Standardization of process for the development of Butterfly pea (Clitoria ternatea L.) flower powder supplemented functional yogurt

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Research Article

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Abstract

The present investigation was carried out to develop functional yogurt enriched with dried Butterfly Pea (*Clitoria ternatea* L.) flower. Initially, physicochemical, microbiological, and sensorial attributes of yogurt prepared from different concentrations (0 to 3%) of Butterfly pea flower were studied. Yogurt supplemented with 1%, w/v butterfly pea flower showed better overall acceptability in sensorial terms and was optimized. The optimized BPF-rich yogurt showed 0.74 ± 0.3% ash content and 16.12 ± 0.02 total soluble solids which were higher than control yogurt. Rheological attributes (viscosity and syneresis), pH, and titratable acidity were similar in optimized BPF-rich yogurt and control yogurt. The DPPH inhibition activity (61.50%) and total phenolic content (87.23 mg GAE/g) and texture of optimized BPF-rich yogurt were better than control. The effect of storage period on free fatty acid, whey separation, acidity, total lactic acid bacteria count, coliform, yeast and mold count, and sensory parameters were studied. A significant increase was observed in free fatty acid value, acidity, and whey separation, while the lactic acid bacteria count was observed to be decreasing significantly ($p < 0.05$) in BPF-rich yogurt during 21 days of storage. Scanning Electron Microscopy analysis revealed better morphological characteristics and hydration properties in optimized BPF-rich yogurt. Ultra-Performing Liquid Chromatography analysis of BPF yogurt showed bioactive compounds such as delphinidin derivatives and cyanidin derivatives exhibiting functional attributes.

Practical Application

Yogurt is a fermented milk product which is having wider consumer acceptability owing to its potential health benefits. Butterfly Pea (*Clitoria ternatea* L.) flower possesses brain-boosting, anti-inflammatory, anti-cancerous, and antimicrobial properties. It also regulates brain development, blood sugar, blood cholesterol and improves skin and hair health. BPF supplemented yogurt is safer and economical and owing to the prevalence of bioactive compounds enhances the flavor, color & appearance, antioxidant properties as well as shelf life of the product.

Introduction

Due to covid-19, people are exceedingly health-conscious about what they are consuming. Parallel to civilization, their lifestyle and eating habits are also changing. Customers prefer food with high nutritional properties. These can be ensured by food preparation without using synthetic additives or preservatives. Common chemical preservatives used in yogurt are sodium benzoate and potassium sorbate (Akpan et al., 2007). Recent studies showed that few plant-derived natural extracts possess medicinal value due to their bioactive components (Loi et al., 2020). Tkaczewska (2020) reported the use of bioactive compounds as additives. Quirós-Sauceda et al. (2014) reviewed the addition of various bioactive compounds as an additive in food products. Thus, this increases the application of natural extracts as additives or preservatives in food. The bioactive compounds show biological activity, such as flavonoids, isoflavones, phenolic acids, carotenoids, etc. Natural pigments and flavors are vital from a biological perspective, and people are demanding natural color and flavor in food products. Therefore, incorporating these natural colors and flavors in food products is of great importance now. Several synthetic additives have been employed in food industries to enhance the color and flavor of food products. Excessive use of synthetic food additives, on the other hand, has been linked to respiratory, dermatological, gastrointestinal, and neurological side effects, as reported by several researchers (Carocho et al., 2014). As a result, the necessity for natural additives has pushed researchers to develop additives derived from natural sources suitable for use in dairy products.

Due to numerous health benefits, milk and milk products play an essential part in the human diet. Milk comprises lactose, vitamins, proteins, and minerals (Barkallah et al., 2017). The bioactive components present in the milk provide a symbiotic microflora. Various bioactive compounds (bioactive peptides, oligosaccharides, and organic acids) are present in milk, particularly in fermented dairy products. The fortification of dairy products with bioactive compounds improves the overall dietary intake while minimizing the adverse effects (Gahruie et al., 2015). Several studies have investigated the bioactive compounds fortified in milk and dairy products (Adinepour et al., 2022; Rashidinejad and Singh, 2021; Wen et al., 2018; Costa et al., 2018; Rashidinejad et al., 2016).

Yogurt is a well-liked fermented milk product in which lactose sugar is converted into lactic acid. The three most common forms of yogurt available on the market are set, stirred, and drinkable. Stirred or Swiss-style yogurts contain flavors, fruit, or other additives that must be stirred before eating to ensure the even distribution of the components. Set yogurt is inoculated with culture, placed in the container immediately, and fermented (Das et al. 2019). Yogurt comprises various functional attributes as they boost immunity. The flavor profile of yogurt includes compounds like diacetyl, 2-butanone, and acetaldehyde (Das et al. 2019). *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophiles* are the most common bacteria in yogurt production (Standard For Fermented Milks, CXS 243–2003). These two microbes can grow separately in milk during the fermentation process. When grown together in a mixed culture, these bacteria form a symbiotic relationship known as protocooperation, in which *L. bulgaricus* produces amino acids and short peptides that encourage the growth of *S. thermophilus*. Simultaneously, *S. thermophilus* creates formic acid, which aids *L. bulgaricus* growth (Standard For Fermented Milks, CXS 243–2003). Since yogurt is rich in minerals like calcium, protein, and vitamins, it can be used as a carrier for the bioactive compounds present in medicinal plants. As a result, some authors recommend fortifying yogurt with natural bioactive compounds as additives to overcome this limitation (Kandylis, et al., 2021; Šeregelj et al., 2021; Dimitrellou et al., 2020; Helal and Tagliazucchi, 2018; Caleja et al., 2016).

Clitoria ternatea L., commonly known as Butterfly pea, belongs to the Fabaceae family. The flower of butterfly pea has a typical blue color, and therefore, they are used in different foods as a blue coloring agent (Thanh et al. 2020). These colors are anthocyanin components that are pH-dependent. Under slight acidic to neutral pH, the blue color changes to light pink (Thanh et al. 2020). The butterfly pea plant has been reported as a plant with medicinal properties (Jeyaraj et al., 2021). The butterfly pea flower (BPF) has anti-cancerous, antioxidant, antidiabetic, anti-inflammatory, and antimicrobial properties (Borikar et al. 2018; Leong et al. 2017; Pasukamonset et al. 2016; Shen et al. 2016; Chayaratanasin et al. 2015). This is due to the presence of anthocyanins (like ternatin) and flavonoids (like kaempferol, quercetin, and myricetin) in BPF (Jeyaraj et al. 2021). The primary compound is ternatin and delphinidin glucoside, a kind of delphinidin (Escher et al. 2020; Pham et al. 2020; Sutakwa et al., 2021; Jeyaraj et al. 2021; Thanh et al. 2020). The primary anthocyanins found in BPF are polyacylated derivatives of delphinidin 3,30,50-triglucoside, known as “ternatin” (Vidana Gamage et al. 2021). Several studies investigated the effect of the addition of BPF in yogurt. Dewi et al. (2019) investigated the effect of BPF as a coloring agent in goat milk yogurt. Sutakwa and Nadia (2021) studied the effect of sucrose on the lactic acid bacteria population of BPF yogurt.

Research focusing on the effect of BPF on the nutritional and sensorial attributes of yogurt has not been addressed before. The objective of the present study was to develop a functional yogurt using BPF powder to enhance the nutritional and sensorial quality of yogurt.

Materials And Methods

2.1 Materials

Fresh cow milk was obtained in clean plastic containers from the Dairy Farm, Banaras Hindu University, Varanasi. A vivid deep blue variety of Butterfly pea flower (*Clitoria ternatea L.*) was procured from the local market. All the Reagents used in this research were of analytical grade and are purchased from Sigma Aldrich, USA, and HI Media Laboratories Pvt. Ltd. (Mumbai, India).

2.2 Microorganism and inoculum preparation

Lactobacillus bulgaricus and *Streptococcus thermophilus* yogurt cultures were procured from Chris Hansen, Denmark. Direct Vat Set Freeze-dried cultures were stored at -20°C and reconstituted in sterilized skim milk prior to use.

2.3 Preparation of Butterfly pea flower powder

Butterfly pea flowers procured from the Department of Horticulture, Banaras Hindu University were initially washed with clean water. Further, flowers were dried in a tray dryer (Khera Instruments Pvt. Ltd. Delhi) at 50–55 ºC till the final moisture content reached 5–6%. Dried flowers were ground and then the powder was sieved (30–40 mesh size) and packed into polyethylene bags and stored at refrigerated temperature for further use.

2.4 Preparation of BPF-rich yogurt

A flowchart for the yogurt preparation is shown in Fig. 1. Milk was standardized at 3.5% fat. Standardized milk was homogenized at 55 ºC at 20 MPa pressure, pasteurized at 85 ºC for 15 min, and then cooled to 60–62 ^{O}C. The dried BPF powder was added to the cooled milk and stirred well for 1 min. After that inoculum was added to the milk at 43 ^{O}C. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* containing inoculums (1:1, v/v) were added to the mixture and stirred for uniform distribution. Then the mixture was placed at 42 ^{O}C for incubation for 4 hrs and cooled. Five different trials comprising BPF powder extract at varying levels (1, 1.5, 2.0, 2.5, and 3%, w/v) and one trial as the control without BPF powder were prepared. All the samples were made in triplicates. All the prepared samples were packed in LDPE cups and were stored at refrigeration temperature (4 ± 1 ^{O}C) for evaluation of their storage stability. During the storage period of 21 days, products were evaluated at the interval of every seven days for physicochemical (acidity, pH, free fatty acids, and whey separation) and microbiological quality (*Lactobacilli, Streptococci*, coliform, and yeast and mold count) as per the standard methods.

2.6 Physicochemical and antioxidant activity analysis of BPF added functional yogurt

Fat was determined by Gerber method (for milk) and Rose Gottleib Method (for yogurt) (AOAC, 2012). Total nitrogen was measured by Kjeldahl method according to AOAC (2012) and protein % was calculated as N% × 6.38. Lactose content of the samples was estimated as per Lane and Eynon method as described in BIS (2001). Total solids contents of the yogurt were determined gravimetrically as per the method given by BIS (2001) using a well-ventilated hot air oven at 100 ºC for 3hrs.

Ash content was determined by adopting the procedure given by AOAC (2012) using a muffle furnace at 550–600 ^{O}C for 2 hrs until ash was carbon-free. Titratable acidity (TA) was determined as per the method of BIS (2001) and the pH was determined by Oakton (Eutech Instruments, Switzerland) laboratory pH meter with a glass electrode. Color intensity was determined by Hunter Color lab using the previous method (Zhang et al, 2019). DPPH inhibition method was used to measure the antioxidant activity of yogurt (Srivastava et al, 2015). The folin-Ciocalteau method was used to measure the total phenolic content of the sample (Tuan Putra et al, 2021). Free fatty acid content was evaluated by AOAC 2012 method.

2.7 Texture profile analysis (TPA)

Textural parameters such as chewiness, gumminess, fracturability, springiness, adhesiveness, cohesiveness, and resilience of yogurt sample were estimated by using a Texture analyzer (TA.XT plus texture profile Analyser, Stable Micro Systems, UK) equipped with a 5 kg load cell. A back extrusion test using a 40 mm cylinder probe was used for the TPA of the samples. The product was subjected to compressive force by probe up to the distance of 30 mm. The conditions set in the Texture analyzer for measuring textural properties were as follows: Pre-Test Speed, 1 mm/s; Post-Test Speed, 1 mm/s; Test speed, 1 mm/s; Trigger force, 10.0 g; Time, 5.0 s. For each evaluation, (3×3×2.5) cm^3 size sample was used during texture analysis. Numerical values of firmness, consistency, cohesiveness, and index of viscosity were measured using the exponent software (version 6.1.1.0). **2.8 Whey separation measurement (Syneresis)**

Whey separation of yogurt samples was measured by the method described by Hassan et al. (2015). 25 ml of set yogurt at 5 ºC was slowly transferred to 50 ml capacity centrifuge tubes causing minimum disturbance to the coagulum. The centrifuge tubes were balanced by adjusting their weights and centrifuged at 3394 RPM in a centrifuge (Remi, India) for 20 min. The quantity of whey separated at the top of the coagulum inside centrifuge tubes was recorded as milliliters. The weight fraction of the supernatant liquid was used as an index of whey syneresis (ml/100 g yogurt). The higher the volume of whey separated, the higher the whey separation.**2.9 Microbiological analysis**

Lactobacillus bulgaricus count, *Streptococcus thermophilus* count, coliform count, and yeast and mold count were analyzed in final products as per the method given by BIS (2001) using MRS agar media for the enumeration of *Lactobacillus bulgaricus*, M17 agar for the enumeration of *Streptococcus thermophilus*, MacConkey agar for the coliform count and potato dextrose agar for yeast and mold count.

2.10 Storage study

All the prepared samples were packed in LDPE cups and were stored at refrigeration temperature (4 ± 1 ºC) for evaluation of their storage stability. During the storage period of 21 days, products were evaluated at the interval of every seven days for physicochemical (acidity, pH, free fatty acids, and whey separation) and microbiological quality (*Lactobacilli, Streptococci*, coliform, and yeast and mold count) as per the standard methods.**2.11 Sensory Evaluation**

Sensory characteristics like flavor, color and appearance, mouthfeel, body and texture, and overall acceptability were investigated by a panel of 10 semi-trained judges at low temperature (10°C). All the six samples were evaluated based on a 9-point hedonic scale for the sensory evaluation with 1 to 9 grades (where, 1 = dislike extremely, 9 = like extremely).

2.12 UPLC (Ultra-Performing Liquid Chromatography) analysis of BPF-rich yogurt

UPLC was carried out using a UPLC system (WATERS ACQUITY UPLC System). PDA Detector was used at 210 nm. Samples were loaded onto a C18-reversed-phase column (BEH C18 2.1x100mm, 1.7µm (BEH C8 2.1x100mm, 1.7µm). The mobile phase consisted of 0.1% TFA in water (solvent A) and 80% Acetonitrile in (solvent B). The samples were eluted with a linear gradient from 4–50% B for 50 min, followed by 50%, 100% B for 4 min, and 100% B for 6 min. The flow rate was 0.350 mL/min. The column temperature was 25 ºC.

2.13 SEM analysis

The microstructure of the yogurt samples was studied using a method described by Wen et al., 2014. The yogurt samples were prepared for scanning electron microscopy (SEM) by taking 0.3 g of yogurt (1 cm below the surface) and mixing it with 3% aqueous agar solution at 45 ºC. After solidification, the gelled samples were cut into 1mm cubes and fixed in 1% glutaraldehyde solution in phosphate buffer at room temperature for 2 h, and then at 4 ºC for 24 h. The samples were washed again with phosphate buffer and dehydrated for 15 min in a graded ethanol series consisting of 50%, 70%, 90%, and 100% ethanol. The samples were then frozen in liquid nitrogen. The dried samples were mounted on aluminum SEM stubs by using a carbon-based tape and coated with gold in an ES-1010 sputter coater (Hitachi, Tokyo, Japan).

2.14 Statistical Analysis

Software Minitab 17 is used to carry out statistical analysis by one-way ANOVA. The level of significance was set at $P < 0.05$.

Results And Discussion

3.1 Proximate analysis of BPF powder

The moisture, protein, ash, % DPPH inhibition activity, TPC and TA of BPF powder were found to be 4.8%, 0.44%, 1.776%, 71.9%, 84.26 mg GAE/g and 0.75%, respectively (Table 1).

Table 1
Physicochemical Analysis of tray dried BPF powder

Attributes	BPF powder
Moisture	4.8%
Protein	0.44%
Ash	1.776%
DPPH	71.9%
Total phenolic content	84.26 mg GAE/g DW
Titrable acidity	0.75%
Calcium	3.09 mg/g
Magnesium	2.23 mg/g
Potassium	1.25 mg/g
Zinc	0.59 mg/g
Sodium	0.14 mg/g

3.2 Sensory evaluation of BPF added yogurt

The colour and appearance scores varied from 6.08 ± 0.12 to 8.81 ± 0.05 (Table 2). The minimum color and appearance score was obtained in T5, while the maximum colour and appearance score was observed in T1. From Table 2, it can be inferred that the maximum flavor score of BPF added functional yogurt was obtained in T1 (8.69 ± 0.045) while the minimum was found in T5 (6.77 ± 0.19). The sensory score for colour and appearance score of the control sample was 8.20 ± 0.05 which was comparable to T1. The maximum sensory score for body and texture of BPF added yogurt was found in T1 (8.8 ± 0.038) and minimum in T5 (7.97 ± 0.040). The sensory score for the mouthfeel of BPF added yogurt was maximum in T1 (8.81 ± 0.05) and the lowest value was obtained in T5 (6.93 ± 0.053). Similarly, the overall acceptability was maximum in trial T1 (8.62 ± 0.033) and minimum in T5 (6.24 ± 0.196). Thus, trial 1 comprising 1%, w/v BPF powder showed better organoleptic properties than control (T0) and other treatments and was chosen for further studies.

Table 2
Comparative values for sensory attributes of functional yogurt incorporated with BPF powder

Trial No	Color and appearance	Body and texture	Flavor	Mouthfeel	Overall acceptance
T1	8.81 ± 0.05	8.8 ± 0.04	8.69 ± 0.05	8.81 ± 0.05	8.62 ± 0.03
T2	8.28 ± 0.05	8.74 ± 0.04	8.26 ± 0.04	8.24 ± 0.04	8.11 ± 0.03
T3	7.78 ± 0.05	8.54 ± 0.07	7.77 ± 0.10	7.84 ± 0.03	7.25 ± 0.05
T4	7.08 ± 0.11	8.22 ± 0.046	7.19 ± 0.04	7.23 ± 0.03	6.93 ± 0.06
T5	6.08 ± 0.12	7.97 ± 0.040	6.77 ± 0.19	6.93 ± 0.05	6.24 ± 0.19

n = 3, SD ± 0.05

3.3 Physicochemical properties of optimized BPF-rich yogurt

The optimized BPF-rich yogurt (1%, w/v BPF powder) comprised; 3% protein, 3.4% fat, 73.4% moisture, 0.891% ash, pH 4.24, titratable acidity 0.76 (Table 3). TS and color parameters (L, a, b values) were higher than the control samples. On contrary, the TA of optimized BPF-rich yogurt was lesser than control yogurt, this may be due to the addition of BPF powder in the yogurt. Viscosity and syneresis of control and optimized BPF-rich yogurt samples were comparable. Higher viscosity in the optimized BPF-rich yogurt sample may be due to BPF polyphenol and casein protein interaction. Lotus (*Nelumbo nucifera*) leaf supplemented yogurt showed similar viscosity behavior in the previous report (Lee et al, 2019).

Table 3
Physiochemical and microbiological analysis of optimized functional yoghurt

Parameters	**Treatments (Mean ± SE)**	
	Optimized Functional Yoghurt (T1)	**Control Yoghurt (To)**
Fat (%)	3.42 ± 0.02	3.40 ± 0.1
Protein (%)	3.25 ± 0.04	3.16 ± 0.02
Ash (%)	0.74 ± 0.3	0.66 ± 0.01
Lactose (g)	3.01 ± 0.01	4.53 ± 0.09
Total Solid (%)	16.12 ± 0.02	14.01 ± 0.05
pH	4.60 ± 0.5	4.57 ± 0.01
Viscosity mPa.s	2.56 ± 0.04	2.16 ± 0.08
Acidity (% Lactic Acid)	0.79 ± 0.1	1.19 ± 0.01
Syneresis (percent)	37.00 ± 2.24	32.28 ± 0.07
Lightness (L*) values	93.76 ± 0.14	72.53 ± 0.13
Redness (a*) values	-2.22 ± 0.05	−1.93 ± 0.03
Yellowness (b*) values	11.26 ± 0.22	7.92 ± 0.02
% DPPH inhibition activity	61.50%	13.66%
TPC (mg GAE/g)	87.23	64.4
Lactobacillus bulgaricus count (log cfu/ g)	8.34 ± 0.13	8.85 ± 0.03
Streptococcus thermophilus count (log cfu/g)	8.68 ± 0.31	8.96 ± 0.05
n = 3, SD ± 0.05		

3.4 TPA of optimized BPF-rich yogurt

Texture attributes are used as parameters to monitor and control product acceptability. The TPA of antioxidant-rich BPFP yogurt was measured in terms of fracturability, cohesiveness, springiness, gumminess, chewiness, and resiliencies using a texture analyzer. The fracturability of control yogurt (8.026 g) was higher than the optimized BPF-rich yogurt (Table 3). The cohesiveness of BPF-rich yogurt increases compared to control. The cohesiveness of control yogurt was 0.370 and in optimized BPF-rich yogurt, it was 0.418. Cohesiveness is an essential textural parameter of yogurt. BPF-rich yogurt had a significant impact on the cohesiveness as compared to control. The high cohesiveness in the BPF-rich yogurt depicts that the BPF-rich yogurt has more strength and is firmer as compared to control (Mousavi et al., 2018). In yogurt, gumminess is a defect. The range of gumminess was 0.750 g for BPF-rich yogurt and − 1.797 g for control (Table 3). The results showed that the gumminess has a slight undesirable effect on the appearance and texture of BPF-rich yogurt. Domagała et al. (2006) also reported the increase in gumminess due to the addition of amaranthus seeds in yogurt. In the present investigation, springiness and resiliencies values were comparable in both control and optimized BPF-rich yogurt. The effect of BPF powder addition to yogurt on their textural properties of springiness is presented in Table 3. There were significant differences in springiness between BPF-rich and control yogurt samples ($p < 0.05$). Springiness indicates the texture integrity of the yogurt which is increased due to the addition of BPF powder. The study by Ayar and Gurlin (2014) is in agreement with our result. The higher chewiness in optimized BPF-rich yogurt may be due to the stickiness effect of BPF, which further enhanced the structure of optimized BPF-rich yogurt. The chewiness is directly related to gumminess as previously reported.

3.5 Antioxidant activity and TPC

The percentage DPPH inhibition activity of the optimized BPF-rich yogurt was (61.50%) higher than control yogurt (13.66) % (Table 3). This may be due to the addition of BPF powder in yogurt and is in correlation with previous findings of Sutakwa et al. (2021) who reported 69.36% DPPH inhibition activity in yogurt added with blue pea flower. The TPC optimized BPF-rich yogurt and control yogurts were 87.23 and 64.4 mg GAE/g, respectively. High TPC in the optimized BPF-rich yogurt was due to the presence of phenolic acid, flavonoid, and anthocyanin pigment (Rabeta and Nabil, 2013). *C. ternatea* floral extracts possess other flavonoids, such as; rutin, epicatechin, and other polyphenolics like; gallic acid, protocatechuic acid, and chlorogenic acid (Azima et al., 2017). Similarly, the addition of *C. ternatea* extracts increased the polyphenolic and antioxidant contents of sponge cakes (Pasukamonset et al., 2018).

3.6 Effect of storage period on sensory quality of optimized BPF-rich yogurt

Sensory scores of the optimized BPF yogurt and control were significantly ($p < 0.05$) affected during the storage (Table 4). BPF yogurt sensory scores during the storage study followed a linear trend and maximum sensory scores for flavor, mouthfeel, and texture were observed on the initial day, while minimum scores on the 21st day of storage. On contrary, minimum sensory scores for colour and appearance, and overall acceptability were observed in the optimized BPF-rich yogurt sample at 0 days, while maximum scores were on the 21st day of storage (Table 4). The storage period of BPF yogurt did not affect the flavour score significantly. Mouthfeel scores decreased slightly in the case of BPF yogurt and were found in the range of 8.69 ± 0.05 to 7.4 ± 0.01 during storage. BPF Yoghurt had texture values in the range of 8.81 ± 0.05 to 7.7 ± 0.05 from the initial day to the 21st day of storage. Overall acceptability scores were increased significantly in BPF yogurt from (8.62 ± 0.03) on the initial day of storage to (8.78 ± 0.01) on the 21st day of storage. However, yogurt was acceptable from sensory throughout the storage period. This might be due to the metabolites produced by inoculum and BPF. A similar trend was observed in the control sample till 14 days of storage, thereafter samples were spoiled and unacceptable (Table 4). From the results obtained during the storage study, it was concluded that there was no major perceivable defect observed in the optimized BPF-rich yogurt up to 21 days of storage period (Table 4). BPF-rich yogurt showed better consistency, taste, flavor, and acidity than control yogurt. This is in agreement with the previous finding of yogurt manufactured from flower-based flour (Vargas et al., 2008).

Table 4
Effect of storage period on sensory scores and physicochemical parameters of optimized functional yoghurt during storage

	Storage Days			
	0	**7**	**14**	**21**
Colour and appearance	8.81 ± 0.05	8.86 ± 0.01	8.95 ± 0.5	8.98.±0.04
Flavour	8.8 ± 0.04	8.6 ± 0.01	8.3 ± 0.05	7.9 ± 0.04
Mouth feel	8.69 ± 0.05	8.45 ± 0.03	8.0 ± 0.01	7.4 ± 0.01
Texture	8.81 ± 0.05	8.8 ± 0.04	8.8 ± 0.04	7.7 ± 0.05
Overall acceptability	8.62 ± 0.03	8.69 ± 0.4	8.75 ± 0.5	8.78 ± 0.01
Whey syneresis	9.92 ± 0.02	11.04 ± 0.03	13.08 ± 0.05	14.44 ± 0.02
FFA content	1.87 ± 0.05.	2.70 ± 0.08	4.20 ± 0.08	6.76 ± 0.03
Acidity	0.76 ± 0.008	0.81 ± 0.008	0.89 ± 0.008	1.13 ± 0.01
pH	4.8	4.2	3.9	3.5
n = 3, SD ± 0.05				

3.7 Effect of storage period on FFA content, acidity, whey separation of BPFP yogurt

The effect of the storage period on BPFP yogurt was evaluated in terms of FFA value, whey separation, pH, and titratable acidity (Table 4). The FFA value for BPF yogurt observed after 1 hr of storage at refrigeration temperature was 1.87 ± 0.05. There was a continuous increase in free fatty acid value after the 14th day of storage (4.20 ± 0.08) and after the 21st day of storage, the increase was significant (6.76 ± 0.03). A similar rise in volatile fatty acid content in herbal yogurt having Korean plant extract was observed with storage (Joung et al., 2016). This might be due to the production of more volatile fatty acids by *L. delbrueckii spp. bulgaricus* strains than *S. thermophilus* strains during yogurt fermentation (Güler and Gursoy-Balci, 2011). Volatile fatty acids in yogurt do not impart much nutritional value but significantly affect the organoleptic properties of yogurt and so consumer acceptance. The supplementation of BPF did not affect the initial pH and TA, which ranged from 4.24 to 4.1 (control) and from 0.76 ± 0.008 to 0.85 ± 0.003 (control) %, respectively (Table 2). During storage, the acidity of BPF yogurt increased significantly ($p < 0.05$) after the 7th day up to 14 days. However, the rise in acidity was lesser in the optimized BPF-rich yogurt sample (1.13 ± 0.01) up to 14 days of storage (1.45 ± 0.01) (Table 4). Further increase in acidity and decreased pH in BPF yogurt may be due to increased metabolism leading to the production of phenolics, flavonoids, and organic acids (Zhang et al., 2019). According to the FSSAI, the acidity of yogurt should be in the range of 0.8–1.25% (FSSAI, 2016), and the results obtained in this study fall within the standard range.

Syneresis or whey separation is the major visible defect that occurs during set yogurt storage. Whey separation for BPF yogurt observed after one hr. of storage at refrigeration temperature was 9.92 ± 0.02 and there was a significant increase after 14th day of storage (13.08 ± 0.03) up to 21 days of storage (14.44 ± 0.02). Whey separation by centrifugal method showed decreased syneresis trend substantially after storage. Syneresis in yogurt gel is dependent upon total solids and protein content.

3.8 Effect of storage on microbiological characteristics

The initial *Lactobacillus bulgaricus* count at refrigeration temperature was 8.85 ± 0.03 (log cfu/g), which significantly decreased after the 7th day of storage (8.35 ± 0.10) with the reduction of 0.50 log cfu/g. After 21 days of storage, the reduction was 3.45 log cfu/g when compared with the initial *lactobacilli* count. The initial *Streptococcus* thermophilus count of 8.96 ± 0.05 decreased significantly ($p < 0.05$) after the 7th day (8.71 ± 0.05) with a reduction of 0.25 log cfu/g. After 21 days of storage (7.48 ± 0.04), the reduction was 1.23 log cfu/g when compared with the initial *streptococci* count. The coliform and yeast and mold were absent in all the yogurt samples during the storage study (Table 5). This may be due to the proper processing of the product and antimicrobial property imparted by lactic acid bacteria (Mishra et al., 2012) and bioactive compounds of BPF (Thuy et al, 2021). This agrees with previous reports, which indicated that high acidity is the main reason for the lower survival of lactic acid bacteria in fermented products having low pH values (Ranadheera et al., 2012).

Table 5
Probiotic, *Escherichia coli*, *Staphylococcus aureus*, Coliform, Yeast & mould, counts in optimized functional yogurt during 21 days of storage

Microorganism	**Storage Days**			
	0	**7th**	**14th**	**21st**
Lactobacillus bulgaricus count (log cfu/ g)	8.85 ± 0.03	8.35 ± 0.10	7.65 ± 0.05	6.40 ± 0.07
Streptococcus thermophilus count (log cfu/g)	8.96 ± 0.05	8.71 ± 0.03	8.21 ± 0.05	7.48 ± 0.04
Escherichia coli	N	N	N	N
Staphylococcus aureus	N	N	N	N
Coliform	10	10	10	10
Yeast & Mold	20	20	20	20
n = 3, SD ± 0.05				

3.9 UPLC analysis of BPF-rich yogurt

UPLC analysis of the BPF-rich yogurt was done to analyze the presence of bioactive compounds in the developed yogurt. Figure 2 depicts the UPLC chromatogram of BPF fortified yogurt. The retention time (RT) of significant peaks in BPF fortified yogurt was observed at 0.77 to 26.16 minutes. Major anthocyanin ternatin was identified, including delphinidin derivatives and cyanidin derivatives, responsible for the blue color of BPF. Compound 1 at RT 0.77 minutes was identified as protocatechuic acid, a phenolic compound. It is a metabolite of cyanidin glucoside. This compound is a dihydroxybenzoic acid derivative that exhibits antioxidant properties and is reported to have hypoglycemic and cardioprotective properties (El-Sonbaty et al., 2019). At RT 1.14 minutes, Peak 2 was identified as p-hydroxybenzoic acid. Peak 3 at RT 1.42 minutes was identified as catechin, a primary antioxidant phenolic compound. Compound 4 was tentatively identified as cyanidin-3-O-rutinoside at RT 4.45 minutes. Cyanidin-3-O-rutinoside and ternatin are responsible for the blue color of BPF (Shen et al. 2016). Compound 5 was identified as apigenin at RT 5.43 minutes. Apigenin is a compound of the flavonoid group. This compound has been found to have anxiolytic, antioxidant, anticancer, anti-inflammatory, antiviral, and antibacterial properties. Compound 6 was recognized as kaempferol, a significant flavonol glycoside present in BPF at RT 5.93 minutes. Kaempferol has a high scavenging ability for free radicals. This compound exhibits analgesic, antihypertension, immune-booster, gastroprotective, cardioprotective, antidiabetic, anticancer, antiestrogenic, neuroprotective, and antiallergic properties (Makasana et al. 2017). The peak at RT 7.64 minutes was identified as diacetyl, a primary flavor component in yogurt. Compound 7 was tentatively identified as a ternatin derivative at RT 9.36. Ternatin is the primary polyacylated anthocyanin responsible for the blue color of BPF. Ternatins are mainly delphinidin-3,3′,5′-triglucoside derivatives (Vidana Gamage et al., 2021). About 15 series (A1-A3, B1-B4, C1-C5, and D1-D3) of ternatin have are there, out of which A1, A2, B1, B2, B3, C2, D1, D2, and D3 are present in BPF (Jeyaraj et al. 2021; Shen et al. 2016). Ternatin exhibits antidiabetic, antioxidant, anti-inflammatory, anti-cancerous, and antimicrobial properties (Fu et al., 2021; Jeyaraj et al., 2021). A small peak of quercetin was detected at RT 11.27 minutes. Another small peak at RT 12.07 minutes was identified as a delphinidin derivative. At RT 21.75 and 24.84 minutes, the major peaks were identified as tyramine and e-tyramine, respectively. These compounds have been reported to be found in fermented foods like yogurt. Tyramine promotes the elevation of blood pressure and has cardioprotective, anti-depressing, and anticancer properties. Tyramine and its derivative have been reported to be effective against migraine and gastric cancer (Gillman, 2018). Peak 13 with RT 26.168 represented n-butyl-p-aminobenzoate (Fig. 2).

3.10 SEM analysis

Scanning electron micrographs (SEM) of control and BPF yogurt are shown in Figs. 3a and b. The surface morphology of the control yogurt differed compared to BPF yogurt which is reported to be spherical, with a smooth surface, less collapsed structure with the presence of slightly dimpled surfaces. Figure 3a. showed a surface, with many small cracks, resembling broken glass or a flake-like structure. On contrary, BPF yogurt showed a smoother surface compared to control yogurt (Fig. 3b). This may be attributed to the fact that the total solid content of BPF yogurt was higher compared to control yogurt (Table 3). Fyfe et al. (2011) reported that low solid feed results in an increased hollow and collapsed surface structure, whereas high solid feed results in a smoother surface. The smother surface enhances the wettability and solubility characteristics, thus increasing the potential hydration properties of BPF yogurt.

Conclusions

Nowadays, consumer demands healthy and nutritious foods to fulfill their requirements and prefer functional foods with health-boosting and disease-preventing properties. The optimized BPF-rich yogurt with 1% (w/v) BPF showed better sensory, textural properties, and antioxidant activities compared to the control yogurt. The optimized BPF-rich yogurt comprised; protein 3%, fat 3.4%, moisture 73.4%, ash 0.891%, pH 4.24, titratable acidity 0.76 and DPPH inhibition activity 61.50% and TPC 87.23 mg GAE/g. TPA analysis of optimized BPF-rich yogurt showed fracturability, cohesiveness, springiness, gumminess, chewiness, and resilience values of 7.543, 54.86, 86.45, 0.750, 0.418, and 0.85, respectively which was better than control yogurt. The optimized BPF-rich yogurt containing antioxidant-rich BPF powder improved its functional properties along with its texture and sensory qualities. BPF powder may be used as a colorant in many foods like health drinks, beverages, confectionery, bakery, and functional foods.

Declarations

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Figures

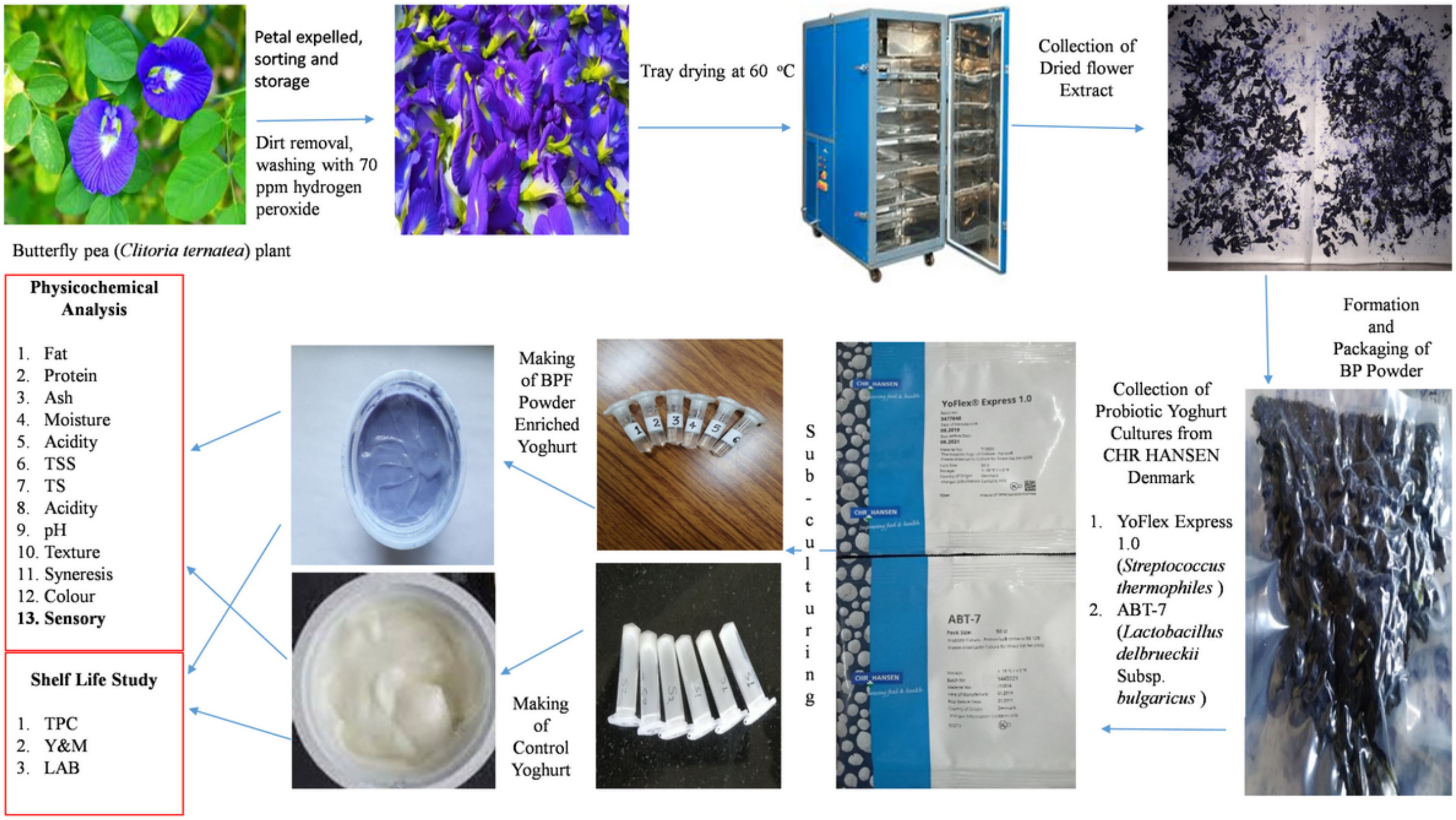


Figure 1

Schematic representation of development of BPF (butterfly pea flower) powder enriched yogurt

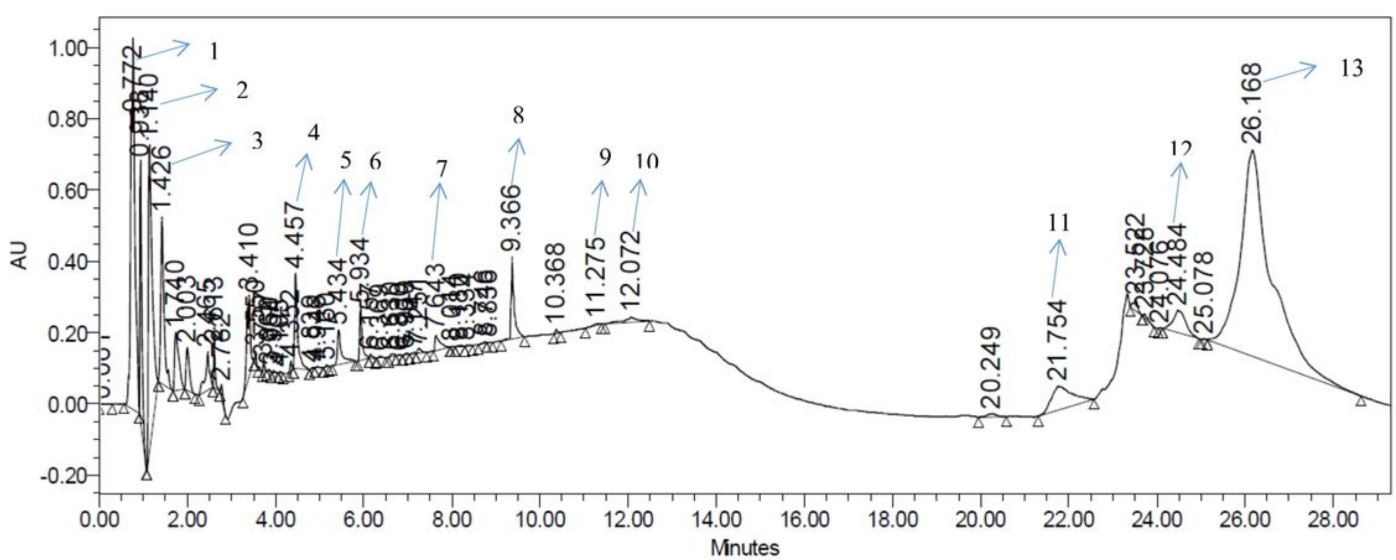
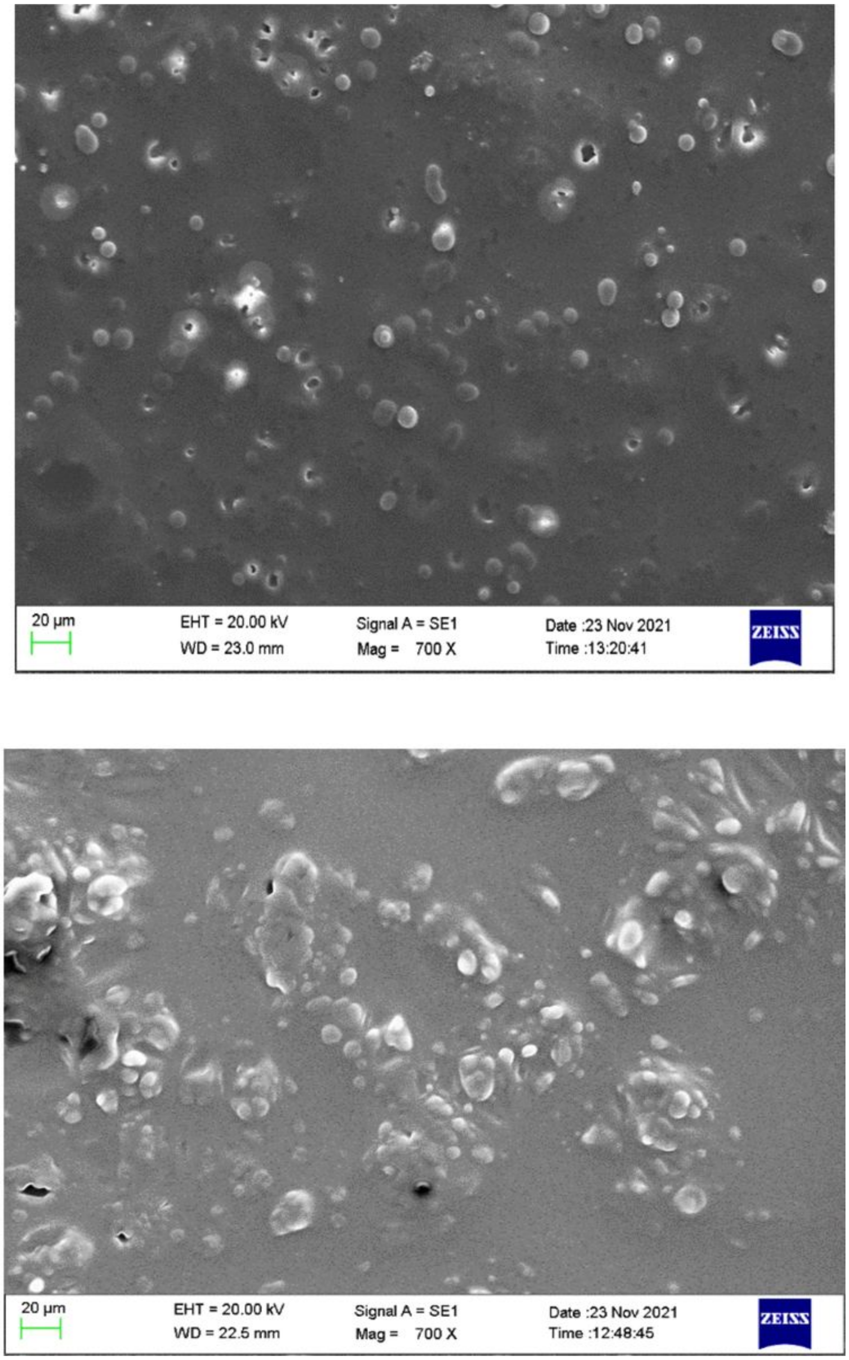


Figure 2

UPLC analysis of butterfly pea flower (BPF) yogurt; Peak 1 (RT 0.77 min, protocatechuic acid), **Peak 2** (RT 1.14 min, p-hydroxybenzoic acid), **Peak 3** (RT 1.42 min, Catechin), **Peak 4** (RT 4.45 min, cyanidin-3-O-rutinoside), **Peak 5** (RT 5.43 min, apigenin), **Peak 6** (RT 5.93 min, kaempferol), **Peak 7** (RT 7.64 min, diacetyl), **Peak 8** (RT 9.36 min, ternatin), **Peak 9** (RT 11.27 min, quercetin), **Peak 10** (RT 12.07 min, delphinidin), **Peak 11** (RT 21.75 min, tyramine), **Peak 12** (**RT** 24.84 min, e-tyramine), **Peak 13** (RT 26.168 min, n-butyl-p-aminobenzoate) **Peak 16** (RT 12.24 min, 2,3 pentandione).



A

B

Figure 3

SEM micrograph of (A) Control Yogurt (B) BPF (Butterfly Pea Flower) rich Yogurt (Mag: 700 nm, size: 20 µm)

Supplementary Files

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- SupplementaryTable28042022.docx