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Optimization of water chestnut (*Trapa bispinosa*) starch, fructo-oligosaccharide and inulin concentrations for low-fat flavoured yogurt consisting of a probiotic *Lacticaseibacillus rhamnosus* strain†

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Water chestnut (*Trapa bispinosa*) starch has technical and functional qualities that are mostly comparable to those of traditional sources. In this study, low-fat flavoured probiotic yogurt was prepared with the addition of *Lacticaseibacillus rhamnosus* (ATCC 7469), mango pulp and water chestnut starch as a fat replacer as well as a biothickener. A central composite face centered experimental design (CCFD) optimization study was carried out using different combinations of fat (0.5–1.5%), starch (1.0–3.0%), fructo-oligosaccharides (0.5–1.5%) and inulin (0.5–1.5%). *L. rhamnosus* exhibited resistance to simulated gastric fluid, intestinal fluid and hydrogen peroxide, along with good cell surface hydrophobicity and antimicrobial properties against *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium* and *Staphylococcus aureus*. Water chestnut starch was found to have a characteristic A-type crystalline pattern with 28% crystallinity, and it was smooth with oval to irregular in shape. The proximate, texture, color, and sensory properties and the viability of *L. rhamnosus* in yogurt did not vary significantly during a 15 day storage period. Thus, the starch from water chestnuts can be explored as a possible fat substitute for formulating low-calorie food products.

Sustainability spotlight

Water chestnut (*Trapa bispinosa*) is an annual aquatic underutilized crop that prefers warm climatic conditions and is a highly nonconventional starch source. It is rich in major constituents such as carbohydrates, proteins and minerals with good amylose content. Extraction of starch from water chestnut and using it as a fat replacer for the development of low-calorie functional products such as yoghurt (consumed widely) will lead to its commercial and sustainable production by marginal farmers. In recent years, consumers are widely looking for low-fat variants of yogurt with desired sensory qualities available in full-fat yogurt without an excessive intake of dietary fat. Thus, in future, the developed low fat functional yogurt can lead to scaling up and commercialization at the industrial level.

1. Introduction

Functional foods are in widespread use due to the classification of obesity as a disease and multiple research findings indicating a link between obesity and coronary heart disease.[1] Fermented dairy products have a long history of providing nutritive value together with beneficial effects on human health.[2] With the increasing awareness of negative impacts of fat and fat rich dairy products on health, consumers are inclining towards non-fat or low-fat milk products. Yogurt is a traditional fermented milk product consumed worldwide and is considered healthy owing to its high content of protein, calcium and beneficial bacteria.[3,4] In recent years, consumers are opting for low fat variants of yogurts that possess the desired sensory qualities of the full fat version while keeping the dietary intake of fat in check. However, reductions of fat levels in products will impair

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their physicochemical and sensory properties, resulting in low consumer desirability. Therefore, thickeners are generally used as alternatives to modify the structure, texture and flavor perception of the food matrix.[5] Starch and prebiotics such as inulin and fructo-oligosaccharides (FOSs) can be used as fat replacers in low-fat dairy products since they possess gelling capacity with water and give mouthfeel and texture similar to fat.[6,7]

Water chestnut (*Trapa natans*) is a free-floating plant that grows in water bodies, is rich in functional polyphenols and belongs to the family Trapaceae.[8] Starch from water chestnut has similar properties to those of the starch extracted from potato and corn sources, which make it potential for commercial applications.[9] The digestibility patterns of water chestnut starch are quite comparable to those of cassava and maize sources.[10]

Yogurt has also been considered the most effective food matrix for the delivery of probiotic bacteria benefiting the host health in adequate amount.[11] Probiotics have various health benefits, including boosting the immune system, inhibition of the growth of pathogenic microorganisms, prevention of diarrhea from various causes, prevention of cancer, and reduction of inflammatory bowel movements.[12,13] Therefore, the incorporation of probiotics in commercial products can help in developing functional food products. However, it has been noted that the viability of probiotics gradually declines in commercial foods. Several factors such as the type of culture used, amount of nutrients supplied, storage temperature and time, and growth retarding factors, may be responsible.[14] A number of studies have been conducted to enhance the growth of probiotic bacteria in commercial food products during storage. Approaches like supplementation with growth factors like prebiotics have been used to enhance the growth of probiotic bacteria in commercial food products during storage.[15] Along with that, degradation of prebiotics generates short chain fatty acids (SCFAs), which stimulates the immune system of the host.[16]

Different research groups have conducted several studies to investigate the use of fat replacers, prebiotics and probiotics in the development of low-fat yogurt.[17,18] This study aims to use water chestnut starch along with prebiotics such as FOS and inulin as a fat replacer and substrate for the viability of incorporated probiotic bacteria during the optimization for production of low fat flavored probiotic yogurt. Incorporation of mango pulp in yogurt can increase its nutritional value, aroma and flavor, which can improve physico-chemical attributes like sensory properties and texture and reduce syneresis in the product. This can also positively influence the physicochemical characteristics like sensory and texture profile and reduce syneresis.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Yogurt cultures (NCDC 263; a commercial mixed culture YC-281) consisting of lyophilized mixed bacteria comprising *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were procured from National Collection of Dairy Cultures (NCDC), NDRI (India). The starter culture was maintained in reconstituted skimmed milk (12 g/100 mL) by sub-culturing once in a fortnight for attaining high activity. The probiotic culture *Lacticaseibacillus rhamnosus* (ATCC-7469) was grown in MRS (HiMedia, India) at 37 °C for 18 h and activated one was maintained in litmus milk at 4 °C for further use. The initial probiotic and functional attributes of *L. rhamnosus* were reconfirmed as per FAO/WHO guidelines.[11] All chemicals used in microbiology and bacteriology preparations were procured from HiMedia, India. All other chemicals and reagents used in the experiments were of analytical grade (Merck, India).

2.2. Probiotic attributes of *Lactobacillus rhamnosus* ATCC-7469

L. rhamnosus (ATCC-7469), used in the present study, is a commercially available Gram-positive rod bacterium having good *in vitro* tolerance against the acidic and bile condition in the gut along with good adhesion properties under the *in vitro* Caco-2 human intestinal cell line.[19,20]

2.2.1. Resistance to simulated gastric fluid (SGF) and intestinal fluid (SIF). Simulated gastric fluid and simulated intestinal fluid were prepared by the method suggested by Fernandez *et al.* with some modifications.[21] Briefly, the SGF was prepared by suspending 0.15 g pepsin in 50 mL MRS broth and the pH was adjusted to 3.5, 4.5 and 6.5 respectively. Simultaneously, SIF was prepared by suspending 0.1% pancreatin and 0.3% ox-bile in 100 mL MRS broth adjusted to pH 6.8 using 0.5 M NaOH. The overnight culture of *L. rhamnosus* was pelleted by centrifugation at 5000 rpm for 5 min at 4 °C, washed in sterile PBS (pH 7.4) twice and re-suspended in PBS. The cell suspensions (10^9 to 10^{10} CFU mL^{-1}) were added to separate pH adjusted SGF. The suspended cultures were incubated at 37 °C and samples were drawn at regular intervals of 0, 30, 60 and 90 min and plated on MRS agar. The colony-forming units per milliliter (CFU mL^{-1}) of the survived bacteria were counted after incubating the plates at 37 °C for 24–48 h. Similarly, the cell suspensions were also added in simulated intestinal fluid (SIF) and survivability was determined by plating after 0, 60, 120, 180 and 360 min.

2.2.2. Cell surface hydrophobicity. The microbial adhesion to hydrocarbon assay to determine the cell surface hydrophobicity of isolates and standard culture using hexadecane (Sigma, India), xylene (Sigma, India) and toluene (Sigma, India) as solvents was done.[22] Cell surface hydrophobicity in terms of percent (H%) was calculated using the following formula:

$$H\% = (1 - A_1/A_0) \times 100 \quad (1)$$

where A_0 and A_1 represent the initial and final absorbance of the suspension and lower aqueous phase at 600 nm.

2.2.3. Resistance to hydrogen peroxide. Resistance of *L. rhamnosus* (ATCC 7469) to hydrogen peroxide was determined by the method suggested by Kullisaar *et al.* with 0.4 mM hydrogen peroxide (30 wt% solution in water) at 37 °C.[23]

2.2.4. Antibiotic sensitivity test. Antibiotic sensitivity test was performed by using commercially available antibiotic discs

(Hexa G-plus 6, HXO 22-1PK, and Hexa Universal-1, HXO 32-1PK) (HiMedia Labs, India). The MRS agar plates were prepared and the overnight culture of *L. rhamnosus* (ATCC 7469) at 37 °C was spread, followed by placing the antibiotic disc on it. The plates were incubated at 37 °C for 12 h and the zone of inhibition was measured.

2.2.5. Determination of DPPH radical scavenging activity. The DPPH (2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; Sigma, India) radical scavenging activity by *L. rhamnosus* was analyzed by a method suggested by Lin & Chang.[24] To determine the DPPH radical scavenging activity, 0.8 mL of intact cells or intracellular cell free extract and 1 mL of freshly prepared DPPH solution (0.2 mM in methanol) were mixed and allowed to react for 30 min at room temperature. PBS or deionized water was taken as the blank sample control and the scavenged DPPH was monitored at 517 nm. The scavenging capacity was calculated using the following equation:

$$\text{DPPH radical scavenging effects (\%)} = [(A_0 - A_1/A_0) \times 100] \quad (2)$$

A_0 and A_1 correspond to the absorbance at 517 nm of the radical (DPPH) in the absence and presence of antioxidants, respectively.

2.2.6. Antimicrobial properties. The antimicrobial property of the probiotic was determined by the agar well diffusion assay method. The targeted pathogenic bacterial strains *Staphylococcus aureus* (ATCC 12600), *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 11229) and *Bacillus subtilis* (ATCC 11774) were grown in nutrient broth at 37 °C. The overnight pathogenic culture was then added to soft nutrient agar (consisting of 0.9% agar) and poured on previously plated and solidified nutrient agar. The overnight culture of probiotic in MRS broth was centrifuged at 6000 rpm for 15 min and the supernatant was added in the wells drawn on the layered nutrient agar. The plates were incubated at 37 °C for 8–12 h. The antimicrobial property was determined by measuring the zone of inhibitions formed by the probiotic strain against the pathogens.

2.3. Extraction of starch from water chestnut

The water chestnuts (*Trapa bispinosa*) used in this study were collected from the local markets of Tezpur and Guwahati, Assam (India), during the months of August–November and stored at −20 °C for further study. The water chestnuts were a locally available double horned variety with length breadth ratios of 1.25 ± 0.2 and 1.02 ± 0.1, respectively (ESI Fig. 1S†). The water chestnut kernels were peeled and the white portion of the kernel was dipped in the 0.2% solution of sodium metabisulfate for 24 h and further ground and made into a slurry by using a blender (Philips, India) and passed through different pore size sieves (BSS no.: 250, 200, 150, 100, 75 and 50) 3–4 times. This was followed by centrifugation (Hettich Zentrifugen EBA 21, Germany) at 3500 rpm for 10 min at room temperature to separate the upper protein layer and whiter starchy materials were collected.

2.3.1. Physicochemical properties of water chestnut starch. Standard AOAC methods were used to determine the moisture, crude fiber and ash content, respectively.[25] The ash content was determined by ignition at 550 °C in a muffle furnace (Optic Ivymen System, SNOL 8, 2/1100, Utena, Lithuania) for 6 h. Fat in the sample was determined using Socs Plus apparatus (Socs Plus, Pelican Equipment, Chennai, India) using *n*-hexane as the solvent, and protein in KelPlus apparatus (KelPlus, Pelican Equipment, Chennai, India), respectively. The pH was determined using a pH meter (pH510, Eutech, Ayer Rajah Crescent, Singapore) calibrated with standard buffer solution (Merck, India), and total soluble solid (TSS) and the titratable acidity were determined by using the method followed by Singh *et al.*[9,26]

Bulk density and tapped density were determined by using the method followed by Olayemi *et al.*[27] Briefly, 20 g of starch powder was taken in a 100 mL graduated cylinder. The true density of the sample was determined by using xylene as the displaced liquid in a 100 mL graduated measuring cylinder. Porosity was calculated from bulk density and true density by using the following formula:

$$E = \left(\frac{\text{true density} - \text{bulk density}}{\text{true density}}\right) \times 100. \quad (3)$$

The hydration capacity was measured according to the method followed by Kornblum with some brief modifications.[28] One gram of sample (Y) with added 10 mL distilled water in centrifuge tubes was kept for 2 h at 8, 20 and 50 °C, respectively. The content of tubes was mixed during the time and further left to stand for 30 min before centrifugation at 3000 rpm for 10 min. The supernatant was decanted and leftover contents were weighed (X). The hydration capacity of the powder was calculated as

$$\text{Hydration capacity} = \frac{X}{Y} \quad (4)$$

The methods given by Gani *et al.* were used for determining the swelling power and solubility of starch powder.[29] The sample starch of five suspensions (1%, w/w) was heated to 50, 60, 70, 80 and 90 °C for 30 min in a flask with mixing every 5 min and cooled to room temperature followed by centrifugation at 5000 rpm for 15 min. The residual volume was calculated after removal of the supernatant and dried at 130 °C for 2 h in an oven.

The morphology of starch granules including the size and shape was observed with a scanning electron microscope (JEOL 6390 LV, Singapore) operating at an acceleration voltage of 15 kV and with different magnifications. Thirty randomly selected granules from the micrographs were measured to determine the size of granules.

The X-ray diffraction pattern was determined according to the method followed by Chiang *et al.*[30] Wide angle X-ray diffractograms of starch and polysaccharide were obtained with an X-ray diffractometer (Rigaku Miniflex, Japan) with a k value of 1.54040 operating at 30 kV acceleration potential and 15 mA current with a copper target. The scanning range was 2–40° of 2θ values with a scan speed of 8° 2θ min^{-1}. The percentage crystallinity was determined as

$$\%\ \text{Crystallinity} = \frac{\text{Area under peaks} \times 100}{\text{Total area}} \quad (5)$$

The infra-red spectrum for starch was obtained with an FTIR spectrometer (Nicolet Impact 410, Canada) equipped with KBr optics and a DTGS detector. The equipment was operated with a resolution of 2.0 cm^{-1} and scanning range of 4000–400 cm^{-1}. Starch samples were mixed and ground with dried KBr (1 : 100 w/w) powder followed by pelleting using compression (≈10 000 psi) and analyzed.

Thermal characteristics of the starch samples were studied using a differential scanning calorimeter (DSC, Shimadzu Model TGA50 DSC60, Japan). Changes in enthalpy (ΔH) and melting point temperature (T_m) of the starch sample were determined.

The pasting profiles of starch were recorded using a Rapid Visco Analyser (RVA Starchmaster 2, Newport Scientific Instruments). The viscosity profiles were recorded using starch suspension. The Std1 profile of Newport Scientific was used, where the samples were held at 50 °C for 2.30 min, cooled from 95 °C to 50 °C at 11.84 °C min^{-1} and held at 50 °C for 2 min. The peak viscosity (PV), hot paste viscosity (HPV), cold paste viscosity (CPV), breakdown (BD) and set back (SB) were recorded.

2.4. The optimal formulation of low-fat yogurt

The optimum low-fat yogurt formulation conditions were investigated using central composite face centered experimental design (CCFD) software. Standardized cow milk with 9.0% solid non-fat (SNF) was divided into 29 equal parts (100 mL each). The independent variables affecting the quality of the end product were the proportions of fat (X_1, 0.5–1.5%), starch (X_2, 1–3%), FOS (X_3, 0.5–1.5%) and inulin (X_4, 0.5–1.5%) as shown in Table 1. The factors such as pH, acidity, syneresis, water holding capacity (WHC) and texture (consistency, firmness, index of viscosity and cohesiveness) were used as quality attributes of low-fat yogurt. A second order polynomial model including linear, squared and interaction terms was fitted to the experimental data. The design of experiments, generation of response surface plots, superimposition of contour plots and statistical analysis were performed using Design Expert software 6.0 (STAT-EASE, Minneapolis, MN, USA).

The sample without any addition was the control. The mixtures were then added with adequate amounts of color and flavor (Marson Industries, India) and pasteurized at 90 °C for 5 min, cooled to 42 °C and inoculated with yogurt culture (NCDC 263) at 3% level. Incubation of the inoculated samples was done at 42 °C for 4–6 h until the pH decreased to 4.6 and stored at 4 °C for further use.

Table 1 Code and level of factors chosen for the trials

	Low	Center	High
Factor	—	0	+
Fat (%, X_1)	−1 (0.5)	0 (1.0)	1 (1.5)
Starch (%, X_2)	−1 (1.0)	0 (1.5)	1 (3.0)
FOS (%, X_3)	−1 (0.5)	0 (1.0)	1 (1.5)
Inulin (%, X_4)	−1 (0.5)	0 (1.0)	1 (1.5)

2.4.1. Titratable acidity, pH, syneresis, water holding capacity (WHC) and texture profile of yogurt. The titratable acidity and pH of the samples were determined as described earlier. Syneresis was determined following the method described by Raju and Pal.[31] while WHC was determined by the method given by Zanhi and Jideani[32] and was expressed as percent pellet weight over original weight of yogurt. Texture was studied using a Texture Analyzer (TA-HDPlus, Stable Microsystems, UK) with the help of a SMS/P75 flat probe fixed to a 5 kg load cell by the back extrusion method. The test was carried out in compression mode. The trigger force used for yogurt was 5 g. The pre-test speed was 5 mm s^{-1} and the test and post-test speed was 1 mm s^{-1}.

2.5. Incorporation of probiotic cultures to optimized low fat yogurt and its storage study

The probiotic *L. rhamnosus* (ATCC 7469) was activated in 100 mL MRS broth at 37 °C for 16 h and centrifuged in a refrigerated centrifuge (SIGMA, 3-18 KS, Osterode, Germany) at 8000 rpm for 15 min at 4 °C. The cell pellet was washed twice with saline water (0.85% NaCl) and re-suspended in mango pulp (Alphonso mango pulp, ADF Food Ltd., India) at a concentration of 10^{10} to 10^{11} CFU mL^{-1}. The probiotic-culture supplemented mango pulp was added to the bottom of the container as a fruit base and the optimized yogurt mix was prepared and poured onto the probiotic-cultured mango pulp. The samples were incubated at 42 °C for 4–6 h and stored in a refrigerator at 4 °C. Proximate nutrient content, texture, color and probiotic viability were analyzed on the 0^{th}, 3^{rd}, 5^{th}, 7^{th}, 11^{th} and 15^{th} days, respectively.

2.5.1. Proximate analysis, texture profile analysis (TPA), color determination of yogurt and probiotic viability of probiotic supplemented low fat yogurt during storage. The physicochemical properties were determined as described earlier. The color of yogurt samples was determined in a Hunter Lab colorimeter (model Color Quest II, Reston, USA), with reflectance mode (RSIN), D65 as the illuminant, CIELab scale (L^*, a^* and b^*), and a 10° observer angle as a reference system. The color scale was expressed as lightness L^* ($L^* = 0$ for black and $L^* = 100$ for white), and the chromaticity parameters a^* (green [−] to red [+]) and b^* (blue [−] to yellow [+]). The total color difference (ΔE) is expressed as

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (6)$$

where (ΔL), (Δa) and (Δb) are the differences of color scale parameter of tested samples with respect to the standard control sample.

The viability of probiotic *L. rhamnosus* in mango pulp was observed on MRS agar by the pour plate method.

2.6. Sensory analysis

A number of 20 semi-trained panelists were selected and the samples were served at 10–12 °C. Samples were randomly

presented to the panelists in the morning session and scored for color, flavor, texture, mouthfeel and overall acceptability using the 9-point hedonic scale, where 9 = excellent and 1 = not acceptable.

2.7. Statistical analysis

All the experiments were carried out in triplicate. Significant differences between means were determined by the *post hoc* Duncan's multiple range test using Statistical Package for Social Sciences SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Probiotic attributes of *Lactobacillus rhamnosus*

The *L. rhamnosus* strain showed resistance to both the SGF and SIF and was viable until 90 min and 360 min, respectively. As shown in Fig. 1(a), the maximum viability was observed at 30 min incubation in different pH medium. After an incubation period of 60 to 90 minutes, a 0.18 log cycle reduction was observed. The probiotic culture was found to be resistant to simulated intestinal fluid (Fig. 1(b)). As the incubation time increased, the count of bacteria in the intestinal fluid decreased to 1.05 log cycles in between 0 and 60 min. However, after 60 min of incubation the count was relatively steady until 120 min, and again it declined gradually until the end of 360 min. The *L. rhamnosus*, our targeted probiotic culture, can survive in sufficient numbers in both the SGF and SIF medium.

L. rhamnosus showed good resistance against hydrogen peroxide. As seen from Fig. 1(c), there was a reduction of 1.5 log cycles of probiotic bacteria even after 480 min of exposure to hydrogen peroxide. Earlier, Kullisaar *et al.* also determined the survival of *Lactobacillus* cells in the presence of 0.4 mM hydrogen peroxide and observed similar results of viability of cells in hydrogen peroxide even after 390 and 450 min, respectively.[23]

The cell surface hydrophobicity of *L. rhamnosus* was found to be effective at 18.45 ± 0.54%, 10.56 ± 0.36% and 4.22 ± 0.04% against toluene, *n*-hexane and xylene, respectively. van der Waals forces, electrostatic forces and various other short-range interactions as well as surface proteins play important roles in microbial adhesion.[33] Based on its adhesion to hydrocarbons it can be further categorized as hydrophobic (good adhesion properties) and hydrophilic (poor adhesion), respectively.[34] Duary *et al.* determined the cell surface hydrophobicity of five *Lactobacillus* strains isolated from human faecal samples; however, the values were comparatively higher than those reported in the present study.[22]

The *L. rhamnosus* strain showed sensitivity against all the tested antibiotics. The formation of zone of inhibitions of 13 ± 1.414, 23 ± 1.514, 17 ± 1.614, 20 ± 1.414, 24 ± 1.214, 21 ± 1.414, 27 ± 1.494, 30 ± 1.414, 12 ± 1.464, 14 ± 1.414 and 15 ± 1.514 mm against ampicillin (10 mcg), chloramphenicol (25 mcg), streptomycin (10 mcg), sulphatriad (300 mcg), tetracycline (25 mcg), bacitracin (10 units), chloramphenicol (30 mcg), penicillin G (10 units), polymyxin B (300 mcg), gentamycin (10 mcg) and neomycin (30 mcg), respectively, shows the sensitivity to different antibiotics.

The scavenging activity of *L. rhamnosus* on DPPH was tested and it was found that the cell lysate (72.43%) had higher scavenging effect than the intact cells (66.67%). Lin and Chang also determined the DPPH scavenging activity of *B. longum* and *L. acidophilus* and observed a relatively lower percentage with 52.1% and 43.2% for intact cells and 41.6% and 20.8% for the intracellular extract in both the strains, respectively.[24]

L. rhamnosus showed inhibitory effect against all the potentially pathogenic microorganisms. The tested strain showed the zone of inhibition of 15 ± 0.80, 14 ± 0.55, 12 ± 0.20 and 7 ± 0.20 mm against *E. coli*, *B. subtilis*, *S. typhimurium* and *S. aureus*, respectively, including a well diameter of 5 mm. Verdenelli *et al.* also observed similar inhibitory effects of *L. rhamnosus* against *E. coli* and *S. aureus*.[35] Earlier, Kullisaar *et al.* observed higher antagonistic activity against *E. coli*, *S. aureus*, *E. faecalis* and *S. sonnei.*[23]

3.2. Physico-chemical properties of the starch extracted from water chestnut

The yield of starch from water chestnut was found to be 12.25 ± 0.20% w/w using the aqueous extraction method. The proximate analysis showed that water chestnut starch contains moisture (dry weight basis) and protein of 10 ± 0.50 and 0.88 ± 0.18%, respectively, which were higher than those of other sources.[9,29] The starch from *T. natans* contains 0.20% and 0.4% protein from three different lakes of Kashmir, India.[9,29] The extracts consist of 98.16% pure starch including 0.16 ± 0.01, 0.28 ± 0.02 and 0.53 ± 0.05% of crude fat, crude fiber and ash, respectively. The amylose content was found to be 25.86 ± 3.11%, which is

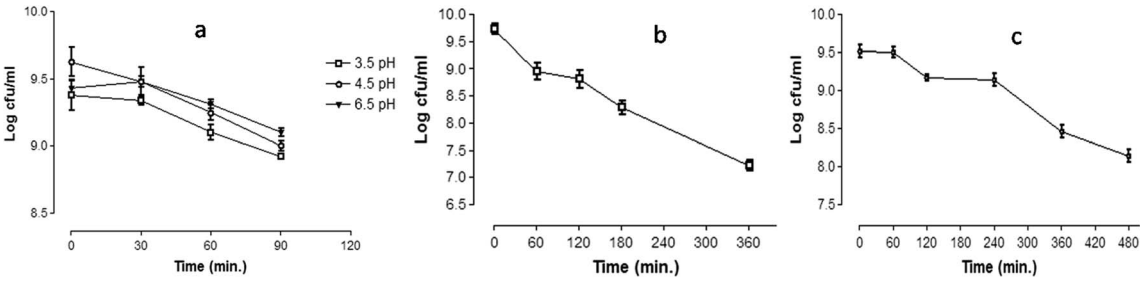

Fig. 1 Resistance of *Lacticaseibacillus rhamnosus* (ATCC-7469) to the simulated gastric juice (a), simulated intestinal fluid (b) and 0.4 mM hydrogen peroxide (c).

slightly lower than that of the water chestnut starch from Anchar lake (30.6%), Wular lake (29.6%) and Dal lake (28.5%) in Kashmir, India.[29] Earlier, Hizukuri *et al.*[36] and Murty *et al.*[37] reported quite lesser amylose contents of 23 and 15% in *Trapa natans* L. var. *bispinosa* Makino starch and *Trapa bispinosa* Roxb. starch, respectively. The pH, titratable acidity (% citric acid), and total soluble solid content of the extracted water chestnut starch were 6.7 ± 0.5 at 30 °C, 0.595 ± 0.01 and 2 °B, respectively. Singh *et al.* reported similar findings, while acidity was lower and total soluble solid content was higher than the soluble solid content of stored water chestnut kernel.[26] This may be due to the difference in variety and climatic growth conditions. The decrease in total soluble solid may be attributed to the inactivation of hydrolyzing enzymes, which are responsible for the breakdown of polysaccharides into simple sugars.

The bulk, tapped and true density for extracted starch were 0.65 ± 0.04, 0.97 ± 0.12 and 1.43 ± 0.22 g mL^{-1}, respectively, resulting in the porosity of 54.6%. The true density was slightly lower than that of potato and maize starch as reported by Singh *et al.*[26] The hydration capacity for water chestnut starch is found to be 1.28 ± 0.01, which is slightly higher than that of maize starch but lower than that of potato starch.[26]

Starches can be categorized as high swelling, moderate swelling, restricted swelling and highly restricted swelling based on their swelling power and solubility.[38] The swelling power and solubility of water chestnut starches were measured at 10° intervals over a temperature range of 50 to 90 °C, which is shown in Table 2. Swelling power is greatly influenced by the heating temperature, while solubility by the leaching of amylose from the starch granules at higher temperature. Swelling power was observed to increase rapidly on increasing the temperature. The highest swelling power of water chestnut starch was obtained above 80 °C due to starch gelatinization. At 50 °C both swelling power and solubility of starch were observed to be minimum.

The shape, size and morphology of water chestnut starch granules are shown in Fig. 2(a). The starch granules are observed to be smooth, oval to irregular in shape and of different size, which thus affects their physicochemical characteristics such as amylose content, pasting temperature, *etc.*[29] The difference in granule shape, size and morphology may be due to the difference in extraction method used and the storage condition.

Pasting properties of water chestnut starch are shown in Table 2, which are affected by the rigidity of starch granules, leaching out of amylose in the solution, starch crystallinity and degree of gelatinization.[39] The peak (PV) and final viscosity (FV) were 1939 ± 7.00 and 3199 ± 11.20 cP for extracted water chestnut starch.

As seen from Fig. 2(b), the X-ray diffraction pattern of native water chestnut starch with major peaks at 2θ values of 17° and 22° and minor peaks at 14.5° and 19° was distinctive of A-type crystalline pattern with 28% crystallinity. These X-ray diffraction patterns were quite similar as reported by Chiang *et al.*[30] in water caltrop (*Trapa quadrispinosa* Roxb.), while C-type patterns were observed by Tulyathan *et al.*[40] in water chestnut *Trapa bispinosa*. On studying the FT-IR spectra of the sample, particular peaks at various wavelengths were observed as seen from Fig. 2(c). Peaks of the native water chestnut starch spectra were recorded at the wavelengths of 1020, 1648, 2934 and 3408 cm^{-1}, which mostly correspond to –C–O stretching, H_2O vibrations and –C–O stretching bonds, –CH stretching and inter and intra molecular hydrogen bonds, respectively. Our results are in accordance with the findings of Correia *et al.* on water-chestnut starch.[41]

The melting points and enthalpy of transition are shown in DSC thermograms, showing curves for gelatinization in Fig. 2(d). Further, no relationship was observed between the enthalpy of transition and % crystallinity. Our results correlate well with Singh *et al.*,[9] where they observed that the melting point and the enthalpy change are found to be 69.6 ± 0.6 °C and 7.3 ± 0.3 J g^{-1}, respectively.

3.3. Optimization of fermentation

3.3.1. Diagnostic checking of fitted models and response surfaces. The effect of different independent variables on the responses, *i.e.*, pH, acidity, syneresis, water holding capacity, consistency, firmness, index of viscosity and cohesiveness was analyzed and is presented in ESI (Table 1S†). The coefficient of

Table 2 Swelling power, solubility and pasting properties of the extracted water chestnut starch[a]

Properties	Parameters	Values*
Swelling power (g g^{-1}) and solubility (%)	50 °C	2.26 ± 0.04^{a} & 0.26 ± 0.10^{a}
	60 °C	3.15 ± 0.45^{b} & 0.53 ± 0.10^{b}
	70 °C	5.24 ± 0.12^{c} & 3.40 ± 0.50^{c}
	80 °C	9.31 ± 0.63^{d} & 6.43 ± 0.11^{d}
	90 °C	15.66 ± 1.24^{e} & 13.57 ± 0.23^{e}
Pasting properties	Peak viscosity (PV)	1939.00 ± 7.00 cP
	Hold viscosity (HV)	1811.00 ± 4.10 cP
	Final viscosity (FV)	3199.00 ± 11.20 cP
	Breakdown (BD)	128.00 ± 0.50 cP
	Set back (SB)	1388.00 ± 1.80 cP
	Pasting temperature (°C)	88.40 ± 0.20

[a] Each value represents mean ± S.D. of triplicates. *Values are mean ± S.D. and $^{a-d}$means in the same column having different superscripts are significantly different at $p \leq 0.05$, as calculated by Duncan's multiple range test (DMRT).

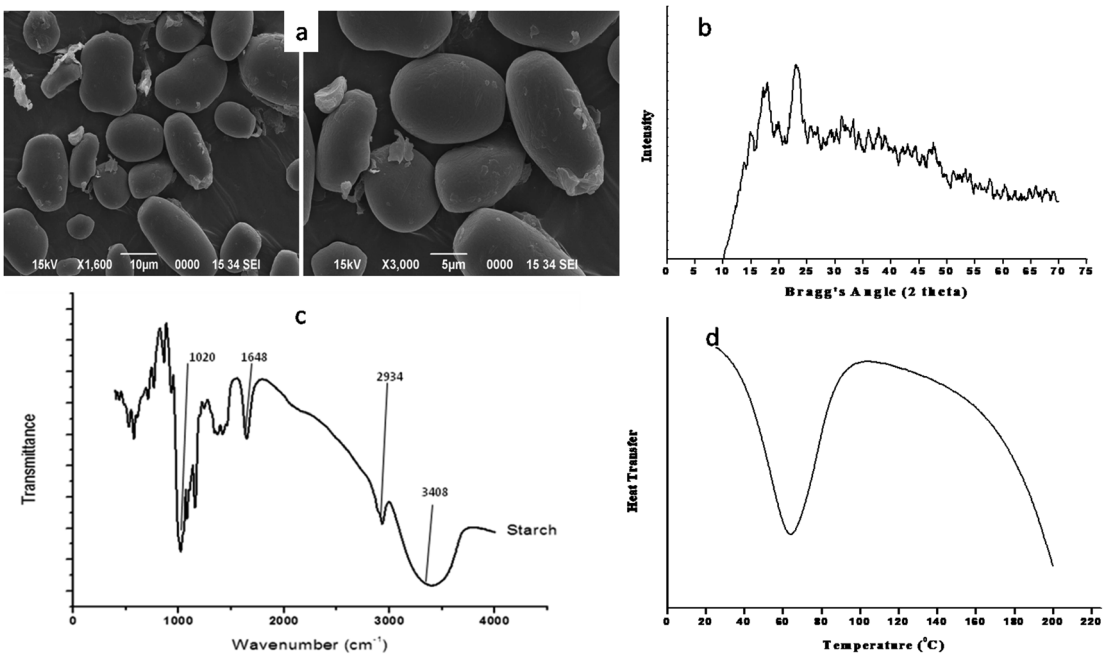

Fig. 2 Morphology and physical properties of water chestnut starch. (a) Scanning electron microscopy images of starch granules, (b) X-ray diffraction pattern, (c) FTIR spectra and (d) DSC thermogram.

regression of the intercept, linear, quadratic and interaction terms of the model were calculated using the least square technique and are shown in Table 2S.† The independent and dependent variables were fitted to the second-order model and tested for adequacy and fitness by ANOVA. The effects of linear, quadratic and interaction terms on each of the responses were observed.

The results show that the models for all the response variables were highly adequate because they have satisfactory levels of R^2 at greater than 90% in three responses and there is a non-significant lack of fit in all the response variables, except for titratable acidity. In these experiments, R^2 for pH, acidity, syneresis, water holding capacity, consistency, firmness, index of viscosity and cohesiveness were observed to be 0.90, 0.77, 0.90, 0.78, 0.81, 0.93, 0.78 and 0.81, respectively, which showed the model to be significantly fitted for the experiment.

3.3.2. Optimization of the process variables. A numerical optimization technique based on the desirability approach was used to determine the workable optimum conditions for fermentation having higher response values. The main criteria for constraint optimization were to maximize WHC, and minimize syneresis. The independent variables were kept in the experimental range for the optimization process. The optimized values of fat, starch, FOS, and inulin obtained were 1.50, 1.00, 0.52 and 1.5, respectively. Fig. 3 shows the various response plots of the interaction of the independent variables on desirability. This combination was further used for the preparation of probiotic mango yogurt and various physicochemical properties were analyzed along with the storage study up to 15 days. From the analysis of the ANOVA data and the statistical parameters, after the removal of the insignificant terms, the final deduced empirical model in terms of coded factors is shown below:

$$\mathrm{pH} = -0.26X_1^2 \quad (7)$$

$$\mathrm{Acidity} = 0.014X_2 - 8.333 \times 10^{-3}X_3 - 0.025X_1^2 \quad (8)$$

$$\mathrm{Syneresis} = -11.95 \times X_1 + 3.08 \times X_1X_3 \quad (9)$$

$$\mathrm{WHC} = 2.72 \times X_1 - 2.28X_2X_4 \quad (10)$$

$$\mathrm{Consistency} = -313.03X_3 + 903.12X_1^2 - 395.60X_1X_2 + 246.85X_1X_4 - 340X_2X_4 \quad (11)$$

$$\mathrm{Firmness} = 54.2X_3 - 108.39X_4 + 89.06X_4^2 - 53.27X_3X_4 \quad (12)$$

$$\mathrm{Index\ of\ viscosity} = 37.06X_1 + 17.4X_3 + 47.01X_4^2 \quad (13)$$

$$\mathrm{Cohesiveness} = 14.68X_2 + 14.04X_3 - 8.84X_4 + 30.30X_4^2 \quad (14)$$

where X_1, X_2, X_3 and X_4 are independent variables and each variable is set in the range of 0.5–1.5%, 1.0–3.0%, 0.5–1.5% and 0.5–1.5%, respectively. The F-value for the lack of fit was insignificant ($p > 0.05$), thereby confirming the validity of the model. The model F values of 9.96, 3.42, 9.62, 3.57, 4.54, 14.85, 3.63 and 4.51, respectively, for the above models imply that the models are significant. There is only a 0.01, 1.40, 0.01, 1.17, 0.39, 0.01, 1.08, and 0.40% chance, respectively, that a model F value could occur due to noise. The value of coefficient of variation (C.V.) was 1.12, 3.17, 9.77, 11.40, 6.22, 16.08, −17.23, and −16.60%, suggesting that the model was reliable and reproducible.

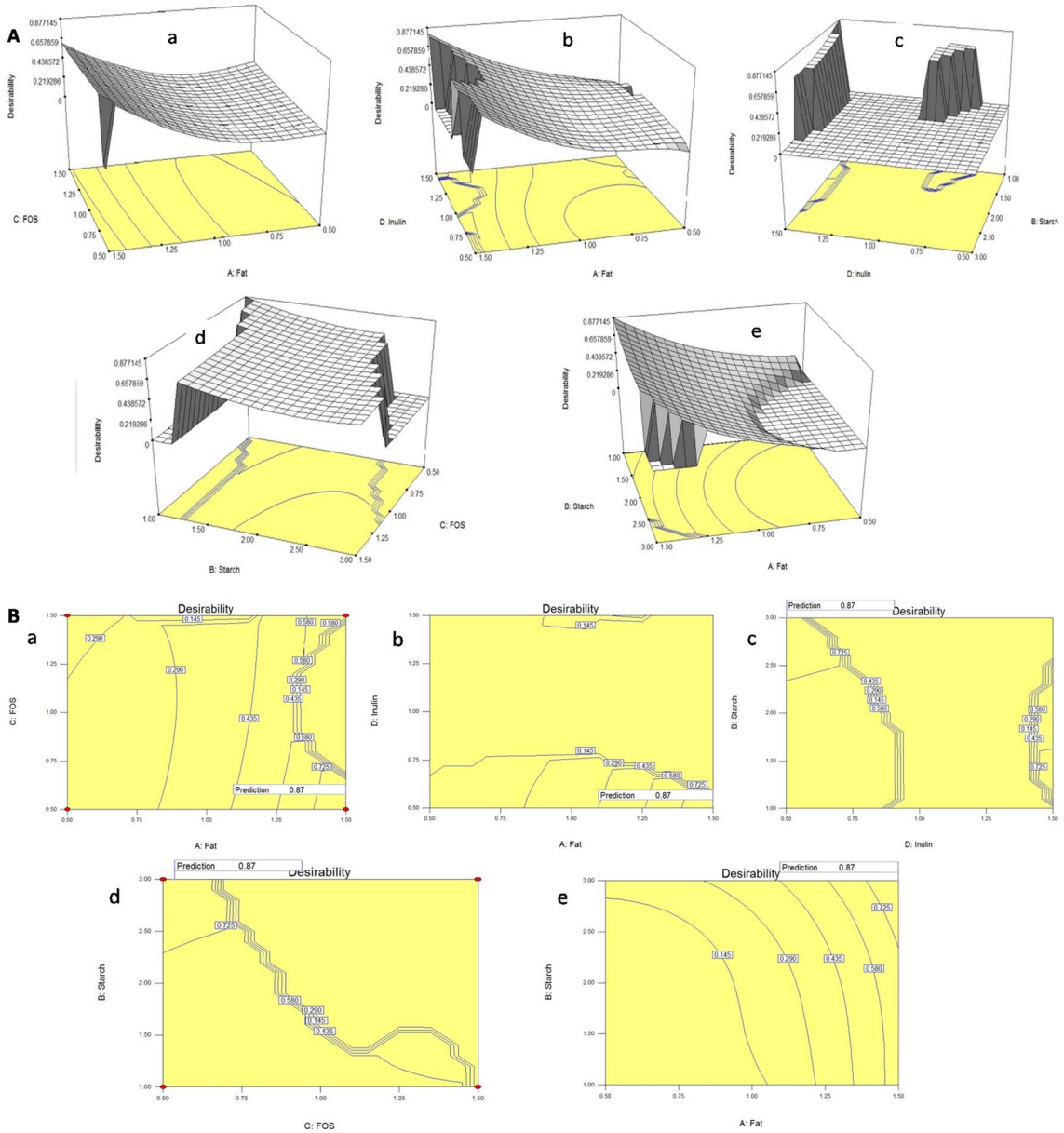

Fig. 3 Response of interaction (3D and contour plots) of independent variables on desirability. (A) 3-D plots of responses of (a) FOS and fat%; (b) inulin and fat%; (c) inulin and starch%; (d) starch and FOS% and (e) starch and fat%. (B) Contour plots of responses of (a) FOS and fat%; (b) inulin and fat%; (c) inulin and starch%; (d) starch and FOS% and (e) starch and fat%.

3.4. Storage study of optimized formulated yogurt

The proximate analysis (Table 3) suggests that during the storage period of the formulated yogurt for 15 days under refrigerated conditions, the addition of starch, FOS and inulin had no significant effect on the protein, fat and ash content of the yogurt samples. Earlier, Aguirre-Mandujano *et al.*[42] also formulated low-fat yogurt consisting of protein content in between 3.43 and 3.91 g per 100 g. The ash content of the samples was in the range between 0.41 and 0.47%, which also showed non-significant changes with the control samples during storage and was almost similar to the values observed by Farinde *et al.*[43] *i.e.* 0.6%, 0.3% and 0.4% in cow's milk, soymilk and commercial yogurt, respectively. The pH of formulated probiotic mango yogurt was lower than that of the control one and decreased gradually during the storage period. The addition of mango pulp, which naturally has an acidic pH, may be the cause of the yogurt's reduced pH value. Our findings are in accordance with the finding of Srisuvor *et al.*,[44] reporting a lower pH value on the addition of inulin. On similar lines, Raju and Pal[31] reported that different bulking agents had a significant effect on the acidity of ASMD (artificially sweetened misti dahi), which increased with maltodextrin and decreased with sorbitol.

Table 3 Storage study parameters of formulated probiotic low fat yogurt[a]

Time	Protein (%)		Fat (%)		Ash (%)		pH		TS (%)	
Days	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample
0	3.59 ± 0.19^{a}	3.87 ± 0.24^{a}	1.51 ± 0.16^{a}	1.55 ± 0.09^{a}	0.39 ± 0.03^{a}	0.43 ± 0.06^{a}	4.72 ± 0.01^{a}	4.49 ± 0.00^{a}	12.33 ± 0.20^{a}	14.88 ± 0.02^{a}
3	3.81 ± 0.26^{a}	3.81 ± 0.23^{a}	1.59 ± 0.22^{a}	1.58 ± 0.08^{a}	0.41 ± 0.01^{a}	0.43 ± 0.02^{a}	4.71 ± 0.01^{a}	4.46 ± 0.01^{b}	12.32 ± 0.12^{a}	14.90 ± 0.03^{a}
5	3.94 ± 0.06^{a}	3.96 ± 0.39^{a}	1.51 ± 0.14^{a}	1.59 ± 0.12^{a}	0.41 ± 0.02^{a}	0.43 ± 0.03^{a}	4.68 ± 0.00^{b}	4.35 ± 0.03^{c}	12.28 ± 0.25^{a}	15.37 ± 0.03^{b}
7	3.83 ± 0.34^{a}	3.98 ± 0.31^{a}	1.59 ± 0.20^{a}	1.57 ± 0.05^{a}	0.39 ± 0.06^{a}	0.43 ± 0.02^{a}	4.52 ± 0.01^{c}	4.36 ± 0.01^{c}	12.78 ± 0.02^{b}	15.42 ± 0.02^{c}
11	3.82 ± 0.11^{a}	3.89 ± 0.18^{a}	1.53 ± 0.16^{a}	1.59 ± 0.13^{a}	0.40 ± 0.06^{a}	0.43 ± 0.04^{a}	4.47 ± 0.01^{d}	4.35 ± 0.00 ^{c}	12.90 ± 0.04^{c}	15.49 ± 0.02^{c}
15	3.75 ± 0.29^{a}	3.86 ± 0.18^{a}	1.58 ± 0.14^{a}	1.58 ± 0.05^{a}	0.41 ± 0.03^{a}	0.44 ± 0.06^{a}	4.43 ± 0.01^{e}	4.18 ± 0.02^{d}	13.26 ± 0.23^{d}	15.52 ± 0.03^{d}

Time	Syneresis (%)		WHC (%)		Consistency (g s)		Firmness (g)		Index of viscosity (g s)		
Days	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample	ΔE
0	17.50 ± 0.70^{a}	10.50 ± 0.41^{a}	48.07 ± 2.02^{a}	55.18 ± 2.90^{a}	5696.80 ± 18.40^{a}	2726.81 ± 7.24^{a}	289.84 ± 1.07^{a}	122.14 ± 1.76^{a}	−398.91 ± 4.50^{a}	−413.29 ± 2.40^{a}	1.33 ± 0.12^{a}
3	17.00 ± 1.41^{a}	10.50 ± 0.70^{a}	48.62 ± 1.94^{a}	59.76 ± 0.68^{b}	5739.18 ± 14.01^{b}	2741.98 ± 5.42^{b}	292.92 ± 2.16^{a}	146.98 ± 1.08^{b}	−415.07 ± 8.45^{b}	−416.20 ± 5.22^{a}	1.35 ± 0.12^{a}
5	19.00 ± 1.20^{a}	11.00 ± 0.01^{b}	54.03 ± 0.43^{b}	59.05 ± 0.63^{b}	5743.08 ± 17.94^{b}	2743.73 ± 5.83^{b}	311.54 ± 1.52^{b}	148.51 ± 0.88^{b}	−419.57 ± 3.42^{b}	−418.09 ± 5.01^{a}	1.45 ± 0.12^{a}
7	19.50 ± 0.70^{b}	11.50 ± 0.80^{b}	55.86 ± 2.10^{b}	59.20 ± 2.09^{b}	5747.74 ± 20.42^{b}	3112.80 ± 8.42^{c}	374.07 ± 2.47^{c}	184.48 ± 1.58^{c}	−419.79 ± 2.47^{b}	−419.91 ± 3.28^{a}	1.57 ± 0.14^{a}
11	20.00 ± 1.41^{b}	12.10 ± 0.10^{c}	58.72 ± 3.57^{c}	59.38 ± 1.73^{b}	5742.17 ± 18.08^{b}	3319.27 ± 4.86^{d}	384.23 ± 1.88^{d}	191.21 ± 1.12^{d}	−418.53 ± 5.46^{b}	−414.52 ± 1.38^{a}	1.63 ± 0.22^{a}
15	20.50 ± 0.70^{c}	12.00 ± 0.30^{c}	58.89 ± 2.26^{c}	59.13 ± 2.63^{b}	5818.15 ± 28.54^{c}	3327.36 ± 10.7^{d}	424.91 ± 3.40^{e}	248.01 ± 1.94^{e}	−420.32 ± 3.58^{b}	−417.00 ± 4.74^{a}	1.67 ± 0.14^{a}

[a] Each value represents mean ± S.D. of triplicates. Values are mean ± S.D. and $^{a-e}$ means in the same column having different superscripts are significantly different at $p \leq 0.05$, as calculated by Duncan's multiple range test (DMRT).

The total solid (TS) content did not vary significantly but was higher in formulated yogurt than that of the control due to the addition of starch and probiotic. Srisuvor *et al.*[44] also reported a gradual increase in TS content of low-fat yogurt on increasing the addition level of inulin and polydextrose. In comparison to the control samples, the syneresis levels in the probiotic mango yogurt significantly (<0.05%) decreased with the addition of starch, FOS, and inulin. Earlier, Milanovic *et al.*[45] reported a decrease in syneresis values in probiotic yogurt samples during 5 days of storage in comparison to the control one. In contrast to syneresis, WHC is an important physical property of yogurt affecting curd stability. Addition of starch, FOS and inulin had a significant effect (<0.05%) on the WHC of the probiotic mango samples. This is due to the fact that the addition of starch, FOS and inulin increases the TS content of the samples and as a result they absorb more amount of water, thereby increasing the WHC values. The experimental verification of decreased syneresis and increased WHC values indicates the validation and adequacy of the developed model equations. The strong correlation between the real and predicted results confirmed that the response model was adequate to reflect the expected optimization.

Yogurt samples were also subjected to texture profile analysis to analyze their consistency, firmness, cohesiveness and index of viscosity. As seen in Table 3 the control samples were observed to have higher consistency values than the probiotic yogurt samples. Due to the addition of prebiotics and starch, the firmness values were observed to be lower in probiotic mango yogurt than in the control. Total color difference (ΔE) of both the samples and control yogurt was calculated for the storage time of 15 days (Tables 3 and 3S†). The increased value of ΔE suggests that the supplements *i.e.* starch, FOS and inulin, and mango pulp along with an artificial colorant (yellow) exhibited color changes different from the control during the storage.

The total bacterial count of probiotic yogurt was 10.5, 9.39 and 9.46 CFU g^{-1} for 0, 7 and 15 days, respectively, while the control yogurt showed 9.5, 8.2 and 7.9 CFU g^{-1} at the aforesaid period. The decrease in counts may be due to the decline in the pH value of the stored samples due to lactose fermentation. Our results are not in agreement with the finding of Srisuvor *et al.*[44] where the numbers of probiotic bacterium and the two yogurt cultures increased during the first 7 days of storage after which the numbers seemed to be stagnant.

3.5. Sensory analysis

From the sensory analysis as shown in Table 4, it has been found that the overall acceptability score of the low-fat flavored probiotic yogurt was almost similar to that of the control. In general, the fat replacer improved various preference characteristics of the yogurt in comparison with the control. The addition of FOS and inulin significantly affected the color, flavor, texture, mouthfeel and overall acceptability.

Table 4 Sensory analysis values of the samples during storage[a]

	Control*					Sample*				
Days	Color	Flavor	Texture	Mouthfeel	Overall acceptability	Color	Flavor	Texture	Mouthfeel	Overall acceptability
0	8.15 ± 0.02^{a}	8.63 ± 0.06^{a}	8.65 ± 0.03^{a}	8.30 ± 0.05^{a}	8.50 ± 0.06^{a}	8.02 ± 0.04^{a}	8.08 ± 0.03^{a}	7.70 ± 0.10^{a}	8.10 ± 0.02^{a}	8.05 ± 0.03^{a}
3	8.15 ± 0.06^{a}	8.80 ± 0.50^{a}	8.60 ± 0.04^{a}	8.20 ± 0.20^{a}	8.33 ± 0.14^{a}	8.10 ± 0.06^{a}	7.90 ± 0.03^{a}	7.58 ± 0.05^{b}	8.10 ± 0.03^{a}	8.00 ± 0.06^{a}
5	7.85 ± 0.04^{b}	8.70 ± 0.05^{a}	8.60 ± 0.09^{a}	7.85 ± 0.05^{b}	8.05 ± 0.20^{a}	8.00 ± 0.09^{a}	7.85 ± 0.08^{a}	7.55 ± 0.02^{b}	8.00 ± 0.08^{a}	7.95 ± 0.04^{a}
7	7.90 ± 0.26^{b}	8.05 ± 0.03^{b}	7.88 ± 0.01^{b}	7.85 ± 0.10^{b}	7.90 ± 0.02^{b}	8.00 ± 0.01^{a}	7.90 ± 0.04^{a}	7.20 ± 0.10^{c}	8.00 ± 0.05^{a}	7.80 ± 0.07^{b}
11	7.55 ± 0.04^{c}	7.97 ± 0.01^{b}	7.80 ± 0.02^{c}	7.50 ± 0.08^{c}	7.90 ± 0.08^{b}	7.50 ± 0.01^{b}	7.62 ± 0.09^{b}	7.00 ± 0.08^{c}	7.25 ± 0.03^{b}	7.60 ± 0.03^{c}
15	7.55 ± 0.05^{c}	8.07 ± 0.06^{b}	7.50 ± 0.05^{d}	7.30 ± 0.01^{d}	7.70 ± 0.10^{c}	7.35 ± 0.02^{b}	7.65 ± 0.01^{b}	7.00 ± 0.06^{c}	7.25 ± 0.09^{b}	7.50 ± 0.01^{d}

[a] Each value represents mean ± S.D. of triplicates. *Values are mean ± S.D. and [a–d]means in the same column having different superscripts are significantly different at $p \leq 0.05$, as calculated by Duncan's multiple range test (DMRT).

4. Conclusion

The physicochemical and sensory problems of fat replacers in low-fat yogurt must be addressed to avoid decreasing its market appeal. A high-quality yogurt should not show any signs of shrinking, breaking down into lumps, or whey-off that might affect the acceptability or preference of the product for the user. In our study, it was evident that water chestnut starch could improve the physical and sensory properties of the formulated yoghurt. The optimization of formulation ingredients *i.e.* starch, fructo-oligosaccharides and inulin along with *Lacticaseibacillus rhamnosus* resulted in probiotic low fat yogurt with better textural and functional properties. The viability of the *L. rhamnosus* probiotic strain was also improved under the storage condition (4 °C) even up to 15 days owing to the utilization of prebiotics *viz.*, FOS and inulin as substrates. Thus, the addition of water chestnut (*Trapa bispinosa*) starch as a potential fat replacer along with prebiotics (fructo-oligosaccharides and inulin) can be useful in the formulation of low-fat probiotic yogurt.

Author contributions

Sangita Borah: investigation; methodology; data curation; formal analysis; visualization; validation; writing – original draft; Tridisha Kakoty: investigation; methodology; data curation; formal analysis; visualization; validation; writing – original draft; Pallab Kumar Borah: formal analysis; visualization; validation; writing – reviewing and editing; Nikhil Kumar Mahnot: formal analysis; visualization; writing – reviewing and editing; Dibyakanta Seth: formal analysis; visualization; writing – reviewing and editing; Falguni Patra: formal analysis; visualization; writing – reviewing and editing; Raj Kumar Duary: conceptualization; visualization; formal analysis; resources; supervision; writing – reviewing and editing.

Conflicts of interest

The authors declare no conflict of interest.

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References

1 C. Lobato-Calleros, O. Martinez-Torrijos, O. Sandoval-Castilla, J. P. Perez-Orozco and E. J. Vernon-Carter, Flow and creep compliance properties of reduced-fat yogurts containing protein-based fat replacers, *Int. Dairy J.*, 2004, **14**, 777–782.

2 A. L. B. Penna, S. Gurram and G. V. B. Canovas, Effect of high hydrostatic pressure processing on rheological and textural properties of probiotic low-fat yogurt fermented by different starter cultures, *J. Food Process Eng.*, 2006, **29**(5), 447–461.

3 M. A. Farag, H. A. Saleh, S. El Ahmady and M. M. Elmassry, Dissecting yogurt: the impact of milk types, probiotics, and selected additives on yogurt quality, *Food Rev. Int.*, 2022, **38**(suppl. 1), 634–650.

4 W. A. D. V. Weerathilake, D. M. D. Rasika, J. K. U. Ruwanmali and M. A. D. D. Munasinghe, The evolution, processing, varieties and health benefits of yogurt, *International Journal of Scientific and Research Publications*, 2014, **4**(4), 1–10.

5 A. E. Blok, D. P. Bolhuis, L. N. Arnaudov, K. P. Velikov and M. Stieger, Influence of thickeners (microfibrillated cellulose, starch, xanthan gum) on rheological, tribological and sensory properties of low-fat mayonnaises, *Food Hydrocolloids*, 2023, **136**, 108242.

6 R. Karimi, M. H. Azizi, M. Ghasemlou and M. Vaziri, Application of inulin in cheese as prebiotic, fat replacer and texturizer: A review, *Carbohydr. Polym.*, 2015, **119**, 85–100.

7 M. C. Rosa, M. R. Carmo, C. F. Balthazar, J. T. Guimaraes, E. A. Esmerino, M. Q. Freitas, M. C. Silva, T. C. Pimentel and A. G. Cruz, Dairy products with prebiotics: an overview of the health benefits, technological and sensory properties, *Int. Dairy J.*, 2021, **117**, 105009.

8 P. Adkar, A. Dongare, S. Ambavade and V. H. Bhaskar, *Trapa bispinosa* Roxb.: a review on nutritional and pharmacological aspects, *Adv. Pharmacol. Pharm. Sci.*, 2014, **2014**, 959830.
9 G. D. Singh, S. Singh, A. S. Bawa and D. C. Saxena, Physicochemical, pasting, thermal and morphological characteristics of Indian water chestnut (*Trapa natans*) starch, *Starch/Staerke*, 2009, **61**, 35–42.
10 D. Qiao, W. Tu, B. Zhang, R. Wang, N. Li, K. Nishinari, S. Riffat and F. Jiang, Understanding the multi-scale structure and digestion rate of water chestnut starch, *Food Hydrocolloids*, 2019, **91**, 311–318.
11 FAO/WHO, *Evaluation of Health and Nutritional Properties of Powder Milk and Live Lactic Acid Bacteria*, Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report, Rome, Italy, 2001.
12 A. Latif, A. Shehzad, S. Niazi, A. Zahid, W. Ashraf, M. W. Iqbal, A. Rahman, T. Riaz, R. M. Aadil, I. M. Khan, F. Ozogul, J. M. Rocha, T. Esatbeyoglu and S. A. Korma, Probiotics: mechanism of action, health benefits and their application in food industries, *Front. Microbiol.*, 2023, **14**, 1216674.
13 V. E. Vera-Santander, R. H. Hernandez-Figueroa, M. T. Jimenez-Munguia, E. Mani-Lopez and A. Lopez-Malo, Health benefits of consuming foods with bacterial probiotics, postbiotics, and their metabolites: a review, *Molecules*, 2023, **28**(3), 1230.
14 N. P. Shah, Probiotic bacteria: selective enumeration and survival in dairy products, *J. Dairy Sci.*, 2000, **83**, 894–907.
15 T. Paseephol and F. Sherkat, Probiotic stability of yogurts containing Jerusalem artichoke inulins during refrigerated storage, *J. Funct. Foods*, 2009, **1**(3), 311–318.
16 A. Rousseaux, C. Brosseau and M. Bodinier, Immunomodulation of B lymphocytes by prebiotics, probiotics and synbiotics: application in pathologies, *Nutrients*, 2023, **15**(2), 269.
17 A. A. Ayana and A. E. Ibrahim, Attributes of low—fat yogurt and kariech cheese made using exopolysaccharides producing lactic acid bacteria, *Am. J. Food Technol.*, 2015, **10**(1), 48–57.
18 S. Hekmat, K. Morgan, M. Soltani and R. Gough, Sensory evaluation of locally-grown fruit purees and inulin fibre on probiotic yogurt in Mwanza, Tanzania and the microbial analysis of probiotic yogurt fortified with *Moringa oleifera*, *J. Health Popul. Nutr.*, 2015, **33**(1), 60–67.
19 M. Succi, P. Tremonte, A. Reale, E. Sorrentino, L. Grazia, S. Pacifico and R. Coppola, Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese, *FEMS Microbiol. Lett.*, 2005, **244**(1), 129–137.
20 E. M. Tuomola and S. J. Salminen, Adhesion of some probiotic and dairy *Lactobacillus* strains to Caco-2 cell cultures, *Int. J. Food Microbiol.*, 1998, **41**(1), 45–51.
21 M. F. Fernandez, S. Boris and C. Barbes, Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract, *J. Appl. Microbiol.*, 2003, **94**, 449–455.
22 R. K. Duary, Y. S. Rajput, V. K. Batish and S. Grover, Assessing the adhesion of putative indigenous probiotic lactobacilli to human colonic epithelial cells, *Indian J. Med. Res.*, 2011, **134**, 664–671.
23 T. Kullisaar, M. Zilmer, M. Mikelsaar, T. Vihalemm, H. Annuk, C. Kairane and A. Kilk, Two antioxidative lactobacilli strains as promising probiotics, *Int. J. Food Microbiol.*, 2002, **72**, 215–224.
24 M. Y. Lin and F. J. Chang, Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356, *Dig. Dis. Sci.*, 2000, **45**(8), 1617–1622.
25 AOAC, *Official Methods of Analysis*, Association of Official Analytical Chemists, Arlington, VA, 16th edn, 1995, vol. 4.
26 G. D. Singh, S. Singh, N. Jindal, A. S. Bawa and D. C. Saxena, Physico-chemical characteristics and sensory quality of Singhara (*Trapa natans* L.): an Indian water chestnut under commercial and industrial storage conditions, *Afr. J. Food Sci.*, 2010, **4**, 693–702.
27 O. J. Olayemi, A. R. Oyi and T. S. Allagh, Comparative evaluation of maize, rice and wheat starch powders as a pharmaceutical excipients, *Niger. J. Pharm. Sci.*, 2008, **7**, 131–136.
28 S. S. Kornblum and S. B. Stoopak, A new tablet disintegrant agent: cross linked polyvinylpyrollidone, *J. Pharm. Sci.*, 1973, **62**(1), 43–49.
29 A. Gani, S. S. Haq, F. A. Masoodi, A. A. Broadway and A. Gani, Physico-chemical, morphological and pasting properties of starches extracted from water chestnuts (*Trapa natans*) from three lakes of Kashmir, India, *Braz. Arch. Biol. Technol.*, 2010, **53**(3), 731–740.
30 P. Y. Chiang, P. H. Li, C. C. Huang and C. C. Wang, Changes in functional characteristics of starch during water caltrop (*Trapa quadrispinosa* Roxb.) growth, *Food Chem.*, 2007, **104**(1), 376–382.
31 P. N. Raju and D. Pal, Effect of bulking agents on the quality of artificially Sweetened misti dahi (caramel colored sweetened yogurt) prepared from reduced fat buffalo milk, *LWT*, 2011, **44**, 1835–1843.
32 N. K. Zanhi and I. A. Jideani, Physico-chemical and sensory qualities of soy and milk solids fortified low-fat yogurt, *Afr. J. Agric. Res.*, 2012, **7**(38), 5336–5343.
33 Y. F. Dufrene, Sticky microbes: forces in microbial cell adhesion, *Trends Microbiol.*, 2015, **23**(6), 376–382.
34 G. I. Geertsema-Doornbusch, H. C. van der Mei and H. J. Busscher, Microbial cell surface hydrophobicity: the involvement of electrostatic interactions in microbial adhesion to hydrocarbon (MATH), *J. Microbiol. Methods*, 1993, **18**, 61–68.
35 M. C. Verdenelli, F. Ghelfi, S. Silvi, C. Orpianesi, C. Cecchini and A. Cresci, Probiotic properties of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from human faeces, *Eur. J. Nutr.*, 2009, **48**(6), 355–363.
36 S. Hizukuri, Y. Takeda, T. Shitaozono, J. Abe, A. Ohtakara, C. Takeda and A. Suzuki, Structure and properties of water chestnut (*Trapa natans* L. var. *bispinosa* Makino) starch, *Starch/Staerke*, 1988, **40**(5), 165–171.

37 V. L. N. Murty, D. Choudhury and P. Bagehi, Physicochemical studies of water chestnut starch (*Trapa bispinosa* Roxb.), *Can. J. Chem.*, 1962, **48**, 2260–2265.
38 I. J. Schoch and E. C. Maywald, Preparation and properties of various legume starches, *Cereal Chem.*, 1968, **45**, 564–573.
39 V. J. Morris, Starch gelation and retrogradation, *Trends Food Sci. Technol.*, 1990, **1**, 2–6.
40 V. Tulyathan, K. Boondee and T. Mahawanich, Characteristics of starch from water chestnut (*Trapa bispinosa* Roxb.), *J. Food Biochem.*, 2005, **29**(4), 337–348.
41 P. Correia, L. Cruz-Lopes and L. Beirao-da-Costa, Morphology and structure of chestnut starch isolated by alkali and enzymatic methods, *Food Hydrocolloids*, 2012, **28**(2), 313–319.
42 E. Aguirre-Mandujano, C. Lobato-Calleros, C. I. Beristain, H. S. Garcia and E. J. Vernon-Carter, Microstructure and viscoelastic properties of low-fat yoghurt structured by monoglyceride gels, *LWT*, 2009, **42**(5), 938–944.
43 E. O. Farinde, T. O. Adesetan, V. A. Obatolu and M. O. Oladapo, Chemical and microbial properties of yoghurt processed from cow milk and soymilk, *J. Food Process. Preserv.*, 2009, **33**, 245–259.
44 N. Srisuvor, N. Chinprahast, C. Prakitchaiwattana and S. Subhimaros, Effects of inulin and polydextrose on physicochemical and sensory properties of low-fat set yoghurt with probiotic-cultured banana puree, *LWT*, 2013, **51**(1), 30–36.
45 S. D. Milanovic, M. D. Caric, M. S. Duric, M. D. Ilicic and K. G. Durakovic, Physico-chemical properties of probiotic yogurt produced with transglutaminase, *Acta Period. Technol.*, 2007, **38**, 45–52.