PRESSURIZED LIQUID EXTRACTION APPLIED ON VEGETABLES BY-PRODUCTS – A PHENOLIC COMPOUNDS INTERACTIONS STUDY

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RESUMO – Subprodutos agroindustriais são considerados boas fontes de compostos fenólicos. A extração líquida pressurizada (ELP) é uma tecnologia verde, atualmente aplicada como técnica de extração acelerada permitindo extrair uma variedade de compostos bioativos de subprodutos vegetais. A farinha de frutas e hortaliças (FFH) gerada na produção de bebida isotônica apresentou 80% de fibra insolúvel de um total de 48,4% de fibras alimentares, 26% carboidratos, 9,5% proteínas e 5% lipídios. Este estudo objetivou caracterizar os compostos fenólicos na FFH por UPLF Q-TOF-MS após ELP (9 condições), e propõe uma discussão sobre a interação dos fenólicos e os macronutrientes. A técnica de extração aplicada propitie o rompimento de interações matriz-analito extraindo diversos compostos fenólicos. Foram identificados tentativamente 90 compostos: ácidos fenólicos (28), flavonoides (32) e outros polifenóis (28). Caracterizar o perfil de fenólicos em resíduos de alimentos como a FFH permite indicar uma melhor aplicação da matriz como nutracêutico ou ingrediente farmacêutico ou alimentar.

PALAVRAS-CHAVE: interação matriz-analito; extração verde; nutracêutico; polifenóis.

ABSTRACT – Agro-industrial by-products were considered good sources of phenolic compounds. Pressurized liquid extraction (PLE) is a “green” technology, currently applied as accelerated solvent extraction technique for a wide variety of bioactive compounds from food by-products. Fruit and vegetable residue (FVR) was generated from the production of an isotonic beverage presenting 80% insoluble dietary fibers from total fibers (48.4%), 26% available carbohydrates, 9.5% proteins and 5% lipids. This study aimed to characterize phenolic compounds in FVR flour by UPLC Q-TOF-MS after PLE (9 conditions), and purposes a discussion about phenolics and macronutrient interactions. Extraction techniques applied propitiate breaking matrix-analyte interactions extracting large number of compounds. Hence, 90 compounds were tentatively identified: phenolic acids (28), flavonoids (32) and other polyphenols (28). Establish the phenolics profile in food by-products such as FVR, can drive a better application as nutraceutical or food or pharmaceutical ingredients.

KEYWORDS: matrix-analyte interactions; green extraction; nutraceutical; ingredients
1-INTRODUCTION

Phenolic compounds are commonly extracted using conventional extraction methods by organic solvents. However, most of them cannot be easily extracted due to complex structures and also by the presence of insoluble bound forms conjugate with cell wall components through ester, ether or glycosidic bonds (Dey et al., 2016). Extraction techniques are worldwide studied to optimize the extraction process and increase efficiency in the extraction of free and bound phenolic compounds (Rodriguez-Pérez et al., in press). Pressurized liquid extraction (PLE) is a “green” technology based on eco-friendly solvent extraction at high temperatures and pressures to prepare solid samples for further clean-up, analytical separation and determination (Herrero et al., 2015; Mustafa & Turner, 2011; Vazquez-Roig & Picó, 2015).

Fruit and vegetable residue (FVR) generated from the production of an isotonic beverage based on the whole processing of 11 vegetable species was used in this work (Ferreira et al., 2015). The FVR presented a rich macronutrient composition, especially fibers (80% insoluble dietary fibers of the total fibers 48.4%) (Roberta et al., 2014). Dietary fibers associated with phenolic compounds antioxidants have become increasingly interesting, as it could be useful for the food industry to enhance the bioactivity and technological properties of products (Ajila & Prasada Rao, 2013; Quirós-Sauceda et al., 2014). Considering the interesting chemical composition of FVR flour and its potential application as new food additive, the aim of this study was to apply an accelerated solvent extraction, such as PLE and to characterize the phenolic compounds from FVR and promote a discussion about phenolics and macronutrient interactions.

2. MATERIAL AND METHODOS

2.1 Extraction of phenolic compounds by Pressurized Liquid Extraction (PLE)

Two gram of sample was mixed with 2 g of sea sand and placed into an 11-mL volume extraction cells. Samples were extracted under the following conditions, time was fixed at 20 min, pressure: 10 MPa, heat-up time: 5 min; static time: 5 min; flush volume: 60%; purge: N₂, 60 s; number of cycles: 1. The extraction parameters (Table 1) were optimized with modifications (Lozano-Sánchez et al., 2014).

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Temperature (ºC)</th>
<th>Proportion ethanol:water (%)</th>
<th>Dielectric constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>50:50</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>50:50</td>
<td>34.7</td>
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<tr>
<td>3</td>
<td>176</td>
<td>85:15</td>
<td>21.6</td>
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<tr>
<td>4</td>
<td>63</td>
<td>15:85</td>
<td>59.1</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>0:100</td>
<td>50.4</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>50:50</td>
<td>26.8</td>
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<tr>
<td>7</td>
<td>63</td>
<td>85:15</td>
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<td>8</td>
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<td>33.4</td>
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<tr>
<td>9</td>
<td>120</td>
<td>100:0</td>
<td>19</td>
</tr>
</tbody>
</table>
2.2 UPLC Q-TOF-MS analysis and data processing

For UPLC-MS analysis, 2 uL of extracts and standards were injected in triplicate onto a UPLC QqTOF-MS/MS system equipped with an electrospray ionization source (ESI) (Xevo G2-S QTOF, Waters Corporation, UK) operating in negative ion mode ESI(-). Chromatographic separation was carried out on an ACQUITY UPLC® HSS T3 C18 column (100 x 2.1 mm, 1.8 µm particle size). The mobile phase gradient elution consisting of acidified water (0.3% formic acid) (pump A), acetonitrile with 0.3% formic acid and 5 mM ammonium formate (pump B). The gradient method were 3% B at 0 min, 50% B at 11.8 min, 85% B at 12.38-13.53 min. Data were collected from m/z 50 to 1000. The capillary and cone voltage were set at 2.0 kV and 30 V, respectively. The dessolvation gas (N2) was set at 600 L.h-1 at a temperature of 450 °C, the cone gas was set at 50 L.h-1, and the source temperature was set at 120 °C. Data acquisition was performed by using MassLynx 4.1 (Waters Corporation, UK).

The raw data of all replicates analysis were processed with Progenesis QI v2.1 (Nonlinear Dynamics, Waters Corporation, UK). The identification of phenolic compounds was performed by searching for polyphenols with MetaScope, a fully integrated search tool that allowed the use of the in-house database PolyphenolsPubChemID by using the following parameters: precursor mass error ≤ 5 ppm, fragment tolerance ≤ 10 ppm, retention time within 0.5 min and retention time limits 0.3−11.0 min. Target analysis was also applied for identification of the phenolic compounds by comparing the run parameters of 35 phenolic standards. The processed data were exported to EZinfo, where Principal Components Analysis (PCA) was elaborated.

3. RESULTS AND DISCUSSION

The most part of real food systems are colloidal by their nature, where the components performed two main functions, nutritional and structure-forming (Semenova, 2007). In food systems, several types of interactions may occur among components, including hydrogen bonds, hydrophobic, Van der Waals, and electrostatic interactions (Soltanizadeh et al., 2014).

The dielectric properties of a solvent are one of the primary features for its selection as the extracting solvent. The general consensus states that the dielectric properties of the solvent used in the extraction process determine its ability to extract nutrients (Singh et al., 2014). Phenolic compounds were extracted from FVR using PLE in different experimental conditions. The transfer of analytes from the matrix is strongly influenced by the solvent polarity and its physical properties, which is correlated to the various parameters of the extraction process (Vazquez-Roig & Picó, 2015).

![Figure 1. Principal Components Analysis (PCA) of the phenolics compounds extracted in the different experimental extraction conditions of fruit and vegetable residue.](image-url)
According to the principal components analysis (PCA) of the phenolic compounds tentatively identified in the FVR, the optimum condition to extract phenolic compounds was clearly obtained in the condition 6 (200 °C, 50% ethanol). In this case, the mid-polarity solvent associated with high temperatures, enhanced diffusion rates and solubility of analytes and improved the extraction efficiency, especially for phenolic acids.

In all experimental conditions, all classes of phenolic compounds are extracted, being flavonoids those more efficiently extracted (Figure 2). Flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds (Balasundram, Sundram, & Samman, 2006). Furthermore, different profile of PLE of flavonoids was obtained in the conditions 2, 5, 6 and 9 (Figure 3).

Flavonoid structures enable biopolymers interactions in hydrophobic environment, but hydrophilic interactions with hydroxyl group are also possible. In fact, it can easily occur during extractions process. Hydrophilic interactions with low molecular components can be established by strong bonds at polymeric internal network. Breaking matrix-analyte interactions and achieving higher diffusion rate is the goal when applied high temperature and pressure (Vazquez-Roig & Picó, 2015). Low molecular size structures and high number of hydroxyl group promote strong links in internal polymeric network. Elevated temperature in conditions 3, 6 and 8 certainly broke biopolymers bounds and the phenolic acids release was effortless.

Figure 2. Normalized abundance by UPLC-Q-TOF-MS of the classes of phenolic compounds extracted in the different experimental extraction conditions of fruit and vegetable residue.

Focusing on application of FVR as nutraceuticals or ingredients for new functional foods, establish phenolics compounds is essential. PLE identified 90 bioactive compounds: phenolics acids (28), flavonoids (32) and other polyphenols (28) (data not shown). The phenolic profile of FVR can be compared with those of berries such as Vaccinium arctostaphylos L. and V. myrtillus L., worldwide known for the health benefits attributed to their polyphenols and high natural anthocyanin contents (Colak et al., 2016). Health benefits of some specific compounds is also well established, for instance the very recent works about hesperidin (Du Preez et al., 2016; Justin et al., 2016; Martin et al., 2016; Mohammadi et al., 2016). This flavanone represents above 50% of flavonoids in all conditions (Figure 3). Remarkably, the extract 5 shows above 90% of this compound. These results strengthen the use of FVR as an alternative source of bioactive antioxidant compounds. Piceatannol is a natural analog of resveratrol and this phytochemical has showed a wide range of biological activities, such as skin protection, vasodilatation and lowering of blood glucose levels, besides to exhibit anti-inflammatory properties (Yamamoto et al., 2016). Piceatannol is a naturally occurring stilbene derivative and represented the major compound in the other polyphenols class present in extracts of FVR (data not shown).
Figure 3. Profile of flavonoid compounds extracted from FVR by PLE.

4. CONCLUSIONS

Fruits and vegetables are considered complex food matrices and application of extraction techniques can propitiate breaking matrix-analyte interactions. In this study, PLE combined to high definition UPLC-MS enabled the extraction and identification of a large number of phenolic compounds of different polarities. The assessment of the phenolic compounds profile obtained after different PLE conditions allowed also point out analyte interactions with macronutrients. All these results together, especially the characterization of the phenolics profile in food by-products such as FVR, can drive a better application as nutraceutical or food or pharmaceutical ingredients.

5. ACKNOWLEDGEMENTS

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6. REFERÊNCIAS BIBLIOGRÁFICAS


