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ORIGINAL PAPER

Gouda cheese with different coagulants and types of milk: physicochemical, biochemical, microbiological, and sensory properties

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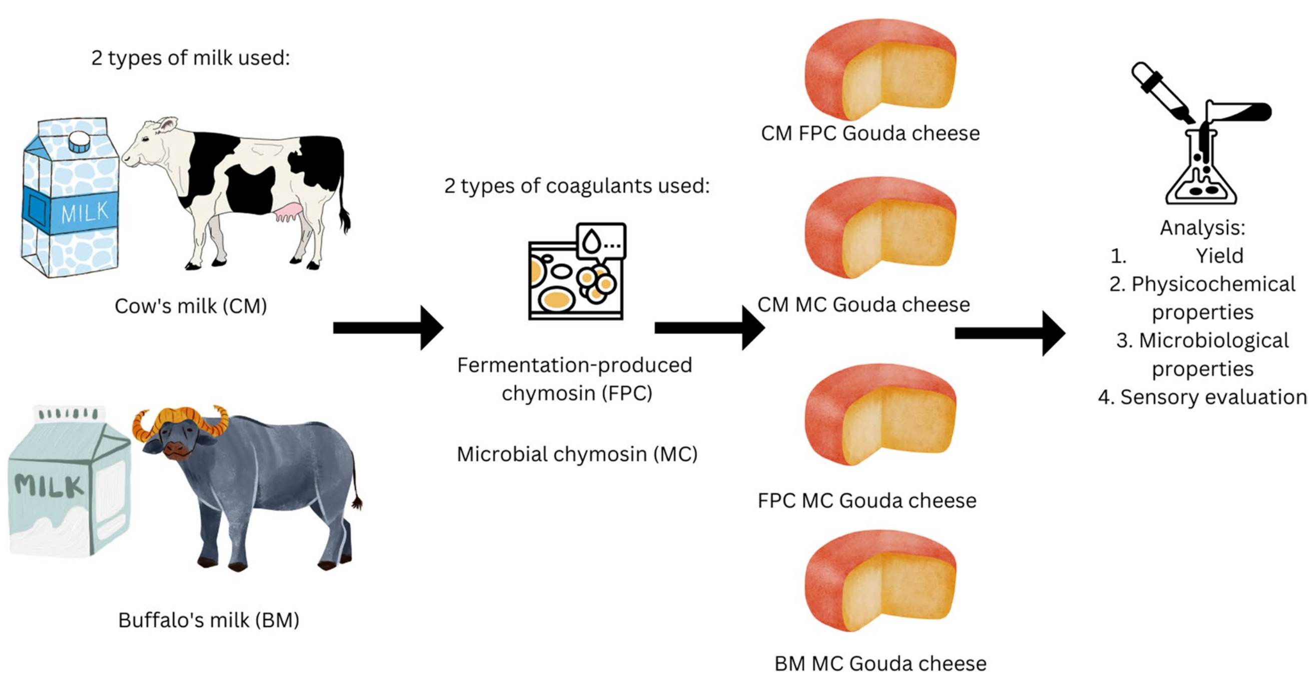
Abstract

The type of coagulant used in cheese production is a significant contributor to proteolysis during ripening. The common application of calf rennet faces restrictions due to ethical, cultural, and dietary concerns. This research determined the physicochemical, biochemical, microbiological, and sensory properties of Gouda cheese using fermentation-produced chymosin (FPC) and microbial coagulant (MC) produced from cow and buffalo milk. Buffalo milk was chosen based on its high-fat content and underutilised application in the cheese industry. Gouda cheese from buffalo milk with FPC has the highest fat (38.5%), moisture (38.77%), cheese production yield (12.88%), as well as the highest monounsaturated and polyunsaturated fatty acids. No yeast or mould are detected in Gouda cheese from buffalo milk FPC or buffalo milk MC. Gouda cheese from buffalo milk FPC has a significantly lower total plate count and lactic acid bacteria count. Based on its overall acceptability score, Gouda cheese from buffalo milk FPC can be a good alternative to traditional Gouda cheese from cow milk.

Graphical abstract



Keywords Semi-hard cheese · Fermentation-produced chymosin · Microbial coagulant · Buffalo milk · Cow milk

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Introduction

Gouda cheese is defined in the United States by the Code of Federal Regulations (CFR). The CFR specifies a maximum moisture content of 45% by weight and a minimum of 46% fat content on a dry-weight basis for Gouda cheeses [1]. Gouda cheese production in the United States increased from 19 to 48 million pounds per year between 2010 and 2014 [1]. In addition, the export of Gouda cheese has also increased drastically since 2008, and it was the cheese with the best potential for export [2]. Generally, Gouda cheese is made from pasteurized cow's whole milk, but some artisan Gouda cheese can be produced using sheep's or goat's whole milk so that the cheese can be aged for a long time [3]. Recently, there has been a rising demand for dairy products produced with buffalo milk because it has a higher nutritional value; it is high in protein, fat, lactose, minerals, total solid content, and non-fat solid contents [4–6]. Buffalo milk can provide more energy per unit volume than cow's milk since it has higher fat and protein content and is more economical for consumers, processors, and producers [5]. However, a previous study reported defects in Gouda cheese made from buffalo milk due to slower acidity development, lower moisture retention, and a lower rate of biochemical changes, which requires modification in processing such as the types of coagulants to improve the sensory characteristics [7].

During the Gouda cheese processing, the curd of Gouda cheese is acidified using a starter culture before the salting and pressing process, and its ripening takes approximately 1–3 months [1, 8]. Chymosin or rennet is a milk coagulant commonly used in Gouda cheese production and is extracted from the abomasum, the inner mucosa found in the fourth stomach chamber of young animals including calf, lamb, and kid [9]. Although animal rennet is a major source of milk-clotting activity, it has drawbacks such as high cost, diet, and religious concerns [9]. Substitutes for cheese-making potential should mimic calf rennet-specific properties: a high ratio of clotting activity to proteolytic activity at the pH and temperature of cheese-making and sufficient thermolability to ensure whey products without remnants of active coagulant [10].

A previous study on Gouda cheese produced by a microbial rennet substitute (*Rhizomucor miehei* proteinase) showed significant differences in proteolysis and bitterness compared with Gouda cheese produced by calf rennet [10]. Fermentation-produced chymosin is, although used to a far lesser extent, is approved in most European countries. However, there is a lack of evidence that cheese yield is affected by fermented chymosin compared with calf rennet [11, 12]. Progress has been made regarding enzyme extraction and fermentation techniques for coagulants to produce Gouda cheese. Therefore, this research aims to determine the physicochemical, biochemical, microbiological, and sensory properties of Gouda cheese produced from cow and buffalo milk using fermentation-produced chymosin and microbial coagulant.

Materials and methods

Materials

Raw buffalo and cow milk samples were procured from Dengkil, Selangor, and kept at 4ºC during transportation to the laboratory. Calcium chloride was purchased from Evachem (Selangor, Malaysia). Mesophilic C101 starter culture was purchased from New England Cheese Making Supply Company (S. Deerfield, MA). Fermentation-produced chymosin (FPC) and microbial coagulant (MC) (CHR Hansen, Denmark). Plate count agar, Man-Rogosa-Sharpe, and potato dextrose agar were purchased from Sigma-Aldrich (St. Louis, MO).

Production of Gouda cheese

Raw fresh buffalo and cow milk were pasteurized for 30 min at 63 °C and cooled down to 4 °C for storage. Four types of Gouda cheese were manufactured using buffalo milk with FPC (BM FPC) and MC (BM MC), and cow milk with FPC (CM FPC) and MC (CM MC). The production of Gouda cheese was conducted following Sulieman et al. [3] with modifications based on our preliminary study. For each cheese type, independent batches of 4 L pasteurized milk were used. The pasteurized milk was heated to 30–33 °C and 3.75 g of C101 starter culture (containing lactose, *Lactococcus lactis* subsp. *lactis,* and *cremoris*) was added, followed by 1.25 g of calcium chloride and left for 30 min. The 3.75 g of fermentation-produced chymosin or microbial coagulant was diluted in 60 mL of distilled water before adding to the milk and left at 30–33 °C for another 40 min for the coagulation process. Once the curd was formed, it was slowly stirred for 15 min for whey protein removal. The whey protein was removed, water (at 60 °C) was added again until the curd reached 34–35 °C and stirred for 10 min. Next, half of the whey protein was removed and replaced with water (at 60 °C) until the curd reached 36–37 °C. Stirring was continued for another 10 min before the curd settled down for 15–20 min. After that, all water was removed, and the curd was transferred into a mould and pressed with 6–8 kg of weight for 30 min on both sides. The step was continued using a higher weight of 12–15 kg for 6–8 h. On the next day, the curd was removed from the mould and immersed in a salt solution of 15% w/w overnight. Finally, the curd was air dried at room temperature (≈25 °C) and packed with vacuum packaging. Gouda was stored at 10–17 °C for 4 weeks for the ripening process. Table 1 shows the formula for Gouda cheese production.

Table 1. The formula for Gouda cheese production.

Yield of Gouda Cheese

The weight of milk used and Gouda cheese produced were recorded. The yield (%) of Gouda cheese produced was calculated by using the formula below [13]:

$$\frac{Weight\ of\ Gouda\ cheese, g}{Weight\ of\ milk, g} \times 100\%$$

Physicochemical properties (pH, proximate, and colour analysis)

The samples were analysed for pH using a benchtop pH meter (Trans BP3001, Mettler Toledo (M), Greifensee, Switzerland). The crude protein analysis was determined from nitrogen content using the Micro Kjeldahl method [14] by multiplying the total nitrogen in the sample with the empirical factor (the conversion factor), 6.38. Next, the Gerber method was used to determine the fat content in milk [14]. The ash content and the moisture content were measured using the oven method [14]. The colour analysis was done by placing a Minolta chromameter (CR-410; Minolta, Ramsey, NJ).

Table 1 The formula for Gouda cheese production

Ingredients	BM FPC	CM FPC	BM MC	CM MC
Type of milk	Buffalo	Cow	Buffalo	Cow
Milk (liter)	4.00	4.00	4.00	4.00
$CaCl_2$ (gram)	1.25	1.25	1.25	1.25
C101 Starter Culture (gram)	3.75	3.75	3.75	3.75
Fermentation produced chymosin (gram)	3.75	3.75		
Microbial coagulant (gram)			3.75	3.75

BM FPC buffalo milk with fermentation-produced chymosin; *CM FPC* cow milk with fermentation-produced chymosin; *BM MC* buffalo milk with microbial coagulant; *CM MC* cow milk with microbial coagulant

Texture analysis

Gouda was cut into 2 cm length × 2 cm height × 2 cm width and left at room temperature for 1 h. Then, using a texture profile analyzer (TA.HD *Plus Connect,* Stable Micro Systems Ltd., Surrey, UK), the cheese cube was compressed at 60 mm/min. The result was recorded as the force to compress the cheese sample up to 75% of its original height within two consecutive compressions. The cheeses were analysed for their hardness, adhesiveness, cohesiveness, gumminess, and chewiness, resilience [15].

Fatty acid analysis properties of cheese

Fatty acid analysis of the cheese was carried out according to Mohsin et al. [16] with GC-FID.

Microbiological properties

About 25 g of the cheese sample was homogenized with 255 mL of 0.1% peptone water in a sterile Stomacher bag for 3 min. Serial decimal dilutions were carried out. Plate count agar (PCA) was used to incubate the culture at 37 °C for 48 hs for total aerobes. For total lactic acid bacteria, Man-Rogosa-Sharpe (MRS) agar was used to incubate the culture aerobically at 37 °C for 48 hs. For total yeast and moulds, potato dextrose agar (PDA) was used to incubate the culture at 25 °C for 7 days [13].

Sensory evaluation

The sensory evaluation of Gouda cheese was carried out using a preference test alongside a 9-point hedonic scale. The panelists were provided water for palate cleansing between the samples [1]. The Gouda cheese samples: BM FPC (buffalo milk with fermentation-produced chymosin), CM FPC (cow milk with fermentation-produced chymosin), BM MC (buffalo milk with microbial coagulant), CM MC (cow milk with microbial coagulant) were cut into 3 cm height × 3 cm length × 3 cm width cubes to be served to a 65-member panel recruited among University Putra Malaysia staff and students to be evaluated organoleptically.

Statistical analysis

Minitab statistical software (Minitab 20.0, Minitab Incorporation, USA) was used to analyze the results. The data were expressed as mean from three replicates ± standard deviation (SD). The significant difference ($p < 0.05$) within means was analyzed by using a two-sample T-test for the raw milk analysis and Analysis of Variance (ANOVA) for the cheese properties analysis. The post hoc test for ANOVA was carried out using Tukey's post hoc.

Results and discussion

Physicochemical properties of raw milk

Milk is an important beverage that provides the important macro- and micronutrients the human body needs. It is recognized for its high levels of protein, fat, lactose, minerals, total solid, and non-fat solid content [4–6]. When compared to cow's milk (CM), buffalo milk (BM) is higher in total solids, protein, fat, and ash with a general composition of 82–83% water, 4–5% protein, 6–12% fat, 0.08% ash, 4–5.5% lactose [17]. The result from Table 2 shows that the protein content (4.40%), fat content (4.67%), and ash content (0.78%) in buffalo milk are significantly higher than in cow milk. In addition, higher moisture content was shown in cow milk (91.26%), while buffalo milk showed comparatively low moisture content (85.95%). Buffalo milk can provide more energy per unit volume than cow's milk because it has higher fat and protein content [5]. This factor also makes it more suitable and economical for consumers, processors, and producers to produce dairy products, especially cheese.

Table 2 also shows a significant difference between the pH values of buffalo and cow milk. The pH of cow milk is slightly higher (6.45) compared to buffalo milk (6.38). Generally, milk has a pH of between 6.4 and 6.8, if the pH of the milk is too high, the bovine might have udder infection or mastitis. Next, the colour analysis of milk was measured in terms of the value of L^* (lightness) with values ranging from 0 to 100, a^* (redness) with values ranging from − 128 to 127, and b^* (yellowness) with values ranging from − 128 to 127. Table 2 shows that cow milk is brighter, yellow, and green than buffalo milk. Through the naked eye, milk appears white. This is because as light hits the casein micelles in the milk, they refract and scatter, resulting in milk appearing white. Carotenoids that are present in the milk can affect the yellowing properties. The colour of milk and dairy products highly depends on their carotenoid concentration. When compared, cow milk is yellowish-white in colour, while buffalo milk has creamy-white colour. This is because the beta-carotene pigment (a type of carotenoid) that is present in buffalo milk is converted into a colourless vitamin A compound, making it appear whiter.

Yield of cheese

Cheese yield is one of the critical things to look at in cheese making because this will affect the profit of the cheesemaker. Cheese yield is affected by many factors like milk quality and composition, pre-treatments, type of coagulant used, curd firmness during cutting, and curd handling process. Table 3 shows the yield of cheese obtained. Cheese produced using buffalo milk gives the highest yield, with a 12.88% yield of cheese made from buffalo milk with

Table 2 Proximate, pH and color analysis of raw milk

Sample	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	pH	Colour		
						L^*	a^*	b^*
BM	4.40 ± 0.20^{a}	4.67 ± 0.06^{a}	0.78 ± 0.01^{a}	85.95 ± 0.08^{b}	6.38 ± 0.01^{b}	69.31 ± 0.74^{b}	-1.31 ± 0.06^{a}	8.90 ± 0.06^{b}
CM	3.16 ± 0.05^{b}	1.97 ± 0.03^{b}	0.57 ± 0.01^{b}	91.26 ± 0.05^{a}	6.45 ± 0.02^{a}	72.93 ± 1.67^{a}	-2.58 ± 0.13^{b}	10.03 ± 0.28^{a}

Values are expressed as mean ± standard deviation (N = 3). Means with different letters within the same column are significantly different ($p < 0.05$)

BM buffalo milk; *CM* cow milk

Table 3 Yield, proximate, pH and colour of Gouda cheese

Sample	Cheese yield (%)	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	pH	Colour		
							L	a*	b*
BM FPC	12.88 ± 0.00^{a}	25.20 ± 0.63^{ab}	38.5 ± 0.00^{a}	3.69 ± 0.02^{c}	38.77 ± 0.29^{a}	5.15 ± 0.01^{b}	74.78 ± 0.54^{b}	0.29 ± 0.04^{c}	19.08 ± 0.18^{c}
CM FPC	7.73 ± 0.00^{c}	26.57 ± 0.31^{a}	36.1 ± 0.00^{b}	4.94 ± 0.03^{a}	33.08 ± 0.53^{d}	5.06 ± 0.00^{c}	76.58 ± 0.18^{a}	0.88 ± 0.01^{a}	21.40 ± 0.10^{b}
BM MC	11.88 ± 0.00^{b}	21.43 ± 0.90^{c}	35.8 ± 0.00^{c}	4.37 ± 0.07^{b}	37.48 ± 0.14^{b}	5.20 ± 0.00^{a}	76.14 ± 0.24^{a}	0.38 ± 0.01^{d}	19.21 ± 0.20^{c}
CM MC	6.00 ± 0.00^{d}	23.87 ± 0.35^{b}	31.8 ± 0.00^{d}	5.02 ± 0.10^{a}	35.39 ± 0.11^{c}	5.03 ± 0.01^{d}	76.24 ± 0.20^{a}	0.75 ± 0.01^{b}	25.85 ± 0.13^{a}

Values are expressed as mean ± standard deviation (N = 3). Means with different letters within the same column are significantly different ($p < 0.05$)

BM FPC buffalo milk with fermentation-produced chymosin; *CM FPC* cow milk with fermentation-produced chymosin; *BM MC* buffalo milk with microbial coagulant; *CM MC* cow milk with microbial coagulant fermentation-produced chymosin (BM FPC). An 11.88% cheese yield is made from buffalo milk with a microbial coagulant (BM MC). However, both cheeses made from cow milk give a significantly lower yield of only 7.73% and 6.00%, respectively. The higher dry matter content is due to higher protein (particularly casein) and fat content in buffalo milk gives a high yield of cheese, has good processability, and produces good quality cheese. Studies have also found that the casein and fat content of milk have a direct effect and are highly correlated with cheese yield [18].

Cheese yield is an essential indicator of profit for the cheesemaker. It is influenced primarily by milk composition, quality, and other factors, including milk pre-treatments, coagulant type, curd firmness at cutting, and curd handling. During the coagulating process in cheese-making, protein, fat, minerals, and vitamins in the milk are concentrated and separated as solid while the whey will be drained off. The ingredient that carries out this process is the rennet or coagulant. Cheese yield is also affected by the coagulating properties of the milk. When the yield of cheese is compared in terms of the type of coagulant used, it can be observed that cheese produced using fermentation-produced chymosin gives a higher yield compared to cheese produced using microbial coagulant. For example, for the two buffalo milk-produced Gouda cheese, cheese made from fermentation-produced chymosin gives a yield of 12.88%, while the cheese made from microbial coagulant gives a yield of 11.88%. This scenario is similar to cow milk Gouda cheese, whereby the cow milk fermentation-produced chymosin cheese (CM FPC) produces a cheese yield of 7.73%.

In comparison, cow milk microbial coagulant cheese (CM MC) gives only a 6.00% yield. Since fermentation-produced coagulant is identical to the traditional animal rennet or calf chymosin, studies found that no differences in cheese yield were observed when cheese produced using fermentation-produced chymosin and animal rennet was compared [19]. However, Barbano & Rasmussen [19] have shown reduced cheese yield when microbial-derived coagulant is used. The result showed that the cheese yield was reduced by 0.54 and 0.74% compared to fermentation-produced chymosin Cheddar cheese yield when *R. miehei* and *R. pusillus* microbial coagulants were used, respectively. Jacob et al. [20] compared the cheese yield of Gouda cheese made from the traditional animal rennet with *R. miehei*-derived microbial coagulant. The result showed a reduction between 0.50% and 1.22% in cheese yield when *R. miehei*-derived microbial coagulant was used.

Physicochemical properties of Gouda cheese

Table 3 shows the result of proximate analysis for the four Gouda cheeses produced. Previous studies reported the protein content of Gouda cheese is found to be between 20 to 25% done by [3, 21]. The result shows that cheese made with buffalo milk using microbial coagulant has a significantly lower protein content of 21.43% than the other three cheeses. Buffalo milk is higher in protein content, especially casein when compared to cow milk. Hence, it was expected that buffalo milk Gouda cheese should have a higher protein content than cow milk Gouda cheese. The significantly lower protein content in BM MC might be due to loss during the coagulation process.

The fat content of the cheeses lies between 30 to 40%. From the result, BM FPC has the highest fat content of 38.5%, while CM MC has the lowest fat content of only 31.8%. Both Gouda cheeses made using buffalo milk have significantly higher fat content than the other two cow milk cheeses when the same type of coagulant is used. For example, BM FPC has 38.5% fat content while CM FPC only has 36.1% fat content; BM MC has 35.8% fat content, while CM MC only has 31.8% fat content. This may be because the loss of buffalo milk fat with whey is relatively less than that in the case of cow milk. Also, both Gouda cheeses made using fermentation-produced chymosin have significantly higher fat content than the other two cheeses made using microbial coagulant. For example, BM FPC has 38.5% fat content while BM MC only has 35.8% fat content; CM FPC has 36.1% fat content while CM MC only has 31.8% fat content. This may be because the loss of fat with whey is relatively lesser when fermentation-produced chymosin is used.

According to the study by Sulieman et al. [3], Gouda cheese has an ash content of between 3.2% and 4.8%. For the ash content of the cheeses, both cheeses made from cow milk using a microbial coagulant and fermentation-produced chymosin show the highest ash content of 5.02% and 4.94%, respectively. This is followed by 4.37% ash by buffalo milk cheese made using a microbial coagulant and 3.69% ash by buffalo milk cheese made using fermentation-produced chymosin. From the result, we can see that all the cheeses met the moisture requirements for CFR of less than 45% for Gouda cheese. The cheeses produced had moisture content in the range of 35% to 38%, with buffalo milk Gouda cheese made using fermentation-produced chymosin having the highest moisture content of 38.77%, followed by buffalo milk Gouda cheese made using microbial coagulant with a moisture content of 37.48%. Both Gouda cheeses made using cow milk have significantly lower moisture content than the two buffalo cheeses, with 33.08% made using fermentation-produced chymosin and 35.39% made using microbial coagulant. This could be due to the higher fat content in buffalo milk.

The pH value of Gouda cheese ranges from 4.9 to 5.6 [1]. According to the result shown in Table 6, the cheeses' pH is in the range of 5.0 to 5.2. Compared to the pH of milk, the pH of the cheese produced showed a decrement. This is because the lactic acid bacteria turn the milk's lactose sugar into lactic acid, lowering the cheese's pH to milk. From the result, both buffalo milk cheese has a higher pH than cow milk cheeses. This could be due to alkalinization due to lactic acid degradation during cheese ripening.

Colour is one of the most essential attributes which affect consumer perception of a product's quality. Together with attributes like flavour and texture, they are the main attributes that contribute to the overall quality of a product. The colour analysis showed that BM FPC has significantly lower lightness compared to the other 3 kinds of cheese. For redness or a^* value, BM FPC, CM FPC, and BM MC have positive a^* value. This indicates that the cheese is on the red side, while CM MC has a negative a^* value indicating it is on the green side. For the b^* value, we can see that the most yellow cheeses are the two cow milk cheeses with 21.40 b^* value and 25.85 b^* value, respectively. Compared to the cow milk Gouda cheeses, the buffalo milk Gouda cheeses are less yellow with only a 19.08 b^* value and 19.21 b^* value. These results aligned with the study done by Milovanovic et al. [22], whereby cow's cheese has higher yellowness (higher b^* value) compared to buffalo's cheese. The paleness of buffalo milk Gouda cheese is due to the lack of carotene content in buffalo milk. This problem could be overcome by adding an annatto to enhance the colour of the cheese to make it more appealing and attractive to the consumer.

Texture analysis

The main factors affecting the texture of Gouda cheese are moisture content, degree of proteolysis, pH, salt, and fat content [23]. Table 4 shows the result obtained for texture profile analysis of the Gouda cheeses prepared. The cheeses were analysed for their hardness, springiness, cohesiveness, gumminess, chewiness, and resilience. Among these parameters, the Gouda cheese results showed that they are all low in springiness, cohesiveness, and resilience. All three parameters are less than 1% in value for all four types of Gouda cheeses. Springiness is the rate at which a product returns to its original position after deformation force was exerted, cohesiveness is the ability of the product to withstand a second deformation relative to how it behaved under the first deformation, and resilience is the ability of a product to return to its original shape. This shows that all the Gouda cheeses produced were unable to return to their original position after deformation, unable to withstand the second deformation well, and unable to return to their original shape after being pressed.

Hardness is the force necessary to attain a given deformation. The result shown in Table 4 shows that CM MC has the hardest texture, followed by CM FPC, BM MC, and BM FPC. Both Gouda cheeses made from cow's milk are shown to have a harder texture than buffalo milk. This is due to the lower moisture content in cows' milk and Gouda cheese. The hardness is also higher when microbial coagulant is used in cheese-making. Gumminess is the multiplication of hardness and cohesiveness. It is the energy required to disintegrate a semi-solid food into a state ready for swallowing. The result shown in Table 4 shows that CM MC has the highest gumminess, followed by CM FPC, BM MC, and BM FPC. Both cow milk Gouda cheeses showed relatively higher gumminess than the other two buffalo milk Gouda cheeses. The trend in the cheese gumminess is like the hardness of the cheeses. This can be due to the lower moisture content in cow's milk and Gouda cheese than in buffalo milk. Also, the gumminess is higher when microbial coagulant is used in cheese-making. The chewiness is the energy required to masticate a solid food to a state ready for swallowing. It is the multiplication of hardness, cohesiveness, and springiness. CM MC has the highest chewiness, followed by CM FPC, BM MC, and BM FPC. Both cow milk Gouda kinds of cheese showed relatively higher chewiness than the other two buffalo milk Gouda cheese. Also, the chewiness is higher when microbial coagulant is used in cheese-making.

Fatty acid methyl ester analysis

The result for fatty acid methyl ester found in 100 g of the four Gouda cheeses produced is shown in Table 5. For saturated fatty acids (SAFAs), CM FPC has the highest amount of saturated fat (23,949.74 mg/ 100 g sample)

Table 4 Texture profile analysis of Gouda cheese

Sample	Hardness, g	Springiness, %	Cohesiveness, %	Gumminess	Chewiness	Resilience, %
BM FPC	3220.33 ± 277.20^{d}	0.25 ± 0.02^{d}	0.13 ± 0.01^{c}	452.67 ± 34.48^{d}	110.51 ± 4.99^{d}	0.02 ± 0.00^{d}
CM FPC	$10,850.43 \pm 125.87^{b}$	0.46 ± 0.01^{c}	0.20 ± 0.00^{b}	2021.70 ± 136.31^{b}	839.18 ± 45.58^{b}	0.03 ± 0.00^{c}
BM MC	7646.59 ± 101.28^{c}	0.52 ± 0.01^{b}	0.19 ± 0.00^{b}	1656.63 ± 36.91^{c}	678.63 ± 11.65^{c}	0.05 ± 0.00^{b}
CM MC	$15,774.18 \pm 70.31^{a}$	0.86 ± 0.01^{a}	0.23 ± 0.01^{a}	3774.71 ± 234.00^{a}	2750.32 ± 36.91^{a}	0.07 ± 0.00^{a}

Values are expressed as mean ± standard deviation (N = 3). Means with different letters within the same column are significantly different ($p < 0.05$)

BM FPC buffalo milk with fermentation-produced chymosin; *CM FPC* cow milk with fermentation-produced chymosin; *BM MC* buffalo milk with microbial coagulant; *CM MC* cow milk with microbial coagulant

Table 5 Fatty acid methyl ester analysis of Gouda cheese

Structure	mg/100 g sample			
	BM FPC	CM FPC	BM MC	CM MC
C 4	650.43	637.46	608.80	640.76
C 6	310.77	420.65	291.82	412.31
C 8	168.96	255.36	158.67	243.89
C 10	362.60	576.67	339.63	538.27
C 11	8.75	7.43	8.85	7.37
C 12	647.50	1380.13	596.93	1255.77
C 13	23.59	17.12	22.49	15.99
C 14	2485.63	3646.14	2307.94	3238.71
C 15	319.67	256.29	293.84	226.84
C 16	10,734.97	10,007.76	9961.19	8742.91
C 17	163.12	156.54	155.04	171.45
C 18	6444.10	6267.02	5986.18	5376.52
C 20	102.62	65.55	96.08	41.66
C 21	0.00	0.00	0.00	0.00
C 22	53.20	51.68	82.53	90.96
C 23	223.58	145.47	204.45	140.02
C 24	59.66	58.47	71.93	75.71
C 14:1	199.72	263.90	185.38	238.01
C 15:1	59.88	65.07	56.98	61.10
C 16:1	604.50	479.79	524.73	357.01
C 17:1	78.06	72.29	71.70	85.06
C 18:1n9t	0.00	0.00	0.00	0.00
C 18:1n9c	12,934.73	9965.96	11,980.56	8674.63
C 20:1n9	125.64	73.00	118.59	41.03
C 22:1n9	0.00	0.00	0.00	0.00
C 24:1	83.41	84.66	109.77	111.93
C 18:2n6t	199.76	202.04	180.60	173.37
C 18:2n6c	1168.90	734.05	1077.52	646.61
C 18:3n6	30.47	34.48	27.65	29.56
C 18:3n3	147.87	90.37	137.44	82.33
C 20:2	0.00	0.00	0.00	0.00
C 20:3n6	26.45	27.30	24.10	0.00
C 20:3n3	81.45	57.35	76.66	80.24
C 20:4n6	0.00	0.00	0.00	0.00
C 20: 5n3	0.00	0.00	41.94	0.00
C 22:2	0.00	0.00	0.00	0.00
C 22:6n3	0.00	0.00	0.00	0.00

BM FPC buffalo milk with fermentation-produced chymosin; *CM FPC* cow milk with fermentation-produced chymosin; *BM MC* buffalo milk with microbial coagulant; *CM MC* cow milk with microbial coagulant

followed by BM FPC (22,759.16 mg/ 100 g sample), CM MC (21,219.13 mg/ 100 g sample) and finally BM MC (21,186.36 mg/ 100 g sample). SAFAs were found to be higher in Gouda cheese produced using fermentation-produced chymosin. Short-chain fatty acids have an important effect on the aroma of cheese [13]. This is because lipolysis of the milk will release short and medium-chain fatty acids as they are predominantly esterified [24]. From the result, the fatty acid that contributed most to the profile of SAFAs in Gouda cheese is palmitic acid, C 16 followed by stearic acid, C 18, and myristic acid, C 14.

BM FPC has the highest amount of monounsaturated fat (14,085.94 mg/ 100 g sample) followed by BM MC (13,047.71 mg/ 100 g sample), CM FPC (11,004.67 mg/ 100 g sample), and finally CM MC (9568.76 mg/ 100 g sample). MUFAs are important in reducing the bad cholesterol levels in our blood, which then helps to lower the risk of getting cardiovascular diseases. MUFAs have been found to reduce inflammation, improve insulin sensitivity, reduce the risk of getting cancer, and lose weight. From the result in Table 9, the four Gouda cheeses produced are high in oleic fatty acid, C 18:1n9c, and Palmitoleic fatty acid, C 16:1. Both fatty acids were found to be higher in buffalo milk Gouda cheese than cow milk Gouda cheese.

For polyunsaturated fatty acids (PUFAs), BM FPC has the highest amount of polyunsaturated fat (1543.90 mg/ 100 g sample) followed by BM MC (1565.92 mg/ 100 g sample), CM FPC (1145.58 mg/ 100 g sample), and finally CM MC (1012.11 mg/ 100 g sample). Polyunsaturated fatty acids are fatty acids that are rich in omega-3 and omega-6 fats. These fatty acids are the essential fatty acids that cannot be generated by our body and must be obtained from foods. PUFAs are important for blood clotting, brain health, muscle strength, nerve function, and cell growth. Alpha-linolenic acid (ALA), Eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are famous sources of omega-3 fats which have a wide variety of health benefits like antihypertension, antioxidant, anticancer, antiaging, antidepression and can protect us against cardiovascular diseases. From the result shown in Table 6, no DHA was found in all four cheeses. Among the omega-3 tested, C 18:3n3 has the highest amount, with both buffalo milk cheeses having much higher content than the cow milk cheeses (in the range of 80 to 150 mg/100 g sample), followed by C 20:3n3 (in the range of 55 to 82 mg/ 100 g sample). C 20: 5n3 was found only in BM MC Gouda cheese (41.94 mg/100 mg sample).

Omega-6 is an important nutrient that helps to maintain bone health, maintain the reproductive system, stimulate hair, and skin growth, and regulate metabolism. Among the omega-6 tested, C 18:2n6c was found to have the highest amount, with both buffalo milk cheeses having much higher content than the cow milk cheeses (in the range of 600 to 1200 mg/100 g sample), followed by C 18:3n6 (in the range of 25 to 31 mg/100 g sample), and then C 20:3n6 (in the range of 24 to 28 mg/100 g sample. However, C 20:3n6 was only detected in BM FPC, CM FPC, BM MC, and not CM MC. Hence, there a more polyunsaturated fatty acid in buffalo milk Gouda cheese than in cow's milk cheese. Among the fatty acid methyl esters detected, SAFAs have

Table 6 Microbiological properties of Gouda cheese

Sample	Week	Yeast and Mold (Log cfu/mL)	Total Plate Count (Log cfu/mL)	Lactic Acid Bacteria (Log cfu/mL)
BM FPC	1	ND	5.09 ± 0.01^{Da}	ND
	2	ND	3.51 ± 0.03^{Cb}	5.55 ± 0.06^{Ca}
	3	ND	2.94 ± 0.12^{Dc}	3.92 ± 0.02^{Cb}
	4	ND	3.17 ± 0.11^{Dd}	2.36 ± 0.02^{Cc}
CM FPC	1	3.39 ± 0.00^{Ac}	8.83 ± 0.03^{Ab}	9.02 ± 0.01^{Ab}
	2	3.89 ± 0.10^{Ab}	8.81 ± 0.05^{Ab}	8.93 ± 0.03^{Ac}
	3	5.66 ± 0.13^{Aa}	8.30 ± 0.13^{Bc}	8.41 ± 0.03^{Bd}
	4	3.57 ± 0.13^{Bc}	9.07 ± 0.03^{Aa}	9.18 ± 0.06^{Aa}
BM MC	1	ND	8.22 ± 0.05^{Bc}	8.32 ± 0.01^{Bc}
	2	ND	8.47 ± 0.05^{Bb}	8.62 ± 0.01^{Ba}
	3	ND	9.15 ± 0.02^{Aa}	8.41 ± 0.02^{Bb}
	4	ND	8.32 ± 0.12^{Bbc}	8.38 ± 0.06^{Bbc}
CM MC	1	3.15 ± 0.03^{Bb}	7.88 ± 0.10^{Cbc}	8.23 ± 0.03^{Cc}
	2	2.67 ± 0.22^{Bc}	8.74 ± 0.01^{Aa}	8.96 ± 0.02^{Ab}
	3	2.53 ± 0.28^{Bc}	7.82 ± 0.11^{Cc}	8.90 ± 0.04^{Ab}
	4	4.18 ± 0.03^{Aa}	8.07 ± 0.04^{Cb}	9.08 ± 0.06^{Aa}

Values are expressed as mean ± standard deviation (N = 3). Means with different letters within the same column are significantly different ($p < 0.05$). (A-D between different types of cheese samples during the same week of storage; a-d between the different weeks of storage within the same cheese

BM FPC buffalo milk with fermentation-produced chymosin; *CM FPC* cow milk with fermentation-produced chymosin; *BM MC* buffalo milk with microbial coagulant; *CM MC* cow milk with microbial coagulant

the highest content compared to MUFAs and PUFAs. Furthermore, it was found that fermented-produced chymosin Gouda cheeses have higher SAFA content, while buffalo milk Gouda cheese has higher MUFAs and PUFA content.

Microbiological properties of cheese

The factors controlling the growth of microorganisms in cheese include water activity, the concentration of salt, oxidation–reduction potential, pH, the presence of NO_3, a relatively low ripening temperature, and the production of bacteriocins by some starter strains [25]. Table 6 shows the microbiological properties of Gouda cheeses over four weeks of storage. From the table, no yeast and mould were detected in both the buffalo milk and Gouda cheese over four weeks of storage. For the cow milk Gouda cheese, the yeast and mould count for CM FPC are significantly higher than CM MC throughout the storage except for the last week, whereby CM FPC has a yeast and mould count of 3.57 Log cfu/mL while CM MC has yeast and mould count of 4.18 Log cfu/mL.

According to the study done by Park et al. [13], the yeast and mould count detected in a six-week Gouda cheese made from pasteurized milk is 3.99 Log cfu/mL. Yeast and mould grow well at low pH of 2–4, where bacteria cannot grow or grow poorly. They have suitable tolerance toward low pH and high salt concentration. Freshly made cheese with low pH is very susceptible to the growth of yeast and mould, and these microorganisms can proliferate to around 10^6 to 10^7 cfu/g during the early ripening stage [25, 26]. When yeast and mould grow on the cheese's surface, they will oxidize lactate to produce carbon dioxide and water. This, in turn, produces ammonia by deaminating amino acid, which increases pH. When this happens, the pH value of cheese will increase from 5.0 to around 7.5, which is more suitable for bacteria growth [26].

BM FPC has a significantly low total plate count over the storage period compared to three other Gouda cheeses. The other three Gouda cheeses have a total plate count of between 7.5 to 9.5 Log cfu/mL. According to the study done by Park et al. [13], the aerobic bacteria count detected in a six-week Gouda cheese made from pasteurized milk is 5.65 Log cfu/mL. Table 6 shows that BM FPC has a significantly low lactic acid bacteria count over the storage period compared to three other Gouda cheeses. This is because the lactic acid bacteria were non-detected during the first week of storage. The other three Gouda cheeses have lactic acid bacteria count between 8.0 to 9.5 Log cfu/mL. Also, it can be observed that the lactic acid bacteria count for BM FPC decreased over the ripening period. This could be due to brine processing inhibiting these microorganisms' growth. According to the study done by Park et al. [13], the lactic acid bacteria count detected in a six-week Gouda cheese made from pasteurized milk is 5.97 Log cfu/mL. As a result, lactic acid bacteria generally grow more rapidly than yeast and mould.

Sensory evaluation

Sensory evaluation has been carried out using a 9-point hedonic scale. The attributes investigated were colour, texture, aroma, flavour, aftertaste, and overall acceptability. From Table 7, the cheese colour does not affect the consumer's affection significantly. For texture, the texture of the two buffalo milk Gouda cheeses is preferred by the consumer and has a higher rating of 6.49 and 6.38, respectively, compared to cow milk Gouda cheeses. Overall, the aroma of all four cheeses falls in the 5 to 6 score range. Only BM MC has a significantly lower score for aroma, with only 4.62. Both cow milk Gouda cheese has the highest rating for flavour, followed by BM FPC and BM MC. BM MC is the least favoured for both aroma and flavour. This might be due to the poor synergetic properties of the cheese caused by excessive moisture retention. Usually, cheese with high moisture content will have defective flavour characteristics when they mature [27]. Finally, the consumer preferred the

Table 7 Sensory evaluation of Gouda cheese

Sample	Colour	Texture	Aroma	Flavor	Aftertaste	Overall Acceptability
BM FPC	6.88 ± 1.31^a	6.49 ± 1.55^a	5.77 ± 2.02^a	4.51 ± 2.19^{bc}	4.02 ± 2.02^b	4.77 ± 2.07^{bc}
CM FPC	6.97 ± 1.22^a	4.43 ± 1.71^b	6.26 ± 1.38^a	5.08 ± 1.85^{ab}	4.88 ± 1.82^a	5.43 ± 1.69^{ab}
BM MC	6.69 ± 1.35^a	6.38 ± 1.56^a	4.62 ± 2.38^b	3.78 ± 2.11^c	3.92 ± 2.12^b	4.38 ± 2.00^c
CM MC	6.86 ± 1.20^a	5.05 ± 1.77^b	6.43 ± 1.72^a	5.74 ± 1.85^a	5.51 ± 1.58^a	5.91 ± 1.66^a

Values are expressed as mean ± standard deviation (N = 3). Means with different letters within the same column are significantly different ($p < 0.05$)

BM FPC buffalo milk with fermentation-produced chymosin; *CM FPC* cow milk with fermentation-produced chymosin; *BM MC* buffalo milk with microbial coagulant; *CM MC* cow milk with microbial coagulant

aftertaste of both cow milk Gouda cheeses to the buffalo milk Gouda cheeses. The bitter aftertaste was expected to be detected by the consumer; however, the consumer could not distinguish them well from cheeses made from microbial coagulant. For overall acceptability, it can be seen that both cow milk Gouda cheeses have higher consumer acceptability compared to the buffalo milk Gouda cheeses. However, from the result, we can say that BM FPC can be a good alternative for traditional cow milk Gouda cheese when the flavour and aftertaste are improved.

Conclusion

In conclusion, buffalo milk has higher ash, protein, and fat content than cow milk, which indirectly makes Gouda cheese produced using buffalo milk appear whiter. Buffalo milk fermentation-produced chymosin can be a good alternative for traditional cow milk Gouda cheese when its flavour and aftertaste have been improved. Therefore, future works need to be carried out to improve the flavour and aftertaste of Gouda cheese from buffalo milk fermentation-produced chymosin to meet the sensory expectation of the consumers. The limitation of this study is the recruitment of untrained panelists in sensory evaluation due to the limitation of time to recruit and train the panelists. Future works can also be conducted on early recruitment of the sensory panelists to train them, hence they can give a better view of sensory evaluation of these Gouda cheese products.

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