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Starter culture influence on the yogurt preservation and safety

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Abstract

This study explores the impact of distinct starter cultures on the physicochemical characteristics and microbiological safety of yogurt during refrigerated storage. Yogurt samples were formulated from standardized, pasteurized cow's milk using YF-L812 and MyYo cultures. Both starter cultures contains *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*; additionally, MyYo includes probiotic strains *Lactobacillus acidophilu*s La-5® and *Bifidobacterium animalis* subsp. *lactis* BB-12®. Analytical assessments included titratable acidity, pH, lipids, total solids and *Enterobacteriaceae* counting. The YF-L812 variant demonstrated consistent acidification kinetics and compositional stability over a period of 6 weeks, while the MyYo formulation maintained acceptable parameters for a duration of 4 weeks. The absence of *Enterobacteriaceae* in all samples complies with the safety standards. The results emphasize the critical role of starter culture selection in modulating post-acidification trends, preserving nutritional and textural attributes, and ensuring microbiological safety. These findings substantiate the efficacy of starter culture selection as a pivotal factor in ensuring the stability and safety of yogurt.

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Keywords: *starter culture*, *shelf-life, yogurt*

1. Introduction

According to the Codex Alimentarius [1], yogurt is defined as a cultured dairy product that is obtained through fermentation of milk using starter cultures containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* or combinations of *S. thermophilus* and other *Lactobacillus* species. These strains are essential for lactic fermentation and contribute to the texture, flavor and shelf life of the final product. Due to their transient presence in the gastrointestinal tract, daily consumption is recommended to achieve potential health benefits [2]. The ongoing pursuit of technologically and biologically efficient starter cultures is a paramount concern in the realm of fermented dairy production. The selection of strains is of high importance, with

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criteria encompassing not only technological performance but also the capacity to ensure microbial safety and maintain nutritional value over time [3]. A variety of factors must be taken into consideration when attempting to produce high-quality yogurt. The most significant of these factors are the acidification rate, proteolytic activity, and synergistic interactions between strains [4]. The interaction between *S. thermophilus* and *L. bulgaricus* is a crucial aspect of the milk coagulation and acidification process. *S. thermophilus* produces formic acid, pyruvic acid, and CO_2, which stimulate the growth of *L. bulgaricus*. In turn, *L. bulgaricus* hydrolyzes casein, releasing peptides and amino acids that enhance the development of *S. thermophilus*, which possesses a lower proteolytic capacity [5]. The performance of starter cultures is influenced by several factors, including the incubation temperature, the treatment of the milk, and the processing conditions [6]. The optimal incubation temperature for *L. bulgaricus* is between 41-42°C, which supports its growth. In addition, pasteurization of the milk substrate eliminates spoilage and pathogenic microorganisms, thereby providing a favorable medium for fermentation [7]. Moreover, *S. thermophilus* is particularly sensitive to hydrogen peroxide produced in the presence of oxygen, so minimizing aeration during processing is essential [8]. The production of high-quality fermented dairy products necessitates the utilization of raw milk that adheres to stringent compositional and microbiological standards. Specifically, the milk must have a minimum density of 1.028 g/cm^3, a titratable acidity not exceeding 16.5–19°T (approximately 0.16–0.19% lactic acid), a minimum protein content of 3.25%, and a resazurin reduction time of at least three hours. Furthermore, the milk should be free of colostrum, antibiotic residues, disinfectants, and signs of mastitis [9]. A comprehensive understanding of the behavior of *S. thermophilus* and *L. bulgaricus*, both in monocultures and mixed cultures, is imperative for advancements in probiotic and functional dairy products [10]. The employment of biologically compatible microorganisms, meticulously selected for their advantageous interactions with milk components, is fundamental to the development of innovative fermented dairy products that exhibit enhanced structural, sensory, and nutritional characteristics [11]. To ensure product safety, it is imperative to assess thoroughly all potential risks in yogurt production. This involves the identification of critical control points and the implementation of preventive measures to eliminate pathogens or harmful substances [12]. Microbial contamination has been shown to have a substantial impact on the quality and shelf life of yogurt. Consequently, adhering to stringent hygiene practices and meticulous handling during the manufacturing process is imperative to ensure the integrity and longevity of the product [13]. Milk is particularly vulnerable to contamination by pathogens such as *Salmonella*, which can compromise food safety and public health [14]. Contamination with pathogens such as *Listeria monocytogenes* and *Escherichia coli* poses a significant health risk, thereby underscoring the necessity for rigorous microbial control measures [15]. Therefore, the judicious selection and implementation of suitable starter cultures, in conjunction with stringent hygienic practices and continuous microbiological monitoring, emerge as pivotal strategies for ensuring the production of yogurt that not only meets safety standards but also fulfills consumer expectations for quality and shelf-life [16]. The present study evaluated the impact of different commercial starter cultures on the physicochemical stability and microbiological safety of yogurt during refrigerated storage. Two formulations were compared in this study: The YF-L812 product contains *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, in addition to MyYo, which also includes the probiotic strains *Lactobacillus acidophilus* La-5® and *Bifidobacterium animalis* subsp. *lactis* BB-12®. Key parameters such as pH, titratable acidity, fat content, total solids, and the presence of *Enterobacteriaceae* were monitored over time. The aim was to determine how culture composition influences acidification behavior, product shelf life, and compliance with food safety standards in yogurt production.

2. Materials and Methods

2.1. Yogurt production and started cultures

The yogurt samples were produced using pasteurized, standardized cow's milk with 1.7% fat content, obtained from a certified dairy processor. The milk was fresh and in accordance with current food safety and hygiene regulations for fermented dairy products [16]. Two commercial starter cultures were utilized in the process. The first was MyYo (Germany), a freeze-dried culture containing the probiotic strains *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* La-5® and *Bifidobacterium animalis* subsp. *lactis* BB-12®. The second culture, YF-L812 (CHR Hansen, Denmark), was a frozen blend of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus,* produced under Hazard Analysis and Critical Control Points (HACCP) guidelines and in accordance with ISO 27205 | IDF 149:2010 standards. Two experimental variants were prepared: sample 1 (YF) was inoculated with 1 g of YF-L812 per liter of milk, and sample 2 (MY) was inoculated with one 5 g sachet of MyYo culture per liter, according to the manufacturer's indications. In both cases, the milk was heated to the temperature of 43–45°C using a laboratory electric heating plate. Following inoculation, samples were subjected to incubation at a temperature of 43 ± 1°C for a duration of 6–8 hours within a calibrated laboratory Incucell incubator (MMM MedCenter, Germany), until the onset of visible coagulation.

2.2. Analysis of physicochemical properties of milk

Prior to processing, a LactoStar physicochemical analyzer (Funke Gerber, Germany) was utilized to assess parameters such as lipids, protein, and lactose content, thereby ensuring consistency across batches. This instrument operates based on infrared absorption and electrical conductivity to rapidly and accurately measure parameters such as lipids content, protein, lactose, total solids, non-fat dry matter, freezing point, density, and pH. Approximately 20 mL of well-homogenized milk was introduced into the analyzer, which performed simultaneous multi-parameter analysis. Prior to analysis, the equipment was calibrated using standardized control samples according to the manufacturer's instructions [17]. All measurements were conducted at a controlled temperature of 40 ± 1°C to ensure accuracy and repeatability.

2.3. Analysis of titratable acidity

Titratable acidity was determined by acid-base titration using 0.1 N sodium hydroxide (NaOH) from Sigma-Aldrich in the presence of ROTH phenolphthalein. The volume of NaOH used was recorded, and the titratable acidity was calculated and expressed as a percentage of lactic acid [18].

2.4. pH analysis

The pH of yogurt samples was measured using a Hanna pH meter (Hanna Instruments, USA) with a temperature-compensated glass electrode. To ensure the accuracy of the measurements, all trials were conducted at room temperature and in triplicate.

2.5. Lipids content

The fat content of yogurt samples was determined using the acid-butyrometric method, which was adapted from the Gerber method for dairy products as specified in ISO 19662:2018. The fat content was subsequently determined by direct measurement on the butyrometer scale and expressed as a percentage.

2.6. Total solids content

The total solids content of the yogurt samples was determined by oven drying, in accordance with STAS 6352-1/88 [19]. Total solids content was calculated based on the difference in weight before and after drying, and expressed as a percentage of the initial yogurt mass.

2.7. Counting and confirmation of *Enterobacteriaceae*

The enumeration of *Enterobacteriaceae* was performed in accordance with ISO 21528-2:2007 [20], employing the colony count method on Millipore Violet Red Bile Glucose Agar

(VRBG). Identification was based on oxidase negativity and glucose fermentation, with confirmation using the Vitek 2 Compact system.

3. Results and Discussion

This section presents the results of physico-chemical and microbiological analyses of milk and yoghurt, with particular emphasis on the influence of starter cultures on yoghurt quality. These results are then reviewed and discussed in relation to the relevant scientific literature and established standards.

3.1. Physico-chemical analyses of milk

The results of the physico-chemical analysis of the milk used in yogurt production performed with LactoStar milk analyzer confirmed that the raw material met the quality requirements for fermented dairy processing.

Table 1. Physico-chemical characteristics of the milk used for yogurt production

Milk parameter	Value	Reference standard (Ordin 184/1972)
Titratable acidity (°T)	18	15–19
pH	6.5	6.4–6.6
Fat content (g%)	1.7	Min. 1.5
Protein content (g%)	3.4	Min. 3.2
Density (g/cm³)	1.03305	1.028–1.033
Non-fat dry matter (%)	9.1	Min. 8.5
Total solids (%)	12.5	11.9–14.2
Moisture (%)	87.2	Max. 87.3
Lactose (%)	4.55	4.8
Ash content (%)	0.08	Max. 0.7
Freezing point (°C)	-0.541	-0.54 to -0.57

As presented in Table 1, yogurt production are in strong agreement with both the Ordin 184/1972 standards. The titratable acidity, measured at 18°T, falls within the standard range of 15–19°T. This assertion is further substantiated by Colinet & Soyeurt (2010) who demonstrated that titratable acidity in cow's milk exhibits comparable variations and functions as a reliable indicator of freshness and microbial activity when evaluated using mid-infrared spectrometry [21]. The pH of the milk, at 6.5, also falls within the accepted range of 6.4–6.6, indicative of adequate freshness and buffering capacity. This observation is consistent with the findings of Litwińczuk et al., (2012) who reported that fresh cow's milk generally exhibits pH values between 6.5 and 6.7, influenced by factors such as the cow's diet, health, and stage of lactation [22]. The fat content, recorded at 1.7%, exceeds the minimum requirement of 1.5% but remains below the average fat concentration of standard whole milk. A study by Ahmida et al., (2021) reported that the average fat content in raw cow's milk can reach approximately 3 %, depending on breed and feeding practices [23]. The protein content was measured at 3.4%, which exceeds the minimum standard of 3.2%. This outcome aligns with the findings reported by Miciński et al., (2013), who observed that protein concentrations in cow's milk typically range from 3.3% to 3.5% [24]. The milk's density, recorded at 1.03305 g/cm³, is at the upper boundary of the standard range (1.028–1.033 g/cm³), signaling a favorable ratio of solids to water. This observation is consistent with the findings reported by Chalupa-Krebzdak et al., (2018), who attributed higher densities to enhanced nutritional quality and reduced dilution [25]. In terms of non-fat dry matter and total solids, the milk sample demonstrated levels of 9.1% and 12.5%, respectively, both of which significantly exceeded their respective minimum thresholds. These values are consistent with those found in a study by Gurmessa & Melaku (2012), where non-fat solids ranged from 8.3% to 9.5%, and total solids ranged from 11.5% to 13.6% [26]. The moisture content was measured at 87.2%, which is slightly below the maximum allowable limit of 87.3%. This ensures that the concentration of dry matter remains sufficient for fermentation without compromising product integrity. The lactose level, at 4.55% (versus the expected 4.8%), falls within the range reported by Araújo et al. (2018), who noted lactose values between 4.4% and 4.9% in cow's milk [27]. The ash content, which reflects the mineral fraction of milk, was notably low at 0.08%, significantly beneath the typical range of 0.6–0.8% [27]. While this deviation might suggest lower

mineral content, it remains within the regulatory limits and does not impair yogurt production. The freezing point of -0.541°C falls within the standard interval of -0.54 to -0.57°C. This parameter, frequently employed for the detection of adulteration, corresponds with values cited in recent dairy quality assessments, thereby confirming the milk's authenticity. The lactose content of 4.55% falls slightly below the anticipated 4.8% threshold but remains within the 4.4–4.9% range thereby facilitating adequate fermentation. The ash content was found to be minimal at 0.08%, falling below the conventional range of 0.6–0.8% [27], but remaining within acceptable limits

3.2. The influence of starter cultures on the yogurt's physicochemical properties

Titrable acidity and pH

As shown in Figure 1, sample 1 (YF) showed a steady increase in titratable acidity and a corresponding decrease in pH over 7 weeks of storage, reaching 140 °T and pH 3.3, respectively. These results align with findings by Matela et al. (2019) [28], who reported pH values between 3.94–4.22 and titratable acidity ranging from 0.69% to 1.81% in commercial yogurts. The most rapid acidification occurred within the first 24 hours, reflecting high fermentative activity. Thereafter, the changes were more gradual, indicating continued but reduced metabolic activity. These results confirm the robust and sustained acidification potential of the YF-L812 culture during cold storage.

Moreover, Figure 1 illustrates that the utilization of MY starter cultures in the production of yogurt resulted in a steady escalation in acidity levels, accompanied by a concomitant decline in pH over a period of 4 weeks. These alterations led to the attainment of 136°T and a pH value of 3.8. These outcomes are analogous to those reported by Canja et al. [29], who observed that rapid acidification by *L. bulgaricus* strains resulted in elevated post-acidification during storage. The observed acidification trend in sample 2 (MY) indicates similar metabolic patterns, underscoring the fundamental role of starter culture type in dictating yogurt's physicochemical evolution.

The correlation between Sample 1 (YF) and sample 2 (MY) is evident in their distinct acidification patterns, as illustrated in the accompanying graphic in Figure 1. Sample 1 (YF) demonstrated a gradual and sustained increase in acidity, resulting in stable yogurt quality over a 6 weeks period. Conversely, sample 2 (MY) demonstrated a more rapid acidification rate, reaching a higher acidity level more rapidly and experiencing a significant pH decrease in the initial weeks. These observations underscore the pivotal role of culture composition in dictating the rate of acidification and the overall stability of yogurt. This observation lends further support to the hypothesis that the enhanced stability exhibited by sample 1 (YF) is attributable to the divergent metabolic activity exhibited by these two distinct cultures.

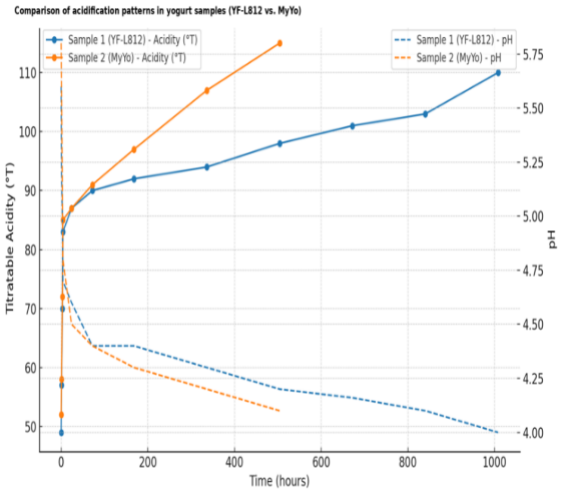

Figure 1. Comparison of acidification patterns in yogurt samples

Lipids content

As illustrated in Figure 2, a gradual decline in fat content was observed for both yogurt samples during the 28-day storage period, with sample 1 (YF) decreasing from 2.20% to 2.08% and sample 2 (MY) from 2.15% to 2.04%. These results are consistent with the findings reported by Dan et al., (2023) who observed that various combinations of *L. delbrueckii* subsp. *bulgaricus* and S. thermophilus influenced yogurt's physicochemical properties, including structure

and moisture retention [30]. These factors can affect fat distribution and stability. The slightly greater fat retention observed in sample 1 (YF) may be indicative of lower lipolytic activity or enhanced matrix integrity. From a food safety perspective, maintaining stable fat content is imperative, as excessive lipolysis can lead to the accumulation of free fatty acids, which may compromise flavor and shelf life. The samples exhibited consistently high fat values, remaining above the regulatory threshold of 2.0%, indicative of both compositional quality and microbial stability throughout storage.

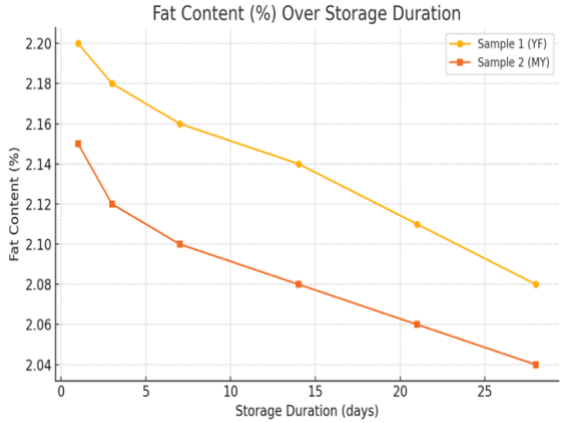

Figure 2. Evolution of fat during storage

Total solids

The total solids content in yogurt samples exhibited a consistent elevation throughout the 28-day period, with a gradual decline over time as showed in Figure 3. These values suggest stable product structure and minimal syneresis. These findings are consistent with those reported by Shrestha et al. (2021) [31], who observed that a yogurt starter fermented at 37°C for 18 hours exhibited a water content of 82.48%, indicating a total solids level of approximately 17.52% [32]. While slightly higher than the values observed in this study, these results reinforce that total solids are a key indicator of yogurt quality and texture [33]. The initial elevated values followed by a slight decrease in both studies may be attributed to ongoing metabolic activity and water redistribution during storage, emphasizing the importance of starter culture composition and fermentation parameters in maintaining physicochemical stability [34].

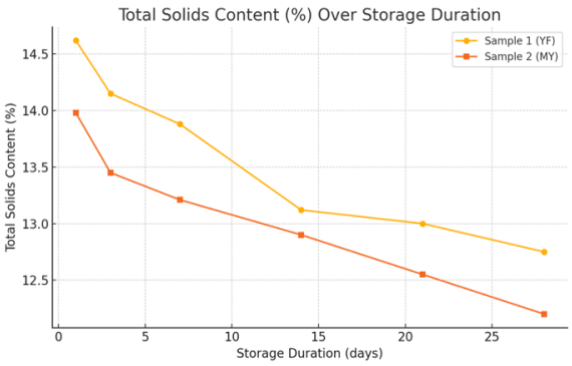

Figure 3. Evolution of total solids content during storage

1. *Enterobacteriaceae*

As presented in Table 2, the *Enterobacteriaceae* detection assay yielded negative results, with no characteristic colonies observed on VRBG agar for either yogurt sample. This finding indicates that additional biochemical confirmation was not necessary. The results are reported in accordance with ISO 7218:2007, with concentrations below the limit of detection (<1 CFU/g), confirming the absence of *Enterobacteriaceae* and ensuring microbiological safety [35]. A comparison of these findings with those from [36] and [37] reveals notable disparities. As indicated in the preceding studies, there was a considerable presence of *Enterobacteriaceae* in a significant proportion of yogurt samples, with some exhibiting levels that surpassed the established acceptable limits. These results suggest the potential for microbiological risks and underscore concerns regarding food safety, particularly in regard to the shelf-life of the products. The presence of these bacteria can contribute to spoilage and a reduced shelf-life, as they indicate that contamination may occur during production or post-processing. Conversely, the absence of *Enterobacteriaceae* in the samples suggests a reduced probability of microbial spoilage, which may account for the extended shelf-life observed in the yogurt samples examined in this study. The microbiological safety of the samples is directly associated with their stability and quality over time, ensuring that the products remain safe for consumption for an extended period, free from

potential contamination that could compromise their safety and freshness.

Table 2. *Enterobacteriaceae* detection

Sample	Colony growth on VRBG Agar	Biochemical confirmation	Final result (CFU/g)
Sample 1	No colonies observed	Not required	CFU/g
Sample 2	No colonies observed	Not required	CFU/g

Conclusions

This study underscores the major role of starter culture selection in determining the physicochemical properties, stability, and microbiological safety of yogurt. Sample 1 (YF) exhibited a gradual increase in titratable acidity and a sustained decrease in pH, maintaining stability for a period of 6 weeks. In contrast, sample 2 (MY) demonstrated rapid acidification, reaching higher acidity levels and a significant pH decline within the first few weeks. This resulted in a reduced shelf-life of only 4 weeks. The accelerated acidification and reduced shelf-life observed in sample 2 (MY) can be partially attributed to the inclusion of probiotic *Bifidobacterium*. This bacterium influenced the fermentation kinetics and led to more pronounced changes in physicochemical parameters such as pH and titratable acidity. In addition, it contributed to maintaining microbiological safety by supporting beneficial microbial activity throughout the storage period. A gradual decline in fat content was observed in both samples, with sample 1 (YF) retaining slightly higher fat levels. The total solids content in both samples exhibited minimal variation, suggesting stable texture and product structure. Of particular significance is the confirmation of the absence of *Enterobacteriaceae* in both samples through rigorous microbiological analysis, thereby ensuring the microbiological safety of the yogurt throughout the storage period. These findings underscore the fundamental role of starter culture composition in regulating acidification rates, enhancing product stability, and ensuring food safety by preventing microbial contamination. Future research endeavors should prioritize the optimization of fermentation conditions, the exploration of the effects of novel probiotic strains, and the evaluation of the long-term microbiological safety and sensory attributes of yogurt, with the objective of further enhancing product stability and health benefits.

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