Isolation, purification and characterization of milk clotting enzyme from leaves of Portulaca Oleracea (purslane) plant against animal and microbial rennet in manufacturing white soft cheese

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Abstract :

The plant is a rich source of protein, especially enzymes. In this study, a milk clotting enzyme (MCE) was produced from *Portulaca oleracea* to be a substitute for commercial MCE. The first steps of partial purification were carried out by precipitation with ammonium sulfate (AS) and were the concentration of ammonium sulfate at 40% gave high the enzyme activity, followed by using sequential chromatographic technique of the most active fraction on sephadex G100 by rate purification to 3.77, Yield with 6.59 % recovery. To the MCE of optimum temperature was 40 °C and the enzyme activity was stable at 20 to 70 °C. The MCE enzyme showed the optima of pH 6 and was more stable in broad from pH 4.0 to 7.0. Effect of calcium chloride at a concentration of 25 mM gave the highest relative activity of the purified MCE. Sodium chloride at a concentration of 2 % gave the highest relative activity of the purified MCE 139.2. The metal ions at 1 and 5 mM gave activators and inhibitors of the purified MCE. On the other hand, soft Cheese was made from pasteurized milk with rennet from microbial and animal sources. tow cheese treatments were made from raw milk and pasteurized milk using purified MCE, the results showed and it was found that the soft cheese making by MCE from plant developed lower Moisture, pH and titratable acidity, while TN, SN and TVFA were high in cheeses manufactured by MCE from *Portulaca oleracea* plant and displayed better flavor and greater acceptability than other treatments cheese. Increased rate of proteolysis in cheese manufactured by MCE from plant extract had a direct relation to accelerated ripening soft cheese.

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Keywords: Milk clotting enzyme, Purification, Proteolytic, *Portulaca oleracea*, soft Cheese.

المستخلص:

يعتبر النبات مصدر غني بالبروتين وخاصة الإنزيمات وفي هذه الدراسة تم إنتاج إنزيم تجبن اللبن من نبات الرجلة (*Portulaca oleracea*) ليكون بديلا لإنزيمات تجبن اللبن التجارية من مصادرها الحيوانية والميكروبية وقد تم إجراء الخطوات الأولى للتنقية الجزئية بالترسيب بأملاح كبريتات الامونيوم وقد أعطى تركيز كبريتات الامونيوم بنسبة ٤٠ % نشاط إنزيمي عالي تلا ذلك استخدام تقنية الكروماتوغرافيا المتسلسلة للجزء الأكثر نشاطا على عامود من السيفادكس G ١٠٠ بمعدل تنقية ٣.٧٧ ومحصول بنسبة استرداد ٦.٥٩ %. وكانت درجة الحرارة المثلى للإنزيم عند ٤٠ درجة مئوية وكان نشاط الإنزيم مستقرا من ٢٠ إلى ٧٠ درجة مئوية. وأظهر إنزيم تجبن اللبن درجة الـ pH المثلى عند ٦ pH وكان أكثر استقرارا في نطاق واسع من ٤.٠ pH الى ٧.٠ pH. وأعطى تأثير إضافة تركيزات مختلفة من كلوريد الكالسيوم أعلى نشاط نسبى للإنزيم عند تركيز ٢٥. كما أعطى كلوريد الصوديوم بتركيز ٢ ٪ أعلى نشاط نسبي لـلانزيم (139.2). كما أعط تأثير إضافة تركيزات مختلفة من أيونات المعادن عند ١ و ٥ مليمول نتائج جيدة كمنشطات ومثبطات لـلإنزيم المنقى جزئيا. من ناحية أخرى، تم تصنيع الجبن الطري من اللبن المبستر باستخدام المنفحة من مصادر ميكروبية وحيوانية. ثم تم إجراء معاملتين للجبن من اللبن الخام واللبن المبستر باستخدام الانزيم المنقى المستخلص من النبات، وأظهرت النتائج أن صناعة الجبن الطري بواسطة الإنزيم المنقى من مصدر نباتى طورت رطوبة ودرجة pH ومحتوى حموضة أقل، بينما كانت TN و SN و TVFA عالية في الجبن المصنوع بواسطة الانزيم المنقى من نبات الرجلة *Portulaca oleracea* وأظهرت نكهة أفضل وقبولًا أكبر من جبن المعاملات الأخرى. وكان لزيادة معدل التحلل البروتيني في الجبن المصنوع بواسطة الانزيم المجبن المستخلص من النبات علاقة مباشرة بتسريع نضج الجبن الطري وظهور النكهة المقبولة.

الكلمات المفتاحية: انزيم تجبن اللبن، التنقية، التحلل البروتينى، نبات الرجلة، الجبن الطرى

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INTRODUCTION

Recently, the demand for intermediate materials has increased.Over recent years witnessing dramatic progress in the production of new enzymes (**Mamo, *et al* 2018).** One of the important enzymes in making cheese is Calf rennet (EC 3.4.23.4), which contains chymosin and is widely used as a by-product of cheese making, this led to a decrease in the numbers of young calves and increase of calf rennet's price, Which led researchers in this field to search for alternatives to animal rennet from non-animal sources to MCE (**Cavalcanti *et al.*, 2004)** which would satisfy to be a good alternative in cheese manufacture and preserve livestock. Moreover, led to much cheese consumption the use of plant coagulant. Recently, the spread of brain diseases in cows has led to reluctance to use animal rennet. **(Roseiro et al., 2003).**

Rennet from Microbial source has proven its efficiency as a suitable alternative to animal rennet, But for a long time there has also been interest in searching for milk clotting enzymes from various plant sources., This is because it is safe, harmless, and low cost **(Tavaria et al., 2001)** such as chymopapain, papain, papaya and carican which has been purified from commercial papaya (*Carica papaya* L), Also, pineapple (*Ananas comosus* L.), Cardosin B (*Cynara cardunculus*) and (*Bromelia plumieri*) **(Goodenough & Owen, 1987., Azarkan et al.,1996; Zimacheve et al.,1994; Maksimenko et al.,1990; Monates et al., 1990)** Recently, the MCE was isolated and purified from Moringa **(Abdeen et al., 2021)** and *Solanum elaagifoulum* (**Kholif et al., 2016).** It was found that proteoses from plant sources are of interest to many people from the medical and nutritional aspects because they are a natural product that is easy to extract by hydrolysis. **(Silva and Malcata, 2005).**

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The plant proteases used in cheese making must have a high clotting power with a weak proteolysis capacity (ratio milk clotting/proteolysis) in order to obtain a good product and free defects to test and off-flavor. The coagulation power of the enzyme depends on this ratio. To hydrolyse specifically κ-casein **(Jacob et al., 2011).** There are also many proteases that are unable to analysis whey proteins or analyzed the peptides into amino acids, which makes few plant proteases suitable for cheese production.

In cheese making from the coagulants from plant have been used since ancient times in Mediterranean, European and African **(Shah et al., 2014).** Also, Spain and Portugal more types of cheeses are made using vegetable coagulant. Traditional cheese making In Nigeria are used extracts from Calotropis procero **(Shah et al., 2014).** Also The extract purified enzyme from seeds of *Solanum dubium and Solanum elaeagnifolium* were used in the manufacture of soft cheese in Sudan and egypt **Guiama, et al., (2010)** and **Kholif et al, (2016**). In Mexico, the proteases from plant are used As an alternative to rennet in the manufacture of soft cheese **(Gutiérrez et al., 2012).**

From the previous information, it was the aim of this study was initiated to isolate purification and characterize the milk clotting enzyme from *Portulaca oleracea* and study the effect of using it in the manufacture of white soft cheese.

Material and Methods

Commercial fine grade salt such as NaCl and $CaCl_2$ was obtained from El-Nasser Company, Alexandria, Egypt. pH-meter 646 with glass electrodes, Ingold, Knick, Germany.The *Portulaca Oleracea* plant used in this study was collected from Umdinar station in Giza Governorate, and then the *Portulaca Oleracea* was powdered using electric grinder. The dry powder (50g/100ml) was macerated with different buffers to determined

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activity of MCE in this plant (**Cheded, 1975).** Fresh cow's and buffalo's milks were obtained from El-Serw Station, Agriculture Research Center, having the average of cow's and buffalo's milks were as follows: T.S 12.41%, fat 4.10 %, protein 3.32 % and ash 0.63 %. Microbial rennet Chy-Max from (Chr Hansen., Holding A/S, Boege, 2970 Hoersholm, Danmark). Animal rennet from El-Serw Station. Cheese starter culture of *Str. Thermophiles* and *L.bulgaricus*. Were obtained from National Research Centre, Egypt

Preparation of crude enzyme extracts with different buffers

To obtain the highest activity of the coagulation enzyme from the *Portulaca Oleracea***,** used the buffers were prepared in pH measurements according to **Gomori (1955)**. Moreover. Three buffers (0.01M acetate, phosphate sodium hydroxide and citrate buffer pH 5 and dist. water pH 6.8).

Extraction of crude enzyme in phosphate sodium hydroxide buffer.

Fifty grams powder from Portulaca Oleracea were soaked in a flask (100 ml) for 24 hr at 5 ºC using 0.1 M phosphate sodium hydroxide buffer pH 5 with shaking for the first 3 hr and solutions were then centrifuged at 8000/g at 4 ºC for 15 min, The aqueous filtrate was used for testing clotting activity was carried out following the method by **Gautama et al (2010)** and **(Abdalla et al., 2011).**

Proteolytic activity (PA)

The PA of MCE was determined by according to **Chopra and Mathur (1983).**

Milk clotting activity (MCA)

The determined of MCA was according to **Arima and Iwasaki (1970)** the substrate (10 % skim milk in 0.01 M $CaCl_2$) was prepared and the pH was adjusted to 6.5. The substrate (2.0 ml) was pre incubated for 5 min at 37 °C, and add 0.2 ml of

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MCE, and the curd formation was observed at 37 °C while manually rotating the test tube from time to time, and The end point was recorded when discrete particles were discernible. One unit milk-clotting is defined as the amount of enzyme that clots 10 ml from substrate within 40 min. Enzyme activity is calculated from the following equation:

$$MCA = \frac{2400}{t_c} \times \frac{V_s}{V_c}$$

Where **MCA** is the milk-clotting units (U/ml), **Vs** is the volume of milk (ml), **Vc** is the volume of clotting enzyme added into the milk (m1), and $\mathbf{t_c}$ is the time span to coagulation (sec)

Protein content was determined calorimetrically at 595 nm using, according to **Bradford (1976).** The proteolytic activity of MCE was determined by according to **Chopra and Mathur (1983).** Specific activity calculation is calculated by divide the determined MCA to the protein content.

Purification of crude enzyme

After obtaining the raw enzyme, the molecular purification stages are as follows: Three steps for achievement of crude enzyme extract purification:

Step 1: Precipitation by ammonium sulfate

Crude enzyme extract was precipitated by different concentrations of ammonium sulfate from 10 to 100 % saturation, according to **Colowick and Kaplan (1955).** Suitable quantity of solid ammonium sulfate was added to the supernatant and then cooling centrifuged at 8000 g for ١٥ min. The precipitate was collected with minimum quantity of 0.1 M phosphate sodium hydroxide at pH 5.

Step 2: dialysis bag

The supernatant fraction was dialyzed in the same buffer and pH using dialysis bag and kept in the refrigerator for 48 hr.

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Step 3: Gel filtration by using sephadex G-100

After the step of dialysis bag the enzyme extracts was purified by gel filtration method using Sephadex G-100 column successively (2.5 x 40 cm) and eluted with same buffer at a flow rate of 0.7 ml min-1. Five ml fractions were collected and assayed for enzyme activity and protein (mg/ml) at 280 nm reported by **Dioxn and Webb (1968).**

Characterization of purified MCE

Optimum pH

The optimum pH of the pure enzyme was determined by replacing different buffers from 0.1 M citrate buffer pH 5 in the clotting assay with the following buffer: 0.1 M citrate buffer (pH 4- 6), 0.1 M phosphate buffer (pH 6-7), 0.1 M Tris-HCl buffer (pH 8-9) and Glysine NaoH buffer (pH 10) according to **Gomori (1955)**. The reaction was carried out using the milk clotting assay procedure.

Optimum temperature and Thermal stability

The optimum temperature of the pure enzyme was determined by incubating the reaction mixture of the clotting assay at different temperatures ranging from 20 to 100 °C for 10 min. The enzyme activity was then assayed at each temperature to define the milk clotting enzyme optimal temperature, In addition to enzyme extract were heat treated for 15, 30, 45 and 60 min in water baths set at different temperatures of 30 to 100°C followed by rapid cooling to 37°C and analyzed immediately for residual enzyme activity **(ahmed et al., 2010)**

Effect of $CaCl_2$ and NaCl concentrations

The effect of the presence of various concentrations of $CaCl_2$ and NaCl which ranged from (0 -50 mM) and (0-10%) respectively, on purified MCE was studied..

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Effect of some salts and chelating agents

The presence of 1 and 5 mM of $MnSO_4.H_2O$, $BaCl_2.2H_2O$, EDTA, $MgCl_2.6H_2O$, $FeCl_2.6H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $MgSO_4.7H_2O$ and $NiSO_4.xH_2O$ effect on enzyme activity was studied..

Production of soft cheese

Soft cheese was manufactured out as illustrated by **Abdel-Salam (2010)** with some modifications from the prepared milk. Cheese milk was heated 63±2 °C for 30 minutes, and cooled to 38 °C, then divided into four portions: the first portion was made from raw milk using clotting plant extract as a control. Other three portion make from pasteurized milk using three coagulation enzymes as follows: animal, microbial and plant (T1, T2 and T3) were applied respectively, the other steps manufacturing of soft cheese were followed. The cheese samples were stored in saline solution a refrigerator and stored carried out for 90 days at 4 °C and cheese was analyzed when fresh, 30, 60 and 90 days of storage period for sensory properties, rheological, chemical and microbiological.

Physicochemical properties

According to **AOAC methods (2012)** it was determined that the treatable acidity (TA), moisture, total nitrogen (TN), soluble nitrogen (SN) and ash contents. The measured of pH values were using a digital laboratory pH meter (Jenway 3510, UK). The fat was determined by Gerber tubes (Gerber Instruments AG, Effretikon, Switzerland). And total volatile fatty acid (TVFA) was determined by method to **Kosikowski (1982),** and the value was expressed as ml of 0.1N NaOH/100g cheese. All tests were conducted in triplicate conducted.

Textural profile analysis:

TPA was performed on cheese samples according to **Glibowski et al. (2008)**

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Microbiological analysis of white soft cheese samples

For microbiological analysis of soft cheese, ten grams of each sample were homogenized in trisodium citrate (3 % w/v) as the first dilution, and then the following serial dilutions were done in sterile saline (0.85 % NaCl w/v). The pouring plate technique was used for enumerating microbes in the samples. The counts of *L.bulgaricus*, *Str. Thermophiles* and total counts were determined using MRS agar medium and the plates were incubated anaerobically at 37 ºC for 72 h. The counts of mold and yeast were detected on acidified potato dextrose agar (pH 3.5) and the plates were incubated aerobically at 25 ^{o}C for 4 days **(APHA, 1994).** Finally, coliform counts were determined using violet red bile agar (VRBA) medium according to the method described by **Mabrouk et al. (2021)**. The plates were incubated anaerobically at 37 ^{o}C for 24 h.

Sensory evaluation:

Organoleptically scored according to **EN ISO 13299, (2016)** method to Cheese samples stored at (0, 30, 60 and 90) days in refrigerated for characteristic of appearance, body and flavor by score (10, 40 and 50 points) respectively. Three replications for each treatment were done and judged cheese samples ordinary consumers and the specialists from members of staff the dairy science, national research center.

Statistical analysis:

Statistical analysis was performed using General Linear Model (GLM) according to **SAS Institute (1990),** Duncan's multiple ranges.

Results and Discussion

Activity of MCE from plant extracted under different buffering conditions

For the extraction of the MCE, *Portulaca Oleracea* plant in dist. water and three extractions buffers were tried to select the

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type of buffer solution that achieves maximum enzyme activity. The results of the effect of extraction methods on the MCE activity are presented in Table 1. Extraction of *Portulaca Oleracea* plant with 0.1 M phosphate sodium hydroxide pH 5 gave the highest MCE activity (233.17 U/ml), Specific activity (10173.82) and Ratio specific MCE/Specific PA (141.33), compared to the dist. water and other buffers. The dist. water, acetate and Citrate buffers gave the highest activity were 101.81, 118.18 and 148.23 U/ml, while Ratio specific MCE/Specific PA 97.90, 103.66 and 125.61 to MCE, respectively. The results obtained agree with **kholif et al., (2016)** and **Gutierrez et al. (2012)** who found that the phosphate buffer solution gave the highest extraction of enzymes from plant sources. These results are in disagreement with **Yousif et al. (1996), Ahmed et al., (2009)** and **Guiama et al., (2010)** who found that extraction with 5 % NaCl in 50 mM acetate buffer (pH 5.0) yielded higher MCA than other buffers.

Table 1. Activity of plant extracted enzyme under different buffering conditions

Type of buffers (0.1 M)	pH	MCE (U/ml)	PA (U/ml)	PC (mg/ml)	Specific MCE	Specific PA	Ratio specific MCE/ Specific PA
distilled Water	6.8	101.81	1.04	0.021	4848.09	49.52	97.90
Acetate	5.0	118.18	1.14	0..019	6220.00	60.00	103.66
Phosphate	5.0	233.17	1.65	0..023	10137.82	71.73	141.33
Citrate	5.0	148.23	1.18	0..020	7411.50	59.00	125.61

PC. Protein content. PA. Proteolytic activity. MCE. milk clotting enzyme.

Purification steps of MCE

Ammonium sulphate

In this study after the centrifugation process under cooling to the crude enzyme, the precipitation process with ammonium

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sulfate comes as the first step in purifying the enzyme, where it is a preliminary ammonium sulphate (AS) saturation fractionation on MCA from *Portulaca Oleracea* plant. Where the crude enzyme extract of 50 ml in the same buffer and pH the above mentioned was using different AS concentrations (10–100 % saturation). The results showed that in (Table 2) 40 % saturation from AS gave the highest MCA, Sp, MCA, yield (%) and rate purification were 394.16, 11944.24, 33.80 and 1.18 respectively, comparison the other concentrations from AS. Although. The results obtained indicated that the degree of saturation of AS greatly affected MCA, specific activity, total activity, Sp. MCA, yield (%) and rate purification. Accordingly, the range of 40 % was selected for potential purification of the MCE from this plant. These results are in conformity with the findings of **Isam et al. (2009)** and **kholif et al. (2016)** who found the highest MCA, and specific activity with saturation range of 35–55%; **Ahmed et al. (2009)** who reported the highest total activity, yield and purification fold with saturation range of 40-55%; **Duarte et al. (2009)** who found the best MCA at saturation of 40-60 %; **Ahmed et al. (2010)** who found the highest MCA, specific activity, total activity, yield (%) and degree of enzyme purification at saturation of 40-50 %. However, **Chaiwut et al. (2007), Demir et al, (2008).**

Dialysis bag

As an alternative to rennet, the proteolytic capacity must be less than the coagulating capacity of the enzyme so that defects such as off-flavor and bitter taste do not occur in the cheese. Therefore, it was necessary to choose the appropriate concentration of ammonium sulfate, which gives the highest coagulating capacity and the lowest proteolytic capacity. In this step, after the enzyme precipitated by AS at 40 % saturation is the best for MCE. The enzyme is stored in the dialysis bag in the

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same buffer and pH at 4 ^{o}C for 24 hr to remove the remaining salts and then determine the activity of MCE .Enzyme activity was determined after step of dialysis bag, where activity gave (373.17 U/ml), yielded 16.04 % and 1.26 rate purification. This results agreement with **kholif et al (2016).**

Sephadex G-100

The third stage of the partially purified of MCE is using chromatographed in a column of Sephadex G-100. Where it showed the purification only one peak with MCE (at fraction 30) as shown in Table 2 and Fig. 1 which was obtained when the dialyzed pooled fraction was loaded on to Sephadex G-100 column equilibrated with 0.1 M phosphate sodium hydroxide buffer pH 5. Purification results of the enzyme from *Portulaca Oleracea* plant using different purification means resulted in 3.77 rate purification with a yield of 6.59 % and specific activity of 38442.5. A simple purification procedure was developed in this study to obtain a very active and stable enzyme from *Portulaca Oleracea* plant. Results are in agreement with **Calvo and Fontecha (2004)** who showed only one peak from extract of hygienized kid rennet past and Albizia julibrissin, respectively while **Egito et al. (2007)** reported several proteolytic bands in Albizia seed extract and one diffuse proteolytic bands from sunflower seed extract. However, the findings of **Abdalla et al. (2010),** who concluded that two peaks with PA, were eluted from purification of Jacaratia.

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Table 2. Purification step of MCE from *Portulaca Oleracea* plant.

Purification steps	Volume (ml)	MCE (U/ml)	PC (mg/ml)	Sp. MCE	TA	TP	Yield (%)	Rate purification
Crude extract	50	233.17	0.023	10137.82	11658.50	1.15	100.0	1.00
Ammonium sulfate 40 %	10	394.16	0.033	11944.24	3941.60	0.33	٣٣.٨٠	١.١٨
Dialysis bage	5	٣٧٣.١٧	0.0٢٩	١٢٨٦٧.٩٣٣	١٨٦٥.٨٥	0.١٤٥	١٦.٠٤	١.٢٦
Sephadex G -100	5	١٥٣.٧٧	0.0٠٤	٣٨٤٤٢.٥	٧٦٨.٨٥	٠.٠٢.	٦.٥٩	٣.٧٧

MCE=Milk clotting enzyme, PC=Protein content, TA=Total activity, TP=Total protein Sp. =Specific activity.

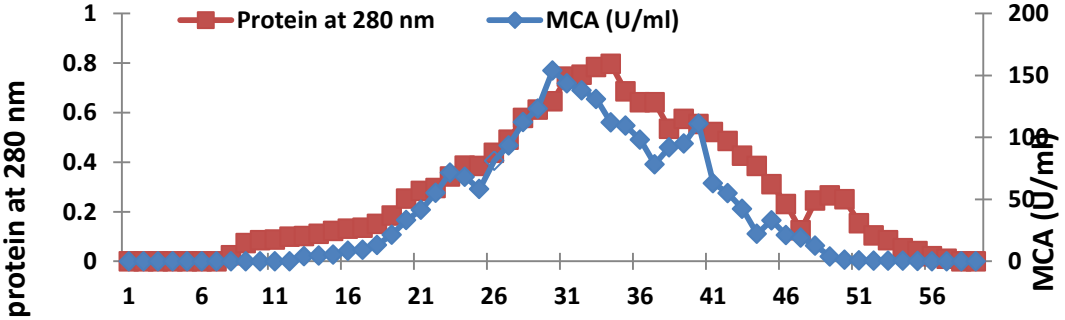

Fig. 1. Gel filtration for the chromatography of MCE from *Portulaca Oleracea* on a Sephadex G-100 column (40 x 2.5 cm) the column was equilibrated with 0.1 M acetate buffer, pH 5.0 at a flow rate of 0.7 ml min-1 and 5 ml fractions

Characterization of purified MCE

Optimum pH

pH always is one of the most important properties in enzyme production, as it directly affects a significantly on enzyme activity. The results indicated that the MCE retains its activity in the pH range from (4 -7) where activity ranges between (53.3 to 62.0 % U/ml) and highest MCA was observed at pH 6 was (100 % U/ ml) (Fig. 2). Similar behavior of optimum pH was reported for MCE from *Bacillus subtilis*

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MTCC 10422; and *Solanum elaeagnifolium* fruit seeds the enzyme optimal activity at pH 6.0 and 5.9 **Kumari Narwal et al., (2016)** and **kholif et al., (2016),** respectively, optimum pH of purified MCE from *Streptomyces pseudogrisiolus* NRC 15 was determined as 6.5 **(El-Sayed 2013)**. Highest MCA of religiosin B was observed at pH 6.0 **(Kumari et al., 2012).**

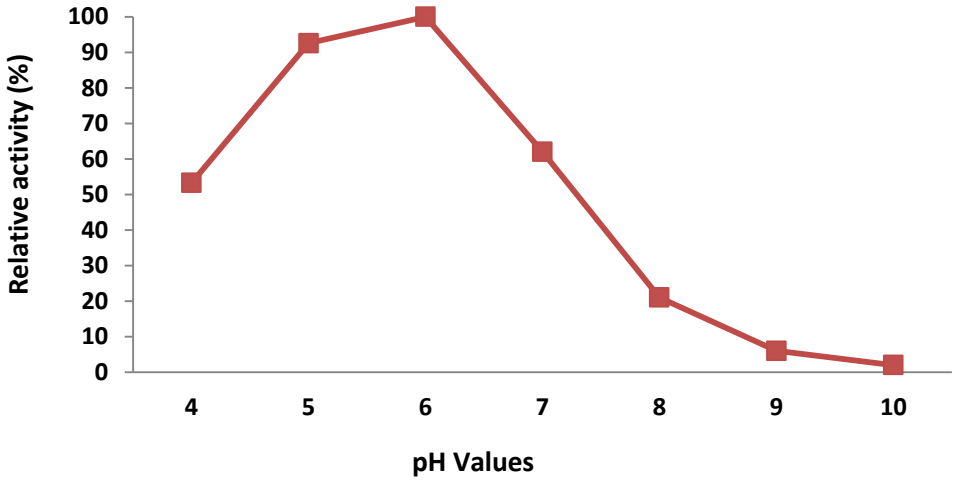

Fig. 2. Effect of pH values on purified MCE from *Portulaca Oleracea* plant

Optimum temperature

Temperature is an important property for determining enzyme function, as it greatly affects enzyme activity. Showed from the results in Fig. 3 the effect of different temperatures at rang from (20 -100 °C) on purified MCA from *Portulaca Olerace*. It was found that the enzyme maintains its activity in a wide temperature range from 20-70 °C, where the activity at 20 °C was 65.54% and at 70°C was 50 %, and the MCE activity increased as the temperature increased from 20 to 40 °C, Where it gave at 40 °C activity 100 %, then the enzyme activity began to gradually decrease until 70 °C, followed by the activity

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rapidly decreased where the enzyme activity decreased sharply until 80 °C , this is due to denaturation of the protein by thermal. The optimal temperature for the MCE activity was at 40 °C. These results to MCA are in agreement with **Kumari Narwal et al. (2016)** and **kholif et al (2016)**; the activity of purified enzyme from *B. subtilis* MTCC 10422 starts and *Solanum elaeagnifolium* fruit seeds increasing with the increase in temperature and gets maxima at 45 and 40 ^{o}C ,respectively. **(El-Sayed, 2013),** the MCE demonstrated activity at 45^{o}C, **(Wang ,2009)**; the highest MCA of our purified MCE was at 36°C and this result differs from the MCE produced by *Nocardiopsis* sp., which showed the maxim activity at 55 ^{o}C. **Cavalcanti *et al.*, (2004)** mentioned that, the maximum MCA was recorded at 65°C for *Rhizomucor miehei* (**Walsh and Li, 2000)** *Bacillus sphaericus* (**El-Bendary *et al.*, 2007**).

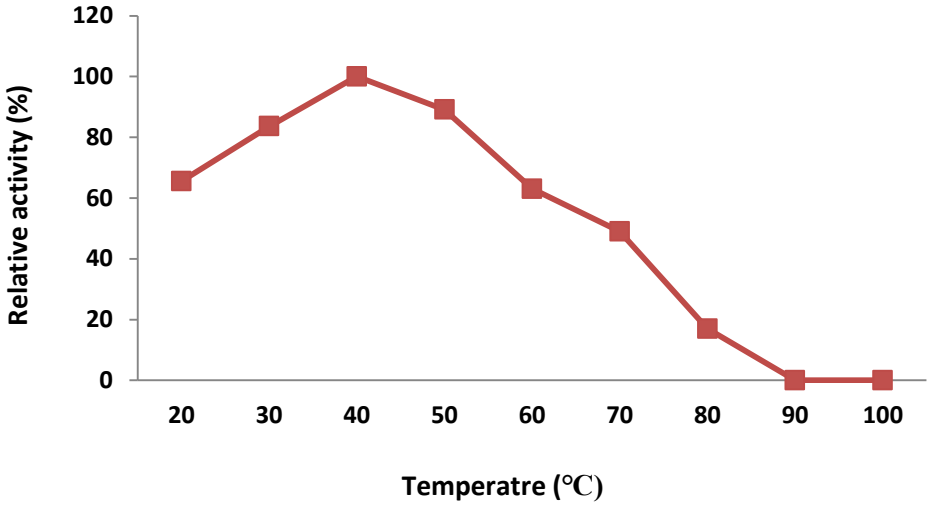

Fig. 3. Effect of temperatures on purified MCE from *Portulaca Oleracea* plant

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Effect of $CaCl_2$ concentrations

Addition of calcium chloride to milk prior to curdling was found to favor not only the rate of reaction but also the extraction of clear whey, so Calcium chloride is an important factor in cheese making. Appropriate concentrations of $CaCl_2$ may be added. To study this effect, $CaCl_2$ was added in different concentrations from 1 to 50mM. As shown in Fig. 4. It was found that the enzyme activity increases with increasing $CaCl_2$ concentration even 25 mM from $CaCl_2$, Where the enzyme gave activity (164 %), then the enzyme activity decreases gradually with increasing the concentration of $CaCl_2$ up to 30mM to give activity (96 %) and then a sharp decline occurred in the activity of the enzyme up to 50 mM to give activity (51 %). These results show similar behavior with **Abdalla et al. (2010).** The clotting activity increased with increasing calcium chloride concentration, while coagulation time decreased, calcium not only creates iso-electric conditions but also creates ion bridges between phosphate moieties of casein micelles **(Sun et al., 2014).**According to **El-Sayed (2013).**

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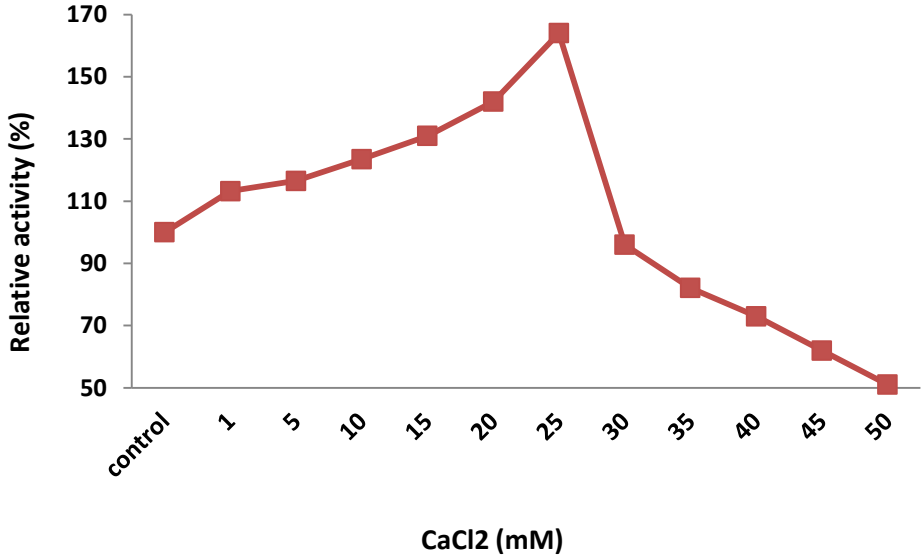

Fig. 4. Effect of Ca Cl_2 concentrations on the purified MCE from *Portulaca Oleracea* plant

Effect of NaCl concentrations

Sodium chloride is one of the most important additives usually used in cheese making, and is usually used in Egypt during the process of cheese manufactured because because it gives a distinctive taste to produce as a preservative.it protects milk against spoilage by microorganisms. Different concentrations of range from (0-10 % NaCl) were used in this study on the purified MCA. Results showed a gradual reduction in MCA observed when increasing the NaCl concentration above 2 %. As shown in Fig. 5 The enzyme activity gradually increased with increasing sodium chloride concentration up to 2 % NaCl. The results showed that at a concentration of 1 % NaCl the enzyme gave an activity of 134.86 %, and at a concentration of 2 % NaCl, the enzyme gave an activity of 139.2. Indicated that the

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enzyme activity decreases gradually with increasing the concentration of sodium chloride and then a sharp decline in the activity of the enzyme up to 10% NaCl to give activity 8 % occurred. The findings are in agreement with the results obtained by **Abdalla et al. (2010)** indicated that with increasing sodium chloride concentration, the clotting activity decreased and coagulation time increased. **Wahba et al. (1995**) found that addition of NaCl to milk resulted in a marked decrease in clotting activity; **Shehata et al. (1996)** proposed that the relative MCA of bacterial-coagulation decrease as the concentration of NaCl in milk increased up to 15 %.

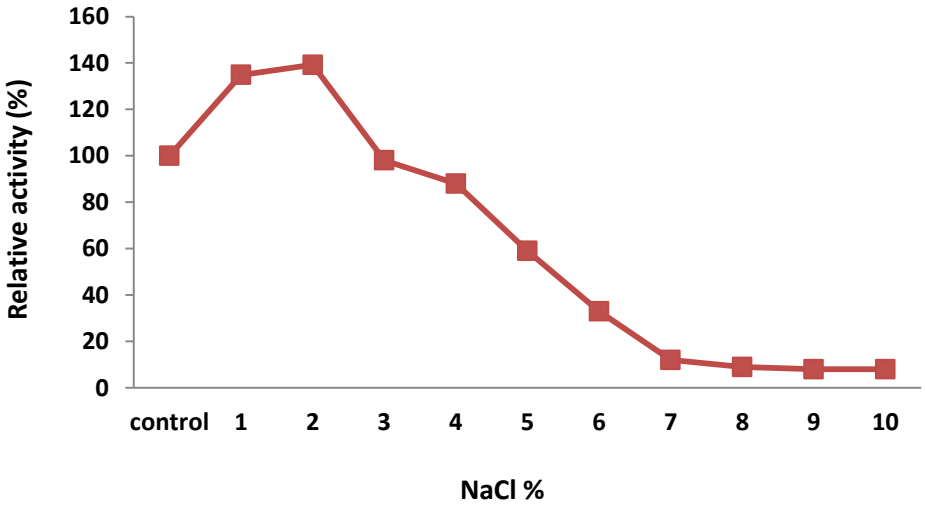

Fig. 5. Effect of NaCl concentrations on the purified MCE from *Portulaca Oleracea* plant

Effect of different metal ions and other materials

There are some ions and minerals that can be activators or inhibitors of enzymes in general. Other divalent cations such as magnesium are also known to can cause coagulation. Hence, the

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effect of monovalent and divalent ions at different concentrations (1 and 5 mM) on the activity of the purified MCE used in this study was investigated (Fig. 6). The results showed the effects of different metal ions on clotting activity always exhibit differently. Some metal ions and other materials were added in concentrations (1and 5 mM), found that at concentration 1mM Ba^{+2} and Fe^{+2} are activators and give activity 135.89 and 110.40. Whereas $Mn^{+2,}$ EDTA, Zn^{+2}, Cu^{+2}, Mg^{+3} and Ni^{+2} are inhibitors of the purified MCA. on the other hand, the Ba^{+2} at 1mM, while Ba^{+2} , Mg^{+2} at 5 mM were the most effective, The Mg^{+3},Ni^{+2} at both 1 and 5mM were the most inhibitors of the purified MCA. This results agreement with **kholif et al., (2016)**, and contrary to our results **El-Bendary et al., (2007)** reported that Fe+2 ion did not show any effect on the enzyme activity.

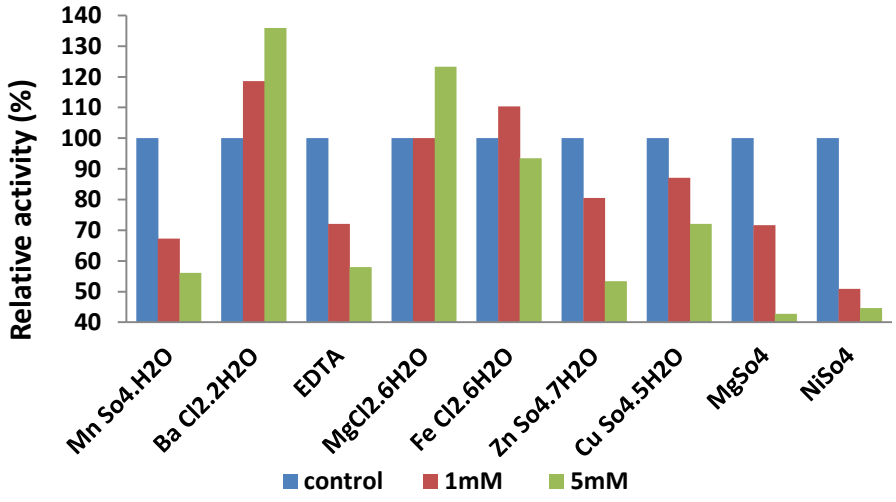

Fig. 6. Effect of different inhibitors and activators on the purified MCE from *Portulaca Oleracea* plant.

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Effect of thermal stability

In order to know the thermal stability of the enzyme, we must study the effect of different temperatures and at different times on the enzyme activity. Where the enzyme was incubated for 15-60 mins and the temperatures from 30-100 °C. The enzyme activity is stable at temperature from 30 to 60 °C on the all incubation times and there were no significant differences in enzyme activity at all times. Then slightly decreased in activity at all times at 60-70 °C. Then it began a sharp decrease in activity of enzyme at incubation at 70 to 80°C at all times of incubation until the disappearance of enzyme activity completely at 90 °C (Fig. 7). This result was similar to the result of **(Isam et al., 2009),** the thermo stability of the enzyme was found to be up to 70 ºC. **Wang et al. (2009)** the activity of enzyme is stable at 40°C for 60 min, and loss of 20 % of enzyme activity when Incubation at 45 °C for 20 min, while it was fully inactivated upon heating for 20 min at 60 °C.

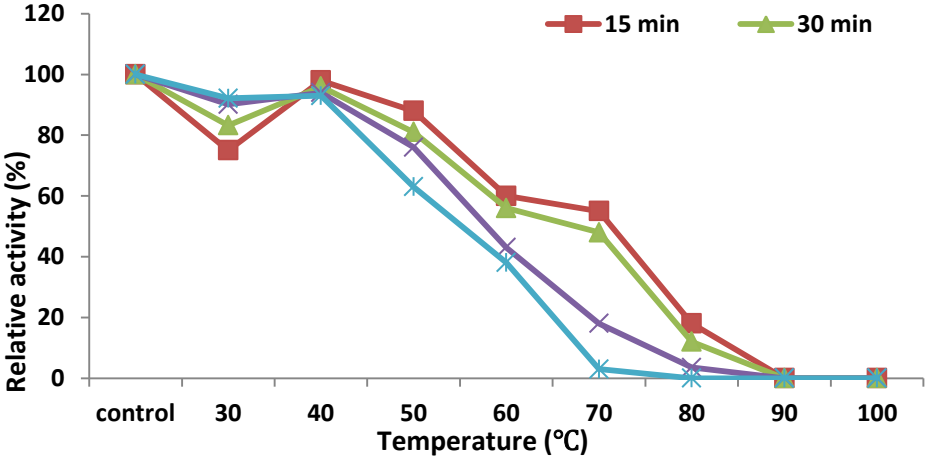

Fig. 7. Effect of thermal stability on the purified MCE from *Portulaca Oleracea* plant

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Chemical of cheese properties

The composition of cheeses samples determined during storage time is presented in Table (3). All samples retained more moisture except soft cheese sample make by microbial rennet (T2) at fresh time. It was also reported that the moisture content of the cheese samples decreased with the increase in storage time until the end storage time. The moisture in all cheese treatments decreased during storage, and the control sample made from raw milk and plant extract had the highest moisture values at the end of the storage period, giving a moisture of 64.06 compared to the other of the samples made from pasteurized milk and different coagulations, giving 63.78, 63.57 and 63.45 for T1, T2 and T3 respectively. In all cases, control treatment making by row milk had lowest pH and on the contrary high acidity values as compared to soft cheese making by pasteruzaid milk during storage and in the end storage period. The pH values gradually decreased under the action of lactic acid starter activity in converting lactase sugars into lactic acid during storage and on the contrary in acidity. TN content of all cheese treatments increased, and the lowest TN content (2.81%) was in the control sample, and the treatment T3 gave the highest TN content (3.01 %) at the end of the storage period. On the other hand, the equivalent TN increased as the ripening period extended. The loss of water and the rise in the cheese's total solids are the causes of the apparent increase. Results in Table (3) mention that, the level of SN in all treatments were high significantly 0.49, 0.52, 0.57 and 0.59 % for T1, T2 and T3 respectively, and control sample were significantly lower 0.49 %, but were increased during ripening period of cheese in all treatments. The TVFA same approach was followed during the storage period. It increased in all treatments with significant differences during storage and its content at the end of the storage period was 17.00,

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16.34, 15.33 and 19.69 for control, T1, T2 and T3 respectively. The ash content represents mainly the salt content of the cheese, as well the minerals from the milk captured into the cheese. Very slight increase of salt concentration for treatments as results of ripening the increase in due to the different in total solid content of the cheese. Fresh and ripened cheese contained (4.09/4.23), (4.08/4.24), (4.02/4.31) and (4.06/4.26) cheese for control, T1, T2 and T3 treatments respectively, these results are similar to what was mentioned **Abou-zeid (2015)** where indicated that the level of soluble nitrogen, non protein nitrogen and TVFA in the control treatment were lower than those of the other treatments.

Table 3. Changes of chemical composition of soft cheese with different coagulations during storage

Parameter	Storage period (Days	Treatments			
		Control	T1	T2	T3
Moisture (%)	Fresh	65.٣٨.$^{a}_{\pm 0.08}$	65..2٤$^{a}_{\pm 0.47}$	65.١٨.$^{a}_{\pm 0.74}$	65.25$^{a}_{\pm 0.62}$
	30	65.1٦$^{ab}_{\pm 0.27}$	64.94$^{b}_{\pm 0.57}$	64.87$^{b}_{\pm 0.24}$	64.39$^{b}_{\pm 0.27}$
	60	64.51$^{b}_{\pm 0.82}$	64.٣٤.$^{c}_{\pm 0.33}$	64.١٣.$^{c}_{\pm 0.19}$	64.02$^{c}_{\pm 0.22}$
	90	64.0٦$^{c}_{\pm 0.56}$	63.78$^{d}_{\pm 0.52}$	63.57$^{d}_{\pm 0.48}$	63.45$^{d}_{\pm 0.56}$
SE		0.29	0.25	0.29	0.30
pH	Fresh	5.85$^{a}_{\pm 0.03}$	5.89$^{a}_{\pm 0.01}$	5.81$^{a}_{\pm 0.01}$	5.75$^{a}_{\pm 0.01}$
	30	5.60$^{b}_{\pm 0.04}$	5.53$^{b}_{\pm 0.04}$	5.59$^{b}_{\pm 0.01}$	5.32$^{b}_{\pm 0.55}$
	60	5.29$^{c}_{\pm 0.04}$	5.34$^{c}_{\pm 0.10}$	5.13$^{c}_{\pm 0.12}$	5.16$^{bc}_{\pm 0.02}$
	90	5.09$^{d}_{\pm 0.10}$	4.75$^{d}_{\pm 0.10}$	4.83$^{d}_{\pm 0.07}$	4.55$^{bc}_{\pm 0.03}$
SE		0.039	0.06	0.035	0.059
Titratable acidity (%)	Fresh	0.88$^{d}_{\pm 0.01}$	0.86$^{d}_{\pm 0.03}$	0.87$^{d}_{\pm 0.01}$	0.83$^{d}_{\pm 0.01}$
	30	1.12$^{c}_{\pm 0.04}$	1.24$^{c}_{\pm 0.09}$	1.34$^{c}_{\pm 0.04}$	1.44$^{c}_{\pm 0.10}$
	60	1.53$^{b}_{\pm 0.15}$	1.72$^{b}_{\pm 0.20}$	1.70$^{b}_{\pm 0.04}$	1.85$^{b}_{\pm 0.20}$
	90	1.82$^{a}_{\pm 0.13}$	1.94$^{a}_{\pm 0.02}$	1.89$^{a}_{\pm 0.10}$	1.97$^{a}_{\pm 0.02}$
SE		0.057	0.039	0.038	0.14
TN (%)	Fresh	١.٩2$^{d}_{\pm 0.07}$	١.٨4$^{d}_{\pm 0.19}$	١.81$^{d}_{\pm 0.10}$	١.٩5$^{d}_{\pm 0.11}$
	30	٢.٣8$^{c}_{\pm 0.08}$	2.٢6$^{c}_{\pm 0.18}$	2.٢4$^{c}_{\pm 0.06}$	٢.52$^{c}_{\pm 0.03}$
	60	٢.٦3$^{b}_{\pm 0.03}$	٢.٧5$^{bc}_{\pm 0.07}$	٢.٨3$^{b}_{\pm 0.04}$	٢.٨9$^{b}_{\pm 0.06}$
	90	٢.٨1$^{a}_{\pm 0.03}$	٢.٩3$^{a}_{\pm 0.11}$	٢.٨2$^{a}_{\pm 0.04}$	٣.٠1$^{a}_{\pm 0.06}$
SE		0.034	0.078	0.048	0.039
SN (%)	Fresh	0.24$^{d}_{\pm 0.02}$	0.٢٨$^{d}_{\pm 0.02}$	0.3٠$^{d}_{\pm 0.05}$	0.٣٣$^{d}_{\pm 0.05}$

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	30	$0.3٦^{c}_{\pm0.02}$	$0.٣7^{c}_{\pm0.08}$	$0.4٣^{c}_{\pm0.05}$	$0.٤٧^{c}_{\pm0.01}$
	60	$0.4٤^{b}_{\pm0.01}$	$0.٤8^{b}_{\pm0.05}$	$0.٥١^{b}_{\pm0.06}$	$0.٥7^{b}_{\pm0.06}$
	90	$0.٤٩^{a}_{\pm0.04}$	$0.٥٢^{a}_{\pm0.06}$	$0.٥7^{a}_{\pm0.07}$	$٠.٥٩^{a}_{\pm0.05}$
SE		0.013	0.033	0.033	0.027
TVFA (0.1 ml NaOH/ 100g)	Fresh	$٩.00^{d}_{\pm2.0}$	$1٠.66^{d}_{\pm1.5}$	$٩.11^{d}_{\pm3.1}$	$١٠.45^{d}_{\pm3.1}$
	30	$1١.33^{c}_{\pm2.5}$	$١٢.33^{c}_{\pm2.5}$	$١١.00^{c}_{\pm2.7}$	$١٣.02^{c}_{\pm1.6}$
	60	$١٤.34^{b}_{\pm2.5}$	$١٥.00^{b}_{\pm2.0}$	$١٣.66^{b}_{\pm8.3}$	$١٦.10^{b}_{\pm2.2}$
	90	$١٧.00^{a}_{\pm2.0}$	$١٦.34^{a}_{\pm6.4}$	$١٥.33^{a}_{\pm5.6}$	$١٩.69^{a}_{\pm2.9}$
SE		1.49	2.04	2.87	3.44
Ash (%)	Fresh	$4.٠9^{d}_{\pm0.02}$	$4.٠8^{c}_{\pm0.02}$	$4.٠2^{c}_{\pm0.02}$	$4.٠6^{d}_{\pm0.03}$
	30	$4.١6^{c}_{\pm0.03}$	$4.١9^{b}_{\pm0.02}$	$4.٠9^{b}_{\pm0.04}$	$4.١٥^{c}_{\pm0.04}$
	60	$4.٢2^{b}_{\pm0.03}$	$4.٢6^{b}_{\pm0.02}$	$4.٢٣^{b}_{\pm0.03}$	$4.٢٤^{b}_{\pm0.03}$
	90	$4.٢3^{a}_{\pm0.03}$	$4.٢4^{a}_{\pm0.04}$	$4..٣١^{a}_{\pm0.04}$	$4.٢٦^{a}_{\pm0.07}$
SE		0.016	0.017	0.026	0.025

Control, soft cheese making from raw milk and plant extraction. T1, T2 and T3 soft cheese making from pasteurized milk and animal rennet, microbial rennet, and plant extract, respectively SE, standard error

a, b, c..: The same small letter in in each column (cheese period) mean no significant difference ($P \leq 0.05$).

Textural profile analysis

In Table 4 shows the calculated rheological parameters of soft cheese treatments which manufactured from pasteruzaid milk by different coagulations compared to those of control cheese manufactured by raw milk and extraction plant when fresh and after 90 days old soft cheese Hardness (N), Cohesiveness (area B/A), Springiness (mm), Gumminess (N) and Chewiness (N.mm) took the same trend between all treatments that were increased by increasing the storage period. Fresh soft cheese treatment T3 with plant extracted had lower hardness, springiness, gumminess, and chewiness and cohesiveness than T1, T2 and control respectively. This was correlated with the moisture content, and correlated with type coagulation enzyme. In general, during the first days of storage, values for the parameters tended to increase gradually at rates that were

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influenced by moisture loss. These results agree with that reviewed by **Abou-zeid (2015), Katsiari et al (2002), Fox et al (2000)** and **Bryant et al (1995)**, and are partially confirmed by those results of **Youssef et al. (2019)** , **Awad et al. (2003**) and **(Kim et al., 2017).** Also **(El-Shibiny et al., 2018)** found that the low moisture content during ripening lade to increase a consequence hardness significantly.

Table 4: Textural profile analysis of soft cheese with different coagulation during storage

Textural properties	Ripening Period (day	Treatments			
		Control	T1	T2	T3
Hardness N	**fresh**	$3.9^{b}_{\pm 0.021}$	$2.8^{b}_{\pm 0.012}$	$1.8^{b}_{\pm 0.04}$	$2.6^{b}_{\pm 0.01}$
	90	$23.9^{a}_{\pm 0.03}$	$9.30^{a}_{\pm 0.02}$	$17.5^{a}_{\pm 0.07}$	$6.60^{a}_{\pm 0.03}$
Cohesiveness (B/A area)	**fresh**	$0.637^{a}_{\pm 0.08}$	$0.579^{a}_{\pm 0.05}$	$0.634^{a}_{\pm 0.02}$	$0.401^{a}_{\pm 0.07}$
	90	$0.105^{b}_{\pm 0.01}$	$0.272^{b}_{\pm 0.02}$	$0.505^{b}_{\pm 0.06}$	$0.343^{b}_{\pm 0.08}$
Springiness Mm	**fresh**	$0.641^{a}_{\pm 0.02}$	$0.642^{a}_{\pm 0.07}$	$0.797^{a}_{\pm 0.07}$	$0.593^{a}_{\pm 0.09}$
	90	$0.546^{b}_{\pm 0.05}$	$0.326^{b}_{\pm 0.04}$	$0.532^{b}_{\pm 0.02}$	$0.583^{b}_{\pm 0.02}$
Gumminess N	**fresh**	$2.484^{b}_{\pm 0.04}$	$1.621^{b}_{\pm 0.04}$	$1.507^{b}_{\pm 0.05}$	$0.507^{b}_{\pm 0.02}$
	90	$2.509^{a}_{\pm 0.02}$	$2.529^{a}_{\pm 0.02}$	$8.378^{a}_{\pm 0.02}$	$2.246^{a}_{\pm 0.06}$
Chewiness N/mm	**fresh**	$1.592^{b}_{\pm 0.02}$	$1.041^{b}_{\pm 0.09}$	$1.404^{b}_{\pm 0.07}$	$0.441^{b}_{\pm 0.02}$
	90	$1.369^{a}_{\pm 0.01}$	$1.824^{a}_{\pm 0.02}$	$4.720^{a}_{\pm 0.02}$	$1.319^{a}_{\pm 0.04}$

Control, soft cheese making from raw milk and plant extraction. T1, T2 and T3 soft cheese making from pasteurized milk and animal rennet, microbial rennet, and plant extract, respectively.

a, b, c..: The same small letter in in each column (cheese period) mean no significant difference ($P \leq 0.05$).

Microbiological analysis

The results of microbial (log cfu/ml) counts (total count, *L.bulgaricus, Str. Thermophiles*, total coliform group, yeasts and molds and Spore forming counts) of soft cheese treated manufactured by different coagulations during storage at 4 ℃ for 90 days are presented in Table 5. The data shows the mean values of some microbiological properties of soft cheese treated

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with different coagulation. The results indicated that in fresh storage, the total counts were 5.28, 4.22, 4.18, and 4.09 log cfu/g and then decreased for all treatments at the end of the storage time: 5.48, 4.18, 3.26 and 3.44 log cfu/g for control, T1, T2, and T3, respectively, as a result of the soft cheeses ageing and salting. **(El-Rebody et al., 2014)** found that the total counts decrease in the cheese samples that have been matured for 120 days ($2.05 \times 10^5 \pm 1.0^3 \times 10^5$). According to **(El Neenay et al., 2013),** LAB was found to be 8×107 cfu/g in fresh of enzyme modified cheese slurry. In other enzyme modified cheese slurry, LAB count ranged from 9.6×10^5 cfu/g to 2×10^8 cfu/g. On the other hand, the total number of *L.bulgaricus* and *Str. Thermophiles* increased at the fresh time and during storage until 30 days, then the number began to decrease until 90 days, as shown in Table 5. while in all treatments of soft cheese manufactured by different coagulation, molds and yeasts were not detected in the fresh product or after 30 days of storage at 4 ℃, whereas they appeared after 60 days in treatment control, T1 and T2 and increased up to 90 days of storage at 4 ℃, While molds and yeasts appeared in the T3 treatment only after 90 days. In all treatments. Additionally, their counts at the end of the storage period were 3.68, 1.36, 1.43 and 1.03 log cfu/g for control, T1, T2 and T3, respectively. By **(El Neenay et al., 2013),** who indicated that by examination of enzyme modified cheese for yeasts and molds, data revealed that the counts were decreased after 2 weeks of the storage period for all treatments.

While coliform bacteria were not found in all samples of soft cheese manufactured by different coagulation during storage time at 4 ℃, this is due to the hygienic conditions set during the manufacturing process and storage, according to **(Saad et al., 2022).**

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Table 5: Microbiological Properties of soft cheese with different coagulation during storage.

Storage days	control	T1	T2	T3
L.bulgaricus				
Zero	$5.45^{b}_{\pm0.52}$	$5.21^{a}_{\pm0.37}$	$5.83^{a}_{\pm0.17}$	$5.45^{a}_{\pm0.42}$
30	$6.74^{a}_{\pm0.06}$	$5.63^{a}_{\pm0.11}$	$5.94^{b}_{\pm0.36}$	$5.75^{b}_{\pm0.40}$
60	$6.35^{b}_{\pm0.27}$	$5.22^{b}_{\pm0.34}$	$5.30^{c}_{\pm0.10}$	$4.88^{bc}_{\pm0.17}$
90	$5.04^{c}_{\pm0.26}$	$4.83^{c}_{\pm0.39}$	$4.80^{d}_{\pm0.17}$	$4.77^{c}_{\pm0.20}$
SE	0.17	0.17	0.11	0.18
Str. Thermophiles				
Zero	$6.29^{b}_{\pm0.19}$	$6.05^{b}_{\pm0.17}$	$5.84^{b}_{\pm0.13}$	$6.17^{a}_{\pm0.10}$
30	$7.07^{a}_{\pm0.29}$	$6.40^{a}_{\pm0.20}$	$6.38^{a}_{\pm0.21}$	$6.47^{a}_{\pm0.19}$
60	$6.55^{bc}_{\pm0.13}$	$5.84^{b}_{\pm0.15}$	$5.72^{c}_{\pm0.44}$	$5.88^{b}_{\pm0.24}$
Zero	$5.17^{d}_{\pm0.19}$	$4.74^{d}_{\pm0.21}$	$4.42^{e}_{\pm0.10}$	$4.32^{d}_{\pm0.26}$
SE	0.13	0.11	0.13	0.14
Total Bacterial Counts				
Zero	$5.28^{b}_{\pm0.25}$	$4.22^{ab}_{\pm0.14}$	$4.18^{b}_{\pm0.14}$	$4.09^{ab}_{\pm0.24}$
30	$5.71^{a}_{\pm0.13}$	$4.37^{a}_{\pm0.44}$	$4.50^{a}_{\pm0.33}$	$4.42^{a}_{\pm0.42}$
60	$5.93^{a}_{\pm0.07}$	$4.64^{a}_{\pm0.50}$	$4.41^{ab}_{\pm0.29}$	$4.37^{ab}_{\pm0.23}$
90	$5.48^{a}_{\pm0.43}$	$4.18^{ab}_{\pm0.12}$	$3.26^{c}_{\pm0.50}$	$3.44^{b}_{\pm0.25}$
SE	0.16	0.19	0.17	0.20
Mold and yeasts counts				
Zero	$0.00^{c}_{\pm0.00}$	$0.00^{c}_{\pm0.00}$	$0.00^{c}_{\pm0.00}$	$0.00^{b}_{\pm0.00}$
30	$0.00^{c}_{\pm0.52}$	$0.00^{c}_{\pm0.41}$	$0.00^{c}_{\pm0.00}$	$0.00^{b}_{\pm0.00}$
60	$1.88^{b}_{\pm0.52}$	$0.63^{b}_{\pm0.41}$	$0.75^{b}_{\pm0.25}$	$0.00^{b}_{\pm0.40}$
90	$3.68^{a}_{\pm0.22}$	$1.36^{a}_{\pm0.30}$	$1.43^{a}_{\pm0.37}$	$1.03^{a}_{\pm0.33}$
SE	0.21	0.22	0.14	0.13
Spore forming counts				
Zero	$0.00^{d}_{\pm0.00}$	$0.00^{d}_{\pm0.00}$	$0.00^{c}_{\pm0.00}$	$0.00^{b}_{\pm0.00}$
30	$0.00^{d}_{\pm0.00}$	$0.00^{d}_{\pm0.00}$	$0.00^{c}_{\pm0.00}$	$0.00^{b}_{\pm0.00}$
60	$1.54^{c}_{\pm0.12}$	$1.03^{c}_{\pm0.05}$	$0.00^{c}_{\pm0.00}$	$0.00^{b}_{\pm0.00}$
90	$2.43^{b}_{\pm0.35}$	$1.50^{b}_{\pm0.42}$	$1.01^{b}_{\pm0.18}$	$0.06^{a}_{\pm0.11}$
SE	0.26	0.24	0.22	0.17
Coliform counts				
Zero	ND	ND	ND	ND
30	ND	ND	ND	ND
60	ND	ND	ND	ND
90	ND	ND	ND	ND

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Control, soft cheese making from raw milk and plant extraction. T1, T2 and T3 soft cheese making from pasteurized milk and animal rennet, microbial rennet, and plant extract, respectively. ND= not detected . SE, standard error

a, b, c..: The same small letter in in each column (cheese period) mean no significant difference ($P \leq 0.05$).

Organoleptic properties of soft cheese

The sensory attributes of soft cheese are the most important criteria that determine demand consumer and increase the acceptability demand on this commodity. The current section of the study deals with the evaluation of these sensory attributes using (15 persons) 10 persons from dairy science department. National research centre, and 5 person's ordinary consumers as panelists representing Product judging panel. The jury was trained on cheese transactions and informed of the characteristics required for judging.

Appearance the first parameter that the consumer experience when tasted the products and the final score is 10 points in figure (٨-A). Indicates that during the whole storage period (90 days) in saline solution at 4 C all treatments of soft cheese got the acceptable score significantly ($p \leq 0.05$). The corresponding scores at fresh time ranged between 6.49 for control manufactured from raw milk and extracted plant to 7.06 for T3 manufactured from pasteruzaied milk and extracted plantat. while The corresponding scores at the end of the storage period were from 7.82 for T1 to 8.72 for T3 and little change in the color was observed, which was still in the acceptable score range. The change in color may be due to a loss a little moisture. However, after 90 days, T3 sample with got the high score significantly ($p \leq 0.05$) comparison to other samples.

In this parameter Body &Texture the whole score is 40 points. The Body &Texture of soft cheese is very important

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parameter that makes the consumer prefer it and because it and determines the structural composition of the cheese. Table figure (٨-B) indicate that these parameters that the texture of the all samples was acceptable significantly ($p \leq 0.05$) at the fresh and the end of the ripening period .

However, we observed a significant differences ($p \leq 0.05$) in texture among the treatments, where the T3 most acceptable (37.42), followed by samples T2 got score (35.09) followed by T1 and control sample at their end storage time. The reason may be due to using the different of coagulations in the samples with a loss of a little moisture, which leads to an increase in the smoothness of texture.

In this parameter the flavor, which has the largest share of consumer acceptance of soft cheese, the whole score is 50 points in fig (٨-C). Indicates that soft cheese samples with all treatments at the zero were acceptable significantly ($p \leq 0.05$) comparison control treatment (39.34) and T3 got high score (41.67) at fresh time comparison with other treatments. On the other hand, preference increases in flavor for all cheese samples during storage time until the end of storage. At the end of the storage period, the samples were evaluated as: 42.47, 46.47, 43.81 and 45.47 for control, T1, T2 and T3 respectively.

Over all, putting the three evaluations (appearance, texture and flavor) all together in fig (٨-D) We end up with a final conclusion which indicates that all samples at zero time got preferred score significantly ($p \leq 0.05$). On the other hand, increase in all attributes during storage time, and after storage for 90 days, the T3 sample maintained the preferred status (91.61) followed by the scores 84.99, 89.83 and 86.72 for control, T1 and T2 samples, respectively. There were significant differences ($p \leq 0.05$) between all samples during the storage time. These

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results were in agreement with **Maarse, (1991), Pino et al., (2007) and EL Ahwal,. et al. (2019).**

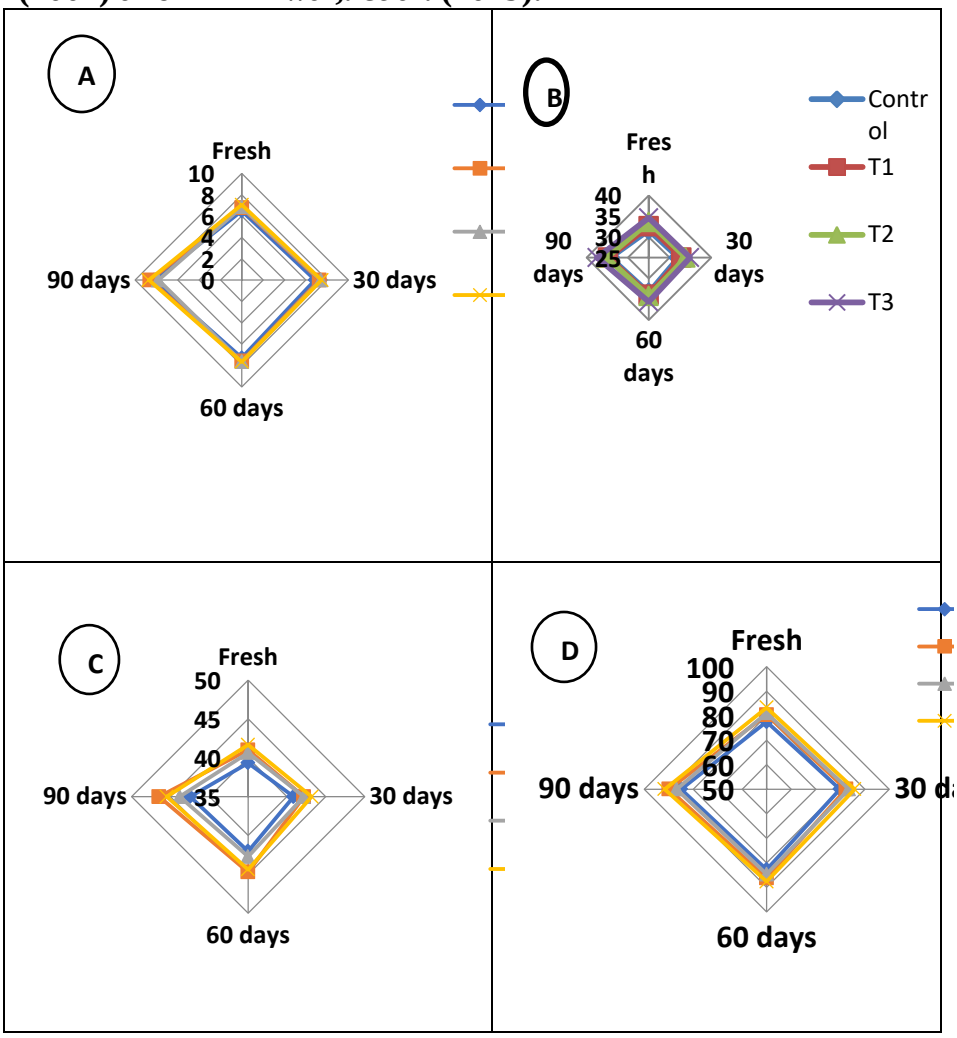

Fig ٨, (A-D). Sensory evaluation of soft cheese with different coagulation during storage.

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Control, soft cheese making from raw milk and plant extraction. T1, T2 and T3 soft cheese making from pasteurized milk and animal rennet, microbial rennet, and plant extract, respectively

Conclusion

This study deals with the isolation, extraction, separation and purification of MCE from *Portulaca oleracea*. The enzyme was purified by economical and inexpensive methods, and the plant source from which this enzyme was extracted is available in large quantities as it is a weed or agricultural waste: it can be used for the production of pure enzyme on a large scale. The enzyme was found to be stable in the presence of inhibitory substances such as metal ions and active over a wide range of temperatures and pH; thus, this enzyme can be used as an effective alternative to animal and microbial rennet. The enzyme is resistant to autolysis and can be stored at 4 °C for a long time without significant loss of its activity. Moreover, the high milk-coagulating activity of the enzyme can pave the way for its use in the manufacture of many types of cheese, as well as other food and biotechnological industries.

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REFERENCES

Abdalla, M. O. M., Dawla, A. A. Ali and Babiker, E. M (2010) Extraction milk-clotting activity measurements and purification of Solanum dubium fresen (Gubbain) for cheesemaking. World J. Dairy &Food Sci., 5(2), 152-159.

Abdalla, M.O.M., S.E.O. Kheir and O.A.O. El Owni (2011) Effect of Extraction Method, Ammonium Sulphate Concentration, Temperature and pH on Milk-Clotting Activity of Solanum dubium Fruit Extract. Advance Journal of Food Science and Technology 3(1), 40-44.

Abdel-Salam, A. M. (2010). Functional foods: Hopefulness to good health. American Journal of Food Technology, 5, 86-99. https://doi.org/10.3923/ajft.2010.86.99

Abdeen, E, M, M., Ibrahim, O, A and Kholif, A, M, M (2021) Utility of Moringa oleifera waste as a coagulant in goat soft cheese production. Heliyon 7 (2021) e07536

Abou-zeid., N (2015). The use of plant enzymes for ripening acceleration of Ras cheese. IIOABJ; Vol. 6; Issue 2; 2015: 15–21

Ahmed, I. A. M., Babiker E. E. and 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 Science and Technology, 43, 759–764

Ahmed, I. A. M., Morishima, I., Babiker E. E. and Mori, N. (2009) Characterization of partially purified milk-clotting enzyme from Solanum dubium Fresen seede, Food Chem. 116, 395-400.

ISSN: 2537-0804 eISSN: 2537- 0855

AOAC (2012). Official methods of analysis, 19th ed. Method 947.05. *Association of Official Analytical Chemists*, Washington

APHA (1994). *Standard methods for examination of dairy products* (16th ed.). Washington, DC., USA: American Public. Health Association.

Arima, K., Ya, J. and Iwasaki, S. (1970) Milk-clotting enzyme from Mucor pusillus var. Lindt. In: E. G. Pearlman & L. Lorand (Ed.), Methods in Enzymology (pp. 446–459). New York: Academic Press.

Awad, S., El Attar, A., Ayad, E. H. E., El-Soda, M., (2003). Characterisation of Egyptian Rascheese. 1. Sensory evaluation, rheological, physico- chemical properties and microbiological analysis. Egyptian Journal of Dairy Science, 31, 289–303.

Azarkan, M., Nijs, M., Smilders , N., Guermant, C., Vincentelli, J. and Looze, Y. (1996) Appl. Bioctechnol. 60, 167-83.

Bryant, A. Z Ustunol., Steffe., J 1995. Texture of Cheddar cheese as influenced by fat reduction. J Food Sci. 60:1216-1219.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annual Biochemistry. 72, 248-254.

Calvo, M. V. and Fontecha J., (2004) Purification and characterization of a pregastric esterase from a hygienized kid rennet paste. J. Dairy Sci., 87, 1132-1142.

ISSN: 2537-0804 **eISSN: 2537- 0855**

Cavalcanti, M.T.H., Teixeira, M. F. S., Lima Filho, J. L. and Porto, A. L. F.(2004) Partial purification of new milk-clotting enzyme produced by Nocardiopsis sp. Bioresource Technology, 93, 29–35.

Chaiwut, P., Nitsawang, S., Shank I. and Kanasawud, P. (2007) A comparative study on properties and proteolytic components of Paoaya peel and latex proteases. Chiang Mai Journal of Sci., 34, 109-118.

Cheded, M. A. (1975) Effect of vegetable rennet on some characters of cheese, M.Sc, Facurty of Agricurture, Ain-Shams Univ.

Chopra, A. K. and Mathur, D. K. (1983) Factors affecting protease production by Bacillus stearothermophilus RM-67. J. Food Protect. 116,1020-1025.

Colowick, S. P. and Kablan, N. O. (1955) Methods in Enzymology, Vol. (1). Academic press, Inc. Elsevier publishers, USA, 835 pp.

Demir, Y., Gugo, A., Duran E. D. and Demir, N. (2008) Cysteine protease (Capparin) capsules of Caper (Capparis spinosa). Food Technology and Biotechnol. 46(3), 286-291.

Dioxn, M. and Webb, E. (1968) Enzymes (2nded.) Longman Green Co. Ltd London and Academic Press, New York, 380 pp.

Duarte, A. R. Duarte, K. A. Moreira, M. T. H. Cavalcanti, J. L. de Lima and A. L. Figueiredo, (2009) Jacaratia corumbensis O. Kuntze anew vegetable source of milk-clotting enzymes. Brazilian Archieves of Biology and Technol., 52: 1-9.

ISSN: 2537-0804 eISSN: 2537- 0855

EL-Ahwal, R.I., Sala, E., Abo El-kher and E.Hattem, H. E (2019) Quality and Shelf Life of Labneh as Affected by Using some Essential Oils J. Food and Dairy Sci., Mansoura Univ., Vol. 10 (4): 135 – 139.

El-Bendary, A. Magda., Moharam, E. Maysa and Ali, H. Thanaa (2007). Purification and characterization of milk clotting enzyme produced by Bacillus sphaericus. Journal of Applied Sciences Research, 3, (8), 695-699.

El Neenay, M., Awed, S. A., Abbas, M., Attia, I. A., (2013). Production of Enzyme Modified Ras Cheese. ALEXANDRIA SCIENCE EXCHANGE JOURNAL, 34(2), 249–254.

El-Rebody, A. A., Amer, A. A., Youssef, M. R., (2014). Assessment Of Sanitary Measures Of Ras Cheese In Manufacturing Dairy Plant In Alexandria Governorate. Alexandria Journal of Veterinary Sciences, 40, 87–94.

El-Sayed, E. M., Saad., M. M., Selim, M. H. and El-Hadedy, D. E. (2013) Isolation of clt Genes Encoding milk clotting enzyme and characterization of milk clotting enzyme from new isolate Streptomyces pseudogriseolus NRC-15Am. J. Biotechnol. Mol. Sci., 3(1): 8-23.

El-Shibiny, S., Abd El-Gawad, M. A., Assem, F. M., El-Sayed, S. M., (2018). The use of nanosized eggshell powder for calcium fortification of cow's and buffalo's milk yogurts. Acta Scientiarum Polonorum, Technologia Alimentaria, 17(1), 37–49.

ISSN: 2537-0804 **eISSN: 2537- 0855**

EN ISO 13299, (2016). Sensory analysis, methodology, general guidance for establishing a sensory profile. Edition 2. https://www.iso.org/standard/58042. Html

Etigo, A. S., Girardet, J. M., Laguna, L. E., Poirson, C., Molle, D., Miclo, L., Humbert, G. and Gaillard, J. L. (2007) Milk-clotting activity of enzyme extract from sunflower and albizia seeds and specific hydrolysis of bovine K-casein. International Dairy J., 17, 816-825.

Fox, P. F., Guinee, T. P., Cogan, T. M., McSweeney, P.L.H., (2000). Cheese Rheology and texture. Pp. 305–333 in Fundamentals of Cheese Science. Aspen Publishers, Inc., Gaithersburg, MD.

Glibowski, P., P. Zarzycki and M. Krzepkowska (2008).The rheological and instrumental textural properties of selected table fats. *International Journal of Food Properties*, 11: 678 – 686.

Gomori,G. (1955) Preparation of Buffers for Use in Enzyme Studies, Handbook of Biochemistry and Molecular Biology.

Goodenough, P. W. and Owen, J. (1987) Chromatographic and eletrophoretic analyses of papaya proteinase. Phytochem. 26, 74-79.

Guiama, V. D. G. Libuoga, E. Ngah, R. G. Baka, K. C. Ndi, B. Maloga, J. M. Bindzi, P. Donn and C. M. Mbofung (2010) Milk-clotting potential of fruit extract from Solanum esculentum, Solanum macrocarpon L. and Solanum melongena. African J. Biotechnol., 9 (12): 1797-1802.

Gutierrez. M. N,. Chavez. G. D. R and Jimenez. C. H (2012) Exploring the milk clotting properties of a plant coagulant

ISSN: 2537-0804 eISSN: 2537- 0855

from the Berries of S. elaeagnifolium var. Cavanilles. Journal of food science, Vol, 71, Nr (1).

Isam, A. M. A., Isao, M., Elfadil. E. B. and Nobuhiro, M. (2009) Characterisation of partially purified milk-clotting enzyme from Solanum dubium Fresen seeds. Food Chemistry, 116, 395–400.

Jacob, M. and Jarosand Rohm, H. (2011) Recent advances in milk clotting enzyme. International Journal of Dairy Technology, 64,14-33.

Katsiari, M.C., Voutsinas, L.P., Kondyli E., (2002). Improvement of sensory quality of low-fat Kefalogra-viera-type cheesewith commercial adjunct culltues. Inter.dairy J 12:757–764.

Kholif, A. M. M., Mahran, G. A., Khorshid, M. A., El-Nawawy, M. A., El-Tanboly, E. E and Abd-Rabou, N. S.(2016) Isolation, Purification and Characterization of Milk Clotting Enzyme from Fruit Seeds of *Solanum Elaeagnifolium* Plant Egypt. J. Food Sci. 44, pp. 1 –27.

Kim, Y. K., Nam, M. S., Bae, H. C., (2017). Characteristics of Gouda cheese supplemented with chili pepper extract microcapsules. Korean Journal for Food Science of Animal Resources, 37(6), 833–839.

Kosikowski, F.V, (1982). Cheese and Fermented Milk Food.2nd ed. Published and Distributed by F.V. Kosikowski and Association, Brooktonal, New York.P.255.

Kumari Narwal, R., Bharat B., Ajay, P., Anil, P. and Sarla, M (2016) Purification, physico-chemico-kinetic characterization and thermal inactivation thermodynamics of

ISSN: 2537-0804 **eISSN: 2537- 0855**

milk clotting enzyme from Bacillus subtilis MTCC 10422. LWT - Food Science and Technology 65 (2016) 652-660

Kumari, M., Anurag, S. and Jagannadham, M.V. (2012) Religiosin B, a milk-clotting serine protease from Ficus religiosa. Food Chemistry 131: 1295–1303.

Mabrouk, A. M.; Heba H. Salama; Hoda S. El Sayed and Samah M. El Sayed (2021). Preparation of synbiotic whey protein gel as a carrier of free and encapsulated probiotic bacteria. J Food Process Preserv. 2021;00:e15612. https://doi.org/10.1111/jfpp.15612.

Maksimenko, A. V., L. A. Nadirashvili, A. D. Romashchenk, G. S. Erkomaishvili and V. P. Torchili (1990) Stabilization of Papaya proteinase complex by chemical modification and copmarativ study of native and modified preparation. Biotekhnologiya, 2, 57-60.

Mamo, J. and Assefa, F. (2018) The Role of Microbial Aspartic Protease Enzyme in Food and Beverage Industries. Journal of Food Quality, 2018, Article ID: 7957269. https://doi.org/10.1155/2018/7957269

Maarse H., (1991). Volatile compounds in foods and beverages. New York: Marcel Dekker Inc. 767

Monates, C., M. Amador, D. Cuevas and F. Cordoba (1990) Subunit structure of Karatasin, the proteinase isolated from Bromelia plumieri (Karatas) Agric. Biol. Chem., 54, 17-24.

Pino J., González M., Ceballos L., Centurión- Yah A.R., Trujillo-Aguirre J., Latournerie- Moreno L. and Sauri-Duch E., (2007) Characterization of total capsaicinoids, colour and volatile compounds of Habanero chilli pepper (Capsicum

ISSN: 2537-0804 eISSN: 2537- 0855

chinense Jack.) cultivars grown in Yucatan. Food Chemistry, 104(4), 1682-1686(2007). doi:10.1016/j.foodchem.2006.12.067.

Roseiro LB, Barbosa M., Ames JM and Wilbey, RA (2003) Cheese making with vegetable coagulants—the use of Cynara L. for the production of ovine cheeses. Int. J. Dairy Technol. 56,76–85.

Saad, M. A., Abd-Rabiu, H. S., Elk tab, E., Rayan, A. M., Abedin, A., Abdelkader, A., Ibrahim, S. F., Hussein, H., (2022). Occurrence of Toxic Biogenic Amines in Various Types of Soft and Hard Cheeses and Their Control by Bacillus polymyxin D05-1. *Fermentation*, *8*(7). https://doi.org/10.3390/fermentation8070327

SAS Institute (1990) SAS User's Guid / STAT Ver. 6.044th Ed. SAS Inst. Inc. Cary, NC. https://doi. org/ 10.4236/psych.2017.810100

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

Shehata, A. E., Fayed, E. A., Ismail A. A. and Salem, M. M. (1996) Production and characterization of bacterial coagulants as calf rennet replace for Egyptian cheese making. Egyptian J. Food Sci., 24(3), 417-449.

Silva, S. V. and Mlalcata, F. X. (2005)' Studies pertaining to coagulant and proteolytic activities of plant proteases from Cynara cardunculus. Food Chemistry, 89, 19-26.

Sun, Q., Wang, X. P., Yan, Q. J., Chen, W., and Jiang, Z. Q. (2014) Purification and characterization of a chymosin from

ISSN: 2537-0804 **eISSN: 2537- 0855**

Rhizopus microsporus var. rhizopodiformis. Applied Biochemistry and Biotechnology, 174, 174e185.

Tavaria, F. K., Sousa, M. J., & Malcata, F. X. (2001) Storage and lyophilization effects of extracts of Cynara cardunculus on the degradation of ovine and caprine caseins. Food Chemistry, 72, 79–88.

Wahba, A., El-Abbassy, F., El Shafei, H. and Awad (1995) Effect of some factors on the activity of milk clotting enzymes. Egyptian J. Food Sci., 23(1-2):27-35.

Walsh, M. K. and Li, X. (2000) Thermal stability of acid proteinases. J. Dairy. Res. 67, 637. DOI: https://doi.org/10.1017/S0022029900004532

Wang, Y., Qiaolin C., Zaheer. A., Xiaoxue. J. and Xiaojia B (2009) Purification and partial characterization of milk-clotting enzyme extracted from glutinous rice wine mash liquor. Korean J. Chem. Eng., 26(5), 1313-1318.

Youssef, A. M., Assem, F. M., Abdel-Aziz, M. E., Elaaser, M., Ibrahim, O. A., Mahmoud, M., Abd El-Salam, M. H., (2019). Development of bionano composite materials and its use in coating of Ras cheese. Food Chemistry, 270, 467–475.

Yousif, B. H., McMahon, J. D. and Shammet, M. K. (1996) Milk-clotting enzyme from Solanum dobium plant. International Dairy Journal, 6, 637–644.

Zimacheve, A. V., Lyevleva, E. V., Huan, P. H., Nhan V. H., and Mosolov, V. V. (1994) A proteinase from the proliferous top of the pineapple fruit (Ananas comosus L) Prikl. Biokhim. Mikrobiol., 30, 215-222 .

ISSN: 2537-0804 eISSN: 2537- 0855