

The Content in Bioactive Compounds of Different Brewers' Spent Grain Aqueous Extracts

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Abstract

In the last decade by-products of food and beverage processing have attracted much attention due to their functionality and potential as food ingredients. Brewers' spent grain is the major by-product of the brewing industry representing a valuable source of bioactive ingredients. The aim of this study was to assess the effect of extraction time and temperature on the efficiency of water as solvent for the extraction of bioactive compounds from brewers' spent grain (BSG). In terms of extraction efficiency, the results from polyphenols, flavonoids and antioxidant activity, showed that the best extraction parameters for aqueous extracts are 90°C and 60 minutes. In comparison with the control, the best extraction method generated 87% of the phenolics and 43.46% of the flavonoids obtained by a methanol extraction. The preliminary results for the aqueous extracts showed that water can be used as extraction solvent, but a longer extraction time and higher temperature are needed in order to have a content in bioactive compounds similar to that of methanolic extracts. The obtained values for polyphenols, flavonoids and antioxidant activity, emphasize the importance and the opportunities of the reuse of this agro-industrial waste.

Keywords: *antioxidant activity, bioactive compounds, brewers' spent grain, extraction, phenolic compounds.*

INTRODUCTION

Large amounts of wastes are generated annually by the food industry, their efficient management and valorisation representing one of the main problems; therefore, the urgent demands for sustainability in the food and agricultural sectors led to their valorisation as a source of bioactive compounds (Galanakis, 2013). A wide spectrum of commercially important products such as additives, nutraceuticals, enzyme, biofuels, biopolymers, and organic acids can be developed from the bioconversion of agro-industrial waste (Galanakis, 2012). For this reason, it is essential to develop new technologies and extraction techniques that fully exploit the high recovery potential of these by-products (Corbo *et al.*, 2014).

Cereal origin wastes represent a potential source of bioactive molecules, including proteins,

polysaccharides, antioxidants, minerals, lipids and vitamins. Brewers' spent grain (BSG) is the major by-product of the brewing industry, representing around 85% of the total generated waste (Kunze, 1996; Niemi *et al.*, 2012). This insoluble residue generated from the production of wort is composed of the barley malt residual constituents and includes the barley grain husk, but also minor fractions of pericarp and fragments of endosperm (Mussatto, 2013). This plant-derived by-product is known to contain significant amounts of valuable components which remain unexploited in the brewing process.

The process of obtaining the brewers' spent grain by-product is schematically represented in figure 1. In the brewery, the malted barley is milled and mixed with water in the mash tun, and the temperature of mash is slowly increased from

37 to 78°C to promote the enzymatic hydrolysis of malt constituents. This enzymatic conversion stage (mashing) produces a sweet liquid known as wort. After the saccharification process is finished, the clear sweet wort is separated from the solid components – the spent grain. The wort is then transferred to the wort kettle, while the spent grain is removed from the lauter tun (Linko *et al.*, 1998)

Nowadays, the advances in scientific research support the idea that diet may fulfil nutritional needs and exert a beneficial role in some diseases (Otlés and Cagindi, 2012). The extraction of value added compounds such as proteins, dietary fibres, polysaccharides, flavour compounds, antioxidants, and other phytochemicals from plant-derived waste is becoming a trend with major effects for the improvement of food structure and consequently with a positive impact on maintaining a healthy lifestyle. The application and development of novel technologies could make possible the production of improved functional products based on valorisation of food processing wastes (Ofori and Hsieh, 2013).

In the last decades, there is great interest in finding new and unconventional sources of compounds with antioxidant properties. This trend is due to the many studies that suggested that there is a strong relation between the consumption of diets rich in phenolic compounds and a reduced risk of cardiovascular and neurodegenerative diseases (Pandey and Rizvi, 2009; Bouallagui *et al.*, 2011).

BSG may be used in the production of novel functional foods and beverages considering the antioxidant health benefits similar to those

exhibited by the barley (McCarthy *et al.*, 2013). The polyphenols of barley have an important role during the malting process as well as preventing the enzymatic oxidation of polyunsaturated fatty acid. Because most of the phenolic compounds of the barley are localized in the grain husk, BSG represents not only a rich source of natural antioxidants but also an inexpensive alternative to synthetic antioxidants (Guido *et al.*, 2005; Bouayed *et al.*, 2010; Mussatto, 2013; Meneses *et al.*, 2013; Farcas *et al.*, 2015).

Thus, for the reasons listed above, the present study's principal objective was to characterize different BSG aqueous extracts in order to establish the optimal extraction parameters. In this sense, the extracts were characterised regarding the contents of total phenols, flavonoids and antioxidant activity.

MATERIALS AND METHODS

The brewers spent grain used in this work was obtained as a by-product from the mashing process of dark lager beer with 100% all grain malted barley (Weyermann Specialty Malting Company, Bamberg – Germany). This biomass was provided by the Microbrewery of the Faculty of Food Science and Technology of UASVM Cluj-Napoca, Romania. Due to the initial high water content (75%) the fresh BSG was preserved by oven-drying at 78°C for 12 h to reach a moisture content of 6% and to avoid a microbial degradation process. Then, the dried samples were packed in sealed polyethylene bags and stored at room temperature until further analyses.

The standard compounds (gallic acid, quercetin) and reagents: 2,2-diphenyl-1-picrylhydrazyl

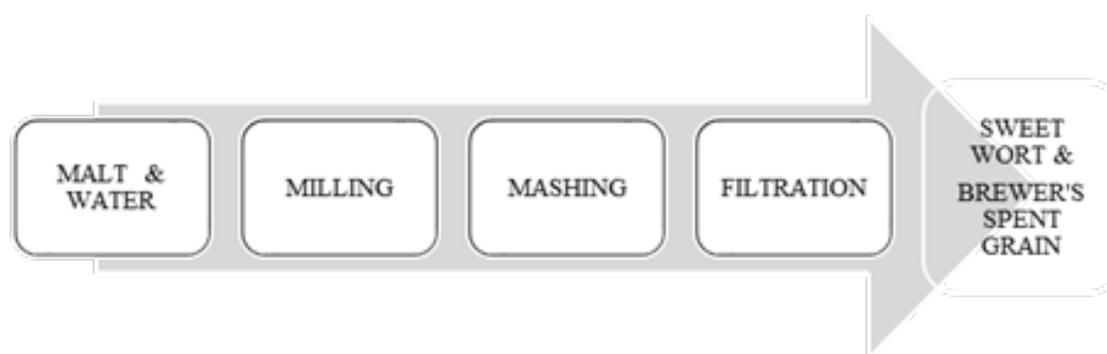


Fig. 1. Schematic representation of the process to obtain BSG from malt (Farcas *et al.*, 2014)

(DPPH), Folin-Ciocalteu, methanol, aluminium chloride, sodium carbonate, sodium nitrite and sodium hydroxide were purchased from Sigma Aldrich or Merck (Darmstadt, Germany).

The BSG aqueous extracts were obtained using different extraction temperatures and extraction times according to the experimental protocol described below:

The volume of recovered extracts were quantified and used for the final calculation. Duplicate extractions were made for each protocol combinations. All spectrophotometric readings were made using a Shimadzu UV-1700 PharmaSpec spectrophotometer (Kyoto, Japan).

The total phenolic compounds assay

The Folin-Ciocalteu method estimates the total content of all phenolics present in the analyzed samples, including flavonoids, anthocyanins and non-flavonoid phenolic compounds (Singleton *et al.*, 1999). Aliquots of 100 μ l sample were mixed with 6 ml distilled water and 0.5 ml Folin-Ciocalteu, followed, after 5 min by the addition of 1.5 ml Na_2CO_3 (7.5% in water) in order to create basic conditions (pH \sim 10) for the redox reaction between phenolic compounds and the reagent. After incubation for 120 min at room temperature, the absorbance was read at 750 nm against the blank, in which the sample was replaced with methanol, based on the gallic acid calibration curve ($r^2=0.9936$). The results were expressed as gallic acid equivalents/100 g fresh weigh (fw).

The total flavonoid assay

The total flavonoid content of the extracts was determined using the aluminium chloride

colorimetric method as described previously in other studies (Zhishen *et al.*, 1999). Aliquots of 1 ml of aqueous extracts were diluted with distilled water to a final volume of 5 ml and mixed with 300 μ l of 5% NaNO_2 and 300 μ l of 10% AlCl_3 . After 5 min of incubation in a dark place, was added 2 ml NaOH 1N and 6.4 ml distilled water. The absorbance was measured at 510 nm against the blank, in which the AlCl_3 was replaced with methanol. The standard curve was performed using different concentrations of quercetin solution ($r^2=0.9989$) and the flavonoid content was expressed as mg of quercetin equivalents for 100 g/fresh weight (fw).

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity

The DPPH scavenging activity assay was performed according to a method reported by Brand-Williams *et al.* (1995). This method is based on the ability of stable free radicals of 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors. A DPPH solution was freshly prepared in methanol. A volume of 3.9 ml of this solution was allowed to react with 100 μ l aqueous extract at room temperature in a dark place. After 30 min, the absorbance was read at 515 nm against the blank (methanol). The antioxidant activity was calculated as follows:

$$\% \text{ DPPH scavenging activity} = [(A_{\text{DPPH}} - A_s) / A_{\text{DPPH}}] \cdot 100, \text{ where}$$

A_{DPPH} was the absorbance of 2,2-diphenyl-1-picrylhydrazyl solution, and A_p the absorbance in the presence of the sample.

Tab. 1. The experimental design of the aqueous extraction parameters: time - temperature variations and extraction steps

Protocol A - variable time	Protocol B - variable temperature	Extraction steps
10 min / 90°C	30°C / 30 min	
20 min / 90°C	50°C / 30 min	1g dried BSG + 20mL H ₂ O
30 min / 90°C	70°C / 30 min	Sonication 10 min ²
45 min / 90°C	90°C / 30 min	Water bath (according to A and B)
60 min / 90°C	-	Ice bath 10 min
		Centrifugation 5 min/6000 rpm
		Filtration (Millipore 0.45 μ m filter)
*Control sample 30 min / 30°C (in methanol)		

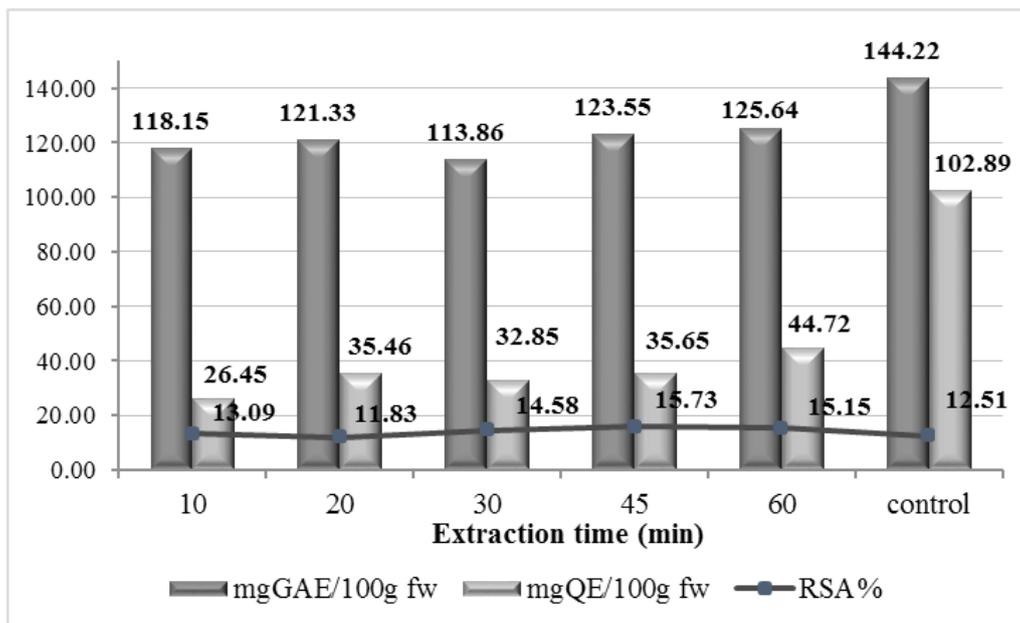


Fig. 2. The total polyphenolics content (mg GAE/100g fw), flavonoids content (mg QE/100g fw) and antioxidant activity (RSA%) – Protocol A

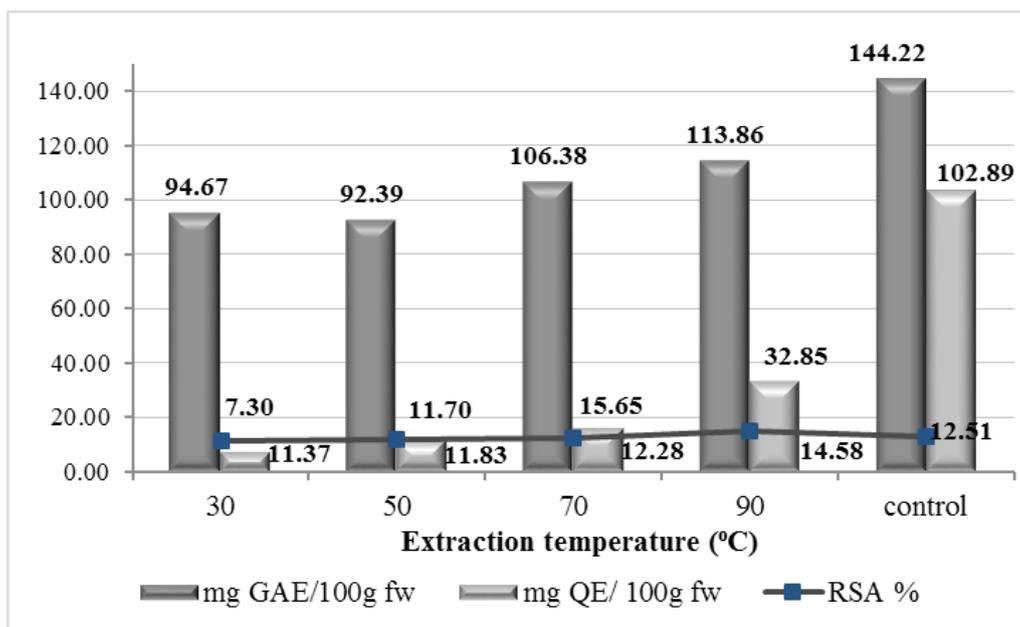


Fig. 3. The total polyphenolics content (mg GAE/100g fw), flavonoids content (mg QE/100g fw) and antioxidant activity (RSA%) – Protocol B

RESULTS AND DISCUSSION

The total phenolic content, flavonoids and antioxidant activity of the aqueous extracts for each extraction from A and B protocol are presented in Figure 1 and 2. To highlight the influence of

temperature and time on the yield of extraction of phenolic compounds, aqueous samples were analyzed compared with a control extract (solvent – methanol, extraction parameters 30 min/30°C).

Depending on the extraction time and temperature, the radical scavenging activity varied between 11.37% and 15.73%, the total phenolics ranged between 92.39 mg GAE/100g fw and 125.64 mg GAE/100g fw, while the flavonoid content was between 7.30 mg QE/100g fw and 44.72 mg QE/100g fw.

The obtained results for the aqueous extracts were comparable with those obtained for the methanolic extracts (control sample), thus demonstrating the opportunity of using water as solvent extraction.

In terms of extraction efficiency, the results from polyphenols, flavonoids and antioxidant activity, showed that the best extraction parameters for aqueous extracts are 90°C and 60 minutes. In comparison with the control, the best extraction method generated 87% of the phenolics and 43.46% of the flavonoids obtained by a methanol extraction.

CONCLUSION

The preliminary results for the aqueous extracts showed that water can be used as extraction solvent, but a longer extraction time and higher temperature are needed in order to have a content in bioactive compounds similar to that of methanolic extracts.

The obtained values for polyphenols, flavonoids and antioxidant activity, emphasize the importance and the opportunities of the reuse of this agro-industrial waste.

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REFERENCES

- Bouallagui Z, Han J, Isoda H, Sayadi S (2011). Hydroxytyrosol rich extract from olive leaves modulates cell cycle progression in MCF-7 human breast cancer cells. *Food and Chemical Toxicology* 49:179-184.
- Bouayed J, Bohn T (2010). Exogenous antioxidants – double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxid. Med. Cell. Longev.* 3:228-237.
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* 28-31.
- Corbo MR, Bevilacqua A, Petrucci L, Casanova FP, Sinigaglia M (2014). Functional Beverages: The Emerging Side of Functional Foods Commercial Trends, Research, and Health Implications. *Comprehensive Reviews in Food Science and Food Safety* 13:1192-1206.
- Farcas A, Tofana M, Socaci S, Mudura E, Scrob S, Salanta L, Muresan V (2014). Brewers' spent grain – A new potential ingredient for functional foods. *Journal of Agroalimentary Processes and Technologies* 20(2):137-141.
- Farcas A, Socaci S, Tofana M, Dulf FV, Mudura E, Diaconeasa Z (2015). Volatile profile, fatty acids composition and total phenolic content of brewers' spent grain by-product with potential use in the development of new functional foods. *Journal of Cereal Science* 64:34-42.
- Galanakis CM (2012). Recovery of high added value component from food wastes: conventional, emerging technologies and commercialized applications. *Trend Food Sci. Tech.* 26:68-87.
- Galanakis CM (2013). Emerging technologies for the production of nutraceuticals from agricultural by-products: a viewpoint of opportunities and challenges. *Food. Bioprod. Processing* 91:575-9.
- Guido LF, Boivin P, Benismail N, Goncalves CR, Barros AA (2005). An early development of the nonenal potential in the malting process. *Eur. Food Res. Technol.* 220:200-206.
- Kunze W (1996). *Technology Brewing and Malting.* VLB Berlin.
- Linko M, Haikara A, Ritala A, Penttila M (1998). Recent advances in the malting and brewing industry. *J. Biotech.* 65:85-98.
- McCarthy AL, O'Callaghan YC, Neugart S, Piggott CO, Connolly A, Jansen MA, Krumbein A, Schreiner M, Fitzgerald RJ, O'Brien NM (2013). The hydroxycinnamic acid content of barley and brewers' spent grain (BSG) and the potential to incorporate phenolic extracts of BSG as antioxidants into fruit beverages. *Food Chem.* 141:2567-74.
- Meneses NGT, Martins S, Teixeira JA, Mussatto SI (2013). Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. *Sep. Purif. Technol.* 108:152-158.
- Mussatto SI (2013). Brewer's spent grain: a valuable feedstock for industrial applications. *J. Sci. Food Agric.* 94:1264-1275.
- Niemi P, Tamminen T, Smeds A, Viljanen K, Ohra-Aho T, Holopainen-Mantila U, Faulds CB, Poutanen K, Buchert J (2012). Characterization of lipids and lignans in brewer's spent grain and its enzymatically extracted fraction. *J. Agric. Food Chem.* 60:9910-9917.

16. Ofori JA, Hsieh Y-HP (2013). Novel technologies for the production of functional foods. In: Bagchi D, Bagchi M, Moriyama H, Shahidi F, editors. *Bio-nanotechnology: a revolution in food, biomedical and health sciences*. New York, N.Y.: John Wiley & Sons, p. 143–162.
17. Otles S, Cagindi O (2012). Safety considerations of nutraceuticals and functional foods. In: McElhatton A, Sobral PJA, editors. *Novel technologies in food science*. New York, N.Y.: Springer, p. 121–36.
18. Pandey KB, Rizvi SI (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* 2:270-278.
19. Singleton VL, Orthofer RM, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Method Enzymol.* 299:152-178.
20. Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoids contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64:555-559.