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Research article

MOZZARELLA CHEESE ENRICHED WITH SPICES: IMPACT ON MUSKY FLAVOUR, NUTRITIONAL PROFILE, TEXTURAL AND MICROSTRUCTURAL PROPERTIES

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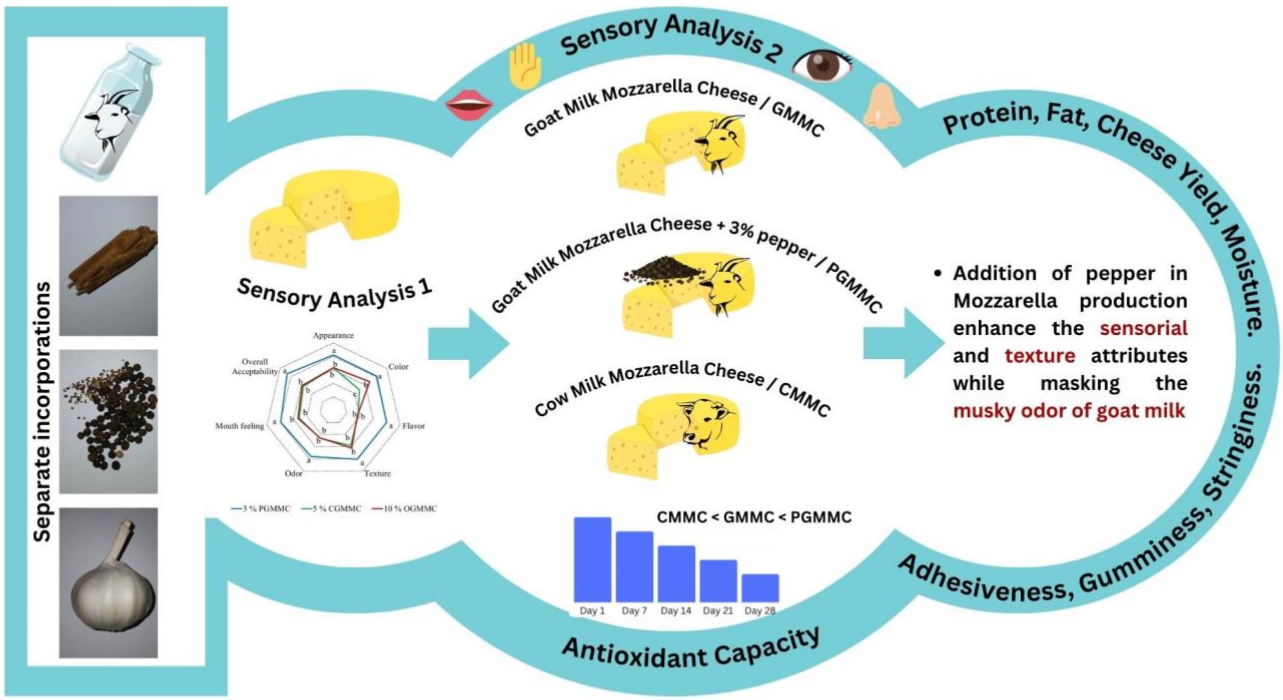
Abstract

Goat milk offers low allergenicity, excellent digestibility, and a rich nutrient profile, but its musky flavour limits consumer acceptance. This study aimed to develop a spice-flavoured goat milk mozzarella cheese to overcome this issue. Preliminary sensory evaluations identified 3% (w/w) pepper (*Piper nigrum*) as the most suitable spice compared to cinnamon (*Cinnamomum verum)* and onion (*Allium cepa*). Selected pepper-incorporated goat milk mozzarella cheese (PGMMC) was developed and compared with conventional goat milk mozzarella cheese (GMMC) and cow milk mozzarella cheese (CMMC) to evaluate flavour masking and product quality. Physicochemical, textural, microstructural, and antioxidant properties were assessed over 28 days of storage. PGMMC showed a higher cheese yield (12.36%), significantly lower fat content (19.94% w/w), and the highest overall acceptance while effectively masking the musky flavour compared to CMMC. PGMMC also exhibited the highest total phenolic content (75.72 mg GAE/100 g), indicating superior antioxidant capacity. Texture analysis revealed GMMC had higher stringiness than PGMMC ($p_{value}<0.05$). Microstructure analysis showed more compact and smooth protein matrices in GMMC and PGMMC compared to CMMC. In conclusion, pepper addition to goat milk mozzarella enhances sensory, textural, and nutritional qualities while successfully masking the undesirable musky flavour, promising an approach to increase consumer appeal.



Graphical abstract

1. Introduction

Goat milk (GM) and cow milk (CM) play a vital role in the dairy industry, where each distinguished by unique attributes such as flavour and nutritional profile. GM and derived dairy products are recognized as healthier foods compared to their CM counterparts due to unique nutritional profiles accompanied by bioactive substances and therapeutic properties (Navamniraj *et al*., 2023). GM contains higher levels of vitamin A and various minerals including potassium, selenium, zinc, calcium, chloride, phosphorus, and copper than CM while GM proteins help to increase the bio-availability of minerals (Lopez-Aliaga *et al*., 2003). Moreover, GM is highly digestible compared to CM which is attributed to the smaller size of fat globules and micelles. Many researchers have claimed that GM has lower allergenic properties thus providing a promising solution for CM allergenicity of infants (Haenlein, 2004). Despite the potential health benefits and functional properties of GM; generally, a lower demand can be observed compared to CM in commercial settings due to the undesirable flavour characteristics. The typical undesirable flavour and aroma of GM is often attributed to the presence of short-chain fatty acids such as caprylic, capric, and caproic acids which limits the further popularization of goat milk among consumers (Posecion *et al*., 2005). Various research efforts have been made to improve the sensory properties of GM and derived dairy products in order to reduce the unpleasant "goaty" flavour (Chen *et al*., 2021).

Production of value-added dairy products such as flavoured or spiced cheese, yoghurt and curd may provide sustainable solutions to overcome such off-flavour properties of fresh goat milk (Kochubei-Lytvynenko *et al*., 2019; Posecion *et al*., 2005).

Cheese made of GM is renowned for its potential health benefits, has garnered increasing attention in recent years. Thus, the present work focuses on the development of a goat milk Mozzarella cheese (GMMC) formulated with spices in attempts to achieve a GMMC that is similarly preferred like cow milk mozzarella cheese (CMMC) alleviating

GM's rancid and musky flavour. The study will help to unveil the opportunities to increase and expand the market of GM offering economic opportunities for farmers and producers, fostering a more sustainable and profitable goat farming and processing industry**.**

2. Materials and methods

2.1. Materials

2.1.1. Sample collection

Cow milk was obtained from jersey-sahiwal cross-breed and goat milk from saanen breed was obtained from the Kottunna Milk Farm situated in the Gampaha district (low country), Sri Lanka. Pepper, cinnamon, and onion powder were purchased from a retail shop at Kiribathgoda, Gampaha district.

2.1.2. Chemical and reagents

All the used chemicals were of analytical reagent grade obtained from the Faculty of Agriculture, Rajarata University of Sri Lanka.

2.2. Methods

2.2.1. Preparation of Mozzarella cheese

Cow milk was obtained from Jersey-Sahiwal crossbred cows while goat milk was obtained from the Saanen breed reared under the same farming conditions in Sri Lanka.

Mozzarella cheese preparation was done following the procedure described by Jana and Tagalpallewar (2017) with several modifications. Pasteurization of milk was done at 72 °C for 15 seconds and cooled the milk to 37 °C. Thermophilic starter culture (CHN-22, Chr.Hansen) with the composition of *Lactococcus cremoris*, *Leuconostoc*, *Lactococcus lactis* subsp. *lactis*, and L*actococcus lactis* subsp. *lactis* biovar diacetylactis was inoculated at a 0.075% w/v rate; afterwards the container was covered with a lid and was kept undisturbed for 45 min at 32 °C. Then, 0.03% (w/w) of rennet powder containing bovine chymosin (chymosin 100%, 650 International Milk-Clotting Units per gram (IMCU g-1); CHY-MAX) was added to the mixture. For the spiced-flavored mozzarella cheeses, selected spices were added before the addition of rennet powder. The closed container was kept for 60 min at 32 °C and cheese curd was cut and allowed to whey drainage for another 30 min at 35-40 °C. The remaining whey was heated to 80-85 °C and the stretching process of cheese in hot whey was practiced for 3–5 min. Then, the cheese was coldly brined (20-23% brine strength) for a sufficient period. Finally, cheese was packed and stored at 4 °C. Sensory evaluations of Mozzarella cheeses were conducted on the 7th day after preparation.

In the experiment, three types of GMMCs, each with three concentrations, were prepared: PGMMC (Pepper-*Piper nigrum* flavoured GMMC) with pepper concentrations of 3%, 6%, and 9% (w/w), CGMMC (Cinnamon-*Cinnamomum verum* flavoured GMMC) with cinnamon concentrations of 3%, 5%, and 7% (w/w), and OGMMC (*Onion-Allium cepa* flavoured GMMC) with onion concentrations of 4%, 6%, and 10%(w/w). After identifying the best spice-incorporated GMMC, additional samples were prepared, including cow milk mozzarella cheese (CMMC) infused with the selected spice and its optimal concentration. Furthermore, CMMC and goat milk mozzarella cheese (GMMC) without any added spices were produced to evaluate and confirm the best spice-incorporated GMMC.

2.2.2. Sensory analysis

Sensory evaluation was conducted employing thirty (30) untrained panelists. Five (05) separate sensory evaluations were carried out during the study period employing the same panelists for each. The tasting panel consist of 45% male and 55% female with age range of 25-60 years. Five consecutive sensory evaluations were carried out for different response type.

2.2.2.1 Sensory test 1

The first sensory evaluation was designed to determine whether pasteurized GM has any unpleasant rancid and musky flavour compared to pasteurized CM. Duo test - Difference test in sensory evaluation was employed for the test.

2.2.2.2. Sensory test 2

The second sensory evaluation was conducted to determine whether GMMC has reduced the undesirable flavour in GM. Duo test - Difference test in sensory evaluation was adapted for the test 2.

2.2.2.3. Sensory test 3

In the third sensory assessment, respondents were asked to score sensory properties including appearance, flavour, colour, texture, aroma, and overall acceptability of cheese types according to a 5-point hedonic scale. The same score was not given for two or more samples.

PGMMC with pepper concentrations of 3%, 6%, and 9% (w/w), CGMMC with cinnamon concentrations of 3%, 5%, and 7% (w/w), and OGMMC with onion concentrations of 4%, 6%, and 10% (w/w) were used in preliminary tests in order to find the ideal spice levels.

Then a third sensory evaluation was conducted to select the best spice-flavoured GMMC among the identified best levels of PGMMC, CGMMC, and OGMMC.

2.2.2.4. Sensory test 4

Subsequently, a fourth sensory analysis was done to ascertain the best concentration level of the selected spice to incorporate into GMMC to mask the musky flavour. Friedman test – Descriptive test in sensory evaluation was employed for the test 4.

2.2.2.5 Sensory test 5

The sensory analysis was to determine whether there was a significant flavour difference between CMMC and GMMC when both had been incorporated with the verified optimum level of the selected best spice. Duo test - Difference test in sensory evaluation was adapted in test 5.

2.3. Laboratory analysis of Mozzarella cheese

As the treatments for further analysis, CMMC without spices (control), GMMC without spices, and 3.0% (w/w) PGMMC were used (Figure 1). Protein, fat, cheese yield, microstructure, and textural and microstructural properties were measured on the 7th day of the maturation period. All other physicochemical properties, antioxidant properties and microbiological properties were measured during the storage period of the 1st, 7th, 14th, 21st, and 28th days.

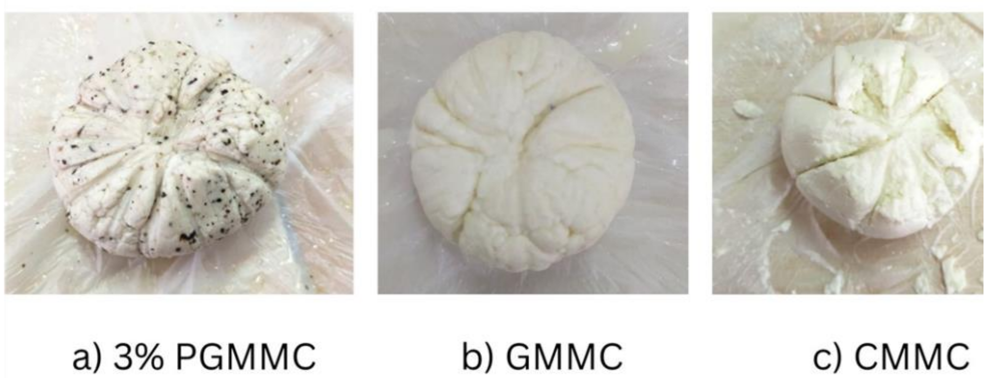


Figure 1. a) 3% Pepper incorporated Goat Milk Mozzarella Cheese; b) Non-spiced Goat Milk Mozzarella Cheese; c) Non-spiced Cow Milk Mozzarella Cheese.

2.3.1.Physiochemical characteristics of Mozzarella cheese

Crude protein and crude fat content were determined by using the Kjeldahl method and the Soxhelt method respectively. The cheese yield was expressed as a percentage of the ratio between the weight of the cheese and of the pasteurized milk sample used at the beginning. The pH of Mozzarella cheese samples was determined at 25˚C by using an electrode immersion pH meter (Thermo Scientific pH 450, Singapore). The titrimetric method was used to determine the titratable acidity, and it was expressed as the lactic acid percentage (w/w) (Gnanarathna *et al*., 2022). Moisture content of the cheese was measured by the oven dry method; 2-3 grams of cheese were dried in 100 ± 2°C for 24 h and expressed as a percentage by loss of weight for the initial weight of the cheese sample. Physiochemical properties were reported as the average of the three replicates.

2.3.2. Antioxidant assays

The ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) method was used to estimate the total antioxidant capacity (Seneviratne *et al*., 2009). By using spectrophotometry, the absorbance reduction at 734 nm was observed. Trolox was used as the standard for the calibration curve. The antioxidant capacity (AOC) was expressed as (µmol Trolox eq/g) Trolox equivalent antioxidant capacity (TEAC). Antioxidant analysis was conducted for three replicates for each treatment.

2.3.3. Folin-Ciocalteu assay for polyphenols

Folin-Ciocalteu's method was used to determine the total phenolic content (TPC) of prepared Mozzarella cheese samples (Seneviratne *et al*., 2009). Gallic acid was used as the standard for the calibration curve. The TPC was expressed as mg GAE/100g.

2.3.4. Instrumental texture analysis

Textural properties of Mozzarella cheese were evaluated instrumentally using a Brookfield texture analyzer (Texture Pro CT V1.8, USA) as described by Hashim *et al*. (2009). Texture properties including hardness, adhesiveness, cohesiveness, gumminess, and stringiness length were calculated using a software program (Texture Pro software, Brookfield Instruments).

2.3.5. Microbiological quality assessment

Total plate count (TPC), coliform count (CC), and yeast and mould count of mozzarella cheese were assessed using Plate count agar, MacConkey agar, and Potato Dextrose Agar (HIMEDIA, India) at 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days respectively, during the storage period.

2.3.6. Microstructure of Mozzarella cheese

Scanning electron microscopic (SEM) images were obtained as the procedure previously described by Jaya (2009) with few modifications. Samples in size of 1 cm × 1 cm × 10 mm were excised from the middle of the Mozzarella cheese samples and were frozen at -18°C. The pre-frozen Mozzarella cheese samples were dried in a laboratory freeze dryer (Alpha 1-4 LS basic, Christ, Germany) for 72 h under the vacuum of 1×10^{-3} kPa and the condenser temperature was -50°C. Once the final moisture content was below 2% (w/w); samples were mounted onto an SEM specimen stub, coated with gold using a sputter coater (Quorum, Germany) at 1×10^{-2} kPa and examined under SEM (ZEISS EVO 18, Germany) operated at 15 kV. Images of the microstructure were obtained in two magnification levels as ×1000 and ×5000.2.2.1 Extraction of phenolic compounds.

2.3. Data analysis

The first, second, and fifth sensory evaluations were tested using the duo-trio test, and 20 responses were the critical value of the test from 30 responses, according to the test table. A Friedman test (alpha = 0.05) was employed to identify whether there were significant differences in cheese types in the third and fourth sensory evaluations.

The experiment design was a complete randomized design (CRD). Cheese yield, fat content, protein content, and textural characteristics were analyzed by the one-way analysis of variance (ANOVA) and mean comparisons were performed by using the Tukey-Kramer HSD test. The rest of the physicochemical parameters and microbiological parameters were analyzed by using the repeated measure ANOVA method and mean comparisons were performed by using the Least square means test. Data was analyzed by SAS software (SAS Institute Inc., Cary, NC, USA.).

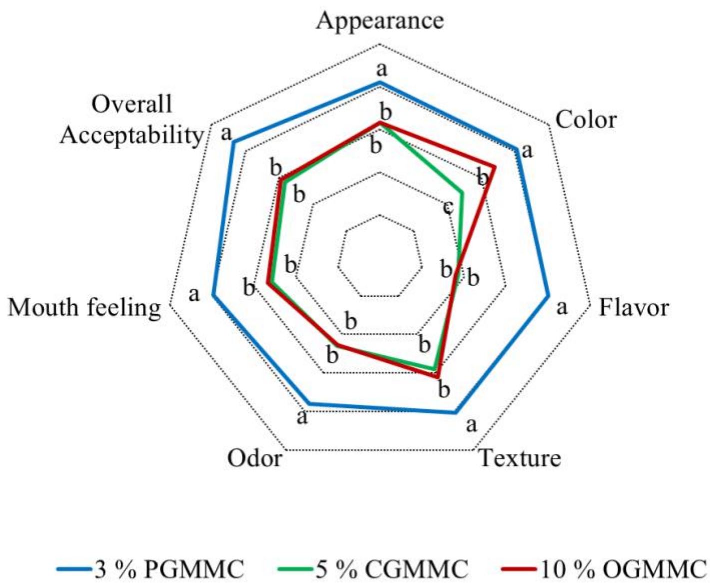


Figure 2. The mean score of tested cheese types for different attributes at third sensory evaluation. Mean scores of an attribute followed by the same letter are not significantly different at $p_{value} < 0.05$. PGMMC: Pepper incorporated Goat Milk Mozzarella Cheese; CGMMC: Cinnamon incorporated Goat Milk Mozzarella Cheese; OGMMC: Onion incorporated Goat Milk Mozzarella Cheese.

3. Results and discussions

3.1. Sensory analysis

The duo test results in the first sensory evaluation demonstrated ($p_{value}<0.05$) that the pasteurized GM sample had a significantly unpleasant musky flavour compared to CM. In the second sensory evaluation, the majority ($p_{value}<0.05$) acknowledged that GMMC had unpleasant musky flavour, but less than pasteurized GM indicating that the Mozzarella cheese production process has contributed to the reduction of musky flavour of GM. According to the results of tested organoleptic properties in the preliminary test of third sensory analysis, the best levels of each PGMMC, CGMMC, and OGMMC were 3%, 5%, and 10% (w/w) respectively. The results of the third sensory analysis indicated that all the tested attributes are statistically significant ($p_{value}<0.05$). The 3% (w/w) PGMMC obtained the most significantly highest rank sums for texture, odour, mouth feel and overall acceptability, establishing it as the most preferred ($p_{value}<0.05$) cheese type (Figure 2). In line with our results, Krumov *et al*. (2010) demonstrated that processed cheese incorporating black pepper exhibited higher sensory performance than the control, suggesting a significant enhancement in sensory quality due to the addition of black pepper. In another study on cottage cheese has shown that, the black pepper addition at level of 1% (w/w) resulted in improved sensory attributes, including flavour, colour, and overall acceptability (Himabindu & Arunkumar, 2017). Further, in agreement with our results, a study conducted on Mudaffara cheese showed that black pepper-added cheese attained the highest scores for taste, odour, and colour, compared to other tested spices even after refrigeration for over four weeks (Ahmed & Foda, 2012). The fifth sensory test results indicated that there was no significant difference between 3% (w/w) pepper-flavoured CMMC and 3% (w/w) PGMMC in preference, suggesting that the 3% PGMMC had no unpleasant musky flavour. Thus, PGMMC added with 3% pepper was used for further evaluation of physiochemical textural and microstructure properties.

3.2. Cheese yield and other physicochemical characteristics of Mozzarella cheese

Both cheese types made of goat milk showed higher ($p_{value}<0.05$) protein content compared to CMMC (Figure 3(A)). Previous studies have shown that the elevated milk fat content in GM would uphold the enhanced protein recovery in goat milk cheese (Pastorino *et al*., 2003).

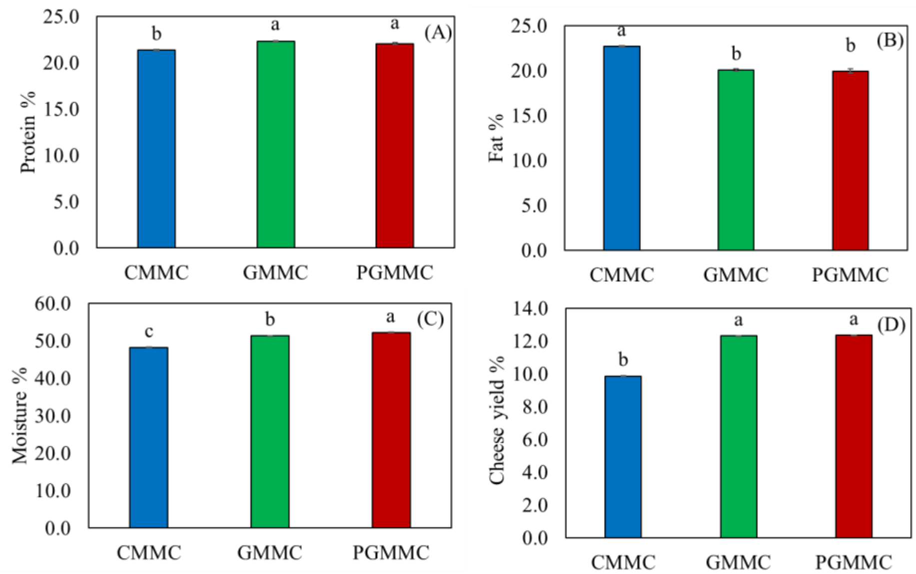


Figure 3. Variation of protein content (A), fat content (B), moisture content (C), and cheese yield (D) in tested cheese types. Means followed by the same letter are not significantly different at α=0.05. CMMC: Cow Milk Mozzarella Cheese, GMMC: Goat Milk Mozzarella Cheese, PGMMC: 3% Pepper incorporated Mozzarella Cheese

As illustrated in Figure 3(B), CMMC displayed the highest fat content (p_{value}<0.05) whereas the fat contents among PGMMC and GMMC were not found to be statistically distinguishable (P > 0.05). The casein-to-protein ratio in milk has been identified as a determinant of fat recovery, but not protein recovery (Pazzola *et al*., 2019). According to Pastorino (2023), higher casein-to-protein ratio is found in CM compared to GM leading to higher fat content in cow milk cheese in accordance with our results. Furthermore, a negative correlation between fat recovery and moisture retention in both GM and CM cheeses was reported by Johnson *et al*. (2001). These findings were also aligned in present study as well; whereas significantly lower fat content and higher moisture content was observed in GMMC than CMMC.

Notably, the PGMMC stands out with the highest (p_{value}<0.05) moisture content (52.25% (w/w)) while GMMC exhibited a significantly higher moisture content (51.35% (w/w)) than CMMC (48.25% (w/w)) (Fig. 2 (C)). Both GMMC and PGMMC had higher (p_{value}<0.05) cheese yields than CMMC (Fig. 2 (D)). Guo *et al*. (2004) found that, fat content, protein content and moisture content of GM have significant positive relationships with the cheese yield. More specifically, a strong correlation has been observed among milk fat content and percentage cheese yield for goat milk cheese (Zeng *et al*., 2007). In agreement with our results, Johnson *et al*. (2001) reported that greater moisture recovery positively correlates with higher cheese yield.

Throughout the storage period, the antioxidant capacities (AOC) of CMMC were found to be higher (p_{value}<0.05) than both GMMCs. This finding is attributed to the fact that cow milk contains a higher number of antioxidant peptide types derived from casein compared to goat milk, which indicates factors that cause variation in the antioxidant capacity of milk among different species (Stobiecka *et al*., 2022). In the present study, PGMMC exhibited consistently higher AOC (p_{value}<0.05) than GMMC, as illustrated in Table 1.

Table 1. Antioxidant capacity (AOC), Total Phenolic content (TPC), pH and titratable acidity of tested cheese types at storage period of 28 days.

Parameter	Day	Mean ± SE		
		CMMC	GMMC	PGMMC
AOC (µmol Trolox eq/g)	1	7.97±0.01^{Aa}	5.30±0.02^{Ca}	6.52±0.02^{Ba}
	7	7.45±0.01^{Ab}	4.89±0.01^{Cb}	5.84±0.01^{Bb}
	14	6.82±0.02^{Ac}	4.75±0.01^{Cc}	5.25±0.02^{Bc}
	21	6.20±0.0^{Ad}	4.14±0.02^{Cd}	4.40±0.02^{Bd}
	28	5.53±0.02^{Ae}	3.10±0.03^{Ce}	3.94±0.01^{Be}
TPC (mg GAE/100 g)	1	55.98±0.17^{Ba}	47.17±0.11^{Ca}	75.72±0.17^{Aa}
	7	46.16±0.17^{Bb}	42.89±0.17^{Cb}	62.39±0.16^{Ab}
	14	41.57±0.17^{Bc}	38.49±0.11^{Cc}	59.56±0.16^{Ac}
	21	38.11±0.11^{Bd}	36.86±0.17^{Cd}	48.05±0.17^{Ad}
	28	34.27±0.17^{Be}	35.34±0.17^{Ce}	40.75±0.11^{Ae}
pH	1	5.92±0.03^{Ca}	6.09±0.01^{Aa}	6.01±0.01^{Ba}
	7	5.63±0.01^{Cb}	5.93±0.01^{Ab}	5.85±0.01^{Bb}
	14	5.52±0.01^{Bc}	5.64±0.01^{Ac}	5.65±0.01^{Ac}
	21	5.43±0.01^{Bd}	5.56±0.01^{Ad}	5.53±0.01^{Ad}
	28	5.32±0.01^{Ce}	5.48±0.01^{Ae}	5.43±0.01^{Be}
Titratable acidity %	1	0.45±0.00^{Be}	0.27±0.00^{Ce}	0.61±0.00^{Ae}
	7	0.55±0.01^{Bd}	0.36±0.00^{Cd}	0.82±0.01^{Ad}
	14	0.78±0.01^{Bc}	0.62±0.00^{Cc}	1.25±0.00^{Ac}
	21	1.03±0.01^{Bb}	0.85±0.01^{Cb}	1.50±0.01^{Ab}
	28	1.27±0.00^{Ba}	1.16±0.00^{Ca}	1.75±0.01^{Aa}

[a] Means in the same row without a common capital letter superscript significantly ($p_{value} < 0.05$) differ for each treatment (cheese type); means in the same column without a common simple letter superscript significantly ($p_{value} < 0.05$) differ among the storage period.

[b]Abbreviations; CMMC: Cow Milk Mozzarella Cheese, GMMC: Goat Milk Mozzarella Cheese, PGMMC: 3% Pepper incorporated Mozzarella Cheese, AOC: Antioxidants capacity, TPC: Total phenolic content, and WHC: Water holding capacity.

The impacts of black pepper on the quality of different cheese types also have consistently reported an increase in antioxidant potential (Ahmed & Foda, 2012). Subsequently, there had been significant ($p_{value}<0.05$) declines in the antioxidant capacities of all three cheese types over the 28-day storage period (Table 1). The total phenolic content (TPC) of PGMMC had the significantly highest values during the storage period. It could be due to black pepper (*Piper nigrum* L.) which is recognized for its rich content of phenolic compounds (Lee *et al*., 2020). Furthermore, CMMC consistently demonstrated higher ($p_{value}<0.05$) TPC than GMMC until the twenty-first day. The TPC of all tested Mozzarella cheese types experienced a significant ($p_{value}<0.05$) decline over the storage period, as indicates in Table 1.

According to Table 1, both GM cheese types consistently exhibited significantly higher pH values compared to the CMMC and there was a significant decline in the pH values of all cheese types over the storage period irrespective of the cheese type. The titratable acidity values of PGMMC over the storage period were the highest ($p_{value}<0.05$) which could have resulted from the higher acid content of pepper (Himabindu & Arunkumar, 2017). CMMC consistently showed higher ($p_{value}<0.05$) titratable acidity than GMMC across the storage period (Table 1). Moreover, a positive gradient in titratable acidity was apparent during the storage for all three cheese types. The activities of starter and non-starter bacteria ferment lactose and metabolize lactic acid, leading to an increase in acidity over the storage period.

3.3. Textural characteristics of cheese

Instrumental texture properties of cheese act as important attributes to be tested together with sensory properties. As depicted in Figure 4(A), CMMC gave the highest (p_{value}<0.05) mean hardness. In agreement with our observation, Półtorak *et al*. (2015) reported that cheeses that had reduced fat content showed the highest hardness values. Further, the current study results could be attributed to the lower pH and moisture content as the hardness being negatively correlated with moisture content and pH level of cheese (Pastorino *et al*., 2003).

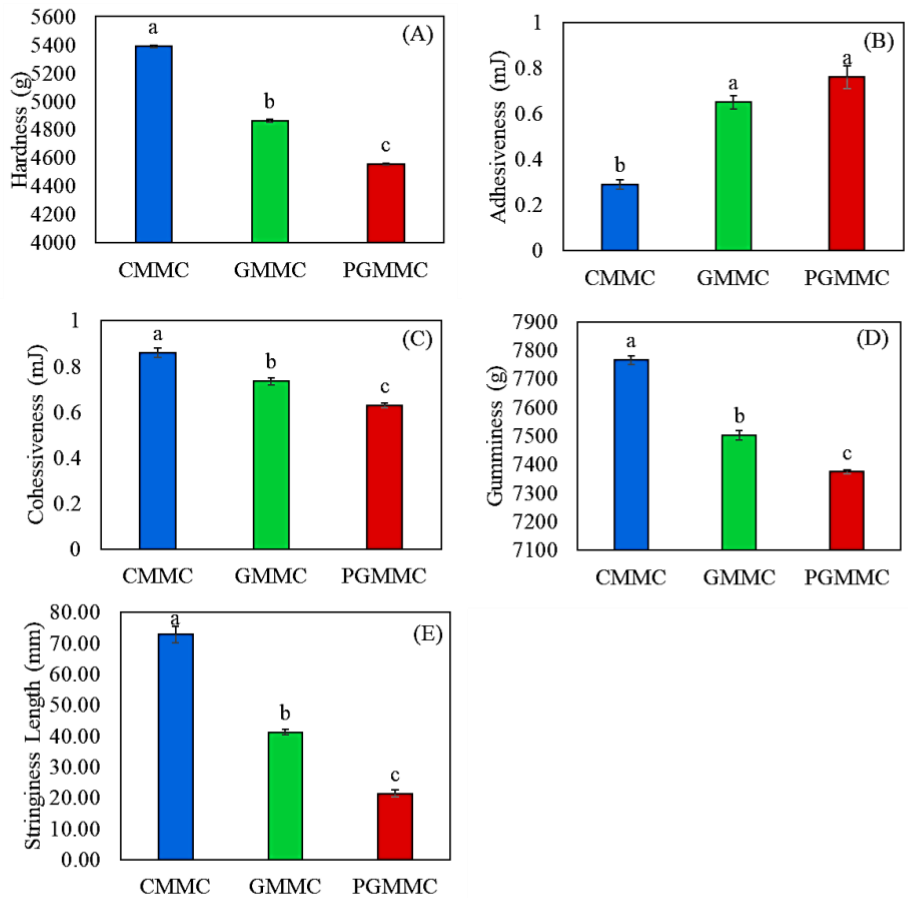


Figure 4. Variation of hardness (A), adhesiveness (B), cohesiveness (C), gumminess (D), and stringiness length (E) in tested cheese types. Means followed by the same letter are not significantly different at p_{value} < 0.05 CMMC: Cow Milk Mozzarella Cheese; GMMC: Goat Milk Mozzarella Cheese; PGMMC: 3 % Pepper incorporated Mozzarella Cheese.

The adhesiveness of both GMMC and PGMMC was significantly higher compared to the adhesiveness of CMMC (Fig. 4B). Moisture content surpassing over 50% has been attributed to the increased adhesiveness of mozzarella cheese which is in line with our results (Kindstedt & Fox, 1993). Yet, the optimum shredability could be obtained with CMMC due to its moderate moisture content (Jana & Tagalpallewar, 2017). Figure 4(C) illustrates both GMMC and PGMMC exhibited (p_{value}<0.05) lower cohesiveness (0.74 mJ and 0.63 mJ) compared to CMMC (0.86 mJ). This is most likely due to small-fat globules in goat milk having interacted more with the casein network, which leads to the weakening of the structure (Logan *et al*., 2017). The highest (p_{value}<0.05) gumminess was found in CMMC while GMMC exhibited higher gumminess than PGMMC (Figure 4D). A review on mozzarella cheese by Kindstedt (1993) described that gumminess is negatively associated with moisture content, assuring our results of higher moisture content in GMMC than in CMMC.

According to Figure 4(E), CMMC exhibited a significantly greater stringiness length compared to others and GMMC showed higher stringiness length (p_{value}<0.05) than PGMMC. The stringiness is expressed quantitatively as stretched length. Based on the review by Jana and Tagalpalllewar (2017), higher fat content and lower moisture content contribute to increased stringiness length in cheese. Thus, a comparatively higher stretch in CMMC is observed due to the higher fat content and lower moisture content in CMMC compared to both PGMMC and GMMC.

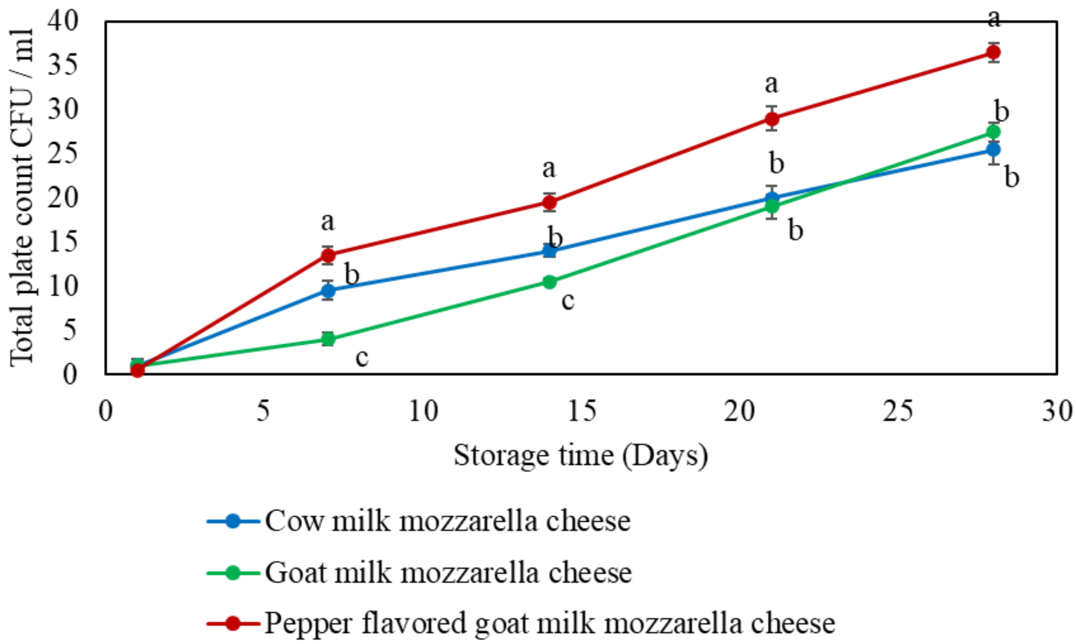


Figure 5. Total Plate Count in tested cheese types during the storage period of 28 days. Means at a storage time followed by the same letter are not significantly different at α=0.05

3.4. Microbiological characteristics of cheese

On the first day of storage, the mean Total plate count (TPC) was 1cfu mL^{-1} in both CMMC and GMMC, while it was 0.5 cfu mL^{-1} in PGMMC. Since the seventh day onwards, PGMMC consistently exhibited higher (p_{value}<0.05) TPC (Figure 5). The CMMC recorded significantly higher TPC values than GMMC before the third week, and on the last day, the GMMC type reported a higher value (p_{value}<0.05). A positive gradient (p_{value}<0.05) is noticeable in all the Mozzarella cheese types throughout the entire storage period (Figure 5). Food safety was ensured with E. coli results, after the 4th week of storage, 1 cfu mL^{-1}, 1 cfu mL^{-1} of and 3 cfu mL^{-1} of E. coli was reported in CMMC, PGMMC and GMMC respectively. According to regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs concern E. coli s an indicator of the degree of hygiene; when the cheese mage with milk and whey which have subjected to heat treatment considered E.coli level as unsatisfactory at >1,000 cfu g^{-1} (Losito *et al*., 2014). Thus, reported level of the current study is far below than the threshold level.

By the end of the fourth week of storage, the mean count of yeast and mould for each tested cheese type was reached to 2.5 cfu mL^{-1}. PGMMC did not exhibit vulnerability to yeast, mould, or E. coli growth due to the positive impact of the antimicrobial activity of pepper (Himabindu & Arunkumar, 2017; Krumov *et al*., 2010).

3.5. Microstructure of Mozzarella Cheese

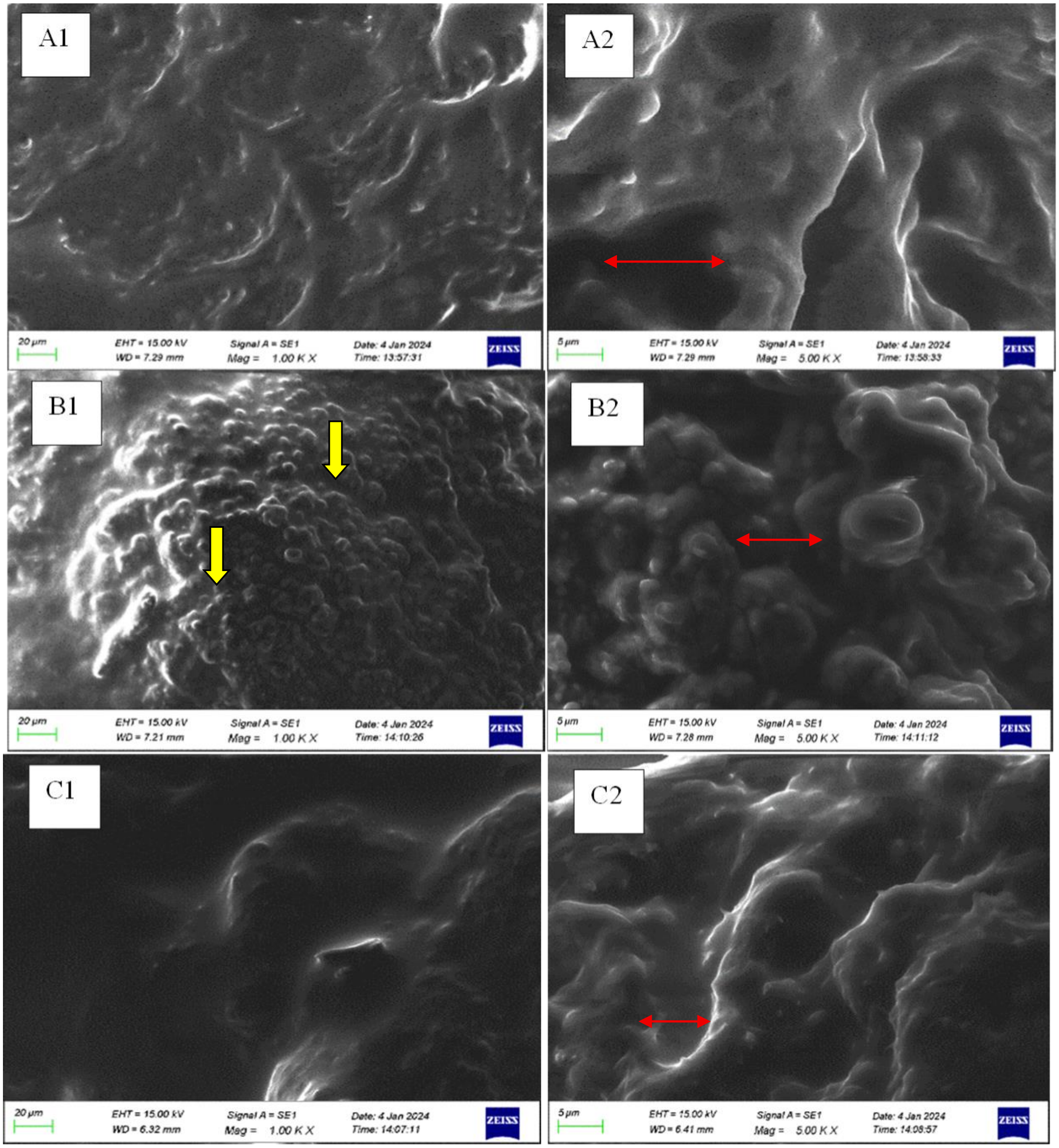


Figure 6. SEMs of CMMC at ×1000 (A1) and ×5000 (A2) magnification, GMMC at ×1000 (B1) and ×5000 (B2) magnification, and PGMMC at ×1000 (C1) and ×5000 (C2) magnification. Yellow colour single head arrows denote the dense protein network and red colour double headed arrow represent the fat globule displaced locations in protein network.

CMMC: Cow Milk Mozzarella Cheese; GMMC: Goat Milk Mozzarella Cheese; PGMMC: 3 % Pepper incorporated Mozzarella Cheese.

A more compact and smooth protein structure was observed in GMMC (Figure 6 B1& B2-yellow colour single headed arrows) compared to CMMC. The protein structure of CMMC (Figure 6 (A1& A2)) appeared less compact than that of GMMC which showed a uniform protein matrix with compressed casein micelles (Cunha *et al*., 2010). Protein matrices of the GMMC and PGMMC had more fat displaced areas compared to protein matrix of CMMC.

The diameter of the displaced fat globules in GM is smaller than that in CM whereas the smaller fat globules facilitate elevated integration into the casein network which was clearly observed through SEM images (Pazzola *et al*., 2019; Weerasingha *et al*., 2022). Similar findings were observed in the microstructure characterization in the present study (red colour double headed arrows represent the variations of the fat globule diameter displaced in the protein matrix of each mozzarella cheese type).

4. Conclusions

There is no difference in flavour between 3% (w/w) PGMMC and 3% (w/w) pepper-flavoured CMMC. Hence, the Mozzarella cheese production process has mitigated the musky flavour characteristic of GM, and pepper flavour has counteracted the residual musky flavour in GMMC. Both GMMC and PGMMC exhibit higher cheese yields compared to CMMC. Compared to CMMC fat contents are lower and protein contents are higher in both GMMC and PGMMC. Nonetheless, there are no significant differences between the PGMMC and GMMC in terms of cheese yield, fat content, or protein content. During the 28-day storage period, PGMMC consistently showed the highest total phenolic content compared to CMMC. When considering the textural properties, adhesiveness is higher in both PGMMC and GMMC compared to CMMC, and cohesiveness, gumminess, and hardness are lower than those of CMMC. The total plate counts of PGMMC would grow significantly higher than those of GMMC and CMMC throughout the 28-day storage period. However, the growth of yeast, mould, and E. coli was not detected in noticeable numbers during the storage period. Based on these finding, it can be concluded that, 3% (w/w) pepper addition would eliminate the musky flavour of the goat milk mozzarella cheese while improving physicochemical, textural and antioxidant properties, and maintaining microbiological safety.

Based on the results, it is evident that GMMC variants exhibit inferior texture properties and this underscores the need for further studies to enhance their texture properties. Additionally, the importance of the development of proper storage and packing methods to extend the shelf life has been pointed out by the research findings. An examination of the impact of the developed product on the nutritional state of the consumer is a timely suggestion because one of its goals that to assist in the mitigation of malnutrition among children, by increasing the consumption of GM.

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