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Changes in Phytate Content of Newly Released wheat Varieties during Different Processing Methods

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Abstract: ***Wheat (Triticumaestivum) is the most important staple food crop and occupies a unique position for more than one third of the world population. Globally, wheat is the leading source of vegetable protein in human food, having a higher protein content than other major cereals, maize (corn) or rice.In terms of total production tonnages used for food, it is currently second to rice as the main human food crop and contributes more calories and proteins to the world diet than any other cereal crops. Wheat is nutritious and provides more nourishment for humans than any other food source. The wheat flour contains various anti-nutrients like phytates, oxalates, polyphenols etc.,.Phytic acid (also known as phytate) is a substance (anti nutrient) found in many types of plant foods, such as grains (wheat, maize, bajra etc.,), legumes (including peanuts and soybeans), nuts, and seeds. It is found thatphytic acid is found predominantly (about 80% of it) in the bran, or outermost shell, of whole grains. The various processing methods like soaking, roasting, sprouting and malting reduces the phytic content in wheat. 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. All the wheat varieties were cleaned, washed well under running tap water to get rid of all the dust, soil particles and foreign matter, dried & ground to flour using aatachakki and processed in three different ways roasting, sprouting and malting and were used for nutritional evaluation of raw and processed wheat varieties and inference. Phytic acid content in unprocessed wheat varieties ranged from 234.15 mg/100g to 253.9 mg/100g and in processed wheat varieties from 167.18 mg/100g to 241.63 mg/100g. Processing treatments resulted in significant decrease in the phytic acid levels. Lowest phytic acid content was found in sprouted WH-1080 variety (167.18 mg/100g).Sprouting significantly ($P \leq 0.05$) reduced the anti nutrient as compared to malting and roasting. There was reduction of 19.21% - 28.6% of phytic acid levels during sprouting. Thus sprouting method was found to be most beneficial in reducing anti nutrient (phytic) of all the wheat varieties.***

Keywords: anti-nutrients, phytic acid, malting, sprouting

1. Introduction

Wheat (*Triticumaestivum)* is the most important staple food nourishment crop and occupies a unique position for more than one third of the world population. It is nutritious and provides more for humans than any other food source. It is one of the least expensive cereals available in fabricated form rich in nutrition. It also contains a diversity of minerals like calcium, iron, zinc, phosphorous, magnesium , potassium etc., (Sikandra 2005, Hussain 2010) vitamins like thiamine (Yadav*et al.*, 2011) and fatty acids like stearic acid, myristic acid, palmitic acid etc., . On an average, wheat contains energy 320 – 350 kcal, carbohydrate 74-78%, protein 10-13%, lipids 1-3% and considerable proportions of vitamins i.e., thiamine 0.3-0.5 mg and riboflavin 0.2-0.45 mg and minerals zinc 2.0- 3.43 mg, iron 2.8- 3.5mg and magnesium 110-130 mg/100g.The wheat flour contains various anti-nutrients like phytates, oxalates, polyphenols etc., (Qazi 2003,,Mallick 2013) the most important out of these are phytic acid. Phytic acid (also known as phytate) is a substance (anti nutrient) found in many types of plant foods, such as grains (wheat, maize, bajra etc.,), legumes (including peanuts and soybeans), nuts, and seeds. It's the storage form of phosphorus, an important mineral constituent which is used in the production of energy as well as the formation of structural elements like cell membranes.It is found thatphytic acid is found predominantly (about 80% of it) in the bran, or outermost shell, of whole grains.

These anti-nutrients are reduced by some cooking treatments like boiling, sprouting, fermentation, roasting, malting etc.,**(**Hemlatha*et al*., 2007, Priyanka*et al*.,2011) These anti-nutrients are reduced to a considerable level and thus the products which are consumed have low levels of these anti-nutrients as the flour have been processed to make the products organoleptically.

2. Review of Literature

Hooda (2002) evaluated the anti-nutritional factors in wheat and found that phytic acid concentration. Qazi*et al*. (2003) studied two wheat varieties and found phytic acid content as 869.2 and 869.4 mg/100 g which reduced upto 280.3 mg/100g after formation of unleavened bread. . Sikandra(2005) also evaluated phytic acid content in wheat flour sample and the result showed that it was 212.67mg/100g to 297.33mg/100g. Manu (2006**)** also studied the phytic acid content in whole wheat flour compared to composite flour with supplementation and found that it was 214.33 mg/100g of whole wheat flour. Singh (2006) also studied the phytic acid levels and the result was 232.53mg/100g & of wheat flour.Nikita *et al*., (2008) analyzed the proximate composition of five wheat varieties grown under organic and inorganic farming conditions and found that the C-306 variety had the lowest amount of phytic acid (240 mg/100 g)while WH- 912 had the highest (269 mg/100 g) grown under inorganic conditions. Rakhi and

Punia, (2013**)** found the phytic acid content of wheat varieties from 206.71 to 240.10 mg/100g.

Rakhi and Punia (2013) found that Phytic acid content of unprocessed wheat varieties ranged from 206.71 to 240.10 mg/100g which after germination reduced to 129.40 to 160.36 mg/100g.

3. Materials and Methods

The present study was carried out in the Department of Foods and Nutrition, CCS Haryana Agricultural University, 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 chapter contains relevant information related to the research design and methodologies used for the present investigation. The research procedures have been clearly described under the following headings and subheadings:

3.1 Procurement of material
3.2 Processing of varieties
  3.2.1 Roasting
  3.2.2 Sprouting
  3.2.3 Malting
3.3 Phytic Acid estimation of raw & processed wheat varieties

3.1 Procurement of Material

Newly Released Wheat varieties (WH 1025, WH 1080) and one conventional varietyC-306 were procured from the Department of Genetics &Plant Breeding, CCS Haryana Agricultural University, Hisar in a single lot. All the wheat varieties were cleaned, washed well under running tap water to get rid of all the dust,soil particles and foreign matter, dried & ground to flour using *aatachakki*available in the departmental laboratory and was used for nutritional evaluation of raw and processed wheat varieties and inference.

3.2 Processing of Varieties

3.2.1 Roasting
The procured wheat grains were washed to remove dust and other foreign particles and was dried under sun to remove excess water and then dried in hot air oven at 50^{o}C for 24 hrs.

3.2.2 Sprouting
Grains were washed to remove soil and dust and then soaked for 12 hrs and after that they were kept in petri dishes which were lined with wet filter paper for germination/sprouting in an incubator at 30^{o}C for 48 hrs. Grains were kept moist by sprinkling distilled water frequently. After 48 hours sprouts can be seen emerging out.

3.2.3Malting
Malting was done by the method of Chaturvedi and Sarojini (1996).There were four major stages involved. These were:

3.2.3.1 Steeping
This involved soaking of seeds in excess water for rehydration and to facilitate germination. 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 muslin cloth using rubber band and the grains were allowed to soak for 6 hrs at 25^{o}C – 30^{o}C in an incubator. The unimbibed water was drained off without removing the cloth.

3.2.3.2 Aeration
For enhancement of enzyme activity aeration was done by removing cloth for a period of 3 hrs in a well ventilated room. During aeration, grains were rotated occasionally by glass rod. After aeration, grains were re steeped with fresh formaldehyde solution for a period of 16 hrs and the mouth of the dish was again covered. At the end of steeping period, water was drained off, cloth was removed and the sample was air rested for a period of 2 hours.

3.2.3.3 Germination
The soaked seeds were sprayed with 25 ml of 0.1 % formaldehyde solution to prevent the mold growth and allowed to germinate in an incubator at 25^{o}C -30^{o}C.The grains were roasted twice daily. A constant humidity was maintained inside the incubator by means of keeping a pan of water. Grain samples were removed from the incubator after 48 and 72 hours.

3.2.3.4 Kilning
The germinated grains were dried in hot air oven at 50^{o}C for 24 hrs. Kilned grains were de vegetated by abrasive action. After kilning, grains with characteristic malt aroma were obtained.

3.3 Phytic acid estimation

Phytic acid content was determined by using the method of Davies and Reid (1979).

3.3.1 Reagents Required
1) **0.5 M HNO_3:** 15.96 ml of 69.5% HNO_3 was diluted to 500 ml with water.
2) **Ferric ammonium sulphate:** 2.5 mg ferric ammonium sulphate was dissolved in water, added a few drops of HCl and made volume to 500 ml with water.
3) **Ammonium thiocyanate:** Tengram of ammonium thiocyanate was dissolved in water and made to 100 ml.
4) **Iso-amyl alcohol**
5) **Sodium phytate:** 30.54 mg sodium phytate was dissolved (5.5% H_2O, 97% purity and containing 12 Na/mole) in 100 ml 0.5 M HNO_3 which gave a solution containing 20 mg phytic acid in 100 ml or 200 mgphytic acid/ml.

3.3.2 Extraction
Five hundred mg sample was extracted with 20 ml 0.5M HNO_3 for 3 h and continuous shaking on a shaker at room temperature. After proper shaking, it was filtered through

Whatman filter paper No. 1. Filtrate was used for the estimation of phytic acid.

3.3.3 Estimation Procedure

One ml HNO_3 extract was taken in a stoppered test tube and made a final volume of 1.4 ml with water. One ml ferric ammonium sulphate solution was added. The content was mixed in the tubes thoroughly and placed in a boiling water bath for 20 minutes. Tubes were cooled down to room temperature under running tap water. Five ml iso-amyl alcohol was added, mixed the contents vigorously and added 0.1 ml ammonium thiocyanate solution. The tubes were shaken well and centrifuged at 3000 rpm for 10 minutes. Color intensity in the alcohol was read at 465 nm against iso-amyl alcohol blank exactly after 15 minutes of addition of ammonium thiocyanate.

For plotting standard curve, 0.4-1.0 ml standard phytate solution containing 80-200 mg phytic acid was taken and made to 1.4 ml with water. 0.341 O.D. corresponded to 160 mg phytic acid.

4. Results

The present study was conducted to assess the nutrient composition of newly released wheat varieties *viz*. WH-1080 & WH-1025 and one conventional variety C 306. Various processing methods like roasting, sprouting & malting were used to check the levels of phytic acid. The results so obtained during the course of investigation were subjected to suitable statistical analysis, tabulated and have been presented systematically in the tables below.

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

Varieties	*Phytic Acid*
WH-1080	234.5 ± 1.14
WH 1025	238.06 ± 2.10
C 306	253.9± 1.18
C.D(P≤0.05)	3.9

Values are mean ± SE of three independent determinations

Table 2: Effect of processing on phytic acid content of wheat varieties (mg/100g , on dry weight basis)

Variety / Treatment	WH-1080	WH 1025	C 306	Variety Mean
Unprocessed (Control)	234.15 ± 1.14	238.06 ± 2.10	253.9 ± 1.18	222.9± 0.25
Roasted	223.72 ± 2.4	226.61 ± 3.4	241.63 ± 1.1	230.41±2.07
Sprouted	167.18 ± 1.1	183.48 ± 1.1	205.11 ± 0.5	215.86 ±0.98
Malted	222.98 ± 1.1	219.73 ± 0.6	229.44 ± 1.1	211.55 ±1.12
Treatment Mean	212.75 ±1.27	216.97 ±2.16	228.52 ±1.05	
CD(P≤0.05)	Variety : 2.87 Treatment : 2.79 Interaction :4.97 (Variety x Treatment)			

Values are mean ± SE of three independent determinations

Phytic acid content in unprocessed wheat varieties ranged from 234.15 mg/100g to 253.9 mg/100g and in processed wheat varieties from 167.18 mg/100g to 241.63 mg/100g. Processing treatments resulted in significant decrease in the phytic acid levels. Decrease in phytic acid levels were highest during sprouting treatment (19.21% - 28.6%) as compared to the roasting (4.45% to 4.83%) and malting treatment (4.77% to 9.63%). Lowest phytic acid content was found in sprouted WH-1080 variety (167.18 mg/100g).

5. Discussion

The present investigation was conducted to evaluate two newly released wheat varieties namely WH-1080, WH-1025 and one conventional wheat variety C-306 for their physico-chemical properties and nutritional composition. The wheat varieties were procured from were procured from the Department of Genetics and Plant Breeding, College of Agriculture, CCS HAU, Hisar in a single lot. Wheat grains were manually cleaned to remove broken seeds, dust and other foreign matter and washed under running water then dried & ground to flour using *Aatachakki*. The varieties were processed using roasting, sprouting and malting methods. The phytic acid content of wheat varieties ranged from 234.5 to 253.9 mg/100g. Wheat variety C-306 had highest (253.9 mg/100g) amount of phytic acid whereas variety WH-1080 had lowest amount (234.5 mg/100g). Evidence has shown that, there are many factors such as genetics, environmental fluctuations, type of soils, year and fertilizer application that can affect the antinutrient composition of cereal grains (Muahamad*et al*. 2010).

Sprouting treatment decreased phytic acid content. There existed significant difference in all the varieties. Sprouting significantly reduced the phytic acid content as compared to malting and roasting. Rakhi and Punia (2013) had results that there was reduction of phytic acid content after germination and heat treatment. The loss of phytic acid during sprouting may have been due to hydrolytic activity of the enzyme phytase, which is reported to be present in various plant foods. Sandberg (1991) and Agte and Joshi (1997) observed that food processing such as soaking and sprouting of whole cereals and legumes activate the endogenous plant phytases as well as bacterial phytases. These reduce the hexaform of phytic acid into lower forms having lower acids and B-complex vitamins. The results of present study showed that sprouting could significantly decrease phytic acid in wheat grain, which is in agreement with the reports about similar treatment on other cereals such as steeping and sprouting of oats or corn (Larsson and Sandberg, 1995; Fageer*et al*. 2004), sprouting and malting of pearl millet (Archana*et al*. 1998), soaking and sprouting of mungbean (Grewal*et al*; 2006, Khattab*et al*. 2009). The observed reduction in phytic acid content during heat treatments may be partly due to heat labile nature of phytic acid and formation of insoluble complexes between phytate and other components (Udensi*et al*. 2007). This decrease could also be related to that fact that these compounds are heat labile and degrade upon heat treatment (Rakic*et al*. 2007).

6. Summary & Conclusions

The phytic acid content of wheat varieties ranged from 234.5 to 253.9 mg/100g. Wheat variety C-306 had highest (253.9 mg/100g) amount of phytic acid whereas variety WH-1080 had lowest amount (234.5 mg/100g). There existed significant difference in all the varieties. Sprouting significantly ($P \leq 0.05$) reduced the antinutrient as compared to malting and roasting. There was reduction of 19.21% - 28.6% of phytic acid levels during sprouting. Thus sprouting method was found to be most beneficial in reducing antinutrient (phytic) of all the wheat varieties.

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