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Functional improvement of synbiotic yogurt enriched with *Lacticaseibacillus rhamnosus* and aloe vera gel using the response surface method

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Abstract

The response surface technique was applied to produce synbiotic yogurt containing *Lacticaseibacillus rhamnosus* and aloe vera gel (AVG) with high functionality (antioxidant and antimicrobial characters), superior physicochemical properties, and desirable sensory attributes. The experiments were planned around a central composite design (CCD) with two independent variables: AVG concentration (0–5%, w/w) and storage time (1–28 days). The AVG concentration and storage time significantly improved the viability of *L. rhamnosus* up to 7.9 cfu/g during the shelf life which is a practical limit for a probiotic. It enhanced the yogurt's antioxidant and antipathogenic activity, proteolytic content, water-holding capacity, and sensory aspects. High concentrations of AVG reduced the yogurt's desirable textural aspects (hardness and gumminess) except for firmness and adhesiveness and to some degree the sensory properties as well. The results showed that adding 5% AVG to probiotic yogurt produced a functional food with 68% desirability that retained its beneficial properties for at least 14 days under refrigerated storage.

Keywords Probiotics, Aloe vera gel, Yogurt, Synbiotic, Response surface methodology

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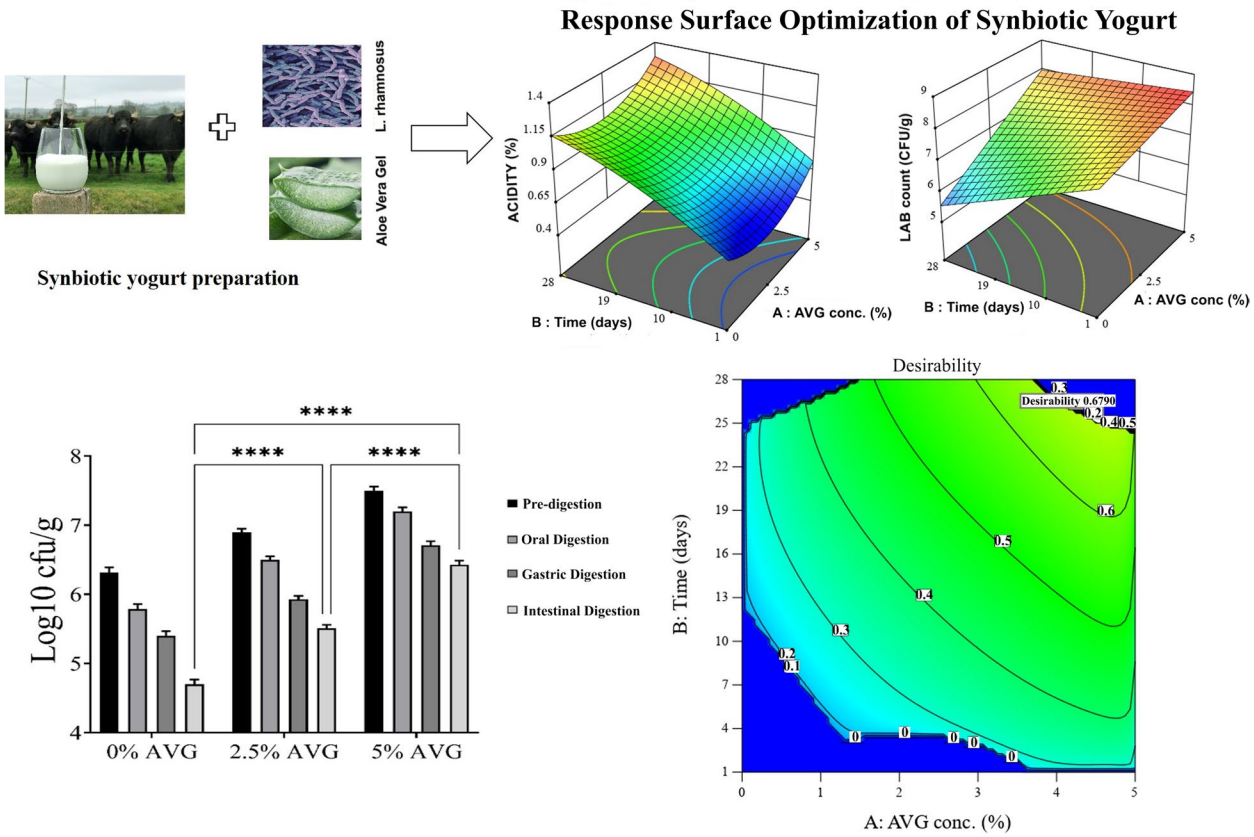
Graphical Abstract



Introduction

Advances in the food industry and increasing consumer awareness of healthy and nutritious foods have accentuated the demand for functional foods providing desirable bioactive and therapeutic properties beyond their nutritional value (Shori 2013). Taking nutrition to the next level, such foods can be readily incorporated into a health-promoting diet to reduce disease (Galanakis 2020). Expanding efforts are being made to improve human health by modulating the intestinal microbiota using prebiotics, live microbial adjuncts, and probiotics. Probiotic organisms require a vehicle to reach the site of action in the human gastrointestinal (GI) tract (Galanakis 2020). The vehicle is generally a food product containing live beneficial bacteria. Probiotic bacteria are used not only because of their health-promoting effects on the intestinal tract but also because of the sensory enjoyment they impart to food and the expanding variety of products formulated with them (Madhu et al. 2012). With its ability to support live cultures, yogurt is the ideal dairy product for introducing probiotics and beneficial functional ingredients into the digestive system as it is eaten worldwide, particularly in South Asia (Hussain et al. 2016; Sengupta et al. 2014). The viability and stability of probiotics during their shelf life are paramount for ensuring a satisfactory minimum level (8–9 log cfu g^{-1}) of viable organisms from the moment they are ingested to the moment they arrive in the gut and manifest their health benefits (da Cruz Rodrigues et al. 2019; Moghanjougi et al. 2020). *Lacticaseibacillus rhamnosus* is one of the best-studied probiotics showing benefits in GI disorders (Tapiovaara et al. 2016) and the strain (NPL 905) used in this study was chosen on the basis of its previous performance in improving shelf life of cottage cheese (Ahmed et al. 2021). To avoid the confounding effect and to optimize the concentration of aloe vera gel, the authors focused only on the one best performing probiotic strain available in their culture collection.

The demand for functional foods with probiotics that boost natural human immunity has risen, especially during the COVID-19 pandemic (Sohrabpour et al. 2021). The pandemic has also highlighted the possibility of

contamination of refrigerated foods with coronavirus, which can withstand near freezing temperatures (Galanakis 2020; Rizou et al. 2020; WHO 2020). Fermented milk products have a long history of being consumed to improve wellbeing, and they are poor coronavirus carriers because of their low pH (Amiri et al. 2020; Kumar et al. 2021). Consumers prefer organic yogurt, which is perceived as healthier and more environmentally friendly than conventional yogurt (Karnopp et al. 2017). Extending the shelf life of probiotic yogurt by adding prebiotics is essential for the sustainable growth of the dairy market (da Cruz Rodrigues et al. 2019). The *Aloe vera* (L.) Burm. f. plant is a well-recognized food additive of prebiotic value and longstanding ethnomedicinal significance in Pakistan. AVG is a beneficial and nutrient-rich product containing prebiotic polysaccharides like acemannan and fructans (Gullón et al. 2015; Tornero-Martínez et al. 2019). It possesses antimicrobial, antiviral, and antioxidant properties (Boudreau & Beland 2006) and synergistically improves probiotics' viability, growth, and metabolic activity in the colon (Mohanty et al. 2018). The polysaccharides in AVG could act as stabilizers, reducing yogurt's tendency to produce whey (syneresis) during storage (Mudgil et al. 2016). The combination of AVG and probiotic bacteria conforms to the consensual definition of a synbiotic by the International Scientific Association of Probiotics and Prebiotics ISAPP (Swanson et al. 2020), as the combination is more beneficial than its constituents (Sanders & Marco 2010).

AVG could improve the longevity of probiotics during transport and storage, preserve them during passage through the gastrointestinal tract (GIT), and enhance the yogurt's physicochemical and organoleptic properties. This study aimed to optimize the formulation of AVG-fortified yogurt to create a synbiotic product with adequate probiotic levels and desirable physicochemical, functional, and sensory qualities. Although incorporating functional components like AVG and probiotics into yogurt improves its nutritional and physiological potential, they could negatively affect its sensory and textural attributes, thus influencing consumer acceptance (Hussain et al. 2016). AVG could also reduce the functional therapeutic limit of viable probiotics needed for health benefits (Hasani et al. 2016). Hence, it is incumbent upon researchers to determine the optimal AVG concentration to retain the qualities of symbiotic yogurt. For this purpose, predictive polynomial quadratic equations and response surface methodology was used to describe AVG's individual and interactive effects during yogurt storage, according to a central composite design with the fermentation process. Response surface methodology (RSM) has been applied for optimizing new product formulations by delineating the effects of independent variables on desirable attributes and is regarded as an effective method for maximizing beneficial properties and reducing unwanted side effects in new products (Selvamuthukumaran et al. 2015).

Materials and methods

Preparation of functional ingredients

Standard yogurt culture Lyofast Y 450B (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) were obtained commercially (Clerici-Sacco SpA, Italy). The probiotic strain *L. rhamnosus* (NPL 905) used in this study was obtained from the in-house culture collection of the National Probiotic Laboratory (NPL) at the National Institute for Biotechnology and Genetic Engineering (NIBGE) and has previously been described (Ahmed et al. 2021). This indigenous probiotic culture was selected based on potential probiotic characteristics, fermentation dynamics, low-temperature viability, and compatibility with starter cultures. Frozen stock cultures of *L. rhamnosus* were revived on De Man, Rogosa, and Sharpe (MRS) agar at 37 °C under anaerobic conditions and subcultured in 100 mL of MRS broth with incubation at 37 °C to log phase. The bacterial suspension was centrifuged at 4000 g for 10 min at 4 °C, the supernatant was discarded, and the probiotic pellet was washed twice with sterile saline (0.5% w/v). The resulting probiotic pellets were suspended in milk at 6×10^8 CFU/ml and cooled to 4 °C until yogurt culturing (Mohan et al. 2020; Zhao et al. 2012).

Low-calorie synbiotic yogurt was made by adding 2.5% or 5% aloe vera gel (AVG) as a prebiotic in a completely randomized design (CRD) experimental approach. Freshly harvested, ripe aloe vera leaves were obtained from a neighboring agricultural station (NIAB, Faisalabad). Whole leaves were cut from the bottom of the plant and rinsed thoroughly with warm distilled water (Pinzon et al. 2018). The leaves were cut open; the gel was scooped out, macerated in a household blender, and sterilized by autoclaving at 121 °C for 16 min to inhibit the growth of its native microbiota (Ahlawat & Khatkar 2011) and stored at 4 °C to prevent oxidation of the polyphenols until use (Amin et al. 2019; Kaur et al. 2015). Total soluble solids were measured with a digital refractometer (PAL-1, Atago, Tokyo, Japan) (Kaur et al. 2015), and pH was determined using a digital pH meter (Hanna Instruments, Rhode Island, USA). The moisture content was estimated by vacuum drying at 60 °C until a constant weight was reached, and the results were expressed on a wet basis (Garcia-Segovia et al. 2010). The crude ash content was determined by incineration in a muffle furnace at 550 °C (Miranda et al. 2009), and crude protein concentration was measured by the Kjeldahl's method (Vega-Gálvez et al. 2011).

Characterization of the synbiotic yogurt included determination of physicochemical parameters, probiotic count, antioxidant, and antimicrobial potential, tolerance to simulated gastrointestinal stress (GIT), and sensory evaluation. The entire experiment was performed three times, and the measurements were done in triplicate.

Synbiotic yogurt preparation

Fresh milk taken from domesticated buffaloes (*Bubalis bubalis*) (6 L) was standardized to the desired fat and SNF (solids-nonfat) values to produce yogurt with the desired set style. The standardized milk was heated at 95 °C for 15 min then cooled to 40 °C in an ice bath. The probiotic yogurt samples were produced by mixing milk samples with a (2% v/v) commercially available freeze-dried starter culture (a 1:1 mixture of *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus*) and 1% (v/v) of *Lacticaseibacillus rhamnosus* suspension. The inoculated milk was divided into three groups to produce synbiotic yogurt supplemented with AVG as a prebiotic and stabilizer: 0% (control), 2.5%, and 5% AVG. The mixtures were put into 200-ml plastic cups and incubated at 42 °C for about 4.5 h until a pH of 4.6 ± 0.1was reached. After fermentation, the yogurt samples were cooled to room temperature for 30 min and transferred to a refrigerator at 4 °C. The samples were stored at 4 °C for > 28 days before determining the functional, physicochemical, textural, and sensory characteristics. Three trials were performed.

Enumeration of *L. rhamnosus* added to yogurt

The total bacterial count in the yogurt samples was measured weekly for 28 days by plating serial dilutions and counting colonies. Yogurt samples (10 g) were homogenized in a stomacher (ProBlend, Synbiosis, USA) for 1 min in 90 mL of sterile water with 1 g L^{-1} peptone. Appropriate dilutions were spread onto MRS agar plates and incubated at 37 °C for 24 h (Mohan et al. 2020).

Physicochemical analysis of synbiotic yogurt

The total ash determination was done using a reported method (Kaur et al. 2015). The total protein content was measured by Kjeldahl's method and fat content by the Gerber method. The pH of yogurt samples was determined using a pH meter (Hanna Instruments, Rhode Island, USA), and titratable acidity was measured as a percentage of lactic acid (Mousavi et al. 2019).

Syneresis measurements

Whey syneresis was measured by placing 30 g of yogurt in a 50 mL volumetric tube made of polypropylene and centrifuged at 3000 g for 15 min at 10 °C. After centrifugation, the separated whey was weighed. Whey syneresis is expressed as the percentage by weight of the whey separated from the sample relative to its initial weight (Kumar et al. 2021).

Determination of water holding capacity (WHC)

WHC of synbiotic yogurt was determined as described by (El-Kholy et al. 2020). A 5 g sample of yogurt was centrifuged at 3282.7 g for 30 min at 10 °C. The supernatant solution was isolated, and the resulting precipitate was weighed.

The WHC was calculated using the following formula:

$$\%\text{WHC} = (\text{original yogurt weight} - \text{supernatant weight})/\text{original yogurt weight} \times 100$$

Viscosity analysis

The viscosity was measured at 25 ± 1 °C using a Brookfield digital rotational viscometer (model DV2T, Ametek, Brookfield, USA). For 50 mL samples, viscosity measurements were carried out after 60 and 70 s, and the mean values from triplicate readings were calculated (Rezaei et al. 2019). Spindle #5 of the viscometer was operated at 16.2 g to achieve the torque values recommended by the manufacturer (10–100%).

Texture analysis

Textural properties, including hardness, cohesiveness, gumminess, and adhesiveness, were measured using a Zwick texture analyzer (TA-XT-Plus (Stable Micro Systems, Surrey, UK) and four cycles of the extrusion test. According to the manufacturer's instructions, the extrusion test used a cylindrical probe with a 40 mm diameter. The probe's penetration rate into the yogurt sample was 10 mm/s, and the penetration depth was 25 mm. All experiments were performed at 5 °C ± 1 °C with three replicates.

Determination of functional attributes

Proteolysis measurement

Proteolysis of yogurt samples during storage was measured by the OPA method (Sáez et al. 2018). Five g of yogurt sample was mixed with 10 ml of 0.72 N trichloroacetic acid with stirring, followed by incubation in an *o*-phthalaldehyde (*o*-PA, Alfa Aesar, Germany) solution containing 2 mL of 40 mg mL^{-1}*o*-Pa) dissolved in methanol, 50 mL of 100 mM sodium tetraborate (Merck,

Germany), 5 mL of 20% (m/v) sodium dodecyl sulfate (Merck, Germany), and 0.2 mL β-mercaptoethanol (Alfa Aesar, Germany) for 10 min at room temperature before reading the absorbance at 340 nm with a UV–visible spectrophotometer (UVD-3200, Labomed Inc. USA).

Antioxidant activity (DPPH method)

The antioxidant activity of the yogurt samples was assayed using DPPH (2,2′-diphenyl-1-picrylhydrazyl radical as described previously (Faraki et al. 2020). Aliquots of the samples (100 µL) were mixed with freshly prepared DPPH solution (0.004% (w/v) in methanol) and allowed to react for 30 min at room temperature. A sample without yogurt was used as a control. DPPH scavenging activity was monitored by the decrease in absorbance at 517 nm, as calculated by the following formula:

$$\text{DPPH scavenging activity (\%)} = (\text{control } A_{517} - \text{sample } A_{517})/\text{control } A_{517} \times 100$$

Effect of AVG on L. rhamnosus viability during simulated yogurt digestion

In vitro digestion was carried out following the INFOGEST protocol (Brodkorb et al. 2019). The simulated salivary (SSF), gastric (SGF), and intestinal (SIF) fluids were prepared and stored at 4 °C. Before in vitro digestion, the fluids were warmed to 37 °C; 2.5 g of each yogurt sample containing different concentrations of AVG and 2.5 g of each control yogurt without AVG were weighed into 50 mL Falcon tubes. The oral phase was made by mixing 13 µL of 0.3 M $CaCl_2{\cdot}2H_2O$, 488 µL of water, and 2 mL of 6.55 mg/mL α-amylase solution (Sigma-Aldrich, USA), in SSF (final activity 75 U/mL). The entire mixture was incubated at 37 °C with stirring at 27.4 g for 2 min. Subsequently, 3 µL of 0.3 M $CaCl_2{\cdot}2H_2O$, 347 µL of water, and 4.55 mL of a 0.07 mg/mL pepsin solution (BioWorld, USA) in SGF (2000 U/mL final) were added. The pH was adjusted to 3 by adding 6 M HCl to start the gastric phase of digestion.

The gastric chyme was stirred (27.4 gm) for two h at 37 °C, then mixed with 20 µL of 0.3 M $CaCl_2{\cdot}2H_2O$, 655 µL of water, 1.25 mL of 160 mM bile extract in SIF, and 8 mL of 22.15 mg/mL pancreatin solution (Merck, Germany) in SIF (100 U/mL final). The pH was adjusted to 7.0 with 1 M NaOH, and the mixture was stirred at 27.4 g for 2 h at 37C. A sample was collected and placed in an ice bath at each phase to stop the enzymatic reaction. At the end of each stage of artificial digestion, yogurt mastication was performed in a stomacher (ProBled Synbiosis, UK) in 0.05 M phosphate-buffered saline (PBS) at high speed for one min. The suspension was serially diluted, plated on MRS agar, incubated at 37 °C for 48 h, and colonies counted. The results were reported as log cfu/g of yogurt.

In vitro growth control of selected pathogens by synbiotic yogurt

The pathogenic bacteria, *Listeria monocytogenes* strain ATCC 7644 and *E. coli* strain ATCC 25,922, were cultured as described (Falah et al. 2021) and centrifuged at 3282.7 g for 10 min at 4 °C. The supernatants were discarded, and the pellets were washed with cold, sterile PBS (pH 7.0) and dissolved in the same buffer to a final bacterial titer of 6 log cfu/g. Each pathogen suspension was mixed (10%, *w*/*v*) with 10 g of the synbiotic yogurt samples with different AVG concentrations. These spiked yogurt samples were kept at 20 °C, and microbial levels were determined on days 0, 14, and 28 using the pour-plate method. The plates were incubated for two days at 37 °C, colonies were counted, and the reduction in cfu of the added pathogens was calculated as a percentage of initial titer.

Sensory evaluation

After refrigerated storage overnight, all yogurt samples underwent a hedonic sensory evaluation (consumer acceptance test) by a panel of fifty untrained healthy individuals who were regularly consuming yogurt in their diet were selected to ensure realism and to understand the product acceptance and consumer behavior (Singh-Ackbarali & Maharaj 2014). The panel consisted of both males and females (21 to 40 y) with experience of having yogurt in their diets and no allergic reactions to milk. The sensory evaluation was performed in individual booths under controlled incandescent lighting and temperature (20 °C). All yogurts (15 mL) were served at 7 °C using a coded plastic container following a type-III balanced incomplete block design ($t=10$, $k=4$, $r=6$, $b=15$, $l=2$), in which ten independent evaluations were obtained for each block, totaling 600 responses (Karnopp et al. 2017). The participants were asked to eat some bread and rinse their mouths with sterile water between tastings. No information about the sample types was provided to the panelists to prevent any biases. A standard 9-point hedonic scale (1 = highly dislike, 5 = neither like nor dislike, and 9 = highly like) was used (Mohan et al. 2020). The panelists were in individual chambers with no contact during the sensory assessment. Before evaluating each sample, the panelists were given water to neutralize the flavor and effects of the previous sample. The

descriptors used in this study were taste (acid/sour, bitter or astringent), appearance (smooth, lumpy or thick), color (creamy, white or yellowish), mouthfeel (light, thick, floury or slimy) and overall acceptance of synbiotic yogurt (Mousavi et al. 2019).

Optimization of fermentation conditions

Response surface methodology (RSM) was used to optimize AVG-enriched yogurt production under the influence of independent variables such as storage time (X1) and AVG concentration (X2) and 20 dependent variables *(L. rhamnosus* cfu, pH, acidity, WHC, fat, protein, ash, viscosity, syneresis, antioxidant activity, proteolysis, anti-pathogenicity, hardness, gumminess, cohesiveness and adhesiveness, taste, mouthfeel, appearance, and general acceptance were observed in this study. The preliminary experiments selected these concentrations, which showed that AVG concentrations of 0–5% and storage times of 1–28 days resulted in palatable yogurt products. The experiments were randomized to minimize variability due to extraneous factors. The experimental design included 13 experiments consisting of eight star-points and five center-points (with two factors and three levels for each variable) to determine the method's repeatability. The face-centered central composite design matrix and experimental results are shown in (Table 1).

The optimal conditions for producing AVG-fortified probiotic yogurt were determined by taking the maximum values of the independent variables (storage time and AVG concentration) and the responses (*L. rhamnosus* cfu, pH, acidity, WHC, fat, protein, ash, viscosity, syneresis, antioxidant activity, proteolysis, anti-pathogenicity, hardness, gumminess, cohesiveness and adhesiveness, taste, mouthfeel, appearance, and general acceptance). While the variables of syneresis and pH were optimal at minimum levels, the variables of taste, appearance, mouthfeel, and overall acceptability were evaluated over a range of levels.

Statistical analysis

This study incorporated RSM into the data analysis using a commercial statistical package, Design-Expert version 13.0.0 (Stat-Ease Inc., Minneapolis, USA). All experiments were run in triplicate, and multiple regression analyses were performed to fit the data to the best model for each response. The correlation coefficients and mismatch test were used to determine the significance of the regression equations (Table 2). The analysis of variance (ANOVA), the regression coefficients of individual linear or quadratic models, and the optimization of the polynomials were significant at $P < 0.01$ and $P < 0.05$.

Results and discussion

Characterization of AVG

In this study, the weight of total solids (TS) of AVG comprised 1.62% of the total eight (w/w), while a value of 1.8% (w/w) for total solids in AVG was reported in a prior study (Kaur et al. 2015). The difference in TS might be due to seasonal variations. In summer, more water evaporation occurs because of the extended daylight length (Boudreau & Beland 2006). The AVG TS content increased the gelation time of milk and acted as a buffer to protect the bacteria from the harmful effects of low pH (Wu et al. 2009). A high TS level was reported to affect apparent viscosity (Wu et al. 2009) but our experiments did not see this. The total soluble solids gave a reading of 1.8°brix. Here, the moisture content of AVG was found to be 95.4%, but seasonal fluctuations and day length affect water availability (Scala et al. 2013; Soares et al. 2019). The ash content in AVG was observed to be 20.7% (w/w) of the dry matter and this was considered significantly high compared to other fractions (Boudreau & Beland 2006). The pH of AVG was 4.3 in this study, while previous studies found the pH of AVG to be 4.4 to 4.7 (Boudreau & Beland 2006; Kaur et al. 2015), with an acidity of 0.27%. The accumulation of organic acids, such as malic acid, in aloe vera pulp, could be a reason for the high acidity of AVG (Kaur et al. 2015). AVG usually contains only a tiny amount of protein (Wu et al. 2009); AVG protein content was 5.41 µg/mL in this study.

Viability of *L. rhamnosus* during refrigerated storage in synbiotic yogurt

A viable probiotic count in yogurt above the recommended level of 6 log cfu/g was the critical qualitative criterion in the end product until expiration (Güler-Akın et al. 2018). RSM plots show that the *L. rhamnosus* (LAB) count was significantly higher ($P < 0.05$) in synbiotic yogurt samples containing 5% AVG concentrations then probiotic yogurt during the initial weeks of storage (Fig. 1A). The number of *L. rhamnosus* diminished during the storage period, although the decline rate was lower in the synbiotic samples than in probiotic yogurt. The maximum count of *L. rhamnosus* (8.41 log cfu/g) was observed on the first day of chilled storage in the synbiotic yogurt sample with 5% AVG, whereas the minimum count (5.31 log cfu/g) was found in the control yogurt sample at the end of the storage period. The higher viable count of *L. rhamnosus* with increasing AVG in yogurt could be ascribed to the presence of prebiotic polysaccharides such as acemannan (Chiodelli et al. 2017). Prebiotics help maintain the metabolic activity of probiotics in the presence of organic acids and mitigate their negative

Table 1 Experimental design matrix for optimization of AVG enriched synbiotic yogurt produced by *L. rhamnosus* according to CCD

Independent variables			Dependent variables (responses obtained from the experiment)									
			Physicochemical analysis								Functional analysis	
Run	X1: AVG conc. (%)	X2: Storage time (days)	pH	acidity (% w/w)	WHC (% w/w)	Syneresis (% w/w)	Protein content (%)	Fat content (%)	Ash content (%)	Viscosity (cp)	Lab count (log cfu/g)	Antioxidant activity (%)
1	2.5	14.5	3.8 ± 0	0.83 ± 0	69 ± 0	37 ± 0	3.4 ± 0	3.4 ± 0	0.4 ± 0	335 ± 0.4	7.4 ± 0	62 ± 0
2	0	14.5	4.1 ± 0	0.91 ± 0	60 ± 0	52 ± 0	3.5 ± 0	3.4 ± 0	0.4 ± 0	1639 ± 0.5	6.9 ± 0	51.7 ± 0
3	2.5	14.5	3.9 ± 0	0.9 ± 0	68 ± 0	36 ± 0	3.2 ± 0	3.4 ± 0	0.34 ± 0.4	4191 ± 0.7	7.1 ± 0.4	63 ± 0
4	2.5	14.5	3.7 ± 0	0.73 ± 0	67 ± 0	36 ± 0	3.3 ± 0	3.4 ± 0	0.33 ± 0	3591 ± 0.8	7.6 ± 0.2	63 ± 0
5	2.5	14.5	3.6 ± 0	0.88 ± 0	69 ± 0	39 ± 0	3.24 ± 0	3.4 ± 0	0.4 ± 0	3979 ± 0.6	7.2 ± 0.2	61 ± 0
6	5	14.5	3.6 ± 0	1.1 ± 0	72 ± 0	29 ± 0	3.2 ±	3.3 ± 0.5	0.4 ± 0	5193 ± 0.2	8.2 ± 0.2	70 ± 0
7	2.5	14.5	4 ± 0	0.83 ± 0	71 ± 0	38 ± 0	3.21 ± 0	3.4 ± 0	0.4 ± 0.3	3667 ± 0.4	7.9 ± 0	59 ± 0
8	0	1	4.4 ± 0	0.56 ± 0	54 ± 0	51 ± 0	3.4 ± 0	3.5 ± 0	0.4 ± 0	1520 ± 0.2	7.5 ± 0	49 ± 0
9	2.5	1	4 ± 0	0.6 ± 0	64	44 ± 0	3.4 ± 0	3.4 ± 0	0.39 ± 0	3025 ± 0	8.1 ± 0	59 ± 0
10	0	28	4 ± 0	1.24 ± 0.1	66 ± 0.1	45 ± 0.1	3.24 ± 0.3	3.41 ± 0.2	0.41 ± 0.2	1121 ± 0	5.31 ± 0.2	55.7 ± 0.3
11	5	1	4.2 ± 0	0.65 ± 0.4	70 ± 0.03	38 ± 0.2	3.36 ± 0.3	3.3 ± 0.2	0.36 ± 0.4	4520 ± 0	8.41 ± 0.3	63.1 ± 0.5
12	2.5	28	3.8 ± 0	0.93 ± 0.1	69 ± 0	34 ± 0	3.2 ± 0.5	3.31 ± 0	0.39 ± 0.3	3635 ± 0.1	6.9 ± 0.1	67 ± 0.3
13	5	28	3.4 ± 0	1.33 ± 0	77 ± 0	30 ± 0	3.11 ± 0	3.16 ± 0	0.41 ± 0	4732 ± 0	7.9 ± 0	72.4 ± 0

Table 1 (continued)

Independent variables	Functional analysis			Texture analysis				Sensory analysis			
Run	Proteolytic activity (%)	inhibition of *E.coli* (%)	Inhibition of *L.monocytogenes* (%)	Hardness (N)	Gumminess (N)	Adhesiveness (N)	Cohesiveness (N)	Taste	mouthfeel	appearance	Overall acceptability
1	0.5 ± 0	7.9 ± 0	8.5 ± 0	33 ± 0	1.1 ± 0	22.3 ± 0	0.64 ± 0.3	7.5 ± 0.5	7.5 ± 0.6	7.3 ± 0.02	7.5 ± 0.5
2	0.42 ± 0	5.32 ± 0.4	5.17 ± 0	43.1 ± 0	1.65 ± 0	28.8 ± 0	0.62 ± 0.2	7.3 ± 0.5	7 ± 0.7	7.2 ± 0.7	7.8 ± 0.5
3	0.5 ± 0	7.43 ± 0.6	7.9 ± 0.4	34.3 ± 0	1.14 ± 0	21.3 ± 0	0.63 ± 0.4	7.5 ± 0.5	7.5 ± 0.3	7.3 ± 0.5	7.5 ± 0.4
4	0.5 ± 0	8.5 ± 0.3	6.7 ± 0	35.6 ± 0	1.2 ± 0	21.5 ± 0.3	0.64 ± 0.6	7.5 ± 0.5	7.5 ± 0.6	7.3 ± 0.2	7.5 ± 0.32
5	0.5 ± 0	7.45 ± 0	7.5 ± 0	37 ± 0	1.23 ± 0	20.6 ± 0.6	0.64 ± 0.5	7.5 ± 0.5	7.5 ± 0.6	7.3 ± 0.2	7.5 ± 0.12
6	0.42 ± 0.8	8.43 ± 0.4	7 ± 0	28.3 ± 0	0.63 ± 0.3	19.5 ± 0.5	0.67 ± 0.4	7.2 ± 0.5	7.6 ± 0.5	7.1 ± 0.2	7.2 ± 0.2
7	0.5 ±	7.4 ± 0	7.6 ± 0	35.7 ± 0	1.25 ± 0	21.7 ± 0.2	0.64 ± 0	7.5 ± 0.5	7.5 ± 0.5	7.3 ± 0.3	7.5 ± 0.4
8	0.4 ± 0.3	4.1 ± 0.3	3.33 ± 0	44.6 ± 0.4	1.47 ± 0.3	30.5 ± 0	0.62 ± 0	8 ± 0.07	8.5 ± 0.5	8 ± 0.3	8.5 ± 0.4
9	0.5 ± 0	6 ± 0	4 ± 0	34 ± 0	0.7 ± 0	22. ± 0	0.6 ± 0	8 ± 0.4	8.1 ± 0.5	7.7 ± 0.6	8 ± 0.5
10	0.44 ± 0	7.34 ± 0.2	6.9 ± 0.1	42.1 ± 0	1.78 ± 0	27.1 ± 0.2	0.64 ± 0.11	7 ± 0.5	6.9 ± 0	6.8 ± 0.3	7.7 ± 0
11	0.51 ± 0	6.4 ± 0.1	6.3 ± 0.2	29.2 ± 0	0.51 ± 0.3	18.9 ± 0.3	0.65 ± 0.32	7.5 ± 0.5	7.8 ± 0.4	7.5 ± 0.4	8 ± 0.31
12	0.51 ± 0	9.2 ± 0.4	9.7 ± 0.3	37 ± 0.2	1.38 ± 0.5	19.2 ± 0.12	0.66 ± 0.62	7 ± 0.4	6.8 ± 0.3	7 ± 0.3	7 ± 0.5
13	0.6 ± 0	13.5 ± 0	12.19 ± 0	29 ± 0	0.74 ± 0	23 ± 0	0.68 ± 0	7.1 ± 0	7.3 ± 0.2	6.9 ± 0.6	7 ± 0.4

Results are means of three independent experiments with three repetitions ± SD ($n = 3$)

Table 2 The analysis of variance (ANOVA) and estimated regression coefficients of predicted linear and quadratic polynomial models for the responses in the optimization of synbiotic yogurt

Coefficients	Physicochemical analysis								Functional analysis		
	pH	acidity (% w/w)	WHC (% w/w)	Syneresis (% w/w)	Protein content %	Fat content %	Ash content %	Viscosity (cp)	Lab count (log cfu/g)	Antioxidant activity %	Proteolytic activity %
Intercept	3.9	0.833	67.4	39.14	3.29	3.398	0.354	3758.8	7.417	61.1	0.47
X_1	-0.2*	0.062	6.5*	-8.48*	-0.1*	-0.1*	-0.002	1694.2*	0.8*	8.17*	0.04*
X_2	-0.2*	0.28*	4*	-3.92*	-0.1*	-0.1*	0.012	70.5	-0.6*	4.05*	0.03
X_1X_2	0	2.13	0	0	0	-0.01	0.008	152.75	0.42*	0	0
$X1^2$	0	0.18*	0	0	0	-0.1*	0.017	-348.4	0	0	0
$X2^2$	0	-0.07	0	0	0	-0.02	0.027*	-434.4	0	0	0
Model (*p*-value)	*	**	***	***	**	***	ns	***	***	***	**
Lack of fit (*p*-value)	0.5 ns	0.1 ns	0.2 ns	0.05 ns	0.6 ns	0.1 ns	0.3 ns	0.7 ns	0.8 ns	0.5 ns	0.3 ns
R^2	0.67	0.90	0.9	0.88	0.64	0.95	0.696	0.968	0.920	0.95	0.54

Coefficients	Functional analysis		Texture analysis				Sensory analysis			
	inhibition of *E.coli* %	Inhibition of *L. monocytogenes* %	Hardness (N)	Gumminess (N)	Adhesiveness (N)	Cohesiveness (N)	Taste	mouthfeel	appearance	Overall acceptability
Intercept	7.60	7.14	35.57	1.13	21.2	0.64	7.48	7.5	7.29	7.46
X_1	1.9*	1.67*	-7.3*	-0.5*	-4.2*	0.02	-0.08*	0.05	-0.08*	-0.3*
X_2	2.3*	2.5*	0.02	0.21*	-0.41	0.02*	-0.4*	-0.6*	-0.42*	-0.47*
X_1X_2	0.97*	0	0	0	1.83*	0	0.15*	0.3*	0.15*	-0.05*
$X1^2$	0	0	0	0	3.39*	0	-0.2*	0	-0.1*	0.15*
$X2^2$	0	0	0	0	-0.08	0	0.073	0	0.1*	0.145*
Model (*p*-value)	***	***	***	***	**	***	***	**	***	***
Lack of fit (*p*-value)	0.2 ns	0.2 ns	0.7 ns	0.1 ns	0.6 ns	0.3 ns	0.2 ns	0.2 ns	0.04 ns	0.2 ns
R^2	0.94	0.86	0.92	0.91	0.21	0.78	0.9	0.9	0.98	0.96

X1: storage time. X2: AVG concentrations

* Significant at $p \leq .05$. ** Significant at $p \leq .01$. *** Significant at $p \leq .001$. *ns* not significant

impact (Nagpal et al. 2012). Synbiotics should improve the shelf life and enhance the health-promoting properties of probiotics (Gullón et al. 2015). Both on the first day and during the storage period, mold and yeast were not detected in any probiotic yogurt samples, presumably due to good hygienic conditions during preparation and storage.

Changes in physicochemical attributes of synbiotic yogurt

Low pH or acidity is an essential parameter for yogurt quality. The pH changes in yogurt samples (probiotic and synbiotic) were monitored at seven-day intervals up to 28 days of chilled storage (Fig. 1B & C). Increasing AVG concentration and storage time decreased the pH. It was ascertained from the ANOVA table that the RSM model for acidity was quadratic and statistically significant ($P \leq 0.05$), but the lack of fit was not significant. In this study, a reciprocal relationship was observed between acidity and the concentration of AVG, most likely because *L. rhamnosus* could utilize AVG to produce more lactic acid. The prebiotic polysaccharides and other growth-promoting substances in aloe vera rejuvenate the probiont's metabolism and the yield of organic acids during refrigerated storage of food products (Mukhekar et al. 2018). The gradual decrease in pH with a concomitant increase in acidity in probiotic yogurt samples could be due to acidification during storage, residual enzyme activity in the starter (Wijesundara & Adikari 2017), or the high amounts of non-protein nitrogen and vitamins that fuel microbial growth (Govindammal et al. 2017). These changes can lead to the deterioration of the flavor of the yogurt.

The response surface plots showed that the protein content of probiotic yogurt made with different percentages of aloe vera gel gradually decreased with increasing storage time (Fig. 1D). At the same time, the ash content of probiotic and synbiotic yogurt continued to increase over the storage period (Fig. S1-A), possibly because of the breakdown of complex organic matter into a more straightforward form by *Lactobacillus* during the fermentation process (Sengupta et al. 2014). The highest percentage of fat was reported in probiotic yogurt. The quadratic model for fats was found to be statistically significant ($P \leq 0.05$), but the lack of fit was not significant relative to the pure error of fat percentage, which reduced steadily with more aloe vera (Fig. S1-B). It could be that the higher moisture content in aloe vera (Yadav & Shukla 2014) helped to increase the moisture content of synbiotic yogurt (Nazni & Komathi 2014). The decreasing proteolytic activity of LAB results in fewer breakdown products translating into an overall lesser protein content (Sengupta et al. 2014).

Changes in syneresis and WHC of synbiotic yogurt

Syneresis occurs when water is released due to gel shrinkage or is extracted from a gel (Ghaderi-Ghahfarokhi et al. 2021). During the post-acidification process, the network of casein micelles in the yogurt fails to stabilize itself, resulting in a deterioration in the quality of the fermented product, including loss of sensorial and textural parameters (Hussain et al. 2016), which makes these yogurts unacceptable to consumers. In this study, the syneresis of probiotic yogurt samples increased with storage, but the increasing concentration of aloe vera gel in yogurt samples during storage decreased whey secretion (Fig. S1C), possibly due to the stabilizing nature of aloe vera (Govindammal et al. 2017). These results contrast with Azari et al. (Azari-Anpar et al. 2017), where increasing the concentration of aloe vera gel increased syneresis. The increased acidity of milk can lower the pH value of casein to its isoelectric point, resulting in syneresis (Vital et al. 2015).

The water-holding capacity (WHC) of the yogurt is also a fetching property since it is indicative of its coagulability (El-Kholy et al. 2020). The highest WHC (77%) was observed in synbiotic yogurt containing 5% AVG on the 28^{th} day of chilled storage, while the lowest (54%) was found in the probiotic yogurt samples on the first day of storage. The high concentration of AVG significantly influenced the WHC of probiotic yogurt ($p < 0.0001$), but the storage time did not (Fig. S1D). The increased WHC of AVG-containing samples could result from the absorption of unbound water (Güler-Akın et al. 2018).

Changes in viscosity of synbiotic yogurt

Viscosity is a qualitative parameter that plays a crucial role in a yogurt's acceptability by consumers. Generally, the viscosity and syneresis of yogurt have an inverse relationship (Bansal et al. 2016). Viscosity reflects the compactness and firmness of yogurt samples, and the more, the better (Hasani et al. 2016). The ANOVA data have shown that the quadratic model for viscosity was statistically significant ($P \leq 0.005$), but the lack of fit was not significant. The viscosity of the yogurt samples was 1121 to 5193 cp, and synbiotic yogurt samples had a higher viscosity than probiotic ones (Fig. S1E). Similar results were reported by (Tahmasebi & Mofid 2021), where high prebiotic content resulted in more thick yogurt. The increase in viscosity in the AVG-yogurt samples can be attributed to casein micelle rearrangement (Allgeyer et al. 2010; Noh et al. 2013) and the formation of a colloidal system by the polysaccharide polymers of AVG (Boudreau & Beland 2006). A yogurt's viscosity is also a function of acid production because an increase in acidity causes the coagulation of milk protein, which increases viscosity (Hill et al. 2017). The exopolysaccharides (EPS)

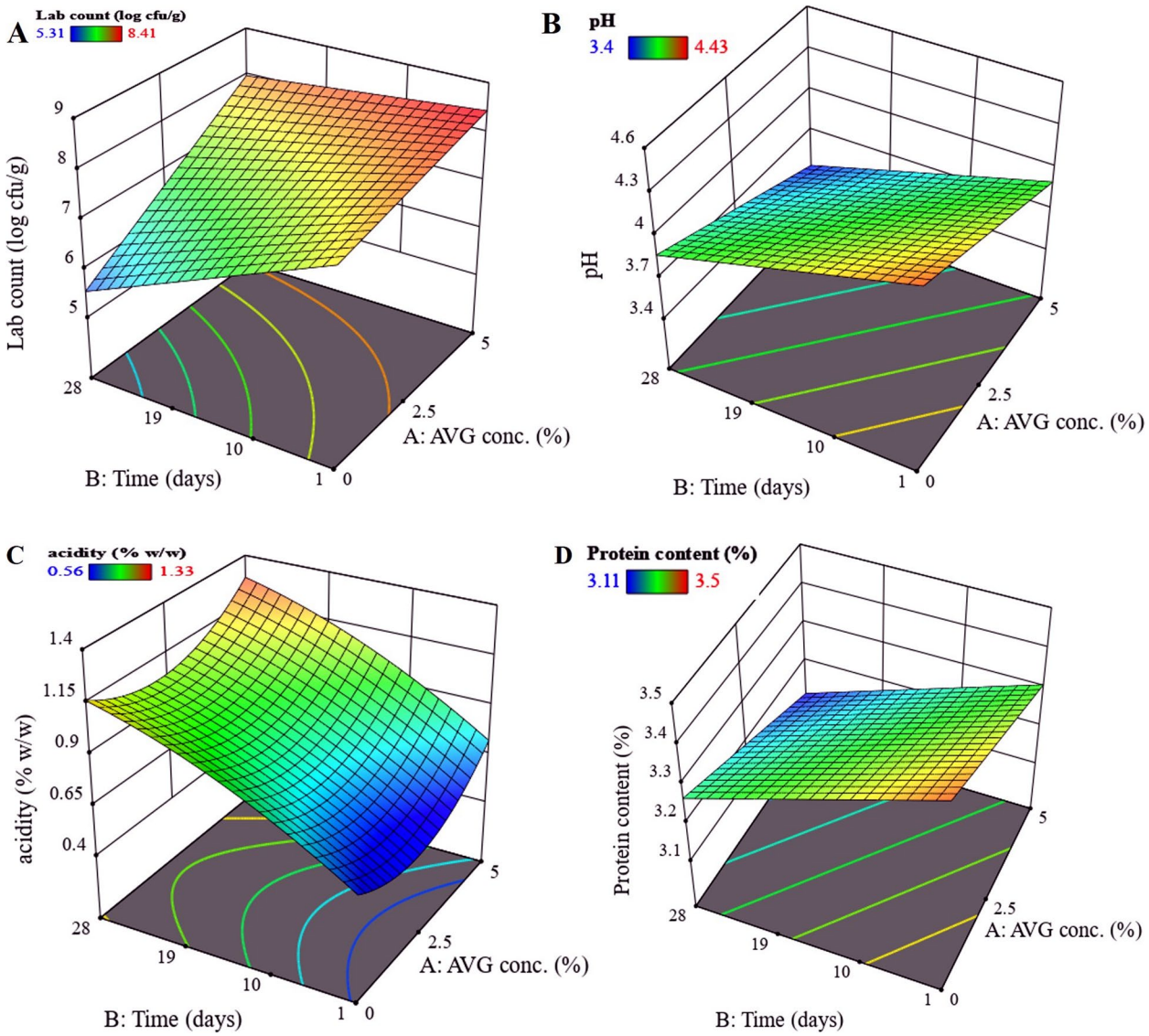


Fig. 1 Three dimensional response surface plot showing effects of interaction between AVG concentration (X1) and storage time (X2) on **A** viability of *L. rhamnosus* (log cfu/g), **B** pH, **C** acidity, and **D** protein content of synbiotic yogurt. The color spectrum from blue to red indicates the range from lowest to highest

produced by probiotic bacteria could also enhance the viscosity of yogurt (Mousavi et al. 2019). In contrast, researchers have reported that increasing the AVG concentration reduced the viscosity of yogurt due to the high concentration of polyphenols in AVG (Azari-Anpar et al., 2017).

Changes in texture profile of synbiotic yogurt

The textural profile parameters, namely hardness, cohesiveness, springiness, and gumminess of probiotic and synbiotic yogurt, are presented in Fig. 2. Hardness is the most frequently evaluated parameter for ascertaining the quality of yogurt texture. Adding AVG to probiotic yogurt samples initially decreased their hardness, while the hardness increased with increasing storage time (Fig. 2A). An overall increase in softness of synbiotic yogurts containing AVG was observed during the first week of storage because of its antimicrobial compounds (Sonawane et al. 2021). These compounds can harm the starter culture (*S. thermophilus* and *L. delbrueckii* sub sp. bulgaricus), reducing the yogurt's hardness and changing its texture (Azari-Anpar et al. 2017). The starter culture species, *L. delbrueckii*, is essential for developing food texture through EPS production, and its inhibition

caused the softness of yogurt in control samples (Chand et al. 2021). This effect on food might be due to the ability of specific LAB spp. to channel a larger part of the available sugars into the biosynthesis of EPS. At the same time, the improvement of hardness during the chilled storage period for synbiotic yogurt can be equated to the ability of adjunct *L. rhamnosus* to produce EPS which binds the free water of yogurt to form a firmer gel and increasing hardness (Han et al. 2016). EPS also helps to improve texture and stabilize the yogurt (Konieczna et al. 2018). The polysaccharides in AVG, including glucomannan, acemannan, and cellulose, contribute to the formation of the gel structure. Reinforcement of gel structure occurs at low temperatures, which improves the hardness of synbiotic yogurt with increasing time (Shahrezaee et al. 2018).

Adhesion refers to the force required to overcome the bond between the surfaces of the coagulate and the remaining material (Fadela et al. 2009). The adhesiveness of synbiotic yogurt samples was lower than probiotic yogurt samples during the first week of storage (Fig. 2C), but the adhesive value improved during the chilled storage period. A decline in the pH of yogurt during storage, which contracts the gel, should increase adhesiveness (El-Kholy et al. 2020).

The last parameter of gumminess has an undesirable effect on the texture and appearance of yogurt samples (Azari-Anpar et al. 2017). The addition of either 2.5 or 5% AVG to the yogurt sample reduced its gumminess (Fig. 2B), possibly because of the loss of matrix proteins through proteolytic activity (Azari-Anpar et al. 2017; Mousavi et al. 2019). Our results demonstrated that the effect of AVG concentration on the cohesiveness of synbiotic yogurts significantly improved with prolonged chilled storage ($P < 0.05$) in comparison to control yogurt without AVG (Fig. 2D).

Functional aspects of synbiotic yogurt

Changes in the proteolytic activity of synbiotic yogurt

The response surface plots showed a significant increase ($P < 0.05$) in proteolysis of probiotic yogurt containing AVG during the storage period in comparison to probiotic yogurt. Yogurt with a high AVG concentration showed more proteolysis than controls on day 28 of storage (Fig. 3A). The increase in proteolysis could be due to the growth and metabolic activity of *L. rhamnosus* during storage, stimulated by the bioactive components of AVG such as aloin, phytosterols, and acemannan (Basannavar et al. 2014). Heightened proteolysis also increases the concentration of amino acids available for the growing probiotic population, thus boosting their numbers. Proteolysis would also improve the digestibility of the synbiotic yogurt and offer more health benefits (Shah 2007). However, the risk that the degradation of peptide products might adversely affect the yogurt's consumer appeal cannot be discounted. Fortunately, *L. rhamnosus* strains possess peptidases that can hydrolyze these peptides, thereby minimizing any bitterness (Batista et al. 2017; Rodriguez-Serrano et al. 2014).

Antioxidant activity of synbiotic yogurt

The antioxidant activity of synbiotic yogurt is one of its chief beneficial attributes (Selvamuthukumaran et al. 2015). In this study, we found that the antioxidant activity of yogurt samples increased with storage time (Fig. 3B). In synbiotic yogurt samples with 5% AVG, the DPPH-mediated radical scavenging was 63.1% on the first day and remained consistently high during the entire storage time compared to the probiotic yogurt at 48.7%. The results supported the hypothesis that AVG improved LAB's metabolic activity and increased the content of phenolic and flavonoid compounds (Al-Dhabi et al. 2020) which ultimately enhanced the antioxidant potential of the synbiotic yogurt (Al-Dhabi et al. 2020). The AVG addition increased the oxidation potential of the antioxidants, superoxide dismutase, and glutathione peroxidase (Cuvas-Limón et al. 2016; Madhu et al. 2012; Mudgil et al. 2016). AVG also possesses vitamins E and C, which act as free radicals scavengers during oxidation reactions (Miranda et al. 2009). The structural breakdown of plant cell walls releases additional antioxidant compounds into the final product matrix (Azari-Anpar et al., 2017). Similar results were reported by (Madhu et al. 2012), where prebiotic compounds improved the antioxidant activity of yogurt.

Synbiotic yogurt's higher antioxidant activity could also be due to the proteolytic action of adjunct probiotic bacteria in the yogurt (Chen et al. 2019). The production of organic acids from microbial metabolic activity during fermentation and refrigerated storage could be another source of antioxidant activity (Shori 2013).

One of the most critical challenges the food industry has to contend with is developing green countermeasures to eliminate oxidants generated by synthetic additives in commercial food processing (Aloğlu & Öner 2011). Increased oxidative stress has been linked to many diseases, including diabetes mellitus, cancer, and CVD (Carocho & Ferreira 2013); synbiotic yogurt with higher antioxidant potential offers an excellent way to counteract this (Muniandy et al. 2016).

Antipathogenic activity of synbiotic yogurt

The contamination of yogurt with *S. aureus*, *L. monocytogenes,* and coliforms has been associated with outbreaks of food poisoning (Bachrouri et al. 2006; Gulmez & Guven 2003), and the pathogen-killing properties of synbiotic and probiotic yogurt can be effective in preventing this (Fig. 3C, D). Our results show that

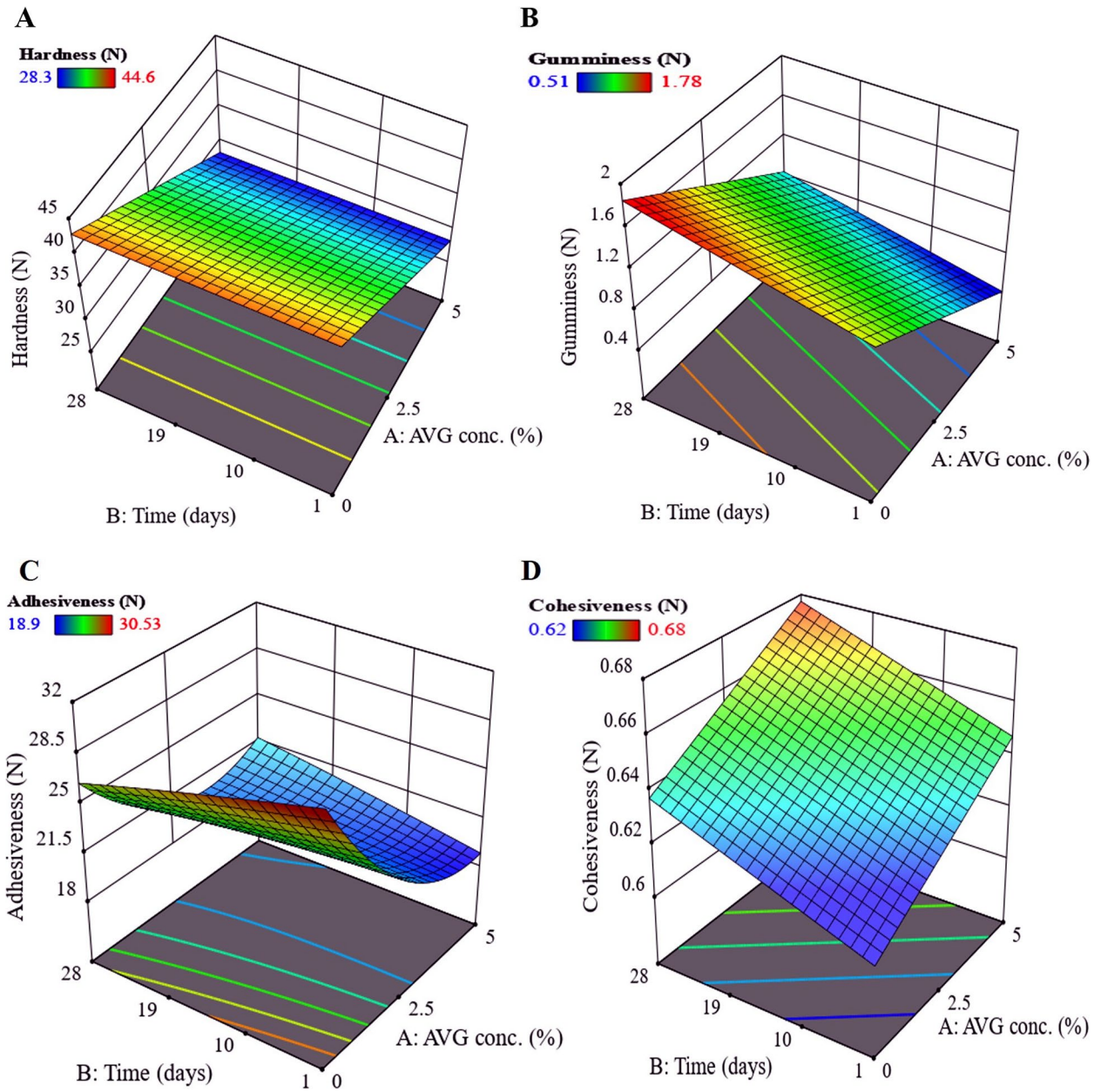


Fig. 2 Three dimensional response surface plot showing effects of interaction between AVG concentration (X1) and storage time (X2) on **A** hardness, **B** gumminess, **C** adhesiveness, and **D** cohesiveness of synbiotic yogurt. The color spectrum from blue to red indicates the range from lowest to highest

both types of yogurts effectively prevented the growth of foodborne pathogens and significantly diminished their numbers during the storage period. *E. coli* counts were significantly reduced by day 28 in the presence of 5% AVG with reference to low or no AVG. Our study confirmed that *L. rhamnosus,* combined with aloe vera gel, could be significantly ($P < 0.01$) deleterious to pernicious food pathogens, thus increasing the stability and shelf-life of yogurt. The antimicrobial activity of *L. rhamnosus* is a subject of interest because of its disease mitigation potential but has been underexplored in food preservation (Kamal et al. 2018). The chemical nature of this biopreservation is multifactorial, involving a host of biochemical moieties like organic acids, inorganic compounds, and proteins (Kariyawasam et al. 2020). Here we observed that the higher concentration of AVG decreased the number of pathogenic bacteria considerably, possibly due to antimicrobial agents like

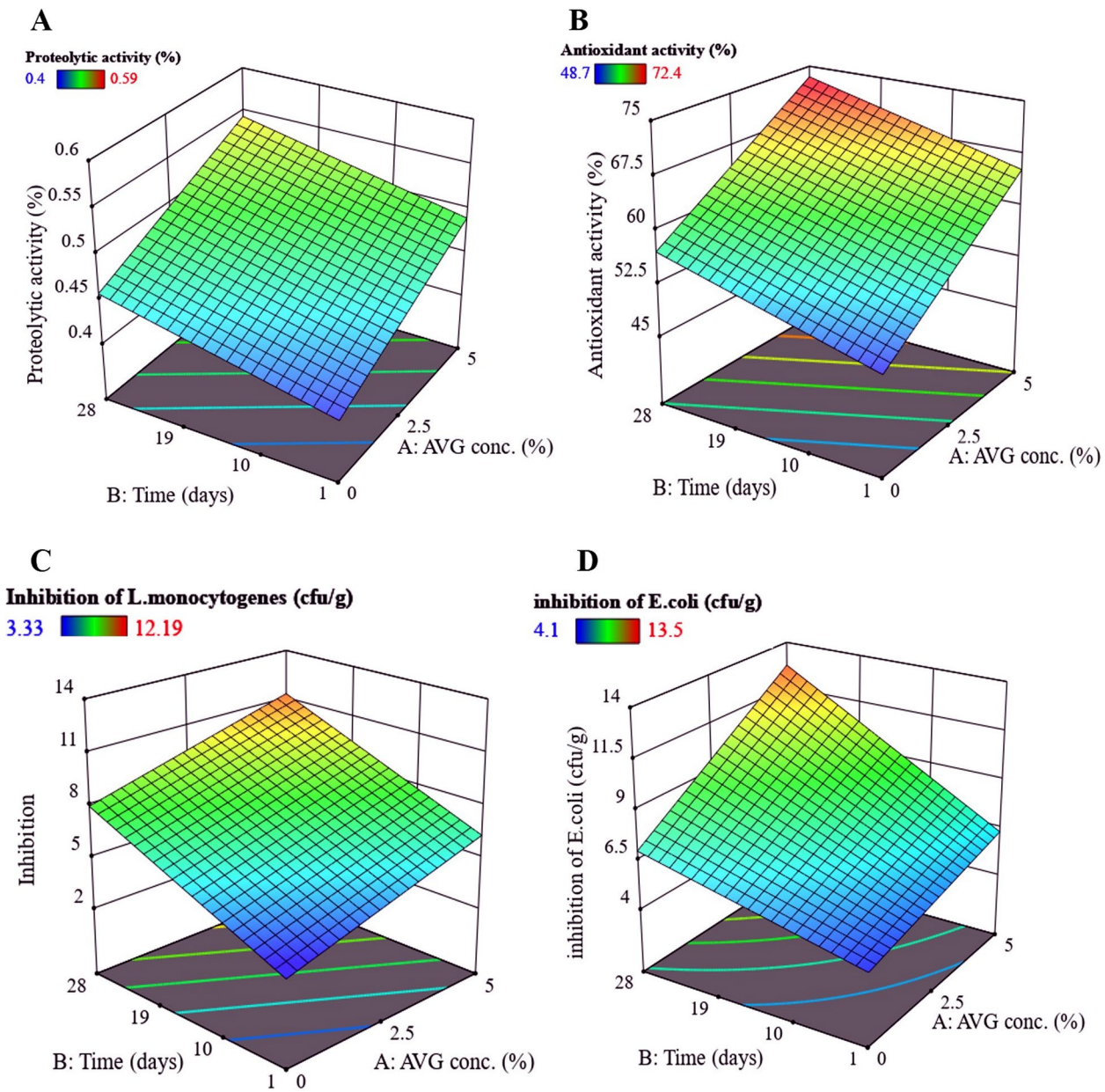


Fig. 3 Three dimensional response surface plot showing effects of interaction between AVG concentration (X1) and storage time (X2) on **A** proteolytic activity, **B** antioxidant activity, **C** inhibition of *L. monocytogenes*, and **D** inhibition of *E. coli* by synbiotic yogurt. The color spectrum from blue to red indicates the range from lowest to highest

ascorbic acid, *p*–coumaric acid, cinnamic acid, and pyrocatechol (Falah et al. 2021).

Survival of L. rhamnosus in yogurt subject to GI tract stress after consumption

The extent to which AVG attenuates the effect of gastrointestinal stress on the transiting *L. rhamnosus* was determined using the Infogest model, which simulates the oro-gastric and intestinal phases of human digestion (Minekus et al. 2014). At the onset of in vitro digestion, the *L. rhamnosus* strain in yogurt fortified with AVG had viable counts of 6–7 log cfu/g, the recommended minimum necessary at the target site (Güler-Akın et al. 2018).

Figure 4 shows that *L. rhamnosus* in the control yogurt decreased by 1 log in the oral phase, whereas the titer remained unaffected in yogurt containing AVG. Probiotic bacteria need to survive the deleterious effects of gastric acid to reach the intestinal tract. The in vitro digestion model subjects the bacteria to a

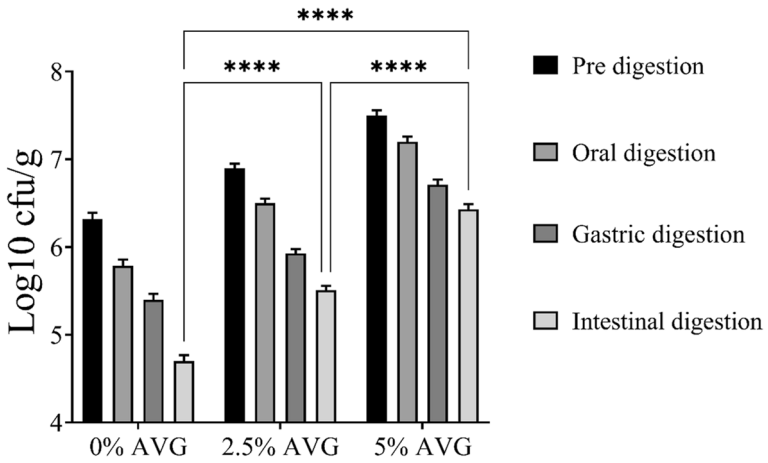


Fig. 4 Effects of AVG concentrations on the viability of *L. rhamnosus* during in vitro digestion. A (***) indicates a statistically significant difference between treatments and AVG concentrations ($p < 0.0001$) as measured by two-way analysis of variance (ANOVA)

gradual decline in pH and exposure to pepsin, simulating the human gastric events associated with ingestion of a semi-solid meal (Madureira et al. 2011). The acidic stomach environment can damage bacterial membranes and DNA. Bile salts in the small intestine also cause membrane damage and protein misfolding, leading to DNA injury by oxidative shock and low intracellular pH, reducing probiotic viability in the upper GI tract (Melchior et al. 2020).

L. rhamnosus in control yogurt showed significantly decreased viability (5.79 log cfu/g) during gastric exposure, which AVG mitigated. Under simulated intestinal conditions, *L. rhamnosus* experienced a slight reduction with 5% AVG compared to lower concentrations or no AVG (control yogurt); having a viable concentration of 6.43 log cfu/ml thus, demonstrating the protective effect of AVG addition on the probiotic strain (Nazirah et al. 2013). Approximately 80% of the added *L. rhamnosus* survived the simulated intestinal condition in the presence of 5% AVG which is substantially higher than the 41% loss of viability in control samples.

Sensory acceptability of synbiotic yogurt

The sensory rating was the average of the scores made by fifty panelists for the different yogurt samples (Fig. S2). The organoleptic rating showed that the synbiotic yogurt with 2.5% AVG received the highest scores. The AVG positively and significantly influenced the sensory attributes of the yogurt ($P < 0.01$). A significant decrease in overall acceptance has been observed in some yogurts containing aloe vera gel (Azari-Anpar et al. 2017) and synbiotic yogurt containing inulin and *L. rhamnosus* (Canbulat & Ozcan 2015). A slight decrease in color and appearance score was found with increasing AVG concentrations because of its color and high moisture content (Hussain et al. 2016). The sensory scores for all parameters, appearance, mouthfeel, taste, and overall acceptance, among the various yogurt samples, decreased slightly with storage, which could be due to lactic acid build-up.

Taste and aroma are vital attributes for the overall acceptance of a yogurt product, and AVG's water-holding ability affects the odor. It was reported that the addition of small amounts of prebiotics did not affect a yogurt's textural, rheological, or sensory qualities, possibly due to its neutral or slightly sweet taste (Allgeyer et al. 2010; Falah et al. 2021). The health advantages of synbiotic yogurt were so noteworthy that consumers chose to eat it despite the lower sensory rating (Azari-Anpar et al. 2017).

Optimization of independent variables of yogurt fermentation

RSM software with central composite design (CCD) was used in this study to determine the optimal conditions for manufacturing synbiotic yogurt with different concentrations of AVG. This model has been successfully used to determine efficient formulation and manufacturing parameters without compromising yogurt quality (Chen et al. 2016). To accomplish CCD, 13 experiments were analyzed, with two independent factors: AVG concentration and storage time. The RSM analysis for the various yogurt products indicated an optimum AVG concentration of 5% and storage time of 14 days. The optimum levels for *L. rhamnosus* viability, pH, acidity, WHC, fat content, protein content, ash content, viscosity, proteolytic activity, syneresis, antioxidant activity, anti-pathogenicity, hardness, gumminess, cohesiveness and adhesiveness, taste, mouthfeel, appearance, and general acceptance with 68% desirability are shown in Fig. 5.

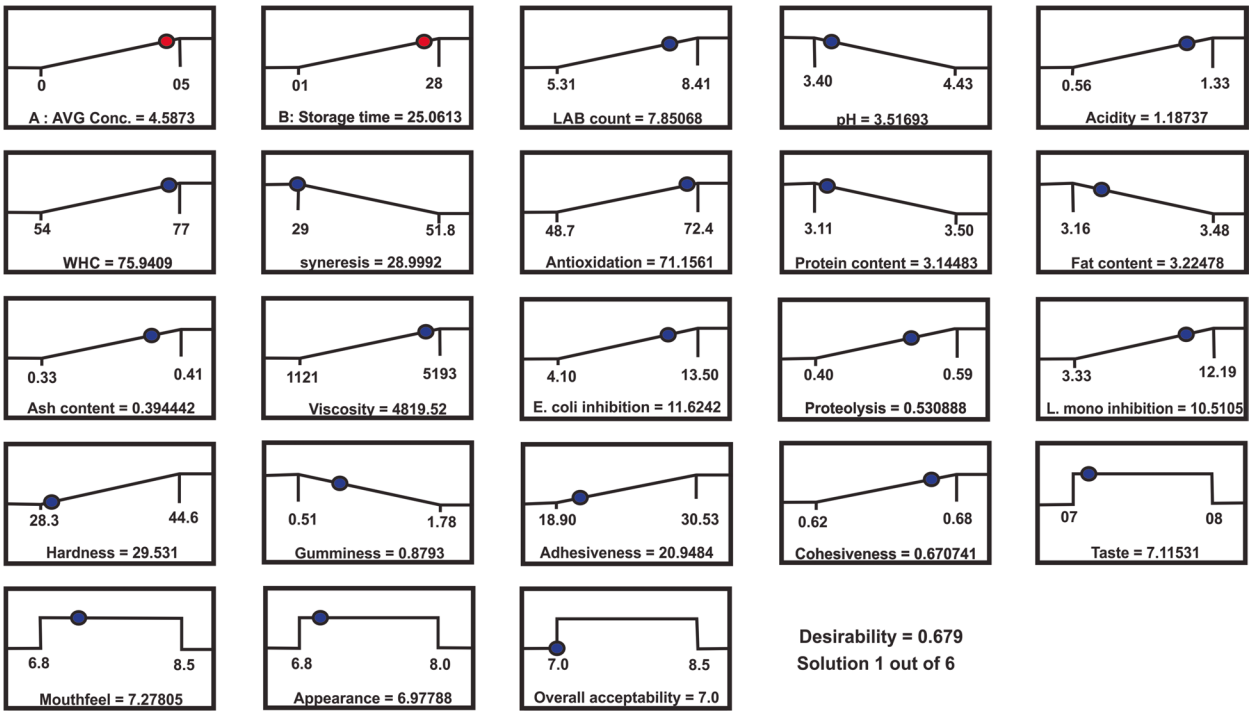


Fig. 5 The overall desirability function based on individual optimal response shown as shaded circle in panels (1: AVG concentration,2: time, 3:LAB count 4: pH, 5: acidity, 6: WHC, 7: syneresis, 8: antioxidant activity, 9: Protein content, 10: Fat content, 11: Ash content, 12: Viscosity, 13: inhibition of *E. coli*, 14: proteolytic activity, 15: inhibition of *L. monocytogenes*, 16: hardness, 17: gumminess, 18: adhesiveness, 19: cohesiveness, 20: Taste, 21: mouthfeel, 22:appearance, 23: overall acceptance) for producing synbiotic yogurt enriched with AVG

Conclusions

The current study aimed to produce synbiotic yogurt (*L. rhamnosus* plus AVG) with maximum viable probiotic count and enhanced physicochemical, antioxidant, antipathogenic, and sensory properties. This was achieved using response surface methodology (RSM) to optimize the AVG concentration and refrigerated storage time. The addition of AVG up to 5% to the probiotic yogurt stimulated the growth of *L. rhamnosus* and increased the total antioxidant and proteolytic content, antipathogenic potential, viscosity, and water retention capacity. Based on the RSM method, it can be concluded that adding 5% AVG enhanced the desirability of synbiotic yogurt by up to 68% and helped to maintain its properties during refrigerated storage for at least two weeks. Even after storage for 28 days, the quality of the synbiotic yogurt was still acceptable. The findings of this research can be utilized to produce a commercially desirable AVG-based synbiotic yogurt.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-023-00153-0.

Additional file 1: Fig. S1. Three dimensional response surface plot showing effects of interaction between AVG concentration (X1) and storage time (X2) on the (A) ash, (B) fat, (C) syneresis, (D) water holding capacity (WHC), and (E) viscosity of synbiotic yogurt. The color spectrum from blue to red indicates the range from lowest to highest. **Fig. S2.** Three dimensional response surface plot showing effects of interaction between AVG concentration (X1) and storage time (X2) on the sensory properties of synbiotic yogurt. The color spectrum from blue to red indicates the range from lowest to highest.

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Authors' contributions

Sadia Ahmed: Major investigation, formal analysis, writing original draft. Asia Noor: part of investigation, validation of results. Muhammad Tariq: validation, resources. Arsalan Zaidi: conceptualization, methodology, resources, writing—review and editing, visualization, supervision. The author(s) have read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human participants were per the institutional and national research committee's ethical standards and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Institutional review committee approved the study (NIBGE). Informed consent was obtained from all individual participants included in the study.

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no competing interest.

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