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Probiotic yoghurt fortified with rosehip seed powder: Viability, physicochemical, and sensorial properties

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Abstract

The impact of rosehip seed powder (RSP) fortification (1, 2, and 3%) on some physicochemical characteristics and bacterial survival of set type probiotic yoghurt was evaluated during 21-d storage at 4°C. Textural, microstructural, total phenolic content, antioxidant activity, and sensorial attributes were also assessed in the samples, with and without RSP fortification. Milk was fermented by yoghurt cultures with the inclusion of the probiotic cultures comprising *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis*. The total solid, protein, and total phenolic contents in yoghurts fortified with RSP were significantly higher than control, and an enhancement in the yoghurt's antioxidant capacity and firmness, while a reduction in yoghurt's adhesiveness and syneresis occurred ($p < 0.05$). However, sensory scores were lower in RSP-fortified yoghurts than in control. Throughout 21-d storage, the yoghurt's titratable acidity increased while its syneresis and pH decreased. The present work also determined that RSP fortification of probiotic yoghurt increased ($p < 0.05$) in *L. acidophilus* and *B. animalis* subsp. *lactis* growth (up to 8.24 and 8.10 log CFU/g, respectively) as compared to their respective controls (7.84 and 7.83 log CFU/g, respectively), and probiotic counts in yoghurts fortified with RSP remained over 6 log CFU/g at the end of storage. The documented knowledge of fortifying probiotic yoghurts with RSP could inspire the dairy industry to generate a novel synbiotic product while utilising wasted rosehip seeds as processing waste.

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Introduction

A member of the Rosaceae family, rosehip is also known as rose haw or rose hep. The rosehip fruit has high nutritious quality, particularly considering the phytochemical profile and biological potential, besides being one of nature's richest fruits in terms of vitamin C (Demir *et al*., 2014). Rosehip plays a significant role in the human diet and the food industry due to its nutritional value, sensorial quality, and availability of bioactive components. Rosehip fruits and seeds have preventative and therapeutic effects against infectious and inflammatory diseases, diabetes, gastrointestinal disorders, cold symptoms, diarrhoea, and urinary tract infections due to the presence of these components (Ilyasoğlu and Ilyaso, 2014). In this case, fresh rosehips are usually consumed as a snacks, and dried rosehips are used to make jam, tea, marmalade, and nectar, among other things.

The rosehip seeds, which amount to approximately 30% of the weight of the fruit, are a waste product in the industrial production, and commonly used as animal feed even though they contain even more nutritionally valuable and biologically active components such as phenolic compounds, carotenoids, vitamin C, minerals, and polyunsaturated fatty acids (Gül and Șen, 2017). There are significant amounts of linoleic and oleic acids in rosehip seed oil. The rosehip seed oil has a wide range of applications in the pharmaceutical and cosmetic industries due to its organoleptic, nutritional, and health-promoting properties (Qadir and Anwar, 2020). Rosehip seeds are also a good source of dietary fibre (Gül and Șen, 2017). Some of the fibres in these by-products could be used as

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functional components since they have properties like water retention, swelling, and gel formation (Lamsal and Faubion, 2009). These bioactive chemicals and dietary fibre-rich by-products could thus be utilised as functional components in dairy products.

Foods with specialised nutritional benefits, such as functional foods containing probiotics, are in growing demand as a result of increased consumer awareness and access to information about healthy lifestyles (Tripathi and Giri, 2014). Probiotic-fortified foods are defined as foods that contain sufficient live bacteria to provide beneficial effects in the host (Saad *et al*., 2013). One of the most significant functional dairy products is probiotic yoghurt, which is popularly consumed worldwide. Yoghurt has long been recognised as the most common probiotic carrier.

More research on yoghurt's health advantages and consumer acceptability has resulted from its widespread popularity and high biological value, medicinal capabilities, and nutritional value (Ehsani *et al*., 2016). Recently, researchers have been looking into adding components like probiotics, prebiotics, or their combination (symbiotic), as well as numerous plant extracts (bioactive ingredients) to make functional yoghurts with higher nutritional, physiochemical, sensorial, and rheological qualities than traditional (standard) yoghurts for therapeutic purposes.

Food products claiming probiotic advantages, on the other hand, must meet the recommended minimum number of 10^6 - 10^7 CFU/g mL at the time of intake. However, some probiotic organisms (*e.g.*, bifidobacteria) do not grow quickly in milk due to a lack of growth-promoting elements (Belletti *et al*., 2009). Numerous research has been conducted as a consequence to add milk in order to promote the growth of probiotic microorganisms, thereby providing an opportunity for innovation (Gustaw *et al*., 2011). Additionally, previous research has shown that adding various ingredients to yoghurt, such as fruit fibre, powdered passion fruit, pineapple, mango, and guava pulps might impact the probiotic viability as well as the yoghurt's physicochemical, textural, and sensorial qualities (do Espírito Santo *et al*., 2012; Bedani *et al*., 2014; Sah *et al*., 2016a). By promoting the growth and activities of the gut microflora, the synbiotic product, which combines probiotic strains and prebiotic components, could multiply the health benefits of yoghurt.

Although there has been some earlier research on the use of a variety of new additives to improve yoghurt quality characteristics and add beneficial qualities, we found no studies on the use of rosehip seed as a yoghurt ingredient. Considering this, the goal of the present work was to assess the viability of probiotic *L. acidophilus* and *B. animalis* subsp. *lactis* in yoghurts fortified with RSP, as well as the physicochemical, textural, sensorial, and microstructural features. Moreover, the influence of rosehip seed fortification on total phenolic content (TPC) and antioxidant activity was also investigated.

Materials and methods

Materials

Rosehip (*Rosa canina* L.) seed powder (RSP) was purchased from Ayhan Ercan Superfoods (İstanbul, Turkey). Pasteurised cow milk (3.1% protein, 3.2% fat, 3.54% lactose, and pH 6.97) was purchased from AK Gıda (Adapazarı, Turkey). Commercial freeze-dried yoghurt starters containing *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*) was obtained from Maysa Gıda (İstanbul, Turkey), and probiotic bacteria (*L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12) was obtained from the culture collection of Department of Food Engineering, Tekirdağ, Turkey. All chemicals used in the present work were obtained from Merck (Darmstadt, Germany).

Production of probiotic yoghurts

Branded and pasteurised milk was used to make yoghurt that resembled homemade yoghurt, without using any additional milk powder. Pasteurised milk was subjected to heat treatment at 85°C for 10 min in a water bath, and then quickly cooled and chilled at 43°C. Yoghurt culture was propagated in 1 L sterilised skim milk at 30°C for 30 min. The propagated culture (2.5 mL/kg milk) and 0.1% (w/w) of each *L. acidophilus* and *B. animalis* subsp. *lactis* suspension as the probiotic strain were inoculated to the milk (Sah *et al*., 2016b). Following that, the inoculated milk was divided into four portions: 0% (control), 1%, 2%, and 3% RSP. The inoculated milk was later transferred to 100 g plastic containers, and incubated at 42°C until the pH dropped to 4.6. For subsequent testing, all of the samples were maintained at 4 ± 1°C.

The pH, titratable acidity, water holding capacity (WHC), and syneresis of samples were evaluated on days 1, 7, 14, and 21 of the cold storage periods. Total solids (TS), protein, TPC, antioxidant activity, textural, colour, microstructural, and sensorial properties were assessed one day after production.

Physico-chemical analysis

The pH value of samples was measured with a pH meter (Interlab, Turkey) after calibration. The titratable acidity and total solids of samples were evaluated according to do Espírito Santo *et al*. (2012). The moisture and protein contents of samples were measured by the oven and Kjeldahl methods (Aziznia *et al*., 2008), respectively. To determine sample syneresis, 5 g of each sample was placed on a different Whatman paper number 43, and left above a glass container for 120 min at 4°C (García-Pérez *et al*., 2005). The weight of the liquid collected in the glass container was estimated as a percentage by dividing it with the initial weight of the yoghurt sample using Eq. 1:

$$Syneresis\ (\%) = \frac{Total\ weight\ of\ separated\ liquid}{Total\ wight\ of\ the\ yoghurt\ sample} \times 100 \quad \text{(Eq. 1)}$$

Sample (10 g) was centrifuged at 5,000 *g* for 10 min at 4°C to determine the WHC of the samples (García-Pérez *et al*., 2005). The precipitate that was obtained from isolating the supernatant solution was weighed, and the WHC was calculated using Eq. 2:

$$WHC = \frac{Initial\ yoghurt\ weight - Supernatant}{Original\ yoghurt\ weight} \times 100 \quad \text{(Eq. 2)}$$

The colour of the yoghurt samples was measured by a chroma meter CR 400 (Konica Minolta Sensing, Inc., Japan), and the data were reported as L^*, a^*, and b^*. The L^* indicated the degree of brightness (0 - 100), a^* indicated red to green, and b^* indicated yellow to blue (García-Pérez *et al*., 2005).

Textural analysis

Firmness, cohesiveness, adhesiveness, and gumminess indices were determined using a texture analyser (TA. HD. PLUS, Stable Micro Systems, Godalming, Surrey) one day after production. The probe was a 25 mm acrylic cylinder, moved speed of 1 mm/s, and test speed of 1 mm/s through 10 mm within the sample. The data were presented as the average of four measurements (Öztürk *et al*., 2018).

Extraction procedure for antioxidant and TPC analysis

Briefly, 5 g of yoghurt was mixed with an adequate amount of methanol solution (25 mL) for extraction (80:20, methanol: distilled water). The mixtures were homogenised using an ultra-turrax homogeniser (IKA Werke M20, Germany) then centrifuged (SIGMA 2-16 KL) at 8,000 rpm and 4°C for 15 min. The obtained supernatants were filtered using Whatman No.1 filter paper, and kept at 4°C for antioxidant activity and TPC analyses.

Determination of antioxidant activity and total phenolic content

The TPC of the samples were determined by a spectrophotometric assay based on the Folin-Ciocalteu method, as described by Hasperué *et al*. (2016). The calibration curve was created using gallic acid in a range of concentrations between 0.500 and 0.100 mg/mL. Reaction absorbance was measured at 720 nm. Results were calculated as milligrams of gallic acid equivalents (GAE) per kilogram of sample yoghurt. The DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity was measured as described in previous study. Briefly, 600 µL of DPPH solution were combined with 300 µL of the extraction sample (1 mM in 95% methanol), and incubated for 35 min at 35°C in the dark. The absorbance was measured at 517 nm, and the values of scavenging effect were expressed as % inhibition. The DPPH scavenging activity was calculated using Eq. 3:

$$DPPH\ scavenging\ activity = \frac{A\ control\ 517 - A\ extract517}{A\ control517} \times 100 \quad \text{(Eq. 3)}$$

where, A control = DPPH working solution with water instead of the sample.

Microstructural analysis

A scanning electron microscope (SEM) was used to examine the microstructure of yoghurt samples, as described by Fu *et al*. (2018). Yoghurt samples were lyophilised using a freeze dryer (Alpha 2-4 LD Plus Christ) after being stored at 4°C for 14 d. The dry sample was coated with aluminium mounted on the stub of SEM system (QUANTA FEG

250, FEI), and micrographs were recorded. Electron accelerating voltage was 2 kV, and the magnification was 1,000×.

Microbiological analysis

Bacterial counts were carried out in duplicate at 1, 7, 14, and 21 d using the spread-plate method, and colony counts given as log CFU/g. To make a one-tenth dilution, 10 g of sample was added to 90 mL of NaCl solution (0.85 g/100 mL); subsequently, other serial dilutions were made from the first dilution.

L. delbrueckii subsp. *bulgaricus* was enumerated using MRS Agar (pH 5.4), and plates were incubated anaerobically at 37°C for 24 ± 1 h. M-17 Agar (pH 7.2) was used for the selective enumeration of *S. thermophilus* by incubation aerobically at 37°C for 24 ± 1 h. Populations of *B. animalis* subsp. *lactis* were enumerated by pour-plating 1 mL of each dilution on MRS agar containing lithium chloride (2 g/L) and sodium propionate (3 g/L) (LP-MRS agar), following 72 h of anaerobic incubation at 37°C (Vinderola and Reinheimer, 1999). MRS-bile Agar (pH 6.2) was used for *L. acidophilus* enumeration, and plates were incubated under anaerobic conditions at 37°C for 48 ± 1 h (Hasani *et al*., 2016). All media were obtained from Merck (Darmstadt, Germany).

Sensorial analysis

The assessments of appearance, colour, taste, odour, after taste, and consistence for yoghurt samples were evaluated by 21 panellists using five-point hedonic scale (1: very bad; 2: bad; 3: medium; 4: good; and 5: very good) (Mousavi *et al*., 2019). All the samples (4 ± 1°C) were presented at the same time and rated as a group. Before analysing each sample, the panellists were given water to wash away the preceding sample's flavour.

Statistical analysis

ANOVA was used to analyse the data in JMP 5.0.1 (SAS Institute) in order to determine significant differences between means of samples and storage days. The Tukey's test was used to compare various groups at $p < 0.05$, and significant differences were shown by different letters.

Results and discussion

Microbial viability

During the storage period (21 d) at 4°C, viable *L. acidophilus*, *B. animalis* subsp. *lactis*, *L. bulgaricus*, and *S. thermophilus* were enumerated, and the findings are presented as log CFU/g in Table 1. As can be seen from the results of the first day of storage, RSP had a beneficial effect on both starter cultures and the counts of probiotic strains in yoghurt samples ($p < 0.05$). The highest count was 9.15 log CFU/g in the yoghurt sample with 3% RSP on the first day, thus indicating a significant ($p < 0.05$) increase in *S. thermophilus* vitality due to the addition of RSP. In this regard, it has been reported that fortifying banana or apple fibre to yoghurt increased *S. thermophilus* counts significantly (do Espírito Santo *et al*., 2012). Similarly, moringa extracts at concentrations of 0.1 and 0.2% increased the viability of *S. thermophilus* in probiotic yoghurt (Zhang *et al*., 2019). During the storage period, *S. thermophilus* count decreased (particularly between day 1 and 14) even though the rate of decrease in the RSP-fortified samples was lower than that in control. At the end of 3-w shelf life, the highest viable numbers of *S. thermophilus* were counted in yoghurts fortified with 2 and 3% RSP. Our findings were similar to those of do Espírito Santo *et al*. (2012) who showed a comparable decrease in *S. thermophilus* counts in yoghurts with passion fruit peel co-fermented by *L. acidophilus* strains between day 1 and 14 of storage. Furthermore, the findings were consistent with those of do Espírito Santo *et al*. (2012) and Demirci *et al*. (2017) who reported a modest decrease in *S. thermophilus* throughout storage of probiotic-cultured yoghurts containing açai and rice bran, respectively. Contrarily, tomato powder and guava pulp had no statistically significant impact on *S. thermophilus* counts in probiotic yoghurts throughout and over the shelf life (Bedani *et al*., 2014; Demirci *et al*., 2020).

With respect to *L. bulgaricus*, the counts were stable, and ranged on average from 8.12 to 8.88 log CFU/g up to 1-w storage ($p > 0.05$), and followed by a decrease in viability at the end of 4-w storage ($p < 0.05$) (Table 1). This decrease was more pronounced in control; in other words, on day 21, samples fortified with 2 and 3% RSP had the highest *L. bulgaricus* counts, whereas control had the lowest. These findings showed that RSP promoted *L. bulgaricus* growth in yoghurt, which was consistent

Table 1. Viable counts (log CFU/g) of probiotic yoghurts with different concentrations of RSP during cold (4 ± 1°C) storage.

Microorganism	Time (d)	RSP			
		0% (Control)	1%	2%	3%
S. thermophilus	1	8.59 ± 0.01^{Ad}	8.66 ± 0.02^{Ac}	9.05 ± 0.01^{Ab}	9.15 ± 0.02^{Aa}
	7	7.25 ± 0.01^{Bd}	7.44 ± 0.01^{Bc}	8.21 ± 0.12^{Bb}	8.33 ± 0.14^{Ba}
	14	6.32 ± 0.01^{Cb}	6.11 ± 0.02^{Cb}	7.15 ± 0.01^{Ca}	7.27 ± 0.14^{Ca}
	21	5.77 ± 0.01^{Db}	6.10 ± 0.01^{Cb}	7.10 ± 0.02^{Ca}	7.05 ± 0.07^{Da}
L. bulgaricus	1	8.12 ± 0.01^{Ad}	8.18 ± 0.01^{Ac}	8.53 ± 0.01^{Ab}	8.87 ± 0.01^{Aa}
	7	8.16 ± 0.01^{Ad}	8.22 ± 0.01^{Ac}	8.57 ± 0.01^{Ab}	8.88 ± 0.01^{Aa}
	14	7.96 ± 0.01^{Bd}	7.84 ± 0.01^{Bc}	8.25 ± 0.01^{Bb}	8.10 ± 0.01^{Ba}
	21	6.56 ± 0.01^{Cc}	6.74 ± 0.01^{Cb}	7.12 ± 0.02^{Ca}	7.08 ± 0.01^{Ca}
L. acidophilus	1	7.84 ± 0.01^{Ad}	8.24 ± 0.01^{Ac}	8.66 ± 0.01^{Ab}	8.87 ± 0.01^{Aa}
	7	7.80 ± 0.01^{Ac}	8.22 ± 0.01^{Ab}	8.55 ± 0.01^{Ba}	8.58 ± 0.01^{Ba}
	14	6.83 ± 0.01^{Bc}	7.01 ± 0.01^{Bb}	7.10 ± 0.14^{Ca}	7.05 ± 0.01^{Cab}
	21	6.80 ± 0.14^{Ba}	6.04 ± 0.02^{Cb}	6.18 ± 0.01^{Db}	6.01 ± 0.01^{Db}
B. lactis	1	7.83 ± 0.01^{Ac}	8.1 ± 0.01^{Ab}	8.26 ± 0.01^{Ab}	8.47 ± 0.02^{Aa}
	7	7.78 ± 0.01^{Ad}	8.07 ± 0.01^{Ac}	8.16 ± 0.02^{Ab}	8.38 ± 0.01^{Ba}
	14	6.9 ± 0.14^{Bb}	7.41 ± 0.01^{Ba}	7.39 ± 0.14^{Ba}	7.75 ± 0.01^{Ca}
	21	6.78 ± 0.01^{Bd}	7.02 ± 0.01^{Ca}	6.96 ± 0.01^{Cb}	6.88 ± 0.01^{Dc}

RSP: yoghurts with rosehip seed powder. Different lowercase superscripts in the same row indicate significant difference between samples for the same period of storage ($p < 0.05$). Different uppercase superscripts in the same column indicate significant difference between means for same type of yoghurt sample at 1st, 7th, 14th, and 21st day of refrigerated storage ($p < 0.05$).

with the pH data. *Siraitia grosvenorii* fruit extract fortification previously showed a similar pattern of *L. bulgaricus* viability in yoghurt (Abdel-Hamid *et al*., 2020). However, adding banana fibre, passion peel powder, pineapple waste powder, apple fibre, and rice bran to probiotic yoghurt had no influence on *L. bulgaricus* viability (do Espírito Santo *et al*., 2012; Sah *et al*., 2016a; Demirci *et al*., 2017). Several other researchers indicated a considerable decrease of *L. bulgaricus* gradually along with storage in probiotic yoghurts fortified with passion fruit peel powder, and yoghurt containing Jerusalem artichoke inulins, similar to our findings on storage (Paseephol and Sherkat, 2009; do Espírito Santo *et al*., 2012).

The viability of *L. acidophilus* and *B. animalis* subsp. *lactis* in the yoghurt formulations during storage period at 4°C are shown in Table 1. On the first day of storage, the fortification of RSP at all concentrations increased the survival of both probiotic counts as compared to control ($p < 0.05$). The highest *L. acidophilus* (8.87 log CFU/g) and *B. animalis* subsp. *lactis* (8.47 log CFU/g) counts were observed in the sample with 3% RSP after 1-df storage period. The increase in *L. acidophilus* and *B. animalis* subsp. *lactis* growth in probiotic yoghurt fortified with RSP could be attributed to several factors. Firstly, rosehip seeds contain prebiotic fibres and compounds (Gül and Şen, 2017) that can promote the growth of probiotic bacteria. Secondly, rosehip seeds contain polyphenols such as flavonoids and tannins (Gül and Şen, 2017) which are not absorbed in the small intestine but reach the colon where they undergo biotransformation by the colon microbiota, thus meeting prebiotic criteria (Gibson *et al*., 2017). Thirdly, rosehip seeds are rich in vitamins and minerals (Demir *et al*., 2014) that are important for bacterial growth and metabolism. By fortifying RSP into the yoghurt, it could provide additional nutrients to support the growth of the probiotics. These combined factors contributed to the favourable conditions for the increased growth of *L. acidophilus* and *B. animalis* subsp. *lactis* in the fortified probiotic yoghurt. In this context, probiotic yoghurt fortified with rice bran or pineapple waste exhibited a considerable increase in *B. animalis* subsp. *lactis*, *L. casei*, and *L. acidophilus* counts in comparison to control (Sah *et al*., 2016b; Demirci *et al*., 2017).

Considering the values obtained over the storage period, probiotic count profile in control and RSP-fortified yoghurts showed a constant pattern ($p > 0.05$) up to the 7th day of storage; however, this behaviour changed dramatically ($p < 0.05$), notably after 7-d storage, and the viable numbers of *L. acidophilus* and *B. animalis* subsp. *lactis* decreased to around 6.01 - 6.80 and 6.78 - 7.02 log CFU/g, respectively, at the end of the assessed storage. The rate of decrease in survival during storage was higher in RSP-fortified yoghurt samples as compared to control (comparing the samples at day 1 and 21 of storage). Many researchers have reported similar behaviours of different probiotic microorganisms (such as steady or increase trend first, and then decrease) during the storage period in the fortified yoghurts (do Espírito Santo *et al*., 2012; Sah *et al*., 2016a; Demirci *et al*., 2017; Mousavi *et al*., 2019).

Combined probiotic cultures in yoghurt can cause poor growth and consequent low storage viability as compared to pure cultures, most likely due to nutrient competition (Ranadheera *et al*., 2012). Furthermore, previous research has found that cell viability loss is primarily caused by two factors: the accumulation of organic acids during growth and fermentation, and a decrease in pH during product storage (post-acidification) (Kailasapathy *et al*., 2008).

In line with previous findings, *L. acidophilus* showed a greater decrease in viability than the bifidobacteria (Vinderola *et al*., 2002; Ranadheera *et al*., 2012). On the 21st day of refrigerated storage, *L. acidophilus* level were considerably lower in yoghurts containing RSP as compared to control. With regard to RSP-fortified yoghurts, *L. acidophilus* counts at the end of 3-w shelf life were similar ($p > 0.05$). In contrast, *B. animalis* subsp. *lactis* counts were higher in RSP-fortified yoghurts as compared to control at the end of shelf life. Hydrogen peroxide has been shown to have little effect on bifidobacteria, unlike *L. acidophilus*. Bifidobacteria may be able to survive longer than *L. acidophilus* in the presence of the well-known hydrogen peroxide producer, *L. bulgaricus* (Dave and Shah, 1997). The number of viable cells of probiotic bacteria remained over 6 log CFU/g between the first and last day of storage, in yoghurt with or without RSP, which is the recommended limit value for a food to exert its probiotic benefits on the host (Maciel *et al*., 2014).

Post-acidification (pH) and titratable acidity

The pH and acidity of yoghurt are crucial indicators of its quality. Table 2 displays the results of post-acidification (pH) and titratable acidity during the storage period of yoghurts. The pH of yoghurts ranged from 4.33 to 4.40 after one day of storage, and no differences were found between the yoghurt samples ($p > 0.05$). Titratable acidity ranged from 0.69 to 0.74% lactic acid in yoghurt samples. A statistically significant increase ($p < 0.05$) in this parameter was observed when the fortification of RSP. This result was similar to those reported by Mousavi *et al*. (2019) who mentioned that adding flaxseed to yoghurt increased the acidity.

The pH of control remained ($p > 0.05$) constant throughout the 21-d cold storage. After the first day of cold storage to day 7, pH values in yoghurts with 2 and 3% RSP decreased; however, yoghurt with 1% RSP remained constant. Between days 7 and 14 of storage, there was no significant difference ($p > 0.05$) in any of the RSP-fortified yoghurts for pH. After the 14th day, the pH of all the RSP-fortified yoghurt samples decreased ($p < 0.05$). After 21-d cold storage, the pH of all samples ranged from 4.11 to 4.32, and as was to be expected, the pH of all fortified yoghurts was significantly lower than that of control. The rosehip seed did, in fact, cause a considerable decrease in the pH of all probiotic yoghurts in this circumstance. Similar pH alterations were mentioned for probiotic yoghurt fortified with pineapple peel powders or passion fruit during cold storage (do Espírito Santo *et al*., 2012; Sah *et al*., 2016a).

All probiotic yoghurts did not have a pH below 4.0, which is typically thought to be harmful to probiotic organisms' survival (Dave and Shah, 1997). The sensitivity of probiotics to low pH may be species specific, as evidenced by the higher survivability of *B. animalis* subsp. *lactis* during storage as compared to *L. acidophilus*.

The acidity levels for all yoghurt samples showed an increase with time from the first day to the end of storage, and it was clear that the considerable increase in acidity of all the products (except 2% RSP) occurred between days 7 and 14 of storage. The metabolic action of the probiotic and starter bacteria during the storage period of the output is responsible for post acidification in probiotic yoghurts (Wang *et*

Table 2. Post-acidification (pH), titratable acidity, WHC, and syneresis during cold (4 ± 1°C) storage of yoghurt samples.

	Sample	Day 1	Day 7	Day 14	Day 21
pH	C	4.33 ± 0.07^{Aa}	4.38 ± 0.02^{Aa}	4.31 ± 0.06^{Aa}	4.32 ± 0.02^{Aa}
	1%	4.43 ± 0.00^{Aa}	4.42 ± 0.02^{Aa}	4.33 ± 0.00^{Aa}	4.11 ± 0.06^{Bb}
	2%	4.45 ± 0.04^{Aa}	4.30 ± 0.00^{Bb}	4.27 ± 0.01^{Ba}	4.15 ± 0.00^{Cab}
	3%	4.40 ± 0.01^{Aa}	4.28 ± 0.00^{Bb}	4.20 ± 0.07^{Bca}	4.16 ± 0.04^{Cab}
Titratable acidity (% lactic acid)	C	0.69 ± 0.00^{Bb}	0.74 ± 0.00^{ABbc}	0.76 ± 0.00^{Ac}	0.76 ± 0.01^{Ac}
	1%	0.71 ± 0.00^{Bab}	0.72 ± 0.00^{Bc}	0.83 ± 0.00^{Ab}	0.83 ± 0.00^{Ab}
	2%	0.72 ± 0.00^{Ba}	0.78 ± 0.00^{Aa}	0.78 ± 0.01^{Ac}	0.81 ± 0.00^{Abc}
	3%	0.74 ± 0.00^{Ba}	0.75 ± 0.00^{Bb}	0.86 ± 0.00^{Aa}	0.88 ± 0.00^{Aa}
Water holding capacity (%)	C	54.65 ± 0.39^{Aa}	56.48 ± 1.01^{Aa}	61.84 ± 0.07^{Aa}	51.73 ± 2.55^{Aa}
	1%	53.67 ± 0.98^{Aab}	51.08 ± 0.21^{Bb}	55.22 ± 1.60^{Ab}	44.23 ± 0.12^{Cb}
	2%	53.33 ± 0.73^{Aab}	54.26 ± 0.36^{Aa}	54.57 ± 0.12^{Ab}	48.53 ± 0.99^{Bab}
	3%	50.91 ± 1.13^{Bb}	56.49 ± 0.35^{Aa}	55.72 ± 0.08^{Ab}	51.43 ± 0.20^{Ba}
Syneresis (%)	C	39.64 ± 0.11^{Aa}	38.69 ± 0.40^{ABa}	38.5 ± 0.49^{ABa}	38.02 ± 0.18^{Ba}
	1%	38.6 ± 0.07^{Ab}	37.02 ± 0.12^{Bab}	36.48 ± 0.02^{Bab}	35.82 ± 0.01^{Cb}
	2%	38.11 ± 0.01^{Ab}	37.93 ± 0.00^{Aab}	35.42 ± 0.01^{Bbc}	34.35 ± 0.10^{Cc}
	3%	37.1 ± 0.01^{Ac}	36.8 ± 0.07^{Ab}	34.15 ± 0.03^{Bc}	33.55 ± 0.03^{Cc}

C: control. Different lowercase superscripts in the same column indicate significant difference between the samples for the same period of storage ($p < 0.05$). Different uppercase superscripts in the same row indicate significant difference between means for same type of yoghurt sample at 1st, 7th, 14th, and 21st day of refrigerated storage ($p < 0.05$).

al., 2019). The titratable acidity of yoghurts fortified with RSP was found to be significantly higher than control at the end of shelf life. The acidity increase from the first to the last day of storage was concurrent with findings of Agil *et al*. (2013) and Demirci *et al*. (2020) who fortified yoghurt with tomato powder and lentil, respectively.

The availability of fermentable compounds in RSP which were metabolised by LAB resulting in organic acid generation might explain the lower pH and higher acidity of the RSP-fortified yoghurts as compared to control.

Syneresis and water holding capacity

Syneresis, an undesired characteristic of yoghurt, is the result of liquid separating from the yoghurt curds (Hongyu *et al*., 2000). Whey separation was significantly decreased with RSP fortification, and the yoghurt with 3% RSP had the lowest syneresis value (37.1%) on the first day of storage. In addition, RSP-fortified yoghurts exhibited lower whey separation as compared to unfortified yoghurt during storage. Water binding abilities of rosehip seed might have contributed to the decrease in syneresis. Our data were consistent with Mousavi *et al*. (2019) who found a decrease in whey separation in probiotic yoghurts, in which flaxseeds were added to them, as well as a constant decrease during storage. Öztürk *et al*. (2018) also reported that oleaster flour significantly lowered yoghurt syneresis.

The WHC of the yoghurt-gel is a desired feature that shows coagulation stability. The milk composition (protein and/or fat globules) and acidity, where the higher the value, the better the yoghurt structure, are the most crucial factors influencing water retention (Srisuvor *et al*., 2013). In the present work, the WHC values of probiotic yoghurts ranged from 50.91 to 54.65% on the initial of storage (Table 2). WHC was significantly decreased by RSP fortifications, with the lowest WHC value found in a yoghurt sample containing 3% RSP. The findings of Ranadheera *et al*. (2012) and Demirci *et al*. (2017) were similar to ours; they demonstrated that adding fruit or rice bran to yoghurt decreased the WHC, respectively. A decrease in WHCs of RSP-fortified yoghurts was determined between the first and last days of cold storage ($p < 0.05$). However, no noticeable difference was found in the WHC of control during this period ($p > 0.05$).

Physico-chemical properties, TPC, and antioxidative activity

The physicochemical properties, TPC, and antioxidant activity of yoghurt formulations were measured on day 1, and the results are given in Table 3. As seen in Table 3, physicochemical properties of RSP-fortified yoghurts were significantly influenced as compared to control. Due to the lack of any fortified component, control had the lowest total solid values (Table 3). Total solid content increased linearly with increasing RSP concentration ($p < 0.05$). These data were in accordance with those of Öztürk *et al*. (2018), Demirci *et al*. (2020), and Abdel-Hamid *et al*. (2020) for yoghurts made with oleaster flour, tomato powder, and *Siraitia grosvenorii* fruit extract, respectively. Protein content of control was lower than RSP-fortified yoghurts regardless of the fortification level of RSP.

Table 3. Physicochemical characteristics, antioxidant capacity, and total phenolic contents of probiotic yoghurt samples at one day after production.

Physicochemical characteristic	RSP			
	0% (Control)	1%	2%	3%
Total solid (%)	12.80 ± 0.14^{D}	12.82 ± 0.02^{C}	13.52 ± 0.01^{B}	15.45 ± 0.01^{A}
Protein (%)	3.32 ± 0.007^{B}	3.51 ± 0.004^{A}	3.48 ± 0.028^{AB}	3.49 ± 0.007^{A}
Total phenolic content (mgGAE/kg)	42.09 ± 2.91^{D}	127.80 ± 28.76^{C}	263.61 ± 31.02^{B}	441.14 ± 27.55^{A}
DPPH (% inhibition)	10.77 ± 0.32^{B}	48.22 ± 1.65^{A}	52.45 ± 0.40^{A}	66.57 ± 0.24^{A}
*L**	90.67 ± 0.04^{A}	89.42 ± 0.01^{B}	89.17 ± 0.22^{B}	88.83 ± 0.05^{C}
*a**	-0.63 ± 0.05^{D}	-0.01 ± 0.00^{C}	0.16 ± 0.00^{B}	0.31 ± 0.01^{A}
*b**	1.20 ± 0.03^{D}	1.98 ± 0.01^{C}	2.14 ± 0.00^{B}	2.34 ± 0.01^{A}

RSP: yoghurts with rosehip seed powder. Different uppercase superscript s in the same row indicate significant difference ($p < 0.05$).

Probiotic yoghurts fortified with RSP indicated remarkably ($p < 0.05$) greater TPC when compared with that of control. It should be mentioned that increasing the level of RSP resulted in a steady increase in phenolic content. The contents varied from 42.9 to 441.14 mg GAE/kg, and these numbers referred to control and 3% RSP samples, respectively.

Probiotic yoghurts fortified with RSP exhibited significantly ($p < 0.05$) higher antioxidative activities when compared with control; however, there were no statistical differences ($p < 0.05$) in RSP-fortified samples. In comparison to control, fortifying 3% RSP improved the DPPH radical scavenging activity by approximately 6-fold. These findings were in accordance with Abdel-Hamid *et al*. (2020) who found that set-yoghurt fermented with *Siraitia grosvenorii* fruit extract had increased antioxidant activity. Furthermore, Sah *et al*. (2016b) and Demirci *et al*. (2020) found that probiotic yoghurt fortified with tomato powder and powder of pineapple waste increased antioxidant activity. The high polyphenol and vitamin C levels of rosehip may account for the enhanced antioxidative activity of probiotic yoghurt after fortification with this fruit seed.

*L**, *a**, and *b** values of probiotic yoghurts fortified with or without RSP stored at 4°C are presented in Table 3. Control had considerably higher *L** value than RSP-fortified yoghurts ($p < 0.05$). The samples with 1 and 2% RSP, which were similar to each other ($p > 0.05$), had lower *L** values than the sample with 3% RSP ($p > 0.05$). Furthermore, RSP-fortified samples showed greater *a** and *b** values than control, and these values increased as the additive content in the yoghurt formulation increased ($p > 0.05$). Vinderola *et al*. (2002) and Demirci *et al*. (2020) found similar *L** value decreases, and *a** and *b** value increases when fortifying yoghurt with cupuassu and tomato powder pulp, respectively.

Textural properties

The texture of yoghurt is a critical indicator for its quality. Figure 1 depicts the impact of RSP fortification on the textural qualities of probiotic yoghurts. The firmness of the samples ranged from 278.50 to 425.29 g. 2 and 3% RSP fortification resulted in higher firmness of yoghurt; however, there was no statistically significant difference between the 1% RSP and control ($p > 0.05$). These two samples had high enough total solid contents, thus giving samples with higher firmness in comparison to other samples. Furthermore, yoghurt texture can be improved by LAB that produce exopolysaccharides. The higher numbers of exopolysaccharides-producing *L. acidophilus* and *S. thermophilus* in fortified samples (on day 7) might also contribute to increasing in their firmness parameter. Our findings were comparable to those of Mousavi *et al*. (2019) who found that adding flaxseed to yoghurt improves its hardness. Contrarily, do Espírito Santo *et al*. (2012) and Sah *et al*. (2016a) reported that the fortification of passion fruit peel or pineapple peel powder increased the firmness of yoghurt samples.

The force required to remove the stuck substance from the mouth during chewing is referred as adhesiveness. It is used as a measure of yoghurt stickiness, and inversely related to yoghurt eating quality (Mudgil *et al*., 2017). Control was found to be more adhesive (-744.89) than the other samples fortified with RSP. Higher water in the gel system, as indicated by enhanced lower syneresis of RSP-fortified yoghurts, was likely responsible for the decrease in adhesiveness following the fortification of RSP to yoghurt. A softer yoghurt gel network is probably caused by more water in the gel system.

In addition, RSP fortification and rate did not affect both cohesiveness and gumminess properties of probiotic yoghurts ($p > 0.05$). The average values for the cohesiveness and gumminess of yoghurt samples fortified with RSP differed between 0.358 - 0.466 and 121 - 154 g, respectively. Mousavi *et al*. (2019) noticed that the fortification of flaxseed decreased the adhesiveness of yoghurt. However, they also showed that gumminess and cohesiveness in flaxseed-fortified yoghurts were higher than those of control.

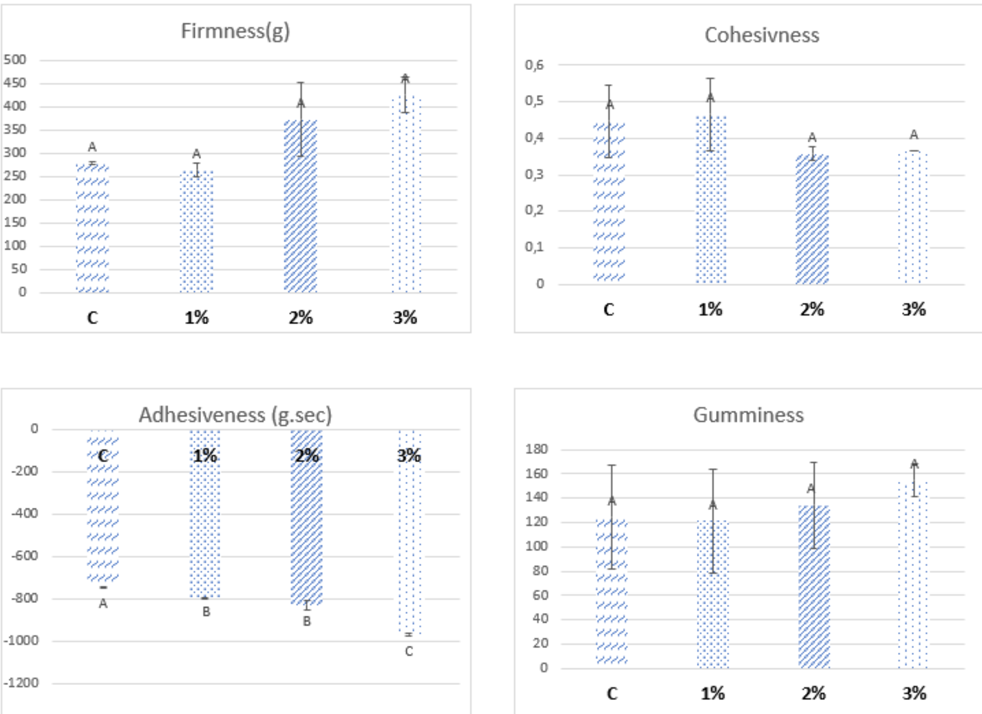

Figure 1. Textural profile of probiotic yoghurt samples. Firmness, cohesiveness, adhesiveness, and gumminess values of yoghurt samples are shown. Different uppercase letters indicate significant difference ($p < 0.05$).

Microstructure of yoghurt

Micrographs were taken to depict differences in the microstructure of yoghurt gel, and to describe how RPS fortification affected its physical and structural qualities. Different gel structures, such as the compactness of the three-dimensional network of casein micelles and pore diameters, are visible in the micrographs (Figure 2). The globular shapes of casein micelle aggregation are interspersed by void zones of the original serum in a three-dimensional network. The microstructures of control and RSP-fortified yoghurts showed striking variations. Control and RSP-fortified yoghurt microstructures differed dramatically. Similar effects were found by Espírito-Santo *et al*. (2013) in yoghurts fortified with passion fruit fibre.

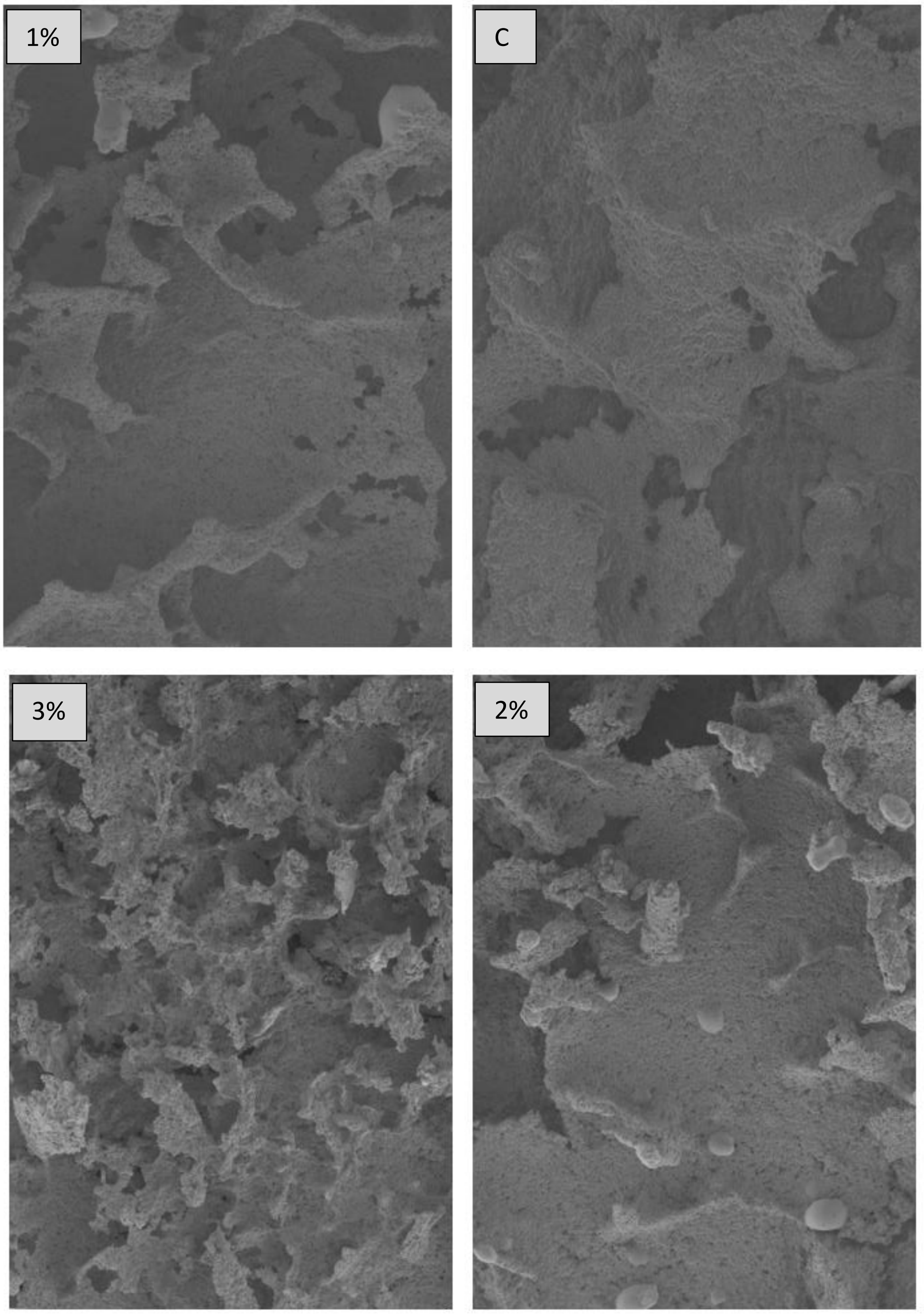

Figure 2. Micrographs of yoghurt samples.

Sensorial properties

Table 4 shows the sensorial attributes of probiotic yoghurts after 24 h of storage at 4 ± 1°C. In general, all yoghurt samples displayed mean scores varying from 3.14 to 4.85. In terms of appearance, texture, and taste, panellists gave RSP-fortified yoghurts a lower score than control ($p < 0.05$). Although there was no statistical difference ($p > 0.05$) in taste amongst RSP-fortified yoghurts, the sample with 3% RSP had lower scores than the others in terms of texture ($p < 0.05$). It was found that there were no differences ($p > 0.05$) between the samples in terms of colour and odour properties (Table 4).

Phenolic compounds in foods play an active role in the formation of bitter and astringent taste (Pedan *et al*., 2019). Rosehip seeds are a remarkable source of high-value oils with functional components such as tocopherols, phytosterols, and phenolics. The fact that the samples fortified with RSP had lower taste rating than control could have been due to the phenolic compounds found in rosehip seeds which led to bitterness.

Table 4. Sensorial attributes of probiotic yoghurt samples.

Characteristic	RSP			
	0% (Control)	1%	2%	3%
Appearance	4.85 ± 0.37^{A}	3.71 ± 0.75^{B}	4.14 ± 0.37^{AB}	3.85 ± 0.75^{B}
Colour	4.85 ± 0.37^{A}	4.14 ± 0.69^{A}	3.71 ± 0.75^{A}	3.71 ± 1.36^{A}
Texture	4.42 ± 0.52^{A}	3.71 ± 0.48^{AB}	3.71 ± 0.48^{AB}	3.57 ± 0.54^{B}
Odour	4.85 ± 0.37^{A}	4.00 ± 1^{A}	4.14 ± 1.06^{A}	3.66 ± 0.81^{A}
Taste	4.85 ± 0.53^{A}	3.85 ± 0.69^{AB}	4.28 ± 0.95^{AB}	3.14 ± 1.16^{AB}

RSP: yoghurts with rosehip seed powder. Different uppercase superscripts in the same row indicate significant difference ($p < 0.05$).

Comparing control with RSP-fortified yoghurts, the higher appearance ratings for control appeared to be associated with higher WHC values. Additionally, the alterations in the colour of yoghurts (less brightness, and higher redness and yellowness) brought about by RSP fortification might be attributed to the decrease in the scores for appearance and colour (although differences for colour were not statistically significant). Sensorial textural scores were consistent with physical property values, and decreased in relation to the decrease in WHC and adhesiveness, particularly with the fortification of 3% RSP.

There was no data about the sensorial acceptance of yoghurts fortified with RSP in the literature; however, in the most recent studies, the fortification of various substances to yoghurt, such as flaxseed and grape seed, was found to lower the sensory score (Mousavi *et al*., 2019).

Conclusion

As a result of fortifying RSP in the studied concentrations (1 - 3%) into the probiotic yoghurt, starter cultures and probiotic bacteria growth were promoted, and bacterial counts remained higher (> 6 log CFU/mL) in all the RSP-fortified yoghurts as compared to control during the overall period of 21 d (except *L. acidophilus* at 21st day). The presence of prebiotic dietary fibres and polyphenols in RSP, as well as being rich in vitamins and minerals, may be associated with factors that promote the growth of probiotic bacteria in yoghurt. On the other aspect, the viable cells of *L. acidophilus* and *B. lactis* in all fortified yoghurts remained adequate (> 6 log CFU/mL) to provide health benefits to the consumer. Moreover, the fortification of RSP in yoghurts containing probiotics improved physical qualities like syneresis, firmness, and adhesiveness, but it reduced sensory parameters like appearance, consistency, aftertaste, and taste. The fortification of RSP might contain compounds that enhanced the gelation properties of the yoghurt matrix, thus leading to reduced syneresis. The prebiotic fibres and polyphenols present in RSP could contribute to the improved physical quality by interacting with proteins and forming a more stable gel network. RSP might have introduced distinct flavours or aromas that were not well-liked by consumers, thus impacting the overall sensory experience. Additionally, the altered texture and increased thickness resulting from the addition of RSP might not align with the sensory expectations of traditional yoghurt, thus leading to reduced scores in sensorial evaluations. As expected, fortifying RSP into yoghurt increased antioxidant activity and TPC. As a whole, fortification with RSP appeared to be a promising alternative for developing new functional products that could be rich in health-promoting compounds, and preferred by health-conscious consumers.

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