Food Research International 46 (2012) 370–377

Contents lists available at SciVerse ScienceDirect

Food Research International

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

# Nutrient distribution, phenolic acid composition, antioxidant and alpha-glucosidase inhibitory potentials of black gram (*Vigna mungo* L.) and its milled by-products

T.K. Girish [a], V.M. Pratape [b], U.J.S. Prasada Rao [a,*]

[a] *Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, 570 020 India*
[b] *Department of Grain Science and Technology, Central Food Technological Research Institute, Mysore, 570 020, India*

ARTICLE INFO

*Article history:*
Received 13 August 2011
Accepted 24 December 2011

*Keywords:*
Black gram (*Vigna mungo* L.)
Nutrient composition
Bioactive compounds
Dietary fiber
Phenolic acids
Antioxidants
α-glucosidase inhibition

ABSTRACT

Black gram belongs to the Leguminosae family. It is one of the less studied legumes, although it is widely used in different parts of the world. Black gram in the form of cotyledon (dhal) is mainly used for the preparation of various food products. During milling of black gram into cotyledon, about 25% of the grain is removed as waste by-products. In the present study, nutrient content, phenolic acid composition, antioxidant activity and α-glucosidase inhibitory properties of total black gram flour and its milled fractions were determined with a view to provide economic importance to these by-products. Protein content in black gram and its fractions ranged from 12 to 42%, while fat content ranged from 0.9 to 3.4%. Germ had the highest content of fat and protein, while seed coat and plumule fractions had the lowest (0.9%). Seed coat had the highest dietary fiber content (78.5%) while cotyledon had the lowest (24.4%). Seed coat, plumule and aleurone layer enriched in seed coat extracts showed a better antioxidant potential compared to other fractions and this may be due to the quantitative and qualitative differences in phenolic acids. Extracts of seed coat, plumule and aleurone layer enriched in seed coat extracts showed good α-glucosidase inhibitory activity. Black gram flour contained phenolic acids like gallic, protocatechuic, gentisic, vanillic, syringic, caffeic and ferulic acids. However, composition and content of these phenolic acids varied in different fractions. Ferulic acid was the major phenolic acid in all the fractions. Protocatechuic acid, ferulic acid, gentisic acid and gallic acid contents in these fractions negatively correlated ($P<0.05$) to $IC_{50}$ values of both free radical scavenging and α-glucosidase inhibitory activities indicating their potential antioxidant and antidiabetic properties. As black gram and its fractions are rich in antioxidant compounds and nutrients, they may have potential applications as nutraceuticals and functional food ingredients in various processed foods for the improvement of health benefits.


## 1. Introduction

Legume seeds are valuable sources of proteins and other nutrients, and they are good source of nutrients for the majority of the world population. It is also reported that legumes have certain phytochemicals like polyphenols, flavonoids, phytosterols that possess health benefits (Kritchevsky & Chen, 2005; Sessa, 2004; Sreerama, Sashikala, & Pratape, 2010). Black gram or black gram lentil (*Vigna mungo* L.) belongs to the Leguminosae family (Reddy, Salunkhe, & Sathe, 1982; Salunkhe, Kadam, & Chavan, 1985). It is one of the less studied legumes although it is widely used in India, Pakistan, Iran, Greece and East Africa (Bhattacharya, Latha, & Bhat, 2004; Chaudhary & Ledward, 1988). Black gram is used for the preparation of different food products. Dehusked cotyledon is used for the preparation of fermented foods such as idli, dosa, and non-fermented foods like cooked dhal, hopper, papad and waries (spicy hollow balls) (Batra & Millner, 1974). Traditionally, sweets prepared with whole black gram flour and jaggery were regarded as nutritious food in India. Whole black gram flour paste either alone or in combination with sandalwood paste or fenugreek paste is used for skin or hair care, respectively. Incorporation of black gram flour was reported to improve the quality of buffalo meat burgers (Modi, Mahendrakar, Narasimha Rao, & Sachindra, 2004) and beef sausages (Chaudhary & Ledward, 1988) and the nutritional quality of biscuit (Patel & Venkateswara Rao, 1995).

Whole black gram is a rich source of protein, fiber, several vitamins and essential minerals such as calcium and iron (Reddy et al., 1982; Salunkhe et al., 1985). Processing of black gram into dehusked cotyledon essentially involves the removal of seed coat, germ, aleurone layer and plumule, and these fractions may consist of a variety of nutrients. Currently, except cotyledon fraction, the other fractions are discarded or used as animal feed. However, the distribution of nutrients and bioactive compounds in these fractions is not known.

Foods rich in nutraceuticals and dietary fiber are gaining importance because of their health benefits. Polyphenols, carotenoids and

* Corresponding author at: Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, 570 020, India. Tel.: +91 821 2514876; fax: +91 821 2517233.
*E-mail address:* prasadarao_ummiti@yahoo.com (U.J.S. Prasada Rao).

0963-9969/$ – see front matter 
doi:10.1016/j.foodres.2011.12.026

dietary fiber have a role in prevention of cardiovascular disease, cancer and diabetes (Lario et al., 2004; Scalbert, Manach, Morand, & Remesy, 2005). By-products from different food processing industries which were traditionally treated as environmental pollutants are being recognized as good sources for obtaining valuable components. By-products from cereal, legume and fruit processing industries have been found to be rich and economically inexpensive sources of bioactive compounds such as antioxidants, dietary fibers and enzymes (Ajila, Bhat, & Prasada Rao, 2007; Ajila, Naidu, Bhat, & Prasada Rao, 2007; Liyana-Pathirana & Sahidi, 2006; Moure et al., 2001; Sessa, 2004). The seed coat (husk) of cereals and legumes possesses large quantities of endogenous antioxidants such as phenolic compounds (Moure et al., 2001; Tsuda, Ohshima, Kawakishi, & Osawa, 1994). Black gram lipids were shown to have cholesterol-reducing effect in both humans and experimental animals (Saraswathi Devi & Kurup, 1972). Distribution of bioactive compounds in plants varies in different tissues. In the present study, the extract of black gram and its milled fractions viz., cotyledon, seed coat, germ, aleurone layer enriched in seed coat fraction and plumule were investigated for the nutritional composition, phenolic acid composition, carotenoid content, and also their antioxidant and α-glucosidase inhibition properties.

## 2. Materials and methods

### 2.1. Materials

Gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), α-amylase (Termamyl), pepsin, pancreatin, celite were purchased from Sigma Aldrich Chemical Co. (St. Louis, USA). Folin–Ciocalteu reagent was obtained from SR Laboratories Limited (Mumbai, India). All other chemicals and solvents were of analytical grade.

### 2.2. Milling of black gram and separation of milled products

Black gram (10 kg) was pitted in Versatile Dhal Mill (CFTRI design) mixed with 30 mL of oil, kept overnight for tempering and dried at 60°C for 8 h. The black gram thus obtained after treatment was milled using Versatile Dhal Mill according to the procedure described by Narasimha, Ramakrishnaiah, Pratape, Sasikala, and Narasimhan (2002). Black gram was milled into cotyledon, seed coat, and mixture of germ, aleurone, seed coat powder, and plumule. The mixture was further separated into different fractions by air classification as described in Fig. 1 (Ajila & Prasada Rao, 2009).

### 2.3. Determination of proximate composition

Moisture, protein, fat, ash and crude fiber contents in whole black gram flour and its milled fractions (BGMF) were determined by AOAC methods (2005). The total carbohydrate content was calculated by the difference method.

### 2.4. Extraction of total polyphenols and determination of total phenolics

Whole black gram flour (1 g) and BGMF (1 g) were extracted with 30 mL of either 80% acetone or 80% ethanol separately and were centrifuged for 15 min at 8000×*g*. The clear supernatants obtained were subjected to total phenolic content estimation using the Folin–Ciocalteu reagent following the procedure described by Swain and Hillis (1959). Gallic acid was used as a standard. The total polyphenol content in the extract was expressed as gallic acid equivalents (GAE).

### 2.5. Determination of anthocyanin content

Monomeric anthocyanin content of the black gram flour and BGMF acetone extracts were measured using a spectrophotometric pH differential method (Wolfe, Xianzhong, & Liu, 2003). Anthocyanin content was expressed as mg cyanidin 3-glucosides equivalent/100 g sample for the triplicate extracts.

### 2.6. Determination of carotenoids

Black gram flour and BGMF (1 g) were homogenized with 40 mL of methanol containing 1 g KOH. The mixture was saponified

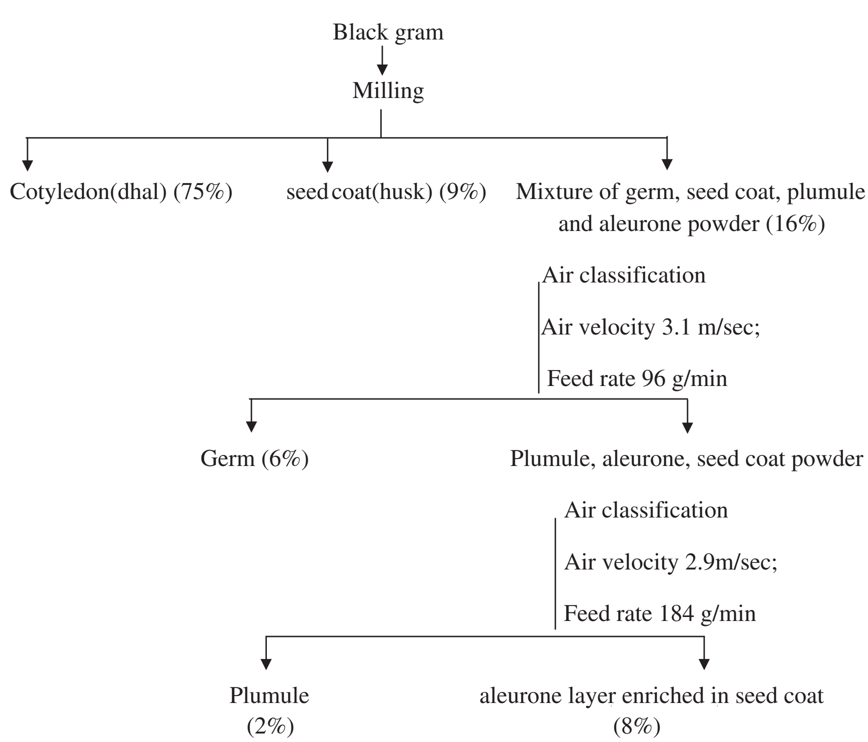


**Fig. 1.** Flow diagram for separation of black gram milled fractions.