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Probiotic and Anthocyanin Rich Purple Sweet Potato Frozen Yogurt

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Abstract

Sweet potatoes are a rich source of diverse bioactive compounds, essential minerals, and dietary fibre and can be rationally targeted for wider human health-focused dietary solutions, especially to address diet-linked non-communicable chronic disease challenges. Therefore, the development of probiotic and anthocyanin-rich purple-fleshed sweet potato-based frozen yogurt to preserve and improve sweet potato-based human health-relevant nutritional qualities for health-targeted food application was studied. Purple fleshed sweet potato (PSP) variety Bhu Krishna was selected based on its high baseline phenolic content and antioxidant activity. Full factorial design with five levels of PSP puree and yogurt resulted in 25 experimental runs employed in this study. Nutritional and microbial data of frozen yogurt was recorded before storage and 10thday of storage at -4°C. Among 25 combinations frozen yogurt samples with a higher percentage of PSP showed significantly *($p<0.05$)* higher nutritional value. The combination, 70:30 showed higher anthocyanin content of 55.24 and 51.14 mg100 g^{-1}, protein content of 6.56 and 5.64%, and a fibre content of 0.69 and 0.33% on zero and tenth day of storage. In addition, the viable beneficial microbial count of formulations was ranged between 5.45 to 7.22 log CFU ml^{-1} at tenth day of storage. Results suggested that beneficial lactobacillus (LAB) based frozen yogurt development was an effective post-harvest processing strategy for higher retention of anthocyanin content and its associated antioxidant and anti-hyperglycaemic functionalities in sweet potatoes. Additionally, such frozen yogurt with probiotic as well as prebiotic potential can be integrated into health-focused dietary solution strategies, especially to improve human gut health and to mitigate chronic oxidative stress-linked non-communicable disease challenges.

Keywords : Frozen yogurt, Purple sweet potato, Probiotic, Viable cell count, Anthocyanins

Introduction

Regular intake of foods abundant in dietary fibre and health protective bioactive compounds is important to counter the risks associated with development of non-communicable diseases (NCD). Hence dietary interventions that include a whole plant-based diet, rich in phenolic bioactive compounds (phenolic acids, flavonoids, alkaloids, stilbenes, tannins, coumarins, and lignans) are needed as effective health strategies to fight NCDs (Bauer et al., 2013). Among vegetables, coloured sweet potatoes are recognized as functional foods rich in carotenoids, flavonoids, anthocyanins, phenolic acids and minerals. The occurrence of different colours in sweet potato pulp is linked to the presence of several bioactive compounds that act as pigments such as anthocyanidins, β-carotene, and flavonoids. In addition to providing colour to the food, the bioactive compounds can beneficially

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contribute to the consumer's metabolism. Although most sweet potatoes have white to off-white flesh, the colour may be cream, yellow, orange and purple. Vitamins and minerals in sweet potatoes make them a healthy choice at mealtime.

Orange sweet potatoes are one of the richest sources of beta-carotene, a carotenoid known for its benefit for eye health and ability to reduce cancer risks. Purple sweet potatoes are a richer source of anthocyanin pigments, which act as antioxidants that can help reduce inflammation and boost the immune system. Purple sweet potatoes have been reported to contain higher anthocyanins as compared to blueberries, blackberries, cranberries and grapes (Bridgers et al., 2010). The intervention to introduce and widen the dissemination of biofortified purple-fleshed sweet potato will result in a significant improvement in overall vitamin A intake and nutritional status. Moreover, purple-fleshed sweet potato is an ideal food and nutrition security crop. The dietary intakes of sweet potatoes are strongly recommended for their undeniable health-promoting properties. They also offer potential applications in food industries for the development of novel value-added food products.

Targeting sweet potatoes in safe and inexpensive dietary support strategies to prevent and manage the early stages of type 2 diabetes and related health risks has significant relevance. So, integrated food products incorporating biofortified purple sweet potatoes and yogurt as ingredients with prebiotic, probiotic, as well as bioactive compounds, linked antioxidant, anti-diabetic, and antihypertensive properties can subdue or eliminate diet-linked health challenges. The present study focuses on the physicochemical, phenolic linked antioxidant and antidiabetic properties of frozen yogurt developed using purple sweet potato puree.

Materials and Methods

Purple sweet potato (PSP) tubers of *var*. Bhu Krishna, grown in the experimental farm of ICAR-Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, Kerala, India, were used for developing frozen yogurt. After harvesting, PSP tubers were graded, sorted, and the diseased and damaged tubers were discarded. The selected tubers were washed in clean water to remove any adhering dirt. Tubers were baked at 180°C for 40 min followed by cooling to room temperature. Cooled tubers were peeled manually and cut into small pieces. A weighed quantity of chopped sweet potatoes were added to a food processor and blended for 5 min. It was then mashed to get a paste like consistency. This purple sweet potato puree was stored at -10°C for further use.

Frozen yogurt preparation

Yogurt was prepared using the ingredients listed in Table 1. Full factorial design was selected for designing the PSP based frozen yogurt. Both yogurt formulation and PSP puree was added in different concentrations such as 30, 40, 50, 60 and 70% using full factorial design which resulted 25 experimental runs as shown in Table 2. The resulted PSP yogurt was stored at -4 °C for further biochemical analysis.

Table 1. Yogurt formulation

Ingredients	Concentration (g $100g^{-1}$)
Fresh skim milk	68.9
Fresh cream	8.0
Skim milk powder	3.89
Sucrose	16.0
Carboxymethyl cellulose	0.2
Starter (*Lactobacillus plantarum*)	3.0
Vanilla flavour	0.01

Table 2. Purple sweet potato frozen yogurt formulations

Treatment	PSP Puree: Yogurt
T1	30:70
T2	30:60
T3	30:50
T4	30:40
T5	30:30
T6	40:70
T7	40:60
T8	40:50
T9	40:40
T10	40:30
T11	50:70
T12	50:60
T13	50:50
T14	50:40
T15	50:30
T16	60:70
T17	60:60
T18	60:50
T19	60:40
T20	60:30
T21	70:70
T22	70:60
T23	70:50
T24	70:40
T25	70:30

Starch and total sugar contents

The starch and total sugar content of sweet potato based frozen yogurt samples were analyzed according to a reported procedure (Moorthy and Padmaja, 2002).

Crude protein content

The crude protein content in the samples was estimated according to the AOAC official method (AOAC, 1960) using KjelTron (Tulin equipments, India) rapid automatic protein analyzer.

Total fat content

Sample (2 g) was added to a wide mouthed boiling tube. Twenty ml of alcohol:diethyl ether mixture (3:1) was added to the sample and stirred well. The tubes were kept in a thermostatic water bath at 55°C for 2 h. It was then centrifuged at 3000 rpm for 10 min and the clear supernatant was decanted to a pre-weighed petri dish. Another lot of alcohol:diethyl ether mixture was added to the tube and extracted for two hours. It was then centrifuged and decanted to the same plate. The extraction was repeated once again for 1 h and the petri dish containing the supernatant was dried in an air oven at 60°C till it reached a constant weight. The quantity of crude fat present in the sample was calculated based on the following formula:

$$\text{Weight of fat} = (\text{Weight of Petri dish + extract}) - (\text{Weight of empty Petri dish}) \quad \ldots \text{Eqn (1)}$$

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100 \quad \ldots \text{Eqn (2)}$$

Total soluble phenolic content

The total soluble phenolic (TSP) content of aqueous and ethanol (12%) extracts of sweet potatoes was determined based on the protocol described by Shetty et al., (1995). The assay was carried out by placing 0.5 ml of the sweet potato extracts into the sample test tubes and diluted for 2 times by adding 0.5 ml of distilled water. For measuring blank, 0.5 ml of distilled water was added instead of the sample extracts. In each test tube, 1 ml of 95% ethanol, 0.5 ml of 50% (v/v) Folin-Ciocalteu reagent, and 5 ml of distilled water were added. Then after adding 1 ml of 5% sodium carbonate, the contents were mixed well by using a digital vortex mixer (Thermo Fisher Scientific, Waltham, MA, USA) and incubated in the dark for 60 min. Samples were remixed again before reading the absorbance using a GENESYS 50 UV-Vis spectrophotometer PROMO (Thermo Fisher Scientific, USA) set at 725 nm wavelength. The absorbance values were converted to total soluble phenolic content and expressed in milligrams gallic acid equivalent (GAE) per gram of fresh weight (FW) based on a standard curve that was established using different concentrations of gallic acid in 95% ethanol.

Alpha amylase enzyme inhibitory activity

The assay protocol used in this study was adopted from the Worthington Enzyme Manual (1993). The buffer used was 0.1 M sodium phosphate (pH 6.9) with 0.006 M sodium chloride added to it. A volume of 500 μl of each sweet potato sample extract was added to test tubes, while the control tubes had 500 μl of buffer only. Additionally, each sample extract had a corresponding sample blank tube, which contained 500 μl of the sample extract, without addition of enzyme. Then 500 μl of porcine pancreatic amylase enzyme (0.5 mg ml^{-1} buffer) was added to all the tubes, except for sample blank and incubated for 10 min at 25°C. After incubation, 500 μl of 1% starch was added to all tubes and incubated for 10 min. The reaction was then stopped by the addition of 1 ml of 3,5-Dinitrosalicylic acid before placing tubes in a boiling water bath for 10 min. After incubation, tubes were removed and allowed to cool at room temperature. The reaction mixture in each tube was then diluted by adding 10 ml of distilled water to adjust the absorbance of the control to 1.0±0.02 and absorbance was measured at 540 nm using a GENESYS 50 UV-Vis spectrophotometer (Thermo Fisher Scientific, USA). The inhibition percentage of α-amylase enzyme activity was calculated based on the absorbance readings and using the following formula (Eqn. 3).

$$\text{\% Inhibition} = \frac{\text{Abs control} - (\text{Abs extract} - \text{Abs sample blank})}{\text{Abs control}} \times 100 \quad \ldots \text{Eqn (3)}$$

Total antioxidant activity

The total antioxidant activity of the sample extracts was measured using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. For the DPPH assay, protocol described by Kwon et al., (2006) was used, where 0.25 ml sample was added to 1.5 ml centrifuge tubes, while 0.25 ml 95% ethanol was added to the control tube. Then 1.25 ml of 60 mM DPPH (in 95% ethanol) was added to all centrifuge tubes and mixed well by vertexing and then incubated for 5 min. The mixture was then centrifuged at 15,115×g for 1 min to pelletize the precipitate. The absorbance of the supernatant was measured at 517nm using a GENESYS 50 UV-Vis spectrophotometer (Thermo Fisher Scientific, USA). The percentage of inhibition for DPPH radicals was calculated using equation (4).

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance extract}}{\text{Absorbance control}} \times 100$$

… Eqn. (4)

The inhibition percentage from the DPPH radical scavenging assay was expressed as mM Trolox equivalents (TE) per gram of sample based on the Trolox standard curve.

Viable cell count

Purple sweet potato based frozen yogurt samples were serially diluted to 10^{-5} followed by 100 μl aliquots of each dilution plated on duplicated MRS agar plates by the spread plate method. Different serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) were made using 100 μl of frozen yogurt samples and sterile water to determine the growth. Later, these plates were incubated for 48 h at 37ºC. Plates containing viable cells of *L. plantarum* were recorded as Log CFU (colony forming units) ml^{-1}.

Anthocyanin estimation

Anthocyanin content of purple sweet potato based frozen yogurt samples was determined using the pH differential method previously reported by Rodriguez and Wrolstad (2001). About 5 g of sample was homogenized with 0.1% acidified methanol followed by centrifugation at 10,000 rpm for 10 min, and extraction was carried out multiple times until the supernatant became colourless or the pellet become white. After collecting all the supernatants, 1 ml of the supernatant was separately diluted with 20 ml of two different buffer solutions (0.025M KCl buffer and 0.4M sodium acetate buffer) separately. The absorbance from anthocyanin was measured using a GENESYS 50 UV-Vis spectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of λ_{max} and 700 nm against distilled water as blank. Then anthocyanin content was calculated using following formula (Eqn.5).

$$A = (A\lambda_{max} - A_{700}) \text{ at pH 1 buffer} - (A\lambda_{max} - A_{700}) \text{ at pH 4.5 buffer}$$

$$\text{Monomeric anthocyanin} = A \times MW \times DF \times 1000 / \text{extinction coefficient}$$

… Eqn (5)

Where, MW (molecular weight) is 449; extinction coefficient is 26900 and DF (dilution factor) is the diluted final volume per sample taken for dilution, i.e., 20 l^{-1}

Statistical analysis

Statistical analysis of data was carried out using Data Expert software (version 15). Significant statistical differences between treatments effect, day effect and treatment × day interactions for all experimental runs were determined using Tukey's least square means separation at the 0.05 probability level.

Results and Discussion

Phenolic-linked antioxidant and antidiabetic properties

Screening food products for phenolic linked antioxidant and antidiabetic properties helps to ensure the human health benefits. Therefore, total soluble phenolic (TSP) content was studied for 25 combinations of PSP frozen yogurt which is varied from 0.54 (T14) to 1.96 (T22) mg GAE 100 g^{-1} FW (fresh weight basis) and the difference among the frozen yogurt formulations were found statistically significant ($p<0.05$). The lowest TSP content was observed in formulations T14 and T17 which contained 50 and 60% of PSP puree content, respectively. Overall, a higher retention of TSP content after ten days of storage at -4ºC was observed in this study. Similar results were reported by Chintha et al., (2021) among different sweet potato cultivars using beneficial lactic acid bacteria-based biotransformation strategy.

The total antioxidant activity of PSP frozen yogurt samples was determined using DPPH free radical scavenging assay. Statistically significant ($p<0.05$) differences in antioxidant activity among PSP frozen yogurt samples were observed (Table 3). The highest total antioxidant activity was observed in T19 (0.43 mM Trolox g^{-1} FW) of PSP frozen yogurt formulation by T16 (0.42 mM Trolox g^{-1} FW) and fermented lowest was observed in samples T11 and T12 (0.12 mM Trolox g^{-1} FW) after 10 days of storage at -4ºC.

To understand the potential anti-diabetic property of PSP frozen yogurt formulations, α-amylase enzyme inhibitory activity was determined using an *in vitro* assay model. Moderate to high α-amylase enzyme inhibitory activity (95.33% to 55.30%) (Table 3). Like the results of TSP content and antioxidant activity, statistically significant ($p<0.05$) differences in alpha amylase activity were observed among PSP frozen yogurt formulations in this study. Moreover, presence of active viable cells of PSP frozen yogurt samples after 10 days of storage at -4ºC was studied. In this study, viable cell count was found stable even after 10 days, higher viable cell count was recorded in T18 (7.22 log CFU ml^{-1}) and lowest in T19 (5.45 log CFU ml^{-1}) (Table 3). Therefore, presence of viable cell count in the PSP frozen yogurt can offer probiotic benefits to the consumers. Similar results about presence of probiotic bacteria (>7.22 log CFU ml^{-1}) in yogurt after 14 days of storage was reported by Sarvari et al (2014).

Proximate composition of purple sweet potato frozen yoghurt formulations

Studying food products for proximate composition like starch, ash, fat and sugar content is crucial to ensure the human health benefits. Overall, the starch content among different PSP frozen yogurt varied from 4.41 (T6) to 9.95 % (T3) at zero day and the formulation showed 4.10 and 8.11% after tenth day of storage at -4°C. At tenth day of storage the starch content ranged between 3.95 (T1) to 8.18% (T20). The difference was found significant ($p<0.05$) among the PSP frozen yogurt formulations both at zero day and tenth day of storage (Table 4). Ash content refers to the inorganic residue obtained after ignition or complete oxidation of organic matter in food stuff. Ash content is useful in assessing the quality grading of products and give the idea of the amount of mineral element present in the product. It ranged from 0.69% (T6) to 1.00% (T4, T15 and T25) at zero day. At tenth day of storage ash content was found ranged from 0.61% (T6) to 0.97 (T15 and T25). Fat content was estimated for different PSP frozen yogurt formulations. Analysis of frozen yogurt before storage showed that the fat content ranged from 11.91% (T24) to 19.54% (T8) (Table 4). Since the purple sweet potatoes are low in fat,

Table 3. Total soluble phenolic content, total antioxidant activity and viable cell count of beneficial microorganisms in purple sweet potato frozen yogurt after storage at -4°C for 10 days

Treatment	PSP Puree:Yogurt	Total soluble phenolic content (mg GAE 100 g^{-1} FW)	α-amylase inhibitory action (%)	Total antioxidant activity (mM Trolox g^{-1} FW)	Viable cell count (log CFUml^{-1})
T1	30:70	0.80 ± 0.00^{g}	55.03 ± 0.09^{x}	0.24 ± 0.10^{f}	5.49 ± 0.01^{t}
T2	30:60	0.66 ± 0.08^{k}	59.32 ± 0.02^{w}	0.17 ± 0.04^{k}	5.87 ± 0.02^{q}
T3	30:50	0.70 ± 0.01^{h}	63.01 ± 0.04^{u}	0.21 ± 0.02^{h}	5.40 ± 0.04^{v}
T4	30:40	0.69 ± 0.05^{i}	67.22 ± 0.05^{s}	0.24 ± 0.00^{f}	5.87 ± 0.00^{q}
T5	30:30	0.65 ± 0.02^{l}	69.38 ± 0.02^{r}	0.21 ± 0.00^{h}	6.23 ± 0.00^{k}
T6	40:70	0.69 ± 0.00	76.21 ± 0.00^{q}	0.18 ± 0.01^{j}	5.50 ± 0.08^{s}
T7	40:60	0.67 ± 0.00^{j}	77.3 ± 0.01^{o}	0.40 ± 0.08^{c}	6.12 ± 0.06^{m}
T8	40:50	0.58 ± 0.10^{q}	60.57 ± 0.00^{v}	0.25 ± 0.06^{e}	6.69 ± 0.05^{g}
T9	40:40	0.63 ± 0.02^{m}	66.01 ± 0.08^{t}	0.40 ± 0.10^{c}	6.48 ± 0.04^{i}
T10	40:30	0.63 ± 0.01^{m}	76.55 ± 0.10^{p}	0.16 ± 0.00^{l}	7.07 ± 0.05^{b}
T11	50:70	0.56 ± 0.00^{r}	78.01 ± 0.03^{m}	0.12 ± 0.02^{m}	5.95 ± 0.11^{o}
T12	50:60	0.59 ± 0.04^{p}	78.04 ± 0.06^{l}	0.12 ± 0.01^{m}	6.00 ± 0.02^{x}
T13	50:50	0.65 ± 0.05^{l}	78.45 ± 0.04^{k}	0.21 ± 0.06^{h}	5.90 ± 0.01^{p}
T14	50:40	0.54 ± 0.01^{s}	77.56 ± 0.08^{n}	0.22 ± 0.02^{g}	6.15 ± 0.00^{l}
T15	50:30	0.60 ± 0.00^{o}	79.78 ± 0.02^{j}	0.28 ± 0.00^{d}	6.75 ± 0.01^{f}
T16	60:70	0.61 ± 0.03^{n}	80.75 ± 0.01^{i}	0.42 ± 0.01^{b}	6.11 ± 0.02^{n}
T17	60:60	0.54 ± 0.00^{s}	80.75 ± 0.00^{i}	0.25 ± 0.00^{e}	7.05 ± 0.04^{c}
T18	60:50	0.59 ± 0.02^{p}	81.25 ± 0.01^{h}	0.43 ± 0.04^{a}	7.22 ± 0.01^{a}
T19	60:40	0.89 ± 0.00^{e}	81.78 ± 0.00^{g}	0.25 ± 0.08^{e}	5.45 ± 0.02^{u}
T20	60:30	0.87 ± 0.04^{f}	82.88 ± 0.05^{f}	0.10 ± 0.09^{n}	6.78 ± 0.00^{e}
T21	70:70	1.25 ± 0.05^{d}	83.79 ± 0.07^{e}	0.17 ± 0.07^{k}	6.57 ± 0.00^{w}
T22	70:60	1.96 ± 0.02^{a}	83.95 ± 0.05^{d}	0.19 ± 0.00^{i}	6.61 ± 0.02^{r}
T23	70:50	1.91 ± 0.00^{b}	88.39 ± 0.00^{c}	$0.22v\pm0.02^{g}$	6.28 ± 0.03^{j}
T24	70:40	1.88 ± 0.05^{c}	88.84 ± 0.02^{b}	0.25 ± 0.01^{e}	6.57 ± 0.00^{h}
T25	70:30	1.91 ± 0.01^{b}	95.33 ± 0.02^{a}	0.40 ± 0.00^{c}	6.89 ± 0.10^{d}

Note: Value=mean ± standard error, different letters in column represent significant differences in total soluble phenolic content, alpha-amylase inhibitory action, total antioxidant activity and viable cell count at 95% level of confidence interval ($p<0.05$).

Table 4. Proximate composition of purple sweet potato frozen yoghurt formulations under storage conditions at -4 °C

Treatment	Starch (%)		Ash (%)		Fat (%)		Sugar (%)	
	0 day	10th day	0 day	10th day	0 day	10th day	0 day	10th day
T1	4.48^{nop}	3.95^{p}	0.91^{abcd}	0.88^{abcd}	18.56^{bcd}	16.21^{bcd}	8.01^{hij}	6.79^{jklm}
T2	4.45^{nop}	4.07^{p}	0.97^{abc}	0.90^{abc}	17.25^{d}	16.84^{d}	6.90^{jklm}	6.09^{klm}
T3	5.90^{klmn}	5.27^{lmno}	0.90^{abcde}	0.89^{abcde}	17.15^{cd}	15.36^{cd}	9.16^{fgh}	8.18^{ghij}
T4	6.34^{hijk}	6.00^{klm}	1.00^{a}	0.92^{ab}	16.65^{def}	15.26^{def}	9.16^{fgh}	8.72^{ghi}
T5	6.12^{ijklm}	5.45^{klmn}	0.89^{abcde}	0.90^{abcde}	15.23^{efg}	14.64^{efg}	8.71^{ghi}	8.74^{ghi}
T6	4.41^{nop}	4.10^{p}	0.69^{g}	0.61^{g}	16.50^{de}	15.32^{de}	8.01^{hij}	7.26^{ijk}
T7	5.41^{klmn}	5.15^{mno}	0.96^{abcd}	0.89^{abcd}	19.84^{abc}	17.95^{abc}	8.71^{ghi}	8.22^{ghij}
T8	4.50^{nop}	4.28^{op}	0.85^{f}	0.79^{f}	19.54^{a}	18.69^{a}	8.33^{ghij}	7.40^{ijk}
T9	6.21^{ijkl}	6.01^{jklm}	0.89^{abcde}	0.82^{abcde}	18.39^{abc}	18.34^{abc}	7.07^{ijkl}	5.23^{m}
T10	6.08^{jklm}	5.23^{lmno}	0.82^{ef}	0.78^{ef}	15.95^{d}	16.47^{d}	6.25^{klm}	5.33^{lm}
T11	8.38^{bcde}	7.38^{defg}	0.87^{abcde}	0.85^{abcde}	18.82^{ab}	18.94^{ab}	9.16^{fgh}	7.14^{ijk}
T12	8.44^{bcd}	7.63^{cdefg}	0.90^{abcde}	0.89^{abcde}	17.53^{def}	15.53^{def}	10.72^{cdef}	9.16^{fgh}
T13	9.95^{a}	8.11^{bcdefg}	0.85^{def}	0.85^{def}	13.53^{efg}	14.21^{efg}	9.84^{defg}	8.01^{hij}
T14	8.71^{b}	7.50^{defg}	0.94^{abcd}	0.89^{abcd}	13.28^{fgh}	13.96^{fgh}	10.75^{bcdef}	9.16^{fgh}
T15	7.76^{bcdefg}	7.14^{fghi}	1.00^{a}	0.97^{a}	15.42^{ghi}	13.29^{ghi}	11.76^{abc}	8.01^{hij}
T16	8.14^{bcdef}	7.08^{ghij}	0.99^{bcdef}	0.83^{bcdef}	15.70^{def}	16.21^{def}	10.55^{cdef}	8.71^{ghi}
T17	8.33^{bcde}	7.77^{bcdefg}	0.98^{abcd}	0.85^{abcd}	14.94^{fgh}	14.10^{fgh}	12.50^{a}	9.54^{efgh}
T18	8.34^{bcde}	7.56^{defg}	0.90^{abcdef}	0.89^{abcdef}	13.50^{hi}	11.21^{hi}	11.80^{abc}	8.01^{hij}
T19	8.65^{bc}	8.04^{bcdefg}	0.90^{abcde}	0.89^{abcde}	14.30^{fgh}	14.01^{fgh}	10.55^{cdef}	8.01^{hij}
T20	8.49^{bcd}	8.18^{bcdef}	0.82^{cdef}	0.85^{cdef}	15.55^{efg}	14.24^{efg}	11.25^{abcde}	7.41^{ijk}
T21	7.92^{bcdefg}	7.63^{cdefg}	0.89^{abcde}	0.90^{abcde}	17.17^{def}	15.11^{def}	11.57^{abcd}	9.16^{fgh}
T22	8.33^{bcde}	7.76^{bcdefg}	0.90^{abcde}	0.90^{abcde}	13.74^{ghi}	13.24^{ghi}	12.47^{ab}	8.01^{hij}
T23	8.22^{bcde}	7.90^{bcdefg}	0.89^{abcde}	0.87^{abcde}	12.80^{ghi}	12.13^{ghi}	12.24^{abc}	8.71^{ghi}
T24	8.04^{bcdefg}	7.50^{defg}	0.94^{abcd}	0.92^{abcd}	11.90^{i}	11.36^{i}	11.78^{abc}	8.01^{hij}
T25	7.73^{bcdefg}	7.14^{fghi}	1.00^{a}	0.91^{abcd}	13.07^{ghi}	11.21^{ghi}	12.18^{abc}	9.54^{efgh}

*Different letters in the superscript represent significant differences in starch, ash and fat content due to significant treatment and day type interaction and sugar content due to significant treatment × day effect at 95% level of confidence interval ($p<0.05$).

the fat content found out in the PSP frozen yogurt is contributed by the yogurt in the proportion of 30% in T24 and 50% in T8. On tenth day, the fat content ranged from 18.69% (T8) to 13.05% (T25) among the different combinations. The fat content in frozen yogurt comes from curd and cream.

The total sugar content in frozen yogurt ranged from 6.25% (T10) to 12.50% (T17) among different combinations for the initial fresh sample. On 10th day, the sugar content ranged from 7.14% (T11) to 9.54% (T17 and T25) (Table 4). Estimating fiber content of food products ensures the prebiotic nature of the developed product. The fiber content in different PSP frozen yogurt formulations ranged from 0.10% (T1) to 0.72% (T19) (Table 5). Enhanced protein content in food products is gaining increasing attention from consumers and food industries due to their diverse health related functions, including the relevance in addressing chronic malnutrition as well as providing dietary benefits against chronic diseases. In the present study, PSP frozen yogurt formulations showed higher protein content, which

Table 5. Fiber, protein, and anthocyanin contents of purple sweet potato frozen yoghurt formulations under storage at -4 ºC

Treatment	Fiber content (%)		Protein content (%)		Total anthocyanin content (mg $100g^{-1}$)	
	0 day	10thday	0 day	10thday	0 day	10thday
T1	0.17^{klmn}	0.10^{n}	4.81^{ijklm}	4.39^{mn}	17.08^{vw}	14.45^{w}
T2	$0.21^{hijklmn}$	0.18^{jklmn}	4.81^{ijklm}	4.29^{mn}	19.69^{tuv}	17.10^{vw}
T3	0.19^{ijklmn}	0.12^{mn}	$5.23^{defghijk}$	$4.51l^{mn}$	20.77^{stu}	17.73^{uv}
T4	$0.18^{hijklmn}$	0.14^{lmn}	4.33^{mn}	4.14^{n}	23.03^{qrs}	22.01^{rst}
T5	$0.37^{defghijk}$	0.22^{hijkmn}	4.81^{ijklm}	4.11^{n}	23.87^{pqrs}	22.05^{qrst}
T6	$0.18^{hijklmn}$	$0.18^{hijklmn}$	5.67^{bcd}	4.51^{lmn}	23.94^{pqrs}	22.63^{qrst}
T7	$0.35^{defghijk}$	$0.30^{efghijklmn}$	5.65^{bcdefg}	4.56^{lmn}	24.09^{pqr}	23.10^{qrs}
T8	$0.39^{defghij}$	$0.35^{defghijk}$	$5.22^{defghijk}$	4.77^{jklm}	24.74^{opq}	23.87^{pqrs}
T9	$0.31^{defghijklmn}$	0.40^{defg}	$5.23^{defghijk}$	4.29^{mn}	27.89^{mno}	26.45^{nop}
T10	0.62^{a}	0.51^{abcd}	4.53^{lmn}	4.41^{mn}	28.79^{lmn}	28.57^{lmn}
T11	$0.30^{defghijklmn}$	$0.22^{hijklmn}$	5.69^{bcdef}	4.56^{lmn}	31.08^{jklm}	29.47^{klmn}
T12	$0.18^{hijklmn}$	$0.23^{ghijklmn}$	$5.27^{defghij}$	5.01^{hijkl}	31.23^{jkl}	29.74^{klm}
T13	$0.26^{fghijklmn}$	$0.27^{fghijklmn}$	$5.20^{efghijk}$	5.12^{ghijk}	32.27^{ijk}	30.16^{klm}
T14	$0.25^{fghijkmn}$	$0.39^{defghij}$	6.39^{a}	$5.58^{bcdefgh}$	35.17^{g}	30.99^{jklm}
T15	0.72^{a}	$0.22^{hijklmn}$	6.08^{abc}	5.34^{defghi}	36.59^{h}	34.15^{hij}
T16	$0.28^{fghijklmn}$	$0.35^{defghijk}$	$5.24^{defghij}$	$5.21^{defghijk}$	36.03^{h}	34.41^{hi}
T17	0.50^{abcd}	$0.29^{efghijklmn}$	5.51^{defgh}	5.15^{fghijk}	41.45^{g}	36.45^{h}
T18	0.44^{cdefg}	0.40^{defg}	6.08^{abc}	5.38^{defgh}	51.02^{d}	40.78^{g}
T19	0.72^{a}	$0.30^{efghijklmn}$	5.45^{defgh}	$5.25^{defghij}$	48.14^{cdef}	42.14^{g}
T20	0.51^{bcde}	$0.23^{ghijklmn}$	5.50^{defgh}	5.67^{bcdefg}	49.30^{bcdef}	47.23^{ef}
T21	0.46^{cdef}	$0.39^{defghij}$	5.57^{cdefgh}	$5.25^{defghij}$	50.96^{bc}	47.54^{def}
T22	$0.34^{defghijk}$	$0.28^{fghijklmn}$	6.16^{ab}	5.58^{cdefg}	50.96^{bc}	49.54^{bcdef}
T23	0.39^{defghi}	$0.33^{defghijklm}$	5.57^{cdefgh}	5.74^{bcde}	51.97^{b}	50.45^{bcd}
T24	0.46^{cdef}	$0.39^{defghij}$	6.10^{abc}	5.54^{cdefg}	52.14^{ab}	50.21^{bcd}
T25	0.69^{ab}	$0.33^{defghijklm}$	4.81^{ijklm}	5.64^{bcdefg}	55.24^{a}	51.14^{bc}

*Different letters represent significant differences in fibre and protein content due to treatment × day effect and anthocyanins content interaction due to treatment and day effect at 95% level of confidence interval ($p<0.05$).

wasin the range of 4.11% (T5) at 10th day to 6.39% (T14) at zero day (Table 5). Statistically significant differences in protein content between frozen yogurt formulations were also observed.

The anthocyanin content of the frozen yogurt samples was found highest before storage (55.24 mg $100g^{-1}$ in T25) and the lowest was recorded in T1 (14.45 mg $100g^{-1}$) (Table 5). Retention of anthocyanins after 10 days of storage at -4ºC was found positively correlated with higher antioxidant and anti-diabetic properties of the yogurt samples.

Conclusion

Healthy snack products with higher nutritional content and higher nutraceutical compounds are on-demand to fulfil the market need of health-conscious consumers. With this work, purple sweet potato (PSP) based frozen yogurt was evaluated to be nutritional frozen dessert with high retention of anthocyanin content even after 10 days of storage at -4ºC. The PSP frozen yogurt development was carried out using full factorial design to understand the effect of variables such as PSP puree and yogurt on the responses like protein, fiber, starch content, anthocyanin level, total soluble phenolics, antioxidant activity and antidiabetic property. Results showed a significant *($p<0.05$)* impact of PSP puree and yogurt on the responses in this study. The optimized PSP frozen yogurt in terms of nutritional was found to be T18 with a higher total antioxidant activity (0.43 mM Trolox g^{-1} FW), antidiabetic property (81.25%), total soluble phenolics (0.59 mM Trolox g^{-1} FW) and higher viable cell count (7.22 log CFU ml^{-1}) coupled with good amounts of starch, protein, fibre and ash content. Among 25 combinations, the combination, 70:30 showed higher anthocyanin content of 54.41 ± 0.15 $mg100g^{-1}$, protein content of 6.56 g, and a fibre content of 0.61 g on zero and tenth day of storage. In addition, the viable microbial count of all the trials was found steady from the zero to tenth day of storage at -4ºC. Results suggested that beneficial *lactobacillus* based frozen yogurt development was an effective post-harvest processing strategy for retention of anthocyanin content and its associated antioxidant and anti-hyperglycaemic functionalities. Additionally, such frozen yogurt with probiotic as well as prebiotic potential can be integrated into health-focused dietary solution strategies, especially to improve human gut health and to mitigate chronic oxidative stress-linked non-communicable disease challenges. Overall, it can be concluded that PSP puree has the potential to produce healthy frozen dessert with higher nutritional characteristics.

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