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Effect of pre-treatments on the antioxidant potential of phenolic extracts from barley malt rootlets

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ARTICLE INFO

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Gallic acid (PubChem CID: 370)
Sodium carbonate (PubChem CID: 10340)
Trolox (PubChem CID: 40634)
DPPH radical (PubChem CID: 2735032)
ABTS Radical (PubChem CID: 16240279)
Potassium Persulfate (PubChem CID: 24412)
Sodium hydroxide (PubChem CID: 14798)
Ethyl acetate (PubChem CID: 14798)
Citric acid (PubChem CID: 313)
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ABSTRACT

In this study, barley malt rootlets (BMR) were subjected to five different pre-treatments (steaming (220 °C), roasting (60 °C), autoclaving (121 °C), microwaving (160–800 W, 30–120 s) and enzyme treatment). Total phenolic content (TPC) and antioxidant activity of the BMR extracts were evaluated for both free and bound phenolics. The free phenolic content for non-treated extract was 1.8 mg/g of dry weight of BMR with 17.5% of antioxidant activity. Among the pre-treatments, autoclaving exhibited the highest values for free phenolics of 3.8 mg/g of dry weight of BMR and 71.6% of antioxidant activity. Pre-treatments did not show any effect on bound phenolic content, but increased antioxidant activity. The highest %DPPH activity for bound phenolics was observed for microwave treatment (160 W, 120 s) with 49.9%. Overall, pre-treatments significantly increased the free phenolic content of BMR phenolic extracts. Additional research is necessary to understand the phenolic profile and the thermal interactions of bound phenolic extracts.

Hypothesis

This study aims at testing the hypothesis that pre-treatments will not significantly affect the 48 antioxidant potential of the barley malt rootlets (BMR) phenolic extracts.

1. Introduction

Barley malt rootlets (BMR) are one of the brewers spent grains obtained by the removal of sprouts from malted barley, together with malt hulls and other parts of malt (Boruff and Van Lanen, 1958; Robbins and Pomeranz, 1963). The sprouts are constituted of acrospires and rootlets with more than 25% of protein and 11 to14% of crude fiber (Kent and Evers, 1994).

The total production of malt across the world is approximately 12MT which constitutes about 420KT of BMR (Robbins and Pomeranz, 1963; Kent and Evers, 1994). These are available at low cost throughout the year and produced in large quantities not only by large but also by small breweries.

Currently, BMR are used as animal feed supplement, while their potential use to produce alpha-amylase (Briggs et al., 2004), activated carbon (Briggs et al., 1981), ethanol (Lewis, 2000), lactic acid (Guido and Moreira, 2013) and xylitol (Coghe, Gheeraert, Michiels, & Delvaux, 2006) were previously investigated. Studies conducted on brewers spent grain (BSG) from different malt varieties, showed that these could be a potential source of ferulic and p-coumaric acids (Moreira et al., 2013). BMR are one among the natural source of antioxidants (Salama, El-Sahn, Mesallam, & Shehata, 1997). Salama et al. (1997) conducted a detailed composition analysis of barley rootlets and reported that BMR have up to 3.49 ± 0.01 g/kg dry matter of phenolic compounds. Antioxidant phenolic compounds from barley malt rootlets if extracted, could potentially reduce the formation of free radicals, thus becoming a

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source for natural antioxidants. Hence, an efficient extraction technique is an essential step in recovering the maximum amount of phenolics.

Solid-liquid extractions are the most commonly used methods with huge history due to their ease and broad applicability. However, with the increased use of solvents, high energy consumptions and degrading quality of thermolabile compounds, the application of emerging food technologies for extraction purposes have emerged (Misra et al., 2017). Among the novel techniques microwave-assisted (MW) extraction, ultrasound-assisted (US) extraction, accelerated solvent extraction and supercritical fluid extraction (SFE) are in recent use for the extraction of antioxidant phenolic compounds from food matrices. Several classes of phenolic compounds have been efficiently extracted from a variety of matrices using these green technologies, such as peanut skins (Ballard, Mallikarjunan, Zhou, & O'Keefe, 2010), quercus bark (Bouras et al., 2015), olive oil wastes and by products (Rosello-Soto et al., 2015), winery wastes and by-products (Barba, Zhu, Koubaa, Sant'Ana, & Orlien, 2016), mushrooms (Rosello-Soto et al., 2016), spent coffee grounds (Budaraju and Mallikarjunan, 2017).

Factors like solvent concentration, temperature of extraction, solid to solvent ratio play a critical role for efficient extraction of phenolic compounds from any food matrix. Thermal treatments can even enhance the yield and the antioxidant activity, by breaking lignocellulose of the cell components by creating disorder structure with or without the removal of inherent components (Duh, Yen, Yen, & Chang, 2001; Lee et al., 2003; Nicoli, Anese, Manzocco, & Lerici, 1997; Niwa, Kanoh, Kasama, & Neigishi, 1988). Enzymatic hydrolysis, steaming, autoclaving, microwave irradiation, and roasting are the most common pre-treatments used to increase the phenolic content, antioxidant activity and extraction efficiencies in various food matrices. The effect of several pre-treatments has been presented in different studies as a part of valorization of brewers spent grain (Ravindran et al., 2018). The effect of each pre-treatment varies according to its mode of action (Ravindran and Jaiswal, 2016).

Enzyme hydrolysis increases the rate of hydrolysis by penetrating the cell walls and is one of the best pre-treatment methods adopted for the high extraction yield of bioactive compounds (Sancho, Bartolome, Gomez-Cordoves, Williamson, & Faulds, 2001). Roasting accelerates the removal of moisture from the material surface, causing the collapse of the surface and trapping the phenolic compounds inside the material; thus, increasing yields. Autoclaving increases the available surface area for extraction solvents to penetrate cell walls by solubilizing the adhesive and removing them (Zhang et al., 2009). Steam treatment has been attractive for the degradation and separation of not only structural cell wall components, such as cellulose, hemicellulose, and lignin, but also antineoplastic constituents from plant biomass (Kurosumi, Kobayashi, Mtui, & Nakamura, 2006). Microwave energy is a useful alternative treatment in processing fruits and vegetables because of its rapid heating rate and its non-thermal effect on enzyme inactivation. Microwaves also reduce the impact of elevated temperature and improves retention of thermolabile compounds and other secondary metabolites.

Each pre-treatment has its advantages in its own way. An efficient pre-treatment strategy is one which is simple, cost effective, devoid of corrosive materials and do not give rise to indigestible or inhibitory compounds (Ravindran and Jaiswal, 2016). Determination of total phenolic content and antioxidant activity as a predictive tool to estimate the antioxidant potential of the barley malt rootlets is of major interest in the current study (Touati, Barba, Louaileche, Frigola, & Esteve, 2016; Granato, Nunes, & Barba, 2017). Thus, the current work focuses on investigating the impact of various pre-treatments on phenolic content and antioxidant potential of BMR phenolic extracts. To the best of our knowledge, this is the first work so far conducted on BMR.

2. Materials and methods

2.1. Barley malt rootlets (BMR)

Barley malt rootlet were procured from Rahr Malting Corporation, Shakopee, Minnesota. The initial moisture content of the sample measured using Instant Moisture analyzer (Model: Smart Turbo™-5, CEM, Matthews, NC) was 2.8% on fresh basis. It was ensured that the moisture content was always < 5% to avoid microbial contamination. BMR were ground to a particle size < 0.5 mm using Udy mill (Cyclone Sample Mill, UDY Corporation, CO, USA) and was kept in a plastic bag and stored at 4 °C until used.

2.2. Chemicals

Acetone, ethyl acetate, methanol and sodium carbonate (Na_2CO_3) were procured from Fischer Chemicals (NJ, USA). DPPH (2.2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid), trolox were procured from EMD Millipore (SanDiego, CA, USA). Folin Ciocalteu reagent, citric acid, α-amylase, viscozyme L were purchased from Sigma Aldrich (St. Louis, MO, USA). Gallic Acid (Chem-Impex, IL, USA), potassium persulfate (Labchem, PA, USA), sodium hydroxide (Ricca Chemicals, USA), concentrated hydrochloric acid (Alfa Aesar, NY, USA) were all used. All the chemicals were of analytical grade.

2.3. Pre-treatment processes

2.3.1. Steaming and roasting

About 5 g BMR sample was steamed as a single layer on a glass plate. Samples were immediately cooled to room temperature and stored at 4 °C until used. Roasting of BMR sample was done on a hot plate (Cimarec™, Iowa, USA) using 5 g of BMR at 60 °C for 3 min. Samples were cooled to room temperature and stored at 4 °C until used.

2.3.2. Autoclaving

Autoclave treatment was carried out by following the standard temperature (121 °C) and pressure (15 psi) for 20 min, which is typically used for sterilization and enzyme inhibition. Approximately 5 g of BMR sample was autoclaved (AMSCO, 3021-S, Gravity, OH, USA). Samples were cooled to room temperature and stored at 4 °C until used.

2.3.3. Microwave

A household microwave oven (800 W, 2450 MHz, Carousel, Model R-220 K, Sharp Inc., Illinois, USA) was used for the study. Microwave output power was measured according to the method of Zhang, Bi and Liu (2007) using the Eq. 1.

$$Q_{abs=(mc_p\Delta T)/t} \quad (1)$$

where, Q_{abs} is the power absorbed by water per unit time (W), m is the mass of water (g); c_p is the specific heat capacity of water (kJ/Kg. K), DT is the temperature rise in the water load (°K). and t is the time microwave power was on (s). Nine different combinations of microwave power and time were performed for the study. Five grams of ground BMR was placed on a glass plate and treated with various combinations of power (160 W, 480 W, 800 W) and time (30 s, 75 s, 120 s) using the household microwave oven. Pre-treated samples were cooled down to room temperature and stored at refrigerator conditions until used.

2.3.4. Enzyme hydrolysis

Enzyme hydrolysis treatment was performed according to the method described by Radenkovs, Klava, Kransnova, and Juhnevica-Radenkova (2014). Ten grams of ground BMR were added to 90 mL of distilled water and 500 μL of α-amylase from *Bacillus amyloliquefaciens*