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DESIGN OF PROBIOTIC YOGHURT WITH THE ADDITION OF ROYAL JELLY

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Abstract

This study aimed to develop a probiotic yoghurt enriched with royal jelly to enhance its nutritional and functional qualities. The effects of incorporating *Lacticaseibacillus casei* RC-1 and royal jelly on the physicochemical, antioxidant, microbiological, and rheological properties of yoghurt were evaluated over 21 days of refrigerated storage. Three yoghurt formulations were designed and tested: control yoghurt with classic starter culture (Yc), yoghurt with classic starter culture and probiotic strain (Ypro), and yoghurt with classic starter culture, probiotic strain and royal jelly (Ypro+RJ). Parameters such as pH, titratable acidity, water content, water holding capacity (WHC), fat content, colour (L*, a*, b*), total polyphenols, antioxidant activity (DPPH, FRAP), and microbial counts were analyzed. Ypro+RJ maintained the highest pH and lowest acidity, indicating reduced acidification. WHC improved in all samples, with Ypro+RJ reaching the highest value. Colour shifts were minimal, suggesting little perceptible difference. Ypro+RJ had the highest polyphenolic content and strongest antioxidant activity, reflecting a synergistic effect between the probiotic and royal jelly. Microbial analysis showed increased lactic acid bacteria (LAB) counts in Ypro and Ypro+RJ, while the total plate count and yeast/fungi counts remained within safe limits. Rheological testing revealed shear-thinning behavior in all samples. Overall, royal jelly addition enhanced the yoghurt's functional properties, boosting antioxidant activity, microbial viability, and storage stability. These results suggest that royal jelly-

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enriched probiotic yoghurt could serve as a next-generation functional food with potential health and consumer benefits.

Keywords: yoghurt, royal jelly, probiotic yoghurt, probiotic royal jelly yoghurt

Introduction

The food industry models and the undeniable demand for healthy foods are increasing globally (Nunes *et al*., 2020). The term "functional food" evolved as a low-cost solution to many chronic health issues and is continuing gaining influence in many areas of science and medicine. From its conception in 1984, "functional food" changed its meaning depending on the different cultures and countries. The purpose of this definition was to increase communication among the scientists, consumers, and other groups and to legitimize the science of functional foods worldwide (Martirosyan and Singh, 2015). Nowadays, the consumers have high expectations for the functional foods products with both health benefits and organoleptic properties. Therefore, the key for the success of functional foods will be the development of the fundamental relationship between functional nutritional role, bioactive characteristics, new technologies and consumer understanding for health associated benefit (Zrnic *et al*., 2023).

Fermented dairy products have been an essential part of the human diet for the last centuries. They are providing not only improved flavors for the final product but also a variety of biological activities and health benefits (Zhang *et al*., 2023). Yoghurt is a fermented dairy product obtained by bacterial fermentation of milk under the influence of the lactic acid bacteria (LAB) *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* Fermentation process includes conversion of lactose into lactic acid, leading to specific texture and taste of the yoghurt. The quality of yoghurt is affected by the composition of milk, fermentation process and conditions, bacterial strains and other factors (Tamime and Robinson, 2007). Yoghurt is a valuable food known as a rich source of proteins, essential fatty acids, vitamin A, vitamin B2, vitamin B12, potassium, calcium and phosphorus (Hadjimbei *et al*., 2022). In addition, *S. thermophilus*, *L. bulgaricus* and *Bifidobacterium* strains are known to be folic acid producers. Thus, it is related to additional health benefits including immunomodulatory, antibacterial, antiviral, anticancer, antioxidant, anti-obesity, anti-hyperthensive and cholesterol-lowering functions (Zhang *et al*., 2023).

The addition of probiotic strains in fermented milk products to increase their functionality has a positive impact on the gut microflora, boosted immune response, improved digestion and potential reduction of the risk of certain diseases. The effectiveness of probiotic yoghurt is determined by the viability of probiotic strains and presence of prebiotic substances which supported the growth of the probiotic microorganisms (Sanders *et al*., 2019).

Lacticaseibacillus casei (formerly *Lactobacillus casei*) is a probiotic strain used to improve the health benefits and nutritional value of fermented milk. It is often used in fermented dairy products such as yoghurt and kefir (Cui and Qu, 2021).

Lactobacillus casei is one of the most commonly used probiotics that was reported to soothe multiple illnesses. *L. casei* promotes a healthy intestinal gut-ecosystem by boosting the growth of beneficial microorganisms and suppressing the growth of harmful bacteria. That positive impact helps reduce the risk of many gastro-intestinal tract disorders. Furthermore, *L. casei* aids digestion through the production of lactic acid, reducing the pH of the intestinal tract, thus making the environment less conductive for the harmful bacteria. It also promotes the digestion of lactose which is beneficial for people with lactose intolerances (Liu *et al*., 2021). It was proved that *L. casei* modulates inflammatory responses in the gut, as well as reduces symptoms of inflammatory diseases such as irritable bowel syndrome (IBS) and Crohn's disease (Rochat *et al*., 2007). *L. casei* demonstrates remarkable survival through the gastro-intestinal tract and positively affects gut microbiota, contributing to improved immunity system and boosted gut health and microbiota modulation (Oozeer *et al*., 2006).

Royal jelly (RJ) is a complex secretion produced by worker bees (*Apis mellifera L*.) that is used as staple food for the queen bee. It is a rich source of proteins, sugars, lipids, vitamins, and minerals that contribute to its wide spectrum of biological activities (Kunugi and Mohammed, 2019). Supplementation of dairy products with RJ, especially fermented ones like yoghurt, increases their nutritional value and functional properties (Mabrouk, 2016). Besides the health benefits (especially the positive impact on gut health) and improved functional properties, the addition of RJ to probiotic yoghurts can increase their textural and organoleptic properties. In this regard, supplementation of fermented milk with RJ improved viscosity, antioxidant activity and water holding ability (Morsy, 2017). Sensory assessments with optimal concentrations showed that RJ-enriched yoghurt was generally well received, leading to balanced sensory appeals and health benefits. Fermented dairy products with the addition of RJ demonstrated improved antibacterial, antioxidant and anticancer activities. These properties are resulted of unique chemical composition of RJ and the synergistic effects with the fermentation process products (Hassan *et al*., 2022)

Therefore, the aim of the present research was to develop a probiotic yoghurt with *Lacticaseibacillus casei* RC-1 and the addition of RJ in order to enhance its nutritional profile, functional properties and overall quality. Specifically, the study aimed to evaluate the effects of incorporation of *L. casei* RC-1 and RJ on the physicochemical, antioxidant, microbiological, and rheological properties of yoghurt during 21 days of refrigerated storage.

Materials and methods

Milk

Fresh milk was delivered by a certified supplier of the University of Food Technology, Plovdiv. The milk (10 mL sample) was analysed using an automatic milk analyzer MilkoScope Expert (Etcon Analytical, Nigeria) at room temperature (20±2°C). The obtained physicochemical parameters of the milk were as follows:

pH 6.6±0.1, fat 3.28±0.20%, solids-non-fat (SNF) 8.60±0.15%, density 1028.8±0.5 kg/m^3, protein content 3.23±0.10%, added water 0.00%, freezing point -0.565±0.005°C, antibiotic test (MilkSafe™ 3BTS kindly provided by CHR Hansen, Denmark) – negative.

Starter culture

Freeze-dried starter culture YF-3331 50 U for yoghurt (CHR Hansen, Denmark) containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* was used.

Probiotic strain

Lacticaseibacillus casei RC-1 (isolated from homemade cheese from the town of Erzurum, Turkey) was kindly provided by Dr. Remzi Cholakov from the Özgazi B. V. Dairy Foods, Etten-Leur, The Netherlands. The probiotic properties of the strain were proved in our previous study (Tumbarski *et al*., 2025a).

Royal jelly

The RJ was purchased from a beekeeper located in the village of Vrabcha, Pernik district, Bulgaria (42°51′N 22°44′E). The RJ was packed in non-transparent vials and stored frozen (-15°C) until use with previously determined characteristics (Tumbarski *et al*., 2025b).

The different types of fermented yoghurts are shown on Figure 1.

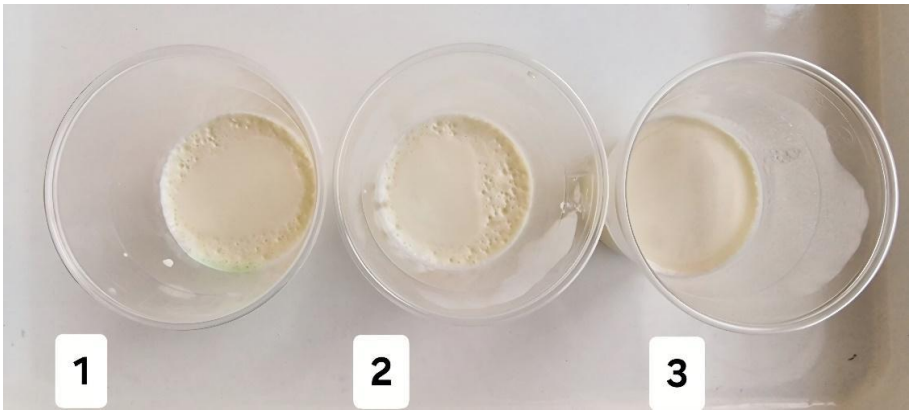

Figure 1. Different types of tested yoghurts.

Where 1 is (Yc) – control yoghurt sample with classic starter culture for yoghurt; 2 is (Ypro) – yoghurt with classic starter culture and probiotic strain and 3 is (Ypro+RJ) – yoghurt with classic starter culture, probiotic strain and RJ.

Culture media

Malt extract agar (MEA) MEA (Scharlab S.L., Spain) was used for cultivation of yeasts and fungi. A quantity of 48 g of MEA base was dissolved in 1 L of deionized water (pH 5.6±0.2).

Plate count agar (PCA) was used to determine the total number of viable aerobic microorganisms The dehydrated agar (total 23.5 g/L) was dissolved in 1 L of deionized water (pH 5.6±0.2), with constantly stirring, heated to boiling.

Chloramphenicol glucose agar (CGA) (Scharlab S.L., Spain) is a selective medium for enumeration of yeasts and fungi. A quantity of 40 g of CGA base was dissolved in 1 L of deionized water (pH 6.6±0.2).

De Man, Rogosa and Sharpe agar (MRS) agar is a selective medium for cultivation and enumeration of lactobacilli. A quantity of 55.2 g of MRS-broth (Merck, Germany) was dissolved in 1 L of deionized water (pH 6.4±0.2), and then 15 g of agar (Scharlab S.L., Spain) was added.

M 17 agar with glycerophosphate (Himedia, India) is a selective medium for cultivation and enumeration of lactic streptococci. A quantity of 52.25 g of M17 agar base was dissolved in 1 L of deionized water (pH 7.1±0.2).

All the culture media were prepared according to the manufacturers' instructions and autoclaved at 121°C for 20 min before use.

Experimental design of probiotic yoghurt

The classical technology for the production of set-type yoghurt was applied. Fresh milk was homogenized at 62–65°C under a pressure of 15-20 MPa using a high-pressure homogenizer. Subsequently, the milk was pasteurized at 92–95°C with a retention time of 15–20 min, followed by immediate cooling to 42°C and division into three portions:

- (Yc) – control yoghurt sample with classic starter culture for yoghurt (3% of YF-3331).
- (Ypro) – yoghurt with classic starter culture (1.5% of YF-3331) and probiotic strain (1.5% of *L. casei* RC-1).
- (Ypro+RJ) – yoghurt with classic starter culture (1.5% of YF-3331), probiotic strain (1.5% of *L. casei* RC-1) and RJ (2%).

All yoghurt samples were poured into plastic cups (100 mL) and incubated at 44°C for 4 h (samples Yc and Ypro) and for 7 h (sample Ypro+RJ). Yoghurts were gradually cooled at 4°C and stored in a refrigerator at the same temperature. During the storage period, the samples were subjected to chemical, microbiological, rheological and sensory analyses on the 1st and the 21st days.

Total phenolic content

The total phenolic content (TPC) was determined using a Folin-Ciocalteu reagent. The reaction mixture contained 1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, Merck, Germany), 0.8 mL of 7.5% sodium carbonate (Sigma-Aldrich, Merck) and 0.2 mL of the tested sample. After incubation at room temperature for 20 min (in darkness), the absorbance was measured by a spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., UK) at 765 nm against a blank (distilled water). The results were presented as mg equivalent of gallic acid (GAE)/g of dry weight sample (Ivanov *et al.*, 2014)

Antioxidant activity

DPPH radical scavenging assay

The reaction mixture containing 2.85 mL of DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Merck, Germany) and 0.15 mL of the tested sample was kept at 37°C for 15 min. The absorbance was measured at 517 nm against a blank (methanol). The antioxidant activity was expressed as mM Trolox equivalents (TE)/g of dry weight sample (Ivanov *et al.*, 2014).

Ferric-reducing antioxidant power (FRAP) assay

The FRAP reagent was freshly prepared with 300 mM acetate buffer with pH 3.6, 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM hydrochloric acid and 20 mM Iron (III) chloride hexahydrate (Sigma-Aldrich, Merck, Germany) in distilled water in a ratio of 10:1:1. The reaction mixture (3 mL of FRAP reagent and 0.1 mL of the samples) was incubated at 37°C for 10 min in darkness. The absorbance was measured at 593 nm against a blank (distilled water). The antioxidant activity was expressed as mM TE/g of dry weight sample (Ivanov *et al.*, 2014).

Determination of pH

pH was determined using a calibrated digital pH-meter WTW pH 7110 (InoLab, Germany) equipped with a standard glass electrode, following the AOAC 981.12 the procedure of the standard. Calibration was performed before each measure with standard buffer solutions at pH 4.0 and 7.0. Measurements were made directly on stirred yogurt samples. Each was measured in duplicate, and the experiment was repeated twice for reproducibility (AOAC International, 2005)

Determination of titratable acidity

The determination of titratable acidity was implemented according to the Bulgarian State Standard BSS 1111:1980. The titratable acidity was determined by adding 10g yoghurt sample, homogenized with distillated water and titration of each sample with 0.1 N NaOH using phenolphthalein as an indicator until the appearance of a pale pink colour persisting over one minute. The results were expressed as % lactic acid.

Determination of fat content

The fat content of the yogurt samples was determined by the Gerber butyrometric method, in accordance with AOAC International, 2005 Official Method 989.05 and ISO 19662:2018 standards.

Determination of moisture content/dry matter

The moisture content/dry matter was determined by the gravimetric method (ISO 5534:2004).

Water-holding capacity

Water-holding capacity (WHC) was determined by centrifugation of 10 g of the yoghurt sample in centrifuge tubes at 4000 rpm for 30 min using ST 802 A centrifuge. After centrifugation, the supernatant was weighed and WHC (%) was determined according to the following equation 1.

$$WHC, \% = \frac{(OYW,g - SW,g)}{OYW,g} \times 100 \quad (1)$$

where: WHC - water-holding capacity (%); OYW – original yoghurt weight (g);SW – supernatant weight (g).

Determination of colour

The colour was measured using a portable colourimeter FRUWR-10QC (China) by the CIELab method. TheCIELab system consists of a lightness component (L*) and two chromatic components, as the a* value represents green (-a) to red (+a), and the b* value represents blue (-b) to yellow (+b) colours. The colourimeter was calibrated using a standard white plate (L* = 96.20, a* = 0.06, b* = -6.20) (Falcao *et al*., 2013). The values of these parameters were read and recorded automatically by the device. In the second stage of the analysis, the colourimeter was calibrated to the colour of the control yoghurt in order to determine the variations in the colour components (ΔL, Δa, Δb) and the total colour variation index (ΔE) by the following equation (2):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (2)$$

Enumeration of characteristic microorganisms in yoghurt

The enumeration of lactobacilli and lactic streptococci in yogurt was implemented according to the Bulgarian State Standard BSS ISO 7889:2005 using the spread-plating method on MRS and M 17 agar media, respectively, and incubation at 37°C for 48 h. The results were expressed as colony-forming units/g (cfu/g).

Enumeration of yeasts and/or fungi in yogurt

The enumeration of yeasts and/or fungi in yogurt was implemented according to the Bulgarian State Standard BSS ISO 6611:2006 using the pour-plating method on a CGA medium and incubation at 25°C for 48 h. The results were expressed as cfu/g.

Total fatty acids and polar metabolites

The levels of polar metabolites and the profile of fatty acids were analyzed using chromatography–mass spectrometry (GC–MS) (Ivanova *et al*., 2023). Metabolite retention times were matched against reference data from the Golm Metabolome Database (GMD) (Hummel *et al.*, 2010) as well as the National Institute of Standards and Technology (NIST 08) libraries (Manion *et al*., 2015).

Sensory analysis

To conduct the sensory analysis at the end of the storage period, 15 unbiased testers over 18 years old were invited. The analysis was performed by testing 100 mL of the three yoghurt samples placed in plastic cups. The yogurt samples Yc (the control), Ypro and Ypro+RJ were evaluated on the following sensory parameters: surface, colour, coagulum appearance, cutting surface, consistency, flavour and aroma. For this purpose, the 9-points Hedonic scale was used: 9 = liked extremely, 5 = neither liked nor disliked, 1 = disliked extremely. The results from the analysis were recorded on individual questionnaire cards and then, a sensory vprofile of each yoghurt type was made using MS Excel 2010 software (Tumbarski *et al.*, 2021).

The sensory evaluation involved voluntary adult participants and did not include vulnerable groups or invasive procedures. According to institutional guidelines, formal ethical approval was not required. All participants provided informed consent prior to participation.

Rheological analyses

The rheological properties of yogurt samples were analyzed using a rotational rheometer HAAKE™ MARS™ iQ Air (Cat. No. 379-0700; Thermo Fisher Scientific Inc., Waltham, MA, USA), operated via HAAKE RheoWin software. The instrument was equipped with a plate–plate measuring geometry (35 mm diameter of the upper plate) and a fixed gap of 1 mm. All measurements were carried out at 25±0.1°C, maintained by the integrated Peltier temperature control system on the lower plate.

The three yoghurt samples (Yc, Ypro, and Ypro+RJ) were equilibrated to room temperature and gently stirred to ensure homogeneity while avoiding air incorporation. Approximately 1.0 mL of each sample was placed on the lower plate, and the upper plate was lowered to the set gap. Excess sample outside the measuring area was removed with a spatula prior to testing.

Oscillatory tests were performed to determine the storage modulus (G′) and loss modulus (G″), which represent the elastic and viscous components, respectively. A frequency sweep test was conducted over the range of 0.1 to 10 Hz.

The apparent viscosity (η) was determined through a flow curve test in controlled shear rate (CSR) mode. The shear rate was ramped from 0.1 to 100 s^{-1}, with a stabilization time of 5 s per step. Flow behavior was assessed based on viscosity vs. shear rate profiles.

All measurements were performed in triplicate, and results were expressed as mean ± standard deviation. The viscoelastic behavior was further evaluated by calculating $\tan \delta = G''/G'$.

Shear stress as a function of shear rate was analyzed and fitted to two nonlinear rheological models by:

- Ostwald–de Waele (Power Law) model, shown in equation 3.

$$\tau = K \cdot \dot{\gamma}^{n} \quad (3)$$

- Herschel–Bulkley model, where is used the following equation 4.

$$\tau = \tau_0 + K \cdot \dot{\gamma}^{n} \quad (4)$$

where: τ is the shear stress (Pa); $\dot{\gamma}$ is the shear rate (s^{-1}); K is the consistency coefficient; n is the flow behavior index; τ_0 is the yield stress.

Statistical analysis

Results from triplicates were presented as mean values ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed using Statgraphics Centurion statistical program version XVI, 2009 (Stat Point Technologies, Inc.,

Warrenton, VA, USA). Mean differences were established by Fisher's least significant difference test for paired comparison with a significance level of $P \leq 0.05$.

Results and discussion

Physicochemical analyses

The results from determination of pH and titratable acidity of the yoghurt samples are presented in Table 1.

Table 1. pH, titratable acidity, moisture content, dry matter, water-holding capacity and fatness of yoghurt samples.

Parameter	Storage time, day	Experimental group		
		Yc	Ypro	Ypro+RJ
pH	**1**	4.30 ± 0.02^{aA}	4.42 ± 0.02^{bA}	4.82 ± 0.02^{cA}
	21	4.12 ± 0.04^{aB}	4.12 ± 0.02^{aB}	4.48 ± 0.04^{bB}
Titratable acidity, % LA*	**1**	0.91 ± 0.01^{aA}	0.91 ± 0.00^{aA}	0.73 ± 0.04^{bA}
	21	1.08 ± 0.01^{aB}	1.02 ± 0.05^{bB}	0.86 ± 0.04^{cB}
Moisture, %	**1**	84.73 ± 2.44^{aA}	86.38 ± 1.02^{aA}	84.99 ± 1.03^{bA}
	21	83.24 ± 2.80^{aA}	83.97 ± 4.03^{aA}	84.21 ± 1.33^{aA}
Dry matter, %	**1**	15.27 ± 2.44^{aA}	13.62 ± 1.02^{bA}	15.01 ± 1.03^{aA}
	21	16.76 ± 2.80^{aA}	16.03 ± 4.03^{aA}	15.79 ± 1.33^{aA}
WHC, %**	**1**	45.20 ± 0.10^{aA}	53.16 ± 0.13^{bA}	48.62 ± 0.10^{cA}
	21	57.48 ± 0.12^{aB}	54.13 ± 0.15^{bB}	62.48 ± 0.12^{cB}
Fatness, %	**1**	3.50 ± 0.10^{aA}	3.50 ± 0.10^{aA}	3.50 ± 0.10^{aA}
	21	3.60 ± 0.10^{aA}	3.60 ± 0.10^{aA}	3.60 ± 0.10^{aA}

*LA – lactic acid; **WHC - water-holding capacity. "a-c" letters point out differences ($P<0.05$) between yoghurt samples. "A-B" letters point out differences ($P<0.05$) between storage days

As seen in Table 1. on the first day of storage the sample Ypro+RJ showed higher pH value compared to the control Yc and probiotic yoghurt sample Ypro, indicating a buffering effect of RJ addition on lactic acid production. This was also reflected in the prolonged coagulation time in this sample (7h). After 21 days of cold storage, Ypro+RJ sustained a remarkably higher pH value compared to the other yoghurt samples. This pH decrease after the addition of RJ was consistent with the findings of Atallah (2016), who reported that yogurt containing RJ showed higher pH values and a reduced rate of acidification over 21 days of storage period.

The analysis of titratable acidity indicated that on the first day 1 of the storage, the yoghurt sample containing RJ (Ypro+RJ) showed a significantly lower value compared to both control and probiotic sample. During the 21 days of cold storage, titratable acidity increased in all samples, as the yoghurt sample Ypro+RJ maintained the lowest value in comparison with other two samples. These findings suggested that RJ moderates the development of acidity, probably due to its buffering capacity and antimicrobial components, consistent with previous reports made by Atallah (2016), who reported titratable acidity values between 0.9 and 1.1% lactic acid in yoghurts containing RJ during storage. Metry and Owayss (2009) found that

yoghurts with addition of RJ showed an increased titratable acidity over 21 days of storage compared to the control, confirming RJ's impact on acidity of yoghurt.

During the 21-day of storage, all yoghurt samples showed a progressive decrease in moisture and an increase in dry matter, respectively, which is a common tendency in fermented dairy products due to evaporation and syneresis. The control yoghurt sample (Yc) showed a decrease in moisture content from 84.73% to 83.24% and a dry matter increased. The sample with probiotic strain (Ypro) had the greatest moisture loss and the highest increase in dry matter, indicating a lower moisture decrease. In contrast, the probiotic yoghurt sample with RJ (Ypro+RJ) demonstrated better stability, with insignificant reduction in moisture and the lowest increase in dry matter. These results demonstrated that the addition of RJ maintained the moisture balance in probiotic yoghurt during storage, potentially due to its bioactive compounds, such as proteins and sugars, which improved water-holding capacity and helped to reduce the syneresis, thus improving yoghurt texture, freshness, and shelf-life. This finding was in line with previous studies that reported improved physicochemical stability in yoghurt after RJ supplementation (Kavas, 2022). Atallah (2016) reported that a probiotic yoghurt with the addition of RJ showed an increasing trend of dry matter content over 21 days of storage, and noted that RJ had the potential to moderate this increase. This finding supported the claim that RJ enhanced water-holding capacity and reduced syneresis.

The analysis of water-holding capacity (WHC) of the three yoghurts during the 21-day storage period showed considerable differences influenced by the addition of probiotics and RJ. On the first day of storage, the probiotic yoghurt (Ypro) displayed a higher WHC compared to the control (Yc) sample, probably due to the production of exopolysaccharides (EPS) by probiotic strains, which are known for improve gel matrix structure and water retention. A yoghurt sample enriched with probiotic strain and RJ (Ypro+RJ) showed a WHC on the first day of storage, which value was intermediate compared to the other two samples. Over 21 days of storage, all samples showed an increase in WHC, but the most significant improvement was observed in the Ypro+RJ sample, where both the control sample and probiotic strain sample showed similar results. These findings were consistent with previously published studies indicating that RJ supplementation improved the water-holding capacity and the stabilization of fermented milk products during storage period (Hassan *et al*., 2022; Kavas, 2022). The higher WHC of the Ypro+RJ sample implies a synergistic effect between probiotics and RJ due to improved protein interactions and likely bioactive contribution of RJ constituents such as proteins and carbohydrates. These results supported the potential of RJ as a functional component in probiotic yoghurt formulations for improving the product's physicochemical properties and quality.

Over 21 days of refrigerated storage, the fat content of the three yoghurt samples (Yc, Ypro and Ypro + RJ) remained remarkably stable, increasing slightly from 3.5% to 3.6 %. This increase is related to moisture loss. Similar studies showed insignificant changes in fat composition when incorporating royal in yoghurt, with variations in values resulting from the water evaporation rather than fat metabolism

(Atallah, 2016; Atallah and Morsy, 2017). It can be concluded that the RJ enrichment supports the innate stability of the yoghurt.

Determination of colour

The results from determination of colour parameters of yoghurt samples during the storage are presented in Table 2.

Table 2. Determination of colour of yoghurt samples.

Parameter	Storage time, day	Experimental group		
		Yc	Ypro	Ypro+RJ
L*	**1**	81.94±1.96^{aA}	83.72±0.29^{bA}	83.33±0.33^{bA}
	21	85.09±0.10^{aB}	85.42±0.36^{aB}	84.15±0.57^{bA}
a*	**1**	0.97±0.06^{aA}	0.84±0.03^{bA}	0.64±0.02^{cA}
	21	3.21±0.06^{aB}	3.12±0.11^{aB}	3.25±0.07^{aB}
b*	**1**	15.62±0.35^{aA}	15.32±0.11^{aA}	15.43±0.19^{aA}
	21	14.1±0.10^{aB}	14.15±0.18^{aB}	14.67±0.23^{bB}
ΔL	**1**	-	1.33±0.26^{aA}	0.56±0.43^{bA}
	21	-	1.03±0.45^{aA}	0.82±0.43^{aB}
Δa	**1**	-	0.12±0.05^{aA}	-0.02±0.03^{bA}
	21	-	-0.07±0.04^{aB}	-0.06±0.05^{aA}
Δb	**1**	-	0.12±0.42^{aA}	-0.12±0.13^{aA}
	21	-	-0.21±0.17^{aA}	0.05±0.04^{bB}
ΔE	**1**	-	1.39±0.27^{aA}	0.58±0.44^{bA}
	21	-	1.07±0.46^{aA}	0.83±0.43^{bA}

"a-c" letters point out differences ($P<0.05$) between yoghurt samples. "A-B" letters point out differences ($P<0.05$) between storage days

The colour is an important factor in consumer acceptance and perception, particularly in functional dairy products, where different ingredients such as probiotics or bioactive components (RJ) may influence visual appearance. Colour was instrumentally evaluated by the CIELab system, measuring lightness (L*), red-green (a*), and yellow-blue (b*) components. Additionally, the absolute colour difference (ΔE) was calculated to assess the perceptible variation during storage. All yoghurt samples demonstrated an increase in L* values over the storage period, resulted as a lighter colour at day 21 compared to day 1. The parameter a* values significantly increased from their initial values by day 21, reflecting a noticeable change to redness. Parameter b* values decreased slightly over time, indicating a reduction in yellow range.

Total overall colour differences (ΔE) between Yc and the experimental samples showed that on day 1, Ypro (ΔE = 1.39) exhibited the highest difference compared to the control, while Ypro+RJ was more visually stable (ΔE = 0.58). On Day 21 of the storage period, ΔE values decreased slightly for Ypro (1.07) and remained low for Ypro+RJ (0.83), confirming minimal colour deviation. According to recent reports, a ΔE value under 3 is unnoticeable to the human eye (Atallah, 2016),

indicating that all yoghurt samples maintained visually stable colour profiles during 21 days storage period.

Total polyphenols and Antioxidant activity

The results from total polyphenolic content and antioxidant activity of the yoghurt samples during the storage for 21 days are shown in Table 3.

Table 3. Total polyphenolic content and antioxidant activity of yoghurt samples.

Parameter	Storage time, day	Experimental group		
		Yc	Ypro	Ypro+RJ
Total polyphenols, mg GAE/100g	**1**	20.32±0.28^{aA}	20.33±0.32^{aA}	20.68±0.11^{bA}
	21	13.01±0.24^{aB}	13.50±0.17^{bB}	13.60±0.30^{bB}
Antioxidant activity, mM TE/100g				
DPPH	**1**	68.95±1.21^{aA}	69.32±1.03^{aA}	69.60±1.20^{aA}
	21	51.72±2.07^{aB}	54.44±2.51^{aB}	54.71±1.46^{aB}
FRAP	**1**	33.18±2.03^{aA}	36.83±1.42^{aA}	41.76±2.07^{aA}
	21	23.80±1.43^{aB}	24.39±1.33^{aB}	24.78±1.30^{aB}

"a-c" letters point out differences (P<0.05) between yoghurt samples. "A-B" letters point out differences (P<0.05) between storage days

As seen from results in Table 3, all samples showed a decrease in total polyphenols during the storage for 21 days, which is caused by degradation of polyphenolic compounds during the fermentation process and storage period. The probiotic yogurt sample with RJ (Ypro+RJ) demonstrated the highest polyphenolic content compared to the other two samples during the entire storage period. The results were in agreement with those obtained by Hassan *et al*. (2022). This indicates that RJ contributed to the higher polyphenolic content.

The antioxidant activity determined by DPPH and FRAP methods decreased in all yoghurt samples after 21 days of storage. The probiotic yogurt sample with RJ (Ypro+RJ) demonstrated highest antioxidant activity in comparison with the other two samples during the entire storage period, which is expected due to the natural decline of antioxidant capacity in fermented products. Ypro+RJ sample sustained the highest antioxidant capacity at the beginning and end of storage period for both assays confirmed by Hassan *et al*. (2022). This implies that the RJ supplementation could enhance and maintain the antioxidant potential of probiotic yoghurt over the storage time.

Total fatty acids

The results from total fatty acids of yoghurts samples during the 21-days of storage are presented at Table 4.

Table 4. Fatty acids composition of yoghurts samples.

Parameter	Storage time, day	Experimental group		
		Yc	Ypro	Ypro+RJ
		Medium chain fatty acids, % of total fatty acids		
Butyric acid, 4:0	**1**	5.18 ± 0.35^{aA}	4.50 ± 0.39^{Ba}	3.23 ± 0.22^{cA}
	21	6.42 ± 0.56^{a}	5.41 ± 0.25^{b}	3.71 ± 0.17^{c}
Caproic acid, 6:0	**1**	4.13 ± 0.28^{aA}	5.60 ± 0.49^{bA}	6.02 ± 0.41^{cA}
	21	5.13 ± 0.45^{a}	6.72 ± 0.31^{b}	6.92 ± 0.32^{b}
Caprylic acid, 8:0	**1**	3.26 ± 0.22^{aA}	2.83 ± 0.25^{bA}	4.86 ± 0.33^{cA}
	21	4.04 ± 0.35^{a}	3.40 ± 0.16^{b}	5.59 ± 0.26^{c}
Capric acid, 10:0	**1**	4.22 ± 0.28^{aA}	3.67 ± 0.32^{bA}	8.69 ± 0.59^{cA}
	21	4.82 ± 0.42^{a}	4.41 ± 0.21^{a}	9.99 ± 0.47^{b}
Lauric acid, 12:0	**1**	4.55 ± 0.31^{aA}	3.96 ± 0.35^{bA}	6.33 ± 0.43^{cA}
	21	5.64 ± 0.49^{a}	4.75 ± 0.22^{b}	7.28 ± 0.34^{c}
		Long chain fatty acids, % of total fatty acids		
Myristic acid, 14:0	**1**	10.43 ± 0.70^{aA}	9.07 ± 0.80^{aA}	3.01 ± 0.20^{bA}
	21	12.93 ± 1.13^{a}	10.89 ± 0.51^{b}	3.46 ± 0.16^{c}
Palmitic acid, 16:0	**1**	25.62 ± 1.73^{aA}	22.29 ± 1.95^{aA}	15.00 ± 1.01^{bA}
	21	28.77 ± 2.52^{a}	26.75 ± 1.25^{a}	17.25 ± 0.81^{b}
Stearic acid, 18:0	**1**	10.39 ± 0.70^{aA}	9.04 ± 0.79^{aA}	9.76 ± 0.66^{aA}
	21	12.88 ± 1.13^{a}	10.85 ± 0.51^{b}	11.22 ± 0.52^{a}
Arachidonic acid, 20:0	**1**	0.26 ± 0.02^{aA}	0.23 ± 0.02^{aA}	0.21 ± 0.01^{aA}
	21	0.32 ± 0.03^{a}	0.27 ± 0.01^{b}	0.24 ± 0.01^{c}
Behenic acid, 22:0	**1**	0.13 ± 0.01^{aA}	0.11 ± 0.01^{aA}	0.14 ± 0.01^{aA}
	21	0.16 ± 0.01^{a}	0.13 ± 0.01^{b}	0.16 ± 0.01^{a}
		Monounsaturated fatty acids, % of total fatty acids		
Caproleic acid, 10:1	**1**	nd*	nd*	2.16 ± 0.15^{aA}
	21	nd*	nd*	1.90 ± 0.09^{aB}
Z-5-dodecenoic acid, 12:1	**1**	nd*	nd*	0.77 ± 0.05^{aA}
	21	nd*	nd*	0.68 ± 0.03^{aB}
Myristoleic acid, 14:1	**1**	0.72 ± 0.05^{aA}	0.88 ± 0.08^{bA}	1.73 ± 0.12^{cA}
	21	0.60 ± 0.05^{aB}	0.78 ± 0.04^{bA}	1.52 ± 0.07^{c}
Palmitoleic acid, 16:1	**1**	1.90 ± 0.13^{aA}	2.32 ± 0.20^{bA}	1.87 ± 0.13^{aA}
	21	0.67 ± 0.06^{aB}	2.04 ± 0.10^{bA}	1.64 ± 0.08^{c}
Oleic acid, 18:1	**1**	24.97 ± 1.68^{aA}	30.32 ± 2.66^{bA}	28.36 ± 1.91^{Ac}
	21	15.50 ± 1.24^{Ba}	20.68 ± 0.97^{bB}	23.50 ± 1.10^{c}
		Polyunsaturated fatty acids		
Linoleic acid, 18:2	**1**	3.43 ± 0.23^{aA}	4.19 ± 0.37^{bA}	5.63 ± 0.30^{cA}
	21	1.40 ± 0.12^{aB}	2.07 ± 0.10^{bB}	2.95 ± 0.10^{cB}
Linolenic acid, 18:3	**1**	0.69 ± 0.05^{aA}	0.84 ± 0.07^{bA}	2.05 ± 0.14^{cA}
	21	0.61 ± 0.05^{aA}	0.74 ± 0.03^{bB}	1.80 ± 0.08^{cB}
Eicosadienoic acid, 20:4	**1**	0.12 ± 0.01^{aA}	0.15 ± 0.01^{bA}	0.18 ± 0.01^{cA}
	21	0.10 ± 0.01^{aA}	0.13 ± 0.01^{bA}	0.16 ± 0.01^{cA}

*nd – not detected. "a-c" letters point out differences (P<0.05) between yoghurt samples. "A-B" letters point out differences (P<0.05) between storage days

The conducted research showed that the addition of RJ influenced the Ypro+RJ yoghurt sample by the composition of medium-chain fatty acids (MCFAs), with observed increases in caproic, caprylic, capric and lauric acids over the storage period. This enhancement may be due to bioactive lipid components in RJ or its modulation of microbial metabolism. In contrast, butyric acid was consistently higher in the control sample (Yc), while the lowest values were noted in Ypro+RJ, which still increased over time. This reduction may result from microbial assimilation during fermentation or storage. Saturated long-chain fatty acids (LC-SFAs), particularly palmitic, myristic, and stearic acids, were most plentiful in the control sample, with lower levels in the RJ-enriched yogurt sample (Ypro+RJ). This change suggests that RJ could reduce hypercholesterolemic fatty acids, potentially through modified lipid biosynthesis or differences in fat origin which led to improvement of the nutritional lipid profile. RJ introduced unique monounsaturated fatty acids (MUFAs) not present in other samples. While total MUFA content slightly declined in Ypro+RJ, the presence of RJ-specific MUFAs supports its contribution to metabolic and cardiovascular health benefits. Stable levels of palmitoleic and myristoleic acids further enhanced the lipid profile (Mantzourani and Kokotou, 2023). Remarkably, RJ improved the profile of polyunsaturated fatty acid (PUFA), particularly in omega-3 and omega-6 fatty acids such as linoleic and linolenic acids. These compounds are known for their anti-inflammatory and cardioprotective effects. Their increased content in RJ-enriched yogurt sample highlights RJ's role in supporting PUFA synthesis and maintaining functional lipid content during the storage period. Moreover, RJ positively modulates the fatty acid composition of probiotic yogurt, enhancing its functional and nutritional value through its own lipids and synergistic interaction with probiotic cultures, which is consistent with the findings of Kavas (2022).

In Table 5 are shown the results of total polar metabolites of yoghurt samples during the 21-day storage period.

Table 5. Total polar metabolites of yoghurts samples.

Parameter	Storage time, day	Experimental group		
		Yc	Ypro	Ypro+RJ
		Amino acids, % of total polar metabolites		
Alanine	**1**	3.27 ± 0.27^{aA}	5.28 ± 0.43^{bA}	1.79 ± 0.15^{cA}
	21	2.84 ± 0.23^{aA}	4.49 ± 0.36^{bA}	1.61 ± 0.13^{cA}
Glycine	**1**	0.86 ± 0.07^{aA}	0.75 ± 0.06^{bA}	1.05 ± 0.09^{cA}
	21	0.75 ± 0.06^{aA}	0.64 ± 0.05^{aB}	0.95 ± 0.08^{bA}
Valine	**1**	2.40 ± 0.20^{aA}	2.08 ± 0.17^{aA}	2.93 ± 0.24^{bA}
	21	2.09 ± 0.17^{aA}	1.77 ± 0.14^{bA}	2.64 ± 0.21^{cA}
Leucine	**1**	4.58 ± 0.37^{aA}	3.96 ± 0.32^{aA}	5.59 ± 0.45^{bA}
	21	3.99 ± 0.32^{aB}	3.37 ± 0.27^{bA}	5.03 ± 0.41^{cA}
Isoleucine	**1**	2.02 ± 0.16^{aA}	1.75 ± 0.14^{aA}	2.46 ± 0.20^{bA}
	21	1.76 ± 0.14^{aB}	1.48 ± 0.12^{bA}	2.22 ± 0.18^{cA}
Proline	**1**	4.99 ± 0.41^{aA}	4.32 ± 0.35^{aA}	4.94 ± 0.40^{aA}
	21	4.34 ± 0.35^{aB}	3.67 ± 0.30^{bB}	4.45 ± 0.36^{cA}
Serine	**1**	3.14 ± 0.25^{aA}	2.71 ± 0.22^{aA}	3.78 ± 0.31^{bA}
	21	2.73 ± 0.22^{aA}	2.31 ± 0.19^{bA}	3.41 ± 0.28^{cA}

Threonine	**1**	2.68±0.22^{aA}	2.25±0.18^{aA}	3.26±0.27^{bA}
	21	2.33±0.19^{aB}	1.91±0.16^{bA}	2.94±0.24^{cA}
Aspartic acid	**1**	4.03±0.33^{aA}	5.94±0.48^{bA}	2.72±0.22^{cA}
	21	3.51±0.29^{aB}	5.05±0.41^{bA}	2.45±0.20^{cA}
Methionine	**1**	1.65±0.13^{aA}	1.45±0.12^{aA}	2.05±0.17^{bA}
	21	1.43±0.12^{aB}	1.23±0.10^{aB}	1.84±0.15^{bB}
Pyroglutamic acid	**1**	6.07±0.49^{aA}	7.76±0.63^{bA}	4.46±0.36^{cA}
	21	5.28±0.43^{aA}	6.60±0.54^{bA}	4.02±0.33^{cB}
Creatinine	**1**	4.34±0.35^{aA}	3.76±0.31^{aA}	2.21±0.18^{bA}
	21	3.78±0.31^{aA}	3.19±0.26^{bA}	1.99±0.16^{cA}
Glutamic acid	**1**	3.54±0.29^{aA}	4.04±0.33^{bA}	2.56±0.21^{cA}
	21	3.08±0.25^{aB}	3.44±0.28^{aB}	2.30±0.19^{bA}
Phenylalanine	**1**	1.46±0.12^{aA}	1.26±0.10^{aA}	1.79±0.15^{bA}
	21	1.27±0.10^{aA}	1.07±0.09^{bA}	1.61±0.13^{cA}
Lysine	**1**	1.39±0.11^{aA}	1.20±0.10^{aA}	1.70±0.14^{bA}
	21	1.21±0.10^{aA}	1.02±0.08^{bA}	1.53±0.12^{cA}
Tyrosine	**1**	0.57±0.05^{aA}	0.49±0.04^{aA}	0.69±0.06^{bA}
	21	0.50±0.04^{aA}	0.42±0.03^{bA}	0.63±0.05^{cA}
		Carbohydrates, % of total polar metabolites		
Galactose	**1**	2.69±0.22^{aA}	2.33±0.19^{aA}	2.51±0.20^{aA}
	21	2.09±0.17^{aB}	2.57±0.21^{bB}	1.86±0.15^{cB}
Glucose	**1**	1.32±0.11^{aA}	1.19±0.10^{aA}	1.53±0.12^{bA}
	21	1.04±0.08^{aB}	0.71±0.06^{bB}	0.86±0.07^{cB}
Lactose	**1**	7.90±0.64^{aA}	7.00±0.57^{aA}	6.10±0.50^{aA}
	21	5.53±0.45^{aB}	6.70±0.54^{bB}	5.96±0.48^{cB}
		Organic acids, % of total polar metabolites		
Pyruvic acid	**1**	3.18±0.26^{aA}	2.87±0.23^{A}	3.67±0.30^{A}
	21	3.43±0.28^{Aa}	3.16±0.26^{bA}	4.18±0.34^{cA}
Lactic acid	**1**	10.62±0.86^{aA}	10.53±0.86^{A}	9.17±0.74^{A}
	21	13.47±1.09^{aB}	12.58±1.02^{aB}	10.45±0.85^{bA}
Oxalic acid	**1**	3.81±0.31^{aA}	3.44±0.28^{A}	4.40±0.36^{A}
	21	6.11±0.50^{aB}	6.79±0.55^{aB}	5.01±0.41^{bA}
Succinic acid	**1**	1.86±0.15^{aA}	1.68±0.14^{A}	2.15±0.17^{A}
	21	2.01±0.16^{aB}	1.85±0.15^{aA}	2.45±0.20b^{A}
Itaconic acid	**1**	2.77±0.23^{aA}	2.51±0.20^{A}	3.20±0.26^{A}
	21	3.00±0.24^{aB}	2.76±0.22^{aA}	3.65±0.30^{bA}
Fumaric acid	**1**	1.31±0.11^{aA}	1.18±0.10^{A}	1.51±0.12^{A}
	21	1.49±0.12^{aB}	1.30±0.11^{aA}	1.72±0.14^{bA}
Malic acid	**1**	4.03±0.33^{aA}	3.91±0.32^{A}	4.99±0.41^{A}
	21	4.56±0.37^{aA}	4.30±0.35^{aB}	5.69±0.46^{bA}
Fumaric acid	**1**	1.57±0.13^{aA}	1.42±0.12^{aA}	1.80±0.15^{bA}
	21	1.69±0.14^{aA}	1.56±0.13^{aA}	2.45±0.20^{bB}
Adipic acid	**1**	2.21±0.18^{aA}	2.60±0.21^{aA}	2.70±0.22^{bA}
	21	2.40±0.19^{aA}	2.51±0.20^{aA}	3.08±0.25^{bB}
α-Hydroxyglutaric acid	**1**	2.26±0.18^{aA}	2.18±0.18^{aA}	2.79±0.23^{bA}
	21	3.94±0.32^{aB}	2.40±0.20^{bB}	3.20±0.26^{cB}
Suberic acid	**1**	1.02±0.08^{aA}	0.92±0.07^{aA}	1.18±0.10^{bA}
	21	1.10±0.09^{aB}	1.02±0.08^{aA}	1.34±0.11^{bA}
Azelaic acid	**1**	2.31±0.19^{aA}	2.09±0.17^{aA}	2.65±0.21^{bA}
	21	2.49±0.20^{aA}	2.29±0.19^{bA}	3.04±0.25^{cB}
Isocitric acid	**1**	4.14±0.34^{aA}	5.13±0.42^{bA}	5.68±0.46^{bA}
	21	4.75±0.39^{aB}	5.84±0.47^{bA}	5.45±0.44^{cA}

"a-c" letters point out differences (P<0.05) between yoghurt samples. "A-B" letters point out differences (P<0.05) between storage days

The Ypro+RJ sample exhibited the highest total polar metabolite content through storage period, followed by elevated levels of branched-chain amino acids (leucine, isoleucine, valine), such as serine, threonine, lysine, and phenylalanine. Thus suggests enhanced proteolytic activity or shifts in microbial metabolism influenced by RJ. Carbohydrate levels, particularly lactose and glucose, declined more markedly in Ypro+RJ, reflecting intensified fermentation and microbial utilization. Among organic acids, the RJ-enriched sample showed increased levels of pyruvate, succinate, itaconic, adipic, and α-hydroxyglutaric acids, while lactic acid remained lower than in the control sample (Yc). These shifts imply that RJ modulates metabolic pathways, promoting the synthesis of alternative fermentation products. These findings are consistent with earlier studies showing that probiotic strains reshape amino acid and organic acid profiles during storage (Kandasamy *et al*., 2024), and align with the metabolically active composition of RJ.

Microbiological analysis

The number of characteristic microorganisms (lactobacilli and lactic streptococci) in yoghurt samples during the storage is shown in Table 6.

Table 6. Characteristic microorganisms (lactobacilli and lactic streptococci) in yoghurt samples.

Parameter	Storage time, day	Experimental group		
		Yc	Ypro	Ypro+RJ
		Lactic acid bacteria, CFU/g		
Lactic streptococci	**1**	2.4×10^{9}	2.1×10^{9}	2.8×10^{9}
	21	1.7×10^{9}	2.6×10^{9}	1.8×10^{9}
Lactobacilli	**1**	1.5×10^{7}	3.3×10^{7}	7.0×10^{7}
	21	2.6×10^{8}	2.0×10^{8}	1.5×10^{8}

As seen from Table 6 the probiotic enriched sample (Ypro) revealed lower streptococci, but higher lactobacilli counts. The yoghurt sample enriched with RJ (Ypro+RJ) maintained the highest streptococci and lactobacilli counts, probably due to the positive impact of RJ supplementation on the LAB growth. After 21 days of storage, in the sample Yc streptococci count decreased, while lactobacilli increased. The sample Ypro showed an increase in streptococci count, while lowering the growth of lactobacilli was observed. Sample with RJ (Ypro+RJ) indicated lowering of streptococci and lactobacilli growth. All samples exhibited lowered viability in streptococci over the storage period; lactobacilli increased in Yc and Ypro samples. The RJ sample started strongest, but converted to similar lactobacilli levels of lactobacilli and streptococci growth, viability remained above the functional level. These observations are consistent with these made by Kavas (2022), who found that implementing 2% RJ improved probiotic viability of the tested samples. Similar to the research made by Atallah and Morsy (2017), the LAB decline over 21 days of storage, but remained more than 10^{6} CFU/g in RJ enriched sample.

The results from total plate count (mesophilic aerobic and facultative anaerobic microorganisms), yeasts and fungi and pathogenic microorganisms (*Listeria monocytogenes*) in yoghurt samples during the 21 days of storage are presented in Table 7.

Table 7. Total plate count (mesophilic aerobic and facultative anaerobic microorganisms), yeasts and fungi and pathogenic microorganisms (*Listeria monocytogenes*) in yoghurt samples.

Parameter	Storage time, day	Experimental group		
		Yc	Ypro	Ypro+RJ
Yeasts and fungi, CFU/g	**1**	< 10	< 10	< 10
	21	6.8×10^2	7.4×10^2	9.5×10^3
Total plate count, CFU/g	**1**	60.00	80.00	6.6×10^2
	21	2.0×10^2	2.3×10^2	3.1×10^3
***L. monocytogenes*, CFU/g**	**1**	-	-	-
	21	-	-	-

As seen from the results in Table 7, on the first day of storage all tested samples showed low number of mesophilic and facultative anaerobic microorganisms (total plate count - TPC) and absence of yeasts and fungi. On the 21st day of storage, all yoghurts showed an increase in the TPC values, which was the most significant in the probiotic-enriched sample Ypro+RJ. The same trend was observed in yeasts and fungal counts, which implied to the statement that nutrient-rich composition of RJ supported their proliferation (Metry and Owayss, 2009). On the other hand, RJ is not a sterile product, and its addition to the product also contributed to the higher microbial counts. *L. monocytogenes* was not detected during the entire storage period for 21 days.

Sensory analysis

The Sensory analysis of the tested yoghurts during the storage period are presented at Figure 2.

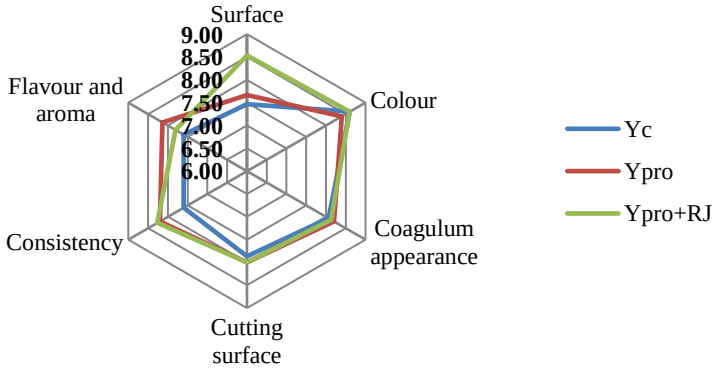

Figure 2. Sensory analysis of the tested yoghurts.

The sensory evaluation revealed in Figure 2. showed that the probiotic yoghurt enriched with RJ (Ypro + RJ) received the highest scores for surface appearance and consistency, implying improved gel structure and creaminess due to RJ's impact on texture. Colour and coagulum appearance were comparable to the control, demonstrating good visual acceptability. Flavour and aroma were slightly lower than in Ypro, but remained acceptable, possibly reflecting the addition of delicate notes from RJ. These findings are in agreement with similarly reported studies made by Kavas (2022), which reported that RJ improves textural and visual appeal without compromising flavour and quality in probiotic dairy products.

Rheological analyses

The summarized results from rheological analyses are presented in Table 8.
At the beginning of storage (day 1), the control sample (Yc) exhibited the highest values of complex viscosity |η∗|$|\eta^*|$|η∗| and dynamic viscosity compared to Ypro and Ypro+RJ, while the tan(δ) value was significantly lower, indicating more elastic behavior. In contrast, the Ypro and Ypro+RJ samples showed substantially lower viscosity values but considerably higher tan(δ).
After 21 days of storage, all samples demonstrated a pronounced decrease in rheological parameters. The most pronounced reduction was observed in Ypro+RJ, which retained minimal viscosity values, whereas Yc and Ypro maintained higher values, although still significantly reduced compared to day 1. Interestingly, the tan(δ) of Ypro+RJ remained high throughout the entire storage period, while in Yc and Ypro the tan(δ) values leveled off around 3, suggesting a more balanced viscoelastic character after 21 days.

Table 8. Change of summarized rheological parameters of the investigated samples over time.

Parameter	Storage time, day	Experimental group		
		Yc	Ypro	Ypro+RJ
|η*|, Pa.s	**1**	23.3 ± 0.2^{aA}	19.4 ± 0.5^{bA}	19.4 ± 0.1^{bA}
Apparent viscosity	**21**	14.6 ± 0.3^{bB}	15.3 ± 0.5^{aB}	4.2 ± 0.1^{cB}
η, Pa.s	**1**	95.5 ± 1.2^{aA}	10.0 ± 0.7^{bA}	8.6 ± 0.5^{cA}
Dynamic viscosity	**21**	3.3 ± 0.2^{bB}	7.6 ± 0.4^{aB}	2.5 ± 0.1^{cB}
tan(δ)	**1**	3.8 ± 0.1^{bA}	30.1 ± 0.5^{aA}	30.1 ± 0.3^{aA}
	21	3.2 ± 0.2^{bA}	3.0 ± 0.1^{bB}	26.6 ± 0.7^{aB}

"a-c" letters point out differences (P<0.05) between yoghurt samples. "A-B" letters point out differences (P<0.05) between storage days

The results from the Herschel–Bulkley and Ostwald–de Waele rheological models are presented in Table 9. The results obtained from the Ostwald–de Waele and Herschel–Bulkley rheological models confirm the pronounced pseudoplastic behavior of all investigated samples.
For the control sample (Yc), the Ostwald–de Waele model showed a decrease in the flow behavior index (n) and an increase in the consistency index (K) after 21 days of storage, indicating stronger structural interactions and enhanced system stability.

The Herschel–Bulkley model confirmed these findings by showing a marked increase in yield stress (τ_0), suggesting a more stable and elastic structure during prolonged storage.

Table 9. Rheological parameters of yoghurt samples.

Parameter	Storage time, day	Experimental group		
		Yc	Ypro	Ypro+RJ
	Ostwald–de Waele			
Power law index	**1**	0.22±0.01	0.73±0.03	0.22±0.01
	21	0.17±0.02	0.16±0.01	0.15±0.03
Consistency index (K, Pa·s^n)	**1**	33.73±1.30	10.16±1.60	10.17±1.02
	21	41.19±0.44	228.43±1.18	74.73±1.01
Coefficient of determination (R^2)	**1**	0.970	0.750	0.748
	21	0.979	0.991	0.935
	Herschel–Bulkley			
Power law index	**1**	0.22±0.01	1.21±0.05	1.23±0.07
	21	0.48±0.02	-	-
Consistency index (K, Pa·s^n)	**1**	26.69±3.83	0.52±0.06	0.52±0.10
	21	4.76±3.83	134.13±0.04	-
τ_0	**1**	5.54±0.39	84.10±4.76	84.10±4.76
Pa.s	**21**	47.22±0.18	74.79±0.17	-
Coefficient of determination (R^2)	**1**	0.972	0.906	0.906
	21	0.996	0.233	-

For the Ypro sample, a relatively high flow behavior index was observed at the beginning of storage, corresponding to a weakly structured system. By the end of storage, however, n sharply decreased, while K increased dramatically, pointing to the formation of a stronger network. The Herschel–Bulkley model also indicated high τ_0 values, but with a low coefficient of determination at day 21, suggesting that this model did not adequately describe the behavior of the sample at the end of storage.

Conclusions

The growing global demand for functional foods is driving innovation in the dairy industry, particularly in the development of enriched probiotic products with added health benefits. Yoghurt, as a widely consumed fermented dairy product, serves as an effective matrix for the delivery of probiotic microorganisms such as *L. casei*, contributing to improved nutritional and therapeutic properties. The incorporation of RJ—characterized by its high nutritional density and well-documented antibacterial, antioxidant, and immunomodulatory activities—into probiotic yoghurt formulations, presents a promising strategy to further enhance both functional and sensory characteristics. The present study demonstrated that the addition of RJ to probiotic yoghurt significantly enhanced its nutritional, functional, and bioactive properties. Among the evaluated formulations, the combination comprising the yoghurt starter culture, *L. casei* RC-1, and RJ (Ypro+RJ) exhibited superior

performance across multiple quality parameters and biological activities over 21 days of refrigerated storage. Overall, these findings indicated that RJ can be successfully incorporated into probiotic yoghurt to enhance its functional value without compromising safety or sensory appeal, making it a promising candidate for the development of next-generation functional dairy products.

References

AOAC International, 2005. AOAC Method 981.12: pH of yogurt. *AOAC Official Methods of Analysis.*

AOAC International, 2005. Official Method 989.05: Fat in Milk (Gerber Method). *AOAC International.*

Atallah, A.A. 2016. The Production of Bio-yoghurt with Probiotic Bacteria, Royal Jelly and Bee Pollen Grains. *Journal of Nutrition & Food Sciences*, **6**(3), 1-7.

Atallah, A.A. Morsy K.M. 2017. Effect of Incorporating Royal Jelly and Bee Pollen Grains on Texture and Microstructure Profile of Probiotic Yoghurt. *Journal of Food Processing & Technology*, **8**(9), 1-4.

Bulgarian State Standard BSS 1111:1980. "Milk and milk products -Determination of acidity". The Bulgarian Institute of Standardization, Sofia, Bulgaria (in Bulgarian).

Bulgarian State Standard BSS ISO 6611:2006. "Milk and milk products –Enumeration of colony-forming units of yeasts and/or moulds - Colony-count technique at 25 degrees C". The Bulgarian Institute of Standardization, Sofia, Bulgaria (in Bulgarian**).**

Bulgarian State Standard BSS ISO 7889:2005. "Yogurt - Enumeration of characteristic microorganisms - Colony-count technique at 37 degrees C". The Bulgarian Institute of Standardization, Sofia, Bulgaria (in Bulgarian**).**

Cui, Y., Qu, X. 2021. Genetic mechanisms of prebiotic carbohydrate metabolism in lactic acid bacteria: Emphasis on *Lacticaseibacillus casei* and *Lacticaseibacillus paracasei* as flexible, diverse and outstanding prebiotic carbohydrate starters. *Trends in Food Science & Technology*, **115**, 486-499.

Falcao, S.I., Freire, C., Vilas-Boas, M.A. 2013. Proposal for physicochemical standards and antioxidant activity of Portuguese propolis. *Journal of the American OilChemists' Society*, **90**(11), 1729-1741.

Hadjimbei, E., Botsaris, G., Chrysostomou, S. 2022. Beneficial Effects of Yoghurts and Probiotic Fermented Milks and Their Functional Food Potential. *Foods*, **11**(17), 2691.

Hassan, A.A., Elenany ,Y.E., Nassrallah, A., Cheng, W., Abd El-Maksoud, A.A., 2022. Royal jelly improves the physicochemical properties and biological activities of fermented milk with enhanced probiotic viability. *Lebensmittel-Wissenschaft & Technologie*, **155**, 112912.

Hummel, J., Strehmel, N., Selbig, J., Walther, D., Kopka, J. 2010. Decision tree supported substructure prediction of metabolites from GC-MS profiles. Metabolomics, **6**, 322-333.

ISO 19662:2018, IDF 238:2018 Milk 2018 Determination of fat content – Acido-butyrometric (Gerber method).

ISO 5534:2004, IDF 4:2004 Cheese and processed cheese — Determination of the total solids content (Reference method), Brussels, Belgium.

Ivanov, I., Vrancheva, R., Marchev, A., Petkova, N., Aneva, I., Denev P., Georgiev, V., Pavlov, A. 2014. Antioxidant activities and phenoliccompounds in Bulgarian fumaria species. *International Journal of Current Microbiology and Applied Sciences*, **3**(2), 296-306.

Ivanova, T., Dincheva, I., Badjakov, I., Iantcheva, A. 2023. Transcriptional and Metabolic Profiling of Arabidopsis thaliana Transgenic Plants Expressing Histone Acetyltransferase HAC1 upon the Application of Abiotic Stress—Salt and Low Temperature. *Metabolites*, **13**(9), 994.

Kandasamy, S., Park, W.S., Bae, I.S., Yoo, J., Yun, J., Hoa, V.B., Ham, J.S. 2024. HRMAS-NMR-Based Metabolomics Approach to Discover Key Differences in Cow and Goat Milk Yoghurt Metabolomes. *Foods*, **13**(21), 3483.

Kavas, N. 2022. Functional probiotic yoghurt production with royal jelly fortification and determination of some properties. *International Journal of Gastronomy and Food Science*, **28**, 100519.

Kunugi, H., Mohammed, A.A., 2019. Royal Jelly and Its Components Promote Healthy Aging and Longevity: From Animal Models to Humans. *International Journal of Molecular Sciences*, **20**(19), 4662.

Liu, Y., Li, Y., Yu, X., Yu, L., Tian, F., Zhao, J., Zhang, H., Zhai, Q., Chen, W. 2021. Physiological characteristics of *Lactobacillus casei* strains and their alleviation effects against inflammatory bowel disease. *Journal of Microbiology and Biotechnology*, **31**(1), 92–103.

Mabrouk, A. 2016. The production of bio-yoghurt with probiotic bacteria, Royal Jelly and Bee Pollen Grains. *Journal of Nutrition & Food Sciences*, **6**(3), 510.

Mantzourani, C., Kokotou, M.G. 2023. Targeted and suspect fatty acid profiling of royal jelly by liquid chromatography-high resolution mass spectrometry. *Biomolecules*, **13**(3), 424.

Martirosyan, D.M., Singh, J. 2015. A new definition of functional food by FFC: what makes a new definition unique? *Functional Foods in Health and Disease*, **5**(6), 209-223.

Manion, R., Huie, R., Levin, D., Manion, J., Huie, R., Levin, R., Burgess, D., Orkin, V., Tsang, W., McGivern, W., *et al*. 2015. NIST Chemical Kinetics Database, NIST Standard Reference Database 17, Version 7.0 (Web Version), Release 1.6.8, Data Version 2015.09.

Metry, W.A. and Owayss, A.A. 2009. Influence of incorporating honey and Royal Jelly on the quality of yoghurt during storage. *Egypt Journal of Food Science*, **37**, 115–131.

Morsy, M., 2017. Effect of incorporating Royal Jelly and bee pollen grains on texture and microstructure profile of probiotic yoghurt. *Journal of Food Processing & Technology*, **8**(9), 1-4.

Nunes, R., Silva, V.L., Consiglio-Kasemodel, M.G., Polizer, Y.J., Saes, M.S.M., Fávaro-Trindade, C.S. 2020. Assessing global changing food patterns: A country-level analysis on the consumption of food products with health and wellness claims. *Journal of Cleaner Production*, **264**, 121613.

Oozeer R., Leplingard A., Mater D.D., Mogenet A., Michelin R., Seksek I., Marteau P., Doré J., Bresson J.L., Corthier G. 2006. Survival of *Lactobacillus casei* in the human digestive tract after consumption of fermented milk. *Applied and Environmental Microbiology*, **72**(8), 5615-7.

Rochat, T., Bermúdez-Humarán, L., Gratadoux, J.J., Fourage, C., Hoebler C., Corthier, G., Langella, P. 2007. Anti-inflammatory effects of *Lactobacillus casei* BL23 producing or not a manganese-dependant catalase on DSS-induced colitis in mice. *Microbial Cell Factories*, **6**(22), 1-10.

Sanders, M.E., Merenstein, D.J., Reid, G., Gibson, G.R., Rastall, R.A. 2019. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nature Reviews Gastroenterology & Hepatology*, **16**(10), 605-616.

Tamime, A.Y., Robinson R.K. 2007. *Tamime and Robinson's Yoghurt: Science and Technology*, CRC Press.

Tumbarski, Y.D., Todorova, M.M., Topuzova, M.G., Georgieva, P.I., Ganeva, Z.A., Mihov, R.B., Yanakieva, V. 2021. Antifungal activity of carboxymethyl cellulose edible films enriched with propolis extracts and their role in improvement of the storage life of kashkaval cheese. *CurrentResearch in Nutrition and Food Science Journal*, **9**(2), 487-499.

Tumbarski, Y., Peykova-Shapkova, I., Ivanova, M., Cholakov, R., Dutkiewicz, A., Grzymajło K. 2025a. Characterization and Selection of Lactobacillus Strains with Potential Probiotic Applications. *Applied Sciences*, **15**(6), 2902.

Tumbarski, Y., Peykova-Shapkova, I., Enkin, D., Ivanov, I., Todorova, M., Ivanova, M., 2025b. Physicochemical properties, antioxidant activity, antimicrobial potential and 10-hydroxy-2-decanoic acid (10-HDA) content of Bulgarian royal jelly. *BIO Web of Conferences*, **170**, 02009.

Zhang, T., Geng, S., Cheng, T., Mao, K., Chitrakar, B., Gao, J., Sang, Y. 2023. From the past to the future: Fermented milks and their health effects against human diseases. *Food Frontiers*, **4**(4), 1747-1777.

Zrnic, M., Gajic, T., Vukolic D. 2023. The future of functional foods: trends, opportunities and obstacles in the food industry. *Academy of applied studies Belgrade: Processing book*, 137-142.