Influence of YoFlex® Premium 5 starter culture on fermentation process and yogurt quality

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Abstract

Introduction. The aim of this study was to investigate the multifunctional properties of the novel yogurt starter culture YoFlex® Premium 5 for its potential application in the production of yogurts with extended shelf life.

Materials and methods. Milk bases were fermented using the yogurt starter cultures YC-X16 and YoFlex® Premium 5. Sample viscosity was measured using a Kinexus Pro+ rotational rheometer; the degree of structural recovery was determined by a calculation method; active acidity was assessed by the potentiometric method; and sensoryeptic properties were evaluated by sensory profiling.

Results and discussion. The YoFlex® Premium 5 yogurt starter culture, compared to YC-X16, halves fermentation time without a pronounced lag phase and fully stabilizes yogurt acidity during the first 7 days of storage. Beyond this period, pH changes become more pronounced, indicating the need for targeted technological measures to limit acidification of the milk-based medium. Yogurt produced with YoFlex® Premium 5 approaches the effective viscosity of the most structured yogurt obtained using YC-X16 on a milk base fortified with 1% skimmed milk powder. Exopolysaccharides produced by YoFlex® Premium 5 not only increase yogurt viscosity but also markedly enhance its thixotropic properties and moisture retention, contributing to a smoother texture and improved mouthfeel. Specifically, the degree of structural recovery of disrupted clots rises from 18.6% in the control and 30% in the YC-X16 + 1% skimmed milk powder sample to 42% in the YoFlex® Premium 5 sample, demonstrating its superior gel-forming ability. Therefore, combining the structuring effect of skimmed milk powder with the thixotropic benefits of exopolysaccharides from YoFlex® Premium 5 is recommended, particularly for stirred yogurt production, where consistency and stability are critical. Furthermore, achieving excellent quality in fresh yogurts fermented with YoFlex® Premium 5 requires additional stabilization during storage for up to 14 days or longer. The use of protective cultures may help maintain yogurt quality over extended storage, representing a promising direction for future research. These findings highlight the potential of YoFlex® Premium 5 to improve both the technological efficiency and sensory attributes of yogurt, making it a valuable tool for industrial applications.

Conclusions. The application of the YoFlex® Premium 5 starter culture in yogurt production shortens fermentation time, improves viscosity and thixotropic properties, and yields an excellent sensory profile, but requires additional measures to stabilize yogurt quality during storage.

Introduction

Yogurt is a widely consumed fermented milk product with proven functional effects on human health (Munteanu-Ichim et al., 2024). Preventing the growth of undesirable microorganisms is important for ensuring yogurt quality and safety and for extending its shelf life (Wang et al., 2025). Although yogurt is generally considered microbiologically stable (Garnier et al., 2017), there has recently been growing interest in its biological preservation using selected microorganisms and their metabolites (Chaves et al., 2021; Siedler et al., 2020). These approaches act mainly through pH reduction, competition for nutrients, and the production of bacteriocins. The use of probiotic lactic acid bacteria strains helps maintain fermentation stability and improves the functional properties of the final product (Nielsen et al., 2021; Prócel et al., 2025; Souza et al., 2023; Stabnikov et al., 2025).

Lactic acid bacteria can perform several additional technological functions in yogurt production. In particular, a significant increase in yogurt viscosity was observed when using Chr. Hansen YoFlex® Premium 1 cultures compared with YF-L901 and YF-L701 cultures, including under conditions with the addition of 1–4% nonfat dry milk (Wang et al., 2023). The possibility of reducing the required amount of nonfat dry milk by 1–2% has been demonstrated due to the structuring effect of exopolysaccharides (EPS) produced as metabolites of bacterial starter cultures (Chaharovskyi and Lukashchuk, 2024). EPS have been shown to improve the sensory properties and stability of yogurt, even at low concentrations (Buldo et al., 2016). Their ability to interact with water molecules and milk proteins enhances the viscosity and mechanical strength of the acid gel while reducing syneresis. In particular, co-fermentation of commercial starter cultures with the probiotic EPS-producing strain *Lactiplantibacillus plantarum* MC5 in compound starter systems positively affected the texture, rheological behavior, and storage stability of yogurt (Zhao et al., 2022). The specific technological functionality of EPS is largely determined by the structure and molecular weight of the biopolymer macromolecules. These molecular differences govern the specific interactions between exopolysaccharides and the protein matrix, resulting in increased viscosity and/or elasticity (thixotropic behavior) of yogurt (Brüls et al., 2023). The use of EPS-producing strains in low-fat and non-fat yogurt has been shown to reduce the need for stabilizers and to minimize post-fermentation mechanical stress on the curd when skim milk powder is incorporated (Zhang et al., 2016). The unique technological role of EPS depends on the structure and molecular weight of the biopolymer macromolecules. These molecular-level differences determine the specificity of EPS interactions with the protein matrix, which can result in increased viscosity and/or elasticity (thixotropy) of yogurt (Brüls et al., 2023). It has been proven that the use of EPS-producing strains in non-fat and low-fat yogurts reduces the need for stabilizers and minimizes the post-fermentation mechanical impact on the curd due to the addition of skim milk powder (Zhang et al., 2016). Therefore, the selection of EPS strains of lactic acid bacteria for use in yogurt production should be based on a preliminary study of the specifics of the influence of each commercial starter culture on the characteristics of the finished product. EPS production, dynamics of acidity changes, consistency and stability of yogurt during storage significantly depend on the bacterial composition of the starter, in particular, on the ratio between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Ivashchenko et al., 2023). A high proportion of *Streptococcus thermophilus* increases the abundance of EPS-producing strains, improves curd structure, but may alter flavor and increase acidity (Lange et al., 2020). Therefore, it is recommended for yogurt culture developers to test the specified ratio in the range of 1:1 to 1:4 by evaluating the fermentation profile and sensory parameters of the yogurt (Dan et al., 2023). EPS also have bioactive properties, including prebiotic, immunomodulatory, and antioxidant properties (Wang et al., 2025). Thus, research and innovation in the biosynthesis and application of EPS contribute to the further development of functional dairy product technologies (Ouarabi et al., 2025).

A new generation of yogurt starter cultures has recently emerged, including YoFlex® Premium 5 (Chr. Hansen). This culture can increase yogurt viscosity even at lower protein levels and reduce the need for stabilizers and skimmed milk powder. It also exhibits reduced post-acidification and positively affects product quality during storage. However, the specific functional and technological properties of YoFlex® Premium 5 require further detailed and comprehensive investigation, which underlines the relevance of the present study.

Materials and methods

Materials

Raw milk that meets regulatory requirements was used to produce the yogurt test samples. As a source of dry nonfat milk solids, skimmed milk powder was used with a fat content of 1.5% and a protein content of 32% (Rybak et al., 2014). Standardized milk samples were fermented by technologically efficient direct vat set cultures from Chr. Hansen: (a) YC-X16 – classic starter culture (*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*); (b) YoFlex® Premium 5 – new culture that produces EPS during fermentation (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*).

Preparation of test samples

Milk mixtures were standardized for fat content (2.5%) and nonfat dry milk solids (NFMS) content (9.5%), were pasteurized and cooled to a temperature of 40-43 °C and were inoculated by direct vat set cultures. Three samples with YC-X16 culture were additionally fortified with skim milk powder (SMP) in an amount of 0.5 to 1.0%. Fermentation of milk mixtures was carried out at the specified temperature until the acidity values reached not higher than pH 4.8.

The designations of samples are given below:

- control (YC-X16);
- sample 1 (YC-X16 with 0.5% SMP);
- sample 2 (YC-X16 with 0.75% SMP);
- sample 3 (YC-X16 with 1.0% SMP);
- sample 4 (YoFlex® Premium 5).

Research procedure

The samples were prepared according to the technological scheme of stirred yogurt production. The pH of samples after inoculation of yogurt cultures was determined every 2 hours of the fermentation process for 8 hours. After fermentation, the samples were stirred, cooled to a temperature of 4±2 ºC, and kept under these conditions for at least 6 hours. After that, the pH of the samples and syneresis were determined and these indicators continued to be recorded during yogurt storage at the specified temperature conditions after 7, 14, and 28 days. The effective viscosity and thixotropic ability of the samples were determined in samples on the 14th day of storage, in accordance with the usual yogurt shelf life. The quality level of the samples according to the complex of sensory indicators was determined in fresh yogurt samples and samples after 14-day storage.

Methods

Active acidity was assessed by the potentiometric method by lab pH-meter pH/mV/Temp Knick Portamess® 911.

The viscosity of yogurt samples was measured on the 14th day of storage at 4±2 °C. Measurements were carried out using a Kinexus Pro+ rotational rheometer (Malvern Instruments Ltd, United Kingdom) with top geometry C25 DIN L0142 SS (cylinder) and bottom geometry PC25 DIN C0350 AL. Before measurement, samples were gently stirred for 30 seconds and brought to a temperature of 10 °C. The effective viscosity of the yogurt samples was determined during both forward and reverse shear sweeps, with the shear rate increasing from 0.1 to 400 s^{-1} and then decreasing from 400 to 0.1 s^{-1}.

The degree of restoration of the structure of yogurt samples was calculated from the value of the effective viscosity at the end of the measurement at a shear rate gradient of γ = 0.1 s^{-1} (reverse stroke), taking the effective viscosity of the practically undestroyed structure at the beginning of the measurement as 100% (γ = 0.1 s^{-1}) (Liang et al., 2022).

Syneresis of yogurt samples was calculated using a modified method described by Arab et al. (2023). For this purpose, yogurt samples cooled to 4 °C were placed in graduated centrifuge tubes and centrifuged at 350×g (model K-24; Sigma Laborzentrifugen GmbH, Germany) for 10 minutes. The clear supernatant was collected and weighed. The syneresis of the yogurt samples was calculated using the equation:

$$\text{Syneresis}(\%) = \frac{\text{weight of supernatant (g)}}{\text{weight of yogurt sample (g)}} \times 100\%$$

Sensory properties of yogurt samples were assessed by sensory profiling on a twenty-five-point descriptive scale according to method (ISO 13299:2016) for sensory attributes: appearance, texture, color, taste, and flavor. The scores obtained for each of the 5 characteristics were recalculated taking into account the weighting factors: taste – 0.3, texture – 0.3, flavor – 0.2, appearance – 0.1, color – 0.1. The quality level of each yogurt sample was assessed according to the following differentiation: 21-25 points excellent; 16-20.9 points – good; 11-15.9 points – satisfactory; 6-10.9 points – poor (practically unacceptable); less than 6 points – very poor (unacceptable).

Statistical analysis

The studies were performed in 3 or more replicates, and the results were expressed as mean±standard deviation. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey HSD tests using the SPSS statistical package. Statistical difference was defined at $p \leq 0.05$.

Results and discussion

Direct vat set cultures activity during milk samples fermentation

The changes in the active acidity of samples during fermentation lasting up to 8 hours is shown in Fig. 1. The end of the fermentation process was recorded by the time of reaching the active acidity pH = 4.8.

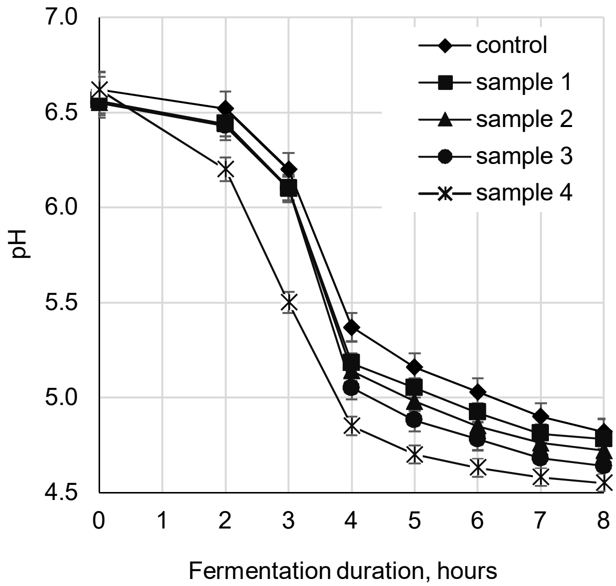


Figure 1. Changes in active acidity during fermentation of yogurt samples

Although all samples had nearly identical initial active acidity (Fig. 1), the pattern of pH changes during fermentation differed. In particular, the control sample with the YC-X16 culture exhibited the longest lag phase and a relatively moderate decrease in pH, reaching a value of 4.8 only at the 8th hour of fermentation. In contrast, sample 4 with the YoFlex® Premium 5 starter culture showed almost no lag phase, reaching a pH of approximately 4.8 by the 4th hour, indicating the high activity and technological advantage of this new culture.

Samples 1-3, containing skimmed milk powder, are close to the control sample in terms of the change in active acidity at the beginning of fermentation, but over time, they show an intermediate value between the control sample and sample 4, and their fermentation process takes place within 6-7 hours. The dynamics of fermentation of samples 1-3 becomes more noticeable with an increase in the content of skimmed milk powder in them from 0.5 to 1.0%, which changes not only the texture, but also affects the kinetics of fermentation. This effect can be explained by a change in the composition, buffer capacity and nutritional content of the medium (Honesová et al., 2024, Karam et al., 2013).

Changes in the active acidity of yogurt samples were also monitored during storage for up to 28 days (Fig. 2).

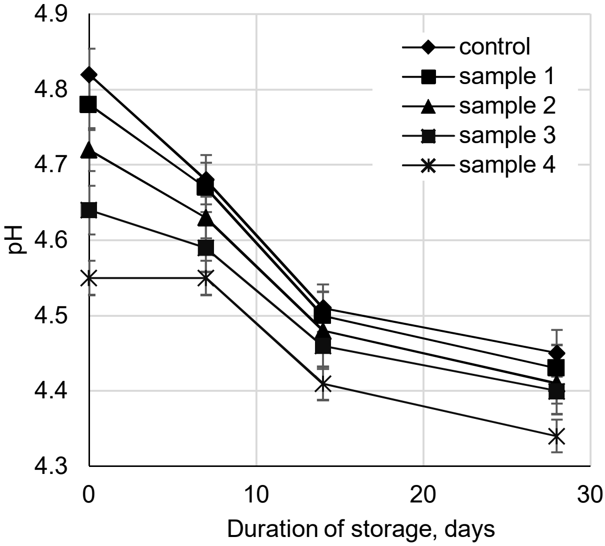


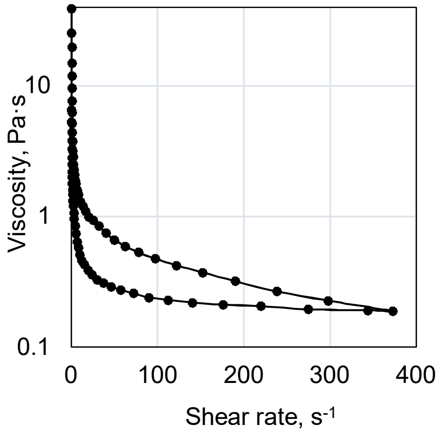
Figure 2. Changes in active acidity during storage of yogurt samples

After fermentation, post-acidification during storage was more pronounced in the control sample and less in samples 1 and 2, due to the slower accumulation of lactic acid. Active acidity decreased by 0.3 (control), 0.28 (sample 1), and 0.24 (sample 2) pH units during the first 14 days of storage. Continued storage from day 14 to 28 showed a slowdown in the decline of lactic acid bacteria activity, likely due to lactic acid accumulation, which increases intracellular acidity (Béal et al., 1999).

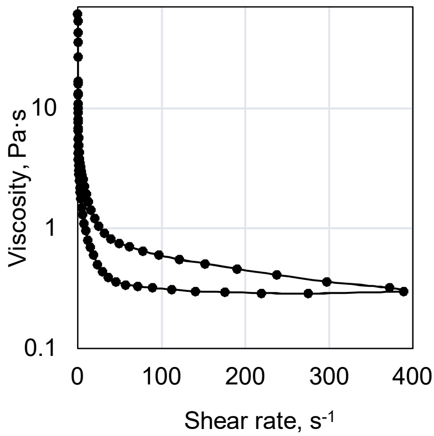
Sample 3 exhibited three stages of pH change: during the first 7 days, acid formation was somewhat inhibited; from day 7 to 14, lactic acid accumulated more actively; and from day 14 to 28, this process slowed again. In contrast, sample 4, with the highly active YoFlex® Premium 5 starter, showed complete stabilization of yogurt acidity during the first 7 days, after which the pH trend resembled that of sample 3. This indicates that this starter requires a modified technological approach to limit acidification during storage beyond 7 days, for example, by reducing the content of available substrates (Deshwal et al., 2021). Nevertheless, the active acidity of all samples remained within the acceptable range for yogurt ($4.0 \leq pH \leq 4.5$) throughout storage (Kang et al., 2019).

Viscosity and syneresis of yogurt samples

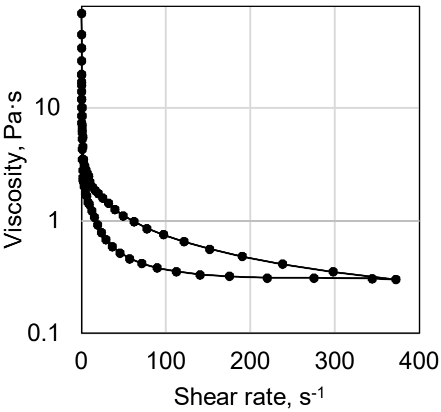
Changes in the effective viscosity of yogurt samples, measured by rotational viscometry with logarithmic scales on both axes, are shown in Fig. 3. Measurements were performed in both forward (0.1 s^{-1} to 400 s^{-1}) and reverse (400 s^{-1} to 0.1 s^{-1}) shear rates to provide a comprehensive assessment of the samples' rheological properties.



a



b



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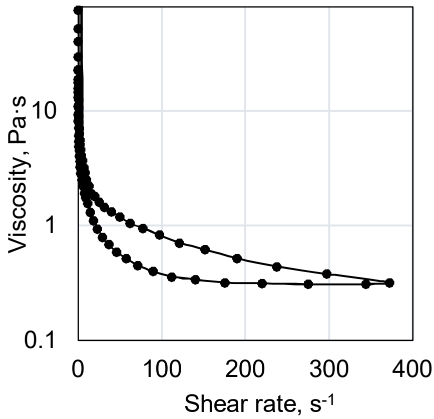

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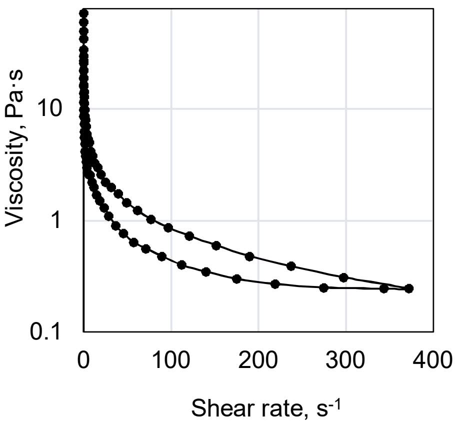

e

Figure 3. Changes in the effective viscosity of yogurt samples during rheometric measurement:
a, control;
b, sample 1;
c, sample 2;
d, sample 3;
e, sample 4

From Figure 3, it is obvious that the significant effect of skimmed milk powder on the effective viscosity of yogurt samples with a practically undestroyed texture at a shear rate of γ=0.1 s^{-1} is observed. Compared with the control (η=35.1132 Pa·s), the effective viscosity of samples 1-3 increased by 1.7-2.1 times with an increase in the content of skimmed milk powder from 0.5 to 1.0%**.** This effect can be explained by the increase in protein content in the milk base, which strengthens the protein matrix of the curd (Isleten et al., 2006) and, consequently, increases the viscosity of the yogurt. Sample 4 shows slightly lower, but similar, viscosity characteristics to sample 3. It can be assumed that this is a consequence of the lower bond energy formed by EPS compared to milk proteins.

While casein gels at pH ≈ 4.6 form energetically stable and practically irreversible clots, with hydrophobic interactions between proteins estimated in the range of 30–80 kJ/mol (De Kruif, 2012), polysaccharide gels, EPS systems, and protein–polysaccharide complexes are dominated by lower-energy hydrogen and hydration bonds (Everett et al., 2005). As a result, the most structured texture was observed in sample 3, which contained 1% skimmed milk powder. Sample 4, produced by fermenting milk with the YoFlex® Premium 5 culture, exhibited a similar textural characteristic.

To study the thixotropic behavior of the yogurt samples, the degree of recovery of the nearly destroyed structure at γ = 400 s^{-1} was calculated during the reverse measurement at a minimum shear rate of γ = 0.1 s^{-1}, and compared with the initial values of effective viscosity (Fig. 4).

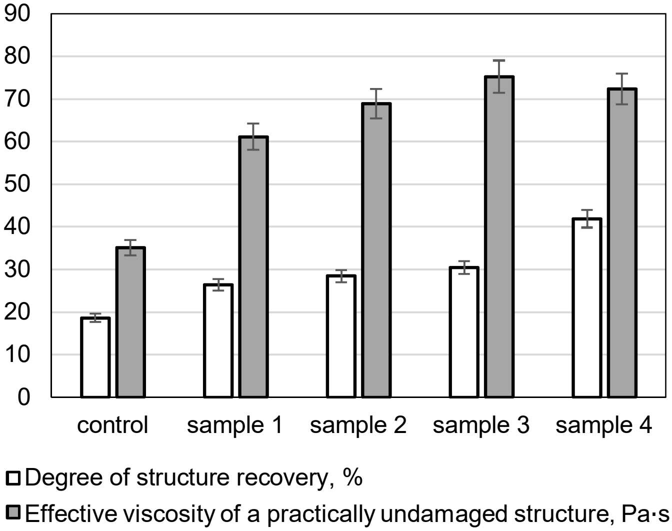

Figure 4. Comparative assessment of the initial values of effective viscosity and thixotropic ability of yogurt samples

As shown in Fig. 4, samples 1–3 containing skimmed milk powder exhibited higher textural integrity and a greater ability to recover the structure at reduced shear rates compared with the control. Data on the effect of skimmed milk powder on yogurt thixotropy are limited, but it is known that increasing the content of nonfat milk solids (NFMS) enhances the viscosity modulus and strength of the protein gel (Honesová et al., 2024; Karam et al., 2013), thereby improving its recovery ability.

As for sample 4, obtained by fermentation of the YoFlex® Premium5 culture, it should be noted its slightly lower effective viscosity at the beginning of the rheometric measurement, compared to sample 3 (72.3105 versus 75.2701 Pa·s). However, the thixotropic ability of sample 4 significantly exceeds the degree of recovery of the effective viscosity of the destroyed texture of all other samples and reaches a rather high value – 41.92%. It can be assumed that EPS form additional low-energy bonds in the structure of protein gel, which have the ability to restore the formed matrix more quickly after destruction (Akin et al., 2009). A similar effect was observed by other scientists (Jurášková et al., 2022; Zheng et al., 2013), who found the formation of a more elastic and rapidly recovering protein gel in the presence of EPS, compared to the protein clot as a control.

In the information environment, there is no clear and unambiguous classification of yogurts according to the levels of "low / medium / high thixotropy" (i.e., by threshold values). Some studies indicate that after a strong shear, the restoration of the structure of yogurts is possible in the range of 20-30% to 40-50% (Chen et al., 2024; Lee et al., 2010). Therefore, it was decided to apply the following criteria to assess the level of thixotropic ability of yogurt by the degree of structure restoration:

- more than 70% - high thixotropy (the texture is almost completely restored after destruction);
- 30-70% - medium thixotropy (partial restoration of the texture);
- less than 30% - low thixotropy (the system is almost not restored).

According to the proposed classification, only samples 3 and 4 show average thixotropy due to the increased content (1%) of skimmed milk powder and in the presence of EPS produced by the YoFlex® Premium 5 culture.

Thus, milk proteins in the composition of skimmed milk powder show high texturing ability, and EPS more effectively improve the thixotropic properties of the gel. Therefore, it is possible to predict a possible synergistic interaction between skimmed milk powder and EPS to combine their positive effects on the textural and mechanical characteristics of the yogurt, in particular to increase its thixotropic ability. This assumption will be tested in further studies.

The synergetic capacity of yogurt samples during storage was also determined (table), taking into account the possibility of modification of the yogurt microstructure by exopolysaccharides produced by lactic acid bacteria (Arab et al., 2023).

Table Syneresis of yogurt samples during storage, %

Sample	Storage duration, days			
	0	7	14	28
Control	$15.90^{a}\pm0.71$	$14.36^{a}\pm0.68$	$14.21^{a}\pm0.60$	$14.32^{a}\pm0.68$
Sample 1	$14.96^{ab}\pm0.52$	$13.73^{ab}\pm0.57$	$13.11^{ab}\pm0.55$	$12.99^{ab}\pm0.65$
Sample 2	$12.83^{b}\pm0.61$	$11.29^{b}\pm0.48$	$10.69^{b}\pm0.49$	$11.26^{b}\pm0.43$
Sample 3	$9.82^{c}\pm0.41$	$9.19^{c}\pm0.44$	$9.22^{c}\pm0.42$	$9.31^{c}\pm0.40$
Sample 4	$9.01^{cd}\pm0.46$	$8.36^{cd}\pm0.39$	$8.27^{d}\pm0.38$	$8.11^{d}\pm0.32$

Note: Values are the means±standard deviation. Means within the same column with different superscripts are significantly different at $p \leq 0.05$

Syneresis was highest in all fresh yogurt samples, which can be attributed to the large number of temporarily formed low-energy bonds capable of causing substantial reconfigurations in the protein gel structure (Brüls et al., 2024). Syneresis decreased during storage, but the variability was minimal, and no clear trends were observed. Therefore, further studies of this parameter will be conducted on a larger set of fermented samples, including those combined with skimmed milk powder (SMP).

It should also be noted that no statistical differences ($p > 0.05$) were observed for the control sample and sample 1 throughout the storage period, indicating that 0.5% SMP had little effect on yogurt syneresis. In contrast, sample 4, fermented with the YoFlex® Premium 5 culture, showed the lowest whey separation, reflecting the high moisture-holding capacity of EPS and associated modifications in the yogurt microstructure (Han et al., 2026). These results confirm the technological effectiveness of the YoFlex® Premium 5 starter culture.

Sensory evaluation of yogurt samples

The results of the sensory evaluation of yogurt samples, carried out by sensory profiling according to the main sensory attributes (appearance, texture, color, taste, and flavor) with subsequent calculation of the total weighted score taking into account the weighting coefficients, are shown in Fig. 5.

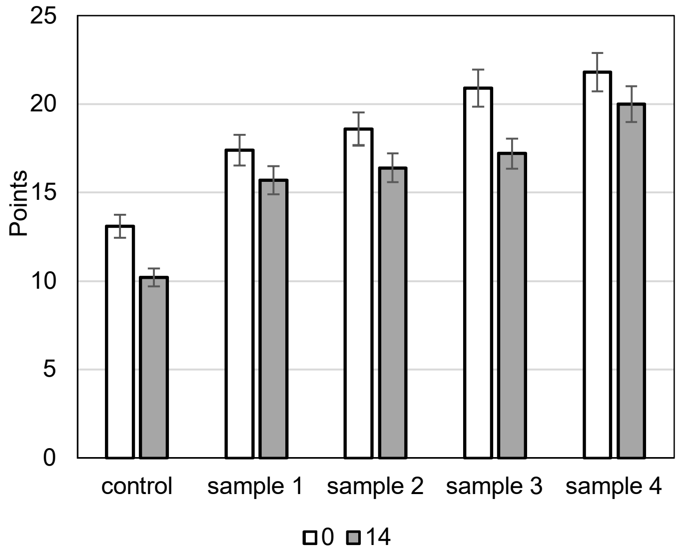

Figure 5. Sensory evaluation of yogurt samples according to a set of quality indicators immediately after production and after 14 days of storage

Based on the data obtained, fresh samples 3 and 4 exhibited excellent quality. During storage up to 14 days, some sensory characteristics (taste, flavor) require improvement, which remains an important task for further research.

Overall, considering the evaluated quality indicators, samples 3 and 4 showed the highest performance, highlighting the technological impact of 1% skimmed milk powder (sample 3) and EPS produced by the YoFlex® Premium 5 culture (sample 4). The use of this new starter culture allows a significant reduction in fermentation time and prevents post-acidification during the first 7 days of storage. Skimmed milk powder effectively structures the yogurt gel but increases production costs compared with yogurt made using YoFlex®

Premium 5. The thixotropic ability of yogurt containing 1% skimmed milk powder (lower dosages are ineffective) was rated as moderate to low, whereas the use of YoFlex® Premium 5 increased the degree of gel recovery to 42%. Therefore, a rational approach for future research may be to combine the functional and technological benefits of the YoFlex® Premium 5 starter culture with varying amounts of skimmed milk powder.

Conclusions

The YoFlex® Premium 5 yogurt starter culture exhibits higher activity than YC-X16, significantly shortening fermentation without a pronounced lag phase and stabilizing yogurt acidity during the first 7 days of storage. Skimmed milk powder at 1% increases yogurt viscosity through protein matrix formation, while exopolysaccharides improve thixotropy, increasing gel recovery from 30% to 42% and reducing syneresis. Combining YoFlex® Premium 5 with skimmed milk powder may further enhance functional and technological properties. Fresh samples with skimmed milk powder or YoFlex® Premium 5 showed excellent sensory quality, but additional stabilization is needed during storage beyond 14 days. Future research should explore synergies between skimmed milk powder and YoFlex® cultures to achieve high viscosity, thixotropy, and stable quality over extended storage using bioprotective cultures.

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