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Changes in Polyphenol Content of Newly Released Varieties of Wheat during Different Processing Methods

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Abstract

Background: Wheat *(Triticum aestivum)* is the most important food crop in India and most important cereal grain. It is widely consumed in northern India. It is the leading source of all the macronutrients like proteins (especially vegetable protein), energy and carbohydrates and micronutrients like minerals (iron, calcium, zinc, potassium etc.) and B vitamins. It is currently second to rice in terms of total production (tonnes) as the main human food crop. The wheat flour besides various nutrients also contains anti-nutrients like phytates, oxalates, polyphenols, tannins etc. Polyphenol are the phytochemicals i.e., compounds which are found abundantly in natural plant food sources that have antioxidant properties. These phytochemicals are found in many types of plant foods, such as grains like wheat, pearl millet, maize etc., legumes like peanuts and soybeans, nuts, and seeds. There are various factors which affect the polyphenol content of plants and these include environmental factors, degree of ripeness at the time of harvesting, processing and storage. Polyphenolic content of the foods are greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall etc.

Methods: The treatments like soaking, roasting, sprouting and malting reduce the polyphenolic content in wheat varieties. Two newly released varieties of wheat (WH-1080 and WH-1025) and one conventional wheat variety C-306 were collected from the Department of Genetics and Plant Breeding, College of Agriculture, CCS HAU, Hisar in a single lot.

All the three wheat varieties obtained were cleaned, washed well under running tap water to get rid of all the dust, soil particles and foreign matter adhered to the grains, dried and ground to flour using conventional flour mill and processed in three different ways roasting, sprouting and malting and then used for nutritional evaluation of raw and processed wheat varieties and interpretation.

Results: Polyphenol content in wheat varieties which were unprocessed ranged from 234.15-338.4 mg/100 g (on dry weight basis) and in processed wheat varieties from 218.98-338.4 mg/100 g. Processing treatments showed significant decrease in the polyphenol levels. Lowest polyphenol content was found in sprouted WH-1080 variety 218.98 mg/100 g. Sprouting significantly ($P<0.05$) reduced the polyphenol content as compared to malting and roasting.

Conclusion: Sprouting treatment was found to be most advantageous in reducing the polyphenol content in all the wheat varieties when compared to other treatments like roasting and malting.

Keywords: Anti-nutrients; Polyphenols; Malting; Sprouting; Roasting; Phytochemicals

Introduction

Wheat (*Triticum aestivum*) is the most important staple form of food crop which is rich in macronutrients and micronutrients. It is especially rich in proteins especially vegetable proteins, energy, carbohydrates, and vitamins and minerals like calcium, phosphorus, magnesium, zinc, iron etc., thus making it a wholesome crop. India is the fourth largest producer of wheat in the world after Russia, the USA and China and accounts for 8.7% of the world's total wheat production. Uttar Pradesh, Punjab and Haryana are the three prominent wheat producing states. These states account for about 60% of the wheat area and produce about three-fourths of the total wheat production in India

Wheat is one of the least expensive cereal grains available amongst rice, pearl millet, maize flour etc., in fabricated form which rich in nutrition as it contains a variety of minerals like iron, calcium, phosphorous, zinc, potassium, magnesium etc. [1,2], vitamins like thiamine [3], and fatty acids like stearic acid, myristic acid, palmitic acid etc. The nutritional content of wheat on an average are energy 320-350 kcal, protein 10-13%, carbohydrate 74-78%, lipids 1-3% and considerable amounts of vitamins i.e., thiamine 0.3-0.5 mg and riboflavin 0.2-0.45 mg and minerals mg, iron 2.8-3.5 mg, zinc 2.0-3.43 and magnesium 110-130 mg/100 g, on dry weight basis. The wheat flour has various anti-nutrients like phytates, oxalates, polyphenols, tannins etc. [4,5]. The most important anti-nutrients found out of these are phytic acid and polyphenols. Polyphenol are the phytochemicals meaning compound found abundantly in natural plant food sources that have antioxidant properties. These are found in many types of

plant foods, such as grains (wheat, maize, pearl millet etc.), legumes (including peanuts and soybeans), nuts, and seeds. There are various factors which affect the polyphenol content of plants and these include environmental factors, degree of ripeness at the time of harvesting, processing and storage. Polyphenolic content of the foods are greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall etc.

These anti-nutrients like phytates, polyphenols, tannins, oxalates are reduced by some cooking treatments which are boiling, sprouting, fermentation, roasting, malting etc. [6,7]. The above mentioned antinutrients are reduced considerably and thus the products which are consumed have low levels of these anti-nutrients as the flour has been processed to make the products organoleptically acceptable.

Review of Literatures

Hooda [8] analysed the anti-nutritional factors in wheat and found that the polyphenol concentration as 355.11 mg/100 g (dry weight basis) in wheat flour samples. Sangwan and Dahiya [9] also evaluated the wheat grains for polyphenol content in and found that the polyphenol concentration is 320.4 mg/100 g (dry weight basis). Varsha [10] also evaluated polyphenol content in wheat flour samples and found that it was 465 mg/100 g. Sikandra [1] in 2005 also evaluated the polyphenolic content in wheat flour samples and the result obtained was 275-342 mg/100 g concentration of polyphenol. Ragee and Kiefer [11] studied the phenolic composition of cereals for food use and found that Mg content in refined flours, Polyphenols was found to be 562 µg/g in hard wheat flour (bread flour) which was more than soft wheat flour about 501 µg/g. Manu [12] in 2006 also evaluated the polyphenol content in whole wheat flour and compared it to the composite wheat flour with supplementation and found that it was 299.20 mg/100 g of polyphenol in whole wheat flour. Singh [13] in the year 2006 also evaluated the polyphenols levels and the result showed that the polyphenol content was 298.39 mg/100 g of wheat flour. Nikita and the group in 2008 analyzed the proximate composition of five wheat varieties which were grown under both organic and inorganic farming conditions and found that on average, the polyphenol content of inorganic wheat was significantly higher than that of organic wheat varieties Among the five varieties a significant variation (275-342 mg) in polyphenol contents was observed; the C-306 and WH-711 varieties had the maximum amount of polyphenols. Rakhi and Punia [14,15] in 2013 while evaluating polyphenol content in wheat varieties found that polyphenol content of unprocessed wheat varieties ranged from 487.16 mg/100 g and 502.33 mg/100 g which decreased to 335.14-385.51 mg/100 g after germination.

Materials and Methods

The investigation or the experiment was conducted in the Department of Foods and Nutrition, I.C. College of Home Science, CCS Haryana Agricultural University, and Hisar. Two newly released varieties of wheat (WH-1080 and WH-1025) and one conventional variety of wheat C-306 were procured from the Department of Genetics and Plant Breeding, College of Agriculture, CCS HAU, Hisar in a single lot.

This section of materials and the methods will provide relevant information related to the research design and the methodologies used for the present investigation. All the research procedures have been clearly described below under the following headings and subheadings: 1) Procurement of material from the store; 2) Processing of raw wheat varieties: Roasting, Sprouting, and Malting; 3) Estimation of polyphenol content in the raw & processed wheat varieties.

Procurement of material from the store

Both the newly released wheat varieties (WH 1025, WH 1080) and one conventional variety C-306 was obtained from the Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, and Hisar in a single lot. All the wheat varieties were first cleaned, washed thoroughly under running tap water so as to get rid of all the dust, soil particles and foreign matter adhered to the grains and then dried & ground to flour using flour mill which was available in the departmental laboratory and then the flour was used for nutritional evaluation of raw and processed wheat varieties and inference.

Processing of raw wheat varieties

Roasting: The wheat grains of all the three varieties which were procured were washed to remove dust and other foreign particles and then were dried under sun to remove excess water and then again dried in hot air oven at the temperature of 50°C for 24 hrs.

Sprouting: Wheat grains of all the varieties after washing and cleaning remove soil and dust were soaked for 12 hrs and after that they were kept in petri dishes or containers which were lined with wet filter paper to promote germination/sprouting and then kept in an incubator at 30°C for 48 hrs. All the grains were kept moist by sprinkling distilled water frequently. After 48 hrs sprouting occurs and the sprouts can be seen emerging out.

Malting: Malting process was done by the standard and approved method of Chaturvedi and Sarojini [16]. There were altogether four major stages involved. These were:

- **Steeping:** This process involves soaking of wheat grains in excess water for rehydration and to facilitate germination in the grains. All the three wheat varieties were soaked in germinating dish by using double the amount of 0.1% formaldehyde solution (to avoid microbial degradation). The mouth of the dish was covered with a cloth preferably muslin cloth by using rubber band and then the grains were allowed to soak for 6 hr at 25-30°C in an incubator. The un-imbibed water left was drained off without removing the cloth.
- **Aeration:** After steeping aeration was done by removing cloth for a period of 3 hrs in a well-ventilated room to enhance the enzyme activity. During aeration process, grains were rotated by glass rod occasionally. After aeration, grains were subjected to steeping with fresh formaldehyde solution for a period of 16 hrs and then the mouth of the dish was again covered. At the end of steeping period, extra water was drained off from the container and cloth was removed and the sample was air rested for a period of 2 hrs.
- **Germination:** The seeds which were soaked were sprayed with 25 ml of 0.1% formaldehyde solution to prevent the mold growth and then were allowed to germinate in an incubator at 25-30°C. The germinated grains were roasted twice daily. A constant humid atmosphere was maintained inside the incubator by means of keeping a pan of water. After 48 hrs and 72 hrs grain samples were removed from the incubator.
- **Kilning:** The grains which were germinated were dried in hot air oven at a temperature of 50°C for 24 hrs. Then the kilned grains were de vegetated by abrasive action. After kilning, grains which had characteristic malt aroma were obtained.

Polyphenol estimation

Total Polyphenols were extracted by the method of Singh and Jambunathan [17].

Defatted sample (500 mg) was refluxed with 50 ml methanol containing 1% HCL for four hours. The extract was concentrated by evaporating on a hot water bath and brought its volume to 25 ml with methanolic–HCL. The amount of polyphenolic compounds was estimated as tannic acid equivalent according to Folin-Davis procedure.

Reagents required

Folin-denis reagent preparation: To 750 ml water, 100 g sodium tungstate, 20 g phosphomolybdic acid and 50 ml phosphoric acid were added and heated and then refluxed for two hours.

Tannic acid (Stock solution): 100 mg of tannic acid was weighed and then dissolved in distilled water and the volume was made up to one liter. In order to use a working standard solution, 20 ml stock solution was diluted to 100 ml with water and the aliquot was made.

Saturated aqueous sodium carbonate solution: Sodium carbonate of amount 350 g was dissolved in one litre of hot distilled water at the temperature of 70-80°C, cooled and filtered through glass wool.

Procedure: In a graduated test tube add 1.5 ml of test solution/ sample and 8.5 ml of distilled water. After thorough mixing, add Folin–Denis reagent and the tubes were well shaken. Exactly after 3 minutes, one ml of saturated sodium carbonate solution was added and the tubes were thoroughly shaken again. After an hour, the absorbance was read at 725 nm on UV-VIS Spectrophotometer -118 using a suitable blank. If the solution was cloudy or precipitates appeared, it was centrifuged before readings were taken. A standard curve was plotted by taking 0.5-4 ml working tannic acid standard solution containing 10-80 µg tannic acid.

Where,

M: Concentration of extract elute obtained from graph;

V: Volume made of Extract (ml);

W: Weight (g) of sample;

V1: Volume of extract aliquot taken (ml).

Results

The analysis was conducted with the objective of assessing the nutrient composition of the two newly released wheat varieties viz. WH-1080 & WH-1025 and one conventional variety i.e., C-306.

Various treatments like roasting, sprouting & malting were used to check the levels of polyphenol. The results obtained during the analysis or experiment was subjected to suitable statistical analysis, tabulated and have been presented systematically in the Table 1.

The polyphenol content of all the three wheat varieties ranged from 307.56-338.4 mg/100 g. The newly released varieties WH-1080 and WH-1025 did not differ significantly (P<0.05) among themselves and thus have similar polyphenol content (307.56 mg/100 g and 311.4 mg/100 g respectively).

The variety C-306 had significantly (P<0.05) higher polyphenol content (338.4 mg/100 g) as compared to the newly released varieties.

Varieties	Polyphenols
WH-1080	307.56+1.8
WH 1025	311.4+1.7
C 306	338.4+2.3
C.D (P<0.05)	4.03
Values represented are mean ± SE of three independent observations.	

Table 1: Polyphenol content of varieties of wheat (mg/100 g, on dry weight basis).

Variety Treatment	WH-1080	WH 1025	C-306	Mean of varieties
Unprocessed (Control)	307.56+1.8	311.4+1.7	338.4+2.3	304.84+0.23
Roasted	267.12+0.15	263.11+1.1	275.85+0.5	249.56 +11.89
Sprouted	218.98+1.1	241.42+2.3	229.51+1.7	294.58 +4.53
Malted	251.48+2.3	245.67+2.3	242.82+1.1	284.64 +4.64
Treatment Mean	261.28+0.21	267.15 +0.16	271.64 +0.29	-
CD(P<0.05)	Variety: 2.77 Treatment: 2.58 Interaction: 5.13			
Values represented are mean ± SE of three independent observations.				

Table 2: Effect of processing methods on polyphenol content of varieties of wheat (mg/100 g, as on dry weight basis).

Polyphenol content in wheat varieties which were not processed ranged from 307.56-338.4 mg/100 g and in processed wheat varieties from 218.98-275.85 mg/100 g. The processing methods resulted in significant decrease in polyphenol content. Highest reduction was observed in sprouting method (22.47-32.17%) and the decrease was significantly (P<0.05) higher than the other two treatments like roasting (13.14-18.48%) and malting (18.23-28.24%). Lowest polyphenol content was observed in sprouted WH-1080 variety (218.98 mg/100 g) (Table 2).

Discussion

The experiment was conducted to evaluate two newly released varieties of wheat namely WH-1080, WH-1025 and one conventional (already checked) wheat variety C-306 for their physico-chemical properties and nutritional composition. All the three wheat varieties were procured from the Department of Genetics and Plant Breeding, College of Agriculture, CCS HAU, Hisar in a single lot. The next step was cleaning of the wheat varieties and it was done manually to remove broken seeds, dust and other foreign matter and then washed under running water which was then dried & ground to flour using flour mill. All the three wheat varieties were processed using treatments like roasting, sprouting and malting using the standard procedures. The variety WH-1080 was found to have the lowest polyphenol content (307.56 mg/100 g) and variety C-306 had highest polyphenol content (338.4 mg/100 g). Many factors such as genetics, type of soils, environmental fluctuations, and year and fertilizer

application influence the anti-nutrient composition of cereal grains as shown by the evidences [18].

Sprouting treatment significantly decreased polyphenol content among all the three wheat varieties when compared to the other two processing methods like roasting and malting. A significant difference in all the varieties was found to exist. Rakhi and Punia [15] found that there was reduction of polyphenol content after germination/sprouting and heat treatment. As polyphenols are present in the periphery of the seed there is possibility of their passing out into soaking water through seed coat. The reduction of polyphenolic compounds (tannins) after heat treatment (blanching) may be due to fact that these compounds, in addition to their predominance in seed coats are water soluble and consequently leach into the liquid medium [19]. This decrease could also be associated to that fact that these anti-nutrients are not heat stable and usually degrade upon heat treatment [20].

Summary and Conclusions

The varietyWH-1080 had lowest polyphenol content (307.56 mg/100 g) and variety C-306 had highest polyphenol content (338.4 mg/100 g). There existed significant difference in all the varieties. Sprouting significantly ($P<0.05$) reduced the anti-nutrient as compared to malting and roasting. A reduction of 22.47-32.17% of polyphenols levels during sprouting was observed. Thus method that was found to be most advantageous in reducing anti-nutrient (polyphenol) of all the processing methods in all the wheat varieties is sprouting.

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