

# The Potential of Night Jasmine (*Nyctanthes arbor-tristis*) Flower Extract as a Functional Ingredient in Yogurt Production: The Effects on Fermentation, Rheology, Sensory, and Antioxidant Properties of Yogurt

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## Abstract

*Nyctanthes arbor-tristis*, commonly known as Harsinghar or Night Jasmine Flower (NJF), holds a prominent place in traditional medicine due to its diverse biological activities. With the growing trend of fortifying yogurt with natural herbs to enhance nutritional and health benefits, this research aimed to optimize herbal yogurt with NJF extract using response surface methodology (RSM). Twenty experiments were conducted with varying percentages of NJF extract, inoculum sizes, and temperatures. The NJF-fortified yogurt was evaluated for sensory, textural, and physicochemical analyses, along with an ESI-MS assessment of bioactive components. Results showed that properties varied with NJF extract percentage, with trial T18 (3.68% NJF extract, 1.5 ml inoculum size, and 41°C temperature) achieving the highest sensory score and acceptability, as well as superior textural and antioxidant properties compared to control yogurt. T18 was identified as the optimized product with protein-3.1%, fat-3.4%, moisture-72.8%, ash-0.87%, pH-4.65, and titratable acidity (TA)-0.72. The antioxidant activity of T18 and CY was 72.32% and 12.62%, respectively, and the total phenolic content was found to be 85.17 mg GAE/g, underscoring its potential as a health-enhancing yogurt variant.

**Keywords:** Functional yogurt, night jasmine flower extract, antioxidant, and functional attributes, shelf-life study

## Introduction

A wide variety of foods that not only provide necessary nutrients but also support longevity and good health have emerged due to the concept of functional foods. As consumers' lifestyles have evolved, they have become more aware of their eating habits and are increasingly drawn to natural, healthy foods. Along with nutrients, consumers are also highly concerned about synthetic additives. In response to consumer preferences and the demand for functional foods, bioactive compounds from natural sources are increasingly being used as additives to enhance the quality, nutritional value, and medicinal properties of food (Fazilah et al. 2018).

The chemical composition of natural plants has gained significant attention due to its potential impact on health. Consequently, the extraction and utilization of natural compounds are becoming more popular (Thanh et al. 2020). Out of the 354,000 species of flowering plants (Qian et al. 2022) documented worldwide, more than 50,000 are said to have medical and pharmaceutical applications (Parekh and Soni, 2020).

One such plant is "*Nyctanthes arbor-tristis*" (NAT), a member of the 'Oleaceae' family, commonly known as "Parijat", "Coral Jasmine", "Harsinghar", and "Queen of the night." It is widely known for its ornamental use and its pleasant fragrance. The name *Nyctanthes* is derived from two Greek words: *Nycta* (night) and *Anthos* (flower), meaning "night flower." Due to its flowers blooming at night and shedding their petals the following day, the tree is also referred to as "the sad tree" or the "tree of sorrow," appearing rather plain during the day (Sharma et al. 2021).

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DOI: 10.2478/ebtj-2025-0009

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NAT is a small tree or shrub with flaky grey bark that can grow up to 10 meters. It is believed to possess variety of therapeutic properties. In India, Indonesia, and Malaysia, NAT flowers are used to induce menstruation, and the bitter leaves are used as a cholagogue, purgative, diaphoretic, and water pill. Tribal communities in India's central region use different parts of the NAT plant to treat wounds, snake bites, colds, bloody diarrhea, and coughing fits. In traditional Indian medicine, NAT is also reported to have anti-allergic, anti-histaminic, and anti-microbial properties among other uses (Agrawal and Pal, 2013).

Dairy-based products have long served as effective carriers of phytonutrients in the modern nutritional food system. A variety of dairy products can be transformed into nutraceuticals by incorporating herbs or their extracts. This integration of herbal extracts into dairy products facilitates the creation of functional foods that are both nutritious and therapeutic (El-sayed and Youssef, 2019). Yogurt, in particular, is a popular functional food due to its superior flavor and excellent nutritional value. An emerging trend involves fortifying yogurt with natural herbal extracts to enhance its nutritional content and health benefits. Studies have shown that yogurt consumption offers several health benefits, including immune system support, the prevention of colon cancer and gastrointestinal inflammation, improved lactose digestion, and better blood sugar regulation (Moore et al. 2018; Amadarshanie et al. 2022). Lactose-intolerant individuals can consume yogurt and other fermented foods as the lactose is converted to lactic acid during fermentation.

There are three main types of yogurts available in the market: set, stirred, and drinkable. All species of *Lactobacillus*, including the *L. delbrueckii subspecies bulgaricus*, which is found in yogurt, have been shown to inhibit harmful bacteria growth, stimulate immune responses, and improve the absorption of food components. *Streptococcus* species, particularly *S. thermophilus*, which is found in yogurt also exhibit probiotic properties (Fernandez and Marette, 2017). Flavor compounds in yogurt include acetaldehyde, diacetyl, ethanol, and 2-butanone, with acetaldehyde and diacetyl being the most prominent (Das et al. 2019).

Compared to milk, yogurt offers greater nutritional benefits due to its higher content of calcium, protein, vitamin B2, vitamin B6, and vitamin B12 than milk does. As yogurt has gained popularity, producers and researchers have explored adding value through probiotics, prebiotics, and plant extracts rich in polyphenols, creating functional yogurt with enhanced health. However, polyphenols may affect the growth and appearance of *Streptococcus* and *Lactobacillus* starter cultures (Fazilah et al. 2018). Extracts from various plants, herbs, and fruits have been used increase yogurt's nutritional and functional value. Herbal nutraceuticals are particularly popular among consumer seeking alternative healthcare options to treat and prevent illness. Consequently, there has been a growing demand for herbal supplements in food products like yogurt (Dabija et al. 2018).

Recently, consumer interest has shifted towards the health benefits of plant-based diets. Combining NJF with yogurt, could offer synergistic health benefits, particularly due to the antioxidant activities of the flower. In this study, NJF powder extract was added to yogurt to develop a functional product with improved organoleptic properties, better physicochemical qualities, and an extended shelf-life.

## Materials and Methods

### 2.1 Materials

*S. thermophilus* and *L. bulgaricus* were employed as the starter cultures for yogurt production and were purchased from Chris Hansen, Denmark. A bright white variety of NJF powder was purchased from a local market in Varanasi (India). Amul cow milk of 3.5% fat and 8.5% solid-not-fat (SNF) was procured from Amul Dairy, Banaras Hindu University campus, Varanasi, India. All the chemicals were analytical-grade and procured from Sigma Chemical Company in St. Louis, Missouri, in the United States.

### 2.2 Control Yogurt (CY)

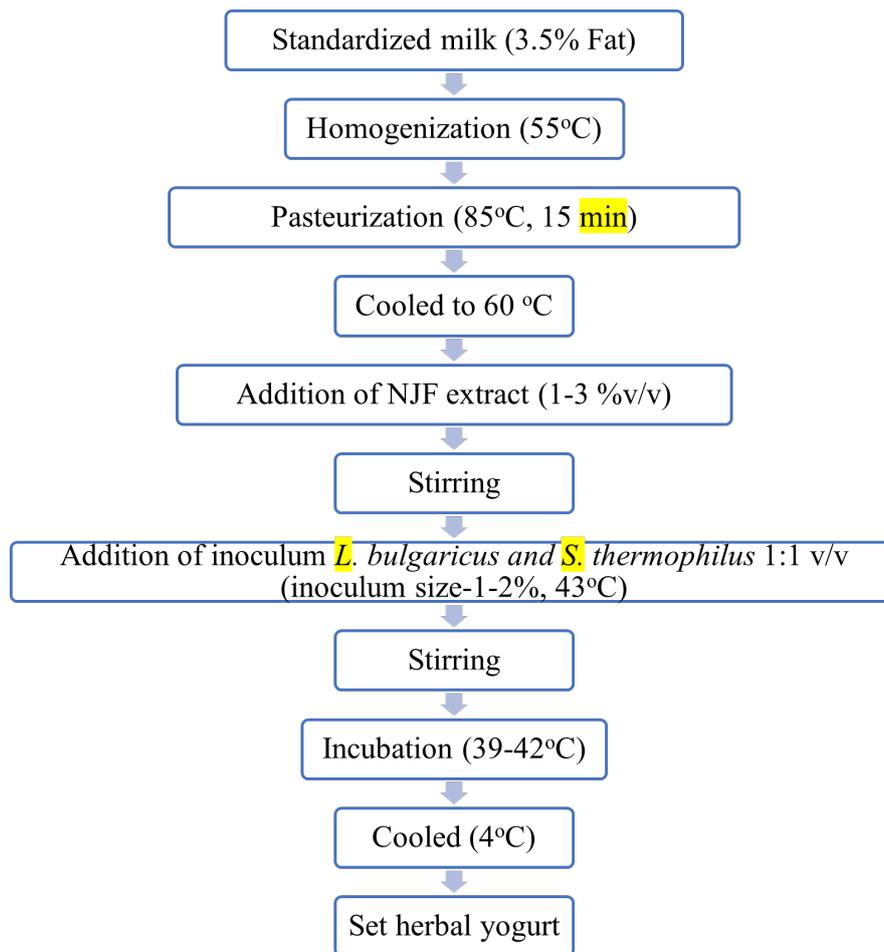
Yogurt must have at least 3.0 % fat, 8.0 % milk SNF, 2.9% milk protein, and 0.6% acidity expressed as lactic acid, according to the Food Safety and Standard Authority of India (FSSAI) guidelines. Milk was homogenized at 17 MPa at 55°C after being pasteurized at 85°C for 30 minutes. It was chilled to 42°C and given 1% inoculation culture. The finished mixture was placed into pre-sterilized low-density polyethylene (LDPE) cups and incubated for three to four hours at a temperature of 40 to 42°C. pH was determined after 4 hours, and the fermentation process was terminated by chilling and storing it at a temperature of 4-6 °C for additional investigation (Pankaj et al. 2022).

### 2.3 Preparation of NJF extract

The NJF powder extract was prepared by the method described by Zhang et al. (2019). 100 mL of boiling, distilled water was added to 2.0 g of NJF powder in a 250.0 ml beaker. After covering the beaker with aluminum foil, it was stirred and allowed to steep for 30 minutes. The blended mixture was filtered through muslin cloth after having been cooled to 60 °C and filtered using a Whatman No. 1 filter paper. A rotary evaporator was used to concentrate the filtered solvent at 50 °C, and the leftover material was freeze-dried and kept at -80 °C until used.

### 2.4 Preparation of yogurt with NJF extract

A flowchart for the yogurt preparation is shown in Fig. 1. Standardized milk was homogenized at 55 °C at 20 MPa pressure, pasteurized at 85 °C for 15 min, and then cooled to 60-62 °C. The dried NJF powder extract (1-3% w/v), was added to the cooled milk and stirred well for 1 min. Then inoculum (1-2%, v/v) containing *L. bulgaricus* and *S. thermophilus* (1:1, v/v) were added to the milk at 43°C. and stirred for uniform distribution. Then the inoculated milk was placed at temperature (39-42 °C) for incubation until the pH reached to 4.6 and stored at 4 °C.



**Figure 1.** Flowchart for preparation of herbal yogurt incorporated with NJF extract.

## 2.5 Design of experiment

RSM was used to determine the optimal parameters, with twenty distinct trials conducted as shown in Table 1. Each sample was stored in LDPE cups at a controlled temperature of  $4 \pm 1$  °C to evaluate storage stability. Over a 21-day period, physicochemical properties (including acidity, pH, free fatty acids, and whey separation) and microbiological qualities (counts of *Lactobacilli*, *Streptococci*, coliforms, and yeast and mold) were assessed at seven-day intervals using standard methods.

## 2.6 Sensory Evaluation

The samples were evaluated using a 9-point hedonic rating test by 50 semi-trained panelists whose ages ranged from 20 to 35. The samples were evaluated for flavor, color, and appearance; body and texture; mouthfeel; and overall acceptability. Paper cups with three numeral codes were used to serve yogurt. Between samplings, panelists rinsed their palates with water (Pankaj et al. 2022).

## 2.7 Physicochemical properties

The physicochemical properties of CY and T18 were determined using standard analytical methods. The gravimetric

method outlined in AOAC (2005) was used to determine the total solids (TS) content, with moisture estimated by difference method (100-TS%). The total mineral (ash) content was determined by dry ashing in the muffle furnace at 550–600 °C for 2 hours until ash was carbon-free (Maurice and Marshall, 2010). The Kjeldahl method was employed to determine the nitrogen content, and the protein % was calculated as  $N\% \times 6.38$  (Sáez-Plaza et al. 2013). The fat content in the samples was calculated using the AOAC (2012) method. The samples' TA was determined following the AOAC (2005) method and reported as a percentage of lactic acid. The pH values were measured using an electronic pH meter, following the method of Ranganna (2001). The pH meter was calibrated with standard buffer solutions of pH 7 and pH 4 prior to measurement.

## 2.8 Antioxidant activity

The determination of antioxidant activity was conducted using the DPPH inhibition method described by Srivastava et al. (2015) with slight modifications. One gram of homogeneously mixed samples (CY and T18) was taken in 10.0 ml of methanol and extracted overnight in the dark at room temperature. The samples were then centrifuged at 6000 rpm for 20 minutes.

**Table 1.** Experimental design and the real values for the variables using RSM

Trial no.	NJF extract (%)	Inoculum size (ml)	Temperature (°C)
T1	2	2.34	41
T2	2	1.5	42.68
T3	1	2	40
T4	3	2	40
T5	2	1.5	41
T6	3	2	42
T7	1	1	42
T8	1	1	40
T9	2	1.5	41
T10	2	1.5	41
T11	2	1.5	41
T12	2	1.5	41
T13	2	1.5	41
T14	2	0.65	41
T15	2	1.5	39.31
T16	3	1	40
T17	1	2	42
T18	3.68	1.5	41
T19	3	1	42
T20	0.31	1.5	41

Subsequently, 0.1 ml of the centrifuged supernatant was taken, to which 0.25 ml of DPPH solution (80 µg/ml methanol) was added, followed by 2 ml of methanol. A control blank was set up with 2.1 ml of methanol without the sample to measure the absorbance of DPPH in the absence of antioxidants. The mixtures were left at room temperature in the dark for 30 minutes. After the reaction time, the absorbance was measured at 515 nm using a spectrophotometer.

### 2.9 Total phenolic content (TPC)

The TPC was determined using the Folin-Ciocalteu method, as described by Makasana et al. (2017) with slight modifications. Initially, 2.0 g of the sample was homogenized in 15 ml of 80% v/v aqueous ethanol at room temperature and centrifuged at 6,000 rpm for 20 min at 4 °C. The supernatant was poured into petri dishes and evaporated to dryness at room temperature. The residue was dissolved in 5 mL of distilled water. For the analysis, 100µl of the sample's extract was diluted with 3 ml of distilled water, and 0.02 ml of Folin-Ciocalteu's reagent was added. After 3 minutes, 2 ml of 20% sodium carbonate was added, and the contents were thoroughly mixed, allowing a blue color to develop. The absorbance was measured at 760 nm using a UV spectrophotometer (Shimadzu, Japan), with gallic acid as a standard. The result was expressed as mg gallic acid/g dry material.

### 2.10 Texture profile analysis (TPA)

Textural parameters of CY and T18 samples, including fracturability, springiness, chewiness, gumminess, adhesiveness,

cohesiveness, and resilience were analyzed using Texture Analyzer (TA.XT plus texture profile Analyzer, Stable Micro Systems, and UK) equipped with a 5 kg load cell. A back extrusion test was performed using a 40 mm cylinder probe. The pretest speed to 1.5 mm/s, test speed to 1.0 mm/s, post-test speed to 10.0 mm/s, with a test distance of 3.5 mm, trigger force auto-set to 25 g, and tare force auto.

### 2.11 Storage study

CY and T18 samples were packed in LDPE cups and stored at a 4±1 °C for 21 days to evaluate their storage stability. Evaluations were conducted every seven days (on days 0, 7, 14, and 21) to assess pH, TA, and microbiological parameters. The pH of the yogurt samples was measured using a pH meter (pH 900, Precisa Co, Dietikon, Switzerland). TA was determined using neutralization titration with 0.1 N NaOH. One g of each yogurt samples was diluted in 10 mL of distilled water. The amount of NaOH required to reach the endpoint (pink color), was used to calculate the percentage of lactic acid, following the method described by Amadarshanie et al. (2022). The microbiological quality of the yogurt samples was assessed by counting *L. bulgaricus* on De Man–Rogosa–Sharpe (MRS) agar, *S. thermophilus* on M17 agar, coliforms on MacConkey agar, and yeast and mold on potato dextrose agar (PDA). The plates were incubated at 37 ± 1 °C for 48 hours. All microbiological counts were performed according to the method described by BIS (2001).

**Table 2.** Sensory attributes of herbal yogurt incorporated with NJF extract.

Trial No.	Color and appearance	Body and texture	Flavor	Mouthfeel	Overall acceptance
T1	8.00±0.2	7.96±0.15	7.50±0.1	8.00±0.1	7.87±0.14
T2	7.50±0.1	7.96±0.05	7.75±0.05	8.25±0.05	7.87±0.06
T3	8.00±0.1	7.96±0.05	8.10±0.1	8.50±0.1	8.14±0.09
T4	7.75±0.05	8.00±0.1	8.16±0.15	8.21±0.10	8.03±0.10
T5	8.46±0.15	8.96±0.05	8.40±0.1	9.00±0.1	8.71 ±0.10
T6	8.06±0.20	8.90±0.1	8.03±0.15	8.53±0.15	8.38±0.15
T7	7.10±0.1	8.10±0.1	8.00±0.1	8.03±0.15	7.81±0.11
T8	8.50±0.1	8.46±0.05	8.10±0.1	9.00±0.1	8.52±0.09
T9	8.10±0.26	8.90±0.1	8.00±0.1	9.03±0.05	8.51±0.13
T10	8.03±0.35	8.83±0.15	8.10±0.1	8.83±0.15	8.45±0.19
T11	8.13±0.15	9.00±0.1	8.16±0.15	8.93±0.05	8.86±0.11
T12	8.00±0.1	8.93±0.11	8.00±0.1	8.90±0.1	8.46±0.10
T13	8.00±0.1	8.96±0.05	8.03±0.05	8.83±0.15	8.46±0.09
T14	8.90±0.1	9.03±0.05	8.06±0.20	9.00±0.1	8.75±0.11
T15	8.96±0.05	8.23±0.20	8.00±0.1	8.90±0.1	8.52±0.11
T16	8.30±0.43	8.96±0.05	9.00±0.1	8.10±0.1	8.59±0.17
T17	8.20±0.37	8.23±0.20	8.00±0.1	9.00±0.1	8.34±0.19
T18	9.00±0.15	9.06±0.1	9.00±0.1	9.10±0.05	9.04±0.10
T19	8.03±0.15	9.00±0.1	9.00±0.1	8.10±0.1	8.53±0.11
T20	8.16±0.1	8.20±0.2	8.86±0.15	8.90±0.1	8.53±0.14

Given application of abbreviation value are mean ± standard deviation. Mean in the same column with control ( $P \leq 0.05$ ) as analysed by analyzed of variance and T1, T2,.....T20 is trial no. one, two ..... trial twenty correspondence.

### 2.12 Electrospray Ionization Mass Spectrometry (ESI-MS) Analysis

Mass spectrometry (ESI-MS) analysis was conducted using a Micromass QTOF-2™ mass spectrometer (Waters, UK). The sample was ionized in positive electrospray ionization mode, with a needle voltage of 3 kilovolts and a cone voltage of 30 volts. The desolvation temperature was set at 350 °C with a nitrogen gas flow rate of 550 L/h, and a collision energy of 20 V was applied. Full scan mass spectrometry covered the  $m/z$  range of 50 to 1000, with a scan time of 0.5 s.

Prior to analysis, an aqueous extract of CY and T18 were prepared according to a method adapted from Flores-Mancha et al. (2021). 50mL each sample was transferred into centrifuge tubes and centrifuged (Avanti® J-26 XPI, Beckman Coulter®, USA) at 10,000× g for 30 minutes at 4°C. The supernatants were filtered through Whatman™ filter paper (GE Healthcare, UK) with a pore size of 125 mm. The filtered extracts were then centrifuged again under the same conditions, followed by filtration through a syringe filter with a 0.2 µm pore size to remove any residual particulates before analysis (Flores-Mancha et al. , 2021).

### 2.13 Statistical Analysis:

All analytical experiments were conducted in triplicate, with the result expresses as mean values ± standard deviation. A one-way ANOVA was performed on the data using Minitab 17 after the initial data processing in MS Excel. Statistical significance was set at  $P < 0.05$ .

## Results and Discussion

### 3.1 Sensory Analysis

A sensory evaluation is crucial for determining the consumer acceptance of yogurt enriched with NJF extract. The analysis demonstrates that the final yogurt product's organoleptic qualities remain largely unaffected by the additives. Fifty semi-skilled sensory panelists evaluated yogurt samples prepared with NJF extract at concentration ranging from 1-3% (w/v) (Table 2). The sensory analysis revealed varied scores across different attributes of the yogurt. Color and appearance scores ranged from 7.10 to 9.00, indicating differences in visual appeal among the trials, with higher scores reflecting a more visually appealing yogurt. Similarly, body and texture scores ranged from 7.96 to 9.06, with higher scores suggesting a smoother and more desirable texture. Flavor scores ranged from 7.50 to 9.00, reflecting differences in taste perception, where higher scores indicated a more enjoyable flavor profile. Mouthfeel scores ranged from 8.00 to 9.10, suggesting varying levels of perceived creaminess or smoothness. Overall acceptance scores ranged from 7.81 to 9.04, with Trial 18 receiving the highest score of 9.04, indicating superior overall satisfaction. This suggests that Trial 18, which incorporated 3.68% NJF extract, an inoculum size of 1.5 ml, and a temperature of 41 °C, was perceived as the most favored among all trials due to its superior sensory attributes across all categories. These findings align with the result of Amadarshanie et al. (2022), who reported similar consumer acceptance for yogurt fortified with 3.5% NJF.

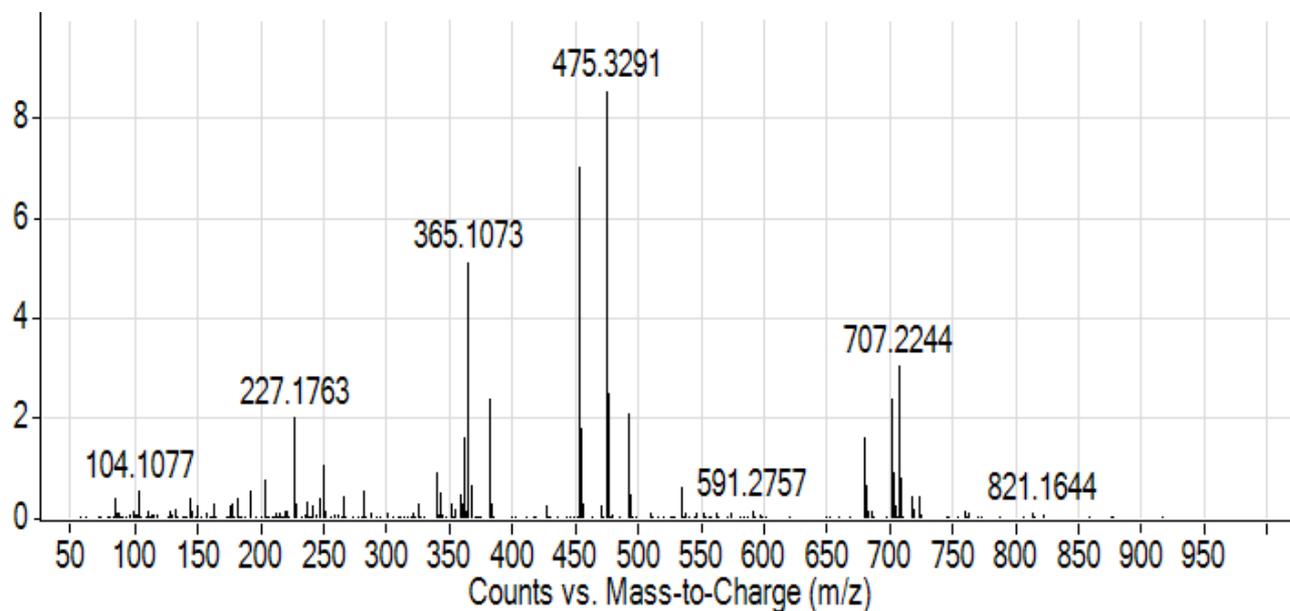
### 3.2 Physicochemical and texture profile analysis

The result of the physicochemical and texture profile analysis is summarized in Table 3. The moisture percentage of the optimized herbal yogurt is 72.8%, compared to 71.4% for the CY, possibly indicating the hydration properties of the NJF extract. The extract may have contributed to increased water retention, resulting in higher moisture content. The slight increase in protein percentage in the optimized herbal yogurt (3.1%) compared to the CY (2.9%) may be attributed to the protein content of the NJF extract. Conversely, the slight decrease in fat percentage in the optimized herbal yogurt (3.1%) compared to the CY (3.5%) could be due to the addition of NJF water extract, which may have displaced some fat content. The lower pH value in the optimized herbal yogurt (pH 4.65) compared to the control (pH 4.69) may be attributed to the acidity of the NJF extract, which might have contributed acidic compounds, resulting in a slightly lower pH value. The higher ash percentage in the optimized herbal yogurt (0.87%) compared to the CY (0.645%) may be due to the presence of minerals and other inorganic compounds present in the NJF extract. The higher antioxidant activity in the herbal yogurt (72.32%) compared to the control (12.62%) is likely due to the presence of bioactive compounds present in the NJF extract, such as 7-hexadecene, 1-octadecene, and hexanedioic acid, as reported by Heendeniya et al. (2020).

as reported by Rani et al. (2012), may have contributed to the higher TPC in the herbal yogurt. The slightly lower TA percentage in the optimized herbal yogurt (0.72%) compared to the control (0.78%) could be due to the acidic nature of the NJF extract, possibly stemming from compounds such as oleanolic acid, nyctanthic acid, and tannic acid, as reported by Rani et al. (2012).

Texture attributes are important parameters for monitoring and controlling product acceptability. The textural properties of control yogurt have hardness of 155g, while the optimized product has a hardness of 135 g. Hardness, which is the amount of force needed to achieve a certain distortion, is the most important factor in assessing yogurt firmness. The association between the phenolic compounds of NJF extract and the casein protein in milk may result in a weaker casein protein network in the optimized sample, which could explain the loss in hardness (Mudgil et al., 2017).

Both control and optimized samples have a springiness of 0.90. According to Mudgil et al. (2017), springiness is influenced by heat, the protein interface, flexibility, and the extent of protein unfolding. The enhanced texture integrity observed in the optimized sample may contribute to the improved springiness. Chewiness is lower for the control (42) than for the optimized sample (49). This increase in chewiness may be at-



**Figure 2.** Mass spectrum of the optimized NJF herbal yogurt showing major fragments at different mass-to-charge ratios (m/z). Key fragments include: m/z 475 (Crocic-3), m/z 707 ( $\beta$ -mono gentiobioside, glycosides), m/z 591 (Arborside C), m/z 227 (6- $\beta$ -hydroxyloganin), and m/z 365 (Carotenoids). The spectrum highlights the significant components detected in the analysis, indicating the presence of Crocic-3, various glycosides, and other key compounds.

This increased antioxidant activity aligns with the higher TPC in the optimized herbal yogurt (85.17mg GAE/g) compared to the control (6.23mg GAE/g). Phenolic compounds such as flavonoids, anthocyanins, and tannins present in the NJF extract,

contributed to the thickness and stickiness imparted by NJF, which improves the yogurt sample's structure (Mousavi et al., 2019). The cohesiveness of the control and optimized samples was 0.36 g and 0.48 g, respectively. Increased cohesiveness with NJF

**Table 3.** Comparative values for physicochemical analysis and textural properties of CY and optimized herbal yogurt (T18)

Attributes	CY	T18
Moisture (%)	71.4	72.8
Protein (%)	2.9	3.1
Fat (%)	3.5	3.1
pH (%)	4.69	4.65
Ash (%)	0.65	0.87
DPPH (% inhibition)	12.62	72.32
TPC (mg GAE/g)	6.23	85.1
TA (%)	0.78	0.72
Hardness (g)	155	135
Springiness (mm)	0.76	0.90
Chewiness (Mj)	42.00	49.00
Cohesiveness(g)	0.36	0.48

All values present as mean  $\pm$  SD of triplicate readings. CY was control sample, T<sub>18</sub> was the optimized NJF herbal yogurt.

addition may be the result of NJF's viscosity, which strengthens the yogurt's structural integrity (Mudgil et al., 2017).

### 3.3 Storage-study

The results of storage study are presented in Table 4. During the span of 21 days at 4°C  $\pm$  1, the pH value underwent significant changes. Throughout the storage period, the pH remained within the acceptable range. Yogurts showed a post-acidification impact, leading to lower pH values during storage. The identical pH trends in both samples suggests that the NJF extract did not affect the yogurt's pH over the storage period. The gradual decrease in pH observed could be attributed to the rapid fermentation process and the metabolism of lactose by lactic acid bacteria, which produce lactic acid from lactose. pH changes during storage are influenced by the type of bacterial culture used and the storage temperature (Bakirci and Kavaz, 2008). A low pH results from an increase in fermenting microorganisms at temperatures above 4°C. However, when yogurts are cooled immediately after incubation, they maintain a higher pH due to reduced bacterial activity (Amadarshanie et al., 2022).

While the addition of NJF extract did not significantly affect the yogurt's acidity compared to the control, the acidity of both samples increased considerably after 15 days of storage at 4 C  $\pm$  1. The acidities of the T18 and the control yogurts, ranged from 0.92 to 1.09 and 0.87 to 0.99, respectively. The primary cause of the increased acidity in yogurt is the conversion of lactose to lactic acid by bacterial cultures (Bakirci and Kavaz, 2008).

A microbiological analysis was conducted to evaluate the T18 and control samples (Table 4). The results, which depend on processing conditions, postprocessing contamination, and sanitation, are crucial for assessing the quality and safety of yogurt for human consumption. The initial *L. bulgaricus* count of CY was 7.63 $\pm$ 0.4 log cfu/g, which significantly decreased to 7.17 $\pm$ 0.10 log cfu/g by day 7, with a reduction of 0.03 log cfu/g. After 21 days, the count dropped further to 6.18 $\pm$ 0.3 log cfu/g, representing a reduction of 0.09 log cfu/g compared to the initial count. In the T18 sample, the initial *L. bulgaricus* count was 8.14 $\pm$ 0.5 log cfu/g, which significantly decreased to 7.73 $\pm$ 0.6 log cfu/g after 7 days, with a reduction of 0.02 log cfu/g. After

21 days, the count was 6.25 $\pm$ 0.3 log cfu/g, with a reduction of 0.11 log cfu/g. The initial *S. thermophilus* count in the CY was 4.57 $\pm$ 0.5 log cfu/g, decreasing to 4.39 $\pm$ 0.5 log cfu/g by day 7, with a reduction of 0.02 log cfu/g. After 21 days the count further of decreased to 3.94 $\pm$ 0.4 log cfu/g, with a reduction of 0.02 log cfu/g. In the T18 sample the initial *S. thermophilus* count was 4.48 $\pm$ 0.4 log cfu/g which decreased significantly ( $p < 0.05$ ) to 4.41 $\pm$ 0.3 log cfu/g by day 7 with a reduction of 0.01 log cfu/g. After 21 days, the count was 4.01 $\pm$ 0.3 log cfu/g, with a reduction of 0.05 log cfu/g. These results align with previous reports, which indicated that high acidity is the main reason for the reduced survival of lactic acid bacteria in fermented products with low pH values (Ranadheera et al. 2012).

According to the guidelines set by the Federal Agency for Food and Drug Administration Control, any 100-mL yogurt sample should have negative coliform counts (Mbaeyi-Nwaoha & Egbuche, 2012). The findings showed that, up to 21 days of storage, the coliform, yeast, and mold counts in both samples remained within acceptable ranges. This is likely due to proper processing practices and the antimicrobial properties imparted by lactic acid bacteria.

### 3.4 ESI-MS Analysis

ESI-MS spectrometry was used to identify bioactive components in the optimized herbal yogurt. Five bioactive compounds were tentatively identified in the hydro-alcoholic extract (50:50 v/v) of the optimized herbal yogurt: Crocin-3 (m/z 475), glycosides including  $\beta$ -mono gentiobioside (m/z 707), Arborside C (m/z 591), 6- $\beta$ -hydroxyloganin (m/z 227), Carotenoids (m/z 365). The corresponding mass spectra are shown in Fig.2. These compounds are known to have significant health benefits.

Crocetin and carotenoids are well-documented in *Nyctanthes* extracts, contributing to its known antioxidant and anti-inflammatory properties (Vankar, 2008). Arborside C and 6- $\beta$ -hydroxyloganin were isolated from *Nyctanthes* as part of an activity-guided fractionation study and have been reported to exhibit antiplasmodial activity against *Plasmodium falciparum* (Tuntiwachwuttikul et al. 2003). Another study highlights that *Nyctanthes arbor-tristis* flowers are rich in various glycosides of crocetin, including  $\beta$ -monogentiobioside ester,  $\beta$ -monogentio-

**Table 4.** Storage study of CY and optimized herbal yoghurt (T18)

1. pH		
Storage Days	CY	T18
0	4.57± 0.5	4.48± 0.4
7	4.39±0.5	4.41± 0.3
14	4.01± 0.2	4.09± 0.5
21	3.94± 0.4	4.01± 0.3
2. Titratable Acidity (% Lactic Acid)		
Storage Days	CY	T18
0	0.87± 0.02	0.92± 0.2
7	0.89± 0.06	0.96± 0.06
14	0.92± 0.04	0.99± 0.05
21	0.99± 0.02	1.09± 0.04
3. Microbial Count		
A. <i>L. bulgaricus</i> count (log cfu/ g)		
Storage Days	CY	T18
0	7.63± 0.4	8.14± 0.5
7	7.17± 0.2	7.73± 0.6
14	6.68± 0.4	6.57± 0.2
21	6.18± 0.3	6.25± 0.3
B. <i>S. thermophilus</i> count (log cfu/g)		
Storage Days	CY	T18
0	4.57± 0.5	4.48± 0.4
7	4.39±0.5	4.41± 0.3
14	4.01± 0.2	4.09± 0.5
21	3.94± 0.4	4.01± 0.3
C. Coliform (log cfu/g)		
Storage Days	CY	T18
0	<10	<10
7	<10	<10
14	<10	<10
21	<10	<10
D. Yeast and Mold (log cfu/g)		
Storage Days	CY	T18
0	<20	<20
7	<20	<20
14	<20	<20
21	<20	<20

All values present as mean ± SD of triplicate readings. CY was control sample, T18 was the optimized NJF herbal yogurt.

bioside-β-D monoglucoside ester, and β-digentiobioside ester of α-croctin. These glycosides, along with other compounds like carotenoids, contribute to the flower's high antioxidant properties (Pandey, 2012). This finding further supports their presence and potential bioactive role in the herbal extract used in T18.

Gupta et al. (2005) reported the antiviral activity of Arborside C against two viruses: Encephalomyocarditis Virus (EMCV) and Semliki Forest Virus (SFV), both of which cause encephalitis. Another study highlighted the antileishmanial activity of Arborside C and 6-β-hydroxyloganin (Tandon et al. 1991). Similarly, the antihistaminic activity of Arborside C was documented by Nirmal et al. (2009). Furthermore, Pandeti and coworkers demonstrated the anticancer activity of 6-β-hydroxyloganin and its derivative, showing moderate activity against MCF-7 and Hep G2 cell lines through caspase-depend

ent apoptosis (Pandeti et al. 2014).

## Conclusion

These findings indicate that the sensorially accepted sample containing 3.68% NJF extract exhibits greater free radical scavenging ability and total phenolic content compared to CY. Additionally, incorporating the extract (3.68% NJF extract) had minimal impact on the physicochemical properties, proximate composition, and texture profile of the yogurt in comparison to CY. As a result, NJF extract-fortified yogurt shows promise as a viable option for creating innovative dairy products with improved functional qualities and appearance. Moreover, considering that no bitterness was observed in the taste of yogurt up to 3.68% NJF extract content, there is an opportunity to explore the feasibility of increasing the NJF

extract percentage beyond this threshold. However, further *in vivo* studies are necessary to validate the potential health benefits of NJF-fortified yogurt.

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