Determination of proximate composition and bioactive compounds of the Abyssinian purple wheat

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Abstract: This study was conducted to investigate valid information's of the Landrace Ethiopia purple wheat and to focus on the future development of the food product with an excellent health benefits. The purple wheat grain was cleaned of foreign materials and impurities with manually by human hand. The samples were ground into flour and sieved through 75 μm sieve and were packaged in airtight plastic bags prior to analyses. The flours were analyzed for their proximate composition and bioactive compounds, the results showed that the purple wheat contained ash (5.5%), crude protein (8.5%), crude fat (3.03%), crude fiber (7.3%), moisture content (10.76%), utilized carbohydrate (64.85%) and dry gluten (0.283 g). It also contained condensed tannin, total phenol and anthocyanin content (25.6 mg/100 g, 253 mg/100 g and 197.4 mg/100 g) of anti-nutritional factors, respectively. Colored-grain wheat is one kind of new germplasm resource of cereal crops, some of which are rich in beneficial anthocyanin.

Subjects: Food Additives & Ingredients; Food Chemistry; Food Engineering; Public Health Policy and Practice

Keywords: purple wheat; anthocyanin; dry gluten; tannin; ash

ABOUT THE AUTHOR

Hagos Hailu Kassegn is a Food science and Technologist instructor at Mekelle University with department of Food Science and post harvest technology with my research focus on sciences at the cereal chemistry, functional food development and process optimization of traditional food preparation methods in order to better sustainable existing of the traditional indigenous knowledge. I have been exploring biological active natural food products from cereal grains, roots and spices. Other research interests include the development of nutrient dense food products for prevention of malnutrition with the special group of people (Infant, children and pregnant) in developing countries; and personal meal diet products and the characterization of essential oils from oil seed plants.

PUBLIC INTEREST STATEMENT

Colored-grain wheat is one kind of new germplasm resource of cereal crops and the grains is rich in purple color and other nutrients. The presence of good source of phenols and low gluten content in purple wheat, which will further be helpful in the preparation of various functional food products. Due to its higher price in the market for its brewing quality in which purple wheat grain is preferable indigenous wheat variety in Ethiopia. Purple wheat can act as an anti free radical activity. It is also useful in curing systemic inflammation. In addition, one of the active ingredients of purple color can inhibit the oxidation of blood pressure and platelet aggregation, maintain the normal osmotic pressure of blood vessels, and reduce the vulnerability of capillaries. It would be potential candidates for various food industrialists/researchers to prepare functional foods.
1. Introduction
The nutritional value of wheat is extremely important as it takes an important place among the few crop species being extensively grown as staple food sources. The importance of wheat is mainly due to the fact that seeds can be ground into flour, semolina, etc., which form the basic ingredients of bread and other bakery products, as well as pastas, and thus it presents the main source of nutrients for the most of the world population. Plant anthocyanins can act as an antioxidant and show antibacterial and anti-cancer activity. They are also useful in curing systemic inflammation (Kong, Chia, Goh, Chia, & Brouillard, 2003). But there is another potentially great benefit to these communities and that is the possibility to ensure such staple crops are nutritionally-balanced and help remove the millions of cases of nutrition-related deficiency disease that afflict them. It should be emphasized that in the past, there has not been a single instance where plants have been bred to improve their nutritional content. If this has occurred, it is purely by accident not designed (Lindsay, 2002; Welch & Graham, 2002).

Pigmented wheat can provide a naturally colored and/or functional food ingredient for the cereal industry. A number of varieties of tetraploid wheat originating from Abyssinia (Ethiopia) have purple colored grains, due to the presence of anthocyanins in cells of the pericarp (Sharman, 1959). Wittmack, Weizenkörner, and Sitzungsber Ges Natuforsch Freunde (1906) were the first who described purple tetraploid wheat's from Abyssinia, Ethiopia. Later on, the purple seed color was successfully transferred into hexaploid wheat (Cop, 1965).

Anthocyanins are chemical compounds of plant origin as well known as pigments responsible for blue, purple, red, or orange coloration of plant tissues and organs. From the structural point of view anthocyanins are glycosides composed of hydroxyl or methoxyl 2-phenylbenzopyrilium skeleton with hydroxyl and methoxy groups in the B-ring. The structural variation extends bounded sugars (the most frequent is pentoses—xylose, arabinose, rhamnose, fructose and hexoses—galactose, glucose), aliphatic, and aromatic acids. They are beneficial for human health and fitness, possessing several unique traits and functions in the organism, including anti-obesity effects and influencing of brain activities (Prior & Wu, 2006). Anthocyanins are competent for effective elimination of oxidative stress in the human body by balancing between oxidants and antioxidants (Temple, 2000).

The most common disadvantage of anthocyanins in food processing is the sensitivity to different factors such as temperature (Ibanoğlu, 2002), light intensity, storage conditions, pH, metal ions, enzymes, oxygen, sulfur dioxide, ascorbic acid, sugars, and co-pigments. Nevertheless, their color stability can be improved by acylation (Bąkowska-Barczak, 2005).

Seeds of cereals are not typical sources of anthocyanins nevertheless blue, red, and purple seeds attract consumers, food producers, and plant breeders (Guo, Zhang, Xu, & Guo, 2013), therefore, relevant knowledge has been cumulated also in maize, wheat, barley, oat, and rice as the most important world food sources. Colored-grain wheat varieties with good genetic stability, excellent stress resistance and high yield are still required (Guo et al., 2013).

The objective of the study was to gather valuable information about proximate components in the unconventional Abyssinian purple wheat, which would be potential candidates for functional foods, as well as to enrich wheat products (bread) with biologically active substances (Figure 1).
2. Materials and methods

2.1. Sample collection and flour preparation

The purple wheat sample grain was purchased from the Maichew market in Southern highland of Tigray, Ethiopia. The samples were cleaned of foreign materials and sieved through 180 μm to remove small grain size and placed in labeled, dry plastic bags and transported to a laboratory of the Department of Food Science and Processing Technology, Haramaya University for analysis. The whole grain flour was milled using pestle and mortar for powder preparation and sieved through 75 μm sieve. The flours were packaged in airtight plastic containers prior to analyses.

2.2. Proximate composition

Proximate parameters (carbohydrate, fats, protein and ash) of the purple wheat were determined using the Association of Official Analytical Chemists (AOAC, 2000) method. The nitrogen content of the samples was determined by micro-Kjeldhal method. The nitrogen value obtained was multiplied by 6.25 to convert it to crude protein. The weight difference methods were used to determine moisture and ash content levels while crude fat of the purple wheat was determined using the AOAC procedure with petroleum ether as solvent. The carbohydrate content was determined by calculation using the different method:

\[
\% \text{Total Carbohydrate} = [100 - \%(\text{Protein} + \text{Fat} + \text{Moisture} + \text{Ash} + \text{Fiber})]
\]

\[
\% \text{Utilized Carbohydrate} = (100 - \%(\text{Moisture} + \text{Crude protein} + \text{Crude fat} + \text{Ash} + \text{Crude Fiber})).
\]

The various proximate parameters were all reported in percentage (AOAC, 2000).

2.3. Gross energy value

The gross energy values (Kcal/100 g samples) of the purple wheat were estimated using the factors for protein (4Kcal/g), fat (9 Kcal/g) and carbohydrate (4 Kcal/g). The equation is

\[
\text{Food energy} = (\% \text{Crude protein} \times 4) + (\% \text{Fat content} \times 9) + (\% \text{Carbohydrate} \times 4)
\]

2.4. Wet and dry gluten determination

The wet gluten content of flour was measured by hand washing using dilute salt (2% NaCl) solution as described in AACC (2000) Method 38-12.01. Wheat flour (3 g) was placed in a porcelain cup and sufficient distilled water was added starting from 2 mL to form the firm ball dough and the dough left to stand at 25°C for 30 min before washing. The dough was kneaded gently in stream of washing water over nylon cloth until starch and all other soluble matter was removed. Washing continues until 2 to 3 drops of wash water obtained by squeezing from gluten mass made no white cloud in clean water contained in clean beaker. The gluten obtained was left to stay in washing water for
about 1 h and pressed as dry as possible between the hands and then roll it into a ball, place it in, a weighed flat bottom weighing dish and the net mass was recorded as wet gluten. The dry gluten content was determined by drying the wet gluten obtained according to AACC (2000) Method 38-12.01. The mass of dry gluten had been taken after drying as wet gluten for 24 h at 100°C.

2.5. Determination of bioactive compounds of purple wheat

2.5.1. Determination of condensed tannin content
The condensed tannin content of the sample was determined according to the modified Vanillin-HCl methanol method as described by Price, Hagerman, and Butler (1980). The Vanillin-HCl reagent was prepared by mixing equal volumes of 8% concentrated HCl in methanol and 1% Vanillin in methanol. The solution of the reagent was mixed just prior to use. About 0.2 g of the ground sample placed in a small conical flask. Then 10 mL of 1% HCl in methanol was added. The conical flask was capped and continuously shaken for 20 min and the content, then centrifuged at 2,500 rpm for 5 min. About 1.0 mL of the supernatant was pipetted into a test tube containing 5 mL of Vanillin-HCl reagent. Absorbance at 450 nm was read on a spectrophotometer after 20 min incubation at 30°C, a blank sample was carried out with each run of the sample. A standard curve was prepared expressing the result as catechin equivalent, i.e. catechin (mg/mL). Tannin content was expressed as catechin equivalent as follows:

\[
Tannin(\%) = \frac{(C \times 10) \times 100}{200}
\]

where \(C\) = Concentration of corresponding to the optical density, 10 = volumes of the extract (mL) and 200 = sample weight.

2.5.2. Determination of total phenol content
The total phenolic content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu reagent according to Singleton, Orthofer, and Lamuela-Raventos (1999). The reaction mixture contained 0.1 mL acidified MeOH extract, oxidized by 0.5 mL Folin-Ciocalteu reagent (1:10 Folin-Ciocalteu:H₂O) and 0.8 mL 7.5% Na₂CO₃. The latter was added 2 min after the extract and the Folin-Ciocalteu reagent were mixed. The blank sample was prepared simultaneously with 0.1 mL H₂O instead of extract. The mixture was heated in a water bath at 50°C for 5 min and cooled to ambient temperature before measuring the absorbance at 760 nm in a type U-1100 spectrophotometer (Hitachi, Tokyo, Japan).

Two readings were made for each extract and the results were expressed as mg Gallic acid equivalents per 100 g dry matter using the respective calibration curve.

2.5.3. Determination of total anthocyanin content
The total anthocyanin content (TAC) was determined following Abdel-Aal and Hucl (1999). The acidified MeOH extract was filled with cavities of 1 cm thickness and measured at 535 nm in a type U-1100 spectrophotometer (Hitachi, Tokyo, Japan). The reading was first adjusted to zero with an empty microcuvette and afterwards by a cuvette with acidified MeOH solely. The results were calculated using the calibration curve and expressed as mg cyanidin-3-glucoside equivalents per kg dry matter (ppm). The optical density of the extract solution was measured at 535 nm. The total anthocyanin concentrations calculated by using the extinction coefficient.

\[
E_{1cm}^{1%} = 98.2 \text{ at } 535 \text{ nm}
\]

3. Data analysis
Descriptive and explanatory statistical data analysis was carried out.
4. Results and discussion

4.1. Proximate composition

The moisture content in this study had found 10.76%, which is more than 10% not acceptable limit for long term storage of flour Adom, Sorrells, and Liu (2005). Moisture content of foods is influenced by type, variety and storage condition. The low moisture content of wheat flour would enhance its storage stability by avoiding mold growth, biochemical reactions Adom et al. (2005) and extend the shelf life of the final product.

The crude protein content of the flour sample shows 8.53% (Table 1). The protein content of wheat flour reported in this study was found to be lower than the protein content of the wheat grains, which may vary between 10 and 18%, according to the report of Sramkova, Gregova, and Sturdik (2009) and the protein content of bread wheat flour 9.9% and also lower than wet and dry gluten content of bread wheat flours 25.0 and 8.9%. The crude protein content differences can be attributed to the geographical location of soil fertility. Since soils with low nitrogen levels can influence protein levels reported by Blumenthal, Baltensperger, Cassman, Mason, and Pavlista (2008). The protein content of the flours in this study suggests that it may be useful in food formulation systems, especially with higher protein content of legume crops. The purple wheat flours also had lower wet and dry gluten content than did the bread wheat flours. The purple wheat flour to be used for bread baking demands extra treatment, additional ingredients and different time of fermentation due to lower content of protein as well as gluten content.

The crude fat content of purple wheat flour had found 3.03. The fat content of the purple wheat flour in this study found higher than 1.8% reported in refined wheat flour (Anonymous, 2008). The differences in fat content may be due to location and varietal differences (Moss, Gore, & Murray, 1999). Diets with high fat content contribute significantly to the energy requirement for humans. The high fat content of purple wheat flour in this study would make it a better source of fat than the other refined wheat flour. High fat flours are also good for flavor enhancers and useful in improving palatability of foods in which it is incorporated (Aiyesanmi & Oguntokun, 1996).

The ash content of the purple wheat flours exhibited 5.5%, which shows in (Table 1). The ash content of purple wheat flours in this study was higher than the ash content of 1.40% refined wheat flour (Leach, McCowen, & Scotch, 1959). The differences in ash content may be due to differences in soil characteristics, climate conditions, genetic variations and milling process which was removing off bran with the refined wheat flour. Ash content is an indication of mineral content of a food. This therefore suggests that purple wheat flour could be important sources of minerals than the common refine wheat flours.

| Table 1. Proximate composition and bioactive compounds of Abyssinian purple wheat flour |
|--------------------------------------------------|
| **Mean results of proximate components of purple wheat** |
| Code | % Moisture | % Crude fiber | % Ash | % Crude protein | % Crude fat | Carbohydrate | Energy, Kcal/g |
|------|------------|---------------|-------|----------------|-------------|--------------|----------------|
| PW   | 10.76      | 7.33          | 5.5   | 8.53           | 3.03        | 64.85/72.2   | 320.77         |
| **Mean results of Anthocyanin, gluten and anti-nutritional factor contents of purple wheat, catechin Eq mg/100 g and %, respectively** |
| Code | Total phenols content | Total con. tannin | Total anthocyanin content | Gluten content |
|------|------------------------|-------------------|--------------------------|----------------|
| PW   | 25.3                   | 25.6              | 197.4                    | Wet (%) 16.7   |
|      |                        |                   |                          | Dry (%) 5.7    |

Note: Proximate and bioactive components reported as the mean of three triplicate, PW is purple wheat flour.
The crude fiber content of brown rice flour 1.23% and refined wheat flour 0.85% reported by Leach et al. (1959) is lower than with the purple wheat flour found in this study. The crude fiber content varied from 6.5 to 8.5% recorded for purple wheat in this research was quite different to the 0.85% reported by Leach et al. (1959). Crude fiber helps in the prevention of heart diseases, colon cancer, diabetes, etc. Purple Wheat flour would be a better source of fiber content; since it had significantly higher crude fiber content as compared to refine wheat flour. Therefore, it will be useful if purple wheat is added to meal diet and used in food formulation to help relieve constipation.

The utilizable carbohydrate content of the purple wheat flour was obtained 72.2% with an exclusive difference of crude fiber content, total carbohydrate content, which it had an exclusive difference of fiber 7.33%. The total carbohydrate content of the purple wheat flour found in this study was 64.85% lower than that of 74.22% for wheat; reported by Ahmed, Lydia, and Campbell (2012). It can be observed that the purple wheat flours used for this study found a high carbohydrate content. Carbohydrates are good sources of energy and that a high concentration of this is desirable in breakfast meals and weaning food formulas. In this regard, therefore, the high carbohydrate content of the purple wheat flour would make it a good source of energy and use in breakfast formulations.

4.2. Chemical analysis of phytochemicals

The total phenol content (TPC) of the purple wheat flour was found 25.3 mg catechin eq/100 g. Black-grained wheat has been reported to have higher TPC than white varieties Li, Shan, Sun, and Corke (2005) and Zong et al. (2006) reported that blue, black-purple, and purple wheat grains have significantly higher antioxidant content than white or red wheat grains. In the present study, we found that purple wheat flour had higher total phenolic content.

The condensed tannin content (CTC) is another category of bioactive compounds as TPC, (Salar, Purewal, & Bhatti, 2016). CTC provides specific flavor to the natural extracts or food formulations prepared from tannin rich plants. Although, the amount of tannin content is lesser as compared to other bioactive components in plant extracts, but their presence in extracts could not be neglected. Whereas, in this study the maximum amount of extracted CTC was 25.6 mg catechin Equivalent (CE) per hundred grams. The results may differ depending on the type of plant, plant part, extraction temperature, extraction time and extraction phase used for recovery of bioactive compounds.

4.2.1. The total anthocyanin content (TAC)

The samples were milled into whole-meal flour using pestle and mortar miller and the anthocyanin was extracted according to Abdel-Aal and Hucl (1999) (Method 1) with no modifications. TAC in anthocyanin-colored purple wheat was found the mean value of 197.4 mg/100 g. The results of TAC in this investigation showed that higher extracted value of anthocynin content in which this higher value is due to the effect of the whole purple wheat flour and the purple color samples used in this investigation.

5. Conclusions

The most abundant bioactive compounds found in purple wheat are phenolic and anthocynin contents located in the outer layer of grain. Purple tetraploid wheat landraces are traditionally used for food and beverages in Ethiopia. Due to their antioxidant activity, anthocyanins contained plants have an increasing interest in nutritionists and food scientists. Anthocyanins in purple wheat grains are expressed in either the pericarp or aleuron layer. The consumption of whole purple wheat flour may be beneficial to human health.
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