



## Research article

Utility of *Moringa oleifera* waste as a coagulant in goat soft cheese productionEl-Sayed M.M. Abdeen<sup>a</sup>, Osama A. Ibrahim<sup>b,\*</sup>, Adel M.M. Kholif<sup>b</sup><sup>a</sup> Animal and Poultry Breeding Department, Desert Research Center, Cairo, Egypt<sup>b</sup> Dairy Science Department, National Research Centre, 33 El Bohouth St., Dokki, Giza P.O. 12622, Egypt

## HIGHLIGHTS

- Milk clotting enzyme was purified from moringa waste resulted during oil extraction.
- Purified milk clotting enzyme from moringa waste could be used as calf substitute.
- Moringa milk clotting enzyme could be used as a coagulant for different milk types.
- No bitterness appeared in goat cheese coagulated with moringa milk clotting enzyme.

## ARTICLE INFO

## Keywords:

Moringa  
Calf rennet substitute  
Goat cheese  
Plant coagulant  
Plant waste

## ABSTRACT

Milk clotting enzyme (MCE) of *Moringa oleifera* from prepared seed cake (PSC) dissolved in acetate buffer pH 5.0 recorded the highest activity compared to other samples, as well as 20–40% saturation of ammonium sulfate precipitated MCE with 28.20% yield and 1.01 purification fold. The proteolytic activity (PA) of crude MCE from *Moringa oleifera* PSC was higher than those of partial purified MCE with 180.81 and 155.47 as MCA/PA ratio, respectively. PSC moringa MCE exhibited their optimal activity at pH 5.0 and 60 °C; it could be capable to coagulate different milk types. Also, goat soft cheeses coagulated with moringa MCE exhibited significantly ( $p \leq 0.05$ ) higher levels of water soluble nitrogen content and total sensorial scores than control cheese. It could be concluded that partial purified MCE from *Moringa oleifera* PSC may prove to be a good candidate in goat cheese production without any appeared defects during their storage period.

## 1. Introduction

Conversion of milk into dairy product such as cheese for longer shelf life retains the milk nutrients that has played a key role in human nutrition (Fox and McSweeney, 2004; Mohamed Ahmed et al., 2010). Calf rennet as a traditional MCE preparation used in cheese production (Harboe et al., 2010). Calf rennet as a conventional MCE was extracted from the suckling calves fourth stomach contains a major portion of chymosin (EC 3.4.23.4), and small portion of pepsin (EC 3.4.23.1); while this proportion is inverted when it was extracted from adult animals (Agudelo et al., 2004). However, global cheese production increases with shorting of calf rennet supply leading to search for calf substitutes from easily and available resources (Mahajan and Badgular, 2012). In addition, calf rennet may be limited for religious reasons, dietary restrictions (vegetarianism), or consumer concern regarding genetically engineered foods (Roseiro et al., 2003). Thus, much research interest has been

directed towards discovering calf substitute for cheese production from MCE preparations of animal, microbial, and plant origin have been studied (Jacob et al., 2011). Much attention has been directed towards natural rennet extracted from plant sources such as *Carica papaya*, *Ficus carica*, *Cynara cardunculus* (Roseiro et al., 2003; Galán et al., 2008), *Cynara scolymus* (Sidrach et al., 2005), and *Solanum dubium* (Mohamed Ahmed et al., 2009) among others. Unfortunately, most of these plant coagulants considered inappropriate due to possess high proteolytic activity leads to produce of short peptides that are responsible for appeared defects in flavor and texture of cheese (Lo Piero et al. 2002; Anusha et al., 2014). Although, an early study showed that the MCE extracted from *Cynara cardunculus* flowers, has been used for sheep cheese production in several areas of Portugal and Spain for years (Verissimo et al., 1995). After three months of ripening, the cheese produced by *Albizia julibrissin* seed extract did not acquire bitterness (Otani et al., 1991). Bruno et al. (2010) also found that the cheese made using *Bromelia hieronymi* fruit

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extract was satisfactory in terms of appearance, body, texture, and flavour. Thus, searching for calf rennet substitute from plants having high ratio of milk clotting activity compared to their proteolytic activity is highly needed to overcome the above mentioned problems.

The ancient Romans, Greeks, and Egyptians ate *moringa oleifera*, which is an edible plant. It is the most nutrient-rich plant which essential for livestock and humans. Thus, several parts of the moringa tree have been utilized in a variety of purposes, including food to combat malnutrition, feed for weight growth and milk production, medication to cure and/or prevent a variety of ailments, and environmental water clarity (Fahey, 2005). Although, moringa has a wide range of applications and uses, but research on its usage as a source of MCE and biochemical features, as well as its technological feasibility for cheese production, is restricted. Thus, the aim of the present study were to purify and characterize of MCE from *Moringa oleifera* waste as an attempt to select the suitable source, cheap and available of plant coagulant as a substitute of calf rennet, and study their effect on chemical, rheological and sensorial properties of goat soft cheese compared to both calf and microbial coagulants, in order to determine its technological suitability for goat cheese production.

## 2. Materials and methods

### 2.1. Raw material and chemicals

*Moringa oleifera* seeds and commercial seed cake (CSC) were obtained and identified from the Egyptian Scientific Society of Moringa, National Research Centre (NRC), Giza, Egypt. Moringa samples including seed kernel (SK), seed husk (SH), and whole seed (WS) were prepared as follow: moringa seeds husk (SH) were removed manually from the whole seeds (WS) to prepare the shelled white kernel (SK), then all of these samples dried at room temperature for two days, and it is ground into a fine powder by laboratory mill, Then it is stored in closed containers at 4 °C until it is use. Also, prepared seed cake (PSC) was prepared from the shelled seeds using cold pressing technique to extract the moringa oil as an attempt to protect the enzyme activity in the seeds, thereafter the seed cake was dried at room temperature for two days, then ground into a fine powder by laboratory mill and stored at 4 °C until use.

Fresh goat, sheep and camel milk were obtained from the herd of Desert Research Center, Egypt; while cow and buffalo milk were obtained from College of Agriculture station, Cairo University. Veal rennet was purchased from Mifad, Misr food additives, Egypt. Microbial coagulant from *M. miehei* (RENIPLUS) was purchased from Caglio star, Spain. Skim milk powder was purchased from BIELMLEK Spoldzielnia mleczrska, Poland. Coomassie brilliant blue G-250 dye was purchased from Bio-Rad, USA. Other chemicals were of analytical grade.

### 2.2. Methods

#### 2.2.1. Detection of milk-clotting activity (MCA) on different *Moringa oleifera* parts

MCA were determined on different *Moringa oleifera* samples such as: SK, SH, CSC, PSC, and WS using distilled water and different extraction buffers at pH 5.0 e.g., sodium acetate, Tris-HCl, and phosphate. The protein content was determined for all extracts and specific activities were calculated.

#### 2.2.2. Extraction and purification of MCE from *Moringa oleifera* PSC

Extraction of MCE from prepared seed cake (PSC) dissolved in sodium acetate pH 5.0. The recovered supernatant was considered as crude extract, and then MCA, proteolytic activity (PA) and protein content (PC) were determined.

MCE of crude extract was partial purified using ammonium sulphate precipitation method according to Colowick and Kaplan (1955). The rich active fraction was dialyzed using acetate buffer pH 5.0 overnight. MCA,

PA and protein content of the recovered fractions were determined. The rich fraction of MCA was considered as a partial purified MCE.

#### 2.2.3. Milk-clotting activity (MCA)

MCA of all prepared fractions were measured according to the method of IDF (1992). MCA is expressed as Soxhlet unit (SU), which calculated using the following equation according to IDF (1992):

$$\text{Soxhlet units/ml} = M \times 2400 / E \times t$$

**M:** substrate volume (ml). **E:** enzyme extract (ml). **t:** clotting time (sec).

One SU of MCA was defined as the amount of enzyme required to clot 1 ml of substrate within 40 min at 35 °C.

#### 2.2.4. Determination of PA

The PA of all prepared fractions was determined according to Chopra and Mathur (1983). The PA unit is defined as the amount of enzyme required to release TCA-soluble fragment giving a blue color equivalent to one µg of tyrosine under the same standard assay condition.

#### 2.2.5. Determination of protein content

Protein content was determined in all prepared fractions was as described by Bradford (1976) procedure using bovine serum albumin (BSA) as a standard.

#### 2.2.6. Specific activity calculation

The specific activity is calculated by divide the determined MCA to the protein content.

#### 2.2.7. Biochemical characterization of MCE from *Moringa oleifera* PSC

Some biochemical properties of partial purified MCE were determined as follows: **Optimum pH:** MCA was measured at different pH values (3–8) using 0.2 M Acetate buffer (pH 3.0–5.0), 0.2 M phosphate buffer (pH 6.0–7.0), and 0.2 M Tris-HCl buffer (pH 8.0). **Optimum temperature:** MCA was determined at different reaction temperatures (30–80 °C) to define their optimum reaction temperature. **Effect of CaCl<sub>2</sub> concentration on MCA:** Skim milk powder as a substrate was dissolved in different concentrations of CaCl<sub>2</sub> ranging from 0.01–0.05% to define the optimal CaCl<sub>2</sub> concentration for MCA. **Effect of NaCl concentration on MCA:** Effect of different concentrations of NaCl ranging from 2.5–20% on MCA were determined. **Determination of MCA on different milk types:** MCA was determined using different milk types such as: cow, buffalo, goat, sheep and camel milk.

#### 2.2.8. Goat soft cheese manufacture

Goat soft cheese was manufactured as described by Abd El-kader (2003). Pasteurized goat milk was divided to four equal portions: the first portion coagulated made with veal rennet (T1); the second portion made with microbial coagulant (T2); while the third and fourth portions were coagulated with crude (T3) and partial purified (T4) MCE from moringa PSC, respectively. The resultant goat soft cheese was packed in plastic cups filled with pasteurized whey and stored at 7 ± 1 °C for 28 days. The whole experiment was repeated in three replicate.

**2.2.8.1. Chemical properties of cheese.** Different cheese samples were chemical analyzed when fresh and after 14, 15, 21 and 28 days of storage at 7 ± 1 °C. Moisture, ash, protein and soluble nitrogen (SN) contents were determined according to AOAC (2000). The pH on warm water macerates was determined using a digital pH-meter with glass electrodes, Ingold, Knick, Germany. Titratable acidity as lactic acid was determined according to Ling (1963).

**2.2.8.2. Texture profile analysis (TPA) of cheese.** TPA of goat soft cheese was carried out using with a Universal Testing Machine (Co metech, B type, Taiwan). A 25-mm-diameter perplex conical-shaped probe was used to perform the TPA analysis at five different points on the sample

**Table 1.** Detection of milk-clotting activity (MCA) on different *Moringa oleifera* samples using different extraction buffers.

| Sample | Extraction buffers |                           |                |                           |                 |                           |                 |                           |
|--------|--------------------|---------------------------|----------------|---------------------------|-----------------|---------------------------|-----------------|---------------------------|
|        | Phosphate pH 5.0   |                           | Acetate pH 5.0 |                           | Tris-HCl pH 5.0 |                           | Distilled water |                           |
|        | MCA*<br>SU/ml      | Specific activity<br>U/mg | MCA*<br>SU/ml  | Specific activity<br>U/mg | MCA*<br>SU/ml   | Specific activity<br>U/mg | MCA*<br>SU/ml   | Specific activity<br>U/mg |
| SK     | 738.46             | 4197.21                   | 342.86         | 2643.52                   | 369.23          | 2296.91                   | 274.29          | 3110.55                   |
| SH     | 66.67              | 869.16                    | 68.57          | 1428.23                   | 60.00           | 565.67                    | 40.00           | 487.19                    |
| CSC    | 96.00              | 707.06                    | 152.38         | 991.66                    | 103.23          | 704.25                    | 79.67           | 697.80                    |
| PSC    | 518.92             | 3085.54                   | 408.51         | 3109.26                   | 369.23          | 2082.62                   | 237.04          | 1695.26                   |
| WS     | 204.26             | 1406.48                   | 274.29         | 2879.11                   | 240.00          | 1754.57                   | 112.94          | 1364.38                   |

**Specific activity** = Enzyme activity/Protein content. **SK**: seed kernel, **SH**: seed husk, **CSC**: commercial seed cake, **PSC**: prepared seed cake, **WS**: whole seed.

\* MCA based on 0.1 g/ml of dried prepared seed cake of moringa in 0.1 M sodium acetate buffer pH 5.0.

surface. The generated plot of force (N) versus time (s) was recorded. The following parameters were determined according to the definition given by the International Dairy Federation (IDF, 1991). From the resulting force-time curve the values for texture attributes were calculated using TPA graphic.

**2.2.8.3. Sensory evaluation.** Sensorial properties of goat soft cheese during their storage period were assessed by the staff members of Dairy Department at National Research Centre, with a maximum score points of 50 points for flavor, 40 points for body and texture, and 10 points for the cheese appearance as described by Pappas et al. (1996).

### 2.2.9. Statistical analysis

Statistical analysis was performed using ANOVA procedure for analysis of variance, and the general linear model (GLM) procedure using SAS software (SAS, 1990). The results were expressed as mean  $\pm$  standard error and the differences between means were tested for significance using Duncan's multiple range tests at ( $p \leq 0.05$ ).

## 3. Results and discussion

MCE was detected in the current investigation in various samples of *Moringa oleifera* in order to determine the appropriate source as a veal rennet substitute. Moreover, its effect on chemical, rheological and sensorial properties of goat soft cheese compared to commercial coagulants were investigated to confirm their suitability as a plant coagulant for goat soft cheese production.

### 3.1. Detection of MCA on different *Moringa oleifera* samples

MCA were determined on different *Moringa oleifera* samples such as: seed kernel (SK), seed husk (SH), commercial seed cake (CSC), prepared seed cake (PSC), and whole seed (WS) using different extraction buffers in order to select the potential source of MCE from moringa samples as shown in Table 1. It could be noticed that SK had the highest MCA and specific activity in all moringa samples dissolved in different extraction buffers except sodium acetate pH 5.0 which showed the highest MCA and specific activity for PSC. However, detected MCA in the line with Tajalsir et al. (2014) who detect MCA on moringa seeds extract; as well as

El-mazar et al. (2012), Egito et al. (2007), Mohamed Ahmed et al. (2009), and Nestor et al. (2012) detect MCA on different plant seeds.

However, PSC sample using the cold pressing method showed MCA more close to SK activity, it could be due to the pressing of the moringa seeds without high temperature protects MCE from losing its activity; as well as this procedure provides the moringa oil with good characteristics together with using the waste as a cheap source of MCE close to seed Kernel MCA. Also, the studies concerning to detect MCA in seed cakes still limited. Therefore, in the present study MCE was partial purified from PSC as a moringa waste during the moringa oil preparation. Also, the biochemical characteristics of the partial purified MCE, as well as their effect on different milk types were investigated.

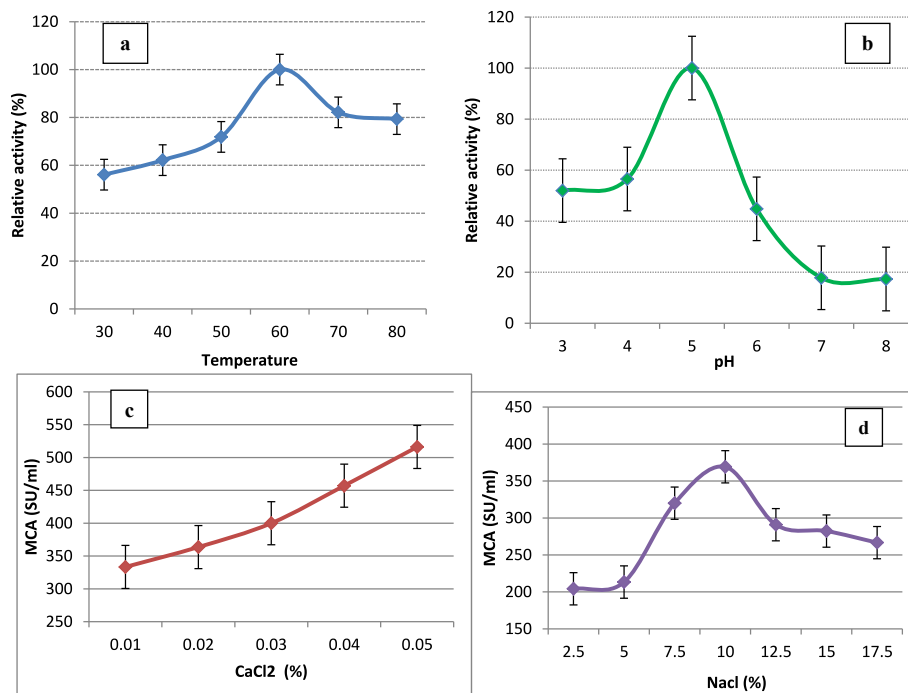
### 3.2. Extraction and partial purification of MCE from *Moringa oleifera* PSC

Table 2 shows that the ammonium sulfate at the level of 20–40% saturation was partial purified the MCE from moringa PSC with 28.20% yield and 1.01 purification fold compared to their crude extract. These results are similar with Tajalsir et al. (2014), who reported that, MCA of *Moringa oleifera* seeds were detected in 20–40% of ammonium sulfate concentrations as well as Nestor et al. (2012) reported that, highest MCA of *Solanum elaeagnifolium* seeds extract recorded at 20% of ammonium sulfate saturation. Moreover, using of ammonium sulfate at low saturation is better from high concentrations that usually used for the purification of MCE from various plant sources (Mohamed Ahmed et al., 2009; Pontual et al., 2012). Ammonium sulfate precipitation (ASP) procedure facilitates for concentration the enzyme extract to a suitable volume that could efficiently use for milk coagulation in cheese manufacture (Tajalsir et al., 2014). However, ASP in one-step has been considered as an economic purification procedure combined with the availability of the plant waste leads to produce MCE in the large scale is possible. Moreover, it could be noted from Table 2, the PA of crude MCE from *Moringa oleifera* PSC was higher than those of partial purified MCE from moringa PSC with 164.72 and 155.47 as MCA/PA ratio, respectively. Similar findings were reported by Abd El-Salam et al. (2017) who observed that PA and MCA/PA ratio of crude MCE from artichoke flowers were higher than the partial purified MCE precipitated by ammonium sulfate. However, MCA and PA in the moringa seed extracts were expected since most of the proteolytic enzymes are concentrated in

**Table 2.** Milk-clotting activity (MCA) and proteolytic activity (PA) of *Moringa oleifera* PSC during purification steps.

| Purification steps | MCA<br>(SU/ml) | Protein<br>(mg/ml) | Specific activity<br>(SU/mg protein) | Yield<br>(%) | Purification fold | PA<br>(U/ml) | (MCA/PA) ratio |
|--------------------|----------------|--------------------|--------------------------------------|--------------|-------------------|--------------|----------------|
| Crude enzyme       | 408.51         | 0.1317             | 3101.82                              | 100          | 1.00              | 2.48         | 164.72         |
| ASP (20–40 %)      | 384            | 0.123              | 3121.95                              | 28.20        | 1.01              | 2.47         | 155.47         |

**PA**: Proteolytic activity; **Specific activity** = Enzyme activity/Protein content; **Total MCA** = Enzyme activity X Fraction volume; **Total protein** = Protein content X Fraction volume; **Yield** = Total activity of purified enzyme/Total activity of crude enzyme X 100; **Purification fold** = Specific activity of purified enzyme/Specific activity of crude enzyme; **ASP**, Ammonium sulfate precipitation.



**Figure 1.** Biochemical characteristics of MCE extracted from *Moringa oleifera* PSC. a: optimum temperature; b: optimum pH; c: calcium chloride; d: sodium chloride.

the seeds which degrade the storage protein during seed germination (Antao and Malcata, 2005).

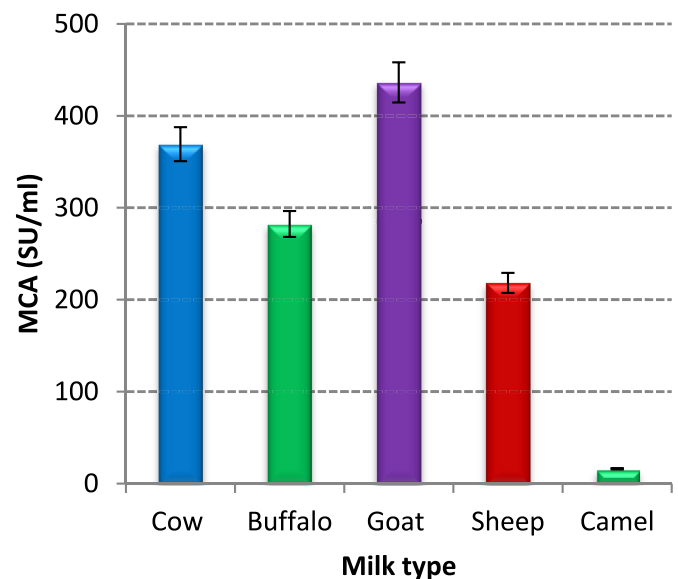
### 3.3. Biochemical characterization of MCE from *Moringa oleifera* PSC

Plant MCE temperature profiles are influenced by plant source, tissue, concentration, and proteolytic enzyme type (Mazorra-Manzano et al., 2013). MCE partially purified from *Moringa oleifera* PSC at different reaction temperatures ranging from 30 to 70 °C were determined. Figure 1a, shows that the MCA was increased as the reaction temperature increased with optimal activity at 60 °C and then it was sharply decreased as the reaction temperature raised over 70 °C. These findings are agreement with MCE from various plants has been reported (Mohamed Ahmed et al., 2009; Mazorra-Manzano et al., 2013; Pontual et al., 2012; Abd El-Salam et al., 2017). However, MCA at  $\geq 50$  °C was higher than at 40 °C, it could be attributed to the protein aggregation and molecular rearrangement in the protein structure (Najera et al., 2003).

The most appropriate response MCA was assessed at a range of pH values from 3 to 8 using various buffers. As demonstrated in Figure 1b, MCE partially purified from moringa PSC demonstrated optimal action at pH 5.0, and then MCA was lowered above pH 7.0. The optimal pH for the reaction (5.0) was comparable to that described by Sidrachl et al. (2005), Nouani et al. (2009) and Abd El-Salam et al. (2017), who reported that the optimal pH activity form different type of artichoke and fig lies in acidic pH values.

Calcium ions are essential in the non-enzymatic phase of milk coagulation (Esteves et al., 2001; Mohamed Ahmed et al., 2010). Thus the effect of different concentration of calcium chloride at the level of 0.01–0.05% on partial purified MCE from PSC of moringa was studied. It could be noticed from Figure 1c, the MCA were gradually increased as the calcium chloride concentration increased. Thus, it was confirmed with partial purified MCE from moringa which required suitable calcium chloride concentration for it is optimal for enzyme activity, in the same line with Abd El-Salam et al. (2017), who reported the Ca<sup>+2</sup> ions dependent in the curd formation by MCE extracted from artichoke flowers.

Sodium chloride is commonly used in food and dairy products as a preservative and flavor ingredient (Walstra et al., 1999; Guinee and Fox, 2004). Thus, the effect of different concentrations of sodium chloride at



**Figure 2.** Milk clotting activity of *Moringa oleifera* PSC on different milk types.

the level of 2.5–17.5% on partial purified MCE from moringa PSC was evaluated. As shown in Figure 1d, it could be noticed that the MCA were increased gradually until 10% of sodium chloride, and then decreased as the sodium chloride level increased. Similar findings by Ahmed et al. (2016) who reported that MCE extracted from *Bacillus stearothermophilus* exhibited their highest MCA activity at the level of 0.1 M sodium chloride while higher concentrations of sodium chloride decreased its MCA.

The effectiveness of partially purified MCE from PSC moringa on several milk types, including cow, buffalo, goat, camel, and sheep milk, was investigated.

As shown in Figure 2, the partial purified MCE could be capable to coagulate the different examined milk types with different coagulation time started of cow, buffalo, goat, sheep followed by camel milk. However, the effect of partial purified MCE towards different milk caseins supported their suitability to be used as a calf substitute in cheese

**Table 3.** Chemical changes of goat soft cheese coagulated with *Moringa oleifera* PSC compared to commercial coagulants during storage at  $7 \pm 1$  °C for 28 days.

| Parameter          | Storage period (day) | Treatments         |                    |                    |                    |
|--------------------|----------------------|--------------------|--------------------|--------------------|--------------------|
|                    |                      | T1                 | T2                 | T3                 | T4                 |
| Moisture (%)       | Fresh                | 68.21 <sup>a</sup> | 69.48 <sup>a</sup> | 68.13 <sup>a</sup> | 67.64 <sup>a</sup> |
|                    | 7                    | 67.82 <sup>b</sup> | 68.62 <sup>b</sup> | 67.64 <sup>a</sup> | 67.24 <sup>a</sup> |
|                    | 14                   | 66.99 <sup>c</sup> | 67.84 <sup>c</sup> | 66.48 <sup>b</sup> | 66.51 <sup>b</sup> |
|                    | 21                   | 66.51 <sup>c</sup> | 66.37 <sup>d</sup> | 65.95 <sup>b</sup> | 66.02 <sup>b</sup> |
|                    | 28                   | 65.29 <sup>d</sup> | 65.68 <sup>e</sup> | 64.99 <sup>c</sup> | 64.30 <sup>c</sup> |
| Total nitrogen (%) | Fresh                | 2.11 <sup>e</sup>  | 2.16 <sup>b</sup>  | 2.25 <sup>d</sup>  | 2.23 <sup>d</sup>  |
|                    | 7                    | 2.24 <sup>d</sup>  | 2.31 <sup>b</sup>  | 2.39 <sup>c</sup>  | 2.53 <sup>c</sup>  |
|                    | 14                   | 2.51 <sup>c</sup>  | 2.53 <sup>ab</sup> | 2.61 <sup>b</sup>  | 2.80 <sup>b</sup>  |
|                    | 21                   | 2.66 <sup>b</sup>  | 2.77 <sup>ab</sup> | 2.79 <sup>a</sup>  | 2.85 <sup>b</sup>  |
|                    | 28                   | 2.81 <sup>a</sup>  | 2.82 <sup>a</sup>  | 2.89 <sup>a</sup>  | 2.93 <sup>a</sup>  |
| WSN (%)            | Fresh                | 0.18 <sup>d</sup>  | 0.16 <sup>d</sup>  | 0.19 <sup>e</sup>  | 0.22 <sup>e</sup>  |
|                    | 7                    | 0.23 <sup>d</sup>  | 0.26 <sup>c</sup>  | 0.29 <sup>d</sup>  | 0.34 <sup>d</sup>  |
|                    | 14                   | 0.38 <sup>c</sup>  | 0.41 <sup>b</sup>  | 0.45 <sup>c</sup>  | 0.52 <sup>c</sup>  |
|                    | 21                   | 0.52 <sup>b</sup>  | 0.54 <sup>a</sup>  | 0.59 <sup>b</sup>  | 0.64 <sup>b</sup>  |
|                    | 28                   | 0.61 <sup>a</sup>  | 0.57 <sup>a</sup>  | 0.67 <sup>a</sup>  | 0.75 <sup>a</sup>  |
| TVFA (%)           | Fresh                | 5.28 <sup>e</sup>  | 6.19 <sup>c</sup>  | 5.88 <sup>e</sup>  | 6.15 <sup>d</sup>  |
|                    | 7                    | 7.55 <sup>d</sup>  | 6.94 <sup>d</sup>  | 7.73 <sup>d</sup>  | 8.67 <sup>c</sup>  |
|                    | 14                   | 9.43 <sup>c</sup>  | 9.91 <sup>c</sup>  | 8.41 <sup>c</sup>  | 10.18 <sup>b</sup> |
|                    | 21                   | 12.80 <sup>b</sup> | 11.23 <sup>b</sup> | 9.34 <sup>b</sup>  | 10.60 <sup>b</sup> |
|                    | 28                   | 16.76 <sup>a</sup> | 15.37 <sup>a</sup> | 12.47 <sup>a</sup> | 15.14 <sup>a</sup> |
| Ash (%)            | Fresh                | 3.75 <sup>a</sup>  | 3.27 <sup>b</sup>  | 3.68 <sup>ab</sup> | 3.80 <sup>a</sup>  |
|                    | 7                    | 3.67 <sup>a</sup>  | 3.37 <sup>ab</sup> | 3.84 <sup>a</sup>  | 3.27 <sup>ab</sup> |
|                    | 14                   | 3.46 <sup>ab</sup> | 3.64 <sup>ab</sup> | 3.59 <sup>ab</sup> | 3.48 <sup>ab</sup> |
|                    | 21                   | 3.35 <sup>ab</sup> | 3.87 <sup>a</sup>  | 3.59 <sup>ab</sup> | 3.42 <sup>b</sup>  |
|                    | 28                   | 3.17 <sup>b</sup>  | 3.48 <sup>ab</sup> | 3.45 <sup>b</sup>  | 3.17 <sup>b</sup>  |
| Acidity (%)        | Fresh                | 0.18 <sup>e</sup>  | 0.19 <sup>d</sup>  | 0.20 <sup>d</sup>  | 0.19 <sup>e</sup>  |
|                    | 7                    | 1.07 <sup>d</sup>  | 1.15 <sup>c</sup>  | 1.17 <sup>c</sup>  | 1.17 <sup>d</sup>  |
|                    | 14                   | 1.21 <sup>c</sup>  | 1.18 <sup>cb</sup> | 1.24 <sup>c</sup>  | 1.28 <sup>c</sup>  |
|                    | 21                   | 1.32 <sup>b</sup>  | 1.28 <sup>b</sup>  | 1.41 <sup>b</sup>  | 1.59 <sup>b</sup>  |
|                    | 28                   | 1.54 <sup>a</sup>  | 1.52 <sup>a</sup>  | 1.77 <sup>a</sup>  | 1.92 <sup>a</sup>  |
| pH                 | Fresh                | 6.13 <sup>a</sup>  | 6.12 <sup>a</sup>  | 6.06 <sup>ab</sup> | 6.02 <sup>a</sup>  |
|                    | 7                    | 5.82 <sup>b</sup>  | 5.74 <sup>b</sup>  | 5.61 <sup>b</sup>  | 5.53 <sup>b</sup>  |
|                    | 14                   | 5.26 <sup>c</sup>  | 5.32 <sup>c</sup>  | 5.27 <sup>c</sup>  | 4.98 <sup>c</sup>  |
|                    | 21                   | 4.99 <sup>d</sup>  | 4.84 <sup>d</sup>  | 4.59 <sup>d</sup>  | 4.24 <sup>d</sup>  |
|                    | 28                   | 4.47 <sup>e</sup>  | 4.53 <sup>e</sup>  | 4.33 <sup>e</sup>  | 4.14 <sup>e</sup>  |

WSN: water soluble nitrogen; TVFA: total volatile fatty acids; T1: liquid calf rennet; T2: microbial rennet powder; T3: crude MCE form prepared seed cake (PSC) of *Moringa oleifera*; T4: partial purified MCE form prepared seed cake (PSC) of *Moringa oleifera*. All parameters are represented as mean of replicates  $\pm$  standard error. Means with different small superscript letters in the same row and different capital superscript letters in the same column are significantly different at  $p \leq 0.05$ .

making. Plant extracts have the ability to hydrolyze k-casein, leading to the development of cohesive curds, and they are also the major enzymes responsible for  $\beta$ -casein hydrolysis (Roseiro et al., 2003).

### 3.4. Chemical changes of goat soft cheese

The chemical changes of goat soft cheese coagulated with plant coagulant from PSC of *Moringa oleifera* (crude and partial purified MCE) compared to both veal and microbial coagulants were showed in Table 3.

The moisture level of goat soft cheese coagulated with moringa MCE was similar to that of cheese coagulated with commercial coagulants, although it was significantly ( $p \leq 0.05$ ) lower than that of cheese coagulated with commercial coagulants. As the storage time proceeded, the moisture content of all goat cheese samples decreased significantly.

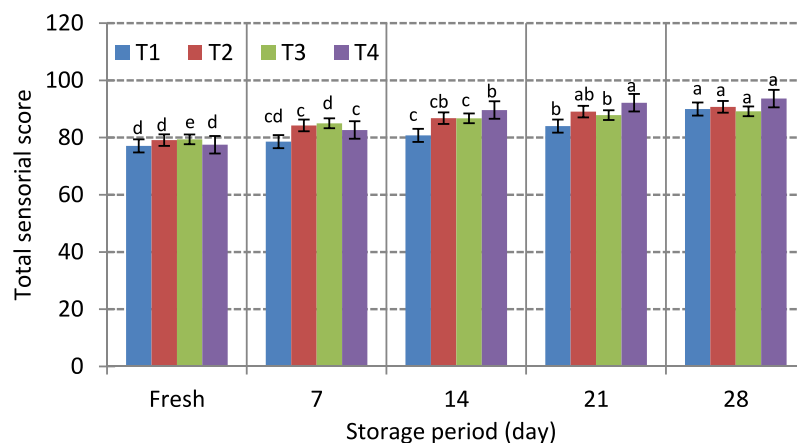
Findings results are in harmony with El-Kholy (2015) and Abd El-Salam et al. (2017). Also, TN contents of goat cheese produced with moringa MCE were slightly higher than the cheese coagulated with both veal and microbial coagulants. TN contents of all resulted goat cheeses were significantly ( $p \leq 0.05$ ) decreased as storage period progressed which could be due to the proteolysis into WSN and hence, loss some of it

in whey (El-Kholy, 2015). Similar results were observed by Abd El-Salam et al. (2017) and Pino et al. (2009) who found that no significant differences between cheese made with veal rennet and plant coagulant concerning protein values. Also, Table 3 shows that the WSN content in the goat cheese made with plant coagulant from PSC of moringa were higher significantly ( $p \leq 0.05$ ) than those coagulated with other commercial coagulants until 21 day of storage, which it could be attributed due to the intense PA of plant coagulant (Tejada and Fernandez-Salguero, 2003; Abd El-Salam et al., 2017). Moreover, WSN contents of all resulted goat cheese were significantly ( $p \leq 0.05$ ) increased as their storage period progressed. Similar finding were reported by Ismail et al. (2010); Hamad and Ismail (2012). Also, Pino et al. (2009) and Abd El-Salam et al. (2017) who observed higher WSN contents of cheese coagulated with plant coagulant than cheese coagulated with veal rennet. It is well known that proteolysis, leads to produce WSN at the early stage, is an important factor for both flavor and texture development of cheese during their ripening period (Sousa et al., 2001; Talib et al., 2009). No significant ( $p \leq 0.05$ ) differences of the ash content of all cheese treatments were observed in both plant and commercial coagulant as mentioned in Table 3. Similar findings were reported by Tejada et al. (2008). Moreover, it could be noted that all goat

**Table 4.** Texture profile analysis of goat soft cheese coagulated with *Moringa oleifera* PSC compared to commercial coagulants during storage at  $7 \pm 1$  °C.

| Textural properties        | Storage period (day) | Treatments         |                    |                    |                    |
|----------------------------|----------------------|--------------------|--------------------|--------------------|--------------------|
|                            |                      | T1                 | T2                 | T3                 | T4                 |
| Hardness<br>N              | fresh                | 5.21 <sup>c</sup>  | 4.87 <sup>c</sup>  | 4.68 <sup>c</sup>  | 5.10 <sup>c</sup>  |
|                            | 14                   | 6.69 <sup>b</sup>  | 8.73 <sup>b</sup>  | 6.65 <sup>b</sup>  | 7.55 <sup>b</sup>  |
|                            | 28                   | 7.34 <sup>a</sup>  | 9.34 <sup>a</sup>  | 7.80 <sup>a</sup>  | 8.08 <sup>a</sup>  |
| Cohesiveness<br>(area B/A) | fresh                | 0.518 <sup>c</sup> | 0.555 <sup>a</sup> | 0.536 <sup>b</sup> | 0.529 <sup>c</sup> |
|                            | 14                   | 0.782 <sup>b</sup> | 0.583 <sup>a</sup> | 0.559 <sup>b</sup> | 0.676 <sup>b</sup> |
|                            | 28                   | 0.873 <sup>a</sup> | 0.585 <sup>a</sup> | 0.655 <sup>a</sup> | 0.973 <sup>a</sup> |
| Springiness<br>mm          | fresh                | 0.667 <sup>c</sup> | 0.641 <sup>c</sup> | 0.742 <sup>b</sup> | 0.642 <sup>c</sup> |
|                            | 14                   | 0.741 <sup>b</sup> | 0.768 <sup>b</sup> | 0.787 <sup>b</sup> | 0.787 <sup>b</sup> |
|                            | 28                   | 0.818 <sup>a</sup> | 0.823 <sup>a</sup> | 0.922 <sup>a</sup> | 0.881 <sup>a</sup> |
| Gumminess<br>N             | Fresh                | 2.700 <sup>c</sup> | 2.699 <sup>b</sup> | 2.513 <sup>c</sup> | 2.726 <sup>c</sup> |
|                            | 14                   | 5.211 <sup>b</sup> | 5.101 <sup>a</sup> | 3.565 <sup>b</sup> | 5.111 <sup>b</sup> |
|                            | 28                   | 6.080 <sup>a</sup> | 5.461 <sup>a</sup> | 5.104 <sup>a</sup> | 6.413 <sup>a</sup> |
| Chewiness<br>(N*mm)        | Fresh                | 1.791 <sup>c</sup> | 1.726 <sup>c</sup> | 1.864 <sup>c</sup> | 1.757 <sup>c</sup> |
|                            | 14                   | 3.799 <sup>b</sup> | 3.927 <sup>b</sup> | 2.800 <sup>b</sup> | 4.019 <sup>b</sup> |
|                            | 28                   | 5.238 <sup>a</sup> | 4.514 <sup>a</sup> | 4.701 <sup>a</sup> | 5.642 <sup>a</sup> |

T1: liquid calf rennet; T2: microbial rennet powder; T3: crude MCE form prepared seed cake (PSC) of *Moringa oleifera*; T4: partial purified MCE form prepared seed cake (PSC) of *Moringa oleifera*. N: Newton mm: Millimeter N,mm: Newton millimeter. All parameters are represented as mean of replicates  $\pm$  standard error. Means with different small superscript letters in the same row and different capital superscript letters in the same column are significantly different at  $p \leq 0.05$ .



**Figure 3.** Sensorial properties of goat soft cheese coagulated with MCE from *Moringa oleifera* PSC compared to commercial coagulants during storage at  $7 \pm 1$  °C for 28 days. 1: sensorial properties of goat soft cheese (Each point presented the collective total scores of all sensorial properties including flavor, body and texture, and appearance), 2: photo of different goat soft cheese. T1: liquid calf rennet; T2: microbial rennet powder; T3: crude MCE form prepared seed cake (PSC) of *Moringa oleifera*; T4: partial purified MCE form prepared seed cake (PSC) of *Moringa oleifera*.



cheese ash contents were significantly ( $p \leq 0.05$ ) increased as their storage period progressed due to the moisture level reduction during their storage period (Kebary et al., 1996).

Moreover, Table 3 shows that, the acidity (%) of goat soft cheese with opposite trends in their pH value which produced with plant coagulant from PSC of *Moringa oleifera* were slightly higher than those coagulated with commercial coagulants, it could be probably due to the PA of moringa extracts as mentioned in Table 2. Acidity of all goat cheeses were significantly ( $p \leq 0.05$ ) increased as their storage period progressed. These findings are in agreement with those noted by Abd el Kader (2003), and Al-Jasser and Al-Dogan (2009). However, pH variation during cheese ripening depends on their buffering capacity which due to the amount of proteins and minerals, the formation of ammonium and/or catabolism of lactic acid (Lemes et al., 2016).

### 3.5. Rheological profile of goat soft cheese

TPA parameters of goat soft cheese made by different coagulants are given in Table 4. The results showed that, all texture properties were increased as their cheese storage period progressed which might be due to its moisture content as described by Calvo et al. (2007). Awad (2016) reported that, the hardness of Karish cheese was increased during their storage period. Also, no significantly ( $p \leq 0.05$ ) differences on hardness, cohesiveness and chewiness were observed using different coagulants. The cheese gumminess was affected by moringa coagulants while gumminess of cheese coagulated with partial purified moringa MCE (T4) was observed the highest values compared to other treatments. Moreover, springiness of T3 which coagulated with crude of moringa MCE had the highest values compared to other coagulants during of all storage intervals.

**Sensorial properties of goat soft cheese:** Total of sensorial scores including flavor, appearance, body and texture of goat soft cheese coagulated with various coagulants during their storage period for 28 days were presented in Figure 3. The results showed that the total sensorial scores of goat soft cheeses coagulated with partial purified MCE form moringa PSC (T4) were higher than other cheese; while the goat cheese coagulated with crude MCE (T3) was similar to the cheese which coagulated with microbial coagulant (T2) after two weeks until 28 days of their storage period.

#### 4. Conclusion

MCE was isolated and purified from PSC of *Moringa oleifera* which produced as a waste during the production of moringa oil resulting in environmental protection from pollution in order to use it as a veal rennet alternative. Moringa MCE exhibited their optimal activity at pH 5.0 and 60 °C; as well as it could be capable to coagulate different milk types. Moreover, MCE from *Moringa oleifera* PSC leads to softer cheese with good flavor. It could be concluded that partial purified MCE from *Moringa oleifera* PSC could be used as a food additive and veal rennet substitute in goat cheese production without any appeared defects until the end of their storage period.

#### Declarations

##### Author contribution statement

El-Sayed M. M. Abdeen: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Osama A. Ibrahim: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Adel M. M. Kholif: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

##### Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

##### Data availability statement

Data included in article/supplementary material/referenced in article.

##### Declaration of interests statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

#### References

Abd El-kader, Y.I., 2003. Changes in the nitrogen fractions of Domiati cheese made with microbial and recombinant rennets during ripening. *Egypt. J. Dairy Sci.* 31, 111–124.

Abd El-Salam, B.A., Ibrahim, O.A., El-Sayed, H.A., 2017. Purification and characterization of milk clotting enzyme from artichoke (*Cynara cardunculus* L.) flowers as coagulant on white soft cheese. *Int. J. Dairy Sci.* 12, 254–265.

Agudelo, R.A., Gauthier, S.F., Pouliot, Y., Marin, H., Savoie, L., 2004. Kinetics of peptide fraction release during in vitro digestion of casein. *J. Sci. Food Agric.* 84, 325–333.

Ahmed, S.A., Wehaidy, H.R., Ibrahim, O.A., El Ghani, S.A., El-Hofi, M.A., 2016. Novel milk-clotting enzyme from *Bacillus stearothermophilus* as a coagulant in UF-white soft cheese. *Biocat. Agric. Biotechnol.* 7, 241–249.

Al-Jasser, M.S., Al-Dogan, A.A., 2009. Properties of white soft cheese produced using rennet substitute extracted from *Solanum dubium* seeds. *Egypt. J. Dairy Sci.* 37, 237–243.

Antão, C.M., Malcata, F.X., 2005. Plant serine proteases: biochemical, physiological and molecular features. *Plant Physiol. Biochem.* 43, 637–650.

Anusha, R., Singh, M.K., Bindhu, O.S., 2014. Characterisation of potential milk coagulants from *Calotropis gigantea* plant parts and their hydrolytic pattern of bovine casein. *Eur. Food Res. Technol.* 238, 997–1006.

AOAC, 2000. *Official Methods of Analysis*, third ed. AOAC, Benjamin Franklin Station, Washington DC, USA.

Awad, S., 2016. Microbial safety criteria and quality of traditional Egyptian Karish cheese. *Afr. J. Microbiol. Res.* 10, 804–812.

Bradford, M.M., 1976. A rapid and sensitivity method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–354.

Bruno, M.A., Lazza, C.M., Errasti, M.E., López, L.M.I., Caffini, N.O., Pardo, M.F., 2010. Milk clotting and proteolytic activity of an enzyme preparation from *Bromelia hieronymi* fruits. *LWT – Food Sci. Technol.* 43, 695–701.

Calvo, M.V., Castillo, I., Díaz-Barcos, V., Requena, T., Fontecha, J., 2007. Effect of a hygienized rennet paste and a defined strain starter on proteolysis, texture and sensory properties of semi-hard goat cheese. *Food Chem.* 102, 917–924.

Chopra, A.K., Mathur, D.K., 1983. Factors affecting protease production by *Bacillus stearothermophilus* RM-67. *J. Food Protect.* 46, 1020–1025.

Colowick, S.P., Kaplan, N., 1955. *Methods in Enzymology*, I. Academic Press, New York.

Egito, A.S., Girardet, J.M., Laguna, L.E., Poirson, C., Mollé, D., Miclo, L., Gaillard, J.L., 2007. Milk-clotting activity of enzyme extracts from sunflower and albizia seeds and specific hydrolysis of bovine  $\kappa$ -casein. *Int. Dairy J.* 17, 816–825.

El-Kholy, A.M., 2015. The use of artichoke (*Cynara scolymus*, L.) extracts for the production of Tallaga cheese. *Int. J. Food Nutr. Sci.* 4, 34–41.

El-mazar, M.M.E., El-Sayed, S.T., Al-Azzouny, R.A., 2012. Screening some local Egyptian seeds extract for milk-clotting activity and physicochemical characterization of *Brassica napus* seed extract. *J. Agric. Food Tech.* 2, 28–34.

Esteves, C.L.C., Lucey, J.A., Pires, E.M., 2001. Mathematical modelling of the formation of rennet-induced gels by plant coagulants and chymosin. *J. Dairy Res.* 68, 499–510.

Fahey, J.W., 2005. *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees Life J.* 1, 1–15.

Fox, P.F., McSweeney, P.L.H., 2004. *Cheese: an Overview in Cheese: Chemistry, Physics and Microbiology*, third ed. Chapman and Hall London.

Galán, E., Prados, F., Pino, A., Tejada, L., Fernández-Salguero, J., 2008. Influence of different amounts of vegetable coagulant from cardoon *Cynara cardunculus* and calf rennet on the proteolysis and sensory characteristics of cheeses made with sheep milk. *Int. Dairy J.* 18, 93–98.

Guinee, T.P., Fox, P.F., 2004. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), *Salt in Cheese: Physical, Chemical and Biological Aspects, Cheese: Chemistry, Physics and Microbiology*, third ed., Vol. 1. Elsevier, Academic Press, San Diego, CA, pp. 207–259.

Hamad, M.N., Ismail, M.M., 2012. Quality of Soft cheese made with Goat's milk as affected with the addition of certain essences. *J. Anim. Prod. Adv.* 2, 121–127.

Harboe, M., Broe, M.L., Qvist, K.B., 2010. In: Law, B.A., Tamime, A.Y. (Eds.), *Technology of Cheese Making*. Wiley-Blackwell, London.

IDF, 1991. *Rheological and Fracture Properties of Cheeses*. Bulletin No. 268 Brussels. International Dairy Federation, Belgium.

IDF, 1992. *Bovine Rennets: Determination of Total Milk-Clotting Activity*. Bulletin No. 157 Brussels. International Dairy Federation, Belgium.

Ismail, M.M., Ammar, E.M.A., El-Shazly, A.A., Eid, M.Z., 2010. Impact of cold storage and blending different lactations of cow's milk on the quality of Domiati cheese. *Afr. J. Food Sci.* 4, 503–513.

Jacob, M., Jaros, D., Rohm, H., 2011. Recent advances in milk clotting enzymes. *Inter. J. Dairy Technol.* 64, 14–33.

Kebarly, K.M.K., Kamaly, K.M., Zedan, A.N., Zaghlool, A.H., 1996. Acceleration ripening of Domiati cheese by acclase and lipozyme enzymes. *Egypt. J. Dairy Sci.* 24, 75–90.

Lemes, A.C., Pavon, Y., Lazzaroni, S., Rozycki, S., Brandelli, A., Kalil, S.J., 2016. A new milk-clotting enzyme produced by *Bacillus* sp. P45 applied in cream cheese development. *LWT-Food Sci. Technol.* 66, 217–224.

Ling, E.R., 1963. *A Text-Book of Dairy Chemistry, Practical*, third ed., 2. Champan and Hall, London, England.

Lo Piero, A.R., Puglisi, I., Petrone, G., 2002. Characterization of “Lettucine”, a serine-like protease from *Lactuca sativaleaves*, as a novel enzyme for milk clotting. *J. Agric. Food Chem.* 50, 2439–2443.

Mahajan, R.T., Badgujar, S.B., 2012. Biological aspects of proteolytic enzymes: a Review. *J. Pharm. Res.* 3, 2048–2068.

Mazorra-Manzano, M.A., Perea-Gutiérrez, T.C., Lugo-Sánchez, M.E., Ramirez-Suarez, J.C., Torres-Llanez, M.J., González-Córdova, A.F., Vallejo-Cordoba, B., 2013. Comparison of the milk-clotting properties of three plant extracts. *Food Chem.* 141, 1902–1907.

Mohamed Ahmed, I.A., Babiker, E.E., Mori, N., 2010. pH stability and influence of salts on activity of a milk-clotting enzyme from *Solanum dubium* seeds and its enzymatic action on bovine caseins. *LWT-Food Sci. Technol.* 43, 759–764.

Mohamed Ahmed, I.A., Morishima, I., Babiker, E.E., Mori, N., 2009. Characterisation of partially purified milk-clotting enzyme from *Solanum dubium* Fresen seeds. *Food Chem.* 116, 395–400.

Nájera, A.I., De Renobales, M., Barron, L.J.R., 2003. Effects of pH, temperature, CaCl<sub>2</sub> and enzyme concentrations on the rennet-clotting properties of milk: a multifactorial study. *Food Chem.* 80, 345–352.

Néstor, G.M., Dely Rubí, C.G., Héctor, J.C., 2012. Exploring the milk-clotting properties of a plant coagulant from the berries of *S. elaeagnifolium* var. *Cavanilles*. *J. Food Sci.* 77, C89–C94.

Nouani, A., Dako, E., Morsli, A., Belhamiche, N., Belbraout, S., Bellal, M.M., Dadie, A., 2009. Characterization of the purified coagulant extracts derived from artichoke flowers (*Cynara scolymus*) and from the Fig tree latex (*Ficus carica*) in light of their

- use in the manufacture of traditional cheeses in Algeria. *J. Food Technol.* 7, 20–29.
- Otani, H., Matsumori, M., Hosono, A., 1991. Purification and some properties of a milk clotting protease from the young seeds of *Albizia julibrissin*. *Anim. Sci. Technol.* 62, 424–432.
- Pappas, C.P., Kondly, E., Voustinas, L.P., Mallatou, H., 1996. Effect of starter level, draining time and aging on the physicochemical, organoleptic and rheological properties of Feta cheese. *J. Soc. Dairy Technol.* 49, 73–78.
- Pino, A., Prados, F., Galan, E., McSweeney, P.L.H., Fernandez-Salguero, J., 2009. Proteolysis during the ripening of goats' milk cheese made with plant coagulant or calf rennet. *Food Res. Int.* 42, 324–330.
- Pontual, E.V., Carvalho, B.E., Bezerra, R.S., Coelho, L.C., Napoleão, T.H., Paiva, P.M., 2012. Caseinolytic and milk-clotting activities from *Moringa oleifera* flowers. *Food Chem.* 135, 1848–1854.
- Roseiro, L.B., Barbosa, M., Ames, J.M., Wilbey, R.A., 2003. Cheese making with vegetable coagulants—the use of *Cynara L.* for the production of ovine milk cheeses. *Int. J. Dairy Technol.* 56, 76–85.
- SAS Institute, 1990. *SAS User's Guid/STAT Ver.6.04*, fourth ed. SAS Inst. Inc., Cary, NC.
- Sidrach, L., García-Cánovas, F., Tudela, J., Rodríguez-López, J.N., 2005. Purification of cynarases from artichoke (*Cynara scolymus L.*): enzymatic properties of cynarase A. *Phytochemistry* 66, 41–49.
- Sousa, M.J., Ardo, Y., McSweeney, P.L.H., 2001. Advances in the study of proteolysis during cheese ripening. *Int. Dairy J.* 11, 327–345.
- Tajalsir, A.E., Ebraheem, A.S., Abdallah, A.M., Khider, F.J., Elsamani, M.O., Ahmed, I.A.M., 2014. Partial purification of milk-clotting enzyme from the seeds of *Moringa oleifera*. *J. Microbiol. Biotechnol. Food Sci.* 4, 58–62.
- Talib, M.A., Abubakar, M.M., Jideani, I.A., Hassan, A., 2009. Use of Jiben seeds extract to manufacture soft white cheese. *Am. J. Appl. Sci.* 6, 551–554.
- Tejada, L., Abellan, A., Prados, F., Maria Cayuela, J., 2008. Compositional characteristics of Murcia al Vino goat's cheese made with calf rennet and plant coagulant. *Int. J. Dairy Technol.* 61, 119–125.
- Tejada, L., Fernandez-Salguero, J., 2003. Chemical and microbiological characteristics of Ewe milk cheese (Lospedroches) made with a powdered vegetable coagulant or calf rennet. *Ital. J. Food Sci.* 15, 125–131.
- Veríssimo, P., Esteves, C., Faro, C., Pires, E., 1995. The vegetable rennet of *Cynara cardunculus L.* contains two proteinases with chymosin and pepsin-like specificities. *Biotechnol. Lett.* 17, 621–626.
- Walstra, P., Geurts, T.J., Noomen, A., Jellema, A., Van Boekel, M.A.J.S., 1999. *Dairy Technology: Principles of Milk Properties and Processes*. Food Science and Technology. Marcel Dekker, New York.