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Valorization of pineapple waste for the extraction of bioactive compounds and glycosides using autohydrolysis

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Fructose (PubChem CID: 5984)
Saccharose (PubChem CID: 5988)
Gallic acid (PubChem CID: 370)
Hydroxybenzoic acid (PubChem CID: 135)
Chlorogenic acid (PubChem CID: 1794427)
Epicatechin (PubChem CID: 72276)
Coumaric acid (PubChem CID: 637542)
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ABSTRACT

Autohydrolysis process, an alternative technology that uses only water as extraction solvent, was evaluated for the extraction of glycosides and polyphenols from pineapple waste. A Box-Behnken design was carried out using different temperatures (150–200 °C), solid-liquid ratio (1:40–1:10 w/v) and reaction time (15–45 min). The best condition for the production of glucose (27.6 g/L) and fructose (33.8 g/L) was 150 °C, 30 min and 1:10 w/v while the highest amount of extracted total polyphenols (1.75 g/L) was obtained at 200 °C, 30 min and 1:10 w/v solid-liquid ratio. In all treatments were detected gallic acid, hydroxybenzoic acid, chlorogenic acid, epicatechin, coumaric acid and caffeic acid. These results indicate autohydrolysis as a valuable alternative for the sustainable extraction of high value-added molecules for further use in industrial, food, cosmetic and health applications.

1. Introduction

The pineapple (*Ananas comosus*) is a perennial plant of the Bromeliaceae family whose fruit is marketed around the world. In 2013, pineapple production was around 210,000 t mainly in Latin America and the Caribbean and Portugal (FAO, 2016). This fruit is eaten fresh or used to make juices, jams and preserves.

Pineapple canning industries produce large quantity of solid waste. The dry matter content of pineapple waste is around 10%, composed of about 96% organic matter and 4% inorganic matter (Abdullah, 2007). According to (Conesa et al., 2015), in the elaboration of pineapple-based food products, this value can be 50%. Other authors reported a waste production from pineapples processing in the range of 25–35% of the weight of the fruit (Seleni et al., 2014). This discrepancy can be due to pineapple variety and the type of processing used.

Worldwide, agricultural industries generate large amount of biomass residues which can cause environmental and pollution concerns (Santana-Méridas, González-Coloma, & Sánchez-Vioque, 2012). Wastes derived from food processing are identified as major resources for the bio-based processes development (Lin et al., 2014) and a sustainable use of these food by-products for the production of value-added products (as chemicals, materials and fuels) could contribute to reduce environmental concerns and improvement of economic growth.

Pineapple waste is an enriched raw material, composed mainly by insoluble fibers, pectins, sugars, protein, vitamins, minerals and phenolic compounds (Díaz-Vela, Totosaus, Cruz-Guerrero, & Pérez-Chabela, 2013). However, in most of the cases research has been focused on recovering proteolytic enzymes such as bromelain, a group of proteases with different applications in the food, textile, cosmetics and others (Coelho-Silvestre et al., 2012). Taking this in consideration, strategies are needed to recover other added value compounds available in pineapple wastes.

Glycosides obtained from these residues have various applications as food additives, prebiotics and biopreservatives (Cuevas, García, Hodaifa, & Sánchez, 2015). Moreover, phenolic compounds are of great interest in the pharmaceutical and food industry due to their biological properties with application on human health. There are few studies related to bioactive polyphenols from pineapple residues. Although we can mention some examples as myricetin, salicylic acid, tannic acid, *trans*-cinnamic acid and *p*-coumaric acid identified in a high dietary

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fiber powder from pineapple shell which is a part of waste and these compounds were reported as potent antioxidants (Larrauri, Rupérez, & Saura-Calixto, 1997). On the other hand, it has been shown that the polyphenols found from pineapple wastes such as ferulic acid and syringic acid, are responsible for antioxidant and antimicrobial activity (Upadhyay, Prava-Lama, & Tawata, 2010).

A wide range of extraction methods have been employed to recover bioactive compounds from agro-industrial wastes (Santana-Méridas et al., 2012). In some investigations the operational conditions are determinant to recovery of bioactive compounds, as the type of solvent, time and temperature of extraction are significant variables on the extraction process. For example, phenolic compounds from pineapple peel were obtained using 25 g of raw material by extraction with reflux, first with 150 mL of n-hexane to remove non-polar compounds and then with 150 mL methanol for 4 h at 60 °C these conditions they influenced on the antioxidant activity by DPPH scavenging capacity method (Li et al., 2014).

Alternatively, aqueous processing (also known as autohydrolysis or liquid hot water) is a recognized environmentally-friendly technology used for the extraction of value-added compounds from several raw materials (such as agricultural residues and wood biomass) (Parajó et al., 2008; Conde, Moure, Domínguez, & Parajó, 2011). This hydrothermal treatment (that uses water as only medium of reaction) has several advantages such as: absence of chemical solvent, no corrosion problems, simple to operate, cost-effective and economical (Ruiz, Rodríguez-Jasso, Fernandes, Vicente, & Teixeira, 2013).

The aim of this work was the find the ideal conditions for bioactive compounds (polyphenols) and glycosides extraction from pineapple wastes by aqueous processing. Time, liquid to solid ratio and temperature were evaluated using a Box-Behnken design. In addition, the antioxidant activity of the liquid extracts was evaluated with ABTS and DPPH techniques.

2. Materials and methods

2.1. Reagents and chemicals

Glucose, fructose, Folin-Ciocalteu phenol reagent, ABTS, 2, 2-diphenyl-1-picrylhydrazyl, gallic, hydroxybenzoic, chlorogenic, epicatechin, coumaric and caffeic acid were purchased from Sigma-Aldrich, Germany, all HPLC grade. Sulphuric acid (96%) and sodium carbonate from Fisher Scientific. Anthrone, methanol and phenol crystallized from PanReac AppliChem. Albumin from Merck. Bradford dye reagent from Alfa Aesar®. Acetonitrile and formic acid grade HPLC from Chem-Lab.

2.2. Raw material

Pineapple waste (composed mainly by core and skin) was kindly provided by a local food company (Oporto, Portugal). Pineapple wastes were placed in aluminum trays and submitted to a drying stage at 60 °C for 48 h in order to avoid degradation. The dried samples were mixed to obtain a representative lot, milled (Cemotec mill model 1090, Sweden) and sieved to obtain a particle size of < 1 mm. Then, samples were placed in a plastic bag and stored in a dry place until further analysis.

2.2.1. Chemical characterization of raw material

2.2.1.1. Total and reducing sugars content. Total sugars determination of pineapple waste was carried out following the methodology reported by Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Glucose was used as standard. In a test tube, 500 µL of sulphuric acid (H_2SO_4 96%) were added to the sample and mixed. From this, 300 µL were taken and placed in a 96 well microplate and in oven at 105 °C for 10 min and then cooled at room temperature. Finally, absorbance was read in an ELISA (Synergy HT, BioTek®, Winooski, VT, USA) microplate analyzer at 480 nm.

The determination of reducing sugars present in pineapple waste was carried out following the methodology reported by Dreywood (1946), also using glucose as standard. In a test tube, 500 µL of diluted sample and 1 mL of the anthrone reagent were added. The tubes were placed in an ice bath for 10 min. Subsequently, the tubes were placed in a water bath at 80 °C for 15 min. Samples were cooled at room temperature. Finally, absorbance was read in an ELISA (Synergy HT, BioTek®, Winooski, VT, USA) microplate analyzer at 620 nm. In both determinations, the pineapple waste samples were diluted one thousand times with distilled water.

2.2.2. Soluble protein content

Soluble protein determination of pineapple waste was carried out in accordance with the methodology reported by Bradford (1976). Albumin was used as standard in a concentration in the range up to 1 g/L. Samples were diluted one hundred times; 50 µL of diluted sample and 500 µL of Bradford reagent were mixed in a test tube. Aliquots of 300 µL were taken and placed in 96 well microplate. Absorbance was read at 595 nm in an ELISA (Synergy HT, BioTek®, Winooski, VT, USA) microplate analyzer.

2.3. Aqueous processing for extraction of glycosides and polyphenolic compounds: experimental Box-Behnken design (BBD)

Box-Behnken design was used for evaluation and optimization of the effect of independent variables (time, solid to liquid ratio (w/v) and temperature) on the release of bioactive compounds and glycosides from pineapple waste by autohydrolysis. Three variables at three levels were used. All treatments were performed in triplicate. Nine central points were used. The condensed matrix of treatments and the variables evaluated were shown in Table 1. To evaluate the proposed model, an Analysis of Variance (ANOVA) test was analyzed for each response variable. A second-order polynomial Eq. (1) that all interaction terms was used to calculate the predicted response:

$$Y = \beta_0 + \sum_{i=l}^{k} \beta_i X_i + \sum_{i=l}^{k} \beta_{ii} X_i^2 + \sum_{i<j}^{k-1} \beta_{ij} X_i X_j + \varepsilon$$

where Y is the response variable, β_0 is constant, $\beta_i X_i$ is linear effect of the independent variable, $\beta_{ii} X_i^2$ is quadratic effect of the independent variable, $\beta_{ij} X_i X_j$ is interaction effects of the independents variables and ε is total error.

Extraction experiments were carried out in steel cylinders (12.5 cm long and 6.45 cm wide) as described by Ruiz, Vicente, and Teixeira (2012) placed inside an oil bath (MC Model, Julabo, Labortechnik GmbH, Seelbach, Germany) with temperature control. Distilled water and pineapple waste were mixed at the different ratios mass/volume shown in Table 1. At the end of the reaction time, the cylinders were placed in an ice bath for cooling down. Subsequently, solid and liquid phases were separated by filtration in order to obtain hydrolysates.

2.4. Hydrolysates analysis: glycosides and polyphenols quantification and identification

Hydrolysates from autohydrolysis assays were analyzed for glycosides and polyphenols content. Glucose and fructose were quantified by HPLC (Jasco AS-2057 plus, Tokyo, Japan) and bomb (Jasco 880-PU, Tokyo, Japan) equipped with refractive index detector (Knauer K-2300, Berlin, Germany) and a column Metacarb 87H (300 × 7.8 mm, Varian, USA) eluted with 0.005 M H_2SO_4, flow rate of 0.6 mL/min in a temperature control chamber (Jhones Chromatography model 7971) at 60 °C (time analysis of 20 min). For polyphenols analysis, it was used an UHPLC (Shimadzu Nexera × 2 LC-30 CE, USA) equipped with autosample (Nexera × 2 SIL-30 AC prominence, USA), diode array detector (SPD-M20A), column oven (CTO-20 AC prominence) and a column Teknokroma ® (Brisa LC2 C18 TR-010481, 25 × 0.46 cm, 5 µm, Barcelona, Spain). Standard solutions of gallic, hydroxybenzoic,