Research article
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Effect of Storage Period on the Nutritional Parameters of Beet Root Supplemented Cookies

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Abstract
This study investigated the impact of storage on the nutritional composition of beetroot cookies. Supplementing products like cookies, cakes, jams, and bread is an effective way to enhance the nutritional value of these foods. Beetroot was dried in a hot air oven, and the resulting beetroot powder was incorporated into the cookie dough. Nutritional assessments were conducted on days 0, 15, 30, and 45 of storage. The results indicated that the addition of beetroot powder enhanced the nutritional profile of the cookies. The moisture content of the cookie increased during the 45-day storage period. Protein, fat, and carbohydrate levels decreased after 45 days. However, fiber and ash contents remained largely unaffected throughout the storage period.

Keywords: Beetroot, Beetroot Powder, Cookies, Nutrition Analysis, Baking

Highlights
- Beetroot powder was used to make supplemented cookies
- Nutrition of the cookies increased with an increase in beetroot powder
- Storage affects the nutritional parameters of cookies differently

1. Introduction
Essential nutrients are provided by food for life, but in addition to this, it also offers bioactive compounds. Fruits, vegetables and grains consumption is linked with decreased cases of CVDs (Cardiovascular diseases), cancer, diabetes, and many age-related functional disorders (Liu 2003). Due to the high content of nutrients, including phytochemicals such as antioxidants, in fruits and vegetables, they are considered essential in dietary management (Dhandevi and Jeewon, 2015). Vegetables refer to all edible plant parts, including fruits, stems, leaves, and seeds. Peels of fruit and vegetables, which are often discarded as waste, contain many beneficial compounds (Khattak and Rahman, 2017). Vegetables can be eaten either in their natural state or in a cooked form. They are low-caloric, hence can have a good effect on human nutrition (Shuaibu et al. 2021). Beetroot, scientifically known as *Beta vulgaris*, belongs to the *Amaranthaceae* family (Abdo et al., 2021). Beetroot is a biennial herbaceous plant with a height of 120 cm. Root color ranges from dark red, white, and yellow to brown (Chhikara et al. 2019). An underground taproot develops in 50-60 days and weighs 100-150 grams (Enaya, 2018). A biennial plant that is cultivated in spring for its flashy root.

A vegetable with low caloric and fat value. They are rich in carbohydrates, starch, fiber, and protein. They are rich in vitamins, both water-soluble and fat-soluble. They contain minerals such as Mn, Mg, K, Na, P, Fe, Zn, and Se (Neelwarne, 2012; Babarykin et al., 2019; Lechner and Stoner, 2019; Chawla et al., 2016). The demand for bakery products has increased due to growing economic and eating habits of people, especially working individuals, due to a lack of time (Srivastava and Singh 2018). The primary factors driving the high demand for bakery goods include the migration of people from rural areas to urban centers, low prices, extended shelf life, appealing taste, and convenient transportation (Lucky et al., 2010). The yield of beetroot in 1994 was around 25.6 t ha^{-1}. Afterwards, it increased from 31.7 in 1996, then from 36.8 to 43.2 t ha^{-1} between 2001 and 2005, and in 2006, it was 41.8 t ha-1 (Sidra et al., 2016).

The pharmacological activities of beetroot include vasodilation, antihypertensive effects, antidiabetic properties, protective effects on the liver, and anticancer properties; it has also been shown to enhance athletic performance (Gheith and El-Mahmoudy, 2018). Recent research suggests that beetroot consumption may lead to improved clinical outcomes for hypertension, diabetes, dementia, and atherosclerosis. It significantly reduces blood pressure (Gilchrist et al. 2014; Presley et al. 2011).

The leading systemized food business in recent times is the bakery industry worldwide. Specifically, cookies prove to be their most popular products due to their suitability, prolonged shelf life, and ready-to-eat nature (Sindhuja et al. 2005), as well as their affordability, good nutritional quality, and availability in numerous flavors (Tripathi and Shankar 2022).

They are valued for their taste, aroma, suitability, and low moisture content (Chung 2007). To improve nutrition, innovative efforts are being made by changing their composition (Khairi et al. 2019). Supplementation of current foods, such as biscuits, cakes, jams, and bread, with functional ingredients is an effective way to increase food nutrition (Kumari et al., 2021).

A wide range of dietary supplements and functional foods is available on the market. Among these, juice and juice mixtures, gels, fermented juices, and dried powders are the most effective (Wiczkowski et al. 2018). The most widely consumed baked products are cookies. They are valued for their taste, aroma, and high shelf life due to their low moisture content. Nowadays, the increased demand for healthier foods has led to the development of cookies with natural ingredients. These cookies offer additional nutritional benefits, providing specific health advantages compared to traditional cookies (Chauhan and Rajput 2018). The present study was conducted to develop beetroot powder-enriched cookies by incorporating beetroot powder at varying concentrations (5% to 30%) into the cookie dough. The effect of storage on the nutritional composition of the cookies was evaluated at 15-day intervals.

2. Materials and Methods

2.1. Study Design

This research was conducted at the Institute of Food and Nutritional Sciences, PMAS Arid Agriculture University, Rawalpindi, to check the effect of storage on beetroot cookies. Different concentrations of beetroot powder (0%, 5%, 10%, 15%, 20%, 25%, and 30%) were used to make cookies, which were then stored for 45 days. The nutritional parameters were checked on 0, 15, 30, and 45 days. Figure (1) provides the pictorial representation of the study.

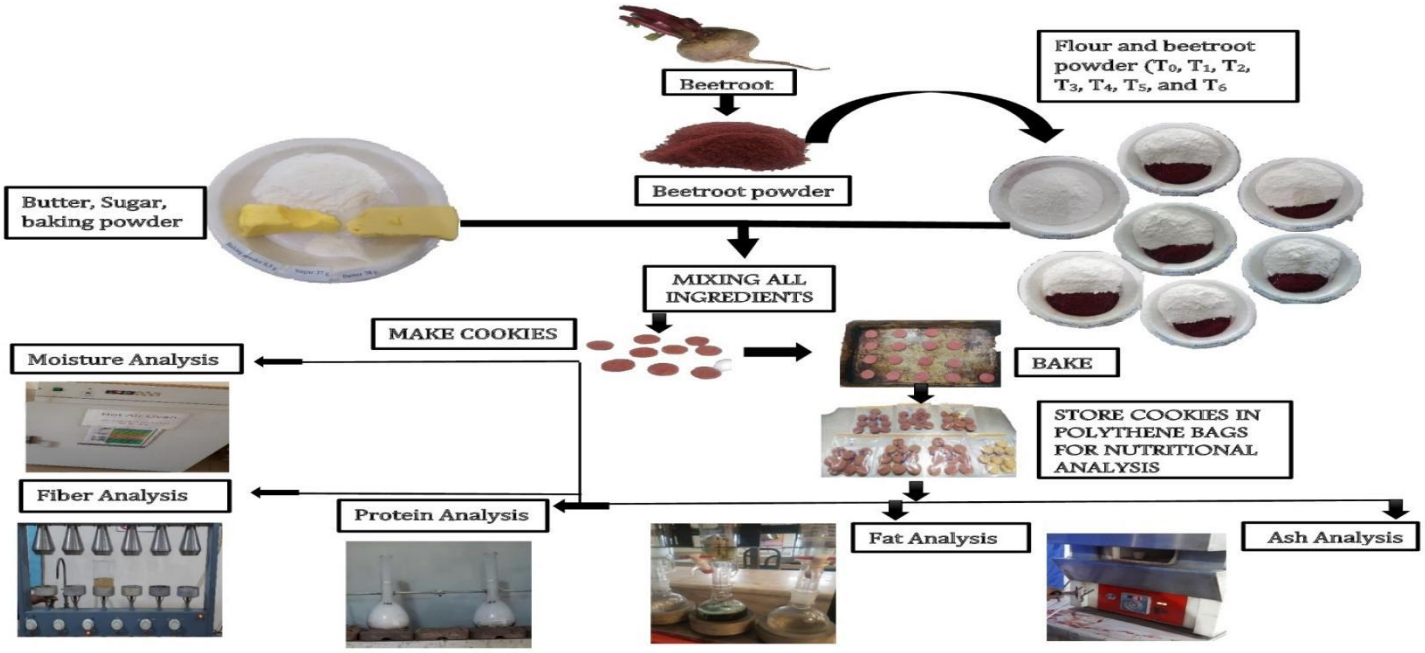

Figure 1: Proposed scheme of study

2.2. Materials Collection

Fresh healthy beetroots were obtained from the local market of Rawalpindi, Pakistan. The ingredients for the cookies, such as sugar, fat, flour, baking powder, and vanilla essence, were obtained from Imtiaz Store, a supermarket in Rawalpindi, Pakistan.

2.3. Beetroot Powder Preparation

Beetroots were washed, peeled, sliced, and subjected to drying. In a hot air oven, they were dried at 110 ℃ for 2 days. The dried beetroot slices were then ground in a domestic grinder and sieved to obtain a fine powder (Salamatullah et al., 2021).

2.4. Cookie Preparation

Beetroot-supplemented cookies were prepared by following the AACC (2000) method with some modifications. Different treatments were used:100:0 (100 g flour, T_o); 95:5 (95 g flour and 5 g beetroot powder, T_1); 90:10 (90 g flour and 10g beetroot powder, T_2); 85:15 (85 g flour and 15g beetroot powder, T_3); 80:20 (80 g flour and 20g beetroot powder,T_4); 75:25(75g flour and 25 g beetroot powder, T_5); 70:30(70 g flour and 30 g beetroot powder, T_6). All other ingredients (sugar, butter, and baking powder) were mixed together for each treatment, and a smooth dough was formed. The dough was then cut into cookie shapes. Cookies were baked at a temperature of 160 ℃ for 10-15 min. The preparation of cookies is explained through a flow chart (Fig.2).

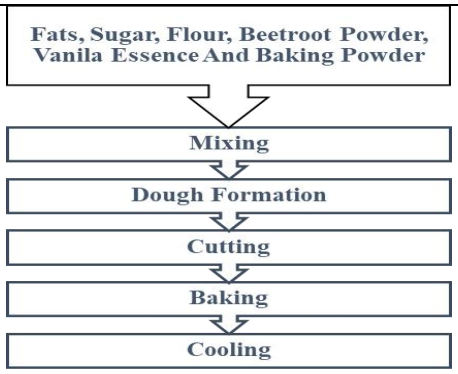

Figure 2: Flow chart of cookie preparations.

2.5. Storage Condition

Cookies were stored in an airtight container labelled as T_0, T_1, T_2, T_3, T_4, T_5, and T_6 at room temperature. Samples from each stored container were analyzed at days 0, 15, 30, and 45.

2.6. Sampling Method

At days 0, 15, 30, and 45, one cookie from each container was selected for analysis.

2.7. Nutritional Analysis of Beetroot Cookies

Moisture, fat, ash, protein, and fibre content of cookies were analysed.

2.7.1. Determination of moisture content

It was determined by using method no. 44-01.01 of AACC (2000). The dish was weighed using a weighing balance. Approximately 2 g of sample was taken and dried in a hot air oven at 130 ± 1.5 ℃ for 50-60 minutes. After the dish was dried, it was removed, cooled in a desiccator, and weighed again.

$$\text{Moisture (\%)} = \frac{weight\ before\ drying - weight\ after\ drying}{weight\ before\ drying - weight\ of\ dish} \times 100$$

2.7.2. Determination of crude fat content

It was determined by method no 30-25.01 of AACC (2000). Approximately 2 g of sample was placed in a thimble, which was then placed in a Soxhlet apparatus. Petroleum ether was used as a solvent in the extraction process. Extraction was done for about 6-8 hours. After the completion of the extraction cycle, the beaker was placed at room temperature to evaporate the solvent. Residue (fat) was weighed.

$$\text{Fat (\%)} = \frac{weight\ of\ fat}{sample\ weight} \times 100$$

Results were expressed as a percentage of dry sample weight.

2.7.3. Determination of crude protein content

Protein was determined by using method no 46-10.01 of AACC (2000). First, digestion was performed in the presence of a digestion mixture in a Kjeldahl flask. Digestion releases ammonia gas. Distillation was done to a known volume using boric acid. Ammonia is measured by titrating the receiving solution with 1 mL of 0.1 N H_2SO_4 and methyl red as an indicator, until a light pink color appears, followed by a blank titration.

$$\text{Nitrogen (\%)} = \frac{(volume\ of\ blank\ titration\ sample - volume\ of\ sample\ titration) \times normality of\ acid \times 14.0067}{sample\ weight} \times 100$$

Protein was determined by multiplying the factor of 6.25 by the calculated nitrogen percentage.

Results were expressed as a percentage of dry sample weight.

2.7.4. Determination of crude ash content

It was determined by method no 08-01.01 of AACC (2000). Crucible was weighed and about 2 g of sample was taken in the crucible and placed in muffle furnace at a temperature of 500-600 ℃ for 5-6 hours. After ignition, the crucible was transferred to a desiccator for cooling.

$$\text{Ash (\%)} = \frac{weight\ of\ crucible\ with\ ash - weight\ of\ empty\ crucible}{weight\ of\ crucible\ with\ residue - weight\ of\ crucible} \times 100$$

Results were expressed as a percentage of dry sample weight.

2.7.5. Determination of crude fibre content

It was determined by Method no 32-10.01 of AACC (2000). Approximately 2 g of sample was taken, and its digestion was performed using H_2SO_4, followed by treatment with a NaOH solution. Afterwards, filtration was done, followed by drying in a hot air oven. Finally, the sample was positioned in muffle furnace at a temperature of 550-600 ℃ for 6-7 hours.

$$\text{Fiber (\%)} = \frac{weight\ before\ ashing - weigh\ after\ ashing}{sample\ weight} \times 100$$

Results were expressed as a percentage of dry sample weight. Fiber is non digestible carbohydrate.

2.7.6. Determination of carbohydrate content

It was determined by the difference method reported by Galla et al. (2017).

Total carbohydrates (%) =100- (Protein (%) +Fat (%) +Ash (%) + Moisture (%) + fiber (%))

Results were expressed as a percentage of dry sample weight. Fiber is an undigestible carbohydrate, while carbohydrate includes both digestible and non-digestible

2.8. Statistical Analysis

All experiments and tests were performed in triplicate. The data were statistically expressed as mean ± standard deviation in the tables. For differences between means, two-way ANOVA was used, followed by the Tukey test.

3. Results and Discussion

Results showed that beetroot powder significantly intensifies the nutritional value of cookies. The increase in the carbohydrate, protein, and fat percentage of cookies is mainly due to the use of other ingredients, such as flour, butter, sugar, and a small amount of egg, to maintain the dough consistency. The effect of treatment and storage for each parameter is discussed below:

3.1 Effect of Treatment and Storage on Moisture Content

Moisture content increased from 2.63% in the T_0 (control) to 5.69 % in T_6 (30% beetroot powder). Statistical analysis showed that storage significantly affects the moisture content of cookies. Moisture content increased from 4.02% on day 0 to 4.19 % on day 45. Means values for treatments and storage are reported in Table (1). Moisture increase during storage may be due to the hygroscopic nature and water-holding capacity of ingredients (beetroot powder, flours) and the nature of the packaging material. These results are consistent with the findings of Butt et al. (2004), who observed an increase in moisture content of palm oil-fortified cookies over time. Jan et al. (2017) also investigated the storage effect on various properties of cookies made with Chenopodium flour. They also observed the same trending pattern of moisture on cookies during the 6-month storage period.

Table 1. Effect of different treatments and storage duration on moisture (%) of cookies

Treatments	Storage period				Mean
	Day 0	Day 15	Day 30	Day 45	
T_0	2.56±0.006^{s}	2.6±0.02^{rs}	2.66±0.02^{qr}	2.72±0.02^{q}	2.63±0.06^{g}
T_1	2.88±0.01^{p}	2.91±0.01^{op}	2.97±0.02^{no}	3.03±0.02^{n}	2.94±0.06^{f}
T_2	3.10±0.02^{m}	3.14±0.02^{m}	3.21±0.02^{l}	3.26±0.02^{l}	3.17±0.07^{e}
T_3	3.8±0.020^{k}	3.87±0.02^{j}	3.93±0.01^{ij}	3.99±0.02^{i}	3.89±0.08^{d}
T_4	4.94±0.02^{h}	4.97±0.03^{h}	5.04±0.02^{g}	5.10±0.02^{g}	5.01±0.07^{c}
T_5	5.28±0.02^{f}	5.34±0.02^{ef}	5.4±0.02^{de}	5.46±0.02^{e}	5.37±0.07^{b}
T_6	5.6±0.02^{c}	5.66±0.02^{bc}	5.72±0.01^{ab}	5.78±0.02^{a}	5.69±0.07^{a}
Mean	4.02±1.2^{d}	4.07±1.2^{c}	4.13±1.2^{b}	4.19±1.2^{a}	

The letters in the superscripts show a significant difference between mean values (Tukey's test)

3.2. Effect of Treatment and Storage on Fat Content

Fat content increased from 21.3 % in the T_0 (control) to 23.95 % in T_6 (30 % beetroot powder). Statistical analysis showed that storage significantly affects the fat content of cookies. The fat content decreased from 22.38% on day 0 to 22.29% on day 45. Fat decreases due to lipid oxidation and environmental factors, such as light, oxygen, and poor packaging quality. Means values for treatments and storage are presented in Table (2). Uthumporn et al. (2015) also reported a decrease in fat content with an increase in eggplant flour in cookies.

Table 2. Effect of Different Treatment and Storage Durations on Fat (%) of Cookies

Treatments	Storage period				Mean
	Day 0	Day 15	Day 30	Day 45	
T_0	21.08±0.01^{r}	21.05±0.01^{rs}	21.02±0.01^{s}	20.98±0.01^{t}	21.3±0.04^{g}
T_1	21.55±0.01^{o}	21.52±0.01^{op}	21.49±0.01^{pq}	21.46±0.01^{q}	21.50±0.03^{f}
T_2	21.8±0.01^{l}	21.77±0.01^{lm}	21.74±0.01^{mn}	21.71±0.01^{n}	21.75±0.03^{e}
T_3	22.27±0.01^{i}	22.23±0.01^{j}	22.2±0.01^{j}	22.16±0.01^{k}	22.21±0.04^{d}
T_4	22.57±0.01^{g}	22.54±0.008^{g}	22.5±0.01^{h}	22.47±0.01^{h}	22.52±0.04^{c}
T_5	23.43±0.01$_{d}$	23.4±0.01^{de}	23.37±0.01^{ef}	23.34±0.01^{f}	23.38±0.03^{b}
T_6	24±0.01^{a}	23.97±0.01^{ab}	23.94±0.008^{bc}	23.91±0.01^{c}	23.95±0.03^{a}
Mean	22.38±1^{a}	22.35±1^{b}	22.32±1^{c}	22.29±1^{d}	

The letters in the superscripts show a significant difference between mean values (Tukey's test).

3.3. Effect of Treatment and Storage on Protein Content

Protein content increased from 6.72 % in the T_0 (control) to 9.52 % in T_6 (30 % beetroot powder). Statistical analysis showed that storage significantly affects the protein content of cookies. Protein content decreased from 8.16 % on day 0 to 8.06 % on day 45. As discussed above, storage increased the moisture level of cookies, which may enhance the activity of the proteolytic enzyme. Additionally, cookies contain reducing sugars, which react with proteins and form complexes, resulting in a decrease in overall protein content. Means values for treatments and storage are revealed in Table (3). Masih et al. (2014) also reported a decrease in protein activity during storage of cookies. Kumari and Sindhu (2019) reported an increase in protein levels with the addition of pumpkin seed powder.

Table 3. Effect of Different Treatment and Storage Duration on Protein (%) of Cookies (which treatments?)

Treatments	Storage period				Mean
	Day 0	Day 15	Day 30	Day 45	
T_0	6.77 ± 0.01^{s}	6.74 ± 0.01^{st}	6.71 ± 0.01^{tu}	6.68 ± 0.01^{u}	6.72 ± 0.03^{g}
T_1	7.26 ± 0.01^{p}	7.22 ± 0.02^{pq}	7.17 ± 0.01^{qr}	7.13 ± 0.01^{r}	7.19 ± 0.05^{f}
T_2	7.46 ± 0.02^{m}	7.44 ± 0.01^{mn}	7.39 ± 0.01^{no}	7.37 ± 0.01^{o}	7.41 ± 0.04^{e}
T_3	8.70 ± 0.01^{g}	8.67 ± 0.01^{gh}	8.64 ± 0.01^{hi}	8.61 ± 0.01^{i}	8.65 ± 0.03^{c}
T_4	8.21 ± 0.01^{j}	8.18 ± 0.01^{jk}	8.15 ± 0.01^{kl}	8.12 ± 0.01^{l}	8.16 ± 0.04^{d}
T_5	9.13 ± 0.01^{d}	9.1 ± 0.01^{de}	9.06 ± 0.01^{ef}	9.05 ± 0.02^{f}	9.08 ± 0.03^{b}
T_6	9.57 ± 0.01^{a}	$9.54\pm0.01_{ab}$	9.51 ± 0.01^{bc}	9.48 ± 0.01^{c}	9.52 ± 0.04^{a}
Mean	8.16 ± 1^{a}	8.12 ± 1^{b}	8.09 ± 1^{c}	8.06 ± 1^{d}	

The letters in the superscripts show a significant difference between mean values (Tukey's test).

3.4. Effect of Treatment and Storage on Ash Content

Ash content increased from 0.93 % in the T_0 (control) to 1.82 % in T_6 (30 % beetroot powder). No statistically significant effect of storage on the ash content of cookies. Ash content changed from 1.37% on day 0 to 1.36% on day 45. This Slight change in ash content may be due to an increase in the moisture content, as discussed above. Water may dilute the mineral (ash) content; minerals are not lost, but their proportion is reduced due to the added water. Means values for treatments and storage are displayed in Table (4). Masih et al. (2014) also reported a non-significant effect on the ash content of cookies.

Table 4. Effect of Different Treatments and Storage Duration on Ash (%) of Cookies

Treatments	Storage period				Mean
	Day 0	Day 15	Day 30	Day 45	
T_0	0.94 ± 0.02^{g}	0.93 ± 0.01^{g}	0.92 ± 0.01^{g}	0.93 ± 0.01^{g}	0.93 ± 0.008^{g}
T_1	$1.12\pm0.01_{f}$	1.11 ± 0.01^{f}	1.12 ± 0.01^{f}	1.12 ± 0.01^{f}	1.12 ± 0.004^{f}
T_2	1.26 ± 0.01^{e}	1.25 ± 0.01^{e}	1.26 ± 0.01^{e}	1.25 ± 0.01^{e}	1.26 ± 0.001^{e}
T_3	1.32 ± 0.01^{d}	1.31 ± 0.01^{d}	1.32 ± 0.01^{d}	1.32 ± 0.01^{d}	1.32 ± 0.004^{d}
T_4	1.45 ± 0.02^{c}	1.44 ± 0.01^{c}	1.44 ± 0.01^{c}	1.42 ± 0.01^{c}	1.44 ± 0.01^{c}
T_5	1.66 ± 0.02^{b}	1.66 ± 0.01^{b}	1.66 ± 0.01^{b}	1.65 ± 0.01^{b}	1.66 ± 0.004^{b}
T_6	1.81 ± 0.02^{a}	1.81 ± 0.0^{a}	1.82 ± 0.01^{a}	1.82 ± 0.01^{a}	1.82 ± 0.005^{a}
Mean	1.37 ± 0.3^{a}	1.37 ± 0.3^{a}	1.36 ± 0.3^{b}	1.36 ± 0.3^{b}	

The letters in the superscripts show a significant difference between mean values (Tukey's test).

3.5. Effect of Treatment and Storage on Fiber Content

Fibre content increased from 0.82 % in the T_0 (control) to 1.40 % in T_6 (30 % beetroot powder). Statistically significant effect of storage on the fibre content of cookies. Fiber content changed from 1.18 % on day 0 to 1.10 % on day 45. Due to the increase in the moisture level of cookies, as discussed above, the percentage of fiber is slightly changed during storage. Means values for treatments and storage are shown in Table (5).

Table 5. Effect of Different Treatment and Storage Duration on Fiber (%) Of Cookies

Treatments	Storage period				Mean
	Day 0	Day 15	Day 30	Day 45	
T_0	0.87 ± 0.01^{l}	0.85 ± 0.01^{lm}	0.81 ± 0.01^{mn}	0.78 ± 0.01^{n}	0.82 ± 0.03^{g}
T_1	1.02 ± 0.01^{k}	0.99 ± 0.02^{k}	0.99 ± 0.02^{k}	1.00 ± 0.01^{k}	1.00 ± 0.01^{f}
T_2	1.12 ± 0.006^{ij}	1.10 ± 0.02^{j}	1.08 ± 0.02^{j}	1.08 ± 0.01^{j}	1.10 ± 0.02
T_3	1.18 ± 0.006^{gh}	1.17 ± 0.01^{ghi}	1.17 ± 0.01^{hi}	1.17 ± 0.01^{hi}	1.17 ± 0.01^{d}
T_4	1.26 ± 0.01^{f}	1.21 ± 0.02^{fgh}	1.17 ± 0.01^{hi}	1.13 ± 0.01^{ij}	1.19 ± 0.05^{c}
T_5	1.36 ± 0.01^{cd}	1.31 ± 0.02^{de}	1.27 ± 0.01^{ef}	1.22 ± 0.01^{fg}	1.29 ± 0.05^{b}
T_6	1.45±0.02a	1.43 ± 0.0^{ab}	1.38 ± 0.02^{bc}	1.34 ± 0.02^{cd}	1.40 ± 0.04^{a}
Mean	1.18 ± 0.1^{a}	1.15 ± 0.1^{b}	1.13 ± 0.1^{c}	1.10 ± 0.1^{d}	

The letters in the superscripts show a significant difference between mean values (Tukey's test).

3.6. Effect of Treatment and Storage on Carbohydrate Content

Carbohydrate content increased from 62.89 % in the T_0 (control) to 64.49 % in T_6 (30 % beetroot powder). Statistically significant effect of storage on carbohydrate content of cookies. Carbohydrate content changed from 64.05 % on day 0 to 63.91 % on day 45. This slight change may be due to Maillard reactions (Maillard, 1912) affecting the reducing sugar level. Also, carbohydrates are found by the difference method; changes in other components may affect carbohydrate levels. Means values for treatments and storage are shown in Table 6.

Table 6. Effect of Different Treatment and Storage Durations on Carbohydrate (%) of Cookies

Treatments	Storage period				Mean
	Day 0	Day 15	Day 30	Day 45	
T_0	62.96±0.01^{o}	62.91±0.01^{op}	62.87±0.01^{pq}	62.82±0.01^{q}	62.89±0.05^{g}
T_1	63.22±0.01^{l}	63.17±0.02^{m}	63.12±0.01^{mn}	63.07±0.01^{n}	63.14±0.06^{f}
T_2	64.16±0.01^{j}	64.11±0.01^{j}	63.97±0.02^{k}	63.92±0.01^{k}	64.04±0.1^{e}
T_3	64.45±0.03^{de}	64.41±0.02^{def}	64.35±0.02^{hi}	64.3±0.008^{i}	64.37±0.006^{d}
T_4	64.48±0.01^{bcd}	64.43±0.01^{efg}	64.39±0.01^{fgh}	64.37±0.01^{gh}	64.42±0.04^{c}
T_5	64.53±0.01^{ab}	64.57±0.01^{a}	64.52±0.02^{abc}	64.47±0.01^{cd}	64.52±0.04^{a}
T_6	64.58±0.01^{a}	64.52±0.02^{abc}	64.46±0.03^{de}	64.39±0.01^{fgh}	64.49±0.07^{b}
Mean	64.05±0.6^{a}	64.02±0.7^{b}	63.95±0.6^{c}	63.91±0.6^{d}	

The letters in the superscripts show a significant difference between mean values (Tukeys test)

Conclusion

The study concluded that beetroot powder acts as a nutritional enrichment supplement, increasing the nutritional value of cookies and making them more nutritious. Cookies have an appealing color and are visually attractive, with no use of artificial color. However, the addition of beetroot and the increase in moisture content during storage make the cookies susceptible to microbial growth. Hence, the storage effect suggests that beetroot cookies can provide nutritional benefits for up to 10 or 15 days. To increase shelf life, the use of packaging material must be effective in limiting the absorption of water from the environment.

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Conflict of Interest: The authors declare that there is no conflict of interest

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