**Selection of pumpkin seed pretreatment techniques for preparation of the pumpkin seed incorporated burfi**

**Abstract**

Burfi is an indigenous milk product made from khoa that is excellent in nutrition. Burfi contains various additives that boost its sensory appeal and customer acceptability. In this context, the nutritional value and health advantages of pumpkin seeds have been investigated for inclusion. Control burfi (C) was prepared without seed incorporation. Burfi (S1) was prepared by incorporating raw seeds. Four batches of burfi were prepared incorporated seeds pretreated with four methods viz. microwave roasted (S2), salt solution soaked and roasted (S3), dry salt roasted (S4) and ghee–coated roasted seeds (S5). Addition of pumpkin seeds in S1, S2, S3, S4 and S5 resulted in significant (P<0.05) increase in level of FFA, fat, protein and ash and significant (P<0.05) decrease in hardness, cohesiveness, springiness, adhesiveness, chewiness and gumminess compared to control. L\* and b\* value decreased significantly (P<0.05) while a\* value increased significant (P<0.05) in all experimental samples. Seeds roasted in dry salt at 85 to 90°C for 15 min (S4) were found to be the most acceptable based on sensory evaluation for use in pumpkin seed incorporated burfi.

**Keywords:** Pumpkin seed, pretreatment, roasting, burfi

**Introduction**

Burfi is a popular milk–based confection in India and has the potential to gain global recognition. It is a highly nutritious, khoa–based indigenous dairy product made from cow or buffalo milk, containing a significant amount of milk solids. On average, 100 g of plain milk burfi provides approximately 400 to 450 kcal, containing around 7 to 10 g of protein, 20 to 25 g of fat (with a significant portion as saturated fat) and 45 to 50 g of carbohydrates, of which about 35 to 40 g are sugars. Burfi also offers modest amounts of essential nutrients, including approximately 200 to 250 mg of calcium and 0.5 to 1.0 mg of iron, making it somewhat beneficial in terms of micronutrients, especially calcium due to its dairy base. Various types of burfi are available in the market, differentiated by the ingredients used, such as plain, mawa, pista, nut, chocolate, coconut and rava burfi, among others. Market samples of burfi often exhibit variations in their physical attributes. However, high–quality burfi is typically characterized by a moderately sweet taste, a soft and slightly greasy texture and a smooth consistency with fine grains (Kamble *et al*., 2019).

The incorporation of fruit seeds or seed powder in burfi production is becoming increasingly popular among consumers due to its unique flavor and enhanced nutritional value. Introducing such ingredients into burfi not only adds value to the product but also enriches it with essential nutrients. This innovation in the dairy industry contributes to product diversification, offering health benefits such as improved growth and immune support. The development of such nutrient–rich dairy products align with the growing consumer demand for healthier and more functional food options (Sharma *et al*., 2023).

Pumpkin, belonging to the genus Cucurbita and the family Cucurbitaceae, is cultivated worldwide for various purposes, including food, animal feed and decoration. While the flesh of many vegetables has long been a staple in the Indian diet, pumpkin seeds have often been discarded as waste despite their high nutritional value. After harvesting, these seeds are commonly used as animal feed, ground into fertilizer or simply discarded, even though they are rich in high–quality oil and protein. With increasing public awareness of sustainable agriculture, clean energy and efficient waste management, pumpkin seeds have the potential to secure a growing market in the snack food industry. Utilizing pumpkin seeds is expected not only to add value to production but also can aids in developing new food products. Hence an opportunity exists to explore pumpkin seeds in burfi for creating value–added products.

Roasting is one of the main processing operations applied to edible seeds and nuts and it can cause various physico‐chemical changes. Roasting involves use of conventional heat treatment similar to various other food processing operations such ascooking, drying, tempering, baking, pasteurization, and sterilization (Megahed, 2001; Rosenberg & Bogl, 1987). The goal of roasting was to improve sensory quality and achieve inactivation of destructive enzymes, which improves the storage and nutritional quality of the product (Griffith *et al*., 1998). Roasting enhances the appearance, colour, taste and aroma of the processed raw material (Mangala *et al*., 1999). The roasting conditions can cause significant changes in color, flavor, fatty acid profile, and bioactive compounds of oil kernel and seed (Kim *et al*., 2002; Yoshida & Kajimoto, 1994).

Consumers today are increasingly aware of the health implications of their diet and prefer dairy products that are not only delicious but also nutritionally enhanced. The integration of natural ingredients such as pumpkin seeds into burfi presents an opportunity to enhance the protein, mineral, fiber and antioxidant content of burfi; improve the sensory appeal (flavour, colour and texture) of burfi and develop a functional dairy sweet with health–promoting properties.

Despite the commercial success of traditional burfi varieties, limited research has been conducted on the incorporation of pumpkin seed in burfi production. The development of such a product could cater to the growing demand for healthier dairy–based sweets while utilizing locally available, cost–effective ingredients, keeping in view the above facts, this investigation has been planned with the objective to select pretreatment of pumpkin seed.

**Materials and methods**

Fresh, raw mixed (cow and buffalo) whole milk received at Anubhav Dairy; Anand was used as the base material for manufacture of burfi. Pumpkin seeds of “True elements” brand, HW wellness solutions Pvt. Ltd. Maharashtra were used. Cane sugar of “Madhur” Shree Renuka sugars Ltd; Mumbai brand of commercial grade (M grade) was used. Tata salt, Tata chemicals Ltd., Gujarat was used.

**Microwave processed pumpkin seeds (S2) :** Then pumpkin seeds were heated in a microwave oven at 200℃ for 25 min and cooled in room temperature.

**Salted and roasted pumpkin seeds (S3) :** Pumpkin seeds were soaked in a 4 % salt solution for 2 h and drained completely using a sieve. Seeds were roasted in sand by taking 1 kg of sand in a ss kadai and adding 100 g seeds. Seeds were roasted 85 to 90°C/15 min till roasted aroma developed and crackling of seeds started.

**Seeds roasted in dry salt (S4) :** Seeds were roasted in dry salt. One kg of salt and 100 g pumpkin seeds were taking in a ss kadai for roasting. Seeds were roasted 85 to 90°C/15 min till roasted aroma developed and crackling of seeds started.

**Roasted seeds coated with ghee (S5) :** One hundred g of pumpkin seeds was coated with a small quantity of ghee (~ 1 g). The ghee coated seeds were roasted in a thick bottomed stainless steel karahi. Ghee coated seeds were roasted 85 to 90°C/15 min till roasted aroma developed and crackling of seeds started.

**Coarse Grinding of Seeds :** After all of the above pretreatment of seeds, half the quantity of seeds was coarsely cut in a nut cutter machine and stored in an airtight container. The remaining half of the seeds were mixed with milk (30:70 ratio of seeds: milk) and finely ground to make a smooth paste and the pasted was stored in an air tight plastic jar.

**Preparation of burfi :** Burfi was prepared from milk standardized to (6.0% fat and 9.0% MSNF). Pretreated seeds (S2, S3, S4 and S5) were incorporated @ 3.0% w/w of milk. Half of the pretreated pumpkin seeds in the form of a paste were added after forewarming of milk (85°C/10 min). The remaining half (coarsely cut pumpkin seeds) were added after pat formation. Sugar was added @ 6 % w/w of milk. The contents were heated in an open pan with continuous stirring. After the desired consistency was obtained the contents were whipped and worked on a low flame for 2 to 4 min. Thereafter, the contents were spread in a clean greased tray, moulded and allowed to set at room temperature for 10 to 12 h. Burfi was cut into square pieces (40 × 30 mm size, 25 mm thickness) and packed in composite polyethylene terephthalate (PET)/low density polyethylene (LDPE) film (50μ thickness) pouches and placed in PET box. Burfi (S1) was prepared using raw pumpkin seed. The burfi sample without the addition of pumpkin seed will serve as a control (C). Six batches of burfi were prepared viz. C, S1, S2, S3, S4 and S5.

**Chemical analysis:** The determine the moisture content of burfi, a gravimetric method based on the (FSSAI 01.039:2022) for cheese was employed. Fat extraction of burfi was determined by Rose–Gottlieb method described in Section (FSSAI 01.073:2022). Protein content in burfi was estimated by Kjeldahl method (FSSAI 01.062:2022). Total carbohydrate was derived by difference. Ash content in burfi was determined as per the method described in FSSAI 01.077:2022. The free fatty acid (FFA) content of burfi was determined using the method described by Deeth *et al*. (1975).

**Colour :** The colour value of pumpkin seed incorporated burfi was measured by chroma meter (Konica Minolta Chroma Meter CR–400, Japan).

**Texture profile analysis:** Texture of the experimental burfi was analysed for Food Texture Analyzer (Loyd Instruments LRX Plus, England). More details here. A two–bite compression test was performed. Data were directly transferred to the NEXYGEN software (Lloyd Instruments) for statistical analysis and computation of specific texture parameters.

**Sensory Evaluation :** A panel of 10 judges familiar with the typical characteristics of dairy–based sweets were selected. Panelists were chosen based on their regular consumption of such products and consistent sensory perception across evaluation sessions. \_\_\_\_\_ The evaluation was carried out using a 9–point hedonic scale scorecard as recommended by Stone and Sidel (2004).

**Statistical analysis:** Statistical analysis was carried out using one-way ANOVA through MS-Excel.

**Results and discussion**

**Effect of Different Pretreatment of Pumpkin Seed on Sensory Score of Pumpkin Seed Incorporated Burfi**

As shown in Table 1 sensory analysis revealed that the flavour scores of samples ranged from 8.09±0.13 (C) to 6.88±0.12 (S3), with the preference order being C > S4 > S1 > S5 > S2 > S3. S4 (dry salt roasted seeds) was significantly (P<0.05) preferred among treated samples and was at par (P>0.05) with the control in flavour. In case of body and texture score, the C scored highest, followed by S5 and S1, while S3 scored the lowest. Although the control was significantly (P<0.05) superior to all treatments, no significant difference (P>0.05) was observed among S1, S2, S3, and S4. In terms of colour and appearance, the control had the highest score (8.31±0.10), while S5 had the lowest (7.02±0.29). Addition of pumpkin seed variants led to a greenish tinge and slight reduction in visual appeal, with S1 and S4 being statistically similar (P>0.05) and relatively better among treatments. The overall acceptability followed the trend: C > S4 > S1 > S5 > S2 > S3. S4 again stood out among experimental samples, scoring 7.68±0.15, and was the most accepted treatment after the control.

**Effect of Different Pretreatment of Pumpkin Seed on Chemical Composition of Pumpkin Seed Incorporated Burfi**

**Moisture :** Moisture content ranged from 18.60±0.54% (S5) to 20.20±0.33% (S2). The control (19.97 ± 0.30 %) was significantly (P<0.05) higher than S3 and S5, while other treatments showed no significant (P>0.05) difference. Variations may be due to processing or batch differences.

**Free fatty acid :** FFA values varied between 0.331±0.01 % (C) and 0.590±0.03 % (S5). FFA levels increased significantly (P<0.05) in all treated samples compared to the control. Pretreatment methods, particularly roasting and ghee–coating could have led to increases in FFA levels significantly (P<0.05). The increase in FFA across treatments indicates lipid hydrolysis or oxidation, possibly induced by thermal pretreatment and prolonged exposure to atmospheric oxygen during roasting. These results suggest a significant impact of processing on the stability of seed lipids. These results are in corroboration with those of Hosseini *et al*. (2017) who reported that roasting regimes decreased kernel PV and increased FFA content which may have implication for the shelf–life of the roasted kernels.

**Fat :** Fat content showed a significant increase in all treatments compared to the control, with values ranging from 21.86±0.29 % (C) to 24.07±0.32 % (S5). Treatments S5 exhibited significantly (P<0.05) higher fat content than the control, reflecting the improved fat extraction or retention due to pretreatments like roasting and ghee coating. The increased fat content enhances the energy density of the burfi. Pooja (2021) also reported that addition of pumpkin seed had significant (P<0.05) effect on the fat content of control kheer sample with increase in levels of pumpkin seed in paneer kheer. Auti (2024) also observed that the fat content in milkshakes was significantly (P<0.05) increased by the addition of roasted pumpkin seed powder compared to control milkshakes.

**Protein :** The protein content significantly increased with all pumpkin seed treatments. The highest protein content was found in S5 (16.08±0.27 %), followed closely by S3 (16.01±0.24 %), both significantly higher than the control (14.96±0.13 %). Other treatments (S1, S2, S4) also showed significantly increased protein levels, though slightly lower than S3 and S5. These findings confirm that pumpkin seed incorporation effectively improves the protein profile of the product. Pooja (2021) reported that addition of pumpkin seed had significant (P<0.05) effect on the protein content of paneer kheer with increase in levels of pumpkin seed in kheer. Auti (2024) also observed that the protein content in milkshakes was significantly (P<0.05) increased by the addition of roasted pumpkin seed powder compared to control milkshakes prepared without addition of pumpkin seeds.

**Ash :** Ash content was significantly higher in all treatments (2.89±0.10 to 2.96±0.09 %) compared to the control (2.35±0.12 %). However, no significant differences were observed among the treated samples themselves. This consistent increase is likely due to the inherent mineral richness of pumpkin seeds and suggests enhanced nutritional quality of the fortified burfi. Pooja (2021) also reported that increase in level of pumpkin seed had significant effect on the ash content of control and experimental paneer kheer sample. Auti (2024) observed that the ash content in milkshakes was significantly (P<0.05) increased by the addition of roasted pumpkin seed powder compared to control milkshakes.

**Carbohydrate :** Carbohydrate content was significantly (P<0.05) reduced in all treated samples compared to the control, decreasing from 40.86±0.34 % (C) to a range of 38.23±0.37 to 38.48±0.45 %. There were non–significant differences (P>0.05) among the treatment groups. The decrease in carbohydrate content could be attributed to increased fat, protein and ash levels in pumpkin seeds add burfi. This modification contributes to a more balanced nutritional profile.

**Effect of Different Pretreatments of Pumpkin Seed on Rheological Properties of Pumpkin Seed Incorporated Burfi**

The rheological attributes of PSIB and control burfi, including hardness, cohesiveness, chewiness, adhesiveness, gumminess and springiness as influenced by the type of pretreatment applied to pumpkin seeds is presented in Table 1.

**Table 1: Effect of different pretreatment of pumpkin seed on rheological property of pumpkin seed incorporated burfi**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Burfi sample** | **Hardness (N)** | **Cohesiveness** | **Chewiness**  **(N mm)** | **Adhesiveness**  **(N mm)** | **Gumminess**  **(N)** | **Springiness**  **(mm)** |
| **C** | 23.19a  ±1.35 | 0.046  ±0.002 | 3.361a  ±0.02 | 0.315d  ±0.03 | 1.067b  ±0.02 | 3.158a  ±0.02 |
| **S1** | 16.99d  ±0.80 | 0.031  ±0.001 | 0.541e  ±0.03 | 0.448c  ±0.02 | 0.527d  ±0.01 | 1.041d  ±0.05 |
| **S2** | 16.57d  ±0.90 | 0.068  ±0.002 | 2.163b  ±0.01 | 0.426c  ±0.01 | 1.120a  ±0.03 | 1.934b  ±0.03 |
| **S3** | 19.12c  ±0.50 | 0.024  ±0.001 | 0.384f  ±0.03 | 0.531b  ±0.03 | 0.451e  ±0.02 | 0.848e  ±0.04 |
| **S4** | 18.82c  ±0.60 | 0.028  ±0.002 | 0.678c  ±0.02 | 0.494b  ±0.01 | 0.543cd  ±0.01 | 1.259c  ±0.03 |
| **S5** | 19.88b  ±0.80 | 0.029  ±0.001 | 0.622d±  0.04 | 0.614a  ±0.02 | 0.572c  ±0.03 | 1.094d  ±0.04 |
| **SEm±** | 0.50 | 0.00 | 0.02 | 0.01 | 0.01 | 0.02 |
| **CD**  **(0.05)** | 1.54 | 0.00 | 0.05 | 0.04 | 0.04 | 0.06 |
| **CV%** | 4.55 | 4.20 | 2.07 | 4.33 | 3.03 | 2.33 |
| Each observation is a mean ± SD of 3 replicate experiments (n=3);  Different superscripts in same column indicate significant differences at (P<0.05) | | | | | | |

**Hardness:** Hardness ranged from 16.57 ± 0.90 N (S2) to 23.19 ± 1.35 N (C). All treated samples were significantly (P<0.05) softer than the control, with S5 (19.88 ± 0.80 N) being the firmest among them, likely due to its lower moisture content. This suggests that pumpkin seed addition generally softens the burfi texture. Hardness of burfi depends upon various factors including moisture content and mineral content.

**Cohesiveness:** Cohesiveness values ranged narrowly between 0.024±0.001 (S3) and 0.068±0.002 (S2). these differences were statistically non–significant (P>0.05). Therefore, seed treatment had no meaningful effect on the internal bonding strength of burfi samples.

**Chewiness:** Chewiness showed significant (P<0.05) variation among treatments, ranging from 0.384±0.03 (S3) to 3.361±0.02 (C) N mm. S2 was the only treatment that moderately retained chewiness (2.163±0.01 N mm), which was significantly (P<0.05) higher than other seed–treated samples.

**Adhesiveness:** Adhesiveness varied significantly across treatments, with the highest value recorded in S5 (0.614±0.02 N mm), indicating a stickier texture. Conversely, the control showed the lowest adhesiveness (0.315±0.03 N mm). The increase in adhesiveness with pumpkin seed treatments may be attributed to the oil content and processing method, especially in S5, which involved ghee coating before roasting.

**Gumminess:** The gumminess of burfi was significantly (P<0.05) influenced by seed pretreatment, ranging from 0.451±0.02 (S3) to 1.120±0.03 N (S2). S3 exhibited the lowest gumminess, possibly due to moisture loss.

**Springiness:** Springiness was highest in the control sample (3.158±0.02 mm) and declined significantly (P<0.05) with all seed treatments. S2 (1.934±0.03 mm) retained the most springiness among treated samples, whereas S3 (0.848±0.04 mm) was the least springy. The reduced springiness in treated samples might be due to seed particles interfering with the gel matrix structure of milk solids in burfi.

**Effect of Different Pretreatment of Pumpkin Seed on Colour Value of Pumpkin Seed Incorporated Burfi**

Colour of burfi samples was expressed in terms of L\*, a\* and b\* values. The effect of different pretreatment of pumpkin seed on colour value of PSIB is presented in Table 2.

**Table 2:** **Effect of different pretreatment of pumpkin seed on colour value of pumpkin seed incorporated burfi**

|  |  |  |  |
| --- | --- | --- | --- |
| **Burfi sample** | **L\*** | **a\*** | **b\*** |
| **C** | 75.46a±0.11 | –1.66a±0.07 | 19.79a±0.15 |
| **S1** | 66.85b±0.15 | –5.06f±0.05 | 16.99e±0.07 |
| **S2** | 65.56d±0.12 | –3.57d±0.06 | 18.62b±0.11 |
| **S3** | 66.07c±0.18 | –3.00c±0.10 | 17.91c±0.09 |
| **S4** | 64.97e±0.09 | –3.78e±0.12 | 17.64d±0.14 |
| **S5** | 63.28f±0.14 | –2.77b±0.08 | 15.24f±0.12 |
| **SEm±** | 0.08 | 0.05 | 0.07 |
| **CD (0.05)** | 0.24 | 0.15 | 0.21 |
| **CV%** | 0.20 | 2.52 | 0.66 |
| Each observation is a mean ± SD of 3 replicate experiments (n=3);  Different superscripts in same column indicate significant differences at (P<0.05) | | | |

**L\* value :** The control (C) burfi had the highest L\* value (75.46±0.11), indicating the lightest colour among all samples. S1 showed a noticeable decrease in lightness (66.85±0.15), likely due to the presence of greenish pigments from raw pumpkin seeds. S2 to S5 further reduced L\* values, with S5 being the darkest (63.28±0.14), probably due to Maillard browning and caramelization during roasting ghee coted seed. Overall, all experimental seed–treated burfi’s were significantly (P<0.05) darker than the control.

**a\* value :** The trend in increasing a\* values (less green) was: S1 < S4 < S2 < S3 < S5 < C. The control (C) had the least green appearance (a\* = –1.66±0.07), while the raw seed treatment (S1) exhibited the most intense green colour with an a\* of (–5.06±0.05). This indicates that the addition of raw pumpkin seeds significantly (P<0.05) enhanced greenness, possibly due to the natural pigments present in the raw seeds. Notably, sample S5, which underwent ghee coating and roasting, had a less green hue (–2.77±0.08), suggesting pigment degradation or browning due to lipid interactions and heat. The differences were statistically significant (P<0.05).

**b\* value :** The control had the highest b\* value (19.79±0.15), indicating a strong yellow hue. Addition of pumpkin seeds generally reduced the yellow intensity, with S5 showing the lowest (15.24±0.12), likely due to browning and pigment degradation during roasting ghee coted seed. S2 (microwave–roasted) maintained relatively high b\* (18.62±0.11), suggesting it preserved more yellow pigments compared to other methods.

Pretreatments resulted in slight variations in physico-chemical properties, including an increase in free fatty acid levels, which could be addressed through process optimization. The rheological profile of burfi was also notably affected. While S4 retained desirable textural attributes such as gumminess and springiness, other treatments showed a reduction in firmness and elasticity. The ghee-coated and roasted treatment (S5) maintained a favourable balance between adhesiveness and hardness, enhancing sensory appeal without compromising texture. Colour characteristics varied significantly depending on the pretreatment. All seed-treated samples appeared darker, with a shift towards red and reduced yellowness, especially in heat and fat-based treatments. Notably, S5 resulted in the darkest and least yellow burfi, likely due to Maillard browning and caramelization. Overall, the type of pumpkin seed pretreatment plays a crucial role in determining the final quality of burfi. With appropriate optimization, the incorporation of pretreated pumpkin seeds presents a promising approach to enhancing the nutritional and sensory profile of traditional milk-based sweets.

**Conclusion:** The study demonstrated that the incorporation of pumpkin seeds, subjected to various pretreatments, significantly influenced the sensory, textural, and visual characteristics of burfi. Among the experimental treatments, the dry salt roasted at 85 to 90°C /15 min (S4) sample was most preferred, showing an overall acceptability score comparable to the control and significantly higher than other treated samples.

**COMPETING INTERESTS DISCLAIMER:**

**Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.**

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**Table 3: Effect of different pretreatment of pumpkin seed on sensory score of pumpkin seed incorporated burfi**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Burfi samples** | **Sensory score (9–point hedonic scale)** | | | |
| **Flavour** | **Body and texture** | **Color and appearance** | **Overall acceptability** |
| **C** | 8.09a±0.13 | 7.96a±0.23 | 8.31a±0.10 | 8.07a±0.11 |
| **S1** | 7.30b±0.05 | 7.18c±0.09 | 7.54bc±0.12 | 7.32c±0.14 |
| **S2** | 7.04b±0.09 | 7.26c±0.16 | 7.34c±0.24 | 7.13cd±0.22 |
| **S3** | 6.88b±0.12 | 7.07c±0.13 | 7.20c±0.21 | 6.96d±0.16 |
| **S4** | 7.62ab±0.08 | 7.34c±0.20 | 7.76b±0.15 | 7.68b±0.15 |
| **S5** | 7.14b±0.13 | 7.65b±0.17 | 7.02c±0.29 | 7.26cd±0.23 |
| **SEm±** | 0.16 | 0.10 | 0.11 | 0.10 |
| **CD(0.05)** | 0.49 | 0.30 | 0.35 | 0.31 |
| **CV%** | 3.72 | 2.42 | 2.62 | 2.35 |
| Each observation is a mean ± SD of 3 replicate experiments (n=3);  Different superscripts in same column indicate significant differences at (P<0.05) | | | | |

**Table 4: Effect of different pretreatment of pumpkin seed on chemical composition of pumpkin seed incorporated burfi**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Burfi sample** | **Constituents (%)** | | | | | |
| **Moisture** | **FFA** | **Fat** | **Protein** | **Ash** | **Carbohydrate** |
| **C** | 19.97a  ±0.30 | 0.331e  ±0.01 | 21.86c  ±0.29 | 14.96c  ±0.13 | 2.35b  ±0.12 | 40.86a  ±0.34 |
| **S1** | 19.65a  ±0.39 | 0.393d  ±0.02 | 23.67ab  ±0.24 | 15.53b  ±0.15 | 2.92a  ±0.09 | 38.23b  ±0.37 |
| **S2** | 20.20a  ±0.33 | 0.504c  ±0.02 | 23.20b  ±0.23 | 15.23b  ±0.17 | 2.89a  ±0.10 | 38.48b  ±0.45 |
| **S3** | 18.66b  ±0.47 | 0.545b  ±0.03 | 23.98a  ±0.17 | 16.01a  ±0.24 | 2.94a  ±0.08 | 38.41b  ±0.29 |
| **S4** | 19.82a  ±0.35 | 0.512bc  ±0.01 | 23.45b  ±0.16 | 15.37b  ±0.21 | 2.91a  ±0.11 | 38.45b  ±0.41 |
| **S5** | 18.60b  ±0.54 | 0.590a  ±0.03 | 24.07a  ±0.32 | 16.08a  ±0.27 | 2.96a  ±0.09 | 38.29b  ±0.32 |
| **SEm±** | 0.23 | 0.01 | 0.14 | 0.12 | 0.06 | 0.21 |
| **CD**  **(0.05)** | 0.72 | 0.04 | 0.43 | 0.36 | 0.18 | 0.65 |
| **CV%** | 2.08 | 4.51 | 1.04 | 1.30 | 3.51 | 0.95 |
| Each observation is a mean ± SD of 3 replicate experiments (n=3);  Different superscripts in same column indicate significant differences at (P<0.05) | | | | | | |

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