

Supercritical Antisolvent Extraction of chlorogenic acids and epicatechin

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ABSTRACT

Coffee berry pulp contains some antioxidant compounds of industrial interest, first of all chlorogenic acids (CGA) and epicatechin (EP). Since they are not soluble in supercritical carbon dioxide (SCCO₂), supercritical antisolvent extraction (SAE) was proposed to extract/fractionate these compounds from coffee berry pulp. SAE process allows the processing of ethanolic extracts of coffee berry pulp with SCCO₂ used as antisolvent to fractionate CGAs, EP and other polyphenolic compounds. The ethanolic solution with 3% w/w of solid was used in SAE experiments. SAE was carried out at different process conditions. Two extracts were obtained: a precipitate (P) and a separator (S) extract, which were analyzed by high performance liquid chromatography. The presence of different compounds in coffee pulp like sugars and colloids interferes with the precipitation of polyphenolics and the material recovered on the precipitation chamber was not a powder. A new procedure will be developed to overcome this problem.

1 - INTRODUCTION

Natural antioxidant compounds are identified as nutraceuticals by their capacity to prevent disease and improve health; their application in food and pharmaceutical products, replacing the synthetic antioxidants, has increased the interest to study new techniques of extraction and to search natural sources of these compounds for industrial applications [1-3]. In several cases wastes from agroindustrial process represent a high value natural matrix.

In the processing of coffee, the green beans represent only 18.5 per cent of coffee fruit, the rest is water, pulp, husk and mucilage with approximatively 20, 41, 4.5 and 16 % respectively [4]. The increase of the production represents an environmental problem for the final disposition of these subproducts [5] since FAO (2003) has projected the world production of coffee in 2010 to 7 millions of tons. Different uses for coffee pulp have been proposed, the most studied has been for the formulation of animal feed [5-7]; but, it presents serious inconvenient for the presence of antinutritional substances, attributed to polyphenolic compounds of high and low molecular weight and caffeine [8-10]. The polyphenolic compounds present in the coffee pulp (tannins, chlorogenic acids, catechins and caffeic acids), can represent between 2 to 6 % of the pulp composition on dry basis [5, 10]. Chlorogenic acid (5-caffeoylquinic acid) (CGA) and (-) - epicatechin (EP) are the major components with 42.2% and 21.6% respectively [6].

Chlorogenic acids are an important group of biologically active phenolic compounds, the most known is 5-caffeoylquinic acid that has demonstrated high antioxidant activity in in-vitro experiments and also as a weight reducer [11]. The opportunity of extract these polyphenolic compounds from the residues of coffee beans production has to be evaluated for

their potential use in formulation of food, pharmaceutical or cosmetic products. The conventional extraction with organic solvents present disadvantages in the extraction of natural compounds, like the contamination of the extract, thermal degradation, no selectivity in extraction and pollution of environment [12].

The supercritical fluid extraction (SFE) of natural compounds represents an alternative to obtain extracts with high purity, more stable and without solvent residue. In many cases the SFE using SCCO₂ has shown its applicability for extraction of antioxidant compounds from natural matrix [3, 13, 14]. However the polar nature of CGAs and EP limits the potentiality of SCCO₂, because of its lipophilic affinity. The extraction of chlorogenic acid from green coffee beans using isopropilic alcohol as cosolvent at 5% w/w has been studied [15]; nevertheless, the yield of extraction was low. The Supercritical Antisolvent Extraction (SAE) can allow us to improve the yield of extraction for polar compounds. In SAE process, the solvent used has to solubilize the compounds of interest and at the same time has to be soluble in SC CO₂ at the operating conditions [16 - 18]. The aim of this research is to identify the potential extraction of CGAs and EP, from coffee pulp using SCCO₂ as antisolvent in a SAE process.

2 - MATERIALS AND METHODS

Materials

The samples of coffee fruit, *Coffea arabica* varieties Typica have been harvest in Malacatos on the south of Ecuador at 1800 m.a.s.l.

Ethanol absolute 99% was from Fluka, acetic acid 96% and methanol grade HPLC were given by Carlo Erba, and water (HPLC grade) from Sigma Aldrich. Standards of chlorogenic acid (5 caffeoylquinic acid) and epicatechin used as standards were acquired from Sigma (Italy).

Ethanolic extract

5 grams of coffee pulp were extracted with 50 mL of absolute ethanol in continuous agitation during 24 hours at room temperature. The resulting suspension was filtered to retain the solid particles, the concentration of the solution was adjusted up to 3% w/w by vacuum evaporation in a rotary evaporator. The extract was diluted in methanol for analysis by high performance liquid chromatography.

HPLC analysis

The analytical separation and quantitative determination of ACG and EP were obtained using an HPLC/UV, equipped with a quaternary pump and degasser. A column (Hypersil BDS C18, 4.6 mm x 250 mm, 5 µm) was used for the separation of polyphenols. The mobile phase consisted in a mixture of two solvents: methanol (A) and aqueous acetic acid 2% v/v (B). 10 µL of solution were injected with a solvent flow of 0.5 mL/min, 30% of solvent A and 70% of B for 30 minutes. For the identification and quantification of the compounds the external standards method was used.

3 - Supercritical Antisolvent Extraction (SAE)

The coffee cherries were hand-pulped and immediately dehydrated on a laboratory oven at 60°C up to humidity less than 10% by weight; then were stored at vacuum on plastic bags for its transportation. The samples were grounded in a knife mill during 1 minute for three times, for prevent thermal damage. A preliminary study of particle size was achieved, the samples with a particle size smaller than 250 µm presented high ethanolic extraction yield of polyphenolic compounds and consequently these were used for all SAE extractions.

Figure 1 shows a schematic representation of the process used; it basically consisted in three steps; in the first one SC CO₂ was pumped into the precipitation chamber with a membrane pump until the operation conditions of pressure and temperature were achieved; in the second one the ethanolic solution was fed with a piston pump through an injector of 180 µm to obtain a high surface contact between the two phases and allow the supersaturation of the insoluble compounds in SC CO₂ and their precipitation at the bottom of the chamber. Ethanol and the compounds dissolved in SC CO₂ pass through a filter of 1 µm and were recovered in a separator by depressurization of CO₂ at atmospheric conditions. The final step consisted of a flow of SC CO₂ to remove the excess of solvent in the precipitates. The precipitate and the extract recovered in the separator were analyzed by HPLC for determine the yield of the separation and fractionation.

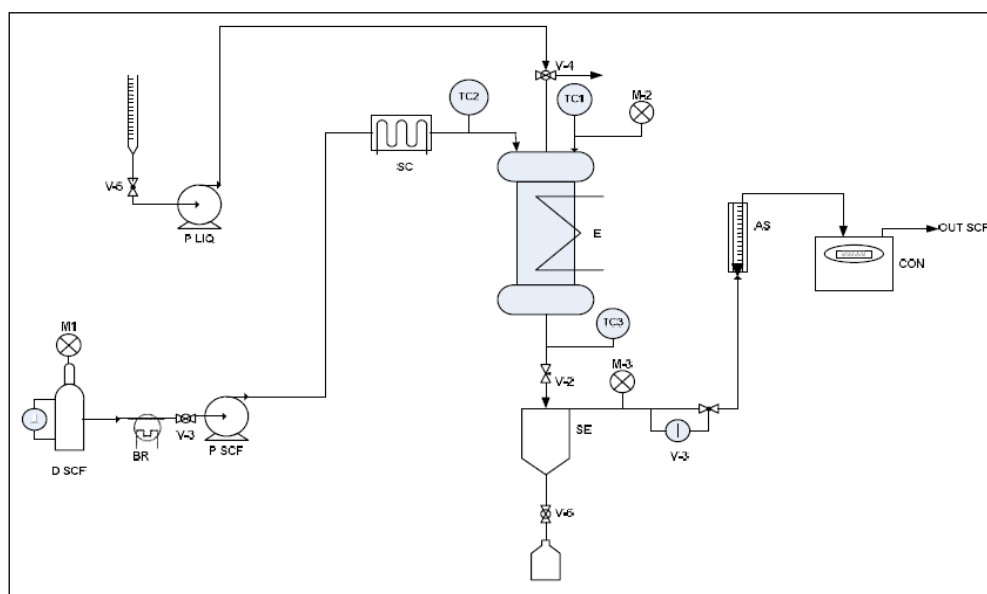


Figure 1. Schematic representation of SAE process

4 - Results and Discussions

The concentration of the solution injected was standardized to 3% w/w, the flow of CO₂ was maintained at 2.38 Kg/h, to obtain a SCCO₂-ethanol molar fraction of 0.98. To prevent degradation of the compounds, the experiments were performed at temperatures between 35 – 40 °C. From literature the Mixture Critical Point (MCP) of the mixture ethanol-CO₂ is located at 71 and 80 bar for these temperatures; therefore, only experiments at pressures higher than 80 bar were performed.

Table 1 shows the change of CO₂ density with the operating conditions and the quantification of CGAs and EP in the precipitated P and separator S extracts, all results are expressed on dry basis. At all tested conditions the highest recovery of CGAs and EP was reached in the separator instead of precipitator extracts. This can be explained by the enhancement of solubility of CGAs and EP in the mixture CO₂-ethanol, compared to the solubility in pure SCCO₂.

Also the simultaneous extraction of different compounds that are present in coffee pulp (like sugars and colloids) can interfere with the precipitation of polyphenolics since the material recovered on the precipitation chamber was not a powder: the collected material had the appearance of a sticky substance stuck on the chamber wall and it was difficult to weight.

Table 1. Composition of ethanolic extract, SAE P and S extracts.

CO ₂ density	T	P	Initial concentration		Recovery in precipitate	Precipitated concentration		Recovery in separator	Separator concentration	
			CGA	EP		CGA	EP		CGA	EP
Kg/m ³	°C	bar	mg/g EtOH ext.	mg/g EtOH ext.	%	mg/g P. ext.	mg/g P. ext.	%	mg/g S. ext.	mg/g S. ext.
815	35	150	30.6	62.7	31.2	21.54	15.02	14.3	58.5	406.3
780	40	150	33.3	66.1	53.9	20.38	18.98	14.1	54.1	308.2
431	35	80	35.5	77.5	50.7	24.42	10.77	14.0	60.8	292.5
281	40	80	28.9	68.6	46.1	26.4	2.38	10.2	76.7	352.9

The highest enrichment of separator extract was achieved at the highest density of SCCO₂ tested, passing of 93.3 mg/g. ethanol extract. to 464.8 mg/g S extract. It corresponds to the increased of solvation power of the CO₂+ethanol mixture with pressure. This yield of extraction can be improved since other polyphenolic compounds, for example isochlorogenic acids, catechins, caffeic and ferulic acids present in coffee pulp can also be extracted. In a future work these compounds will be analyzed for their quantification, and also a methodology to eliminate the interference in the SAE process will be implemented.

5 - Conclusions

Using SAE is possible to fractionate compounds with high polarity like CGAs and EP using SCCO₂ as antisolvent. In this work the conditions for a better separation corresponds to a high density of SCCO₂ and the components were both recovered in the separator. It is necessary to implement a further step to eliminate interfering compounds in the starting ethanolic solution for a better separation.

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