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Effect of *Nigella sativa* Meal Protein Isolates Supplementation on the Physical and Sensory Characteristics of Cookies During Storage

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Abstract: The mandate of current study was to explore the supplementation effect of *Nigella sativa* meal protein isolates in wheat flour for the development of low cost protein dense cookies in order to reduce the threat of protein energy malnutrition. The cookies diameter, thickness and spread factor indicated significant differences among treatments however, non-momentous effect was observed due to storage. The mean values for spread factor of cookies T_0, T_1, T_2, T_3, T_4 and T_5 were 37.56±0.18, 36.07±0.62, 35.08±0.20, 34.68±0.28, 32.28±0.13 and 30.84±0.12, respectively. The highest color scores 7.18±0.21 were assigned to T_0 (control) followed and lowest to T_5 (6.15±0.32). In case of flavor, the highest score 7.08±0.26 was attained by T_0 whereas the lowest was assigned to T_5 6.20±0.19. Similarly, the maximum overall acceptability scores 7.14±0.17 were assigned to T_0 whilst, the minimum 6.19±0.21 for T_5. Overall acceptability scores gradually declined from 6.98±0.35 to 6.52±0.44 during storage.

Key words: Protein isolates, *Nigella sativa*, physical parameters, sensory evaluation

INTRODUCTION

Protein energy malnutrition is one of the major nutritional threats in developing economies due to escalating population and scarce resources. In Pakistan, people are mainly relying on wheat and resultant products to meet the energy needs. However, lysine is limiting amino acids along with methionine and tryptophan that demands exploration of some unconventional protein sources. In this context, *Nigella sativa* meal holds potential to cope with the deficiency as it contains higher protein levels. Product development is not only restricted to create innovative food items but also covers the concept of reformulation. Among different value added foods, baked products are an appropriate choice for the supplementation of protein isolates from nontraditional edible sources (Bakke and Vickers, 2007). Purposely, an array of attempts have already been made to develop various composite flour formulations by supplementing wheat flour with raw or defatted soy, wheat germ and flax and sunflower meals at industrial scale (Junqueira *et al*., 2008). The cereal based edibles made from composite flours are widely accepted and commercialized in many parts of the world to improve the nutritional and sensory response of the finished product. The protein content of wheat plays an important role in the baking performance (Al-Kahtani, 2003). Besides, considerable interest has also been generated regarding wheat flour fortification with high value protein materials to improve the protein and essential amino acid pool of the formulated product. Considering the dilemma of PEM, blending of protein isolates from indigenous raw materials with wheat flour is not only a rational approach but also economical too. Globally, the baked products are widely consumed by the masses thus fortification with protein rich source is a rational approach to improve their nutritional status. The addition of nonconventional proteins has been appreciated by the nutritionists and other scientific sectors to cope with PEM. The supplementation of partially purified proteins is acceptable than raw and defatted sources owing to their beneficial effects on baking (Shahzadi *et al*., 2005; Kenawi *et al*., 2009; Pasha *et al*., 2011). The cookies are considered as ideal vehicle for fortification due to less moisture content and longer shelf life than other baked items (Akubor, 2003; Khattak *et al*., 2003; Hood and Jood, 2005; Sharif *et al*., 2009). In an experiment, full fat and defatted rice brans were substituted in wheat flour @ 5, 10 and 15% to prepare composite cookies. The cookies spread was increased by the addition of full fat rice bran however, supplementation of defatted bran resulted decrease in spread factor (Sekhon *et al*., 1997). Sensory evaluation is used to analyze the product quality by taste, smell, texture and other allied attributes (Hussain *et al*., 2006; Mohsen *et al*., 2009). The sensory profiling is an important tool for product development, improvement, determination of consumer acceptance and shelf life studies. The sensory properties of food are also important just like chemical and microbiological attributes as they serve as diagnostic tool to assess the quality (Kuti *et al*., 2004). The sensory parameters are also used to determine the palatability of foods that has strong influence on the dietary consumption. The color

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serves as a sign for the doneness of food correlated with changes in aroma. In this connection, flavor is the synthesis of taste and smell of the food, crispness indicates the crunchy perception whilst, texture is closely related to the structure and composition (Butt *et al.*, 2004; Alvarez-Jubete *et al.*, 2010).

MATERIALS AND METHODS

Procurement of raw materials: *Nigella sativa* seeds were procured from Barani Agriculture Research Institute (BARI), Chakwal. Commercial straight grade flour (CSGF) and remaining ingredients for cookies preparation were acquired from the local market, Faisalabad.

Preparation of protein enriched cookies: The cookies were prepared from composite flour blends of *Nigella sativa* meal protein isolates and straight grade flour following the protocols of AACC (2000) Method No. 10-50D with slight modifications. *Nigella sativa* protein isolates were evaluated in different combinations by replacing wheat flour @ 5, 10 15, 20 and 25% for the development of protein enriched cookies (Table 1). For creaming purposes, vegetable shortening and sugar were mixed in Hobart Mixer (Model N-50, Hobart Corp. Troy, Ohio, USA) for 25-30 min. Afterwards, remaining ingredients were added and kneaded for further 5 min to homogenous mass. The dough was rolled on a sheeting board to uniform thickness and cut by 50 mm diameter round scorn cutter. The cookies were baked at 175±5°C for 15 min. Finally, cookies were sealed in aluminium foil wraps, packed in boxes and stored for two months. The resultant cookies were evaluated for various physical and sensory characteristics.

Physical analysis: The width, thickness and spread factor of cookies were estimated at 0, 15, 30, 45 and 60 according to the method described in AACC (2000).

Width (W): The width of cookies was measured by placing six biscuits horizontally (edge to edge) and rotated at 90°angles for duplicate reading.

Thickness (T): The thickness of cookies was measured by placing six cookies on one another and the duplicate reading was recorded.

Spread factor (SF): The spread factor was calculated according to the following formula:

$$SF = W/T \times CF \times 10$$

Where:

CF = Correction factor at constant atmospheric pressure

Table 1: Treatments used in the study

Treatments	CSGF (%)	NSMPI (%)
T_1	100	0
T_2	95	5
T_3	90	10
T_4	85	15
T_5	80	20
T_6	75	25

NSMPI = Nigella sativa meal protein isolate,

CSGF = Commercial straight grade flour

Sensory evaluation: The cookies were evaluated for color, flavor, taste, texture and overall acceptability at 0, 15, 30, 45 and 60 days by trained taste panel using 9-point hedonic scale according to the guidelines of Meilgaard *et al.* (2007). The individual rating as liked extremely-9, liked very much-8, liked moderately-7, liked slightly-6, neither liked nor disliked-5, disliked slightly- 4, disliked moderately-3, disliked very much-2 and disliked extremely-1 were used to find out the most suitable composition of cookies for commercialization. In each session, panelists were seated in separate booths equipped with white fluorescent light in the Sensory Evaluation Laboratory at the National Institute of Food Science and Technology. The panelists were requested to express their opinion about the end product by assigning scores to respective attributes. During sensory session, the cookies made from different flour blends were presented in glass plates with secret codes randomly. Mineral water and unsalted crackers were supplied to the panelists to rinse their mouths and neutralized the taste buds. The names of the panelists were concealed to maintain the secrecy.

Statistical analysis: The obtained data for each parameter was analyzed through completely randomized design (CRD) by using Statistical Package (Costat 2003, Co-Hort, v 6.1). The level of significance was determined through analysis of variance (ANOVA) technique according to the principles outlined by Steel *et al.* (1997).

RESULTS AND DISCUSSION

Development of cookies: The cookies were assessed for physical and sensorial attributes to find out the suitability of *Nigella sativa* meal protein isolates.

Physical analysis: In the present project, protein enriched cookies were evaluated for their physical characteristics like diameter, thickness and spread factor.

Diameter: The mean squares for cookies diameter indicated significant differences among various treatments however, non-momentous effect was observed due to storage. The means (Table 2) depicted

Table 2: Effect of treatments and storage on diameter (cm) of cookies

Treatments	Storage (Days) 0	15	30	45	60	Mean
T_0	4.67±0.24	4.66±0.23	4.64±0.23	4.65±0.26	4.67±0.22	4.66±0.13[a]
T_1	4.62±0.23	4.63±0.24	4.62±0.23	4.64±0.23	4.61±0.23	4.62±0.11[a]
T_2	4.57±0.22	4.56±0.22	4.57±0.21	4.56±0.28	4.55±0.23	4.56±0.08[b]
T_3	4.55±0.22	4.56±0.23	4.56±0.22	4.55±0.22	4.56±0.24	4.55±0.06[b]
T_4	4.32±0.21	4.33±0.21	4.31±0.21	4.32±0.14	4.34±0.19	4.32±0.11[c]
T_5	4.24±0.12	4.26±0.13	4.23±0.13	4.23±0.21	4.24±0.21	4.24±0.12[d]
Mean	4.49±0.19	4.50±0.17	4.48±0.17	4.49±0.18	4.49±0.18	-

Means carrying the same letters in a column are not significantly different

T_0 = 100% Straight grade flour, T_1 = 95% SGF and 5% NSMPI, T_2 = 90% SGF and 10% NSMI, T_3 = 85% SGF and 15% NSMPI, T_4 = 80% SGF and 20% NSMPI, T_5 = 75% SGF and 25% NSMPI

Table 3: Effect of treatments and storage on thickness (cm) of protein enriched cookies

Treatments	Storage (Days) 0	15	30	45	60	Mean
T_0	1.24±0.24	1.25±0.25	1.24±0.24	1.23±0.24	1.24±0.24	1.24±0.07[f]
T_1	1.27±0.25	1.27±0.25	1.28±0.26	1.27±0.26	1.29±0.27	1.28±0.09[e]
T_2	1.30±0.27	1.29±0.27	1.30±0.29	1.31±0.27	1.30±0.28	1.30±0.07[d]
T_3	1.30±0.28	1.32±0.26	1.31±0.26	1.32±0.26	1.33±0.27	1.31±0.11[c]
T_4	1.34±0.29	1.33±0.27	1.33±0.25	1.34±0.27	1.33±0.26	1.33±0.06[b]
T_5	1.37±0.26	1.38±0.28	1.37±0.27	1.38±0.28	1.37±0.27	1.37±0.05[a]
Mean	1.30±0.47	1.31±0.46	1.305±0.44	1.308±0.53	1.31±0.44	-

Means carrying same letters in a column do not differ significantly

T_0 = 100% Straight grade flour, T_1 = 95% SGF and 5% NSMPI, T_2 = 90% SGF and 10% NSMPI, T_3 = 85% SGFand 15% NSMPI, T_4 = 80% SGF and 20% NSMPI, T_5 = 75% SGF and 25% NSMPI

a decreasing trend in the diameter of cookies due to increased supplementation level. The maximum diameter was observed in T_0 as 4.66±0.13 cm however, minimum value for this attribute was noted in T_5 4.24±0.12 cm during 60 days storage. Whilst, T_1, T_2, T_3 and T_4 have diameter 4.62±0.11, 4.56±0.08, 4.55±0.06 and 4.32±0.11 cm, respectively. The current results for diameter are in harmony with the findings of McWatters (2003), observed a decreasing trend for the diameter of cookies prepared from soy based blends. Furthermore, Sharif *et al.* (2009) elucidated that diameter of the cookies increases by the addition of defatted rice bran. The results of present study are close to the observations of Grover and Singh (1994) that elevated level of defatted soy flour reduces the diameter of cookies. However in contrast to the instant findings, the diameter of cookies was increased by the supplementation of flax (Hussain *et al.*, 2006). The current results are comparable with the work of Kamaljit *et al.* (2010), who concluded that pea flour incorporation decreases the diameter of cookies in comparison to control. According to the findings of Akubor (2003), cookies prepared from plantain and cowpea flour exhibited a declining tendency for diameter with the proportionate increase in cowpea.

Thickness: The mean squares regarding cookies thickness indicated substantial variations among the treatments whereas, storage and interaction imparted non-significant impact. The thickness measured for control cookies (T_0) was 1.24±0.07 cm that increased with the addition of protein to 1.28±0.09, 1.30±0.07, 1.31±0.11, 1.33±0.06 and 1.37±0.05 cm in T_1, T_2, T_3, T_4 and T_5, respectively (Table 3). The current findings are in line with the previous results of Sharif *et al.* (2009), established an increasing tendency in the thickness of cookies due to the addition of defatted rice bran. Earlier, Grover and Singh (1994) have reported that supplementation of wheat flour with 5-25% defatted soy flour increased the thickness of cookies. The results of instant study are further strengthened by the observations of Tsen *et al.* (1976), they highlighted gain in the thickness of cookies by fortification of wheat flour with soy protein isolate @ 50%. The instant results are also in harmony with the outcomes of Kamaljit *et al.* (2010), who examined that pea flour incorporation increases thickness of the cookies in comparison to control. Conversely, Hood and Jood (2005) have claimed that thickness decreases with the increased level of fenugreek in cookies. Moreover, Taylor *et al.* (2008) unveiled that gluten development contributes towards an expansion in the thickness of cookies.

Spread factor: The ratio between thickness and diameter of the cookies is called as spread factor. It reflects the viscous behavior of batter during baking. The mean squares for spread factor of cookies depicted significant variations with respect to treatments however,

Table 4: Effect of treatments and storage on spread factor of protein enriched cookies

	Storage (Days)					
Treatments	0	15	30	45	60	Mean
T_0	37.66±1.13	37.36±1.12	37.42±1.12	37.80±1.13	37.66±1.13	37.56±0.18[a]
T_1	37.38±1.09	36.46±1.09	36.02±1.08	36.38±1.09	35.74±1.07	36.07±0.62[b]
T_2	35.15±1.06	35.35±1.06	35.15±1.05	34.81±1.04	35.00±1.05	35.08±0.20[c]
T_3	35.00±1.05	34.55±1.03	34.81±1.04	34.47±1.03	34.29±1.03	34.68±0.28[d]
T_4	32.24±0.96	32.56±0.98	32.41±0.97	32.24±0.32	32.39±0.34	32.28±0.13[e]
T_5	30.95±0.71	30.87±0.68	30.88±0.72	30.65±0.73	30.95±0.38	30.84±0.12[f]
Mean	34.37±2.69	34.52±2.44	34.44±2.40	34.39±2.62	34.33±2.39	-

Means carrying same letters in a column do not differ significantly

T_0 = 100% Straight grade flour, T_1 = 95% SGF and 5% NSMPI, T_2 = 90% SGF and 10% NSMPI, T_3 = 85% SGFand 15% NSMPI, T_4 = 80% SGF and 20% NSMPI, T_5 = 75% SGF and 25% NSMPI

storage imparted non-significant effect. The highest spread factor 37.56±0.18 was recorded for control group (T_0), whereas the mean values for T_1, T_2, T_3, T_4 and T_5 were 36.07±0.62, 35.08±0.20, 34.68±0.28, 32.28±0.13 and 30.84±0.12, respectively. The spread factor during storage varied from 34.37±2.69 to 34.33±2.39 from initiation till the termination of the trial (Table 4). The present findings are in corroboration with the research investigation of Sharif *et al.* (2009) and Sudha *et al.* (2007), they alluded that spread ratio of cookies reduces by the proportionate increase in the defatted rice bran. Likewise, Grover and Singh (1994) observed that higher level of defatted soy in cookies resulting significant reduction in spread ratio. Afterwards, Kamaljit *et al.* (2010) deduced that pea flour incorporation decreases the spread ratio of cookies in comparison with control. The present results for spread ratio are also in agreement with the work of Arshad *et al.* (2007), who reported that cookies spread decreases by the supplementation of defatted wheat germ. One of the researchers groups, Hussain *et al.* (2006) revealed a reduction in the spread factor of cookies by increasing the level of flaxseed in straight grade flour. Earlier, it has been probed that spread ratio is affected by the water present in flour and other ingredients during dough mixing (Shrestha and Noomhorn, 2002).

Sensory evaluation of cookies: Sensory evaluation is one of the important aspects for quality assessment usually performed to get the panelists opinion towards the developed product. It also serves as a tool to estimate the consumer's acceptance. In current study, the hedonic response of cookies was determined on fortnightly basis by a trained panel. For the intention, cookies were scored for color, flavor, taste, texture, crispness and overall acceptability to assess the effect of treatments and storage on these traits. The mean squares indicated significant effect of treatments and storage on the sensory attributes of cookies.

Color: The color is an imperative parameter for evaluating the baking performance of cookies that not only reflects suitability of the raw materials but also provides information about formulation and quality of the product (Pasha *et al.*, 2011). It is one of the desirable features for any product to be accepted by the consumers. The results pertaining to mean scores explicated that the addition of *Nigella sativa* meal considerably decreased the color scores. The color scores indicated the maximum rating 7.18±0.21 for T_0 (control) followed by T_3 (7.08±0.10), T_2 (7.02±0.13), T_1 (6.94±0.16), T_4 (6.67±0.29) and T_5 (6.15±0.32). The color scores of cookies were reduced significantly from 7.10±0.33 to 6.58±0.49 during storage from 0 to 60th day (Table 5). It is obvious from the results that cookies prepared by supplementing 20 and 25% of protein isolates did not perform better. The color serves as a sign for doneness of end product in baking and is correlated with changes in aroma and flavor (Hussain *et al.*, 2006). The decrease in color score was observed with increasing level of protein isolates in the cookies. This may be primarily due to the color differences of protein isolates as compared to straight grade flour (Mridula *et al.*, 2007). The present findings are in corroboration with the research investigation of Pasha *et al.* (2011), who observed similar effect on color of cookies prepared by the supplementation of mungbean protein isolate. Similarly, Awan *et al.* (1995) assessed a decreasing trend in the color score of mothbean based biscuits. The current observations are in agreement with the work of Hussain *et al.* (2006), who recorded a gradual decline in color of biscuits during storage. Furthermore, the color fading is also due to the absorption of moisture from the atmosphere and oxidation; such changes are associated with dextrinization of starch and maillard reaction involving the interaction of reducing sugars with proteins (Mepba *et al.*, 2007). The current results are in close harmony with the exploration of Elahi (1997), recorded a proportionate decrease in color scores of cookies during storage.

Flavor: Flavor is an important criterion for the liking or disliking of product. In case of flavor, the highest score 7.08±0.26 was given to T_0 followed by T_3 7.01±0.11, T_2

Table 5: Effect of treatments and storage on color of protein enriched cookies

	Storage (Days)					
Treatments	0	15	30	45	60	Mean
T_0	7.52±0.36	7.22±0.36	7.15±0.35	7.03±0.35	6.97±0.34	7.18±0.21^{a}
T_1	7.15±0.35	7.04±0.35	6.93±0.34	6.89±0.33	6.72±0.33	6.94±0.16^{b}
T_2	7.19±0.35	7.09±0.35	7.03±0.35	6.92±0.34	6.87±0.34	7.02±0.13^{ab}
T_3	7.22±0.36	7.12±0.35	7.07±0.35	7.01±0.35	6.96±0.34	7.08±0.10^{ab}
T_4	6.96±0.34	6.86±0.33	6.73±0.31	6.57±0.31	6.23±0.30	6.67±0.29^{c}
T_5	6.54±0.32	6.39±0.30	6.11±0.30	5.98±0.29	5.75±0.29	6.15±0.32^{d}
Mean	7.10±0.33^{a}	6.95±0.30^{ab}	6.84±0.38^{bc}	6.73±0.41^{c}	6.58±0.49^{d}	-

Means carrying same letters in a column do not differ significantly

T_0 = 100% Straight grade flour, T_1 = 95% SGF and 5% NSMPI, T_2 = 90% SGF and 10% NSMPI, T_3 = 85% SGFand 15% NSMPI, T_4 = 80% SGF and 20% NSMPI, T_5 = 75% SGF and 25% NSMPI

Table 6: Effect of treatments and storage on flavor of protein enriched cookies

	Storage (Days)					
Treatments	0	15	30	45	60	Mean
T_0	7.35±0.30	7.22±0.36	7.18±0.35	6.93±0.33	6.69±0.32	7.08±0.26^{a}
T_1	6.89±0.34	6.82±0.33	6.76±0.33	6.61±0.32	6.56±0.31	6.73±0.14^{b}
T_2	7.08±0.31	7.02±0.34	6.93±0.29	6.87±0.33	6.86±0.33	6.92±0.15^{ab}
T_3	7.12±0.35	7.09±0.35	7.03±0.31	6.95±0.33	6.68±0.34	7.01±0.11^{a}
T_4	6.65±0.32	6.58±0.31	6.42±0.23	6.27±0.29	6.04±0.26	6.39±0.25^{c}
T_5	6.42±0.29	6.35±0.30	6.22±0.27	6.04±0.26	5.97±0.30	6.20±0.19^{c}
Mean	6.91±0.34^{a}	6.84±0.33^{a}	6.76±0.37^{b}	6.61±0.38^{b}	6.47±0.37^{c}	-

Means carrying same letters in a column do not differ significantly

T_0 = 100% Straight grade flour, T_1 = 95% SGF and 5% NSMPI, T_2 = 90% SGF and 10% NSMPI, T_3 = 85% SGFand 15% NSMPI, T_4 = 80% SGF and 20% NSMPI, T_5 = 75% SGF and 25% NSMPI

6.92±0.15, T_1 6.73±0.14 and T_4 6.39±0.25 whereas, the lowest score was assigned to T_5 6.20±0.19. The storage study eluded that flavor of the cookies changed considerably during two months and scores decreased from 6.91±0.34 to 6.47±0.37 (Table 6). The flavor perception is combination of taste and smell along with texture, influenced by the appearance. The bakery products rapidly stale that transforms rich aroma and flavor of the fresh product to bland or off flavor (Jia *et al.*, 2011). The present findings regarding this attribute are in agreement with the results of Mohsen *et al.* (2009), who found a considerable reduction in the flavor scores of soy isolate fortified cookies. The flavor deterioration was due to enhanced free fatty acid, alcoholic acidity and lipase activity during storage. Similarly, Awan *et al.* (1995) observed a decreasing trend in the flavor score of mothbean supplemented biscuits. Likewise, Kamaljit *et al.* (2010) expounded that flavor scores of pea flour incorporated cookies decreases with increased level of supplementation. The development of off flavor as a result of oxidation is related to the presence of moisture that further accelerated by the presence of pro-oxidants (Sharif *et al.*, 2005). The present results are further strengthened by the findings of Haque *et al.* (2002), who observed a decreasing trend in flavor scores of cookies due to the addition of corn, rice, oat and wheat bran @ 20%.

Taste: The taste is detected through the taste buds and influenced by texture, flavor and composition of the food (Sultan *et al.*, 2011). The maximum taste scores were assigned to T_0 7.12±0.18 trailed by T_3, T_2, T_1, T_4 and T_5 as 7.05±0.15, 7.02±0.09, 6.94±0.06, 6.43±0.04 and 6.16±0.01, respectively. The storage caused noticeable decline in taste scores from 6.99±0.31-6.53±0.49 at 0 and 60th day, correspondingly (Table 7). It is evident from the results that cookies containing 15% protein were appreciated by the judges whilst, the higher level of NSMPI adversely affected the flavor scores. The present results are also in accordance with the work of Sharif *et al.* (2005), who observed that the taste of cookies decreases by the addition of rice bran. Likewise, the supplementation of pea and mungbean flour also affected the taste of cookies (Kamaljit *et al.*, 2010; Pasha *et al.*, 2011). In another research study, Rababah *et al.* (2006) evaluated cookies supplemented with soy protein isolates and recorded significant effect for taste scores.

Crispiness: It depicts the crunchy perception and brittleness of the products. The means for crispiness of cookies indicated maximum scores for T_0 (6.94±0.07) followed by T_3 (6.84±0.07), T_2 (6.80±0.06), T_1 (6.72±0.06), T_4 (6.64±0.06) and T_5 (6.53±0.05). Storage also showed decrease in the crispiness scores from 7.08±0.05-6.43±0.05 during two months. The maximum

Table 7: Effect of treatments and storage on taste of protein enriched cookies

	Storage (Days)					
Treatments	0	15	30	45	60	Mean
T_0	7.32±0.37	7.22±0.36	7.14±0.36	7.06±0.35	6.85±0.34	7.12±0.18[a]
T_1	7.08±0.35	7.01±0.35	6.95±0.34	6.87±0.34	6.76±0.33	6.94±0.06[b]
T_2	7.16±0.36	7.11±0.35	7.06±0.35	7.01±0.34	6.93±0.34	7.02±0.09[ab]
T_3	7.12±0.35	7.09±0.34	7.03±0.33	6.98±0.32	6.86±0.32	7.05±0.15[ab]
T_4	6.75±0.33	6.63±0.32	6.47±0.31	6.28±0.30	6.05±0.29	6.43±0.04[c]
T_5	6.49±0.30	6.31±0.29	6.25±0.28	5.95±0.27	5.78±0.25	6.16±0.01[d]
Mean	6.99±0.31[a]	6.89±0.35[ab]	6.81±0.37[b]	6.69±0.46[c]	6.53±0.49[d]	-

Means carrying same letters in a column do not differ significantly

T_0 = 100% Straight grade flour, T_1 = 95% SGF and 5% NSMPI, T_2 = 90% SGF and 10% NSMPI, T_3 = 85% SGFand 15% NSMPI, T_4 = 80% SGF and 20% NSMPI, T_5 = 75% SGF and 25% NSMPI

Table 8: Effect of treatments and storage on crispiness of protein enriched cookies

	Storage (Days)					
Treatments	0	15	30	45	60	Mean
T_0	7.24±0.08	7.10±0.07	6.92±0.08	6.79±0.06	6.65±0.06	6.94±0.07[a]
T_1	7.08±0.05	6.86±0.06	6.72±0.07	6.59±0.06	6.37±0.07	6.72±0.06[c]
T_2	7.12±0.06	6.95±0.05	6.79±0.07	6.66±0.07	6.49±0.08	6.80±0.06[b]
T_3	7.15±0.07	6.97±0.08	6.83±0.06	6.70±0.08	6.54±0.07	6.84±0.07[b]
T_4	7.03±0.06	6.77±0.08	6.63±0.07	6.48±0.07	6.31±0.05	6.64±0.06[d]
T_5	6.83±0.03	6.71±0.07	6.48±0.06	6.39±0.06	6.24±0.06	6.53±0..05[e]
Mean	7.08±0.05[a]	6.89±0.06[b]	6.73±0.06[c]	6.601±0.06[d]	6.43±0.05[e]	-

Means carrying same letters in a column do not differ significantly

T_0 = 100% Straight grade flour, T_1 = 95% SGF and 5% NSMPI, T_2 = 90% SGF and 10% NSMPI, T_3 = 85% SGFand 15% NSMPI, T_4 = 80% SGF and 20% NSMPI, T_5 = 75% SGF and 25% NSMPI

scores were assigned to the freshly prepared cookies that decreased significantly after two month storage (Table 8). In the present study, decline in crispiness scores was observed with progressive increase in the EPI level 20% and above. The modifications in physical and chemical characteristics cause adverse effects on product quality during storage. The cookies are hygroscopic in nature and loss of crispiness occurs due to moisture uptake (Manley, 2002). Addition of processed soy protein isolates with wheat flour @ 25% resulted decline in crispiness scores (Mohsen *et al.*, 2009). Likewise, crunch of cookies decreases with the gradual increase in the supplementation level of mungbean protein (Pasha *et al.*, 2011). The results of the present study are in accordance with the investigation of Butt *et al.* (2004), who observed alteration in the crispiness scores of cookies due to the increased moisture absorption during storage.

Texture: In the current study, texture was significantly affected by the treatments and storage span. The maximum scores for texture 7.19±0.15 were assigned to control (T_0) whilst, the minimum scores 6.41±0.19 were attained by T_5. The mean scores for T_1, T_2, T_3 and T_4 were 6.96±0.12, 7.10±0.11, 7.03±0.11 and 6.66±0.21, respectively. The storage resulted a significant reduction in scores from 7.10±0.21 (0 day) to 6.68±0.20 (60 day) as indicated in the Table 9. The texture is considered as an important quality attribute associated with product freshness. It is perceived as a sign of food safety and an indicator of quality (Mridula *et al.*, 2007). The results of present study are in harmony with the findings of Awan *et al.* (1995), who reported a decreasing trend in the texture scores of mothbean supplemented biscuits. Likewise, Kamaljit *et al.* (2010) explicated that texture scores of pea supplemented cookies also decreased in dose dependent manner. Similar decreasing tendency for this trait was observed in cookies prepared by the addition of mash and mungbeans (Riaz *et al.*, 2007). The declining trend in texture scores was possibly due to moisture absorption from the atmosphere that has an inverse association with texture (Sharif *et al.*, 2005).

Overall acceptability: It is evident from the data that maximum scores 7.14±0.17 were given to T_0 whilst, the minimum 6.19±0.21 for T_5. Nevertheless, T_1, T_2, T_3 and T_4 attained scores by 6.86±0.16, 6.95±0.16, 7.07±0.14 and 6.31±0.24, respectively. Overall acceptability scores gradually declined from 6.98±0.19-6.52±0.12 during storage of cookies (Table 10). The current results regarding overall acceptability are in conformity with the earlier findings of Pasha *et al.* (2011), who explicated that acceptability scores of legumes enriched cookies decreases by increasing supplementation level. Likewise, Kamaljit *et al.* (2010) delineated that acceptability scores of cookies decreased with pea flour enrichment. The present outcomes are also comparable with the work of Hussain *et al.* (2006), they prepared

Table 9: Effect of treatments and storage on texture of protein enriched cookies

	Storage (Days)					
Treatments	0	15	30	45	60	Mean
T_0	7.35±0.22	7.29±0.22	7.21±0.22	7.13±0.21	6.97±0.21	7.19±0.15^{a}
T_1	7.11±0.21	7.05±0.21	6.96±0.21	6.87±0.21	6.83±0.20	6.96±0.12^{b}
T_2	7.23±0.22	7.16±0.21	7.12±0.21	7.06±0.21	6.94±0.21	7.10±0.11^{ab}
T_3	7.18±0.22	7.09±0.21	7.04±0.21	6.98±0.21	7.89±0.21	7.03±0.11^{ab}
T_4	6.96±0.21	6.79±0.20	6.63±0.20	6.51±0.20	6.43±0.19	6.66±0.21^{c}
T_5	6.77±0.20	6.56±0.20	6.41±0.19	6.29±0.19	6.05±0.18	6.41±0.19
Mean	7.10±0.21^{a}	6.99±0.27^{ab}	6.89±0.20^{bc}	6.80±0.20^{cd}	6.68±0.20^{d}	-

Means carrying same letters in a column do not differ significantly. T_0 = 100% Straight grade flour, T_1 = 95% SGF and 5% NSMPI, T_2 = 90% SGF and 10% NSMPI, T_3 = 85% SGFand 15% NSMPI, T_4 = 80% SGF and 20% NSMPI, T_5 = 75% SGF and 25% NSMPI

Table 10: Effect of treatments and storage on overall acceptability of protein enriched cookies

	Storage (Days)					
Treatments	0	15	30	45	60	Mean
T_0	7.34±0.27	7.25±0.27	7.17±0.22	7.02±0.19	6.93±0.13	7.14±0.17^{a}
T_1	7.10±0.16	6.96±0.12	6.83±0.16	6.76±0.16	6.68±0.12	6.86±0.16^{d}
T_2	7.17±0.22	7.03±0.17	6.93±0.18	6.85±0. 17	6.77±0.13	6.95±0.16^{c}
T_3	7.20±0.25	7.12±0.25	7.08±0.20	7.01±0.18	6.94±0.14	7.07±0.14^{b}
T_4	6.65±0.15	6.42±0.11	6.28±0.14	6.17±0.15	6.03±0.09	6.31±0.24^{e}
T_5	6.45±0.13	6.32±0.11	6.21±0.14	6.07±0.12	5.90±0.08	6.19±0.21^{f}
Mean	6.98±0.19^{a}	6.85±0.17^{b}	6.74±0.17^{c}	6.63±0.15^{d}	6.52±0.12	-

Means carrying same letters in a column do not differ significantly. T_0 = 100% Straight grade flour, T_1 = 95% SGF and 5% NSMPI, T_2 = 90% SGF and 10% NSMPI, T_3 = 85% SGFand 15% NSMPI, T_4 = 80% SGF and 20% NSMPI, T_5 = 75% SGF and 25% NSMPI

cookies by the addition of flax and observed a significant effect on the overall acceptability scores. Similarly, cookies with high hedonic response were prepared by blending soy and sorghum (Mridula *et al*., 2007), chickpea and wheat (Rababah *et al*., 2006), wheat, fonio and cowpea (McWatters *et al*., 2003) and soybean, chickpea and isolated soy protein (Taha *et al*., 2006). In a study conducted by Butt *et al*. (2007), declining trend in overall acceptability scores of baked products was observed with the passage of time. From the current findings, it is concluded that supplementation of wheat flour with *Nigella sativa* meal protein isolates up to 15% is suitable for cookies preparation without any adverse hedonic response. It is worth mentioning that the resultant cookies have potential to provide ample amount of protein thus can be used as dietary intervention to alleviate protein energy malnutrition.

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