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Preliminary Study on Antioxidant Activity and Polyphenols Content in Discharged Waste from Beer Production

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Abstract

Polyphenols are a class of chemicals with many beneficial effects on human health, due to their physiological function including antioxidant, anti-mutagenic and cancer preventive activities. There are economic advantages in extracting polyphenols from natural waste resulted from food industry processes (such as beer, wine or olive oil production). They can be reintroduced in industrials like pharmaceutical, cosmetic products or as food preservatives.

The aim of this study was to analyze total polyphenols content and antioxidant activity in every stage of beer beverage process, starting with the analysis of raw materials (malt and hop) and ending with the resulted waste (brewers' spent grain). Total antioxidant activity was evaluated by indirect spectrophotometric method, which utilized 2,2-diphenyl-1-picrylhydrazyl (DPPH) as generated system for free radical DPPH•. Total polyphenols content was determined using Folin Ciocalteu method. Finally, a correlation was made between antioxidant capacity and total polyphenol content of the samples taken into study.

Keywords: natural waste, polyphenols, brewers spent grain, DPPH method, antioxidant activity

1. Introduction

Agro-industrial residues are the most abundant and renewable resources on earth. Accumulation of this biomass in large quantities every year results not only in the deterioration of the environment, but also in the loss of potentially valuable material which can be processed to yield a number of valuable added products, such as food, fuel, feed and a variety of chemicals [20].

Brewery waste is a typical example of such unexploited potential. The most common by-products are spent grain, spent hops and surplus yeast, which are generated from de main raw material used for beer elaboration, the barley malt, hop and yeast. They represent large potential resources for use in biotechnological processes, as for example in fermentative processes for the production of value-added compounds (ethanol, xylitol, lactic acid, among others), as substrate for microorganism cultivation, or simply as raw materials for extraction of compounds such proteins, sugars, acids and antioxidants [19].

Polyphenol compounds, the most abundant antioxidants in human diet, represent a large and complex group of phytochemicals, constituents widely distributed in plants, fruits and vegetables. They are divided into several classes according to the number of phenol rings contained and to the structural elements that bind these rings to each other.

The main groups of polyphenols are: flavonoids, phenolic acids, tannins (hydrolysable and condensed), stilbenes and lignans [6]. Their physiological functions have a great potential on prevention of chronic diseases such as cancer and

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arteriosclerosis, osteoporosis, neurodegenerative diseases and diabetes mellitus [16].

Polyphenols compounds are antioxidants that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. The potential health benefits of polyphenols compounds are getting more and more recognition, as reports indicate that these compounds inhibit the harmful effects of reactive oxygen species, which act as oxidants [9], thus protecting macromolecules, such as proteins, lipids and DNA, from oxidative degradation.

Beer is a very complex beverage and contains various classes of polyphenolic compounds, from simple phenols to complex oligomeric proanthocyanidins. Polyphenols that occur in beer in relatively high concentrations are: the phenol tyrosol, the benzoic acid derivative p-hydroxybenzoic acid, the cinnamic acids p-coumaric and ferulic acid, (+)-catechin and (-)-epicatechin, the proanthocyanidin dimers procyanidin B3 and prodelphinidin B3 and the flavanone isoxanthohumol [4].

About 80% of phenolic compounds present in beer are derived from barley malt and the remaining come from hops [8]. Those phenolic compounds in malting barley include polyphenols (benzoic and cinnamic acid derivatives), flavonoids, proanthocyanidins, tannins, and amino phenolic compounds all of which are known to inhibit nonenzymatic lipid peroxidation and widely recognized as having important antioxidant and antiradical properties [25].

Composition and levels of phenolic compounds in beer vary strongly being influenced by the raw materials utilized, the brewing process and type of beer, and also change during storage. Alcohol-free beer usually has lower levels of phenolic constituents due to losses during the alcohol removing process [4].

Raw materials and by-products resulted from brewing

Polyphenols and phenolic acids present in malt are natural antioxidants, capable of delaying, retarding or preventing oxidation processes, and therefore thought to have a significant effect in malting and brewing as inhibitors of oxidative damage [12].

Brewer's spent grain (BGS) is a by-product of the brewing process, consisting of the solid residue remaining after mashing and lautering. It consist primarily of grain husks and other residual compounds not converted to fermentable sugars by the mashing process [28].

The chemical composition of BSG varies according to barley variety, harvest time, malting and mashing condition and the type and quality of secondary raw materials added in the brewing process [23].

BGS is produced in the largest quantity, corresponding to around 85 % of the total generated and it is estimated that about 200 t of wet spent grain with 70 to 80 % water content are produced per 10.000 hl of produced beer [15].

Despite the fact that it is produced in large quantities during the whole year, BSG has received little attention as a marketable commodity and is mainly used as animal feed [27]. This under-utilization is attributed to its high moisture content (causing transportation and storage difficulties), complex composition, the stigma of being labeled a waste material and potential for rapid microbiological degradation [13].

In hot climates, BSG can spoil within seven to ten days [7] because it contains above 70% water [19].

BSG contains hydroxycinnamic acids including ferulic acid, p-coumaric acid and caffeic acid, which have shown bioactivity in the pure form (antioxidant, anti-inflammatory, anti-atherogenic and anti-cancer). Phenolic extracts from BSG have also shown antioxidant potential, by protecting against oxidant-induced DNA damage, possibly by Fe chelation [1]. This opens up new possibilities for use of this brewery by-product.

Ferulic acid exhibits a number of potential application such as natural antioxidant, food preservative and antimicrobial agent, anti-inflamatory agent and as a food flavor precursor; p-coumaric acid exhibits antioxidant and chemoprotectant properties [2,3].

Ferulic acid (4-hidroxy-3-methoxy-cinnamic acid) was found to be the most abundant hydroxycinnamic acid being present at concentrations ranging from 1860 to 1948 mg/g, while the p-coumaric (4-hydroxycinnamic acid) levels ranged from 565 to 794 mg/g [10].

The hop, *Humulus lupulus* L, is essential in brewing. It is added to beer in small quantities for aroma and bitterness and to provide antifungal and antibiotic properties. These characteristics are mainly attributed to bitter constituents (humulones, lupulones, soft and hard resins) and ethereal oils.

Hop and the hop bract part contain large amounts of polyphenols, which have strong antioxidant properties; procyanidin B-2, B-3 and C-2, polymer structures composed of catechin monomers, are recognized to be the most abundant [5].

Only 15 % of the hops constituents end up in the beer, 85 % will became spent hop material [11]. A fraction of the hop components end up in the trub, mainly when hop powered, pellets or extracts are used in the brewing process.

The hot trub is a precipitation product of the wort boiling process that include: insoluble hop materials, condensation products of hop polyphenols and wort proteins and isomerized hop acids adborbed on the trub solids [11].

Unlike spent grains, the direct use of spent hops as feed supplement is not desirable due to the presence of 2-methyl-3-buten-2-ol, which is the product of bitter acid degradation and has hypnotic-sedative properties [19].

The aim of the present study was to assess the content in polyphenols and the antioxidant activity of raw materials and by-products (BSG) from beer production in order to highlight the potential of last one to be used in food products as a source of bioactive compounds.

2. Materials and Methods

The BSG used in this work was obtained from a process employing 100 % malt, without addition of other cereal adjuncts. The materials, supplied by the microbrewery of the Faculty of Food Science and Technology were stored in the freezer until required for analysis.

All chemicals were obtained from Sigma-Aldrich or Merck (Darmstadt, Germany). All spectrophotometer readings were made using a Shimadzu UV-1700. Results are presented as the mean of two replications with standard deviation.

*Determination of total phenolic compounds.*Total phenolic contents of all sample extracts were determined using Folin-Ciocalteu colorimetric method [24].

Preparation of samples extracts. Ten grams sample were placed in a homogenizer with 10 ml of methanol and were thoroughly mixed for one minute. Then, the samples were centrifuged at 2000 g for 15 minutes at 4°C. The supernatant was collected and stored in air tight glass vials covered with aluminum foil and kept at -20 ºC.

*Protocol assay.*First, 0.1 ml of methanolic extract was transferred to a 10 ml volumetric flask containing 6 ml distilled water, to which was subsequently added 0.5 ml of undiluted Folin-Ciocalteu reagent. After 4 minutes of repose in the dark, 1.5 ml of Na_2CO_3 (7.5% in water) were added in order to create basic conditions (pH ~10) for the redox reaction between phenolic compounds and Folin- Ciocalteu reagent; the volume was made up to 10.0 ml with distilled water. After incubation for 120 min at room temperature, the absorbance was read at 750 nm, against the blank, in which the standard or sample were replaced with methanol.

Sample dilution was done when the recorded absorbance value exceeded the linear range of gallic acid curve.

Standard curve was performed using concentrations of 0, 0.25, 0.50, 0.75, 1 mg/ml of gallic acid. Total phenolic content of samples were expressed as gallic acid equivalents, mg GAE/ kg ± standard deviation of two analysis.

*Antioxidant capacity.*A commonly used method for quantification of antioxidant activity is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, which measures the ability of the test compound to scavenge the DPPH stable radical [26]. The reactions for free radicals capture induce a change in sample color, from blue to yellow and a relative decrease in absorbance. The antioxidant activity is express as antiradical activity of samples.

$$Inhibition\,(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Samples were centrifuged at 6000g for 15 min at 4 °C and aliquots of 0.01 ml of the supernatant were mixed with 3.9 ml of methanolic DPPH (0.025 g/l) and 0.090 ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min.

Absorption of the samples was measured on a Shimadzu UV-1700 at 515 nm against blank of methanol without DPPH.

3. Results and discussion

For all the samples taken into this study (hop, malt, trub, beer, BSG) the total phenolic content and antioxidant activity was determined.

A standard curve for total phenolic content determination was performed using concentrations of 0, 0.25, 0.50, 0.75, 1 mg/ml of gallic acid, obtaining an r^2 of 0.999 (fig. 1).

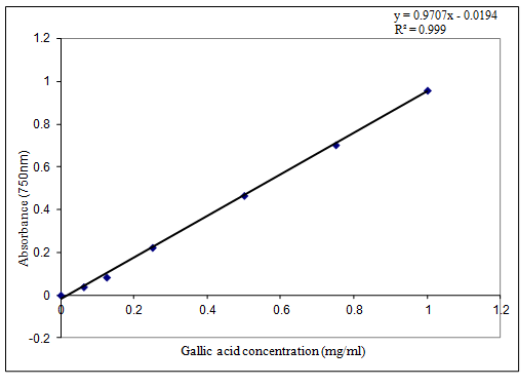

Figure 1. Gallic acid calibration curve

Composition and levels of phenolic compounds in beer vary strongly depending on the used raw materials, the brewing process and type of beer. Also, the total phenolic content of beer may suffer changes during storage.

As figure 2 shows, the highest content of polyphenols (2392 mg GAE/kg) was identified in the hops sample, but only a fraction of them will be found in the finished beer. This happens mostly because the hop compounds end up in trub in the form of insoluble materials, condensation products of hop polyphenols and wort proteins and isomerized hop acids adsorbed on trub solids. This fact also explains the high quantity of polyphenols from the trub and high antioxidant activity (fig. 3) which could come from the development of such non-enzymatic browning products, as Maillard products, that can also act as antioxidants (particularly melanoidins). The values obtained in this study are in accordance with those found in literature [14, 21].

Huige affirmed in 2006 that a large fraction of the hop components end up in the trub, mainly when pellets hop powered (which were used in this study) or extracts are used in the brewing process.

Samaras et al., 2005 [22] found that ferulic acid reacts with Maillard reaction intermediates derived from glucose and proline at high temperatures, leading to higher antioxidant activity and an increase of antioxidant properties of Maillard reaction products with heating time.

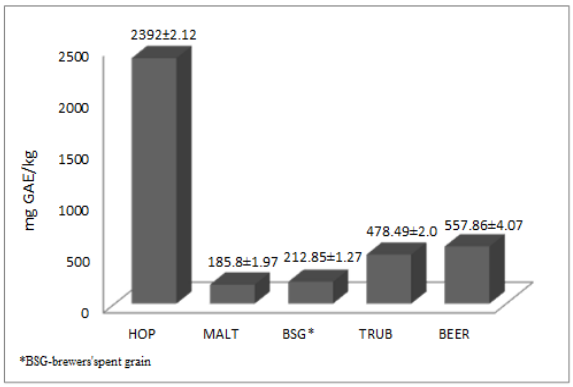

Figure 2. Total Polyphenols content in raw materials (malt and hop) and resulted waste from beer beverage (mean of two replicates ± standard deviation)

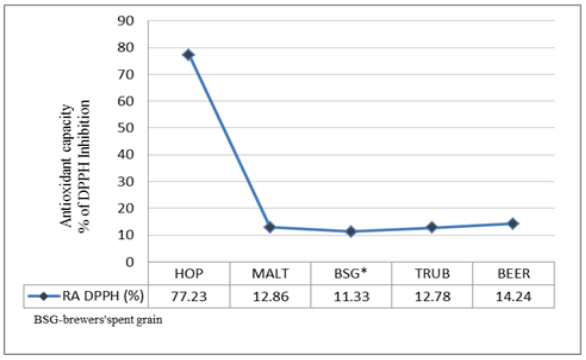

Figure 3. Antioxidant capacity of raw materials (malt and hop) and resulted wastes from beer beverage

BSG contains a relatively high amount of polyphenols of 212.85 mg GAE / kg, considering the fact that the sample has a moisture content of 70%. Similar values have been reported in the literature for malt and BSG by Waters et al. in 2012. Higher content of polyphenols in BSG can be explained by the fact that antioxidants are not evenly distributed in malt grains; for example, p-coumaric acid was present in the lowest amount in the center of the barley kernel and rapidly increased toward the outer layers, such as lignified husk [17].

The presence of the natural antioxidants in malting barley and screening of malting barley variety with the highest level of radical scavengers seems very important to produce beers with high levels of antioxidant activity [18].

McCarthy et al., 2013 [1], have demonstrated in a recent study that phenolic extracts from BSG have antioxidant potential, by protecting against oxidant-induced DNA damage, possibly by iron ions chelation. This opens up new possibilities for use of this brewery by-product.

The literature shows that incorporation of BSG in human foods has resulted in increased protein and fibre contents of the products, where the changes in organoleptic properties are controllable. Phenolic component of BSG has potential bioactive effects, which are worth pursuing given that the inclusion of BSG into human foodstuffs is viable and beneficial [1].

4. Conclusion

The main objective of this study was to evaluate the total polyphenol content and antioxidant activity of discharged waste from beer production, in order to highlight their potential for the extraction of natural antioxidants and their and their efficiency for incorporation in functional foods.

As previously mentioned, BSG consists predominantly of the husk-pericarp-seed coat and is largely made up of cell walls. Since most of the phenolic compounds of the barley grain are contained in the husk, BSG is a potentially valuable source of phenolic compounds and the obtained results confirm this.

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All the researches were carried out in The Food Quality and Safety Testing Laboratory from the Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine University, Cluj-Napoca.

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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