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**Darko BAĆOVIĆ², Olga KOPITOVIĆ*¹,
Dušica RADONJIĆ¹, Miljan VELJIĆ¹, Slavko MIRECKI¹**

ROLE OF *Streptococcus thermophilus* (CNCM I-2980) IN FERMENTATION PERFORMANCE, PRODUCT QUALITY, AND SENSORY CHARACTERISTICS OF PROBIOTIC YOGURT

SUMMARY

Optimizing yogurt fermentation is essential for improving production efficiency while maintaining product quality and consumer acceptance. Using starter cultures such as *Streptococcus thermophilus* can reduce fermentation time while preserving the chemical and sensory qualities of probiotic yogurt. This study aimed to evaluate the effect of a starter culture containing *Streptococcus thermophilus* (CNCM I-2980) on fermentation duration, chemical composition, and sensory characteristics of two probiotic yogurts, in comparison with a control low-fat yogurt produced using a standard yogurt culture. The experimental probiotic yogurts were produced by supplementing the starter cultures with *Streptococcus thermophilus* (CNCM I-2980). Technological parameters, with particular emphasis on fermentation duration, as well as chemical quality parameters and sensory characteristics, were analysed and compared with those of the control yogurt. Statistical analysis was used to determine differences among the products. The control yogurt exhibited a significantly longer fermentation time than both experimental yogurts ($p < 0.05$), demonstrating enhanced fermentation activity linked to *Streptococcus thermophilus* (CNCM I-2980). Significant differences ($p < 0.05$) were observed in lactose content and solids-not-fat content between the control and experimental yogurts. Sensory evaluation showed that both experimental yogurts achieved higher overall scores than the control. The results demonstrate that the inclusion of *Streptococcus thermophilus* (CNCM I-2980) in starter cultures can effectively shorten yogurt fermentation time while maintaining or improving chemical quality and sensory properties, representing a valuable technological approach for improving efficiency and product quality in the dairy industry.

Keywords: probiotic yogurt, starter cultures, chemical and sensory properties, fermentation

¹Olga Kopitović (corresponding author: jokanovicolga@gmail.com), Dušica Radonjić, Miljan Veljić, Slavko Mirecki, Biotechnical Faculty, University of Montenegro, Podgorica, MONTENEGRO;

²Darko Baćović, Šljukić Co., Dairy “Sma”, Ozrinići bb, 81400 Nikšić, MONTENEGRO

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INTRODUCTION

Yogurt is a semi-solid fermented dairy product produced from standardized milk through the activity of a symbiotic culture of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Abayneh, 2020). Probiotic cultures employed in fermented dairy production comprise bacterial strains primarily isolated from the human gastrointestinal tract, most commonly from the genera *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, and *Streptococcus* (Tratnik, 1998). Currently, more than 40 species of the genus *Streptococcus* are known, some of which are pathogenic. However, *Streptococcus thermophilus*, one of the most important and widely used representatives of this genus, has been granted the status of “Generally Recognized as Safe” (GRAS) in the United States and Qualified Presumption of Safety (QPS) in the European Union due to its long history of safe use in food production (Ramya Iyer *et al.*, 2010). Among all *Streptococcus* species, only *S. thermophilus* is utilized in the dairy industry, where it plays an indispensable role in starter cultures for the production of yogurt and certain types of cheese (Samaržija, 2015). As a milk starter culture, *S. thermophilus* rapidly converts lactose into lactic acid, resulting in a rapid decrease in pH and subsequent coagulation of milk proteins, primarily casein. In addition, this bacterium contributes significantly to desirable processing and quality attributes of yogurt, including flavor, acidity, viscosity, and water-holding capacity (Sun *et al.*, 2011). Milk fermentation is the most critical stage in yogurt production, as it directly determines the quality and sensory characteristics of the final product. At the same time, fermentation significantly affects production economics due to energy consumption, labour requirements, and overall process duration. Consequently, shortening fermentation time can lead to reduced production costs (Alline *et al.*, 2018). The ratio and composition of starter cultures have a pronounced influence on fermentation time, pH development, viscosity, and other physicochemical and sensory properties of yogurt. Therefore, identifying effective approaches to reduce fermentation time may not only improve production efficiency but also enhance product quality. Dan *et al.*, (2017) reported that varying ratios of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in starter cultures critically influence yogurt aroma and overall flavor. Furthermore, variations in bacterial proportions influence key fermentation parameters, including acidity, viscosity, water-holding capacity, coagulation rate, and the formation of flavor compounds (Dan *et al.*, 2023). In line with this, Zhou *et al.*, (2019) reported that supplementing a commercial starter culture with *Lactobacillus helveticus* H9 markedly reduced milk fermentation time and enhanced the diversity of volatile flavor compounds, particularly benzaldehyde and acetoin. Fermentation time was reduced from 5.00 ± 0.01 h in the control sample to 3.75 ± 0.01 h with the addition of the *L. helveticus* H9 inoculum. Similarly, Ge *et al.*, (2024) investigated the symbiotic interaction between *S. thermophilus* CICC 6038 and *L. delbrueckii* subsp. *bulgaricus* CICC 6047 and reported enhanced acidification activity, improved rheological properties, richer volatile compound profiles, and more

desirable sensory characteristics in yogurts produced with the symbiotic culture compared to monocultures. Although sensory profiles were generally similar, the symbiotic culture produced yogurt with superior texture and viscosity while significantly shortening fermentation time and increasing biomass and acid production. Zajác *et al.*, (2020) reported differences in fermentation behavior between thermophilic starter cultures YoFlex® YF-LL812 and Delvo® Fresh YS-241, with pH 4.4 reached after 16 and 15 hours of fermentation, respectively. Statistically significant differences ($p < 0.05$) were observed in all textural parameters, including hardness, consistency, cohesion, and viscosity, with yogurt produced using Delvo® Fresh YS-241 exhibiting slightly superior characteristics.

The objectives of this study were to determine whether the addition of a starter culture containing *Streptococcus thermophilus* (CNCM I-2980) could shorten yogurt fermentation time, to evaluate its impact on the chemical quality of the resulting products, and to assess changes in their sensory properties. The significance of this research lies in its potential for direct application in industrial production, leading to reduced production costs and improved product quality. Such an approach could encourage the production of probiotic yogurt containing *S. thermophilus* (CNCM I-2980) at the “Srna” Dairy, as well as its broader implementation in other dairies in Montenegro and beyond.

MATERIAL AND METHODS

Experimental design

The experimental study was conducted over a period of 30 consecutive days. Each day, bulk raw milk obtained from milk collection centers was evenly divided into three separate lots, from which three yogurt batches were produced. Milk intended for each batch was standardised to a fat content of 1% to ensure uniform raw material quality and to minimize variability arising from differences in chemical composition. Key technological parameters of the production process were monitored over ten consecutive production days to identify potential differences in fermentation dynamics and technological behavior among the batches. For chemical analysis, 30 samples were collected from each yogurt batch, ensuring a statistically relevant basis for evaluating the chemical quality of the final products. In parallel, one representative sample from each batch was prepared for sensory evaluation following standard protocols for the assessment of fermented dairy products.

Starter cultures used

Concentrated lyophilised starter cultures produced by Danisco were used in this study, as follows:

YO-MIX™ 885 LYO 500 DCU (starter culture A) represents a basic starter culture for the production of standard yogurt. It consists of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The recommended dosage is 10–20 DCU per 100 L of milk, applied by direct inoculation. This culture is responsible for rapid acidification and the development of a creamy texture.

YO-MIX™ 207 LYO 250 DCU (starter culture B) contains *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*. It is used for the production of probiotic yogurt at a recommended dosage of 15–25 DCU per 100 L of milk and provides a smooth and thick texture with a typical yogurt flavor.

YO-MIX™ 495 LYO 100 DCU (starter culture C) consists of selected strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. It is added at a dosage of 10–20 DCU per 100 L of milk and is used in the production of yogurt and other fermented milk beverages.

YO-MIX™ 883 LYO 500 DCU (starter culture D) is based on a patented strain of *S. thermophilus* (CNCM I-2980). This strain contributes to improved texture and enhanced organoleptic properties of fermented dairy products. It can be used alone or in combination with other cultures. In the present study, starter culture D was combined with starter cultures B and C to produce two experimental probiotic yogurts.

Experimental probiotic yogurts

Cow's milk standardised to a fat content of 1% was used as the raw material for yogurt production. Three yogurt products were manufactured using the same technological process, with the only difference being the composition of the starter cultures:

Product 1 (control): Probiotic yogurt produced using starter culture A, representing standard low-fat yogurt.

Product 2: Probiotic yogurt produced using a mixture of starter cultures B and D in a 1:1 ratio. The inclusion of starter culture D aimed to assess its effect on technological performance, chemical quality, and sensory characteristics.

Product 3: Probiotic yogurt produced using a mixture of starter cultures C and D in a 1:1 ratio, with the same objective as for Product 2.

Chemical analysis

Chemical quality analysis of the control yogurt (Product 1) and the two experimental probiotic yogurts (Products 2 and 3) involved determining fat, protein, solids-not-fat, and lactose contents. Analyses were performed using a MilkoScan FT120 analyser based on FTIR spectrophotometry, in accordance with IDF Standard 141C:2000.

Sensory analysis

Sensory evaluation was carried out by a panel of five trained evaluators, including three experts from the “Srna” dairy (two food technologists and one laboratory technologist) and two members of the Biotechnical Faculty (a professor and an assistant professor of Dairy Technology). Each evaluator received coded, representative samples of all three yogurt products. Sensory analysis was performed according to the method described by Filajdić *et al.*, (1988), which involves scoring taste, odour, colour, consistency, and appearance. Each sensory attribute was evaluated on a scale from 0 to 5. The mean score for each attribute was calculated and multiplied by the corresponding correction coefficient. The maximum possible total score was 20 points.

Technological parameters

Technological parameters were monitored for all three yogurt products during ten consecutive production days. Data were recorded for starter culture inoculation time and temperature, product acidity, and fermentation duration. Fermentation was terminated when an acidity of 33 °SH was reached. Fermentation duration was calculated based on the time elapsed between starter culture inoculation and fermentation termination and was expressed in minutes.

Statistical analysis

All experiments were conducted in triplicate. Statistical analysis was performed using Statistica 7 software (Dell Software, StatSoft, 2004). Descriptive statistical parameters, including mean value ($\bar{X}$), minimum (Xmin), maximum (Xmax), and standard deviation (SD), were calculated. One-way analysis of variance (ANOVA) was applied to assess differences in quality parameters and fermentation duration among the products. Statistical significance was assessed using Tukey's post hoc test, with results considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Technological parameters

Technological parameters were monitored to determine whether the addition of a starter culture containing *Streptococcus thermophilus* (CNCM I-2980) affects the technological aspects of the production of two probiotic yogurts (Products 2 and 3). The results were compared with those obtained for the standard yogurt (Product 1). Table 1 presents the average fermentation duration of all three products.

Table 1. Duration of products fermentation (minutes)

Statistical parameters	PRODUCTS		
	1 (culture A)	2 (culture B+D)	3 (culture C+D)
n	10	10	10
$\bar{X}$	351.5 ^a	246 ^b	234 ^b
Xmin	330	230	195
Xmax	400	260	260
SD	22.491	9.068	21.187

*Mean values marked with the same letter do not differ statistically significantly ($p < 0.05$)

The average fermentation duration of the standard yogurt (Product 1) was 351.5 minutes. In comparison, fermentation durations of 246 minutes and 234 minutes were recorded for Products 2 and 3, respectively. The fermentation of the standard yogurt was significantly longer than that of the yogurts produced with mixed starter cultures ($p < 0.05$). The results of this study are consistent with those reported by several authors. Dan *et al.*, (2023) demonstrated that adjusting the ratios of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus*

thermophilus in starter cultures accelerates milk coagulation. Similarly, Zhou *et al.*, (2019) reported that supplementing a commercial starter culture with *Lactobacillus helveticus* H9 enhanced milk fermentation compared to the control, resulting in shorter fermentation times and a broader diversity of volatile flavor compounds. Ge *et al.*, (2024) further showed that the symbiotic interaction between *S. thermophilus* CICC 6038 and *L. delbrueckii* subsp. *bulgaricus* CICC 6047 improved fermentation efficiency, sensory quality, and yogurt stability. Specifically, this symbiotic culture yielded yogurt with superior texture and viscosity, increased biomass, faster acidification, and markedly reduced fermentation time. In line with these findings, Zajac *et al.*, (2020) observed minor differences in fermentation dynamics when comparing thermophilic starter cultures YoFlex® YF-LL812 and Delvo® Fresh YS-241, with pH 4.4 achieved after 16 and 15 hours, respectively. Compared with the other groups, the addition of *B. coagulans* exerted a positive effect on the appearance and texture of yogurt products (Shenyan Ma *et al.*, 2021).

The chemical quality of experimental products

To evaluate the potential effect of the addition of a starter culture containing *Streptococcus thermophilus* (CNCM I-2980) on the chemical quality of the newly produced probiotic yogurts (Products 2 and 3), their chemical composition was analysed and compared with that of the standard yogurt (Product 1).

Table 2 presents the chemical composition of Product 1 (standard low-fat yogurt) and the two probiotic yogurts produced using mixed starter cultures (Products 2 and 3). A total of 90 samples were analysed, comprising 30 samples of each product. The analyses included the determination of fat, protein, lactose, and solids-not-fat (SNF) contents.

Table 2. Chemical quality of the experimental products

PRODUCTS	statistical parameters	n	QUALITY PARAMETERS			
			Fat, %	Proteins, %	Lactose, %	SNF, %
1	$\bar{X}$	30	1.03^a	3.38^a	4.21^a	9.21^a
	Xmin		0.85	3.18	4.04	8.91
	Xmax		1.22	3.51	4.44	9.55
	SD		0.107	0.001	0.033	0.208
2	$\bar{X}$	30	1.00^a	3.37^a	4.11^b	9.04^b
	Xmin		0.91	3.22	3.93	8.84
	Xmax		1.11	3.48	4.35	9.34
	SD		0.065	0.017	0.011	0.137
3	$\bar{X}$	30	1.03^a	3.38^a	4.10^b	9.07^b
	Xmin		0.95	3.2	3.89	8.88
	Xmax		1.21	3.59	4.28	9.31
	SD		0.050	0.015	0.066	0.061

Mean values marked with the same letter do not differ statistically significantly ($p < 0.05$)

The milk fat content of the experimental probiotic yogurts was 1.00% in Product 2 and 1.03% in Products 1 and 3. No statistically significant differences in milk fat content were observed among the products ($p>0.05$). Similarly, no statistically significant differences were found in protein content, with Product 2 containing 3.37% protein and Products 1 and 3 containing 3.38% protein ($p>0.05$). The results confirm that the incorporation of starter culture D into starter cultures B and C did not alter the fat or protein content of the yogurt, which remained comparable to that of the standard product (Product 1). In contrast, the addition of starter culture D led to a reduction in lactose levels in Products 2 and 3 (4.11% and 4.10%, respectively), as well as a decrease in solids-not-fat (SNF) content (9.04% and 9.07%, respectively), relative to Product 1. The presence of starter culture D promoted more intensive lactose degradation during fermentation in Products 2 and 3, leading to a lower lactose concentration. Since lactose is a major component of SNF, its reduction consequently resulted in lower SNF values. The lactose and SNF contents were statistically significantly higher in Product 1 than in Products 2 and 3 ($p<0.05$).

One of the goals of this study was to assess whether the addition of starter culture D containing *S. thermophilus* (CNCM I-2980) affects the chemical composition of probiotic yogurts. The experimental yogurts contained approximately 1% milk fat. This value is higher than that reported for probiotic yogurts produced in Great Britain (Hussain *et al.*, 2009) and for cow's milk probiotic yogurts used as a reference in studies comparing physicochemical and microtextural properties of goat yogurt (Zhang *et al.*, 2015). The milk fat content of the experimental products was lower than that reported for probiotic yogurts studied by Hassan and Amjod (2010), Torba yogurt (Kesenkas, 2010), probiotic yogurts supplemented with Gobdin fruit paste (Ertem and Çakmakçı, 2017), and yogurts enriched with barley β -glucan (Elsanhoty and Ramadan, 2018). In contrast, the protein content of the experimental yogurts was comparable to values reported by Ertem and Çakmakçı (2017) for Gobdin fruit paste yogurt, Elsanhoty and Ramadan (2018) for barley β -glucan-enriched yogurt, and Zhang *et al.*, (2015) for cow's milk probiotic yogurt. However, protein levels were lower than those reported for probiotic yogurts from Great Britain (Hussain *et al.*, 2009), Hassan and Amjod (2010), and Torba yogurt (Kesenkas, 2010). Lactose content in the experimental yogurts was higher than in barley β -glucan-enriched yogurt (Elsanhoty and Ramadan, 2018). Data on solids-not-fat (SNF) were not available in the cited studies for comparison. Overall, the incorporation of *Streptococcus thermophilus* (CNCM I-2980) into the starter cultures did not adversely influence the chemical composition of the yogurt of the experimental probiotic yogurts, confirming its suitability for industrial application while maintaining the desired fat, protein, lactose, and SNF levels.

Sensory evaluation of the experimental product

To evaluate the influence of the strain *Streptococcus thermophilus* (CNCM I-2980) on the taste, odor, color, consistency, and appearance of Products 2 and 3, a sensory evaluation was conducted. The results were compared with those

obtained for the standard yogurt (Product 1). The outcomes of the sensory analysis for Products 1, 2, and 3 are summarized, and Table 3 presents the categorization of product quality based on the scores obtained.

Table 3. Product quality categorization based on sensory analysis

Quality categories	Points range	PRODUCT (points scored)		
		1 (culture A)	2 (culture B+D)	3 (culture C+D)
Excellent	17.6-20.0		18.84	18.51
Very good	15.2-17.5	16.09		
Good	13.2-15.1			
Acceptable	11.2-13.1			
Unacceptable	<11.2			

As shown in Table 3, the probiotic yogurts supplemented with *Streptococcus thermophilus* (CNCM I-2980) exhibited superior sensory quality compared to the standard yogurt. Based on the scores obtained, Product 2 (18.84) and Product 3 (18.51) were classified in the “Excellent” category, whereas the standard yogurt (Product 1) received a score of 16.09 and was classified as “Very Good.” Sensory evaluation revealed that the incorporation of starter culture D not only maintained but also enhanced the sensory qualities of Products 2 and 3.

Different compositions of starter cultures have a significant influence on the sensory characteristics of yogurt. Dan *et al.*, (2017) emphasized that variations in the ratio of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* within starter cultures have a critical impact on yogurt aroma and overall flavor. Building on this, Ge *et al.*, (2024) examined the symbiotic interaction between *S. thermophilus* CICC 6038 and *L. delbrueckii* subsp. *bulgaricus* CICC 6047, reporting enhanced rheological properties, a more complex profile of volatile compounds, and superior sensory attributes compared to monoculture fermentations. Although sensory profiles were generally similar, the symbiotic culture resulted in yogurt with superior texture and viscosity. Zajac *et al.*, (2020) also reported differences in textural parameters, including firmness, consistency, cohesiveness and viscosity, between thermophilic starter cultures YoFlex® YF-LL812 and Delvo® Fresh YS-241, with yogurt produced with Delvo® Fresh YS-241 showing slightly better characteristics. Sensory characteristics significantly influence yogurt acceptability; however, their impact varies among consumers. For the majority (62%), a clear relationship between sensory profile and product liking was observed, whereas for a smaller group (38%) this relationship was not significant. Impact of sensory differences on consumer acceptability of yoghurt and yoghurt-like products. Furthermore, distinct consumer subgroups with different preference patterns were identified, highlighting market heterogeneity and the importance of precise sensory

characterization in product development (Bayarri *et al.*, 2011). Overall, the above findings emphasize that the choice of starter culture significantly affects the sensory properties of yogurt, including aroma, taste, texture and viscosity, which are crucial for the quality and acceptability of the product among consumers.

CONCLUSIONS

The present study demonstrates that the incorporation of *Streptococcus thermophilus* (CNCM I-2980) into starter cultures significantly reduced the fermentation time of probiotic yogurts without compromising their chemical composition or sensory quality. The experimental products containing mixed starter cultures exhibited fat and protein contents comparable to those of standard low-fat yogurt, while lactose and solids-not-fat levels were slightly lower, reflecting enhanced lactose degradation during fermentation. Sensory evaluation further revealed that the addition of this strain improved flavor, aroma, texture, and overall acceptability, with probiotic yogurts rated as “Excellent” compared to the “Very Good” classification of the control yogurt.

These findings confirm that *S. thermophilus* (CNCM I-2980) is an effective technological tool for enhancing yogurt production efficiency while maintaining high product quality. Its application can reduce production costs by shortening fermentation time and may contribute to the development of probiotic dairy products with improved sensory and functional characteristics. The results of this study support the potential implementation of this starter culture in industrial yogurt production, both at the “Srna” Dairy and in other dairy facilities in Montenegro and beyond.

AUTHOR CONTRIBUTIONS

Darko Baćović: organization of tasks in the dairy, conceptualization and writing original draft; Olga Kopitović: Methodology, software writing – review and editing; Miljan Veljić: Software; statistical analysis. Dušica Radonjić: Laboratory methodology, writing; Slavko Mirecki: Conceptualization, review and editing, investigation.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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