Zagazig J. Agric. Res., Vol. 51 No. (6) 2024 2109-2122

Food, Dairy and Home Economic Research

Available online at http://zjar.journals.ekb.eg
http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

MANUFACTURE OF WHITE SOFT CHEESE USING AQUEOUS ENZYMATIC EXTRACT OF MORINGA SEEDS

Esraa A.E. Ali*, A.A. Abd El-Galeel and E.M. Abd El Wahed

Food Sci. Dept., Fac. Agric., Zagazig Univ., Egypt

Received: 08/10/2024; Accepted: 30/10/2024

ABSTRACT: In this study, white soft cheese (Tallaga cheese) made from buffalo's milk using seed extract from Moringa oleifera, compared to traditional cheese (Tallaga) made with rennet (as control). All cheeses were stored at 7°C ±1 for 28 days for ripening. The samples were analyzed for each periodically during the storage period at 7°C±1 up to 28 days for gross chemical composition, ripening indices and tyrosine, tryptophan and organoleptic properties. Results indicated that white soft cheese (control) contained high moisture content. Also, casein breakdown, synersis, acidity (as lactic acid), tyrosine, tryptophan contents in cheese made with partial purified aqueous extract of Moringa seeds was significantly increased compared to control cheese. While organoleptic properties were more pronounced in control cheese. Also, the milk-clotting activity and the influence of temperature on the milk clotting activity for both calf rennet and partial purified aqueous extract of Moringa seeds was studied.

Key words: Moringa seeds, rennet, tyrosine, proteolysis, tryptophan, white soft cheese.

INTRODUCTION

Using calf rennet for milk coagulation is the basic step in cheese making. However, the worldwide increase in cheese production coupled with the reduced supply and increasing prices of calf rennet has created the demand for alternative milk clotting enzymes as an appropriate rennet substitute **(Anusha *et al*., 2014; Shah *et al*., 2014)**. Chymosin (EC 3.4.23.4) the main enzymatic component of calf rennet is characterized by its high specificity for κ-casein and low general proteolytic activity. This enzyme predominantly cleaves Phe-105-Met 106 bond in κ- casein, thus inducing the coagulating of milk **(Piers *et al*., 1999; Vishwanatha *et al*., 2010)**. Microbial milk coagulants and genetically engineered chymosin remain the major categories of milk coagulating enzymes **(Barry *et al*., 2010)**. Although, the biotechnological preparation of recombinant chymosin has diminished the cost of this product, certain sectors of the population are against genetically engineered food and still prefer a natural alternative. Plants are used as a source of proteases due to their easy availability, efficient purification processes and isolation of natural coagulants. In addition, plant coagulant usage increases the acceptability by vegetarian population and has an advantage of improving their nutritional intake. An inherent drawback of vegetable enzymes is that they usually possess high proteolytic activity leading to undesirable flavour and cheese textures **(Sousa *et al*., 2001)**. This could be explained on the basis that enzyme preparation had low ratio of milk clotting to proteolytic activity **(Anusha *et al*., 2014; Shah *et al*., 2014).** Therefore, the search of a rennet substitute having a high ratio of milk clotting/ proteolytic activity is extremely needed to be taken into consideration for the production of cheese with better quality. Moringa (*Moringa oleifera*) is a versatile and extraordinarily nourishing vegetable tree having a variety of potential usages. *M. oleifera* is one of the famous medicinal plants; it is cultivated in all countries of the tropics for its variety of food and medicinal purposes (**Alegbeleye, 2018; Matic *et al*., 2018**). Moringa tree and leaves

* **Corresponding author: Tel.:** +201212814344
E-mail address: ea0273975@gmail.com

have been testified to be a rich source of highly digestible protein, calcium, potassium, β-carotene, vitamin C, and protein and thus boost the shelf life of fat containing foods due to antioxidant compounds, for example, phenolics, ascorbic acid, carotenoids, and flavonoids (**Ashok and Pari, 2003; Mukunzi *et al*., 2011; Mbikay, 2012; Mishra *et al*., 2012; Dalei *et al*., 2016**) and a variety of medicinal properties some of which include: antimicrobial effect (**Lipipun *et al*., 2003**), hypoglycemic activity (**Kar *et al*., 2003**), radioprotective effects (**Rao *et al*., 2001**), regulation of thyroid hormone (**Tahiliani and Kar, 2000**) and hypocholesterol activity. For example, more importantly, the dried seed extract contains natural coagulants which have been used in several industries. For example, the seed extract is used as a non-toxic natural polypeptide for sedimenting mineral particles and organics in the purification of drinking water, for cleaning vegetable oil, or for sedimenting fibers in the juice and beer industries (**Foidl *et al*., 2001**).

Tajalsir *et al*. (2014) used partially purified milk clotting enzyme from the seeds of *Moringa oleifera*. The high specificity of this enzyme in terms of its high ratio of milk-clotting to proteolytic activity could pave the way for its use in cheese industry as alternative to calf rennet.

The aim of the present work was to use *Moringa oleifera* seeds extracts, which produces the best enzymatic clotting properties, and presents the excellent applicability of its extract in cheese making process.

MATERIALS AND METHODS

Materials

Skim Milk Powder

Arla ®, skimmed milk powder, made from fresh pasteurized buffalo's milk

Spray dried – low heat.

Producer: Arla foods. AB. Mejerivagen, Sweden.

Ingredients: Fat 1.25% max., ash 8% max., protein 34%min., moisture 4% min., Lactose 52% min.

Milk

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

Vegetable rennet enzyme

Moringa seeds (*Moringa oleifera*): were obtained from National Research Centre (NRC), Giza, Egypt. The seeds were carefully cleaned and then coarsely grounded using an electric grinder and kept in polyethylene bags at refrigerator (4-5°C) until being used for enzyme extraction.

Microbial rennet

CHY-MAX® Powder Extra NB

(Activity≈2200IMCU/g, Dosage: 0.027 g ≈60 IMCU/L milk), diluted with distillated water before using, was obtained from Chr. Hansen Inc. Laboratories,

Milwaukee, WI, by Misr food additives (MIFAD), Egypt, was diluted with distillated water to a standard rennet solution before using.

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 Aqueous Enzymatic Extract of Moringa Seeds

Enzyme extraction was done according to the optimum conditions with the procedure described by **Ahmed *et al*. (2010) and Tajalsir *et al*. (2014)**, using sodium acetate buffer (pH 5.0). *Moringa oleifera* was prepared and 7.0 g finely ground by grinder and extracted with 50 ml of the extractant for 4h. with stirring. The extract was filtrated through cheesecloth and centrifuged at 12000×g for 20 min. The supernatant was dialyzed overnight at 40°C against 0.1 mmol/L sodium acetate buffer, pH 5.0. The solution was centrifuged to remove any solid particles and then the activity was measured. Ammonium sulfate fractionation was

carried out following the method described by (**Ahmed *et al.*, 2010**). Milk-clotting and proteolytic activities in the enzymatic extract were determined.

Evaluation of Milk Clotting Activity (MCA)

Skim milk clotting activity was determined according to the method described by **Badgujar and Mahajan (2009)**. The substrate (10% reconstituted skim milk in 0.01 M $CaCl_2$) was prepared and the pH was adjusted to 6.5. The substrate (100 ml) was pre-incubated at different temperatures for 5 min, the temperature was adjusted to,35°C, 40°C, 45°C, 50°C,55°C 60°C, 65°C, 70°C and 75°C in case of Moringa seeds extract and rennet using a thermostatically controlled water bath. Briefly, 1 ml by volume of the aqueous extract was added into 100ml of the milk samples and unit of milk clotting activity (MCU) was determined by rotating the test tube at regular interval times and checking for visible clot formation on the wall of the test tubes. The following formula was used for calculating the milk clotting unit (MCU) reported by **Anusha *et al*. (2014)**, Results are expressed as MCU/ml.

Unit of skim 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 extract

Proteolytic Activity

The proteolytic activity of coagulant with rennet and aqueous extract of Moringa seeds extracts allows for the evaluation of the rate of the degradation rate of casein. It consists of measuring, the increase of non-protein nitrogen (NPN) in tri-chloroacetic acid (TCA) at 12% of the final mixture **Dako *et al*. (2009)** as modified by **Abdel Raouf *et al*. (2017)**.

Effect of Temperature on Milk Clotting Properties of Partially Purified Extract

A temperature at range of 60-70°C was used to determine the optimum temperature for clotting activity using 1ml of enzyme extract in case of Moringa seeds extract.

Salt content

Salt content was estimated as described by **Bradley *et al*. (1992)**.

Protein content

Was determined by the official Kjeldahl method described in **AOAC (2019)**.

Syneresis

The syneresis was measured by placing a 100 ml of the coagulated sample on a filter paper placed on the top of a funnel. After 2 hr. of drainage, the volume of the whey was collected and measured described by **Isanga and Zhang (2009)**. The index of syneresis is calculated as follows:

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

Where:

S: Syneresis

V1: Volume whey after drainage.

V2: Initial volume sample.

Making white Soft Cheese (Tallaga Cheese)

Fresh buffalo's milk was standardized to 5.5% fat, heated at 90°C/5 min, then cooled to 65°C partial purified aqueous enzymatic extract of Moringa seeds and 40°C for calf rennet with adding 0.02% of $CaCl_2$ and 4% of NaCl. The milk was divided to two equal portions as follows: The first portion was renneted with rennet, the second portion was produced with Moringa seeds extract using as coagulant (Dosage 5.0± 0.1ml /100ml milk) (Dosage of extracts were calculated with reference to the partial purification of Moringa seeds extracts. White soft cheese was made from each portion by the conventional method of making Tallaga cheese according to Abou-Donia (2008). The resultant white soft cheese produced cheeses were packaged in plastic bags containing pasteurized whey (4% salt) and stored in refrigerator (7±1°C) for 28 days. Tallaga cheese samples were taken when fresh, then after 7, 14, 21 and 28days at (7±1°C).

The experiment was repeated in triplicates and each analysis in duplicate and average results were tabulated.

Statistical Analysis

The data were analyzed by ANOVA according to the appropriate experimental designs and reported as means (±standared deviations) which

were separated by Duncan's Multiple Range Test at P ≤ 0.05 **(Cochran and cox, 1992)** and least significant difference (LSD) test using SPSS computer program, version 20 (SPSS Inc., Chicago, IL, USA). Triplicate measurements were performed for each analysis.

RESULTS AND DISCUSSION

Milk Clotting Activity

Table 1 demonstrates that when the reaction temperature rose from (35–75°C), the enzyme activity increased as well at 65°C, there was more clotting activity than at 60°C. According to research by **El-Sayed *et al*. (2013)**, who found that the refined rape seed extract's milk clotting activity increased gradually as temperature rose, peaking at 60°C. The underlying theory behind the thermophilic be haviour of plant protease is that elevated temperatures may cause conformational changes in protein structure. It becomes susceptible to proteolysis as a result (**Na'jera *et al*., 2003**). **Ahmed *et al*. (2010) and Pontual *et al*. (2012)** have reported that the MCA of different plants can withstand high temperatures under certain conditions. Furthermore, a number of variables, including plant sources, tissues, concentrations, degree of purification, and enzyme type, affect the temperature profile of the MCE derived from plant extracts (**Mazorra *et al*., 2013**). For the proteases employed in the making of cheese, milk clotting activity (MCA) is the most significant characteristic.

The data in the same Table showed how temperature settings affected the clotting times of extract from Moringa seeds and rennet. The data displayed in the same Table shows that temperature has a substantial (P≤0.05) impact on the clotting time. When the setting temperature was raised above 35°C, the clotting time for rennet was significantly (P≤0.05) reduced up to 40°C. When the temperature rose over 40°C, there was a substantial increase in the clotting time (P≤ 0.05). The milk clotting time was also significantly (p≤0.05) lower at 75°C. The clotting time for extract from Moringa seeds was considerably (P≤0.05) 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 **Abdel Galael and Elzawahry (2005),** who found that no clotting was observed at 54°C in the case of rennet and that the clotting time was significantly (p≤0.05) decreased in the case of rennet up to 42°C and up to 56°C for *Solanum dobium.*

The extract from partial purified aqueous extract of Moringa seeds was the best clotting activity at 65°C, and the temperature-dependent decline in milk clotting activity (MCA). These findings concur with those made public by **Aworh and Muller (1987)**, who noted that at 53°C, chymosin inactivated in gelled milk. Furthermore, MCA from Sodom apple (*Calotropis procera*) extract was found to be more active at 65°C than at 35°C by **Aworh and Nakai (1986)**. These findings showed that extract from Moringa seeds could withstand higher temperatures than rennet. Additionally, **El-Sayed *et al*. (2013)** demonstrated that the pure rape seed extract's milk clotting activity rose gradually with temperature, peaking at 60°C.

Plant proteases 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 **Ahmed *et al*. (2010) and Pontual *et al*. (2012)**. Furthermore, according to **Mazorra-Manzano *et al*. (2013)**, the temperature 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.

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.

Chemical Composition of White Cheese

Moisture content

Additional plant-based coagulants used in cheese treatments include the aqueous enzymatic extracts of Moringa seeds. The results showed that Tallaga cheese that had been coagulated with rennet had a significantly (p≤0.05) higher moisture content than cheese made with aqueous enzymatic extract of Moringa seeds. Additionally, in the same Table 2 shows that throughout ripening storage period up to 28 days, the moisture content of both control cheese and

Table 1. Effect of temperature on milk clotting activity of rennet and Moringa seeds partially purified aqueous extracts

Temperature (°C)	Properties					
	Calf Rennet			Moringa seeds		
	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]	-	-	-
40	465[d]	516.13[a]	100[a]	-	-	-
45	482[c]	497.63[a]	96.47[b]	-	-	-
50	890[a]	290.21[b]	56.23[d]	-	-	-
55	-	-	-	-	-	-
60	-	-	-	1200[b]	40.00[c]	77.5[c]
65	-	-	-	930[d]	51.61[a]	100[a]
70	-	-	-	1100[c]	43.64[b]	84.55[b]
75	-	-	-	1250[a]	38.4[d]	74.4[d]
Mean	581.75	448.44	86.9	1120	43.41	84.11

[a,b,c,d] Means in the same column with different superscripts differ significantly ($p \leq 0.05$).
C.T: Clotting time (Sec)
M.C.A: Milk Clotting Activity (m/ml)
R.A: Relative activity (%) was calculated as 100% at pH and increase or decrease according to clotting time(Sec)

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

Components (%)	Storage period (days)	Treatment		Mean effect
		Control	Moringa seeds extract	
Moisture %	**Fresh**	A 68.47[a]	A 66.54[c]	67.51
	7	A 67.93[a]	B 64.39[c]	66.16
	14	B 66.06[a]	C 63.50[c]	64.78
	21	C 64.21[a]	D 62.05[b]	63.13
	28	D 63.05[a]	E 61.44[c]	62.25
Acidity (as lactic acid%)	**Fresh**	D 0.20[a]	C 0.32[b]	0.26
	7	C 1.25[a]	B 1.37[ab]	1.31
	14	BC 1.45[a]	AB 1.55[a]	1.5
	21	AB 1.60[a]	A 1.75[d]	1.68
	28	A 1.69[a]	A 1.81[c]	1.75
Fat/DM %	**Fresh**	E 48.76[b]	E 47.95[c]	48.36
	7	D 60.11[a]	D 55.20[d]	57.66

Components (%)	Storage period (days)	Treatment		Mean effect
		Control	Moringa seeds extract	
Salt/M%	14	C 62.35[a]	C 62.46[a]	62.41
	21	B 64.00[a]	B 63.13[b]	63.57
	28	A 64.83[a]	A 64.57[a]	64.7
	Fresh	D 6.00[b]	E 6.36[a]	6.18
	7	D 6.26[d]	D 6.99[c]	6.63
	14	C 6.99[b]	C 7.70[a]	7.35
	21	B 7.99[b]	B 8.38[a]	8.19
	28	A 8.40[b]	A 8.79[a]	8.60
TN%	Fresh	A 1..76[d]	A 2.18[b]	1.97
	7	B 1.70 [c]	AB 2.01[a]	1.86
	14	C 1.54[c]	BC 1.75[a]	1.65
	21	DC 1.50[b]	CD 1.53[b]	1.52
	28	D 1.46 [a]	D 1.31[a]	1.39
SN%	Fresh	A 0.46[a]	D 0.50 [a]	0.48
	7	A 0.49 [a]	A 0.51 [a]	0.5
	14	A 0.53 [a]	BC 0.57 [a]	0.55
	21	A 0.56 [a]	AB 0.65 [a]	0.61
	28	A 0.61 [a]	A 0.67 [a]	0.64
NPN%	Fresh	D 0.26	A 0.42[a]	0.34
	7	C 0.36	A 0.45 [a]	0.41
	14	B 0.40	A 0.51 [a]	0.46
	21	A 0.44	A 0.55[b]	0.50
	28	A 0.50[a]	A 0.62[b]	0.56

[a,b,c,d] Means in the same column with different superscripts differ significantly ($p \leq 0.05$).
[A,B,C,D] Means in the same row with different superscripts differ significantly ($p \leq 0.05$).

experimental cheese gradually decreased to a significant level ($p \leq 0.05$) with storage period progressed up to 28 days. Tallaga cheese prepared with the aqueous enzymatic extract of Moringa seeds had a considerably ($p \leq 0.05$) lower moisture content than the control cheese.

This might be the result of an increase in acidity that concentrated on the curd and caused a lower of moisture. It is expected that this will return to that the coagulums structure contain aqueous extract of Moringa seeds. These outcomes concur with those of **Sanjuan *et al*. (2002) and Galan *et al*. (2008)**, who found that cheese prepared with vegetable coagulant had a significantly lower moisture content than cheese made with calf rennet.

Additionally, the current study's findings concur with those of **Abdel-Galeel and El-Zawahry (2005) and Talib*et al*. (2009)**, who found that cheese made using extract from *Solanum dobium* seeds had a lower moisture content than cheese manufactured with calf rennet.

According to **Zakaria *et al*. (2020)**, the moisture content of cheese treated with 2, and 4% extract of Moringa seeds was lower than that of cheese in the control group.

Titratable acidity (as lactic acid%)

Also Table 2 shows the average acidity of Tallaga cheese as percentage of lactic acid as a results of using the aqueous extract of Moringa seeds in the experimental cheese. IT could noticed that titratable acidity of all cheeses were significantly ($p \leq 0.05$) increased throughout ripening period .Also results show that titratable acidity of cheese manufactured using aqueous extract of Moringa seeds was significantly ($p \leq 0.05$) higher than control cheese when fresh and during ripening period up to 28 days. The general trend of these results is in agreement with that reported by **Vioque *et al.* (2000) and Abdeen *et al*. (2021)**. These findings were also noted by **Abdel Kader (2003)** for Domiati cheese made with microbial rennet. **Al Jasser and Al Dogan (2009)** also observed higher acidity for white soft cheese produced using rennet substitute from *solanum dobuim* seeds compared to control cheese. Also, these results are in agreement with that reperted by **Abdel Galeel and El Z-awahary (2017)** who found that activity in Edam cheese made with added aqueous extracts of cardoon or Ginger was significantly ($p \leq 0.05$) higher than control cheese until the end of storage period.

Fat /dry matter% (F/DM%)

The average fat/dry matter (%) for both kind of cheese treatment throughout storage period up to 28 days were displayed in Table 2. It has been noted that as the storage period increases, the fat content (%) and fat/DM % progressively increased in both cheese treatments.

The cheese prepared with Moringa seeds extract, as shown in this table, had considerably ($p \leq 0.05$) lower fat/DM (%) at the end of storage period than the control cheese (C). These findings are consistent with those of **Elkholy (2015),** who found that fat content significantly ($p \leq 0.05$) increased in all cheese treatments during storage period, which might be due to moisture content reduction during storage. Also, these results are in agreement with obtained by **Mahami *et al*. (2011)**, they found that protein, fat, ash and phosphorus contents in all the cheese increased directly with increase of Moringa seeds extract concentration.

Salt/Moisture content %

The average salt/moisture percentage of cheese prepared from extract from Moringa seeds and control was displayed in Table 2. These results demonstrate that, over the course of storage for up to 28 days, salt/M% of the cheese treatments raised regularly and considerably ($p \leq 0.05$). This Table also clearly shows that, during storage period (salt/M)% in cheese manufactured using Moringa seeds extract increased significantly ($p \leq 0.05$) comparison to control cheese (C). The rate at which the proteins in the experimental cheeses can bend water may be the cause of this (**Guinee and Fox, 2004**).

Additionally, we may state that the increase of whey and curd concentration may be the cause of the decreased moisture (**Abd El-Halim, 2007**). 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 cheese made with

Moringa seeds extract. 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. **Kaya (2002)** states that osmotic dehydration of the product results from a mutual diffusion process between moisture loss and salt uptake. There is a possibility that the elevated moisture content is associated to casein proteolysis (Algarni, 2016).

Ripening Indices

Proteolysis

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 increased with Moringa seed extract followed by cheese made with 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 **Badawi and Kebary's (1996) and ElKholy (2015)** reports for Tallaga cheese and Domiati cheese. The same Table revealed that throughout the ripening period, cheese prepared with aqueous extract of Moringa seeds had a greater total protein (%) than the control cheese. The outcomes correspond with **Elkholy (2015)** findings.

Additionally, the cheese sample made with aqueous 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 **Zakaria *et al*. (2020)**.

Soluble nitrogen content (SN%)

Table 2 showed the average of soluble nitrogen content of both control cheese made with rennet (as control cheese) and cheese made aqueous extract of Moringa cheese. These data clearly show that as the storage period increased the soluble nitrogen content increased of all cheese treatment. Cheese made with aqueous extract of Moringa seeds was significantly ($p \leq 0.05$) higher than control cheese, which due to the intence proteolytic activity of Moringa seeds extract. These results in agreement with that reported by Pino *et al*. (2009) who found cheese made with plant coagulant had statistically higher amount of SN % content than cheese manufactured using rennet.

Non-protein nitrogen content (NPN%).

The average of NPN content of Tallaga cheese made with calf rennet (control) or using aqueous enzymatic extract of Moringa seeds as ripening period advanced up to 28 days are displayed in Table 2 The NPN content of all cheeses had significantly ($p \leq 0.05$) increased as ripening period progressed up to 28 days. But the values were significantly ($p \leq 0.05$) higher in cheese made by using aqueous enzymatic extract of Moringa seeds. These results are in agreement with same studies carried out on cheese made from ewe's milk which has shown high levels of non-protein nitrogen in cheeses manufactured using plant coagulant compared to cheese made using calf rennet **Tejada and Fernandz-Salguero (2003), Prado *et al*. (2007) and Galan *et al*. (2008)**. Also, these results are in agreement with **Prasad and Alvarez (1999) and Talib *et al*. (2009)** who reported that SN/TN and NPN//TN contents increase with progress of ripening period, as a result of protein degradation leading to the formation of water-Soluble nitrogen compound and some of which losses in the pickling solution leading to increase in nitrogen content in whey.

Tyrosine and Tryptophan Contents

Table 3 shows the values of soluble tyrosine and tryptophan content of Tallaga cheese made using rennet (as control) or cheese made using aqueous extract of Moringa seeds when fresh and up to 28 days. It can be noticed that the amount of soluble tyrosine and tryptophan significantly with an increase at the end of storage period the results indicated that cheese made by using aqueous of Moringa seeds was significantly ($p \leq 0.05$) higher content soluble tyrosine and tryptophan contents compared to

Table 3. Change in tyrosine and tryptophan Mg/100 g cheese 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
Control	B 25.13[d]	A 34.36[ab]	B 8.01[c]	A 15.50[a]
Moringa seeds extract	B 29.40[b]	A 38.12[a]	B 11.22[b]	A 19.12[a]
Pickling per. Mean	27.27	36.24	9.62	17.31

[a,b,c,d] Means in the same column with different superscripts differ significantly (p≤0.05).
[A,B,C,D] Means in the same row with different superscripts differ significantly (p≤0.05).

control cheese made using calf rennet up to the end of storage period. This can be attributed to the action of proteolytic enzymes of plant coagulant which affect the protein degradation resulting in releasing more tyrosine and tryptophan contents. Which is considered as ripening index for cheese ripening. These given by **El-Shafei (2015)**. The obtained requests agree with these obtained by **Ali (2010)** who found that the increase of soluble tryptophan either in control or the treatment were clear and highly significant different during the storage period of cheeses. Also, these are in accordance with that obtained by **Abd El-Salam *et al.* (1993) and El-Shafei (2015)**.

Additionally, soluble tyrosine and tryptophan contents gradually increased at the time of picking passed (**Kholif *et al*., 2010; El-Alfy, 1988 and 2004; Abd-Rabou and El Senaity, 2005)**.

Syneresis of treated cheese

Table 4 makes it evident that the cheeses syneresis rate increased steadily over the course of the minute and reached 120 minutes at 37°C. 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. (**Wu *et al*., 2001; Abd El-Gelil and El-Zawahary, 2004**).

Organoleptic Properties

Table 5 displays significant differences (P≤ 0.05) in the storage period of up to 28 days between Tallaga cheeses made with extracts from Moringa seeds 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, in that order.

Furthermore, it was noted that Tallaga cheese produced with aqueous enzymatic extract of Moringa seeds was substantially (p≤0.05) less expensive than the other experimental cheeses and control cheese.

Conclusion

From this study it was concluded that partially purified extract of *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.

It is concluded that partial purified extract of Moringa seeds was satisfactory substitute for rennet and suitable for cheese making with acceptable quality characteristics

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

Syneresis gm/gm	Treatment	
	Control	Moringa seeds extract
10 min	B 1.80[d]	AB 1.90[d]
30 min	C 3.45[c]	A 4.20[c]
60 min	B 4.60[b]	AB 5.50[b]
120 min	D 5.20[a]	B 6.70[a]
Mean	3.76	4.58

[a,b,c,d] Means in the same column with different superscripts differ significantly ($p \leq 0.05$).
[A,B,C,D] Means in the same row with different superscripts differ significantly ($p \leq 0.05$).

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	
		Control	Moringa seeds extract
After manufacture	Appearance (10)	A 8.50[a]	A 8.30[a]
	Body and texture (40)	A 39.56[a]	C 33.65[a]
	Flavour (50)	A 47.90[a]	C 45.50[a]
	Total (100)	95.96	87.45
7 days	Appearance (10)	A 8.37[a]	A 8.17[a]
	Body and texture (40)	A 38.60[b]	C 33.10[b]
	Flavour (50)	A 47.68[a]	D 43.82[c]
	Total (100)	94.65	85.09
14 days	Appearance (10)	A 7.99[a]	A 7.60[b]
	Body and texture (40)	A 38.53[b]	D 32.00[c]
	Flavour (50)	D 43.50[b]	C 44.80[b]
	Total (100)	90.02	84.4
21 days	Appearance (10)	B 7.20[b]	C 6.33[c]
	Body and texture (40)	A 37.27[c]	C 33.20[d]
	Flavour (50)	C 42.25[c]	B 43.50[d]
	Total (100)	86.72	83.03
28 days	Appearance (10)	A 7.15[b]	B 6.30[c]
	Body and texture (40)	A 36.69[c]	D 30.56[e]
	Flavour (50)	A 41.15[d]	BC 40.60[e]
	Total (100)	84.99	77.46

[a,b,c,d] Means in the same column with different superscripts differ significantly ($p \leq 0.05$).
[A,B,C,D] Means in the same row with different superscripts differ significantly ($p \leq 0.05$).

REFERENCES

Abd El-Gelil, A.A. and A.R. El-Zawahary (2004). Milk clotting enzyme by using *Solanum dobium* plant as rennet substitute, 1- Preliminary studies on the enzyme extract from *Solanum dobium* plant on milk clotting. Zagazig J. Agric. Res., 6: 2999-3017.

Abd El-Gelil, A.A. and A.R. El-Zawahary (2017). Changes in physico chemical characteristics, proteolysis and organoleptic properties of edam cheese made with added aqueous extract of cardoon (*cynara cardunculus*) and ginger (*zingiber officinale*) during ripening . Zagazig J. Agric. Res., 44 (5): 1797-1808.

Abd El-Halim, S.E. (2007). Improvement of Domiati cheese quality produced from heat-treated buffalo′s milk using different starter cultures. M.Sc. Thesis, Fac. Agric., Ain Shams Univ., Egypt.

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, M.H., E. Alichanidis and G.K. Zerfiridis (1993). Domiati and Feta Type Cheese. In: Cheese: Chemistry, Physics and Microbiology, (Ed): Fox, P. F. Elsevier Appl. Sci., London, UK, 301–335. https: //link. springer.com/ chapter/10.1007/978-1-4615-2648-3-11.

Abdeen, E.M. (2021).Utility of Moringa oleifera waste as a coagulant in goat soft cheese production. J. Homepage, 2405-8440.

Abdel-Galeel, A.A. and A.A. El-Zawahry (2005). Milk clotting enzyme by using *Solanum dobium* plant as rennet substitute: II- Quality and ripening characteristics of Gouda cheese as affected by replacing calf rennet with aqueous extract of *Solanum dobium*. J. Agric. Sci. Mansoura Univ., 30 (12): 7765-7780.

Abdel-Raouf, H.H., A.A. El-Neshwy, A.M. Rabie and S.A. Khalifa (2017). Evaluation of rennet substitute from artichoke (*Cynara scolymus* L.) flowers extracts: study the factors affecting the activity of milk clotting. Food Sci. Dept., Fac. Agric., Zagazig Univ., Egypt.

Abd-Rabou, N.S. and M.H. El-Senaity (2005). Quality of Edam cheese with using rennin like fungal enzyme. J. Agric. Sci. Mansoura Univ., 30 (7): 3999-4004.

Abou-Donia, S.A. (2008). Origin, history and manufacturing process of Egyptian dairy products; An overview. Alex. J. Food Sci., Technol., 5: 51-62.

Ahmed, M.I.A., E.E. Babiker and N. Mori (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 of Food Sci. and Technol., 43: 759–764.

Al-Jasser, M.S. and A.A. Al-Dogan (2009). Properties of white soft cheese produced using rennet substitute extracted from *Solanum dubium* seeds. Egypt. J. Dairy Sci., 37: 237-232.

Alegbeleye, O.O. (2018). How functional is *Moringa oleifera*? A review of its nutritive, medicinal, and socioeconomic potential. Food and Nutr. Bulletin, 39 (1): 149–170.

Algarni, E.H.A. (2016) Soft cheese supplemented with thyme, cumin, and turmeric to increase shelf life during storage period. Adv. Environm. Bio., 10, 227-236. http://www. aensiweb.net/AENSIWEB/ aeb/aeb/ 2016/ December /227-236.pdf

Ali, A.R.M. (2010). Using of non-traditional methods for making soft cheese. MSc. Thesis, Fac. Agric. Moshtohor, Benha Univ., Egypt.

Anusha, R., M.K. Singh and O.S. Bindhu (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 (2019). Official methods of analysis: Association of analytical chemists.

Ashok, K. and L. Pari (2003). Antioxidant action of *Moringa oleifera* Lam. (drumstick) against antitubercular drugs induced lipid peroxidation in rats. J. Med. Food. Fall., 6 (3): 255-259.

Aworh, C. and S. Nakai (1986). Extraction of Milk clotting enzyme from Sodom *Calotropis*

procera. J. Food Sci., (51): 1569-1670.

Aworh, O.C. and H.G. Muller (1987). Cheese-making properties of vegetable rennet from Sodom apple (*Calotropis procera*). Food Chem., (26): 71-79.

Badawi, R.M. and K.M.K. Kebary (1996). Accelerated ripening of cheese by partial *Lyzed lactococci*. Menofiya J. Agric. Res., 21: 63-68.

Badgujar, S.B. and R.T. Mahajan (2009). Proteolytic enzymes of some laticiferous plants belonging to Khandesh Region of Maharashtra, India. J. Pharma. Res., 2: 1434-1437.

Barry, A.L. and A.Y. Tamime (2010). Tochnology of Cheese Making. Blackwell Publishing, Ltd., 2: 8-12.

Bradley, R.L., J.E. Arnold, J.D.M. Barbano, R.G. Semerad, D.E. Smith and B.K. Vines (1992). Chemical and physical method: In (standard Method for the Examination of Dairy products), 16th Ed., (Ed.) Marshall RT, P 433. Ame. Public Health Assoc., (APHA). New York, U.S.A.

Cochran, W.G. and G.M. Cox (1992). Experimental Designs (2nd Ed.). New York, NY: John Wiley and Sons.

Dako, E., A. Nouani, A. Morsli, N. Belhamiche, S. Belbraout, M.M. Bellal and A. Dadie (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.

Dalei, J., V.M. Rao, D. Sahoo, M. Rukmini and R. Ray (2016). Review on nutritional and pharmacological potencies of *Moringa oleifera*. Eur. J. Pharm. Med. Res., 3 (1): 150 – 155.

El-Alfy, M.B.A.S. (1988). Trials on accelerating cheese ripening. Ph.D. Thesis, Moshtohor, Benha Branch, Zagazig Univ., Egypt.

El-Alfy, M.B., M.F. Younis, A.M. Abd El-Aty, S.H.G. Osman and W.A. Gafour (2004). The implication of using soybean milk and probiotic for Feta-like cheese making. Ann. Agric. Sci., Moshtohor, 42 (4):1727–1742.

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-Sayed, S.T., M.M.A. Elmazar and R.A. Al-Azzouny (2013). Purification and Characterization of a Novel Milk-Clotting Enzyme from Brassica napus Seeds. Aust. J. Basic and Appl. Sci., 482-493.

El-Shafei, S. (2015). Technological study on Goats milk, Thesis Ph.D. Benha Univ., Egypt.

Foidl, N.; Makkar, H.P.S. and Becker, K. (2001). The potential of *Moringa oleifera* for agricultural and industrial uses What development potential for Moringa products? October 20th - November 2nd, Dar Es Salaam.

Galan, E., F. Prodos, A. Pino, L. Tejada and J. Fernandez-Salguero (2008). Influence of different amounts of vegetable coagulant from cardoon *Cynara cardunculus* and calf rennet on proteolysis and sensory characteristics of cheeses made with sheep milk. Int. Dairy J., 18: 93-98.

Gaya, P., M. Medina, M.A. Rodricuer-Marin and M. Nunez (1990). Accelerated ripening of ewe's milk Manchego cheese: The effect of elevated ripening temperatures. J. Dairy Res., 44: 159-188.

Guinee, T.P. and P.F. Fox (2004). Salt in Cheese: Physical, Chemical and Biological Aspects. Pages 207–259 in Cheese Chem., Physics and Microbiol., Vol. 1. General Aspects. 3rd ed. P.F. Fox, P.L.H. McSweeney, T.M. Cogan, and T.P. Guinee, ed. Elsevier Academic Press, Oxford, UK.

Inanga, J. and G. Zhang (2009) .production and evqluation of some physicochemical parameter of peanut milk yoghurt LWT- food Sci. and Technol.,42:1132-1138.

Kar, A., B. Choundhary and N. Bandyopadhyay (2003). Comparative evaluation of hypoglycemic activity of some Indian medicinal plants in alloxan diabetic rats. J. Ethnopharmacol., 84 (1):105-8.

Kaya (2002) Effect of salt on hardness and

whiteness of Gaziantep Cheese during short-term brining. J. Food Eng., 52(2):155–159.

Kebary, K.M.K., A.I. Hamed, A.N. Zedan and A.A.F. ElBeheary (2006). Manufacture of low fat Domiati cheese using Novagel. Egyptian J. Dairy Sci., 34: 175-184.

Kholif, A.M., G.A. Mahran, M.A. El-Nawawy, A.A. Ismail, M.M.E. Salem and W.M. Zaky (2010). Use of Some Lactobacillus Strains to Improve Soft Cheese Quality. World Appl. Sci. J., 11 (7): 766-774.

Lipipun, V., M. Kurokawa, R. Suttisri, P. Taweechotipatr, P. Pramyothin, M. Hattori and K. Shiraki (2003). Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection in vitro and in vivo. Antivir Res. Nov; 6 (3):175-80, 2003.

Mahami, T., F.C.K. Ocloo, S.T. Odonkor, C. Owulah and S.A. Coffie (2011). Preliminary study on the influence of moringa seed extracts supplementation on the yield and quality of cottage cheese. Int. J. Recent Trends in Sci. and Technol., 2: 4-8.

Matic, I., A. Guidi, M. Kenzo, M. Mattei and A. Galgani (2018). Investigation of medicinal plants traditionally used as dietary supplements: A review on *Moringa oleifera*. J. Public Health in Afr., 9 (3): 191–199.

Mazorra-Manzano, P.G., M.E. Lugo-Sa´nchez, J.C. Ramirez-Suarez, M.J. Torres-Llanez, A.F. Gonza´lez-Co´rdova and B. Vallejo-Cordoba (2013). Comparison of the milk-clotting properties of three plant extracts. Food Chem., 141 (3): 1902–1907.

Mbikay, M. (2012). Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A review. Frontier in Pharmacol., 3, 24.

Mishra, S.P., P. Singh and S. Singh (2012). Processing of *Moringa oleifera* leaves for human consumption. Bulletin of Environment, Pharmacol. and Life Sci., 2 (1): 28–31.

Mukunzi, D., J. Nsor-Atindana, Z. Xiaoming, A. Gahungu, E. Karangwa and G. Mukamurezi (2011). Comparison of Volatile profile *Moringa oleifera* leaves from Rwanda and China using HS-SPME. Pak. J. Nutr., 10: 602– 608.

Na'jera, A.I., M. De Renobales and L.J.R. Barron (2003). Effects of pH, temperature, $CaCl_2$ and enzyme concentrations on the rennet-clotting properties of milk: a multifactorialstudy. Food Chem., 80:345-352.

Nunez, M., B. Fernandez del Pozo, M.A. Rodriguez-Marin, P. Caya and M. Medina (1991). Effect of vegetable and animal rennet on chemical, microbiological, rheological and sensory characteristics of La Serena cheese. J. Dairy Res., 58: 511-519.

Piers, M.S., G.A. Orellanc and S.A. Singh (1999). Rennet coagulation of casein micelles and heated casein micelles action of Ca+2 and pH. Food Hydrocoll., 13: 235-238.

Pino, A., F. Prados, E. Galan, P.L.H. McSweeney and J. Fernandez-Salguero (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., B.E. Carvalho, R.S. Bezerra, L.C. Coelho, T.H. Napoleão and P.M. Paiva (2012). Caseinolytic and milk-clotting activities from *Moringa oleifera* flowers. Food Chem., 135: 1848–1854.

Prado, A., A. Pino and J. Fernandez-Salguere (2007). Effect of powdered vegetable coagulant from cardoon (*Cynara cardunculus*) in the accelerated ripening of Manchego cheese. Int. J. Food Sci. and Technol., 42: 556 - 561.

Prasad, N. and V.B. Alvarez (1999). Effect of salt and chymosin on the physicochemical properties of feta cheese during ripening. Ibid, 82: 1061.

Rao, A., P. Devi and R. Kamath (2001). In vivo radioprotective effect of *Moringa oleifera* leaves. Indian J. Exp. Biol., 39 (9):858-863.

Sanjuan, E., R. Millan, P. Savedra, M.A. Carmona, R. Gomez and J. Fernandez-Salguere (2002). Influence of animal and vegetable rennet on the physicochemical characteristics of Los Pedroches cheese during ripening. Food Chem., 78: 281-289.

Shah, M.A., S.A. Mir and M.A. Paray (2014). Plant proteases as milk-clotting enzymes in cheesemaking: A review. Dairy Sci. and Technol., 94: 5-16.

Sousa, M.J., Y. Ardo and P.L.H. McSweeney

(2001). Advances in the study of proteolysis during cheese ripening. Int. Dairy J., 11: 327-345.

Tahiliani, P. and A. Kar (2000). Role of *Moringa oleifera* leaf extract in the regulation of thyroid hormone status in adult male and female rats. Pharmacol Res., 41(3):319-323.

Tajalsir, A.E., A.S.A. Ebraheem, A.M. Abdallah, F.J. Khider, M.O. Elsamani and I.A.M. Ahmed (2014). Partial purification of milk-clotting enzyme from the seeds of *Moringa oleifera*. J. Microb., Biotech. Food Sci., 4: 58-62.

Talib, M.A., M.M. Abubakar, I.A. Jideani and A. Hassan (2009). Use of Jiben seeds extract to manufacture soft white cheese. Ame. J. Appl. Sci., 6 (4): 551–554.

Tejada, L. and J. Fernandez-Salguero (2003). Chemical and microbiological characteristics of ewe's milk cheese (Los Pedroches) made with a powder of vegetable coagulant or calf rennet. Ital. J. Food Sci., 15: 125-132.

Vioque, G.M.R., E. Sanchez, C. Mata, L. Tejada and J. Fernandez-Salguro (2000). Chemical and microbiological characteristics of ewe's milk cheese manufactured with extract from flowers of *Cynara cardunculus* and *Cynara humilis* as coagulant. J. Agric. Food Chem., 48: 451-456.

Vishwanatha, K.S., A.G. Appu Rao and S.A. Singh (2010). Production and characterization of milk clotting enzymes from *Asperigillas oryzae*, MTCC 53 App. Microbial. Biotechnol. 85:1849- 1859.

Wu, H., G.J. Hulbert and J.R. Mount (2001). Effects of ultrasound on milk homogenization and fermentation with yogurt starter. Innovative Food Sci. and Emerg. Technol., 1: 211-218.

Zakaria, S.A., H.A. Abdelsalam, A.A. Aburigal, A.E. Sulieman (2020). Physicochemical, Microbiological and Sensory Characteristics of White Cheese Made by Adding *Moringa oleifera* Seeds Extract. Acad. J. Life Sci., 6 (7): 76-82.

صنـاعـة الجبـن الطـري باستخدام المستخلص الإنـزيمـي المـائـي لبـذور المـورينجـا

إسراء أيمن الهادي علي – علي عبد الرحمن عبد الجليل- السيد محمد عبد الواحد

قسم علوم الأغذية – كلية الزراعة – جامعة الزقازيق - مصر

في هذه الدراسة تم تصنيع الجبن الأبيض الطري (جبن الثلاجة) من اللبن الجاموسي باستخدام المستخلص المائي المنقي جزئيا لبذور المورينجا ومقارنتها بالجبن الكنترول (جبن الثلاجة) المصنع باستخدام المنفحة الجافة. كل المعاملات تم تخزينها علي درجة الثلاجة 7°م ±1°م لمده 28 يوم للتسوية. تم تحليل عينات الجبن بصفة دورية خلال التخزين علي 7°م±1°م لمدة 28 يوم للتحليلات الكيماوية، والتحلل البروتيني (مؤشرات التسوية) والتيروسين والتربتوفان، وانفصال الشرش والخواص الحسية للجبن الناتج. أوضحت النتائج المتحصل عليها أن جبن المقارنة (كنترول) كان ذو محتوى عالي من الرطوبة مقارنة بجبن التجربة. أيضًا تحلل الكازين والحموضة(مقدره كنسبة مئوية لحامض اللاكتيك) ومحتوي التيروسين والتربتوفان وانفصال الشرش في الجبن المصنع بالمستخلص المائي لإنزيم بذور المورينجا كان أعلي محتوي عند ($p\leq0.05$) مقارنة بجبن المقارنة (كنترول). بينما أوضحت النتائج ان الخواص الحسية كانت أعلي في جبن المقارنة عن جبن التجربة أيضاً تم دراسة تأثير درجة الحرارة علي خواص التجبن لكل من إنزيم المنفحة المجفف والمستخلص الإنزيمي المائي لبذور المورينجا. وقد أوضحت النتائج بأن استخدام المستخلص الإنزيمي المائي لبذور المورينجا كان بديلا مقبولا للمنفحة ا لصناعة الجبن الطري بخواص جودة مقبولة.

المحكمـــون:

1- أ.د. حازم حامد عبد الرؤوف فايد أستاذ الألبان المتفرغ - كلية التكنولوجيا والتنمية – جامعة الزقازيق.

2- أ.د. صلاح احمد عبد الجليل خليفه أستاذ الألبان الغذائية – كلية الزراعة – جامعة الزقازيق.