Evaluation of the chemical properties of cookies prepared by utilizing wheat germ oil and defatted wheat germ.

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Abstract: The research work was conducted to characterize the wheat germ and assess its suitability for the preparation of cookies. The cookies made by using WGO was evaluated for chemical composition. The wheat flour was supplemented with DFWG flour at the levels of 0, 5, 10, 15, 20 and 25% to prepare different flour blends. The cookies were prepared from these flour blends and were tested for their chemical characteristics and mineral profile. The moisture content of the cookies prepared from different levels of normal shortening replacement with WGO was not affected significantly with the variation in the level of WGO however; moisture content of WGO based cookies increased significantly during the storage period of two months. The crude protein, crude fat, crude fiber, ash and NFE content of WGO based cookies were neither affected significantly by the treatment nor the storage intervals. The TBA value of cookies was significantly decreased by the incorporation of WGO in the cookies formulation while significant increase was observed in this parameter throughout the storage period. The highest TBA value (0.11 mg melonaldehyde/kg) was observed in the control treatment (100% normal shortening) while the lowest TBA value (0.05 mg melonaldehyde/kg) was possessed by cookies prepared from 100% WGO. The rancidity of cookies increased with the passage of time was evident from the higher TBA value (0.13 mg melonaldehyde/kg) at the end of study.

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1. Introduction

Wheat (*Triticum aestivum*) is a major cereal crop grown in many parts of the world. On global basis, wheat and rice accounts for over 50% of the total cereal production in world (Lookhart and Bean, 2000). The wheat grain consists of three distinct parts, the bran, the germ and the endosperm. The distribution of these parts in wheat kernel is as: 82 to 83% endosperm, 12 to 14% bran and 2 to 4% germ (Posner, 2000). The wheat germ is the embryo of wheat, which has reproductive function and separated during milling from endosperm because it adversely influences the keeping quality as well as the processing quality of the wheat flour. In the recent years, with the development of new technologies, the most commercial milling of wheat into flour aims at the maximum extraction of the endosperm without minimum germ contamination. Resultantly, there is a substantial deposit of the wheat germ in the world. Keeping in view the total wheat production of Pakistan, 0.5 million tones wheat germ production in Pakistan was estimated during the year 2007-08 (GOP, 2008). The wheat grain consists of three distinct parts, the bran, the germ and the endosperm. The distribution of these parts in wheat kernel is as: 82 to 83% endosperm, 12 to 14% bran and 2 to 4% germ (Posner, 2000). The wheat germ is the embryo of wheat, which has reproductive function and separated during milling from endosperm because it adversely influences the keeping quality as well as the processing quality of the wheat flour. In the recent years, with the development of new technologies, the most commercial milling of wheat into flour aims at the maximum extraction of the endosperm without minimum germ contamination. Resultantly, there is a substantial deposit of the wheat germ in the world. Keeping in view the total wheat production of Pakistan, 0.5 million tones wheat germ production in Pakistan was estimated during the year 2007-08 (GOP, 2008).

Most of the nutrients in the wheat grain with the exception of starch are concentrated in the germ. It is an excellent source of vitamins, minerals, dietary fiber, calories, proteins, and some functional micronutrients (Shurpalekar and Rao, 1977). The wheat germ not only possesses proteins of high biological value but also its oil is of desirable fatty acid profile and richest natural source of tocopherols (Barns and Tayler, 2006). Due to its composition, the wheat germ is praised as "the natural nutrient treasure-house and life source of mankind" because of its high nutritive value and palatability. The wheat germ contains about 11-14% oil (Singh and Rice, 1980), which can be extracted from wheat germ either by solvent extraction (90% recovery) or by mechanical pressing (50% recovery) (Gomez and Ossa, 2000). The oil in wheat germ has been found to reduce plasma and liver cholesterol in animals (Kahlon, 1989) due to presence of highly beneficial polyunsaturated fatty acids and bioactive compounds like octacosanol and tocopherols (Saito and Yamauchi, 1990).

The wheat germ oil comprises of high content of polyunsaturated fatty acids among which about 80%, mostly linoleic (18:2) and linolenic (18:3) fatty acids (Wang and Johnson, 2001). These fatty acids are essential and also are precursors of a group of hormones called prostaglandins, which play an important role in muscle contractions and in the proper healing of inflammatory processes (Coultate, 1995). Grundy and Denke (1990) estimated that increasing linoleic acid intake lowers cholesterol about half as much as saturated fatty acids increase it. The wheat germ oil contains the highest content of natural antioxidants i.e. tocopherols up to about 1850 mg/kg oil (Davis *et al.,* 1980). Wheat germ oil is more stable to oxidation or rancidity than many other oils due to the antioxidant properties of tocopherols (Haas, 2006). The enrichment of cereal-based foods for improvement in nutritional quality has received considerable attention. Wheat bread and cookies are widely consumed in many developing countries, and, therefore, offer a valuable supplement for nutritional improvement; however cookies are suggested as a better use than bread because of their ready-to-eat form, wide consumption and relatively extended shelf-life (Lorens *et al*. 1979).

On the other hand, after extraction of oil from wheat germ, the defatted wheat germ is also highly nutritive value protein material, which contains about 30% protein (Ge *et al.* 2000). The cereals possess some essential amino acids in relatively low concentrations. The first limiting amino acid in wheat is lysine, which demands that wheat should be supplemented with high lysine-rich proteins to provide a balanced diet (Pichardo *et al.,* 2003). The endosperm contains higher gluten content which is low in lysine however; albumins and globulins are rich in lysine and are located mainly in the bran and the germ parts of wheat kernel (Lookhart and Bean, 2000). The amino acid content of defatted wheat germ protein is higher than the FAO/WHO pattern for essential amino acids (Semenyuk and Dyakonova, 1986). The lysine content of defatted wheat germ has been 5.3 to 6.3% and it also possesses higher levels of minerals (Matz, 1991). The population is suffering with malnutrition mainly due to protein deficiency. The prevalence of varying degree of protein deficiency in some vulnerable groups of population, due to intake of low quality and quantity of protein has been reported in Pakistan (Anon, 2004). The cereals contribute more than 60% to the total protein intake and provide calories to the people of Pakistan. The consumption of animal proteins in Pakistan is low because of its limited supply and high prices. Thus the incorporation of defatted wheat germ protein in bakery products can provide the required essential amino acids to cope this deficiency.

In Pakistan, little or no efforts have been made to eliminate or reduce the risk of diseases through diet therapy. There is a need to introduce such raw materials rich in nutrients in daily diet chart which can reduce the threat of protein deficiency, cancer and cardiovascular diseases. The wheat germ utilization in food products has not yet been explored. Keeping in view the nutritional importance and availability of wheat germ, the present research was conducted.

2. Materials and methods

The research work was conducted to characterize the wheat germ and assess its suitability for the preparation of cookies. The cookies made by using WGO was evaluated for chemical composition.

2.1 Extraction of WGO

The oil was extracted from wheat germ through solvent extraction technique by using n-hexane. The extracted oil was heated at 40°C to remove the last traces of solvent. The crude oil recovered in this way was kept in desiccator over anhydrous calcium chloride for twenty four hours, so that the traces of moisture (if present) could be removed. The percentage recovery of oil was calculated by the formula:

$$\text{Wheat germ oil (\%)} = \frac{\text{Wt.of oil (g)}}{\text{Wt.of wheat germ sample (g)}} \times 100$$

2.2 Characterization and utilization of defatted wheat germ (DFWG)

2.2.1. Preparation of DFWG flour

The defatted wheat germ (DFWG) residue after oil extraction from collected germ was smashed and passed through a 200-mesh sieve to obtain DFWG flour, which was packed in polypropylene bags for further studies.

2.2.2. Flour blends formulation

Table.1. Formulation of DFWG supplemented flour blends

Treatments	Wheat Flour (%)	DFWG Flour (%)
T_0	100	0
T_1	95	5
T_2	90	10
T_3	85	15
T_4	80	20
T_5	75	25

The flour blends were prepared by incorporating DFWG flour in to wheat flour at different concentration levels for the preparation of high protein cookies. The wheat flour was replaced with DFWG flour for blends formulation at different levels as given here in Table 1.

The choice of the above levels of DFWG was based on the report of Dreuiter (1978) who reported the maximum level (25%) of wheat flour substitution for an acceptable baked product.

2.3 Chemical analysis of DFWG supplemented cookies

2.3.1. Proximate analysis

The cookie samples were analyzed for moisture, ash, crude protein, crude fat, crude fiber and nitrogen free extract (NFE) on fortnightly basis for a period of two months according to their respective methods of AACC (2000).

2.3.2. Mineral contents

The minerals (calcium, magnesium, iron, phosphorous, potassium, zinc) of the cookies were determined at storage intervals of 0, 15, 30, 45 and 60 days according to the method given in AOAC (2000).

2.3.3. Thiobarbiteuric acid (TBA) value

The thiobarbiteuric acid value (TBA) is based on reaction between the secondary oxidation products of fats and oils and 2-thiobarbituric acid forming condensation products whose absorbance is measured at 530 nm. The TBA value of the cookies was recorded using the method of Pokorny and Dieffenbacher (1989).

3. Results and discussions

3.1. Chemical composition of DFWG supplemented Cookies

3.1.1. Moisture content

Table 2. Mean squares for proximate composition of DFWG supplemented cookies

SOV	df	Moisture	Crude protein	Crude fat	Crude fibre	Ash	NFE
Treatments (A)	5	1.139^{**}	201.159^{**}	40.231^{**}	1.998^{**}	1.769^{**}	95.908^{**}
Storage intervals (B)	4	2.312^{*}	0.556^{NS}	0.039^{NS}	0.001^{NS}	0.002^{NS}	0.959^{*}
A x B	20	0.003^{NS}	0.053^{NS}	0.002^{NS}	0.0006^{NS}	0.0001^{NS}	0.005^{NS}
Error	60	0.028	0.863	0.024	0.015	0.002	0.331

**Significant (P < 0.01)
*Significant at (P < 0.05)
^{NS} Non significant

Table 3. Effect of treatments and storage on moisture contents (%) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					
	0	15	30	45	60	Mean
T_0	6.37	6.39	6.42	6.75	7.10	**6.60^{f}**
T_1	6.49	6.51	6.55	6.85	7.21	**6.72^{e}**
T_2	6.63	6.64	6.69	7.02	7.47	**6.89^{d}**
T_3	6.78	6.80	6.86	7.15	7.60	**7.03^{c}**
T_4	6.90	6.92	6.97	7.28	7.63	**7.14^{b}**
T_5	7.05	7.08	7.14	7.39	7.74	**7.28^{a}**
Mean	**6.70^{e}**	**6.85^{d}**	**7.05^{c}**	**7.29^{b}**	**7.60^{a}**	

Means carrying same letters within a column or row do not differ significantly (P < 0.01)
T_0 = Cookies prepared from 0% DFWG flour supplementation
T_1 = Cookies prepared from 0% DFWG flour supplementation
T_2 = Cookies prepared from 0% DFWG flour supplementation
T_3 = Cookies prepared from 0% DFWG flour supplementation
T_4 = Cookies prepared from 0% DFWG flour supplementation
T_5 = Cookies prepared from 0% DFWG flour supplementation

The statistical results regarding moisture content of cookies prepared from different flour blends presented in Table 2 indicated that the treatments and storage intervals significantly affected the moisture content of the cookies. However, interaction between storage intervals and treatments was found for non significant effect on this attribute (Table 2). The moisture content of the cookies prepared from different flour blends indicated that the moisture content ranged from 6.72 to 7.46% among different treatments (Table 3). It is obvious from the results that the moisture content was found to be significantly higher in T_5 (25% DFWG flour). The cookies containing 0% DFWG (T_0) showed significantly lower content of moisture (6.72%) as compared to cookies prepared from other treatments of (Table 3). The moisture content of cookies in the present study was observed to be increased during different storage intervals. The moisture content at the initiation of the experiment was 6.70% which increased to 7.60% after expiring of 60 days storage. Increase in the moisture content of cookies may be attributed to the hygroscopic nature of the cookies and change in the relative humidity during storage as the polypropylene bags repeatedly opened for drawing of samples for analysis at each storage interval. The moisture content was found significantly higher in the cookies when supplemented with DFWG flour irrespective of the level of supplementation. Ge *et al.* (2000) demonstrated that DFWG enhanced moisture when added in the wheat flour for the production of cookies which are supportive to the findings of the present study.

3.2 Crude protein

The statistical results for protein content of cookies prepared from different flour blends given in Table 2 revealed that the protein content in the cookies differed significantly among the treatments. However, the protein content did not differ significantly due to storage intervals and the interaction between treatments and storage intervals. The protein content of cookies prepared from different flour blends shown in Table 4 indicated that the protein content in the cookies significantly increased from 11.55% to 15.95% with the increase in the highest level of DFWG flour in flour blends. The cookies containing 25% DFWG flour (T_5) in flour blends possessed the highest protein content (15.95%), while the lowest protein content (11.55%) were observed in the cookies prepared from control treatment (0% DFWG flour). It is obvious from the results that DFWG flour addition had shown positive impact on the protein content of cookies. The results regarding protein content also revealed that the protein content of cookies prepared from different flour blends did not differ significantly during the storage. However, a non significant decrease was observed during the last 30 days of storage. At the beginning the protein content was 13.86% which decreased to 13.70% and 13.57 % after 30 and 60 days of storage, respectively, but protein content between these two storage intervals was found non significantly different.

Table 4. Effect of treatments and storage on crude protein (%) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					Mean
	0	15	30	45	60	
T_0	11.71	11.62	11.55	11.47	11.42	**11.55^f**
T_1	12.5	12.48	12.35	12.3	12.27	**12.38^e**
T_2	13.45	13.31	13.21	13.14	13.09	**13.24^d**
T_3	14.26	14.23	14.15	14.06	13.98	**14.14^c**
T_4	15.11	15.09	15.02	14.93	14.87	**15.00^b**
T_5	16.14	16.08	15.9	15.86	15.79	**15.95^a**
Mean	**13.86^a**	**13.80^a**	**13.70^a**	**13.63^a**	**13.57^a**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

3.3. Crude fat

The statistical results presented in Table 2 indicated that the fat content in cookies prepared from different flour blends varied significantly due to variation in different treatments. However, the storage intervals and the interaction between treatments and storage intervals was found to be non significant for this parameter. The crude fat content in cookies prepared by substituting straight grade flour with DFWG flour in different proportions is given in Table 5. The crude fat content in the cookies prepared from control straight grade flour (T_0) was 13.89%, which decreased gradually as the proportion of DFWG flour increased in the cookies. The DFWG flour incorporation to straight grade flour reduced the fat content of cookies from 13.89 to 13.63%. This decrease in fat content was due to presence of lower fat content (0.5%) in DFWG which reduced the overall fat content of cookies. The effect of storage on the fat content of cookies has been given in Table 4.10. The fat content was found to be the highest in freshly prepared cookies, when analyzed at the initiation of study. However, the fat content decreased non-significantly as a function of storage interval and observed to be 13.71% at the expiry of study i.e. 60 days. Since the formulation of cookies contained additional amount of fat which contributed the higher amount of fat than the raw materials used for the preparation of cookies.

Table 5. Effect of treatments and storage on crude fat (%) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					Mean
	0	15	30	45	60	
T_0	13.95	13.92	13.86	13.88	13.83	**13.89^a**
T_1	13.79	13.75	13.78	13.75	13.71	**13.76^b**
T_2	13.77	13.71	13.7	13.73	13.69	**13.72^c**
T_3	13.72	13.73	13.69	13.66	13.67	**13.69^d**
T_4	13.68	13.75	13.64	13.68	13.67	**13.68^d**
T_5	13.64	13.61	13.62	13.63	13.66	**13.63^e**
Mean	**13.76^a**	**13.75^a**	**13.72^a**	**13.72^a**	**13.71^a**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

3.4. Crude fiber

The analysis of variance for crude fiber of cookies prepared from different flour blends has been shown in Table 2. It is obvious from the results that significant differences existed in the crude fiber content due to treatments i.e. flour blends however; storage intervals possessed non significant effect on this attribute. Similarly, the interaction between both of these variables was found to be non significant for crude fiber content.

The crude fiber content of cookies prepared from different flour blends is given in Table 6. The results revealed that the crude fiber differed significantly among different treatments and was found to be the highest (3.25%) in the cookies prepared from T_5 (25%DFWG flour) followed by T_4(3.17%), which contained 20% DFWG flour. The crude fiber content of the cookies prepared from control T_0 (0% DFWG flour) was found to be the lowest (2.33%) as compared to all other treatments. It has been observed that there was a significant increase in the crude fiber content of cookies when supplemented with DFWG flour irrespective the level of incorporation in wheat flour. The results further exhibited non significant effect of storage on crude fiber of cookies (Table 4.7). The crude fiber content of cookies was 2.85, 2.83, 2.85, 2.84 and 2.83% at 0, 15, 30, 45 and 60 days, respectively. Butt *et al.* (2003) stated that the fiber content in the biscuits was non significantly influenced by the storage intervals which confirms the results of present study.

Table 6. Effect of treatments and storage on crude fiber (%) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					Mean
	0	15	30	45	60	
T_0	2.33	2.32	2.34	2.35	2.31	**2.33^e**
T_1	2.56	2.54	2.55	2.53	2.57	**2.55^d**
T_2	2.76	2.75	2.74	2.76	2.73	**2.74^c**
T_3	3.01	2.99	3.02	3.01	2.97	**3.00^b**
T_4	3.19	3.17	3.19	3.16	3.18	**3.17^a**
T_5	3.27	3.24	3.26	3.25	3.27	**3.25^a**
Mean	**2.85^a**	**2.83^a**	**2.85^a**	**2.84^a**	**2.83^a**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

3.5. Ash content

The results pertaining to the analysis of variance for ash content of cookies prepared from different flour blends are given in Table 2. The statistical results showed that the storage intervals did not significantly affect the ash content. However, the treatments possessed highly significant effect on the ash content of cookies. The results further revealed that interaction between treatments and storage intervals showed non significant effect on the ash content of cookies.

The ash content of cookies shown in Table 7 revealed that the ash content ranged from 0.74 to 1.69%. The highest ash content (1.69%) was found in T_5 (25% DFWG flour) while the cookies prepared from control treatment T_0 (0% DFWG flour) showed the lowest level of ash content. The results exhibited significant increase in the ash content of cookies prepared from incorporation of different levels of DFWG flour. The higher content of ash in the treatments containing DFWG flour is due to presence of higher amount of inorganic matter in DFWG flour as compared to straight grade flour. DFWG flour has shown higher level of ash content i.e. 4.52% as compared to straight grade flour (0.74%), which certainly helped to increase the ash content of cookies supplemented with DFWG. The ash content of cookies (Table 7) also showed a non significant effect among storage intervals. The ash content at the start of experiment was 1.22% which differed non significantly with the ash content of cookies tested at the expiry of the experiment i.e. after 60 days of storage. Butt *et al.* (2003) also observed similar non significant effect of storage on the ash content of cookies and cakes, respectively. The stability of ash content during storage period indicated that there was non significant loss of minerals in the cookies during the storage.

Table 7. Effect of treatments and storage on ash contents (%) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					
	0	15	30	45	60	Mean
T_0	0.76	0.75	0.75	0.74	0.73	**0.74^f**
T_1	0.95	0.95	0.93	0.92	0.92	**0.93^e**
T_2	1.13	1.12	1.11	1.11	1.1	**1.11^d**
T_3	1.29	1.28	1.27	1.28	1.26	**1.27^c**
T_4	1.5	1.48	1.47	1.47	1.48	**1.48^b**
T_5	1.69	1.68	1.67	1.67	1.66	**1.67^a**
Mean	**1.22^a**	**1.21^a**	**1.20^a**	**1.19^a**	**1.19^a**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

3.6. Nitrogen free extract (NFE)

The results regarding analysis of variance for NFE content of cookies prepared from different flour blends presented in Table 2 indicated that NFE content of cookies was affected significantly due to the substitution levels of DFWG flour as well as the storage intervals. However, the interaction between treatments and storage intervals exhibited non significant effect on NFE content of the cookies.

The NFE content of cookies (Table 4.13) was found the highest (62.93%) in T_0 (0% DFWG flour) followed by T_1 (61.56%) which contained 5% DFWG flour. The lowest content of NFE (56.23%) was found in T_5 (25% DFWG flour). The NFE content ranged 59.15% to 62.93% in all the treatments of cookies. It is obvious from the results (Table 8) that NFE content of the cookies decreased with the increase in the level of DFWG flour in the formulation of cookies. The results presented in Table 4.13 further showed that the NFE content of the cookies was also increased significantly during first 30 days storage but it was remained unchanged during the next 30 days of storage period. The NFE content increased significantly from 59.15% (0 days) to 59.74% (30 days) but the results showed a slight reduction in the coming days and the NFE content at the final of study period (59.54%).

Table 8. Effect of treatments and storage on NFE (%) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					
	0	15	30	45	60	Mean
T_0	62.58	62.93	63.03	63.11	63.01	**62.93^a**
T_1	61.21	61.56	61.76	61.75	61.52	**61.56^b**
T_2	59.86	60.17	60.45	60.36	60.22	**60.21^c**
T_3	58.43	58.85	59.07	59.00	58.82	**58.83^d**
T_4	57.03	57.40	57.68	57.57	57.44	**57.42^e**
T_5	55.81	56.26	56.46	56.37	56.26	**56.23^f**
Mean	**59.15^b**	**59.52^{ab}**	**59.74^a**	**59.69^a**	**59.54^{ab}**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

3.7. Calcium

The analysis of variance for calcium content of cookies prepared from different flour blends has been presented in Table 9. It is obvious from the statistical results that the calcium content in cookies varied significantly due to variation in treatments while, the storage intervals and the interaction between treatments and storage intervals exhibited non significant effect on the calcium content of cookies.

It is evident from the results in Table 10 that calcium content was found to be significantly higher in the cookies prepared from DFWG blended flours as compared to the cookies prepared from the control treatment (0% DFWG). The calcium content ranged from 41.95 mg/100g to 52.96 mg/100g among the cookies prepared from different flour blends. It is obvious from the results that the calcium content was found to be significantly the highest (52.96 mg/100g) in the cookies containing 25% DFWG flour, while the lowest calcium content (41.95 mg/100g) was observed in the control treatment that was prepared from wheat flour without DFWG flour addition. The results depicted that the calcium content increased gradually among the treatments with the increase in the level of DFWG flour supplementation in the flour blends. The increase in the calcium content is due to the higher content of calcium in DFWG as compared to straight grade flour. The results presented in Table 2 showed that DFWG flour contained 45.93 mg/100g calcium content as compared to the 32.9 mg/100g of straight grade wheat flour. The results in Table 10 showed that the calcium content of the cookies differed non significantly throughout the storage period. The calcium content of the cookies ranged from 50.27 mg/100g to 50.09mg/100g at the beginning and end of study, respectively. Since the DFWG contained higher amount of calcium, it resulted towards the increase in calcium content of cookies.

Table 9. Mean squares for mineral content of DFWG supplemented cookies

SOV	df	Calcium	Iron	Potassium	Phosphorous	Magnesium	Zinc
Treatments (A)	5	253.061^{**}	7.375^{**}	91626.74^{**}	60232.99^{**}	4498.994^{**}	28.806^{**}
Storage intervals (B)	4	0.0938^{NS}	0.167^{NS}	0.031^{NS}	20.428^{NS}	5.034^{NS}	0.077^{NS}
A x B	20	0.003^{NS}	0.001^{NS}	0.002^{NS}	6.828^{NS}	3.003^{NS}	0.028^{NS}
Error	60	0.852	0.243	16.624	82.253	7.816	0.069

**Significant ($P < 0.01$); ^{NS} Non significant

Table 10. Effect of treatments and storage on calcium contents (mg/100g) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					
	0	15	30	45	60	Mean
T_0	42.12	42.03	41.96	41.88	41.79	**41.95^{e}**
T_1	50.77	50.74	50.7	50.66	50.62	**50.69^{d}**
T_2	51.31	51.26	51.22	51.17	51.14	**51.22^{cd}**
T_3	51.95	51.9	51.88	51.84	51.82	**51.87^{bc}**
T_4	52.42	52.4	52.36	52.31	52.29	**52.35^{ab}**
T_5	53.05	53.01	52.97	52.91	52.88	**52.96^{a}**
Mean	**50.27^{a}**	**50.22^{a}**	**50.18^{a}**	**50.12^{a}**	**50.09^{a}**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

3.8. Iron

The statistical results for iron content of cookies prepared from different flour blends presented in Table 9 indicated that the iron content of the cookies differed significantly by the differences in the treatments as well as storage intervals. However, the interaction between treatments and storage intervals was found to be non significantly different for this parameter.

The results presented in Table 11 depicted that the iron content of the cookies prepared from different flour blends was not significantly affected by different storage intervals. The highest iron content (2.27 mg/100g) was found in the freshly prepared cookies which gradually reduced non significantly to 2.25 mg/100g and 2.22 mg/100g at 30 and 60 days of storage, respectively. The iron content of cookies ranged from 0.19 mg/100g to 3.11 mg/100g among different treatments (Table 11). It is obvious from the results that the cookies prepared from T_0 (0% DFWG flour) contained the lowest content of iron (0.19 mg/100g) as compared to the cookies made from other treatments, and iron content increased significantly with the increase in the level of DFWG flour supplementation in the flour blends. The cookies prepared from the flour blends containing 25% DFWG flour were found to possess the maximum iron content (3.11 mg/100g) followed by the cookies with 20% DFWG flour (2.92 mg/100g). The higher iron content already present in DFWG helped to improve the iron content of cookies prepared by the incorporation of DFWG flour as compared to the control cookies.

3.9. Potassium

The statistical results regarding potassium content showed that potassium content in the cookies varied significantly due to variation in treatments while, the storage intervals exhibited non significant effect on the calcium content of the cookies (Table 9). The interaction between treatments and storage intervals was also found to be non significant for the potassium content. The results presented in Table 12 further revealed that the potassium content of the cookies was not significantly affected during the storage intervals. The potassium content of the cookies at initial day was 210.87 mg/100g which was 210.52 mg/100g at the final day of storage.

Table 11. Effect of treatments and storage on iron contents (mg/100g) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					
	0	15	30	45	60	Mean
T_0	0.23	0.21	0.19	0.19	0.14	**0.19[f]**
T_1	2.21	2.18	2.18	2.17	2.1	**2.17[e]**
T_2	2.48	2.45	2.44	2.46	2.45	**2.44[d]**
T_3	2.71	2.66	2.69	2.59	2.57	**2.64[c]**
T_4	3.01	2.91	2.88	2.87	2.94	**2.92[b]**
T_5	3.10	3.11	3.14	3.11	3.07	**3.11[a]**
Mean	**2.27[a]**	**2.25[a]**	**2.25[a]**	**2.23[a]**	**2.22[a]**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

The potassium content of cookies prepared from different flour blends was found to be significantly higher in the cookies prepared from the flour blends containing 25% DFWG flour (T_5) (Table 12). The potassium content of T_5 was 305.94 mg/100g followed by the cookies prepared from T_4 (275.43 mg/100g), while the lowest potassium content (97.67 mg/100g) was observed in the control cookies prepared without DFWG flour supplementation. The results clearly indicated that potassium content increased linearly with the increase of the DFWG flour level in the wheat flour blends. The higher potassium content of cookies prepared from DFWG blended flours is attributed to significantly higher potassium content present in DFWG than wheat flour. The results of the present study are in concordance with the previous findings of Hooda and Jood (2005) who observed an increase in the potassium content of cookies with the addition of fenugreek seed flour in the cookies production.

Table 12. Effect of treatments and storage on potassium contents (mg/100g) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					
	0	15	30	45	60	Mean
T_0	97.84	97.73	97.66	97.59	97.54	**97.67[f]**
T_1	151.5	151.37	151.33	151.26	151.19	**151.33[e]**
T_2	195.8	195.72	195.63	195.56	195.47	**195.63[d]**
T_3	238.3	238.21	238.1	238.06	237.97	**238.12[c]**
T_4	275.6	275.51	275.42	275.36	275.28	**275.43[b]**
T_5	306.2	306.02	305.94	305.86	305.72	**305.94[a]**
Mean	**210.87[a]**	**210.76[a]**	**210.68[a]**	**210.61[a]**	**210.52[a]**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

3.10. Phosphorous

The statistical results regarding phosphorous content of the cookies prepared from different flour blends presented in Table 9 revealed that the phosphorous content was not significantly affected by the storage intervals, however; the treatments significantly affected the phosphorous content. The interaction between treatments and storage intervals also showed non significant effect on this parameter. The results further revealed that storage intervals showed non significant effect on the phosphorous content of cookies. The phosphorus content of the freshly prepared cookies was 204.14 mg/100g which was 199.47 mg/100g at the end of study in the cookies.

The phosphorous content in the cookies prepared from different flour blends shown in Table 13 indicated that the phosphorous content was significantly higher (282.42 mg/100g) in the cookies prepared from T_5 (25% DFWG flour). The cookies prepared from control treatment (T_0) showed the lowest content of phosphorous (110.72 mg/100g) as compared to the cookies prepared from all other treatments. It is obvious from the results that phosphorous content of the cookies increased progressively with the increase in the level of DFWG flour in the cookies. This increase is attributed to the higher phosphorus content (955.8 mg/100g) in the parent DFWG flour used for incorporation in wheat flour (Table 2).

Table 13. Effect of treatments and storage on phosphorus contents (mg/100g) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					Mean
	0	15	30	45	60	
T_0	112.80	112.35	111.25	109.60	107.60	**110.72^f**
T_1	157.70	156.65	153.65	151.50	150.50	**154.0^e**
T_2	196.75	195.45	192.35	191.60	189.50	**193.13^d**
T_3	230.17	228.54	227.55	226.35	224.50	**227.42^c**
T_4	243.15	241.65	239.45	238.50	237.90	**240.13^b**
T_5	284.25	283.25	282.35	281.45	280.80	**282.42^a**
Mean	**204.14^a**	**202.98^a**	**201.1^a**	**199.83^a**	**199.47^a**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

3.11. Magnesium

The results pertaining to the analysis of variance for magnesium content of cookies prepared from different flour blends presented in Table 9 indicated that the magnesium content of the cookies differed significantly due to the differences in the treatments and storage intervals. However, the interaction between treatments and storage intervals was found to be non significant for this parameter.

The results in Table 14 depicted that the storage intervals showed non significant effect on the magnesium content of the cookies. The maximum magnesium content i.e. 66.68 mg/100g was found in the freshly prepared cookies which gradually reduced to 63.03 mg/100g at the end of study (60 days). The results regarding magnesium content of cookies in Table 14 indicated that the magnesium content ranged from 37.90 mg/100g to 89.33 mg/100g among the cookies prepared from different treatments. It is obvious from the results that cookies of T_0 contained the lowest content of magnesium (37.90 mg/100g) as compared to the cookies of other treatments, which increased significantly with the increase in the level of DFWG flour supplementation in the flour blends. The cookies prepared from T_5 (25% DFWG flour) contained the highest content of magnesium (89.33 mg/100g) followed by the cookies with 20% DFWG flour (75.09 mg/100g).

3.12. Zinc

The statistical data for the zinc content of cookies prepared from different flour blends (Table 9) showed that the treatments had significant effect on the zinc content where as storage intervals as well as the interaction between treatments and storage intervals showed non significant effect on the zinc content of cookies. The results in Table 15 showed that the cookies prepared from the treatment T_5 (25% DFWG flour) contained the highest zinc content (4.81 mg/100g) followed by 4.24 mg/100g and 3.71 mg/100g in cookies of T_4 (DFWG flour 20%) and T_3 (DFWG flour 15%), respectively. The lowest zinc content (0.96 mg/100g) was observed in cookies of T_0 (0% DFWG flour). It was observed that as the amount of the DFWG flour level increased the zinc percentage also increased progressively in the cookies. The results presented in Table 15 further revealed that zinc content of cookies remained unchanged during the storage period.

The results for the zinc content in the present investigation are closely in agreement with the earlier findings of Pirman (2001) who reported that zinc content in DFWG supplemented bread increased with the addition of DFWG in the bread formulation and was observed 3.50mg/100g on average. The findings of Hirofumi and Yoshio (1998) also indicated that wheat flour and DFWG contained 0.87 mg/100g and 2.6 mg100g zinc content, respectively further supported the present investigations, which improved the zinc content of the resultant product as is observed in the present study.

Table 14. Effect of treatments and storage on magnesium contents (mg/100g) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					Mean
	0	15	30	45	60	
T_0	41.35	39.9	37.5	36.25	34.5	**37.90^f**
T_1	53.65	52.5	50.25	50.1	48.5	**51.0^e**
T_2	62.15	61.35	59.85	58.5	57.25	**59.82^d**
T_3	71.95	70.75	78.75	76.5	75.55	**74.70^c**
T_4	79.25	79.2	77.5	77.45	76.5	**77.98^b**
T_5	91.75	91.25	89.5	88.3	85.85	**89.33^a**
Mean	**66.68^a**	**65.83^a**	**65.56^a**	**64.52^a**	**63.03^a**	

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

Table 15. Effect of treatments and storage on zinc contents (mg/100g) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					Mean
	0	15	30	45	60	
T_0	1.05	1.02	0.98	0.97	0.78	**0.96**[f]
T_1	2.15	2.1	2	1.88	1.87	**2.0**[e]
T_2	2.8	2.79	2.75	2.65	2.63	**2.72**[d]
T_3	3.75	3.73	3.7	3.68	3.67	**3.71**[c]
T_4	4.2	4.15	4.3	4.28	4.26	**4.24**[b]
T_5	4.9	4.88	4.8	4.75	4.74	**4.81**[a]
Mean	**3.14**[a]	**3.11**[a]	**3.08**[a]	**3.03**[a]	**3.01**[a]	

Means carrying same letters within a column or row do not differ significantly (P < 0.01)

3.13. Thiobarbiteuric acid (TBA) value

The statistical results regarding TBA value of cookies prepared from different flour blends are presented in Table 16. The statistical results indicated that treatments and storage intervals exhibited significant effect on the TBA value of cookies. However, the interaction between treatments and storage intervals exhibited non significant effect on this parameter. The results for the TBA values of cookies prepared from different treatments are presented in Table 16, which indicated that TBA value of cookies decreased significantly with the addition of DFWG flour in the formulation of cookies. The TBA value ranged from 0.070 mg melonaldehyde/kg to 0.098 mg melonaldehyde/kg among different treatments. The results revealed that maximum TBA value (0.098 mg melonaldehyde/kg) was exhibited by T_0 (0% DFWG) while, T_4 (20% DFWG flour) possessed the lowest TBA value (0.070 mg melonaldehyde/kg). The treatments T_2, T_3 and T_5 were found statistically at par for the TBA value. The TBA value of cookies given in Table 16 revealed that TBA value increased from 0.068 to 0.098mg melonaldehyde/kg from 0 to 60 days of storage, respectively. In fresh cookie samples TBA value was observed as 0.068 mg melonaldehyde/kg which increased to 0.077 and 0.098mg melonaldehyde/kg after 30 and 60 days storage, respectively. The results showed that TBA value of the cookies increased slowly in the first 15 days of storage but increased progressively as the storage prolonged showing the development of rancidity in the cookie samples in the later stages of storage.

The increase in TBA value of the cookies prepared from different flour blends during present study depicted the development of rancidity which is more in cookies prepared from straight grade flour but was comparatively less in the cookies prepared from incorporation of DFWG flour. This increase in TBA value due to incorporation of DFWG flour might be due to the increase in free fatty acids formation because of higher lipase activity. As obvious from the results that DFWG incorporated cookies contained less amount of crude fat this may be reason that the cookies prepared from the flour blends experienced comparatively less rancid as compared to the control treatment. However, higher temperature of the storage room, moisture, heat and light are the key factors that further accelerate the TBA value during the storage of cookies.

Table 16. Effect of treatments and storage on TBA value (mg melonaldehyde/kg) of DFWG supplemented cookies

Treatments	Storage Intervals (days)					Mean
	0	15	30	45	60	
T_0	0.08	0.08	0.09	0.11	0.13	**0.098**[a]
T_1	0.07	0.08	0.08	0.09	0.1	**0.084**[b]
T_2	0.07	0.07	0.08	0.08	0.09	**0.078**[c]
T_3	0.06	0.07	0.07	0.09	0.09	**0.076**[c]
T_4	0.06	0.06	0.07	0.09	0.10	**0.070**[c]
T_5	0.06	0.06	0.07	0.08	0.08	**0.070**[d]
Mean	**0.069**[d]	**0.070**[d]	**0.077**[c]	**0.090**[b]	**0.098**[a]	

Means carrying same letters within a column or row do not differ significantly (P < 0.01)

4. Conclusion:

The results portrayed that incorporation of DFWG and WGO in the cookies improved overall chemical as well as nutritional parameters of cookies.

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