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Chemical characteristics and antioxidant activity of different sunflower hybrids and their utilization in bread

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The research work was conducted to characterize the sunflower hybrids and assess their suitability for the preparation of bread. These seeds were divided into two groups one used as such while other after dehulling. The data obtained for all the studied quality attributes were statistically analyzed. It was found that moisture, ash, fat, protein and all minerals contents increased after dehulling while crude fiber contents decreased. It was also observed that seeds with hull or shell had less phenolic contents and also lowers antioxidant activity. Also a direct correlation between total phenols and antioxidant activity (R² = 0.9954, p 0.0001) was observed. Fatty acid and tocopherol contents were also increased after dehulling but no significant increase was observed. The breads prepared with dehulled seeds up to 14% level of supplementation in wheat flour found to be acceptable with respect to all sensory attributes. In the present study, significant improvement in the proximate composition (ash, fat, crude protein and crude fiber) of sunflower seeds supplemented wheat flours was observed. This reflects the potential for use of sunflower into wheat flour for enhancement of these nutrients/chemical constituents.

Key words: Sunflower seeds, phenols, minerals, tocopherols, fatty acids, bread.

INTRODUCTION

The sunflower is one of the major oilseeds crops ranking fourth with a worldwide production of about 10.6 million metric tonnes in 2006 (FAO-STAT, 2008). The sunflower is an annual plant native to the Americas in the family Asteraceae. Per 100 g the seed is made-up to enclose protein 20.78 g, total lipid (fat) 51.46 g, ash 3.02 g, carbohydrate 20 g and fiber 8.6 g with total energy of 2445 kj (USDA, 2008). It has also been investigated that some elements can decrease the risk of some types of cancer, e.g., selenium. USDA (2008) provided the composition for mineral contents in sunflower seeds as per 100 g seeds containing calcium 78 mg, iron 5.25 mg, magnesium 325 mg, phosphorus 660 mg, potassium 645 mg, sodium 9 mg, zinc 5 mg, copper 1.80 mg, manganese 1.95 mg and selenium 53.0 mcg.

This oxidative damage in human body is involved in the

onset of large number of diseases such as auto-immune diseases, inflammation, cardiovascular-neuro-logical diseases, cancer and aging (Kregel and Zhang, 2006; Wang and Maldonado, 2006). An adequate intake of natural antioxidants could protect the onset of oxidative damage in cells (Ozsoy et al., 2008). Sunflower polyphenols such as caffeic, chlorogenic and ferulic acids exert a high antioxidative potential, which might be beneficial both from a technofunctional and biofunctional point of view (Maier et al., 2009).

Clinical studies show that higher unsaturated fat diets may be preferable even to low-fat diets because they lower total cholesterol, low density lipoprotein (LDL) or bad cholesterol and triglycerides, while maintaining beneficial high density lipoprotein (HDL) cholesterol, which is needed to carry the "bad" cholesterol away. Almost 90% of the fat in sunflower seeds is good unsaturated fat. Due to the role of tocopherols as natural antioxidant, it is believed that tocopherols reduce various human diseases especially caused by oxidative stress including cancer, cardiovascular and coronary heart diseases (Adams and

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Table 1. Formulation of sunflower seed flour supplemented flour blends.

Treatments	Wheat flour (%)	SS Flour (%)
T ₀	100	0
T_1	94	6
T_2	90	10
T_3	86	14

Best, 2002). The importance of antioxidant containing foods is increasing with increasing threats of cardio-vascular diseases. The antioxidant potential of oil seed crops especially sunflower is of great concern with ever increasing use of this oils seed in different food products.

The utilization of bakery products as the supplementation vehicle for different nutrients is progressing day by day. The bakery products in particular bread and cookies are used as main supplementation vehicle for nutrition purposes. Bread prepared from refined flour is nutritionally much poorer and does not adequately meet the requirements for many macro- or micro-nutrients. Also, wheat protein lacks the balance of essential amino acids-lysine, threonine and valine. Therefore, there have been many on-going investigations on enhancing the nutritive value of bread to fulfill the expanding demands of modern dietary habits, considering the products' protein, mineral, vitamins and/or fiber contents. Various bread types enriched with combinations of whole oil seeds are being readily accepted by consumers. Therefore the main aim of the present research was to evaluate different sunflower hybrids for their antioxidant potential as well as the improvement of the nutritional and functional properties of wheat bread by sunflower seeds supplementation.

MATERIALS AND METHODS

Sunflower seeds of different hybrids (FH-37, FH-106, FH-237, FH-259, FH-331, FH-369) were purchased from the Oil Seed Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. These sunflower seeds were divided into two parts. Half of the seeds were used as such (with hull) while the rest half was dehulled for further studies. Seeds were dehulled manually.

The whole sunflower seeds and sunflower seeds after dehulling were analyzed for their proximate parameter that is moisture, crude protein, crude fat, crude ash, crude fiber and NFE content as per their respective methods described (AACC, 2000).

The seeds were further analyzed for mineral content that is calcium, zinc, iron, phosphorous, potassium and magnesium by using AOAC (2000) method. Determination of minerals was done using the 1% digest solution in Atomic Absorption Spectrophotometer (Model: Varian, AA-240).

Determination of total phenolics and antioxidant activity

Total phenolics were determined colorimetrcially using Folin-Ciocalteau reagent (Taga et al., 1984). The absorbance was

measured at 750 nm using a spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid standard and the results were expressed as milligrams gallic acid equivalents (GAE) per 100 g extract. Stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was used to determine the radical scavenging capacity (Hatano et al., 1988). The decrease in absorbance was measured at 515 nm against a blank (without extract) with a spectrophotometer. Antioxidant activity using a β -carotene/linoleic acid system was also carried out according to the method of Taga et al. (1984). The bleaching rate of the β -carotene solution was determined by the measurement of the difference between the initial reading in spectral absorbance at 470 nm at time 0 min and after 60 min.

Characterization of sunflower oil for fatty acid profile and tocopherol contents

Fatty acids profile of sunflower oil was estimated by the Method No. Ce 1f-96 as described in AOCS (1997). The fatty acid methyl ester were extracted with HPLC grade hexane and analyzed by GC (Model 3900 Varian USA) immediately using fused capillary column (Silica 30 m \times 0.25), a flame ionization detector (FID) and nitrogen gas carrier (3.5 ml/min). GC split ratio was 100%, injector and detector temperature was 260 °C and column oven temperature was 222°C for 7.5 min. The fatty acid methyl esters (FAMEs) were injected manually. The fatty acids were identified by chromategraphy retention time by comparing with standard fatty acids provided by sigma. The tocopherol content of the sunflower oil was analyzed by using high performance liquid chromatography (HPLC) Method No. Ce 8 -89 as described in AOCS (1997). Analytical separation of oil components on the column was achieved by using a mobile phase consisting of hexane: isopropyl alcohol (99:1 v/v) on isocratic mode. Total run time and flow rate were 15 min and 1.3 ml/min, respectively. The HPLC system (Perkin Elmer-series 200) consisted of a separations module and fluorescence detector. The detector was set at 290 nm excitation wavelength and 400 nm emission wavelengths. The column temperature was 35 °C. The injection volumes of the both, individual standards and the oil sample were 20 µl. A standard calibration curve was prepared for each tocopherol standard $(\alpha, \beta, \gamma \text{ and } \delta)$ to calculate the amount of these tocopherol isomers present in the oil sample.

Preparation of seed flour

The sunflower seeds (dehulled) were ground in china chakki and grounded samples were was smashed and passed through a 200-mesh sieve to obtain flour, which was packed in polypropylene bags for further studies. Whole seeds were rejected due to their black color of hull and presence of higher amount of crude fiber. The wheat flour was replaced with sunflower seed flour for blends formulation at different levels (Table 1). The choice of the levels of sunflower seeds four was based on the report of Skrbic and Filipcev (2008).

Preparation and evaluation of bread

The bread dough formula was: Flour (refined) (100%), compressed yeast (2%), salt (2%), sugar (3%), shortening 5% and sunflower seed was tested at (6, 10, 14% levels). Percentages are based on flour weight. The bread-making performances of flours (control and blends) were determined using straight dough AACC method (2000).

The subjective evaluation of bread samples was carried out for

Table 2. Proximate composition (%) of whole and dehulled seeds of different sunflower hybrids.

Parameter	Hybrid	FH-37	FH-106	FH-237	FH-259	FH-331	FH-369
Moisture	WS	4.88 ± 0.80c	5.72 ± 0.28ab	$3.06 \pm 0.83d$	5.60 ± 0.55ab	6.32 ± 0.74a	6.20 ± 0.00a
Moisture	DS	$6.34 \pm 0.25 d$	$6.66 \pm 0.76c$	6.13 ± 0.28e	$6.37 \pm 0.23d$	7.72 ± 0.15a	7.01 ± 0.16ab
Ash	WS	2.95 ± 0.62c	3.07 ± 0.14ab	2.97 ± 0.84c	3.13 ± 0.02a	2.68 ± 1.06d	2.97 ± 0.18bc
ASII	DS	3.54± 0.84c	3.90 ± 0.04ab	3.76 ± 0.21b	$3.83 \pm 0.61b$	$3.03 \pm 0.18d$	3.94 ± 0.23a
Fat	WS	32.93 ± 1.38ab	29.67 ± 2.43abc	26.47 ± 1.80c	27.18 ± 78bc	32.13 ± 3.14a	34.95 ± 3.61a
Γαι	DS	39.27 ± 8.03d	42.31 ± 0.54c	42.21 ± 0.50c	37.36 ± 5.87d	51.58 ± 8.45a	$46.60 \pm 0.86b$
Protein	WS	19.32 ± 0.52bc	19.87 ± 1.29bc	22.96 ± 0.02a	19.22 ± 2.44bc	21.91 ± 0.05ab	17.18 ±1.10c
Protein	DS	23.60 ± 3.75bc	22.64 ± 3.82 bc	24.45 ± 2.10b	26.69 ± 2.47a	26.86 ± 1.53a	18.64 ± 3.82d
O	WS	31.25 ± 0.92b	30.86 ± 1.99b	28.84 ± 3.84bc	35.90 ± 0.09a	25.86 ± 0.45c	27.45 ± 0.45bc
Crude fiber	DS	14.30 ± 4.74c	16.73 ± 3.10ab	16.53 ± 0.85b	16.81 ± 1.25a	14.77 ± 5.88c	11.78 ± 5.95d
NEE	WS	7.68 ± 0.36e	10.81 ± 2.71c	13.71 ± 3.95a	8.98 ± 2.32d	11.11 ± 3.32bc	11.26 ± 4.98b
NFE	DS	5.95 ± 1.54c	5.76 ± 0.90cd	6.92 ± 0.26b	6.95 ± 1.44b	8.04 ± 0.18a	6.03 ± 1.34c

WS: Whole seed; DS: Dehulled seeds. Values are mean ± SD. Any two means not sharing same letter differ significantly from each other.

the external sensory characteristics that is, volume, color of crust, symmetry of form, evenness of bake, character of crust and internal characteristics like grain, color of crumb, aroma, taste and texture by a trained panel of five judges using score card. The products were scored by the judges according to the method described by Matz (1972). Based on the results of sensory evaluation of breads, three best bread showing suitability for product preparation, along with control were selected for further analysis. The bread samples were analyzed for ash, crude protein, crude fat and crude fiber according to their respective methods of AACC (2000).

Statistical analysis

The data were interpreted by analysis of variance (ANOVA) using M-Stat C software package as described by Steel et al. (1997). The Duncan's Multiple Range test was used to determine the level of significance that existed between the mean values.

RESULTS AND DISCUSSION

Six hybrids of sunflower grown in Pakistan were selected for the present studies to characterize for proximate composition and technological (bread baking) properties.

Proximate composition of sunflower seeds

The highest moisture content in whole seeds (6.32%) and in dehulled seeds (7.72%) was found in the seeds of sunflower hybrid FH-331. While whole seeds of FH-369 (3.13%) and dehulled seeds of FH-259 (3.94%) contained the highest ash contents. The highest crude fat content in whole seeds (34.95%) and in dehulled seeds

(46.60%) was found in the seeds of sunflower hybrid FH-369. While whole seeds of FH-237 (22.96%) and dehulled seeds of FH-331 (26.86%) contained the highest crude protein contents. The highest crude fiber contents (35.90%) in whole seeds and in dehulled seeds (16.81%) were found in the seeds of sunflower hybrid FH-259. The data showed that significantly, the highest NFE (13.71%) was recorded in whole seeds of sunflower hybrid FH-237 and the highest NFE (8.04%) in dehulled seeds was found in the seed of FH-331. On account of proximate composition, FH-259 was found to be best (Table 2).

It is clear from results that after dehullling there is a significant increase in all constituent except crude fiber and nitrogen free extract which is high in hull of the seeds. Similar finding were also found by some other scientists. There is an increase of moisture contents from 5.50 to 6.54%, ash content 3.49 to 7.05%, fat content from 37.47 to 62.35% and protein content from 18.72 to 19.30% of respectively whole seeds to dehulled seeds among different sunflower seeds. There is decrease of fiber contents from 28.36 to 13% and for nitrogen free extract 6.11 to 3.50% when sunflower seeds were dehulled (Srilatha and Krishnakumari, 2003). Similarly Bhagya and Sastry (2003) also studied the similar affect of dehulling on Niger seeds.

Mineral contents of sunflower seeds

The iron, zinc, copper, manganese, calcium, sodium, potassium and magnesium contents (Table 3) ranged

Table 3. Mineral contents (mg/100 g) of whole and dehulled seeds of different sunflower hybrids.

Parameter	Hybrid	FH-37	FH-106	FH-237	FH-259	FH-331	FH-369
Fe	WS	6.25 ± 3.54c	7.34 ± 4.81b	6.51 ± 1.84c	6.65 ± 2.69c	8.85 ± 2.40a	5.57 ± 0.14d
16	DS	6.4 ± 0.85 d	8.34 ± 5.94b	7.76 ± 3.96c	$7.58 \pm 0.57c$	10.19 ± 3.54a	6.82 ± 1.13d
Zn	WS	10.190 ± 6.45c	12.251 ± 1.23b	12.355 ± 4.00b	12.649 ± 2.98b	14.198 ± 5.71a	9.049 ± 1.09d
211	DS	11.761 ± 2.84d	15.443 ± 2.28b	13.561 ± 1.00c	13.948 ± 7.98c	17.855 ± 4.99a	9.533 ± 6.41e
Co	WS	6.91 ± 1.6cd	7.37 ± 1.6b	7.28 ± 2.5bc	7.09 ± 0.7bcd	8.73 ± 2.4a	6.83 ± 0.1d
Со	DS	7.42 ± 1.1c	10.04 ± 4.0b	7.38 ± 1.4cd	7.7 ±0.6c	12.17 ± 0.7a	$6.92 \pm 2.0d$
N 4	WS	2.9 ± 2.26b	2.48 ± 0.00cd	2.68 ± 1.13bc	2.91 ± 1.27b	3.72 ± 1.41a	2.11 ± 2.69d
Mn	DS	2.97 ± 2.40cd	3.13 ± 2.69bc	2.93 ± 0.14cd	3.49 ± 2.12b	6.99 ± 1.27a	2.50 ± 2.55d
	WS	3.23 ± 0.00b	3.19 ± 0.32b	3.67 ± 0.00ab	3.9 ± 0.32a	3.45 ± 0.32ab	3.67 ±0.00ab
Ca	DS	3.45 ± 0.32ab	3.45 ± 0.32ab	3.97 ± 0.00ab	4.10 ± 0.00a	3.75 ± 0.32ab	3.99 ± 0.00ab
	WS	44.67 ± 0.58a	45.36 ± 0.03a	44.132 ± 0.21a	45.86 ± 1.10a	44.05 ± 2.87a	44.02 ± 0.95a
Na	DS	45.79 ± 0.88ab	45.71 ± 0.58ab	44.43 ± 0.06b	46.27 ± 0.76ab	46.65 ± 1.36a	44.80 ± 0.52ab
.,	WS	5.63 ± 0.08b	6.81 ± 0.08a	5.67 ± 0.07b	5.63 ± 0.10b	5.28 ± 0.06c	4.68 ± 0.15d
K	DS	5.90 ± 0.10cd	7.0 ± 0.10a	$5.98 \pm 0.09c$	6.37 ± 0.10b	$5.68 \pm 0.06d$	5.14 ± 0.10e
	WS	12.75 ± 0.35b	9.08 ± 0.05c	16.18 ±1 .18a	13.80 ± 1.00b	7.34 ± 0.46c	16.36 ± 0.78a
Mg	DS	15.68 ± 0.03c	13.45 ± 0.65d	18.06 ± 0.23a	16.78 ± 0.63b	8.21 ± 0.49e	17.75 ± 0.16ab

WS: Whole seed; DS: Dehulled seeds. Values are mean ± SD. Any two means not sharing same letter differ significantly from each other

from 5.57 to 8.85 mg/100 g and 6.4 to 10.19 mg/100 g, 9.049 to 14.198 mg/100 g and 9.533 to 17.855 mg/100 g, 6.83 to 8.73 mg/100 g and 6.92 to 12.17 mg/100 g, 2.11 to 3.72 mg/100 g and 2.50 to 6.99 mg/100 g, 3.19 to 3.90 mg/100 g and 3.45 to 4.10 mg/100 g, 44.02 to 45.86 mg/100 g and from 44.43 to 46.65 mg/100 g, 4.68 to 6.81 mg/100 g and from 5.14 to 7.0 mg/100 g and 7.34 to 16.36 mg/100 g and 8.21 to 18.06 mg/100 g between the whole sunflower seeds and dehulled seeds, respectively among different sunflower hybrids. On account of mineral content, FH-331 was found to be the best.

Up to now, no previous study was found on the affect of dehulling on sunflower seeds. But in our study, mineral contents in whole seeds were found relatively lower than the dehulled seeds of the respective sunflower hybrids. The higher mineral content in dehulled seeds as compared to whole seeds in the present study may be ascribed to the fact that hull contain less ash contents as described earlier. USDA (2008) gave the following composition for mineral contents in sunflower seeds. Per 100 g seeds contains calcium 78 mg, iron 5.25 mg, magnesium 325 mg, phosphorus 660 mg, potassium 645 mg, sodium 9 mg, zinc 5.mg, copper 1.80 mg, manganese 1.95 mg and selenium 53.0 mcg. Our results are different from USDA values. The difference may be due to the differing climatic and soil conditions and analytical

techniques used also because USDA values are average of several calculations.

Total Phenolic contents and antioxidant activity

The results given in Table 4 also indicate that the total phenolic contents ranged from 2185.76 to 2965.90 mg/100 g and 3162.02 to 3614.34 mg/100 g between the whole sunflower seeds and dehulled seeds, respectively among different sunflower hybrids. The dehulled seeds exhibited higher content of total phenolics than the respective whole seeds of different sunflower hybrids. The highest phenolics content in whole seed (2965.90 \pm 0.44 mg/100 g) and in the dehulled seeds (3614.34 \pm 0.23 mg/100 g) was found in seeds of FH-369.

Various scientists have worked about contents of phenolic compounds in sunflower seeds (Pedrosa et al., 2000). Comparison with the data obtained in the present study is hardly possible because of differing analytical methodologies, the development of novel sophisticated techniques and differences in the sample material and origin. Fiska et al. (2006) determined the total phenolic contents in sunflower seeds and found to be 2700 mg/100 g on dry weight basis. Our results are corresponding as determined by Weisz et al. (2009) by

Parameters	Hybrid	FH-37	FH-106	FH-237	FH-259	FH-331	FH-369
TPC (mg/100 g)	WS	2233.12 ± 0.23d	2571.67 ± 0.35b	2965.90 ± 0.44a	2185.76 ± 0.33d	2865.14 ± 0.36a	2404.31 ± 0.29c
	DS	3162.26 ± 0.21c	3428.34 ± 0.36b	3225.82 ± 0.63c	3162.02 ± 0.42c	3433.56 ± 0.30b	3614.34 ± 0.23a
DPPH radical	ws	58.33 ± 1.443c	66.18 ± 2.453a	65.35 ± 0.173a	59.49 ± 1.358c	60.33 ± 1.212bc	55.39 ± 0.750c
scavenging (%)	DS	65.56 ± 0.504d	69.79 ± 1.047bc	68.58 ± 2.988c	66.47 ± 0.931d	70.86 ± 2.794b	72.34 ± 2.287a
Beta Carotene	ws	52.38 ± 0.76cd	51.12 ± 1.54d	59.95 ± 0.26ab	56.93 ± 1.78abc	55.94 ± 1.39bc	50.38 ± 2.68d
inhibition (%)	DS	58 71 + 1 927d	60 15 + 0 718bcd	61 36 + 6 349h	59 06 + 0 151d	63 95 + 7 067a	63 15 + 1 927a

Table 4. Total phenolics (mg/100 g) and antioxidant activity (%) of whole and dehulled seeds of different sunflower hybrids.

summarizing individual amounts of all constituents and ranged from 2938.8 to 4175.9 mg/100 g dry matter (DM) for the dehulled kernels. They also noticed the TPCs of the sunflower kernels were found to be higher than those determined in the shells.

The free radical scavenging activity ranged from 55.39 to 66.18% and 65.56 to 72.34% between the whole sunflower seeds and dehulled seeds, respectively among different sunflower hybrids. The highest free radical scavenging activity in whole seed $(66.18 \pm 1.45\%)$ and in dehulled seeds $(72.34 \pm 2.28\%)$ was found in the seeds of FH-369. Our results for the DPPH assay are somewhat similar to the findings of such studies. Free radical scavenging activity of the aqueous extract was found to be 58.8% in striped sunflower seeds via DPPH assay (Giada and Mancini-Filho, 2008).

Antioxidant activity via beta carotene bleaching assay ranged from 50.38 to 59.95% and 58.71 to 63.95% between the whole sunflower seeds and dehulled seeds, respectively among different sunflower hybrids. The highest antioxidant activity (59.95 \pm 0.26%) was found in the whole seed sample of FH-369 and the highest antioxidant activity in the dehulled seeds (63.95 \pm 1.06%) was

found of FH-369. Our results are similar to the earlier findings of some scientists. The antioxidant activity from sunflower residue was found to be near 70% by Matthaus et al. (2002). And also the antioxidant activity of sunflower seeds determined by Velioglu et al. (1998) was 72.9%.

Relationship between total phenolics and antioxidant activity

There is a significant relationship between accumulation of high amount of phenolic compounds and antioxidant activity ($R^2 = 0.9954$, p=0.0001). It is clear from the results that there is a direct relationship between total phenolics contents and antioxidant activity of different sunflower hybrids. It means if seeds have more phenolics contents they also have high antioxidant activity and vice versa. Different results have been reported on the aspect of relationship between phenolic content and antioxidant activity. A parallel increase between phenol content and antioxidant activity was found during germination of Pangium edule (Andarwulan et al., 1999). Tsaliki et al. (1999) also observed an increase in the antioxidant activity of lupin seed. Also a linear positive relationship

existed between the anti-oxidant activity and total phenolic acids content of the tested *Ocimum* gratissimum accessions (Javanmardi et al., 2003).

Characterization of sunflower oil for fatty acid profile and tocopherol contents

The results regarding fatty acid profile (Table 5) shows that palmitic acid contents ranged from to 3.5 to 9.1% and 5.3 to 11.3%, palmitoleic acid contents from 0.3 to 6.5% and 2.7 to 6.9%, stearic acid contents from 3.5 to 9.7% and 5.4 to 15.6%. oleic acid contents from 33.6 to 46% and 34.8 to 49.6% and linoleic acid contents seeds ranged from 38.6 to 53.9% and 24.6 to 43.9% respectively in whole seeds and dehulled among different sunflower hybrids. FH-369 and FH-37 was found to be good sources of oleic and linoleic acid respectively. It is clear from the above study that seeds after dehulling have more fatty acids contents for palmitic acid, palmitoleic acid, stearic acid and oleic acid but linoleic acid contents decreased after dehulling to substantial amount. The results of the present study are incomplete in line with the findings of Perez-Vicha et al. (1998). They investigated a set of 387 intact-seed

Table 5. Fatty acid contents (%) of whole and dehulled seeds of different sunflower hybrids

Fatty acid (%)	Hybrid	FH-37	FH-106	FH-237	FH-259	FH-331	FH-369
Palmitic acid	WS	5.2 ± 0.51c	9.1 ± 0.01a	4.7 ± 0.04 cd	8.5 ± 0.66ab	$7.6 \pm 0.08b$	3.5 ± 0.02d
raimilic acid	DS	$7.1 \pm 0.28c$	11.3 ± 0.93a	$6.8 \pm 0.01c$	$9.9 \pm 0.05b$	9.5 ± 0.41b	$5.3 \pm 0.08d$
Palmitoleic acid	WS	0.9 ± 0.04d	3.8 ± 0.01b	1.4 ± 0.01c	1.9 ± 0.07c	6.5 ± 0.13a	0.3 ± 0.01e
Paimiloleic acid	DS	4.6 ± 0.11d	$5.4 \pm 0.02c$	4.8 ± 0.41d	6.1 ± 0.11b	6.9 ± 0.08a	2.7 ± .04e
Stearic acid	WS	3.5 ± 0.31f	5.8 ± 0.01d	6.7 ± 0.07c	5 ± 0.02e	7.6 ± 0.21b	9.70 ± 0.61a
Steame acid	DS	$5.4 \pm 0.04 f$	11.2 ± 0.14c	$9.5 \pm 0.09d$	8.1 ± 0.07e	13 ± 0.38bc	15.6 ± 0.08a
Olaia aaid	WS	35.8 ± 0.84e	41.3 ± 0.92b	33.6 ± 0.99e	39.7 ± 1.02bcd	38.6 ± 0.99cd	46 ± 0.92a
Oleic acid	DS	37.5 ± 0.95d	45.1 ± 0.87b	34.8 ± 1.02e	42.1 ± 1.05c	40.1 ± 0.37c	49.6 ± 0.64a
	WS	53.9 ± 1.11a	39.1 ± 0.02c	49.6 ± 1.33a	43.5 ± 0.68b	38.6 ± 0.08c	39.1 ± 0.28c
Linoleic acid	DS	43.9 ± 0.55a	26.1 ± 0.83cde	43.3 ± 0.92a	32.1 ± 0.49b	27.4 ± 0.27c	24.6 ± 0.31e

Table 5. Fatty acid contents (%) of whole and dehulled seeds of different sunflower hybrids

Fatty acid (%)	Hybrid	FH-37	FH-106	FH-237	FH-259	FH-331	FH-369
Palmitic acid	WS	5.2 ± 0.51c	9.1 ± 0.01a	4.7 ± 0.04cd	8.5 ± 0.66ab	$7.6 \pm 0.08b$	3.5 ± 0.02d
Faimilio aciu	DS	7.1 ± 0.28c	11.3 ± 0.93a	$6.8 \pm 0.01c$	$9.9 \pm 0.05b$	$9.5 \pm 0.41b$	$5.3 \pm 0.08d$
Palmitoleic acid	WS	0.9 ± 0.04d	3.8 ± 0.01b	1.4 ± 0.01c	1.9 ± 0.07c	6.5 ± 0.13a	0.3 ± 0.01e
Paimitoleic acid	DS	4.6 ± 0.11d	$5.4 \pm 0.02c$	4.8 ± 0.41d	6.1 ± 0.11b	$6.9 \pm 0.08a$	2.7 ± .04e
Stearic acid	WS	3.5 ± 0.31f	5.8 ± 0.01d	6.7 ± 0.07c	5 ± 0.02e	7.6 ± 0.21b	9.70 ± 0.61a
Stearic acid	DS	5.4 ± 0.04f	11.2 ± 0.14c	$9.5 \pm 0.09d$	8.1 ± 0.07e	13 ± 0.38bc	15.6 ± 0.08a
Olaia aaid	WS	35.8 ± 0.84e	41.3 ± 0.92b	33.6 ± 0.99e	39.7 ± 1.02bcd	38.6 ± 0.99cd	46 ± 0.92a
Oleic acid	DS	$37.5 \pm 0.95d$	45.1 ± 0.87b	34.8 ± 1.02e	42.1 ± 1.05c	40.1 ± 0.37c	49.6 ± 0.64a
Linglein anial	WS	53.9 ± 1.11a	39.1 ± 0.02c	49.6 ± 1.33a	43.5 ± 0.68b	38.6 ± 0.08c	39.1 ± 0.28c
Linoleic acid	DS	43.9 ± 0.55a	26.1 ± 0.83cde	43.3 ± 0.92a	32.1 ± 0.49b	27.4 ± 0.27c	24.6 ± 0.31e

samples of sunflower for fatty acid profile. Their results revealed that Palmitic acid was found in the range of 3 to 35.5% for whole seeds and 3.9 to 35.8% for dehulled seeds. Palmitoleic acid was observed in the range of 0.0 to 8.6% and 0.0 to 9%, Stearic acid in the range of 1.4 to 30.3% and 1.7 to 28.5%, oleic acid in the range of 7.7 to 90.7 and 9.1 to 90.5% and linoleic acid was observed in the range of 1.8 to 74.5 and 1.9 to 64.4% (Table 2). Fayyaz and Ahmad (2003) reported the percentage of palmitic acid in the range of 6.57 to 7.67 in different sunflower hybrids.

It is obvious from the results that total tocopherol contents (Table 6) ranged from 348.61 to 687.05 mg/100 g and from 415.85 to 721.68 mg/100 g, alpha tocopherol contents ranged from 331.18 to 652.70 mg/100 g and

393.16 to 668.98 mg/100 g, beta-tocopherol contents ranged from 15.69 to 28.17 mg/100 g and 17.93 to 38.21 mg/100 g, gamma-tocopherol contents ranged from 1.27 to 5.82 mg/100 g and from 3.31 to 14.95 mg/100 g and the delta-tocopherol contents ranged from 0.35 to 2.91 mg/100 g and from 1.45 to 4.36 mg/100 g respectively in whole and dehulled seeds among different sunflower hybrids. It is clear from the results that after dehulling there is a small increase in tocopherol contents of different sunflower hybrids. FH-237 was found to contain maximum tocopherol contents. It is clear from the results that after dehulling there is a small increase in tocopherol contents of different sunflower hybrids. Significant variations (389 to 1873 $\mu g/g$ oil) in the total tocopherol concentration of sunflower seed oil have been reported

Table 7. Means for external and internal characteristics of bread.

External characteristics								
Treatments	Volume	Color of crust	Form symmetry	Evenness of bake	Crust character			
T0	8 ±0.00a	6.6 ± 0.89a	4 ± 0.00a	2.8 ± 0.45a	2.8 ± 0.55a			
T1	7.2 ± 0.45ab	6.4 ± 1.14a	3.6 ± 0.55ab	2.4 ± 0.55b	2.6 ± 0.55ab			
T2	7.2 ± 1.10ab	6.6 ± 1.52a	3.6 ± 0.55ab	$2.2 \pm 0.45b$	2.5 ± 0.45ab			
Т3	6.8 ± 0.84 b	6.6 ± 1.52a	$3 \pm 0.71b$	2.2 ± 0.45b	2.5 ± 1.00b			
		Internal ch	naracteristics					
Treatments	Grain	Color of crumb	Aroma	Taste	Texture			
T0	12 ± 1.22a	$7.8 \pm 0.45c$	$7.2 \pm 1.10c$	15.8 ± 3.56a	12.8 ± 0.84a			
T1	12.6 ± 0.89a	7.8 ± 0.84 ab	8 ± 0.71ab	16.4 ± 2.07a	12.6 ± 0.84a			
T2	12 ± 1.00a	$8.8 \pm 0.84b$	8.8 ±0.45b	16.8 ±1.64a	12.4 ± 1.14ab			
T3	11.4 ± 1.52a	9.2 ±0.84a	9.6 ± 0.55a	18.2 ± 1.48a	10.8 ± 1.92b			

Values are mean ± SD. Any two means not sharing same letter differ significantly from each other

(Nolasco et al., 2004). The total tocopherol concentration in crude oil obtained from whole sunflower seeds typically varies between 447 and 900 μg g oil (Gunstone et al., 1994) with extreme values varying from 389 to 1873 μg g oil (Velasco et al., 2002). Alpha-tocopherol typically represents most of 90% of tocopherol content of sunflower seed oil. According to Fisk (2006) tocopherol values ranged from 214 mg total tocopherol kg^{-1} to 392 mg total tocopherol kg^{-1} . The ratio of the four tocopherol isomers remained constant in the seed and oil body preparations $(\alpha:\!\beta:\!\mu:\!\infty)$ approximately 94:5:0.5:0.5). This work provides evidence that an intrinsic population of tocopherol molecules exists in the oil bodies of mature sunflower seeds.

In cultivated material, an average tocopherol content of 669.1 mg kg⁻¹ seed have been reported, made up of 92.4% alpha-tocopherol, 5.6% beta tocopherol, and 2.0% gamma-tocopherol (Velasco et al., 2002). The results of the present study are consistent with the results reported by Rossi et al. 2007) who reported alpha tocopherol content 475 mg/100 g in sunflower seed oil.

Sensory evaluation of wheat bread supplemented with sunflower seeds

The present study regarding sensory evaluation of pan breads prepared from dehulled sunflower seeds supplemented wheat flour indicated that all the external and internal sensory attributes were affected as the level of supplementation of sunflower seeds increased. The scores (Table 7) assigned to taste, aroma and color of crust of breads prepared from freshly prepared composite flour was increased while score for all other parameters decreased than control.

The sensory scores for appearance, texture and flavor of breads have been reported to be decrease by the

incorporation of non wheat flours in wheat flours (Shittu et al., 2007). The results of the present study are in conformity with the work of Skrbic and Filipcev (2008) who found that addition of different levels of sunflower seeds in bread negatively affected the volume. But these results for flavor of bread are in contrast with Shittu et al. (2007) for flavor and taste and similar to Skrbic and Filipcev (2008).

In the present study, assignment of higher score by the panelists to the crust and crumb color, flavor and taste of breads may be attributed to the lighter yellow color and presence of more phenolic compounds in dehulled seeds. The decrease in bread firmness and reduction in volume scores may be ascribed to the decrease in gluten forming proteins and increase in dietary fiber contents contributed by sunflower seeds, which negatively affected the formation of gluten network and depressed the loaf volume due to lack of gas retention capacities of the composite flour. The decrease in character of crust, evenness of bake, and texture and grain scores of breads prepared from dehulled seeds supplemented wheat flour may be attributed towards the coarser grain structure of sunflower seeds flour and more fiber contents of sunflower flour which resulted in the formation of leathery texture and coarser bread grain. The increase in taste and aroma of breads by the increase in level of sunflower flour supplementation might be due to more flavoring compound present in sunflower seeds. The breads prepared with dehulled seeds up to 14% level of supplementation in wheat flour found to be acceptable with respect to all sensory attributes. The breads with sunflower flour supplementation will be superior in nutritional quality and provide more health benefits than normal wheat bread.

In the present study, assignment of higher score by the panelists to the crust and crumb color, flavor and taste of breads may be attributed to the lighter yellow color and

Table 8. Effect of sunflower seeds supplementation on proximate composition of bread.

Supplementation level	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)
T0	14.36 ± 0.56d	1.75 ± 0.21d	$0.58 \pm 0.33c$	$0.92 \pm 0.06d$
T1	14.59 ± 0.74c	$6.30 \pm 0.57c$	$1.47 \pm 0.04b$	1.18 ± 0.01c
T2	$15.05 \pm 0.50b$	11.00 ± 0.71b	$2.40 \pm 0.02a$	1.27 ± 0.01b
Т3	15.62 ± 0.17a	13.40 ± 0.71a	2.44 ± 0.03a	1.38 ± 0.01a

WS: Whole seed: DS: Dehulled seeds. Values are mean \pm SD. Any two means not sharing same letter differ significantly from each other

presence of more phenolic compounds in dehulled seeds. The decrease in bread firmness and reduction in volume scores may be ascribed to the decrease in gluten forming proteins and increase in dietary fiber contents contributed by sunflower seeds, which negatively affected the formation of gluten network and depressed the loaf volume due to lack of gas retention capacities of the composite flour.

The decrease in character of crust, evenness of bake, and texture and grain scores of breads prepared from dehulled seeds supplemented wheat flour may be attributed towards the coarser grain structure of sunflower seeds flour and more fiber contents of sunflower flour which resulted in the formation of leathery texture and coarser bread grain. The increase in taste and aroma of breads by the increase in level of sunflower flour supplementation might be due to more flavoring compound present in sunflower seeds.

The breads prepared with dehulled seeds up to 14% level of supplementation in wheat flour found to be acceptable with respect to all sensory attributes. The breads with sunflower flour supplementation will be superior in nutritional quality and provide more health benefits than normal wheat bread.

Selection of sunflower seeds supplemented breads

Selection of breads prepared from maximum acceptable levels of sunflower seeds in wheat flours was made on the basis of sensory acceptability. The results of the sensory studies revealed that breads prepared from composite flours of dehulled seeds at all the levels of supplementation were found to be acceptable for their overall acceptability. The breads prepared from composite flours of dehulled seeds in wheat flour were selected for further studies. The bread prepared from 100% wheat flour was also used as control.

Chemical composition of wheat breads supplemented with sunflower seeds

The chemical composition provides basic information about the components and quality of the products. It is evident form the results (Table 8) that addition of

sunflower seeds at 14% contributed towards more increase in ash content (1.38%). The ash content significantly increased from 0.92% at 0% in control, 1.18 at 6%, 1.27% at 10% and 1.38% at 14% level of supplementation. Results also indicated that addition of sunflower seeds at 14% contributed towards more increase in fat content (13.40%). The fat content significantly increased from 1.75 at 0% (control), 6.30 at 6, 11 at 10 and 13.40% at 14% level of supplementation. Also the addition of sunflower seeds at 14% contributed towards more increase in crude fiber content (2.44%). The crude fiber content significantly increased from 0.58 at 0% (control), 1.47 at 6, 2.40 at 10 and 2.44 at 14% level of supplementation. The results for supplementation levels at 10 and 14% were statistically found to be similar. Addition of sunflower seeds at 14% contributed towards more increase in crude protein content (15.62%). The crude protein content significantly increased from 14.36 at 0% (control), 14.59 at 6%, 15.05% at 10% and 15.62% at 14% level of supplementation.

The supplementation of sunflower in wheat flour significantly improved the nutrient profile of the breads. The results of the present study are in line with the earlier study conducted by Skrbic and Filipcev (2008) in which they found significant improvement in the proximate composition (ash, fat, crude protein and crude fiber) of sunflower seeds supplemented wheat flours.

Conclusion

In the present study there is a significant increase in total phenolics, antioxidant activity and total and individual tocopherol contents after dehulling. The scores for overall crust and crumb color, flavor and taste of breads increased with dehulled seeds supplementation. The breads prepared with dehulled seeds up to 14% level of supplementation in wheat flour found to be acceptable with respect to all sensory attributes. It was concluded that the sunflower seeds flours supplemented wheat flour significantly improved the chemical composition (ash, crude fat, crude fiber and crude protein) of breads. Therefore sunflower seeds should be included in daily diet plan through its incorporation into wheat flour used for production of breads and the baking industry should focus on the fortification of bakery products with

sunflower seeds.

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REFERENCES

- AACC (American Association of Cereal Chemists) (2000). Approved methods of American Association of Cereal Chemists. St. Paul, Minnesota, USA.
- Adams AK, Best TM (2002). The role of antioxidants in exercise and disease prevention. Phys. Sports Med., 30: 37-44.
- Andarwulan N, Fardiaz D, Wattimena GA, Shetty K (1999). Antioxidant activity associated with lipid and phenolic mobilization during seed germination of Pangium edule Reinw. J. Agric. Food Chem., 47: 3158-3163.
- AOAC (Association of Official Analytical Chemists) (2000). Official methods of Analysis. Washington D.C. 13th edition.
- AOCS (American Oil Chemist's Society) (1997). Official Methods and recommended Practices of the American Oil Chemist's Society. Washington D.C. 5th edition.
- Bhagya S. Sastry MCS (2003). Chemical, functional and nutritional properties of wet dehulled niger (*Guizotia abyssinica* Cass.) seed flour. Lebensm.-Wiss. u.-Technol., 36: 703–708.
- FAO-STAT (2008). Food Bulletin. Website: http://faostat.fao.org/site/567/default.aspx. visited on: 2008/06/05.
- Fayyaz H, Ahmad RA (2003). Effects of seasonal variations on oil and fatty acid profile of sunflower. Heliantrus, 26(38):159-166.
- Fisk LD, White DA, Carvalho A, Gray DA (2006). Tocopherol An Intrinsic Component of Sunflower Seed Oil Bodies. J. Am. Oil Chem., Soc., 83: 341–344.
- Giada MDLR, Mancini-Filho J (2008). Antioxidant capacity of the striped sunflower (*Helianthus annuus* L.) seed extracts evaluated by three *in vitro* methods. Int. J. Food Sci. Nutri., 12: 1-7.
- GOP (2009). Economic survey 2008-09. Government of Pakistan, Finance Division, Economic Advisor's Wing, Islamabad, Pakistan.
- Gunstone FD, Harwood JL and Padley FB (1994). The Lipid Handbook (Second Edition) (Chapman & Hall, London). p. 130.
- Hatano T, Kagawa H, Yasuhara T, Okuda T (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull., 36: 1090-1097.
- Javanmardi J, Stushnoff C, Locke E, Vivanco JM (2003). Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food Chem., 83(4): 547-550.
- Kregel KC, Zhang HJ (2006). An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am. J. Physiol., 292: 18-36.
- Maier T, Schieber A, Kammerer DR, Carle R (2009). Residues of grape (*Vitis vinifera* L) seed oil production as a valuable source of phenolic antioxidants. Food Chem., 112: 551-559.

- Matthaus B (2002). Antioxidant activity of extracts obtained from residues of different oilseeds. J. Agric. Food Chem., 50: 3444-3452.
- Matz SA (1972). Bakery Technology and Engineering. The AVI Pub. Co., Westport, Connecticut.
- Nolascoa SM, Aguirrezábalb LAN, Crapistec GH (2004). Tocopherol Oil Concentration in Field-Grown Sunflower is accounted for by Oil Weight per Seed. J. Am. Oil Chem. Soc., 81:1045-1051.
- Ozsoy N, Can A, Yanardag R, Akev N (2008). Antioxidant activity of Smilax excelsa leaf extracts. Food Chem., 110: 571-583.
- Pedrosa MM, Muzquiz M, Garcia-Vallejo C, Burbano C, Cuadrado C, Ayet G (2000). Determination of caffeic and chlorogenic acids and their derivatives in different sunflower seeds. J. Soc. Food Agric., 80: 459-464.
- Perez-Vicha B, Velascob L, Fernandez-Martineza JM (1998). Determination of seed oil content and fatty acid composition in sunflower through the analysis of intact seeds, husked seeds, meal and oil by near-infrared reflectance spectroscopy. J. Am. Oil Chem. Soc., 75: 547-555.
- Rossi M, Alamprese C, Ratti S (2007). Tocopherols and tocotrienols as free radical-scavengers in refined vegetable oils and their stability during deep-fat frying. Food Chem., 102: 812-817.
- Shittu TA, Raji AO, Sanni LO (2007). Bread from Composite Cassava-Wheat Flour: I Effect of Baking Time and Temperature on some Physical Properties of Bread Loaf. Food Res. Int., 40: 280-290.
- Skrbic B, Filipcev B (2008) Nutritional and sensory evaluation of wheat breads supplemented with oleic-rich sunflower seed. Food Chem., 108(1): 119-129.
- Srilatha K, Krishnakumari K (2003). Proximate composition and protein quality evaluation of recipes containing sunflower cake. Plant foods for Human Nutri., 58: 1-11.
- Steel RGD, Torrie JH, Dickey DA (1997). Principles and Procedures of Statistics - a biometrical approach (3rd edition). McGraw Hill Book Co. Inc., New York, USA.
- Stefansson BR (2007). Oilseed crops. The Canadian Encyclopedia (Historica Foundation, Toronto). Available at: http://www.thecanadianencyclopedia.com. Accessed July 18, 2009.
- Taga MS, Miller EE, Pratt DE (1984). Chia seeds as a source of natural lipid antioxidants. J. Am. Oil Chem. Soc., 61: 928-931.
- Tsaliki E, Lagouri V, Doxastakis G (1999). Evaluation of the antioxidant activity of lupin seed flour and derivatives (*Lupinus albus* ssp. *Graecus*). Food Chem., 65: 71-75.
- USDA (2008). Accessed on, 2009/07/25,http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list nut edit.pl
- Velasco L, Fernandez-Martinez JM, Garcia-Ruiz R, Dominguez J (2002). Genetic and environmental variation for tocopherol content and composition in sunflower commercial hybrids. J. Agric. Sci., 139: 425-429.
- Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. J. Agric. Food Chem., 46(10): 4113-4117.
- Wang JS, Maldonado MA (2006). The ubiquitin-proteasome system and its role in inflammatory and autoimmune diseases. Cell Molecular Immunol., 3: 255-261.
- Weisz GM, Kammerer DR, Carle R (2009). Identification and quantification of phenolic compounds from sunflower (*Helianthus annuus* L.) kernels and shells by HPLC-DAD/ESI-MSn. Food Chem., 115: 758-765.