

RESEARCH ARTICLE

Effect of coagulating enzymes and types of milk on the physico-chemical, proximate composition and sensory attributes of iron fortified Mozzarella cheese

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ABSTRACT

Mozzarella cheese is an extremely versatile product that has been widely used by the food sector as an essential ingredient in pizza, cheeseburger, cheese-based salads, etc. The main aim of the present investigation is to study the effect of coagulating enzymes and types of milk on the physico-chemical, proximate composition and sensory attributes of iron fortified mozzarella cheese. Iron-fortified mozzarella cheese was prepared using chymosin (control group) and kiwifruit crude extract (treatment group) from three different types of milk (cow milk 100%, goat milk 100%, and cow: goat milk 50:50 ratio). SDS-PAGE of kiwifruit crude extract exhibited protein bands at 24.5 kDa, 20.3 kDa, and 18.8 kDa. The milk clotting time of kiwifruit crude extract exhibited a shorter coagulation time in the presence of CaCl₂. Highly significant ($p < 0.01$) difference between enzymes, types of milk, and the interaction effect between enzymes and types of milk were noted for pH. Similarly, a highly significant difference ($p < 0.01$) was observed between types of milk and interaction effect between enzymes and types of milk while a significant difference ($p < 0.05$) was observed between enzymes for titratable acidity. The highest moisture and fat content were noted in the control group for goat and mixed milk cheese while fat, ash, and TS were comparably higher in the treatment group in mixed and goat milk cheese samples. The sensory score of mixed milk cheese enjoyed the maximum ratings by the taste panel members. Based on the findings, the present study suggests that mixed milk iron fortified mozzarella cheese (treatment group) was adjudged the best product compared to cow milk or goat milk mozzarella cheese.

Keywords: Kiwi fruit; Mozzarella cheese; Physico-chemical attributes; Proximate composition; Sensory evaluation.

INTRODUCTION

Mozzarella cheese is a soft, unripened, white cheese belonging to the Pasta-filata family (Jana and Mondal, 2011) which has its origin in the Battipaglia region of Italy (Citro, 1981). The Mozzarella cheese has unique functional properties for its ability to melt and stretch on heating and is used by the food sector as an important ingredient (Hammad et al., 2017) in pizza, cheeseburger, cheese-based salads, etc. (Vogt et al., 2015).

Cheese is a milk product derived from the whole, skimmed or partly skimmed milk produced by coagulating the milk protein casein. It may be produced from cows, buffaloes, goats, or sheep milk with a variety range of flavours, textures, shapes and sizes. The milk is initially acidified

prior to addition of non-animal rennet or coagulating agents followed by partial whey draining. The product may contain starter cultures of harmless lactic acid and flavour producing bacteria and cultures of other harmless microorganisms, safe and suitable enzymes and sodium chloride. It may vary in shape and sizes and is available in the market in the form of blocks, slices, cut, shredded or grated cheese. Mozzarella cheese should contain maximum of 60% of moisture and minimum of 35% of fat on dry-matter basis as per the standard laid down by Prevention of Food Adulteration Act & Rules (1955 Amended 2006).

Traditionally, mozzarella cheese is made from water buffalo milk's which is organoleptically superior and nutritionally better (Jana and Mandal, 2011; Vogt et al., 2015), however, it is also prepared from cow's milk, ewe's milk, and goat's

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milk in many western and European countries with proper modifications in the processing technique.

Iron deficiency has been reported to be the common form of micronutrient deficiency in foods along with vitamin A and iodine deficiency (Gonmei and Toteja, 2018). According to WHO (2015), the maternal and neonatal mortality rate in developing countries due to iron deficiency (anaemia) has been reported to be 3.0 million deaths in 2013 and is also an important contributor to overall global mortality. To combat iron deficiency, food fortification has been considered the most practical and cost-effective prevention programme (Hurrell, 2002). As such, dairy products can offer a good vehicle for iron fortification due to their high nutritional value and low iron content.

Since immemorial times, rennet has been used as the main milk coagulating enzyme for cheese production. However, FSSAI (2009) has prohibited the use of animal rennet as a coagulating agent in India due to religious considerations and an increase in the vegetarian population. Hence, food scientists have started to find alternative sources of coagulating enzymes derived either from plants or microbes. Many fruits have active proteolytic enzymes like actinidin (*Actinidia deliciosa*), ficin (*Ficus carica*), and papain (*Carica papaya*) which can act upon the casein of milk. Actinidin, a cysteine protease obtained from kiwifruit (*Actinidia deliciosa*) is found to be fully compatible with the manufacturing conditions of cheese with fewer flavour defects in dairy products as compared to other counterparts such as ficin or papain. Puglisi et al. (2012) reported the use of actinidin in the manufacture of mozzarella cheese with improved sensory attributes in the product.

Hence the present study was aimed to develop appropriate technology for the production of iron-fortified mozzarella cheese from cow's milk, goat's milk, and mixed milk (cow and goat milk 50:50 ratio) using kiwifruit as a protease source and to study its effect on the physico-chemical, proximate and sensory attributes of iron-fortified mozzarella cheese.

MATERIALS AND METHODS

Raw materials

Fresh cow's milk was collected from the Instructional Livestock Farm Complex (ILFC), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India, and goat's milk was procured from nearby localities, viz., Tetelia and Tapesia villages, Sonapur, Kamrup (M), Assam, India, respectively. A cold chain was maintained for the transportation of milk samples from the place of collection to the laboratory and kept in a refrigerator until processing. CHY-MAX

Powder Extract NB containing active milk clotting enzyme chymosin was supplied by CHR HANSEN Pvt. Ltd, Denmark, and 80IMCU/l were used as milk coagulating enzymes for the control samples. Fresh kiwifruits (*Actinidia chinensis*) were procured from Dirang, Arunachal Pradesh, as well as from Nayantara Supermarket, Six Miles, Guwahati, Assam, India. Ferric chloride hexahydrate was procured from Sigma, New Delhi, India, while citric acid of analytical grade, calcium chloride (CaCl₂), and sodium chloride (NaCl) was obtained from Fisher Scientific, India, respectively.

Protein profiling of kiwifruit extract by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Preparation of the crude Kiwifruit extract

The crude extracts were prepared from a fresh homogenate of kiwifruits (*Actinidia chinensis*) following Lo Piero et al. (2011) method. The homogenates were centrifuged in a high-speed refrigerated centrifuge (HERMLEZ36 HK, Germany) at 24,400g for 20min, the supernatant was again subjected to ultracentrifugation (Beckman Coulter, Model: Optima L-100K) at 1,50,000g for 1hour at 4°C. The resultant supernatant was collected for protein profiling using SDS-PAGE.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The protein profiling of the crude extract prepared from kiwifruit was done by SDS-PAGE following the method of Laemmli (1970). Briefly, the test was performed on 4% stacking gel and 12% resolving gel under reducing conditions. Samples were prepared by mixing the respective samples with 2X sample buffer (0.125M Tris HCl, 4.0% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue, pH 6.8) and heated at boiling temperature for 5min. After mixing, each sample was expected to have a final denatured protein concentration of 2mg/ml. Each well of the gel was loaded with 20µl of heat-treated samples and a pre-stained protein marker (Thermo Scientific, USA) was loaded in one well parallel to the samples. Electrophoresis was carried out at a constant current of 20mA for 2 hours or till the indicator dye (bromophenol blue) reached the bottom of the resolving gel. After electrophoresis, the separated proteins were visualised after staining the gel with Coomassie Brilliant Blue stain in a gel documentation system (Biorad, USA.)

Milk clotting activity

The milk clotting activity was measured by the method described by Uchikoba and Kaneda (1996) based on the appearance of clotted flakes on the side of the test tubes.

Preparation of iron-fortified mozzarella cheese

Iron-fortified mozzarella cheese was prepared from cow's milk (100%), goat's milk (100%), and mixed cow and goat milk (50:50) using chymax powder (control group) and kiwifruit crude extract (treatment group) as per standard technique laid down by Kanawjia et al. (2011) with slight modifications. Immediately after procurement, the milk was subjected to filtration followed by standardization to a 3-4% fat level. The standardized milk was heated at 72°C/2min followed by cooling to 4°C. CaCl₂ was added @ 0.02g 100ml⁻¹ prior to acidification. Acidification was accomplished by the addition of 20% (w/v) citric acid to achieve a pH of 5.2–5.4 followed by the addition of FeCl₃·6H₂O @1g/L. The temperature of the acidified milk was subsequently raised to 29°C and CHY-MAX Powder Extract NB was added @80 IMCU/L of milk (control). For treatment groups, kiwifruit extract was used as a coagulating agent @150 µg/ml. The coagulated milk was then allowed to sit for 15 min or until complete coagulation of the milk could be observed. The curd mass was then cut with sterile cheese knives and allowed to remain undisturbed in whey for 5min. Slowly, the temperature of the curd was raised from 29°C to 38°C within 45min along with continuous stirring of the coagulated mass for uniform cooking and then held at 38°C for 30min. Whey was drained off and the curd mass was collected and stretched in hot water (85°C/10min) and molded into a ball. The cheese balls were then immersed in 20% w/v chilled brine solution for 2h followed by surface drying under refrigerated conditions (7-9°C/6h).

Sensory evaluation

The freshly prepared iron-fortified Mozzarella cheese samples were evaluated organoleptically for different quality attributes such as appearance, colour, body & texture, flavour, saltiness, and overall acceptability by a 7-membered semi-trained panel using a 9-point hedonic scale (Larmond, 1987) with 1 being extremely disliked and 9 being extremely liked. Sensory evaluation of the cheese samples was done on the day of preparation by offering it to the semi-trained panel members in the form of thin slices with even cutting of edges to look alike.

Titrateable acidity and pH of the cheese samples

AOAC's (2000) titrimetric method was used for determining the titrateable acidity of cheese samples and the pH of the cheese slurry was measured by a digital pH meter (Metrohm 780).

Evaluation of physicochemical attributes

The total solid (TS), moisture, and ash content of the different cheese samples were determined according to AOAC (2000). The protein content of cheese samples was analyzed by Pelican KELPLUS Classic DX VA automatic

protein estimation unit (Pelican Equipments, Chennai). Protein percentage was calculated by multiplying the nitrogen content by factor 6.38. Ether extract of the oven-dried cheese sample was determined by an automatic Pelican Socplus–SCS04E fat extraction system (Pelican Equipment's, Chennai).

Statistical analysis

The experiment was conducted in a factorial Randomized Block Design (RBD), considering 2 enzymes and 3 types of milk. Two-way ANOVA was performed in the generalized linear model (Proc GLM) $Y_{ijk} = \mu + E_i + M_j + ExM_{ij} + e_{ijk}$

where,

Y_{ijk} = Dependent variable (k^{th} observation in i^{th} enzyme and j^{th} milk).

μ = General effect.

E_i = Effect due to i^{th} enzyme ($i=1,2$).

M_j = Effect due to j^{th} milk ($i=1,2,3$).

ExM_{ij} = Interaction effect due to i^{th} enzyme and j^{th} milk.

e_{ijk} = Effect due to non-assignable causes.

Pair-wise comparison between the means was done by following the method of least significant difference (LSD). Data were analyzed using SAS 9.3 software.

RESULTS AND DISCUSSION

Electrophoretic analysis of kiwifruit crude extract

Crude kiwifruit extract was used for protein profiling using SDS-PAGE. The electrophoretic pattern of the kiwifruit crude extract is depicted in Fig. 1. The protein profile of the column elutes yielded three main bands showing an apparent molecular mass of 24.5 kDa, 20.3 kDa, and 18.8 kDa, respectively (Fig. 1, Lane 2 and 3). The theoretical molecular weight of actinidin is 24.0 kDa (Nieuwenhuizen et al., 2007)

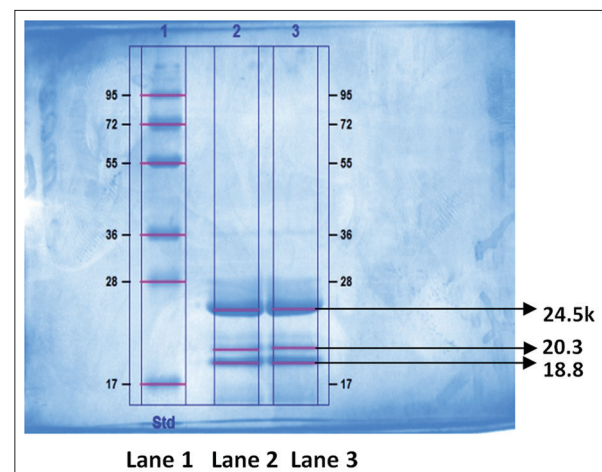


Fig 1. SDS-PAGE of Kiwifruit extract (Lane 1: Protein ladder ranging from 17 kDa to 95 kDa, Lane 2 and 3: Kiwifruit crude extract).

which agrees with the findings of the present study. However, the additional bands of molecular mass at 20.3 kDa and 18.8 kDa might probably be due to the degradation products of the main band formed during the purification procedure. Puglisi et al. (2014) reported two bands showing an apparent molecular mass of 31.7 KDa and 24.0 KDa in kiwi juice aqueous solution, where the molecular weight of one of the band (24.0 KDa) corroborate the findings of the present study. On the contrary, Richards (2014) observed a higher molecular mass of actinidin at 26.0 kDa along with multiple protein bands of molecular mass of 22, 18, and 16 kDa hypothesized to be from the thaumatin-like protein (TLP), which is abundant in kiwifruit and has been attributed to the disruption of three disulfide bonds of actinidin by reducing agents.

Milk clotting activity

The milk clotting activity of kiwifruit crude extract exhibited by the presence of clotted flakes on the side of the test tubes, and it was first observed at 43min and 47min, respectively, in the presence and absence of CaCl_2 , using 80 μg of actinidin, which reflects that presence of CaCl_2 shortens the clotting time than that observed in the absence of the salt. As the protein concentration increased (120, 150, and 200 μg) required time to clot the milk decreased both in the presence and absence of CaCl_2 . In the present study, the clotting time ranged between 13 to 43min (with CaCl_2) and between 17 to 47min (without CaCl_2) with varying concentrations of actinidin. These findings support the findings of Guinee et al. (2007) who demonstrated enhancement of rennet clotting time in the manufacturing of cheese due to the addition of CaCl_2 . The milk clotting times of kiwifruit crude extract in the present study are higher than the reports of Puglisi et al. (2014) who reported that the clotting time of aqueous solution of kiwifruit extracts were 16+0.15, 12+0.08, 3.5+0.016 and 1+0.016 in absence of CaCl_2 ; and 15+0.10, 10+0.05, 2.5+0.016 and <1 in presence of CaCl_2 at a protein concentration of 40, 120, 250 and 380 μg , respectively. Shorter clotting time might be due to the presence of other kiwi proteases that might be present in the actinidin mixture, thus enhancing the availability of actinidin targeted casein residues thereby degrading the substrates and reducing the coagulation time. Shorter clotting time indicates the high proteolytic activity of the kiwifruit extract and is undesirable as it produces soft-bodied cheese. Piero et al. (2011) reported that pure actinidin induces milk coagulation within 24min using 40 μg of protein content, while on the other hand, Alirezai et al. (2011) reported a higher clotting time of actinidin at different pH, temperature, and CaCl_2 concentration. Based on the time taken to coagulate the milk by chymosin in the control group (approximately 30min) and keeping the time factor constant, the final concentration of kiwifruit

extract was standardized at 150 $\mu\text{g}/\text{ml}$ in presence of CaCl_2 in the treatment group for the preparation of iron-fortified mozzarella cheese from different types of milk.

pH

In the present investigation, the pH of the treated samples was significantly lower ($p < 0.01$) than the control group, except for goat milk mozzarella cheese samples. Based on the different types of milk used for the preparation of iron-fortified mozzarella cheese, the pH of goat milk cheese samples (5.45 ± 0.01) was significantly lower ($p < 0.01$) than cow (5.70 ± 0.05) and mixed milk cheese samples (5.74 ± 0.10) in the control group while in the treatment group cow's milk cheese samples (5.10 ± 0.07) were significantly lower ($p < 0.01$) than goat milk (5.56 ± 0.01) and mixed milk cheese samples (5.54 ± 0.02), respectively (Table 1). Similar pH values were reported by Sameen et al. (2002) and Sulieman et al. (2012) for cow's milk cheese. However, the pH values of control groups were slightly higher in the present study which may be attributed to a higher cooking temperature that tends to increase syneresis, reduce curd moisture and lactose and lactate contents, resulting in lower lactate to protein ratio and higher buffering capacity in cheese, thereby increasing the pH values of the end product. The mean pH value of iron-fortified goat milk mozzarella cheese were 5.45 ± 0.01 and 5.56 ± 0.01 for the control and treatment groups, respectively, which are in close agreement with the findings of El-Tahra et al. (2008) and Paz et al. (2016) but significantly lower than cow's milk and mixed milk iron-fortified mozzarella cheese. On the contrary, Delgado et al. (2011) reported a slightly lower pH of goat milk mozzarella cheese (5.18), which might probably be due to a soft pressing of the curd that facilitates whey retention and its subsequent acidification.

Titrateable acidity

The titrateable acidity expressed as per cent lactic acid varied between 0.43 ± 0.02 to 0.75 ± 0.01 (treatment group) and 0.47 ± 0.01 to 0.70 ± 0.01 (control group), respectively (Table 1). Analysis of variance revealed highly significant ($P < 0.01$) differences between types of milk and interaction effect between types of milk and enzymes used, while significant ($P < 0.05$) differences were observed between

Table 1: Effect of enzymes and types of milk on pH* and titrateable acidity (%LA)* of iron fortified mozzarella cheese

Types of Milk	pH		Titrateable Acidity (% lactic acid)	
	Control	Treatment	Control	Treatment
Cow	5.70+0.05 ^{Aa}	5.10+0.07 ^{Ba}	0.51+0.01 ^{Aa}	0.59+0.01 ^{Ba}
Goat	5.45+0.01 ^b	5.56+0.01 ^b	0.70+0.01 ^{Ab}	0.75+0.01 ^{Bb}
Mixed	5.74+0.10 ^{Aa}	5.54+0.02 ^{Bb}	0.47+0.01 ^{Ac}	0.43+0.02 ^{Bc}

n=5; *Mean+SE

Means with common superscript column-wise (lowercase letter) and row-wise (capital letter) do not differ significantly ($p > 0.05$).

the different enzymes used in the experiment. Low lactic acid percent in mixed milk cheese samples compared to cow and goat milk cheese samples in both treatments and control group may be associated with a loss of lactose in the finished product. The findings of the present study agree with the findings of many researchers (Pizaia et al., 2003; Indumathi et al., 2013; Seth and Bajwa, 2015; Waheed et al., 2017). However, Sulieman et al. (2012) have reported much lower titratable acidity in cow's milk mozzarella cheese ($0.203 \pm 0.01\%$) and goat milk mozzarella cheese ($0.13 \pm 0.01\%$), respectively. On the contrary, El-Tahra et al. (2008) reported higher acidity of cow's milk mozzarella cheese and it was attributed to the incorporation of 0.5 and 1.0% of whey protein to the cow's milk.

Chemical proximate composition

The effect of variables (enzyme and milk) on proximate composition are presented in Table 2.

Moisture

The highest moisture content in all the iron-fortified cheese samples was recorded in the control group compared to the treatment group for the different types of milk used for the preparation of the cheese samples. A highly significant difference ($p < 0.01$) was observed between the enzymes used for the preparation of iron fortified goat milk mozzarella cheese. High moisture content in control goat's milk cheese samples (47.15 ± 0.20) might probably be due to a less amount of whey being expelled out during the manufacturing process. Another reason might probably be due to an increase of the cooking temperature which may increase the rate of syneresis outside the curd piece leading to the development of a dehydrated protein layer that inhibits further movement of moisture out of the curd piece and reduces whey loss and increases the moisture content in the product. The findings of the present study can be co-related to a higher TS content in goat milk cheese for the treated group. These findings agree with the findings of Rasovic et al. (2013) and Karki and Ojha (2018). On the contrary, Waheed et al. (2017) reported higher moisture content ranging between 49.45 ± 0.12 to 51.52 ± 0.16 using plant coagulant and between 61.00 to 62.90% using different forms of iron (Khalifa, 1996), while Indumathi et al. (2013) reported low moisture content in iron-fortified Gouda cheese samples which might probably be due to

different processing techniques used in the preparation of mozzarella and gouda cheeses. The variation in the moisture content might also be due to the salting method employed (Guinee et al., 2000), manufacturing technique for preparation of mozzarella cheese (El-Owinand Osman, 2009), type of acidulants used to bring down the pH (Seth and Bajwa, 2015), brining of cheese (Guinee et al., 2007) and cooking temperature (McSweeney, 2007).

Protein

A non-significant difference was recorded for the different types of enzymes and milk used as well as the interaction effect between types of milk and enzymes used for the protein content. The results are in close agreement with the findings of Karki and Ojha (2018) who have reported protein content of $47.65 \pm 1.42\%$ and $48.53 \pm 1.58\%$ in the rennet and kiwi juice coagulated mozzarella cheese samples, respectively. On the contrary, other researchers have reported lower protein content in different types of mozzarella cheese prepared from cow, goat, and mixed milk than the findings of the present study (Suleiman et al., 2012; Queiroga et al., 2013) which can be attributed to the differences in the protein content of the raw milk used, loss of some amount of salt soluble protein during dipping in brine solution and denaturation of protein due to heat treatment.

Fat

The fat content in the control and treated groups ranged between 11.83 ± 0.07 to 13.17 ± 0.03 percent and 9.44 ± 0.55 to 13.22 ± 0.44 percent, respectively. The highest fat content was recorded in iron-fortified mixed milk (13.22 ± 0.44) and the lowest in goat's milk (9.44 ± 0.55). A highly significant difference ($p < 0.01$) was observed between goat milk and mixed milk cheese samples in the control group and between goat milk and cow and mixed milk samples in the treatment group while a non-significant difference was found for the different enzymes used. The results are in close agreement with the findings of Srbinovski et al. (2001), however, Imm et al. (2003), El-Tahra et al. (2008), and Bhattarai and Acharya (2010) reported a higher fat content in mozzarella cheese compared to the present findings. Low-fat content in iron-fortified goat milk mozzarella cheese sample in the present investigation might probably be due to small-sized fat globules that

Table 2: Effect of enzymes and types of milk on proximate composition* of iron fortified mozzarella cheese

Types of Milk	Proximate Composition (%)									
	Moisture		Protein**		Fat**		Ash**		Total Solid	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Cow	44.40+0.56	44.06+0.51	49.94+1.67	49.32+0.25	12.52+0.16 ^{ab}	12.38+0.22 ^a	3.89+0.16	4.11+0.05	55.60+0.55	55.94+0.51
Goat	47.15+0.20 ^a	42.39+0.21 ^b	47.78+0.31	49.83+0.42	11.83+0.07 ^a	9.44+0.55 ^b	3.86+0.03	4.69+0.70	52.85+0.20 ^a	57.38+0.43 ^B
Mixed	45.82+1.06	45.25+0.77	51.21+1.31	50.43+0.38	13.17+0.03 ^b	13.22+0.44 ^a	4.27+0.27	4.09+0.08	54.18+0.98	54.75+0.77

n=5; *Mean+SE; ** On dry matter basis

Means with common superscript column wise (small letter) and row wise (capital letter) does not differ significantly

might have leaked out during stretching of the curd in hot water.

Ash

The results of the mean ash content of the iron-fortified mozzarella cheese samples revealed non-significant ($p>0.05$) between the different types of milk used, enzymes and interaction effect between types of milk and enzymes. Numerically higher ash content in the treated groups might probably due to better binding of the iron within the protein matrix in the cheese samples. The present findings agree with the findings of several other researchers (Imm et al., 2003; EL-Tahra et al., 2008; Bhattarai and Acharya, 2010; Indumathi et al., 2013; Jalili, 2016; Waheed et al., 2017; Liu et al., 2018). Niro et al. (2014) on the contrary have reported high ash content ranging from 6.10 to 8.22 g/100g of DM of mixed ewe and goat milk cheese during the entire storage period and it was attributed to the progressive loss of moisture during the storage period.

Total solids

In the present investigation, the highest total solid content was recorded in the kiwifruit extract coagulated goat milk mozzarella cheese and was significantly higher ($p<0.01$) than the control group, while the other results in terms of TS content were non-significant. The highest total solid content in goat milk cheese samples can be correlated to the low moisture content in the corresponding cheese samples. As the amount of total solid increases, moisture content decreases and vice versa. The amount of whey expelled out in the treatment group was lower than the control group, resulting in the retention of more total solids in the iron-fortified cheese samples coagulated with actinidin. Many researchers have also reported similar findings (EL-Tahra et al., 2008; El-Owni and Osman, 2009; Delgado et al., 2011; Karki and Ojha 2018). However, Suleiman et al. (2012) reported significantly lower total solid content in mixed milk mozzarella cheese compared to cow's milk and goat's milk mozzarella cheese on the 1st and 15th day of the storage period.

Sensory evaluation of iron-fortified mozzarella cheese

The sensory profile is the most unique characteristic that contributes to the overall acceptability of any food

product. The organoleptic properties of iron-fortified mozzarella cheese prepared from cow's, goat's and mixed milk are determined by different quality attributes viz., appearance, colour, body, and texture, flavour, saltiness, and overall acceptability, which are presented in Table 3. Mixed milk iron-fortified mozzarella cheese prepared with kiwifruit crude extract showed the highest rating for all the parameters compared to cow milk or goat milk cheese alone. In the treatment group, mixed milk cheese samples were significantly higher than cow milk cheese samples for appearance ($p<0.05$) and saltiness ($p<0.01$) attributes. Body & texture, flavour, and overall acceptability were significantly higher ($p<0.01$) for mixed milk cheese samples compared to cow milk and goat milk cheese samples. On the contrary, in the control group, only body & texture revealed a highly significant ($p<0.01$) difference between mixed milk and goat milk cheese samples while other parameters were found to be non-significant. From the results, it could be concluded that the taste panel members relished the cheese samples coagulated with kiwifruit crude extract. Lower panel ratings in iron-fortified cow and goat milk mozzarella cheese might probably be due to less preference towards iron addition in cow and goat milk that might have given a metallic taste to the product. Other probable reasons might be the variation in milk sources, breakdown of fat and protein by residual enzymes, chemical and microbiological characteristics of raw milk used and proteolysis, lipolysis, and fermentation of carbohydrates which might affect the taste of the end product. Goat milk cheese tends to produce a soft body due to small size fat globules that result in personal disliking by the panel members resulting in a low rating for the body and texture attribute of the product. Results of the present study are comparable to the results of Waheed et al. (2017) who did not observe any significant differences in flavour and taste of cheese prepared with plant coagulant and control samples, and, overall acceptability increased with an increase in the concentration of plant protease extract with a rating of 8.00 ± 0.61 at 10% of papaya leaves extract. The results of this study also corroborate the findings of Bhattarai and Acharya (2010) who reported superior overall acceptability, flavour, texture, and taste for cow's milk and mixed milk cheese samples over buffalo milk cheese.

Table 3: Effect of enzymes and types of milk on organoleptic properties* of iron fortified mozzarella cheese

Types of Milk	Organoleptic Properties											
	Appearance		Colour		Body & Texture		Flavour		Saltiness		Overall Acceptability	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Cow	7.67±0.11	7.09±0.17 ^a	7.71±0.10	7.23±0.15	7.66±0.33 ^a	7.03±0.19 ^a	7.63±0.21	7.09±0.17 ^a	7.57±0.16 ^a	7.46±0.05 ^a	7.65±0.09	7.18±0.07 ^a
Goat	7.80±0.10	7.57±0.06 ^{ab}	7.88±0.05	7.56±0.08	6.86±0.23 ^b	6.43±0.05 ^a	7.77±0.07	7.46±0.07 ^a	8.11±0.13 ^b	8.01±0.14 ^b	7.64±0.12	7.40±0.04 ^a
Mixed	7.80±0.32	8.00±0.09 ^b	7.71±0.34	8.03±0.14	7.86±0.29 ^a	8.03±0.11 ^b	7.83±0.24	7.94±0.11 ^b	7.80±0.23 ^{ab}	8.20±0.03 ^b	7.80±0.28	8.04±0.07 ^b

n=5; *Mean±SE

Means with common superscript column wise does not differ significantly.

CONCLUSION

The study was conducted to develop production technology for the preparation of iron-fortified mozzarella cheese using kiwifruit crude extract as an enzyme source. Based on the experimental results, kiwifruit extract can be successfully used as a coagulating enzyme for preparing mozzarella cheese with an optimum level of kiwifruit extract to be used at 150µg/ml of milk for complete coagulation within 27min (with CaCl₂). The optimum level of FeCl₃ (@ 1g/l) could be safely used without showing any off-flavour in the product. Based on chemical proximate composition and sensory analysis, mixed milk mozzarella cheese (treatment group) was the best product compared to cow milk or goat milk iron-fortified mozzarella cheese.

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Authors' contributions

Masuk Raquib and Trishna Borpuzari conceptualized and designed the work. The first author executed the experimental work and carried out the laboratory analysis. Mineswar Hazarika, Saurabh Kumar Laskar, Girindra Kumar Saikia, and Razibuddin Ahmed Hazarika provided the necessary guidelines and contributed critically to revise the manuscript.

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