Journal of Cereal Science 69 (2016) 358–362

Contents lists available at ScienceDirect

Journal of Cereal Science

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

# Preparation of protein and mineral rich fraction from grain amaranth and evaluation of its functional characteristics

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ARTICLE INFO

*Article history:*
Received 28 January 2016
Received in revised form
27 April 2016
Accepted 2 May 2016
Available online 3 May 2016

*Keywords:*
Antioxidant activity
Fatty acids
Phytic acid content
Protein digestibility

ABSTRACT

Grain amaranth was fractionated to prepare a seed coat rich fraction along with the fine seed coat, middling and flour fractions. The nutritional content of the coarse seed coat fraction and its antioxidant potential were evaluated. It was observed that, the coarse seed coat fraction contained highest protein (17.81 g/100 g), dietary fiber (25.78 g/100 g), free sugar (2.25 g/100 g), calcium (1115 mg/100 g), sodium (279 mg/100 g), magnesium (178.4 mg/100 g) and potassium (398.8 mg/100 g) contents compared to all other fractions. The carbohydrate and protein digestibility of all the fractions were more than 80%. A slight decrease in linoleic acid and a concurrent increase in palmitic acid contents were observed in coarse seed coat fraction. The total phytic acid increased and total polyphenols contents decreased in the coarse seed coat fraction compared to the native grain. The DPPH, ABTS and total antioxidant activities are comparatively high in this particular fraction. The study indicated a possibility of preparation of a protein, fiber and mineral rich fraction from grain amaranth with good antioxidant potential which can be used as a functional food ingredient.



## 1. Introduction

Grain Amaranth, one of the important pseudocereals, is known for its nutritional advantages. High content of good quality protein and lipids and presence of good amount of minerals like calcium, brands grain amaranth to be focused as the “cereal of future” (Taylor et al., 2014). It contains about 14–15 g/100 g protein, 7–8 g/100 g lipids and 11–16 g/100 g dietary fiber. In addition, amaranth contains high concentrations of calcium, phosphorous, iron, potassium and zinc and forms a good source of essential amino acids (Alvarez-Jubete et al., 2010; Gamel et al., 2004; Mota et al., 2016). Amaranth protein also exhibits hypotriglyceridemic effect (Escudero et al., 2006) and its lysine content is also high. The amount of lipids present in amaranth is comparatively higher than the other cereals. Within the unsaponifiable matter, squalene is the main component (about 8% of seed oil). Squalene is known for its chemopreventive effects on colon cancer, hypocholesterolemic action and helps to control hypertension (He et al., 2002; Pogojeva et al., 2006). Amaranth shows reasonably good antioxidant activity, comparable to wheat and its 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity is higher than wheat and foxtail millet (Asao and Watanabe, 2010). It was also reported that grain amaranth shows a considerable Angiotensin-Converting Enzyme inhibitory activity (Asao and Watanabe, 2010). Amaranth seeds have been recommended for people with celiac disease as it is a gluten-free food (Alvarez-Jubete et al., 2010).

Despite of all these nutritional advantages, the food uses of grain amaranth is still limited. The grains are mainly popped and used in sweet like preparations along with jaggery in parts of north India. A few attempts have been made to use it as composite flour in some bakery products like pasta wherein 5–10% of the amaranth flour has been used (Sudha and Leelavathi, 2012). However, the food preparations from grain amaranth are neither widespread nor very popular. The typical taste and texture of grain and also very small grain size may be the major constrains. Single grain of amaranth is relatively small in size with 0.9–1.7 mm diameter and its 1000-kernel weight ranges from 0.6 to 1 g (Berghofer and Schoenlechner, 2002). The embryo is large and encloses the perisperm like a ring,

*Abbreviation:* AAS, atomic absorption spectrometry; ABTS, 3-ethylbenzothiazoline-6-sulphonic acid; CSC, Coarse seed coat; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FF, fine flour; FSC, fine seed coat; MF, middling fraction.

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http://dx.doi.org/10.1016/j.jcs.2016.05.002
0733-5210/

accounting for about 25% of the grain weight. In contrast to the other two pseudocereals, quinoa and buckwheat, it is not necessary to remove the thin and smooth seed coat (Berghofer and Schoenlechner, 2002). Unlike other grains, the starch is present in the perisperm of the grain. Thus, the distribution of nutrients in the grain may vary largely from other cereals or pseudocereals. Hence, fractionating amaranth grain needs special attention. A few reports are available on milling of this grain using barley pearler and successive sieving (Betschart et al., 1981; Sequra and Bressani, 2002). Milling the grain amaranth using roller mill was explored by Nanka (1998) wherein a vario-technical roller mill was used. Schoenlechner (2000) used a technical scale roller mill in combination with a plansifter using a single break system. However, milling grain amaranth using multiple break systems to obtain a fraction rich in protein, mineral and dietary fiber with lower starch contents will be highly desirable. Thus, the main objective of this study was to fractionate grain amaranth to prepare a stream rich in protein, dietary fiber and minerals which can be used as a potential ingredient in different designer foods.

## 2. Materials and methods

### 2.1. Materials

Grain amaranth (K432) procured from University of Agricultural Sciences, Bangalore, was cleaned to remove the foreign particles and stored in polyethylene bags till use. The initial moisture content of the sample was determined by air oven method (AACC, 2000) which was found to be 11.5 ± 1 g/100 g.

### 2.2. Reagents and standards

Termamyl, pepsin, pancreatin, amyloglucosidase, gallic acid, DPPH, ABTS, trolox, and fatty acid methyl esters mix (GLC-10) were procured from Sigma Chemical Co., St. Louis, MO, USA. Phytic acid/total phosphorus kit was purchased from Megazyme International Ireland Ltd, Bray, Ireland. Reference mineral standard solutions for atomic absorption spectrometry (AAS) were procured from Merck Specialities Pvt. Ltd, Mumbai, India. All other chemicals used were of analytical grade.

### 2.3. Milling

Preliminary trials indicated that tempering the grains with additional water to raise the moisture content to 16 g/100 g was desirable. Accordingly, 5 kg of grain amaranth was tempered with additional water to raise the moisture content up to 16 g/100 g and rested for 24 h before milling. The tempered sample was milled in a laboratory scale roller mill (Buhler, MLU-202) to prepare four streams namely flour, middling, fine seed coat and coarse seed coat fractions. The middling and seed coat fractions were further pulverized to prepare a flour of less than 250μ and used for further studies. The whole amaranth grains pulverized to 250 μ size served as control sample.

### 2.4. Nutrient composition

Moisture, fat, protein and ash contents of amaranth fractions were determined according to AACC (2000) methods and the soluble, insoluble and total dietary fiber contents were estimated by the method of Asp et al. (1983). The ash contents were dissolved in dilute HCl and the solutions were used for estimation of calcium by precipitating as calcium oxalate, whereas iron, zinc, sodium, magnesium and potassium contents were estimated by atomic absorption spectroscopy. The polyphenol contents were determined as per the procedure of Singleton et al. (1995).

### 2.5. Phytic acid

Phytic acid content was determined using Megazyme kit (K-PHYT 05/07, Megazyme, Ireland).

### 2.6. Free sugars

For the extractions of the free sugars, defatted flours (5 g) of all fractions including control sample were suspended in 20–30 ml of 70% ethanol and refluxed at 97 °C for 2 h. The suspension was centrifuged for 15 min at 4000 rpm. The supernatant was collected and the residue was re-suspended in fresh 70% ethanol and refluxed again. The procedure was repeated for minimum four times. The supernatants were pooled, volume was measured and used as free sugar extract. The free sugar content of the extract was estimated by phenol sulphuric acid method (Ford, 1981). Briefly, to 0.5 ml of the sample 0.5 ml of distilled water and 1 ml of 5% phenol was added. To the mixture, 5 ml of concentrated sulphuric acid was added and the absorbance was read at 490 nm. Glucose solution (100 mg/100 ml) was used as a standard.

### 2.7. Carbohydrate digestibility

The carbohydrate digestibility of defatted fractions was estimated according to Mouliswar et al. (1993). Briefly, 100 mg of defatted sample was cooked in boiling water bath for 15 min with 15 ml of water and 0.1 ml Termamyl. To the solution, 15 ml of 0.2 M Glycine − HCl buffer of pH 2 with 15 mg pepsin was added and incubated at 37 °C for 2 h. The pH of the solution was adjusted to 6.8 with 0.2 M NaOH. To that, 15 ml of 0.05 M phosphate buffer containing 15 mg of pancreatin was added and incubated at 37 °C for 2 h. The pH of the solution was adjusted to 4.8 using 0.1 M HCl followed by the addition of 15 ml acetate buffer (0.05 M) containing 15 mg amyloglucosidase and incubated at 55 °C for 2 h. The final volume of the solution was made up to 100 ml with water. The reducing sugars were estimated by dinitrosalicylic acid method.

### 2.8. Protein digestibility

The protein digestibility of defatted samples were estimated according to Mouliswar et al. (1993). Briefly, 100 mg protein equivalent defatted sample was incubated with 50 ml of 0.1 M HCl containing12.5 mg of pepsin for 3 h. The pH of the solution was adjusted to 8 by 0.5 N NaOH, followed by the addition of 0.05 M phosphate buffer containing 6 mg pancreatin and incubated at 37 °C for 24 h. Final volume of the solution was made up to 100 ml with water and centrifuged at 5000 rpm for 15 min. The protein content of the digested sample was estimated by Lowry's method (Schacterle and Pollack, 1973).

### 2.9. Fatty acid profile

The fatty acid composition of amaranth was analysed as per Liang (2010). The fatty acid methyl esters were fed to a capillary column (VF-1 ms, 30 m × 0.25 mm ID, DF-0.25 μm thickness coated stationery phase) fitted to gas chromatography (Model GC-2010 Plus, Shimandzu Corporation, Tokyo, Japan) equipped with a flame ionization detector. The oven temperature was programmed from 140 to 240 °C at 4°C/min with an initial hold at 140 °C for 5 min. The injector and the detector were maintained at 260 °C. The flow rate of carrier gas $N_2$ was set at 1.44 mL/min. The fatty acids were identified with reference to the retention time of standard fatty acid methyl esters under the same condition. The fatty acids