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ULTRASOUND-ASSISTED AND ENZYME-ASSISTED EXTRACTION OF FRUCTANS AND PHENOLICS FROM PARSNIP (*PASTINACA SATIVA* L.)

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Abstract
Parsnip (Pastinaca sativa L.) is a vegetable that gained attention due to its significant content of fructans, besides macronutrients and other biologically active compounds. The major compound of fructan class is inulin, an ingredient with prebiotic properties used in pharmaceutical and functional food industries.
The main purpose of this study was to evaluate the extraction yield of fructans and phenolics in fresh and dried parsnip root following non-conventional techniques (ultrasound and enzyme -assisted extraction) and to compare it to that of the traditional method. Extraction was performed in water, as eco-friendly solvent. Ultrasound-assisted extraction was optimized for different extraction time (5, 10 and 15 minutes) at ultrasonic amplitude of 70%. The enzyme cellulase was used in the pre-treatment experiments. Enzyme-assisted extraction was optimized for different incubation temperature (40^o, 50^o and 55^{o}C). Quantitative analysis of the investigated compounds was done using standard spectrophotometric methods.
The results showed good extraction yields in ultrasonic and enzyme pre-treated extracts compared to those of samples extracted by traditional methods. High levels of inulin and phenolic compounds were obtained in ultrasonic extracts which undergoes an enzymatic treatment for 3h at 40^{o}C.

Keywords: cellulase, fructans, parsnip, phenolics, ultrasounds

1. INTRODUCTION

Fructans are considered reserve carbohydrates widely spread in about 36,000 plant species in quantities that vary according to several factors like soil moisture (Ohaneye et al., 2019), soil salinity (Kirtel et al., 2018) and temperature (Puangbut et al., 2012) . Fructans are found in almost all species included in the Asteraceae family, some of them with a particular economic importance, the most famous being chicory root and Jerusalem artichoke (De Carvalho and Figueiredo-Ribeiro, 2001).

Pastinaca sativa, commonly known as parsnip is a plant which belongs to Apiaceae family. The root of this plant is used for culinary purpose and as food ingredient, due to its flavor and high content of dietary fibre (Southgate, 2001; Ostertag et al., 2002). The results of some studies have shown that the root contains 26.8 g/100g DM dietary fibre, 1.3 g/100g DM fructans and 5.9 g/100g DM crude proteins. Besides these compounds, the parsnip contains several vitamins and minerals, being low in calories, fat and sodium (Kalala et al., 2017).

Fructans are polysaccharides composed of 1 to 70 units of fructose, usually bound by a terminal sucrose molecule. The structure of fructans is diverse. The linear form characterized by $\beta(2\rightarrow6)$

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fructosyl-fructose linkages is found in levan-type fructans, while the β(2→1) linkage is characteristic of inulin-type fructans (Carabin and Flamm, 1999). The difference between carbohydrates consists in their degree of polymerization, the inulin being characterized by a large variation in the number of monosaccharide unit (Carabin and Flamm, 1999).

Fructo-oligosaccharides cannot be digested by humans, so they are considered to be non-caloric components resistant to the hydrolytic activity of salivary and small intestine enzymes. Bifidobacteria present in the large intestine degrade fructo-oligosaccharides, which results in short chain fatty acids with particular importance in the body (Luo & et al., 1996). Some of the beneficial effect are that short-chain fatty acids lower the pH, reduce the rotting process in the intestine, and increase the bifidobacteria population (Choque Delgrado et al., 2010).

Ultrasound-assisted extraction is a non-conventional method succesfully applied to extract diverse compounds showing some benefits. Its use shortens extraction time, reduces the solvents consumption and presents high reproducibility (Chemat et al., 2017). All studies have shown that this method is a green and economical alternative to conventional methods such as maceration, Soxhlet extraction or Clevenger distillation, to be applied for food and natural products (Chemat et al., 2017). On the other hand, the enzyme-assisted extraction might become an useful tool particularly for the food industry. By using the enzymes such as cellulase, pectinase or hemicellulase, the release of bioactive compounds has been shown to be improved, due to breakage of the cell wall which facilitates the release of cellular content (Puri et al., 2012).

The aim of the present study was to comparatively evaluate the content of inulin and phenolics extracted from fresh and dried parsnip root by the aid of conventional and non-conventional methods. Ultrasonication time and enzyme incubation temperature were investigated for recovering high yields of bioactive compounds. Combination of non-conventional extractive technologies was also tested as this may result in enhanced yield of extraction of bioactive compounds compared to conventional methods.

2. MATERIALS AND METHODS

2.1. Plant material and chemical reagents

The parsnip root samples purchased from a local market in Sibiu, Romania were washed, peeled, cut into slices and grated. Plant material was divided in two parts, one was used in the form of fresh sample, the other was oven-dried at 35^{o}C for 24 hs, up to a moisture content of about 2.8%. Dried sample was grinded for 3 min at 2000 - 5000 rpm using a grind-mill (Retsch GM 200). The samples have been stored in the freezer until analyses were performed.

Cellulase from *Aspergillus niger*, activity >60.0000 U/g, was used for enzyme-assisted extraction. All chemical reagents were of analytical grade.

2.2. Extraction procedures

2.2.1. Maceration

The maceration process was done according to the method proposed by Apolinario et al., 2017, with some modification. Briefly, approximately 2.8 g of plant material was mixed with 20 ml of distilled water. The extraction was performed in water bath at 80^{o} C for 2 hs. The mixture was filtered and the residue was re-extracted with 20 ml distilled water for another 2 hs. The filtrates were combined for analysis.

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2.2.2. Ultrasound-assisted extraction

Ultrasound-assisted extraction was done using the Branson SLPe-150 CE sonifier, at 70% amplitude and room temperature. The investigated extraction times were 5,10 and 15 min, respectively.

2.2.3. Enzyme-assisted extraction

The enzyme-assisted extraction was performed according to the method described by Strati et al., 2015, using cellulase solution of 122.5 U/g. The samples were incubated under stirring in a water bath at different temperatures, as follow: 40^o, 50^o and 55^{o}C for 3 hs. After incubation, each test tube was immersed in boiling water for 3 min to inactivate the enzyme. The samples were filtered and the residue was extracted by the conventional and non-conventional methods.

2.3. Determination of inulin and total phenolics content

The inulin and total phenolics contents were determined spectrophotometrically, according to the method proposed by Pencheva et al., 2012 and Singleton and Rossi, 1965, respectively. The results were expressed in mg/100 ml for inulin and mg of gallic acid equivalents (GAE) / 100 g DM for phenolics content.

2.4. Statistical analysis

All measurements were performed in duplicate. Results were presented as mean±standard deviation.

3. RESULTS AND DISCUSSIONS

Following the conventional method of extraction of specific bioactives (phenolics, fructans) from parsnip root samples, 1.268 mg GAE/g of total phenolics and 109.081 mg/100g of inulin have been obtained from fresh sample, and 2.284 mg GAE/g of phenolics and 44.148 mg/100g of inulin for dried sample, respectively.

Regarding the optimization of extraction temperature using the ultrasound-assisted approach by testing three different points (5, 10 and 15 minutes), our investigation showed an optimum extraction time of parsnip phenolics and inulin from fresh and dried samples of 5 minutes of ultrasound treatment at 70% ultrasonic amplitude. This condition was used for subsequent extractions.

The highest amount of total phenolics (3.385 mg GAE/g) was found in dried sample that undergoes ultrasonication for 5 min. Fresh sample showed the highest amount of inulin (119.81 mg/100 ml) under similar extraction conditions.

As shown in Figures 1 and 2, fresh samples of *Pastinaca sativa* root contain lower amounts of phenolics compared to dried samples, while opposite was found in case of inulin content.

In order to improve the extraction yield and to recover high amounts of bioactive compounds, enzyme pre-treatment of samples was performed, followed by conventional and ultrasound-assisted extraction.

Figures 3 and 4 show the content of total phenolics and inulin in samples that have been treated with cellulase for 3 hs at 40^o, 50^o and 55^{o}C and control samples (non treated with enzymes). In all cases, the obtained results showed higher amounts of bioactives in enzyme ultrasonic extracts than those obtained through maceration. The highest contents (4.326 mg GAE/g, 156.674 mg/100ml inulin) were found in dried samples treated with cellulase for 3 hs at 40^{o}C, followed by ultrasonication for 5 min at room temperature.

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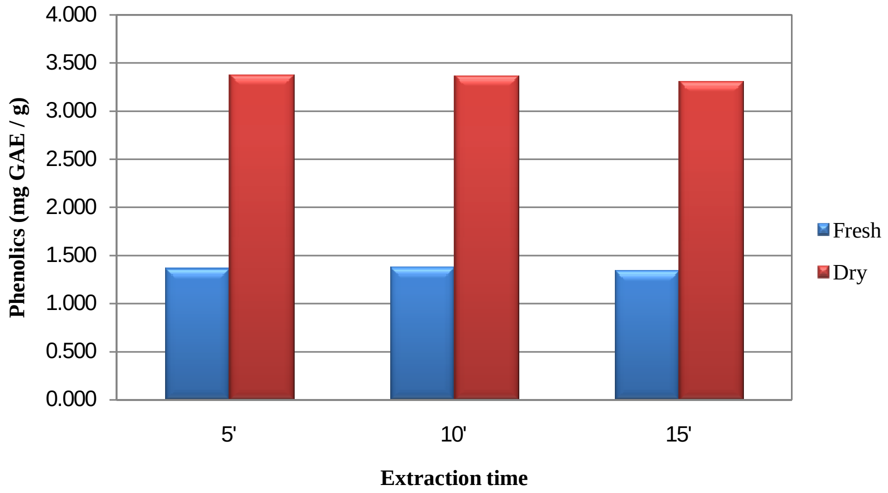

Figure 1. Total phenolics content of Pastinaca sativa root samples (fresh and dried) according to different extraction time using ultrasound-assisted extraction

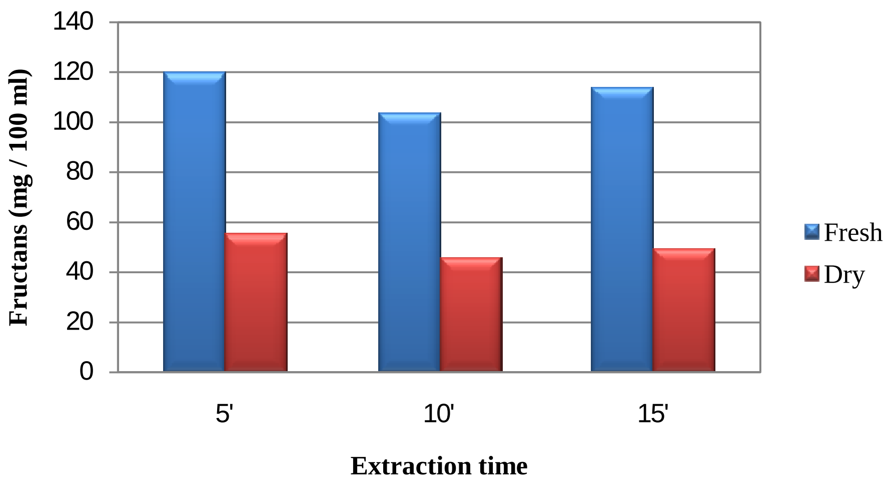

Figure 2. Inulin content of Pastinaca sativa root samples (fresh and dried) according to different extraction time using ultrasound-assisted extraction

High content of total phenolics indicates good antioxidant properties of the prepared aqueous extract of parsnip root.

To our knowledge, literature is scarce in studies on phenolics and fructans extracted from parsnip under ultrasonication and enzymatic pre-treatment. However, there are some studies on content of fructo-oligosaccharides (FOS) and total dietary fiber content. Jovanovic-Malinovska et al. reported a concentration of 0.39 g/100g FOS on fresh material. The total dietary fiber content in parsnip is about 30.4% on dry matter, according to Castro et al., 2012.

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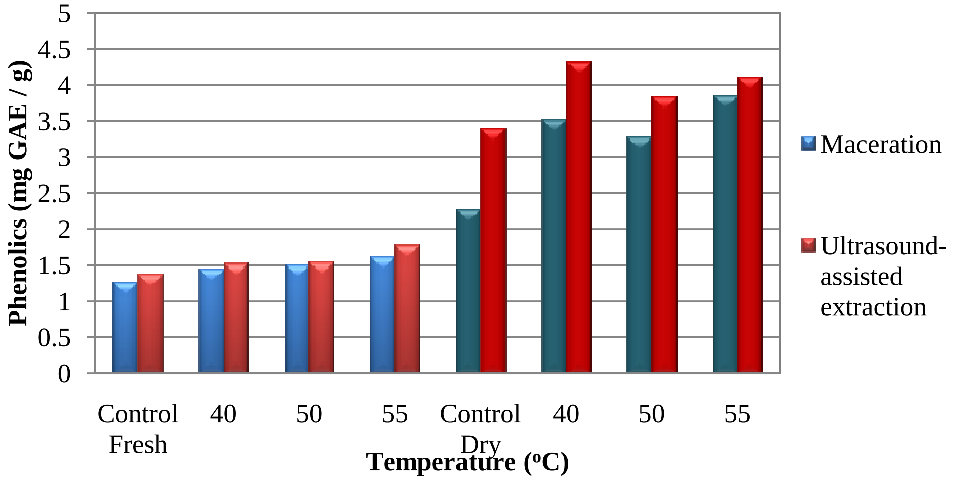

Figure 3. Total phenolics content of Pastinaca sativa root samples (fresh and dried) according to different incubation temperature using cellulase pre-treatment

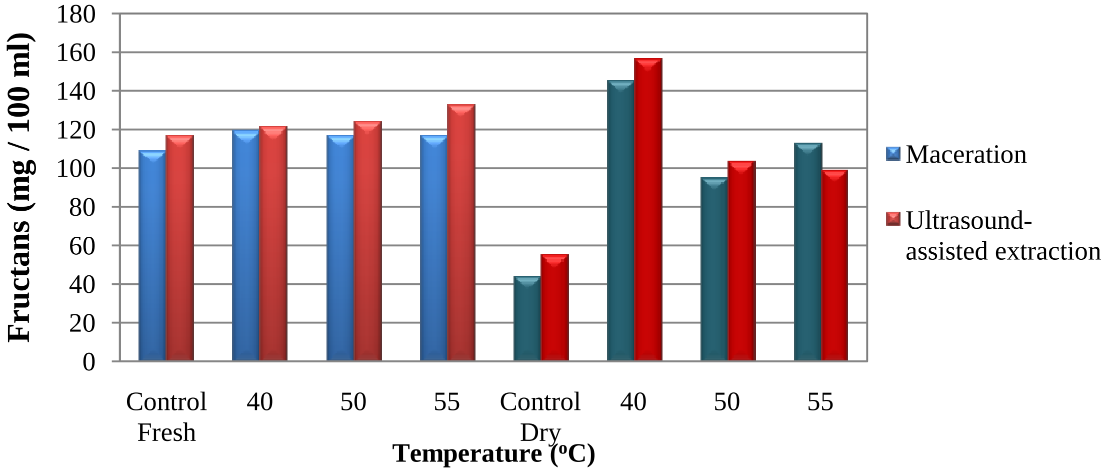

Figure 4. Inulin content of Pastinaca sativa root samples (fresh and dried) according to different incubation temperature using cellulase pre-treatment

4. CONCLUSIONS

Parsnip (*Pastinaca sativa* L.) root contains considerable amounts of phenolics and inulin, known for their health benefits. Extraction of these bioactive molecules was performed in water by using conventional (maceration) and non-conventional techniques (ultrasound-assisted and cellulase-assisted extraction). Ultrasound-assisted extraction was optimized for different extraction time (5, 10 and 15 min), while enzyme-assisted extraction was investigated for different incubation temperature (40, 50 and 55°C).

High levels of phenolic compounds (4.326 mg GAE/g) and inulin (156.674 mg/100ml) were obtained in ultrasonic extracts which undergoes an enzymatic pre-treatment for 3 hs at 40°C compared to conventional method.

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Crude aqueous extracts of parsnip root may found useful application for food supplements, food ingredients and pharmaceutical industries.

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