Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

The Effect of Some Plant Enzymes on the Chemical Composition, Proteolysis and Lipolysis in Soft Cheese

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Abstract
This research was conducted with the aim of studying the effect of some plant enzymes on chemical composition**,** proteolysis and lipolysis in soft cheese (Tallaga)**.** On the other hand enable of the possibility of applying these enzymes in cheese making. This study explained that purified plant aqueous extracts of coagulants obtained from cardoon flowers extract (CFE) or Moringa seeds extract (MSE) were used in soft cheese (Tallaga) making, compared to calf rennet (C.R.) as control cheese. Organoleptic, proteolysis and Physico-Chemical proteolysis of soft cheese (Tallaga) were estimated at up to 28 days. Cheese made with cardoon flowers aqueous extract showed lower levels in moisture content and total nitrogen percent compared to MSE and control cheese respectively. Also, casein breakdown, Syneresis, acidity (as lactic acid percentage), Tyrosine and Tryptophan contents in cheese manufactured with CFE was significantly increased than that made with MSE and control cheese respectively. While total volatile fatty acids (TVFA) and organoleptic properties were more pronounced in control cheese, followed by cheese made with CFE and MSE. According to these results, it is concluded that purified cardoon flowers and Moringa seeds extracts are suitable milk clotting for cheesemaking.

Keywords: Moringa oleifera, Cynara carduculus, Tryptophan, Total volatile fatty acids

Introduction

The most common enzyme preparation for milk coagulation is calf rennet, however over the past 50 years, researchers have looked for rennet alternatives due to the coagulant's scarce availability and expensive cost[1]. In order to meet the need for milk coagulants for the manufacture of cheese, it is therefore not only conceivable but also imperative to search for substitute coagulants from conveniently and locally obtainable incomes, such as plant enzymes extract. Several plant extracts were used by researchers to investigate the coagulation potential of bovine milk [2,3]. High milk-clotting activity (MCA) on κ-casein and low non-specific proteolytic activity (PA) are characteristics of quality plant rennet [4]. Actually, the texture and flavour of cheese are correlated with the MCA/PA ratio.

Suitable texture and a significant discount in bitterness are produced by a high value of the latter, and these are crucial factors in determining how satisfied customers are with cheese [5].

Aqueous extracts from cardoon flowers have long been used to produce goat and sheep cheeses, particularly in Portugal and Spain. Other plant coagulants were also utilised in the milk gelation and cheese- manufacture processes. Traditionally, cardoon extract-infused cheeses were handmade on a small scale. Nonetheless, in the Mediterranean countries' regional areas, they have a significant socioeconomic role in the dairy and agricultural sectors [6]. Thistle varieties known as cardoon (*Cynara cardunculus* L.) are mainly found growing in stony, arid environments[7]. Some Mediterranean, West African, and European regions have produced traditional sheep cheeses by using the flower pistils as a natural rennet alternative[8,9,10].

Plant source cardoon has been utilised as a coagulant for milk [11]. Numerous studies have demonstrated that cardosins A and B, two aspartic proteases, can be extracted and purified from thistle flowers[12,13].

As far as we are aware, not many research have looked into how temperature and pH buffer affect the enzymatic activities of *Cynara cardunculus* rennet [14,15]. Therefore, assessing PA and MCA at various rennet pH values is intriguing.

In some Mediterranean countries, aspartic proteinases from thistle flowers of different species in the genus Cynara L are traditionally used to make cheese [16]. This is one of the few uses of plant-derived enzymes for this purpose [17]. These enzymes have strong proteolytic activity that ultimately indications to the widespread break of caseins, giving cheeses that are extremely respected for their taste and quality and have a soft, buttery smoothness, as well as a honest aroma and a slightly piquant, creamy flavour. They also exhibit high clotting activity, similar to chymosin [18] (cleaving K-casein at the peptide bond Phe105-Met106).

The main application for cardoon is in the production of several Portuguese and Spanish cheeses. [19,20,21]. A variety of traditional cheeses are also made using C humilis L, the other species of Cynara [4,22].. These plant proteinases are natural enzymes that can also be utilised to make cheeses aimed at the vegetarian market, which makes their usage as milk coagulants intriguing [5]. The flowers of cardoon flawers are simple to handle in order to extract proteinases.

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Receive Date: 04 September 2024, **Revise Date:** 29 October 2024, **Accept Date:** 02 December 2024
DOI: https://doi.org/10.21608/ejchem.2024.318252.10350

Cardosins A and B, aspartic proteases that resembled chymosin and pepsin, respectively, in terms of specificity and activity, were identified as the cause of the MCA of *Cynara cardunculus* extracts [6]. The primary disadvantage of cardosins is their low yields, weak and creamy texture, and excessive bitter taste in the cheeses they create, making their industrial use as cow rennet alternatives all but nonexistent. Their increased proteolytic activity is the primary cause of this [23]

A fascinating source of milk clotting enzymes, such as aspartic enzyme in various sections, is the Moringa oleifera plant [24]. According to [25], M. oleifera's milk clotting activity was limited to the extract from the seeds; the remaining portions were either lacking in milk clotting activity or had very little of it. Proteases found in abundance in *Moringa oleifera* seed extract have a particular clotting activity that is 200 times higher than that of flower extract. Based on these findings, it can be said that M. oleifera seed extract produces adequate milk clotting activity for the production of cheese [26]. [27] shown that the use of *Moringa oleifera* seed extracts in cottage cheese preparation increased the cheese's output. Similarly, using *Moringa oleifera* seeds as a coagulant increased the cheeses' sensory attributes, such as texture, appearance, flavour, taste, and colour [28].

Moringa oleifera seeds were utilised to make partially purified milk clotting enzyme (MCE) by [26]. Because of this enzyme's high specificity and great ratio of MCE to PA, it may eventually replace renin enzyme (C.R) in the cheese manufacturing. From this study it was concluded that partially purified aqueous extract of Cardoon (*Cynara cardunculus*) flower and *Moringa oleifera* seeds are a viable vegetable source of coagulant that can be used to make Tallaga cheese without negatively affecting its texture or flavour. Additionally, it demonstrates that the enzyme is completely attuned with the physical and chemical parameters used in the production of Tallaga cheese. (55 and 65°C for cardoon and moringa respectively). Henceforth, these enzymes can be regarded as a conceivable alternative for natural calf rennet in the coagulation of milk, resulting in the creation of novel dairy products. Through the results obtained from the study, it became clear that the cheese made using cardoon (artichoke) flowers T1 was faster in the cheese ripening process compared to the cheese made using Moringa seeds T2. It was also noted that the cheese obtained from cardoon flowers T1 was better in terms of sensory evaluation compared to Moringa T2. It can be recommended to use artichoke flowers to produce soft white cheese (Tallaga cheese) used at a concentration of 1% per 100 ml of milk as an alternative to calf rennet.

Materials and methods

Materials:

Skim milk powder:

Reconstituted skim milk was purchased from Obour Land Company for Food Industries (Obour City, Cairo, Egypt). This milk was then used to test the clotting activity of extracts from cardoon flowers and partially purified moringa seeds.

Milk:

Fresh buffalo's milk was obtained from the Laboratory of dairying Experimental Agricultural Research Centre, Faculty of Agriculture, Zagazig University

Preparing cardoon flowers and Moringa seeds for enzymatic extraction process:

The *Cynara cardunculus* L (cardoon flower) was acquired from the Crops Research Institute at the Agric. Research Centre in Giza, Egypt. Cardoons were air dried for two weeks at room temperature. Before being utilised for enzyme extraction, the flowers were meticulously washed, coarsely powdered with an electric grinder, and stored in polyethylene bags at a temperature of 4-5°C. Moringa seeds (*Moringa oleifera*): were obtained from National Research Centre (NRC), Giza, Egypt. Before being utilised for enzyme extraction, the seeds were thoroughly washed, coarsely ground with an electric grinder, and stored in polyethylene bags at a temperature of 4-5°C.

Calf rennet:

The source of the calf rennet was Chr. Hansen's Laboratories in Copenhagen, Denmark. Before use, it was diluted to a normal rennet solution using distiullated water.

Salt: Clean food grade salt (NaCl) was used.

Calcium Chloride: pure Calcium Chloride used in cheese making, it was purchased from El-Gomhoria company, Cairo, Egypt.

Methods:

Partial purification of crude Moringa seeds enzyme extract

Using sodium acetate buffer (pH 5.0), enzyme extraction was carried out under ideal circumstances, following the guidelines provided by[26]. After preparing 7.0 g of finely crushed *Moringa oleifera,* 50 ml of the extractant was used to extract the plant for four hours while stirring. After filtering the extract via cheesecloth, it was centrifuged for 20 minutes at 12000×g. Overnight at 40°C, the supernatant was dialysed against 0.1 mmol/L sodium acetate buffer (pH 5.0). After centrifuging the mixture to get rid of any solid particles, the activity was calculated. The procedure for ammonium sulphate fractionation was followed [26]. Proteolytic and MCA in the extract were identified.

Partial purification of crude Cardoon flowers extract

The upper purple-colored pistils of *C. cardunculus* L were cut, divided from the remainder of the plant, and then carefully selected out to eliminate waste. The resulting crude extracts were then used. All freshly harvested flowers were promptly stored to dry for around 30 days at ambient temperature (20–25°C) and shielded from the sun. In a Beckman Coulter (Aventi J-E) centrifuge, 2 g of material (fresh or lyophilised) was homogenised for 2 hours in 50 mL of 0.1 M acetate buffer (pH5.5). The sample was then centrifuged at 8000xg for 10 minutes at 4°C. [14] state that crude extracts was subsequently

filtered through a Whatman No. 40. Ultimately, they were kept at 4°C and utilised that same day, or they were frozen at -20°C to be employed later.

Evaluation of milk clotting activity (MCA):

We measured milk clotting activity using the technique outlined as described as [29]. pH 6.5 was achieved by preparing the substrate (10% reconstituted skim milk in 0.01 M CaCl2). A thermostatically controlled water bath was used to regulate the temperature to 40°C, 45°C, 50°C, and 55°C in the case of cardoon extract and 60°C, 65°C, 70°C, and 75°C for the substrate (100 ml) during the 5-minute preincubation period. A unit of milk clotting activity (MCU) was calculated by spinning the test tube at regular intervals and looking for visible clot formation on the test tube walls after 1 ml by volume of the purified extract was applied to 100 ml of the milk samples. The milk clotting unit (MCU) as reported by [30] was calculated using the following formula; the results are represented as MCU/ml.

Unit of milk clotting activity (U/ml) = (2400/t) × (S/E),

Where, t is the time required for clot formation,

S is the volume of skim milk,

E is the volume of purified enzyme extract

Effect of temperature on milk clotting properties of partially purified extract:

The ideal temperature for clotting activity was found to be between 50 and 60°C, using 1 millilitre of enzyme extract for cardoon flower extract and between 60 and 70°C for Moringa seed extract, using 5 millilitres of enzyme extract.

Syneresis:

A funnel was used to hold filter paper, and 100 millilitres of the coagulated sample was poured on it to measure the syneresis. The volume of the whey was collected and measured following a two-hour drainage period. Here is how the index of syneresis is determined:

$$S\,(\%) = \frac{V_1}{V_2} \times 100$$

Where:

S: Syneresis

V1: Volume whey after drainage.

V2: Initial volume sample.

Tallaga cheese making:

Fresh buffalo's milk was uniform to 5.5% fat, heat treated at 90°C/5 min, then it was cooled to 65°C for plant based coagulants and 37°C for calf rennet with adding 0.02% $CaCl_2$ and 4% salt. The milk was alienated to three equal quantities as follows: The first quantity was renneted with calf rennet, the second quantity was renneted with cardoon flowers extract and third quantity was renneted with Moringa seeds extract using the enzyme extracts as coagulant where 1% of Cardoon flower extract and 5% of Moringa seeds extract were added to every 100 ml of milk (Dosage of extracts were calculated with reference to the partial purification profiles of cardoon and Moringa seeds extracts. White soft cheese was prepared from each portion by the predictable method of making Tallaga cheese rendering to [31]. The resulting soft white cheese was kept in the refrigerator (5±2°C) for four weeks, packed in plastic cups that were filled with whey from the same treatment. Samples of fresh Tallaga cheese were taken, as well as after 7, 14, 21, and 28 days of pickling at 5±2°C. The experiment was conducted three times in total, with duplicate analyses performed for each, and average results reported.

Cheese analysis:

Samples of the fresh cheese were collected as well as after 7, 14, 21, and 28 days of storage. The [32]technique was followed in the chemical analysis to evaluate the levels of moisture, total solids, fat, titratable acidity (as lactic acid%), salt concentrate, and total nitrogen (TN)%. Total volatile fatty acids (TVFA) were calculated using Kosikowski's (1986) method, whereas soluble nitrogen (SN) and non-protein nitrogen (NPN)% were determined in accordance with [33]. The measurements of tryptophane and tyrosine were made using the [34] method.

Organoleptic evaluation:

The employees of the food science department of Zagazig University's faculty of agriculture evaluated the organoleptic qualities of cheese samples. According to [31], the maximum score points for appearance, body & texture, and flavour were 10, 40, and 50, respectively.

Statistical analysis:

Software from [35] was used to perform analyses of variance (ANOVA) on all of the study's findings. The least significant differences (LSD) at $p<0.05$ were used to collect differences between means. Every measurement was done three times.

RESULTS AND DISCUSSION

Milk Clotting Activity

The data in the Table1 showed how temperature settings affected the clotting times of extracts from Moringa seeds, cardoon flowers, and calf rennet. The data displayed in the table shows that temperature has a substantial ($P\leq0.05$) impact on the clotting time. When the setting temperature was raised above 35°C, the clotting time for calf rennet was significantly ($P\leq0.05$) reduced up to 40°C. When the temperature rose over 40°C, there was a substantial increase in the clotting time ($P\leq0.05$). Additionally, it was evident from the data in the Table that the extract of cardoon flowers had the highest milk clotting time at 55°C, which was substantially ($p\leq0.05$) higher than the other extracts. The milk clotting time was also significantly ($p\leq0.05$) lower at 75°C. The clotting time for extracts from Moringa seeds was considerably ($P\leq0.05$) longer when the setting temperature was raised to 65°C. However, in the instance of calf rennet, no clotting was seen at 55°C; instead, the calf rennet attained its peak activity at 40°C. These findings are consistent with those of [36], who discovered that no clotting

was observed at 54°C in the case of calf rennet and that the clotting time was significantly (p≤0.05) decreased in the case of CR up to 42°C and up to 56°C for *Solanum dobium*.

Cardoon flowers extract exhibits optimal clotting activity at 55°C under the same temperature conditions; above this temperature, clotting activity decreases. However, the extract from Moringa seeds exhibits the best clotting activity at 65°C, and the temperature-dependent decline in milk clotting activity (M.C.A). These findings concur with those made public by[37], who noted that at 53°C, chymosine inactivated in gelled milk. Furthermore, MCE from Sodom apple (*Calotropis procera*) extract was found to be more active at 65°C than at 35°C by [38]. These findings showed that extracts from cardoon flowers or Moringa seeds could withstand higher temperatures than calf rennet. Additionally, [38]demonstrated that the pure rape seed extract's milk clotting activity rose gradually with temperature, peaking at 60°C.

Plant protease's thermophilic characteristic may be explained by the possibility that high temperatures cause a conformational shift in the protein's structure. Furthermore, these findings concur with those of[24]. Furthermore, according to[40], the temperature (profile) of MCE derived from plant extracts depends on a number of variables, including the kind of enzyme, concentration of tissues, degree of purification, and plant sources.

Table (1): Effect of temperature on milk clotting activity of calf rennet, cardoon flowers and Moringa seeds partially purified aqueous extracts.

Temperature (°C)	Properties								
	Calf Rennet			Cardoon			Moringa		
	C.T sec	M.C.A m/ml	R.A%	C.T sec	M.C.A m/ml	R.A%	C.T sec	M.C.A m/ml	R.A%
35	490^{b}	489.80^{a}	94.90^{c}	1820^{a}	131.87^{g}	31.59^{i}	-	-	-
40	465^{d}	516.13^{a}	100^{a}	1715^{b}	139.94^{g}	33.53^{h}	-	-	-
45	482^{c}	497.63^{a}	96.47^{b}	1460^{c}	164.38^{f}	39.38^{g}	-	-	-
50	890^{a}	290.21^{b}	56.23^{d}	880^{f}	272.73^{c}	65.34^{d}	-	-	-
55	-	-	-	575^{i}	417.39^{a}	100^{a}	-	-	-
60	-	-	-	700^{h}	342.86^{b}	82.14^{b}	1200^{b}	40.00^{c}	77.5^{c}
65	-	-	-	850^{g}	282.35^{c}	67.65^{c}	930^{d}	51.61^{a}	100^{a}
70	-	-	-	1020^{e}	235.29^{d}	56.37^{e}	1100^{c}	43.64^{b}	84.55^{b}
75	-	-	-	1183^{d}	202.87^{e}	48.61^{f}	1250^{a}	38.4^{d}	74.4^{d}
Mean	581.75	448.44	86.9	1133.7	243.30	58.29	1120	43.41	84.11

^{a,b,c,d} Means in the same column with different superscripts differ significantly (p≤0.05).
C.T: Clotting time (Sec)
M.C.A: Milk Clotting Activity (m/ml**)**
R.A: Relative activity (%)

chemical composition of processed cheese:

The mean chemical composition of Tallaga cheese, which is prepared from buffalo's milk and has powdered calf rennet as a control (C), is displayed in Table (2). Additional plant-based coagulants used in cheese treatments include the aqueous extracts of cardoon flowers (T1) and Moringa seeds (T2). The results showed that Tallaga cheese that had been coagulated with calf rennet had a significantly (p≤0.05) higher moisture content than any of the other cheeses in the trial. Additionally, this Table shows that throughout the course of the 28-day storage period, the moisture content of cheese from all treatments declined gradually and significantly (p≤0.05). Tallaga cheese prepared with the aqueous extract of cardoon flowers had a considerably (p≤0.05) lower moisture content than the other experimental cheeses and the control cheese. This might be the result of an increase in acidity that concentrated on the curd and caused an outburst of moisture. It is possible to deduce that the coagulum's structure contains extract from cardoon flowers. These outcomes concur with those of [21,41], who found that cheese prepared with vegetable coagulant had a significantly lower moisture content than cheese made with calf rennet.

[42] reported similar findings, observing that cheese coagulated using calf rennet had a considerably lower moisture content than cheese prepared with artichoke (*Cynara scolymus* L) flower extract (p≤0.05). However, [41]discovered that cheese prepared using calf rennet had a higher moisture content than cheese made with vegetable coagulant derived from *Cynara cardumculus*.

These results also demonstrate that cheese created with extract from Moringa seeds (T2) had a considerably ($p \leq 0.05$) higher moisture content compared to cheese produced with extract from cardoon flowers (T1). The findings are consistent with those of [42,43]. Additionally, the current study's findings concur with those of [42,44], who discovered that cheese created using extract from *Solanum dobium* seeds had a lower moisture content than cheese manufactured with calf rennet.

According to[45], the moisture content of cheese treated with 2, 3, and 4% extract of Moringa seeds was lower than that of cheese in the control group.

Acidity %

Table (2) displays the average lactic acid (%) and acidity of Tallaga cheese during storage as a function of extracting cardoon flowers (T1) and Moringa seeds (T2). The titratable acidity of every cheese treatment was found to have increased significantly ($p \leq 0.05$) during the course of the 28-day ripening period.

Competed to the control cheese (C), the experimental cheeses (T1 and T2) had a significant ($p \leq 0.05$) decrease. These data also show that, as the storage period went on, the percentage of lactic acid (%) in the cheese manufactured with calf rennet (C) was considerably ($p \leq 0.05$) higher than that of all the experimental cheeses. Furthermore, treatment (T1) was shown to have a considerably ($p \leq 0.05$) higher lactic acid (%) than treatment (T2). This could be because the extract from cardoon flowers has a stronger proteolytic activity. [46]also mentioned these results in relation to Domiati cheese that had both recombinant and microbial rennet (MR). [47]also noted that, in contrast to control cheese, white soft cheese made with a substitute made of *Solanum dobium* had a greater acidity level.

fat/dry matter (%)

The average fat/dry matter (%) for each kind of cheese treatment throughout a 28-day storage period were displayed in Table 2. It has been noted that as the storage period increases, fat/DM % progressively increased in all cheese treatments.

The cheese prepared with cardoon flower extract (T1), as shown in this table, had considerably ($p \leq 0.05$) higher and fat/DM (%) over the course of storage than T2 and control cheese (C), respectively. These findings are consistent with those of [42], who discovered that throughout the storage period, the fat content significantly ($p \leq 0.05$) rose in all cheese treatments. This could be because the moisture content decreased during storage. Furthermore, these findings concur with those of [27], who discovered that increases in the concentrations of extract from Moringa seeds directly corresponded to increases in the cheese's protein, fat, ash, and phosphorus contents.

Salt/Moisture content %

The average salt/moisture percentage of cheese prepared from extract from Moringa seeds (T2) and cardoon flowers (T1) was displayed in Table 2. These results demonstrate that, over the course of storage for up to 28 days, salt/M% of all cheese treatments raised regularly and considerably ($p \geq 0.05$). This table also clearly shows that, during storage period (salt/M) % in cheese manfuctured using (T2) increased significantly ($p \leq 0.05$) comparison to (T1) and control cheese(C), respectively. The rate at which the proteins in the experimental cheeses can bend water may be the cause of this [48].

Additionally, we may state that the increase of whey and curd concentration may be the cause of the decreased moisture [49]. Additionally, these findings showed that as ripening progressed, the salt/moisture percentage progressively increased significantly ($p \leq 0.05$). This could be explained by the moisture content dropping over the storage time. Furthermore, it was observed that the salt/M% in the cheese samples designated as control (C) was considerably ($p \leq 0.05$) lower than that of T1 and T2, respectively. This might be because the control cheese samples (C) had more moisture in them than the cheese treatments did. The increased ability of the proteins in the control cheese (C) to bind water may be the cause of this.

The variations in the final cheeses' moisture content, however, are more likely to be the cause of the variation observed in salt/M% across all cheese treatments. [50] states that osmotic dehydration of the product results from a mutual diffusion process between moisture loss and salt uptake. Due to adventitious microflora or residual coagulant enzymes (cardosins), there is a possibility that the elevated moisture content is associated to casein proteolysis [51].

Total Nitrogen (TN) %

Total nitrogen percentage (TN) %of all the cheese treatments displayed a significant difference ($p \leq 0.05$), as presented in Table 2. The cheese with the greatest TN content was created with Moringa seed extract (T2), followed by cheese made with cardoon flowers extract (T1) and calf rennet as the control.

Additionally, this table shows that throughout the storage period, the TN% content of all cheeses reduced significantly ($p \leq 0.05$). This decrease may have been caused by the degradation of proteins into SN and the subsequent loss of some water-SN from the SN that was formed from the soluble proteins. These findings corroborated those of [52,42] reports for Tallaga cheese and Domiati cheese. The same Table revealed that throughout the ripening period, cheese prepared with extract from Moringa seeds (T2) had a greater total protein (%) than all other cheese treatments. The outcomes correspond with [42]findings. Additionally, the cheese sample made with the extract of moringa seeds had the greatest protein content ever measured, whereas the control samples had the lowest protein level. These outcomes concur with the findings published by [42].

The rate of ripening:

Proteolysis:

Soluble nitrogen/Total nitrogen percentage (SN/TN%)

The average soluble nitrogen content of Tallaga cheese produced from various treatments was displayed in Table 2. These data clearly show that as the storage period increased, the soluble nitrogen content increased progressively across all cheese treatments. Additionally, when comparing cheeses coagulated by Moringa seed extract (T1) to control cheese (C), the SN/TN% in cheeses prepared with cardoon flower extract (T1) was considerably ($p \leq 0.05$) greater. Owing to the strong proteolytic activity of the plant coagulant, it was observed that the SN/TN% in cheese produced with coagulant plants T1 and T2 was significantly ($p \leq 0.05$) higher than that of control cheese (C). These findings concur with those of [53]. Also, it could be observed that SN/TN% of T1 was higher than T2 as storage period progressed.

In general, cheese manufactured with plant coagulant exhibited greater amounts of SN than cheese made with calf rennet (C). In comparison to cheese manufactured using calf rennet, [53], [54], [55] found that cheese prepared with CFE had higher quantities of water-SN. As the storage period extended, there was a substantial ($p \leq 0.05$) rise in the SN/TN% of all cheese treatments. Similar findings were reported by[56], who discovered that cheese prepared with plant coagulant had statistically higher amounts of SN than cheese manufactured using calf rennet.

These findings also align with the findings of [57,44], who observed that as the ripening period progressed, the contents of SN/TN%, NPN/TN%, and TVFA increased. This was attributed to protein degradation, which produced water-soluble nitrogen compounds, some of which were lost in the pickling solution and increased the nitrogen content in whey.

Non-Protein Nitrogen percentage (NPN%, NPN/TN%)

The average NPN/TN% readings as the ripening process advanced to 28 days are displayed in Table (2). All cheeses had a notable increase in NPN/TN% levels. When compared to control cheese (C), the NPN/TN% of cheese produced with plant coagulants T1 and T2 grew significantly ($p \geq 0.05$) as the ripening period went on. However, throughout the ripening stage, the values in the cheese prepared with CFE (T1) were considerably ($p \geq 0.05$) greater than those in T2, which was made with MSE (T2). These findings are consistent with some research done on ewe's milk cheese, which indicated that cheese prepared using plant coagulant had higher amounts of NPN than cheese made with calf rennet.
[53, 54 ,41].

Total Volatile Fatty Acids (TVFA):

Total volatile fatty acids (TVFA) were the subject of all Tallaga cheese treatments during the storage period, as demonstrated by the results presented in Table (2). These results demonstrate that the lowest ($P \leq 0.05$) TVFA values were found in Tallaga cheese produced from cordon flower extract (T1) and Moring seed extract (T2). In contrast to the control cheese (C).

The findings demonstrated that during the course of the 28-day ripening period, the TVFA contents of the cheese treatments progressively increased. Until the end of the storage period, Control Cheese had the highest TVFA concentration of any experimental cheese. These findings may be explained by an increase in moisture content, which raises water activity and, in turn, produces an increase in lipolytic bacteria and lipase activity [58]. The overall pattern of these data is consistent with that reported by [59], who discovered that cheese produced with animal rennet [60, 61].displayed greater lipolysis than that prepared with vegetable rennet.

Table (2): Chemical analysis of white cheese (Tallaga) prepared with plant extract during storage at 7±1°C for 28 days.

Components (%)	Storage period (days)	Treatment C	Treatment T1	Treatment T2	Pickling per. Mean
Moisture %	Fresh	A 68.47^{a}	A 64.58^{d}	A 66.54^{c}	66.53
	7	A 67.93^{a}	B 62.46^{d}	B 64.39^{c}	64.93
	14	B 66.06^{a}	C 61.55^{d}	C 63.50^{c}	63.7
	21	C 64.21^{a}	CD 61.13^{c}	D 62.05^{b}	62.46
	28	D 63.05^{a}	D 60.71^{d}	E 61.44^{c}	61.73
Acidity (as lactic acid%)	Fresh	D 0.38^{a}	D 0.32^{ab}	C 0.20^{b}	0.3
	7	C 1.42^{a}	C 1.37^{a}	B 1.25^{ab}	1.35
	14	BC 1.63^{a}	B 1.55^{a}	AB 1.45^{a}	1.54
	21	AB 1.84^{a}	A 1.75^{b}	A 1.60^{d}	1.73
	28	A 1.95^{a}	A 1.81^{b}	A 1.69^{c}	1.82
Fat/DM %	Fresh	E 48.76^{b}	C 46.37^{d}	E 47.95^{c}	47.69
	7	D 60.11^{a}	B 56.46^{c}	D 55.20^{d}	57.26
	14	C 62.35^{a}	B 56.58^{b}	C 62.46^{a}	60.46
	21	B 64.00^{a}	A 57.64^{d}	B 63.13^{b}	61.59
	28	A 64.83^{a}	A 57.69^{c}	A 64.57^{a}	62.36

Components (%)	Storage period (days)	Treatment C	Treatment T1	Treatment T2	Pickling per. Mean
Salt/M%	Fresh	D 6.00^{b}	D 6.53^{a}	E 6.36^{a}	6.3
	7	D 6.26^{d}	C 7.20^{b}	D 6.99^{c}	6.82
	14	C 6.99^{b}	B 7.78^{a}	C 7.70^{a}	7.49
	21	B 7.99^{b}	A 8.51^{a}	B 8.38^{a}	8.29
	28	A 8.40^{b}	A 8.78^{a}	A 8.79^{a}	8.66
TN%	Fresh	A 2.09^{b}	A 2.18^{b}	A 2.54^{a}	2.27
	7	B 1.96^{a}	AB 2.01^{a}	B 2.03^{a}	2
	14	C 1.52^{a}	BC 1.75^{a}	BC 1.88^{a}	1.72
	21	D 1.45^{c}	CD 1.53^{b}	CD 1.63^{a}	1.54
	28	E 1.27^{a}	D 1.31^{a}	D 1.50^{a}	1.36
NPN%	Fresh	D 0.26^{b}	A 0.46^{a}	A 0.42^{a}	D 0.28^{b}
	7	C 0.36^{a}	A 0.55^{a}	A 0.45^{a}	C 0.40^{a}
	14	B 0.40^{a}	A 0.56^{a}	A 0.51^{a}	BC 0.41^{a}
	21	A 0.44^{a}	A 0.67^{a}	A 0.55^{b}	AB 0.49^{b}
	28	A 0.50^{a}	A 0.74^{a}	A 0.62^{ab}	A 0.54^{b}
SN %	Fresh	A 0.46^{a}	A 0.55^{a}	D 0.50^{a}	A 0.47^{a}
	7	A 0.49^{a}	A 0.56^{a}	A 0.51^{a}	A 0.50^{a}
	14	A 0.53^{a}	A 0.59^{a}	BC 0.57^{a}	CD 0.55^{a}
	21	A 0.56^{a}	A 0.66^{a}	AB 0.65^{a}	AB 0.57^{a}
	28	A 0.61^{a}	A 0.71	A 0.67^{a}	AB 0.65^{a}
TVFA (ml 0.1N of NaOH/100 gm)	Fresh	E 16^{a}	E 8^{b}	E 6^{c}	10
	7	D 20^{a}	D 12^{c}	D 16^{b}	16
	14	C 24^{a}	C 20^{b}	C 20^{b}	21.33
	21	B 32^{a}	B 28^{b}	B 24^{c}	28
	28	A 36^{a}	A 32^{b}	A 30^{c}	32.67

The means (±SD) of identical letter values in the same column do not differ significantly.
C: Cheese made using calf rennet as a control cheese.
T1: cheese made using Cardoon (*Cynara cardunculus*) flowers extract.
T2: cheese made using moringa seeds extract.

Tyrosine and Tryptophan contents

Table (3) displays the values of the tryptophan and soluble tyrosine contents of Tallaga cheese produced using calf rennet as the control (C), cardoon flower extract (T1), and moringa seed extract (T2) both when the cheese is fresh and up to 28 days after it is stored. These findings demonstrate that the control and experimental cheeses' soluble tyrosine and tryptophan contents rose during the course of the 28-day storage period. Observations reveal that as the storage period increased, the amount of soluble Tyrosine and Tryptophane grew significantly ($P \leq 0.05$) throughout time. Tryptophan and tyrosine amounts were higher in all cheese treatments than in the control group.

Additionally, the results showed that the tryptophane and soluble tyrosine contents of the cheese prepared with cardoon flower extract were significantly ($P \leq 0.05$) higher than those of the control and other experimental cheeses. This is explained by the plant coagulant's proteolytic enzymes, which influence protein degradation and release more tyrosine and tryptophane, which is thought to be a ripening indicator for cheese. These findings are consistent with[62].

The results obtained are consistent with those of [63], who demonstrated that over the cheese storage period, there was a clear and significant difference in the growth of soluble tryptophan in both the control and treatment groups. Furthermore, our findings concur with those of [43,62]. Additionally, it was observed by[53], [54], [55], and others that cheese manufactured using cardoon extract had higher levels of water-soluble nitrogen than cheese made with calf rennet.

Additionally, soluble tyrosine and tryptophan concentrations increased progressively at the pickling[64]. These findings are consistent with those of [65,66,67]

Table (3): Change in tyrosine and tryptophan in white soft cheese (Tallaga) after ripening at 7±1°C for 28 days.

Treatment	Tyrosine		Tryptophan	
	After manufacture	Age 28	After manufacture	Age 28
C	B 25.13^{d}	A 34.36^{ab}	B 8.01^{c}	A 15.50^{a}
T1	B 34.16^{a}	A 40.20^{a}	A 14.40^{a}	A 22.54^{a}
T2	B 29.40^{b}	A 38.12^{a}	B 11.22^{b}	A 19.12^{a}
Pickling per. Mean	29.56	37.56	11.21	19.05

The means (±SD) of identical letter values in the same column do not differ significantly.
C: Cheese made using calf rennet as a control cheese.
T1: cheese made using Cardoon (*Cynara cardunculus*) flowers extract.
T2: cheese made using moringa seeds extract.

Syneresis of treated cheese:

Table (4) makes it evident that the cheese's syneresis rate increased steadily over the course of the minute and reached 120 minutes at 37°C. The results also showed that, in comparison to T2 and Control cheese, the syneresis rate in T1 was considerably ($p \leq 0.05$) higher. It's clear that cheese prepared with Cynara aqueous extracts had a higher syneresis rate, which suggests that the coagulum's structure held less liquid during the cheese's 120-minute dewheying process. These outcomes concur with the findings published by [21]. Additionally, it was shown that the hardness of cheese produced using vegetable coagulant had a negative link with both the soluble nitrogen content and the non-protein nitrogen value. The increased casein breakdown proteolysis activity could be the cause of this. Syneresis rate: The effect of liquid separating from the curd is an undesired feature in crude extract artichokes[68,69].

Table (4): Effect of extracted enzymes and rennet on the syneresis (Tallaga) during storage at 7±1°C.

Syneresis gm/gm	Treatment		
	C	T1	T2
10 min	B 1.80^{d}	A 1.95^{c}	AB 1.90^{d}
30 min	C 3.45^{c}	A 4.4^{b}	A 4.20^{c}
60 min	B 4.60^{b}	A 5.90^{a}	AB 5.50^{b}
120 min	D 5.20^{a}	A 7.30^{a}	B 6.70^{a}
Mean	3.7	4.89	4.58

The means (±SD) of identical letter values in the same column do not differ significantly.
C: Cheese made using calf rennet as a control cheese.
T1: cheese made using Cardoon (*Cynara cardunculus*) flowers extract.

T2: cheese made using moringa seeds extract.

Organoleptic properties

Table 5 displays significant differences (P≥0.05) in the storage period of up to 28 days between Tallaga cheeses made with cardoon flowers (TI) and extracts from Moringa seeds (T2) against those made with calf rennet treatment (C), which served as the control. These findings showed that, up to 28 days, control Tallaga cheese (C) received the highest marks for organoleptic qualities, followed by treatment T1 and treatment T2, in that order. Additionally, it was noted that Tallaga cheese prepared with extract from moringa seeds scored lower on organoleptic qualities than cheese manufactured with cardoon flower extract.

Furthermore, it was noted that Tallaga cheese produced with extract from moringa seeds (T2) was substantially (p≤0.05) less expensive than the other experimental cheeses and control cheese. These findings could be explained by the cardoon flower extract's proteolytic activity, which increases ripening-related proteolysis and increases the amount of SN and NPN in the cheese that is produced.

Table (5): Organoleptic properties of white soft cheese (Tallaga) prepared with plant extracts during storage at 7±1°C for 28 days.

Storage period (days)	Properties	Treatments		
		C	T1	T2
After manufacture	Appearance (10)	A 8.50[a]	B 7.90[a]	A 8.30[a]
	Body and texture (40)	A 39.56[a]	B 35.62[b]	C 33.65[a]
	Flavour (50)	A 47.90[a]	C 45.52[b]	C 45.50[a]
	Total (100)	95.96	89.04	87.45
7 days	Appearance (10)	A 8.37[a]	B 7.24[b]	A 8.17[a]
	Body and texture (40)	A 38.60[b]	B 36.98[a]	C 33.10[b]
	Flavour (50)	A 47.68[a]	C 44.60[c]	D 43.82[c]
	Total (100)	94.65	88.82	85.09
14 days	Appearance (10)	A 7.99[a]	B 7.00[b]	A 7.60[b]
	Body and texture (40)	A 38.53[b]	C 34.18c	D 32.00[c]
	Flavour (50)	D 43.50[b]	B 46.30[a]	C 44.80[b]
	Total (100)	90.02	87.48	84.4
21 days	Appearance (10)	B 7.20[b]	A 7.56[b]	C 6.33[c]
	Body and texture (40)	A 37.27[c]	C 32.96[b]	C 33.20[d]
	Flavour (50)	C 42.25[c]	C 42.69[d]	B 43.50[d]
	Total (100)	86.72	83.21	83.03
28 days	Appearance (10)	A 7.15[b]	A 7.10[c]	B 6.30[c]
	Body and texture (40)	A 36.69[c]	C 31.25[d]	D 30.56[e]
	Flavour (50)	A 41.15[d]	C 40.30[e]	BC 40.60[e]
	Total (100)	84.99	78.65	77.46

The means (±SD) of identical letter values in the same column do not differ significantly.

C: Cheese made using calf rennet as a control cheese.

T1: cheese made using Cardoon (*Cynara cardunculus*) flowers extract.

T2: cheese made using moringa seeds extract.

Conclusion

From this study it was concluded that partially purified aqueous extract of Cardoon (*Cynara cardunculus*) flower and *Moringa oleifera* seeds are a viable vegetable source of coagulant that can be used to make Tallaga cheese without negatively affecting its texture or flavour. Additionally, it demonstrates that the enzyme is completely attuned with the physical and chemical parameters used in the production of Tallaga cheese. (55 and 65°C for cardoon and moringa respectively). Henceforth, these enzymes can be regarded as a conceivable alternative for natural calf rennet in the coagulation of milk, resulting in the creation of novel dairy products. Through the results obtained from the study, it became clear that the cheese made using cardoon (artichoke) flowers T1 was faster in the cheese ripening process compared to the cheese made using Moringa seeds T2. It was also noted that the cheese obtained from cardoon flowers T1 was better in terms of sensory evaluation compared to Moringa T2. It can be recommended to use artichoke flowers to produce soft white cheese (Tallaga cheese) used at a concentration of 1% per 100 ml of milk as an alternative to calf rennet.

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