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Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction: Experimental data and economic evaluation

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ABSTRACT

Flame vine (*Pyrostegia venusta*) (FV), ice-cream-bean (*Inga edulis*) (IC), dog's knot (*Heteropterys aphrodisiaca*) (DK), and common bean (*Phaseolus vulgaris* L.) (BE) extracts were obtained by supercritical and subcritical fluid extraction (SFE) at 323 K and 35 MPa using pure carbon dioxide (CO_2), carbon dioxide with ethanol (CO_2 + EtOH) and carbon dioxide with water (CO_2 + H_2O). The extraction efficiency was evaluated by factoring in the solvent system and plant matrix influence in extraction yield (EY), antioxidant activity (AA), total phenolic content (TPC) and total flavonoid content (TFC). The manufacturing costs of crude extracts (COM_{EY}) and phenolic-rich fractions (COM_{TPC}) were also evaluated. The highest EY was achieved using CO_2 + H_2O, except for IC extracts. The BE extracts showed the highest TPC (338 mg GAE/g extract, dw), and the highest TFC was obtained for IC extracts using CO_2 (260 mg CE/g extract, dw). AA achieved higher values for FV extracts using CO_2 + EtOH (0.096 mL/mg). The lowest COM_{EY} and COM_{TPC} were obtained for FV and BE extracts using CO_2 + H_2O in a 0.5 m^3 extraction column.

1. Introduction

Some natural antioxidants are composed of phenolic compounds commonly found in plant materials; these substances provide a primary defense to the human body by eliminating free radicals. Antioxidants interfere with or deter the formation of free radical chain reactions, thus preventing the formation of hydroperoxides and protecting the cells of the human body from the attack of free radicals, which undergo oxidation with natural oxygen (Karakaya and Kavas, 1999). The levels of these individual compounds vary depending on the source and can be altered by processing conditions, storage, and the extracting solvent (Kalt, 2005). Phenolic compounds may be useful in food processing due to their high antioxidative activities (Rice-Evans et al., 1997) and abundance in the plant kingdom. They are of particular interest for applications in the areas of functional foods and nutraceuticals (Shi et al., 2005) because they act as preserving agents to protect the human body system against degenerative diseases caused by oxidative damage, including aging, cataracts, cancer and cardiovascular, neurodegenerative and inflammatory diseases (Nijveldt et al., 2001).

Pyrostegia venusta leaves have been used in traditional folk medicine as an infusion or decoction and are administered orally as a general tonic for the treatment of coughs and common diseases of the respiratory system related to infections, such as bronchitis, flu and colds (Roy et al., 2011; Veloso et al., 2010), as well as treatment of white patches and infections on the skin (diarrhea, vitiligo and coughs) (Moreira et al., 2012; Roy et al., 2011). The leaves contain phenolic compounds, syringyl groups, flavonoids (quercetin), catechin and biochanin, sterols (cholesterol, β-sitosterol, stigmasterol) (Duarte and Jurgensen, 2007; Santos and Blatt, 1998; Roy et al., 2011), allantoin (Moreira et al., 2012) and rutin (Santos and Blatt, 1998). Furthermore, all the anti-inflammatory actions obtained are also supported by the presence of acacetin-7-*O*-β-glucopyranoside and β-sitosterol (Shen et al., 2010; Veloso et al., 2012).

Inga edulis leaves have been reported to contain high levels of phenolic compounds and antioxidative properties (Pompeu et al., 2012; Silva et al., 2007a; Dias et al., 2010; Souza et al., 2008), with high pharmacological potential, in particular antiulcer activity (Pompeu et al., 2012). Decoctions of the leaves and bark are used as an astringent for diarrhea and as a lotion for arthritis and rheumatism (Silva et al., 2007a; Pompeu et al., 2012). Their leaves are also used in folk medicine due to their diuretic and anti-inflammatory properties (Silva et al., 2007a; Souza et al., 2007). Studies on the chemical profile of *I. edulis* leaf extracts revealed the presence

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of gallic acid, catechin, epicatechin, quercetin, myrecitin, myricetin-3-O-α-L-rhamnopyranoside, quercetin-3-O-α-L-rhamnopyranoside, quercetin-3-O-α-L-glucopyranoside (Souza et al., 2007; Dias et al., 2010), procyanidins B1, procyanidins B2, delfinidin and cyanidin (Dias et al., 2010).

A root infusion of *Heteropterys aphrodisiaca* O. Mach is used in Brazilian traditional medicine as a tonic or stimulating treatment, for nervous debility and muscle and bone weakness (Monteiro et al., 2011; Pott and Pott, 1994). On the other hand, *H. aphrodisiaca* is one of the most famous aphrodisiacs in Midwestern Brazil (Pott and Pott, 1994). Its root has beneficial effects on memory, sexual vitality (Galvão et al., 2002) and vasodilatation properties. This plant is traditionally used as antioxidant, as an anti-rheumatic and to increase fertility (Monteiro et al., 2008). Qualitative analysis of the plant extract revealed glycosides, polyphenols, tannins, alkaloids, saponins and anthracene (Galvão et al., 2002; Paula-Freire et al., 2013). Paula-Freire et al. (2013) also detected the flavonoids catequin and taxifolin, and triterpenoids in *H. aphrodisiaca* roots.

Another raw material that is rich in antioxidants is *Phaseolus vulgaris* L., which is a valuable source of bioactive compounds, among other components; recent studies suggest that dry beans are rich in tocopherols, flavonoids, polyphenols, and phenolics (Boschin and Arnoldi, 2011; Sutivisedsak et al., 2011, 2010; Tsuda et al., 1994). Its polyphenolic compounds are responsible for its anticancer activity (Xu and Chang, 2012), mainly due to proanthocyanidins, the most widely distributed flavonoids in beans (Beninger and Hosfield, 2003). Additionally, a high consumption of beans is believed to reduce the incidence of cardiac diseases, diabetes, colon cancer and hypertension (Ross et al., 2009; Valdez-Ortiz et al., 2012; Campos-Vega et al., 2009).

Conventional extraction methods of phenolic compounds can be found in the scientific literature. These techniques have limitations in obtaining solvent-free extracts (Daood et al., 2002), presenting disadvantages such as degradation or loss of target compounds, consumption of large volumes of organic solvents, and long extraction times. Supercritical and subcritical fluid extraction has great advantages including high selectivity, low viscosity, high diffusivity and high solvent power. Supercritical fluid extraction (SFE) has shown increasing importance in international markets, generating technological improvements mainly in sectors connected to the food, pharmaceutical, and chemical industries (Moura et al., 2009).

In spite of the well-known advantages of the process, such as high-quality products, SFE has economic constraints due to the high investment cost inherent to high pressure processes (Martinez et al., 2007). However, recent studies have shown that supercritical extraction may be economically viable to obtain vegetable extracts (Leitão et al., 2013; Prado et al., 2012). To transfer this technology to industry, an analysis of its economic feasibility must be performed.

In this context, the use of simulators allows estimating the manufacturing cost of a product, in addition to facilitating the design and transfer of process technology. Therefore, the use of simulators as an alternative to setting up a process and maintaining operating conditions allows a reduction of the costs and time consumed in laboratory and pilot plant efforts (Petrides et al., 2002).

This work was based on a preliminary study published by Veggi et al. (2011), who investigated the effect of ethanol addition on antioxidant activity and rapid cost estimation of the extracts obtained by supercritical CO_2 extraction from five Brazilian plant matrices. In this context, the current study aimed to perform an economic feasibility study and chemical profile evaluation of SFE using carbon dioxide (CO_2), carbon dioxide with ethanol (CO_2 + EtOH) and carbon dioxide with water (CO_2 + H_2O) to obtain phenolic-rich extracts from four different Brazilian plants. The efficiency of the solvent extraction systems was determined considering the extraction yield, total phenolic and flavonoid contents, antioxidant activity and cost of manufacturing. The optimum raw material and solvent system combination was determined by principal component analysis and Pearson correlation's matrix. The processes and cost models were developed using the simulation software SuperPro Designer® 6.0 (Intelligen Inc., Scotch Plains, NJ). The qualitative composition of the extracts was determined by thin layer chromatography.

2. Materials and methods

2.1. Chemical reagents

All chemicals used to prepare the reagent solutions were of analytical reagent grade. Dry carbon dioxide (99.9%) was purchased from Gama Gases (Campinas, Brazil). Sodium nitrite (99.5%), aluminum chloride (99.9%), sodium hydroxide (99.5%), glacial acetic acid (99.7%), ethanol (99.5%) and anhydrous sodium carbonate (99.5%) were obtained from Ecibra (Santo Amaro, Brazil). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), rutin (⩾95.0%), quercetin dehydrate (⩾98.0%), catequin (⩾99.0%), β-sitosterol (99.0%) and gallic acid (⩾95.0%) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Folin-Ciocalteu reagent was obtained from Dinamica (São Paulo, Brazil). DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was purchased from Sigma–Aldrich (Steinheim, Germany). Thin layer chromatography (TLC) 20 cm × 20 cm silica gel aluminum plates with and without a 254 nm fluorescent indicator, methanol (99.8%), hexane, ethyl acetate, chloroform, and anisaldehyde (97.5%) were obtained from Merck (Darmstadt, Germany).

2.2. Raw material characterization and preparation

The raw materials FV, IC, and DK were purchased from Superextra (São Paulo, Brazil). The BE (BRS graphite genotype) used in this work were donated by Embrapa Arroz e Feijão (Santo Antônio de Goiás, Brazil). All raw materials were ground in a knife mill (Marconi, model MA 340, Piracicaba, Brazil) for 10 s at 21,500 rpm. Each material was packed and identified in plastic bags and stored in a domestic freezer (Double Action, Metalfrio, São Paulo, Brazil) at 258 K. Particle size analysis was performed using a vibratory sieve system (Model N1868, Bertel, Caieiras, Brazil) using sieves from 16 to 80 mesh (Model ASTME-11, W.S. Tyler, Wheeling, WV, USA). Particles of 24-48 mesh were selected for the SFE assays. The mean particle diameter (d_p) was obtained by the Standard S319.3 method (ASAE, 2000) according to Eq. (1), as follows:

$$d_p = \log^{-1}\left[\frac{\sum_{i=1}^{n}(w_i \log \bar{d}_i)}{\sum_{i=1}^{n} w_i}\right] \quad (1)$$

The moisture was determined by the xylol distillation method (Jacobs, 1973). The real particle density (ρ_r) was determined by pycnometry with helium gas (Micrometrics, Multivolume Pycnometer 1305, Norcross, GA, USA) in the Analytical Center of the Institute of Chemistry at University of Campinas (Campinas, SP, Brazil). The apparent bed density (ρ_a) was calculated using the mass of the sample loaded into the extraction cell and its internal volume. The moisture and apparent bed density were obtained by 2 replicates, while the mean particle diameter and real particle density were determined using 10 replicates.

2.3. Extraction procedure

SFE assays were conducted using two units: (i) The Spe-ed system (Applied Separations, model 7071, Allentown, USA) was used for extraction with pure CO_2; (ii) The SFE-I system equipped with

Table 1
Raw material, bed characterization and extraction parameters used in SFE assays at 323 K and 35 MPa.

	CO_2				CO_2 + EtOH				CO_2 + H_2O			
	DK	BE	IC	FV	DK*e*	BE*e*	IC*e*	FV*e*	DK*w*	BE*w*	IC*w*	FV*w*
Raw material characterization												
Moisture (%)[a]	13 ± 3	13.29 ± 0.06	11.0 ± 0.6	10 ± 2	13 ± 3	13.29 ± 0.06	11.0 ± 0.6	10 ± 2	13 ± 3	13.29 ± 0.06	11.0 ± 0.6	10 ± 2
d_p (10^{-4} m)[b,c]	9.97	9.91	9.96	9.97	9.97	9.91	9.96	9.97	9.97	9.91	9.96	9.97
ρ_r (kg/m^3)[b,d]	1204 ± 40	1450 ± 2	1421 ± 15	1244 ± 12	1204 ± 40	1450 ± 2	1421 ± 15	1244 ± 12	1204 ± 40	1450 ± 2	1421 ± 15	1244 ± 12
Bed characterization												
F (g)[a,e]	3.21 ± 0.03	5.56 ± 0.02	3.35 ± 0.04	2.82 ± 0.04	10.09 ± 0.001	10.10 ± 0.04	10.07 ± 0.03	10.06 ± 0.02	10.07 ± 0.04	10.11 ± 0.07	10.05 ± 0.03	10.06 ± 0.02
ρ_a (kg/m^3)[a,f]	490 ± 3	845 ± 2	507 ± 2	426 ± 4	415 ± 4	721 ± 2	370 ± 3	352 ± 2	415 ± 3	721 ± 4	370 ± 2	352 ± 3
Porosity	0.592 ± 0.1	0.417 ± 0.1	0.643 ± 0.1	0.656 ± 0.1	0.655 ± 0.1	0.503 ± 0.1	0.739 ± 0.1	0.716 ± 0.1	0.655 ± 0.1	0.503 ± 0.1	0.739 ± 0.1	0.716 ± 0.1
Volume (cm^3)	6.53	6.51	6.51	6.57	24.13	13.86	27.01	28.39	24.13	13.86	27.01	28.39
Extraction parameters												
Total time (min)	40 ± 2	40 ± 2	40 ± 2	40 ± 2	75 ± 2	75 ± 2	75 ± 2	75 ± 2	75 ± 2	75 ± 2	75 ± 2	75 ± 2
Q_{CO2} (10^{-4} kg/s)[a,g]	0.54 ± 0.01	0.54 ± 0.01	0.54 ± 0.02	0.54 ± 0.01	1.12 ± 0.03	1.11 ± 0.02	1.12 ± 0.02	1.11 ± 0.02	1.12 ± 0.01	1.11 ± 0.02	1.11 ± 0.01	1.11 ± 0.02
$Q_{modifier}$ (10^{-5} kg/s)[a,h]	–	–	–	–	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
S/F total (kg solvent/kg raw material)[i]	50 ± 1	50 ± 0	50 ± 1	50 ± 0	50 ± 1	50 ± 0	50 ± 1	50 ± 0	50 ± 1	50 ± 0	50 ± 1	50 ± 0

e: CO_2 + EtOH; *w*: CO_2 + H_2O.
[a] Values presented with standard deviation of two determinations.
[b] Values presented with standard deviation of 10 repetitions.
[c] Mean particle diameter (d_p).
[d] Real density of particles (ρ_r).
[e] Raw material mass (F).
[f] Bed apparent density (ρ_a).
[g] CO_2 flow rate (Q_{CO2}).
[h] modifier flow rate ($Q_{modifier}$).
[i] Solvent to feed ratio (S/F); DK (dog's knot); BE (common bean); IC (ice-cream-bean); FV (flame vine).

a 415 cm^3 extraction vessel (3.4 cm diameter and 37.5 cm height, internal dimensions) was used for the extraction using CO_2 with 10% (w/w) of modifiers (ethanol and distilled water). The raw material was placed inside the extractor vessel with the aid of a nylon cell presenting approximately the same diameter as the vessel. To fill the extraction vessel completely, the empty space of the extraction vessel was filled with glass beads with a diameter of 8–10 mesh. The bed characteristics and extraction parameters used in the SFE assays are described in Table 1. The temperature and pressure (323 K and 35 MPa) used for the extractions were selected from the literature because moderate temperature and high pressure generally are associated with low thermal degradation and high solubility of the target compound (Saldaña et al., 1999). The ethanol was removed from the extracts using a rotary evaporator (Heidolph, Laborota 4001, Viertrieb, Germany) connected to a vacuum pump (Heidolph, Rotavac, Viertrieb, Germany). The removal of water from the extracts was performed by freeze-drying in a bench lyophilizer (Liotop L101, Liobras, São Carlos, Brazil). Extraction yields (EY) were calculated as the ratio between the total extract mass and the raw material mass loaded into the extractor on a dry weight (dw) basis. The assays were carried out in duplicate.

2.4. Analyses of the extracts

2.4.1. Thin layer chromatography (TLC)

Identification of flavonoids, terpenoids and antioxidant compounds was performed according to the methodology presented by Wagner and Bladt (2001). The standards used were quercetin, gallic acid, and β-sisterol. The standards were applied on the plates at a concentration of 2 mg/cm^3. The samples were diluted in ethanol, methanol and water. It was observed that SFE extracts were well-solubilized at a concentration of 10 mg/cm^3 in ethanol for CO_2 and CO_2 + EtOH and in methanol for the CO_2 + H_2O extracts. All extracts showed precipitate when diluted in water. The CO_2 + H_2O extract also presented precipitation when diluted in ethanol. The sampling was performed drawing up about 10 μL of each extract solution into the Kimble micro-pipettes by capillary action. The micro-pipettes were touched 2–3 times on the TLC plate to draw the solution onto the plate resulting in a TLC spot of 1–2 mm of diameter. The mobile phases were selected according to the class of the compounds of interest to be detected: hexane and ethyl acetate at 80:20 (v/v) for the CO_2 and CO_2 + EtOH extracts; and chloroform, methanol and water at 60:30:10 (v/v) for the CO_2 + H_2O extracts. The flavonoid and alkaloid plates were observed using an ultraviolet chamber (Mineralight® lamp, model UVGL-58 Multiband UV-254/366 nm, Upland, USA) at 254 and 366 nm. To visualize the terpenoids, the plates were heated at 373 K until the compounds could be fully observed in the ultraviolet chamber. The DPPH plate was exposed for 30 minutes to natural light to verify the presence of yellow spots against the purple background, which indicated the presence of compounds with antioxidant activity.

2.4.2. Total phenolic content (TPC)

Total phenolic content was determined by the Folin–Ciocalteu method described by Singleton and Joseph (1965), with some adaptations for plant extracts suggested by Singleton et al. (1999). The sample solutions were diluted in ethanol at a concentration of 0.10 mg/cm^3 for the CO_2 and CO_2 + EtOH extracts and in methanol at a concentration of 1.0 mg/cm^3 for the CO_2 + H_2O extracts. The standard curve calibration solutions were prepared using gallic acid (0.05–0.50 mg/cm^3). A 1:10 dilution of Folin–Ciocalteu reagent was made in distilled water. The sodium carbonate was diluted with distilled water to a final concentration of 75 mg/cm^3. The reactions were conducted in the dark by mixing 2 cm^3 of the new dilutions (calibration and sample) with 10 cm^3 of the Folin–Ciocalteu solution. After 1 minute had elapsed and before 8 minutes, 8 cm^3 of saturated sodium carbonate solution was added to the mixture, which was placed in a thermal bath (Marconi model MA 127/BO, Piracicaba, São Paulo, Brazil) at 50 °C for 5 minutes; the absorbance was then immediately read at a 760 nm wavelength on a UV–visible spectrophotometer (Hitachi, model U-3010, Tokyo, Japan). TPC was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract in dw. The assays were carried out in triplicate.

2.4.3. Total flavonoid contents (TFC)

Flavonoid content was determined using the colorimetric method developed by Zhishen et al. (1999). The sample solutions were diluted as described previously in Section 2.4.2. An aliquot of 1 cm^3 of the diluted sample and standard solution was added to a 10 cm^3 volumetric flask containing 4 cm^3 of distilled water. At time zero, 0.3 cm^3 of 5% $NaNO_2$ was added to the flask. After 5 minutes, 0.3 cm^3 of 10% $AlCl_3$ was added to the flask. At 6 minutes, 2 cm^3 of 1 M NaOH was added to the mixture. Immediately, 2.4 cm^3 of distilled water was added to the flask and the solution was mixed vigorously. The absorbance of the mixture was determined at 510 nm on a UV–visible spectrophotometer (Hitachi, model U-3010, Tokyo, Japan). The total flavonoid content was expressed as milligrams of catequin equivalents (CE) per gram of extract in dw. The assays were carried out in triplicate.

2.4.4. DPPH free radical scavenging activity

The antioxidant activity was determined by the free radical-scavenging activity of the extracts using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical solution (60 μM) in ethanol according to Kordali et al. (2005). The standard calibration curve was prepared at different concentrations of DPPH (0–60 μM). The sample solutions were diluted in ethanol for the CO_2 and CO_2 + EtOH extracts and in methanol for the CO_2 + H_2O extracts, at a concentration of 0.05–0.20 mg/cm^3. Subsequently, in the dark, an aliquot of 0.1 cm^3 of each dilution of the extract was transferred to tubes with 3.9 cm^3 of DPPH solution and vigorously mixed in a shaker tube. Ethanol was used as a blank to calibrate the spectrophotometer. The readings were taken immediately after mixing the reagents and after 90 minutes of reaction; the absorbance was measured at 517 nm using a UV–visible spectrophotometer (Hitachi, model U-3010, Tokyo, Japan). All assays were carried out in triplicate. The results were expressed in three different ways.

(i) Scavenging ability (SA) expresses the percentage of inhibition of DPPH radical, as shown in Eq. (2):

$$\%SA = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \cdot 100 \quad (2)$$

where $Abs_{control}$ and Abs_{sample} are the absorbance values of the control and the sample, respectively.

(ii) DPPH values were calculated using a linear regression equation in the range of 50–400 μM of Trolox standards. The relative scavenging capacity of the extracts was expressed as Trolox equivalent antioxidant activity (TEAA) (μmol TE g^{-1} extract, dw).

(iii) The inverse half maximal effective concentration (EC_{50}^{-1}), where EC_{50} is the concentration of extract needed to decrease 50% of the maximal absorbance observed for the DPPH control at 0 min of reaction time, was obtained by linear regression of a dose-response curve plotting percent

inhibition and extract concentration. Three different dilutions (0.05–0.20 mg/cm^3) were prepared in vials and evaluated in triplicate.

2.5. Economic evaluation

The process, scale-up and economic simulation was carried out using the commercial software SuperPro Designer® version 6.0 (Intelligen Inc., Scotch Plains, NJ, USA). Process simulations of CO_2 (Fig. 1a) and CO_2 + EtOH (Fig. 1b) were performed according to Prado et al. (2009). The methodology was modified for the $CO_2 + H_2O$ (Fig. 1c) solvent system because freeze-drying equipment was needed to remove the water from the extract. The simulations using CO2 as a solvent were performed for an industrial-scale unit with one CO2 reservoir, a heat exchanger, one CO2 pump, two extraction columns, one flash tank, and one

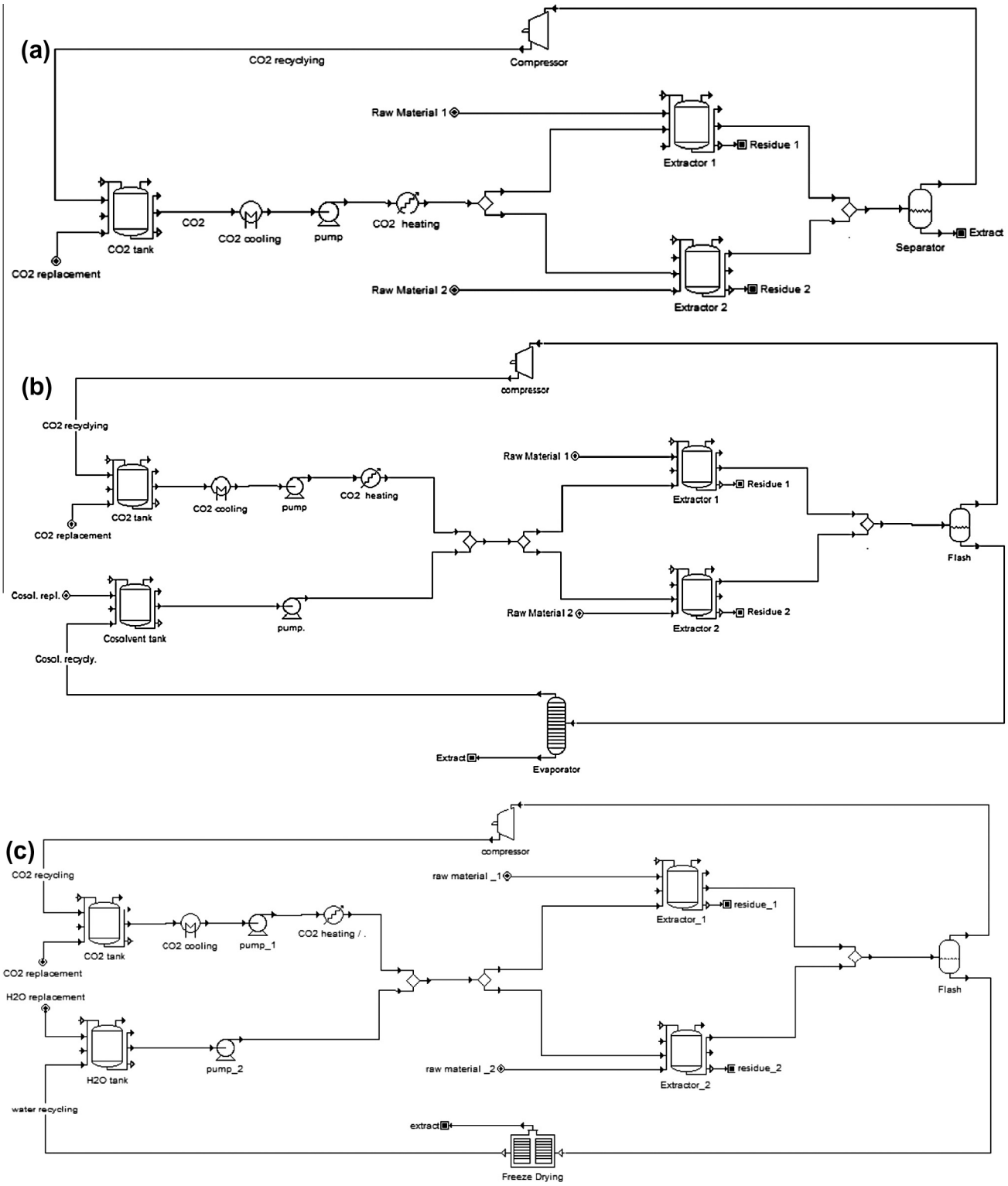

Fig. 1. Flowsheet of the SFE using CO_2 (a), CO_2 + EtOH (b) and $CO_2 + H_2O$ (c), designed by the SuperPro Designer® 6.0 simulator used for economic analysis.

Table 2
Economic parameters used for COM estimation.

Industrial SFE units[a]	Chinese market
2 extractors of 5 L	US$ 95,000.00
2 extractors of 50 L	US$ 380,000.00
2 extractors of 500 L	US$ 1,500,000.00
Depreciation rate	10%/year
Industrial Freeze Dryer units[a]	
Drying area (m^2)	Chinese market
20	US$ 38,000.00
200	US$ 150,000.00
300	US$ 192,000.00
1500	US$ 500,000.00
2000	US$ 590,000.00
3000	US$ 750,000.00
Labor	US$ 4.00/h
2 extractors of 5 L[b]	1 operator
2 extractors of 50 L[b]	2 operators
2 extractors of 500 L[b]	3 operators
Raw materials	
Flame vine (FV)[c]	US$ 15.50/kg
Ice-cream-bean (IC)[c]	US$ 50.00/kg
Bean (BE)[d]	US$ 2.20/kg
Dog's knot (DK)[e]	US$ 26.00/kg
Pre-Processing	US$ 40.00/ton
CO_2 (2% loss)[a]	US$ 2.30/kg
Ethanol	US$ 0.716/L
Water	US$ 0.04/kg
Utilities	
Electricity[f]	US$ 0.092/kW h
Cooling water	US$ 5.15/ton
Steam	US$ 20.00/ton

[a] Direct quotation.
[b] Prado et al. (2009).
[c] Superextra Brasil (2012).
[d] Agrolink (2012).
[e] Santosflora (2012).
[f] CPFL (2011).

CO2 compressor for recycling CO2. In the case of solvent systems using ethanol and water as modifiers, an extra pump and solvent tank was needed. Moreover, an evaporator was necessary to remove ethanol from the extracts obtained by CO_2 + EtOH, while the water removal was performed by freeze-drying equipment. In all SFE processes, the mass of extract is reported as dw and the CO_2 and modifiers are recycled.

The economic data fed to the simulator are presented in Table 2. The scale-up was evaluated for extraction columns with capacities of 0.005, 0.05 and 0.5 m^3, all with the same process design for each solvent system flowsheet. The scale-up criterion assumed that the industrial-scale unit has the same performance as the laboratory-scale unit when the solvent to feed mass ratio (S/F) and operational conditions (temperature, pressure, extraction time, porosity and apparent bed density) are kept constant.

A series of factors influence the COM and can be divided into the following categories: direct costs, fixed costs, and general expenses. The direct costs take into account factors that depend directly on the production rate, such as the raw material, the utilities, and the operation costs. The fixed costs do not directly depend on the production rates and are considered even when the operation is interrupted; some examples are taxes, insurance, and depreciation. General expenses are those deemed necessary to keep the business in operation, and these include administration and marketing costs, and selling, as well as research and development expenses (Turton et al., 2003).

The process was designed to run 7920 h per year, which corresponds to 330 days per year of continuous 24 h per day shifts (Rosa and Meireles, 2005). The number of operators needed per shift varies according to the capacity of the plant (Table 2). Labor charges and labor not directly associated with production were estimated by the simulator (Vieira et al., 2013; Leitão et al., 2013). The raw material cost is related to the acquisition of the plant matrix, as well as CO2 and modifiers (when used) lost during the process. CO2 loss is mainly due to depressurization of the extractor at the end of each batch, while ethanol and water loss is due to the distillation and lyophilization processes. Pre-processing costs are related to the drying and comminution of raw material. The cost of utilities is primarily due to heat exchange agents and the electricity consumed in the process. Utilities needed for the operation of each part of the equipment were estimated by the simulator energy balance. The cost of waste treatment may be neglected because the residue of the SFE process is a harmless bioresidue, which may be incorporated into the soil. According to Boechat et al. (2012), soil incorporation of organic residue material is a desirable disposal alternative for waste treatment with negligible costs when compared to other disposal methods. In addition, the waste material is usually beneficial for the soil and for crop production. Organic materials contain nutrients needed for crop growth and improve soil tilth, increase water holding capacity, lessen wind and water erosion, improve aeration, and promote soil biological activity (Piotrowska et al., 2011). The CO2 lost during system depressurization needs no treatment because in small quantities it is not toxic. Transportation costs still need to be added to the estimated COM. The COM was estimated for the crude extract (COM_{EY}, US$/kg of extract) and for the phenolic-rich fraction (COM_{TPC}, US$/g of phenolic compounds) of all SFE assays.

2.6. Statistical analysis

The data are presented as the mean ± standard deviation (SD) and were subjected to analysis of variance (one way ANOVA) by SAS for Windows, version 9.2 (SAS Institute Inc.) using Tukey's test at a level of 5% statistically significant differences (p-value < 0.05). A Pearson correlation test was used to determine the correlations between results. Principal component analysis (PCA) was performed by XLSTAT for Windows, trial version 2012 (Addinsoft, Paris, France) using the correlation matrix containing auto-scaled data (Souza et al., 2011; Cavalcanti et al., 2012; Cruz et al., 2013). The PCA score plot was used to assess the effect of extraction method and raw material used on the output parameters (EY, TPC, TFC, EC_{50}^{-1}, SA, TEAA, COM_{EY} and COM_{TPC}).

3. Results and discussion

3.1. Raw material and bed characterization

The results of moisture content, real particle density, porosity and apparent bed density of all raw materials, as well as the extraction parameters used in all SFE assays, are presented in Table 1.

3.2. Extraction yield (EY)

Table 3 presents the extraction yields obtained by SFE assays using CO_2, CO_2 + EtOH and CO_2 + H_2O. The CO_2 and CO_2 + EtOH results were previously reported by Veggi et al. (2011), who observed that supercritical CO_2 achieved lower yields than those obtained with the addition of modifiers, except for IC (1.51%, dw). Notably, the extraction yields for BE, FV, BE*e* and FV*e* reported by Veggi et al. (2011) were erroneously presented out of order; the correct values are reported here in Table 3. The extracts obtained by CO_2 + H_2O showed higher yields than CO_2 + EtOH, except for IC (1.31%, dw). According to Durling et al. (2007), CO_2 + H_2O is non-homogeneous solvent mixture that, at the operational conditions used, is considered a near-critical solvent instead a supercritical

Table 3
Extraction yield (EY), total phenolic content (TPC), total flavonoid content (TFC), DPPH scavenging ability (SA), inverse half maximal effective concentration (EC_{50}^{-1}) and Trolox equivalent antioxidant activity (TEAA) in SFE assays at 323 K and 35 MPa.

Solvent system	Sample	EY (% dw[1])	TPC (mg GAE/g extract. dw[1])	TFC (mg CE/g extract. dw[1])	EC_{50}^{-1} (mL/mg)	SA (%. dw[1])	TEAA (μmol TE/g extract. dw[1])
CO_2	DK	$0.78^{2} \pm 0.02^{b.B}$	$244 \pm 40^{a.A}$	$133 \pm 12^{b.A}$	$0.987 \pm 0.004^{d.B}$	$8.6 \pm 0.2^{a.B}$	$760 \pm 12^{b.A}$
	BE	$0.53^{2,*} \pm 0.01^{b.C}$	$164 \pm 26^{a.B}$	$143 \pm 6^{b.A}$	$0.249 \pm 0.000^{a.A}$	$9.9 \pm 0.1^{d.C}$	$851 \pm 9^{b.B}$
	IC	$1.51^{2} \pm 0.01^{a.B}$	$196 \pm 35^{a.A}$	$260 \pm 1^{a.A}$	$0.664 \pm 0.001^{c.A}$	$9.1 \pm 0.1^{b.C}$	$800 \pm 4^{b.AB}$
	FV	$1.18^{2,*} \pm 0.08^{ab.B}$	$233 \pm 23^{a.A}$	$145 \pm 14^{b.A}$	$0.648 \pm 0.002^{b.C}$	$12.3 \pm 0.9^{c.A}$	$1034 \pm 62^{a.A}$
CO_2 + EtOH	DK*e*	$2.44^{2} \pm 0.02^{b.B}$	$102 \pm 54^{a.AB}$	$48 \pm 5^{b.B}$	$2.232 \pm 0.014^{d.C}$	$19 \pm 11.4^{a.A}$	$1503 \pm 831^{a.A}$
	BE*e*	$1.54^{2,*} \pm 0.01^{c.B}$	$93 \pm 12^{a.C}$	$138.5 \pm 0.6^{a.A}$	$0.806 \pm 0.000^{b.B}$	$9.9 \pm 1.1^{c.B}$	$852 \pm 81^{a.B}$
	IC*e*	$2.75^{2} \pm 0.02^{a.A}$	138 ± 103^{aA}	$130 \pm 5^{a.B}$	$1.552 \pm 0.004^{c.C}$	$18 \pm 6^{b.A}$	$1442 \pm 405^{a.A}$
	FV*e*	$2.39^{2,*} \pm 0.06^{b.B}$	$274 \pm 2^{a.A}$	$128 \pm 2^{a.A}$	$0.096 \pm 0.000^{a.A}$	$11.9 \pm 0.4^{d.C}$	$1007 \pm 32^{a.A}$
CO_2 + H_2O	DK*w*	12 ± 2^{bA}	$15 \pm 9^{d.B}$	$14 \pm 2^{c.C}$	$0.139 \pm 0.000^{a.A}$	$2.1 \pm 0.7^{d.C}$	$283 \pm 53^{b.A}$
	BE*w*	$2.67 \pm 0.09^{c.A}$	$338 \pm 3^{a.A}$	$109 \pm 10^{a.B.}$	$1.390 \pm 0.001^{d.C}$	$25 \pm 5^{a.A}$	$1930 \pm 302^{a.A}$
	IC*w*	$1.31 \pm 0.02^{c.B}$	$110 \pm 11^{c.A}$	$58 \pm 6^{b.C}$	$0.800 \pm 0.001^{c.B}$	$2 \pm 2^{b.B}$	$245 \pm 107^{b.B}$
	FV*w*	18 ± 2^{A}	277 ± 13^{bA}	$130.5 \pm 0.9^{a.A}$	$0.258 \pm 0.000^{b.B}$	$2 \pm 1^{c.B}$	$304 \pm 73^{b.B}$

e: CO_2 + EtOH; *w*: CO_2 + H_2O; Results are presented as mean ± standard deviation. [a–d] Lowercase letters represent significant difference at 5% level of significance for samples evaluated for different raw materials using the same solvent. [A–C] Capital letters represent significant difference at 5% level of significance for samples evaluated for the same raw material using different solvents; DK (dog's knot); BE (common bean); IC (ice-cream-bean); FV (flame vine).
[1] Dry weight.
[2] Values reported by Veggi et al. (2011).
[*] These values of extraction yield were erroneously reported out of order by Veggi et al. (2011) and the correct values are reported here on this table.

fluid. Even for this solvent, FV and DK extracts showed very high yields. Using conventional extraction methods, Marques et al. (2007) found that water was the best solvent for the recovery of extracts from DK. According to some studies, water has been used as a modifier for the isolation of polar compounds. Iheozor-Ejiofor and Dey (2009) used water as a modifier, achieving a higher recovery of the target compounds due to the ability of water to interact with the groups at the ends of the polar molecules. In addition, water may increase the density of the fluid mixture (de Lucas et al., 2007) or even soften and swell the matrix structure, favoring the diffusion process (Li et al., 2003).

3.3. Thin layer chromatography (TLC)

3.3.1. Flavonoid detection

Fig. 2 presents the chromatographic plates of SFE extracts obtained from the plants at 35 MPa and 323 K. According to Wagner and Bladt (2001) in Fig. 2A, C, and D, the fluorescent bands (blue, red, bright green, violet and yellow) indicate the presence of flavonoids in the extracts using CO_2, CO_2 + EtOH and CO_2 + H_2O, respectively. No compounds were detected for the BE extract. It was not possible to detect a band for quercetin as identified by Souza et al. (2007) in the IC extract or for β-sisterol in the FV extract, as reported by Vivek et al. (2002). Therefore, under UV-254 illumination (Fig. 2B), the highlighted black spots, particularly for the extracts of BE, IC, FV and DK, confirm the presence of flavonoids. This observation contradicts the results of Menaker et al. (2004) and Catchpole et al. (2004), who reported that polar compounds such as flavonoids are not extractable by SFE using pure CO_2. The blue color is characteristic of phenolic carboxylic acids and, according to Pettit et al. (2003), indicates the presence of different structures of flavonoids in the ethanolic extract of the plant. According to Wagner and Bladt (2001), the green band is characteristic of the flavonoid kaempferol glycoside, and the violet band indicates the presence of flavonols. No band was detected for the IC extract using quercetin as identified by Souza et al. (2007) or for the FV extract using β-sisterol as obtained by Vivek et al. (2002). In Fig. 2D, a blue fluorescent band for the BE extract and a yellow fluorescent band for the IC extract can be observed. The profile composition for the CO_2 + H_2O extracts was quite different from those observed for CO_2 + EtOH and CO_2; it was not possible to characterize the large amount of compounds in these extracts.

3.3.2. Antioxidant activity (DPPH) detection

Fig. 2E, F, and G show the chromatography plates for the extracts using CO_2, CO_2 + EtOH, and CO_2 + H_2O, respectively, after exposure to DPPH, causing a shift from violet (characteristic of the radical) to yellow. After 1 hour of development of the plate shown in Fig. 2E, complete yellow bands indicating the presence of antioxidant compounds in the extracts were observed. The IC extract showed the highest concentration of antioxidants, followed by FV, BE and DK. In Fig. 2F, the bleaching of the bands indicating the presence of antioxidant compounds in the IC and FV extracts was of greater intensity. However, the clearing plaques characteristic of antioxidant compounds were more intense for the pure CO_2 extracts (Fig. 2E) than for the CO_2 + EtOH ones. Fig. 2G shows bleaching of the bands, indicating the presence of antioxidant compounds. Additionally, in the BE, FV and IC extracts, yellow spots were observed, though at a lower intensity. Comparing the behavior of the three extracts (CO_2, CO_2 + EtOH and CO_2 + H_2O) on the DPPH plates, an intense white band is present in the SFE extracts, confirming high antioxidant activity when pure CO_2 is used.

3.3.3. Terpenoid detection

The profile composition of the extracts using CO_2, CO_2 + EtOH, and CO_2 + H_2O for the detection of terpenoids (anisaldehyde) can be seen in Fig. 2H, I, and J, respectively. In Fig. 2H, fluorescent bands of red-brown, violet and violet-blue characterize alcohols, terpenes, and saponin glycosides. In Fig. 2I, the orange-brown and red-brown fluorescent bands typical of terpene alcohols and saponin glycosides can be observed. Although the bands obtained for the plates of the CO_2 + EtOH extracts are different from those observed with CO_2, the colors are characteristic of the same class of compounds. In Fig. 2J, strong gray, green and brown colors were observed under visible light; when subjected to UV-365 nm illumination, these compounds showed brown and purple bands, indicating the presence of terpenes.

3.4. Total phenolic content (TPC)

The phenolic content of the extracts obtained using CO_2, CO_2 + EtOH and CO_2 + H_2O are presented in Table 3. Flame vine extracts showed an increase in total phenolic content (TPC) with the incorporation of ethanol and water as modifiers (273.8 and 277.4 mg GAE/g extract, respectively) compared to CO_2 extracts

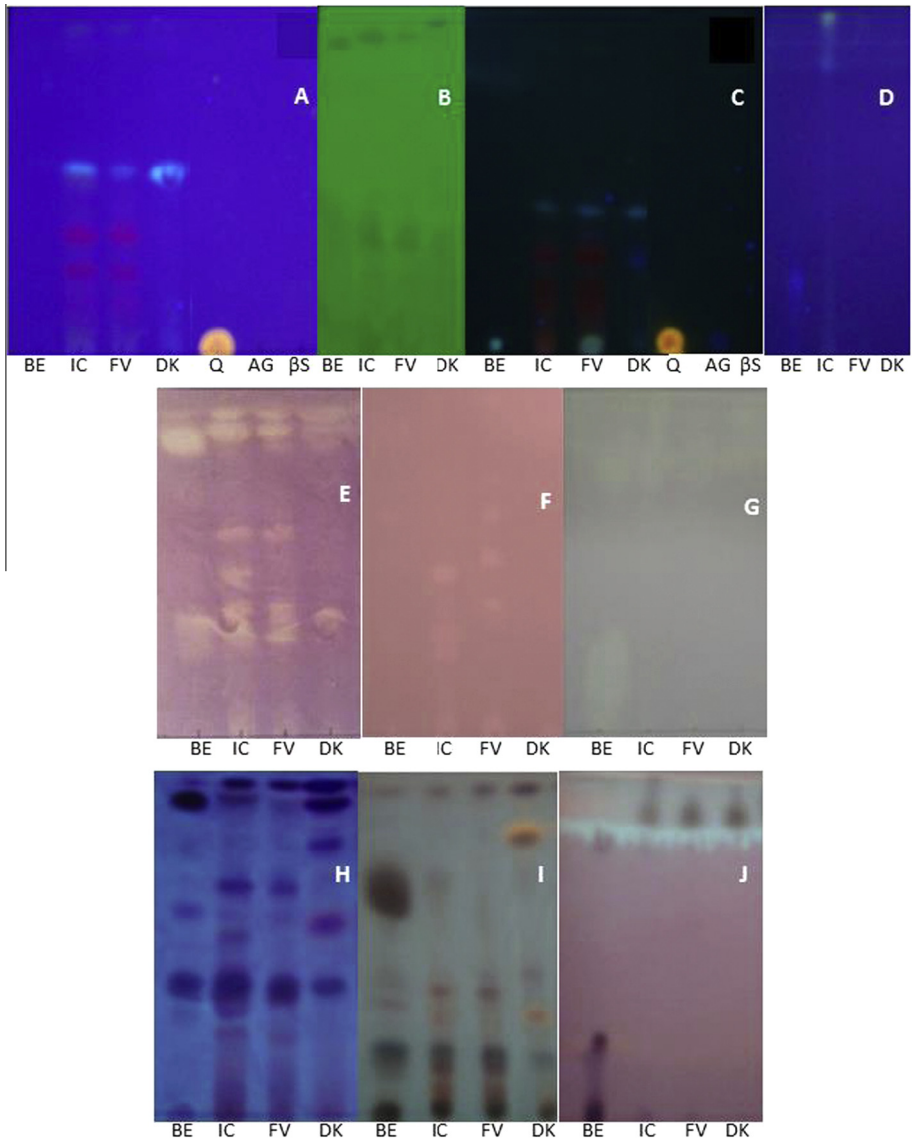

Fig. 2. Chromatographic plates of SFE extracts of bean (BE), ice-cream-bean (IC), flame vine (FV) and dog's knot (DK); gallic acid (AG) and β-sisterol (BS) standard sprayed with flavonoid reagent (A, B, C, D) under UV-365 nm (A, C, D) and UV-254 nm (B); DPPH reagent under visible light (E, F, G) and terpenoid reagent under 365 nm UV (H, I, J) for extracts obtained with CO_2 (A, B, E, H), CO_2 + EtOH (C, F, I) or CO_2 + H_2O (D, G, J).

(233.3 mg GAE/g extract) due to the enhanced polarity of the modifiers. Santos and Blatt (1998) studied the flavonoid, phenolic and proanthocyanidin contents from FV leaves from the forest and cerrado, reporting lower TPC values (17.2 and 18.7 mg GAE/g extract) using the Soxhlet extraction method than the present study. The TPC was determined by Folin-Dennis methodology. The low TPC concentration can be explained by the use of high temperature (373 K), which can degrade heat-sensitive compounds such as phenolics, and the low quantity of solvent used (5 cm^3). On the other hand, higher TPC values were found for IC extracts than those reported in the literature obtained using low-pressure solvent extraction (LPSE) (Silva et al., 2007a,b; Souza et al., 2008). A decrease in TPC was observed in IC extracts using CO_2, CO_2 + EtOH, and CO_2 + H_2O (195.9, 137.6, and 110.98 mg GAE/g extract), respectively. Phenolic recovery was not effective in the presence of ethanol and water; different classes of compounds may have been extracted when these cosolvents were added. According to Silva et al. (2007a), an increase in the ethanol percentage in water (86.8%) used for LPSE had a positive effect on the TPC of IC extracts at 323 K and 30 minutes of extraction. DK extracts have also shown a decrease in TPC using CO_2, CO_2 + EtOH, and CO_2 + H_2O, respectively, as described before for IC extracts. Marques et al. (2007) studied the anatomic and physicochemical characteristics of DK roots. They also evaluated the phenolic recovery from DK roots obtained by decoction using different solvents (water, ethanol/water, methanol/water and acetone/water). According to the authors, all extracts have shown lower TPC values than those obtained in the present study. Water was considered the best solvent, achieving a 45% yield with the highest TPC (10.2%), indicating that higher solvent polarity is associated with higher phenolic yield, which is not in accordance with our results.

TPC recovery from beans using CO_2 + H_2O (338.21 mg GAE/g of extract) was more efficient than CO_2 + EtOH (93.1 mg GAE/g extract) or CO_2 (163.41 GAE mg GAE/g extract). Furthermore, this solvent system indicated high concentrations of antioxidant compounds, flavonoids and terpenoids in TLC analysis (Fig. 2D, G, J) in BE extracts when compared with CO_2 and CO_2 + EtOH. The high TPC found in BE extracts using water can be explained by the presence of proanthocyanidins. According to Ranilla et al. (2007), bean skins with dark colors such as red, brown and black tend to have higher levels of proanthocyanidins.

3.5. Total flavonoid content (TFC)

Table 3 shows the TFC of the extracts obtained using CO_2, CO_2 + EtOH and CO_2 + H_2O at 35 MPa and 323 K. All raw materials exhibited a decrease in TFC with the progressive incorporation of ethanol and water. The FV and BE extracts showed a non-significant ($p > 0.05$) difference among the solvents used except for BE extracted with CO_2 + H_2O, which presented the lowest TFC value. On the other hand, IC and DK extracts exhibited a noticeable and significant difference ($p < 0.05$) among the solvents used. The addition of ethanol almost halved the amount of flavonoids in IC extracts compared to pure CO_2, whereas for DK extracts this difference was almost 3-fold lower. When water was used as a modifier, these values were further reduced. Santos and Blatt (1998) reported a TFC for extracts from FV leaves obtained by Soxhlet extraction with methanol/water (70:30, v/v) for 3 hours, using a solvent-to-feed ratio of 125 mL/g. The authors achieved approximately 5.3–6.2 mg of rutin equivalent (RE)/g of dry material. These results are much lower than the TFC values obtained in this work, corroborating the hypothesis that polar solvents have a deleterious effect on flavonoid recovery. The TFC of supercritical IC extracts was higher than those reported in the literature (Silva et al., 2007a,b; Souza et al., 2008). Silva et al. (2007a) reported that an increase of ethanol concentration in water slightly improved the TFC, in agreement with the results of this study.

3.6. Antioxidant activity

The antioxidant activity of extracts obtained using CO_2, CO_2 + EtOH and CO_2 + H_2O at 35 MPa and 323 K was evaluated by DPPH assay, whose results are presented in three different forms in Table 3: scavenging ability (SA), inverse half maximal of effective concentration (EC_{50}^{-1}) and Trolox equivalents of antioxidant activity (TEAA). The purpose of presenting the results in three different ways is to make the comparison between our results and previous/future literature data easier. Veggi et al. (2011) reported a lower SA than the results obtained in the present work for CO_2 and CO_2 + EtOH solvent systems because SA was obtained at 30 min of kinetic reaction time, while the present work lasted 90 min for all SFE assays because CO_2 + H_2O is still very unstable at 30 min, making it necessary to extend the reaction time. All raw materials presented significant differences among antioxidant activity results ($p < 0.05$), showing the highest values of SA for FV using pure CO_2; IC and DK using CO_2 + EtOH; and BE using CO_2 + H_2O (12.3, 18, 19 and 25%, respectively). The incorporation of ethanol as a modifier increased the antioxidant activity of IC and DK extracts, while decreasing it for FV and BE. On the other hand, the addition of water demonstrated a deleterious effect on the antioxidant activity of all extracts, except for BE, which presented the highest antioxidant activity. This behavior can be explained due to the high antioxidant properties of the proanthocyanidins, which are greater in these materials compared to natural sources of phenolic compounds (Shan et al., 2005). The results obtained by Ranilla et al. (2007) indicate that the major phenolic compound in BE skins is proanthocyanidins, and these polymers are responsible for antioxidant capacity, instead of total flavonoids or flavonols. According to Mattei et al. (2001), the hydro-alcoholic extract of DK showed high antioxidant properties. IC leaves were considered an interesting source of antioxidants by Silva et al. (2007b). For the FV extract, a greater EC_{50}^{-1} was found using pure CO_2 extract (0.648 mL/mg), thus presenting a higher antioxidant activity.

3.7. Economic evaluation

The process economic evaluation largely depends on the identification of the appropriate process scheme and process parameters for the possible alternative extraction protocols. Several alternative combinations of extraction and separation conditions can be selected to design an SFE process; these combinations are normally focused on the pressure and temperature conditions of the extractor and separators (Rosa and Meireles, 2005; Prado et al., 2012). According to the Association for the Advancement of Cost Engineering International, the COM estimation can be divided into five classes (1–5). Class 5 is based on the lowest level of project definition, while the estimate class 1 is closer to the final definition of the project, that is, closer to the real value of COM (AACEI, 1997). The SuperPro Designer® software (version 6.0, Intelligent, Inc.) estimates a COM that can be classified as cost class 2-3 (Turton et al., 2003). Thus, the small amount of experimental data and the relatively low level of project definition make it possible to obtain initial estimates of COM. For accurate cost estimation, the performance of an industrial-scale extractor should be estimated (Albuquerque and Meireles, 2012).

Table 4 shows the cost of manufacturing estimated for the production of crude extracts (COM_{EY}, US$/kg of extract) and the phenolic-rich fractions (COM_{TPC}, US$/g of extract). The time of

Table 4
Cost of manufacturing of crude extracts (COM_{EY}) and phenolic-rich fraction (COM_{TPC}) of all SFE assays at volumetric capacities of 0.005, 0.05 and 0.5 m^3 estimated for the SFE extracts.

Solvent system	Sample	$COM_{EY}^{0.005}$ (US$/kg of extract)	$COM_{EY}^{0.005}$ (US$/kg of extract)	$COM_{EY}^{0.05}$ (US$/kg of extract)	$COM_{TPC}^{0.005}$ (US$/g of phenolic compound)	$COM_{TPC}^{0.005}$ (US$/g of phenolic compound)	$COM_{TPC}^{0.05}$ (US$/g of phenolic compound)
CO_2	DK	$4120 \pm 112^{b.A}$	$3853 \pm 105^{a.A}$	$3599 \pm 98^{a.A}$	$1714 \pm 47^{a.A}$	$1603 \pm 44^{ab.B}$	$1497 \pm 41^{ab.B}$
	BE	$1304 \pm 348^{b.B}$	$920 \pm 246^{c.B}$	$833 \pm 234^{c.AB}$	$808 \pm 216^{c.A}$	$570 \pm 153^{b.B}$	$516 \pm 145^{c.A}$
	IC	$3856 \pm 759^{b.AB}$	$3570 \pm 703^{ab.B}$	$3252 \pm 319^{ab.B}$	$2000 \pm 394^{a.B}$	$1852 \pm 365^{a.B}$	$1687 \pm 166^{a.B}$
	FV	$2324 \pm 153^{a.A}$	$2132 \pm 133^{bc.A}$	$1928 \pm 127^{bc.A}$	$1001 \pm 66^{b.A}$	$918 \pm 58^{b.A}$	$830 \pm 55^{bc.A}$
CO_2 + EtOH	DK*e*	$1586 \pm 68^{a.A}$	$1265 \pm 54^{b.C}$	$1189 \pm 51^{b.B}$	$1795 \pm 77^{b.B}$	$1431 \pm 61^{b.B}$	$1345 \pm 57^{b.B}$
	BE*e*	$715 \pm 38^{a.B}$	$424 \pm 23^{c.B}$	$356 \pm 19^{d.B}$	$774 \pm 42^{d.A}$	$456 \pm 25^{c.B}$	$385 \pm 21^{c.A}$
	IC*e*	$2296 \pm 6^{a.A}$	$1984 \pm 5^{a.B}$	$1911 \pm 5^{a.B}$	$2316 \pm 6^{a.C}$	$2001 \pm 5^{a.B}$	$1927 \pm 5^{a.B}$
	FV*e*	$1451 \pm 13^{a.A}$	$1092 \pm 10^{b.B}$	$998 \pm 18^{c.B}$	$530 \pm 5^{c.B}$	$399 \pm 4^{d.B}$	$364 \pm 7^{c.B}$
CO_2 + H_2O	DK*w*	$2664 \pm 194^{b.A}$	$614 \pm 45^{c.B}$	$353 \pm 26^{c.C}$	$16917 \pm 1229^{c.C}$	$3901 \pm 284^{b.A}$	$2243 \pm 163^{b.A}$
	BE*w*	$6403 \pm 340^{a.A}$	$1232 \pm 66^{b.A}$	$551 \pm 30^{b.A}$	$1893 \pm 101^{b.A}$	$364.2 \pm 20^{c.A}$	$163 \pm 9^{c.A}$
	IC*w*	27617 ± 446^{bB}	$7581 \pm 123^{a.A}$	$5027 \pm 82^{a.A}$	$249956 \pm 404^{a.A}$	$6861 \pm 111^{a.A}$	$4550 \pm 74^{a.A}$
	FV*w*	$1948 \pm 102^{b.B}$	$413 \pm 22^{c.A}$	$209 \pm 11^{c.C}$	$703 \pm 37^{c.C}$	$149.0 \pm 8^{c.B}$	$75 \pm 4^{c.C}$

e: CO_2 + EtOH. *w*: CO_2 + H_2O; Results are presented as mean ± standard deviation. $^{a–d}$ Lowercase letters represent significant difference at 5% level of significance for samples evaluated for different raw materials using the same solvent. $^{A–C}$ Capital letters represent significant difference at 5% level of significance for samples evaluated for the same raw material using different solvents; DK (dog's knot); BE (common bean); IC (ice-cream-bean); FV (flame vine).

extraction was defined according to the time of the laboratory experiments. In the process where pure CO_2 was used as solvent, the extraction time was 40 min, resulting in 5940 batches per year. For the extracts obtained with CO_2 + EtOH, the extraction time was 75 min, resulting in 3168 annual batches. The time of the extractions for the solvent mixture CO_2 + H_2O was 75 min; nonetheless, with the addition of the freeze-drying step, the total process time increased to 24 hours. For this reason, there was a reduction in the number of batches to 330 per year. According to Table 4, a lower COM_{EY} was obtained with CO_2 + EtOH and CO_2 + H_2O working in higher extraction capacity, mainly due to the increase in the extraction yield when modifier was added. A higher recovery of extracts using CO_2 + H_2O, except for IC, resulted in lower costs even at a longer process time.

The high apparent bed density of the BE provided a higher demand for raw materials; however, due to its low cost, US$ 2.20/kg (Agrolink, 2012), lower COM_{EY} values were estimated for BE extracts. Additionally, the increase in the EY caused by the use of water as a modifier was not enough for the annual extract productivity to overcome the effect of the time of extraction. For FV extracts, the impact of the extraction yield can be observed in the COM_{EY}. Due to the low cost of the raw materials, a small COM_{EY} was estimated, especially for extracts obtained with CO_2 + H_2O. For these extracts, the EY had a high impact on the annual production of extract, demonstrating the viability of this process. Similar behavior was obtained for DK extracts. Regarding the IC extracts, the lowest extraction yield and high cost of raw material, US$ 50.00/kg (Santosflora, 2012), directly affected its economic viability, resulting in high estimated costs; the estimated COM values for the extraction with CO_2 + H_2O were very high.

In this context, it can be observed that the price of raw material has a great influence on the final COM_{EY}. So, for most of the plants evaluated in this work, a smaller COM_{EY} were estimated for the process that uses CO_2 + H_2O; this is particularly true for the FV and DK extracts, which presented COM values of US$ 208.7/kg and US$ 353.3/kg of extract, respectively, for the 0.5 m^3 extractor.

The estimated COM_{TPC} is related to the total phenolic content of each extract. The use of modifiers resulted in a smaller extraction yield and polyphenol content for IC extracts. This behavior led to an increase in the cost. On the other hand, the use of water as a modifier resulted in an increase of the extraction yield of DK, but not in the polyphenol content, which caused an increase in the COM_{TPC} of this extract. The polyphenol content for BE extracts was shown to be higher with the use of water as a modifier, causing a decrease in the COM_{TPC}. For the FV extracts, higher amounts of TPC were observed for all three solvent systems, but with low variation, which implicates a low COM_{TPC}.

The increase in the extraction yield and phenolic content has a detrimental effect on the cost of manufacturing, decreasing COM_{EY} and COM_{TPC}, respectively. However, this effect can only be evaluated for a single solvent system because the incorporation of ethanol and water require the use of different equipment, invalidating any comparison between the two. It can be observed that an increase in the size of the extraction vessels results in a significant ($p < 0.05$) decrease of the COM_{EY} and COM_{TPC}. In this context, for the three solvent systems, the manufacturing cost of Brazilian extracts by SFE becomes economically feasible when extraction vessels of 0.05 to 0.5 m^3 are used. However, one can conclude that increasing the batch size or number of batches per year increases the annual plant throughput and consequently leads to a more economical process. This behavior can be observed in the COM_{EY} estimated for FV, DK and BE extracts. Moreover, the cost of the equipment used to estimate the processes were budgeted from Chinese manufacturers, which have SFE unit costs much lower

Table 5
Share of costs in COM of Brazilian plant extracts obtained by SFE at volumetric capacities of 0.005, 0.05 and 0.5 m^3: fixed capital of investment (FCI), cost of raw material (CRM), cost of labor (COL), cost of utilities (CUT) and cost of quality control (CQC).

Solvent system	Economic parameter	DK			BE			IC			FV		
		0.005 m^3	0.05 m^3	0.5 m^3	0.005 m^3	0.05 m^3	0.5 m^3	0.005 m^3	0.05 m^3	0.5 m^3	0.005 m^3	0.05 m^3	0.5 m^3
CO_2	CRM[a]	63.6	77.7	82.9	22.6	36.8	46.7	74.0	83.8	87.0	57.2	74.2	80.6
	COL[b]	7.0	1.6	0.3	15.6	5.1	1.0	4.0	0.9	0.2	8.7	2.1	0.4
	CUT[c]	4.0	4.5	5.1	15.1	25.0	31.6	3.9	2.6	2.7	4.3	5.2	6.0
	FCI[d]	21.6	14.5	11.1	38.2	28.0	18.3	16.0	11.8	9.8	25.0	16.4	12.0
	CQC[e]	3.8	1.7	0.6	8.5	5.1	2.4	2.1	0.9	0.4	4.8	2.1	1.0
	COP ($\times 10^3$)[f]	822	7692	71,844	290	2046	18,300	1536	14,228	140,114	629	5759	52,198
	Production[g]	198	1984	19,841	230	2306	23,066	406	4063	40,637	270	2702	27,020
CO_2 + EtOH	CRM[a]	45.4	66.7	76.4	11.2	22.9	32.0	58.3	76.0	82.7	37.7	60.8	72.7
	COL[b]	11.0	3.2	0.6	17.9	7.3	1.5	8.1	2.1	0.3	13.1	4.2	0.7
	CUT[c]	5.2	7.6	8.8	14.7	30.0	42.0	3.4	4.4	5.0	5.3	8.4	10.1
	FCI[d]	31.0	19.2	13.0	44.5	32.6	21.2	24.8	15.3	11.2	35.1	22.4	14.7
	CQC[e]	7.4	3.3	1.2	11.7	7.2	3.3	5.4	2.2	0.8	8.8	4.2	1.8
	COP ($\times 10^3$)[f]	449	3580	34,098	226	1341	11,243	677	5848	56,306	356	2679	24,620
	Production[g]	282	2827	28,653	316	3165	31,656	294	2941	29,420	243	2468	24,681
CO_2 + H_2O	CRM[a]	5.3	19.0	38.1	0.9	3.1	7.3	9.0	29.0	51.4	3.8	14.3	30.6
	COL[b]	26.4	10.0	2.0	27.5	10.9	2.7	25.4	8.6	1.6	27.0	10.7	2.3
	CUT[c]	21.6	12.7	12.6	23.4	18.4	27.0	20.6	10.5	8.8	21.7	12.8	12.4
	FCI[d]	35.7	47.2	39.3	36.8	54.5	51.7	34.6	42.1	32.1	36.3	50.2	45.1
	CQC[e]	11.0	11.1	8.0	11.4	13.1	11.3	10.4	9.8	6.1	11.2	12.0	9.6
	COP ($\times 10^3$)[f]	374	863	4963	352	678	3033	397	1089	7219	363	757	3908
	Production[g]	143	1434	14,342	55	551	5510	14	143	1430	188	1854	18,926

DK (dog's knot); BE (common bean); IC (ice-cream-bean); FV (flame vine).
[a] Cost of raw material (%).
[b] Cost of labor (%).
[c] Cost of utilities (%).
[d] Fixed capital of investment (%).
[e] Cost of quality control (%).
[f] COP: operational cost (US$/year).
[g] (kg/year).

than those obtained from American or European markets, consequently decreasing the COM of the process. Thus, investment in a 0.5 m^3 unit is entirely feasible, even with the extraction process using $CO_2 + H_2O$ operating for 24 hours. However, the producer would have to be sure of raw material availability and market demand.

Additional economic parameters are presented in Table 5. The cost of raw material (CRM) and the cost of utilities (CUT) increased with increasing extractor size, while the fixed capital of investment (FCI) decreased, which justifies the feasibility of the processes for larger sizes. These results are in agreement with the literature (Prado et al., 2012; Santos et al., 2010; Leitão et al., 2013). Thus, for these raw materials, the investment cost cannot be considered as the main factor responsible for the high cost of manufacturing of the products. The cost of labor (COL), CUT and cost of quality control (CQC) represent a small fraction of COM_{EY} when compared to CRM and FCI. For a given raw material, the FCI and CRM for the three solvent systems are significantly different ($p < 0.05$). For IC, DK and FV extracts using CO_2 and CO_2 + EtOH, the CRM fraction was higher than the FCI. On the other hand, the extracts obtained with $CO_2 + H_2O$ have a higher FCI than CRM, even for BE extracts. This behavior is caused by the addition of freeze-drying equipment, which has higher costs than distillers. Furthermore, the longer extraction time for the $CO_2 + H_2O$ process decreases the productivity; hence, smaller amounts of raw materials are used throughout the year compared to the other processes. This behavior was observed for BE extracts only in extractors with a capacity of 0.5 m^3.

The extract selling prices found in the market were around US$ 21.00/kg (KFO France Ltd., 2012) for dry FV extract, US$ 60.00 and US$ 20.00 (Chá and Cia, 2012) for dry and liquid DK extract and US$ 28.00 (KFO France Ltd., 2012) for dry BE extract. No commercial prices for IC were found in the market. Although these values are much lower than the estimated COM_{EY}, some factors must be taken into account before claiming that the SFE process is not economically viable. The extraction method used and composition of the extracts in the market is not known, thus, it is hard to compare its applicability to SFE extracts.

Table 6
Loadings, eigenvalues and percentage of cumulative variance for the first two principal components (PCs) of SFE experiments.

Variable	PC_1	PC_2
EY	−0.036	0.794
TPC	−0.491	−0.202
TFC	−0.361	−0.172
EC_{50}^{-1}	−0.110	−0.754
SA	−0.616	−0.707
TEAA	−0.616	−0.707
$COM_{EY}^{0.005}$	0.861	−0.263
$COM_{EY}^{0.005}$	0.824	−0.438
$COM_{EY}^{0.05}$	0.729	−0.486
$COM_{TPC}^{0.005}$	0.928	0.122
$COM_{TPC}^{0.05}$	0.974	−0.068
$COM_{TPC}^{0.5}$	0.953	−0.202
Eigenvalues	5.813	2.828
Variance (%)	48.446	23.565
Cumulative variance (%)	48.446	72.011

3.8. Principal component analysis (PCA)

PCA was applied to the whole data set of the three solvents and four raw materials. The loadings, eigenvalues and percentages of cumulative variance are shown in Table 6. The dimensionality of the data was reduced from 12 partially correlated variables to 2 uncorrelated principal components, PC_1 and PC_2, accounting for 72.01% of the variation. The absolute value of the loadings is an indicator of the participation of the analyzed parameters in the PCs. PC_1 correlates inversely with the variables in the decreasing order SA > TEAA > TPC > TFC > EC_{50}^{-1} > EY, while it is highly correlated with cost of manufacturing. PC_2 has shown high correlation with antioxidant activity (EC_{50}^{-1} > SA > TEAA). The graphic representation of the scores and loadings is shown in Fig. 3a and b, respectively. The relationships among the analyzed parameters, as well as the similarities and differences between the SFE experiments, can be identified from the PCA plot. The parameters SA, TEAA and EC_{50}^{-1} were significantly correlated with each other ($p < 0.05$), which could be seen from the PCA loading plot (Fig. 3b) and Pearson's correlation coefficients (Table 7). As expected, all manufacturing costs have shown significant correlations between one other (0.765–0.978). The extraction yield appears to be inversely correlated to TPC, TFC, SA, TEAA and EC_{50}^{-1} (from −0.012 to −0.457), indicating

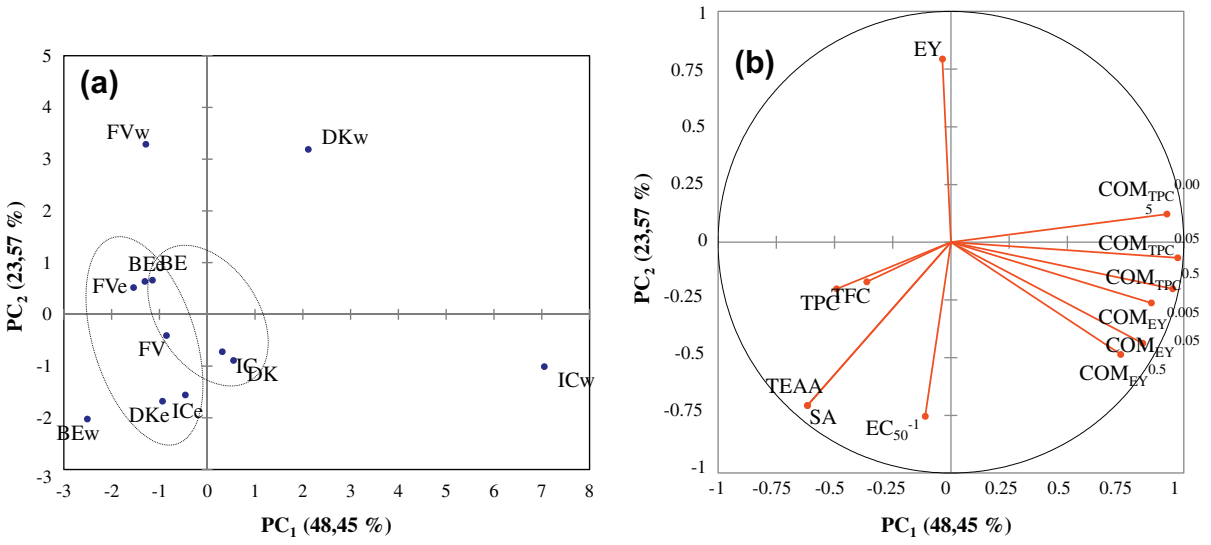

Fig. 3. Principal component analysis of SFE experiments of: (a) bean (BE), ice-cream-bean (IC), flame vine (FV) and dog's knot (DK) performed using CO_2 (−), CO_2 + EtOH (subscript *e*) and $CO_2 + H_2O$ (subscript *w*), and (b) extraction yield (EY), total phenolic content (TPC), total flavonoid content (TFC), DPPH scavenging ability (SA), inverse half maximal effective concentration (EC_{50}^{-1}) and Trolox equivalent antioxidant activity (TEAA).

Table 7
Pearson's correlation coefficients of the variables observed for SFE experiments.

Variáveis	TPC	TFC	EC_{50}^{-1}	SA	TEAA	$COM_{EY}^{0.005}$	$COM_{EY}^{0.05}$	$COM_{EY}^{0.05}$	$COM_{TPC}^{0.005}$	$COM_{TPC}^{0.05}$	$COM_{TPC}^{0.5}$
EY	−0.012	−0.278	−0.355	−0.457	−0.457	−0.168	−0.386	−0.460	0.116	−0.045	−0.156
TPC		0.457	−0.074	0.360	0.360	−0.128	−0.073	−0.050	−0.532	−0.556	−0.545
TFC			−0.174	0.104	0.104	−0.283	0.022	0.163	−0.580	−0.451	−0.347
EC_{50}^{-1}				**0.688**	**0.688**	0.040	0.108	0.140	−0.145	0.004	0.118
SA					**1.000**	−0.326	−0.296	−0.252	−0.543	−0.485	−0.410
TEAA						−0.326	−0.296	−0.252	−0.543	−0.485	−0.410
$COM_{EY}^{0.005}$							**0.871**	**0.721**	**0.810**	**0.833**	**0.807**
$COM_{EY}^{0.05}$								**0.968**	**0.622**	**0.769**	**0.828**
$COM_{EY}^{0.5}$									0.469	**0.666**	**0.765**
$COM_{TPC}^{0.005}$										**0.955**	**0.872**
$COM_{TPC}^{0.05}$											**0.978**

Bold values represent significant difference at 5% level of significance (p-value < 0.05).

that supercritical extracts with higher phenolic content and antioxidant activity were the same with a lower extraction yield. In addition, the parameters TPC and TFC are weakly correlated (0.457) with one other, which indicates a possible co-extraction of phenolic acids or proanthocyanidins. TPC and TFC also presented a weak correlation with antioxidant activity parameters SA, TEAA and EC_{50}^{-1} (−0.074 to 0.360 and −0.074 to 0.104, respectively), indicating possible co-extraction of non-phenolic antioxidants.

The score plot (Fig. 3a) shows the chemical and economic aspects of all SFE experiments. The subscripts *e* and *w* observed in the score plot indicate the extracts obtained by SFE using ethanol and water as modifiers, respectively. The samples obtained with pure CO_2 have no subscript. SFE using pure CO_2 and CO_2 + EtOH showed similar chemical behavior, while water presented no similarity with the raw materials analyzed. Loadings of all analyzed parameters indicated that the SFE experiments with high TPC, TFC, SA, TEAA and EC_{50}^{-1} were those situated in the bottom-left side of the score plot, while the SFE assays with a high cost of manufacturing are located in the bottom-right side. Thus, SFE experiments with high TPC, TFC, SA, TEAA and EC_{50}^{-1} were those obtained using CO_2 from DK, IC and FV, respectively; CO_2 + EtOH from dog's knot and ice-cream-bean (DK*e* and IC*e*); and $CO_2 + H_2O$ from bean (BE*w*). These same SFE assays were also those that presented the lowest cost of manufacturing.

4. Conclusions

Supercritical and subcritical fluid extraction of all raw materials yielded superior polyphenolic and flavonoid contents than those reported in the literature data using conventional extraction methods, suggesting that all plant matrices studied are good sources of phenolic compounds. The highest antioxidant activities were obtained using ethanol and/or water as a modifier for BE*w*, DK*e* and IC*e*, whose lack of correlation with phenolic and flavonoid contents is most likely due to potential co-extraction of other antioxidant compounds, such as phytosterols or vitamins. The aqueous extracts obtained from BE*w* and FV*w* using water as a co-solvent showed the highest phenolic content. Further research about the influence of other processing conditions such as solvent flow rate, solvent-to-feed mass ratio, extraction time, temperature and pressure should be performed to optimize the recovery of antioxidant compounds obtained from those raw materials and to evaluate the economic feasibility. The economic evaluation clearly demonstrates that cost of raw materials has a significant impact on the expenses for the production of extracts. Although the cost of equipment had a great influence on the manufacturing cost, extraction yield has been presented as a more important factor in the economic evaluation of the processes studied. The lowest manufacturing costs were achieved for FV*w* extracted with water as a co-solvent, followed by extracts of DK*w* and Be*e* obtained using water and ethanol, respectively. The aqueous DK*w* extract presents itself as the most viable commercial extract due to its high phenolic content and low cost.

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