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Formulation of antioxidant rich muffins using pumpkin (*Cucurbita moschata* Duchesne ex Poir.) pulp, seeds and different cereal flours

Shabnam Rana, Anju K. Dhiman and Deepika Kathuria*[◆]

Department of Food Science and Technology, YS Parmar University of Horticulture and Forestry, Solan-173230, Himachal Pradesh, India

*Dairy Chemistry Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India

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Abstract

Pumpkin fruit is a rich source of β-carotene whereas, its seeds are an excellent source of protein, unsaturated fatty acid, vitamin E, etc. Due to the higher amount of functional ingredients and low economic value, it possesses great potential in the food industry. Present investigation aimed to utilize pumpkin pulp and seeds to a maximum extent along with different cereal flours for the development of muffins. Also, the storage stability was evaluated under refrigerated conditions for 20 days. Pumpkin pulp possessed 9.20 mg/100 g ascorbic acid, 8.86 mg/100 g β-carotene, 3.65 mg GAE/g total phenols and 25.57% antioxidant activity whereas, seed kernel has 11.73% of protein. The utilization of pumpkin pulp @ of 70% was found desirable to develop pumpkin muffins with whole wheat, rice and barley flours whereas up to 80 % with semolina. In addition, about 20% whole seed and 25% of seed kernel flour can be replaced by cereal flour to prepare muffins. The addition of pulp and seed flour increases nutritional and bioactive components. The developed muffins can be stored safely in aluminium laminated pouches (ALP) under refrigerated conditions for 20 days with minimum losses in nutritional value. Hence, bakery products can be prepared by incorporating pumpkin pulp and seed flour with improved nutritional and antioxidant properties at minimum production cost.

1. Introduction

Fruits and vegetables play a key role in human health and wellbeing. It constitutes of bioactive components which imparts defensive ability to the human body against cardiovascular disease, ischemic heart disease, diabetes, genitourinary diseases, bone-related disease, neurodegenerative disease, inflammatory disease, etc. (Chellammal, 2022). Pumpkin, commonly known as Sitaphal or kashiphal belongs to the family Cucurbitaceae and the genus, *Cucurbita*. The word pumpkin originated from the Greek word *Pepon* which means large melons (Marr *et al.*, 2004). It is tropical and subtropical vegetable crop that grows extensively in countries like Peru, Sri Lanka, Mexico, Brazil, India, etc. (Kripanand *et al.*, 2016). Worldwide, *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita ficifolia* and *Cucurbita turbaniformis* are the five common species of pumpkin (Lee *et al.*, 2003). Pumpkin comes in varied shapes, sizes and colours. The nutritional and health-protective value of the fruit, as well as proteins and oil from the seeds, has piqued the interest of agriculture, food-processing, pharmaceutical, and feed industries in recent years. Pumpkin and its seeds have been recently recognized as a functional food among people due to their increased interest in remaining healthy. It is a rich source of bioactive ingredients such as

vitamin E, ascorbic acid, carotenoids, zeaxanthin, selenium, phytosterols and linoleic acid. The oil component of seeds possesses saturated and unsaturated fatty acids such as palmitic, stearic, oleic, linolenic and gadoleic acid. Carotenoids possess potential function in preventing cancer (Vijaylakshmi *et al.*, 2022). In addition, pumpkin being rich in these nutrients exert antioxidant, antiviral, anticarcinogenic, and anti-inflammatory activity as well as they are known to reduce 16 respiratory infections thus, reducing the impact of COVID-19 (Hamid *et al.*, 2021; Kaushik *et al.*, 2022). The development of functional bakery products such as bread, biscuit, cake, muffins, etc., will not only improve the functional food quality by delivering bioactive components at the appropriate level for physiological effectiveness but will also provide a product that meets the consumer's requirements in terms of appearance, taste and texture.

Though, the popularity of functional foods is rapidly increasing in the food industry especially in bakery and/or confectionery products. Among different bakery products, muffins are considered as the most popular among children, adults, etc. Muffins are sweet baked products that are popular among consumers due to their soft texture and distinct flavour (Ureta *et al.*, 2014). Muffins have a typical porous structure and high volume which give them spongy texture. Several studies have been conducted in recent years to improve the nutritional value of muffins such as fiber rich, sugar free muffins, antioxidants rich and fat free muffins by using fruit pulp. Arifin *et al.* (2019) used pumpkin puree as a butter replacer @ 30% for the preparation of muffins with best textural properties (hardness and chewiness). Marinopoulou *et al.* (2020) developed

Corresponding author: Dr. Deepika Kathuria

Dairy Chemistry Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India

E-mail: deepukathuria@gmail.com

Tel.: +91-9756533626

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Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

wheat bread with enhanced phenolic content and antioxidant capacity by incorporating black and green olive pulp @ 15%. Further more, bread was formulated using watermelon puree/juice by substituting wheat flour up to 30% represented good rheological and sensory quality as observed by Sadji *et al.* (2018). Muffins prepared from raspberry and cranberry pomace in different baking conditions did not affect the texture and microstructure quality of control muffins (Mildner-Szkudlarz *et al.*, 2016). With the change in environment and lifestyle, people are more concerned about their health and food product ingredients and their affect in body. As pumpkin is a precursor of provitamin A hence, the study aimed at developing muffin with maximum utilization of pumpkin pulp and seed flour.

2. Materials and Methods

2.1 Material

Fully ripe pumpkins were purchased from the vegetable market, Solan. To conduct the study, the raw ingredients such as whole wheat flour, semolina, rice flour, barley flour, sugar, milk powder, refined oil, baking powder and baking soda were procured from the local market, Solan. All the chemicals used for analysis were of analytical grade.

2.2 Preparation of pumpkin pulp

Ripe pumpkin pulp was prepared using the standardized method given by Dhiman *et al.* (2018a). The ripe pumpkin was washed, cut into halves in order to remove the seeds, fluffy portions. The halves were divided into large slices and pressure cooked using 5% water. The mixture was allowed to cool at room temperature, followed by grinding in a mixer cum grinder. The pulp thus obtained was strained through a sieve and used further for the preparation of muffins.

2.3 Preparation of pumpkin seeds kernel and whole seed flour

Pumpkin seed kernels and whole seeds were roasted in a pan on gas stove till the seeds appeared light brown in colour. The seeds were allowed to cool at room temperature and ground using a mixer cum grinder separately. The powders thus obtained were sieved using 36 mesh metallic sieve and packed in aluminium laminated pouches (ALP) for further use in the study.

2.4 Preparation of pumpkin muffins with different cereal flours

The preliminary trials were performed to select the recipe for the preparation of pumpkin muffins using different cereal flours. The muffins were prepared using cereal flours including whole wheat/semolina/rice/barley (100 g), refined oil (15 ml), sugar (25 g), milk powder (20 g), baking powder (1.5 g) and baking soda (0.5 g). The cereal flour, sugar, baking powder, baking soda and milk powder were sieved in a bowl using 36 mesh size sieve. To this, oil was added with continuous mixing, followed by the addition of pumpkin pulp. Then the mixture was beaten well by the cut and fold method until a smooth batter was attained (approximately for 3 min). The cups of muffin were greased well with oil and batter was poured into cups, followed by garnishing with pumpkin tutti-frutti. The baking oven was preheated at 170°C and then muffin cups were placed in an oven and baked at a temperature of 170°C for 25-30 min. The whole wheat, rice and barley flour were replaced with

pumpkin pulp in 70:30 ratio whereas semolina in 80:20 ratio. The pumpkin muffins prepared using whole wheat, semolina, rice and barley flour were referred as T₁, T₂, T₃ and T₄, respectively. Pumpkin muffins with different cereal flour were analysed for physicochemical and sensory characteristics.

2.5 Preparation of pumpkin muffins supplemented with seed flour

The best treatment for pumpkin muffin selected under Section 2.3 was considered for the preparation of muffins supplemented with whole seed and seed kernel flour. The whole seed and seed kernel flour were used at different concentrations (5 to 30%) for replacing cereal flour of selected recipe. The best concentration was selected on the basis of sensory characteristics and was analysed for physicochemical characteristics.

2.6 Storage study of pumpkin muffins prepared using pulp and seed flour

The pumpkin muffins prepared using different cereal flours as well as supplemented with pumpkin seed flour selected under Section 2.4 and 2.5 were prepared, packed in aluminum laminated pouches and stored under refrigerated conditions. The quality of muffins was examined at a storage interval of 0, 10 and 20th day on the basis of physicochemical characteristics and sensory evaluation.

2.7 Physicochemical and sensory analysis

The weight of pumpkin fruit was determined using a top pan balance, whereas the length and width of ripe pumpkin were measured using a thread and scale. The weight of the peel/seed/core/fluffy portions of the fruit was subtracted to calculate pulp recovery. The colour of pumpkin flesh was assessed visually using the Royal Horticultural Society Color Chart. In case of muffins, dough yield was expressed in % by measuring the weight of baked product and weight of batter. Baking loss was also calculated in percent by measuring the loss of product weight after baking and weight of batter.

Ripe pumpkin fruit was tested for various chemical properties. The moisture content was determined using a hot air oven by calculating the weight loss due to water evaporation (AOAC, 2010). Ash content was determined in muffle furnace to obtain carbon free white ash. Fat content was analyzed in soxhtrontulin equipments (AOAC, 2010). Protein content was determined in Kjeldrtonulin Equipment where distilled solution was titrated with 0.02 N H₂SO₄ till light pink colour persisted at the end (AOAC, 2010). Fiber content was estimated using fibrotron instrument (Tulin equipments). Left residue was dried in hot air oven at 105°C till constant weight was achieved, then weighed and placed in muffle furnace at 600°C for 4 h (AOAC, 2010). b-carotene optical density was recorded at 452 nm and concentration was calculated using the standard curve as per the procedure given in Rangana (2009). Ascorbic acid was analyzed by titration method using 2,6-di-chlorophenol-indophenol as per method given in book Rangana (2009). Antioxidant activity was analysed as per the method given by Brand-Williams *et al.* (1995). The optical density of a total phenol sample was measured in a spectrophotometer at 765 nm. The concentration was calculated using the gallic acid standard curve and expressed as mg/100 g of sample using the method proposed by Singleton and Rossi (1965).

A panel of ten semi-trained judges rated pumpkin muffins based on their colour, texture, flavour, and overall acceptability on a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely).

2.8 Statistical analysis

The data was represented as mean and Standard deviation. The data for the physicochemical and sensory characteristics of different treatments of muffins were analysed by ANOVA and the level of significance were examined using Duncan's multiple range test ($p < 0.05$) using SPSS software. Independent t-test was used to observed the statistical difference between means of two treatments only.

3. Results

3.1 Physical and chemical characteristics of ripe pumpkin fruit and pulp

Table 1 represents the physical characteristics of ripe pumpkin fruit. The ten randomly selected samples of ripe pumpkin fruit had an average weight, length and diameter of 4380 g, 36.78 cm and 63.21 cm, respectively. The results were almost in the range as suggested by Dhiman *et al.* (2018b), Sharma *et al.* (2018). The firmness in the ripe pumpkin fruit was observed to be 21.38 lbs/inch². The percentage of flesh, peel and seeds was recorded to be 72.89, 4.35 and 13.7%, respectively and therefore, ratio of flesh:peel:seed was observed to be 77:4:19. Further, the pulp recovery from pumpkin flesh was found to be 83.46%. The visual colour of pumpkin peel and flesh was yellow orange group 19 A and yellow-orange group 15 A, respectively. The findings are in conformity with the results of Dhiman *et al.* (2018b) for pumpkin fruit.

Table 2 represents the nutritional characteristics of pumpkin fruit, pulp, whole seeds and seed kernels. The fruit and pulp possess TSS between 5.56 to 8.05°Brix including total sugar between 3.39 to 3.44%. Pumpkin whole seeds and seed kernel contain significantly higher values for fat, protein and ash content. However, fiber content was found to be maximum in pumpkin whole seed. The fat content, *i.e.*, 34.21 and 47.34% in whole seeds and seed

kernels is in line with the results of Elinge *et al.* (2012) and Nyam *et al.* (2013). The value for ash in whole seed and seed kernel were found to be higher than the results of Achu *et al.* (2005), while they are in accordance with the findings of Dhiman *et al.* (2018a). The ascorbic acid was noticed to be highest in seed kernel, followed by fruit, whole seed and minimum in pulp. Fresh fruit and pulp are rich in b-carotene and total phenols compared to whole seed and seed kernel. The antioxidant activity was recorded to be slightly higher in whole seed and seed kernel compared to fresh fruit and its pulp. The results for functional components such as ascorbic acid, b-carotene, total phenols and antioxidant activity in the present study were observed to be higher than the values reported by Dhiman *et al.* (2018a; 2018b); Sharma *et al.* (2018); Singh *et al.* (2016) and Zduñia *et al.* (2016).

Table 1: Physical characteristic of ripe pumpkin fruit

Characteristics	Pumpkin fruit (mean ± SD)
Weight (g)	4380 ± 370.53
Length (cm)	36.78 ± 1.39
Width (cm)	63.21 ± 0.17
Firmness(lbs/inch ²)	21.38 ± 0.36
Flesh (%)	72.89 ± 0.12
Seed (%)	4.35 ± 0.25
Peel (%)	13.7 ± 0.04
flesh:peel:seed	77:4:19
Pulp recovery (%)	83.46 ± 0.04
Colour of peel	yellow orange group (19A)
Colour of flesh	yellow orange group (15A)

Note: No. of treatments: 4, Replications: 4, Data presented in table are the mean and standard deviation.

Table 2: Chemical characteristic of ripe pumpkin fruit and pulp

Characteristics	Pumpkin fruit (mean ± SD)	Pumpkin pulp (mean ± SD)	Pumpkin seed (mean ± SD)	Pumpkin seeds kernel (mean ± SD)
Moisture (%)	87.29 ± 0.45	88.91 ± 0.12	5.6 ± 1.60	4.49 ± 0.06
TSS (°Brix)	8.05 ± 0.12	5.56 ± 0.07	-	-
Titrateable acidity (%)	0.06 ± 0.00	0.04 ± 0.00	-	-
pH	4.67 ± 0.25	5.25 ± 0.07	-	-
Total sugar (%)	3.44 ± 0.25	3.39 ± 0.32	-	-
Reducing sugars (%)	2.69 ± 0.13	2.13 ± 0.03	-	-
Fat (%)	0.43 ± 0.02	0.28 ± 0.02	34.21 ± 0.89	47.34 ± 0.45
Protein (%)	1.52 ± 0.02	1.37 ± 0.02	33.13 ± 0.82	45.54 ± 0.68
Fiber (%)	0.56 ± 0.06	0.51 ± 0.03	13.59 ± 0.01	0.87 ± 0.03
Ash (%)	0.77 ± 0.06	0.78 ± 0.04	4.91 ± 0.02	4.37 ± 0.01
Ascorbic acid (mg/100 g)	14.02 ± 0.12	9.20 ± 0.08	11.16 ± 0.08	16.17 ± 0.11
b-carotene (mg/100 g)	14.96 ± 0.08	8.86 ± 0.10	3.26 ± 0.03	1.68 ± 0.02
Total phenols (mg GAE/g)	3.94 ± 0.06	3.65 ± 0.22	0.66 ± 0.02	0.75 ± 0.02
Antioxidant activity (%)	28.91 ± 0.04	25.57 ± 0.43	38.29 ± 0.04	40.45 ± 0.10

Note: No. of treatments: 4, Replications: 4, Data presented in table are the mean and standard deviation.

3.2 Development of pumpkin muffins prepared using different cereal flour

3.2.1 Physicochemical characteristics of pumpkin-based muffins

It is evident from Table 3 that the pumpkin muffins prepared from different cereal flours exhibit a non-significant effect ($p>0.05$) for upper diameter, lower diameter, height and weight whereas significant differences for dough yield and baking loss. The value for upper diameter, lower diameter, height and weight ranged from 5.05 to 5.42 cm, 3.45 to 3.72 cm, 2.02 to 2.45 cm and 30.55 to 36.94 g, respectively. According to Ramya and Anitha (2020), the weight ranged between 38.12 to 41.31 g for muffins developed from coconut flour. The height of muffin developed using 20 to 30% of pumpkin puree was ranged from 5.00 to 5.13 cm as noticed by Arifin *et al.* (2019). Pumpkin muffins prepared using semolina possessed a

significantly minimum dough yield (87.14%), while maximum baking loss (12.48%). The chemical characteristics of pumpkin muffins prepared using different cereals are also presented in Table 3. The pumpkin muffin prepared using barley reflected maximum value for protein (7.66%), fat (18.65%), fiber (2.07%) content and pumpkin muffin prepared using whole wheat flour exhibited higher value for functional components such as ascorbic acid (5.15 mg/100 g), b-carotene (23.67 mg/100 g), total phenols (10.17 mg GAE/g) and antioxidant activity (64.51%). The protein, ash, b-carotene content found in pumpkin muffins using whole wheat flour and rice flour possess non-significant ($p<0.05$). Awuni *et al.* (2017) developed bread by incorporating 46% orange fleshed sweet potato puree in existing 100% wheat flour bread which contained 260 mg/100 g of b-carotene. The papaya (*Carica papaya* L.) puree at 50% levels was used by Madiha *et al.* (2018) for the preparation of cupcakes having dietary fiber (2.55%), vitamin A (291.33 mg/100 g) and C (35.90 mg/100 g).

Table 3: Physicochemical characteristics of pumpkin muffins prepared using different cereal flours

Characteristics	Pumpkin muffin with whole wheat flour (mean \pm SD)	Pumpkin muffin with semolina (mean \pm SD)	Pumpkin muffin with rice flour (mean \pm SD)	Pumpkin muffin with barley flour (mean \pm SD)
Upper diameter (cm)	5.17 \pm 0.22 ^a	5.05 \pm 0.12 ^a	5.15 \pm 0.12 ^a	5.42 \pm 0.16 ^b
Lower diameter (cm)	3.67 \pm 0.16 ^b	3.45 \pm 0.12 ^a	3.65 \pm 0.12 ^a	3.72 \pm 0.16 ^b
Height (cm)	2.35 \pm 0.12 ^{bc}	2.02 \pm 0.08 ^a	2.25 \pm 0.12 ^b	2.45 \pm 0.12 ^c
Weight (g)	33.69 \pm 0.20 ^c	30.55 \pm 0.28 ^a	32.47 \pm 0.52 ^b	36.94 \pm 4.48 ^d
Dough yield (%)	93.13 \pm 0.02 ^c	87.14 \pm 0.02 ^a	91.60 \pm 0.08 ^b	93.72 \pm 0.02 ^d
Baking loss (%)	6.89 \pm 0.02 ^b	12.80 \pm 0.04 ^d	8.48 \pm 0.04 ^c	6.29 \pm 0.02 ^a
Moisture (%)	34.61 \pm 0.02 ^a	41.86 \pm 0.55 ^b	35.43 \pm 1.20 ^a	41.36 \pm 1.95 ^b
Protein (%)	5.13 \pm 0.05 ^a	7.5 \pm 1.61 ^b	4.42 \pm 0.06 ^a	7.66 \pm 0.55 ^b
Fat (%)	18.29 \pm 0.02 ^c	17.45 \pm 0.12 ^b	16.55 \pm 0.07 ^a	18.65 \pm 0.09 ^d
Ash (%)	1.41 \pm 0.01 ^{ab}	1.31 \pm 0.03 ^a	1.53 \pm 0.08 ^b	1.52 \pm 0.14 ^b
Fiber (%)	0.56 \pm 0.02 ^a	1.97 \pm 0.07 ^c	0.70 \pm 0.06 ^b	2.07 \pm 0.10 ^c
Ascorbic acid (mg/100 g)	5.15 \pm 0.09 ^c	4.85 \pm 0.07 ^b	4.34 \pm 0.19 ^a	4.27 \pm 0.05 ^a
b-carotene (mg/100 g)	23.67 \pm 0.12 ^a	22.95 \pm 0.06 ^a	22.66 \pm 0.44 ^a	21.36 \pm 3.14 ^a
Total phenols (mg GAE/g)	10.17 \pm 0.06 ^c	9.38 \pm 0.18 ^b	7.54 \pm 0.55 ^a	9.27 \pm 0.24 ^b
Antioxidant activity (%)	64.51 \pm 0.21 ^d	52.38 \pm 4.80 ^c	43.49 \pm 2.25 ^b	35.46 \pm 1.18 ^a

The values with same lower case letter on superscript in the same row are non-significant at 0.05% level of significance.

3.2.2 Selection of treatment for the preparation of pumpkin muffins for supplementation with seed flours

The pumpkin muffins prepared using different cereal flours were subjected to sensory evaluation to select the recipe for supplementation with seed flour. The results of the sensory evaluation are detailed in Figure 1 which elucidate that the pumpkin muffins prepared using rice flour (T₃) obtained the highest overall

acceptability score (8.16), followed by T₂ (8.11), T₁ (8.05) and T₄ (8.00). The values with same lower case letter on chart labels are non-significant at 0.05% level of significance. The score for colour and texture was observed to be non-significant ($p>0.05$) among all the treatments. The score of 8.16, 8.23, 8.06 and 8.03 were awarded to treatment T₁, T₂, T₃ and T₄ for flavour, respectively. The maximum score of 8.26 and minimum of 8.00 was recorded in treatments T₂ and T₄, respectively for texture. Based on higher overall acceptability score, T₃ (pumpkin muffins with rice flour) was selected to conduct the study on the supplementation of pumpkin muffins with seed flours.

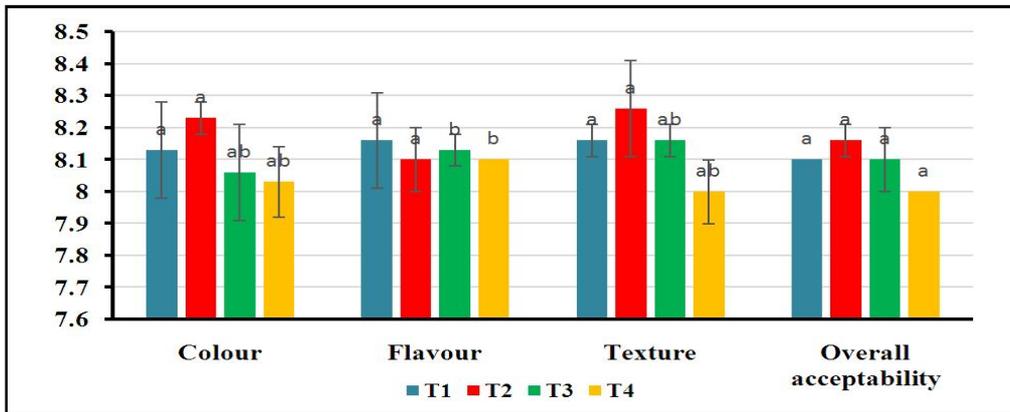


Figure 1: Sensory scores of pumpkin muffins prepared using different cereal flour.

3.3 Development of pumpkin muffins supplemented with seed flours

3.3.1 Sensory characteristics of pumpkin muffins supplemented with seed flour

Figure 2 elucidates the sensory score of pumpkin muffins supplemented with whole seed and seed kernel flour. The values with same lower case letter on chart labels are non-significant at 0.05% level of significance. In case of pumpkin muffins supplemented with whole seed flour, the results revealed that treatment T₅ (20% pumpkin seed flour) got a score of 8.86, 8.73, 8.76 and 8.76 for colour, flavour, texture and overall acceptability, followed by score of 8.63, 8.53, 8.50 and 8.53 in treatment T₄ (15% pumpkin seed flour). On the other hand, the sensory scores

for pumpkin muffins supplemented with seed kernel flour showed that the maximum overall acceptability score was obtained by muffins under treatment T₆ (25% pumpkin seed kernel flour), followed by T₄, T₅, T₁, T₃, T₂, and T₇. Furthermore, the panellists preferred samples from treatment T₆ (25% pumpkin seed kernel flour) despite the fact that the scores for all parameters in all treatments were well above the acceptable limits. Biaek *et al.* (2016) created muffins for children by partially replacing wheat flour with pumpkin seed flour (17, 33, and 50%) and discovered that muffins containing 33% pumpkin seed flour were rated as tasty and very tasty by more than 71% of the children. Nyam *et al.* (2013), on the other hand, discovered that 5% pumpkin rind bread had the best overall acceptability and sensory attributes, followed by 5% pumpkin seed bread.

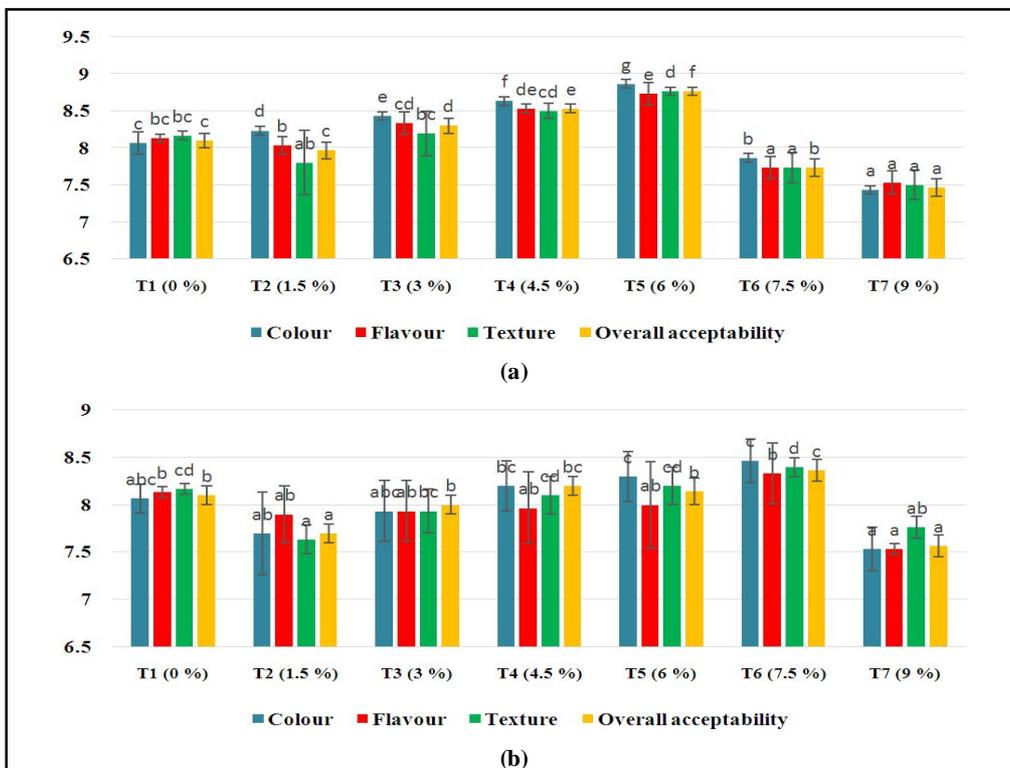


Figure 2: Sensory scores of pumpkin muffins supplemented with (a) whole seed and (b) seed kernel flour.

3.3.2 Physicochemical characteristics of pumpkin muffins supplemented with seed flours

It is evident from Table 4 that the pumpkin muffins prepared from whole seed and seed kernel flours vary significantly from each other in all physical and chemical characteristics. The non-significant differences ($p < 0.05$) were recorded for the upper and lower diameter as well as the height of pumpkin muffins. The weight and dough yield were found to be higher whereas there was lower baking loss in pumpkin muffins supplemented with seed kernel flour which might be due to higher nutritional components. The protein and fat content were observed to be higher in pumpkin muffins

supplemented with seed kernel flour compared to whole seed flour, however, fiber was higher on supplementation with whole seed flour. The function properties including ascorbic acid and total phenols were observed to be higher in pumpkin muffins supplemented with seed kernel flour whereas, pumpkin muffins supplemented with seed kernel flour exhibit higher value for b-carotene and antioxidant activity. Mittal (2018) developed pumpkin pie by supplementing pumpkin seed and seed kernel flour by replacing wheat flour in the ratio of 30:70 and 20:80, respectively and indicated the b-carotene content of 3.21 and 2.85 mg/100 g, respectively.

Table 4: Physicochemical characteristics of pumpkin muffins supplemented with seed flour

Parameters	Pumpkin muffin with whole seed flour (mean \pm SD)	Pumpkin muffin with seed kernel flour (mean \pm SD)
Upper diameter (cm)	5.15 \pm 0.12 ^a	5.25 \pm 0.12 ^a
Lower diameter (cm)	3.55 \pm 0.12 ^a	3.55 \pm 0.12 ^a
Height (cm)	2.55 \pm 0.12 ^a	2.35 \pm 0.12 ^a
Weight (g)	32.21 \pm 0.52 ^a	34.28 \pm 0.90 ^b
Dough yield (%)	92.24 \pm 0.04 ^a	94.28 \pm 00.6 ^b
Baking loss (%)	7.76 \pm 0.02 ^b	5.73 \pm 0.02 ^a
Moisture (%)	35.70 \pm 0.08 ^a	35.84 \pm 0.01 ^b
Protein (%)	9.76 \pm 0.11 ^a	11.73 \pm 0.01 ^b
Fat (%)	21.92 \pm 0.07 ^a	25.51 \pm 0.02 ^b
Ash (%)	1.57 \pm 0.00 ^a	1.59 \pm 0.00 ^b
Fiber (%)	3.49 \pm 0.04 ^b	0.88 \pm 0.00 ^a
Ascorbic acid (mg/100 g)	6.12 \pm 0.03 ^a	7.57 \pm 0.03 ^b
b-carotene (mg/100 g)	25.37 \pm 0.02 ^b	25.24 \pm 0.03 ^a
Total phenols (mg GAE/g)	7.96 \pm 0.02 ^a	8.20 \pm 0.02 ^b
Antioxidant activity (%)	44.37 \pm 0.01 ^b	44.01 \pm 0.02 ^a

Notes: No. of treatments: 4, Replications: 3, Data presented in table are the mean and standard deviation. The values with same lower case letter on superscript in the same row are non-significant at 0.05% level of significance.

3.4 Chemical characteristics of pumpkin muffins during storage

The quality of pumpkin muffins revealed during storage studies of 0, 10 and 20th day under refrigerated conditions are given in Table 5. The data represents an increase in the moisture content of muffins during storage. However, a non-significant increase was recorded in muffins T₃, T₄ and T₅. Further, among different treatments mean maximum value of 42.80% was observed in T₂ and a minimum of 35.17% in T₁ when muffins were stored at refrigerated conditions. However, protein, fat, ash and fiber content were noted to decrease during storage. A non-significant decrease in protein content was recorded in each treatment except T₆. Significant decrease in fat and ash content was recorded with an increase in storage time. The non-significant effect was recorded in case of fiber content in treatment T₂, T₃ and T₄. Among different treatments, the mean maximum

value of 11.73, 25.51 and 1.59% was noticed in T₆ for protein, fat and ash content, respectively. However, treatment T₅ exhibits maximum per cent for fiber content. The effect of storage on ascorbic acid (Table 5) revealed that the mean maximum value was recorded under analysed in T₆ with a value of 7.57 mg/100 g while the minimum value of in T₄ (4.13 mg/100 g) was recorded in T₄ for storage under refrigerated conditions. Except for T₂, there was a decreasing trend in ascorbic acid content during refrigerated storage. An appraisal of data revealed that there was a decreasing trend of b-carotene and total phenol content in muffins during storage however, the effect was significant in T₅ and T₆ for b-carotene and total phenol, whereas T₄ and T₅ for total phenols. The mean value in T₁, T₄, T₅, and T₆ was found to decrease significantly after 20 days of refrigerated storage. The overall effect of treatments on antioxidant activity depicts the highest value of 64.51% in T₁ while lowest value of 35.46% in T₄ at 0 day of storage.

Table 5: Effect of chemical characteristics of pumpkin muffins during storage

Parameters	Treatment	Storage interval (days)		
		0	10	20
Moisture (%)	T ₁	34.61 ± 0.02 ^{aA}	35.22± 0.06 ^{aB}	35.68± 0.19 ^{aC}
	T ₂	41.86 ± 0.55 ^{bA}	43.02± 0.48 ^{bB}	43.53± 0.54 ^{bB}
	T ₃	35.43 ± 1.20 ^{aA}	36.32± 1.16 ^{aA}	37.00± 0.83 ^{aA}
	T ₄	41.36 ± 1.95 ^{bA}	41.92± 1.74 ^{bA}	42.36± 1.68 ^{bA}
	T ₅	35.73 ± 0.03 ^{aA}	35.72 ± 0.48 ^{aA}	32.66 ± 6.36 ^{aA}
	T ₆	35.84 ± 0.01 ^{aA}	36.12 ± 0.08 ^{aB}	36.47 ± 0.17 ^{aC}
Protein (%)	T ₁	5.13 ± 0.05 ^{aA}	5.10± 0.06 ^{aA}	4.99± 0.09 ^{aA}
	T ₂	7.50 ± 1.61 ^{bA}	7.47± 1.60 ^{bA}	7.38± 1.58 ^{bA}
	T ₃	4.42 ± 0.06 ^{aA}	4.40± 0.05 ^{aA}	4.33± 0.04 ^{aA}
	T ₄	7.66 ± 0.55 ^{bA}	7.62± 0.54 ^{bA}	7.54± 0.54 ^{bA}
	T ₅	9.76 ± 0.10 ^{cA}	9.71 ± 0.09 ^{cA}	9.64 ± 0.11 ^{cA}
	T ₆	11.73 ± 0.01 ^{dC}	11.69 ± 0.00 ^{dB}	11.63 ± 0.02 ^{dA}
Fat (%)	T ₁	18.29 ± 0.02 ^{cC}	18.07± 0.04 ^{cB}	17.97± 0.06 ^{cA}
	T ₂	17.45 ± 0.12 ^{bB}	17.34± 0.06 ^{bA}	17.07± 0.05 ^{bA}
	T ₃	16.55 ± 0.07 ^{aC}	16.33± 0.04 ^{aB}	16.14± 0.05 ^{aA}
	T ₄	18.50 ± 0.13 ^{dC}	18.40± 0.05 ^{dB}	18.21± 0.10 ^{dA}
	T ₅	21.92 ± 0.07 ^{eB}	21.69 ± 0.01 ^{eA}	21.54 ± 0.11 ^{eA}
	T ₆	25.51 ± 0.02 ^{fB}	25.28 ± 0.19 ^{fAB}	25.07 ± 0.04 ^{fA}
Ash (%)	T ₁	1.41 ± 0.01 ^{abC}	1.36± 0.01 ^{abB}	1.28± 0.02 ^{bA}
	T ₂	1.31 ± 0.03 ^{aB}	1.24± 0.06 ^{aA}	1.15± 0.02 ^{aA}
	T ₃	1.53 ± 0.08 ^{bcB}	1.43± 0.04 ^{bcAB}	1.33± 0.05 ^{bA}
	T ₄	1.52± 0.14 ^{bcA}	1.46± 0.15 ^{bcdA}	1.31± 0.14 ^{bA}
	T ₅	1.57 ± 0.00 ^{cB}	1.56 ± 0.00 ^{cdA}	1.55± 0.00 ^{cA}
	T ₆	1.59 ± 0.00 ^{cB}	1.58± 0.00 ^{dB}	1.56± 0.01 ^{cA}
Fiber (%)	T ₁	0.56 ± 0.02 ^{aB}	0.54± 0.01 ^{aB}	0.49± 0.02 ^{aA}
	T ₂	1.97 ± 0.06 ^{dA}	1.95± 0.06 ^{dA}	1.91± 0.05 ^{dA}
	T ₃	0.70 ± 0.06 ^{bA}	0.68± 0.07 ^{bA}	0.64± 0.07 ^{bA}
	T ₄	2.07 ± 0.10 ^{eA}	2.01± 0.11 ^{eA}	1.99± 0.11 ^{dA}
	T ₅	3.49 ± 0.04 ^{fB}	3.47 ± 0.01 ^{fB}	3.41 ± 0.01 ^{eA}
	T ₆	0.88 ± 0.00 ^{cB}	0.87 ± 0.00 ^{cA}	0.86 ± 0.01 ^{cA}
Ascorbicacid (mg/100 g)	T ₁	5.15 ± 0.09 ^{cB}	4.95± 0.15 ^{baB}	4.87± 0.08 ^{cA}
	T ₂	4.85 ± 0.07 ^{bA}	4.45± 0.45 ^{aA}	4.36± 0.47 ^{bA}
	T ₃	4.34 ± 0.19 ^{aB}	4.21± 0.22 ^{aAB}	3.94± 0.07 ^{cA}
	T ₄	4.27 ± 0.05 ^{aC}	4.16± 0.04 ^{aB}	3.96± 0.03 ^{cA}
	T ₅	6.12 ± 0.03 ^{dC}	5.99 ± 0.06 ^{cB}	5.82 ± 0.06 ^{dA}
	T ₆	7.57 ± 0.03 ^{eB}	7.48 ± 0.06 ^{dAB}	7.32 ± 0.15 ^{eA}

b-carotene (mg/100g)	T ₁	23.67 ± 0.12 ^{abA}	23.45 ± 0.18 ^{abcA}	23.51 ± 0.11 ^{abA}
	T ₂	22.95 ± 0.06 ^{abA}	22.80 ± 0.15 ^{abA}	22.74 ± 0.16 ^{abA}
	T ₃	22.66 ± 0.44 ^{aA}	22.45 ± 0.43 ^{aA}	22.84 ± 1.02 ^{abA}
	T ₄	21.36 ± 3.14 ^{aA}	21.07 ± 3.16 ^{aA}	21.02 ± 3.14 ^{aA}
	T ₅	25.37 ± 0.02 ^{bB}	25.32 ± 0.03 ^{cB}	25.19 ± 0.04 ^{bA}
	T ₆	25.24 ± 0.03 ^{bB}	25.19 ± 0.03 ^{bcB}	25.09 ± 0.04 ^{bA}
Total phenols (mg GAE/g)	T ₁	10.17 ± 0.06 ^{dA}	10.25 ± 0.63 ^{cA}	9.92 ± 0.61 ^{dA}
	T ₂	9.38 ± 0.18 ^{cA}	9.35 ± 0.48 ^{bcA}	8.97 ± 0.51 ^{cA}
	T ₃	7.54 ± 0.55 ^{aA}	7.94 ± 0.99 ^{aA}	7.51 ± 0.99 ^{aA}
	T ₄	9.27 ± 0.24 ^{cB}	8.94 ± 0.05 ^{abAB}	8.66 ± 0.22 ^{bcA}
	T ₅	7.96 ± 0.02 ^{abC}	7.74 ± 0.11 ^{aB}	7.49 ± 0.13 ^{aA}
	T ₆	8.20 ± 0.02 ^{bA}	8.65 ± 1.00 ^{abA}	7.91 ± 0.13 ^{abA}
Antioxidant activity (%)	T ₁	64.51 ± 0.21 ^{dC}	62.87 ± 0.04 ^{eB}	61.35 ± 0.09 ^{eA}
	T ₂	52.38 ± 4.80 ^{cA}	53.94 ± 0.04 ^{dA}	51.38 ± 0.09 ^{dA}
	T ₃	43.49 ± 2.28 ^{bA}	41.53 ± 2.40 ^{bA}	41.37 ± 0.01 ^{bA}
	T ₄	35.46 ± 1.17 ^{aC}	33.45 ± 1.11 ^{aB}	31.42 ± 0.05 ^{aA}
	T ₅	44.37 ± 0.01 ^{bB}	43.95 ± 0.03 ^{cA}	42.51 ± 0.49 ^{cA}
	T ₆	44.01 ± 0.02 ^{bB}	43.68 ± 0.11 ^{cA}	42.19 ± 0.59 ^{cA}

Notes: No. of treatments: 4, Replications: 3, Data presented in table are the mean and standard deviation. The values with same lower case letter on superscript in the same column for each parameter are non-significant at 0.05% level of significance. The values with same upper case letter on superscript in the same row for each parameter are non-significant at 0.05% level of significance.

4. Discussion

4.1 Effect of storage on chemical characteristics of pumpkin muffins

During 20 days of storage of pumpkin muffins under refrigerated conditions, an increase in moisture content was might be due to the absorption of moisture by muffins. Almost the same trend of increase in moisture content has been observed by Pasha *et al.* (2002) in cookies, Nagi *et al.* (2012) in biscuits and Sharma *et al.* (2016) in muffins. On the other hand, the decrease in nutrients like protein might have been caused due to denaturation of thermally unstable protein during storage. The decrease in fat content of muffins during storage may be attributed to the hydrolysis of triglycerides and the production of free fatty acid. The slight decrease in ash content might have occurred due to biochemical activities by micro-organisms. These changes may be attributed to thermally induced hydrolysis of complex carbohydrates (Bushway *et al.*, 1985). A similar decreasing order for protein, fat, ash and fiber content has been reported by Butt *et al.* (2004), Mushtaq *et al.* (2010) in cookies and Mittal (2018) in pumpkin pie and puff pastry during storage. In case of functional components, the loss of ascorbic acid during storage could be attributed to its oxidation to dehydro ascorbic acid, which is then hydrolyzed to 2,3-diketogluconic acids. Furthermore, the decrease in b-carotene content with an increase in storage period might be due to high susceptibility of carotenoids to auto-oxidative degradation during the processing and storage of food (Sharma *et al.*, 2000). Almost the same trend of decline in b-carotene content during storage has been observed by Butt *et al.* (2004), Mahmood *et al.* (2008) in

cookies, Nagarajaiah and Prakash (2015) in cookies and Mittal (2018) in pumpkin pie and puff pastry. The total phenols reduced during storage may be due to the formation of polymeric compounds complexed by the interaction of phenols with protein and their precipitates. These changes might have resulted in decrease in the percent of antioxidant activity.

5. Conclusion

Pumpkin pulp and seed muffins are outstanding bakery option among all age groups in terms of nutritional value. Pumpkin muffins are favorable alternative for junk food. Pumpkin pulp is an excellent source for the discovery of new products. Hence, it can be concluded that the ripe pumpkin which otherwise is processed to a limited extent, can be successfully and conveniently utilized for the development of functional and good quality muffins at a remunerative cost. b-carotene rich muffins are fine for children having deficiency in vitamin A. Pumpkin crop with wide prospective to produce product using pulp, seed. There are abundant possibilities to discover new product from pumpkin.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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