Pressurized Liquid Extraction of Antioxidant Compounds from Purple Corn (*Zea mays* L.) Waste: technical and economic viability studies

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ABSTRACT

Purple corn (*Zea mays* L.), a plant native to Peru, has been used by civilizations since pre-Incan times to make beverages and dye fabrics. Many studies have demonstrated that purple corn is a rich source of antioxidants that capture free radicals and prevent many degenerative diseases. The drink "chicha morada" is made by boiling purple corn in water, and the wastes from "chicha morada" production are currently discarded. Thus, the objective of this work was to evaluate the economic viability of obtaining antioxidant compounds from "chicha morada" production waste using innovative technologies, such as pressurized fluid extraction (PLE). PLE was carried out at three temperatures (313, 323 and 333 K), three pressures (2, 4 and 6 MPa) and three static extraction times (SET) (5, 10 and 15 min.) using ethanol as a solvent for a total of twenty-seven different extraction conditions. Extracts were evaluated for their global yield (X_0) and antioxidant capacity by measuring radical scavenging activity using the DPPH method. A five minute SET was found to be the best condition for both global yield and antioxidant capacity. Therefore, the effects of pressure and temperature on global yield and antioxidant capacity were evaluation with a SET of 5 min. An optimal tradeoff between extraction global and antioxidant compound yield was obtained at 333 K and 6 MPa. To determine the extraction time that minimizes the cost of manufacturing (COM), an overall extraction curve (OEC) was determined, and an economic evaluation along the OEC that considers 3 scenarios for process scale-up was performed. SuperPro v8.5 software was used to estimate the COM. The economic evaluation indicated the feasibility of an industrial PLE plant with a capacity greater than or equal to 0.05 m^3 for extraction of purple corn waste.

Keywords: Pressurized fluid extraction; Antioxidant, Anthocyanins, Purple corn.

INTRODUCTION

Recently, general interest in consuming food with health benefits has increased. Thus, the search for new fruits and vegetables that contain bioactive compounds with antioxidant activity has widened. Antioxidants are compounds that are able to scavenge free radicals produced by chemical reactions within our cells as well as radicals produced by environmental pollution, UV radiation, alcohol consumption, and consumption of food with chemical residues, preservatives, hormones, saturated fats, and many other compounds. Free radicals are responsible for diseases, such as coronary and respiratory diseases, cancer, and diabetes [1, 2].

Purple corn (*Zea mays* L.) is a native variety of corn from the Peruvian Andes that has been used since pre-colonial Peru for beverage preparation and dyeing of textile fibers [3]. Studies have shown that the anthocyanins in purple corn help fight cervical cancer [4]. Other studies report that there is a significant amount of phenolic compounds (phenols, anthocyanins, phenolic acids and flavonoids) in purple corn that can be used by the pharmaceutical industry [5]. Currently, "chicha morada," a beverage obtained by boiling purple corn in water, is the most consumed product of purple corn. The purple color characteristic of anthocyanins is observed intensively in waste from "chicha morada" production. This fact suggests that purple corn waste from "chicha morada" production could be a potential source of phenolic compounds. Food processing wastes represent an important source of bioactive compounds [6-10]. In many cases, these wastes are not reused and are discarded in sanitary landfills. Therefore, sustainable and environmentally friendly treatment of wastes and byproducts is becoming a very important issue to the food industry.

Pressurized liquid extraction (PLE) is an attractive and sustainable alternative for extraction of phenolic compounds. PLE involves increasing system pressure, which allows for the use of temperatures above the solvent's boiling point. The use of high temperatures increases mass transfer and extraction rates [11]. Additionally, PLE uses less solvent, can be performed in short times compared to conventional extraction; and enables the use of GRAS (Generally recognized as safe) solvents such as ethanol and water [12]. The PLE process has been shown to be highly efficient for the extraction of phenolic compounds with antioxidant activity [13]. Therefore, this work aimed to evaluate pressurized liquid extraction of antioxidants from "chicha morada" production waste, the influence of temperature, pressure and static extraction time on global yields and antioxidant activities and the economic feasibility of the PLE process with ethanol as the solvent.

MATERIALS AND METHODS

To select a raw material, social importance, availability, current demand, economic viability, ecological impact and potential yield of target compounds were considered. A matrix rich in bioactive compounds and functional properties was desired to provide high value products. Purple corn (*Zea mays* L.) cultivated in Cuzco city (Kculli variety) was obtained from the Central Market of Cuzco (Cuzco, Cuzco, Peru). Waste from "chicha morada" production, consisting of grains and cobs, was collected and stored in a domestic freezer. Size reduction of samples was performed using a knife grinder (Marconi, model MA340, Piracicaba, Brazil). Samples were then packed in a plastic bag and stored in a domestic freezer (Metalfrio, DA420, São Paulo, Brazil) at -18ºC.

PLEs were performed using a home-made PLE system described by Rodrigues et al. [14], which consists of: an HPLC pump (Thermoseparation Products, model 3200 ConstaMetric P/F, Fremont, USA), a manometer, an extraction vessel (Thar Designs, CL 1373, Pittsburg, USA), an electric heating jacket, a blocking valve and a backpressure valve (Figure 1).

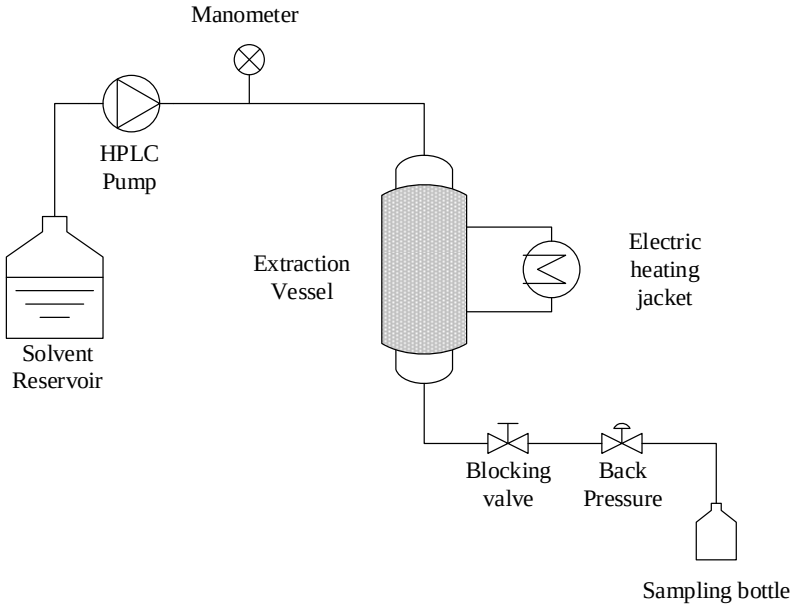

Figure 1 – Flow diagram of the homemade PLE unit used for the extractions.

A 6.3 cm^3 extraction vessel (internal dimensions: 2.0 cm diameter and 2.0 cm height) was completely filled with raw material. Extractions were performed with varying temperatures (313, 323 and 333 K), pressures (2, 4 and 6 MPa) and static extraction times (5, 10 and 15 min). A full factorial design (3×3×3) consisting of 27 experiments was used. Extractions were performed using ethanol (99.5% purity, Dinamica, Campinas, Brazil) at a flow rate of 2.6×10^{-5} kg/s. The rate between the mass of solvent (S) and mass of raw material (F), S/F, was kept constant (S/F = 8). The global extraction yield ($X_{0,\,S/F}$) was calculated as the percentage (%) of extract ($m_{extract}$) obtained from the total initial mass of raw material in dry base (M_{RM}) used to form the extraction bed as shown in Equation 1.

$$X_{0,S/F}\,(\%) = \frac{m_{extract}}{m_{RM}} \times 100 \qquad (1)$$

The radical scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) was used to determine the antioxidant activity (AA) of extracts according to the method of Kordali et al. [15]. A DPPH solution was prepared by dissolving DPPH in ethanol to a concentration of 60 µM. From this DPPH solution, a standard calibration curve with different DPPH concentrations (0, 10, 20, 30 40, 50 and 60 µM) was prepared. Then, different dilutions of PLE extracts in ethanol (5, 3 and 2 mg/cm^3) were prepared in tubes. A 0.1 cm^3 aliquot of each extract dilution was transferred to tubes with 3.9 cm^3 of DPPH solution (60 µM) and mixed in a shaker tube in the dark. A 0.1 cm^3 aliquot of ethanol was used as the control. Pure ethanol was used as a blank to calibrate the spectrophotometer. Readings were taken immediately at the beginning of the reaction and after 30 min at 517 nm in a UV-vis spectrophotometer (Femto, model 800XI, São Paulo, Brasil). The antioxidant activity of the extracts was

calculated by the percentage of inhibition (%PI) with an extract concentration of 0.2 mg/cm^3 according to Equation 2.

$$\%PI = \frac{(Abs^{Control} - Abs^{Amostra})}{Abs^{Control}} \times 100 \quad (2)$$

After determining the condition that resulted in the best global yield and antioxidant activity, a kinetic extraction, that is, a determination of the overall extraction curve (OEC) was performed. This OEC was subsequently used in the economic study. For this study, the cost of manufacturing (COM) at each timepoint on the OEC was estimated. SuperPro Designer v8.5 software was used for process simulation and economic evaluation. The PLE process flow diagram is shown in Figure 2. To estimate the COM for the crude extract, two scenarios were considered: a raw material cost of US$ 0.96/kg and, because a waste product from the food industry is being used, a raw material cost of US$ 0.00.

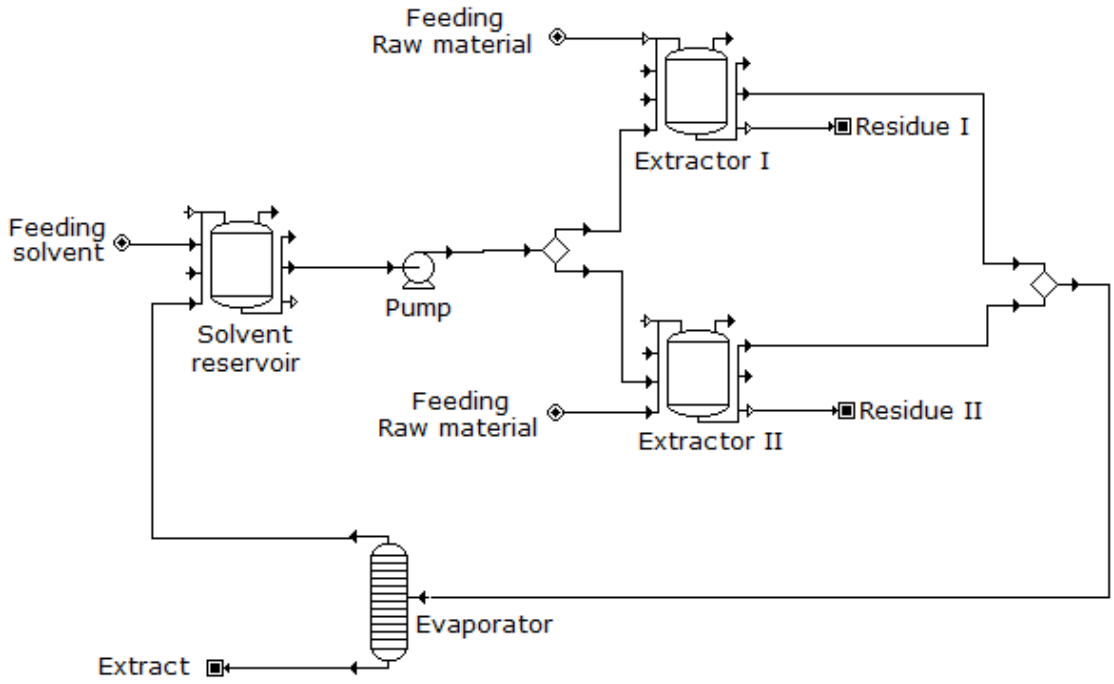

Figure 2 – Flows diagram for the PLE process designed in SuperPro Designer for the economic evaluation.

The main factors that contribute to the cost of manufacturing (COM) are similar to those described by Turton et al. [16]: the fixed cost of investment (FCI) and the operating cost, which consists of the cost of the raw material (CRM), cost of utilities (CUT) and cost of operating labor (COL). The PLE process was designed to operate 7,920 h per year, which corresponds to 330 days per year of continuous operation, 24 h per day. For scale-up, the procedure assumed that the industrial-scale unit had the same performance as the laboratory-scale unit. This study considered extractors with capacities of 0.05, 0.3 and 0.5 m^3.

RESULTS

Figure 3 shows the global yield isotherms and antioxidant activity of extracts. From the Analyzes of Variance (ANOVA), it was concluded that no variable had a statistically significant effect (p>0.05) on global yield and antioxidant yield. Nevertheless, an analysis of the effects of the main variables (temperature, pressure, static extraction time) was performed. A static extraction time of 5 min had a positive influence on global extraction yield and the AA of extracts. Thus, a static extraction time (SET) of 5 min was fixed, and the influence of temperature and pressure on global yield and AA of extracts was evaluated (Figure 3).
Figure 3 shows that increasing temperature (313-333 K) significantly influenced global yield ($X_{0,S/F=8}$) but did not influence the AA of extracts. However, temperatures above 353 K can lead to degradation by hydrolysis [13, 17]. The effect of pressure on the PLE process is not consistent. Studies developed by Luthria [18] and Muckhopadhyay et al. [19] showed that extraction pressure does not significantly influence the PLE of phenolic compounds.

In this study, the increasing pressure (2-6 MPa) resulted in an increase and then a decrease in the concentration of compounds with AA in the extracts. The high global yield and low concentration of antioxidant compounds in extracts obtained at 4 MPa can be explained by co-extraction of other polar compounds at this pressure. The results obtained is this work agree with those obtained by Cardoso et al. [17], who studied anthocyanin extraction by PLE from *Solanum stenotomun* skin: high global yield was obtained at 333 K, 4-6 MPa with a 5 min SET; however, only an extraction condition of 333 K, 6 MPa with a 5 min SET provided extracts rich in AA compounds.

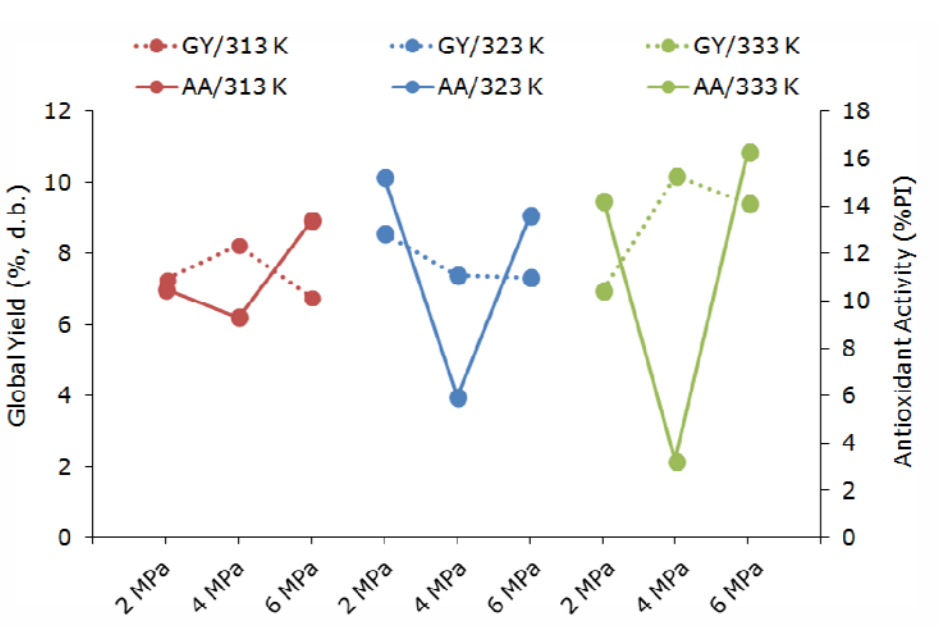

Figure 3 – Global yield ($X_{0,S/F=8}$) and antioxidant activity (%PI) of PLE extracts obtained from purple corn waste with a 5 min static extraction time.

Figure 4 shows the manufacturing cost (US$/kg of extract) of purple corn waste extracts obtained by PLE at 333 K and 6 MPa using extractor vessels with capacities of 0.05, 0.3 and 0.5 m^3. The COM of extracts decreases as the scale increases; however, when extractor vessels with capacities of 0.3 and 0.5 m^3 are used, the COM of extracts remained constant. The lowest COM of purple corn waste extracts, obtained from raw materials with a cost of US$ 0.96 is observed for a 30 min extraction. The COM of extracts obtained with this extraction condition is greater than the extract's selling price on the international market (US$ 18.05/kg powder extract), making the process economically unfeasible.

An evaluation of the factors that affect the COM of extracts obtained at different scales was performed. Figure 5 shows that the COM of extracts is mainly affected by CRM, followed by CUT, COL and lastly FCI. FCI showed the lowest influence on the COM of purple corn waste extracts. A new economic analysis was performed assuming a CRM of zero (US$ 0.00) because the raw material is a waste that does not require any treatment except the grinding. The COM of extracts obtained with a 0.05 m^3 extractor vessel decreases to a value close to the selling price. For larger extractor vessels, the COM further decreases, resulting in an economically feasible process. The lowest COM is obtained for an extraction time of fifteen minutes.

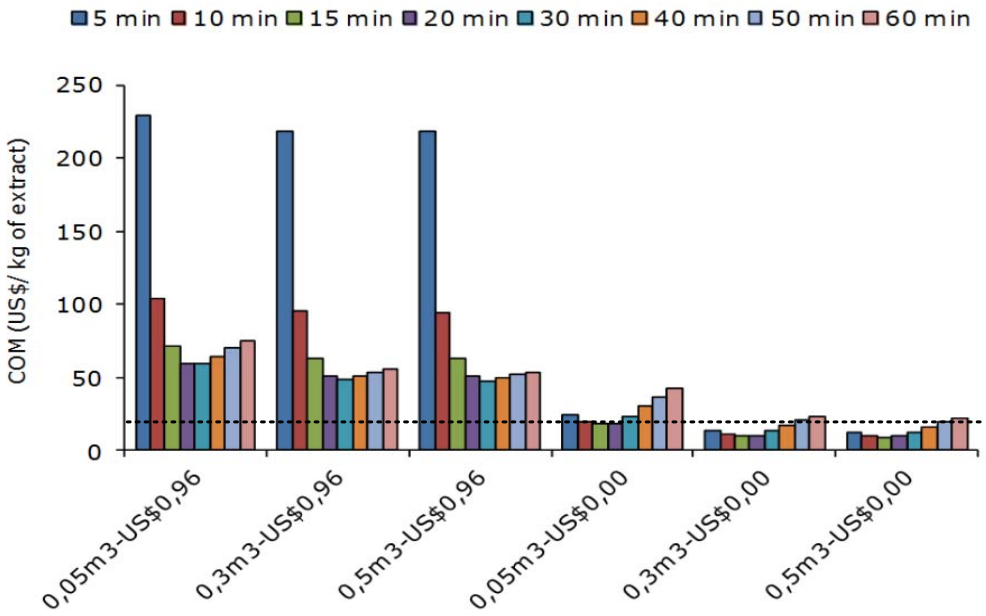

Figure 4 – COM estimation for purple corn waste extracts obtained by PLE from raw materials costing US$ 0.96/kg and US$ 0.00/kg. The dotted line represents the selling price of purple corn extract on the international market (US$ 18.05/kg powder extract).

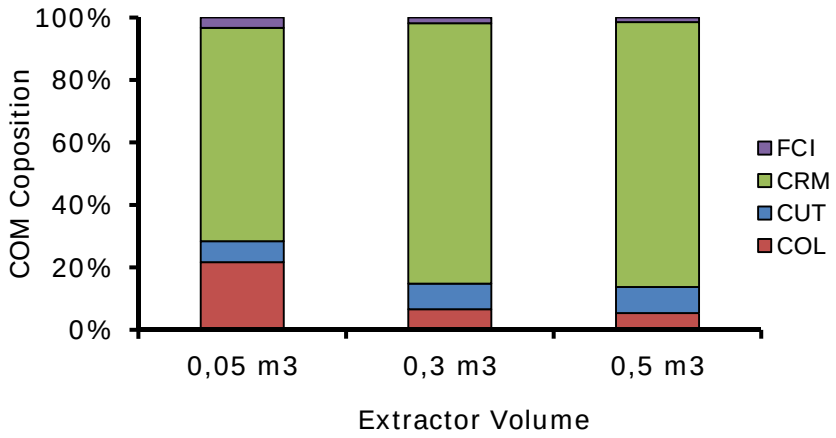

Figure 5 – Contribution of each factor to the COM for purple corn waste extracts obtained by PLE with 30 min of extraction using 0.05, 0.3 and 0.5 m^3 extractor vessels. FCI = Fixed cost of investment, CRM = Cost of raw material, CUT = Cost of utilities and COL = Cost of operating labor.

CONCLUSION

From an analysis of the global yield and AA of purple corn waste extracts obtained by PLE at different temperatures, pressures and static extraction times; it can be concluded that 333 K, 6 MPa and 5 min is the extraction condition that provides the best results. Obtaining purple corn waste extracts by PLE becomes economically feasible at 15 min of dynamic extraction for a raw material cost of US$ 0.00.

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