Obtaining bioactive compounds from Brazilian ginseng roots using pressurized water

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ABSTRACT

Interest in industrial processes employing environmentally friendly transformation methods with low residue generation has been increasing, especially during the current century. The term "biorefinery" applies to the integral use of biomass to produce value-added products with the lowest generation of residue possible. The aim of this work was to quantitatively analyze all products from Brazilian ginseng roots (BGR) obtained using pressurized water while considering the possibility of the integral use of BGR. The effects of the extraction temperature (353-453 K) and static extraction time (5-15 min) on the beta-ecdysone content, emulsification index, total reducing sugars content, and total phenolic content were evaluated. Analysis of variance (ANOVA) was used to determine the influence of the parameters under investigation. Although the static extraction time did not significantly affect the response variables, the effects of temperature were statistically significant (p-value ≤ 0.05) for all of the response variables. The highest beta-ecdysone content (0.6 ± 0.1 %, dry basis – d.b.) and emulsification index (60 %) were achieved at 353 and 393 K, respectively, while the maximum content of total reducing sugars (66.3 %, d.b.) was obtained at 453 K. The highest total phenolic content (60 % of gallic acid equivalent) was obtained at 453 K, which was the highest temperature studied. Thus, it is possible to alter the product profile obtained from BGR using pressurized water technology, indicating that this vegetable matrix can be explored in the context of a biorefinery.

INTRODUCTION

Sub/supercritical fluids have traditionally been used in single unit operations, e.g. extraction, fractionation, and particle formation. Laboratory investigations on the potential value of multiple unit processing using pressurized fluids is frequently accomplished by analyzing the efficacy of one single process followed by another and by determining the practicality of arranging these processes in a tandem manner to achieve the desired end product [1, 2].

Subcritical water offers tremendous opportunities for chemical reactions and extractions, as both a benign catalyst and solvent. Even in the absence of catalysts or additives, subcritical water can be used for hydrolytic purposes. Higher extraction rates can be achieved and a greater variety of components from biomass can be obtained, which may not be possible under conditions of normal temperature and pressure. Subcritical water can be used both to extract polar substances and to decompose natural biopolymers (cellulose,

protein, starch) to produce valuable compounds, such as saccharides, aromatic organic acids, and amino acids [3, 4]. In an effort to increase chemical yields from a given plant material, coproducts in the form of valuable phytochemicals could be extracted prior to hydrolysis at or near the biorefinery site.

Ginseng is the common name for a variety of *Panax* plants, particularly *Panax ginseng* (Asian ginseng) and *Panax quinquefolium* (American ginseng). They are among the most precious roots widely used in health foods. The traditionally medicinal species of the *Pfaffia* genus, such as *Pfaffia glomerata*, which belong to the Amaranthaceae family, are common Brazilian substitutes for the *Panax* genus plants, which are known widely as Brazilian ginseng roots (BGR). There has been significantly less research into the chemical constituents of BGR than there has been for both Asian and American ginseng [5]. Accordingly, the goal of this work was to quantitatively analyze all of the products derived from BGR through extraction using pressurized water.

MATERIALS AND METHODS

Plant material and pressurized liquid extraction process (PLE)

BGR was cultivated, treated and stored according to procedures described in a previous work [6]; the PLE process was performed according to methodology described in the same work [6].

Products characterization

Beta-ecdysone content

Beta-ecdysone quantification was performed by HPLC according to the methodology described by Rostagno et al. [7].

Emulsification index (E_{24})

The emulsification indexes of the BGR extracts were determined according to the Cooper & Goldenberg [8] methodology, with modifications: 2 mL of soybean oil were added to the same volume of extract in an aqueous solution (50 $mg.mL^{-1}$). The solution was then mixed under vortex for 2 min using a tube mixer (Phoenix, model AP 56, Araraquara, Brazil) and left to stand for 24 hours. The E_{24} (%) was calculated by dividing the measured height of the emulsion layer by the total height of the mixture and multiplying by 100.

Total reducing sugars content

The total amount of reducing sugars was determined using the Somogyi-Nelson colorimetric method [9, 10]. The extracts were hydrolyzed in dilute acid to ensure the presence of all the oligosaccharide content. The dry extracts were then diluted until a final concentration of 1 mg of dry extract/1 mL of distilled water was obtained. A glucose calibration curve was established, with results expressed as milligrams of glucose equivalents/g dry extract. A spectrophotometer (Femto, model 800 XI, São Paulo, Brazil) was used for this purpose.

Aqueous extract pH

Before freeze-drying, the pH values of the aqueous extracts were measured using a pH-meter (Digimed, model DM-22, São Paulo, Brazil) calibrated with buffers at pH 4.01 and 6.86.

Total phenolic content

The total phenolic content was estimated using the Folin-Ciocalteau method for total phenolics based on a colorimetric oxidation/reduction reaction of phenols [11].

Statistical analysis

Analysis of variance (ANOVA) was performed using Minitab®16 (Minitab Inc. International, State College, USA) with a significance level of 5 % (p-value ≤ 0.05).

RESULTS

The static extraction time did not significantly affect the all-response variables (p-value > 0.05).

Beta-ecdysone content and surfactant properties

Beta-ecdysone is the most important bioactive compound present in BGR extracts and is responsible for their therapeutic effects [12]. The emulsification properties of products from BGR are attributed to the presence of saponins in its composition. Saponins are glycosides containing one or more sugars on a triterpene or steroid aglycone backbone, also called sapogenins. The sugars are the hydrophilic portion of the molecule, and the sapogenin is the

lipophilic portion of the molecule [13, 14]. The experimental beta-ecdysone content and emulsification index (E_{24}) of the BGR extracts are shown in Figure 1.

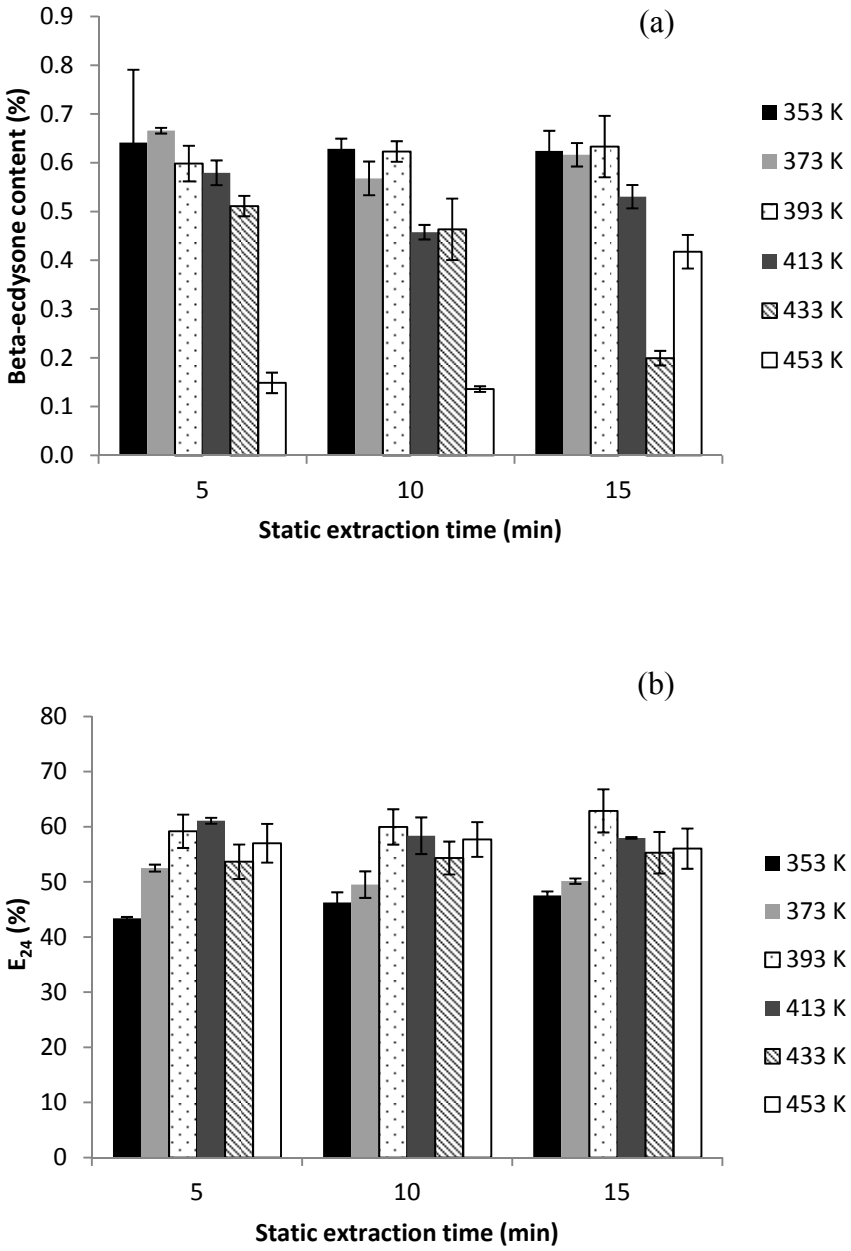

Figure 1: (a) Beta-ecdysone content and (b) Emulsification index of BGR extracts obtained for different temperatures and static extraction times.

Temperature had a significant effect on the beta-ecdysone content (p-value < 0.0001); this finding can be observed in Figure 1a, which shows that beta-ecdysone content decreased with increasing temperature, mainly above 393 K. This may be due to the degradation of beta-ecdysone at these higher temperatures. Statistically, the temperature had a significant influence (p-value < 0.0001) on the E_{24}, and Figure 1b shows that there was no significant decrease of E_{24} with the increase in temperature.

Although it is possible to obtain two distinct products from the same plant material (one extract with beta-ecdysone and one extract with surfactant properties), the residue from the extraction processes is still a rich source of polysaccharides. The polysaccharides may be processed into a number of value-added materials. Considering the economic benefits associated with increasing chemical output from the biomass of BGR, the sugars and phenolic compounds content was investigated.

Total reducing sugars content

Temperature had a significant influence (p-value = 0.0248) on the total reducing sugar content of the BGR extracts. It is shown in Figure 2 that the glucose equivalent content increases with increasing the temperature, up to 433 K, for static extraction times of 5 and 10 minutes. For the static extraction time of 15 minutes, the glucose equivalent content increases only up to 413 K. This may be due to the prolonged contact time between the solvent and plant material at high temperature. Above these temperatures, the glucose equivalent content decreases. This may be due to degradation of the sugars present in the extract, which is implicated by decreasing pH values for temperatures above 413 K, indicating the formation of compounds with an acid character (i.e., organic acids) [15, 16].

Total phenolic content

Statistically, the temperature had a significant influence (p-value = 0.0002) on the total phenolic content, and its effect is shown in Figure 3. The total phenolic content increases slightly with increasing temperature and static extraction time. At a temperature of 453 K, however, the total phenolic content increases dramatically, reaching 60 % gallic acid equivalents. This may be a result of lignin degradation because water under sub- and supercritical conditions effectively decomposes lignin and its derivatives due to the high reactivity of its functional groups at 423-623K. The lignin content present in BGR is 4.2 ± 0.15 % (results not shown). Lignin decomposition in sub/supercritical water begins with hydrolysis to form phenolic compounds. The products formed by the thermal decomposition of lignin include phenolic compounds, volatile liquid and gaseous hydrocarbons, and both carbon monoxide and carbon dioxide [17]. The phenolic compounds generated by these methods are important intermediates in the chemical industry for a diverse range of products, such as pharmaceuticals, dyes, and antioxidants.

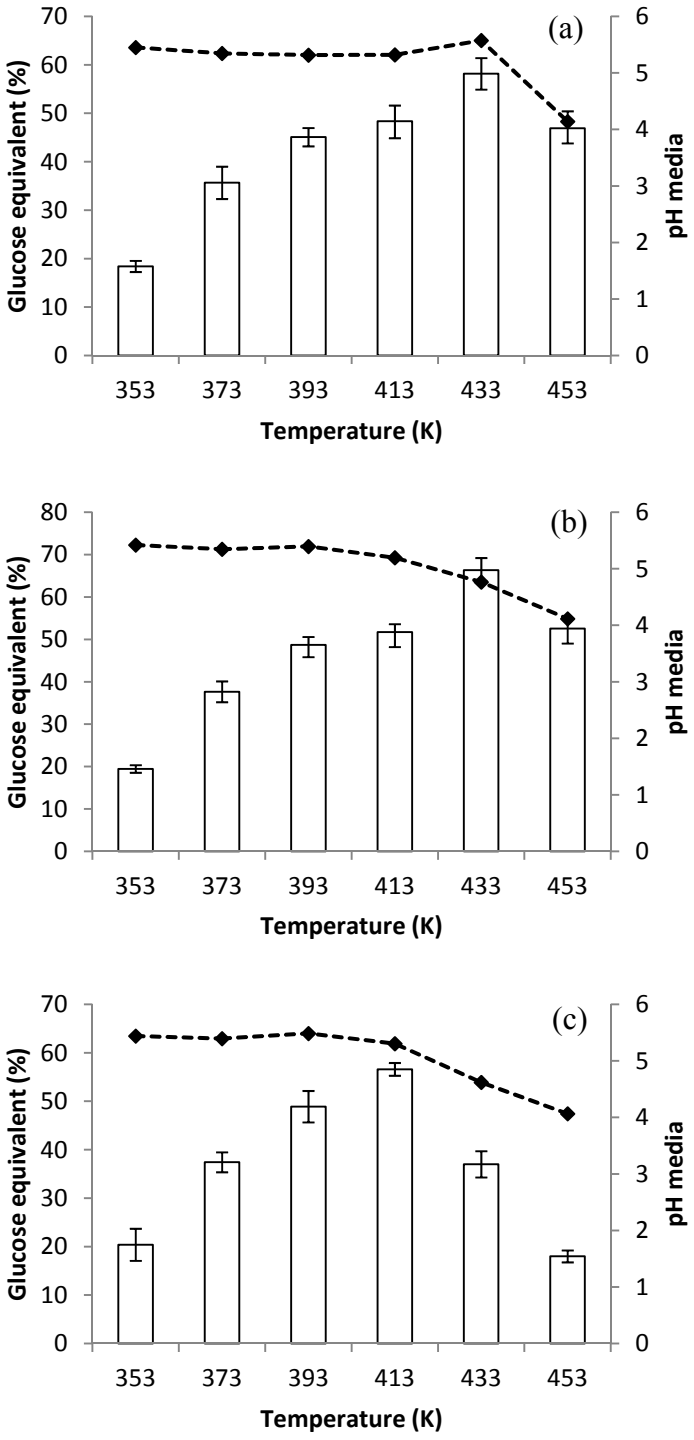

Figure 2: Total reducing sugar content (bars) and pH of the media (line) of the extracts obtained from Brazilian ginseng roots as a function of the temperatures studied at static extraction times of (a) 5 min, (b) 10 min and (c) 15 min.

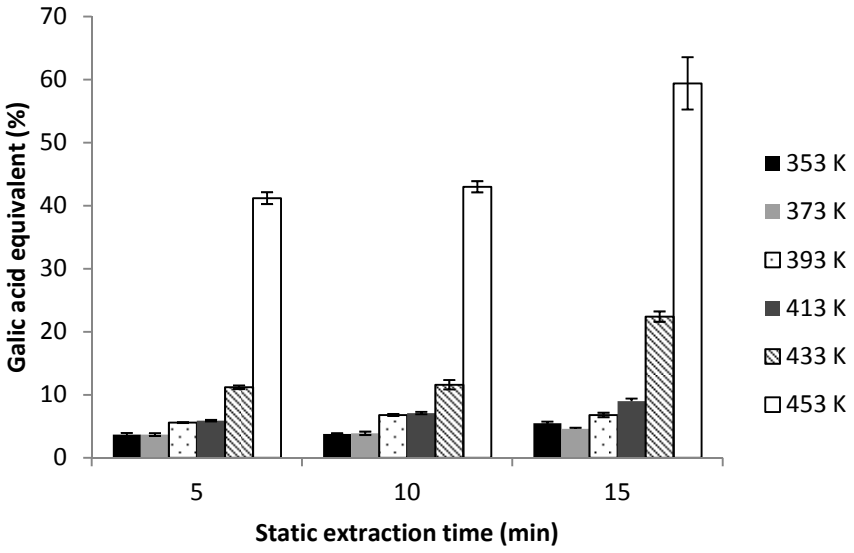

Figure 3: Total phenolic content of the extracts obtained from Brazilian ginseng roots for different temperatures and static extraction times.

CONCLUSION

The subcritical water extraction processes described above demonstrate great potential for the recovery of multiple products with different properties from the same raw material, in this case BGR. Processing raw materials using a single pressurized fluid offers the opportunity to obtain unique product profiles, as demonstrated in the results of this study. However, the combination of two or more solvents appears to be a more versatile and promising avenue for increasing extraction yields, provided the appropriate optimization for each step of processing is applied.

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