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The role of microbial coagulants on the physicochemical, proteolysis, microstructure and sensory properties of low-fat Edam cheese manufactured from ultrafiltered buffalo milk

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ABSTRACT

This work investigates the influence of using microbial coagulants, including *Rhizomucor miehei* (MCR) protease and *Cryphonectria parasitica* (MCC) protease, on the quality characteristics of low-fat Edam cheese made from ultrafiltered buffalo milk (LFUE). Concurrently, a benchmark with calf rennet (CR) has been also performed. Throughout a 90-day ripening period, the cheeses were assessed for their physicochemical features, proteolysis, texture, free amino acid and free fatty acid content, microstructure, and sensory attributes. The study revealed that both microbial coagulants had no significant impact on the physicochemical composition and firmness of the cheeses while slightly affected the free fatty acids. Cheeses made with microbial coagulants displayed higher proteolysis, with MCR and MCC cheeses exhibiting greater levels of water-soluble nitrogen and 12% trichloroacetic acid-soluble nitrogen than CR cheese. MCR and MCC cheeses exhibited more extensive breakdown of αs- and β-caseins, as indicated by the SDS-PAGE electrophoretogram, compared to CR cheese during ripening. As for the proteolytic activity, the microbial coagulant contributed to shaping the free amino acid content, microstructure, and sensory qualities of the cheeses. Notably, MCC cheese outperformed MCR or CR cheeses in terms of free amino acid levels. MCR and MCC cheeses resulted in smooth microstructures with uniform protein networks as observed by microscopy, while CR cheese displayed rough, granular surfaces. With the highest scores for appearance, body, texture, and flavor, MCC cheese demonstrated superior sensory properties compared with MCR and CR cheeses.

1. Introduction

Due to increased consumer awareness of the nutritional value of food, there is a growing demand for food items that could offer better health benefits (Garza-Cadena et al., 2023). Consumers are turning to low-fat foods to prevent chronic diseases, such as cardiovascular issues, hypertension, obesity, diabetes, and cancer, which are associated with excessive consumption of saturated fats (de-Magistris & Lopez-Galan, 2016; McCarthy, Wilkinson, & Guinee, 2017). Thus, low-fat cheeses, including Edam, Gouda, and Cheddar, are becoming more popular due to the adverse health effects of fat. To some extent, fat affects the water-to-protein ratio and determines the firmness and elasticity of cheese by increasing its moisture-retaining properties. Full-fat cheese also has a smooth texture due to fat content and is evenly distributed within the casein matrix, contributing to the cheese's overall flavor quality (Küçüköner & Haque, 2006). Unfortunately, when fat is removed in low-fat cheeses, casein takes on a larger role in texture development, resulting in a firmer texture. This reduction in fat content leads to changes in the biochemistry, microbiology, texture, functionality, and sensory properties of low-fat cheeses (Ibanez Alfaro, 2016; Küçüköner & Haque, 2003). To overcome the negative effects of reducing fat, various approaches have been proposed, including

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the addition of ingredients or modifications to the manufacturing process, such as adding an adjunct starter culture (Ayyash, Abu-Jdayil, Hamed, & Shaker, 2018; Costa et al., 2010), coagulants (Govindasamy-Lucey, Lu, Jaeggi, Johnson, & Lucey, 2010; McCarthy et al., 2017), stabilizers and fat replacers (El-Aidie, Ghita, El-Dieb, & El-Garhi, 2019; Jooyandeh, Goudarzi, Rostamabadi, & Hojjati, 2017; Nateghi et al., 2012; Sahan, Yasar, Hayaloglu, Karaca, & Kaya, 2008), and using polysaccharide-producing cultures to increase moisture retention (Costa et al., 2010).

Due to growing consumer awareness about health and nutrition, cheeses made through the ultrafiltration (UF) technique are believed to have greater nutritional value than those made from regular milk (El-Aidie and Khalifa, 2024). This is because UF, thanks to its effective molecular sieving effect, enables to selective concentration of native whey proteins in the cheese curd, leading to improved yields (El-Garhi, El-Aidie, Rashid, & Hayee, 2018; Soltani, Saremnezhad, Faraji, & Hayaloglu, 2022). However, the high concentration of whey proteins in the UF retentate can inhibit rennet activity, leading to delayed proteolysis and thus causing textural and functional defects in hard and semi-hard cheeses (Benfeldt, 2006; Hayes, McSweeney, & Kelly, 2002; Soltani, Boran, & Hayaloglu, 2016). These latter changes occur as the complexes formed between β-LG and κ-casein reduce the accessibility of κ-casein for chymosin (Hinrichs, 2001; Masotti, Cattaneo, Stuknytė, & De Noni, 2017; Treblin et al., 2022). Additionally, the quantity of milk-clotting enzyme retained in the curd affects casein breakdown, influencing the cheese's structure and flavor (Børsting, Qvist, & Ardö, 2014). In UF cheese production, all the coagulant is retained in the curd, which can increase proteolytic activity and improve proteolysis during the ripening process compared to traditional cheeses (Soltani, Sahingil, Gokce, & Hayaloglu, 2019).

Rennet is a typical milk-coagulating enzyme preparation used in the manufacture of most cheese types. For many years, calf rennet has been widely used as the primary agent in milk coagulation and is considered the natural source of chymosin protease (E.C. 3.4.23.4). Chymosin cleaves the Phe_{105}-Met_{106} bond in κ-casein, releasing caseinomacropeptide, which destabilizes the casein micelles, thus inducing milk coagulation (Vreeman et al., 1986). Due to the increase in cheese production and decrease in animal production worldwide, there has been a shortage of calf rennet and its high cost in the market. Calf chymosin has been replaced by proteinases from other animals or microorganisms, and their use has increased (Gumus & Hayaloglu, 2019; Hayaloglu, Karatekin, & Gurkan, 2014). Particularly, microbial coagulants, obtained by fermentation, can be produced in large quantities at a lower cost than animal rennet. The most used microbial coagulants are proteases from *Rhizomucor miehei, Rhizomucor pusillus, and Cryphonectria parasitica*. *Cryphonectria parasitica* proteases cleave the Ser_{104}-Phe_{105} bond in κ-casein, while *R. miehei* cleaves the Phe_{105}-Met_{106} bond (García et al., 2012). However, the higher heat stability of derivatives from *R. miehei* can result in excessive proteolysis and shorter ripening. The strong proteolytic activity of microbial coagulants during cheese-making can result in the loss of protein breakdown products in the whey, affecting both cheese yield and flavor. To face this issue, cheese-making processes employing UF techniques are a promising option.

Proteolysis is a crucial biological reaction that occurs during cheese ripening (Amiri, Kohneshahri, & Nabizadeh, 2022). Enzymes from milk, coagulant, starter, non-starter and secondary starter bacteria play a role in breaking down the proteins in cheese (Fox & McSweeney, 1996). The initial degradation of casein is thought to be caused primarily by residual coagulants, such as calf or microbial rennet, while the subsequent formation of smaller peptides, free amino acids, and flavor components is due to the enzymes produced by the starter culture, secondary starter, and non-starter bacteria (Fox, Singh, & McSweeney, 1994; Soltani et al., 2016). The type and proteolytic power of the coagulant used can affect the rate of proteolysis and the softness of low-fat cheese (Gumus & Hayaloglu, 2019). Increasing the amount of coagulant added to the cheese milk can accelerate proteolysis in various cheeses, including Cheddar, Mozzarella, Meshanger, and Iranian White (Dave, McMahon, Oberg, & Broadbent, 2003). The use of coagulants with a higher ratio of proteolytic to milk clotting activity than calf rennet or chymosin, such as *Rhizomucor pusillus* (Sheehan, O'Sullivan, & Guinee, 2004), *Rhizomucor miehei* (Soltani et al., 2016), and *Endothia parasitica* (Yun, Barbano, & Kindstedt, 1993), has also been shown to enhance proteolysis (Fox, Guinee, Cogan, & McSweeney, 2017). According to Moschopoulou (2017), microbial rennets exhibit a slightly elevated proteolytic activity, resulting in increased breakdown of cheese proteins during ripening. This process contributes to a leaner texture and a tendency towards enhanced maturation. Soltani et al. (2019) found significant differences in pH, fat, salt, protein content, and proteolysis rate in UF-white cheeses when using a blend of camel chymosin and microbial rennet (*R. miehei*). The study concluded that the lower proteolytic activity of camel chymosin resulted in a denser protein network and a harder texture in UF-white cheese. Furthermore, Gumus and Hayaloglu (2019) observed that increasing the level of calf chymosin in the mixture of calf and camel chymosins for milk coagulation resulted in more severe proteolysis and a softer texture of Turkish Beyaz Peynir (a type of white-brined cheese). Finally, McCarthy et al. (2017) concluded that adding more bovine chymosin, as a coagulant, along with an adjunct culture could increase proteolysis while improving the rheological and functional quality of reduced-fat, reduced-salt Cheddar cheese.

However, making Edam cheese from low-fat buffalo milk concentrated using UF is quite a challenge. Because reducing the fat content can significantly impact the texture and mouthfeel of the cheese, UF low-fat cheeses often have a firmer texture and may lack the creaminess associated with full-fat varieties. Overall, challenges arise from the need to balance reducing fat content while maintaining the quality, flavor, and texture characteristics that consumers associate with Edam cheese. Microbial rennet, which aids in the hydrolysis of the protein and results in a smoother protein network, can be used to accomplish this. To the best of our knowledge, there is no report so far evaluating the effect of using microbial coagulants with varied proteolytic activity on the quality properties of Edam cheese manufactured from low-fat buffalo milk concentrated by UF. Therefore, this current study aims to timely investigate the influence of selected microbial coagulants, *Rhizomucor miehei* (*R. miehei*, MCR) protease and *Cryphonectria parasitica* (*C. parasitica*, MCC) protease, on the physicochemical, proteolysis, microstructure, texture profiles and sensory properties of low-fat Edam cheese made from buffalo milk and concentrated by UF during 90 days of ripening. Herein, a benchmark with CR has been also done.

2. Materials and methods

2.1. Materials

The fresh buffalo raw milk was procured from the Animal Production Research Station in Sakha, Egypt, and its characteristics were measured as follows: pH of 6.65, titratable acidity of 0.19% lactic acid, protein content of 3.73%, fat content of 5.3%, ash content of 0.78%, and total solids of 14.15%. The LD-culture CH-N22 is a mixture of several strains including *Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroides* subsp. *cremoris, and Lactococcus lactis* subsp. *lactis* bv. *diacetyltactis*. This culture is used as a Mesophilic Aromatic Culture that generates

CO_2 and is produced by CHR HANSEN in Denmark. In cheese manufacturing, three types of coagulants were employed: Calf rennet (Naturen MO, sourced from Chr. Hansen in Copenhagen, Denmark, with a chymosin content of over 80%, 180 IMCU mL^{-1}), as well as two microbial rennets, *Rhizomucor miehei* protease (Fromse® 220 IMCUg^{-1}) TL, sourced from DMS Food-Specialties in France, and *Cryphonectria parasitica* protease (Surecurd 600 IMCUg^{-1}), sourced from the Dairy Ingredients Division of Pfizer Inc. in the USA. An alkaline extract of Annatto seeds was obtained from Pfizer in Milwaukee, Wisconsin. Fine-grade commercial salt (NaCl) was purchased from the local market. Calcium chloride was obtained from El-Nasr Company in Cairo, Egypt. All other chemicals and reagents used in this study were purchased from Merck in Darmstadt, Germany.

2.2. Methods

2.2.1. Edam cheese production

Edam cheese was prepared using the modified method outlined by Küçüköner and Haque (2003). Initially, buffalo milk was standardized to 1.5% fat and later concentrated using UF with a carbon membrane (pore size of 0.05–0.1 mm). The milk was concentrated to four times its original volume at 50–52 °C. The resulting retentate milk was then heat-treated at 72 °C for 15–30 s and rapidly cooled to 37 °C. Afterwards, the starter culture (1% w/w), Anatto color (160 μL), and 0.02% food-grade calcium chloride were added, and the mixture was held for 20–30 min. To investigate the impact of microbial coagulants on Edam cheese quality, three batches were prepared using Calf rennet (**CR**) as the control, and *R. miehei* protease (**MCR**) and *C. parasitica* protease (**MCC**) as microbial rennet at levels of 25, 4, and 2.5 mL per 100 L of milk, respectively. The coagulant amount used in cheese production was calculated based on the relative milk-coagulating activity specified by the supplier to coagulate the milk within 45 min. Before addition, the coagulants were diluted with distilled water (1:20) and immediately added to the milk. The milk retentate was then set at 32 °C for 30 min and cut into ca. 1 cm^3 size cubes, followed by resting at the same temperature for an additional 20 min. The curds were then cooked by raising the temperature to 38 °C, allowing them to simmer until the acidity was 0.25–0.30% lactic acid. Cheese curd was pressed in molds covered with cheese cloth at 10–15 kg cm^{-2} for 3 h. The cheese was turned and re-pressed overnight, with the curd temperature maintained at 15–20 °C. Subsequently, cheese balls were immersed in a brine solution containing 20% w/v NaCl for 20 h at 10 ± 1 °C. The cheeses were allowed to form a rind before being coated with wax. The final product was stored at 10 ± 1 °C and 85% relative humidity, and cheese samples for CR, MCR, and MCC were taken at 0, 30, 60, and 90 days of storage for analysis. Three replicates were conducted for each treatment.

2.2.2. Chemical composition

The cheese samples were grated and analyzed to determine their titratable acidity by titration method, moisture content by the oven drying method at 105 °C for 3–4 h, fat content by Gerber method, protein content by Kjeldahl method, and ash content by muffle furnace method according to standard methods AOAC (2012). Salt content was estimated by titration with $AgNO_3$ according to Bradley et al. (1993). To measure the pH, grated cheese (25 g) was homogenized with distilled water (25 mL), and the pH values were determined using a digital pH meter (Hi 8014-Italy).

2.2.3. Proteolysis assessment during ripening

2.2.3.1. Soluble nitrogen fractions. Water soluble nitrogen (WSN) and 12% trichloroacetic acid soluble nitrogen (TCA–SN) fractions of the cheeses were analyzed by the method outlined by Hayaloglu, Guven, Fox, and McSweeney (2005) and expressed as a percentage (%) of total nitrogen.

2.2.3.2. Polyacrylamide gel electrophoresis (SDS-PAGE). The casein fractions of all cheeses were analyzed using polyacrylamide gel electrophoresis (SDS-PAGE) following the method described by Yun et al. (1993), using a constant acrylamide concentration of 15% for the running gel. Cheese (1 ± 0.05 g) was ground and combined with electrophoresis sample buffer in a 20 mL tissue homogenizer (model 3431-kl0; Thomas, Swedesboro, NJ), then thoroughly homogenized. 0.1 mL of the homogenate cheese was transferred into a 2 mL sample vial, followed by the addition of 0.9 mL of electrophoresis sample buffer containing 0.8% (wt/vol) dithiothreitol. Vials were capped, inverted, boiled for 5 min, cooled to 22 °C, and stored at −20 °C. Before electrophoresis, frozen samples were thawed to room temperature and then immersed in boiling water for 5 min. After cooling to room temperature, 8 μL of each cheese sample were loaded onto the gel. α and β-caseins were quantified by scanning Coomassie blue R-250 stained gels using a video densitometer (model 620; The 1-0 Analyst; Bio-Rad Laboratories, Rockville Centre, NY) with a 600 nm filter for the fluorescent cool white light source.

2.2.3.3. Free amino acids determination. The content of free amino acids (FAAs) was measured using High-Performance Capillary Electrophoresis with the Jeol-AminoTag JLC-500V AA analyzer (Jeol Ltd., Tokyo, Japan), following the method described by McCarthy et al. (2017). The analysis was performed on samples collected at 90 days of storage and compared with the results obtained from the control sample (CR) at 0-day-old.

2.2.4. Lipolysis

The method described by Jahreis et al. (1997) was employed for the identification and quantification of free fatty acids. Initially, 2 g of cheese fat was extracted using 15 mL of Fokh's reagent (chloroform/methanol = 2:1, v/v). The extracted lipids were then filtered through anhydrous Na_2SO_4. Subsequently, fatty acid methyl esters (FAMEs) were prepared via transesterification with potassium methylate. Specifically, 0.5 mL of potassium methylate solution (5% wt/wt in methanol) was added to the fat solution in a Pyrex tube. The tube was securely capped, vortexed, and heated at 60 °C for 15 min in a drying cabinet. After cooling, 1.5 mL of sulfuric acid (2% wt/wt in distilled water) was added, followed by another round of vortexing. Finally, fatty acid analysis was conducted via gas–liquid chromatography, in this process, 1 μL of FAMEs was injected into a GC–MS autosampler (7890 A GC System Agilent) equipped with an MSD detector, utilizing a ZB-5 fused silica capillary column. Identification of fatty acids was achieved by quantifying them in mg g^{-1} total lipids relative to the internal standard, methyl tricosanoate (23:0), obtained from Sigma. Prior to transesterification, a 1.00 mL solution of internal standard (1 mg mL^{-1}) was added to all samples, followed by evaporation of the solvent under N_2 flow.

2.2.5. Texture analysis

The texture of the cheese was evaluated by measuring its firmness at 15 °C using a cone penetrometer (Stanhope Seta, Surrey, UK), as described by El-Aidie et al. (2019). The penetrometer had a regular weight of 47.5 g and a supplement of 35 g, making a total of 82.5 g. The scale was calibrated in 35 units, with each unit further broken down into 10 parts of 0.1 mm each. The cheese sample was placed on the base, and the cone was lowered until it touched the surface of the cheese. The button was then pushed for 5 S to record the depth of penetration in units of 0.1 mm. The penetration value (inverse firmness) was calculated by averaging the penetration

depths (in mm) obtained from the measurement taken at three different locations on the surface of the cheese sample.

2.2.6. Microstructure analysis by scanning electron microscopy (SEM)

At the end of the 90-day ripening period, the cheese's fine structure was examined using scanning electron microscopy. Small cheese cubes (ca. 1 mm^3) were prepared using the thin-sectioning technique described by Taneya, Kimura, Izutsu, and Bucheim (1980). These were fixed in a 2% glutaraldehyde solution (pH 6.8) buffered with sodium cacodylate for 2 h, then post-fixed in a mixture of osmium tetroxide and 2% tannic acid (pH 6.0). After stepwise dehydration in ethanol and transfer into acetone, the samples were dried using a Balzer union critical point dryer and subjected to a sputtering process with gold. The samples were examined using a scanning electron microscope (JEOL JSM-6700F, Japan) with an accelerating voltage of 10 kV, following the method outlined by Fu et al. (2018).

2.2.7. Sensory analysis

During different storage periods, cheese samples underwent organoleptic evaluations conducted by a team of five trained panel members. These individuals were permanent staff members of the Dairy Research Department and possessed extensive familiarity with the characteristics of the cheese being assessed. White plastic plates with random 3-digit numbers were used to blind the samples, and water was provided for mouth rinsing. The panel assessed the appearance, texture, flavor, and defects using the IDF (1987) sensory assessment guide for cheese. Each characteristic, such as appearance, texture, and flavor, was rated on a scale from 0 to 10, with 0 indicating the lowest quality and 10 indicating the best quality. The importance of each attribute was considered by multiplying the scores for appearance, texture, and flavor by factors of 1, 4, and 5, respectively. The total score was then calculated by adding the scores for each characteristic, with an ideal cheese receiving a perfect score of 100 (Kondyli et al., 2022).

2.2.8. Statistical analysis

The statistical analysis was performed using SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to assess the differences between the mean values of different groups, and the Tukey HSD post-hoc test was used to identify significant differences between groups. The results were presented as means ± standard deviation (SD), and statistical significance was set at $P < 0.05$. Pearson's correlation coefficient was used to evaluate the correlation between different parameters. The data were checked for normality and homogeneity of variance using the Shapiro–Wilk test and Levene's test, respectively. Additionally, principal component analysis (PCA) was conducted to evaluate the structural correlations among variables and visualize the relationship between LFUE cheese treatments. The data was analyzed using OriginPro software (OriginPro9.1.0, OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Cheese composition

The chemical composition of produced cheeses with MCR and MCC compared to CR during 90 days of ripening is reported in Table 1. We observed no significant influence of either MCR and MCC or CR on the chemical composition of low-fat UF Edam cheese (LFUE) when fresh. These outcomes agree with those of earlier studies conducted by several researchers (Kim, Gunasekaran, & Olson, 2004; McCarthy et al., 2017; Sheehan et al., 2004; Yasar & Guzeler, 2011), who found that the type of coagulant had no impact on the chemical composition of different type of cheese. This contrasts with the results of Hayaloglu, Guven, and Fox (2002), Dave et al. (2003), O'Mahony, Sousa, and Mcsweeney (2003) who observed that the chemical composition of Mozzarella, Cheddar, and Hallumi cheese was influenced by the type of coagulant. As the ripening progressed, the moisture content of all cheese samples declined significantly ($P < 0.05$) after 30 days and continued to drop through the end of the ripening period. This is most likely caused by the cheese's moisture evaporating during ripening. These results are comparable to that detected by D'Incecco et al. (2022) for long hard ripening cheese. However, there were numerically small but statistically significant increases in fat, protein, salt/moisture and ash by the end of the ripening period, as reported in Table 1. These findings are in agreement with Gumus and Hayaloglu (2019). On the other hand, pH values dropped in all cheese samples towards the end of ripening due to a greater accumulation of fermentation products such as lactic acid and other organic acids. These results agreed with those reported by Gumus and Hayaloglu (2019), who detected significantly lower pH values in a white-brined cheese made with a mixture of camel and calf chymosin during ripening.

Table 1
Chemical composition of low-fat Edam cheese made from ultrafiltered buffaloes' milk with microbial coagulants during 90 days of ripening.[a]

Variables	Ripening time (d)	Low fat UF Edam cheese		
		CR	MCR	MCC
Moisture (%)	0	47.5 ± 1.1^{Ab}	48.2 ± 0.4^{Ab}	47.9 ± 0.7^{Ab}
	30	46.6 ± 0.7^{Aab}	47.4 ± 1.2^{Aab}	47.1 ± 1.2^{Aab}
	60	46.1 ± 0.5^{Aa}	46.8 ± 1.5^{Aa}	46.5 ± 0.6^{Aa}
	90	45.6 ± 1.2^{Aa}	46.4 ± 1.6^{Aa}	46.1 ± 0.4^{Aa}
Fat (%)	0	12.5 ± 0.0^{Aa}	12.4 ± 0.1^{Aa}	12.5 ± 0.1^{Aa}
	30	12.6 ± 0.1^{Aab}	12.4 ± 0.3^{Aab}	12.6 ± 0.1^{Aab}
	60	12.7 ± 0.1^{Abc}	12.6 ± 0.0^{Abc}	12.7 ± 0.4^{Abc}
	90	12.7 ± 0.3^{Ac}	12.7 ± 0.1^{Ac}	12.8 ± 0.1^{Ac}
*Fat/DM (%)	0	23.8 ± 0.6^{Aa}	23.9 ± 0.4^{Aa}	24.0 ± 0.2^{Aa}
	30	23.5 ± 0.6^{Aa}	23.6 ± 0.8^{Aa}	23.8 ± 0.7^{Aa}
	60	23.6 ± 0.3^{Aa}	23.7 ± 0.7^{Aa}	23.8 ± 0.6^{Aa}
	90	23.4 ± 0.9^{Aa}	23.7 ± 0.7^{Aa}	23.8 ± 0.4^{Aa}
Protein (%)	0	30.9 ± 0.3^{Aa}	30.3 ± 0.7^{Aa}	30.4 ± 0.1^{Aa}
	30	30.9 ± 0.2^{Aab}	30.4 ± 0.3^{Aab}	30.6 ± 0.1^{Aab}
	60	31.2 ± 0.3^{Abc}	30.7 ± 0.3^{Abc}	30.8 ± 0.4^{Abc}
	90	31.4 ± 0.3^{Ac}	30.9 ± 0.3^{Ac}	31.1 ± 0.3^{Ac}
Salt/moisture (%)	0	4.9 ± 0.4^{Aa}	5.1 ± 0.5^{Aa}	4.6 ± 0.2^{Aa}
	30	5.0 ± 0.2^{Aab}	5.5 ± 0.3^{Aab}	5.0 ± 0.1^{Aab}
	60	5.2 ± 0.1^{Aab}	5.1 ± 1.2^{Aab}	5.2 ± 0.4^{Aab}
	90	5.3 ± 0.2^{Ab}	5.7 ± 0.2^{Ab}	5.3 ± 0.4^{Ab}
Ash (%)	0	4.3 ± 0.1^{Aa}	4.2 ± 0.1^{Aa}	4.3 ± 0.1^{Aa}
	30	4.3 ± 0.1^{Aab}	4.3 ± 0.0^{Aab}	4.4 ± 0.1^{Aab}
	60	4.4 ± 0.1^{Ab}	4.3 ± 0.1^{Ab}	4.4 ± 0.1^{Ab}
	90	4.4 ± 0.1^{Ab}	4.4 ± 0.1^{Ab}	4.4 ± 0.1^{Ab}
pH values	0	6.0 ± 0.1^{Ac}	6.0 ± 0.0^{Ac}	6.0 ± 0.0^{Ac}
	30	5.7 ± 0.6^{Ab}	5.6 ± 0.0^{Ab}	5.6 ± 0.1^{Ab}
	60	5.4 ± 0.0^{Aa}	5.4 ± 0.0^{Aa}	5.4 ± 0.0^{Aa}
	90	5.4 ± 0.0^{Aa}	5.4 ± 0.0^{Aa}	5.3 ± 0.0^{Aa}

[a] Mean ± SD in each column within ripening time with different lowercase letters differ significantly ($P < 0.05$). The values are mean ± SD for n = 3. Mean ± SD in each row with different uppercase letters are significantly ($P < 0.05$) different. CR: Calf rennet; MCR: microbial coagulant (*Rhizomucor miehei*); MCC: microbial coagulant (*Cryphonectria parasitica*) used in manufacture of low fat UF Edam cheese. *Fat/DM: (Fat/Dry matter).

3.2. Proteolysis assessment during ripening

3.2.1. Soluble nitrogen fractions

Fig. 1 displays the water-soluble nitrogen (WSN) levels in LFUE cheese produced using various coagulants during 90 days of

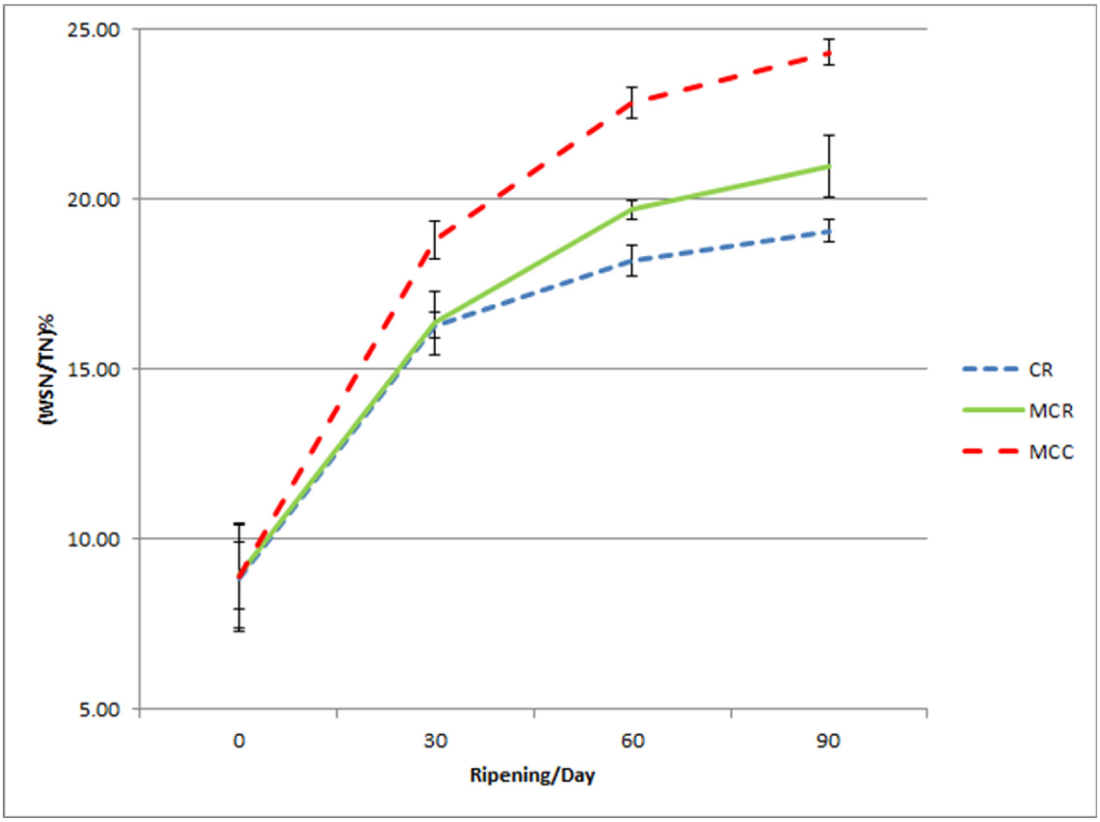

Fig. 1. Changes of WSN/TN during ripening of low fat UF Edam cheese made using microbial coagulants.

storage. No significant differences were found in the cheese samples at 0 days, and their WSN levels were relatively similar, ranging from 8.88 to 8.94 for CR, MCR, and MCC samples. On day 30, significant differences ($P < 0.05$) were observed as the level of WSN increased sharply in all cheese samples, with a more pronounced increase in MCC compared to CR and MCR, which had similar levels of WSN. The WSN in all cheese samples showed a steady increase from day 30 until the end of the ripening period. However, the increase was more pronounced in MCC and MCR samples than in CR cheese sample. This suggests that the microbial protease activity during the ripening period was more intense in MCC and MCR cheeses than CR cheese. A similar trend was observed in Iranian ultrafiltered white cheese by Soltani et al. (2019), where higher values of WSN were found throughout the ripening period when using a higher level of *Rhizomucor miehei* compared to cheeses made using camel chymosin. According to Fig. 2, the TCA-SN levels in all cheese samples increased as the ripening process advanced, because of the release of intermediate and lower molecular weight peptides. The cheese made with MCC had a higher average concentration of TCA-SN compared to those made with MCR and CR. Previous studies by Hayaloglu et al. (2014), Moynihan et al. (2014), and Soltani et al. (2019) have reported significant differences in WSN or TCA-SN levels in Malatya, Mozzarella, and Iranian ultrafiltered white cheeses, respectively, caused by different coagulant enzymes. Similarly, Kandarakis, Moschopoulou, and Anifantakis (1999) reported comparable trends during the ripening process for WSN or TCA-SN in Feta cheese produced using calf or fermentation-produced rennet.

3.2.2. SDS-PAGE

Fig. 3 illustrates the SDS-PAGE electrophoretogram results for the cheese samples. The degradation of α_{s1}- and β-caseins occurred slowly in the first 30 days of ripening. After this period, the hydrolysis of αs_1-casein and the formation of its degradation products intensified significantly, especially after 60 days of ripening. In the MCC and MCR cheese presenting microbial coagulants, there was a larger degradation of α_{s1}-casein, along with higher levels of more mobile peptides. The CR cheese showed a similar trend but to a lesser extent. The results indicate that the type of coagulant significantly impacted the rate at which αs_1- and β-caseins degraded in the LFUE cheese during the maturation process. McCarthy et al. (2017) also observed a comparable pattern in the degradation of αs1- and β-caseins in half-salt, half-fat Cheddar cheese that was produced using bovine and camel chymosin. The observed α_{s1}-casein degradation patterns align with previous research on Kashar cheese using various coagulants (Yasar & Guzeler, 2011). On the other hand, the β-casein degradation increased during storage of both MCC and MCR cheese but it was more extensive in the MCC cheese. The use of microbial enzymes in LFUE cheese led to higher proteolytic activity and significant effects ($P < 0.05$) on the degradation of β- and α_{S1}-casein. These results are consistent with those found by Cepoglu (2005) in white cheese production. Previous research has shown that *C. parasitica* protease has a greater ability to hydrolyze β-casein compared with calf rennet and *R. miehei* (Bogenrief & Olson, 1995; Yun et al., 1993). Interestingly, the concentration of coagulants did not affect the patterns of β-casein degradation in cheese, as reported by Hayaloglu et al. (2014). However, using protease from calf rennet and *R. miehei* resulted in variations in the urea-PAGE patterns of Hallumi cheese after 60 days of ripening. Kubis, Sousa, Walsh-O'Grady, Kelly, and McSweeney (2001) and Dave et al. (2003) found no relationship between rennet concentration and β-casein degradation while a direct correlation was found between rennet concentration and α_{s1}-casein degradation in Cheddar and Mozzarella cheese.

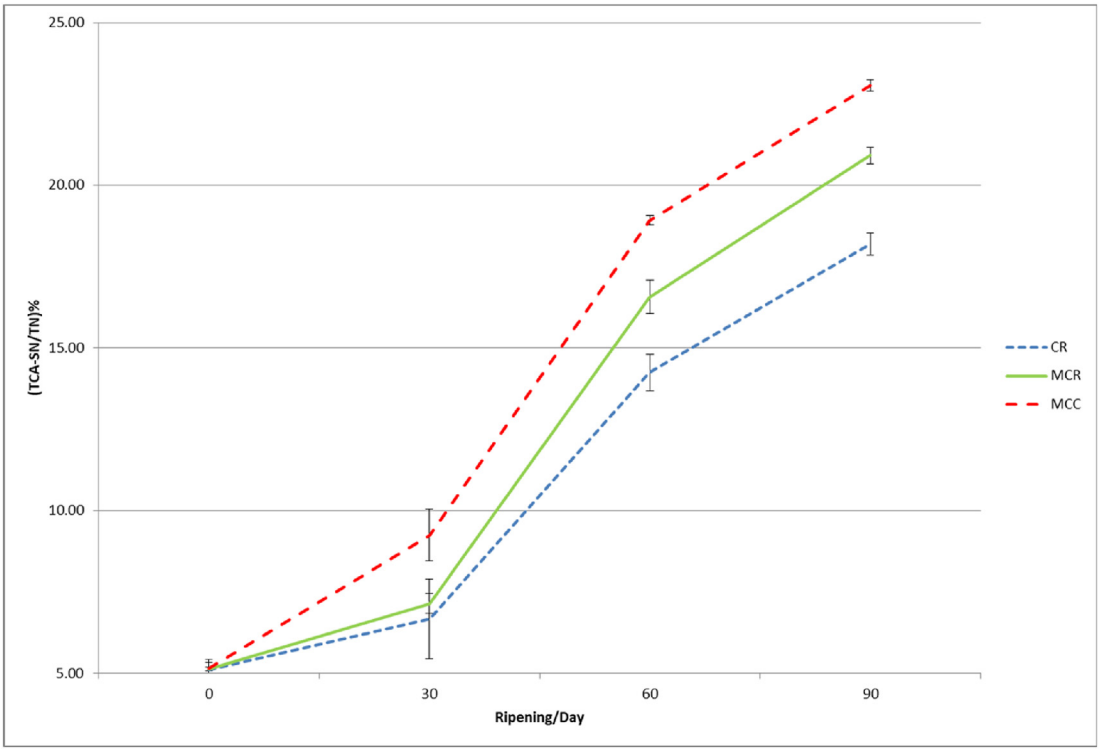

Fig. 2. Changes of TCA-SN/TN during ripening of low fat UF Edam cheese made using microbial coagulants.

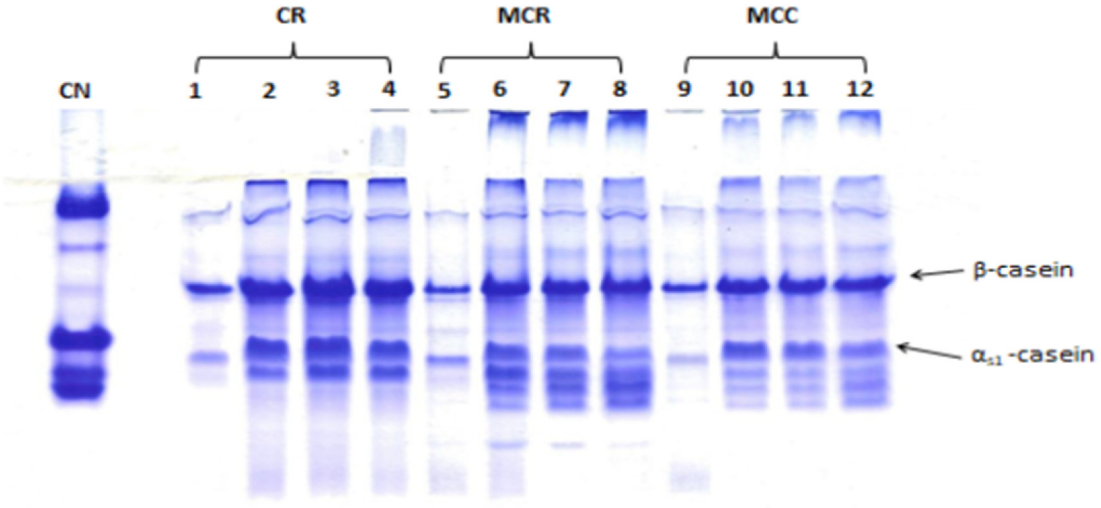

Fig. 3. The electrophoretic patterns of low-fat Edam cheese made from ultrafiltered buffalo milk using different coagulants. Lanes 1–4 represent cheese with CR, lanes 5–8 represent cheese with MCR, and lanes 9–12 represent cheese with MCC at 0, 30, 60, and 90 days of ripening, respectively.

3.2.3. *Free amino acids*

Free amino acids (FAA) levels of LFUE cheese made using microbial coagulants at the end of ripening are presented in Table 2. The results show that the FAA content of the cheese varies significantly ($P < 0.05$) depending on the type of coagulant used. At the end of ripening, all cheese samples exhibited a significant rise in FAA levels, surpassing those found in fresh cheese. This is attributed to the activity of primary and secondary proteolysis depending on the concentrations of residual coagulant and microorganism-derived enzymes, leading to increased amino acid content, as documented in the literature (Gumus & Hayaloglu, 2019; Soltani et al., 2019). In mature cheese, a higher release of amino acids such as alanine, aspartic acid, isoleucine, leucine, threonine, and valine was observed with various coagulants. However, data demonstrate that LFUE cheese made with *C. parasitica* (MCC) had higher FAA content compared to other coagulants at the end of ripening, possibly due to its higher proteolytic activity. The increase in FAA levels during ripening aligns with findings reported by Yasar and Guzeler (2011).

3.3. *Lipolysis*

Table 3 presents the free fatty acids (FFA) profile of LFUE cheese made using microbial coagulants during different ripening periods.

Table 2
Free amino acids values (mg 100 g^{-1} cheese) of low fat Edam cheese made from ultrafiltered buffalo milk with microbial coagulants.[a]

Amino acids	*Fresh cheese	**Ripened Low fat Edam cheese		
		CR	MCR	MCC
Aspartic	3.3^{a}	13.3^{b}	14.4^{c}	22.7^{d}
Glutamic	3.7^{a}	4.2^{b}	6.2^{c}	7.4^{d}
Serine	1.6^{a}	3.9^{b}	5.6^{c}	6.7^{d}
Glycine	2.4^{a}	4.9^{b}	7.8^{c}	9.6^{d}
Histidine	2.7^{a}	5.0^{b}	6.0^{c}	7.0^{d}
Arginine	3.4^{a}	4.7^{b}	6.3^{c}	7.2^{d}
Threonine	6.7^{a}	9.1^{b}	15.2^{c}	17.1^{d}
Alanine	1.5^{a}	50.3^{b}	98.1^{c}	105.6^{d}
Proline	0.7^{a}	5.7^{b}	12.2^{c}	15.7^{d}
Tyrosine	1.2^{a}	5.6^{b}	12.3^{c}	13.9^{d}
Valine	1.3^{a}	8.7^{b}	11.8^{c}	15.9^{d}
Methionine	1.5^{a}	6.2^{b}	10.0^{c}	15.4^{d}
Cystine	0.6^{a}	2.5^{b}	4.9^{c}	5.0^{d}
Isoleucine	1.8^{a}	10.6^{b}	19.8^{c}	19.9^{d}
Leucine	0.9^{a}	5.5^{b}	14.4^{c}	17.9^{d}
Phenylalanine	4.5^{a}	6.8^{b}	8.0^{c}	9.1^{d}
Lysine	1.1^{a}	2.9^{b}	4.3^{c}	7.0^{d}

[a] Mean values with different lowercase letters ($^{a-d}$) in each row are significantly ($P < 0.05$) different. CR: Calf rennet; MCR: microbial coagulant (*Rhizomucor miehei*); MCC: microbial coagulant (*Cryphonectria parasitica*) used in manufacture of low fat UF Edam cheese. *Fresh cheese: cheese sample with Calf rennet (control). **Ripened cheese: cheese samples at the end of ripening period (90 days).

Significant differences ($P < 0.05$) were found in the levels of various fatty acids among the different coagulants. The concentration of FFA increased in LFUE cheese as the cheese ripening progressed with the different coagulants used. Caprylic acid (C_8) levels increased significantly during ripening in both MCR and MCC treatments. Among the long-chain saturated fatty acids, stearic acid (C_{18}) showed the highest percentage in various cheese treatments, reaching its maximum level at the end of ripening, with higher values observed in CR treatment. The elevated levels of fatty acids could potentially be attributed to the activity of microbial enzymes and esterases, as documented by Collins, McSweeney, and Wilkinson (2003). However, arachidic acid (C_{20}), which is another long-chain saturated fatty acid, disappeared during cheese ripening with MCC, which could be credited to its utilization by certain organisms in the cheese, as evidenced by Albenzio et al. (2013). Data revealed that oleic acid ($C_{18:1}$) had the highest percentage among the estimated unsaturated fatty acids, with variations observed among cheese coagulants and a noticeable decrease during ripening in MCR and MCC treatments. Linoleic acid ($C_{18:2}$) content was higher than linolenic acid ($C_{18:3}$), and both declined during ripening in all cheese samples. The reduction in unsaturated fatty acids during ripening may be attributed to the conversion of these fatty acids into lower forms by the action of cheese lipolytic enzymes, as documented by Hamdy, Abdelmeged, and Abd Elmontaleb (2022). The established scientific role of unsaturated fatty acids, such as oleic acid (omega-9), in lowering LDL cholesterol has been well-documented, as well as the recognized importance of linoleic acid (omega-6) and α-linolenic acid (omega-3) in healthy nutrition.

3.4. Texture analysis

Firmness, which is a determinant parameter for cheese quality, is inversely related to penetrometer reading expressed in mm. Fig. 4 illustrates the impact of different coagulants on the penetrometer readings of LFUE cheese. The firmness of the various treatments was nearly similar, as indicated by the non-significant differences in penetrometer readings of 12.6, 12.7, and 12.5 for CR, MCR, and MCC treatments, respectively. The findings from this study agree with previous research conducted by Sheehan et al. (2004) and Yasar and Guzeler (2011), as they also found no significant differences in the textural parameters of Mozzarella and Kashar cheese, despite being made with different coagulants. During the ripening period, the firmness of all treatments showed a gradual increase, indicated by decreasing penetrometer readings, reaching values of 11.0, 11.2, and 10.8 for CR, MCR, and MCC cheese, respectively, at the end of ripening. The rate of firmness increase was significant in all treatments throughout ripening, with no significant differences observed among the treatments at the same age. This can be attributed to the reduction in moisture content during ripening. These findings are consistent with the results reported by El-Garhi et al. (2018) and El-Aidie et al. (2019), who observed increased firmness in cheese with lower moisture content.

3.5. Microstructure

The SEM images of LFUE cheese made with various coagulants at 90 days of ripening are shown in Fig. 5. The results revealed significant effects of coagulant type on the porous structure of the casein network in the cheese samples, as observed in the sectional view. As for cheese produced using calf rennet (Fig. 5A), it exhibited larger globular aggregates and smaller pores compared to cheese produced using *R. miehei* (Fig. 5B) and cheese produced using *C. parasitica* (Fig. 5C). Furthermore, the individual identity of curd particles was clearly visible in cheese with calf rennet, indicating incomplete curd fusion. This could be attributed to reduced proteolytic activity of calf rennet, resulting in a more compact protein network with fewer spaces (Moynihan et al., 2014). In cheese made with *R. miehei* revealed minimal visible curd particles, likely

Table 3
Free fatty acids values of low-fat Edam cheese made from ultrafiltered buffaloes' milk with microbial coagulants during ripening.[a]

Coagulant	Ripening time (day)	Fatty acids %									
		Saturated fatty acids (SFA)%							Unsaturated fatty acids (USFA)%		
		C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
CR	30	6.4^{abA}	0.5^{aA}	0.0^{aA}	6.6^{bA}	2.0^{aA}	38.1^{cA}	3.1^{cA}	34.7^{aA}	7.1^{aA}	0.9^{aA}
	60	6.8^{abA}	0.5^{aA}	0.1^{aA}	8.2^{bA}	2.1^{Aa}	38.8^{cA}	3.3^{cA}	34.4^{aA}	6.9^{aA}	0.6^{aA}
	90	8.1^{abA}	0.5^{aA}	0.0^{aA}	5.1^{bA}	1.9^{Aa}	40.3^{cA}	3.3^{cA}	33.3^{aA}	6.7^{aA}	0.5^{aA}
MCR	30	8.3^{aB}	0.2^{aA}	7.4^{bB}	2.2^{aA}	2.0^{Aa}	34.3^{bA}	1.4^{bA}	35.3^{aA}	8.1^{aA}	0.7^{aA}
	60	9.6^{bA}	0.2^{aA}	7.4^{bB}	3.4^{aA}	2.5^{Aa}	35.1^{bA}	1.4^{bA}	32.1^{aA}	7.3^{aA}	0.7^{aA}
	90	11.4^{aB}	0.3^{aA}	5.4^{bB}	2.9^{aA}	1.9^{Aa}	36.8^{bA}	1.6^{bA}	31.5^{aA}	7.0^{aA}	0.6^{aA}
MCC	30	3.6^{aA}	5.6^{bB}	1.0^{aA}	8.1^{bA}	2.8^{bA}	30.5^{aA}	–	35.0^{aA}	9.2^{bA}	0.7^{aA}
	60	5.2^{aA}	6.0^{bB}	1.1^{aA}	9.8^{bA}	2.9^{bA}	32.9^{aA}	–	32.2^{aA}	9.1^{bA}	0.7^{aA}
	90	8.9^{aA}	6.2^{bB}	0.9^{aA}	7.0^{bA}	2.7^{bA}	33.1^{aA}	–	30.8^{aA}	8.7^{bA}	0.6^{aA}

[a] Mean values with different letters ($^{a-c}$) between treatments are significantly ($P < 0.05$) different, Letters ($^{A-B}$) refer to significant differences between ripening time of low fat UF Edam cheese. CR: Calf rennet; MCR: microbial coagulant (*Rhizomucor miehei*); MCC: microbial coagulant (*Cryphonectria parasitica*) used in manufacture of low fat UF Edam cheese.

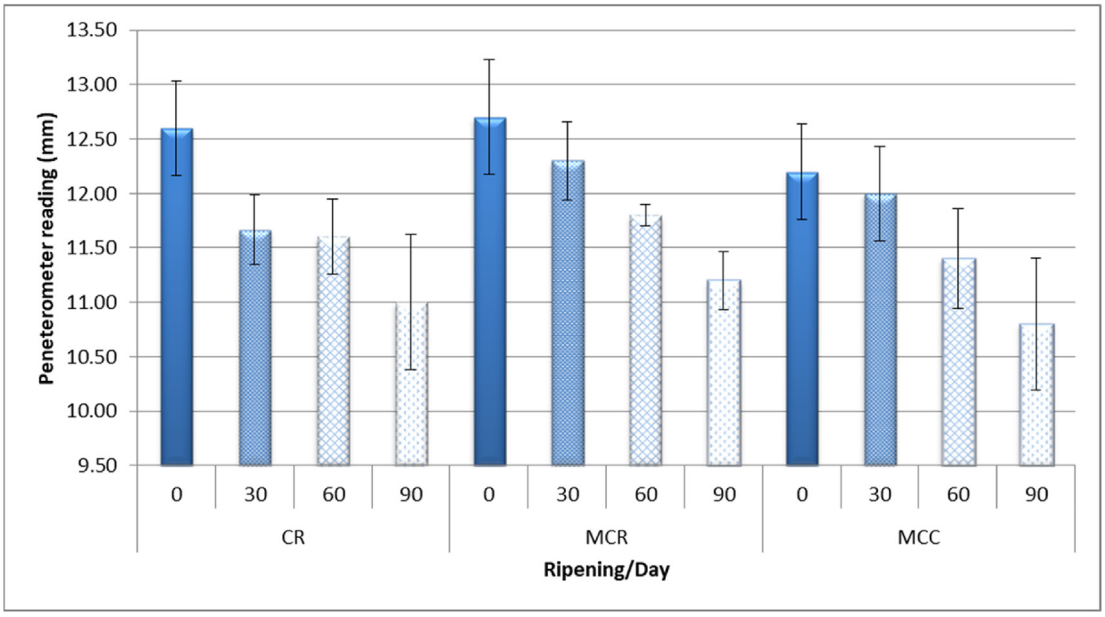

Fig. 4. Penetrometer readings (expressed in mm) of low-fat Edam cheese made from ultrafiltered buffaloes' milk with microbial coagulants during ripening. CR: Calf rennet; MCR: microbial coagulant (*Rhizomucor miehei*); MCC: microbial coagulant (*Cryphonectria parasitica*).

indicating relatively lesser curd fusion. Conversely, in the case of using *C. parasitica*, the individual identity of curd particles was not visible, possibly indicating complete curd fusion (Fig. 5C). Similar findings have been reported by several researchers (El-Aidie et al., 2019; Hayaloglu et al., 2014; Madadlou, Khosroshahi, & Mousavi, 2005). The use of different coagulants was found to greatly impact the texture of the cheese, with noticeable elongation of void spaces in certain cheeses. When *R. miehei* was used as a coagulant (Fig. 5B), some void spaces appeared elongated, and even more elongation pattern was observed when *C. parasitica* was used (Fig. 5C), as compared to cheese made with Calf rennet (Fig. 5A). This elongation of void spaces is considered a positive indicator of body and texture development in cheese, resulting in a smoother protein matrix in MCC cheese compared to other treatments. Additionally, the protein matrix of MCR and MCC cheese remained smooth due to higher proteolysis during the later stages of ripening, which further improved the body and texture of the cheese. These findings are consistent with previous studies by Anderson and Mistry (1994), who observed that the microstructure of cheeses changed considerably from one week to three months, with elongation of void spaces being a typical indication of body development during cheese ripening.

3.6. Sensory analysis

It is likely that the appearance of cheese was not influenced by the type of coagulant used, both when fresh and after 60 days of ripening, as reported in Table 4. However, there was a slight effect observed at the end of the ripening period, as MCR or MCC cheese had higher appearance scores than CR. The body and texture of all cheese samples showed significant ($P < 0.05$) improvement as the ripening period progressed. Initially, the fresh and mature cheeses were firm and elastic, but towards the end of ripening, the CR cheese became slightly firm, while the MCR or MCC cheeses became smooth. Furthermore, the texture of MCC cheese surpassed that of other treatments, especially from the 30th day of ripening until the end of the ripening period. Different coagulant types significantly influenced the flavor of the cheese samples, with flavors increasing notably during ripening ($P < 0.05$). Table 4 reveals that fresh cheese samples had relatively neutral flavors until 30 days of ripening, except for MCC cheese which exhibited a slightly ripened flavor. However, after 60 days of ripening, all cheese samples showed a progressive increase in flavor scores, with CR cheese displaying a slight flavor and MCR or MCC cheeses exhibiting mild flavors. By the end of the 90-day ripening period, MCR cheese or MCC scored higher in flavor compared to CR cheese and were characterized by a well-developed mature flavor. The elevated scores for texture and flavor in MCR and MCC cheeses could be attributed to the rate of proteolysis and the formation of free amino acids, free fatty acids, and flavor compounds, which may have contributed to the enhancement of sensory properties. LFUE cheese made with different coagulants showed similar initial total scores. However, after 30 days of ripening, MCC cheese had the highest scores, followed by MCR and CR cheese. MCC cheese maintained the highest total scores (96.3) at the end of ripening, surpassing MCR cheese (95) and CR cheese (91).

3.7. Principal component analysis (PCA) for LFUE cheese

To gain insight into the microbial coagulants influencing the quality attributes of low-fat Edam cheese produced from ultrafiltered buffalo milk (LFUE), a Principal Component Analysis (PCA) was conducted. This analysis included variables such as chemical composition (moisture, fat, fat/DM, protein, salt/moisture, ash, pH), proteolysis indicators (SN/TN, NPN/TN), firmness, and sensory characteristics (appearance, flavor, texture and total score) as shown in Fig. 6A. PCA also provide an overview of the similarities and differences among treatments, both in their free amino acids content after 90 days of ripening (Fig. 6B) and in their free fatty acids content during the 30–90 days of ripening period (Fig. 6C). Fig. 6A shows that the first two principal components, (PC1 78.37% and PC2 9.81%), accounted for 81.18% of the total variation. In Fig. 6A, variables such as Salt/moisture, Fat, Ash, TP, SN/TN, NPN/TN, and organoleptic properties show positive correlation with PC1, particularly in relation to CR made from calf rennet, MCR, and MCC produced with microbial rennet at 60 and 90 days of ripening.

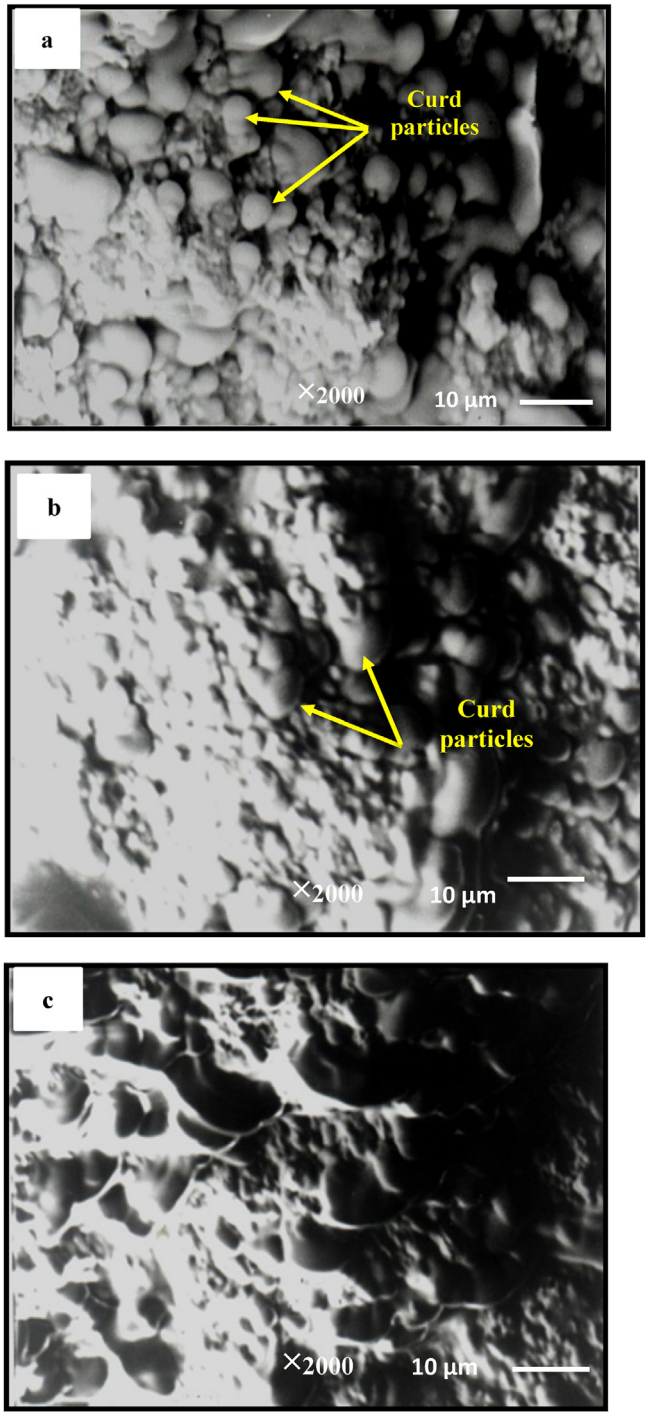

Fig. 5. Micrographs of low-fat Edam cheese made from ultrafiltered buffalo milk with microbial coagulants at the end of ripening. A: Calf rennet; B: microbial coagulant (*Rhizomucor miehei*); C: microbial coagulant (*Cryphonectria parasitica*).

Conversely, the remaining variables exhibit negative correlation with PC1 and are associated with CR, MCR, and MCC at 0 and 30 days of ripening. From the same plot it was noted that MCC and MCR cheese presenting microbial coagulants at 60 and 90 days of the ripening periods tend to be gathered at the top right of Fig. 6A, while LFUE cheese made from CR (control) were located at the bottom right of Fig. 6A. This gathering indicates that LFUE cheese made from microbial coagulants has similar properties. In general, LFUFE cheese produced using *Rhizomucor miehei* and *Cryphonectria parasitica* exhibits a tendency to form tighter aggregates when compared to those made with calf rennet. The notable resemblance between MCR and MCC treatments observed between days 60 and 90 may be attributed to their comparable elevated levels of proteolysis indicators and organoleptic properties. While the control treatments were located at the bottom right of Fig. 6A due to their low values for those indicators.

Table 4
Results of organoleptic assessment of low-fat Edam cheese made from ultrafiltered buffalo milk with microbial coagulants during ripening.[a]

Quality attributes	Ripening time (d)	Low-fat Edam cheese		
		CR	MCR	MCC
Appearance (10)	0	7.0 ± 1.7^{Aa}	7.0 ± 0.0^{Aa}	7.0 ± 1.0^{Aa}
	30	8.0 ± 0.0^{Aab}	8.0 ± 1.7^{Aab}	8.0 ± 1.7^{Aab}
	60	9.0 ± 1.7^{Abc}	9.0 ± 1.0^{Abc}	9.0 ± 0.0^{Abc}
	90	9.0 ± 1.0^{Ac}	10.0 ± 0.0^{Ac}	10.0 ± 0.0^{Ac}
Texture (40)	0	30.0 ± 0.0^{Aa}	30.0 ± 1.0^{Aa}	30.0 ± 0.0^{Aa}
	30	32.0 ± 1.7^{Ab}	32.0 ± 1.0^{Ab}	34.0 ± 0.0^{Ab}
	60	36.0 ± 1.7^{Ac}	35.0 ± 1.0^{Ac}	36.0 ± 1.7^{Ac}
	90	37.0 ± 1.7^{Ad}	38.0 ± 0.03^{Ad}	39.0 ± 1.0^{Ad}
Flavor (50)	0	40.0 ± 3.61^{Aa}	41.0 ± 4.3^{Aa}	41.0 ± 1.0^{Aa}
	30	42.0 ± 0.0^{Ab}	43.0 ± 1.7^{Ab}	44.0 ± 0.0^{Ab}
	60	45.0 ± 1.0^{Ac}	45.0 ± 0.02^{Ac}	45.0 ± 1.7^{Ac}
	90	45.0 ± 2.6^{Ac}	47.0 ± 0.02^{Ac}	47.0 ± 1.7^{Ac}
Total score (100)	0	82.0 ± 1.7^{Aa}	82.0 ± 3.0^{ABa}	82.0 ± 3.5^{Ba}
	30	82.0 ± 2.6^{Aa}	83.0 ± 1.7^{ABa}	86.0 ± 1.7^{Ba}
	60	87.3 ± 4.1^{Ab}	89.0 ± 2.0^{ABb}	90.0 ± 2.0^{Bb}
	90	91.0 ± 2.7^{Ac}	95.0 ± 0.01^{ABc}	96.3 ± 1.2^{Bc}

[a] Mean ± SD in each column within ripening time with different lowercase letters differ significantly ($P < 0.05$). The values are mean ± SD for n = 3. Mean ± SD in each row with different uppercase letters are significantly ($P < 0.05$). CR: Calf rennet; MCR: microbial coagulant (*Rhizomucor miehei*); MCC: microbial coagulant (*Cryphonectria parasitica*) used in manufacture of low fat UF Edam cheese.

Fig. 6B illustrates the analysis of LFUE cheese after 90-day ripening period, denoting the variation in free amino acids across principal components P1 (97.09%) and P2 (1.62%), which collectively account for 98.71% of the total variability. Notably, LFUE cheese produced using MCR and MCC exhibited a striking similarity, both positioned on the positive side of PC1. Furthermore, this cheese variant displayed higher concentrations of various free amino acids compared to CR and the control group, located within the negative coordinates of PC1. Specifically, in the PCA plot, MCC after 90 days of ripening, situated in the top-right quadrant, demonstrated increased levels of amino acids such as aspartic, histidine, threonine, alanine, valine, methionine, phenylalanine, and lysine. These findings suggest increased proteolytic activity in matured LFUE cheese prepared with *C. parasitica* (MCC). These observations were correlated with elevated levels of SN/TN and NPN/TN in Fig. 6A, indicating their contribution to the maturation process of cheese. The PCA emphasized and illustrated the results of free fatty acids at 30, 60, and 90 days of ripening, as clearly shown in Fig. 6C. The initial two factors, PC1 and PC2, elucidated 76.18% of the variance in the variables. In the PCA classification, treatments were grouped into three categories, with the first group situated to the right of PC1 (Fig. 6C). This group encompassed the MCC treatments, which exhibited the highest values for both α-linolenic (C18:3) and myristic (C14) fatty acids at 30 and 60 days of ripening, while MCC at 90 days had the highest values for capric (C10), linoleic (C18:2), and palmitic (C16) fatty acids. The CR treatment at 30, 60, and 90 days of maturity was included in the second group, which is located at the upper left of Fig. 6C. This group exhibited the highest levels of stearic (C18), oleic (C18:1), and arachidic (C20) fatty acids. Finally, the third group was located at the bottom left of Fig. 6C, including the MCR treatment, which has high levels of caprylic (C8) and lauric (C12) fatty acids. This suggests that using microbial coagulants, especially *Cryphonectria parasitica*,

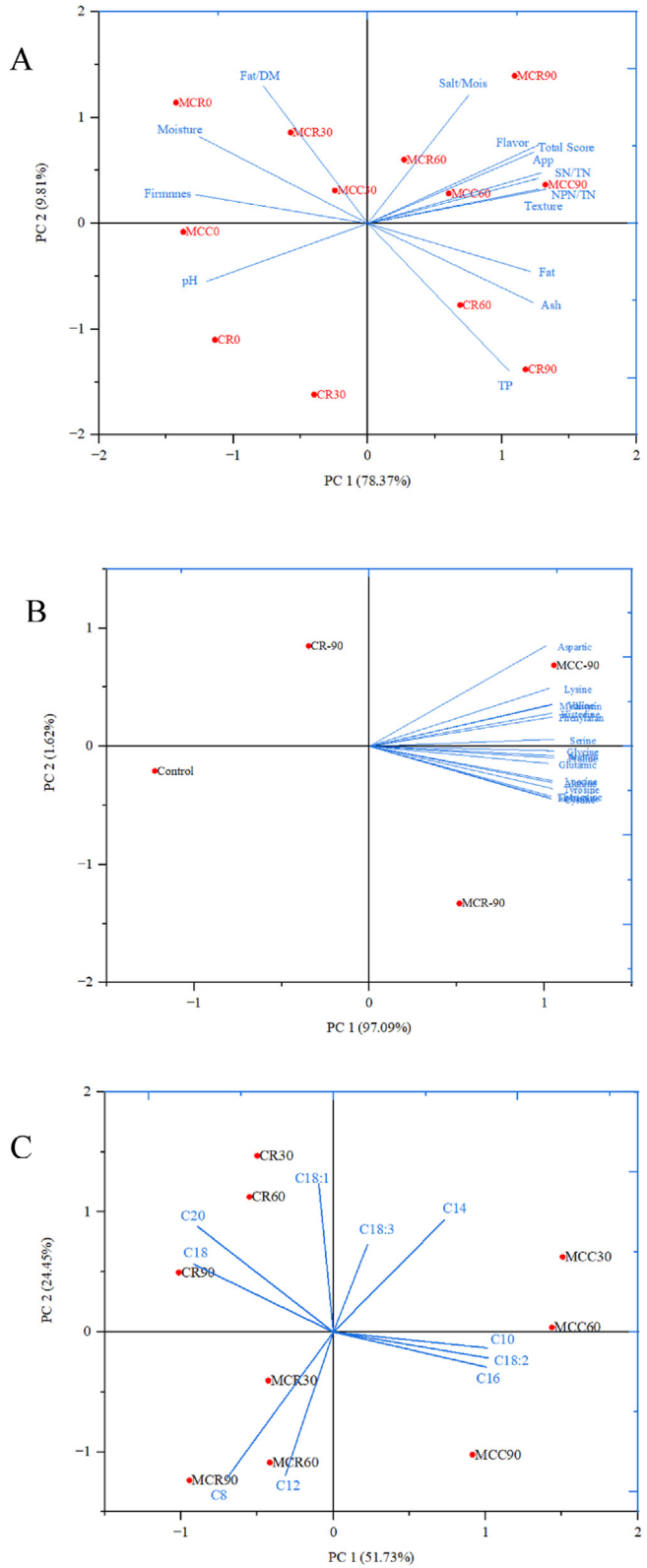

Fig. 6. Principal component analysis (PCA) model of variables: (A) Chemical composition (moisture, fat, Fat/DM, protein, Salt/moisture, ash, pH), proteolysis (SN/TN, NPN/TN), firmness, and sensory attributes, (B) Free amino acids values, and (C) Free fatty acids values of LFUE cheese treatments made from microbial coagulants during ripening periods, (0–90 days) for (A); after 90 days for (B). and at (30–90 days) for (C). CR (control): Calf rennet; MCR: microbial coagulant (*Rhizomucor miehei*); MCC: microbial coagulant (*Cryphonectria parasitica*) used in manufacture of low fat UF Edam cheese. 0, 30, 60, and 90 for CR, MCR, and MCC = ripening periods (days). DM: dry matter ratio; SN/TN: soluble nitrogen/total nitrogen ratio; NPN/TN: non-protein nitrogen/total nitrogen ratio; App: Appearance.

in the manufacture of low-fat Edam cheese made from ultrafiltered buffalo milk (LFUE) resulted in improved final product quality compared with calf rennet.

4. Conclusion

The study investigated the impact of various coagulants on the chemical composition, firmness, proteolysis, microstructure, and sensory characteristics of LFUE cheese. It found that the choice of coagulant did not significantly affect the chemical composition and firmness, while only minor effects were observed on the FFA content. Microbial coagulants resulted in higher proteolysis, as evidenced by elevated levels of water-soluble nitrogen (WSN) and trichloroacetic acid-soluble nitrogen (TCA-SN) during ripening, and greater degradation of αs- and β-caseins compared to calf rennet. LFUE cheeses with microbial coagulants, particularly *R. miehei* and *C. parasitica*, exhibited smoother protein networks and superior sensory attributes, including appearance, body, texture, and flavor, surpassing those made with calf rennet. These findings suggest that microbial coagulants offer potential as effective alternatives to traditional calf rennet in LFUE cheese production, enhancing the sensory qualities and overall quality of the final product. This research provides valuable insights for cheese manufacturers and researchers seeking to optimize LFUE cheese production processes.

CRediT authorship contribution statement

Safaa A.M. El-Aidie: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Roberto Castro-Muñoz:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition. **Basim Abu-Jdayil:** Writing – original draft, Conceptualization. **Samia M. El-Dieb:** Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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