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INFLUENCE OF STARTER CULTURE AND RENNET IN MOZZARELLA CHEESE PROCESSING

SUMMARY

Mozzarella is well-known for its unique stretchy texture and rich flavor, making it a popular choice in various culinary applications, particularly in pizza. The aim of this study was to evaluate the influence of starter-culture and rennet on physico-chemical properties of mozzarella cheese, manufactured by an established and a new modified technology (V1: LYOFAST+MAXIREN XDS and V2:TCC-50+CHY-MAX M200). Faster fermentation was observed in V1, averaging 226 min ± 33.8, while in V2 it lasted 266 min ± 19.4. The acidity decreased in both variants, but a more pronounced downward trend in V1, and after the addition of the rennet MAXIREN XDS, a slight increase of pH was noticed. Chemical analysis demonstrated differences between the two variants, particularly in protein and fat content. V1 exhibited lower protein content compared to V2. Conversely, fat in dry matter showed significant variations, with V1 having lower values compared to V2. Additionally, V1 showed a moderately lower salt content, correlating with a lower moisture content. Higher mozzarella yield with average value of 11,90±0,20 % was achieved for V2, whereas for V1 was 11,63±1,67 %.

Keywords: mozzarella cheese, starter culture, rennet, physicochemical properties, yield

INTRODUCTION

Mozzarella is a popular unripened, soft, and white cheese known for its melting and elastic properties, making it ideal for pizzas. This is due to its high moisture content and proteins found in the curd that, when heated, form a stretchy structure. This type of cheese is representative of the "pasta filata" category of cheeses and is produced using either the direct acidification process or the more

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common use of starter culture. The direct acidification process relies on the acidification of the milk with an acid like vinegar or lemon juice. In contrast, the starter culture method relies on using a lactic acid bacteria culture to acidify the milk. The former method is more traditional and results in softer cheese, while the latter is faster and produces cheese with a more elastic texture. The use of starter cultures is crucial in producing mozzarella as it determines the cheese's sensory properties and helps create a desirable acidic environment (Varnam and Sutherland, 1994). Industries with higher capacity use concentrated cultures, lyophilized concentrated-frozen, or deep-frozen starter cultures. The amount added to a certain volume of milk is defined by the producers (Matijević *et al*., 2015). Different starter cultures are used in mozzarella production, such as *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Enterococcus faecium* and/or *E. faecalis*, *Streptococcus thermophilus*, *L. helveticus* and *L. Delbrueckii* subsp. *Lactis* (Parente *et al*. 2017). *Streptococcus thermophilus,* which is most frequently used in the manufacturing of mozzarella, works together with *Lactobacillus delbrueckii* or *L. helveticus* to promote the growth of lactobacilli during fermentation. This starter culture rapidly generates acidity, forming a consistent curd that prevents whey separation by enzymatically breaking down lactose. Its beta-galactosidase richness enables lactose hydrolysis swiftly, particularly in an aerotolerant environment alongside *L. helveticus*. The use of *S. thermophilus* yields a cheese with reduced proteolysis, contrasting with *L. bulgaricus*, which releases multiple amino acids from casein, stimulating *S. thermophilus* growth. The interaction between these cultures supports mozzarella production (Hutkins and Ponne, 1991).

Moreover, rennet is essential to cheese production. Different types of rennet can be used depending on the cheese being made and the desired texture. *Kluyveromyces lactis* is a yeast variety known for its ability to produce beta-galactosides, enzymes that break down lactose into fermentable sugars (Johnson and Erasun, 2011). Additionally, it is capable of converting lactose into lactic acid. Alternatively, CHY MAX M 200 is a pure chymosin form obtained through submerged fermentation using the *Aspergillus niger* var. Awamori. The starter culture and rennet type determine cheese flavor, texture, and other characteristics. Additionally, other factors, such as the milk composition and the conditions under which it is processed, can also affect the final product. Moreover, the technology used in the manufacturing process of mozzarella cheese has a substantial influence on its physicochemical characteristics (Law and Tamime, 2010). Various authors have examined different categories of coagulants and their impact on the qualitative characteristics of cheeses. During the evaluation of several coagulants for the manufacturing of Halloumi cheese, it was demonstrated that Maxiren powder resulted in significantly higher levels of fat and protein losses in the whey compared to other coagulants that were evaluated (El-Zoghby and Abdel-Kader, 2000). In study by Myagkonosov *et al.* (2023) on milk-clotting enzymes for semihard cheese production, it was observed that the use of Fromase

and 'Pepsin' brands resulted in significantly higher fat and dry matter losses in whey compared to Naturen, Chy-max Extra, and Chy-max Supreme clotting enzymes, even when the same amount of enzymes was used. This could potentially affect the quality and yield of the cheese.

This study aims to evaluate the impact of starter culture and rennet on the physicochemical properties of mozzarella cheese manufactured using an established technology and a new modified technology.

MATERIAL AND METHODS

In this study two variants of mozzarella cheese were examined:

a) Variant V1 - inoculated with the LYOFAST starter culture, yogurt culture *Streptococcus thermophilius* 70% and *Lactobacillus helveticus* 30%, and MAXIREN XDS rennet (which is a chymosin from the milk yeast *Kluyveromyces lactis* which is isolated from the microflora of kefir-technology applied in dairy)

b) Variant V2 - inoculated with starter culture TCC-50, which represents strains of the bacteria *Streptococcus thermophilius* and *Lactobacillus helveticus,* and followed by rennet CHY-MAX M 200

Both variations were produced on an industrial scale at a dairy facility located in Gostivar, North Macedonia. Variant 1 was already present in the dairy plant, whereas variant 2 was a newly introduced one.

The temperature and pH value were controlled throughout the technological process until the appropriate pH value was reached. Quality and chemical composition of mozzarella cheese were analysed. Analyzes of the two variants were made in four repetitions at industry level.

After 48 hours from the day of production, the following analyzes were conducted on the final products of both variants: active acidity (pH), protein content, dry matter and salt.

The following methods were used:

•for chemical-physical analyzes of mozzarella: Active acidity – pH-meter 330i WTW; Total proteins – method according to Kjeldahl (ISO 8968:1 2001); Total fat – method according to Gerber; Dry matter - by drying at a temperature of 102 °C; Moisture – gravimetric procedure; Fat in dry matter - gravimetric procedure; Salt - method according to Mohr.

Mozzarella yield was calculated according to the ratio of mass of cheese [kg] to 100 kg of milk, expressed in percentage.

Technological production procedure

The milk was pasteurized using a plate pasteurizer. The capacity of the pasteurizer was 3,000 L/h, and the milk was pasteurized at a temperature of 68 °C for a duration of 15 seconds.

Once the pasteurization process was completed, the milk was cooled to a temperature of 36 °C for variant V1 and approximately 40 °C for variant V2. The milk was then transferred to an 800 L capacity tank, where it was maintained at a temperature of 36 °C for variant V1 and around 40 °C for variant V2. Table 1 illustrates coagulation processes for V1 and V2, with differences in cooling temperatures and specific amounts of cultures and rennet added to pasteurized milk, as prescribed by the producers of rennet and starter culture.

Table 1. Process of coagulation for V1 and V2

V1:	**V2:**
Pasteurized milk – cooling to 36 °C	Pasteurized milk - cooling to 40 °C
Addition of 2.4 L of LYOFAST yogurt culture /800 L of pasteurized milk	Addition 2 L TCC 50/800 L pasteurized milk
Addition 40 ml to 45 ml of rennet to 800 L of pasteurized milk	Addition of 130 ml CHY MAX M rennet to 800 L of pasteurized milk

The milk was left to coagulate in the tank for about 40 minutes before the curd was cut into 2x2 cm pieces using a special knife shown in Figure 1. The cheese grains were then processed and dried.

Figure 1. Cutting and mixing of cheese coagulum

The cheese curd was then placed on special tables designed for whey draining (Figure 2), where it remained for approximately 3.5 hours while the whey was collected in tanks for further processing. Once the curd reached a pH of around 5.0, a stretch test was performed to determine its desired stretchability, as shown in Figure 3.

Figure 2. Whey draining

The curd was then transferred to a mozzarella machine, where it was mixed manually with water steam at a temperature of 100 °C for a period of 10 to 15 minutes. The steam used for steaming the cheese was a 3% NaCl solution. The next stage involved molding the cheese, which is illustrated in Figure 4.

Figure 3. Stretch test

Figure 4. Cheese molding

After the cheese was molded, it was cooled using cold water and then squeezed. Finally, it was packed in polyethylene (PE) bags and stored in a finished products chamber at a temperature of 2 to 4 °C with a shelf life of 7 days. The final appearance for both variants V1 and V2 is shown in Figure 5.

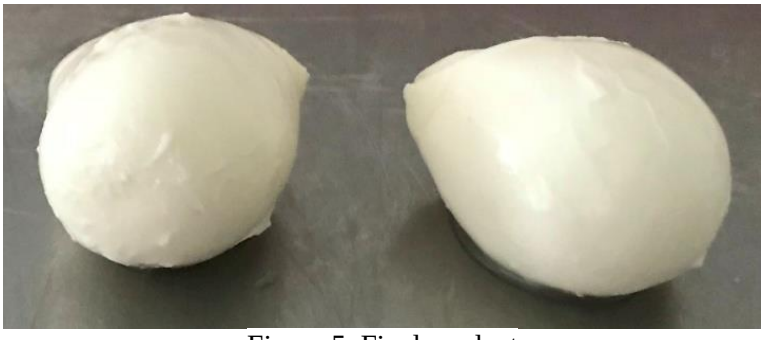

Figure 5. Final product

RESULTS AND DISCUSSION

The results of the fermentation process comparison between variants V1 and V2 are presented in Table 2. Variant V1, which utilized the LYOFAST starter culture, demonstrated a significantly faster fermentation process compared to V2, inoculated with TCC-50. V1 exhibited an average duration of 226 minutes ±33.8, while V2 took 266 minutes ±19.4 to complete fermentation. Notably, the shortest fermentation time observed was 200 minutes when using the yogurt culture.

Table 2. Fermentation process duration

	V1 [min]	V2 [min]
$\bar{x}$	226	266
min	200	255
max	274	295
SD	33.8	19.4

The initiation temperature for fermentation in V1 was slightly lower, around 37-38°C, compared to V2. Moreover, V1 also displayed a higher standard deviation (33.8) in fermentation duration compared to V2 (19.4), indicating greater variability in the process. Graphs 6 and 7 show the dependence of fermentation duration on temperature for the variants V1 and V2.

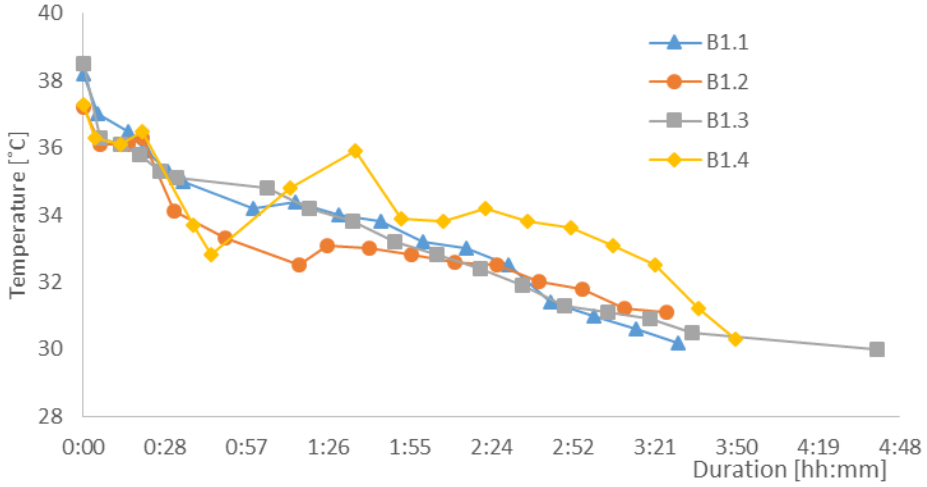

Figure 6. Temperature dependence for duration of fermentation for V1

Of particular interest was the observation that the longest fermentation duration (295 minutes) was recorded in the variant inoculated with TCC-50. This suggests that TCC-50 led to a slower fermentation process compared to the yogurt culture used in V1. Variability between repetitions was also evident, primarily attributed to the use of the LYOFAST starter culture and the storage period of the prepared working culture. The propagation process for obtaining the

working culture with viable bacteria likely contributed to the variability observed in V1.

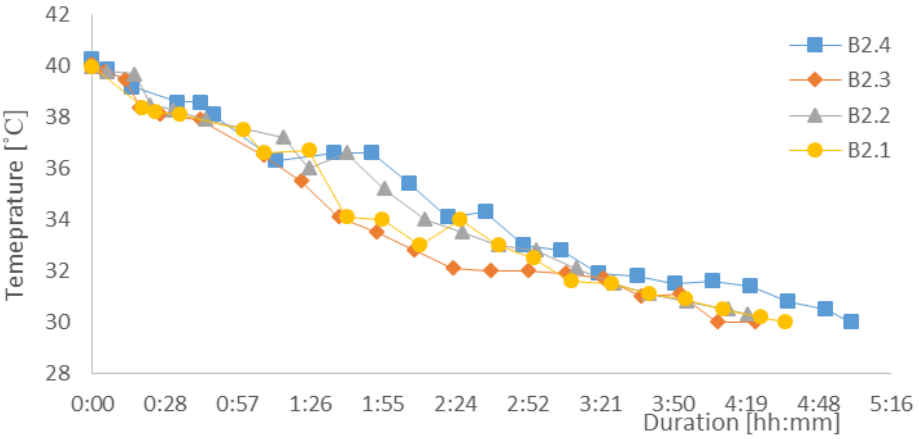

Figure 7. Temperature dependence for duration of fermentation for V2

In contrast, the TCC-50 starter culture, being a direct culture, exhibited more uniform fermentation among the four replicates. Although it had a longer fermentation period due to a higher number of active lactic acid bacteria in the latent (lag) phase, the direct inoculation approach allowed for a more standardized product.

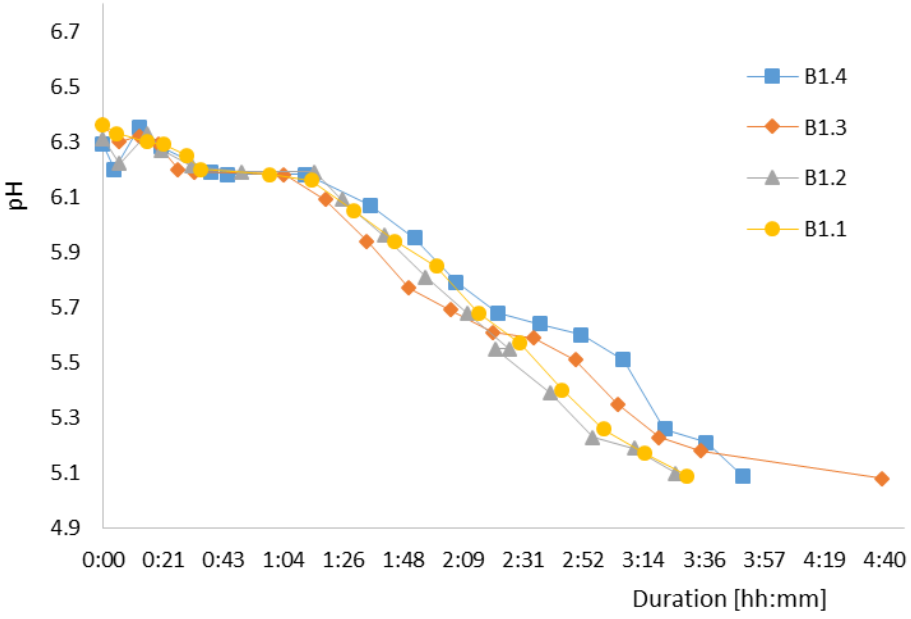

Figure 7. Dynamics of pH during the fermentation for V1

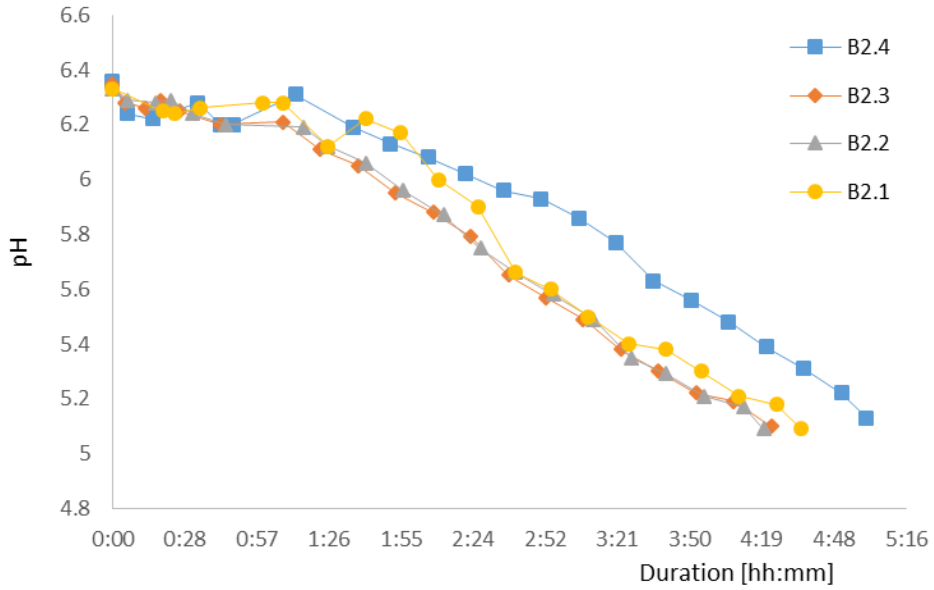

Figure 8. Dynamics of pH during the fermentation for V2

The manufacture of mozzarella is greatly influenced by pH, which affects how malleable the curd becomes in hot water or brine. Lowering curd pH (usually to 5.4–5.1) by lactose–lactic acid conversion is essential for plasticization in pasta filata cheese-making. (Guinee *et al*, 2002).

The figure 7 and a figure 8 illustrate how the duration of fermentation varies with active acidity for V1 and V2 variants, from the moment of inoculation with the starter culture to the final fermentation at pH 5.1. The acidity decreased in both variants, but a more pronounced downward trend in V1, and after the addition of the rennet MAXIREN XDS, a slight increase of pH was noticed.

The minimum initial active acidity in the variant inoculated with the LYOFAST starter culture was 6.29, with an average temperature of 37°C. In TCC-50, the initial pH ranged from 6.33 to 6.36, with an average temperature of 39.5°C. Following the inoculation of the starter culture in both variants, a reduction in active acidity was observed within a temperature range of 36°C to approximately 39°C in V1 and around 39.5°C in V2. The addition of rennet occurred ten minutes after the starter culture was added, when the milk's pH was approximately 6.3. After introducing the rennet, both the temperature and pH continued to decrease.

Upon adding the CHY MAX M 200 rennet (V2) at a temperature of about 40°C, the active acidity slightly decreased. In contrast, for V1, the active acidity increased slightly after adding the MAXIREN XDS rennet at a temperature of about 37°C, and then, after a short time, it decreased again. Throughout the whey pressing process, a continuous decrease in active acidity was observed in both variants, indicating characteristic behavior. Upon reaching a pH of 5.1, the

steaming of the cheese mass commenced, followed by its formation and placement in a cold saline solution to halt the fermentation process. The results on pH are in line with the findings of Guinee et al. (2002), who investigated the effects of Ca content and pH, as well as their interaction, on the texture and heat-induced functionality of Mozzarella cheese. In comparison, Santa *et al*. (2021) reported that the average pH of the cut baskia in kashkaval, belonging to pasta filata cheeses, was 5.32±0.006. Additionally, the average active acidity of the baskia before stretching under hot brine was 5.28±0.005.

Chemical characteristics of two variants of mozzarella are presented in Table 3. The protein content showed lower values (21,20±1,38%) for V1, compared to V2 (22,74±1,19%) without significance differences, while fat in dry matter (V1: 30,85±2,36 %, V2: 37,36±1,70 %.) shows significant differences.

Furthermore, the study found that V1 had a moderately lower salt content (0.417±0.042%) compared to V2 (0.469±0.052%). This lower salt content in V1 was also correlated with a lower moisture content, which had an average value of 62,99±0.61%. In contrast, V2 exhibited higher levels of salt (0.469±0.052%) and moisture (66.29±0.78%) compared to V1.

Table 3. Chemical parameter for two variants of mozzarella cheese

Parameter	**Variant mozzarella**	$\bar{x}$	**min**	**max**	**SD**	**CV**
Protein	V1	21,20	20,05	23,20	1,38	6,5 %
	V2	22,74	21,50	24,35	1,19	5,2 %
Fat	V1	11,41	10,58	12,25	0,69	6,1 %
	V2	12,59	12,07	12,81	0,35	2,8 %
Moisture	V1	62,99	62,28	63,75	0,61	1,0 %
	V2	66,29	65,57	67,05	0,78	1,2 %
Dry matter	V1	37,01	36,25	37,72	0,61	1,6%
	V2	33,71	32,95	34,43	0,78	2,3%
Fat in dry matter	V1	30,85	28,04	33,79	2,36	7,7 %
	V2	37,36	35,14	38,87	1,70	4,5 %
Salt	V1	0,417	0,371	0,463	0,042	10 %
	V2	0,469	0,416	0,533	0,052	11 %

Significant differences in moisture, fat, and protein composition exist between the mozzarella variants (V1 and V2) examined in this study and the findings reported by Imm *et al*. (2003). The fat content of variations V1 (11.41%) and V2 (12.59%) was much lower compared to the 21.13% reported in the study conducted by Imm *et al*. (2003). Similarly, the variants exhibited a protein content that was lower than 26.07% reported in the study conducted by Imm *et al.,* (2003). In a another study conducted by Sameen et al. (2008), the fat content of mozzarella cheese was 16.50±0.50, while the protein content was found to be 14.78±0.78. In comparison to other types of cheese, such as white cheese examined in the study conducted by Jandrić and Savić (2019), the fat content on the first day of manufacture ranged from 20.43% to 22.10%.

The obtained yield for both variants is summarized through the descriptive statistics of all repetitions in Table 4. An evaluation of the yield of fresh mozzarella was conducted, with the V2 mozzarella variant showing a higher yield, averaging 11.90±0.20%. In contrast, the V1 variant had an average yield of 11.63±1.67%. The highest yield value was achieved for V2 at 12.19%, while the lowest value was recorded for V1 at 11.49%.

Table 4. Yield for variants V1 and V2

	V1		**V2**	
	Yield %	**L/kg**	**Yield %**	**L/kg**
$\bar{x}$	11,63 %	8,60	11,90 %	8,40
min	11,49 %	8,40	11,76 %	8,21
max	11,90 %	8,71	12,19 %	8,50
SD	0,19 %	0,14	0,20 %	0,14
Cv	1,67 %	1,65 %	1,68 %	1,66 %

To produce 1 kg of mozzarella in V1, an average of 8.6 L of milk was required, while variant 2 needed an average of 8.4 L. This suggests that implementing the technology used in producing V2 mozzarella would be more economically viable.

Earlier research by Owni and Osman (2009) found that mozzarella cheese produced from heat-treated milk had a higher yield (13.2%) compared to mozzarella produced from raw milk, which had an average yield of 11.65%. The findings of Patel *et al*. (1986) confirmed that thermal treatment of milk resulted in better retention of proteins and mineral salts, leading to higher total dry matter and yield. The increase in yield in the V2 mozzarella variant is likely attributed to denaturation and precipitation of the whey, as well as higher water retention in the soft-formed cheese, resulting in a higher content of moisture and proteins in the product.

In another study, Srbinovska *et al.* (2001) analyzed mozzarella produced from goat's milk, with fat content at 12.50±0.61%, protein at 14.04±0.21%, and total solids at 34.7±0.54%. The yield for this type of mozzarella was 18.13±0.43%, and only 5.5 L of goat's milk was needed to produce 1 kg of mozzarella, showing a significant difference when compared to the results obtained from cow's milk (8.5 L for 1 kg of mozzarella).

Various factors impact the yield of dairy products, particularly those related to the technological quality of milk and animal-related factors, such as genetics, nutrition, physiological and health status. Additionally, the actual production steps (raw material handling and curd coagulation conditions, salting, maturation, etc.) and equipment including hygiene conditions, play a crucial role (AbdEl-Gawad and Ahmed, 2011; Cipolat-Gotet *et al*., 2015; Sales *et al*., 2016). Moreover, an increase in yield can be achieved with homogenized milk, which retains more protein and fat in the curd (Kalit, 2015), while non-specific proteolytic activity can lead to a decrease in cheese yield (Matijević *et al*., 2015).

CONCLUSIONS

In summary, the objective of this study was to assess the influence of starting culture and rennet on the physicochemical characteristics of mozzarella cheese. Two variations were examined: V1, which was inoculated with LYOFAST starter culture and MAXIREN XDS rennet, and V2, which was inoculated with TCC-50 starter culture and CHY-MAX M 200 rennet. The comparison of the fermentation processes between V1 and V2 indicated that V1, which employed the LYOFAST starter culture, demonstrated a notably accelerated fermentation process in contrast to V2. The chemical examination revealed variations in the protein and fat composition, while V2 had a greater fat in dry matter, protein and salt content. Furthermore, it was seen that V2 exhibited a greater yield in comparison to V1, hence suggesting the potential economic feasibility of using the technique utilised in V2 for the manufacture of mozzarella. In conclusion, variant V2 proves to be a more appropriate option for improving mozzarella production in a dairy plant. Despite its longer fermentation period owing to a higher count of active lactic acid bacteria in the latent phase, the direct inoculation approach led to a more standardized product. Considering these factors, it is recommended for dairy plant to adopt the rennet and starter culture utilized in the second experiment for improved mozzarella manufacturing.

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