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Enhancing Beetroot Jam with Stevia: Antioxidant Properties and Quality Assessment During Storage

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Abstract: *Beta vulgaris* L., generally known as beetroot, is a nutrient-rich vegetable with medicinal characteristics, making it an excellent prospect for value-added products such as jam. This study aimed to develop a low-calorie beetroot jam utilizing natural sweetener i.e., stevia to enhance its appeal to health and diet-conscious consumers. Over the course of 21 days, the jam's physico-chemical qualities (moisture, ash, pH, acidity, and total soluble solids), microbial analysis (total plate count), antioxidant capacity (DPPH and total phenolic content), and organoleptic qualities (color, taste, aroma, flavor, and overall acceptability) were assessed. The jam was kept in pre-sterilized glass jars. Results showed a remarkable reduction in moisture content (32.06% to 29.46%), ash content (0.89% to 0.83%), and pH (3.39 to 3.30), while total soluble solids and acidity increased from 66.85°B to 67.46°B and 0.75 to 0.83 respectively during storage. In addition, there was a notable improvement in DPPH (84.87% to 88.28%). Total phenolic content also increased (34.60 mg GAE/g to 38.90 mg GAE/g), with increasing concentration of stevia. Statistics verified that, physicochemical and sensorial properties of the low-calorie beetroot jam were considerably affected by keeping intervals ($p<0.05$). This study emphasizes beetroot's potential for developing low-calorie value-added products like jam.
Keywords: Beetroot, Stevia, Low-calorie jam, DPPH, total phenolic content

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1. Introduction

The demand for fruits and vegetables has surged recently, largely due to their rich fiber and vitamin content, which are essential for maintaining a healthy diet. Additionally, they include healthy substances that could aid in the prevention of conditions like diabetes, heart disease, and cancer. However, many fruits and vegetables have limited consumption because they are seasonal and prone to rapid spoilage. In order to prevent this, preservation techniques such as jam-making have been employed to increase their shelf life and increase their availability throughout the year.

Sugar, a type of carbohydrate, includes different kinds such as glucose, fructose, and sucrose. While fructose, which is present in fruits, provides healthy fiber, vitamins, and minerals, glucose is necessary for brain function. Fruits, vegetables, and sugarcane, a primary source of sucrose, are common sources of natural sugars. Sadly, there has been a significant increase in the intake of processed sweets, which has resulted in major health problems like obesity, type 2 diabetes, and cardiovascular illnesses. Due to its high calorie content, excessive sugar intake can lead to overeating and weight gain. It can also result in blood sugar spikes and falls, which can make you tired and irritable. In the upcoming years, experts anticipate that the prevalence of these health problems would rise globally (Vilela *et al*., 2015).

Beetroot exhibits significant anti-proliferative activity among vegetables. It is highly nutritious, providing a good source of folate, manganese, and dietary fiber. Beetroot contains approximately 10% sugar, 1.6% protein, 8% carbohydrates, and a range of vitamins and minerals, with water making up the remaining content (Ceclu and Nistor, 2020). The beetroot skin is also rich in nutrients. Known for its health benefits, beetroot supports overall wellness and may help alleviate various conditions such as hypertension, cardiovascular diseases, digestive issues, inflammation, and even certain types of cancer (Deepika *et al*., 2019).

The jam belongs to the group of jellied products. It has a gelatinous texture created by cooking fresh, frozen, or partially processed fruit or fruit pieces with the right amounts of acid, sugar, and pectin. Boiling can be done in vacuum apparatus at lower temperature (60–75^{o}C) and lowered pressure (atmospheric pressure) or in open vessels (duplicators) with vigorous stirring at around 100^{o}C (Figuerola *et al*., 2007)..

Traditional jams typically contain high levels of sucrose, making them unsuitable for individuals who are overweight, obese, or have conditions like diabetes and hyperglycemia (Witek et al., 2022). As noted by Parsayee et al. (2013) these jams, which have around 65% dry matter, can contribute to obesity and other health issues due to their excessive sugar content. Natural sweeteners are now used in place of sugar in many recipes. There are many non-caloric natural sweeteners, but stevia (*Stevia rebaudiana*) is the most popular due to its several positive health effects. Stevia (*Stevia rebaudiana*) is a natural sweetener, gained remarkable scientific interest in today's society. It is a small perennial shrub from the Asteraceae family, originally found in northern Paraguay and nearby regions of Brazil. The plant was first documented by the scientist Moisés Santiago Bertoni in 1887 (Ahmad et al., 2020).

Stevia is primarily known for having a high concentration of steviol glycosides in its leaves, which are used as a calorie-free, non-sucrose sweetener in a variety of food products. Glycosides are organic compounds made of two parts: an a glycine, a non-sugar group, and a glycine, a carbohydrate (Hossain et al., 2017). Plants like stevia include carbohydrate-based compounds with 200–300 times more sweetness than regular sugar. Stevia leaves also contain lipids, dietary fiber, essential oils, water-soluble vitamins, minerals, and phenolic compounds in addition to the sweet compounds. Numerous advantages of consuming stevia as a powder on human health have been demonstrated by recent studies. Stevia is a natural alternative to added sugars, offering a low-calorie sweetness without the adverse effects associated with high sugar intake. The FDA describes added sugars as those introduced during food processing, including sucrose, dextrose, table sugar, syrups, honey, and sugars derived from plant sources (Wibbens et al., 2023). The health advantages of stevia include anti-diabetic, anti-obesity, anti-tumor, anti-hypertensive, anti-microbial, anticaries, non-cariogenic, regulating blood pressure, and antioxidant properties. As a result, Stevia *(Stevia rebaudiana*) is gaining recent scientific interest due to its non-caloric properties (Shinduja et al., 2021). Based on the above considerations, this study focused on creating a low-calorie beetroot jam by substituting sucrose with stevia. The aim was to develop a healthier alternative by reducing calorie content while offering multiple health benefits.

2. Materials and Methods

2.1. Materials

Fresh, mature beetroot and other required constituents likes stevia powder, sugar, citric acid, and pectin were purchased from the nearby local market of Bahawalpur. All analytical-grade chemical reagents were procured from Sigma-Aldrich (Tokyo, Japan).

2.2. Proximate analysis of beetroot

Moisture content

The moisture content was determined in hot air oven at 105°C by following the equation as described in AOAC (2006);

% Moisture = wt. of wet sample – wt. of dry sample × 100/wt. of wet sample

Ash

The ash content of beetroot was evaluated using muffle furnace at 550°C using following formula;

$$\text{Ash} = \frac{W_2 - W_0}{W_1}$$

Where W_0 = weight of crucible, W_1 = weight of sample, W_2 = weight of crucible and ash.

Crude Fat

The crude fat content of the sample was determined using the Soxhlet extraction method, as per the procedure described by AOAC (2006). The percentage of crude fat was calculated by comparing the weight of the extracted fat to the initial weight of the sample.

Crude fat = Wt. of dried ether soluble material × 100/Wt. of sample

Crude protein

The protein content was analyzed following the method described by AOAC (2019). The nitrogen content was determined and then used to calculate the protein percentage using a conversion factor.

% Nitrogen = T_S - T_B × Normality of acid × 0.014 × 100 /Weight of sample

Table 1: Treatment plan for development of beetroot jam with stevia powder

Treatments	Stevia Concentration %
T_o	Control
T_1	0.5
T_2	1.0
T_3	1.5
T_4	2

Total Carbohydrate

The total carbohydrate content in the samples was determined by difference, calculated by subtracting the values for protein, fat, ash, and moisture from 100. This analysis was conducted using the phenol-sulfuric acid method as outlined in the AOAC (2019) guidelines.

2.3. Beetroot pulp

Fully ripe, high-quality beetroots free from spoilage were cleaned thoroughly with water. After peeling, they were cut into smaller pieces. The pieces were then cooked over heat and mashed using a hand masher until a smooth consistency was achieved (Rana et al., 2022).

2.4. Preparation of jam

The jam was prepared with different concentration of stevia powder (0.5%, 1%, 1.5%, and 2%) as described in treatment plan (Table 1). In the preparation of beetroot jam, beetroot was washed thoroughly with clean water, peeled and sliced into 2-4 equal sizes, and then blended using hand masher to obtain the beetroot pulp. Beetroot jam was prepared with production steps: pour the beetroot pulp into the pan and then cook the beetroot pulp at 80-100°C for 15 min. After pulping, pectin and citric acid were added. After that jam was heated at a high flame to make it viscous. Stevia powder was incorporated into it. The jam was allowed to cool, and the resulting product exhibited a brix value of 46.1Bx. The final product was transferred into a glass jar that had been thoroughly cleaned, dried, and sterilized. The jam was placed in a cold and dry preferably at room temperature for subsequent analysis. In the process of producing control jam, the substitution of stevia with sugar was implemented.

2.5. Chemical analysis

Moisture content

Moisture content was measured using the direct oven drying method as per AOAC (2006). A 5 g sample of beetroot jam was placed in a pre-weighed dish and dried at 105°C until a stable weight was achieved, usually after 4-6 hours. After drying, the sample was cooled in a desiccator and weighed again. The moisture content was calculated by comparing the initial and final weights of the sample.

Ash content

The sample was dried at 100°C for 1 hour until complete removal of moisture and then charred using an electric heater. Following this, it was placed in a muffle furnace at 550°C for 5 hours, according to AOAC (2019) guidelines.

$$\text{Ash} = \frac{W_2 - W_0}{W_1}$$

pH

The pH of beetroot jam will be calculated by following the method as described in AOAC (2019). A 10 g sample of the jam was dissolved in 90 mL of distilled water to create a homogeneous solution. The pH meter electrode was immersed in the solution, and the pH reading was recorded after stabilization.

Titratable acidity

The Titratable acidity will be determined by following the method of AOAC (2019). The specific amount of beetroot jam was taken and diluted with distilled water to a defined volume (100mL). the titration was carried out with NaOH using phenolphthalein indicator. The following expression was used;

Titratable Acidity(%)=Volume of NaOH(mL)×Normality of NaOH×Equivalent weight of acid/ Wt. of Sample

Total soluble solids

The total soluble solids (TSS) in beetroot jam were measured with refractometer, and it was calibrated before use. A small amount of the jam was placed on the refractometer prism. The Brix reading was taken after ensuring that no air bubbles were present. The TSS content was expressed in degrees Brix (°Brix), indicating the percentage of soluble solids in the sample (AOAC, 2006).

2.6. Microbiological analysis

The total plate count (TPC) for yeast and mold in beetroot jam was determined following the method outlined by Srivastava et al. (2018). A 10 g sample of the jam was homogenized in 90 mL of sterile diluent, and serial dilutions (10^1 to 10^6) were prepared. From each dilution, 0.1 mL was plated on nutrient agar and incubated at 30-37°C for 24-48 hours. Colony-forming units (CFU) were counted for plates with 30-300 colonies, and TPC was calculated as CFU per gram of beetroot jam using the appropriate formula.

TPC (CFU/g)= Number of colonies × Dilution factor/ Volume plated(mL)

Table 2: Proximate composition of beetroot

Nutrients	**Composition %**
Moisture	87.5±1.5
Crude fat	0.27±0.02
Crude protein	1.4±0.05
Ash	1.32±0.04
Total carbohydrates	7.54±0.3

2.7. Determination of antioxidant activity

A 0.5 g sample of beetroot jam was mixed with 100 mL of methanol and centrifuged at 5°C for 5 minutes at 5,000 rpm. The total phenolic content (TPC) was assessed using a modified Folin-Ciocalteu method. In this procedure, 100 µL of the extract or standard was combined with 750 µL of diluted Folin-Ciocalteu reagent and 750 µL of a 60 g/L sodium carbonate solution. After incubating for 90 minutes, absorbance was measured at 725 nm. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry weight (Aziz *et al*., 2023).

Table: 3 Impact of various treatments and storage conditions on the pH of Jam

Treatments	pH			
	0 day	**7 days**	**14 days**	**21 days**
T_o	3.38±0.03	3.35±0.02	3.30±0.05	3.29±0.02
T_1	3.4±0.02	3.37±0.01	3.32±004	3.28±0.04
T_2	3.39±0.04	3.35±0.02	3.32±0.04	3.30±0.03
T_3	3.4±0.02	3.38±0.04	3.35±0.03	3.33±0.03
T_4	3.4±0.01	3.37±0.05	3.32±0.02	3.30±0,02
Mean	**3.39^a**	**3.36^b**	**3.32^c**	**3.30^c**

2.8. Sensory analysis

The sensory evaluation of beetroot jam was conducted by trained panelists using a 9-point hedonic scale. The jam was placed in transparent glass jars and presented to judges for evaluation of its different attributes such as color, taste, aroma, flavor, and overall acceptability. The sensory evaluation performa was filled by each judge, assigning score (1-9) to each treatment.

2.9. Statistical analysis

The data obtained was subjected to statistical analysis following the method of Iftikhar *et al*. (2009). The obtained data was analyzed using a two-factor design with the Tukey HSD test (P<0.05) to compare the treatments. The statistical parameters were calculated using Statistics 8.1 program. The experiments were conducted in triplicate to derive the standard deviation and mean values.

3. Result and Discussion

3.1. Proximate analysis

Proximate composition of beetroot was analyzed including moisture content, ash, crude fat, carbohydrate, and crude protein. The result for all proximate analyses is represented in Table 2. The results indicate that beetroot contains 87.5% moisture, 0.27% crude fat, 1.32% ash, 1.4% protein, and 7.54% total carbohydrate. The results were found to be similar as prescribed by Kale et al. (2018). Earlier research has been conducted on the beetroot to determine its composition and antioxidant properties. The results for moisture content and crude fat of beetroot are in close agreement with Durga et al. (2020) who conducted a detailed study on production technology of beetroot powder by using different drying methods. They concluded that raw beetroot contained high moisture content i.e., 88% and 0.2% crude fat. Due to high moisture content, the beetroot vegetable is very susceptible to spoilage. They also elucidated that a small variation in moisture content among different varieties of beetroot is due to soil conditions, environmental conditions, growing conditions, harvesting periods, and storage temperature. Furthermore, environmental conditions, including temperature fluctuations, sunlight exposure, and moisture levels during growth, can impact the accumulation of crude fat in beetroot.

The results for the ash content of beetroot are in accordance with Kale et al. (2019). A comprehensive investigation was undertaken to assess the physical and chemical composition of beetroot (*Beta vulgaris* L.). They concluded that raw beetroot contained high ash content i.e., 1.4% due to several influencing conditions during its growth and cultivation. The results for the crude protein of beetroot are in close line with Perumpuli et al. (2018) who showed development of low sugar vegetable jam from beetroot (*Beta vulgaris* L.) and studied their physicochemical, sensory, and nutritional properties. They concluded that raw beetroot contained 1.35% protein content. The small variations were observed due to microbial degradation and enzymatic activity.

The results for total carbohydrates of beetroot are closely related to Sakhare et al. (2019) who worked on technology development, organoleptic evaluation, and proximate composition of beetroot candy by means of different sweeteners (*Beta vulgaris* L.). They determined that raw beetroot contained 7.59% total carbohydrates.

Table: 4 Impact of various treatments and storage conditions on the acidity of Jam

Treatments	**Acidity%**			
	0 day	**7 days**	**14 days**	**21 days**
T_o	0.80±0.01	0.82±0.03	0.84±0.02	0.86±0.01
T_1	0.76±0.01	0.78±0.02	0.80±0.01	0.84±0.01
T_2	0.72±0.02	0.76±0.03	0.81±0.01	0.83±0.01
T_3	0.73±0.02	0.76±0.01	0.81±0.03	0.83±0.02
T_4	0.75±0.01	0.75±0.01	0.78±0.03	0.80±0.02
Mean	**0.75^b**	**0.77^b**	**0.80^a**	**0.83^a**

To = Control, beetroot jam with 50 % sucrose; T_1 = Beetroot jam with 0.5 % stevia powder; T_2 = Beetroot jam with 1.0 % stevia powder; T_3 = Beetroot jam with 1.5 % stevia powder; T_4 = Beetroot jam with 2 % stevia powder

Table: 5. Impact of various treatments and storage conditions on the TSS of Jam

Treatments	TSS (°Brix)			
	0 day	**7 days**	**14 days**	**21 days**
T_o	66.08 ± 1.3^{d}	66.10 ± 2.5^{d}	66.12 ± 2.0^{d}	66.14 ± 2.5^{d}
T_1	67.02 ± 2.3^{c}	66.04 ± 3.5^{c}	66.07 ± 3.0^{d}	67.08 ± 3.5^{bc}
T_2	67.05 ± 2.0^{bc}	67.08 ± 3.0^{bc}	67.70 ± 3.1^{ab}	68.05 ± 1.7^{a}
T_3	67.06 ± 3.5^{bc}	67.05 ± 1.8^{bc}	67.70 ± 4.2^{ab}	68.02 ± 1.5^{a}
T_4	67.08 ± 2.7^{bc}	67.70 ± 3.0^{ab}	68.02 ± 4.0^{a}	68.04 ± 2.0^{a}
Mean	**66.85^{c}**	**67.00^{bc}**	**67.12^{b}**	**67.46^{a}**

To = Control, beetroot jam with 50 % sucrose; T_1 = Beetroot jam with 0.5 % stevia powder; T_2 = Beetroot jam with 1.0 % stevia powder; T_3 = Beetroot jam with 1.5 % stevia powder; T_4 = Beetroot jam with 2 % stevia powder

Table: 6 Impact of various treatments and storage conditions on the moisture of Jam

Treatments	Moisture (%)			
	0 day	**7 days**	**14 days**	**21 days**
T_o	33.70 ± 2.0^{a}	33.63 ± 2.2^{ab}	32.89 ± 2.0^{abc}	32.89 ± 3.0^{abc}
T_1	32.70 ± 1.5^{abcd}	32.62 ± 2.0^{abcd}	31.86 ± 1.0^{cde}	31.86 ± 1.0^{cde}
T_2	29.70 ± 1.5^{f}	29.63 ± 1.0^{f}	29.26 ± 1.0^{fg}	28.22 ± 1.4^{g}
T_3	31.70 ± 1.8^{de}	31.62 ± 1.5^{e}	31.28 ± 1.5^{e}	30.86 ± 2.0^{e}
T_4	32.50 ± 2.0^{bcd}	26.35 ± 2.3^{h}	26.50 ± 2.0^{h}	23.50 ± 2.0^{i}
Mean	**32.06^{a}**	**30.77^{b}**	**30.35^{c}**	**29.46^{d}**

To = Control, beetroot jam with 50 % sucrose; T_1 = Beetroot jam with 0.5 % stevia powder; T_2 = Beetroot jam with 1.0 % stevia powder; T_3 = Beetroot jam with 1.5 % stevia powder; T_4 = Beetroot jam with 2 % stevia powder

Table: 7 Impact of various treatments and storage conditions on ash of Jam

Treatments	Ash%			
	0 day	**7 days**	**14 days**	**21 days**
T_o	0.90±0.01	0.88±0.03	0.87±0.01	0.86±0.01
T_1	0.89±0.02	0.87±0.02	0.85±0.03	0.83±0.02
T_2	0.90±0.02	0.88±0.01	0.85±0.03	0.84±0.02
T_3	0.88±0.01	0.86±0.02	0.83±0.01	0.80±0.03
T_4	0.91±0.03	0.80±0.01	0.86±0.02	0.84±0.02
Mean	**0.89^{a}**	**0.85^{b}**	**0.85^{b}**	**0.83^{b}**

To = Control, beetroot jam with 50 % sucrose; T_1 = Beetroot jam with 0.5 % stevia powder; T_2 = Beetroot jam with 1.0 % stevia powder; T_3 = Beetroot jam with 1.5 % stevia powder; T_4 = Beetroot jam with 2 % stevia powder

Table: 8 Impact of various treatments and storage conditions on DPPH of Jam

Treatments	DPPH (%)			
	0 day	**7 days**	**14 days**	**21 days**
T_0	82.00 ± 3.0^{j}	82.16 ± 5.0^{ij}	83.00 ± 4.2^{ij}	83.50 ± 5.0^{i}
T_1	85.03 ± 4.0^{h}	85.27 ± 4.2^{h}	85.80 ± 2.7^{h}	86.00 ± 3.1^{gh}
T_2	85.02 ± 3.5^{h}	87.30 ± 4.0^{fg}	88.03 ± 5.0^{def}	89.20 ± 5.5^{bcd}
T_3	86.20 ± 3.3^{gh}	87.45 ± 3.5^{efg}	88.80 ± 3.2^{cde}	90.60 ± 3.9^{b}
T_4	86.11 ± 2.9^{gh}	88.70 ± 3.0^{cdef}	89.90 ± 3.5^{bc}	92.10 ± 3.0^{a}
Mean	**84.87^{d}**	**86.17^{c}**	**87.10^{b}**	**88.28^{a}**

To = Control, beetroot jam with 50 % sucrose; T_1 = Beetroot jam with 0.5 % stevia powder; T_2 = Beetroot jam with 1.0 % stevia powder; T_3 = Beetroot jam with 1.5 % stevia powder; T_4 = Beetroot jam with 2 % stevia powder

Table: 9 Impact of various treatments and storage conditions on the TPC of Jam

Treatments	TPC (mg GAE/g)			
	0 day	**7 days**	**14 days**	**21 days**
T_0	6.00 ± 2.0^{i}	5.80 ± 1.0^{ij}	5.50 ± 1.7^{ij}	5.20 ± 2.0^{j}
T_1	34.60 ± 2.4^{ef}	34.00 ± 2.2^{fg}	33.60 ± 1.3^{gh}	33.00 ± 2.0^{h}
T_2	35.20 ± 2.3^{de}	34.70 ± 1.6^{def}	34.00 ± 2.2^{fg}	33.70 ± 1.0^{gh}
T_3	38.10 ± 2.0^{b}	36.20 ± 1.3^{c}	35.40 ± 2.0^{d}	35.00 ± 2.8^{de}
T_4	38.90 ± 1.5^{a}	37.40 ± 2.0^{b}	36.30 ± 1.0^{c}	36.66 ± 2.2^{c}
Mean	**30.56^{a}**	**29.62^{b}**	**28.96^{c}**	**28.71^{c}**

To = Control, beetroot jam with 50 % sucrose; T_1 = Beetroot jam with 0.5 % stevia powder; T_2 = Beetroot jam with 1.0 % stevia powder; T_3 = Beetroot jam with 1.5 % stevia powder; T_4 = Beetroot jam with 2 % stevia powder

Table: 10. Impact of various treatments and storage conditions on the TPC of Jam

Treatments	TPC (CFU/g)			
	0 day	**7 days**	**14 days**	**21 days**
T_0	6.05 ± 1.3^{i}	6.20 ± 0.5^{hi}	7.16 ± 0.5^{ef}	9.00 ± 1.2^{a}
T_1	5.70 ± 0.7^{j}	6.20 ± 1.4^{hi}	7.35 ± 1.0^{de}	8.50 ± 0.6^{b}
T_2	6.10 ± 1.0^{hi}	6.29 ± 0.7^{h}	6.99 ± 1.1^{fg}	8.65 ± 0.6^{b}
T_3	6.11 ± 0.5^{hi}	6.20 ± 0.9^{hi}	6.81 ± 0.8^{g}	8.20 ± 0.3^{c}
T_4	6.11 ± 0.8^{hi}	7.03 ± 0.7^{fg}	7.50 ± 0.6^{d}	$8.081.0^{a}$
Mean	**6.01^{d}**	**6.38^{c}**	**7.16^{b}**	**8.48^{a}**

To = Control, beetroot jam with 50 % sucrose; T_1 = Beetroot jam with 0.5 % stevia powder; T_2 = Beetroot jam with 1.0 % stevia powder; T_3 = Beetroot jam with 1.5 % stevia powder; T_4 = Beetroot jam with 2 % stevia powder

3.2. Physicochemical analysis of beetroot jam

3.2.1. pH

The pH is an important factor to obtain optimum gel condition in jam. . The statistical analysis revealed that the pH of the control treatment (T_0) was significantly affected during the storage. At 0 days, pH values varied from 3.4 to 3.38 out of different treatments and throughout the storage. The pH significantly differed throughout storage days and non-significantly ($p>0.05$) between different treatments (Table 3). The most significant reduction in pH values was recorded at the end of storage period. pH manifested a decreasing trend with respect storage days. Maximum pH was documented at 0 days and minimum at the end of 3rd week of storage (21 days). Numerous factors may contribute to a pH drop during storage. One hypothesis is that the breakdown of carbohydrates will result in an increase in acidity. Moreover, this pH drop may also be caused by the breakdown of pectin. These findings are consistent with those of Wasif et al. (2015), who reported a decline in the pH of blended apple and olive fruit jam during storage.

3.2.2. Titratable acidity

The results regarding titratable acidity are shown in table 4. The statistical analysis elucidated that the storage has significant impact on the acidity of treatments. At 0 days, titratable acidity values varied from 0.80 to 0.72 between different treatments. The titratable acidity has significantly differed throughout storage days and non-significantly ($p>0.05$) between different treatments. Minimum titratable acidity was documented at 0 days and maximum at the end of 3rd week of storage (21 days). The acidity of beetroot jam may increase during storage due to microbial activity, particularly from bacteria or yeast that can produce organic acids. Additionally, enzymatic reactions, such as pectinase activity, can break down pectin, leading to the release of more acids. Oxidative degradation and the breakdown of ascorbic acid can also contribute to acidity changes over time. The findings completely agreed with Basmaa et al. (2022) who investigated the titratable acidity of apple jam after the incorporation of sweetener and concluded an increasing trend (0.81% to 0.85%) as noticed in the present study.

3.2.3. TSS (Total Soluble Solids)

The results regarding TSS of beetroot jam are presented in Table 5. The statistical analysis elucidated that there were slight changes that happened throughout keeping period. At 0 days, TSS values varied from 66.08 to 67.08°B among different treatment at 0 day. The total soluble solids significantly differed throughout storage days as well as among different treatments. The most significant reduction in total soluble solids values was recorded concerning days. Minimum TSS was documented at 0 days and maximum at the end of 3rd week of storage (21 days). A rise in total soluble solids (TSS) may be linked to the hydrolysis of polysaccharides, particularly pectin, into simpler sugars in the presence of acid during storage. The increase in total soluble solids (TSS) of beetroot jam during storage can be attributed to several factors. Firstly, the concentration of sugars can rise due to the evaporation of water, enhancing the sweetness. Secondly, enzymatic activity may break down pectin and other polysaccharides, releasing additional soluble sugars. Finally, microbial activity can lead to the production of organic acids, which may further enhance the perception of sweetness over time (Ansari et al., 2017). These changes contribute to the overall improvement in TSS observed during storage. The results are comparable with Khan et al. (2012) who demonstrated an increase in TSS of strawberry jam during storage.

3.2.4. Moisture content

Moisture plays a vital role in determining the shelf life and freshness of food products. Items with high moisture levels typically have a shorter shelf life. In this study, all samples exhibited a decrease in moisture content over the 21-day storage period (Table 6). Statistical analysis indicated significant differences in moisture levels among various treatments and throughout the storage duration. Maximum moisture was documented at 0 days and minimum at the end of 3rd week of storage (21 days). During storage, the mean moisture content value significantly ($p<0.05$) decreased from 32.06% to 29.46%. The results are in close line with Sutwal et al. (2019) who studied low-calorie apple jam with stevia as a sweetener and used Potassium metabisulfite (KMS), as a preservative added to optimized jam, which serves dual functions that impact moisture content. As a firming agent, it enhances jam quality by stabilizing its structure and reducing moisture loss by strengthening bonds between components. Additionally, KMS acts as a glazing agent, forming a waxy coating that acts as a barrier, limiting water loss from the jam. This dual role, both in reinforcing the jam's structure and creating a protective coating, effectively reduces moisture loss during storage, contributing to the preservation of the jam's quality (Basmaa et al., 2022). The decrease in moisture content of beetroot jam during storage can be attributed to several factors. Primarily, water evaporation occurs due to prolonged exposure to air and temperature fluctuations, leading to increased viscosity and concentration of soluble solids. Additionally, osmotic pressure changes can draw water out of the jam as sugar concentration increases, further contributing to moisture loss. Finally, microbial metabolism may also facilitate water loss as microorganisms consume available moisture, resulting in a drier product over time (Farhan et al., 2024).

3.2.5. Ash

Ash is an inorganic material, resulting after burning the organic material. The retrieved ash may not have precisely the same structure as the mineral elements in the actual food. The produced ash is homogeneous in hue, gray or white, free of flammable coal fragments or even bonded particles (Bhardwaj *et al*., 2019). The results regarding ash content of beetroot jam are presented in Table 7. The statistical analysis revealed that the storage has significant ($p<0.05$) impact on ash content. The maximum ash content was recorded at 0 day in T_4 (0.91) while the minimum ash content was recorded in T_3 (0.80). The ash content varied significantly between storage days and non-significantly between different treatments.

These results are comparable with Ashaye *et al*. (2009) who noticed a decline in ash content of rosella jam throughout keeping period. The decrease in ash content of beetroot jam during storage can be attributed to leaching and evaporation processes. Over time, soluble minerals may leach out of the jam due to moisture loss, leading to a reduced concentration of ash. Additionally, prolonged storage can result in the breakdown of certain compounds, which may alter the mineral composition. The overall changes in composition can lead to a measurable decrease in ash content, reflecting alterations in the jam's nutrient profile (Dhanda et al., 2024). The reduction in ash content in beetroot jam during storage can be further explained by the chemical changes that occur over time. As the jam undergoes microbial fermentation, some minerals may be utilized by microorganisms for metabolic processes, leading to a decrease in the overall ash content. Additionally, oxidation reactions may alter the mineral composition, with some minerals becoming less stable and precipitating out of the solution. These combined factors can result in a lower measured ash content in the jam over extended storage periods (Haider et al., 2024).

3.3. Antioxidants analysis

3.3.1. DPPH

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating the antioxidant capacity of various substances, as it measures the ability of antioxidants to neutralize free radicals by donating electrons or hydrogen atoms.

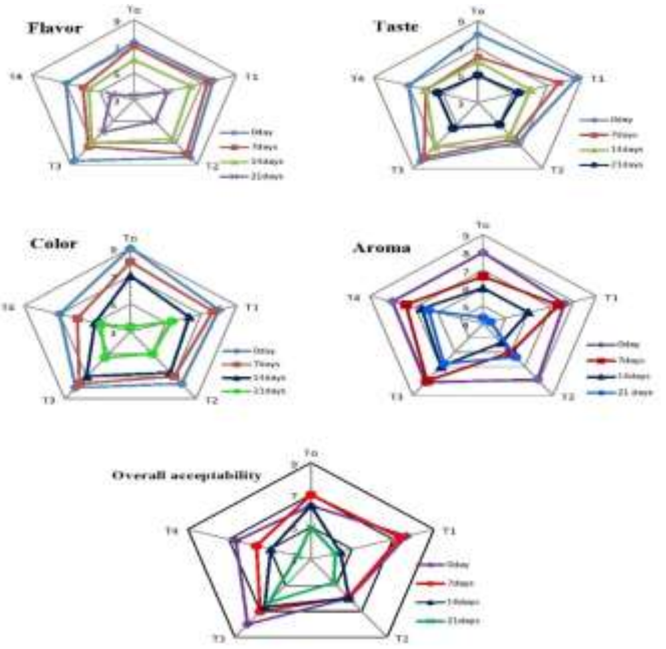

Fig. 1. Sensory Characteristic

The antioxidant characteristics of beetroot jam prepared with stevia powder were examined using two assays: total phenolic count and DPPH, which scavenges free radicals activities. The statistical analysis demonstrated a significant difference ($p<0.05$) in DPPH (2, 2-diphenyl-1-picrylhydrazyl) during the storage (Table 8). The maximum mean value was recorded at 21st day (88.28%) and the minimum mean value was recorded at 0 day (84.87%). Between treatments T_0 showed the minimum value 82.0 at 0 day and maximum by T_4 (86.11), while at 21st day T_4 showed the maximum value (92.1) followed by T_3 (90.60). The increase in antioxidant activity during storage can be attributed to Stevia's natural antioxidant properties. The increase in DPPH radical scavenging activity of beetroot jam during storage can be attributed to the ongoing release of bioactive compounds, such as antioxidants, as the matrix of the jam breaks down. Over time, the degradation of cellular structures may liberate phenolic compounds, enhancing the jam's ability to scavenge free radicals. Additionally, the presence of other natural antioxidants may become more effective as the jam ages, leading to improved overall antioxidant capacity measured by the DPPH assay (Brzezinska-Rojek et al., 2023). The results obtained in this study follow the findings of Kanwal *et al*., (2017) who recorded the antioxidant activity of guava jam and concluded that antioxidant activity increased throughout the keeping period as recorded in current study.

3.3.2. TPC (total phenolic content)

Total Phenolic Content (TPC) quantifies the concentration of phenolic compounds in a sample, which are important phytochemicals known for their antioxidant properties, potentially contributing to health benefits by reducing oxidative stress. The overall phenolic content in a product is subject to variation based on factors such as the source, cultivation circumstances, composition, and concentration of various interfering chemicals present in the raw material. The outcome is contingent upon the extraction technique and solvent employed. The results regarding total phenolic content of beetroot jam are shown in Table 9. The statistical analysis demonstrated a significant difference ($p<0.05$) in total phenolic count during the storage. The maximum tpc value was recorded for T_4 at 0 day (38.9) while the minimum value for T_1 (34.6) having stevia powder in it. A decreasing trend in total phenolic content was observed during the storage. At 0 day tpc was 30.56 which were reduced to 28.71. The reduction in total phenolic content of beetroot jam during storage can be attributed to various factors, including oxidation and degradation processes. Exposure to oxygen can lead to the oxidative degradation of phenolic compounds, reducing their concentration. Additionally, heat and light during storage may further accelerate the breakdown of these sensitive compounds. Microbial activity could also contribute to the depletion of phenolic compounds as they are utilized for metabolic processes, resulting in an overall decrease in total phenolic content over time (Jakubczyk et al., 2023). In addition to oxidation and degradation, the reduction in total phenolic content of beetroot jam during storage can also be attributed to thermal instability, as some phenolic compounds are sensitive to heat and may break down over time. Enzymatic activity, particularly from polyphenol oxidase, can further facilitate the degradation of phenolics, leading to lower concentrations. Furthermore, reactions with sugars or other components in the jam matrix may result in the formation of less active or inactive compounds, contributing to the overall decline in total phenolic content (Kanwal et al., 2017).

3.4. Microbial analysis

3.4.1. TPC (total plate count)

The results regarding total plate counts are shown in Table 10, determined that the viability of microbes in jam varied from 6.01 to 8.48 log CFU/g throughout the storage. The maximum tpc value was recorded for T_0 (9.00 log CFU/g) while minimum for T_4 (8.08 log CFU/g) after 21 days of storage. The total plate count is significantly influenced by both days and treatments and the most significant rise in tpc was recognized in all treatments concerning days. The highest increase in total plate count was documented after 21 days of storage and a minimum of 0 days. The total plate count (TPC) of beetroot jam may increase during storage due to microbial growth facilitated by favorable conditions such as moisture, temperature, and pH. Over time, the breakdown of sugars and other components can provide additional nutrients for microorganisms, promoting their proliferation. Additionally, if the jam is not properly sealed or stored, exposure to air and contaminants can introduce more microorganisms, leading to higher counts. These factors combined contribute to an increase in TPC over the storage period (Petkova et al., 2022). The results of present stdy are in close aggrement with Dhanda et al. (2024) who concluded that bacterial population in jam increased during the storage due to decrease in acidity as the jam ages, creating a more favorable environment for microbial growth.

3.5. Sensory quality characteristics

The sensory evaluation of beetroot jam, conducted using a 9-point hedonic scale, indicated that flavor, taste, color, aroma, and overall acceptability were optimized when sugar was replaced with 0.5-2% stevia. The flavor scores declined over time, with T3 initially rated highest (8.66) and maintaining a leading score (6.0) by the end of storage, while control samples scored lower due to the presence of sucrose. The reduction in flavor during storage is likely due to oxidative changes, enzymatic breakdown of phenols, and loss of volatile compounds, aligning with findings from Dubey et al. (2023) and Priya et al. (2010) on similar products. For taste, T3 again received the highest rating (8.33) on the first day, with a mean score of 7.16 at the end of storage, suggesting that changes in acidity, pH, and sugar composition affected taste. This result is consistent with Wasif et al. (2015), who observed similar trends in apple and olive jam.

Color analysis showed that T3's color score decreased by the 21st day, possibly due to browning reactions, pigment degradation, and pH fluctuations. This trend aligns with observations from Khan et al. (2015) on color changes in stored apple jams. Aroma scores also declined, with T3 maintaining a higher aroma rating (6.80) by the end of storage, attributed to the degradation of aroma compounds and photochemical reactions, consistent with Perumpuli et al. (2018). Overall acceptability followed a decreasing trend across treatments, with T3 scoring highest (8.00 initially and 6.33 at storage end). This reduction reflects the cumulative decline in color, flavor, and texture over time, echoing findings from Muhammad et al. (2012) on diet apple jam. Overall, T3 consistently demonstrated the highest quality across sensory parameters, even after extended storage.

The sensory evaluation of beetroot jam reveals a significant decline in flavor, taste, color, aroma, and overall acceptability during storage, with treatment T3 consistently achieving the highest scores, indicating its preference among panelists throughout the study.

Conclusion

The use of stevia, a natural sweetener, in beetroot jam production has shown to be successful in preserving the desired qualities of traditional jam while providing a reduced calorie choice. Because of its low calorie content, it is appropriate for people with diabetes, those following restricted diets, and people trying to control their weight. However, using high-intensity sweeteners in place of sugar completely can often lead to issues with unwanted qualities including texture and appearance. In order to satisfy consumer preferences and preserve the health benefits of low-calorie beetroot jam with stevia, more research is necessary to improve the texture and aesthetic appeal of the product.

Conflict of interest

There is no conflict of interest between the authors.

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