Iranian Journal of Microbiology

Volume 17 Number 3 (June 2025) 420-433
DOI: http://doi.org/10.18502/ijm.v17i3.18825

ORIGINAL ARTICLE

In vitro inhibition of *Streptococcus mutans* by probiotic yogurt fortified with *Lactobacillus paracasei* and *Sargassum angustifolium* protein hydrolysate: a functional yogurt for teeth decay prevention

Sara Jafarirad[1], Leila Nateghi[1*], Masoumeh Moslemi[2], Kian Pahlevan Afshari[3], Ali Ahmadi Hassan Abad[4], Kianoush Khosravi-Darani[5]

[1]Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

[2]Halal Research Center of IRI, Iran Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran

[3]Department of Animal Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

[4]Department of Food Science and Technology, Faculty of Agriculture, Shiraz University, Shiraz, Iran

[5]Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Received: August 2024**, Accepted:** March 2025

ABSTRACT

Background and Objectives: Probiotic yogurts enriched with *Lactobacillus paracasei (L. paracasei)*, protein hydrolysates derived from *Sargassum angustifolium* macroalgae (SAPH), and encapsulated SAPH were formulated to inhibit *Streptococcus mutans (S. mutans)*, the primary bacterium responsible for dental caries.

Materials and Methods: The yogurt samples were evaluated for physicochemical, microbiological, and sensory characteristics.

Results: On day 21, the yogurt supplemented with *L. paracasei* demonstrated the greatest titratable acidity (97.35°D), the lowest pH value (4.24), reduced syneresis, and enhanced antioxidant, antihypertensive, and rheological properties. In terms of antibacterial activity, the lowest *S. mutans* count was detected in formulations containing free SAPH, either alone or in combination with *L. paracasei*. Conversely, yogurts formulated with encapsulated SAPH exhibited higher survival rates of both *L. paracasei* and *S. mutans* compared to those containing the free form of SAPH.

Conclusion: The findings indicated that although the probiotic yogurt containing free SAPH was more effective in reducing *S. mutans* levels within the yogurt matrix, the encapsulated form achieved an acceptable level of antibacterial activity while contributing to improved sensory acceptance.

Keywords: Dental caries; *Sargassum*; Calcium alginate; Encapsulation; *Lactobacillus paracasei*; *Streptococcus mutans*

[*]Corresponding author: Leila Nateghi, Ph.D, Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran. Tel: +98-9125878775 Fax: +98-2136724767 Email: leylanateghi@yahoo.com; leylanateghi@iauvaramin.ac.ir

INTRODUCTION

Development of innovative functional and health-promoting foods have received great attention primarily due to the well-documented therapeutic effects of bioactive compounds (1-3). To improve shelf life natural and synthetic additives are incorporated into the food matrix to prevent chemical deterioration and microbial spoilage. In this regard, natural additives are of interest because they prolong the shelf life without any adverse effect in the body (4). There are versatile natural additives that can be used in food formulations (5). For example, herbal extracts, essential oils, bioactive peptides, probiotics, and some biopolymers have shown several functional properties (6).

Peptides are produced by breaking the covalent bonds in proteins. Sequences of amino acids determine their functionality e.g. anti-diabetic, antimicrobial, antioxidant, and antihypertension activities in the laboratory and vital systems (7). Hydrolyzation of peptide bonds by mineral acids such as HCl is the most frequent method implemented for producing peptides from proteins (8). To the contrary, the hydrolysates produced by enzymatic methods have predominantly high biological activities. Indeed, enzymatic hydrolysis is controllable but acid hydrolysis may extensively hydrolyze the peptide bonds through which the biological activity is reduced (9). Plant-derived protein hydrolysates have demonstrated considerable potential in the prevention of various health conditions, including cardiovascular diseases, diabetes, hypertension and several forms of cancer (10). In recent years, peptides derived from marine plants have been studied increasingly due to their interesting health benefits (11). For example, bactericidal effects were reported for the bioactive peptides derived from the protein extracted from *Sargassum angustifolium* macroalgae (12).

Bioactivity of peptides depends on the composition and the sequence of their amino acid, the molecular weight, and the hydrophobic/hydrophilic features (13). Addition of bioactive peptides to food formulations may affect the organoleptic attributes by reducing the palatability. Moreover, the bioactivity may be affected by the food matrix and through passage in the gastrointestinal tract in the presence of pepsin and trypsin as proteolytic enzymes. Therefore, it is necessary to protect the bioactive peptides against harsh conditions in the environment before their absorption in the intestine (14). This can be done by encapsulation by which the bioactive peptides are entrapped within encapsulating agents such as biopolymers (15, 16). Encapsulation can mask the bitterness of peptides and reduce further interactions between the food ingredients (14).

Functional foods with antimicrobial potential can reduce body infections other than their nutritional benefits. Tooth decay is one of oral disorders mainly occurred by accumulation of microorganisms and plaque formation in the mouth. *Streptococcus mutans* is part of the normal flora of the mouth and is responsible for formation of dental caries. It can adhere to the surface of teeth through synthesis of extracellular polysaccharides by its glucosyltransferase. Plaque formation, resulting from the accumulation of a high concentration of *S. mutans*, initiates tooth demineralization due to the production of organic acids by the bacteria, ultimately leading to tooth decay and the onset of dental caries (17). Therefore, inhibiting the growth of *S. mutans* effectively prevents the progression of dental decay. Our recent research demonstrated that bioactive peptides generated by Alcalase from the protein extracted from *S. angustifolium* exhibited significant potential in preventing the growth of *S. mutans* (9). Therefore, addition of such bioactive peptides to staple foods in order to control the mouth microflora is of interest. However, use of encapsulated peptides is preferred to mask their unpalatable taste in the matrix. In this study, we aimed to fortify a probiotic yogurt containing *Lactobacillus paracasei* with the bioactive peptides derived from *S. angustifolium* in two forms: free and encapsulated in calcium alginate (CaAlg) and chia seed gum exudate (CSGE). Subsequently, the functional properties of the formulation were assessed in the laboratory, including its ability to suppress angiotensin-I converting enzyme, exert antioxidant effects, and antagonize the growth of *S. mutans*.

The aim of this study was to evaluate the in vitro inhibitory influence of probiotic yogurt, enriched with *L. paracasei* and *S. angustifolium* protein hydrolysate, on *S. mutans* as a potential approach for preventing tooth decay. Furthermore, the physicochemical characteristics including pH, acidity, color, syneresis, and viscosity of the yogurt were assessed.

MATERIALS AND METHODS

Materials. *S. angustifolium* macroalgae layer was collected from Chabahar shores. Alcalase ® 2.4 L

(≥ 2.4 U/g))Novozymes Co-Denmark), CaAlg (Sigma-Aldrich Co-Germany) and CSGE (from local market (Tehran, Iran)) were purchased. Lyophilized cultures of *S. mutans* (ATCC 25175), *L. paracasei* L26 (Cinnagen Co-Tehran, Iran) and DSM (Moorebank, Australia) were provided, respectively.

Extraction of algae protein. The algal protein was extracted through an alkalization-precipitation method (Jafarirad et al., 2023). *S. angustifolium* protein isolate (SAPI) was powdered by freeze-drying and stored at 4°C until hydrolysis.

Hydrolysis of SAPI. The hydrolysis of SAPI was performed using an enzymatic procedure, as outlined by Rezvankhah et al. (13). To denature the protein structure, a 2% (w/v) protein solution was initially heated to 90°C (10 min), and then cooled to 35-40°C (pH 7.0). The enzymatic hydrolysis was carried out using an enzyme-to-substrate ratio of 2% (w/w). Temperature was set at 60°C and hydrolysis was continued for 90 min according to the optimum condition for Alcalase activity (the highest antioxidant and antimicrobial potency of the hydrolysates) determined in our previous study (9). The tempretaure of peptide solution reached to room temperature and centrifuged at 15,000×g (20 min) to remove unhydrolyzed protein residues. The hydrolysates were collected further and freeze-dried in the laboratory. *S. angustifolium* protein hydrolysate (SAPH) was refrigerated.

Encapsulation of SAPH. CaAlg and CSGE were used for encapsulation of SAPH according to the methods of Raei et al. (18). For CaAlg encapsulation, 3 g CaAlg was dissolved in 100 mL distilled water, and stirred vigorously for 10 min. Then, solution of SAPH (10% w/v) was prepared at pH 5.5 and stored overnight in refrigerator to get well-hydrated. Two solutions were mixed at ratio of 1:10 (SAPH: CaAlg) and stirred at 1000 rpm for 1 h followed by ultrasound homogenization under 100 W power for 1 min. The mixture was poured further into a syringe and added drop-wise to $CaCl_2$ solution (3% w/v). The final mixture was stored at 4°C for 30 minutes. Subsequently, the resulting microbeads were collected by centrifugation at 6,000×g for 15 minutes and subjected to freeze-drying. For CSGE encapsulation, 0.1 g of the extracted gum was dissolved in 100 mL of distilled water and stirred at 500 rpm for 2 hours to ensure complete hydration. Then, solution of SAPH (10% w/v) was prepared and mixed with CSGE at ratio of 1:10. The mixture was stirred at 800 rpm for 1 h followed by sonication at 100 W for 1 min. The formed microcapsules were separated by centrifugation at 15000×g for 30 min followed by freeze-drying.

Yogurt preparation. Probiotic yogurt was prepared as stated by the protocol outlined by Ghasempour et al. (19). Initially, the total solids content of raw milk (containing 1.5% fat) was adjusted to 10% (w/v), followed by pasteurization at 90°C for 5 minutes. Following homogenization, the mixture was cooled to 43°C, after which starter cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*), along with *Lactobacillus paracasei* (10^9 CFU/mL), were added. Subsequently, free and encapsulated forms of SAPH—using calcium alginate and chia seed gum as encapsulation matrices -were incorporated into the milk. The inoculated mixture was then incubated at 43°C until the pH decreased to 4.7. The yogurt samples were inoculated with *S. mutans* (10^6 CFU/mL) and mixed for 3-5 minutes to ensure homogenization. Subsequently, 100 g portions were transferred into packaging containers and stored at 4°C. The growth of *S. mutans* was assessed on days 1, 7, 14, and 21 of the storage period.

Physiochemical properties of probiotic yogurts. Titratable acidity of the yogurt samples was measured by titration with 0.1 N NaOH, using phenolphthalein as the pH indicator (20). The results were expressed (°D). The pH of the yogurt samples was measured using a digital pH meter (Milwaukee Mi 151, Romania). Syneresis of the samples were determined according to the method of Ghorbanzade et al. (21). with some modifications. After centrifugation of yogurt samples at 2500 ×g for 5 min, whey was transferred into a graduated cylinder. The extent of syneresis was calculated using Equation 2, as detailed below.

$$\text{Syneresis (\%)} = \frac{\text{Total weight of separated liquid (g)}}{\text{Total weight of yogurt (g)}} \times 100$$

Also, samples were analyzed for color parameters, including redness/greenness (a*), yellowness/blueness (b*), and lightness (L*). The measurements were conducted using a CR-300 Minolta Colorimeter (Osaka, Japan) (19, 22).

The DPPH radical scavenging activity of the yogurt samples was evaluated based on a procedure

by Ghasempour et al.with small modification (19). Solution of 10 g of yogurt in 2.5 mL of water was homogenized (pH was adjusted to 4.0 using 0.1 M HCl). After incubatation in a water bath (at 45°C for 10 min), sample was centrifuged at 4 °C at 5,000×g (10 min) neutralized to pH 7.0 using 0.1 M NaOH, and sent to second round of centrifugation at 4,000×g (10 min). For the DPPH assay, 250 µL of the final supernatant was mixed with 3 mL of a 60 mmol/L ethanolic DPPH solution and kept in the dark at 25°C for 30 minutes. After incubation, the mixture was centrifuged at 4,000×g (10 min) and the absorbance was measured at 517 nm. The DPPH radical scavenging activity was then quantified by Equation 3.

$$\text{DPPH } (\%) = \frac{A_c - A_s}{A_c - A_b} \times 100$$

A_b, A_c, and As represent the absorbance values of the blank, the control and the sample respectively.

Ferric reducing antioxidant power (FRAP). To perform the FRAP assay, a working reagent was freshly formulated according to the modified method by Ahmadi et al. (23). The solution consisted of TPTZ (8 mmol/L), acetate buffer (300 mmol/L), and $FeCl_3$ (20 mmol/L), combined in a volumetric ratio of 1:10:1 (v/v/v). A 250 µL aliquot of yogurt extract was mixed with 3 mL of the FRAP reagent and incubated (at 37°C for 10 min). The absorbance was measured at 593 nm using a spectrophotometer (Perkin Elmer Lambda-2 UV–Visible Spectrophotometer, Germany), with distilled water used as a blank. A calibration curve was constructed using ferrous sulfate solutions at 1 to 10 mmol/L. The results are expressed as mmol $FeSO_4$ /g yogurt (24, 25).

Angiotensin converting enzyme (ACE) inhibition. The ACE inhibitory activity of the yogurt was evaluated based on procedure of Rezvankhah et al. (8), with slight modifications. To prepare the reaction mixture, a basic solution was first made by adding HEPES buffer (50 mM) and a zwitterionic sulfonic acid buffer to a 300 mM NaCl solution (pH 8.3). Subsequently, 250 µL of the solution within the concentration range of 0.5-2 mg/mL, was combined with 30 µL of ACE enzyme solution (0.25 units/mL) and incubated (37°C for 15 min). After incubation, the produced hippuric acid was extracted using 1 mL of ethyl acetate and centrifuged (at 1200×g for 5 min). The ethyl acetate of supernatant was evaporated by boiling. The hippuric acid extract was added to 1 mL water, and the absorbance was measured at 228 nm using a spectrophotometer. For the control, the enzyme was mixed with the HEPES-HCl buffer containing 300 mM NaCl (pH 8.3), but without the sample. The blank solution served as a reference, representing activity in the absence of the enzyme. ACE inhibition was determined using Equation 4.

$$\text{ACE inhibition activity } (\%) = \frac{A_C - A_S}{A_C - A_B} \times 100$$

Where, A_B, A_S and A_C are representing the absorbance of blank, sample and control, respectively.

Viscosity. Apparent viscosity measurements of the yogurt samples were conducted using a Brookfield viscometer (model LVDV-II + Pro, Brookfield Laboratories, Inc., USA) (19). The experiments were done at 15°C with spindle no. 63 at 30 rpm for 20 s. The samples were stirred manually in a clockwise direction for 60 seconds prior to the test. Apparent viscosity is expressed as centipoise (CP).

Microbial experiments. Viability of *L. paracasei* in the yogurts was determined by MRS-vancomycin agar after 72 h incubation at 37°C under anaerobic condition. For inoculation, 1 mL of yogurt was added to 9 mL peptone water 0.1%, and dilution was continued to reach detectable colonies on the plate (19).

Survival of *S. mutans* in the yogurt was checked in Brain Heart Infusion (BHI) broth at 37°C. Briefly, 100 µL of yogurt was added to 100 µL of BHI broth and bacterial survivability was monitored during time by spectrophotometer at 550 nm. Non-inoculated BHI broth was used as blank (26).

Organoleptic attributes. The sensory characteristics of the functional yogurts were assessed by method of Rezvankhah et al. (8) with some modifications. Flavor, texture, odor, color, and overall acceptance were evaluated by scoring from 1 to 5. The lowest score and the highest score indicated the worst and the best characteristics, respectively.

Statistical analysis. The treatments were designed using the factorial method. The data are expressed as the mean ± standard deviation. Statistical analysis was accomplished using one-way ANOVA, and

mean comparisons were made using the Duncan test. A significance level of $p \leq 0.05$ was considered. All experiments were conducted in triplicate.

RESULTS

pH and acidity. The pH and acidity of the yogurt samples are presented in Table 1. As observed, the pH of all samples declined, while the acidity increased during the storage period. The yogurt containing free SAPH and the probiotic yogurt containing *L. paracasei* and free SAPH had higher pH than other samples.

Syneresis. The syneresis values of the yogurt samples are shown in Fig. 1. In our study, whey separation increased significantly during storage. The yogurt containing free SAPH had the highest syneresis. The yogurt containing *L. paracasei* showed the lowest syneresis.

Color changes. According to Table 2 the incorporation of SAPH in its free form into the yogurt resulted

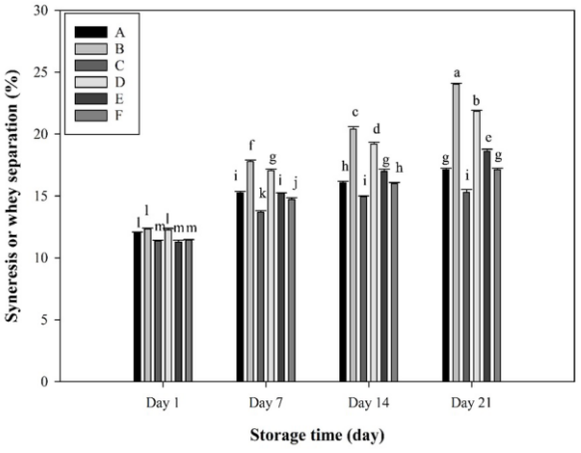

Fig. 1. Syneresis of yogurt samples during 21 days of storage. A: Control yogurt free of *L. paracasei* and SAPH; B: Yogurt containing free SAPH; C: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL); D: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and free SAPH; E: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CSGE; F: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CaAlg

Table 1. pH and acidity of yogurt samples

		pH		
Sample	**Day 1**	**Day 7**	**Day 14**	**Day 21**
A	4.66 ± 0.00^{aA}	4.52 ± 0.01^{bB}	4.41 ± 0.01^{bC}	4.34 ± 0.01^{cD}
B	4.69 ± 0.00^{aA}	4.60 ± 0.00^{aB}	4.53 ± 0.01^{aC}	4.50 ± 0.00^{aC}
C	4.61 ± 0.00^{bA}	4.47 ± 0.01^{bB}	4.32 ± 0.00^{cC}	4.24 ± 0.00^{dD}
D	4.68 ± 0.01^{aA}	4.58 ± 0.01^{aB}	4.51 ± 0.01^{aC}	4.48 ± 0.00^{aC}
E	4.60 ± 0.01^{bA}	4.51 ± 0.00^{bB}	4.47 ± 0.00^{aB}	4.41 ± 0.01^{bC}
F	4.61 ± 0.00^{bA}	4.47 ± 0.01^{bB}	4.41 ± 0.00^{bC}	$4.37 \pm 0.01b^{cC}$
		Acidity (°D)		
A	72.41 ± 1.42^{bC}	82.49 ± 0.87^{cB}	88.96 ± 0.92^{bA}	91.65 ± 1.27^{bA}
B	71.14 ± 0.44^{bD}	78.89 ± 0.75^{dC}	82.68 ± 0.34^{eB}	84.35 ± 0.46^{dA}
C	75.25 ± 1.31^{aD}	85.01 ± 0.9^{5aC}	92.81 ± 0.22^{aB}	97.35 ± 0.27^{aA}
D	71.77 ± 1.08^{bC}	79.44 ± 0.70^{dB}	82.48 ± 1.62^{eA}	84.06 ± 1.21^{dA}
E	75.09 ± 0.52^{aC}	83.02 ± 0.29^{bB}	84.75 ± 1.08^{dB}	88.33 ± 1.29^{bcA}
F	75.39 ± 0.92^{aD}	85.23 ± 0.41^{aC}	87.29 ± 0.25^{cB}	90.53 ± 0.99^{bA}

*Different small letters show significant differences in columns ($p \leq 0.05$)

**Different capital letters show significant differences in rows ($p \leq 0.05$)

***A: Control yogurt free of *L. paracasei* and SAPH; B: Yogurt containing free SAPH; C: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL); D: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and free SAPH; E: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CSGE; F: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CaAlg

in a decrease in lightness (L*), while blue-yellow (b*) and the red-green (a*) values increased.

Antioxidant activity. Results of DPPH free radicals scavenging and FRAP in the samples are illustrated in Figs. 2a and 2b. According to Fig. 2a, antioxidant activity of the samples increased significantly during storage. The highest antioxidant activity was observed in the probiotic yogurt containing *L. paracasei*. Addition of *L. paracasei* and SAPH especially in encapsulated form to yogurt led to increased scavenging of DPPH free radicals. The increasing trend of antioxidant potency was confirmed by the results of FRAP (Fig. 2b).

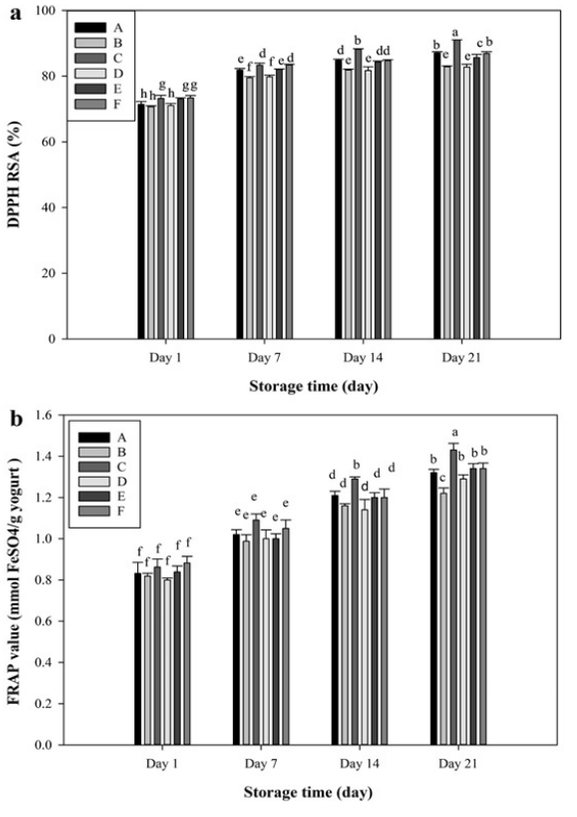

Fig. 2. Antioxidant activity of yogurt sample during 21 days of storage, a) DPPH free radical scavenging, b) Ferric Reducing Antioxidant Power (FRAP). A: Control yogurt free of *L. paracasei* and SAPH; B: Yogurt containing free SAPH; C: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL); D: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and free SAPH; E: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulat*ed by CSGE; F: Probiotic yogurt containing L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CaAlg

Antihypertensive potency. According to Fig. 3, the strongest ACE inhibition was observed in the control and the probiotic yogurt that included *L. paracasei.*

Viscosity. Apparent viscosity of the yogurts is presented in Fig. 4. As seen in the figure, the yogurt with *L. paracasei* exhibited the highest viscosity. The lowest viscosity was obtained for samples B which was incorporated with free SAPH. Apparent viscosity in other samples specially those containing free and encapsulated SAPH was significantly lower than the probiotic yogurt. As mentioned above, optimum condition for SAPI hydrolysis was used in our study (i.e., Alcalase activity at 60ºC for 90 min), through which the highest hydrolysis by producing low molecular weight peptides occurred.

Microbial growth. Antimicrobial effect of free and encapsulated SAPH on *L. paracasei* and *S. mutans* growth is depicted in Figs. 5a and 5b, respectively. During storage, growth of both microorganisms was significantly decreased ($p<0.05$). Interestingly, viability of *S. mutans* and *L. paracasei* in the yogurts containing free SAPH was significantly lower than other samples which shows the high antibacterial potential of SAPH.

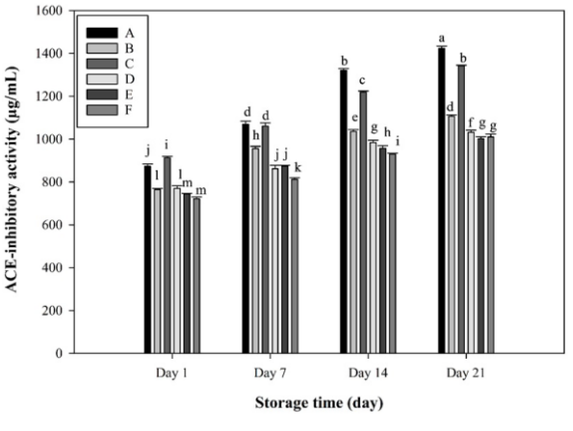

Fig. 3. Angiotensin converting enzyme (ACE) inhibition by yogurt samples during 21 days of storage. A: Control yogurt free of *L. paracasei* and SAPH; B: Yogurt containing free SAPH; C: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL); D: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and free SAPH; E: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CSGE; F: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CaAlg

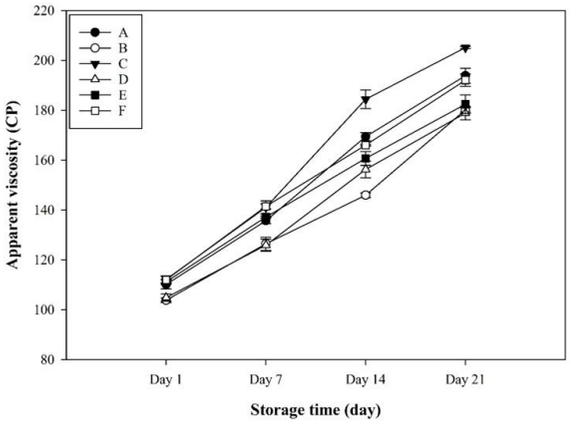

Fig. 4. Apparent viscosity of yogurt samples during 21 days of storage. A: Control yogurt free of *L. paracasei* and SAPH; B: Yogurt containing free SAPH; C: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL); D: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and free SAPH; E: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CSGE; F: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CaAlg

Table 2. Color parameters of yogurt samples

Sample	L*	a*	b*
A	89.49 ± 0.24[a]	-0.27 ± 0.02[f]	6.52 ± 0.04e
B	87.90 ± 0.36[d]	0.78 ± 0.05[a]	8.05 ± 0.03[a]
C	89.61 ± 0.48[a]	-0.04 ± 0.05[e]	6.48 ± 0.23[e]
D	88.59 ± 0.14[c]	0.45 ± 0.02[b]	7.63 ± 0.09[c]
E	88.96 ± 0.20[c]	0.35 ± 0.02[c]	7.87 ± 0.02[b]
F	89.12 ± 0.07[b]	0.12 ± 0.04[d]	7.17 ± 0.04[d]

*Different small letters show significant differences in columns ($p \leq 0.05$)

**A: Control yogurt free of *L. paracasei* and SAPH; B: Yogurt containing free SAPH; C: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL); D: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and free SAPH; E: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CSGE; F: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CaAlg

The highest growth of *S. mutans* was observed in the control yogurt while the lowest count was achieved for the yogurt containing free SAPH. On the other hand, inoculation of *L. paracasei* showed a significant inhibition against *S. mutans.*

Organoleptic attributes. The sensory attributes, such as texture, odor, color, flavor, and overall acceptance, were evaluated and the results are presented in Table 3. Accordingly, the highest score of flavor was achieved for the probiotic yogurt fortified with CaAlg-SAPH, while the lowest score was achieved for the yogurt containing free SAPH. The lower score was achieved by addition of free SAPH to the simple and probiotic yogurts. Naturally, yogurt is white and thick, and other colors give artificial feeling to the consumer. Regarding odor, no significant differences were observed between the samples. The lowest score of texture was observed in the yogurt fortified with free SAPH. It might be associated with the high syneresis of this sample (Fig. 1). The highest score of overall acceptance was given by the panelists to the probiotic yogurts fortified with encapsulated SAPH in both CaAlg and CSGE. No significant difference

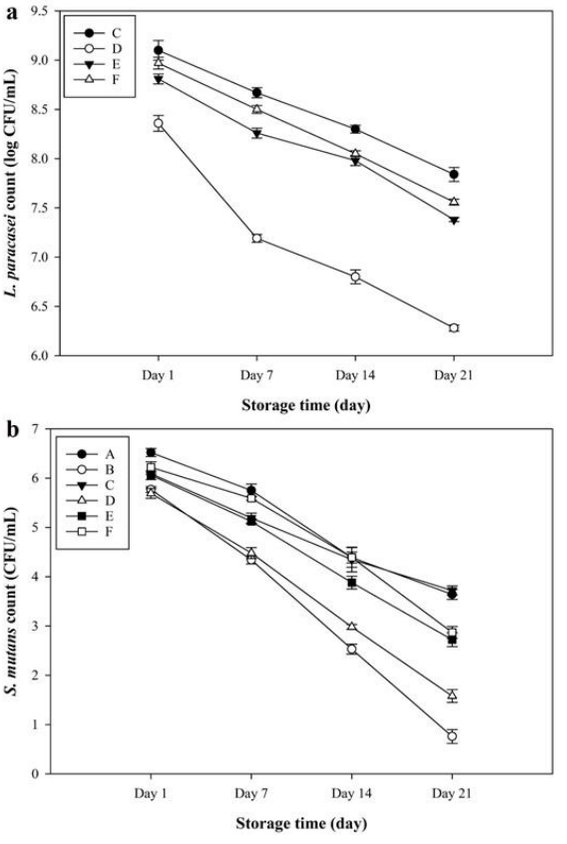

Fig. 5. Viability of *L. paracasei* and *S. mutans* in yogurt samples during 21 days of storage. A: Control yogurt free of *L. paracasei* and SAPH; B: Yogurt containing free SAPH; C: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL); D: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and free SAPH; E: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CSGE; F: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CaAlg

Table 3. Sensory scores of yogurt samples

Samples	Flavor	Color	Odor	Texture	Overall acceptance
A	4.90 ± 0.31^{b}	5.00 ± 0.00^{a}	4.60 ± 0.51^{a}	4.30 ± 0.48^{a}	4.50 ± 0.52^{a}
B	4.10 ± 0.31^{c}	4.70 ± 0.08^{b}	4.80 ± 0.42^{a}	3.60 ± 0.51^{b}	3.90 ± 0.31^{b}
C	4.60 ± 0.51^{b}	5.00 ± 0.00^{a}	4.80 ± 0.42^{a}	4.50 ± 0.52^{a}	4.50 ± 0.52a
D	4.30 ± 0.48^{b}	4.70 ± 0.08^{b}	4.70 ± 0.48^{a}	4.20 ± 0.42^{a}	4.10 ± 0.31^{ab}
E	4.90 ± 0.31^{b}	5.00 ± 0.00^{a}	4.50 ± 0.52^{a}	4.60 ± 0.51^{a}	4.60 ± 0.51^{a}
F	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	4.80 ± 0.42^{a}	4.50 ± 0.52^{a}	4.70 ± 0.48^{a}

*Different small letters show significant differences in columns ($p \leq 0.05$)

**A: Control yogurt free of *L. paracasei* and SAPH; B: Yogurt containing free SAPH; C: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL); D: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and free SAPH; E: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CSGE; F: Probiotic yogurt containing *L. paracasei* (10^9 CFU/mL) and SAPH encapsulated by CaAlg

was found in the overall acceptance between the probiotic yogurts fortified with free and encapsulated SAPH.

DISCUSSION

During fermentation of all samples pH decreased, while acidity increased over the storage period, due to lactic acid production. The yogurt containing free SAPH and the probiotic yogurt containing *L. paracasei* and free SAPH had higher pH than other samples. The increased pH in yogurt fortified with protein hydrolysates is primarily due to their buffering properties, the existence of basic amino acids, potential antimicrobial effects, and interactions with minerals. These factors collectively reduce acidification during fermentation, leading to a higher final pH. It has been reported that the protein hydrolysates contain peptides and amino acids that enhance the yogurt's buffering capacity, making it more resistant to pH reduction during fermentation. Unlike intact proteins, hydrolysates exhibit higher solubility and interact with lactic acid, reducing its impact on lowering pH (27). The findings indicate that the incorporation of protein hydrolysates can impact the fermentation process and the final pH of yogurt, which is consistent with the findings of this study. Numerous studies have explored the effect of protein hydrolysate supplementation on the pH levels of yogurt. This was in agreement with the study of Mashayekh et al. (28). They found that the incorporation of bioactive peptides into yogurt increased the pH compared to control during storage. They suggested that a reduction in microbial growth leads to an increase in pH, as there is less organic acid production by the starter bacteria (28). Similar results were observed for acidity so that the probiotic yogurt containing *L. paracasei* and the probiotic yogurt containing *L. paracasei* and free SAPH had the highest and the lowest acidity on day 21, respectively. Indeed, addition of probiotics to yogurt led to production of more acids, but incorporation of SAPH in free form reduced acid production by inhibition of probiotics and starter bacteria. The effect of whey protein hydrolysate on the pH of yogurt was examined by Fang & Guo (29), who found that the presence of protein hydrolysates resulted in a higher pH compared to the control. Varedesara et al. used grape seed protein hydrolysates in yogurt. The effect of grape seed protein hydrolysate on yogurt properties showed that the treated yogurt had higher pH, along with lower acidity compared to the control sample (30).

Wang et al. described that hydrolysis releases bioactive peptides, some of which contain basic amino acids (e.g., lysine, arginine and histidine). These amino acids can neutralize acids produced during fermentation, leading to a higher pH compared to non-enriched yogurts (31). Also Sah et al. reported that hydrolysates may interact with calcium and phosphate ions in milk, forming complexes that influence acid-base balance. These interactions can reduce the availability of free hydrogen ions, stabilizing pH at a higher level (32).

Another investigation focused on fortifying milk-based yogurt with protein hydrolysates derived from brewer's spent grain. The findings revealed a notable decrease in pH during the second hour of fermen-

tation, potentially attributed to the isoelectric point of the extract and the pH conditions during the extraction process (33). These studies suggest that the incorporation of protein hydrolysates can alter the fermentation dynamics and pH levels of yogurt, potentially due to factors such as buffering capacity, amino acid composition, and interactions with lactic acid bacteria.

Syneresis is associated with whey separation and can be considered as a main problem of dairy products. It can be controlled by incorporation of stabilizers, inoculation of microorganisms producing exopolysaccharides, and exerting appropriate process conditions. The yogurt containing free SAPH had the highest syneresis. The yogurt containing *L. paracasei* showed the lowest syneresis, which was due to production of exopolysaccharides by the probiotic a bacterium in the absence of the antibacterial effect of SAPH. Inclusion of encapsulated SAPH in yogurt led to decreased syneresis compared to free SAPH. In fact, encapsulation causes controlled release of SAPH in the matrix so that activity of probiotic cells was not affected remarkably. Moreover, it is assumed that CaAlg and CSGE increased the water-binding capacity of yogurts. However, acidity is one of the main parameters affecting the syneresis. In this regard, Mashayekh et al. reported that syneresis of yogurt increased throughout storage which might be related to the increasing acidity (27). While yogurts enriched with Clover Sprout Protein Hydrolyzate (CPH) and *Lactobacillus casei* reduced syneresis during storage (34).

The addition of protein hydrolysates and probiotic bacteria to yogurt can influence its physicochemical properties during storage, particularly concerning syneresis. Incorporating certain protein hydrolysates may disrupt the casein gel network in yogurt, leading to increased syneresis (35). Conversely, some hydrolysates enhance the hydrophilic properties of yogurt by increasing protein content, resulting in improved viscosity and reduced syneresis. The specific impact depends on the type and concentration of hydrolysate used (30). Certain probiotic strains produce Exopolysaccharide (EPS) during fermentation, which can improve the water-holding capacity of yogurt and reduce syneresis. However, the extent of this effect varies among strains (36). Probiotic bacteria can interact with milk proteins, potentially affecting the gel structure and water retention properties of yogurt. These interactions can either mitigate or exacerbate syneresis, depending on the specific strains and fermentation conditions (37). In summary, the impact of adding protein hydrolysates and probiotic bacteria on yogurt syneresis during storage is multifaceted. The effects depend on the types and concentrations of hydrolysates and probiotics used, as well as their interactions with the yogurt matrix. Careful selection and optimization of these additives are essential to minimize syneresis and maintain yogurt quality during storage (38).

Addition of SAPH in free form to the yogurt led to reduction of lightness (L*) while blue-yellow (b*) and red-green (a*) parameters increased. It is clear that SAPH was derived from the brown alga *S. angustifolium*. Therefore, it increased the brownness of the yogurt. Use of probiotics did not have significant effects on L* and b*, while it increased a* value. Encapsulation of SAPH caused slight reduction in lightness, which could be related to the covering effect of CSGE and CaAlg. The yogurt containing *L. paracasei* and CaAlg-SAPH showed the lowest b* among the samples containing SAPH.

In present study, the observed increase in antioxidant activity over the storage period may be associated with ongoing enzymatic activity of *L. paracasei* and starter bacteria in favor of antioxidant peptides production (13, 39). Furthermore, CaAlg and CSGE consist of functional groups with strong reducing power. In agreement with these findings, Bhatnagar et al. (40) reported that use of *L. paracasei* in soymilk led to generation of isoflavones and bioactive peptides with strong antioxidant activity. Karimi et al. (41) investigated the antioxidant capacity of bioactive peptides in yogurt and reported that the antioxidant activity of dough, a traditional Iranian fermented dairy beverage, increased in response to higher concentrations of bioactive peptides. The maximum FRAP value was recorded in the probiotic yogurt supplemented with *L. paracasei*. Reduction of ferric ions in the matrix is closely related to acidity. Indeed, conversion of ferric ions (Fe3+) to ferrous ions (Fe2+) is increased in acidic environment. Therefore, addition of *L. paracasei* to yogurt led to production of more organic acids and provided a potent reducing media. The extent of hydrolysis, the nature of the protein hydrolysate, and the selected probiotic strain are key determinants in modulating these health-promoting effects (34). Recent studies have investigated the impact of adding probiotic bacteria and protein hydrolysates to yogurt,

particularly concerning enhancements in antioxidant properties measured by DPPH radical-scavenging activity. Incorporating Quinoa protein hydrolysates (QPH) into yogurt fermented by *L. plantarum* significantly improved the yogurt's antioxidant capacity. Notably, yogurt supplemented with 1% QPH exhibited a higher DPPH radical-scavenging ability compared to that with 2% QPH (42). Incorporation of Grape Seed Protein Hydrolysate (GPH) into stirred yogurt led to improvements in both physicochemical characteristics and sensory attributes. GPH produced using alcalase enzyme demonstrated higher degrees of hydrolysis and antioxidant properties. Yogurt fortified with GPH showed increased DPPH radical-scavenging activity, indicating improved antioxidant potential (30). Throughout the storage period, the enrichment of yogurt with Clover Sprout Protein Hydrolysate (CPH) enhanced its antioxidant activity, thereby contributing to the improvement of overall product quality (34).

ACE contributes to increased blood pressure. It has been reported that plant-derived peptides show a remarkable ACE inhibition activity (8). In the study of Bhatnagar et al., *L. paracasei* was introduced as a potential indigenous lactic acid culture with ACE inhibition activity in soymilk (40). High rate of proteolysis and production of bioactive peptides by probiotics has been reported by Heydari et al. (43). The authors found that three probiotic strains of *Bifidobacterium lactis*, *L. casei* and *L. acidophilus*, were superior to yogurt common starter cultures in production of bioactive peptides (42). As illustrated in Fig. 3, ACE inhibition by the control and the probiotic yogurt containing *L. paracasei* was higher than other samples until the end of storage. It might be due to the antimicrobial effect of SAPH on starter bacteria and *L. paracasei* that limited their metabolism in the products. Nonetheless, ACE inhibition was observed in all samples, and the role of the bioactive peptides is of great importance in this regard. ACE inhibition by bioactive peptides was reported by Daza-Rodríguez et al. (44). The authors reported that several factors such as peptide length, its molecular weight, and hydrophobicity of C-terminal affect the potency of ACE inhibition (44). Recent studies have explored the impact of incorporating probiotics and protein hydrolysates into yogurt, particularly concerning their antihypertensive properties. These health benefits are predominantly linked to the inhibition of angiotensin-converting enzyme (ACE), a key regulator in maintaining blood pressure homeostasis (45). Probiotics have been suggested to exert antihypertensive effects via multiple biological pathways, such as modulating vascular oxidative stress, enhancing the production of short-chain fatty acids, improving endothelial function, and attenuating inflammatory responses. Together, these mechanisms support the regulation of blood pressure and the management of hypertension (45). Lactotripeptides specifically Isoleucine-Proline-Proline (IPP) and Valine-Proline-Proline (VPP) are bioactive peptides generated through the enzymatic hydrolysis of milk proteins, and have been recognized as natural inhibitors of angiotensin-converting enzyme (ACE). Evidence from clinical trials indicates that, when incorporated into a balanced diet and healthy lifestyle, these peptides may contribute to the regulation of blood pressure (46).

An investigation into the functional properties of probiotic Greek yogurt (PGY) and its variant supplemented with roselle extract (PGYR) demonstrated notable improvements in antioxidant and antihypertensive activities upon the inclusion of the extract. Specifically, the antihypertensive potential rose markedly from 35.68% in PGY to 81.36% in PGYR, indicating a substantial enhancement. The findings suggest that fortifying probiotic yogurt with roselle extract significantly amplifies its blood pressure-lowering effects (47).

In summary, the fortification of yogurt with specific probiotics and protein hydrolysates can enhance its antihypertensive potential. The effectiveness of these functional yogurts depends on factors such as the strains of probiotics used, the types of protein hydrolysates incorporated, and their interactions within the yogurt matrix (48).

The greatest apparent viscosity was detected in the yogurt supplemented with *L. paracasei*, which could be attributed to the shear-thickening behavior of the yogurt matrix, likely resulting from intensified bacterial activity and maximal cell proliferation during fermentation. Bacteria cells produce exopolysaccharides by which the viscosity increases. The lowest viscosity was obtained for samples B which was incorporated with free SAPH. Apparent viscosity in other samples specially those containing free and encapsulated SAPH was significantly lower than the probiotic yogurt. Therefore, lower viscosity in the samples containing SAPH owing to the existence of short chain peptides was observed compared to the

samples free of SAPH.

Similar results were obtained with those of other researchers. Incorporating Quinoa protein hydrolysates (QPH) into yogurt fermented by *L. plantarum* significantly improved the yogurt's viscosity (42).

S. mutans is one of the main causes in development of teeth decay. In particular, the yogurt containing CaAlg-SAPH showed lower inhibition against both bacteria than the sample fortified with CSGE-SAPH. It could be due to the more efficient encapsulation by CaAlg that provided a controlled release of SAPH in the matrix. Antibacterial effect of bioactive peptides has been reported by other scientists. Le et al. (49) stated that growth of *L. paracasei* was inhibited by fish gelatin hydrolysates in fermented milk. However, cell viability is closely related to acidy in the environment and cell integrity is lost to some extent under acidic condition during storage (50).

Among all samples, the control yogurt exhibited the highest proliferation of *S. mutans*, whereas the formulation enriched with free SAPH demonstrated the most pronounced inhibitory effect, resulting in the lowest bacterial count. The highest growth of *S. mutans* was observed in the control yogurt while the lowest count was achieved for the yogurt containing free SAPH. Our findings are in accordance with results of Nunpan et al. (51) who studied the effect of two prebiotics galactooligosaccharides and fructooligosaccharides on *L. acidophilus* growth and inhibition of *S. mutans*. They found that the growth rate of *S. mutans* was significantly decreased in the presence of *L. acidophilus* and the prebiotics (51). Antimicrobial effects of bioactive peptides isolated from soybean was reported by Mashayekh et al. (28). Accordingly, addition of soybean bioactive peptides to yogurt reduced the quantity of *Escherichia coli* and *Staphylococcus aureus* in the samples so that more bacterial reduction was observed by increasing the concentration of peptides. While Chen et al., reaported that incorporating Quinoa protein hydrolysates (QPH) into yogurt fermented by *L. plantarum* significantly increased the yogurt's bacterial growth (42).

Probiotics, when administered in appropriate amounts, can benefit the host's health by preventing dental caries through two primary methods: directly targeting potential pathogens and indirectly hindering the ecological pressure that favors pathogen selection (52).

While direct evidence linking protein hydrolysates to the prevention of dental caries remains limited, certain studies have showen the synergistic advantages of synbiotics for oral health. For instance, prebiotics of glucomannan hydrolysate support the growth of probiotics while they suppress cariogenic microorganisms by reducing the incidence of dental caries (53).

The shelf life and survival of probiotic bacteria are critical factors in controlling tooth decay. Research indicates that probiotics can reduce *S. mutans* levels in the oral microbiota, thereby decreasing plaque formation and caries development (54). However, their efficacy depends on their stability and ability to survive throughout storage and consumption. Previous studies have demonstrated that probiotic yogurt containing *Lactobacillus* strains can improve oral health by producing antimicrobial compounds that inhibit pathogenic bacteria (55).

When comparing our findings with recent literature, studies such as the one for Manmontri et al. (56) confirm that probiotic dairy products significantly lower *S. mutans* populations. Another study by Bauer et al. (57) reported that marine-derived peptides have potent antibacterial effects, supporting our results on the inhibitory action of *S. angustifolium* hydrolysates. These findings align with our observation that free SAPH exhibited greater antimicrobial properties compared to encapsulated SAPH, which ensured controlled release while preserving probiotic viability.

Overall, this study highlights the potential of functional yogurts in oral health applications. Future clinical trials should be conducted to confirm these in vitro results and evaluate their impact on long-term dental health outcomes.

The sensory attributes of yogurt, including texture, odor, color, flavor, and overall acceptability, were systematically evaluated. Numerous studies have explored the impact of probiotics and hydrolysates on the sensory qualities of yogurt. For instance, one study focused on the influence of probiotic bacteria on the sensory traits and microbial contamination of yogurt. The findings revealed that yogurt samples fortified with 1% and 2.5% plant extracts exhibited superior taste, color, and overall acceptability (58). Similarly, the inclusion of quinoa protein hydrolysates notably enhanced bacterial proliferation and the production of organic acids such as citric and lactic acids, thereby improving the yogurt’s flavor and overall quality (42). Another investigation assessed

the effect of adding corn starch and Chavil (Ferulago angulata) extract to low-fat probiotic yogurt, analyzing both its physicochemical and sensory properties. The study observed a gradual decline in the sensory scores over time. However, the yogurt containing 0.1% Chavil extract achieved the highest color score, while the sample with 2% corn starch garnered the highest ratings for texture, taste, and overall acceptability throughout the storage period (59, 60). These results collectively suggest that the incorporation of probiotics and hydrolysates can enhance the sensory characteristics of yogurt (58, 61).

CONCLUSION

Addition of *L. paracasei* to yogurt increased the acidity, viscosity, antioxidant potency, and antihypertension activity compared to control. However, fortification of yogurt with SAPH in free form decreased the growth of *L. paracasei* and *S. mutans* in the samples. Encapsulation of SAPH led to controlled release of bioactive peptides by which lower reduction in population of *S. mutans* and *L. paracasei* was observed. Considering the insignificant difference between overall acceptance of the samples containing free and encapsulated SAPH, the probiotic yogurt fortified with free SAPH was selected as the best functional yogurt so that the highest loss of *S. mutans* and the minimum acceptable count of *L. paracasei* (i.e., $<10^6$ CFU/mL) was observed in this sample until the end of storage. It is suggested that the intake of probiotic yogurt containing *L. paracasei* and SAPH may play a role in the prevention of dental decay in individuals. However, the development of a clinical trial to further explore this potential benefit is strongly advised.

REFERENCES

1. Gharehyakheh S, Elhami Rad AH, Nateghi L, Varmira K. Production of GABA-enriched honey syrup using *Lactobacillus* bacteria isolated from honey bee stomach. *J Food Process Preserv* 2019; 43(8): e14054.
2. Sharafi S, Nateghi L. Optimization of gamma-aminobutyric acid production by probiotic bacteria through response surface methodology. *Iran J Microbi*ol 2020; 12: 584-591.
3. Zarei F, Nateghi L, Eshaghi MR, Abadi MET. Optimization of gamma-aminobutyric acid production in probiotics extracted from local dairy products in west region of Iran using MRS broth and whey protein media. *Appl Food Biotechnol* 2018; 5: 233-242.
4. Shariati Z, Jouki M, Rafiei F. Flavored functional drinking yogurt (Doogh) formulated with *Lactobacillus plantarum* LS5, cress seed gum, and coriander leaves extract. *Food Sci Nutr* 2020; 8: 894-902.
5. Khorshidian N, Yousefi M, Khanniri E, Mortazavian AM. Potential application of essential oils as antimicrobial preservatives in cheese. *Innov Food Sci Emerg Technol* 2018; 45: 62-72.
6. Keivanfar L, Nateghi L, Rashidi L, Pourahmad R, Rashidi Nodeh H. Comparing two different extraction techniques on chemical composition and antioxidant property of three essential oils of Ferulago contracta, Rosmarinus officinalis and Lavendula sublepoidota. *J Food Meas Charact* 2023; 17: 3579-3591.
7. Mirzaee H, Ahmadi Gavlighi H, Nikoo M, Udenigwe CC, Khodaiyan F. Relation of amino acid composition, hydrophobicity, and molecular weight with antidiabetic, antihypertensive, and antioxidant properties of mixtures of corn gluten and soy protein hydrolysates. *Food Sci Nutr* 2022; 11: 1257-1271.
8. Rezvankhah A, Yarmand MS, Ghanbarzadeh B, Mirzaee H. Development of lentil peptides with potent antioxidant, antihypertensive, and antidiabetic activities along with umami taste. *Food Sci Nutr* 2023; 11: 2974-2989.
9. Jafarirad S, Nateghi L, Moslemi M, Afshari KP. Functional properties of the bioactive peptides derived from *Sargassum angustifolium* algae. *J Food Meas Charact* 2023; 17: 6588-6599.
10. Arise RO, Idi JJ, Mic-Braimoh IM, Korode E, Ahmed RN, Osemwegie O. In vitro Angiotesin-1-converting enzyme, α-amylase and α-glucosidase inhibitory and antioxidant activities of Luffa cylindrical (L.)M. Roem seed protein hydrolysate. *Heliyon* 2019; 5(5): e01634.
11. Zhang X, Cao D, Sun X, Sun S, Xu N. Preparation and identification of antioxidant peptides from protein hydrolysate of marine alga Gracilariopsis lemaneiformis. *J Appl Phycol* 2019; 31: 2585-2596.
12. Nazarudin MF, Alias NH, Balakrishnan S, Wan Hasnan WNI, Noor Mazli NAI, Ahmad MI, et al. Chemical, Nutrient and Physicochemical Properties of Brown Seaweed, Sargassum polycystum C. Agardh (Phaeophyceae) Collected from Port Dickson, Peninsular Malaysia. *Molecules* 2021; 26: 5216.
13. Rezvankhah A, Yarmand MS, Ghanbarzadeh B, Mirzaee H. Characterization of bioactive peptides produced from green lentil (Lens culinaris) seed protein concentrate using Alcalase and Flavourzyme in single and sequential hydrolysis. *J Food Process Preserv* 2021; 45(11): e15932.

14. Emam-Djomeh Z, Rezvankhah A (2020). Chapter Three -Targeted release of nanoencapsulated food ingredients. In: Release and Bioavailability of Nanoencapsulated Food Ingredients. pp. 79-120. https://doi.org/10.1016/b978-0-12-815665-0.00003-5
15. Sheybani F, Rashidi L, Nateghi L, Yousefpour M, Mahdavi SK. Development of ascorbyl palmitate-loaded nanostructured lipid carriers (NLCs) to increase the stability of Camelina oil. *Food Biosci* 2023; 53: 102735.
16. Yousefi S, Rajaei P, Nateghi L, Nodeh HR, Rashidi L. Encapsulation of sesamol and retinol using alginate and chitosan-coated W/O/W multiple emulsions containing Tween 80 and Span 80. *Int J Biol Macromol* 2023; 242: 124766.
17. Javadpour K, Shekarchizadeh H, Goli M, Moradiyan Tehrani H. Antimicrobial efficacy of *Teucrium polium* L. extracts for dental caries: green extraction techniques and bioactive compounds. *Food Sci Nutr* 2025; 13(1): e4718.
18. Raei M, Rajabzadeh G, Zibaei S, Jafari SM, Sani AM. Nano-encapsulation of isolated lactoferrin from camel milk by calcium alginate and evaluation of its release. *Int J Biol Macromol* 2015; 79: 669-673.
19. Ghasempour Z, Javanmard N, Mojaddar Langroodi A, Alizadeh-Sani M, Ehsani A, Moghaddas Kia E. Development of probiotic yogurt containing red beet extract and basil seed gum; techno-functional, microbial and sensorial characterization. *Biocatal Agric Biotechnol* 2020; 29: 101785.
20. Mirani A, Goli M. Optimization of cupcake formulation by replacement of wheat flour with different levels of eggplant fiber using response surface methodology. *Food Sci Technol* 2022; 42: e52120.
21. Ghorbanzade T, Jafari SM, Akhavan S, Hadavi R. Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt. *Food Chem* 2017; 216: 146-152.
22. Sadeghzadeh Benam N, Goli M, Seyedain Ardebili SM, Vaezshoushtari N. The quality characteristics of dough and toast bread prepared with wheat flour containing different levels of Portulaca oleracea leaf powder. *Food Sci Technol* 2022; 42: e60820.
23. Ahmadi A, Shahidi SA, Safari R, Motamedzadegan A, Ghorbani-HasanSaraei A. Evaluation of stability and antibacterial properties of extracted chlorophyll from alfalfa (*Medicago sativa* L.). *Food Chem Toxicol* 2022; 163: 112980.
24. Shahabi Mohammadabadi S, Goli M, Naji Tabasi S. Optimization of bioactive compound extraction from Eggplant peel by Response surface Methodology: Ultrasound-Assisted solvent Qualitative and Quantitative effect. *Foods* 2022; 11: 3263.
25. Jabbari N, Goli M, Shahi S. Optimization of bioactive compound extraction from saffron petals using ultrasound-assisted acidified ethanol solvent: Adding value to food waste. *Foods* 2024; 13: 542.
26. Song JH, Kim SK, Chang KW, Han SK, Yi HK, Jeon JG. In vitro inhibitory effects of Polygonum cuspidatum on bacterial viability and virulence factors of *Streptococcus mutans* and *Streptococcus sobrinus*. *Arch Oral Biol* 2006; 51: 1131-1140.
27. Pimentel TC, Costa WKAD, Barão CE, Rosset M, Magnani M. Vegan probiotic products: A modern tendency or the newest challenge in functional foods. *Food Res Int* 2021; 140: 110033.
28. Mashayekh F, Pourahmad R, Eshaghi MR, Akbari-Adergani B. Improving effect of soy whey-derived peptide on the quality characteristics of functional yogurt. *Food Sci Nutr* 2023; 11: 3287-3296.
29. Fang T, Guo M. Physicochemical, texture properties, and microstructure of yogurt using polymerized whey protein directly prepared from cheese whey as a thickening agent. *J Dairy Sci* 2019; 102: 7884-7894.
30. Varedesara MS, Ariaii P, Hesari J. The effect of grape seed protein hydrolysate on the properties of stirred yogurt and viability of *Lactobacillus casei* in it. *Food Sci Nutr* 2021; 9: 2180-2190.
31. Wang L, Wang N, Zhang W, Cheng X, Yan Z, Shao G, et al. Therapeutic peptides: Current applications and future directions. *Signal Transduct Target Ther* 2022; 7: 48.
32. Sah BNP, Vasiljevic T, McKechnie S, Donkor ON. Antioxidative and antibacterial peptides derived from bovine milk proteins. *Crit Rev Food Sci Nutr* 2018; 58: 726-740.
33. Naibaho J, Jonuzi E, Butula N, Korzeniowska M, Föste M, Sinamo KN, et al. Fortification of milk-based yogurt with protein hydrolysates from brewers' spent grain: Evaluation on microstructural properties, lactic acid bacteria profile, lactic acid forming capability and its physical behavior. *Curr Res Food Sci* 2022; 5: 1955-1964.
34. Rashidi S, Ariaii P, Esmaeili M, Bagheri R. Evaluating the properties of the hydrolyzed clover sprout protein obtained by enzymatic hydrolysis and its effect in free and micro-encapsulated form on the properties of probiotic yogurt. *J Food Meas Charact* 2024; 18: 2700-2714.
35. Arab M, Yousefi M, Khanniri E, Azari M, Ghasemzadeh-Mohammadi V, Mollakhalili-Meybodi N. A comprehensive review on yogurt syneresis: Effect of processing conditions and added additives. *J Food Sci Technol* 2023; 60: 1656-1665.
36. Szopa K, Pawlos M, Znamirowska-Piotrowska A. Effect of storage time and bacterial strain on the quality of Probiotic Goat's milk using different types and doses of collagens. *Molecules* 2023; 28: 657.
37. Panesar PS, Shinde C. Effect of storage on Syneresis,

pH, *Lactobacillus acidophilus* Count, Bifidobacterium bifidum count of Aloe vera fortified probiotic yoghurt. *Curr Res Dairy Sci* 2012; 4: 17-23.
38. Gantumur MA, Sukhbaatar N, Jiang Q, Enkhtuya E, Hu J, Gao Ch, et al. Effect of modified fermented whey protein fortification on the functional, physical, microstructural, and sensory properties of low-fat yogurt. *Food Control* 2024; 155: 110032.
39. Ren Y, Li L. The influence of protease hydrolysis of lactic acid bacteria on the fermentation induced soybean protein gel: Protein molecule, peptides and amino acids. *Food Res Int* 2022; 156: 111284.
40. Bhatnagar M, Attri S, Sharma K, Goel G. *Lactobacillus paracasei* CD4 as potential indigenous lactic cultures with antioxidative and ACE inhibitory activity in soymilk hydrolysate. *J Food Meas Charact* 2018; 12: 1005-1010.
41. Karimi N, Pourahmad R, Taheri S, Eyvazzadeh O. Effect of addition of bioactive peptide obtained from enzymatic hydrolysis of yogurt whey on qualitative properties of doogh. *Iran Food Sci Technol Res J* 2022; 18: 249-263.
42. Chen A-X, Meng F-B, Li J-J, Chen X-C, Li Y-C, Jiang L-S. Quinoa protein hydrolysates improved the qualities and volatiles of yogurt fermented by *Lactobacillus plantarum*. *Front Sustain Food Syst* 2024; 8: 10.3389/fsufs.2024.1432631.
43. Heydari S, Hosseini SE, Mortazavian AM, Taheri S. Extraction of bioactive peptides produced in probiotic yoghurt and determination of their biological activities. *Int Dairy J* 2023; 139: 105544.
44. Daza-Rodríguez B, Martínez AR, Padilla AM, Lázaro JM. Food-derived bioactive peptides with Angiotensin-Converting enzyme Inhibiting Effect: A systematic review. *J Pharmacol Pharmacother* 2023; 14: 14-24.
45. hang Y, Zheng T, Ma D, Shi P, Zhang H, Li J, et al. Probiotics *Bifidobacterium lactis* M8 and *Lactobacillus rhamnosus* M9 prevent high blood pressure via modulating the gut microbiota composition and host metabolic products. *mSystems* 2023; 8(6): e0033123.
46. Chen Z, Liang W, Liang J, Dou J, Guo F, Zhang D, et al. Probiotics: functional food ingredients with the potential to reduce hypertension. *Front Cell Infect Microbiol* 2023; 13: 1220877.
47. Juandini PA, Arief II, Wulandari Z, Abidin Z. Characteristics, Antioxidant, and Antihypertensive activities of Probiotic greek yogurt with roselle extract addition. *Trop Anim Sci J* 2024; 47: 235-241.
48. Saleh AS, Zhang Q, Shen Q. Recent research in antihypertensive activity of food Protein-derived Hydrolyzates and Peptides. *Crit Rev Food Sci Nutr* 2016; 56: 760-787.
49. Le Y, Lou X, Yu Ch, Guo Ch, He Y, Lu Y, et al. Integrated metabolomics analysis of *Lactobacillus* in fermented milk with fish gelatin hydrolysate in different degrees of hydrolysis. *Food Chem* 2023; 408: 135232.
50. Sah BN, Vasiljevic T, McKechnie S, Donkor ON. Effect of refrigerated storage on probiotic viability and the production and stability of antimutagenic and antioxidant peptides in yogurt supplemented with pineapple peel. *J Dairy Sci* 2015; 98: 5905-5916.
51. Nunpan S, Suwannachart C, Wayakanon K. Effect of prebiotics-enhanced probiotics on the growth of *Streptococcus mutans*. *Int J Microbiol* 2019; 2019: 4623807.
52. Abikshyeet P, Mishra P, Bhuyan L, Kumar V, Mahapatra N, Adhikary T. Probiotics: Dawn of a new era in dental caries management. *J Pharm Bioallied Sci* 2022; 14(Suppl 1): S34-S38.
53. Bernaś AA, Kłosek S. The use of probiotic Preparations in caries prevention and Treatment. *J Health Study Med* 2024; 2024: 39-58.
54. Beattie RE. Probiotics for oral health: A critical evaluation of bacterial strains. *Front Microbiol* 2024; 15: 1430810.
55. Hoxha R, Evstatieva Y, Nikolova D. Physicochemical, Rheological, and sensory characteristics of yogurt fermented by lactic acid Bacteria with probiotic potential and bioprotective Properties. *Foods* 2023; 12: 2552.
56. Manmontri C, Nirunsittirat A, Piwat S, Wattanarat O, Pahumunto N, Makeudom A, et al. Reduction of *Streptococcus mutans* by probiotic milk: a multicenter randomized controlled trial. *Clin Oral Investig* 2020; 24: 2363-2374.
57. Bauer M, Glowacka M, Kamysz W, Kleczkowska P. Marine Peptides: Potential basic structures for the development of Hybrid compounds as Multitarget therapeutics for the Treatment of Multifactorial diseases. *Int J Mol Sci* 2024; 25: 12601.
58. Yang S-Y, Yoon K-S. Effect of probiotic lactic acid Bacteria (LAB) on the quality and safety of greek yogurt. *Foods* 2022; 11: 3799.
59. Aghajani A, Mirzaei F, Kermani M, Saedi Asl MR, Pedram Nia A. Physicochemical and sensory evaluation of low-fat probiotic yogurt containing corn starch and ferulago angulata (Chevil) extract. *J Food Sci Technol* 2023; 14: 51-67.
60. Azarashkan Z, Motamedzadegan A, Ghorbani-HasanSaraei A, Biparva P, Rahaiee S. Investigation of the physicochemical, antioxidant, rheological, and sensory properties of ricotta cheese enriched with free and nano-encapsulated broccoli sprout extract. *Food Sci Nutr* 2022; 10: 4059-4072.
61. Shafizadeh A, Golestan L, Ahmadi M, Darjani P, Ghorbani-HasanSaraei A. Enrichment of set yoghurt with flaxseed oil, flaxseed mucilage and free or encapsulated *Lacticaseibacillus casei*: effect on probiotic survival and yoghurt quality attributes. *Food Sci Technol Int* 2024; 30: 97-106.