Polyphenol recovery by CPE

3.2.1. Effect of surfactant concentration and equilibrium temperature

The surfactant concentration and the equilibrium temperature play a key role in the CPE as well as in the extraction efficiency [22,23]. The values of the recovery as well as of the volumetric ratio (ϕ_C) in function of the percentage of Triton X-114 and of equilibrium temperature are shown in Fig. 2(a) and (b), respectively. It was observed that the increase in the surfactant percentage improved the capacity of the coacervate phase to capture polyphenols showing a logarithm profile.

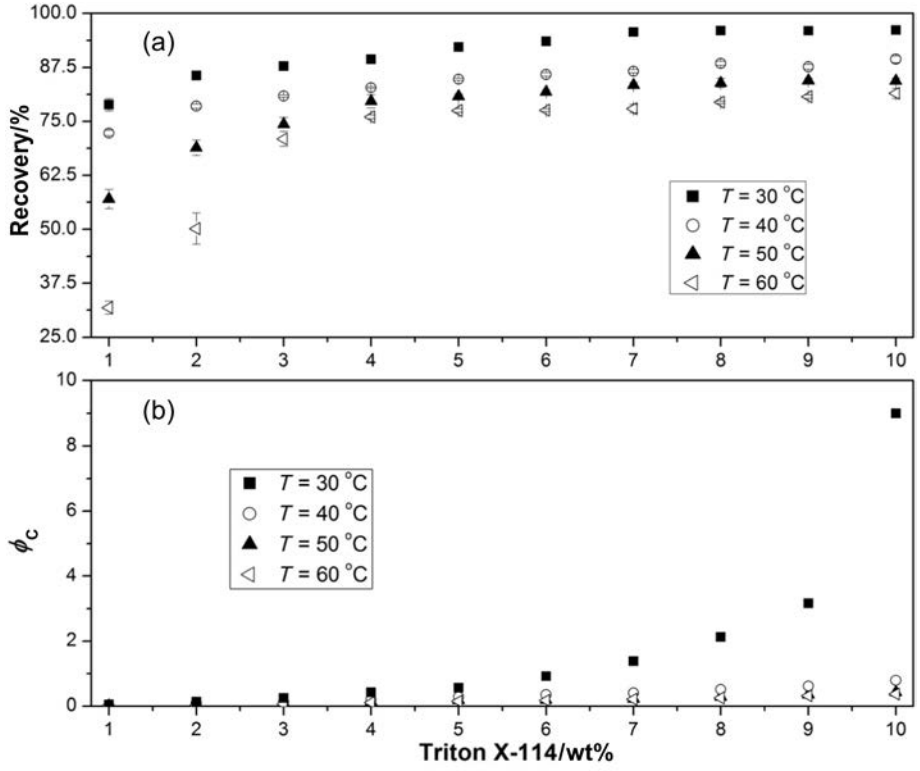

Fig. 2. Effect of surfactant and equilibrium temperature on the polyphenol recovery (a) and volumetric ratio (b) by CPE. In these experiments values of pH and percentage of extract and chloride salt were fixed in 3.25 and 80 wt%, respectively. No sodium chloride was added.

Also, the Recovery increased sharply in the range of 1 wt%–4 wt% then keeping approximately constant to Triton X-114 percentage higher than 4 wt% regardless the equilibrium temperature. The latter showed a negative effect on the Recovery, being more intense at lower surfactant concentration.

The use of 1 wt% Triton X-114, changing the temperature from 30 °C to 60 °C, led to a reduction of the polyphenol recovery in the coacervate phase from 78.83% to 31.85%. Unlike as commented by Wang *et al.* [38] and Han *et al.* [39] the optimum equilibrium temperature (30 °C) was near the cloud point one (18–24.8 °C). In addition, previous studies observed that during the CPE process the high temperatures can degrade the phenolic components [23,26,39].

With regard to the ϕ_C values, it was observed that they were favored with the increase in the Triton X-114 percentage. On the other hand, the change of temperature from 30 °C to 60 °C in the system composed of 10 wt% Triton X-114 reduced the ϕ_C values more than 24-fold. The increase of the temperature causes the breakage of hydrogen bonds between the surfactant and water molecules, thus leading to the dehydration of the coacervate phase [41]. Therefore, we have kept for the experiments the Triton X-114 percentage and equilibrium temperature in 7 wt% and 30 °C, respectively. In this condition similar yield ($p > 0.05$) and concentration factor (solute concentration in the coacervate and diluted ratio) were higher than the system using 10 wt% Triton X-114 under the same equilibrium temperature.

3.2.2. Effect of extract concentration

The influence of the percentage of camu-camu extract was investigated in this study and the results are shown in Fig. 3. It was observed that the extract concentration had little effect on Recovery for the CPE systems, showing values of approximately 95.71%. When the levels of surfactant concentration and equilibrium temperature were selected, the micellar system reached its maximum performance (or near maximum), so that the initial concentration of polyphenols had no impact on Recovery. Similar results have been reported to crystal violet dye [42], eosin dye [43], and phenolic components from OMW [20]. However, the term ϕ_C reached the minimum value in the system for 90 wt% extract. Like the coexistence curves shown in Fig. 1, the polyphenols existing in the extract facilitate the CPE phase separation, then giving a reduction for ϕ_C under the same condition of Triton X-114 and equilibrium temperature.

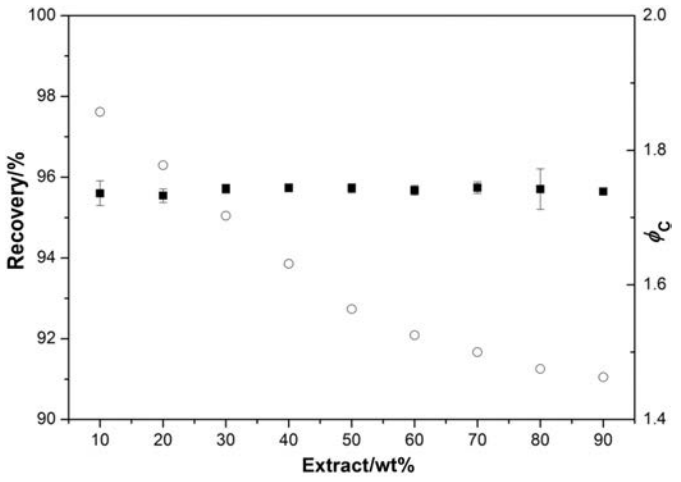

Fig. 3. Effect of extract on the polyphenol recovery (■) and volumetric ratio (○) by CPE. The percentage of Triton X-114, equilibrium temperature, and pH were fixed in 7 wt%, 30 °C, and 3.25, respectively. No sodium chloride was added.

3.2.3. Effect of pH

The pH of the system plays an important role since it acts on the micelle–solute interactions as well as it can influence the economic viability of the CPE [34]. The influence of this parameter was investigated in the system formed by 7 wt% Triton X-114, 80 wt% extract at an equilibrium temperature of 30 °C. It can be seen in Fig. 4 that the pH was not significant for the ϕ_C. However, it reduced the recovery of the polyphenols in the coacervate phase mainly when it was increased. This behavior can be attributed to the dissociation of the phenolic components in alkaline environments. In this case, they interact strongly with the water molecules rather than micelle one [16]. Nonetheless, the reduction of %Recovery was lower than those results showed by other similar molecules reported in literature [23,33,44]. Probably, this occurred due to the greater percentage of surfactant used. In summary, the change of the system pH was not favorable to the polyphenol recovery, then the chosen option was that in which that the pH was not adjusted.

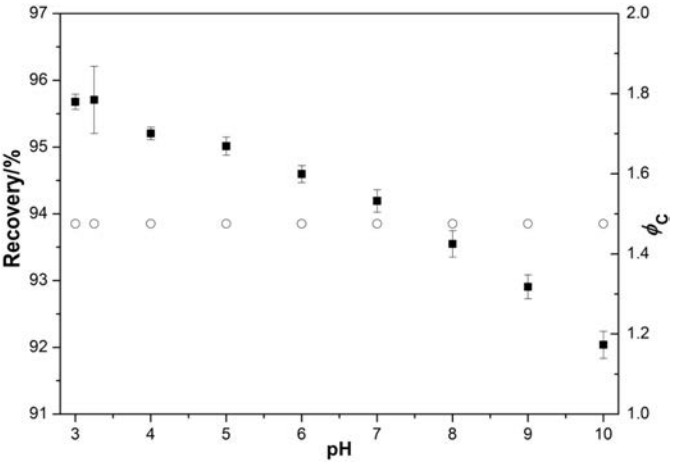

Fig. 4. Effect of the pH on the polyphenol recovery (■) and volumetric ratio (○) by CPE. The equilibrium temperature, the percentage of Triton X-114 and extract were fixed in 30 °C, 7 wt% and 80 wt%, respectively. No sodium chloride was added.

3.2.4. Effect of sodium chloride concentration

In order to investigate the effect of the sodium chloride percentage on the recovery as well as distribution ratio of the CPE system, sodium chloride was added in a range from 0 to 10 wt%, for a system composed of 7 wt% Triton X-114, 80 wt% extract, without adjusting the pH and with an equilibrium temperature of 30 °C. It was observed that addition of salt to the system strongly reduced the polyphenol recovery. This result is quite different from those shown by Ma *et al.* [16], Santalad *et al.* [45], Stamatopoulos *et al.* [26] and Vichapong *et al.* [27]. It can be seen in Fig. 5 that changing the sodium chloride percentage from zero to 10 wt%

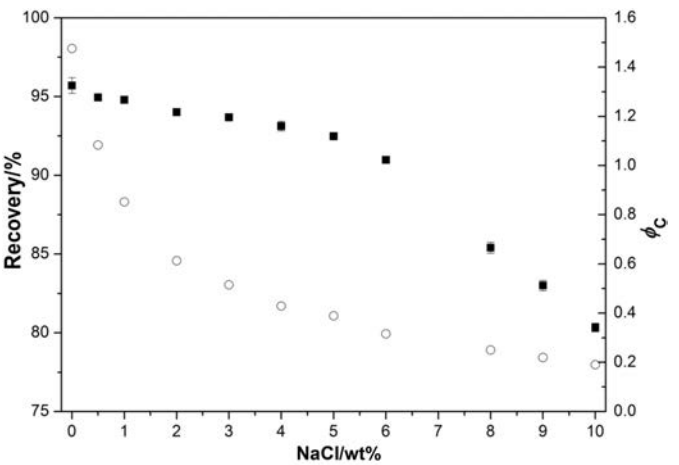

Fig. 5. Effect of sodium chloride on the polyphenol recovery (■) and volumetric ratio (○) by CPE. The equilibrium temperature, pH, percentage of Triton X-114 and extract were fixed in 30 °C, 3.25, 7 wt% and 80 wt%, respectively.

reduced the Recovery from 95.71% to 80.33%. Also, the increase in the salt concentration was unfavorable to the volumetric ratio as well. As commented before in Section 3.1, this behavior is also explained by the salting-out effect leading to dehydration in the coacervate phase, then reducing the ϕ_C and, consequently, increasing the solute concentration in the surfactant-rich phase. Dotted line in Fig. 5 represents non-ideal conditions for the CPE since the densities for the diluted and coacervate phases are quite near. After the incubation time, inversion at phase positions for sodium chloride concentration equal or higher than 8 wt% was observed.

Considering the Recovery as the target the results showed that 7 wt% Triton X-114 and 80 wt% extract incubated at 30 °C was the best condition. Therefore, a validation experiment was carried out in this condition given a Recovery and a concentration factor of 95.33 ± 0.19 and 15.14 ± 0.05, respectively. On the other hand, focusing the concentration factor in the validation experiment the use of 6 wt% NaCl gave a value of 35.15 ± 0.27. Thus, it is evidence that CPE with Triton X-114 is a powerful technique to recover and concentrate the polyphenols of camu-camu residues.

3.2.5. HPLC chromatograms

The chromatograms of camu-camu extract and diluted phase after CPE are shown in Fig. 6. It can be seen that six peaks were identified in the extract that were gallic acid, (+)-catechin, vanillic acid, syringic acid, vanillin and quercetin, However, they were not identified in the diluted phase but in the coacervate phase. Thus, they were estimated by a mass balance in which the concentrations of these six compounds were in (mg per 100 g of residues): gallic acid (9.4); (+)-catechin (0.7); vanillic acid (0.3); syringic acid (0.4); vanillin (0.8); quercetin (1.1). It is important to comment that the two major components (peaks) were not identified in this study. However, they showed retention times of 6.91 min and 7.83 min, respectively. Additionally, a fraction of these components were in the diluted phase (lower curve in Fig. 6).

4. Conclusions

The application of the non-ionic surfactant Triton X-114 in order to recover polyphenols occurring in the camu-camu depulping residue by cloud point extraction was successful. The system formed by 7 wt% Triton X-114, 80 wt% extract, without need of pH adjustment and operating at 30 °C reached the maximum extraction efficiency (95.71%). The salting-out promoted by the sodium chloride addition allowed a higher concentration of polyphenols in the coacervate phase due to the reduced volumetric ratio, even though it reduced considerably the process efficiency. Also, the experiments carried out discharged the changes in the pH as a tool for recycling the surfactant. Finally, further studies will be necessary in order to identify the phenolic compounds recovered.

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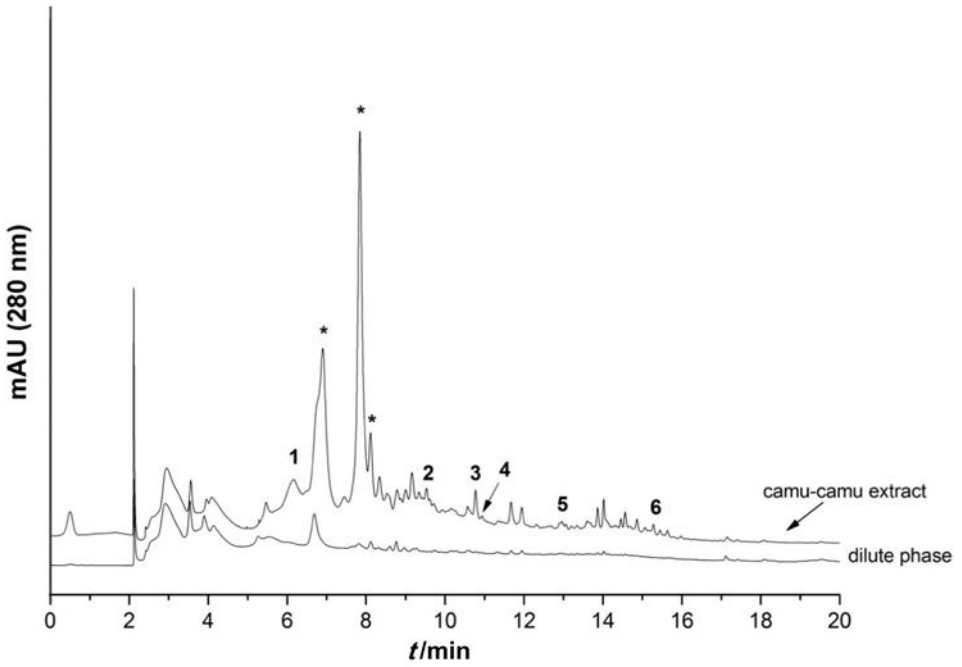

Fig. 6. HPLC chromatograms of camu-camu residue extract (A); and diluted phase after CPE (B). Peaks 1, 2, 3, 4, 5 and 6 correspond to gallic acid, (+)-catechin, vanillic acid, syringic acid, vanillin and quercetin, respectively.* — Not identified.

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