Indian J Dairy Sci 78(4): 320-327
https://doi.org/10.33785/IJDS.2025.v78i04.004

RESEARCH ARTICLE

Response surface optimization of *Prunus napaulensis* yoghurt for improved nutrition and sensory attributes

Phareichon Kashung[1], Karuthapandian Devi[2] (✉), Chand Ram Grover[3]

Received: 01 April 2025 / Accepted: 15 July 2025 / Published online: 23 August 2025
© Indian Dairy Association (India) 2025

Abstract: This study aimed to optimize the process parameters for developing yoghurt enriched with *Prunus napaulensis*, a nutrient-rich indigenous fruit, using Response Surface Methodology (RSM) to enhance its nutritional and sensory attributes. The yoghurt was formulated with varying levels of fruit pulp (6-8%), inoculum (1-2%), incubation time (4-8 hours), and incubation temperature (37- 42°C). The optimization targeted pH and titratable acidity as key responses. A Central Composite Rotatable Design (CCRD) was employed, and the statistical analysis revealed significant models with R^2 values of 0.9493 and 0.9663 for pH and titratable acidity respectively. The formulation, with 7% fruit pulp, 1.5% inoculum and incubation at 39°C for 6 hours yielded a product with desirable pH and acidity. Sensory evaluation indicated that the fruit yoghurt exhibited improved colour and texture due to natural pigments and fibre content, though overall acceptability remained comparable to traditional yoghurt. Physicochemical analysis confirmed enhanced nutritional value, notably increased protein, carbohydrates, vitamins, minerals and anthocyanin content, making it a superior alternative to standard yoghurt. However, the fruit yoghurt demonstrated lower water holding capacity and higher syneresis, impacting texture. Microbial analysis confirmed product stability and safety throughout a 21- day storage period. In conclusion, *Prunus napaulensis* offers significant potential for developing value-added dairy products, providing year-round availability of the fruit. The study supports its commercial viability and underscores its role in diversifying yoghurt products while aligning with consumer preferences for health-conscious foods. Further investigations should explore the long-term health benefits and market acceptance of the developed fruit yoghurt.

Keywords: Descriptive sensory analysis, Fruit yoghurt, Nutrition profile, *Prunus napaulensis,* Response surface methodology, Rheological properties, shelf life study

[1]Food Science and Nutrition Department, Avinashilingam Institute for Home Science and Higher Education for women, Comibatore, Tamil Nadu- 641043
Email: phareichonk@gmail.com
(✉)[2]Food Science and Nutrition Department, Avinashilingam Institute for Home Science and Higher Education for women, Comibatore, Tamil Nadu- 641043
Email address: devi_fsn@avinuty.ac.in
[3]I/c Synbiotic Functional Foods & Bioremediation Research Laboratory, Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana
Email address: chand.Ram@icar.gov.in

Introduction

Prunus napaulensis (Ser.) Steud. or Meghalaya cherry belonging to *Rosaceae* family is a remarkably untapped fruit grown in the north-eastern region of India. The local name varies according to the region. In Meghalaya, in Khasi dialect, the fruit is referred to as *'Sohiong,' 'Theikanthei'* in *Tangkhul* dialect in Manipur. The ripe fruits are consumed fresh and exhibit exceptional quality with distinct flavour, taste and colour. The fruit is rich in nutrients and has a potential for extraction of natural edible food colour that can be used in food industry. It is nutritionally rich in anthocyanin (313- 358 mg), potassium (485- 530mg), zinc (2.10- 2.42mg), copper (1- 1.5mg), manganese (5.62- 7.7 mg), iron (2.32- 9.60mg) and ascorbic acid (50.04- 58.38mg). It also contains nitrogen (69.95- 70mg), phosphorus (87.53- 115mg), sulphur (787.50- 115mg) and fibre (1.7- 2.5mg) (Rymbai et al. 2016).

Commercially, yoghurt is available in a variety of flavour such as mix berries, mango, strawberry, blueberries etc.; as well as in its structural characteristics like firm/set yoghurt, stirred yoghurt, drinking yoghurt, frozen yoghurt and dried or powdered yoghurt. Fruits are generally incorporated into yoghurt to enhance the nutritive as well as the organoleptic properties of the yoghurt. It also has the potential to influence the fermentation behaviour and the resulting by-products such as lactic acid and other organic acids namely, acetic acid, citric acid and malic acid depending to the type of sugar content of the fruit. Therefore, the changes in various aspects like physicochemical, nutritive value and sensory attributes are expected in the yoghurt (Ritu et al. 2016).

In this present study, *Prunus napaulensis* fruit pulp was added in the development and optimization of yoghurt by Response

Surface Methodology (RSM). Due to its short post-harvest life and easy deterioration even under refrigerated conditions, development of value added food is an effective way to preserve the fruit (Aparna et al. 2018). Therefore, efforts were undertaken to develop *Prunus napaulensis* fruit yoghurt aiming to provide year round availability of the fruit.

Materials and methods

Prunus napaulensis fruit was procured from the local market in Ukhrul, Manipur. Fresh milk was procured from the local marker in Coimbatore. Yoghurt starter cultures *Lactobacillus bulgaricus* (NCDC004) and *Streptococcus thermophilus* (NCDC075) were procured from ICAR-National Dairy Research Institute, Karnal-132001, India which was maintained in 10% skim reconstituted milk power (RSMP).

Preliminary preparation of Materials

The fruit was cleaned and stored by removing stalks and leaves, blanched in hot water for 3 minutes, cooled, and dried to remove residual moisture. The blanched fruit was then sliced separating the fruit from the stone. The fruit was then pulverized and pasteurized at 95°C for 15 min. The pasteurized pulp was filled in sterile air tight jars and stored in refrigerator at 4°C until further use.

Development of Fruit Yoghurt with *Prunus napaulensis*

The stirred fruit yoghurt was prepared according to Pagthinathan et al. 2016 with some modifications dictated by the experimental design RSM., Fresh milk procured was standardized to fat 3.5% and solids not fat (SNF) at 9.5% by Pearson method. The standardized milk was heated to 70°C and homogenized by 2 phases: 2000psi and 500psi. The mix was then heated at 90°C for 30 mins and cooled to 45°C. The following ingredients combinations (independent factors) determined by RSM was prepared with 8% sugar constant. The yoghurt was then allowed to cool and varying concentrations of fruit pulp was added, stirred and stored in refrigerator for further analysis.

Experimental Design

The statistical software package design expert 13.0.5.0 Stat-Ease Inc. Minneapolis, MN was used for the construction of the experimental design and analysis of the data. Response surface methodology was employed for the development and optimization of the fruit yoghurt. Central Composite Rotatable Design (CCRD) was used to evaluate the effect of the four variables: Fruit pulp (6-8% w/v), Inoculum level (1-2% v/v), Incubation time (4-8h) and Incubation temperature (37-42°C) on the two responses titratable acidity and pH. The CCRD consisted of 16 factorial points, 8 axial points and 6 central points which dictated 30 sets of experimental runs along with the real and coded values of the independent variables ranges of the experimental data are shown in table 1 which were subjected to multiple regression analysis.

Determination of pH and titratable acidity of fruit yoghurt

The pH values of the fruit yoghurt prepared was measured using a digital pH meter which was calibrated with fresh standard buffers pH 4.0 and pH 7.0.

The titratable acidity of yoghurt sample was determined as per the protocol described by IS:1479, Part I, ISI (2016). Titratable acidity was expressed as % lactic acid.

Descriptive Sensory Analysis

A quantitative descriptive sensory analysis standardized by ICAR-NDRI, Dairy Microbiology Division was used for assessing the organoleptic properties of the prepared fruit yoghurt. Descriptive attributes of appearance, flavour, texture and overall acceptability on a 10 point scale (0-not acceptable to 10- highly acceptable) was evaluated by 50-semi trained panel members.

Physico Chemical Analysis

Titratable acidity, pH, TSS and SNF were determined according to the AOAC methods (2023). The fruit yoghurt along with control was analysed for moisture, protein, fat, total solids, ash, minerals and vitamins namely calcium, iron, potassium, magnesium, zinc, and vitamin C were analysed according to the FSSAI methods (2016). The carbohydrate content was calculated by the difference method. Water Holding Capacity (WHC) was performed according to the method of Elsayed et al. (2020). The syneresis of the fruit yoghurt was determined according to the method given by Ragab et al. (2023).

Rheological properties

The rheological parameters of the fruit yoghurt were determined according to method described by Ragab et al. (2023) with slight modifications using Brookfields R/S+ Rheometer with CC14 probe. Measurements were taken at 0.5 to 100rpm.

Microbial analysis

Microbial analysis was performed according to Salih et al. (2020) with some modifications. Media MRS and M17 agar was used for enumerating *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, respectively. The petri plates were incubated at 37°C for 24hr. Yeast and mold were enumerated using Rose-Bengal Chloramphenicol (RBC) Agar incubated at 25°C for 48 to 72 h. Violet Red Bile Agar was used for enumerating coliform. The plates were incubated at 37°C for 48h. This method was repeated on days 0, 1, 3, 7, 14 and 21.

Statistical analysis

All experiments were performed in triplicates and reported as mean±SD. Design expert version 13.0.5.0 Stat-Ease, USA was applied for the optimization. Descriptive sensory analysis and physicochemical analysis were analysed using Independent sample t-test of the SPSS statistical package (SPSS, Inc., Chicago, USA) version 25.

Results and Discussion

Optimization of fruit yoghurt with *Prunus napaulensis*

The ANOVA data and the model statistics of the response variables significant at 95% confidence level and its correlation coefficients are shown in Table 1 (S1). A linear model was suggested for the pH response, while a quadratic model was suggested for the titratable acidity response. Significant p value (<0.005) observed for all models indicated the significance of the models and non-significance in the lack of fit. The R^2 of both the responses were recorded at 0.9493 and 0.9663 for pH and titratable acidity, respectively indicating the significance of the fitness of the polynomial models used for analysing the effect of the independent variables on the responses. The multiple coded equations in terms of coded factors generated for the responses are given below;

$$\text{pH } Y = +4.63 - 0.0658 * A + 0.1125 * B - 0.1167 * C + 0.2250 * D$$

$$\text{Titratable acidity } Y = +0.7600 + 0.0496 * A + 0.0379 * B + 0.0454 * C - 0.0546 * D + 0.0381 * AB + 0.0269 * AC - 0.0081 * AD - 0.0081 * BC + 0.0069 * BD - 0.0644 * CD + 0.0293 * A^2 - 0.0407 * B^2 - 0.0532 * C^2 - 0.0282 * D^2$$

Effects of the variables on pH and acidity of the fruit yoghurt

pH and titratable acidity play a crucial role in the sensory attributes when developing a fermented food product. Keeping this in mind, the quantity of *Prunus napaulensis* fruit pulp, inoculum and milk were incorporated in appropriate levels. The regression coefficients estimates of different variables on the responses of the developed fruit yoghurt are presented in Table 1 (S1).

The pH of the developed fruit yoghurt ranged from 3.98 to 5.13. The lowest pH was observed in the product formulation containing 6% fruit pulp, 1% inoculum incubated at 37°C for 8h whereas the product formulated by fruit pulp of 6%, inoculum at 2% incubated at 42°C for 4h reported the highest pH 5.13. 3D plots depicting the effect of fruit pulp (%), inoculum level (%), incubation time (h) and incubation temperature (°C) on the pH and titratable acidity (%LA) are presented in figure 1(S2).The change in the pH could have resulted from the addition of the fruit which have a pH range of 3.53±0.06 (Kambhampati et al. 2018). As well as the incubation time and temperature; primarily, the bacterial cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) used in the yoghurt preparation produces lactic acid as a by-product by breaking down lactose during fermentation. The amount of lactic acid present affects the pH values; higher production of lactic acid results in the pH

Table 1 Experimental ranges of independent in terms of actual and coded factors of fruit pulp, inoculum level, incubation time and incubation temperature and dependent variables in the preparation of fruit yoghurt with *Prunus napaulensis*

		Actual values				Coded values				Response 1	Response 2
Std	Run	Factor 1 Fruit pulp (%)	Factor 2 Inoculum level (%)	Factor 3 Incubation time (h)	Factor 4 Incubation temperature (ºC)	Factor 1 Fruit pulp (%)	Factor 2 Inoculum level (%)	Factor 3 Incubation time (h)	Factor 4 Incubation temperature (°C)	pH	Titratable acidity (% LA)
19	1	7	0.5	6	39.5	0.000	-2.000	0.000	0.000	4.36	0.53
9	2	6	1	4	42	-1.000	-1.000	-1.000	1.000	4.9	0.59
7	3	6	2	8	37	-1.000	1.000	1.000	-1.000	4.48	0.76
21	4	7	1.5	2	39.5	0.000	0.000	-2.000	0.000	4.9	0.46
20	5	7	2.5	6	39.5	0.000	2.000	0.000	0.000	4.8	0.69
22	6	7	1.5	10	39.5	0.000	0.000	2.000	0.000	4.4	0.66
13	7	6	1	8	42	-1.000	-1.000	1.000	1.000	4.79	0.55
17	8	5	1.5	6	39.5	-2.000	0.000	0.000	0.000	4.77	0.77
26	9	7	1.5	6	39.5	0.000	0.000	0.000	0.000	4.57	0.72
1	10	6	1	4	37	-1.000	-1.000	-1.000	-1.000	4.61	0.61
11	11	6	2	4	42	-1.000	1.000	-1.000	1.000	5.13	0.62
5	12	6	1	8	37	-1.000	-1.000	1.000	-1.000	3.98	0.72
16	13	8	2	8	42	1.000	1.000	1.000	1.000	4.89	0.7
2	14	8	1	4	37	1.000	-1.000	-1.000	-1.000	4.38	0.57
27	15	7	1.5	6	39.5	0.000	0.000	0.000	0.000	4.7	0.79
29	16	7	1.5	6	39.5	0.000	0.000	0.000	0.000	4.7	0.8
4	17	8	2	4	37	1.000	1.000	-1.000	-1.000	4.49	0.69
3	18	6	2	4	37	-1.000	1.000	-1.000	-1.000	4.72	0.58
24	19	7	1.5	6	44.5	0.000	0.000	0.000	2.000	4.98	0.57
28	20	7	1.5	6	39.5	0.000	0.000	0.000	0.000	4.69	0.74
15	21	6	2	8	42	-1.000	1.000	1.000	1.000	4.91	0.5
23	22	7	1.5	6	34.5	0.000	0.000	0.000	-2.000	4.17	0.75
6	23	8	1	8	37	1.000	-1.000	1.000	-1.000	4.17	0.86
14	24	8	1	8	42	1.000	-1.000	1.000	1.000	4.6	0.57
12	25	8	2	4	42	1.000	1.000	-1.000	1.000	5.01	0.76
18	26	9	1.5	6	39.5	2.000	0.000	0.000	0.000	4.42	1.01
10	27	8	1	4	42	1.000	-1.000	-1.000	1.000	4.74	0.52
8	28	8	2	8	37	1.000	1.000	1.000	-1.000	4.36	0.97
25	29	7	1.5	6	39.5	0.000	0.000	0.000	0.000	4.67	0.75
30	30	7	1.5	6	39.5	0.000	0.000	0.000	0.000	4.69	0.76

decrease. The rate and extend of the fermentation resulting is pH reduction is also influenced by time and temperature of incubation. Higher temperature accelerates the bacterial growth resulting in increased acid production and pH reduction; and longer incubation time permits more time for lactose fermentation producing more lactic acid thus lowering the pH of the fruit yoghurt. The titratable acidity (% LA) of the developed yoghurt ranged from 0.46 to 1.01 %LA. The highest titratable acidity was reported in the formulation with 9% fruit pulp, 1.5% inoculum incubated at 39.5°C for 6h whereas, The lowest titratable acidity value was observed in the formulation with 7% fruit pulp, 1.5% inoculum incubated at 39.5°C for 2 h. Pagthinathan et al. (2016) reported the effect of change in titratable acidity in yoghurt on addition of fruit; an increase in titratable acidity was observed on addition of fruits attributing to the organic acids present in the fruit. Additionally, the influence of fermentation conditions like temperature and time on titratable acidity was also reported.

Optimization of fruit yoghurt

Numerical optimization determined the optimal variable ratios efficiently, making it a practical approach for analyzing multifactor, multi-response system. The optimized levels of the variables are: 7% fruit pulp, 1.5% inoculum level, incubation at 6h and incubation time of 39.5°C with a desirability of 1. The predicted responses were validated by using the optimized variables which predicted pH 4.6393 and recorded pH 4.56±0.2 (n=3) and predicted titratable acidity (%LA) of 0.76 and recorded 0.7±0.1 (n=3). The observed response reveal that there was no significant difference between the predicted and the experimental response, hence, confirming the model adequacy.

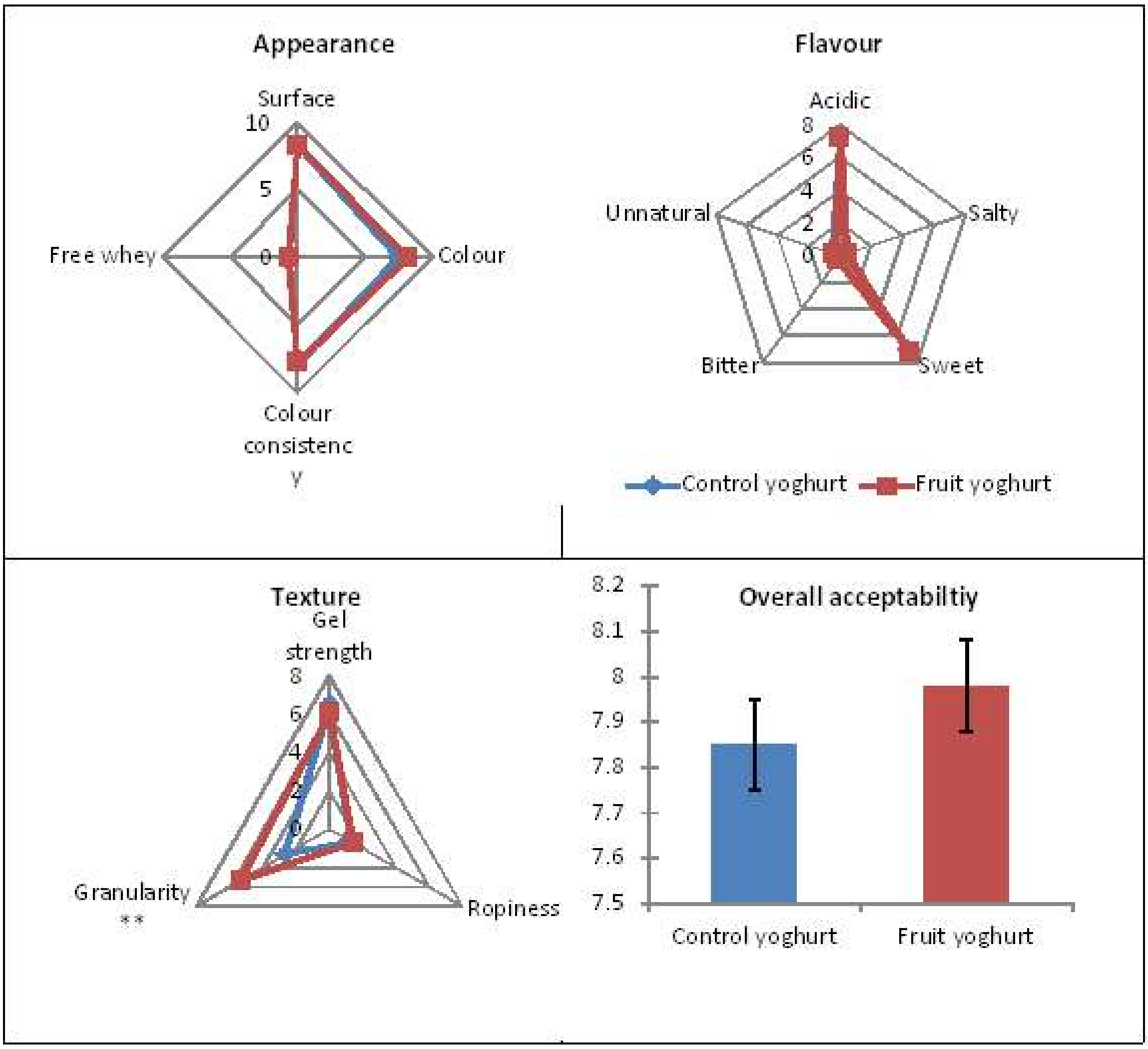

Figure 1 Descriptive sensory evaluation of the fruit yoghurt using *Prunus napaulensis*

The values show the mean and standard deviation (n= 50) **significant(p<0.05)

Sensory evaluation

Statistical analysis for the Descriptive sensory evaluation for the developed fruit yoghurt (Figure 1) describes the assessment of various attributes such as appearance, flavour, texture and overall acceptability. In appearance, even if there was no statistical significant difference in the surface and colour consistency between the fruit yoghurt and control yoghurt; the fruit yoghurt exhibited a slightly higher score in colour which can be due to the colour pigment present in the fruit pulp. Regarding flavour, there was no significant difference observed in acidic, salty, sweet, bitter and unnatural flavour between the control and fruit yoghurt. In terms of texture, the fruit yoghurt exhibited a higher score in granularity as compared to yoghurt resulting due to the addition of the fruit pulp. But no significant difference was observed in gel strength or Ropiness between the two yoghurts. There was no significant difference in the overall acceptability scores between control yoghurt and fruit yoghurt.

Physico chemical analysis

The results indicated significant nutritional enhancement and comparable physicochemical properties (Table 2) between the control and developed fruit yoghurt. The Physico chemical parameters including pH, total soluble solids, total solids and titratable acidity of the fruit yoghurt was comparable to that of control yoghurt with $p<0.05$ and were observed to be within the levels prescribed by FSSAI (2016). No significant difference was recorded in moisture, fat and total sugar ($p<0.05$) between the two sample while an increase in energy content (84.7±2.3 Kcal), protein (4.1± 0.15g) and carbohydrate (8.2± 0.2g) with $p<0.05$ was observed in the fruit yoghurt. The fruit yoghurt contained significant amounts of Vitamin C (4.4± 0.1mg) potassium (262± 1.5mg) magnesium (8.5± 0.06mg) and zinc (0.4± 0.01mg) with differences being highly significant ($p<0.00$) as compared to the control yoghurt. The fruit yoghurt was also observed to have substantial amounts of fibre (1.73± 0.15g) and anthocyanin (95.6± 1.5mg) which were absent in the control yoghurt. Thus, the incorporation of *Prunus napaulensis* significantly enhanced the nutritional profile of yoghurt, increasing energy, carbohydrates,

Table 2 Physico-chemical properties and Nutritional composition of control and fruit yoghurt with *Prunus napaulensis*

Parameters	Control	Fruit Yoghurt	't' value	P value
pH	4.5±0.1	4.56±0.2	0.323	0.763^{NS}
TSS	11.8±0.2	11.5±0.5	1.000	0.374^{NS}
Titratable Acidity (%)	0.72±0.09	0.7±0.1	0.247	0.817^{NS}
Total solids	14.0±0.45	14.2±0.25	-0.559	0.606^{NS}
Solids non-fat (SNF)	9.9±0.58	10.16±0.2	-0.476	0.659^{NS}
Water Holding Capacity (%)	81.16±1.25	74.33±1.60	5.798	0.004**
Syneresis (%)	17.33±1.15	24.22±0.8	-8.360	0.001**
Moisture (%)	84.1±8.4	82.1±1.1	0.391	0.716^{NS}
Energy (Kcal)	72.2±2.6	84.7±2.3	-6.168	0.004**
Carbohydrate (g)	5.4±0.4	8.2±0.2	-9.823	0.001**
Protein (g)	3.3±0.26	4.1±0.15	-4.725	0.009**
Fat (g)	4.09±0.14	4.06±0.15	0.246	0.818^{NS}
Total Sugar (g)	4.8±0.2.6	4.6±0.1	1.225	0.288^{NS}
Ash (%)	0.75±0.09	0.46±0.01	5.109	0.007**
Calcium (mg)	149±0.7	141±0.5	15.250	0.000**
Iron (mg)	0.16±0.05	0.5±0.01	-9.722	0.001**
Vitamin C (mg)	BDL	4.4±0.1	-12.965	0.000**
Potassium (mg)	129±2.08	262±1.5	-89.443	0.00**
Magnesium (mg)	BDL	8.5±0.06	-242.890	0.000**
Zinc (mg)	BDL	0.4±0.01	-48.379	0.000**
Anthocyanin (mg)	BDL	95.6±1.5	-108.476	0.000**
Fibre (g)	BDL	1.73±0.15	-19.654	0.000**

The values show the mean and standard deviation (n=3) BDL= Below Detection Limit NS= non-significant **significant($p<0.05$)

protein, iron, vitamins, minerals, anthocyanin and fibre content, while maintain similar physicochemical parameters to that of control yoghurt; suggesting that *Prunus napaulensis* yoghurt is a nutritionally superior alternative to traditional yoghurt (Ritu et al. 2016, Sheikh et al. 2022).

Water Holding Capacity (WHC) and Syneresis Index

The control yoghurt exhibited higher WHC (81.16 % ±1.25) compared to the fruit yoghurt (74.33 % ±1.60) indicating that the control yoghurt can retain more water within its matrix. WHC is affected by the protein network and the interaction between casein micelles and other ingredients added. Higher WHC results in firmer, creamier texture which is desirable in yoghurt (Elsayed et al. 2020, Ragab et al. 2023). The lower WHC of the fruit yoghurt can be attributed to the addition of the fruit, the impact of the water content and fruit particles disrupting the gel matrix of the yoghurt. This results in weakened gel structure, leading to increased water mobility and decreased water holding capacity.

Syneresis is inversely related to Water Holding Capacity and is used to indicate the structural integrity of yoghurt (Elsayed et al. 2020). Syneresis of fruit yoghurt was significantly higher (24.22% ±0.8) compared to control yoghurt (17.33% ±1.15). Increased syneresis is observed in fruit yoghurts due to the addition of fruits which can affect the texture resulting to excess water release (whey separation) (El-Diasty and Abdelkhalek 2024). The addition of *Prunus napaulensis* fruit pulp in yoghurt decreased the Water Holding Capacity and increased the syneresis which can impact the texture, consistency and shelf life of the fruit yoghurt. Since higher syneresis and lower WHC can result in watery texture in yoghurt, additives like pectin and inulin (Zhou et al. 2024) and other stabilizers like arabic gum, guar gum (Salih et al. 2020) and gelatin (Pagthinathan et al. 2016) can help reduce the syneresis in fruit yoghurt and enhancing the water binding capacity, thus improving the stability and sensory quality of the fruit yoghurt (Zhou et al. 2024).

Rheological analysis

As shear rate increases from 0.506s^{-1} to 100.003s^{-1}, the shear stress increased from 15.678 Pa to 34.139 Pa in control yoghurt while the fruit yoghurt exhibited a lower shear stress range compared to control yoghurt (9.823 to 20.648Pa) as displayed in figure 2. This indicates that the control yoghurt exhibited a shear-thinning behaviour (pseudo plasticity), which is a characteristic of yoghurt. With the increase in shear rate, the resistance to flow (viscosity) decreases (Ragab et al. 2023) While the fruit yoghurt also exhibited shear thinning behaviour, the stress values were consistently lower than control yoghurt, indicating a weaker gel network and lower resistance to deformation which may have resulted by the addition of fruit pulp. The use of food-grade stabilizers and hydrocolloids such as pectin,guar gum, gum arabic (Salih et al. 2020), gelatin (Pagthinathan et al. 2016)and inulin (Zhou et al. 2024) can help reinforce the gel network and improving the viscosity and gel strength. Addition of milk protein concentrates or whey protein isolates (Elsayed et al. 2020, Zhou et al. 2024)) can strengthen the gel network and counteract the weakening effects of fruit pulp. Additionally the use of exopolysaccharides (EPS) producing probiotic strains may enhance the gel strength while preserving the sensory qualities of the yoghurt (Tiwari et al. 2021). Zhou et al. (2024) reported that shear thinning is a desirable attribute in yoghurt for consumer acceptability due to its smooth texture during consumption. Shukla et al. (2024) reported the relation of shear-thinning behaviour and improved sensory attributes, such as creaminess and smoothness which are critical for consumer satisfaction.

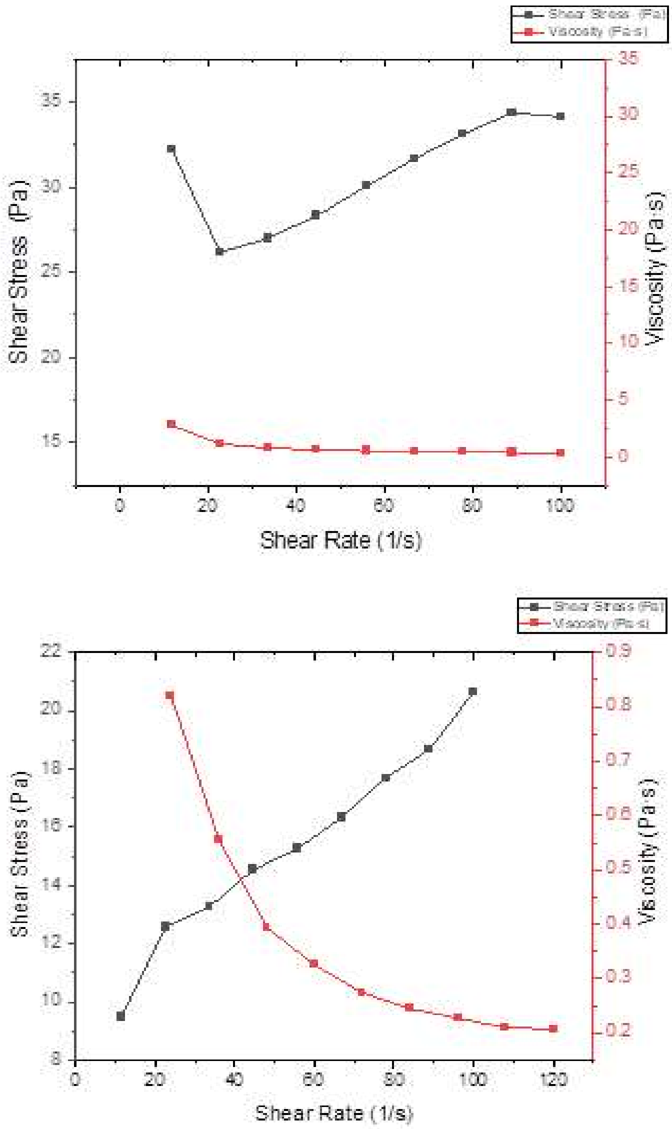

Fig 2. Rheological behaviour of (a) control yoghurt and (b) fruit yoghurt: shear stress (Pa) and velocity (Pa·s) as a function of shear rate (1/s).

Table 3 Microbial viability and quality assessment of fruit yoghurt during storage

DAY	Yeast and Mold (cfu/mL)	Coliform (cfu/mL)	*Streptococcus thermophilus* (cfu/mL)	*Lactobacillus bulgaricus* (cfu/mL)	pH	Titratable acidity (%LA)
Day 0	ND	ND	2.18 x10^7 ±6.658	2.37 x10^7 ±7.506	4.6±0.0	0.79±0.0
Day 1	ND	ND	2.29 x10^7 ±8	2.47 x10^7 ±9.609	4.6±0	0.8±0.0
Day 3	ND	ND	2.58 x10^7 ±11.02	2.58 x10^7±8.66	4.5±0.0	0.83±0.01
Day 7	ND	ND	2.76 x10^7 ±9.074	2.66x10^7 ±8.737	4.04±0.1	0.91±0.02
Day 14	ND	ND	2.93x10^7 ±6.03	2.94 x10^7 ±7.55	3.93±0.14	1.12±0.02
Day 21	1 x10^4±1	ND	TNTC	TNTC	3.6±0.02	1.31±0.02

The values show the mean and standard deviation (n=3) ND= not detected, TNTC= too numerous to count

The viscosity of the control yoghurt decreases significantly from 30.9961 Pa·s at 0.506 s^{-1} to 0.3414 Pa·s at 100.003 s^{-1} indicating a classic shear–thinning property where yoghurt becomes more viscous under mechanical stress. The fruit yoghurt exhibited a lower viscosity starting at 19.4208 Pa·s at 0.506 s^{-1} and drops to 0.2065 Pa·s at 100.003 s^{-1} which is considerably lower than the control yoghurt at every shear rate. Overall, the control yoghurt demonstrated a higher shear stress and viscosity compared to the fruit yoghurt, indicating a more robust gel structure. The lower viscosity implies a smoother and less resistant flow which can be attributed to the presence of the fruit disrupting the protein matrix by the fruit component such as water and fibre content, thus reducing the overall viscosity (Acurio et al. 2024, Ragab et al. 2023))

Effect of storage on microbial viability and quality of fruit yoghurt

A progressive decrease in pH was observed during the storage period (Table 3) from an initial value of 4.6 on day 0 to 3.6 on day 21 indicating an increase in acidity during the storage period. A steady increase in Titratable acidity (%LA) was observed from 0.79% on day 0 to 1.31% on day 21 which was consistent with the lactic acid production by the lactic acid bacteria (LAB) during fermentation. Yeast and mold were not detected until Day 21 observed at a count of 1x10t cfu/mL, which could be due to the acid-tolerant nature of yeast and mold signalling a potential spoilage (Aleksic et al. 2024, Salih et al. 2020). The detection of Coliform bacteria were not detected throughout the storage period of 0 to 21 days indicating that the product remained free from coliform contamination, which may be attributed to the hygienic practices during the production and storage. According to the Food Safety and Standards Authority of India, 2020, the coliform count should not exceed 10 cfu/mL. The initial count of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* at day 0 started at 2.18 x 10^7 cfu/mL and 2.37 x 10^7 cfu/mL respectively which steadily increased to 2.93 x 10^7 cfu/mL and 2.94 x 10^7 cfu/mL respectively on day 14 and further became too numerous to count (TNTC) on day 21. This increasing microbial growths throughout the storage period aligns with the production of lactic acid by the lactic acid bacteria which is a characteristic of yoghurt or fermented milk products in turn contributing to the decrease in pH over time (Sheikh et al. 2022, Salih et al. 2020)

Conclusions

The development and optimization of fruit yoghurt enriched with *Prunus napaulensis* through Response Surface Methodology (RSM) demonstrated significant potential for enhancing the nutritional profile and sensory attribute of yoghurt product. The study confirmed that the inclusion of *Prunus napaulensis* fruit pulp not only improves nutritional value, but also contributes to the sensory characteristics of the yoghurt. The optimized parameters-7 % fruit pulp, 1.5% inoculum, incubation for 6 hours at 39.5°C- yielded a product with desirable pH and titratable acidity values. F values were significant in both the responses with R^2 of 0.9198 and 0.9663 for pH and titratable acidity, respectively proving the fitness of the polynomial regression models analysis the effect of the two responses. The predicted responses were similar to the experimental results thus validating the accuracy of the model. Sensory evaluation revealed that the fruit yoghurt showed slight enhancement in colour and texture due to the natural pigments and components of the fruit, overall acceptability remained comparable to the traditional yoghurt. Thus, appealing to consumer preferences. The shelf life study confirmed the stability of the product, making it suitable for commercial application. This study underscores the potential of *Prunus napaulensis* as a valuable ingredient for dairy product innovation, contributing to the diversification of yoghurt offerings in the Indian market.

Future research should explore the long-term health benefits of *Prunus napaulensis*, along with its market viability and acceptance, paving the way for its commercial introduction. This study contributes significantly to the on-going efforts to diversify dairy products and enhance their nutritional value, aligning with the global trend towards healthier eating.

Acknowledgement

The author(s) would like to express their gratitude to the participants for their support and contributions to this research.

Funding

The author(s) would like to acknowledge the financial support from Indian Council of Social Science Research (ICSSR) [ICSSR/RFD/24-25/HLTH/ST/385] to carry out the study

Conflict of interest

The author(s) have no competing interests to declare.

References

Acurio Arcos L, Masabanda W, García M, Garzón J, Espín C (2024) Physicochemical, rheological, sensory, and proximal properties of yogurt flavored with Aloe vera gel. Emir J Food Agric 36:1–12

Aleksic B, Udovicki B, Kovacevic J, Miloradovic Z, Djekic I, Miocinovic J, Tomic N, Smigic N. (2024) Microbiological Assessment of Dairy Products Produced by Small-Scale Dairy Producers in Serbia. *Foods* 13(10):1456

Aparna K, Manas RS, Sowmya M, Premi DM, Dasgupta M, Sreedhar M (2018) Nutrient and antioxidant composition in value-added products made with underutilized Prunus (Prunus nepalensis) fruits. J Pharmacogn Phytochem 7(4):1550-1556

Bureau of Indian Standards (BIS) (2016) IS 1479-1: Methods of Test for Dairy Industry, Part 1: Rapid Examination of Milk. Bureau of Indian Standards, New Delhi

El-Diasty M, Abdelkhalek A (2024) Effect of Spirulina Powder on Physical and Chemical Composition of Yoghurt Produced from Milk of Different Somatic Cell Counts. Zagazig Vet J 52:451–464

Elsayed A, Ismail A, Aly AA, Atallah AA (2020) Quality and microstructure of freeze-dried yoghurt fortified with additives as protective agents. *Heliyon* 6(10)

Food Safety and Standards Authority of India (2020) *Food safety and standards (food products standards and food additives) regulations, 2011: Milk and milk products*

FSSAI (2016) Manual of Methods of Analysis of Foods: Milk and Milk Products

Kambhampati V, Sabyasachi M, Rama CP (2018) Physicochemical characterization and mass modeling of Sohiong (Prunus nepalensis L.) fruit. J Food Meas Characterization 12(2):923-936

Latimer GW (2023) Official methods of analysis of AOAC International. 22nd ed. AOAC Publications, New York

Pagthinathan M, Nafees M, Jeganathan N (2016) Development and investigation of Palmyrah fruit pulp (PFP) added yoghurt. Int J Sci Res Innov Technol 3(5):15-30

Ragab ES, Zhang S, Korma SA, Buniowska-Olejnik M, Nasser SAA, Esatbeyoglu T, Lv J, Nassar KS (2023) Physicochemical and rheological properties of stirred yoghurt during storage induced from high-intensity thermosonicated goat and cow milk. Fermentation 9(1):42

Ritu G, Hafsa H, Naveen KS, Prashant K (2016) Development and quality evaluation of yoghurt fortified with pineapple, apple, and sweet lemon juice (fruit yoghurt). Int J Eng Res Technol 5: 621-629

Rymbai H, Patel RK, Deshmukh NA, Jha, Verma VK (2016) Physical and biochemical content of indigenous underutilized Sohiong (Prunus nepalensis Ser.) fruit in Meghalaya, India. Int J Minor Fruits, Medicinal Aromatic Plants 2(1):54-56

Salih ZA, Siddeeg A, Farga AA, Mousa RMA, Husam SA, Zubair ASA (2020) Physicochemical, microbial and sensory characteristics of yoghurt processed by adding various formulations of gum arabic and guar gum. Annu Res Rev Biol 35(11):33-44

Sheikh S, Siddique F, Ameer K, Ahmad R, Hameed A, Ebad A, Mohamed Ahmed I, Shibli S (2022) Effects of white mulberry powder fortification on antioxidant activity, physicochemical, microbial, and sensorial properties of yogurt produced from buffalo milk. Food Sci Nutr 11: 204–215

Shukla V, Villarreal M, Padilla-Zakour O I (2024) Consumer acceptance and physicochemical properties of a yogurt beverage formulated with upcycled yogurt acid whey. Beverages 10(1)18.

Tiwari S, Kavitake D, Bruntha D P, Shetty P H (2021) Bacterial expolysaccharides for improvement of technological, functional and rheological properties of yoghurt. Int J Biol Macromol 183:1585-1595

Zhou Z, Zhang L, Liu T, Hu G, Hu H, Aziz T, Zhang M, Wu J, Yang Z, Yang Z, Albekairi T (2024) Physicochemical properties of yoghurt supplemented with polymerized whey protein and inulin. *LWT* 210:116888