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ORIGINAL RESEARCH PAPER

IMPACT OF STORAGE TEMPERATURE ON THE SHELF LIFE AND SENSORY QUALITY OF SET YOGURT WITH BIOPROTECTIVE CULTURE

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Abstract: Bioprotective cultures like *Lactobacillus rhamnosus* naturally inhibit spoilage fungi through nutrient competition and antifungal metabolite production, offering a clean-label alternative to synthetic preservatives. This study investigated the impact of bioprotective cultures on set yogurt quality under storage temperatures of 5, 10, and 25 ºC. Physicochemical and sensory parameters were monitored over storage. The *p*H decreased gradually across all temperatures. Titratable acidity (°SH) increased over time, with higher values observed at elevated temperatures. Sensory analysis revealed higher overall acceptance (SE = 8) at lower temperatures during early storage. Statistical models demonstrated that temperature influenced more than 70 % of the variations in *p*H, ºSH, and sensory acceptance, with coefficients of determination (R^2) ranging from 0.72 to 0.85. Yogurt with FQ9 stored at 10 ºC exhibited an extended shelf life of up to 40 days. This study highlights the effectiveness of bioprotective cultures in improving yogurt shelf life and quality while meeting clean-label demands, offering sustainable solutions for the dairy industry.

Keywords: *Lactobacillus rhamnosus, FQ9, quality, set yogurt, shelf life, storage temperature*

INTRODUCTION

Yogurt is a widely consumed fermented milk product with well-documented probiotic benefits [1, 2]. However, the dairy industry faces significant challenges due to contamination by yeast and molds, which are naturally present in the environment and can lead to product spoilage. Preventing dairy food waste necessitates a multifaceted approach, including reducing microbial contamination, applying advanced processing technologies, utilizing biocontrol strategies, implementing proactive monitoring, and employing data-driven decision tools [3]. Spoilage in yogurt and cultured dairy products is primarily caused by fungi, including yeasts and molds like *Torulaspora delbrueckii*, *Clavispora lusitaniae*, and *Penicillium* spp. These fungi thrive in low *p*H and temperature conditions, leading to off-odors, off-flavors, and body defects [4, 5]. This microbial activity can significantly impact the sensory qualities and shelf life of dairy products, making it imperative to address contamination effectively. Consumers, on the other hand, demand safe, natural products and seek to extend shelf life without compromising on a "clean label." Clean label foods are recognized for containing natural ingredients, undergoing minimal chemical processing, and excluding artificial additives. These products align with values such as sustainability, fair trade, and environmental responsibility [6, 7]. The clean label trend reflects a growing consumer awareness and preference for transparency and health-conscious choices in food production.

Bioprotective cultures, which are live microorganisms added to foods, offer a promising solution to control microbial growth without altering the technological and sensory qualities of the products. They act through mechanisms such as displacement, competition for nutrients, and production of metabolites [8]. These cultures are particularly common in dairy products, especially those using lactic acid bacteria like *Lactobacillus paracasei* and *Lactobacillus rhamnosus*. These strains have demonstrated effectiveness in inhibiting spoilage fungi in yogurt by competing for essential nutrients, specifically the depletion of the essential trace element manganese, as a key factor in preventing fungal growth [9, 10]. The manganese transporter gene (mntH1) was identified as crucial for this bioactivity, with its expression varying by strain and environmental factors. This mechanism offers a natural and effective alternative to artificial preservatives, aligning with consumer demand for healthier food preservation methods [10]. Bioprotective cultures produce various antifungal metabolites, including organic acids, fatty acids, cyclopeptides, reuterin, hydrogen peroxide, and volatile compounds like diacetyl. These compounds are essential for inhibiting spoilage microorganisms and enhancing the shelf life and safety of dairy products [11, 12]. The production of these metabolites is the most studied mechanism of action of bioprotective cultures, highlighting their critical role in modern food preservation strategies.

Furthermore, the development and application of bioprotective cultures in dairy processing align with the industry's goals of maintaining product quality while adhering to consumer demands for natural and sustainable food products. By leveraging these cultures, the dairy industry can effectively reduce spoilage, extend shelf life, and meet the growing market demand for clean label products. This approach not only addresses the technical challenges of spoilage but also supports the economic and environmental goals of sustainable food production. This study investigated the impact of

incorporating bioprotective cultures in the industrial production of set cow yogurts, focusing on maintaining quality across different storage temperatures (5 °C, 10 °C, and 25 °C). The research monitored physicochemical and sensory changes throughout the storage period to determine the optimal storage temperature for preserving yogurt quality.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. The solvents used for titratable acidity, including sodium hydroxide and phenolphthalein were purchased from Sigma-Aldrich, (Sigma-Aldrich, St. Louis, Missouri, United States) with the purity of ≥ 98%. Distilled water was used throughout the experiments. *p*H buffer solutions (*p*H 4.00 and *p*H 7.00) were used for calibration of the *p*H meter (Sigma-Aldrich, St. Louis, Missouri, United States).

Set yogurt production

The subject of this study was set yogurt samples produced from cow's milk in a dairy industry located in the Pelagonia region. The yogurt production process involved heating the cows' milk to 90 °C for 15 seconds, then cooling it to 42 °C before fermentation. Two different batches were prepared for comparison: one batch was fermented using only a starter culture (control), while the other batch was fermented with both the thermophilic starter culture (Chr. Hansen) and the bioprotective culture FRESHQ® 9 (Chr. Hansen). The starter culture used for fermentation was a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. The addition of the starter culture and bioprotective culture adhered to the manufacturer's recommended guidelines. The yogurt samples were packaged in 400-gram plastic cups with shrink sleeves and sealed with aluminium lids. After inoculation with the respective cultures, the samples were incubated until they reached a *p*H of 4.6 ± 0.1, which took approximately 250 minutes. Following fermentation, the yogurt products were cooled to 4 °C and maintained at this temperature for 24 hours. After that, samples were stored at three different temperatures 5, 10, and 25 °C to simulate various storage conditions. Quality changes were meticulously monitored over time. The study assessed various parameters, including physicochemical properties (*p*H and titratable acidity) and sensory characteristics.

Samples were collected and analyzed at various time intervals. Specifically, those stored at 25 °C were monitored for up to 16 days post-production, while samples kept at 5 and 10 °C were observed for up to 49 days. This comprehensive study aimed to highlight the practical benefits and effectiveness of incorporating bioprotective cultures in dairy production. By extending the shelf life and preserving the quality of yogurt under diverse storage conditions, the research underscores the potential advantages of using bioprotective cultures in enhancing dairy product stability and safety.

*p*H and titrable acidity

The *p*H of the samples was measured with a *p*H meter Testo 206 (Testo SE & Co. KGaA, Lenzkirch, Germany). Before measurement, the *p*H meter was calibrated using standard buffer solutions at *p*H 4.00 and *p*H 7.00. The *p*H probe was then inserted directly into the sample, and the reading was recorded once it stabilized. The *p*H meter was rinsed with distilled water and wiped dry between measurements to prevent cross-contamination. The titratable acidity of the yogurt was determined using the Soxhlet-Henkel method (°SH). The acidity was calculated using the following equation:

$$°\mathrm{SH} = a \times 2 \times F \quad (1)$$

where a represents the volume (mL) of 0.1 M sodium hydroxide used in the titration, and F is the factor of the 0.1 M sodium hydroxide solution.

Sensory analysis

Set yogurts were evaluated by a sensory panel consisting of 20 semi-trained participants. Approximately 20 g of each sample, cooled to 4 ± 1 °C, was placed in pre-labeled cups in a randomized order. The panellists rated attributes such as flavor, mouth feel, appearance, texture, and overall acceptance on a 9-point scale, where 1 indicated "extremely dislike" and 9 indicated "extremely like." The average score for each attribute was calculated after all evaluations were completed. The sensory assessment was conducted in accordance with European Union ethical and food research guidelines. Informed consent was obtained from all participants, who reported no known food allergies and signed an informed consent form prior to the evaluation. At the conclusion of the evaluation period, sensory panel data were analyzed to assess the preferences and overall acceptability of the yogurt samples. Statistical analyses were conducted to identify any significant differences in the sensory attributes between the two batches.

Statistical analysis

The regression model that is used in this work is more commonly used for the analysis of food products [13]. With this model, the relationship between both independent and dependent variables has the form:

$$z = b_0 + b_1x + b_2y + b_3x^2 + b_4xy + b_5y^2 \quad (2)$$

where x and y are the independent variables; z is the dependent variable; and b are the coefficients of the model.

The evaluation of the model was done through the coefficient of determination (R^2), the coefficients of the model, their standard error (SE), p-value, and Fisher's test (F). Analyses of the coefficients of the model and of the residuals were made.

Data for the three main characteristics of yogurt, *p*H, °SH, and sensory analysis (SA), were reduced with the Principal Component Analysis (PCA) method [14]. PCA is a method for reducing the volume of input variables. Through PCA, relationships between features recorded in the dataset are found and interpreted. To determine the optimal storage time and temperature, a linear programming algorithm was implemented through the linprog function. An “interior-point-legacy” method was used. The algorithm reaches a solution after traversing the interior of the dataset. The

"interior-point-legacy" algorithm offers a systematic approach to solving the problem of solving the set optimization task. Matlab 2017b software system (The Mathworks Inc., Natick, MA, USA.) as well as the Statistica 12 program (TIBCO Software Inc., Palo Alto, CA, USA) were used. All data were processed at a significant level of $p \leq 0.05$.

RESULTS AND DISCUSSION

*p*H

The *p*H of yogurt is a critical factor for assessing both its safety and quality during storage, as it influences microbial stability, flavor, and texture. Regular monitoring of *p*H ensures the product remains within safe acidity levels while maintaining its sensory attributes, such as taste and mouthfeel [15]. The *p*H value of all analyzed products decreased gradually from the first day (4.56 ± 0.01) after production until the end of the storage period, across all three storage temperatures and for both batches (control yogurt and yogurt with bioprotective culture) ($p < 0.05$) (Figure 1).

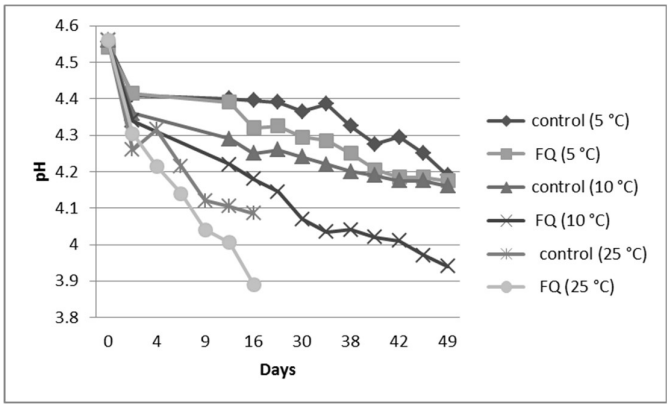


***Figure 1**. Changes in pH value during storage*

From the presented results in Figure 1, it can be noted that at refrigerated temperature at 10 °C there was significantly reduction of the *p*H at yogurt produced with bioprotective sulture (FQ 10 °C). Over-acidification or post-production acidification refers to the reduction in *p*H that occurs after fermentation and during storage under refrigerated conditions [16]. Yogurt cultures, such as *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, remain metabolically active even at low temperatures, producing small amounts of lactic acid through lactose fermentation [17]. This ongoing acid production leads to a noticeable decrease in *p*H. Post-acidification during storage is attributed to the activity of β-galactosidase, which remains functional at temperatures between 0 °C and 5 °C [18].

Titratable acidity

The titratable acidity (TA) increased over the storage period at all temperatures in both batches (Figure 2). At a storage temperature of 10 °C, the TA values for the batch with

bioprotective culture FQ-9 and the control batch remained at similar levels. This observation aligns with the findings of Jia *et al*. [19], who reported that increasing the proportion of *Lactobacillus rhamnosus* GG resulted in decreased values for titratable acidity, water-holding capacity, and viscosity index. A slight post-acidification was observed in samples produced with bioprotective cultures stored at 5 °C, potentially due to factors such as interrupted cold chain, extended fermentation times, slow cooling, or the influence of starter cultures.

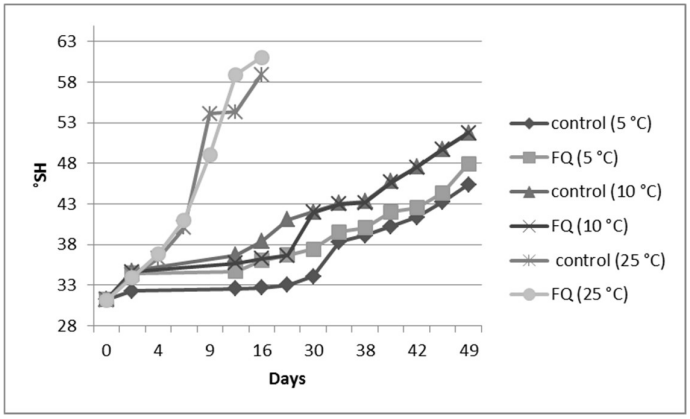


Figure 2. *Changes in °SH values during storage*

Sensory analysis

The sensory analysis aimed to assess quality changes in the products over time. Sensory attributes, including flavor, mouthfeel, appearance, texture, and overall acceptance, were evaluated, with only the overall acceptance presented in Figure 3. These findings highlight the critical role of bioprotective cultures in prolonging yogurt shelf life and preserving its quality under varying storage conditions. Similar results were reported by Lestari *et al*. [20], who found that incorporating probiotics such as *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis* (BB-12®) as DVS cultures enhanced the microbiological properties of yogurt while maintaining sensory characteristics comparable to conventional yogurt.

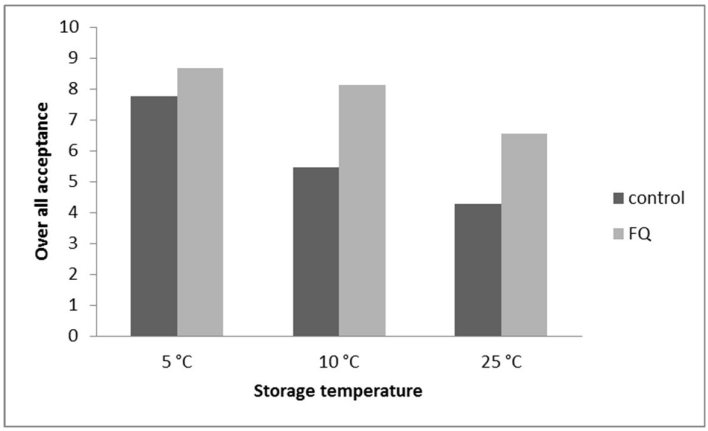
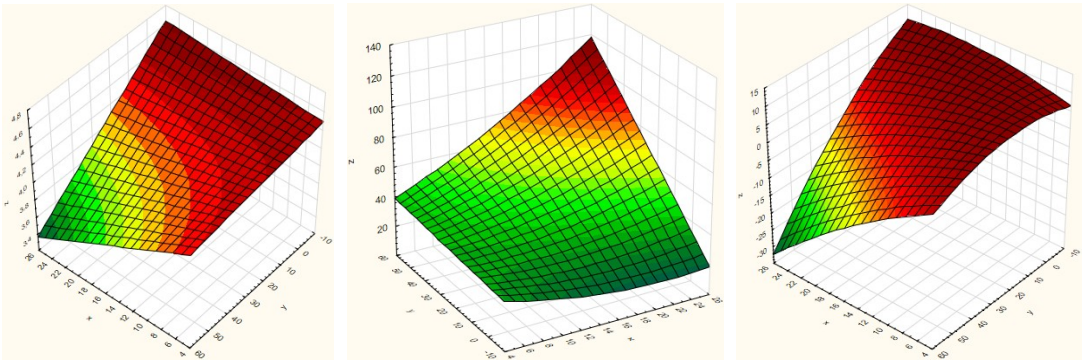


Figure 3. *Overall acceptance of set yogurt*

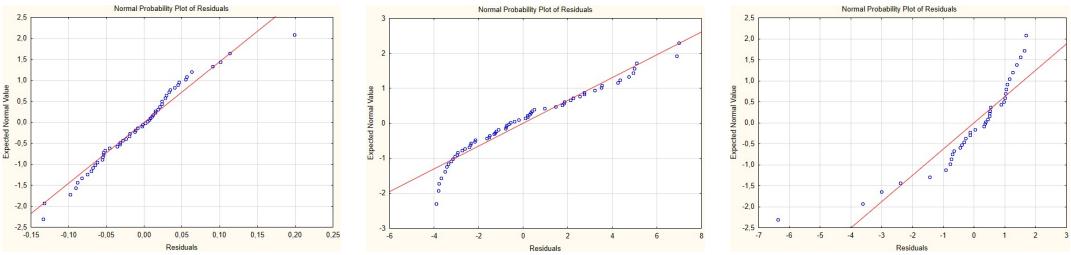
Figure 4 shows an overview of the obtained models for active acidity (*p*H), titratable acidity (°SH), and overall sensory analysis (SA) for the control sample. x and y denote the temperature and day of storage, and the dependent variable z represents any of the studied characteristics, *p*H, °SH, and SA. It can be seen that in all three models, high values of the dependent characteristics are obtained when the two independent variables are at their lowest levels. This means that at a lower temperature and at the beginning of the storage period, yogurt shows the best characteristics compared to the remaining days of storage.



a) *p*H=f(T, d) b) SH=f(T, d) c) SA=f(T, d)

Figure 4. *Models for control sample – general view*

An analysis of residuals was performed for the resulting models for the control sample. The results are shown in Figure 5. It can be seen that for all three models, the initial and final values of the residuals deviate from the normal probability plot. The models would have strong predictive power in their middle parts.



a) *p*H=f(T, d) b) °SH=f(T, d) c) SA=f(T, d)

Figure 5. *Models for control sample – general view*

The models for the control sample have the form:

*p*H=f(T, d) $$pH = 4.46 - 0.0002T^2 - 0.0007Td \quad (3)$$

°SH=f(T, d) $$SH = 38.49 - 1.52T - 0.14d + 0.05T^2 + 0.06Td \quad (4)$$

SA=f(T, d) $$SA = 6.12 + 0.57T + 0.24d - 0.02T^2 - 0.004d^2 - 0.03Td \quad (5)$$

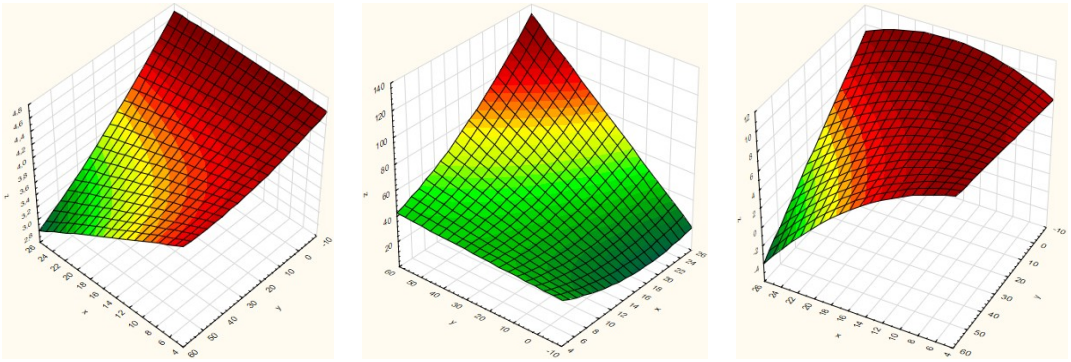
It can be seen that in the three models, the coefficients before T have higher values. This means that the storage temperature has a greater influence on the change in product characteristics than the storage period.

The obtained models were evaluated for the control sample. It is presented in Table 1. The temperature values influence more than 70 % of the change in yogurt characteristics. The obtained value of the coefficient of determination is R^2 = 0.73 - 0.85. The standard error value is SE = 0.07 - 3.02. Degrees of freedom were determined, and according to Fisher's test, in all cases, F >> Fcr and p < 0.00 It follows that the obtained models describe the experimental data with sufficient accuracy.

Table 1. *Assessment of control sample models*

Model	R^2	F	Fcr	p-value	SE
*p*H=f(T, d)	0.73	F(2, 59)=78.98	3.15	0.00	0.07
°SH=f(T, d)	0.85	F(4, 57)=80.37	2.53	0.00	3.02
SA=f(T, d)	0.80	F(5, 56)=45.33	2.38	0.00	1.48

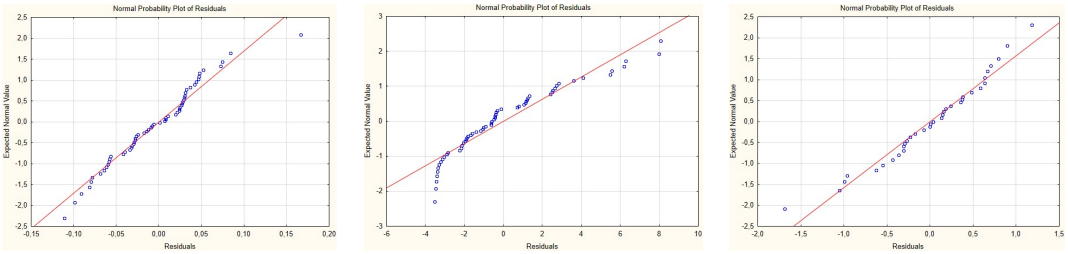
Figure 6 shows an overview of the obtained models for active acidity (*p*H), titratable acidity (°SH), and overall sensory analysis (SA) for the sample with bioprotective culture (FQ9). x and y denote the temperature and day of storage, and the dependent variable z represents any of the studied characteristics, *p*H, °SH, and SA. It can be seen that in the *p*H and SA models, high values of the dependent characteristics are obtained when the two independent variables are at their lowest levels. This means that at a lower temperature and at the beginning of the storage period, yogurt shows the best characteristics compared to the remaining days of storage. In the °SH model, high values of the dependent variable are observed when the temperature is high and the storage period is longer.



a) *p*H = f(T, d) b) SH = f(T, d) c) SA = f(T, d)

Figure 6. *Models for FQ9 sample – general view*

An analysis of the residuals was performed for the resulting models for the FQ9 sample. The results are shown in Figure 7. It can be seen that for all three models, the initial and final values of the residuals deviate from the normal probability plot. The models would have strong predictive power in their middle parts.



a) pH = f(T, d) b) SH = f(T, d) c) SA = f(T, d)

Figure 7. *Models for FQ9 sample – general view*

Sample models with FQ9 have the form:

pH=f(T, d) $$pH = 4.49 - 0.006d - 0.0002T^2 + 0.0001d^2 - 0.001Td \quad (6)$$

°SH=f(T, d) $$SH = 46 - 2.78T - 0.18d + 0.09T^2 + 0.07Td \quad (7)$$

SA=f(T, d) $$SA = 6.33 + 0.56T + 0.07d - 0.02T^2 - 0.01Td \quad (8)$$

In all three models, the coefficients associated with T are larger than those for d, indicating that storage temperature has a greater impact on product characteristics than storage period.

The resulting models were evaluated for the FQ9 sample. It is presented in Table 2. The temperature values influence more than 70 % of the change in yogurt characteristics. The obtained value of the coefficient of determination is R^2 = 0.72 - 0.85. The standard error value is SE = 0.06 - 3.04. Degrees of freedom were determined, and according to Fisher's test, in all cases, F >> Fcr and p < 0.00. It follows that the obtained models describe the experimental data with sufficient accuracy.

Table 2. *Assessment of FQ9 sample models*

Model	R^2	F	Fcr	p-value	SE
pH = f(T, d)	0.90	F(4, 57) = 129.46	2.53	0.00	0.06
SH = f(T, d)	0.85	F(4, 57) = 78.98	2.53	0.00	3.04
SA = f(T, d)	0.72	F(4, 57) = 10.45	2.53	0.00	1.50

The storage time of yogurt supplemented with FQ9 has been determined. Data for pH, SH, and SA reduced to the first principal component were used. This component describes over 95 % of the variance in the data.

A model of this type was obtained:

$$PC_1 = 5.02 - 2.83T - 0.19d + 0.09T^2 + 0.07Td \quad (9)$$

For this model, R^2 = 0.85; F(4, 57) = 79.65 >> Fcr = 2.53; p < 0.00; and SE = 3.07. These results show that the obtained model describes the experimental data with sufficient accuracy. An optimization algorithm revealed that using bioprotective cultures at a storage temperature of 10 °C can extend the yogurt's shelf life to up to 40 days. Under these conditions, the limit values of pH = 4 are reached, °SH = 50, and SA = 8 [21].

This model was selected for its accuracy in analyzing the interaction of key variables in food product preservation. The regression model was specifically chosen due to its

ability to effectively represent the relationship between the principal components and storage conditions, ensuring high enough accuracy for food product analysis. The obtained result complements the findings of studies in the available literature. Bansode *et al.* [22] showed how the initial *p*H, storage temperature, and preservatives affect how long yogurt is safe, which reinforces the idea that maintaining a *p*H of 4 is important for product quality. Anwar *et al.* [23] mentioned that the way yogurt is stored and the length of time it ferments can change its physicochemical properties, like its acidity and *p*H. This is similar to how time and temperature can make food last longer on the shelf. Jakubowska *et al.* [24] also pointed out the effect of storage time and temperature on yogurt quality, with the result that lower temperatures preserve its characteristics for a longer period, as also concluded from the optimization algorithm that storage at 10 °C can extend the shelf life by up to 40 days. These sources together support the model and findings obtained in this work.

Figure 8 shows the resulting model in general and the optimal storage temperature and time for yogurt with FQ9. The optimum value is found in low values for temperature and high levels of storage day. This is indicative of the fact that the lower the temperature, the longer the shelf life of the product can be extended before the limits of its significant characteristics are reached.

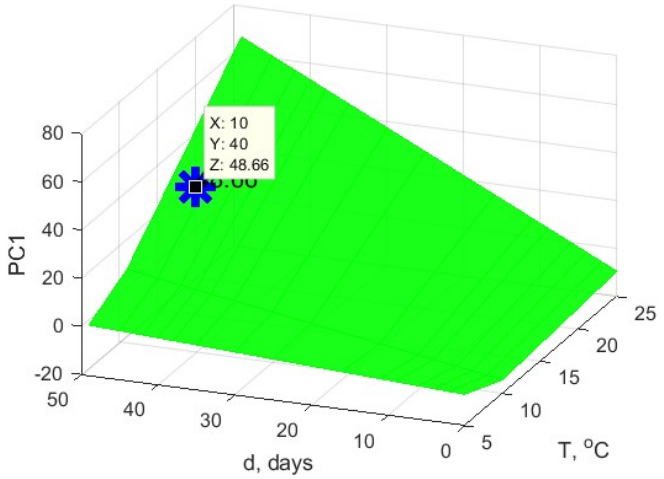


Figure 8. *Determination of optimal storage temperature and time of yogurt with FQ9*

CONCLUSIONS

Bioprotective cultures effectively extend yogurt shelf life while maintaining its quality, addressing both technical challenges and consumer demand for natural food preservation methods. This study highlights the critical role of temperature, which influences over 70 % of yogurt's key characteristics. Lower storage temperatures significantly extend the product's shelf life, delaying the point at which essential quality parameters are compromised. Notably, yogurt produced with the bioprotective culture FQ9 demonstrated a prolonged shelf life compared to the control at all tested storage temperatures. Even when stored at slightly elevated temperatures 10 °C (4-8 °C, as recommended by manufacturers), yogurt with FQ9 maintained its shelf life for up to 40

days, preserving crucial quality parameters (pH ≥ 4, °SH ≤ 50, SE = 8). These findings underscore the potential of bioprotective cultures as a sustainable, clean-label solution for spoilage prevention and shelf-life extension in the dairy industry, providing both technical and consumer-focused benefits. For a more reliable insight into the shelf life of the product, microbiological analyses should be conducted in parallel in future research.

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