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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 75µm 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/100g, 253 mg/100g and 197.4 mg/100g) 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.

Keywords: Purplewheat; anthocyanin; dry gluten; tannin; ash

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 et al., 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 and 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 (Sharma, 1959). Wittmack *et al.,* 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 (Copp, 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 hydroxyled or methoxyled 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 and 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 *et al*., 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.

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 flour 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 difference method

% Total Carbohydrate= [100−% (Protein+Fat+Moisture+Ash+Fiber)]

%utilized Carbohydrate= (100 -% (Moisture + Crude Protein + Crude Fat + Ash+Crude Fiber). The various proximate parameters were all reported in percentage (AOAC, 2000)

2.3. Grass 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 (4kcal/g). The equation is

Food energy= (% crude protein x 4) + (%fat content x 9) + (5carbohydrate x 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 No. 38-10. Wheat flour (3 g) was placed in a porcelain cup and sufficient distilled water was added starting from 2mL to form the firm ball dough and the dough left to stand at 25^{o}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 1hr 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 No. 38-10. The mass of dry gluten had been taken after drying as wet gluten for 24 hrs at 100^{o}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 *et al.*, 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 minutes and the content, then centrifuged at 2500 rpm for 5 minutes. About 1.0 ml of the supernatant was pippetted into a test tube containing 5 ml of Vanillin-HCl reagent. Absorbance at 450nm was read on a spectrophotometer after 20 minutes 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:

$$\text{Tannin}(\%) = \frac{(\text{C} \times 10) \times 100}{200}$$

Where: C= Concentration of corresponding to the optical density

10 = volumes of the extract (ml)

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 *et al.,* 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 760nm 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 535nm. The total anthocyanin concentrations calculated after Francis (1982) using the extinction coefficient. $\left(E_{1\text{cm}}^{1\%} = 98.2 \text{ at } 535 \text{ nm}\right)$

1. Data Analysis

Descriptive and explanatory statistical data analysis was carried out.

3. Results and Discussion

3.1. Proximate Composition

The moisture content of purple wheat flour was lower than 13.3% for wheat flour as reported by Dwyer, 1995. The moisture content in this study found 10.76%, which is more than 10% not acceptable limit for long term storage of flour Adom *et al*., 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%-18%, Sramkova *et al*., 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 (Blumenthal *et al*., 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%). Singleton *et al*., 1999 had reported 2.80% and 1.80% fat content in brown rice and refined wheat flours, respectively. The fat content of purple wheat flour from 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 *et al*., 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 and 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 (1.40%) of refined wheat flour Leach *et a*l., 1959. The differences in ash content may be due to the 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.

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 a*l., 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 and Lydia, 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.

3.2. Chemical Analysis of Phytochemicals

The total phenol content (TPC) of the purple wheat flour was found 25.3mg catchin eq/100g. Black-grained wheat has been reported to have higher TPC than white varieties Li et al., 2005. 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, 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 catchin 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.

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/100g. 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 milling and extraction method.

5. Conclusions

Purple wheat grain is reaching source of bioactive compounds with potential health benefits, but the nutritional properties will only be fully exploited if whole-wheat products are available. The most abundant bioactive compounds found in purple wheat are phenolic and anthocynin contents located in the outer layer of grain. Most of the phenolic compounds in bran bind to carbohydrates, and can survive gastrointestinal digestion reaching the colon intact, where they provide an antioxidant environment. Purple tetraploid wheat landraces are traditionally used for food and beverages in Ethiopia (Geleta et al. 2009). However, the purple wheat flour protein and dry gluten content were exhibited the lowest value as compared to durum wheat, which may be an alternative diet for those with the gluten allergies and diabetic victims.

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8. Competing Interests

The authors declare no competing interest.

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ABOUT THE AUTHORS

Hagos Hailu Kasegn 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 anthocyanins and other nutrients. The presence of bioactive properties 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 rich in anthocyanin content, can act as an antioxidant and show antibacterial and anti-cancer activity. It is also useful in curing systemic inflammation. In addition, one of the active ingredients of anthocyanin can inhibit the oxidation of low-density lipoprotein 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.

Figure 1. Uncleaned Abyssinian purple wheat at market with farmer ready for sale

Photo credit of Hagos Hailu

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/100g and %, respectively.

Code	Total Phenols content	Total con. Tannin	Total Anthocyanin content	Gluten content	
				Wet (%)	Dry (%)
PW	25.3	25.6	197.4	16.7	5.7

Note: proximate and bioactive components reported as the mean of three triplicate, PW is purple wheat flour

Author's photo

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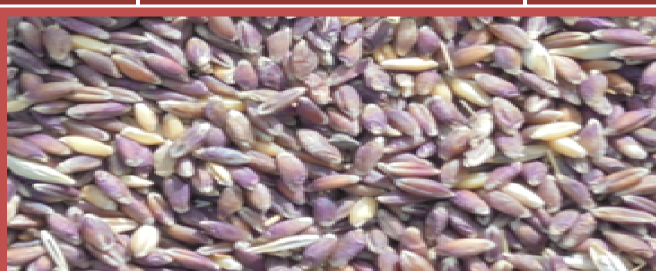

Abyssinian Purple Wheat(photo credity of Hagos Hailu)