SUPERCRITICAL ANTISOLVENT FRACTIONATION OF BIOACTIVE COMPOUNDS FROM MANGO BY-PRODUCTS

Meneses Miguel[a,b]*, Guamán María[a], Adami Renata[b], Scognamiglio Mariarosa[b], Caputo Giuseppe[b] and Reverchon Ernesto[b]

a. Departamento de Ciencias Agropecuarias y de Alimentos
Universidad Técnica Particular de Loja
San Cayetano alto s/n. AP 1101608, Ecuador

b. Department of Industrial Engineering, University of Salerno
Via Ponte don Melillo 84084 Fisciano, Italy

Email: mameneses@utpl.edu.ec

Abstract. In this study the supercritical antisolvent fractionation (SAF) process was used to fractionate and recovery polyphenolic compounds from mango by-products. The liquid extract was prepared by liquid-solid extraction in acetone and purification by adsorption/desorption in C18 column with ethanol. The SAF process was performed changing the pressure (8 – 15 MPa) and temperature (35 – 40 ºC) of SC-CO_2, the effect was evaluated over the chemical composition and antioxidant power of the precipitated recovered. The best condition, 40 ºC and 150 bar, allows to obtain a precipitate as powder with a content of polyphenols of 911.94 mg GAE/g extract with an EC50 less than 0.05 mg/mL for antioxidant power, and increasing of concentration of polyphenolic compounds of 348.6 mg/kg mangiferin, 6.5 mg/kg isomangiferin, 5262.0 mg/kg quercetin 3-O glucoside, 750.2 mg/kg quercetin and 137.6 mg/kg kaempferol. The SAE preserves the polyphenolic compounds; moreover the antioxidant power was improved possibly by the micronization of compounds that can increase the dissolution velocity in the DPPH assay.

Keywords : mango by-products, antioxidants, supercritical antisolvent extraction

1. Introduction

Epidemiological evidences suggest that diet rich in fruits and vegetables, both source of antioxidant polyphenols provide health benefits such as prevention of cardiovascular diseases and certain types of cancer [1]. In the development of functional ingredients and nutraceuticals the by-products of fruits processing are being recognized as raw materials by its high content of bioactive compounds with antioxidant activity and important dietary fiber content [2, 3]. In the processing of mango, the peels and seeds are the main biowastes produced. Depending on the cultivar and product manufactured these by-products represent between 35 – 60 % of the whole fruit [4]. The phytochemical compounds in the extracts of *Mangifera indica* Linn have been reported to possess antiviral, antibacterial, analgesic, anti-inflammatory, and immune modulatory activities [5]. These health benefits are related to the presence of phenolic compounds [2, 6, 7] among them xanthones (mangiferin) and flavonols (quercetin) as main compounds.

Different investigations have proposed the exploitation of kernel, a mango by-product, as a source of fat, natural antioxidants, starch, flour and feed, in addition for mango peels the achievement include the production of biogas [8], dietary fiber with a high antioxidant activity [9] and the use as a source of pectin and phenols [10]. Furthermore, mango peels have been shown to be a rich source of flavonol O- and xanthone C-glycosides [6, 11] gallotanins and benzophenone derivatives [12].

The extraction of antioxidant compounds from mango peels is usually carried out with organic solvents such as methanol, ethyl alcohol, acetone and water in pure form and also as mixture. The traditional technology used in the extraction of phenolic compounds in some cases limits its applications or made necessary post processing with the consequent cost increase and detrimental in quality. In recent years the

supercritical fluid extraction had been applied for obtaining natural and safety antioxidants [13]. The advantages of CO_2 as supercritical fluid are knows, non-toxicity, non-residual solvent in the final product, good characteristics in food and pharmaceutical applications, cheap and reduction in waste solvent production [14].

Previous studies have shown that the extraction of polar antioxidant compounds required high pressure and the presence of organic co solvents such as ethyl alcohol, methanol, water, due to the non-polar nature of CO_2. Supercritical antisolvent precipitation (SAS) is a technique that has been extensively used to study the production of micronic and submicronic particles of polar compounds with controlled particle size and distribution [15-17]. In the SAS technique the diffusivities can be up to two orders of magnitude higher than those of liquid antisolvent and it is possible the completely elimination of liquid solvent and its recovery from antisolvent by depressurize until gas phase in a separator. In different cases the SAS has been used for the extraction/fractionation of polar solution of natural extracts, for example the selective antisolvent extraction of antioxidant compounds from grape by-products [18] allows the recovery of phenolic compounds presents in the feed solution at moderate conditions of pressure and temperature (11 MPa and 40 °C). Similarly SAS procedure has been used to perform the coprecipitation of green tea extracts [19] and rosemary antioxidants [20] from liquid solution to obtain protection against degradation; in both cases the process allowed the selective precipitation of 90% of polyphenols compounds together to a biodegradable polymer. Eventually some compounds can be co-extracted with the liquid solvent this permits the fractionation of soluble compounds in SC-CO_2 [21]. Berardini, Knodler, Schieber and Carle [10] have proposed the extraction of pectin and polyphenols from mango peels using adsorption technology to separate them; it is a relevant step for SAS of fruit extracts since the presence of pectin makes it difficult of fractionate and micronize [18, 22].

In this work, SC-CO_2 was used as antisolvent to recovery the antioxidant compounds presents in a mango peels extract avoiding the denaturation of radical scavenging activity. The mango extract was obtained by two steps; a liquid–solid extraction and adsorption/desorption in C18 column. The supercritical antisolvent fractionation technique gave rise to a powder of concentrated antioxidants and ethyl alcohol. The process was evaluated by quantification of phenolic compounds and their antioxidant power in the unprocessed and processed SAE extract.

2. Materials and methods

2.1 Materials

Mango by-products (*Mangifera indica* L. cv. Tommy Atkins and Haden) corresponding to peels with added pulp were provided by Ecuadorian fruit processing industry, these by-products were dehydrated at 37 °C and grinding until 350 µm of particle size.

All reagents were of analytical grade and the polyphenol standards used were of HPLC grade.

2.2 Extraction of polyphenol compounds

5 g of mango by-products were put in contact with 0.5 g of ascorbic acid and extracted with 50 mL of aqueous acetone (80%, v/v) for 6 h under stirring at room temperature (25 ºC). Then the solution was centrifuged and the supernatant separated and the solid residue extracted once again at the same conditions. The organic solvent of supernatants was removed by vacuum evaporation in a rotary evaporator at 30°C, the remained aqueous solution was dissolved 1:4 in deionized water.

For purification of polyphenols a 5g C18 cartridge was activated with 25 mL of ethanol and wetted with 30 mL of deionized water before that the extract was adsorbed in the column. Then the extract was washed with 30 mL of deionized water and finally the phenolic compounds were desorbed from the column with ethanol, this procedure was optimized to avoid artifacts. The purified solution was kept at -4 ºC until perform the subsequent experiments and analysis.

2.3 Supercritical Antisolvent Fractionation, SAF

The schematic representation of the SAF equipment used for experiments is shown in figure 1. Two pumps were used, a high pressure pump (LEWA, mod LDB1 M210S) for delivering CO_2 and a HPLC pump (Gilson, mod 305) for liquid solution. The precipitator chamber was a stainless steel vessel of 0.4 L in volume (id 50 mm), it is provided with a 180 µm nozzle in the top for liquid injection and a stainless steel filter (1 µm

porosity) in the bottom to collect the powder material. A separator located downstream the pressure reduction valve was used to recovery the liquid solvent.

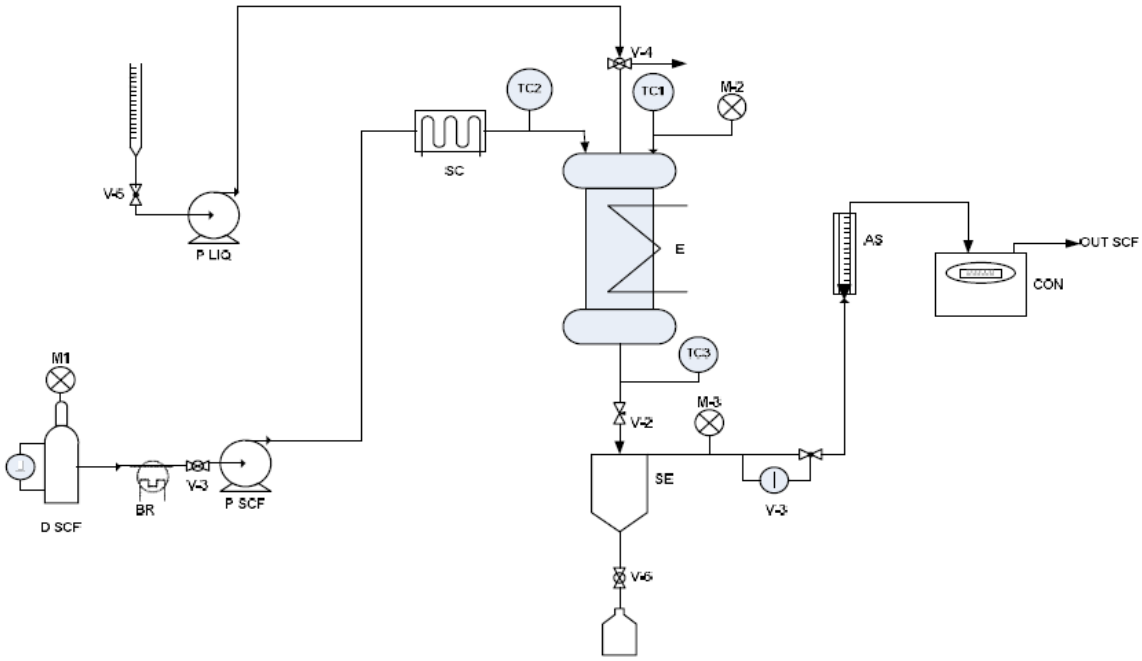

Figure 1. Schematic representation of SAF process

For SAF experiments at begin CO_2 was pumped to the precipitator until reach the operating condition of pressure, then the pressure was regulated by a micrometric valve. When the volumetric flow of CO_2 was stable, pure solvent was sent through the nozzle to the precipitation chamber in a volume enough to reach steady state of solvent and antisolvent. At this point the solvent flow was stopped and began the delivery of ethanolic solution from C18 cartridge desorption. At the end, a purge in the precipitator with pure CO_2 was performed maintaining the operating conditions to wash the ethanol solubilized. If the final purge with pure CO_2 was not performed the solvent contained in the antisolvent condensed during depressurizing and dissolves or modifies the precipitate. The precipitated compounds were collected in the filter while the ethanol and solubilized compounds were recovered in the separator operated at 3 MPa.

The recovery yield was determined by weighting the total amount of precipitated compounds collected in the precipitator and the compounds condensed with ethanol in the separator, both fractions related to the total amount of extract dissolved in ethanol.

2.4 Analytical analysis

HPLC analysis. The separation and identification of phenolic compounds was realized in an Agilent HPLC, a Synergi Hydro-RP column (150 mm x 3.0 mm i.d., 4 µm, Phenomenex) with a C18 ODS guard column (4.0 mm x 2.0 mm i.d.) operated at room temperature. The elution program used was according the method explained by Berardini et al. [6]. The system was left to stabilize for 5 min between consecutive injections. The injection volume was 10 µL with a solvent flow 0.6 mL/min and the monitoring was performed at 370 nm. The compounds were identified by comparison with the relative retention time of polyphenol standards.

DPPH assay. The method described by Brand-Williams [23] based on the reduction of DPPH radical was used with some modifications. For each extract concentrations between 1 – 0.05 mg/mL were prepared in methanol and physiologic solution. An aliquot of 150 µL of extract solution was added to 2.85 mL of DPPH solution (1.1 ± 0.02 units in absorbance). The decrease in absorbance was determined at 515 nm after 24 h of reaction as described by Thaipong et al. [24]. For each extract the percentage of DPPH inhibition was plotted

and antiradical activity was defined as the concentration of extract necessary to decrease the initial DPPH concentration by 50%.

Total phenolic content. The Folin Ciocalteu method adapted by Thaipong et al. [24] was used to determine the total phenol content. 150 µL of methanolic extract solution were mixed with 2400 µL of water and 150 µL of 0.25N Folin-Ciocalteu. The mixture was allowed to react for 3 minutes and then 300 µL of 1N Na2CO3 solution was added and well mixed. The solution was incubated at room temperature in dark for 2 h. The absorbance was measured at 725 nm and the results were expressed as gallic acid equivalents (mg GAE/ g of extract) using a calibration curve.

3. Results and discussion

3.1 Extraction of polyphenols

Preliminary extraction test were performed using absolute ethanol, however, in this arrangement, the process was relatively unsuccessful because several compounds contained in the vegetable matrix interfered with extraction. Sugars and pectin contained in high quantities in mango peels [10], exerted not only a barrier and competition for extraction of polyphenolic compounds but also present operational problems in SAF experiments [18, 22]; therefore a step of purification was mandatory as mentioned by Floris et al. [18]. The ethanolic mango extract desorbed from C18 cartridge had a total phenol content of 888.63 ± 102.38 mg GAE/g extract and polyphenol content of 100.83 ± 11.42 mg/kg for mangiferin, 4.39 ± 0.27 mg/kg for isomangiferin, 4579.43 ± 1597.48 mg/kg for 3-O-quercetin galactoside, 418.51 ± 159.05 mg/kg for quercetin and 54.03 ± 11.63 mg/kg for kaempferol.

3.2 SAF extraction

The concentration of the solution injected was standardized to 5 mg/mL; the flow of CO_2 was maintained at 2.38 kg/h to obtain a CO_2 molar fraction of 0.98. Temperature of process is a sensible parameter when polyphenolic compounds are processed, to avoid thermal degradation of antioxidant compounds the temperature was selected over critical value for CO_2 31 °C until 40 °C. For select the pressure range was necessary to take into account the phase diagrams for CO_2-ethanol and considering pressures near and over mixture critical point [25]. Therefore the experiments were carried out at 35 – 40 °C and 8 – 15 MPa.

Table 1 shows the experimental conditions, the extraction yield and mangiferin content. At all tested conditions the highest recovery of polyphenols was reached in the precipitator instead of separator, in the form of micronized powder. The fast extraction of ethanol in SC-CO_2 produces supersaturation of the polyphenols and their precipitation [18]. The dry powder represents additional advantages over conventional liquid forms due to lower storage cost and higher concentration as mentioned by Meterc, et al. [26].

Table 1. Experimental conditions in SAF process

Experiment	Operating conditions	Precipitator		Separator	
		% yield (w/w)	Mangiferin mg/kg	% yield (w/w)	Mangiferin mg/kg
1	40 °C, 8 MPa	53	299	18	ND
2	35 °C, 8 MPa	58	274	20	3
3	40 °C, 10 MPa	62	256	15	ND
4	35 °C, 10 MPa	52	247	15	ND
5	40 °C, 15 MPa	61	348	13	25.7
6	35 °C, 15 MPa	61	318	15	19.6

The operation pressure also influences the solubility in CO_2 of other compounds contained in the liquid solution. The higher was the pressure the larger was the solubility of the mango polyphenolic compounds. This eventually induces the fractionation of the compounds between the precipitator and the separator. The HPLC analysis of the SAF fractions permits to calculate the concentration of phenolic compounds as showed in table 2 for 40°C and 15 MPa. After SAF processing there was no significant change in the total phenol content. The total phenolic content for unprocessed extract, SAF processed and condensed extract was 888.63, 838.83 and 72.75 mg/g of extract respectively.

Table 2. Concentration of phenolic compounds in SAF process (40 °C, 15 MPa).

Phenolic compounds	Ethyl alcohol extract	SAF precipitated	SAF condensed
Phenolic compounds (mg/kg of extract)			
Mangiferin	100.83	348.6	25.7
Iso Mangiferin	4.39	6.5	ND
3-O-Quercetin galactoside	4579.43	5262	287.9
Quercetin	418.81	750	180.5
Kaempferol	54.03	137.6	52.6
Total phenol content (mg GAE/g of extract)	888.63	838.83	72.75

3.3 Antioxidant activity

The free radical scavenging activity of unprocessed and processed mango extract was measured in ethanol and aqueous solution as shown in figure 2. For each sample, seven concentrations were tested. The radical scavenging activity shows high dependence on the solvent used to dissolve unprocessed and processed samples. It was observed that the DPPH free radical-scavenging activity in aqueous solution is higher for processed than that of unprocessed mango extract. It last probably depends on the increasing of dissolution velocity in water of antioxidant compounds for the microparticles obtained in SAF process. Similar results were found by Yang, et al. [27] for the increase of solubility of polymeric procyanidins precipitated by SAS and it was demonstrated also in solubility analysis of unprocessed and processed extracts.

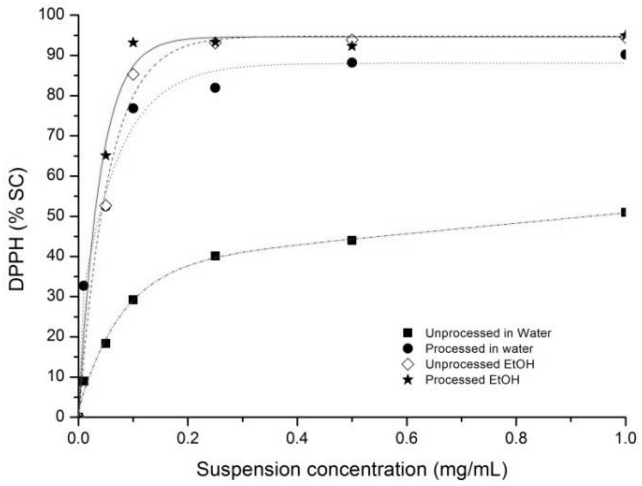

Figure 2. Radical Scavenging Activity of SAE mango extract, 40 °C and 15 MPa

The EC50 values of DPPH radical-scavenging activities were <0.05 mg/mL for processed and unprocessed samples in ethanol, however for water solutions these were 0.93 mg/mL for unprocessed and 0.037 mg/mL for processed. Lower EC50 means a higher free radical-scavenging activity of the compounds dissolved. Therefore, the processed mango extract possess a high potential to be applied as natural antioxidant reagent. Reducing the drug particles size is an effective and widely used approach to increase its solubility, by enlarging the effective surface area.

4. Conclusions

SAF was very effective to extract and recovery polyphenols from mango by-products without detrimental in the total phenol content and antioxidant activity. The dried powder extract represents additional advantages and the possibility of control the dimension of particles of extract. The SAF process can be easily incorporated in the industry, it is very fast and completed in a single step. The dry extract obtained can be used in the formulation of functional foods or nutraceuticals.

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