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Microbiological, antioxidant, and sensory properties of probiotic yoghurt enriched with different strawberry varieties

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Abstract

The influence of different strawberry varieties on the microbiological, antioxidant and sensory properties of probiotic yoghurt, containing *Bifidobacterium animalis* subsp. *lactis* Bb12, were monitored for 21 days. For this purpose, strawberry pulp (15 %) containing strawberry and sugar in a 1:1 ratio was used in the yoghurt formulation. Higher total phenolic content and ferric reducing antioxidant power (FRAP) were detected in Albion and Camarosa varieties than Rubygem whereas Albion variety had the highest total anthocyanin content ($p<0.05$). Colour parameters (L*, a*, b*) of probiotic yoghurts differed significantly with the incorporation of strawberry pulp. The viable counts of yoghurt starter bacteria in the experimental yoghurts remained above 7.64 log cfu/g throughout the storage period. Addition of probiotic yoghurt with strawberry pulp enhanced the viability of *S. thermophilus* and *L. bulgaricus* depending on the variety of strawberry. A similar effect on *B. lactis* viability was observed in fruit probiotic yoghurt samples except the 1st day of storage. The counts of *B. lactis* were above 8.5 log cfu/g throughout the storage period. The highest total phenolic content, antioxidant activity (FRAP) and total anthocyanin content were found in Albion variety added probiotic yoghurt during the storage period. Sensory characteristics of probiotic yoghurt were not generally influenced by the addition of strawberry while sample enriched with Camarosa variety showed the lowest overall acceptability scores on 14th day.

Keywords: strawberry; yoghurt; probiotic bacteria; viability; antioxidant activity

Introduction

Recently, consumers' curiosity and tendency towards more nutritious and healthy foods has increased. Therefore, researchers have begun to look for ways to improve functional foods while taking food safety into consideration. Functional dairy products include fermented dairy products, especially yoghurt. Yoghurt is one of the most consumed groups of fermented dairy products worldwide due to its nutritional and health-promoting properties. Yoghurt has beneficial effects on mainly promoting intestinal microflora and releasing of a range of bioactive peptides that have functional properties such as antihypertensive, antioxidant, antithrombotic, opioid, antimicrobial, cytomodulatory and immuno-modulatory (Mann et al., 2017). Incorporating probiotic microorganisms into yoghurt production is the most commonly used method to obtain functional dairy products. Probiotics are defined by FAO/WHO as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'. The definition of a prebiotic is "a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota".

It is a new trend to use fruits or vegetables to improve the functional properties of dairy products (Gurkan et al., 2019; Ujiroghene et al., 2019). In particular, the inclusion of fruits in yoghurt makes it popular due to their prebiotic properties, since they contain dietary fibres such as fructooligosaccharides. Added fruits also effectively increase the acceptance of the product and have the advantage of containing high levels of vitamins, minerals, phenolic compounds and improving the antioxidant profile. There are some studies on the possible prebiotic effects (Coman et al., 2018; Dimitrellou et al., 2020; Gallina et al., 2018) and antioxidant activity-enhancing effects of fruits (Cuşmenco and Bulgaru, 2020; Durmus et al., 2021; Raikos et al., 2019).

Strawberry is the most commonly added fruit to dairy products due to its sensory properties such as flavour and colour, as well as its nutritional properties and the presence of phenolic compounds, especially anthocyanins, and antioxidant activity (Oliveira et al., 2015; Sengül et al., 2014). Anthocyanins found in strawberries give their colour, and the anthocyanin responsible for the bright red colour is pelargonidin 3-O-glucoside. In addition, preventing role in intracellular oxidation and acting as a protective agent against acute liver injury and cardiovascular disease of anthocyanins has been reported (Ünal and Okatan, 2023). Strawberry (*Fragaria* x *ananassa*) is one of the most important fruits belonging to the *Rosaceae* family and has many varieties. Some studies have confirmed that chemical profiling, total phenolic content, and antioxidant properties differ depending on the strawberry variety (Chaves et al., 2017; Mandave et al., 2014).

Although the use of strawberry on some characteristics of yoghurt have been studied, the effect of different varieties of strawberry on viability of both traditional yoghurt starter bacteria and probiotic bacteria and some functional properties of yoghurt have not been investigated. The aim of this study was to evaluate the influence of 3 different strawberry varieties (Rubygem, Camarosa and Albion) on the physicochemical and antioxidant characteristics, starter culture survival, and sensory properties of probiotic yoghurt during 21 days of storage.

Materials and methods

Strawberry, strains and ingredients

The study was carried out on fruits of Rubygem, Camarosa and Albion strawberry varieties provided from a commercial producer in Köprübaşı district of Manisa province, Turkey. The freeze-dried direct-vat-set yoghurt starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (YoFlex) and *Bifidobacterium animalis* subsp. *lactis* Bb12, as probiotic bacteria, containing 10^{11} and 10^{10} cfu/g, respectively, were obtained from Chr. Hansen A/S, Hørsholm, Denmark. The skim milk powder was kindly provided by Pınar Dairy Products, Pınarbaşı, İzmir, Turkey.

Strawberry yoghurt manufacture

Firstly, strawberries were mixed by a blender to obtain puree. Strawberry puree and an equal amount of sucrose were heated at 85 °C for 20 min and cooled to 5 °C. The strawberry pulps were added to yoghurts at a ratio of 15 g pulp to 100 g yoghurt.

For yoghurt manufacture, the milk was standardized with skim milk powder to obtain 140 g/L of nonfat milk solids and heated at 85 °C for 30 min. After cooling to 43 °C, the yoghurt starter culture and *B. lactis* Bb12 was added to milk base according to the manufacturer's instructions and incubated at 40 °C until a pH 4.7 was reached. After fermentation, the yoghurt samples were cooled to room temperature for 30 min and transferred to a refrigerator at 4 °C for one night. Then, yoghurt was divided into four equal parts as follows: (P) plain control probiotic yoghurt (without strawberry puree), (A) probiotic yoghurt added with 15 % Albion strawberry variety, (C) probiotic yoghurt added with 15 % Camarosa strawberry variety, (R) probiotic yoghurt added with 15 % Rubygem strawberry variety. After mixing yoghurt and strawberry pulp they were put into 200 mL plastic containers and stored at 4 °C for 21 days.

Physicochemical analysis

The total soluble solid content of the fruit juice was determined by using a digital refractometer (PR-1; Atago, Tokyo, Japan) and expressed in percent. The titratable acidity was determined by titrating 10 mL of juice with 0.1 N NaOH up to pH 8.1. The results were expressed as gram citric acid per 100 mL of fruit juice. The determination of total solids, fat, titratable acidity, and protein contents in yoghurt were made according to the methods described in the AOAC (2000). The pH values of both fruit juice and yoghurts were determined using a pH meter (Hanna Instruments Model pH: 211; Woonsocket, RI, USA).

The colour analysis of yoghurt samples was performed by a Minolta CR-400 Colorimeter (Japan) and the L*, a* and b* colour dimensions, which are lightness (L*), redness (a*) and yellowness (b*), were expressed in accordance with the CIE Lab. System.

Microbiological analysis

The counts of *S. thermophilus* were enumerated using M-17 agar (Merck, Darmstadt, Germany) after incubating the plates aerobically at 37 °C for 72 h. *Lactobacillus delbrueckii* subsp. *bulgaricus* was enumerated using MRS agar (Merck) adjusted to pH 5.2, with anaerobic incubation at 42 °C for 72 h. *Bifidobacterium animalis* subsp. *lactis* was enumerated on a TOS-MUP agar after incubating the plates anaerobically at 37 °C for 72 h (Turgut and Çakmakçı, 2018).

Total phenolic content, total antioxidant activity, and total anthocyanin content

The antioxidant properties were determined according to Raikos et al. (2019). Total phenolic contents of strawberry varieties and probiotic yoghurt samples were determined by the Folin-Ciocalteu method. The phenolic content was compared to a gallic acid standard curve while the total phenolic content of the samples was expressed as milligrams gallic acid equivalents (GAE) per litre of sample whereas the results were expressed per 100 mL for fruit samples. The equation for the gallic acid calibration curve was y=0.0012x+0.037 and the correlation coefficient was R^2=0.9989. Preparation of yoghurt extracts was performed according to Singleton et al. (1999). For this purpose, 10 g of yoghurt sample was centrifuged (Sigma Centrifuge, Model 3-16K, SciQuip Ltd., Newtown, Wem Shropshire, England) at 9383xg for 25 min, filtered using Whatman no. 1 filter paper (Global Life Sciences Solutions, Buckinghamshire, UK) and the supernatant was used in the method.

The antioxidant activity of both strawberry varieties and probiotic yoghurt samples were determined by ferric reducing antioxidant power (FRAP) method. In the FRAP assay, reductants (“antioxidants”) in the sample reduce Fe (III)/tripyridyltriazine complex to a blue ferrous form, with an increase in the absorbance at 593 nm. The results are expressed in µmol Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (TE)/g, with reference to a Trolox (25-500 µmol) standard curve. The probiotic yoghurt extracts were obtained by adding 2.5 mL distilled water to 10 g of yoghurt sample. After mixing, pH value was adjusted to 4.0 by 0.1M HCl and acidified yoghurt was incubated at 45 °C for 10 min in a water bath. After centrifugation at 4 °C for 10 min (5000xg), the pH value of the supernatant was adjusted to 7.0 by 0.1M NaOH. Then the supernatant was again centrifuged at 4 °C for 10 min (5000xg) and the obtained clear supernatant was frozen (-20 °C) and used for the antioxidant analysis (Shori and Baba, 2011).

Total anthocyanin contents of the strawberry and yoghurt extracts were measured using the pH differential method. Results are expressed as milligrams of pelargonidin 3-glucoside (P 3-G) equivalents per 100 g of weight. Yoghurt extracts for the anthocyanin analysis were obtained by diluting the yoghurt sample five times with a mixture of 95 % ethanol/water (80/20). After centrifugation (4 °C, 10 min, 500 rpm) and filtration (with a pore size of 0.45µm) steps the supernatant was frozen (-20 °C) and used for the analysis.

Sensory properties

Sensory evaluation of the probiotic yoghurt samples was carried out according to Martin-Diana et al. (2003). Samples were evaluated for their taste, aroma, consistency, appearance, overall acceptability by the experienced academicians from the Department of Dairy Technology (Ege University, Izmir, Turkey). The evaluation is based on the five-point hedonic scales (1, dislike extremely; 5, like extremely). Yoghurts, coded with three digits, were presented to the panellists in individual plastic containers.

Statistical analyses

The experiments, including the yoghurt production, were repeated three times. The data obtained were processed by one-way ANOVA using the general linear model (GLM) procedure in SPSS (version 11.05; SPSS Inc., Chicago, IL, USA). The means were compared using the Duncan multi comparison test at the p<0.05 level.

Results and discussion

Characteristics of strawberry samples

Some characteristics of strawberry varieties are given in Table 1. Rubygem variety had significantly lower total soluble solids (p<0.05) and titratable acidity (p<0.01) but higher pH value than those of other varieties.

While the total phenolic substance content was found to be higher in the Camarosa variety than in other strawberry samples, no statistical difference was found between Camarosa and Albion varieties. Similarly, Chaves et al. (2017) detected higher total phenolic content in the Camarosa variety than in the Albion variety.

The highest antioxidant activity and total anthocyanin content was determined in Albion variety whereas there were no significant differences between other two varieties in terms of these parameters. The results obtained in this study do not corroborate those of a previous work developed by Chaves et al. (2017). This difference could be explained by the influence of harvest period as also indicated by Mandave et al. (2014).

Physicochemical properties of yoghurt samples

The total solids, fat and protein contents of probiotic yoghurts ranged between 16.25-21.38 %, 2.38-2.53 %, and 4.53-5.05 %, respectively. It was observed that the addition of strawberry provided a significant difference (p<0.05) in total solids (TS), fat and protein content of probiotic yoghurts compared to the control sample. However, strawberry variety had no significant effect (p>0.05) on the mentioned

Table 1. Characteristics of strawberry samples

Variety	Total solids (%)	Titratable acidity (g citric acid/100 mL)	pH	Total phenolic content (mg GAE[x]/100 g)	Antioxidant activity (μmol TE[y]/g)	Total anthocyanin content (mg pelargonidin-3-glucoside/100 g)
Albion	10.47±0.38 [a*]	0.84±0.05 [a**]	3.67±0.01 [b*]	131.48±8.84 [ab*]	51.20±6.35 [a*]	27.62±1.93 [a*]
Camarosa	10.90±0.26 [a]	0.95±0.07 [a]	3.65±0.02 [b]	140.83±4.60 [a]	48.28±4.87 [ab]	22.48±1.58 [b]
Rubygem	9.13±0.67 [b]	0.57±0.05 [b]	3.75±0.04 [a]	120.14±6.25 [b]	34.99±1.12 [b]	20.91±1.65 [b]

[a-b] Means ± standard deviations in the same column with different superscript lowercase letters are significantly different ($p<0.05$)
**, **, Nonsignificant or significant at $p<0.05$, or 0.01, respectively.*
[x]Gallic acid equivalent, [y] Trolox equivalents

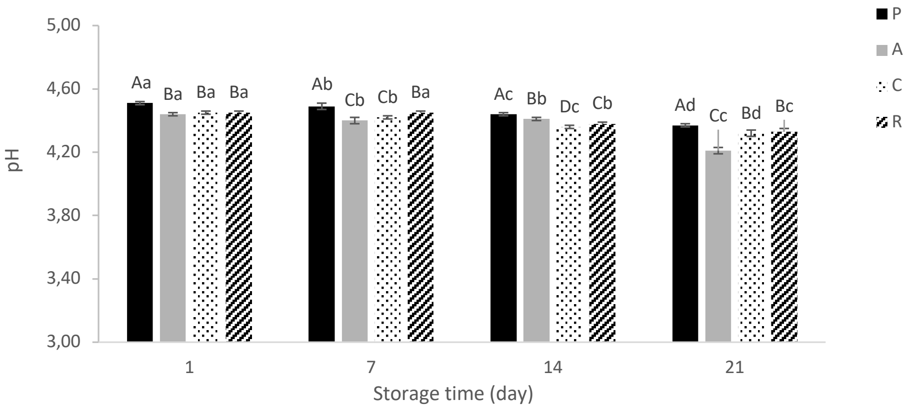

Figure 1. Changes in pH values in plain probiotic yoghurt produced without strawberry pulp (P), probiotic yoghurt produced with Albion strawberry pulp (A), probiotic yoghurt produced with Camarosa strawberry pulp (C), probiotic yoghurt produced with Rubygem strawberry pulp (R) during 21 days of storage

physicochemical properties. As expected, the addition of strawberry pulp increased the TS of probiotic yoghurts which is also observed by Jaster et al. (2018). On the other hand, fat and protein contents significantly decreased by the corporation of strawberry pulp.

In line with our findings, Cuşmenco and Bulgaru (2020) found that yoghurts produced with different fruit additions had higher TS and lower protein and fat content. Consistent with these results, Jaster et al. (2018) found that TS content of yoghurts increased with increasing concentrations of strawberry pulp. The lower fat content in strawberry probiotic yoghurts compared to plain yoghurt is attributed to the low fat content of strawberry pulp. Similar findings were obtained by Sengül et al. (2014). They also stated that as the strawberry pulp concentration increased, the fat and protein content of yoghurts decreased.

The pH values of the probiotic yoghurts and their changes during 21 days of storage are given in Figure 1. The addition of strawberry pulp significantly decreased the pH values of the samples throughout the storage period ($p<0.05$) which is parallel to the result obtained by Cuşmenco and Bulgaru (2020), Jaster et al. (2018) and Yousef et al. (2013). Similarly, Yedikardas (2010) found that production with fibres of apricot, sugar beet and strawberry decreased the pH value of yoghurts during storage.

Although there were no significant differences ($p>0.05$) among the pH values of strawberry pulp added yoghurts at the beginning of storage, yoghurt supplemented with Rubygem variety generally had the highest pH values on the other storage days. This result is compatible with the fact that the pH value of Rubygem variety strawberry is higher than other strawberry varieties. On the other hand, the lowest pH value was found for the yoghurt produced with Albion strawberry pulp at the end of storage. The different pH values of strawberry yoghurts may also be explained by the sugar contained in the strawberry varieties (Kamber and Harmankaya, 2019). The pH values of all experimental yoghurts significantly decreased throughout the storage period ($p<0.05$).

Color is an important factor affecting consumer preference especially in dairy products with fruits. The L* (Lightness), a* (redness-greenness), and b* (yellowness-blueness) values of the probiotic yoghurts at 4°C for 21 days are evaluated and given in Table 2. In general, the addition of strawberry pulp had a statistically significant influence on all color values ($p<0.05$). Although the L* values of the plain control yoghurt were the highest among all samples during 14 days, the difference was not statistically significant ($p>0.05$). It is an expected result that the addition of strawberry pulp causes a decrease in the whiteness value of the yoghurts due to pigmentation. The highest L* value was detected in control yoghurt whereas sample produced with Camarosa strawberry pulp had the lowest value at the end of the storage.

Plain control probiotic yoghurt had the lowest a* and the highest b* values during whole storage period ($p<0.05$). The results showed that production with different strawberry varieties caused significant differences in a* and b* values of yoghurts. The highest a* value were detected in sample supplemented with Albion variety whereas yoghurt containing Rubygem variety had the lowest values throughout the storage. Strawberry probiotic yoghurts manufactured with Albion and Camarosa varieties had the lowest b* values during 21 days. Strawberry yoghurts showed the loss of redness (a*) during storage most probably due to degradation of anthocyanins or/and the formation of yellow and brown polymerization compounds (Ścibisz et al., 2019).

Similarly, Jaster et al. (2018) found that the addition of strawberry pulp reduced the L* values of yoghurt samples. They stated that this was due to the redness coming from the strawberries. In parallel with the results of this study, they found that the a* values of control yoghurts without strawberry pulp were negative, and the addition of strawberry pulp increased the a* values of the samples.

Microbiological characteristics

The changes in the viable counts of *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Bifidobacterium lactis* are given in Table 3. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* counts were kept above 7.47 log cfu/g in the control sample and above 7.64 log cfu/g in strawberry yoghurts. These results are in agreement with the Codex Alimentarius Commission (2011), which stablishes that the counting of lactic acid bacteria must be over 10^7 cfu/g.

The promoting effect of addition of strawberry pulp on the viability of *S. thermophilus* differed according to strawberry variety on different storage days. This may be due to the phenolic compounds content that may act as prebiotic and enhance the viability of *S. thermophilus*. Phenolics have been identified as prebiotics (Gibson et al., 2017) and recent *in vitro* studies have examined the potential prebiotic effects of phenolics (Coman et al., 2018; Rodríguez-Costa et al., 2018).

The highest counts of *S. thermophilus* were found in the sample enriched with Albion strawberry pulp at the beginning of the storage; whereas the highest counts were found in the sample enriched with Camarosa strawberry pulp on 21st day. Although some fluctuations in *S. thermophilus* numbers were observed during storage, viability generally decreased at the end of storage compared to the beginning. This may be due to a decrease in pH and therefore an increase in acidity; that was also reported by Szołtysik et al. (2020) who studied fruit yoghurt. Shah (2006) verified that high acidity can affect starter microorganisms, mainly the *Streptococcus*. Jaster et al. (2018) also determined a significant reduction in the counts of lactic acid bacteria for the yoghurt samples enriched with concentrated strawberry pulp.

L. bulgaricus counts were significantly higher in yoghurts enriched with strawberry varieties compared to the control sample during storage ($p<0.05$). Although there no significant differences among probiotic strawberry yoghurts in terms of *L. bulgaricus* viability for a week, the highest count was determined in Rubygem variety added sample at the end of the storage. Kamber and Harmankaya (2019) found that the highest *L. bulgaricus* viability was in strawberry-added yoghurt compared to yoghurt samples enriched with apricot, banana and peach pulp. Some other researchers also found that *L. bulgaricus* viability was higher in strawberry-added yogurt compared to plain yoghurt (Jaster et al., 2018; Kowaleski et al., 2020).

All experimental yoghurts had the recommended Bifidobacteria levels (≥10^6 cfu/mL) throughout the storage period for being a functional food and providing health benefits (FAO, 2013). Although the pH value decreased during storage in all yoghurt types, Bifidobacteria managed to survive at a high rate. The reason for this is the tolerance of the Bb12 strain to low pH values, which was also reported by Jungersen et al. (2014). Kowaleski et al. (2020) also detected a minimum of 8 log cfu/g Bifidobacteria in yoghurt with 26 % strawberry added during 35 days of storage.

Strawberry pulp showed a prebiotic effect, varying depending on the strawberry variety, during storage except for the 1st day. This prebiotic effect has been attributed to the capability of polyphenols and insoluble fibres present in strawberry to selectively stimulate the proliferation of beneficial microflora in the gut (Fernandez and Marette, 2017). The prebiotic effect of strawberries may also be due to ellagic acid, one of the plant phenolics obtained from

Table 2. *Colour parameters of probiotic yoghurts during 21 days of cold storage*

	Product	Day 1	Day 7	Day 14	Day 21
L*	P	$70.24±2.75^{Aab}$	$68.07±6.00^{Abc}$	$63.34±2.85^{Ac}$	$74.56±2.09^{Aa}$
	A	$66.49±4.02^{Aa}$	$64.10±1.34^{Aa}$	$61.51±5.58^{Aa}$	$67.76±3.58^{Ba}$
	C	$68.21±4.81^{Aa}$	$62.03±3.04^{Ab}$	$58.68±3.30^{Ab}$	$62.16±1.70^{Cb}$
	R	$68.74±3.92^{Aab}$	$62.32±4.14^{Ab}$	$62.02±4.40^{Ab}$	$70.71±4.31^{ABa}$
a*	P	$-1.49±0.20^{Db}$	$-1.25±0.21^{Db}$	$-1.22±0.24^{Db}$	$-1.88±0.09^{Da}$
	A	$6.83±0.36^{Aa}$	$6.24±0.18^{Ab}$	$5.45±0.30^{Ac}$	$4.87±0.19^{Ad}$
	C	$5.72±0.34^{Ba}$	$4.99±0.04^{Bb}$	$4.26±0.16^{Bc}$	$3.83±0.12^{Bd}$
	R	$4.55±0.20^{Ca}$	$3.93±0.17^{Cb}$	$3.47±0.20^{Cc}$	$3.04±0.19^{Cd}$
b*	P	$6.20±0.34^{Ab}$	$6.11±0.41^{Ab}$	$6.01±0.47^{Ab}$	$8.12±0.35^{Aa}$
	A	$2.98±0.23^{Db}$	$3.26±0.17^{Cb}$	$3.09±0.39^{Cb}$	$5.12±0.34^{Ca}$
	C	$3.41±0.25^{Cb}$	$3.34±0.28^{Cb}$	$3.29±0.26^{Cb}$	$4.91±0.38^{Ca}$
	R	$3.88±0.11^{Bb}$	$3.89±0.31^{Bb}$	$4.04±0.27^{Bb}$	$6.13±0.35^{Ba}$

$^{a–d}$ Means ± standard deviations in the same row with different superscript lowercase letters are significantly different (p<0.05)
$^{A–D}$ Means ± standard deviations in the same column with different superscript uppercase letters are significantly different (p<0.05)
P = Plain probiotic yoghurt produced without strawberry pulp, A = Probiotic yoghurt produced with Albion strawberry pulp, C = Probiotic yoghurt produced with Camarosa strawberry pulp, R = Probiotic yoghurt produced with Rubygem strawberry pulp

ellagitannins found in high concentrations in strawberries. Ellagic acid and its derivative urolithins have been shown to have a prebiotic effect both *in vitro* and *in vivo* studies (Muthukumaran et al., 2017).

The storage period significantly affected *B. lactis* counts. Lower viability was observed in all experimental samples at the end of the storage when compared to the 1st day. Since Bifidobacterium species are known to grow more slowly below pH 5.0, this can be attributed to increased acidity.

Total phenolic content, total antioxidant activity, and total anthocyanin content

Changes in the total phenolic content, ferric reducing antioxidant power, and total anthocyanin content of probiotic yoghurts during refrigerated storage are given in Table 4. The highest phenolic contents were determined in probiotic yoghurts enriched with Albion and Rubygem varieties for the first week whereas Albion added yoghurt had the highest values in the rest of the storage. On the other hand, the plain control probiotic yoghurt without strawberry had the lowest phenolic content throughout storage, as expected.

The total phenolic content remained constant in sample A whereas some fluctuation was observed for the other strawberry probiotic yoghurts throughout the storage period. Similar fluctuations in the phenolic content during storage has been reported for salal berry added yoghurt beverage and was attributed to the formation of compounds that react with the Folin-Ciocalteu reagent (Raikos et al., 2019). The authors stated that amino acids with phenolic side chains, such as tyrosine may be released by the proteolysis of milk proteins, which could contribute to the increase in total phenol content. On the other hand, the possible interaction of polyphenols with milk proteins can form insoluble complexes which reduce the total free polyphenol content (Oliveira et al., 2015).

The antioxidant activity of probiotic yoghurt samples was expressed by the ferric reducing power (FRAP) assay. This method evaluates the presence of reducers, such as antioxidants, that cause reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. The addition of strawberry pulp significantly increased the reducing power of probiotic yoghurt as found in some other studies (Jaster et al., 2018; Sengül et al., 2014). Strawberry variety was found to have a significant effect on antioxidant activity. The probiotic yoghurt enriched with Albion variety pulp showed the highest antioxidant activity for 14 days whereas control sample had the lowest values ($p<0.05$). The high amount of phenolic content and antioxidant activity of probiotic yoghurt enriched with Albion variety is related to the fact that both parameters of this strawberry variety are higher than other varieties. Although some fluctuations were observed in the antioxidant activity of experimental yoghurts, FRAP values of the samples C and R significantly ($p<0.05$) increased on 21st day which is parallel to Sengül et al.'s (2014) finding.

Anthocyanins are known to affect antioxidant power, color characteristics, and stability of high acid foods. The total anthocyanin content of experimental probiotic yoghurts was

Table 3. *Changes in the viable counts (log cfu/g) of S. thermophilus, L. delbrueckii subsp. bulgaricus and B. lactis during refrigerated storage of probiotic yoghurts*

Product	log cfu/g			
	Day 1	**Day 7**	**Day 14**	**Day 21**
		S. thermophilus		
P	8.28 ± 0.07^{Ba}	7.93 ± 0.15^{Bb}	7.89 ± 0.05^{Bbc}	7.77 ± 0.08^{Bc}
A	8.64 ± 0.08^{Aa}	8.44 ± 0.05^{Ab}	7.78 ± 0.05^{Cc}	7.81 ± 0.02^{Bc}
C	8.10 ± 0.08^{Cb}	7.99 ± 0.14^{Bb}	7.79 ± 0.07^{Cc}	8.41 ± 0.06^{Aa}
R	8.02 ± 0.02^{Cab}	7.87 ± 0.18^{Bb}	8.10 ± 0.03^{Aa}	7.64 ± 0.06^{Cc}
		L. delbrueckii subsp. bulgaricus		
P	7.98 ± 0.20^{Bb}	7.71 ± 0.19^{Bc}	7.47 ± 0.06^{Cd}	8.81 ± 0.08^{Ca}
A	9.16 ± 0.04^{Aa}	8.93 ± 0.04^{Ac}	9.03 ± 0.00^{Ab}	9.11 ± 0.03^{Ba}
C	9.23 ± 0.01^{Aa}	8.82 ± 0.10^{Ac}	9.06 ± 0.01^{Ab}	9.04 ± 0.02^{Bb}
R	9.17 ± 0.01^{Ab}	8.98 ± 0.03^{Ac}	8.92 ± 0.05^{Bc}	9.30 ± 0.11^{Aa}
		B. lactis		
P	9.37 ± 0.03^{Aa}	9.29 ± 0.03^{Bb}	9.18 ± 0.07^{Bc}	9.05 ± 0.01^{Bd}
A	9.09 ± 0.04^{Dc}	9.91 ± 0.02^{Aa}	9.14 ± 0.04^{Bb}	8.94 ± 0.01^{Cd}
C	9.24 ± 0.03^{Ba}	9.19 ± 0.08^{Cab}	9.04 ± 0.03^{Cc}	9.14 ± 0.02^{Ab}
R	9.19 ± 0.01^{Cb}	9.15 ± 0.04^{Cc}	9.45 ± 0.03^{Aa}	8.92 ± 0.02^{Cd}

$^{a-d}$Means ± standard deviations in the same row with different superscript lowercase letters are significantly different ($p<0.05$)
$^{A-D}$Means ± standard deviations in the same column with different superscript uppercase letters are significantly different ($p<0.05$)
P = Plain probiotic yoghurt produced without strawberry pulp, A = Probiotic yoghurt produced with Albion strawberry pulp, C = Probiotic yoghurt produced with Camarosa strawberry pulp, R = Probiotic yoghurt produced with Rubygem strawberry pulp

analyzed and no values were detected in the control sample as expected. The anthocyanin content of strawberry yoghurts changes between 1.86-3.77 mg pelargoidin-3-glucoside/100 g. Jaster et al. (2018) also determined similar anthocyanin content (1.60-2.99 mg pelargoidin-3-glucoside/100 g) for strawberry enriched yoghurt. Ścibisz et al. (2019) and Oliveira et al. (2015) detected higher amount of total anthocyanin content than our results for strawberry yoghurt probably due to the different strawberry variety and addition rate or manufacture process. In our study, the strawberry yoghurts had higher amount of total anthocyanin content than those of strawberry fruit varieties. This may be probably due to the evaporation of water during the preparation of strawberry puree. Another reason may be the preservation of anthocyanins within the structure of yoghurt. Oliveira et al. (2015) reported that anthocyanins in milk system might form complexes with macromolecules that protect anthocyanins from degradation.

The highest total anthocyanin content was determined in Albion variety added probiotic yoghurt during 21 days whereas there were no significant differences between other strawberry yoghurts. This is in parallel with the anthocyanin contents of the strawberry varieties. In addition, the high anthocyanin content of sample A is also reflected in the color characteristics of the sample, and it is compatible with the fact that the a* value, which represents the red colour, is significantly higher than other strawberry yoghurts. It was also reported by Ünal and Okatan (2023) that the colour of strawberries is due to the anthocyanins.

Total anthocyanin contents of strawberry added probiotic yoghurts gradually decreased throughout the storage period which is parallel to the results obtained by Oliveira et al. (2015), Raikos et al. (2019) and, Ścibisz et al. (2019). This may be due to the degradation of anthocyanins by oxidation and other chemical reactions depending on reduction of pH throughout the storage time. It has been reported that lactic acid bacteria synthesize ß-glucosidase that can hydrolyse the glycosidic bonds of anthocyanins into anthocyanidins which are unstable and easily degraded (Acar and Yüksekdağ, 2023). Moreover, hydrogen peroxide produced by lactic acid bacteria can also facilitate the breakdown of anthocyanins, leading to their degradation and colour loss (Ścibisz and Ziarno, 2023).

Sensory evaluation

Sensory attributes were evaluated as taste, aroma, texture, appearance, and overall acceptability. The results for the experimental yoghurts are given in Figure 2 A (d 1), B (d 7), and C (d 14), and D (d 21). No significant differences were noted among samples in terms of taste, aroma, texture, and appearance during storage ($p>0.05$). Experimental probiotic yoghurts exhibited similar attributes in terms of overall acceptability except 14th day. The overall acceptability of yoghurt enriched with the Camarosa variety was lower than the other samples on day 14, probably due to its lowest pH value. Najgebauer-Lejko et al. (2021) also specified that there were no significant differences in terms of taste and odour scores between the control and fruit enriched yoghurts.

Storage time generally had no significant effect on sensory parameters in all yoghurt samples. However, overall acceptability scores of the samples enriched with the Camarosa and Albion varieties decreased significantly at the end of storage compared to the 1st day. Similar results were obtained in other studies on strawberry yoghurt (Sengül et al., 2014; Turgut and Çakmakçı, 2018).

Table 4. *Changes in the total phenolic content, total antioxidant activity, and total anthocyanin content of probiotic yoghurts during 21 days of cold storage*

Product	Day 1	Day 7	Day 14	Day 21
	Total phenolic content (GAE mg/L)			
P	175.61±11.23^{Cab}	176.25±11.42^{Bab}	169.80±2.39^{Cb}	185.00±3.19^{Ca}
A	221.46±17.22^{Aa}	211.25±7.89^{Aa}	218.75±1.59^{Aa}	211.67±6.12^{Aa}
C	198.75±8.62^{Ba}	187.71±1.43^{Bb}	192.50±7.10^{Bab}	185.63±3.93^{Cb}
R	205.21±3.29^{ABa}	204.79±8.75^{Aa}	194.37±7.37^{Bb}	196.04±2.92^{Ba}
	FRAP (µmol TE/g)			
P	0.213±0.00^{Da}	0.207±0.04^{Db}	0.192±0.05^{Dc}	0.187±0.00^{Dc}
A	0.707±0.00^{Aa}	0.488±0.00^{Ab}	0.446±0.00^{Ac}	0.394±0.00^{Cd}
C	0.365±0.00^{Cb}	0.355±0.01^{Cc}	0.351±0.00^{Cc}	0.484±0.00^{Ba}
R	0.421±0.00^{Bc}	0.433±0.00^{Bb}	0.408±0.00^{Bd}	0.488±0.00^{Aa}
	Total anthocyanin content (mg pelargoidin-3-glucoside/100 g)			
P	0.00±0.00^{C}	0.00±0.00^{C}	0.00±0.00^{C}	0.00±0.00^{C}
A	3.77±0.06^{Aa}	3.40±0.02^{Ab}	3.20±0.04^{Ac}	3.00±0.01^{Ad}
C	2.24±0.04^{Ba}	2.09±0.00^{Bb}	2.03±0.03^{Bb}	1.87±0.01^{Bc}
R	2.34±0.04^{Ba}	2.06±0.03^{Bb}	2.02±0.02^{Bb}	1.86±0.00^{Bc}

$^{a-d}$ Means ± standard deviations in the same row with different superscript lowercase letters are significantly different ($p<0.05$)
$^{A-D}$ Means ± standard deviations in the same column with different superscript uppercase letters are significantly different ($p<0.05$)
P = Plain probiotic yoghurt produced without strawberry pulp, A = Probiotic yoghurt produced with Albion strawberry pulp, C = Probiotic yoghurt produced with Camarosa strawberry pulp, R = Probiotic yoghurt produced with Rubygem strawberry pulp

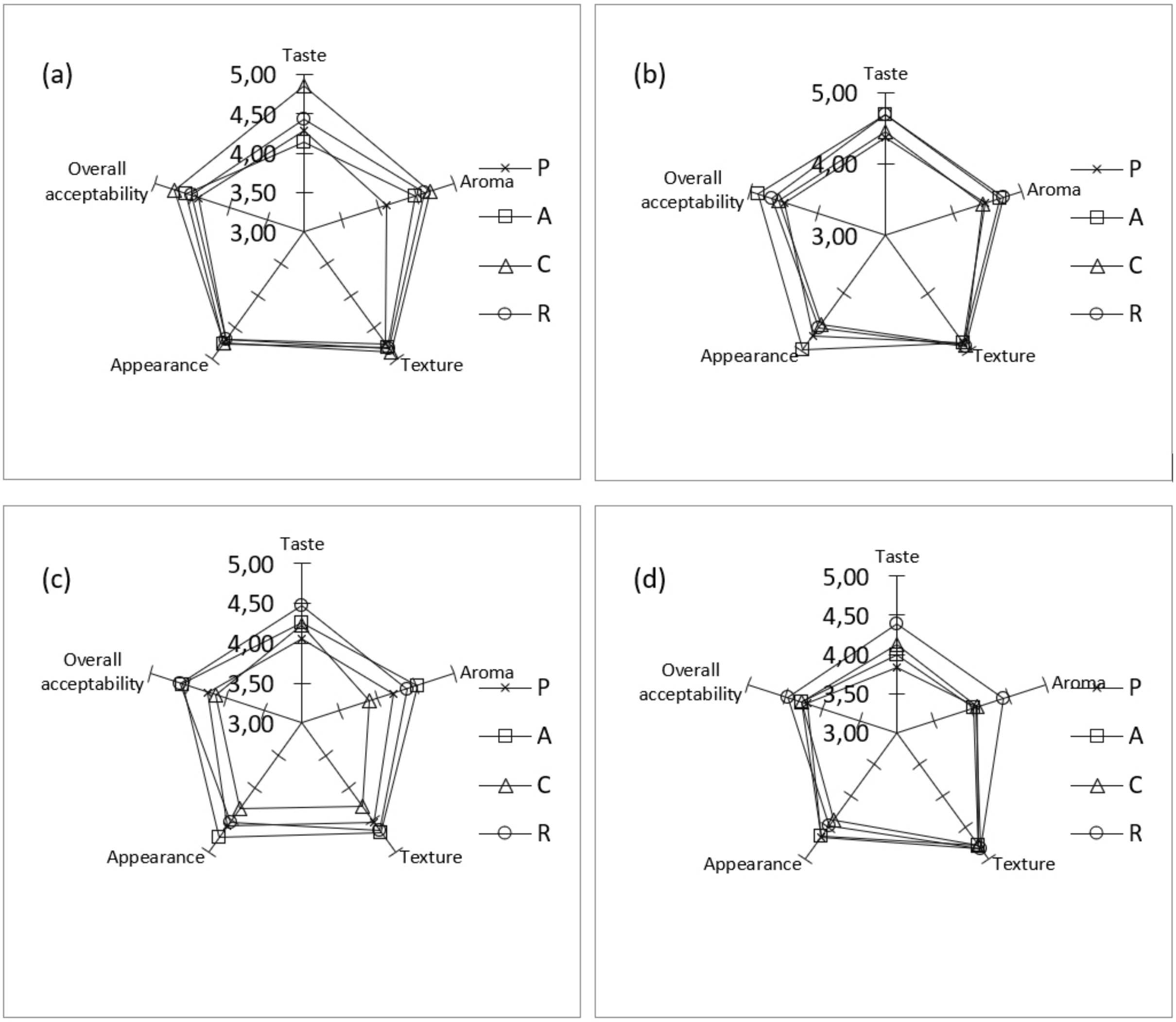

Figure 2. *Sensory scores of (a) 1-d-old yoghurts, (b) 7-d-old yoghurts, (c)14-d-old yoghurts, (d) 21-d-old yoghurts; P=Plain probiotic yoghurt produced without strawberry pulp, A=Probiotic yoghurt produced with Albion strawberry pulp, C=Probiotic yoghurt produced with Camarosa strawberry pulp, R=Probiotic yoghurt produced with Rubygem strawberry pulp*

Conclusions

The present study demonstrated that supplementation of probiotic yoghurt with different strawberry varieties provided high viable counts of both yoghurt starter bacteria and probiotic bacteria. The viability *S. thermophilus* and *L. bulgaricus* was above the recommended level by Codex Alimentarius Commission (10^7 cfu/g) for lactic acid bacteria in yoghurt. *B. lactis* maintained the recommended minimum level of probiotic bacteria throughout storage to demonstrate its health benefits. Strawberry pulp showed a prebiotic effect varying depending on the variety except the beginning of the storage. Strawberry addition improved the antioxidant properties of probiotic yoghurt compared to plain yoghurt during 21 days of storage. Strawberry probiotic yoghurt with Albion variety had higher total phenolic and anthocyanin content and exhibited superior antioxidant activity when compared to other strawberry yoghurts. Strawberry pulp enriched probiotic yoghurts demonstrated high sensory performance throughout the storage however Camarosa variety added probiotic yoghurt had lower overall acceptability scores than the other strawberry yoghurts on 14th day.

In conclusion, it seems that strawberry variety can play a significant role in providing functional properties to yoghurt. Considering the strawberry varieties studied in this study, Albion seems more advantageous than other varieties. Future studies are needed to examine some other health effects of products by taking into account the variety of fruits used in the production of dairy products in order to increase food functionality.

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Mikrobiološka, antioksidacijska i senzorska svojstva probiotičkog jogurta obogaćenog različitim sortama jagoda

Sažetak

U radu je praćen utjecaj različitih sorti jagoda na mikrobiološka, antioksidacijska i senzorska svojstva probiotičkog jogurta koji sadrži *Bifidobacterium animalis* subsp. *lactis* Bb12, tijekom 21 dana. U tu svrhu u formulaciji jogurta korištena je pulpa jagode (15 %) koja sadrži jagodu i šećer u omjeru 1:1. Viši ukupni sadržaj fenola i antioksidacijska snaga reduciranja željeza (FRAP) otkriveni su kod sorti albion i camarosa nego kod rubygema, dok je sorta albion imala najveći ukupni sadržaj antocijana ($p<0,05$). Parametri boje (L*, a*, b*) probiotičkih jogurta značajno su se razlikovali ovisno o pulpi jagode. Broj bakterija jogurtne kulture u eksperimentalnim jogurtima ostao je iznad 7,64 log cfu/g tijekom cijelog razdoblja skladištenja. Dodavanje pulpe jagode u probiotičke jogurte povećalo je održivost *S. thermophilus* i *L. bulgaricus* ovisno o sorti jagode. Sličan učinak primijećen je i na *B. lactis* u uzorcima voćnog probiotičkog jogurta osim prvog dana skladištenja. Broj bakterija *B. lactis* bio je iznad 8,5 log cfu/g tijekom cijelog razdoblja skladištenja. Najveći ukupni sadržaj fenola, antioksidacijska aktivnost (FRAP) i ukupni sadržaj antocijana tijekom perioda skladištenja utvrđeni su u probiotičkom jogurtu s jagodom sorte albion. Dodatak jagode općenito nije utjecao na senzorske karakteristike probiotičkog jogurta, dok je uzorak obogaćen sortom camarosa pokazao najniže ukupne ocjene prihvatljivosti 14. dana.

Ključne riječi: jagoda; jogurt; probiotičke bakterije; održivost; antioksidativno djelovanje

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