Food Chemistry 239 (2018) 385–401

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analysis of polyphenols in brewer's spent grain and its comparison with corn silage and cereal brans commonly used for animal nutrition

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ARTICLE INFO

Article history:
Received 3 May 2016
Received in revised form 15 June 2017
Accepted 21 June 2017
Available online 23 June 2017

Keywords:
Agricultural and agro-industrial waste
Alkaline hydrolysis
Antioxidant capacity
Bioactive compounds
HPLC-DAD

ABSTRACT

Brewer's spent grain (BSG) could be tested as an alternative source of polyphenols in animal nutrition. Proper extraction and analytical methods are critical for quantification. Thus, extraction for BSG, corn silage, and brans of rice, corn, and wheat were studied for the highest yield of polyphenols. A method for 18 phenolic monomers by HPLC-DAD was developed, validated, and applied to samples. An aqueous solution of NaOH (0.75% w/v) using integral samples for extraction resulted in the highest values for colorimetric measurements in all analyzed sources. Method by maceration showed the highest phenolic yield when applied in corn silage and BSG. However, for brans the best method was microwave assisted. Results from HPLC-DAD analysis clearly showed that native structures of phenolic compounds were simplified to its monomers allowing quantification and sample discrimination. BSG had the highest concentration of polyphenols and could be a promising and innovative source for animal feed studies.

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1. Introduction

Brewer's spent grain (BSG) is a beer industry residue that represents more than 85% of the total produced by-products (Mussatto, Dragone, & Roberto, 2006). In 2014, Brazil has produced a total of 14 billion liters of beer reaching the third place in the global production ranking, just behind China and the United States of America (Isozaki, 2015). Worldwide breweries are capable to produced more than 30 million tons of brewer's spent grain per year and at a least 2 million of tons are produced just in Brazil (Niemi et al., 2012). This scenario emphasizes the relevance in the inclusion of this residue in the food production chain, as has already occurred with other wastes produced in large amounts by agroindustry.

Among the agricultural wastes that are annually produced in large amounts and used for animal consumption there is the corn silage. It is rich in cellulose, hemicellulose and lignin and widely used to feed ruminants (Kuzmanović et al., 2015). Cereal brans are also important by-products obtained from corn, wheat, and rice flour production (Brewer, Kubola, Siriamornpun, Herald, & Shi, 2014; Zilic et al., 2013). All those cereals grains were produced worldwide in 2015's recorded values of 1,336.6 billion tons, 729.1 million tons, and 494.7 million tons, respectively (FAO. Cereal Supply, Agriculture Organization of the United Nations (FAO), & Italy, 2016). Cereal brans are common ingredients in animal feed and some previous works in literature have already reported polyphenol profile and quantification results (Setyaningsih, Saputro, Palma, & Barroso, 2015; Vaher, Matso, Levandi, Helmja, & Kaljurand, 2010; Wang et al., 2014). Thus, comparisons of these materials with new ingredients in animal diet are important to evaluate the relevance of it as source of polyphenols.

It is noteworthy that corn silage is one of the main items of forage in the diet of cattle in many parts of the world and brewer's spent grain could be tested as an alternative source of polyphenols in animal nutrition because its chemical composition contains high levels of fiber and protein (Mussatto et al., 2006), which could suggest putative benefits for ruminants. The advantages of such substitution would entail the use of an agro-industrial waste of low commercial value to replace a dietary ingredient with a higher market value. Furthermore, this waste could be a promising source of polyphenols with greater antioxidant activity than corn silage.

As already reported by Masisi, Beta, and Moghadasian (2016) cereal by-products obtained from agricultural and agroindustry activities are promising sources of bioactive compounds. Most

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http://dx.doi.org/10.1016/j.foodchem.2017.06.130
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phenolic compounds can act as strong antioxidants (Jun, Shin, Song, & Kim, 2015) being helpful to promote human health against chronic diseases (Del Rio et al., 2013). Moreover, it could improve food quality either by direct application as food stabilizers (McCarthy et al., 2013) or indirectly by the animal diet increasing deposition in tissues (Castillo, Pereira, Abuelo, & Hernández, 2013; Fruet et al., 2016). All future applications of these plant matrices require previous studies on the evaluation of extraction conditions and polyphenols characterization.

Due to the polyphenol's chemical diversity and its interaction with other matrix constituents, extraction from vegetable sources are complex and the initial steps in samples preparation requires the evaluations of some important conditions (Masisi et al., 2016). Solvent composition and the energy supplied during extraction are some factors that greatly affect analyte recovery (Acosta-Estrada, Gutiérez-Uribe, & Serna-Saldívar, 2014; Wanyo, Meeso, & Siriamornpun, 2014). Since antioxidant capacity is mainly linked to the concentration of these compounds, it could also be affected as a property of the final extract mixture.

As a final remarkable consideration to this work, the use of phenolic compounds in ruminant diet was already reported as a promising strategy to improve animal well being for dairy cattle or as a supplementation strategy for antioxidant fortified milk and meat (Castillo et al., 2013; Fruet et al., 2016; Paraskevakis, 2015). The dose-response effect of these compounds, as well as their mechanism of action in ruminant animals has still not been fully elucidated. Therefore, there is a growing interest in the chemical elucidation and quantification of these compounds in food destined for animal consumption, which has a polyphenol profile that is still unclear.

The main purpose of this works is the evaluation of differ extraction conditions for characterization of polyphenols content or the identification of major monomers that could be present in brewer's spent grain. Moreover, corn silage and brans of corn, wheat, and rice were also evaluated for comparison purposes. Since this vegetable sources are the major ingredients used for animal feed. Extraction was carefully studied to determine the contribution of different solvents and the effect of microwave energy in the polyphenol's yield and profile. The method used for separation and quantification of phenolic compounds in extracts of cereal wastes by HPLC-DAD is detailed described.

2. Materials and methods

2.1. Chemicals and standards

4-Hydroxybenzoic acid (99%); caffeic acid (98%); catechin (98%); chlorogenic acid (95%); epicatechin (98%); gallic acid (98%); kaempferol (90%); kaempferol-3DGlp (97%); myricetin (96%); p-coumaric acid (98%); protocatechuic acid (97%); quercetin (95%); resveratrol (99%); sinapic acid (99%); syringic acid (95%); trans-cinnamic acid (99%); trans-ferulic acid (99%); vanillic acid (97%) were purchased from Sigma–Aldrich (St. Louis, Missouri, United States). HPLC-grade methanol used for mobile phase was obtained from Merck (Darmstadt, Germany). HPLC-grade acetonitrile and formic acid used for mobile phase was obtained from Sigma–Aldrich (St. Louis, MO, U.S.A.). HPLC-grade water was obtained from a Milli-Q system (Millipore, Bedford, Massachusetts, U.S.A.). Polytetrafluoroethylene syringe filter and membrane (PTFE) was from Allcrom (São Paulo, Brazil).

2.2. Samples

The corn silage was obtained from a commercial hybrid corn variety. The silage was ensiled using double plastic bag in micro-silos with a volume of forty liters. It was compressed and sealed immediately after harvest. Silos were opened after 60 days. At this date, the dry matter content was 34.61%.

Rice, corn, and wheat bran were purchased directly from a specialized company (Cooperativa Agrícola Mista Ltda – CAMNPAL, Nova Palma, Brazil) in feed production for ruminants. It had a dry matter content of 88.94%, 88.12%, and 87.23% for rice, corn, and wheat, respectively.

The brewer's spent grain was obtained from a medium-sized brewery. All amount used in this work was collected from the same production lot and with a dry matter content of 18.97%.

Samples of corn silage and brewer's spent grain were pre-dried in a forced ventilation oven (55 °C; 72 h) with a final dry matter content of 94.18% and 95.84%, respectively. All samples were finely milled in a refrigerated analytical mill (Marconi, São Paulo, Brazil) during 1 min at 27,000 rpm, standardized to 0.5 mm of particle diameter using a sieve system, and stored at −20 °C until the extraction experiments. Samples were studiedin two forms: one with its initial fat content and named as integral samples; and another after fat removal with ethyl ether using exhaustive extraction (Soxhlet apparatus), 4 h, lipid content in grams per 100 g of dry matter corresponding to the 3.2% corn silage; 19.9% rice bran; 3.9% corn bran; 4.2% wheat bran; 8.2% brewer's spent grain and named as defatted samples.

2.3. Extraction of phenolic compounds by maceration and microwave-assisted extraction

Maceration and microwave-assisted extractions were tested using the following five raw materials: corn silage; rice bran; corn bran; wheat bran, and brewer's spent grain. All of them were evaluated as integral and as defatted samples. The following solvents were tested: 50% methanol; 50% acetone, and 0.75% NaOH aqueous solution.

The microwave equipment (Synthos 3000, Anton-Paar, São Paulo, Brazil) equipped 16-carrousel containers (Rotor 16) was set up as detailed by Moreira et al. (2013) with some adaptations. For all solvents being tested, one gram of each sample was transferred to polytetrafluorethylene (PTFE-TFM) based tubes in a solid to liquid ratio of 1:20 w/v. Temperature was maintained and monitored at 100 °C under stirring (magnetic stirring bar, 200 rpm) in all the containers during the whole extraction time (15 min).

For maceration, powdered samples were mixed with each solvent (50% methanol; 50% acetone, and 0.75% NaOH aqueous solution) at a solid to liquid ratio of 1:10 and kept at room temperature (around 20 °C) for 24 h under constant stirring (magnetic stirring bar, 200 rpm) at dark (Brewer et al., 2014; Jun et al., 2015).

All the extracts obtained by both methods were centrifuged (15 min, 4000 rpm, MTD III Plus, Logen Scientific). For the extracts obtained with 0.75% of sodium hydroxide aqueous solution, the pH value in supernatantwas adjusted to 6.5 with a 6 M hydrochloric acid solution. Supernatants were filtered (cellulose, 0.45 μm) and stored at −20 °C for further analysis.

2.4. Total phenolics and flavonoids

Total phenolic content was determined by the Folin-Ciocalteu reagent (FCR) colorimetric method (Singleton, Orthofer, & Ramuela-Raventos, 1999). Quantification was performed by a calibration curve using Gallic acid as an authentic phenolic standard (0–70 mg L^{-1}; $Y = 0.013x + 0.013$; $R^2 = 0.999$). Results were expressed by equivalence as milligrams of gallic acid equivalent (GAE) per gram of sample.

Total flavonoid content was determined by a colorimetric method (Bao, Cai, Sun, Wang, & Corke, 2005). Quantification was done using quercetin as the authentic standard for flavonoids (0–80 mg L^{-1}; $Y = 0.002x + 0.0223$; $R^2 = 0.999$). Results were

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