

## Energy Consumption Versus Antioxidant Activity of Pressurized Fluid Extracts from *Pfaffia glomerata* Roots

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Conventional extraction techniques have been applied to obtain antioxidant extracts from *Pfaffia glomerata* roots, most of the times, using polar extracting solvents. Even if these techniques are able to provide extracts with antioxidant activities, more environmentally friendly techniques are nowadays preferred. Among them, supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) with green solvents have been widely applied to natural bioactive compounds extraction. The limitation of the use of pure supercritical CO<sub>2</sub> for obtaining antioxidant extracts from *Pfaffia glomerata* roots was already demonstrated. When high amounts of modifier are added, the formation of a gas-expanded liquid is observed. This extracting solvent combines the advantages of the solvation properties of typical liquids and the transport properties of supercritical fluids, being an intermediate process between SFE and PLE, which can be called as pressurized fluid extraction (PFE). In this work, PFE of Brazilian ginseng (*Pfaffia glomerata*) roots were performed in order to obtain antioxidant extracts with potential applications in the pharmaceutical and food areas. Several CO<sub>2</sub>+ethanol mixtures (90:10 %, 50:50 % and 0:100 %, w/w) as extracting fluid were assayed. The effects of other two process parameters including pressure (10–20 MPa) and temperature (323–363 K) on the extraction yield, antioxidant activity and energy consumption per unit of manufactured product were investigated. PFE process was simulated using the SuperPro Designer simulation platform. The use of 10 % (w/w) of ethanol produced extracts with the highest antioxidant activity. On the other hand, higher temperature and ethanol percentage resulted in higher extraction yield and lower energy consumption per unit of manufactured product, while pressure did not affect any response variables.

### 1. Introduction

Ginseng species is one of the most appreciated natural sources of bioactive compounds in the world. The most known Ginseng species belongs to the *Panax* genus, which have been used for thousands years by folk medicine (Lu et al., 2008).

Species of the genus *Pfaffia* (Amaranthaceae) have been commercialized as substitutes for *Panax* (ginseng, Araliaceae). Due to the similar morphology of their roots to those of ginseng, they are popularly known as “Brazilian ginseng”. In Brazil, different species have been described, being *Pfaffia glomerata* the most important specie. Since besides similarity in appearance Brazilian ginseng roots (*Pfaffia glomerata*) extracts have also similar effects to ginseng, large amounts of this plant material are being exported for production of their extracts (Júnior, 2005).

Conventional extraction techniques have been applied to obtain antioxidant extracts from *Pfaffia glomerata* roots, most of the times, using polar extracting solvents (Flores et al., 2009). Even if these techniques are able to provide extracts with antioxidant activities, more environmentally friendly techniques are nowadays preferred (Straccia et al., 2012). Among them, supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) with green solvents have been widely applied to natural bioactive compounds extraction.

Supercritical fluid extraction (SFE) is still a somewhat novel technique, in which the characteristics of the final product can be easily altered by changing the process parameters such as temperature, pressure, and co-solvent (Dos Santos et al., 2013). Carbon dioxide is the most used solvent in SFE because it is safe, nontoxic, and generally available at a reasonable cost (Pereira and Meireles, 2010). Pressurized liquid extraction (PLE) has received different names, such as accelerated solvent extraction (ASE), enhanced solvent extraction (ESE), among others. PLE is a solid-liquid extraction process performed at high temperatures (323 - 473 K) and high pressures (1 - 20 MPa), its main advantages over traditional extraction methods being dramatic decreases in the amount of solvent used and the extraction time (Sun et al., 2012).

The limitation of the use of pure supercritical CO<sub>2</sub> for obtaining antioxidant extracts from *Pfaffia glomerata* roots and other ginseng species was already demonstrated (Wang et al., 2001). In all studies, the addition of modifiers (methanol, dimethylsulfoxide or ethanol) for supercritical CO<sub>2</sub> extraction, greatly enhanced the extraction compared to that of pure CO<sub>2</sub>, the latter of which yielded negligible quantities of saponins, the most important antioxidant compounds class present in these species (Wood et al., 2006). When high amounts of modifier are added the formation of a gas-expanded liquid is observed. This extracting solvent combines the advantages of the solvation properties of typical liquids and the transport properties of supercritical fluids, being an intermediate process between SFE and PLE, which can be called as pressurized fluid extraction (PFE). First used to extract environmental contaminants (Deuster et al. 1997), this process was already used successfully for extract bioactive compounds, such as anthocyanins (Seabra et al., 2010), curcuminoids (Braga and Meireles, 2007), polyphenols (Chang et al., 2000) from natural sources.

In this work, PFE of Brazilian ginseng (*Pfaffia glomerata*) roots were performed in order to obtain antioxidant extracts with potential applications in the pharmaceutical and food areas. Several CO<sub>2</sub>+ethanol mixtures (90:10 %, 50:50 % and 0:100 %, w/w) as extracting fluid were assayed. The effects of other two process parameters including pressure (10-20 MPa) and temperature (323-363 K) on the extraction yield, antioxidant activity and energy consumption per unit of manufactured product were investigated. PFE process was simulated using the SuperPro Designer simulation platform.

## **2. Material and methods**

### **2.1 Chemical and reagents**

All the chemicals used to prepare reagent solutions were of analytical reagent grade. Carbon dioxide (99.9 %) was purchased from Gama Gases Especiais Ltda. (Campinas, Brazil). Ethanol (99.5 %) was purchased from Ecibra (Santo Amaro, Brazil).

### **2.2 Raw material preparation**

Brazilian ginseng roots (*Pfaffia glomerata*) were cultivated in the experimental field of CPQBA (Campinas, Brazil), where they were collected on March 25, 2004, being 3 y old. They were washed and dried in a forced air circulation dryer at 313 K for 5 d. The dried roots (8.9 % moisture) were then comminuted in a pulse mill (Marconi, model MA 340, Piracicaba, Brazil) for few seconds. Next, the particles of higher size were milled again, this time using a knife mill (Tecnal, model TE 631, Piracicaba, Brazil) for 2 s at 18,000 rpm and finally, they were separated according to their size using sieves (Series Tyler, W.S. Tyler, Wheeling, IL). The milled roots were stored in freezer (Metalfrío, model DA 420, São Paulo, Brazil) at 263 K. For the extraction assays particles of 7.9 µm of diameter, according to ASAE methodology (ASAE, 1993), were used. The moisture content of the dried roots was determined by the AOAC method (Method 4.1.03) (AOAC, 1997).

### **2.3 Pressurized fluid extraction (PFE) process**

In Table 1, a summary of the process conditions in all experiments performed is shown. A home-made unit, described by Pasquel et al. (2000) was used for the experiments that use CO<sub>2</sub> and ethanol mixtures, while another recently finished unit was used in the experiments that only ethanol is used. This latter unit consists of a HPLC pump (Thermoseparation Products, Model ConstaMetric 3200 P/F, Fremon, USA), a 6.57 cm<sup>3</sup> extraction cell (Thar Designs, Pittsburg, USA) containing a sintered metal filter at the bottom and upper parts, a electrical heating jacket and a back pressure regulator (BPR) valve (Model n°26-1761-24-161, Tesco, Elk River, USA). A more detailed description of this unit can be found elsewhere (Santos et al., 2012a). Different amounts of raw material was placed in each equipment, then, the amount of total solvent was adjusted to keep the solvent mass to feed mass ratio constant (S/F = 50) in all experiments. The criterion adopted in the present study has proved to be efficient for comparison of results obtained in different equipments (Prado et al., 2011). The static and dynamic extraction periods always were 5 min and 1 h, respectively. The assays were done in duplicate.

Table 1: Summary of the pressurized fluid extraction process conditions in all experiments performed

|      | Temperature (K) | Pressure (MPa) | Mass fraction of Ethanol (%) |
|------|-----------------|----------------|------------------------------|
| E 1  | 323             | 10             | 10                           |
| E 2  | 323             | 10             | 50                           |
| E 3  | 323             | 10             | 100                          |
| E 4  | 323             | 20             | 10                           |
| E 5  | 323             | 20             | 50                           |
| E 6  | 323             | 20             | 100                          |
| E 7  | 363             | 10             | 10                           |
| E 8  | 363             | 10             | 50                           |
| E 9  | 363             | 10             | 100                          |
| E 10 | 363             | 20             | 10                           |
| E 11 | 363             | 20             | 50                           |
| E 12 | 363             | 20             | 100                          |

#### 2.4 Determination of extraction yield

After extraction ethanol was removed using rotary evaporator (Heidolph, model Laborota 4001WB, Viertrieb, Germany), and the flasks containing the extracts were then weighed in analytical balance. The extraction yield of the experiments was calculated as the ratio between the total mass of extract and the initial mass of dry raw material fed into the extractor. After ethanol removal the extracts were analyzed in terms of antioxidant activity.

#### 2.5 Determination of antioxidant activity of extracts

The determination of antioxidant activity (AA) of the extracts was based on the coupled oxidation of  $\beta$ -carotene and linoleic acid. The technique developed by Marco (1968) consisted of measuring the bleaching of  $\beta$ -carotene resulting from oxidation by the degradation products of linoleic acid. In short, the substrate of reaction was prepared using 10 mg of  $\beta$ -carotene (97 %, Sigma-Aldrich, St. Louis, USA), 10 cm<sup>3</sup> of chloroform (99 %, Ecibra, Santo Amaro, Brazil), 60 mg of linoleic acid (99 %, Sigma-Aldrich, St. Louis, USA) and 200 mg of Tween 40 (99 %, Sigma-Aldrich, St. Louis, USA). This solution was concentrated in rotary evaporator (Laborota, model 4001, Vertrieb, Germany), with vacuum control (Heidolph Instruments GmbH, Vertrieb, Germany) and a thermostatic bath at 323 K, being then diluted in 50 cm<sup>3</sup> of distilled water. The oxidation reaction was conducted using the following procedure: to each 1 cm<sup>3</sup> of substrate, 2 cm<sup>3</sup> of distilled water and 0.05 cm<sup>3</sup> of extract diluted in ethanol (99.5 %, Ecibra, Santo Amaro, Brazil) were added. The dilution used for AA determination was 0.02 g of extract/cm<sup>3</sup> of solvent. The mixture was placed in thermal bath (model TE 159, Tecnal, Piracicaba, Brazil Marconi, model MA159/300, Piracicaba, Brazil) at 313 K, and the product of reaction was monitored using a spectrophotometer (Femto, model 800 XI, São Paulo, Brazil) at 0, 1, 2 and 3 h of reaction, using absorbance readings at 470 nm. The AA was determined in duplicate for each extract and calculated following the same calculation procedure done by Santos et al. (2010).

#### 2.6 Determination of energy consumption per unit of manufactured product

Simulations for the determination of the energy consumption per unit of manufactured product employing each PFE experimental process conditions were carried out using the SuperPro Designer 6.0<sup>®</sup> process simulator. This software allows for mass and energy balance estimation for all streams of the process. The results were normalized in order to determine the energy consumption (in terms of cost) per unit (1 kg) of manufactured product (PFE extract).

The PFE process developed in the SuperPro Designer consists of two pumps (one for CO<sub>2</sub> and one for ethanol), one extractor, two heat exchangers, a flash tank, an evaporator and a condenser (Figure 1). The extraction procedure consists placing a known mass of dried and milled *Pfaffia glomerata* roots in contact with supercritical carbon dioxide + ethanol.

#### 2.7 Statistical analysis

Statistical analyses were performed using analyses of variance (ANOVA). The mean values were considered significantly at  $p < 0.05$  and very significantly at  $p < 0.01$ . Statistica software (release 7, StatSoft, Tulsa, USA) was used to calculate the effects of the temperature, pressure and fraction of ethanol (%) on extraction yield, antioxidant activity and energy consumption per unit of manufactured product.

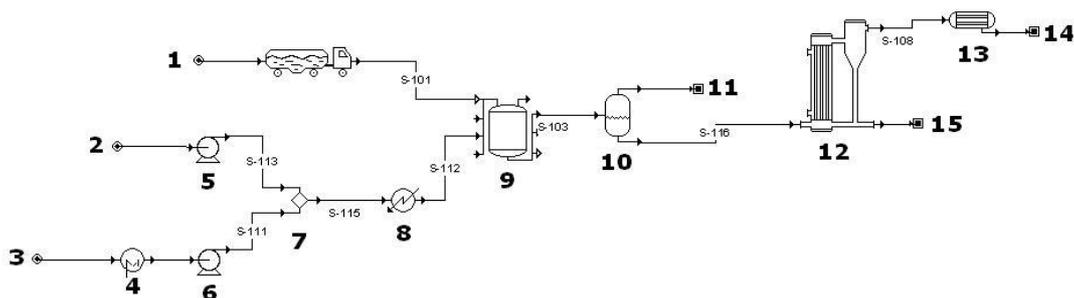


Figure 1: Scheme for the pressurized fluid extraction process developed in SuperPro Designer<sup>®</sup> process simulator. 1- Raw material flow; 2 - Ethanol flow; 3- CO<sub>2</sub> flow; 4 - Heat exchanger for cooling; 5 - Ethanol pump; 6 - CO<sub>2</sub> pump; 7- Mixer; 8 – Heat exchanger for heating; 9 - Extractor; 10 - Flash tank; 11 - CO<sub>2</sub>-rich phase flow; 12 - Evaporator; 13 - Condenser; 14 - Ethanol-rich phase flow; 15 - Extract flow

### 3. Results and discussion

The effects of temperature, pressure and ethanol concentration on the extraction yield, antioxidant activity and energy consumption per unit of manufactured product were evaluated. The experimental values at various experimental conditions are presented in Table 2. The use of 10 % (w/w) of ethanol produced extracts with the highest antioxidant activity. On the other hand, higher temperature and ethanol percentage resulted in higher extraction yield and lower energy consumption per unit of manufactured product.

Table 2: Process results of extraction yield, antioxidant activity and energy consumption per unit of manufactured product

|      | Extraction Yield (%) d.b. | Antioxidant Activity (%) after 3 h of reaction | Energy consumption (US\$/kg of extract) |
|------|---------------------------|--|---|
| E 1  | 0.26 ± 0.01               | 51 ± 2   | 206 ± 7                                 |
| E 2  | 1.32 ± 0.04               | 40 ± 1   | 49 ± 2                                  |
| E 3  | 1.95 ± 0.05               | 20.5 ± 0.9                                     | 41.1 ± 0.4                              |
| E 4  | 0.34 ± 0.02               | 55 ± 3   | 167 ± 4                                 |
| E 5  | 1.41 ± 0.03               | 45 ± 2   | 48.1 ± 0.3                              |
| E 6  | 2.46 ± 0.09               | 24 ± 2   | 33 ± 0.5                                |
| E 7  | 0.43 ± 0.01               | 51 ± 2   | 124 ± 5                                 |
| E 8  | 2.12 ± 0.05               | 42 ± 1   | 30.5 ± 0.8                              |
| E 9  | 4.9 ± 0.1                 | 20 ± 1   | 16.0 ± 0.2                              |
| E 10 | 0.46 ± 0.08               | 52 ± 1   | 123 ± 2                                 |
| E 11 | 2.3 ± 0.8                 | 41 ± 1   | 30.1 ± 0.3                              |
| E 12 | 5.2 ± 0.2                 | 22 ± 1   | 16 ± 1                                  |

Statistical analysis to evaluate the influence of the studied parameters at the results is shown in Table 3. The extraction yield and energy consumption per unit of manufactured product were significantly affected by extraction temperature and mass fraction of ethanol. Only mass fraction of ethanol was significant ( $p < 0.05$ ) with respect to the antioxidant activity. Mass fraction of ethanol was very significantly ( $p < 0.01$ ) for all response variables, while temperature was very significantly ( $p < 0.01$ ) only for energy consumption per unit of manufactured product. Pressure showed no influence on the response variables analyzed at the range studied.

Studying the SFE from *Paffia glomerata* roots using pure CO<sub>2</sub> Leal et al. (2010) observed that pressure had different influences on the extraction yield at different temperatures. During SFE using CO<sub>2</sub> without any cosolvent, the effect of temperature and pressure on supercritical CO<sub>2</sub> solubilization power depends on the solute vapor pressure and solvent density, being the extraction yield strongly affected by the CO<sub>2</sub> density. As the temperature increases at a constant pressure, the solute vapour pressure increases; however, the solvent density decreases. When any cosolvent is added to CO<sub>2</sub> it is not easy to predict the

behaviour. The cosolvent effect is normally to increase the concentration of the target compound. A decrease in the cosolvent effect of self-associating cosolvents (cosolvents with both H-bond donor and acceptor properties like ethanol) may occur at high cosolvent concentrations. Seabra et al. (2010) studying the effect of several CO<sub>2</sub>+cosolvents mixtures as extracting fluid has demonstrated that the main process parameter is in particular the liquid phase composition, corroborating our findings.

*Table 3: The P-values obtained with ANOVA realized to evaluate the influence of different parameters on pressurized fluid extraction process*

| Parameter                       | Extraction Yield (%) d.b. | Antioxidant Activity (%) after 3 h of reaction | Energy consumption in terms of cost: US\$/kg of extract |
|---------------------------------|---------------------------|--|---|
| A: Temperature                  | 0.020704                  | 0.558842                                       | 0.008044  |
| B: Pressure                     | 0.238933                  | 0.163454                                       | 0.401148  |
| C: Mass fraction of Ethanol (%) | 0.008656                  | 0.000451                                       | 0.000017  |

Since the increase in extraction temperature has not statistically influenced the antioxidant activity of the extracts the degradation of the bioactive compound extracted was minimum at the range studied. Undoubtedly, the higher extraction yields were obtained causing the decrease in antioxidant activity, probably due to a lower selective extraction resulting in the extraction of other compounds with no and/or low antioxidant activity. Similar results were found by Santos et al. (2012b) when studying the Pressurized Organic Solvent Extraction of *Pfaffia glomerata* using ethyl acetate as extracting solvent. According with the authors, the increase in the content of non-saponin compounds in the extracts with the increase in the extraction temperature can also help with the decrease in the antioxidant activity. As a higher mass of extract is obtained by the increase of extraction temperature and/or adding more cosolvent lower energy consumption per unit of manufactured product is achieved. Condition E1 presented the higher Energy consumption per unit of manufactured product even using the lowest conditions of pressure and temperature as the lowest extraction yield was obtained. Probably condition E8 (Temperature: 363 K; Pressure: 10 MPa; Mass fraction of Ethanol: 50 %) represent the best set of results as extraction yield is comprised in a middle range, in comparison with the other conditions, its antioxidant activity is only 23 % lower than the highest result achieved and energy consumption per unit of manufactured product is one of the lowest obtained.

Although simple statistical tools were used in this analysis, the optimization of this extraction process represents clearly a multi-objective problem as optimal conditions for extraction yield and energy consumption per unit of manufactured product are not the best conditions for antioxidant activity.

#### 4. Conclusions

The use of 10 % (w/w) of ethanol produced extracts with the highest antioxidant activity. On the other hand, higher temperature and ethanol percentage resulted in higher extraction yield and lower energy consumption per unit of manufactured product, while pressure did not affect any response variables. Mass fraction of Ethanol was significant for all response variables studied, and temperature was significant only for extraction yield and energy consumption per unit of manufactured product. Best results for extraction yield and antioxidant activity were opposite. Therefore the best result for the analyzed conditions comprised the highest extraction yield obtained with reasonable antioxidant activity and lower energy consumption per unit of manufactured product as possible, being condition E8 (Temperature: 363 K; Pressure: 10 MPa; Mass fraction of Ethanol: 50 %).

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