Research Article

Development and Nutritional Evaluation of Multigrain Bread Supplemented with Sunflower Seed (*Helianthus annuus*) Flour

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Abstract

Oilseeds have gained much acceptance due to their high nutritive value but not much therapeutic dietary options are available to utilize them. The purpose of this study was to incorporate sunflower seeds to produce therapeutic bakery product. Sunflower seed flour was supplemented to develop multigrain bread using varying cereal flours in addition to refined wheat flour. One control and five experimental samples were prepared. Organoleptic evaluation was done by a panel of 10 judges. Multigrain bread supplemented with 7.5 percent sunflower seed flour was found to be highly acceptable. The proximate parameters of moisture, protein, crude fat, crude fibre and total ash were found to be highest for highly acceptable bread sample i.e. 31.73%, 10.61%, 2.92%, 11.36% and 3.29% respectively while the carbohydrate content of control sample was more (55.18%). Total antioxidant activity (12.13%), total phenols (31.54 mg/100g) and flavonoids (17.53 mg/100g) were maximum in highly acceptable bread sample. The developed multigrain bread had high contents of omega 3 fatty acids (186.16 mg/100g), omega 6 fatty acids (13701.40 mg/100g), phosphorus (68.74 mg/100g), magnesium (28.13 mg/100g) and copper (0.12 mg/100g) as compared to the control sample.

Keywords: Sunflower seeds, therapeutic dietary option, multigrain bread, organoleptic evaluation, nutritive value

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Introduction

The supplementation of bakery products (especially bread and cookies) with oilseeds is a classical route to ameliorate nutritional deficiencies. The enriched products have improved organoleptic properties and high nutritive value in addition to the provision of health benefits. The market of oilseeds supplemented food products is on hike as people urge to adopt robust lifestyle practices. Bread is the most commonly consumed food product due to its economical price and ease of usage [1]. The nutritional quality of bread made from refined wheat flour is poor as it lacks some essential amino acids and inadequately meets the requirements of macro and micro nutrients. The amino acids lysine, threonine and valine are deficient in wheat [2]. Oilseeds such as sunflower seeds and pumpkin seeds can be supplemented in conventional recipes and bakery products. Sunflower seed is an ideal low carbohydrate and high protein raw or roasted snack option. It can also be used as a confectionary nut [3].

Sunflower belongs to the family Asteraceae and is grown all over the world due to its nutritional and medicinal properties. Its notable nutritional, medicinal and culinary advantages have resulted in hike in the popularity and usage of the sunflower and its constituent parts worldwide. The sunflower seed provides a wide range of nutritious components like proteins, fiber, unsaturated fats, vitamin E, iron, selenium, folate, copper, zinc and phytochemicals. It comprises almost 20 % proteins in the form of 11 s globulins and napin type 2s albumins [4]. Sunflower seeds (per 100 g) provide 14 grams of carbohydrates, 7 grams of which comprise of fibre. It has significant amount of glutamic acid, aspartic acid, cysteine and arginine possessing a sound amino acid profile and a low anti nutritional content. The essential amino acids in the seed include phenylalanine, tyrosine, methionine, leucine and cysteine. The seeds contain 35-42 percent oil which has 55-70 percent of linoleic acid being the predominant unsaturated fatty acid followed by 20-25 percent of oleic acid. Alpha tocopherol accounts for 90 percent of the total tocopherols. A substantial amount of phosphorus, magnesium and copper is also present in the seeds [5]. The antioxidant power of defatted sunflower seed shells is majorly due to the content of its phenolic compounds [6]. Therefore, taking into consideration the advantageous effects of sunflower seeds, the present study is framed with the following objectives to supplement sunflower seeds in multigrain bread for nutritional enhancement and to evaluate the chemical composition and sensory parameters of supplemented bread.

Materials and Methods

Procurement and processing of sunflower seeds

Sunflower (*Helianthus annuus*) seeds (hybrid PSH 1962) were procured from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

Processing of sunflower seeds

Sunflower seeds were washed thoroughly under running water to eliminate any foreign particles and dust. After sun drying the clean seeds, they were roasted at 60°C for 10-15 minutes. The roasted seeds were then ground in an electric grinder to obtain fine flour. Sunflower seed flour (in raw form) was used in the development of sunflower seed flour supplemented multigrain bread which was further subjected to nutritional evaluation. Schematic presentation of processing of sunflower seeds is shown in **Figure 1**.

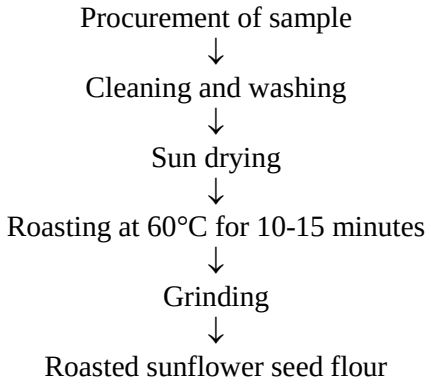

Figure 1 Schematic presentation of processing of sunflower seeds

Development and standardization of sunflower seed flour supplemented multigrain bread

Selection and procurement of ingredients

For the standardization of bread, all the ingredients like refined wheat flour, whole wheat flour, barley flour, oats flour, rice flour, maize flour, compressed yeast, refined oil, powdered sugar and salt were obtained in bulk from the local market whereas sunflower seeds were procured from Punjab Agricultural University, Ludhiana.

Preparation of bread

Five experimental samples of multigrain bread were developed using standardized recipe and sunflower seed flour was incorporated at different levels i.e. 2.5 percent, 5 percent, 7.5 percent, 10 percent and 12.5 percent (**Table 1**). On the other hand, control sample of bread was developed using 100 percent refined wheat flour. All the samples were prepared in the bakery laboratory of Department of Food and Nutrition, College of Community Science, Punjab Agricultural University, Ludhiana.

Organoleptic evaluation of the developed sunflower seed flour supplemented multigrain bread

The developed samples were organoleptically evaluated by a semi-trained panel of 10 judges from Department of Food and Nutrition, College of Community Science, Punjab Agricultural University, Ludhiana. The panel members were served with one control and five experimental samples. Different codes were given to the bread samples so that an unbiased evaluation of the samples was made. Sensory evaluation of the developed samples was carried out to determine the most suitable level of sunflower seed flour incorporated in multigrain bread.

Chemical Analysis

After the development and organoleptic evaluation of all samples of breads, the multigrain bread with highest acceptability along with its corresponding control were weighed, homogenized and oven dried at 105°C. Dried samples were stored in air tight plastic bags for further nutritional evaluation. Estimation of proximate composition i.e. moisture, crude protein, crude fat, crude fibre, total ash, carbohydrate, energy and estimation of minerals i.e. phosphorus, magnesium and copper was done using standardized methods of AOAC [7] while the total antioxidant activity was measured using DPPH method given by Dehshahri *et al* [8]. Estimation of total phenols was done by the

following method of Singleton *et al* [9] and flavonoids by the method of Zhishen *et al* [10]. Omega 3 and omega 6 fatty acids were analyzed using procedure given by Appelqvist [11].

Table 1 Ingredients and method used to prepare bread

Product	Ingredients used		Method
	Control	**Supplemented samples**	
Bread	Refined wheat flour (250g) Fresh yeast (7.5g) Powdered sugar (15g) Calcium powder (1.25g) Gluten powder (2.5g) Salt (5g) Oil (25ml) Water (135ml)	Refined wheat flour (118.75/112.5/106.25/100/93.75g) Wheat flour (37.5g) Barley flour (37.5g) Oats flour (25g) Rice flour (12.5g) Maize flour (12.5g) Sunflower seed flour (6.25/12.5/18.75/25/31.25g) Fresh yeast (7.5g) Powdered sugar (15g) Calcium powder (1.25g) Gluten powder (2.5g) Salt (5g) Oil (25ml) Water (135ml)	• Fresh yeast was dissolved with sugar in lukewarm water (60-70 ml) to prepare flying ferment. • Refined flour was sieved twice along with flour. • Dough was prepared by adding flying ferment and remaining water. • Oil was added to dough and dough was placed for proofing at a temperature of 43-50ºC for 20-25 minutes. • Dough was deflated and pressed gently to expel carbon dioxide. • Bread tin was greased and the dough placed in it for one hour for final proofing. • It was baked at 200 ºC for 25 minutes.

Statistical Analysis

The data was analyzed using various statistical tools such as mean and standard deviation. To test significant differences between various samples of developed multigrain bread, F test and Duncan's Multiple Range Test (DMRT) were applied. Student's t test (for two means) was used to compare the nutritional composition of the developed bread samples. All the data processing was performed using SPSS (Statistical Package for the Social Sciences) software [12].

Result and Discussion

Organoleptic evaluation of bread samples

Organoleptic scores of bread samples are presented in **Table 2**. Samples supplemented with 2.5 percent (E1) and 7.5 percent (E3) sunflower seed flour obtained the highest scores of 8.00 each. The taste of E3 was better than E1. The scores of taste of the developed test samples improved up to 7.5 percent level of supplementation and decreased on further increment in the level of sunflower seed flour. The control (C) grabbed the highest scores for the attributes of appearance, colour and texture when compared to all six bread samples. The colour and flavor of the control and test samples revealed no significant difference up to 7.5 percent supplementation of sunflower seed flour while the difference in textural and taste attributes of all the six samples was statistically non-significant. Multigrain bread supplemented with 7.5 percent sunflower seed flour was found to be highly acceptable.

Proximate composition of developed bread samples

The proximate composition of control and highly acceptable developed sample is given in **Table 3**. It was observed that except the carbohydrate content, all other parameters of proximate composition were higher in the developed sample containing 7.5 percent sunflower seed flour than the control sample. The moisture content of the highly acceptable developed product (31.73%) was significantly higher ($p<0.01$) than the control sample (29.41%) which is thought to be due to the inclusion of multigrain flours and sunflower seed flour. The moisture content increased due to integration of grains which absorbed the moisture during bread making and released it during baking process [13].

There was a significant increment in the amount of crude protein i.e. from 9.13 percent in control to 10.61 percent in most acceptable sample. The crude fat content of control and highly acceptable developed product was 1.26 percent and 2.92 percent while the crude fibre significantly increased ($p<0.01$) from 2.52 percent to 11.36 percent. Malik *et al* [14] reported that the crude protein content of barley flour (11.65%), oats flour (9.60%) and maize flour (9.78%) was

higher than wheat flour (9.55%). The crude fat and crude fibre content of these flours was also more than the wheat flour. So the multigrain bread developed by using combination of different flours had higher proximate parameters of protein, fat and fibre than the control sample. Dasappa *et al* [15] prepared whole wheat bread by using multigrain mix of soyabean, fenugreek seeds, oats, sesame seeds and flaxseeds at varying levels (5, 10, 15 and 20%) and found that substitution of multigrain mix at 15 percent level of whole wheat flour significantly improved the protein, fat and fibre contents by 1.5, 5 and 2.5 times respectively. Nadeem *et al* [3] found that addition of sunflower seeds (at 6, 10 and 14% levels) increased the crude fat, crude fibre and total ash contents of breads. The fat content of whole wheat bread (control) was 1.75 percent which increased to 13.40 percent when sunflower seeds were supplemented at 14 percent level. There was a significant increase in crude protein from 14.36 percent in control sample to 15.62 percent in 14 percent sunflower seed supplemented bread.

Table 2 Organoleptic evaluation of the developed sunflower seed flour supplemented multigrain bread

S. No	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
C	8.30^{a} ±0.48	8.40^{a} ±0.52	7.90^{a} ±0.74	7.90^{a} ±0.57	7.90^{a} ±0.74	7.90^{a} ±0.57
E1	8.00^{ab} ±0.47	8.10^{a} ±0.74	7.90^{a} ±0.74	8.10^{a} ±0.32	8.00^{a} ±0.47	8.00^{a} ±0.67
E2	7.90^{ab} ±0.57	8.00^{a} ±0.82	7.80^{a} ±0.92	8.00^{a} ±0.47	8.10^{a} ±0.47	7.90^{a} ±0.74
E3#	7.90^{bc} ±0.52	8.00^{a} ±0.74	7.80^{a} ±0.63	8.10^{a} ±0.00	8.20^{a} ±0.00	8.00^{a} ±0.47
E4	7.00^{c} ±0.82	6.70^{b} ±0.82	7.10^{a} ±0.63	7.70^{ab} ±0.48	7.80^{a} ±0.48	7.00^{b} ±0.67
E5	7.10^{c} ±1.10	6.90^{b} ±0.88	7.20^{a} ±1.20	7.30^{b} ±1.06	7.70^{a} ±0.48	7.20^{b} ±0.79
F Ratio	5.482	8.354	1.925	2.600	0.893	4.585
P value	0.00**	0.00**	0.105^{NS}	0.035*	0.492^{NS}	0.001**
C.D.	0.61	0.67	0.73	0.51	0.43	0.58

Means with different notations (a, b, c and d) indicate significant difference at 5% level of significance.
C (Control) = 100% refined flour
E1 = 47.5% refined flour, 15% wheat flour, 15% barley flour, 10% oats flour, 5% maize flour, 5% rice flour + 2.5% sunflower seed flour
E2 = 45% refined flour, 15% wheat flour, 15% barley flour, 10% oats flour, 5% maize flour, 5% rice flour + 5% sunflower seed flour
E3 = 42.5% refined flour, 15% wheat flour, 15% barley flour, 10% oats flour, 5% maize flour, 5% rice flour + 7.5% sunflower seed flour
E4 = 40% refined flour, 15% wheat flour, 15% barley flour, 10% oats flour, 5% maize flour, 5% rice flour + 10% sunflower seed flour
E5 = 37.5% refined flour, 15% wheat flour, 15% barley flour, 10% oats flour, 5% maize flour, 5% rice flour + 12.5% sunflower seed flour
Values are Mean ± S.D. * Values are significant at 5% level, ** Values are significant at 1% level, NS – Non significant
most acceptable level of supplementation (at 7.5 percent level) when compared to control sample

Table 3 Proximate composition of multigrain bread developed using sunflower seed flour (dry weight basis)

Treatment	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Total Ash (%)	Carbohydrates (%)	Energy (Kcal/100 g)
Control	29.41±0.52	9.13±0.90	1.26±0.14	2.52±0.27	2.50±0.30	55.18±1.34	268.58±2.78
Experimental	31.73±0.23	10.61±0.28	2.92±0.22	11.36±0.48	3.29± 0.22	21.46±1.33	324.48±3.53
t- test	19.20**	2.71*	4.44**	27.51**	3.71*	25.39**	32.75**

Values are Mean ± S.D., * Values are significant at 5% level, ** Values are significant at 1% level
Experimental – multigrain bread supplemented with 7.5 percent sunflower seed flour

The total ash content of the highly acceptable developed product (3.29%) was significantly more ($p<0.05$) than the control (2.50%).The ash contents of barley, oats, maize and rice flours used in the developed multigrain bread was more than the wheat flour i.e. 2.2 percent, 1.7 percent, 2 percent, 0.76 percent and 0.66 percent respectively [14]. The carbohydrate content of highly accepted sample (21.46%) showed a significant reduction ($p<0.01$) with 62 percent less than the control (55.18%). The total carbohydrate content of bread is mainly dependent on the amount of bread flour or refined flour [16]. The addition of sunflower seeds replaced the percentage of refined flour thus reducing the carbohydrate content of the highly acceptable developed product. The energy content of 100 g of developed product (324.48 Kcal/100g) was 20.80 percent higher than the control sample (268.58 Kcal/100g). Ugwuona and Obeta [17] found that bread fortified with 20 percent whole and defatted sesame seeds reduced the carbohydrate level from 55.44 percent to 50.37 percent (for whole sesame seeds) and 48.68 percent (for defatted ones). Also, the addition of sesame seeds upgraded the content of other proximate parameters like crude protein, crude fibre, lipid and total ash.

Skrbic and Filipcev [2] supplemented high oleic acid sunflower seeds at different levels (8%, 12% and 16%) in breads prepared from refined flour and whole wheat flour. The amount of protein and total ash increased non-significantly and that of crude fat and crude fibre significantly as the percentage of sunflower seeds in breads increased from 8 percent to 16 percent. The values of crude protein, crude fibre, crude fat and ash were higher in whole wheat breads as compared to refined flour breads.

Mineral content

The mineral content of control and highly acceptable developed bread sample is given in **Table 4**. There was a non-significant increase in copper and magnesium contents of organoleptically most accepted developed sample in comparison to the control sample. The amount of phosphorus in highly acceptable product was significantly high ($p<0.01$) than the control. This increase in mineral content may be attributed to the inclusion of multigrain flours and sunflower seed flour in the developed bread. Skrbic and Filipcev [2] reported that addition of sunflower seeds at 16% level in white bread and at 12 and 16 percent level in wheat bread significantly increased the copper content. Supplementation of sunflower seeds increased the copper, manganese and zinc contents of whole wheat breads.
The cake formulated by adding 15 percent sunflower seeds had considerably high amount of magnesium (111 mg/100 g), manganese (0.92 mg/100 g), iron (1.89 mg/100 g) and potassium (253 mg/100 g). Except for calcium and sodium, all other minerals increased with increasing percentage of sunflower seed flour (at 5%, 10% and 15% of flour) in the cake [18]. Nadeem *et al* [3] used sunflower seeds (with and without hull) at 6, 10 and 14 percent levels to prepare wheat bread and found that supplementation of dehulled sunflower seeds enhanced the overall nutritional quality of whole wheat breads.

Table 4 Mineral content of multigrain bread developed using sunflower seed flour (on dry weight basis)

Treatment	Phosphorus (mg/100g)	Magnesium (mg/100g)	Copper (mg/100g)
Control	37.28 ± 0.10	22.83 ± 15.84	0.08 ± 0.01
Experimental	68.74 ± 0.19	28.13 ± 0.07	0.12 ± 0.03
t- test	257.78**	0.58 ^{NS}	2.29 ^{NS}

Values are Mean ± S.D., ** Values are significant at 1% level, NS – Non significant
Experimental – multigrain bread supplemented with 7.5 percent sunflower seed flour

Total antioxidant activity

The total antioxidant activity of control and highly acceptable developed sample calculated by the 2,2-di-phenyl-2-picryl-hydrazyl (DPPH) method is given in **Table 5**. The antioxidant activity of the multigrain bread supplemented with 7.5 percent sunflower seed flour had significantly high ($p<0.01$) DPPH scavenging potential or antioxidant activity than the control sample. The difference between the two samples was nearly 59 percent which may be due to different cereal flours and sunflower seeds in the formulated bread that have good antioxidant potential. Brindzova *et al* [19] found that the bakery products supplemented with other cereals in addition to wheat flour exhibit high antioxidant properties as compared to the control sample. Harris and Kris [20] reported that thermal processing of grains enhances the availability of antioxidants. Hung *et al* [21] stated that refined wheat flour bread has three folds reduced antioxidant activity because most antioxidant components are present in the bran and aleurone layers. Aludatt *et al* [22] observed the supplementation of wheat flour at 5, 10 and 15 percent levels with barley flour and protein isolate from barley increases the total antioxidant activity of rusks. Angioloni and Collar [23] reported that the value addition of various cereals like oats, rye, buckwheat and wheat flour in bread enhanced the total antioxidant potential. Additionally, the black hulls of oats and β glucan present in barley have extra ordinary free radical chelating properties. Cereal flours have high phenolic acids that chelate free radicals exhibiting antioxidant properties and prevent lipid peroxidation [24].

Bioactive compounds

The bioactive compounds of control and highly acceptable developed bread sample are given in Table 5. Multigrain bread supplemented with 7.5 percent sunflower seed flour had more amount of total phenolic and total flavonoid content than the control sample. The total phenols (in gallic acid equivalent) and total flavonoids (in quercetin equivalent) in control sample were 27.06 mg/100 g and 2.82 mg/100 g while that in organoleptically most liked bread sample were 31.54 mg/100 g and 17.53 mg/100 g. Sunflower has the highest amount of phenolic components as compared to flaxseeds, pumpkin seeds, poppy seeds and sesame seeds [25]. Adom and Liu [26] analyzed the phytochemical composition of different grains and discovered that corn had the highest value of total phenols

followed by wheat, oats and rice. Nyam and Tan [16] reported that addition of 5 percent pumpkin seeds in bread increased the total phenolic content from 15.21 mg/100 g to 35.66 mg/100 g.

Sayed-Ahmad *et al* [27] observed that chia supplementation (at 2, 4 and 6% levels) enhanced the total phenolic content of wheat bread. The highest content of total phenolics was found in whole grain flours and cereal grains of rye (4190 mg/kg) and wheat bran (4527 mg/kg). Bread made from refined wheat flour and whole rye grain had 111 and 765 mg phenolics per Kg. After ferulic acid, the most prevailing phenols found in cereal products were sinapic acid, p-coumaric acid and ferulic acid dehydrodimers [28]. Gawlik-Dziki *et al* [29] observed that the antioxidant activity of phenolic compounds is not destroyed even after baking process. Oats contain distinctive components named avenalumic acids and avenanthramides (26-27 mg/Kg) which exhibit high antioxidant properties

Table 5 Total antioxidant activity and bioactive compounds of multigrain bread developed using sunflower seed flour (on dry weight basis)

Treatment	Total antioxidant activity (% inhibition)	Total Phenols (mg GAE/100g)	Flavonoids (mg QE/100g)
Control	7.61 ± 0.23	27.06 ± 3.22	2.82 ± 1.28
Experimental	12.13 ± 0.37	31.54 ± 3.94	17.53 ± 5.15
t- test	9.42	6.68	4.80
P value	0.01**	0.01**	0.01**

Values are Mean ± S.D., ** Values are significant at 1% level, NS – Non significant
Experimental – multigrain bread supplemented with 7.5 percent sunflower seed flour

Omega 3 and omega 6 fatty acids

The data (**Table 6**) revealed that addition of sunflower seeds in multigrain bread enhanced the contents of essential fatty acids. Omega 3 fatty acid content in control sample was 148.69 mg per 100 g which increased to 186.16 mg per 100 g in highly acceptable developed sample and the amount of omega 6 fatty acid increased by 93 percent from 7104.94 mg per 100 g (in control) to 13701.40 mg per 100 g (in the sample supplemented with 7.5% sunflower seed flour). Skrbic and Filipcev [2] proclaimed that addition of high oleic acid sunflower seeds at 16 percent level non-significantly improved the contents of omega 3 and omega 6 fatty acids in refined flour bread from 47.7 mg per 100 g to 50.1 mg per 100 g and 509 mg per 100 g to 1973 mg per 100 g. Similarly omega 3 and omega 6 fatty acids in whole wheat bread increased from 56.4 mg per 100 g to 59 mg per 100 g and from 732 mg per 100 g to and 2209 mg per 100 g respectively.

Osuna *et al* [30] reported that the wheat breads developed using flaxseed flour at 5, 10 and 15 percent levels had significantly higher levels of n-3 fatty acid. The addition of chia flour at the rate of 7.8 g per 100 g (F7.8) and 11 g per 100 g (S11) in wheat bread enhanced the linoleic and linolenic acid content. The amount of linoleic acid in control was 0.86 which increased to 1.02 and 1.10 in F7.8 and S11 while alpha linolenic acid increased from 0.03 in control to 1.21 in F7.8 and 1.85 in S11 samples [13]. Romankiewicz *et al* [31] studied the effects of adding chia seed flour at various percentages i.e. 2, 4, 6 and 8 percent to whole wheat bread. The proportion of alpha linolenic increased but linoleic acid decreased with increase in addition of chia seed flour.

Table 6 Omega 3 and Omega 6 fatty acid composition of multigrain bread developed using sunflower seed flour (on dry weight basis)

Parameter	Control	Experimental	t-test
Omega 3 fatty acid (mg/100 g)	148.69 ± 1.26	186.16 ± 0.86	45.33**
Omega 6 fatty acid (mg/100 g)	7104.94 ± 4.75	13701.40 ± 2.28	1855.99**

Conclusion

The study concluded that enrichment of sunflower seed flour up to 7.5 percent level in multigrain bread was found to be highly acceptable. Not only the organoleptic properties of the bread improved but its nutritional value also enhanced after the addition of sunflower seed flour. The highly acceptable developed product had higher amounts of protein, fat, fibre, ash, energy, total antioxidant activity, total phenols, flavonoids, omega 3 and omega 6 fatty acids as well as minerals i.e. phosphorus, magnesium and copper as compared to the control sample. Thus, value addition of sunflower seed flour in bakery products can be a consequential proposition to improve their nutritive value. The consumption of such products routinely can enhance the nutritional status of individuals and pose therapeutic properties as well.

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Publication History
Received 01.02.2020
Revised 04.03.2020
Accepted 11.04.2020
Online 30.04.2020