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Optimization of fat extraction technique in khorasan wheat using different solvent system

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Abstract

Wheat (*Triticum aestivum* L.) is one of the leading cereal grains produced and consumed worldwide. Khorasan wheat is an inimitable among all the cereal grains grown in many parts of the world as a staple food. The aim of this study was to find out a suitable method for total fat extraction in khorasan wheat by using different combination of solvents. Different set of experiments namely E_1, E_2, E_3 and E_4 for fat extraction from khorasan wheat were designed. The results of our study clearly indicated that among all the tested methods, E_4 showed highest extracted fat content from khorasan wheat (2.59%). While fat content from E_1, E_2, E_3 were found to be 2.09, 1.65 and 1.87 % respectively. Additionally in khorasan wheat, protein content was found to be 14.07 %, while micronutrients (phosphorous, potassium and vitamin-B 3) were found to be 359 mg/100gm, 410 mg/100gm and 5.88 mg/100gm respectively. The present investigation revealed that concentration of crude protein, crude fiber, Vitamins (vitamin-B3) and minerals (Phosphorous and Potassium) were higher in Khorasan than the other wheat, and it must be utilized in diet to combat nutritional imbalance and mineral deficiency.

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Introduction

Khorasan wheat is a unique cereal grain grown in many parts of the world as a staple food. Wheat is the basis for numerous food products and is one of the most important sources of energy for living population. Worldwide, people eat wheat products more than any other kind of food. The wheat products strongly influence nutritional balance, because they contain all the necessary nutrients including proteins, fats and vitamins (Morris and Bryce, 2000). Wheat was one of the first cereal grains to be cultivated and spread to all parts of the world because of its good adaptability to various climates and soils (Kent and Ever, 1994). Studies involving dietary fat in different cereals are of immense importance, because consumers, industrial food processors and governmental agencies have a strong interest in these studies. Consumers are highly conscious about the intake of total fat, saturated fat and cholesterol for improving health (Chao *et al.*, 1991). In 1879 Franz von Soxhlet invented the Soxhlet extraction technique for the quantification of fat in the cereals (Soxhlet,1879), and since then, it has been frequently used for the extraction of lipids in agricultural chemistry before becoming the most useful apparatus for solid–liquid extraction in various fields (Schmarr *et al.,* 1996; Rao *et al.,* 2007). Currently, Soxhlet technique is very common and widely used as a reference method in many laboratories for the extraction of fat from various food materials. But as Soxhlet method is a controlled process of extraction and time consuming, extraction hours varies from 8-12 hours. The determination of fat content is one of the most common analysis performed in a food industry, (Lumley and Colwell, 1991). With an ever-increasing range of processed, composite and novel foods available, the analyst faces challenging task of selecting an appropriate method for fat determination.

The nutritional value of wheat is enormously important as it takes an important place among the few crop species being grown as staple food. Khorasan is considered as one of the important cereal grains for human nutrition and offers good source of dietary carbohydrates, proteins, vitamins, minerals and fibers (Sofi *et al.,* 2013). The nutritional quality is linked to the chemical composition and the presence of specific bioactive compounds which satisfy the nutritional needs of consumers and contributes to their health and welfare (D'Egidio and Pagani, 2010). The nutritional importance of khorasan is mainly due to the fact that its seeds can be ground into flour, semolina, etc., which form the basic ingredients of bread and other bakery products. Therefore, it offers the key source of nutrients to the most of the world population (Zuzana *et al.,* 2009). Over three billion people are currently malnourished and this global crisis in nutritional health is the result of dysfunctional food systems that do not consistently supply enough of the essential nutrients. Protein deficiency is a major dietary problem faced by the people worldwide, particularly from the under developed and developing countries. (Welch, 2005; Bashir *et al.,* 2015).

Therefore, the aim of this study was to optimize the fat extraction methods from khorasan wheat using different combination of solvent system and to investigate the nutritional values of khorasan wheat cultivated in India.

Material and methods

The samples of khorasan wheat for the study were purchased from the local market and cleaned well to make free from foreign material. The clean seeds were pulverized and stored in polyethylene bags at 22°C for further analysis.

Total fat content determination

Four series of experimental design were carried out (E_1, E_2, E_3 and E_4). Based upon the results each method was modified.

E_1: In this experimental design 5 g of khorasan wheat were taken and mixed with seventeen-fold volume of the chloroform and methanol mixture (2:1). The resulting solution was homogenized for 5 minutes, and filtered by vacuum pump. After filtration two-fold volume of methanol and chloroform (2:1) was added

and the lower layer of the solution was transferred into separating funnel. To this solution 25 ml of distilled water was added and separating funnel was shook for a few minutes and then left to stand for the separation of the layer. Bottom layer of the solution was transferred into another separating funnel, to which again a mixture of the chloroform – methanol - water in a ratio of 3:48:47 was added and shook for few minutes until layer gets separated. The lower layer from funnel was transferred into empty dried and weighed into titration flask and the solvents were evaporated over vacuum evaporator and fat percentage was calculated by gravimetric method. First series of analysis was repeated with the modification in homogenization time of 10, 20, 30, 40 minutes respectively.

E_2: Samples were treated with twenty-fold volume of methanol - chloroform (2:1) instead of seventeen-fold volume as in previous experiments were added. The homogenization was carried out for 1 hour.

E_3: In this experiment, 5g of samples were loaded into a porous cellulous thimble and placed into the thimble holder. 150 ml of the solvent (n-hexane) with couple of boiling chips were added. Solvents were heated on a heating mantle to get vaporized and pass through the thimble and extract soluble compounds. This process of continuous vaporization and condensation was carried out for 4 hours and the extracted solvent containing the solute were transfer into a pre dried and weighed beaker. After that, the extracted components were isolated by the evaporation and fat percentage was calculated by gravimetric method (Soxhlet, 1879).

E_4: 8g of samples were weighed and placed into the volumetric bottle and 10 ml of ethanol was added. To the homogenous mixture, 8 ml of formic acid and 12 ml of hydrochloric acid and water solution (7:3) were added and the homogenization process was continued for 5 minutes. Subsequently the volumetric flask was put into the water bath heated at the temperature of 75 °C for 20 minutes. The solution was allowed to cool down and the extraction process continued by addition of 18ml ethanol and 50ml hexane ad homogenized for 5minutes to form 2 passes. The hexane layer on the top was filtered through the filter paper into the pre-dried and weighed evaporation flask. To this extracted layer 30ml of hexane were added and homogenized for next 5 minutes, separated again and the hexane layer on the top was filtered through the filter paper into the same evaporation flask with first extract. This extraction with 30ml of hexane was repeated two times and finally, the hexane extract was evaporated on the vacuum evaporator with the temperature of 50°C (International Organization for Standardization, 7302).

Total fat content expressed as percent fat;

$$\frac{(M3 - M2) \times 100}{M1}$$

M_1 - weight of the sample (g); M_2 - weight of the flask (g); M_3 - weight of the flask after extraction (g)

Proximate analysis

Proximate composition of the khorasan wheat was determined using AOAC (Association of official agricultural chemist) methods. Moisture content (% MC) was determined by drying samples in an oven at 105°C for 5 hours and weighed till the constant drying. Crude protein percentage (% CP) was determined by Kjeldahl method IS: 7219-1973 and the percentage nitrogen obtained was used to calculate the % CP using the relationship: % CP = % N X 6.25. Fiber was determined by using AOAC 985.29. Ash percentage was determined by incinerating the samples in a muffle furnace at 550°C for four hours. The ash was cooled in a desiccator and weighed. Carbohydrate was calculated by difference. Energy were calculated by using formula (9*Fat) + 4 (protein% + carbohydrate %).

Determination of total minerals

Elemental analysis was performed by using Thermo Atomic Absorption Spectroscopy iCE 3000 series equipped with Flame and (vapor generation assembly) VGA detector. In this method, 1.0 - 2.0g of samples were weighed into silica crucible and charred on hot plate or flame and after completion of

charring, the sample kept inside muffle furnace for ashing at 550±50 °C for 4-6 Hours. After cooling, ash were transferred into beaker and 5ml of nitric acid and 1ml of 30% hydrogen peroxide (H_2O_2) were added and placed over the hot plate till clear solution attained. The 50ml volume was made up by adding Milli Q deionized water. The dilute filtrate solution was used for the analysis of elements of interest (Fe, Ca, Mg, Zn, Mn, Co and K) using suitable hollow cathode lamps. Ammonium vandate was used to determine phosphorous along with ammonium molybdate using method (AOAC 965.17).The standard conditions for each elements are presented in Table 1.

Vitamin analysis

Vitamin analysis were performed by using Thermo scientific HPLC (High Performance Liquid chromatography) Quaternary Auto Sampler equipped with RI, UV, PDA and fluorescence detector (Suparna *et al.,* 2015).

Sample preparation for vitamin B complex

15g khorasan wheat samples were taken into a 100mL volumetric flask, 60ml mobile phase (0.6 g of pentane sulphonic acid + 0.4125 g of heptane sulphonic acid + 20ml of glacial acetic acid + 250ml of methanol and make up to 1000ml with deionized water). Samples were placed on water bath for 20 minutes and then it was placed in an ultrasonic bath for 5 minutes to dissolve. Sample solution centrifuged and supernatant was taken to filter out by using 0.45-µm-syringe filter.

Sample preparation for vitamin E analysis

5g of samples were taken into Erlenmeyer flask, 40ml (95% ethanol) and 50 mg pyro-gallic acid was added to each flask. Subsequently, Glass beads were added to promote even boiling. 10 ml (50% KOH solution) were added to each flask and swirled. Reflux of the flask were done for 45 min and all the flask were swirled after 10 minutes intervals, 10ml glacial acetic acid solution was added into each flask to neutralize the KOH. Quantitatively transferred solution into each 100 ml volumetric flask and make the volume by using 50:50 THF (Tetra hydro furan): Ethanol. Samples kept for at least 1 hour at room temperature and preferably overnight in refrigerator to allow fatty acid salts formed during saponification to precipitate. Determination of Vitamin-E was done by using mobile phase (98 % methanol + 2 % H_2O). The standard conditions for each vitamins are presented in Table 2.

Results and discussion

Lipids are present only in a small extent in khorasan, but they have a significant effect on the quality and the texture of foods.

Table 1. Showing standard condition for Atomic absorption methods.

Element	Wavelength	Gas	Detector
Ca	422.9	Acetylene/nitrous oxide	Flame AAS
Fe	248.3	Air/Acetylene	Flame AAS
Mg	285.2	Air/Acetylene	Flame AAS
Zn	213.9	Air/Acetylene	Flame AAS
Mn	279.5	Air/Acetylene	Flame AAS
Co	240.7	Air/Acetylene	Flame AAS
K	766.5	Air/Acetylene	Flame AAS

This is because of the ability of lipids to associate with proteins due to their amphipathic nature and starch forming inclusion complexes (Cornell, 2003). In our study, Fig.1 shows that, experimental series (E_1) with different homogenization time *viz.*, 5 minutes, 10 minutes, 20 minutes, 30 minutes and 40 minutes, fat percent were measured 1.46, 1.71, 2.09, 1.39 and 0.89% respectively.

These results suggest that the time of homogenization strongly influenced the results of the fat content determination. The total fat content in khorasan after 20 minutes were found to be 2.09%, which was close to the statements mentioned on the package of the sample. While experimental design E_2, E_3 and E_4 showed that, among all tested methods E_4 was found to have more fat content i. e; 2.59%. While the other tested methods E_2 and E_3 were found to be 1.87 and 1.65% fat content respectively as shown in Fig. 2. Our present findings were comparable with previously published data (Amal *et al.,* 2012).

Proximate analysis

The Proximate Analysis is linked to the nutritional quality and the presence of specific elements or bioactive compounds suitable to satisfy the nutritional needs of consumers and they contribute to their welfare and health (D'Egidio and Pagani, 2010).

Nutritional analysis of khorasan in Fig.3 showed that , the most prominent feature of khorasan was its protein content compared to the other nutritional values, which were found to be 10.51%, 1.44%, 14.07%, 2.59%, 2.07% and 71.39% moisture, ash, protein, fat, crude fibre and carbohydrate respectively.

Table 2. Showing standard condition for HPLC (High Performance Liquid chromatography).

HPLC Condition	Vitamin-B Complex	Vitamin- E
Column	RP C_{18}, 5µm, 250 x 4.6 mm	RP C_{18}, 5µm, 250 x 4.6 mm
Flow rate	1 ml/min	1 ml/min
Injection volume	10µl	10µl
Temperature	Ambient Temperature	Ambient Temperature
Detection	UV- VIS at 280 nm	UV-VIS at 282 nm
Retention Time	40 min	30 min

Our present findings are in line with (Analytical Report No. 88011589, 1988) performed by Medallion Laboratories who executed a complete nutritional analysis of Khorasan and compared it with common types of wheat (Amal *et al.,* 2012). The most striking superiority of khorasan wheat was found in its protein level which showed 40 percent higher value than the average wheat, like Canadian durum (10.6 - 12.7 %) and Egyptian durum (12.66 - 14.40 %) (Nadia *et al.,* 2009). Because of its higher percentage of Protein & fat content, which produce more energy than carbohydrates, khorasan can be described as a "high energy grain".

Table 3. Minerals and Vitamin composition of Khorasan wheat.

Parameters	Results (mg/100g)	Parameters	Results (mg/100g)
Calcium (Ca)	19.73±0.41	Potassium (K)	410±0.82
Iron (Fe)	3.28±0.27	Thiamine (Vit-B_1)	0.53±0.06
Magnesium (Mg)	130±1.95	Riboflavin (Vit-B_2)	0.20±0.19
Phosphorus (P)	359±0.73	Niacin (Vit-B_3)	5.88±0.81
Zinc (Zn)	3.55±0.16	Vitamin B_6	0.17±0.26
Manganese (Mn)	2.88±0.49	Vitamin E	0.56±0.52

Mean value ± standard deviation.

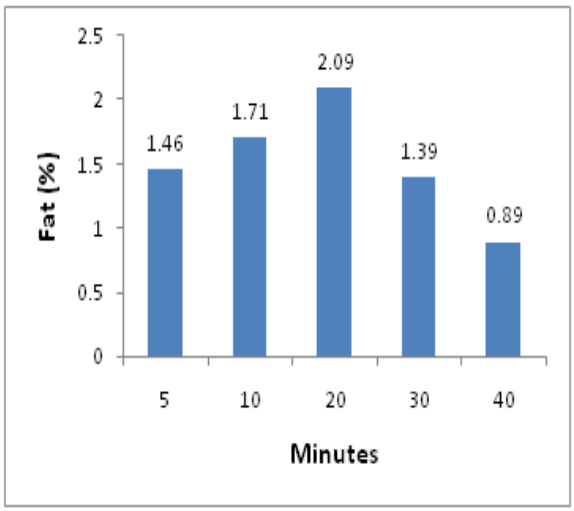

Fig. 1. Showing fat % after different hominization time.

Elemental and vitamin analysis

Differences in the composition of vitamin B among the wheat's are not huge as shown in table 3, especially for thiamine, which amounted to about 0.53 mg per 100 g. Riboflavin was relatively high in einkorn and common wheat (about 0.5 mg per 100 g) but was low (0.20 mg per 100 g) in the khorasan. On the other hand, khorasan wheat had higher concentrations of niacin (5.88 mg per 100 g) compared to einkorn, spelt SK0263, and common wheat was 2.5 mg per 100 g (Abdel-Aal *et al.,*1995). Ranhorta *et al.,* 1995 analyzed three B vitamins-thiamine, riboflavin and niacin and found that only niacin was higher (5%) in spelt wheat compared with

hard red winter wheat. Among the fat-soluble vitamins (vitamin A, E, and D), there were no significant differences among wheats (Zlatica K. *et al.,* 2008). Among all the micronutrients, mineral analysis of khorasan (Table 3) showed that Potassium and Phosphorus concentration were higher 410 and 359 mg/100gm respectively.

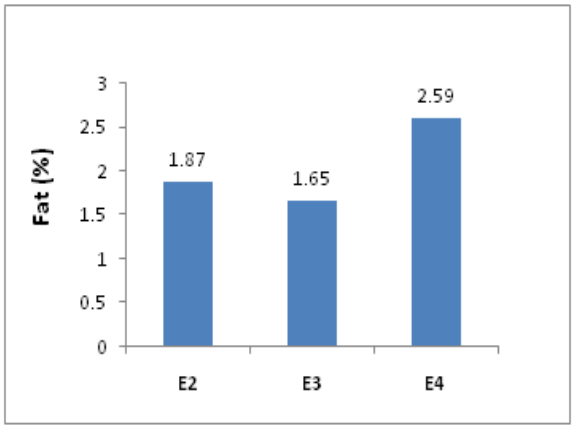

Fig. 2. Showing fat % after experiment design E_2, E_3 and E_4.

Additionally, Iron, Zinc and Manganese were found to be lowest i.e; 3.28, 3.55 and 2.88 mg/100gm respectively. These results were comparable to the (Piergiovanni, 2009) study, which showed in kamut 490.8 mg/100gm and 445 mg/100gm potassium and phosphorus respectively. These results were also comparable to the other varieties of wheat *viz.* norba, farvento, rio and forenza.

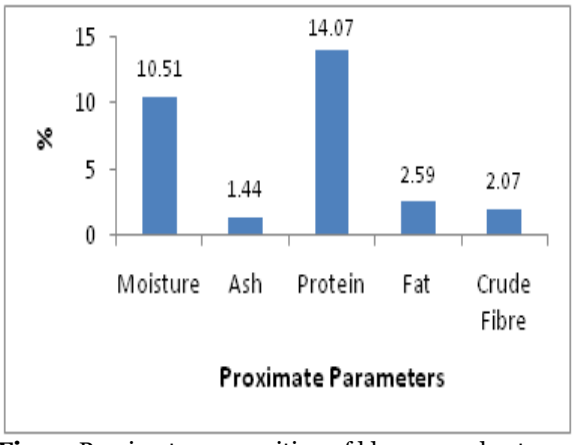

Fig. 3. Proximate composition of khorasan wheat.

Conclusion

The conclusion of the present study is based on following observations: since, the present investigation was aimed to study the optimization of fat extraction method and to investigate the nutritional characteristics of khorasan. Among all the fat extraction methods used in this study, E_4 method in which addition of hydrochloric acid with homogenization of 5 minutes showed the superior results. The present investigation revealed that concentration of crude protein, crude fiber, Vitamins (vitamin-B3) and minerals (Phosphorous and Potassium) were higher in Khorasan than the other wheat, and it must be utilized in diet to combat nutritional imbalance and mineral deficiency. This investigation will be useful for breeders, growers, traders, millers and bakers. Keeping in view the results obtained from the study could be helpful for the future wheat grain improvement programs and can be designed with more ease.

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