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STUDY ON THE SPECIFIC FEATURES OF LACTIC CULTURES USED IN THE PRODUCTION OF FERMENTED DAIRY PRODUCTS

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Abstract

This paper aims to examine the specific characteristics of lactic cultures used in the fabrication of fermented dairy products, highlighting both their essential role in the fermentation process and their impact on the quality and organoleptic properties of the final products made available for consumer use. The study focused on analysing the influence of storage temperature on the viability and fermentative activity of thermophilic and mesophilic cultures, stored under optimal freezing and at elevated temperatures, followed by assessment of coagulation time and sensory quality in yogurt and fermented milk (sana). Although differences in coagulation time were minor, higher storage temperatures slightly accelerated the process without altering organoleptic properties. Fermentation dynamics were further analysed through titratable acidity measurements. Results indicated that thermophilic cultures acidified more rapidly in the initial hours after inoculation, while mesophilic cultures showed slower, progressive acidification. Data was represented graphically to highlight these comparative patterns. Additionally, the study explored whey valorisation as a strategy for product diversification. Sweet whey was fermented to obtain kefir prototypes in three variants: plain, honey-enriched, and mint-flavoured. Sensory evaluations and consumer feedback through questionnaires confirmed positive acceptance, suggesting that whey-based kefir represents a promising alternative for expanding the range of functional dairy products.

Key words*: lactic cultures, thermophilic cultures, mesophilic cultures, fermented dairy products, whey-based kefir.*

INTRODUCTION

Fermented dairy products represent a category of foods with a significant impact on nutrition and public health, constituting an essential component of the daily diet (Oprea et al., 2019). These products are obtained through technological processes that rely on carefully selected lactic cultures and raw milk tested for antibiotic residues (Baraitareanu et al., 2021). Such cultures may consist either of a combination of bacterial strains characteristic of each product type, or of a single microbial species, thereby conferring superior organoleptic qualities and introducing unique attributes to the products, such as natural preservation, enhanced flavour, improved digestibility, and the development of a distinctive texture (Guzun et al., 2001).

Lactic cultures, composed of carefully selected microorganisms, play a crucial role in the manufacture of fermented dairy products. When inoculated into high-quality raw material, namely milk, they initiate and regulate fermentation while simultaneously shaping sensory attributes such as aroma, taste, and texture, ensuring food safety (Mitrea et al., 2003; Petcu, 2006; Banu, 2009; Cartașev & Rudic, 2016; Goran et al., 2016). The incorporation of starter lactic cultures into production processes has revolutionized the dairy industry by improving product consistency and expanding the diversity of available products for consumers (Cartașev & Rudic, 2016; Banu, 2009).

Fermented dairy products are widely appreciated for their dietary benefits and therapeutic properties. Their high nutritional value results from the raw material in a form that is more readily assimilated by the body (Gajaila & Gajaila, 2022). Proteins undergo partial hydrolysis during manufacturing, facilitating their absorption in the digestive tract (Cartașev & Rudic, 2016; Oprea et al., 2020).

Starter lactic cultures are also classified according to their optimal growth temperature, into mesophilic and thermophilic cultures. Mesophilic cultures consist of Leuconostoc and *Lactococcus* species and may be composed of a single strain or a combination of multiple

strains. Thermophilic cultures contain one or more *Lactobacillus* species and a *Streptococcus* species (Dobrea, 2008).

To fully exploit all products and by-products of milk processing, the utilization of whey, an underutilized by-product of cheese production, must also be considered. Whey, a greenish-yellow turbid liquid, can be classified as sweet or acid whey, depending on the production method (Guzun et al., 2001). Adequate consumption of whey contributes significantly to overall health, as it retains proteins, carbohydrates, fats, vitamins, and probiotic bacteria from milk after processing (Besediuk et al., 2024).

The primary objective of this study is to analyse the behaviour of lactic cultures used in fermented dairy production, highlighting their influence on the qualities and organoleptic characteristics of the final product.

In the context of sustainability and innovation within the fermented dairy sector, this study also aims to valorise sweet whey, a scarcely utilized by-product available in significant quantities at the end of milk processing. Based on preliminary consumer preference surveys indicating a strong interest in kefir, another objective of this research is to develop a new fermented dairy product using whey as the primary raw material, adapting traditional kefir technology to create a new product while optimizing resource use.

MATERIALS AND METHODS

The research was conducted at a sanitary and veterinary authorized milk processing facility located in the southern region of the country, which produces a wide range of dairy products, with the focus of this study placed on fermented dairy products.

The materials used in this study included: raw ingredients (milk, whey, lactic cultures); packaging materials; measuring equipment; laboratory glassware; and chemical reagents. Additionally, auxiliary materials such as honey and finely crushed dried mint were used.

The methodological approach adopted in this study comprised organoleptic evaluation and physicochemical analysis.

Organoleptic assessment involves the evaluation of parameters related to the appearance, consistency, colour, odour, and taste of the finished fermented dairy product. It is important to note that this evaluation should be conducted in a clean, well illuminated environment, free from extraneous odours (Savu & Petcu, 2002).

Physicochemical evaluation includes the analysis of product integrity and freshness. To determine the freshness of the products, acidity was measured using the Thörner method: a portion of the sample is titrated with sodium hydroxide solution in the presence of phenolphthalein, which serves as an indicator, until a sudden colour change to pink occurs, that must remain visible for one minute. The resulting acidity is expressed in Thörner degrees (°T) and corresponds to the volume of 0.1 N sodium hydroxide solution (in millilitres) required to neutralize the acidity present in 100 ml of the product (Savu et al., 2013).

In the facility where the study was conducted, the standard method of product manufacturing is batch inoculation in vats, however, for the purpose of the experiments, the direct fermentation method in the retail packaging was employed.

RESULTS AND DISCUSSIONS

Inoculation of lactic cultures at different storage temperatures

This study evaluates the effect of storage temperature on the performance of lactic cultures, focusing on coagulation time and sensory characteristics of fermented dairy products.

Yogurt samples were prepared with pasteurized milk inoculated with YoFlex Express (thermophilic culture) which was subjected to three storage conditions: optimal freezing at 18°C (batch 1), laboratory room temperature at 19°C for 6 hours (batch 2), and thermostatic room temperature at 41°C (batch 3).

Fermented milk samples (sana) were inoculated with Flora Danica (mesophilic culture) which was subjected to the same conditions as the previous three batches: -18°C (batch 4), 19°C (batch 5), and thermostatic room temperature at 29°C (batch 6).

The exposure of cultures to elevated cultures was limited to 6 hours to avoid compromising bacterial viability. The inoculation was performed directly in the retail packaging, 6 batches of 6 samples each.

Following the dosing of the culture in accordance with the technical specifications of the lactic cultures, the procedures of inoculation, homogenization, sealing and labelling of the samples were carried out. The containers were transferred to the thermostatic chambers where the fermentation process progressed differently across batches, highlighting the impact of storage temperature on culture performance. Coagulation times varied slightly (Tables 1 and 2), on the order of minutes, yet these variations reflected subtle modifications in fermentation dynamics and microbial activity.

Table 1. Variation of coagulation time according to the storage temperature of the *YoFlex Express* culture (thermophilic culture)

Batch number	Type of sample	Inoculated culture	Storage temperature of the culture	Coagulation time
Batch 1	Yogurt	YoFlex Express	-18°C	4 hours
Batch 2	Yogurt	YoFlex Express	19°C	3 hours, 40 minutes
Batch 3	Yogurt	YoFlex Express	41°C	3 hours, 20 minutes

Table 2. Variation of coagulation time according to the storage temperature of the *Flora Danica* culture (mesophilic culture)

Batch number	Type of sample	Inoculated culture	Storage temperature of the culture	Coagulation time
Batch 4	Sana	Flora Danica	-18°C	10 hours, 30 minutes
Batch 5	Sana	Flora Danica	19°C	10 hours, 15 minutes
Batch 6	Sana	Flora Danica	29°C	10 hours, 10 minutes

As observed, higher storage temperatures of the cultures corresponded to shorter coagulation times, indicating enhanced fermentative activity. While such deviations did not markedly alter the process, they underscore the significance of storage conditions in maintaining long-term viability of lactic acid bacteria.

Following the analysis of coagulation time, the samples from all six batches were subjected to organoleptic evaluation to assess potential variations in appearance, consistency, colour, odour, and taste resulting from differences in the storage temperatures of the cultures. Notably, samples inoculated with mesophilic cultures exhibited membrane bulging due to CO_2 production (Figure 1), in contrast with thermophilic cultures, where no such effect was observed (Figure 2).

Figure 1. Samples with mesophilic culture (Original)

Figure 2. Samples with thermophilic culture (Original)

Upon opening the containers of the three batches, the appearance of the curd was evaluated before and after homogenization. All samples exhibited curd with appropriate consistency, porcelain-like fracture (Figure 3), uniform white colour, and a pleasant, slightly acidic flavour and aroma characteristic of yogurt.

After homogenization (Figure 4), the texture became creamy, and no gas bubbles were observed in the thermophilic yogurt samples, in agreement with previous reports (Savu et al., 2013; Guzun, 1998).

Figure 3. Porcelain-like fracture (Original)

Figure 4. After homogenization (Original)

Organoleptic analysis of the final three batches, consisting of fermented milk (sana), revealed CO_2 bubble formation upon opening and

breaking of the curd (Figure 5), as well as a fine coagulum with a more fluid consistency after homogenization (Figure 6). The samples retained a uniform white colour and a pleasant, slightly acidic flavour and aroma, free of off-flavours, again consistent with data from the literature (Savu et al., 2013; Guzun, 1998).

Figure 5. Curd disruption (Original)

Figure 6. After homogenization (Original)

Overall, the results of the sensory evaluations across all six batches indicate that short-term deviations in storage temperature of lactic cultures did not significantly alter the sensory characteristics of the final products, which remained consistent with those produced under optimal conditions. While minor fluctuations at the inoculation stage appear negligible, long-term deviations during storage may substantially affect both the viability of the cultures and the sensory attributes of the products.

Titratable acidity of fermented dairy products produced with thermophilic and mesophilic lactic cultures

This section presents a comparative analysis of the evolution of titratable acidity thermophilic and mesophilic lactic cultures, with the aim of highlighting differences in acidification behaviour between the two. Both culture types are fundamental to the dairy industry, as the rate band extent both acidification during fermentation can vary.

For this study, two experimental batches were prepared, each comprising 6 samples. The first batch was obtained by inoculating pasteurized milk, directly in retail containers, with the thermophilic culture YoFlex Express. The second batch was inoculated with the mesophilic culture Flora Danica.

Following the dosage and inoculation steps in accordance with the technical specifications, the samples were homogenized, sealed, and labelled according to their respective batches.

They were then transferred to thermostatic chambers specific to each group. The first batch underwent incubation at 41.1 °C (± 0.5 °C), while the second batch was incubated at 29°C (± 0.5°C).

After placing the sample batches in their respective thermostatic chambers, titratable acidity was measured throughout the fermentation process at regular hourly intervals using the Thörner method.

The titratable acidity for each experimental sample was calculated according to the reference formula specific to the titration method. The results, expressed in Thörner degrees (°T), were organized (Tables 3 and 4), providing a chronological representation of acidity changes.

Table 3. Titratable acidity values in the batch inoculated with *YoFlex Express* (thermophilic culture)

Time interval from inoculation	Sample number	Type of sample	Inoculated culture	Acidity value (°T)
1 hour	1	Yogurt	YoFlex Express	28°T
2 hours	2	Yogurt	YoFlex Express	50°T
3 hours	3	Yogurt	YoFlex Express	58°T
4 hours	4	Yogurt	YoFlex Express	68°T
5 hours	5	Yogurt	YoFlex Express	81°T
6 hours	6	Yogurt	YoFlex Express	89°T

Table 4. Titratable acidity values in the batch inoculated with *Flora Danica* (mesophilic culture)

Time interval from inoculation	Sample number	Type of sample	Inoculated culture	Acidity value (°T)
1 hour	1	Sana	Flora Danica	22°T
2 hours	2	Sana	Flora Danica	43°T
3 hours	3	Sana	Flora Danica	52°T
4 hours	4	Sana	Flora Danica	62°T
5 hours	5	Sana	Flora Danica	78°T
6 hours	6	Sana	Flora Danica	87°T

For comparative visualization, the quantified acidity values were plotted graphically (Figure 7). The graph shows a steady increase in titratable acidity over six-hour fermentation period for both batches: batch 1 (blue line) for yogurt (thermophilic culture) and batch 2 (orange line) for fermented milk (sana), (mesophilic culture). This upward trend reflects progressive microbial activity responsible for fermentation.

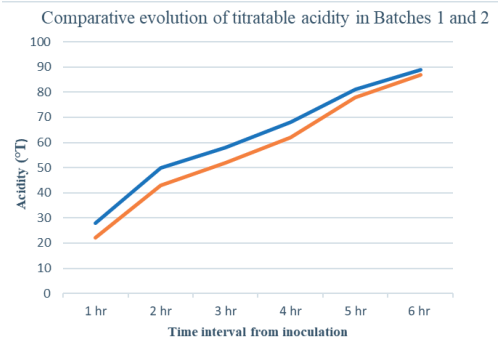

Figure 7. Comparative graphical representation of the titratable acidity of batches 1 and 2

Comparatively, batch 1 exhibited a faster and more pronounced increase in acidity during the initial hours of fermentation, while in contrast batch 2 showed a slower rate of acidification. Throughout the fermentation period, batch 1 consistently reached higher acidity values. The difference was most pronounced during the early stages and decreased toward the end, with batch 2 approaching acidity levels similar to batch 1 as the curves converged.

Both batches are capable of achieving high titratable acidity; however, the thermophilic culture in batch 1 is more efficient in rapidly producing elevated acidity in the early hours, requiring careful monitoring to prevent excessive acidification of the final product.

Production technology and sensory characteristics of a whey- based kefir (new product) – from concept to prototype

This section presents the results of the efforts undertaken to develop an innovative fermented dairy product: a kefir formulated using sweet whey as the substrate. The study aimed to inoculate the sweet whey with selected probiotic cultures, producing a whey-based kefir while promoting full utilization of industrial by-products, reducing ecological impact, and contributing to the diversification of dairy products on the market (Petcu et al., 2020).

The primary raw material used as a substrate for the new product was sweet whey obtained in the study facility as a by-product of the cheese-making process.

The initial step in formulating the product involved the collection of sweet whey, followed by thermal conditioning to reach the optimal range for the subsequent inoculation stage. Inoculation was performed according to the dosing and usage instructions specified in the technical sheet, followed by homogenization to ensure to ensure mixture uniformity and transfer to the thermostatic chamber to initiate controlled fermentation.

For the evaluation and monitoring of the fermentation process, after transferring the containers to the thermostatic chamber, the whey-based kefir was subjected to periodic determination of titratable acidity using the Thörner method. The obtained values were calculated according to the reference formula, expressed in Thörner degrees (°T), and summarized in Table 5.

Table 5. Titratable acidity values during the fermentation of whey-based kefir

Time interval from inoculation	Type of sample	Acidity value (°T)
1 hour	whey-based kefir	18°T
2 hours	whey-based kefir	24°T
3 hours	whey-based kefir	33°T
4 hours	whey-based kefir	39°T
5 hours	whey-based kefir	55°T
6 hours	whey-based kefir	74°T

The graph in Figure 8 shows an exponential increase in acidity, reflecting consistent activity of the lactic cultures as fermentation progresses. Initially, titratable acidity increases slowly during the first two hours after inoculation, remains relatively stable between hours 2 and 4, and accelerates toward the end of the monitoring period, indicating heightened microbial activity. This accelerated increase contributes to achieving the optimal fermentation point for the whey-based kefir, significantly influencing its taste and texture, which become progressively more acidic or viscous as fermentation advances.

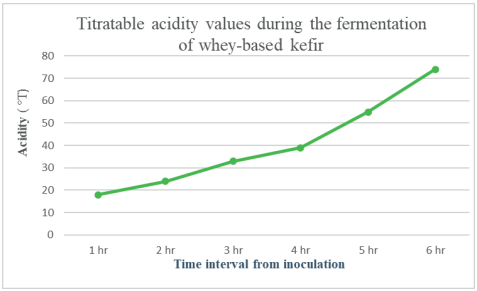

Figure 8. Graphical representation of titratable acidity during the fermentation of whey-based kefir

Following completion of the fermentation process and removal of the samples from the thermostatic chamber, three experimental variants of whey-based kefir (Figure 9) were prepared to diversify the product range.

The first variant was plain kefir without additives, serving as the control. The second variant included finely chopped dried mint and salt, designed to provide a refreshing, slightly salty flavour profile. The third variant incorporated linden honey with additional essential oils, aiming to create a product with sweet aromatic notes.

Figure 9. From left to right: sample of honey, plain kefir, mint kefir (Original)

Considering the objective of this study to propose an innovative dairy product and assess its potential acceptability, it was deemed appropriate to design and implement a structured questionnaire in two stages. The first stage aimed to evaluate the receptivity of potential consumers to the concept of whey-based kefir, while the second stage targeted individuals who participated in the actual product testing to assess their perceptions.

For a clear and concise interpretation of the responses collected, the data were synthesized and presented graphically in the form of charts, thereby facilitating analysis of the response distribution and highlighting general trends expressed by the participants.

From a total of 20 questions included in the questionnaire, four relevant items were selected Their results, following completion by 50 respondents, 15 of whom participated in the tasting of the newly developed products, were summarized and represented in the form of diagrams, providing a graphical illustration of the statistical outcomes.

The results presented in Figure 10 highlight that participants demonstrated a high level of interest in the concept of a whey-based kefir. Approximately half of the respondents (48%) assigned the maximum score (5 - very interesting), while 36% awarded a score of 4. No participant assigned scores of 1 or 2, suggesting a promising potential for the newly developed product.

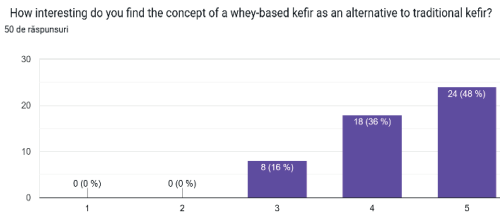

Figure 10. Schematic representation of the responses regarding the new product concept

The diagram in Figure 11 illustrates participants perspectives on the use of whey in the development of a why-based kefir as a novel product. The concept of innovation and sustainability was highly valued by a substantial proportion of respondents (62%), while 38% rated it as moderate, indicating an overall favourable attitude among all participants.

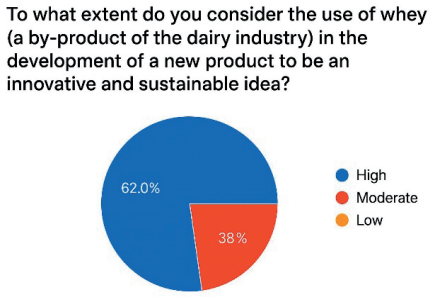

Figure 11. Schematic representation of the responses regarding the sustainable use of whey

The diagram in Figure 12 highlights the opinions of individuals who participated in the tasting of the newly developed whey-based kefir variants (plain, honey-flavoured, mint-flavoured) regarding the perception of acidity. Among the 15 responses, a significant proportion of 86.7% evaluated the acidity as moderate, while 13.3% perceived it as high. These results suggest a positive sensory evaluation in terms of acidity, providing valuable insights for adjusting the formulation in line with consumer preferences.

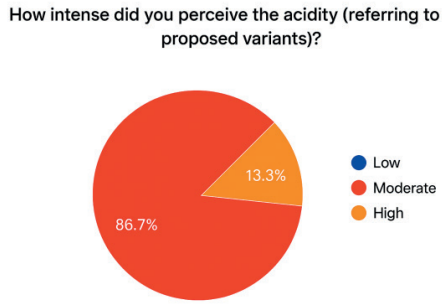

Figure 12. Schematic representation of the responses regarding the perception of acidity, addressed to the tasting participants

The results presented in Figure 13 emphasize consumers preference for the flavoured experimental variants of whey-based kefir, with honey and dried mint each receiving 53.3% of the votes, while the plain variant without additives was chosen by only 20% of participants. This data highlights the higher consumption preference for flavoured products among consumers.

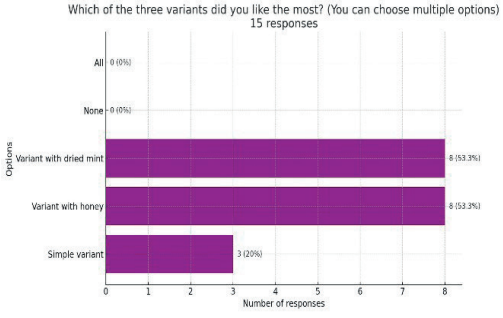

Figure 13. Schematic representation of the responses regarding the preferred choice of the whey-based kefir variant, addressed to the tasting participants

CONCLUSIONS

The findings of this study highlight the influence of lactic cultures on the fermentation process and on the sensory characteristics of fermented dairy products, as well as the potential for whey valorisation through the development of a whey-based kefir as a new product in the dairy industry.

From the analysis of six batches of fermented dairy products produced using two types of lactic cultures (thermophilic and mesophilic), stored under optimal and also exposed to elevated temperatures for six hours, it was observed that storage temperature did not significantly affect coagulation time or the sensory properties of the final products.

Titratable acidity determinations of products obtained with thermophilic and mesophilic cultures revealed differences in acidification dynamics. Thermophilic cultures exhibited a more rapid acidification during the initial hours after inoculation, while mesophilic cultures showed a slower but consistent fermentation.

The whey inoculated with lactic cultures could be used to develop a new product: whey-based kefir, was supported by both experimental results and sensory evaluation, including a consumer survey. The concept received favourable feedback, with over half of respondents expressing positive acceptance: 48% awarding the highest score (5) and 36% scoring it 4. Acidity was also positively rated, with 86,7% of participants considering it “moderate”. Among the three experimental product variants, the flavoured kefirs with honey and dried mint were the most appreciated, each achieving a preference rate of 53.3%, confirming their consumption potential.

Further nutritional investigations of whey-based kefir could provide additional insights for its integration into the fermented dairy market, while continued research may ultimately support a patent application.

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