ANALYSIS OF FLAVONOIDS IN MALT AND BREWERS' SPENT GRAIN BY UV-VIS SPECTROPHOTOMETRY USING DIFFERENT EXTRACTION TECHNOLOGIES

Farcas Anca*, Tofana Maria*, Socaci Sonia*, Scrob Stancuta*, Salanta Liana*, Pop Carmen*

* University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Science and Technology, 3-5 Mănăştur Street, 400372, Cluj–Napoca, Romania,
e-mail: farcas.ancuta@yahoo.com

Abstract

The main goals of this research was to determine the total flavonoids content in raw materials and discharged waste from beer production as well a comparative study between two different extraction methods. This study also provides new arguments justifying the fact that brewers'spent grains can be exploited as a natural and inexpensive alternative source of antioxidants. The total flavonoid content of the extracts was determined using aluminium chloride colorimetric method. Two extraction technologies of flavonoids were investigated and compared including, ultrasonic extraction and marinated extraction. A calibration curve was performed using different concentrations of standard quercetin solutions (r^2 = 0.9872) in order to quantify the total flavonoid content of each malt and brewers'spent grain samples.The absorbance was measured at 500 nm using a Shimadzu UV-1700 PharmaSpec spectrophotometer and the results were expressed as mg of quercetin equivalents/g.

Key words: flavonoids, brewers' spent grain, malt, ultrasonic extraction, marinated extraction, spectrophotometry.

INTRODUCTION

In the last few years, great attention has been paid to the bioactive compounds since these compounds have the ability to promote a number of benefits for human health. Additionally, there is still a growing interest in finding natural resources with antioxidant activity to effectively replace the synthetic antioxidants, which have been related to toxic and carcinogenic effects (Bouayed and Bohn, 2010). Researches have been intensified in order to find natural resources like agricultural and agro-industrial residues as potential sources of bioactive compounds.

Flavonoids constitute one of the most common forms of phenolic compounds present in plants. To date, more than 6000 different flavonoids have been described and the number continues to increase.

Flavonoids are polyphenolic compounds comprising of 15 carbons, with 2 aromatic rings connected by a 3-carbon bridge. According to the modifications of the central C-ring, they can be divided into different structural classes including flavonols, flavones, flavan-3-ols, flavanones, isoflavones, and anthocyanidins (Harborne and Williams, 2000).

Multiple health benefits have been proposed for flavonoids, although the biochemical and physiological mechanisms are not fully defined. They are well-recognized antioxidant molecules with proven antioxidant activity in numerous biological systems (Lotito et al., 2000; Rice-Evans C., 2001).

The identification of potential targets for the beneficial health effects of flavonoids exponentially increased over the years. Results of population studies suggest that adopting flavonoid-rich diets may protect against cardiovascular disease (Hertog et al., 1995; Huxley et al., 2003). Mechanisms by which these compounds exert their cardiovascular protective effects are still unknown. It is widely hypothesized that dietary flavonoids improve cardiovascular health and may help to prevent cardiovascular diseases by inhibiting pathogenic processes such as oxidative stress (lipid and protein peroxidation), inflammation, endothelial dysfunction, and platelet activation (Nijveldt et al., 2001). Flavonoids can also chelate metal ions and often decrease metal ion pro-oxidant activity (Mira et al., 2002).

Brewers' spent grain (BSG), the main low-value solid waste, is the major by-product of the brewing industry, representing around 85 % of the total by-product generated. This material is basically composed of the barley malt residual compounds and includes: the barley grain husk in the greatest proportion, and also minor fractions of pericarp and fragments of endosperm and other residual compounds not converted to fermentable sugars in the mashing process (Xiros and Christakopoulos, 2009).

BSG is available at low or no cost throughout the year and is produced in large quantities not only by large but also small breweries. 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 (Kunze, 1996). It is generally incorporated into animal feeds currently making them a low-value product, maximally marketed between €1 and 6/tone, or alternatively BSG is composted, incinerated, dumped or anaerobically fermented (Fillaudeau L. et al., 2006).

Scientific research has revealed that BSG has highly desirable nutritional characteristics from a human dietary standpoint. Typical BSG compositions vary but always include high levels of dietary fiber, protein and particularly essential amino acids, as well as appreciable levels of minerals, polyphenols, vitamins, and lipids. These quality characteristics, in addition to its low cost and high levels of availability, make BSG appropriate as a food ingredient.

Figure 1 is a schematic representation of the process rezulting in the production of brewers' spent grain from barley.

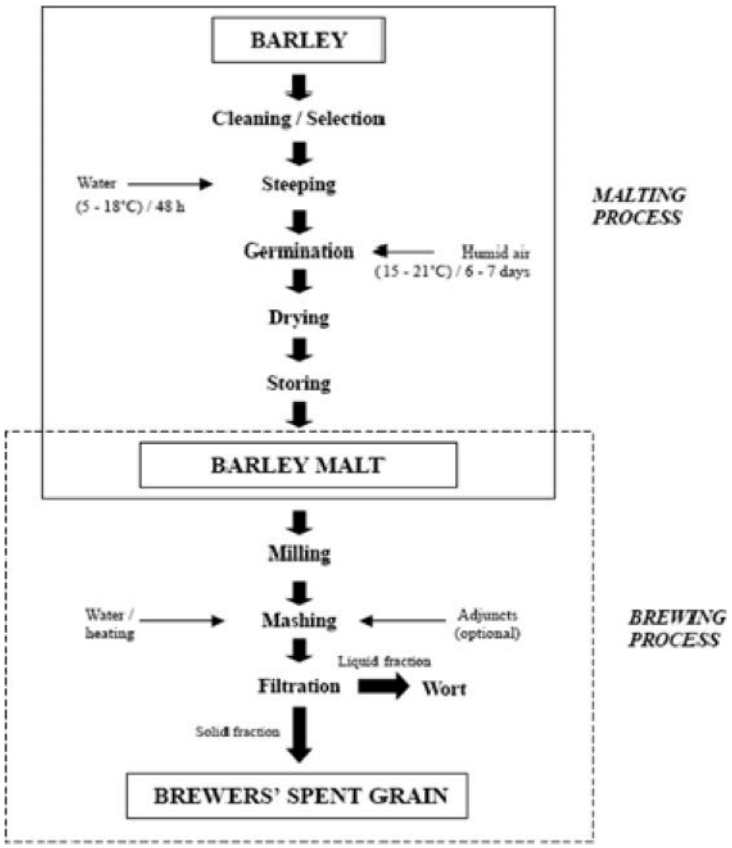

Fig. 1. Schematic representation of the process to obtain BSG from natural barley (Mussatto et al., 2006)

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 (Santos et al., 2003) but always include high levels of dietary fibre, protein and particularly essential amino acids, as well as appreciable levels of minerals, polyphenols, vitamins and lipids (Mussatto S.I. et al., 2006).

BSG may provide a number of benefits when incorporated into human diets such as for the prevention of certain diseases including cancer, gastrointestinal disorders, diabetics, and coronary heart disease (Aman, 1994; Jacobs, 1998).

Phenolic compounds 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 (Bonoli et al., 2004; Karabín et al., 2006). About 80% of phenolic compounds present in beer are derived from barley malt and the

remaining come from hops. Polyphenols identified in barley include anthocyanins, flavonols, phenolic acids, catechins and proanthocyanidins (Goupy et al., 1999).

MATERIAL AND METHOD

All the materials (malt, brewers' spent grain) were supplied by the microbrewery of the Faculty of Food Science and Technology of USAMV Cluj-Napoca. The BSG used in this work was obtained from a process employing 100 % malt, without addition of other cereal adjuncts. Caramelized and black malt are added in smaller quantities (5-10 %) to obtain darker colors and to enhance flavor characteristics. The reagents used were purchased from Sigma-Aldrich or Merck (Darmstadt, Germany) and spectrophotometric readings were made using a Shimadzu UV-1700 PharmaSpec spectrophotometer.

Fresh brewers'spent grain samples were preserved in two ways: by lyophilization using laboratory freeze dryer Alpha 1-2 Lyo Display Plus and by oven-drying at 78 ºC for 12 hours. Then the dried sample was packed in sealed polyethylene bags and stored.

Two extraction technologies of flavonoids were investigated and compared including, ultrasonic extraction and marinated extraction.

a. Ultrasonic Extraction: 0.5 grams of ground sample were accurately weighed and placed with 25 mL of ethanol–water (70:30, v/v) solvent in a vessel. The sealed vessel was placed in the ultrasonic bath for extraction for an hour at room temperature (25 °C). Then the extract was centrifuged at 4,000 rpm for 30 min; the supernatant was diluted to 25 mL in a volumetric flask (25 mL) with the solvent.

b. Marinated Extraction: 0.5 grams of ground sample were accurately weighed and placed in a sealed vessel by adding 25 mL of the ethanol–water (70:30, v/v) solvent, followed by the extraction for 48 h at room temperature (25 °C). Then the following works were done as the description in ultrasonic extraction.

Determination of Flavonoids

The total flavonoid content of the extracts was determined using aluminium chloride colorimetric method (Zhu H. et al., 2010) and the results were expressed as mg of quercetin equivalents/g.

An aliquot of 2 mL of extract was accurately removed in a volumetric flask (10 ml) and mixed with 0.6 ml of $NaNO_2$ (5%) solution. After 6 min, 0.5 ml of the $Al(NO_3)_3$ (10%) solution was added to the volumetric flask, shaken and left to stand for another 6 minutes. Finally, 3.0 ml of the NaOH (4.3%) solution were added into the volumetric flask, followed by the addition of water to the final volume of 10 ml, shaken and allowed to stand

for 15 min at room temperature, before measuring the absorbance at 500 nm; as reference solution was used the sample solution without coloration.

Preparation of Standard Solution

To calculate total flavonoid values for malt and BSG extracts a standard solution of quercetin was prepared as follows: 100 mg quercetin was accurately weighed and dissolved in ethanol–water (70:30, v/v) and then the solution was diluted to 50 mL in a volumetric flask by the same solvent. Two milliliters of this solution was removed and diluted to 25 mL in a volumetric flask by ethanol–water (70:30, v/v) solvent. Standard curve (fig.1) was performed using concentrations of 0, 0.25, 0.50, 0.75, 1 mg/ml of quercetin solution (r=0.9872).

RESULTS AND DISSCUSIONS

Barley grain is reported to be an excellent source of phenolic compounds including phenolic acids (benzoic and cinnamic acid derivatives), flavonoids, tannins, proanthocyanidins, and amino phenolic compounds, which are widely recognized as having important antioxidant and antiradical properties (Hernanz et al., 2001). There are more than 50 proanthocyanidins reported in barley, and they include oligomeric and polymeric flavan-3-ol, catechin (c), and gallocatechin (gc). The most abundant proanthocyanidins in barley are dimeric proanthocyanin B3 and procyanidin B3. Major trimers include T1 (gc–gc–c), T2 (gc–c–c), T3 (c–gc–c), and T4 or procyanidins C2 (c–c–c) (Friedrich et al., 2000).

The concentration in flavonoids from the five analyzed samples, were calculated using a calibration curve based on different concentrations of quercetin (Figure 2).

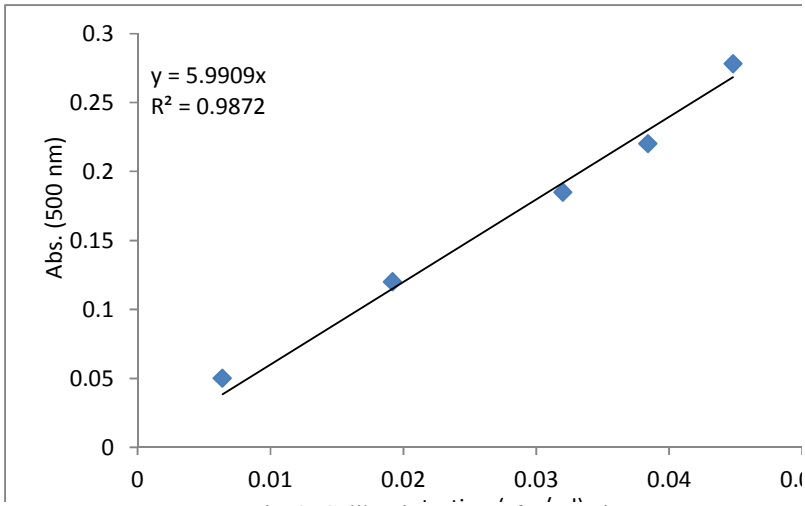

Fig. 2. Calibration curve of quercetin

As can be seen in figure 3, all the analyzed samples contained appreciable amounts of flavonoids, varying from 5.11 to 17.55 mg QE/g, depending not only on sample type but also on the extraction method. The richest sample in flavonoids was the black malt sample, 17.55 mg QE/g, while the other two malt sample (caramel and base) had the lowest content of flavonoids. Comparing the efficiency of the extraction methods (ultrasonic / marinated) some statements can be made. For all the three malt samples ultrasonic extraction had the highest effiency and therfore, in this case it was the most appropriate method for the extraction of flavonoids. The greater difference between the efficency of the extraction method was noticed in the case of black malt sample, when the efficiency of ultrasonic extraction was 1.8 times higher than that of marinated extraction.

Paradoxically, in case of brewers' spent grain, the traditional extraction method by marinating had better results than ultrasonic extraction for both samples: 9.71 ± 0.12 mg QE/g for lyophilized BSG sample respectively 9.44 ± 0.12 mg QE/g for dried BSG sample. Although, the differences between the two extraction methods, in the case of BSG sample, was smaller than for the malt samples. There are also other studies which confirm the presence of flavonoids in brewers'spent grain. Depending on the used extraction solvent, there have been reported values for flavonoid content varying between 0.98 and 4.61 mg QE/g (Meneses et al., 2013).

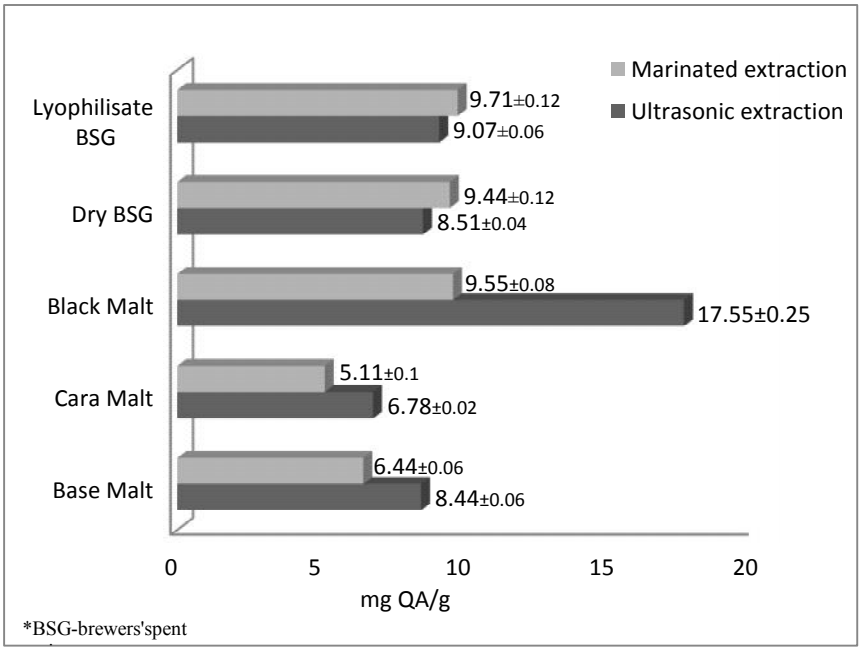

Fig. 3. Total flavonoids content in malt and BSG with a comparative study between two different extraction methods (mean of three replicates ± standard deviation)

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 (Aoif McCarthy et al., 2013). In 2012, McCarthy has done research on brewers' spent grains content in phenolic compounds, and finally concluded that it is a suitable target for development as a health promoting food supplement. Specifically, they have been reported to have DNA-protective effects by acting as powerful antioxidants.

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 natural antioxidant compounds.

CONCLUSIONS

BSG is still traditionally supplied to local farmers for elimination, and the development of economically viable technologies for valorization of this agroindustrial by-product has been encouraged.

The nutritional characteristics, in addition to its low cost and high levels of availability, make BSG appropriate as a food ingredient or raw material for the production of added-value products. Total reuse of this by-product is not only interesting from an economic point of view, but also environmentally, since the elimination of industrial by-products represents a solution to pollution problems.

Acknowledgments

The research was financially supported by programm Innovation-subprogramm Inovation Checks – PNII, CCCDI-UEFISCDI, project number PN-II-IN-CI-2013-1-0018. All the analyses were carried out in The Food Quality and Safety Testing Laboratory (L.I.C.S.A.) from the Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine University, Cluj-Napoca.

REFERENCES

1. Aman P, JX Zhang, G. Hallmans, E. Lundin, 1994, Excretion and degradation of dietary fiber constituents in mileostomy subjects consuming a low fiber diet with and without brewer's spent grain. J Nutr 124:359–63.
2. Bonoli M, V. Verardo, E. Marconi, M.F. Caboni, 2004, Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometer study among extraction methods of free and bound phenolic compounds. J Agric Food Chem 52:5195–200.
3. Bouayed J., T. Bohn, 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.
4. Fillaudeau, L., P. Blanpain-Avet, G. Daufin, 2006, Water, wastewater and waste management in brewing industries. Journal of Cleaner Production 14, 463-471.
5. Friedrich W., A. Eberhardt, R. Galensa, 2000, Investigation of proanthocyanidins by HPLC with electrospray ionization and spectrophotometry. Eur Food Res Technol 211:56–64.

6. Goupy P., M. Hugues, P. Boivin, M.J. Amiot, Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J Agric Food Chem, 1999, 79:1625–34.
7. Harborne J.B., C.A. Williams, 2000, Advances in flavonoids research since 1992. Phytochemistry 55:481–504.
8. Hernanz D., V. Nuñez, A.I. Sancho, C.B. Faulds, G. Williamson, B. Bartolomé, C. Gómez-Cordovés, 2001, Hydroxycinnamic acids and ferulic acid dehydrodimers in barley and processed barley, J. Agric. Food Chem. 49 4884–4888.
9. Hertog M.G., D. Kromhout, C. Aravanis, H. Blackburn, R. Buzina, F. Fidanza, S. Giampaoli, A. Jansen, A. Menotti, S. Nedeljkovic, 1995, Flavonoids intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 155:381–386.
10. Huxley R.R., H. Neil, 2003, The relation between dietary flavonol intake and coronary heart disease mortality: A meta-analysis of prospective cohort studies. Eur J Clin Nutr 57:904–908.
11. Jacobs D., K. Meyer, L. Kushi, A. Folsom, 1998, Whole-grain intake may reduce the risk of ischemic heart disease death in post-menopausal women: the LoweWomen's Health Study. Am J Clin Nut 66:1264–76.
12. Karabín M., P. Dostálek, P. Hofta, 2006, Review of methods for estimation of antioxidant activity in brewing industry, Chem. Listy 100, 184-189.
13. Kunze W., 1996, Technology Brewing and Malting, VLB Berlin.
14. Lotito S.B., L. Actis-Goretta, M.L., Renart, M. Caligiuri, D. Rein, H. Schmitz, F.M. Steinberg, C.L. Keen, C.G. Fraga, 2000, Influence of oligomer chain length on the antioxidant activity of procyanidins. Biochem Biophys Res Commun 276:945–951.
15. McCarthy A.L., Y.C. O'Callaghan, A. Connolly, C.O. Piggott, R.J. Fitz-Gerald, N.M. O'Brien, 2012, Phenolic extracts of brewers' spent grain (BSG) as functional ingredients—assessment of their DNA protective effect against oxidant-induced DNA single strand breaks in U937 cells. Food Chem 134(2):641–646.
16. Meneses N.G.T., S. Martins, J. A. Teixeira, S.I. Mussatto, 2013, Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains/Separation and Purification Technology 108, 152–158
17. Mira L, M. Tereza Fernandez, M. Santos, R. Rocha, M. Helena Florenncio, K.R. Jennings, 2002, Interactions of flavonoids with iron and copper ions: A mechanism for their antioxidant activity. Free Radic Res 36:1199–1208.
18. Mussatto SI, G. Dragone, I.C. Roberto, 2006, Brewers' spent grain: generation, characteristics and potential applications. J Cer Sci 43(1):1–14.
19. Nijveldt RJ, E. van Nood, DEC van Hoorn, P.G. Boelens, K. van Norren, PAM van Leeuwen, 2001, Flavonoids: A review of probable mechanisms of action and potential applications. Am J Clin Nutr 74:418–425.
20. Rice-Evans C., 2001, Flavonoid antioxidants. Curr Med Chem 8:797–807.
21. Santos M., J.J Jimenez, B. Bartolome, C. Gomez-Cordovez, M.J. Del Nozal, 2003, Variability of brewers' spent grain within a brewery. Food Chem., 80: 17-21.
22. Xiros C., P. Christakopoulos, 2009, Enhanced ethanol production from brewer's spent grain by a *Fusarium oxysporum* consolidated system. Biotech. for Biofuels, 2:4.
23. Zhu H., Y. Wang, Y. Liu, Y. Xia, T. Tang, 2010, Analysis of Flavonoids in *Portulaca oleracea* L. by UV–Vis Spectrophotometry with Comparative Study on Different Extraction Technologies, Food Anal. Methods 3:90–97.