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Development and nutritional evaluation of multigrain cake-rusk supplemented with pomegranate peel powder

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Abstract
The growing trend of enhancing the nutritional value and bioavailability of baked goods by incorporating natural product components like pomegranate peel is gaining traction. Pomegranate peel, known for its medicinal and antioxidant properties, is often overlooked as a by-product in the pomegranate processing industry. In a recent study, a multigrain cake-rusk was developed by adding pomegranate peel powder (PPP) to a mix of wheat flour, chickpea flour, and pearl millet flour. As PPP content increased, sensory evaluations indicated a decrease in color, aroma, texture, taste, and overall acceptability scores. Proximate composition analysis showed that PPP-enriched cake-rusk had significantly higher levels of protein, fat, ash, and crude fiber compared to a control cake-rusk made only with wheat flour. Dietary fiber content also increased with greater PPP incorporation. Furthermore, the PPP-enriched cake-rusk exhibited higher levels of total phenols, flavonoids, and antioxidant activity. In terms of digestibility, PPP-added cake-rusk had lower *in-vitro* protein and starch digestibility than the control. Microbial analysis revealed reduced bacterial and fungal counts with increased PPP content, highlighting improved antimicrobial properties, aligning with previous studies on pomegranate waste. Over time, during storage, all cake-rusk samples saw declining organoleptic scores, with the control cake-rusk maintaining higher scores than the PPP-assisted version.

Keywords: Antimicrobial, multigrain cake-rusk, organoleptic, pomegranate peel powder, proximate composition

1. Introduction

The use of entire natural fruit components and their by-products in baked goods to improve nutritional value and bioavailability while keeping costs in check is on the upswing. Pomegranate (*Punica granatum*), a fruit from the Punicaceae family, has its origins in the Middle East but is now widely cultivated in Western countries. India, following Iran, is a major producer (Tharshini, 2016) [27]. Over centuries, different parts of the pomegranate fruit, particularly the peels, have been harnessed for their therapeutic properties, assisting in the treatment of conditions such as inflammation, fever, bronchitis, diarrhea, dysentery, vaginitis, urinary tract infections, and even malaria. These effects are attributed to beneficial phenolic components like ellagitannins and ellagic acid (Ahmed *et al*., 2014) [1]. Pomegranate peel, constituting about 50% of the fruit's weight, boasts various medicinal attributes, including accelerated wound healing, immune regulation, antibacterial effects, and anti-atherosclerotic and antioxidant properties. Pomegranate peels are rich in high molecular weight phenolics, ellagitannins, proanthocyanidins, intricate polysaccharides, flavonoids, and various trace elements. Despite being discarded after juice extraction, pomegranate peels have the potential to become one of the most valuable by-products in the food industry (Moorthy *et al*., 2015; Akhtar *et al*., 2015) [13, 2].

Pomegranate peel powder stands out with significantly elevated levels of lysine, leucine, aromatic amino acids (phenylalanine and tyrosine), threonine, and valine when compared to the standard protein pattern. This results in amino acid scores surpassing 100 (Rowayshed *et al*., 2013) [18]. The incorporation of pomegranate peel powder into food products based on wheat, millet, and pulses proves to be both cost-effective and advantageous in augmenting the amino acid composition, thus making a valuable contribution to human nutrition.

In addition to its established nutritional and nutraceutical importance, pomegranate peel also possesses appealing properties for stabilizing food systems and enhancing physiological attributes. Successful applications of pomegranate peel powder and extracts in various food items, including edible oils, bakery products, jellies, and meat products, have been documented (Ventura *et al*., 2013) [30].

The practice of using composite flours in the baking industry has a longstanding tradition and continues to attract significant attention in modern times. Wheat, although a primary source of protein and energy, falls short in providing certain essential amino acids. To improve the nutritional profile of wheat-based products, the common approach is to incorporate various legumes and grains. Among these alternatives, chickpeas (*Cicer arietinum* L) shine as legumes rich in protein, dietary fiber, carbohydrates, folate, and essential trace minerals like iron, molybdenum, and manganese (Meng *et al*., 2010) [11]. Similarly, pearl millets are highly nutritious and, in terms of energy, protein, vitamins, and mineral content, can rival or even surpass other major cereals. They serve as a unique source of pro-vitamin A and minerals such as calcium, magnesium, and phosphorus (Kaur *et al*., 2014) [9]. Pearl mill*et al*so exhibits anti-allergic properties and is recommended for addressing conditions like severe constipation, stomach ulcers, and weight loss, owing to its high fiber content. However, its use is primarily confined to traditional consumers and individuals from lower economic backgrounds, largely because it is predominantly used as whole flour in food preparation. Hence, the current study aims to develop and conduct a nutritional analysis of multigrain cake-rusk enriched with pomegranate peel powder.

2. Materials and Methods

In the current study for the development of the cake-rusk, wheat grains (Triticum aestivum, C-306), pearl millet (Pennisetumglaucum86-M-86) and chickpea (*Cicer arietinum* HC-5) were procured in a single lot from the Department of Genetics and Plant Breeding, CCS HAU, Hisar (29°08'58.7"N 75°41'39.1"E). Pomegranate (Kandhari Variety) was procured in bulk from the fruit market of Hisar (29°09'45.6"N 75°44'18.2"E).

2.1 Processing of Raw Material

The wheat, pearl millet and chickpea were cleaned and ground in an electric grinder (Cyclotec, M/s Tecator, Hoganas, Sweden). The flour obtained was sieved through a 60-mesh sieve and packed in airtight plastic containers for further use. The pomegranate fruits were first washed thoroughly after that fruit was peeled and cut into small pieces. Then the cut pieces of peel were dried in a tray drier (Khera Industry, K.I.180 and Delhi, India) at a temperature of 50 °C until it reached a moisture contentof 10% (Mphahlele *et al*. 2016) [15]. The dried peel was ground into a fine powder using an electric grinder (Cyclotec, M/s Tecator, Hoganas, Sweden). The powder obtained was sieved through a 60 mesh sieve and was stored in an airtight HDPE bags (350 µm) at -18 °C (Remi Deep Freezer RRFD-250).

2.2 Production of Cake-rusk

Wheat flour (WF), chickpea flour (CF), pearl millet flour (PMF) and pomegranate peel powder (PPP) blended in a blender (Waring Commercial Products, Stamford, USA). The dough was mixed with a constant (15%) percentage of CF and PMF whereas the levels of PPP varied from 6%, 8%, 10% and 12%. The formulation of biscuit flour is given in Table 1. The dough was kneaded in BREDD HS20 spiral mixer and circular cake-rusk were made using cookies making machine (Cookies dropping machine TDM-N5, India). Cake-rusk were baked at 160 °C till brown colour in an oven (Luxurious Elector-thermal Film Baker, NFD-40F, Guangzhou China).

Table 1: Flour blends for development of multigrain cake rusk

Ingredients (%)	Control	Type-I	Type-II	Type-III	Type-IV
Wheat Flour (WF)	100	64	62	60	58
Chickpea Flour (CF)	0	15	15	15	15
Pearl Millet Flour (PMF)	0	15	15	15	15
Pomegranate Peel Powder (PPP)	0	6	8	10	12

2.3 Sensory evaluation of cake-rusk

The sensory evaluation in terms of color, appearance, aroma, texture, taste and overall acceptability was carried out using 9 points hedonic scale by a panel of 20 semi-trained judges of Chaudhary Charan Singh Haryana Agricultural University, Hisar, India.

2.4 Proximate composition of different flours and developed Cake-rusk

Proximate composition (moisture, ash, crude fat, protein and fiber) was determined by employing the standard method of analysis (AOAC, 2000). Total, soluble and insoluble dietary fiber constituents were determined by the enzymatic method (Furda, 1981) [5].

2.5 *In vitro* starch and protein digestibility different flours and developed Cake-rusk

In vitro starch digestibility was calculated by the method of Singh *et al*. (1982) [23]. Fifty mg of defatted sample was dispersed in 1.0 ml of 0.2 M phosphate buffer (pH 6.9) and mixed with 0.5 ml of pancreatic amylase. The mixture was incubated at 37 °C for 2 hours with shaking. After incubation, 2 ml of dinitrosalicylic reagent was quickly added and the mixture was heated for 5 minutes in a boiling water bath. After cooling and filtration, the absorbance was measured at 550 nm. A blank was run without enzyme, and a standard curve was prepared using maltose. The starch digestibility was calculated by dividing the concentration from the graph by the weight of the sample. *In vitro* protein digestibility calculated by the method of Mertz *et al*. (1983) [12]. Two hundred fifty mg of sample was combined with 20 ml of pepsin reagent in a centrifuge tube. The tube was sealed and placed in a shaker-incubator at 37 °C for 3 hours. After cooling, 5 ml of 50% TCA was added to the solution and centrifuged at 10,000 rpm for 10 minutes at room temperature, followed by filtration. A 10 ml aliquot was dried and digested for nitrogen determination using the micro kjeldahl method. Protein digestibility was calculated using the provided formula.

Protein digestibility (%) = (Digested protein/ Total protein) × 100 ……………………………………………………..…(1)

2.6 Total phenol, total flavonoids and antioxidant activity of different flours and developed Cake-rusk

The phenolic compounds were extracted following the procedure described in Ozgoren *et al*. (2019) [17]. The total phenolic content of the samples was determined using the Folin-Ciocalteu method (Singleton *et al*. 1999) [24]. The absorbance of the reaction mixtures were measured at 760 nm using a T80 UV/VIS Spectrometer (Thermo Scientific, GENESYS 50). The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g on a dry basis. Total flavonoids was determined by aluminium chloride method colorimetric method (Zhishen *et al*. 1999) [32]. The same extracted used in total phenol was used in estimation of total flavonoids. Then, 0.5 ml of 5% $NaNO_2$ was added to each test tube and mixed for 5 minutes. Subsequently, 0.6 ml of 10% $AlCl_3$ was added and mixed. After 6 minutes, 2 ml of 1N NaOH was added and thoroughly mixed. The volume was then made up to 10 ml by adding 2.1 ml of distilled water. The resulting pink color's absorbance was measured at 510 nm against a blank. The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable DPPH free radical, was determined by the method followed by Tadhani *et al*. (2007) [25]. A standard series of Trolox with known concentrations ranging from 10 to 40 µg was prepared. This involved taking aliquots of 0.1, 0.2, 0.3, and 0.4 ml, and adjusting the volume to 1.0 ml with methanol. These standard solutions were treated in the same manner as the samples. The absorbance of each solution was measured at 517 nm, and a calibration curve was constructed by plotting the absorbance values against the corresponding concentrations.

2.7 Organoleptic evaluation of cake-rusk during storage
Organoleptic evaluation of stored multigrain cake-rusk supplemented by pomegranate peel powder was periodically carried out an interval of 30 days by a panel of twenty semi-trained judges for colour, appearance, aroma, texture, taste and overall acceptability using a nine-point Hedonic scale.

2.8 Statistical Analysis
The statistical analysis followed a one-factor completely randomized design (CRD) with three replications. To assess the significance of differences (p<0.05) among the means, Duncan's multiple range tests were employed. The data analysis was conducted using IBM® SPSS® Statistics 19.0 software (IBM, Armonk, USA).

3. Results and Discussion
3.1 Organoleptic Acceptability of Cake-rusk
The comparison between the control and the developed cake-rusk is depicted in Figure 1. The average rating for color exhibited a significant decrease as the concentration of PPP increased (Table 2), a result in line with previous research by Ureta *et al*. (2014) [29] on cakes, Ismail *et al*. (2014) [6] on cookies, and Topkaya and Isik (2019) [28] on muffin cakes.
Scores for aroma significantly declined from 8.30 (indicating 'Liked very much') in Type-I to 5.25 in Type-IV as the PPP content increased. In the case of texture, as the content of PPP in the multigrain cake-rusk increased, the scores dropped from 8.40 (reflecting 'Liked very much') to 5.30, ranging from 'Liked very much' to 'Neither liked nor disliked.' This trend of decreasing texture scores with higher PPP content aligns with findings reported by Topkaya and Isik (2019) [28] and Viuda-Martos *et al*. (2012) [31].
Taste scores for the PPP-supplemented multigrain cake-rusk (PPPSMCR) also significantly decreased with increasing PPP content, ranging from 8.40 ('Liked very much') to 5.45 ('Neither liked nor disliked'). Similar observations regarding reduced organoleptic acceptability scores due to the addition of pomegranate peel were reported by Topkaya and Isik (2019) [28], Tharshini *et al*. (2018) [26], and Ismail *et al*. (2014) [6] in the context of muffins, nankhtai, cake-rusk, and cookies, respectively. The mean scores of the sensory characteristics of Type-IV were significantly lower than those of other supplemented and control cake-rusk. Type-IV cake-rusk fell within the category of 'Neither liked nor disliked,' prompting further analysis to concentrate solely on Type-I to Type-III cake-rusk.

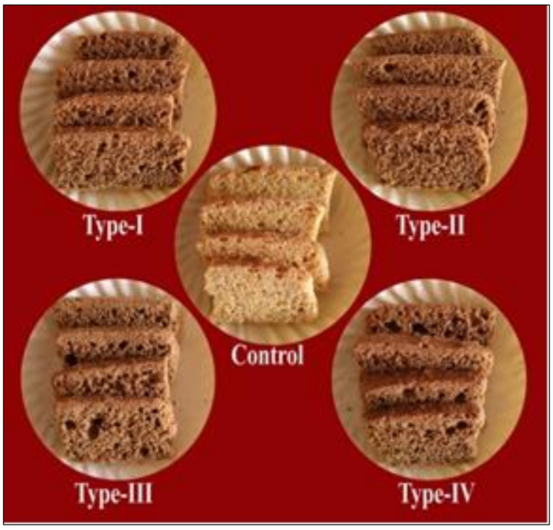

Fig 1: Pomegranate peel powder-supplemented cake-rusk

Table 2: Mean score of sensory characteristics of developed PPP-supplemented cake-rusk

Cake-rusk	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Control (RF 100%)	8.40±0.20	8.30±0.13	8.30±0.16	8.40±0.22	8.40±0.22	8.34±0.23
Type-I	8.20±0.13	8.00±0.18	8.10±0.18	8.20±0.18	8.30±0.16	8.16±0.12
Type-II	7.60±0.16	7.40±0.21	7.60±0.22	7.70±0.20	7.60±0.23	7.58±0.27
Type-III	7.40±0.21	7.25±0.26	7.40±0.26	7.40±0.23	7.40±0.21	7.37±0.22
Type-IV	5.10±.022	5.20±0.23	5.25±0.18	5.30±0.13	5.45±0.27	5.26±0.18

Values are mean ± SE of ten observations

3.2 Proximate composition of cake-rusk
Compared to the control (with 3.02% moisture), Type-I, Type-II, and Type-III multigrain cake-rusk exhibited significantly higher moisture contents of 3.54%, 3.72%, and 3.88%, respectively (Table 3). This increased water-holding capacity observed in the PPPSMCR can be attributed to the higher dietary fiber content in PPP as opposed to wheat. The moisture retention ability of the cake-rusk is directly influenced by their dietary fiber content, resulting in a significantly higher moisture content (Topkaya and Isik, 2019) [28].
The protein and fat contents in the control cake-rusk were

significantly lower than in the PPPSMCR (Table 3). The protein and fat contents in cake-rusk made from 100% refined flour were 9.82% and 20.52%, respectively. Type-I cake-rusk had a protein content of 11.23%, significantly higher than that of 100% refined flour cake-rusk. Similarly, Type-II cake-rusk had a protein content of 10.79%, which was also significantly higher than that of 100% refined flour cake-rusk. The protein content of Type-I cake-rusk was significantly higher than that of Type-II and Type-III cake-rusk, indicating that an increase in the percentage of pomegranate peel powder led to a decrease in protein content. Previous research by Topkaya and Isik (2019) [28] indicated that the protein content of pomegranate peel powder (PPP) is lower than that of wheat flour. However, in this study, the PPPSMCR showed significantly higher protein content compared to the control cake-rusk due to the presence of chickpea and pearl millet flour in addition to PPP. Lu *et al*. (2022) reported that cake-rusk incorporating chickpea flour had a higher protein content compared to whole wheat flour cake-rusk. Similarly, Singh *et al*. (2020) [20] reported that cake-rusk incorporating pearl millet flour displayed a higher protein content. Type-I cake-rusk exhibited a significantly higher protein content compared to Type-II and Type-III cake-rusk. This difference can be attributed to the fact that Type-II and Type-III cake-rusk contain a relatively higher proportion of PPP, resulting in comparatively lower protein content.

The fat content of PPPSMCR was significantly higher compared to the control cake-rusk. The fat content of cake-rusk increased from 21.72% to 23.05% with an increase in PPP. This higher fat content in the developed cake-rusk can be attributed to the higher fat content of PPP compared to wheat flour, as reported by Topkaya and Isik (2019) [28]. Similarly, Lu *et al*. (2022) reported that cake-rusk incorporating chickpea flour and pearl millet flour (Siddiq and Prakash, 2015) had higher fat content compared to wheat. The ash and crude fiber contents of the control cake-rusk made with wheat flour were determined to be 1.79% and 0.72%, respectively. These values were found to be significantly lower than those of the PPP supplemented multigrain cake-rusk. The ash and crude fiber contents of the PPPSMCR increased as the proportion of pomegranate peel powder (PPP) increased, as shown in Table 2. Type-III cake-rusk displayed higher ash and crude fiber contents compared to Type-II and Type-I cake-rusk. These findings align with previous studies (Essa and Mohamed, 2018 [4], Topkaya and Isik, 2019) [28] that reported higher ash and crude fiber content in multigrain products supplemented with pomegranate peel powder compared to control products developed using a single type of grain flour.

3.3 Dietary fiber: In Table 3, it is evident that the PPPSMCR showed notably higher (p<0.05) total, soluble, and insoluble dietary fiber contents in comparison to the control cake-rusk. With an increase in the level of PPP incorporation from 6 to 10 percent, there was a significant rise in the total, soluble, and insoluble dietary fiber content within the multigrain cake-rusk. Among the PPPSMCR variations, Type-III cake-rusk displayed a significantly (p<0.05) higher total, soluble, and insoluble dietary fiber content. These findings are in line with prior studies by Essa and Mohamed (2018) [4], Topkaya and Isik (2019) [28], and Tharshini (2016) [26], which highlighted variations in dietary fiber contents in various value-added baked products due to differences in the extent of supplementation with pomegranate peel powder. This difference is because the fiber content of PPP is higher compared to that of wheat, which leads to the observed changes in dietary fiber content.

3.4 Total phenol, flavonoids and anti-oxidant activity

The total phenol, flavonoid, and antioxidant activity levels in all varieties of multigrain cake-rusk with pomegranate peel powder were markedly higher (p≤0.05) than those in the control cake-rusk. As the level of PPP substitution in the cake-rusk increased, there was a notable and significant (p≤0.05) rise in the levels of total polyphenols, flavonoids, and radical scavenging activity. These outcomes are in line with prior research by Essa and Mohamed (2018) [4], Topkaya and Isik (2018), and Ismail *et al*. (2016) [7]. The heightened levels of total phenols, flavonoids, and antioxidant activity in the PPPSB can be attributed to the presence of pomegranate peel powder (Essa and Mohamed, 2018) [4]. The observed increase in the overall phenolic content and antioxidant activity levels of the cake-rusk is primarily due to the difference in total phenolic content and antioxidant activity values between pomegranate peel (PP) powder and wheat flour. PPP is rich in various polyphenols that play a pivotal role in conferring antioxidant properties to PPP-supplemented cake-rusk (Negi *et al*. 2003) [16]. Similar findings of enhanced antioxidant activity and total phenol content due to the addition of PPP were reported by Topkaya and Isik (2019) [28], Altunkaya *et al*. (2013) [3], Salgado *et al*. (2012) [20], and Ismail *et al*. (2014) [6].

Table 3: Biochemical composition of PPP-supplemented multigrain cake-rusk supplemented with pomegranate peel powder

Cake-rusk Parameter	Control	Type-I	Type-II	Type-III	CD (p≤0.05)
Moisture*	3.02±0.04	3.54±0.05	3.72±0.06	3.88±0.08	0.22
Protein	9.82±0.05	11.23±0.11	10.79±0.06	10.35±0.08	0.28
Fat	20.52±0.05	21.72±0.01	22.95±0.02	23.05±0.09	0.18
Ash	1.79±0.01	2.23±0.05	2.28±0.02	3.39±0.06	0.15
Crude fiber	0.72±0.05	2.24±0.08	3.24±0.09	3.82±0.11	0.29
Total fiber	8.42±0.08	9.40±0.10	9.88±0.20	10.35±0.14	0.46
Soluble fiber	1.70±0.14	2.20±0.06	2.40±0.12	2.78±0.17	0.44
Insoluble fiber	6.72±0.17	7.20±0.06	7.48±0.11	7.57±0.15	0.44
Total phenols (mg GAE/100 gm)	58.19±0.60	104.53±0.56	112.75±0.61	125.68±1.17	2.59
Total flavonoids (mg RE/100 gm)	39.75±0.75	105.69±0.92	122.75±0.46	145.84±0.43	2.23
Total Antioxidants	22.30±0.43	35.71±0.43	37.76±0.37	40.36±0.26	1.26
Protein digestibility (%)	72.49±0.01	72.04±0.03	71.85±0.03	70.51±0.05	0.12
Starch digestibility (mg maltose release/ g meal)	51.37±0.08	50.04±0.03	49.56±0.07	49.21±0.06	0.22

Values are mean ± SE of three independent determinations
*On a fresh weight basis

3.5 *In-vitro* protein and starch digestibility

The *in-vitro* protein and starch digestibility of the control cake-rusk made from wheat flour were significantly (p<0.05) higher in comparison to all types of PPPSMCR (Table 3). Among the multigrain cake-rusk variations, Type-I and Type-II displayed significantly higher *in vitro* protein digestibility than Type-III, although no significant difference was observed between Type-I and Type-II. Similarly, a significant (p<0.05) decrease in the *in-vitro* starch digestibility of PPPSMCR was noted with an increase in the level of supplementation with pomegranate peel powder. These findings are in accordance with studies conducted by Tharshini (2016) [26]. The disparities in starch and protein digestibility between the control and value-added baked products can be attributed to variations in the starch, protein, and antinutrient contents of the raw flour and pomegranate peel powder used in the product development. Additionally, the biological utilization of protein primarily depends on its digestibility (Tharshini *et al*. 2018) [26].

3.6 Changes in quality characteristics of stored cake-rusk

The initial sensory scores of the freshly baked cake-rusk for each type were excellent (Figure 2). However, as the storage duration increased, there was a gradual decline in the sensory scores. Throughout the storage period, the control group consistently maintained higher sensory attributes compared to the cake-rusk enriched with PPP. Notably, among the PPPSMCR varieties, Type III exhibited the lowest sensory scores. These findings are consistent with prior research conducted by Ismail *et al*. (2016) [7] and Tharshini (2016) [27], which reported that products supplemented with PPP and made from various composite flours could be stored for up to 90 days.

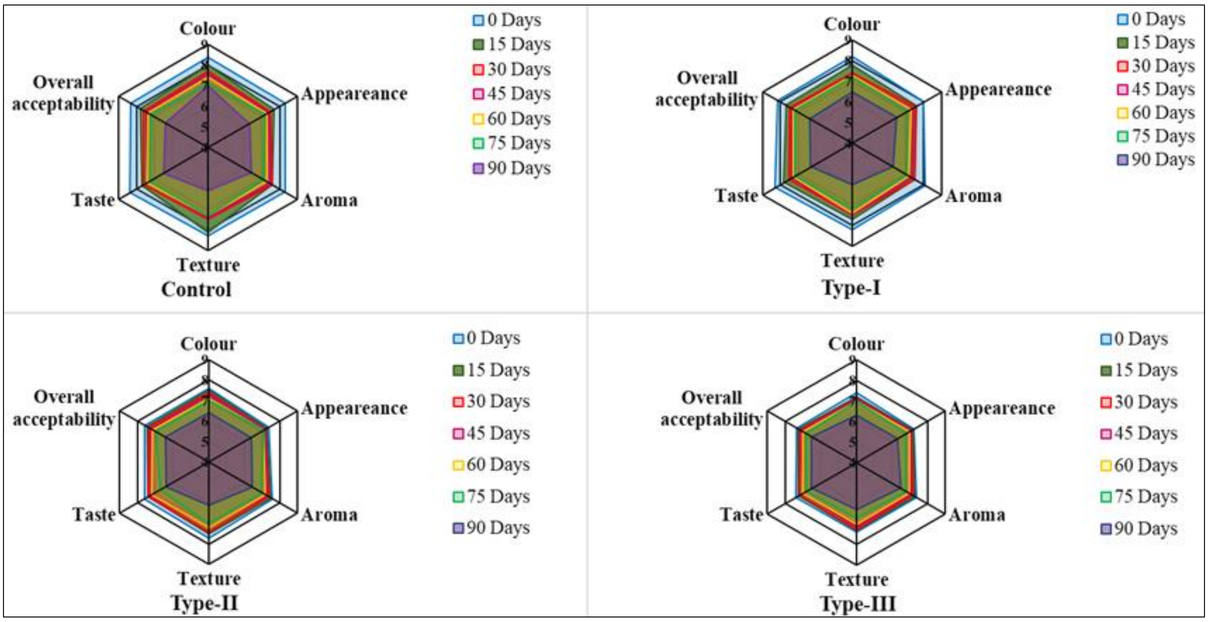

Fig 2: Organoleptic characteristics of pomegranate peel powder enriched and control cake-rusk during storage

4. Conclusion

In summary, the addition of pomegranate peel powder (PPP) to multigrain cake-rusk shows great potential for improving their nutritional content and antioxidant properties. Despite a decrease in sensory ratings as PPP content increased, all PPP-enhanced multigrain cake-rusk samples remained acceptable, indicating their potential for consumer approval. The inclusion of PPP led to higher protein, fat, ash, and crude fiber levels in the multigrain cake-rusk, making them a valuable source of essential nutrients and dietary fiber. Furthermore, the elevated levels of total phenols, flavonoids, and antioxidant activity in the PPP-enriched multigrain cake-rusk underscore the health benefits associated with their consumption. While the *in-vitro* digestibility of protein and starch in the PPP-enhanced multigrain cake-rusk was lower than in the control, the overall nutritional and antioxidant advantages outweigh this limitation. This study also revealed that the supplementation of pomegranate peel powder effectively curbs the growth of bacteria and fungi in stored cake-rusk. Overall, incorporating pomegranate peel powder into multigrain cake-rusk offers an innovative and sustainable approach to enhance the nutritional value and health-promoting qualities of baked goods.

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