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Research Article

Production, characterization, food application and biological study of powder of pumpkin (*Cucurbita maxima*) parts (peel, flesh and seeds)

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Abstract

Present study was conducted to explore and utilize the excellent nutritional profiles of pumpkin peel, flesh and seeds. Pumpkin parts are good sources of protein, fat, fiber and ash contents. Pumpkin (*Cucurbita maxima*) three fractions (peel, flesh and seeds) were separated, dried and grinded into powder. Replacement of white flour with pumpkin powders at 0, 5, 10 and 15% levels were tested for their effect on physical, sensory, chemical and nutritional characteristics of biscuits. Increase in thickness and decrease in width and spread factor of the biscuits was observed by increasing the percentage of pumpkin powders from 0-15%. Ash, fiber and fat contents of biscuits were increased significantly by replacing white flour with pumpkin powders. Pumpkin seeds flour 15% replacement provided optimal values for ash (1.65±0.020%), fat (32.46±0.06%) and fiber contents (1.24±0.009%). Replacement level from 5-10 % of all three types of powders got good scores, close to the control for color, taste, texture, flavor and overall acceptability. Pumpkin seeds powder supplemented biscuits exhibited a remarkable increase in protein quality of biscuits which can be compared with casein-based diet formulation. 15% replacement level of pumpkin seeds powder resulted in 63.38±0.45% BV, 87.17±0.6% TD, 55.47±0.46% NPU and 2.12±0.05 PER of biscuits and these results were close to the diet based on casein (67.19±0.24% BV, 95.82±0.72% TD, 63.85±0.19% NPU and 2.53±0.12a% PER values). Utilization of pumpkin parts in the form of powders, extracts and functional food products should be the key strategy to improve humans' health.

Keywords: acceptability; biscuits; chemical composition; digestibility; hardness; protein quality

Introduction

Nowadays, fruits and vegetables industries produce bulky quantities of waste by products, which are not actually waste but possesses huge economic potential. These by products are mainly composed of skin with

lower percentages of seeds and pulp. All these by products are comprised of interesting chemical composition and therefore these can be measured as excellent raw materials for the production of value-added foodstuffs [1]. Kaur *et al.* [2] reviewed food usage and nutritional status of pumpkin (*Cucurbita*), sideways a range of potential health benefits. *Cucurbita* (pumpkin) belongs to family *Cucurbitaceae*. It is heat-sensitive plant possessed with edible fruit, which has adequate amounts of bio actives, reported for abundant health benefits, namely, antioxidants, antidiabetics, hypotensive, anticarcinogenic, hyper protective actions. Pumpkin is a rich source of all micro and macro nutrients, vitamins and amino acids. The recent search and demand of consumers for health encouraging food products has unlocked a competition for different food formulations containing bioactive compounds.

Pumpkin is a fellow of the family *Cucurbitaceae*. It includes *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita mixta*, *Cucurbita ficifolia* and *Telfairia occidentalis* Hook. *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata* are three economically important species, harvested all over the world with high production [3]. Cultivation of pumpkin is observed in Asia (China and Subcontinent), Europe (France and Portugal), Western America and also spread from Mexico to Chile and Argentina. Cultivation of pumpkin can be done from sea level to high altitude. It is annual vine having a number of large fruits and is well known for its edible fruit, seeds and green [4]. Pumpkin seeds are considered its most important fraction due to their low-fat and high-protein proportion [5]. After the seeds another important fraction of pumpkin is its edible fruit. Mature fruits are used in desserts, confectionary and beverages (alcoholic and non-alcoholic) on the other hand immature fruit is prepared as eatable vegetable. Pumpkin is a respectable source of carbohydrates, mineral and vitamins. It is also a decent source of β carotene. In America different parts of pumpkin fruit are used as food throughout its cultivation area [6].

Different scientists in past incorporated pumpkin powders in different bakery products to check the level of suitability, changes in physico-chemical composition, effects on sensory parameters and nutritional quality of bakery products. Production of flours of peels and pulp of pumpkin and their physico-chemical analysis revealed that these parts are good sources of nutrients and their incorporation in biscuits preparation imposed high impact in biscuit industry [7]. Al-Demery [8] replaced wheat flour with pumpkin flour at 5, 10, 15 and 20% level to evaluate physic-chemical properties of bread. Pongjanta *et al.* [9] replaced 10, 20, 30, 40 and 50% pumpkin powder to prepare cakes and cookies. Kulkarni and Joshi [10] prepared biscuits in which pumpkin powder replaced white flour. Giami *et al.* [11] supplemented wheat flour with fluted pumpkin seeds flour at 5, 10, 15, 20 and 25% level to check quality and sensory attributes of cookies. Bhat and Bhat [12] incorporated pumpkin powder into wheat flour for preparation of cake. Turkosy and Ozkaya [13] supplemented wheat flour with pumpkin pomace powder to check cookie quality. Functional properties, sensory evaluation and nutritious quality of biscuits fortified with puree from pumpkin peel were explored and promising results were found [14].

Biscuits and cookies play much important role in diet of humans, which are normally taken with tea. On the other hand, infants utilize biscuits as their weaning food. While young school going children consume biscuits as snacks in their schools [15]. Biscuits are very popular across the world as

these are consumed by every age group of people and these are baked items with low moisture contents so exhibit longer shelf life, available in a variety of flavors and tastes, also these are ready to eat food items and now a days enriched with different economic sources of micro and macro nutrients **[16]**. Wheat flour, fat, sugar, egg and water are the necessary basic ingredients used in the development of biscuits. Among these ingredients white refined wheat flour which is utilized in bulk quantity does not acquire a healthy nutritional profile and is deficient in many necessary micronutrients and dietary fiber **[17]**. Utilization of composite flour technology to develop enriched and supplemented baked food products has gained much importance in recent times and is attracting food products developers and researchers to explore new food items **[18]**. Juikic *et al.* **[19]** investigated composite flours developed from press cake obtained after extraction of oil from pumpkin seeds, as alternate of white flour for the production of biscuits and concluded that press cake from pumpkin seeds residues after oil extraction can be utilized successfully as a purposeful and nutritional substitute for wheat flour. A successful combination of white flour and pumpkin flour for production of biscuits would be nutritionally beneficial. Biscuits developed by replacing white flour with pumpkin flour were not only nutritious but also impacted positive health effects on consumers **[20]**. In an experiment conducted on rats the effect of two limiting amino acids addition was checked on the biological value and digestibility of pumpkin seed cake protein remained after oil extraction **[21]**.

The main objectives of this study were, production of powders from pumpkin three fractions i.e., peel, flesh and seeds, characterization of these powders, food application of these pumpkin powders and biological studies of these powders at 0, 5, 10 and 15% replacement levels with white flour in biscuits preparation.

Materials and Methods

Procurement of raw materials

Ripe pumpkins (n=50) comprised of an average weight of 5±0.5 kg, were purchased from resident marketplace of District Sargodha, Pakistan. Albino rats with an average body weight 190-210 g of whichever sex were acquired from National Institute of Health Sciences, Islamabad, Pakistan. All chemicals used in this research work were of reagent grade and purchased of Sigma-Aldrich company, Germany. Remaining ingredients for development of biscuits were purchased from resident market of District Sargodha, Pakistan.

Preparation of pumpkin parts (peel, flesh and seeds) powders

Purchased mature pumpkin fruits were washed, manually peeled and separated into three fractions i.e., peel, flesh and seeds. Slicing of flesh portion into 2×3 inches pieces was done with knife. After slicing blanching at 94 °C for 2 minutes was performed to inactivate enzymes. Powder of each fraction was prepared by conventional hot air-drying method at 60 °C for 24 hours in hot air oven (BIOBASE HAS-T105 China). Grinding of dried parts was done with common spice grinder (NIMA NM-8300 Japan), to obtain fine quality powder as described by Pongjanta *et al.* **[9]** with some modifications. Final powders were filled in polyethylene sacks and stored at room temperature.

Preparation of composite flours

The composite flours containing different percentages of white flour, pumpkin peel powder, pumpkin flesh powder and pumpkin seeds powder were prepared as presented in (Table 1).

Preparation and storage of biscuits

Biscuits with 0, 5, 10 and 15% replacement levels of each pumpkin peel, pumpkin flesh and pumpkin seeds powder were developed

rendering to the method designated by Sultan [22] with roughly modifications. Prepared biscuits were packed in polythene bags and stored inside laboratory shelf at ambient conditions for further analyses.

Physico-chemical analyses of white flour, pumpkin peel, flesh and seeds powders and developed biscuits

White flour, pumpkin peel powder, pumpkin flesh powder, pumpkin seeds powder and biscuits were scrutinized to determine moisture, fat, fiber, protein and ash content according to standard methods given in AACC [23]. Width, thickness and spread factor of prepared biscuits were also determined by following the standard methods elaborated in AACC [23].

Sensory evaluation of developed biscuits

Freshly prepared and stored biscuits were subjected to sensory evaluation to calculate scores of colors, texture, taste, flavor and overall acceptability for 28 days' study, with weekly intervals, by 10 judges panel as described by Larmond [24] with slight modifications.

Biological studies of developed biscuits

The prepared biscuits from different composite flours were used for biological study as described by Hulse *et al.* [25]. Diet plan given in (Table 2) was used at 5% protein level to check true digestibility (TD), net protein utilization (NPU), biological value (BV), protein efficiency ratio (PER) and feed efficiency (FE) according to the procedures elaborated by Pellet and Young [26]. Diet blends were prepared based formulations described by Giami [27].

Statistical analysis

All investigates were executed in triplicate to get triplicate determinations and results were stated as means ± standard deviations. The statistical analysis was done using analysis of variance. Duncan's multiple-range test was used to differentiate between the mean values.

Results and Discussion

Proximate analysis of White flour, pumpkin peel, flesh and seeds powders

White flour, pumpkin peel powder, pumpkin flesh powder and pumpkin seeds powder were examined for proximate composition and data has been presented in Table 3. From the Table 3 chemical composition of white flour can be seen as moisture contents 13.40%, ash contents 1.05%, fat 0.95%, fiber 0.70% and protein 10.15%. These results were very close to chemical composition of white flour given by Awan *et al.* [17], in which moisture 12.46%, ash 0.85%, fat 1.18 %, fiber 0.58 % and protein was 11.58%. Results supportive to our study were found in research work conducted by Anitha *et al.* [28] when they compared proximate conformation of wheat flour and pumpkin powder.

Table 1. Composition of composite flours

Treatments	White Flour %	Pumpkin peel powder %	Pumpkin flesh powder %	Pumpkin seeds powder %
T_0	100	-	-	-
T_1	95	5	-	-
T_2	90	10	-	-
T_3	85	15	-	-
T_4	95	-	5	-
T_5	90	-	10	-
T_6	85	-	15	-
T_7	95	-	-	5
T_8	90	-	-	10
T_9	85	-	-	15

Table 2. Composition of samples, casein and protein free diets (Ingredients g/100 g)

Diet*	Sample	Corn oil	Salt mixture	Vitamin mixture	Cellulose	Starch	Casein
A	75.42	8	5	1	1	9.58	-
B	77.64	8	5	1	1	7.36	-
C	78.01	8	5	1	1	6.99	-
D	79.24	8	5	1	1	5.76	-
E	80	8	5	1	1	5	-
F	81.17	8	5	1	1	3.83	-
G	82.24	8	5	1	1	2.76	-
H	76.46	8	5	1	1	8.54	-
I	77.17	8	5	1	1	7.83	-
J	77.52	8	5	1	1	7.48	-
K	-	8	5	1	1	78.75	6.25
L	-	8	5	1	1	85	-

Diet*; A=100% white flour, B=5% pumpkin peel powder, C=10% pumpkin peel powder, D=15% pumpkin peel powder, E=5% pumpkin flesh powder, F=10% pumpkin flesh powder, G=15% pumpkin flesh powder, H=5% pumpkin seeds powder, I=10% pumpkin seeds powder, J=15% pumpkin seeds powder, K= casein diet, L= protein free diet

The results of proximate composition of pumpkin peel powder were; moisture 10.80%, ash 3.63%, fat 1.72%, fiber 3.10% and protein 1.90%. These results do not differ too much as described by Kim *et al.* **[29]** in his research work, in which chemical composition of three parts (peel, flesh and seeds) of pumpkin was analyzed. Kim's findings for pumpkin peel were as; moisture 75.679%, ash 1.120 %, fat 0.869%, fiber 2.235% and protein 1.654%. In Table 1 the values of moisture, ash, fat, fiber and protein in pumpkin flesh are 14.20, 1.35, 1.25, 1.85 and 1.25% respectively, which are similar to those given by Kim *et al.* **[29]**; moisture 84.043 %, ash 1.053%, fat 1.088%, fiber 0.420% and protein 1.131% in pumpkin flesh. The results for chemical composition of pumpkin seeds powder from (Table 3) indicated moisture 5.75%, ash 1.80 %, fat 7.40%, fiber 2.55% and protein 3.75% which are also similar to those described by Kim *et al.* **[29]** in which moisture, ash fat, fiber and protein contents in pumpkin seeds were 2.751, 4.422, 52.434, 16.154 and 27.48% respectively. Supportive results were obtained when Mala *et al.* **[30]** compared proximate composition of fresh pumpkin and pumpkin powder. Similar results supporting this research work were also observed in findings of Kundu *et al.* **[31]** when they studied the proximate composition of pumpkin flour during incorporation in wheat flour. From these given results it was clear that pumpkin seeds contained more amount of protein, ash, fiber and fat as compared to pumpkin peel and flesh. While pumpkin flesh contains lesser contents of protein, ash, fat and fiber as compared to pumpkin peel and seeds.

Physical characteristics of developed biscuits

Data of physical characteristics of biscuits has been presented in (Table 4). It was evident from the Table 4 that width of biscuits was decreased significantly by increasing level of all three types of pumpkin parts powders, while thickness was increased and due to this decrease in width and increase in thickness spread factor was also decreased. Similar results were obtained when Turksoy and Ozkaya **[13]** supplemented wheat flour with pumpkin pomace powder to check cookie quality. Giami *et al.* **[11]** added fluted pumpkin seeds powder with 5, 10, 15, 20 and 25% level in wheat flour for cookie

preparation and observed a decrease in width, thickness and spread factor of cookies. Supportive results were also obtained when Mala *et al.* [30] studied physico-chemical composition of muffins incorporated with pumpkin powders. Results similar to our study were found in the findings of Anitha *et al.* [28] when the studied the effect of mixing pumpkin powder with wheat flour and observed a significant decrease in width and increase in thickness of developed biscuits.

McWatters *et al.* [32] reported that during mixing of ingredients, more water absorption occurs, which increases the dough viscosity due to which spread factor of cookies is reduced. In case of pumpkin peel, flesh and seeds powders fiber percentage is greater than white flour (Table 1) and this fiber may be the reason of decrease in spread factor of biscuits. When wheat flour is replaced with some non-wheat flours for biscuits preparations, width is reduced due to more water absorption during mixing [33]. Same were the results in case of pumpkin peel, flesh and seeds powders incorporation in white flour. Seker *et al.* [34] reported that increasing fiber percentage during cookie preparation reduced the spread factor of cookies.

Table 3. Proximate composition of raw materials

Raw Materials	Moisture %	Ash %	Fat %	Fiber %	Protein %
White flour	13.56±0.02 b	1.06±0.02d	0.98±0.03d	0.72±0.01a	10.11±0.03a
Pumpkin peel powder	10.79±0.03c	3.61±0.04a	1.77±0.04b	3.14±0.03a	1.90±0.03b
Pumpkin flesh powder	14.29±0.05a	1.36±0.03c	1.24±0.03c	1.82±0.01c	1.24±0.04c
Pumpkin seeds powder	5.74±0.04d	1.82±0.05b	7.38±0.11a	2.54±0.04d	3.73±0.05d

Means with different alphabetical letters in a column are statistically significant while means accompanied with same alphabetical letters in a column are statistically non-significant (P>0.05).

Table 4. Physical analyses of developed biscuits

Treatments	Width (mm)	Thickness (mm)	Spread factor
T_0	257.33±1.45a	45.67±0.75bc	59.17±0.95a
T_1	236.17±2.62cd	44.90±0.67c	52.57±0.41c
T_2	222.33±1.86f	48.47±1.04a	45.80±0.52e
T_3	204.33±2.19gh	48.90±0.47a	41.85±0.01g
T_4	230.73±1.69de	47.13±0.55ab	48.57±0.12d
T_5	210.23±3.26g	47.93±0.61a	43.85±0.05f
T_6	198.17±1.31h	48.50±0.75a	40.97±0.18g
T_7	246.40±2.39b	44.13±0.55c	55.92±0.02b
T_8	239.67±3.23c	45.67±0.72bc	52.27±0.66c
T_9	225.97±1.74ef	47.33±0.69ab	47.77±0.61d

Means with different alphabetical letters in a column are statistically significant while means accompanied with same alphabetical letters in a column are statistically non-significant (P>0.05).

Chemical composition of developed biscuits

The results of chemical composition of biscuits with different levels of pumpkin peel, flesh and seeds powders have been elaborated in (Table 5). Each fraction of pumpkin powder (peel, flesh and seeds) was added by replacing 5, 10 and 15% white flour and a control with 100% white flour was used for preparation of biscuits. From the Table 5 it was clear that by increasing the level of pumpkin peel powder ash, fat and fiber

contents were significantly increased, while moisture and protein contents were slightly decreased. The addition of pumpkin flesh powder also caused significant increase in ash, fat and fiber contents and decrease in moisture and protein contents as protein contents in pumpkin flesh powder were lesser than in white flour. Similarly, the addition of pumpkin seeds powder resulted increment in ash, fat and fiber and decrement in moisture and protein contents. In case of pumpkin seeds powder, increase in fat contents was greater than other two pumpkin fractions, which was due to presence of greater fat contents in pumpkin seeds powders (Table 1). And in case of pumpkin peel powder, increase ash contents are greater than in other two types of powders, as pumpkin peel powder contains more amount of ash contents i.e. (Table 1). Al-Demery **[8]** replaced wheat flour with pumpkin flour at 5, 10, 15 and 20% level to evaluate physic-chemical properties of bread and similar results were obtained where carbohydrate and protein contents were decreased while ash, fiber and moisture contents were increased.

Kulkarni and Joshi **[10]** reported that moisture and protein contents were lower whereas ash and fiber contents were higher in biscuits, in which pumpkin powder replaced white flour. According to Kulkarni and Joshi **[10]** the decrease in moisture contents with increasing level of pumpkin powder was due to more amount of carbohydrate contents in pumpkin powder, which replaced refined white flour. Pongjanta *et al.* **[9]** replaced 10, 20, 30, 40 and 50% pumpkin powder to prepare cakes and cookies and reported increase in fat and carbohydrate contents. Giami *et al.* **[11]** supplemented wheat flour with fluted pumpkin seeds flour at 5, 10, 15, 20 and 25% level to check quality and sensory attributes of cookies and reported slight increase in moisture, fat, ash and protein contents where as crude fiber contents were slightly decreased. Some more similar results were found when Bhat and Bhat **[12]** incorporated pumpkin powder into wheat flour for preparation of cake and reported that moisture, crude fiber, ash and β carotene contents were increased and crude protein, crude fat and carbohydrates were decreased. Supportive results were also obtained when Mala *et al.* **[30]** studied physico-chemical composition of muffins incorporated with pumpkin powders.

Table 5. Chemical composition of developed biscuits

Treatments	Moisture %	Ash %	Fat %	Fiber %	Protein %
T_0	7.04±0.022a	0.57±0.012i	30.36±0.03h	0.37±0.012h	6.62±0.019a
T_1	6.80±0.012c	1.68±0.015c	30.88±0.05g	0.86±0.009e	6.44±0.018bcd
T_2	6.70±0.006d	1.88±0.009b	30.99±0.04fg	1.25±0.015b	6.38±0.007cde
T_3	6.58±0.018e	2.20±0.024a	31.07±0.04ef	1.57±0.009a	6.32±0.015def
T_4	6.90±0.055b	1.09±0.018h	31.13±0.03e	0.56±0.015g	6.25±0.023efg
T_5	6.84±0.027bc	1.18±0.009g	31.30±0.06d	0.77±0.009f	6.15±0.018g
T_6	6.69±0.052d	1.32±0.009f	31.46±0.02c	0.92±0.015d	6.22±0.140fg
T_7	6.14±0.026f	1.45±0.009e	32.07±0.05b	0.85±0.017e	6.54±0.023ab
T_8	5.93±0.028g	1.55±0.012d	32.16±0.03b	1.13±0.018c	6.49±0.046abc
T_9	5.75±0.027h	1.65±0.020c	32.46±0.06a	1.24±0.009b	6.45±0.023bcd

Means with different alphabetical letters in a column are statistically significant while means accompanied with same alphabetical letters in a column are statistically non-significant (P>0.05).

Sensory evaluation of developed biscuits

Sensory evaluation of biscuits was performed for color, taste, flavor, texture and overall acceptability. The results elaborated in (Table 6), indicated highest score of color for biscuits with 100% white flour. On the other hand, 5% and 10% replacement of white flour with pumpkin peel, flesh and seeds powders also got reasonably high score for color of biscuits by panelists, whereas 15% replacement of white flour with pumpkin parts powders got low scores for color. These low scores for 15% replacement level indicates that color of biscuits got dark due to more pumpkin peel, flesh and seeds powders especially in case of pumpkin flesh powder. Similar results for color were obtained when Giami *et al.* [11] prepared cookies with 5, 10, 15, 20 and 25% replacement levels of wheat flour with fluted pumpkin seeds powder and reported that acceptable cookies with 5 and 10% replacement levels got fairly high scores for color very close to the control sample (100 % wheat flour). Pongjanta *et al.* [9] reported that 10% replacement of wheat flour with pumpkin powder expressed best scores for color of prepared biscuits. Kulkarni and Joshi [10] reported that biscuits prepared with 2.5% replacement level of pumpkin powder scored maximum numbers for color.

The data for quality scores of tastes, flavor and texture revealed that T_0 obtained maximum scores from judges where as T_3, T_6 and T_9 were disliked by judges by giving minimum scores. From results it was clear that 5 and 10% replacement level of pumpkin peel, pulp and seeds powders, with white flour also got fairly high scores for taste, flavor and texture, which mean that these treatments were also liked by judges. Giami *et al.* [11] prepared cookies with 5, 10, 15, 20 and 25% replacement levels of wheat flour with fluted pumpkin seeds powder and reported that acceptable cookie with up to 15% replacement levels were very similar in terms of taste, flavor and texture to control cookies with 100% wheat flour. Pongjanta *et al.* [9] reported that 10% replacement level of pumpkin flour with wheat flour for preparation of cookies got good results for taste, flavor and texture. According to Kulkarni and Joshi [10], 2.5% replacement level of pumpkin powders obtained maximum numbers for taste, flavor and texture. El-Demery [8] reported that 5 and 10% substitution of pumpkin powder for bread preparation got good scores for taste, flavor and texture.

The mean scores for overall acceptability for each treatment and control sample have been presented in (Table 6). From the Table 6, it was clear that judges liked 5 and 10% replacement level of pumpkin peel, pulp and seeds powders in biscuits preparation as these treatments got scores close to the control sample. From Table 6 it was clear that as replacement level of pumpkin peel, pulp and seeds powders was increased from 10 to 15% scores for overall acceptability of biscuits were decreased. These results do not differ too much from those described by Giami *et al.* [11] who prepared cookies with 5, 10, 15, 20 and 25% replacement levels of wheat flour with fluted pumpkin seeds powder and reported that overall acceptability of cookies was close to control sample up to 15% replacement level. Similarly, Pongjanta *et al.* [9] reported that 10% replacement level of pumpkin flour with wheat flour for preparation of cookies got good scores for overall acceptability. Similar results were obtained for overall acceptability when Turksoy and Ozkaya [13] supplemented wheat flour with pumpkin pomace powder to check cookie quality. Results similar to our study were found in the findings of Anitha *et al.* [28] when they studied the effect of mixing pumpkin powder with wheat flour and noticed that 10% replacement level got good scores for sensory parameters. Different results were obtained when Mala *et al.* [30] incorporated pumpkin powder in wheat flour

muffins and declared that 20% replacement level was acceptable for good sensory characteristics.

Effect of storage on sensory characteristics of developed biscuits

Sample biscuits along with control were packed in polythene bags and stored for 28 days at ambient conditions inside a laboratory shelf were analyzed for sensory characteristics at 0, 7, 14, 21 and 28 days of intervals, to check color, taste, flavor, texture and overall acceptability. There was no quality deterioration observed by judges during this period of storage. Similar results of storage studies were obtained when Awan *et al.* [17] prepared biscuits from composite flour containing moth-bean flour.

Table 6. Sensory evaluation of developed biscuits

Treatments	Color	Taste	Flavor	Texture	Overall acceptability
T_0	7.40±0.221a	7.40±0.221a	7.50±0.224a	7.30±0.213ab	7.40±0.221a
T_1	6.90±0.180ab	6.70±0.213b	7.20±0.200a	6.80±0.249bc	7.30±0.153a
T_2	6.60±0.221b	6.30±0.213b	6.60±0.221b	5.80±0.200de	6.40±0.221b
T_3	5.40±0.163c	5.00±0.149c	6.10±0.180b	5.20±0.200f	5.20±0.200c
T_4	6.80±0.200b	6.50±0.167b	6.30±0.153b	6.70±0.213c	7.10±0.180a
T_5	6.50±0.167b	6.17±0.141b	6.36±0.232b	6.30±0.213cd	6.25±0.271b
T_6	5.30±0.153c	5.06±0.186c	5.42±0.159c	5.30±0.153ef	4.87±0.198c
T_7	7.40±0.163a	7.50±0.224a	7.60±0.163a	7.60±0.163a	7.60±0.163a
T_8	6.60±0.163b	6.40±0.163b	6.60±0.163b	6.40±0.221c	6.30±0.153b
T_9	5.40±0.145c	5.20±0.200c	5.20±0.200c	5.00±0.149f	5.30±0.153c

Means with different alphabetical letters in a column are statistically significant while means accompanied with same alphabetical letters in a column are statistically non-significant (P>0.05).

Biological studies of experimental biscuits

The results regarding biological studies of experimental biscuits have been presented in (Table 7). It was evident from the results that rats fed with 100% white flour had less weight gain (7.61±0.04 g) and this diet had low PER (1.15±0.02), low NPU (35.34±0.14) low TD (73.63±0.09) and Low BV (47.92±0.07) as compared to pumpkin parts flour supplemented diets. A slight increase in nutritional quality of biscuits supplemented with pumpkin peel powder and pumpkin flesh powder was observed as compared with biscuits containing 100% white flour. Whereas pumpkin seeds powder supplemented biscuits had a remarkable increase in protein quality of biscuits which can be compared with casein-based diet formulation. In diet formulations, 15% replacement level of pumpkin seeds powder resulted in 63.38±0.45% BV, 87.17±0.6% TD, 55.47±0.46% NPU and 2.12±0.05 PER of biscuits and these results were close to the diet based on casein (67.19±0.24% BV, 95.82±0.72% TD, 63.85±0.19% NPU and 2.53±0.12a% PER values). It was clear from the Table 7 that by increasing the level of supplementation of pumpkin parts powders nutritional value of biscuits was increased. No data in literature was found regarding protein quality of biscuits supplemented with pumpkin peel and pumpkin flesh powder, however some data was found regarding protein quality of whole pumpkin powder and pumpkin seeds powder by animal studies. Giami *et al.* [11] used pumpkin seeds flour at 0, 5, 10, 15 and 20% replacement levels with wheat flour and cookies supplemented with 15-20% replacement level were nutritionally comparable with casein-based diet and cookies supplemented with 5-10 % pumpkin seeds flour resulted

higher BV, TD, NPU and PER values as compared to cookies with 100% wheat flour. Results of present study had close resemblance with that of Sharma *et al.* [35], according to them BV, TD and NPU values of pumpkin seeds were 67, 92 and 62, respectively. They further analyzed amino acids profile of pumpkin proteins and resulted that Sulphur containing amino acids of cucurbit proteins were comparable with whole egg protein. Digestibility of pumpkin seed cake crude protein is rather high, on average 88% (apparent) and 94% (true), irrespective of the addition of limiting amino acids. After the addition of limiting amino acids the biological value of proteins NPU and PER increased [21]. According to Bressani and Arroyae [36] *Cucurbita farinose* (pepitoria pumpkin) flour proteins efficiency is 80 % close to the skim milk proteins. According to Cravioto *et al.* [37] PER value of raw pumpkin seed had increased from 2.5 to 2.75 with supplementation of lysine and further addition of soya bean meal increased this value up to 2.91. Pumpkin seed kernel flour had good quality protein; as expressed by their high TD, BV and NPU [38]. The quality of proteins from fluted pumpkin seeds was investigated using male Wistar albino rats. The proteins were found to have high true digestibility (TD) and feed conversion ratio (FCR), net protein utilization (NPU) and protein efficacy ratio (PER) [39]. The Curcubitaceae seeds had good protein quality as judged by the PER (0.75-1.36) and (NPU) (46.10-69.10) [40]. In vivo the protein quality of the Pumpkin rind, flesh and seeds was assayed by animal feeding experiments and supportive results were observed [41]. Pumpkin is one of the important vegetables, known for its nutritional and medicinal properties. Powders of constituent's parts (peel, flesh and seeds) of pumpkin improved quality of biscuits [42].

Table 7. Biological studies of experimental biscuits

Diet*	Weight gain (g)	Protein intake (g)	Protein efficiency ratio (PER)	Net Protein Utilization (NPU)	True Digestibility (TD)	Biological Value (BV)
A	7.61±0.04j	6.64±0.04i	1.15±0.02fg	35.34±0.14i	73.63±0.09h	47.92±0.07i
B	8.23±0.04i	6.93±0.02h	1.19±0.02f	37.24±0.28h	75.05±0.06g	49.54±0.06h
C	9.39±0.06g	6.89±0.05h	1.35±0.03e	37.99±0.04g	75.4±0.05g	50.44±0.13g
D	9.82±0.14f	7.14±0.1g	1.37±0.02e	40.93±0.14e	75.4±0.05ef	53.67±0.21e
E	8.27±0.06i	7.56±0.05f	1.09±0.04g	34.22±0.22j	75.68±0.1fg	45.33±0.19j
F	8.75±0.06h	7.83±0.03e	1.13±0.04fg	37.18±0.1h	76.78±0.07e	48.3±0.1i
G	10.48±0.06e	7.94±0.05e	1.34±0.05e	39.92±0.41f	76.39±0.99ef	52.46±0.47f
H	15.19±0.1d	8.38±0.13d	1.78±0.05d	48.94±0.05d	82.52±0.19d	59.44±0.13d
I	17.29±0.1c	8.85±0.15c	1.92±0.02c	51.68±0.26c	84.6±0.44c	61.28±0.09c
J	19.33±0.05b	9.29±0.03b	2.12±0.05b	55.47±0.46b	87.17±0.6b	63.38±0.45b
K	26.59±0.35a	10.9±0.05a	2.53±0.12a	63.85±0.19a	95.82±0.72a	67.19±0.24a

Diet*; A=100% white flour, B=5% pumpkin peel powder, C=10% pumpkin peel powder, D=15% pumpkin peel powder, E=5% pumpkin flesh powder, F=10% pumpkin flesh powder, G=15% pumpkin flesh powder, H=5% pumpkin seeds powder, I=10% pumpkin seeds powder, J=15% pumpkin seeds powder, K= casein diet

Conclusion

The results of this study revealed that increasing the level of supplementation of pumpkin parts (peel, flesh and seeds) powders, increases the nutritional quality of biscuits but color, flavor, taste, texture and overall acceptability of biscuits was acceptable up to 10 % replacement level. Among pumpkin peel, flesh and seeds the pumpkin seeds powder replacement with

white flour increased the protein quality of biscuits most. From present study it can be concluded that pumpkin powders could be used to replace white flour for the production of nutritional bakery products.

Authors' contributions

Conceived and designed the experiments: MY Quddoos & A Mehmood, Performed the experiments: MA Iqbal & MA Jamil, Analyzed the data: A Rafique, K Iftikhar & MA Majeed, Contributed materials/ analysis/ tools: MA Murtaza & S Noreen, Wrote the paper: A Hussain & T Kausar.

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