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TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF BY-PRODUCTS FROM CEREAL AND LEGUME MILLING INDUSTRIES

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ABSTRACT

Agro-industrial waste is often utilized as feed for animals or as fertilizers on farms, but they may be rich sources of certain health-promoting compounds like antioxidants. The objective of this research was to examine total phenolic content and antioxidant activity by DPPH radical scavenging method of certain legume and cereal industries by-products. Four types of by-products namely bengal gram seed coat, bengal gram brokens, rice brokens and wheat bran were studied for this purpose. The results showed that bengal gram seed coat extract possess much higher amount of phenolic content as well as antioxidant activity followed by bengal gram brokens, wheat bran and rice brokens. This suggested that these by-products could be used as functional ingredients for the development of health-promoting food sources.

Key words: Antioxidant activity, By-products, Cereal, Legume, Total phenolic content.

INTRODUCTION

Antioxidants are recognized as reducing oxidative damage associated with many diseases, including cardiovascular disease, cancer, atherosclerosis, diabetes, immune deficiency disease and ageing (Wang *et al*., 2007). Oxygen radicals induce oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing. The most effective way to eliminate free radicals which is the cause of oxidative stress is with the help of antioxidant. However, there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) because of their possible activity as promoters of carcinogenesis. Because of which, there is growing interest toward natural antioxidants from herbal sources.

By-products of plant food processing represent a major disposal problem for the industry concerned, as this is becoming much more expensive. Due to increasing production, disposal represents a growing problem since the plant material is usually prone to microbial spoilage, which is limiting its further exploitation. On the other hand, costs of drying, storage and shipment of by-products are economically limiting factors (Schieber *et al.*, 2001). Therefore, agro-industrial waste is often utilized as feed for animals or as fertilizers on farms. But, in today's lifestyle, by-products are considered to be a promising source of functional compounds (Laufenberg *et al.*, 2003) and in addition they also contain plant secondary metabolites, i.e. polyphenols that are increasingly being recognized for their potential benefits for human health. In the food industry, the recovery and modification of the by-products is becoming increasingly important. To alleviate hunger and to overcome malnutrition, there is an increasing demand in developing countries to explore these by-products for different types of nutrients and phytochemical properties. It is estimated that million tones of by-products are generated annually from cereal and legume mills in the country. These comprises of seed coat/husk, powder, large and small brokens, shriveled and under-processed grains which are presently disposed off only as feed grade material, fetching low remunerative prices (Ramakrishnaiah *et al.*, 2004). The importance of the antioxidant constitutes of these by-products in the maintenance of health is also increasingly of interest among food manufactures

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and consumers, as the future trend toward developing functional foods. Moreover, literature on the health beneficial effects of these by-products is scanty.

The purpose of the present study is to evaluate the amount of total phenolic content in bengal gram seed coat, bengal gram brokens, rice brokens and wheat bran as well as their antioxidant activities using 2, 2'-diphenyl-1-picrylhydrazl (DPPH) free-radical scavenging test. By this an attempt has been made to provide information on natural antioxidants present in cereal and legume by-products for increasing their use in the development of health-promoting food sources.

MATERIALS AND METHODS

Collection of samples: From different kind of by-products of milled bengal gram two were selected namely; seed coat and brokens (broken seeds + seed coat) of bengal gram. The samples were collected from legume milling industries. The samples of rice brokens and wheat bran were procured from rice and wheat milling industries.

Processing and Preparation of samples

(i) Bengal gram seed coat

Bengal gram seed coat
↓
Cleaning and washing under running water
↓
Dipping in rolling boiling water for 10 min
↓
Spreading over filter paper
↓
Drying (70°C, 6 h)
↓
Grind to fine powder and stored in airtight plastic bottles

Fig 2.1 Flow diagram for bengal gram seed coat processing

(ii) Bengal gram brokens

Bengal gram brokens
↓
Cleaning and washing under running water
↓
Dipping in rolling boiling water for 10 min
↓
Spreading over filter paper
↓
Drying (80°C, 7 h)
↓
Grind to fine powder and stored in airtight plastic bottles

Fig 2.2 Flow diagram for bengal gram brokens processing

(iii) Broken rice

Broken rice
↓
Cleaning and washing under running water
↓
Spreading over filter paper
↓
Drying (60°C, 6 h)
↓
Grind to fine powder and stored in airtight plastic bottles

Fig 2.3 Flow diagram for broken rice processing

(iv) Wheat bran

Wheat bran
↓
Cleaning and sieving
↓
Stored in an air tight plastic bottles

Fig 2.4 Flow diagram for wheat bran processing

Preparation of extracts: The samples for antioxidant activity were extracted by the method of Xu *et al*. (2007). The air dried sample was accurately weighed in 0.5 g aliquots and then 5 ml of acetone/water (50:50, v/v) extraction solvent was added. The mixtures were shaken at 300 rpm for 3 h at room temperature on an orbital shaker. The mixtures were again extracted for another 12 h in dark. The extracts were centrifuged at 3000 rpm for 10 min and the supernatant was removed in new tubes. The precipitated residues were re-extracted with 5 ml of extraction solvent. The two extracts from both extractions were combined and stored at 4°C in the dark until analysis.

Determination of total phenolic content: Total phenolic contents were estimated by the method of Singleton and Rossi (1965). A dilute of each extract (1 ml) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5ml, 1N) and aqueous Na_2CO_3 (15 ml, 1M) in a 100 ml volumetric flask. The mixtures were incubated for 8 min at room temperature. A dose of distilled water was added to make a volume 100 ml. the mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 765 nm against a reagent blank, which was composed of the same reagents except that 1 ml of distilled water was used in lieu of sample extract. The results were expressed as gallic acid equivalents (mg GAE/g of sample) through the calibration curve of gallic acid (50 to 500 mg/liter).

Determination of DPPH radical scavenging activity (DPPH RSA): DPPH scavenging activity was carried out the method of Hudec *et al.* (2007). An aliquot of 0.4 ml of 2.5 mg/l DPPH in ethanol was added to 7.6 ml of sample extracts. The mixture was shaken well for 1 min and incubated for 30 min at room temperature. The absorbance was recorded at 517 nm against ethanol blank. A control was measured using the same procedure except ethanol was used instead of extracts (at zero min). The percent of DPPH radical discoloration of the sample was calculated according to the equation (%) discoloration:

% radical scavenging activity=

$$A_{control} - A_{sample} \text{ X100 } / A_{control}$$

Where,

($A_{control}$) = absorbance for the control

(A_{sample}) = absorbance for the sample

Statistical analysis: All assays were carried out in triplicates. Appropriate statistical analyses were carried out as per the methods described by Sheoran and Pannu (1999).

RESULTS AND DISCUSSION

Mean values for the total phenolic content of four different by-products of legume and cereals are shown in Fig. 1. The amount of total phenolics was found to be different in different by-products and varied from 0.44 to 13.47 mg GAE/g of dry weight. The highest amount of phenolic contents was detected in bengal gram seed coat (13.47 mg GAE/ g) and lowest in rice brokens (0.44 mg GAE/g). The results of total phenolic content, showed the variation among different by-products and this variation was significant (P > 0.05).

The DPPH radical scavenging activity in different extracts of by-products is shown in Fig. 2. Significant differences in scavenging percentage between extracts were observed and the results clearly indicate that all the extract exhibited high antioxidant activity except rice brokens. Among the different by-products, bengal gram seed coat showed the highest antioxidant activity, followed by bengal gram brokens, wheat bran and rice brokens.

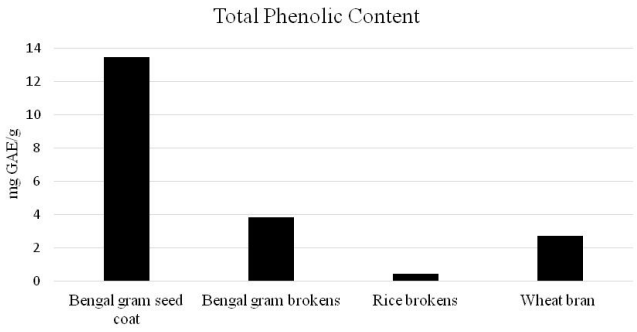

FIG 1: Total Phenolic Content of of by-products

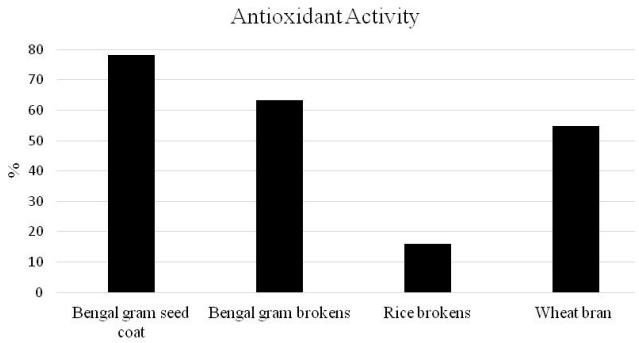

FIG 2: DPPH radical scavenging activity of by-products

Phenolic compounds are a class of antioxidant agents which act as free radical terminators. And DPPH method has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Porto *et al.*, 2000; Seow *et al.* 2012). DPPH· is considered to be a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid autoxidation. Antioxidants react with DPPH·, reducing the number of DPPH free radicals to the number of their available hydroxyl groups. It is visually noticeable as a discoloration of DPPH· from purple to yellow. When the total phenolic content and DPPH activity were analyzed, the higher amount was observed in bengal gram seed coat. Whereas, rice brokens showed lower amount of both. These results could be attributed to genetic background, grain physical properties and the seed coat colour, since the seed coat is the structure richer in phenolic compounds (Segev *et al.*, 2010). Most of the antioxidants of cereal and legumes decreased during milling as these are mainly present in bran layer which is removed during milling, which caused lower concentration of antioxidant activity in rice brokens. Earlier studies also revealed that seed coat, plumule and aleurone layer enriched in seed coat extracts showed a better antioxidant potential compared to other fractions milling and which may be due to the quantitative and qualitative differences in phenolic acids (Sreeramulu *et al.*, 2009; Vaher *et al.*, 2010; Girish *et al.*, 2012).

CONCLUSION

Simplest things make the most significant difference in our lives. By-products are simple items that can deliver fantastic health benefits in our daily life. The by-products of cereal and legume milling industries are often utilized as feed for animals or as fertilizers on farms. But from the present study it can be concluded that these by-products possess a good amount of phenolic content and antioxidant activity. As in recent years, the demand of natural antioxidants is increased, because of adverse health effects of synthetic antioxidants, these by-products could be represented as one such class of antioxidants. These by-products can be used not only as accessible source of natural antioxidants but also as an ingredient for functional food and control of degenerative diseases and so to enhances health benefits which may combat degenerative disorders such as obesity, heart diseases, cancer, gall stones, diabetes, constipation etc. Food products like bread, biscuits, noodles, *chapati*, *papad* or *idli* which are mainly prepared using refined cereals or legumes can be incorporated with these by-products to increase their antioxidant levels. Further investigation on the isolation and identification of antioxidant component(s) in these by-products may lead to chemical entities with potency of clinical use.

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