Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

2026, **27** (1), pp. 001 – 011

https://doi.org/10.29081/ChIBA.2026.654

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry

ISSN 1582-540X

ORIGINAL RESEARCH PAPER

CHANGES IN THE QUALITATIVE PROPERTIES OF SET YOGURT FOLLOWING THE APPLICATION OF BIOPROTECTIVE CULTURES

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Received: July, 18, 2025
Accepted: March, 04, 2026

Abstract: Sour milk, or "set yogurt" as defined by European terminology, is the most popular fermented dairy product in the region and has enjoyed growing global popularity over time. Access to international markets requires effective product protection, which can be achieved through the use of bioprotective probiotic cultures. This study aimed to investigate whether the addition of bioprotective cultures induces any changes in the qualitative properties of set yogurt initially inoculated with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. The research material consisted of set yogurt samples supplemented with three different bioprotective cultures, along with a reference control. The study evaluated *p*H, fatty acid profiles, microbiological analyses and sensory characteristics, assessed by two independent sensory panels. The sensory attributes analyzed included appearance, aroma, consistency, spoon consistency, mouthfeel, taste/acidity, and aftertaste. Statistical analysis of the results demonstrated that the addition of bioprotective cultures did not significantly affect the qualitative properties of set yogurt during storage period.

Keywords: *fatty acids, pH, probiotics, reference control, sensory analyses*

INTRODUCTION

Set yogurt is one of the oldest and most popular fermented dairy products, with a tradition of consumption worldwide [1]. It is a fermented product with addition of lactic acid bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. During the process of fermentation, bacteria decompose lactose into the lactic acid that causes milk gelation, development of characteristic acid taste and increased bioavailability of certain nutrients. Besides its high nutritional value, set yogurt is well known for its probiotic and functional properties that can contribute to improvement of digestive health and immune system [2]. However, traditional production of set yogurt is facing certain challenges like unwanted micro flora growth, variations in texture and flavor and limited shelf life. Considering these challenges, bioprotective cultures have shown up to attention, not only easing the fermentation, but also provide additional product protection from spoilage and contamination [3]. Bioprotective cultures are specific probiotic and/or lactic acid bacteria with antimicrobial properties, such as the ability to produce bacteriocins, organic acids, or hydrogen peroxide, which can inhibit the growth of pathogenic and spoilage microorganisms.

The use of bioprotective cultures can influence the qualitative properties of set yogurt, including its chemical composition, physicochemical characteristics, texture, aroma, and microbiological stability [4]. These cultures can slow down the degradation of proteins and lipids. Additionally, their use can lead to an increase in bioactive components, such as peptides with antimicrobial activity, antioxidants, and enzymes, which enhance the nutritional value and functionality of the product. As a result, set yogurt produced with bioprotective cultures is favored by consumers due to its beneficial effects on intestinal microflora and its potential to strengthen the immune system [5]. For all these reasons, interest in the use of bioprotective cultures in industrial dairy production is growing, especially due to the increasing demand for natural, "clean label" products (i.e., without added preservatives) [6, 7]. Understanding their influence on set yogurt is crucial for improving product quality and stability, as well as for developing competitive products with an extended shelf life [8]. Incorporating bioprotective cultures in yogurt production offers a promising approach to enhancing product safety and shelf life, while also meeting consumer demand for clean-label products [9]. Furthermore, bioprotective cultures are recognized as a sustainable solution for spoilage prevention and shelf-life extension in the dairy industry, providing both technological advantages and consumer-oriented benefits.

This study aimed to monitor changes in the qualitative properties of set yogurt with the addition of bioprotective cultures, with a special focus on their effects on the physical and chemical content, microbiological stability, and sensory properties of the set yogurt. This research will provide a deeper understanding of the benefits that come from bioprotective culture usage, and it will give directions for further research in this area.

MATERIALS AND METHODS

Chemicals and reagents

The subject of this study was cow's milk set yogurt produced in a dairy facility located in the Pelagonia region. Before production began, the raw cow's milk intended for set yogurt was analyzed. The results indicated average chemical quality, with the following composition: milk fat 2.96 %, protein 3.18 %, lactose 4.55 %, and total solids non-fat 8.43 %. The average microbiological quality of the raw milk was 3,363,000 CFU·mL^{-1} (ISO 21187:2011, Bactocount IBC, Bentley Instruments, Marœuil, France) with a somatic cell count of 463,000 cells·mL^{-1} (ISO 13366-2:2010, Bentley Somacount CC 150, Bentley Instruments, Marœuil, France). Although these counts exceed EU regulatory limits, they represent the actual raw milk quality under the studied conditions and were verified using standardized methods. The production process, in accordance with dairy product specifications, began by heating the cow's milk to 90 °C for 15 seconds, followed by cooling to 42 °C for the addition of the starter and bioprotective cultures. Four different batches were prepared for comparison: one batch was fermented using only a thermophilic starter culture (reference control, Chr. Hansen), while the other three were fermented using the same starter culture in combination with one of three different bioprotective cultures, FQ® 9 (*culture composition: Lactobacillus rhamnosus, Chr. Hansen*), FQ® 10 (culture composition: *Lactobacillus rhamnosus*, Chr. Hansen), or FQ® 11 (culture composition: *Lactobacillus rhamnosus*, Chr. Hansen).
The standard starter culture used for fermentation was a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. After inoculation, the mixture was transferred to a filling machine and dispensed into 400-gram plastic cups, which were then sealed with aluminum lids. The filled and sealed cups were placed in plastic trays, arranged on pallets, and transferred to a warm chamber for fermentation at 40 °C for approximately 4 hours, or until the product reached a *p*H of 4.6 ± 0.1. Upon completion of fermentation, the products were transferred to a cold room at 4 °C and stored for 45 days in a specifically marked area designated for test production.
Quality changes were monitored over time. The study has been considering physicochemical properties (*p*H and fatty acids profile), microbiology analyses, and sensory characteristics.

*p*H

*p*H value has been determined in the following time period: first, 7th, 14th, 21st, 28th, 35th, 37th, 40th, 42nd, and 45th day after production. *p*H value has been determined by a *p*H meter Seven Easy (Mettler Toledo, Switzerland). The instrument has been calibrated by standard buffer solutions with *p*H 4.00 and *p*H 7.00 by immersing the probe into the product sample. Reading was recorded after stabilization of the measured value, usually after few seconds. All measurements were performed in a duplicate and values obtained were expressed as average values in order to provide better accuracy of the results.

Fatty acids profile

The content of saturated fatty acids and the overall fatty acid profile in set yogurt samples was determined on days 7, 21, 35, and 45 after production. All analyses were conducted in accordance with the requirements of MKC EN ISO/IEC 17025:2018. The fatty acid profile was determined using gas chromatography with a flame ionization detector (GC-FID), following the Association of Official Analytical Collaboration AOAC 996.06 method (modified). Separation was achieved using an HP-88 capillary column (J&W 112-8867; 60 m × 250mm × 0.20 mm; Agilent Technologies, USA). The carrier gas was nitrogen at a constant flow rate of 1.0 mL/min. The injector and detector temperatures were set at 250 °C, and identification of individual fatty acids was based on the comparison of retention times with those of standard mixtures.

Microbiology analyses

Microbiological analyses were conducted on all four products on the second day after production, in accordance with the dairy testing plan. The tests included enumeration of yeasts and molds (ISO 21527-1:2008), *Escherichia coli* (ISO 16649-2:2001), and *Enterobacteriaceae* (ISO 21528-2:2017).

Sensory analysis

Samples were evaluated by two sensory panels each one consisting of 8 trained panellists in the following time period: 1-st, 7-th, 14-th, 21-st, 28-th, 35-th, 37-th, 40-th, 42-nd and 45-th day after production.

1. Sensory panel from the dairy company of 8 experienced panellists in dairy products sensory analyses, and
2. Sensory panel of 8 panellists from the Faculty of Biotechnical Sciences with previous training and experience in sensory evaluation within educational and research activities.

Hedonic scale questionnaire has been created for this sensory evaluation. Questions and the scale were adapted to provide simple, yet precise and representative evaluation of the most relevant sensory properties of the set yogurt samples.

The evaluation was performed with three-degree grade scale:

Grade 1 – The product significantly differs from expected qualitative requirement (lowest grade).

Grade 2 – The product partially satisfies qualitative requirements, with small variations.

Grade 3 – The product fully satisfies expected qualitative requirement (highest grade).

Sensory analyses focused on seven key sensory properties: aroma, appearance, consistency (overall visual and texture evaluation), spoon consistency, mouth feel, flavor and acidity, aftertaste.

The products were previously coded and served under identical conditions to avoid any subjective circumstances. Panellists have fulfilled the questioners individually and independently, following the standards for sensory analyses.

After the sensory evaluation, the results were processed statistically. For every individual sensory parameter as well as for every individual product sample, the average value has been calculated to determine the level of product acceptance under panellist's perception.

Statistical analysis

The results were displayed in tables and graphically by using standards statistical descriptive methods: arithmetic mean - $\bar{x}$, standard error (SE) and standard deviation (SD). Statistical significance among tested categories was analyzed at significance level of 5 % ($p<0,05$) by using variance analyses (ANOVA) method with post-hoc Tukey test. The results have been processed by Microsoft Office Excel and statistical software SPSS 20. Additionally, principal component analysis (PCA) [10], was used as a tool for dimensionality reduction and data visualization. It creates an orthogonal coordinate system based on the variations in the original data, arranging the axes according to the total variance. The main criterion for selecting principal components is that they explain more than 95 % of the variance in the experimental data, which ensures that a significant part of the information is preserved. If the covariance matrix is diagonal, the variables are independent, which allows visualization of the data by their mean square error. In the case of a non-diagonal matrix, it can be transformed so that its eigenvectors form the diagonal. This method was used to evaluate the influence of the day of storage on the characteristics of yogurt. Before processing, the data were normalized in the interval [0;1]. Table 1 shows the combinations of days of storage - yogurt type.

Table 1. *Yogurt/ Day combinations table*

Yogurt / Day	REF	FQ10	…	FQy
7	D7REF	D7FQ10	…	D7FQy
21	D21REF	D21FQ10	…	D21FQy
…	…	…	…	…
Dx	DxREF	DxFQ10	…	DxFQy

RESULTS AND DISCUSSION

*p*H

Growth of the lactic acid bacteria in set yogurt causes accumulation of organic acids, mainly lactic acid. As consequence of this increased content of lactic acid in food matrix acidification with *p*H constantly decreasing while concentration of hydrogen ions (H^+) is increasing [11]. During the 45 days storage period, *p*H value was monitored for the set yogurt produced with standard yogurt culture and with three bioprotective cultures (FQ9, FQ10 and FQ11) (Table 2).

The results observed indicate a general trend of gradual *p*H decrease in all tested samples, suggesting prolonged fermentation and increased acidity during storage. On the first day after production, the *p*H values of all samples were similar, with the highest *p*H observed in the sample with FQ10 (4.58), while the reference control had a *p*H of 4.44.

Table 2. *Set yogurt pH change to samples with and without bioprotective cultures during storage*

Days after production	Control	FQ9	FQ10	FQ11
1	4.44^{a}	4.42^{c}	4.58^{a}	4.34^{b}
7	4.44^{a}	4.56^{a}	4.31^{c}	4.37^{a}
14	4.31^{b}	4.46^{b}	4.22^{e}	4.27^{c}
21	4.26^{ef}	4.41^{c}	4.34^{b}	4.21^{d}
28	4.28^{cd}	4.40^{c}	4.19^{f}	4.21^{d}
35	4.27^{de}	4.28^{d}	4.28^{d}	4.20^{d}
37	4.28^{cd}	4.27^{de}	4.27^{d}	4.18^{e}
39	4.27^{de}	4.26^{e}	4.20^{f}	4.18^{e}
42	4.29^{c}	4.27^{de}	4.14^{g}	4.21^{d}
45	4.25^{f}	4.21^{f}	4.09^{h}	4.15^{f}

*Values with different superscripts indicate statistically significant differences ($p < 0.05$)

During the storage period, the reference control and the samples with FQ9 and FQ10 showed a gradual decrease in *p*H values, reaching levels below 4.30 by day 21. In contrast, the sample with FQ11 exhibited a more rapid *p*H decline, reaching 4.27 as early as day 14, and indicating more intensive lactic acid bacteria activity. From day 21 to day 45, all samples showed stabilized *p*H values, with only minor fluctuations. At the end of the storage period (day 45), the lowest *p*H was recorded in the sample with FQ10 (4.09), while the highest was in the reference control (4.25). Differences between samples were statistically significant ($p < 0.05$), as indicated by different superscripts in Table 2.

Fatty acids profile

Milk fat consists of 400 different fatty acids with different chain length, out of which majority are saturated fatty acids (SFA) and less monounsaturated (MUFA) as well as polyunsaturated fatty acids (PUFA). This diversity of fatty acids creates one very complex composition [12, 13]. Cow's milk fat containes approximately 70 % SFAs, 25 % MUFAs, and 2-5 % PUFAs [14]. Figure 1 shows the influence of the day of storage on the fat composition of yogurt SFA. The two principal components used together describe over 95 % of the variance in the experimental data.

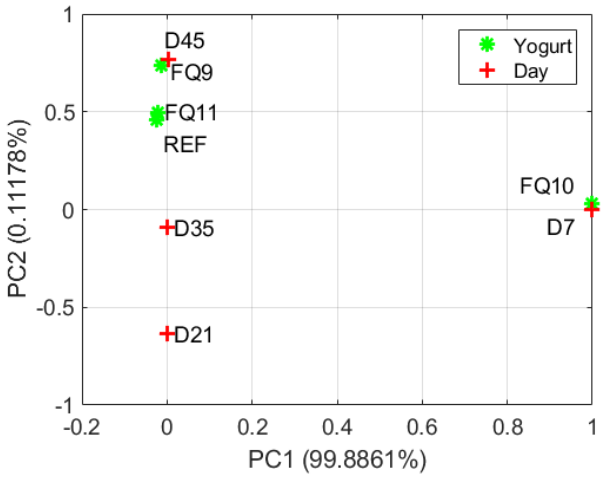

Figure 1. *Effect of storage time on the saturated fatty acid (SFA) composition of yogurt samples with and without bioprotective starter cultures*

It can be seen that on day 7, there are differences compared to the other samples in FQ10. During the remaining days of storage, no significant differences are observed. The largest change is observed on day 45 for all samples.

Figure 2 shows the influence of the day of storage on the fat composition of yogurt MUFA. The two principal components used together describe over 95 % of the variance in the experimental data. It can be seen that in the initial days of storage there are no significant changes in the fat composition of yogurt. Such changes are observed on day 35 for samples FQ9 and FQ10. On day 45, significant changes are observed in samples REF and FQ11.

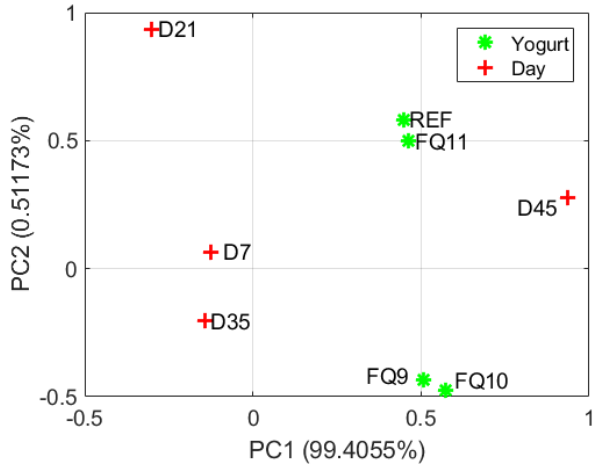

Figure 2*. Effect of storage time on the monounsaturated fatty acids (MUFA) composition of yogurt samples with and without bioprotective starter cultures*

Figure 3 shows the influence of the day of storage on the fat composition of yogurt PUFA. The two principal components used, together describe over 95 % of the variance in the experimental data. It can be seen that on day 7, differences in the amounts of fat composition compared to the other samples are observed in FQ9. On day 21, changes occur in REF and FQ10. On day 35, changes are observed for FQ11. On day 45, there is no change in the samples and they retain their characteristics.

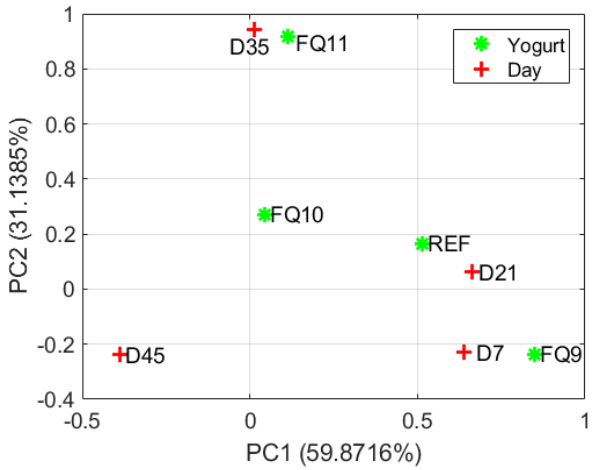

***Figure 3**. Effect of storage time on the polyunsaturated fatty acids (PUFA) composition of yogurt samples with and without bioprotective starter cultures*

Table 3 shows distribution of fatty acids according to their classification to saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). In (SFA), reference control shows higher values (5.404 %) compared to samples with bioprotective cultures. Additionally, set yogurt produced with bioprotective cultures FQ9 (5.393 %), FQ10 (5.398 %), and FQ11 (5.400 %) shows almost identical content, but it is still lower than reference control. This indicates that bioprotective cultures didn't cause significant changes in SFA compared to reference control. On the other hand, monounsaturated fatty acids (MUFA) at the reference control are 4.466 %, while set yogurt with bioprotective cultures FQ9, FQ10 and FQ11 show slight values increase (4.471 % to 4.473 %). Even if the differences are minimal, it can indicate to mild influence of bioprotective cultures in improvement of fatty acids profile. Additionally, (PUFA) in reference control is 0.735 %, while FQ10 and FQ11 show mild increase to 0.744 % and 0.741 %. FQ9 is similar to reference control (0.730 %).

***Table 3.** Content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (%) in set yogurt with and without bioprotective cultures*

Group fatty acids	Reference control [%]	SE	FQ9 [%]	SE	FQ10 [%[	SE	FQ11 [%]	SE
SFA	5.404[a]	0.782	5.393[a]	0.782	5.398[a]	0.783	5.400[a]	0.782
MUFA	4.466[a]	1.152	4.471[a]	1.152	4.473[a]	1.153	4.471[a]	1.152
PUFA	0.735[b]	1.411	0.730[b]	1.410	0.744[b]	1.412	0.741[b]	1.410

*Values with different superscripts indicate statistically significant differences in the same row ($p < 0.05$),
SE – Standard Error

Sensory analysis

Food quality, especially in dairy products, is not defined only through its chemical content or microbiological safety, but more and more is based on consumer's perception. Sensory properties - as flavor, texture, aroma and mouth feel sensation are playing the major role in acceptance and repeated buying of the product. These sensory properties are among the most important factors with influence on the consumer's decision, and their

understanding and objective measurement is of significant meaning for maintaining and improvement of the dairy products quality [15]. Table 4 presents the individual summary scores for each test product, as evaluated by the sensory panel from the Faculty of Biotechnical Sciences.

Table 4. *Results of the sensory evaluation of set yogurt conducted by the panel from the Faculty of Biotechnical Sciences*

Sample	After taste	Flavor	Mouth feel	Consistency	Spoon consistency	Appearance	Aroma
Reference	2.46	2.44^{ab}	2.33	2.40	2.29^{b}	2.00^{b}	2.43^{ab}
FQ 9	2.51	2.59^{a}	2.44	2.54	2.55^{a}	2.24^{a}	2.47^{a}
FQ 10	2.36	2.48^{ab}	2.28	2.43	2.53^{a}	2.19^{a}	2.42^{ab}
FQ 11	2.39	2.34^{b}	2.29	2.38	2.33^{b}	2.09^{ab}	2.29^{b}

*Values with different superscripts indicate statistically significant differences in the same column ($p < 0.05$)

Statistically significant differences ($p < 0.05$) were observed in specific sensory attributes, including flavor, spoon consistency, appearance, and aroma. Among all samples, the product containing the bioprotective culture FQ9 received the highest scores across all evaluated sensory attributes.

Table 5 presents the summary sensory scores for all test products as evaluated by a trained dairy sensory panel. Statistically significant differences ($p < 0.05$) were observed only for the attribute “consistency”. Interestingly, this panel favored different products for different sensory attributes: FQ10 received the highest scores for appearance and aftertaste; FQ11 was best rated for spoon consistency, mouthfeel, and consistency; while the reference control achieved the highest score for flavour.

Table 5. *Results of the sensory evaluation of set yogurt conducted by the panel from the Dairy plant*

Sample	After taste	Flavor	Mouth feel	Consistency	Spoon consistency	Appearance	Aroma
Reference	2.75	2.80	2.80	2.70^{b}	2.83	2.05	2.68
FQ 9	2.65	2.60	2.68	2.79^{ab}	2.85	2.18	2.53
FQ 10	2.78	2.63	2.88	2.85^{ab}	2.88	2.45	2.70
FQ 11	2.63	2.58	2.90	2.93^{a}	2.95	1.95	2.58

*Values with different superscripts indicate statistically significant differences in the same column ($p < 0.05$)

When comparing the results from both sensory panels (Tables 4 and 5), it becomes evident that no single test product was superior across all evaluated sensory attributes. This diversity in panel preferences highlights a balanced sensory performance among all yogurt samples. Notably, products containing bioprotective cultures consistently performed well and, in many cases, outperformed the control sample. This supports the conclusion that the use of bioprotective cultures does not negatively impact the qualitative characteristics of set yogurt. Although the reference control received the highest score for flavour (2.80), the scores of the bioprotective culture samples were comparable: FQ9 - 2.60, FQ10 - 2.63, and FQ11 - 2.58, indicating that the addition of bioprotective cultures did not significantly compromise flavour quality.

Microbiological analysis

Microbiological analyses conducted on all four products on the second day after production revealed no detectable counts of yeasts and molds, *Escherichia coli*, or *Enterobacteriaceae*. These findings indicate compliance with hygienic standards and reflect good sanitary conditions during dairy processing.

CONCLUSIONS

The incorporation of bioprotective cultures, particularly *Lactobacillus rhamnosus*, offers dual advantages - extending shelf life and providing probiotic benefits without compromising product quality. Throughout the 45-day storage period, no significant changes were observed in the fatty acid profile or pH trends between the control and test samples. Although a gradual *p*H decline occurred in all samples, it remained within acceptable limits. Sensory analysis also revealed no consistent differences, with all products maintaining favorable attributes. These findings confirm that the use of bioprotective cultures does not negatively affect the qualitative properties of set yogurt, even beyond its regular shelf life. Their application represents a practical and natural strategy for enhancing the stability and safety of industrial yogurt production. Further research is recommended to explore the relationship between the dosage of bioprotective cultures and post-acidification behavior.

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