Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Acceleration RAS Cheese Ripening Using Extracts of Some Plants

Adel M. M. Kholif[1*], Ola M. A. K. Shalabi[2], Monira M. Basiony[3] and Mohamed M. El-Metwally[3]

[1]*Dairy Science Department, National Research Centre, 33 El-Bohouth, Dokki, Giza, Egypt.*

[2]*Dairy Department, Faculty of Agriculture, Mansoura University, Mansoura 33516, Egypt.*

[3]*Dairy Technology Department, Animal Production Research Institute, Agricultural Research Center, Egypt.*

Abstract

PROTEASE ENZYME from the fruit of *Solanum nigrum* and Pineapple plant, which both has the capacity of proteolysis to acceleration ripening of Ras cheese ripening was obtained by fractional precipitation by ammonium sulfate (AS) of the most active fraction at concentration on 60 and 70 % respectively, followed by using sequential chromatographic column of technique by sephadex G 100 to the most active fraction, results indicated to rate purification (RP) was 7.28 and 4.80 folds with 17.35 and 19.76 % recovery to protease enzyme from *S. nigrum* and Pineapple, respectively. The optimum temperature of the enzymes was found to be 50 and 60 °C and the enzyme activity (EA) was stable at 20 to 80 °C. The enzymes showed the optimum pH of 7 and 8, and quite stable in broad pH range of 6.0 to 9. The effect of calcium chloride at a concentration of 10 and 20 mM gave the highest relative activity of the purified proteases about 121.54 and 133.21 as a relative activity. Sodium chloride at a concentration of 3 and 2 % gave the highest relative activity of the purified proteases. Ras cheese was manufactured by adding the above enzymes with different concentrations, and it was found that the Ras cheese treated with plant enzymes developed higher soluble protein and Added plant proteases contributed to the breakdown of casein and total nitrogen (TN), soluble nitrogen (SN) and total volatile fatty acids (TVFA) were high in cheeses treated with protease, and displayed better flavor and greater acceptability than control cheeses. Increased rate of proteolysis in enzymes-treated cheese had a direct relation to accelerated ripening Ras cheese.

Keywords: Proteases enzyme activity, Purification, *Solanum nigrum*, pineapple, acceleration ripening.

Introduction

A plant is a rich source of enzymes, especially proteolysis of enzymes, the protease from proteolytic enzymes and indicates to proteases and peptidases, there are also many parts of plants and their seeds that have been used as a source of enzymes. [1]. Enzymes extracted from plants as a substitute are used to replace traditional enzymes. Bromelain as a proteolytic enzyme is present in all parts of pineapple such as the crown, stems, fruit, skin and tubers [2]. So proteases are a type of enzyme that catalyzes the hydrolysis of polypeptides and peptide bonds in proteins [3]. Because protease enzymes the most important segment and largest sector used in the commercial and industrial aspects [4], these enzymes are used in food, detergents leather industry, as biocatalysts in organic synthesis, processing and, among many other industries, as therapeutics because it has a major and important role in several physiological and metabolic processes throughout an organism [5]. Concern is increasing with the large number of agricultural wastes such as peels, seeds, leaves, stems, or wastes resulting from the food industry, and the lack of industries to use them [6]. The use of such waste from agriculture and industry is important for countries that seek sustainable development [7]. Also, protease of plant source from S. of the Solanaceae family commonly known as black nightshade usually grows in more different types of soils, including shallow, dry, stony, or deep soils, and It can be grown in agricultural areas characterized by a tropical and subtropical climate by sowing the seeds during April and May. Other protease from pineapple is the most popular of all

*Corresponding authors: Adel M. M. Kholif, adelkholif@hotmail.com, am.kholif@nrc.sci.eg, Mobile: +02-01141166455.
(Received 05 October 2024, accepted 02 January 2025)
DOI: 10.21608/EJVS.2025.325761.2406

fruits and it is very an important ingredient in the food and dairy industry [8]. However, 75 % (bark, leaves, stem and crown) is agricultural waste, while only 25 % of this fruit can be used in industry or as a marketable product, while [9]. Brazil, Philippines and Costa Rica are responsible for one third of total pineapple production [10].

Bromelain has proved to contain high proteolytic activity; Crude enzyme can be easily obtained by extraction and purification from fruits and waste parts of the pineapple. It is a good candidate, since the purified protease is has activity and the extraction is feasible, bromelain also has a wide range in terms of temperature, thermal stability and pH. The antioxidant [11]. Proteases used as milk coagulants in cheese manufacturing must show high clotting activity and low proteolytic activity (ratio: milk-clotting/proteolysis). This ratio depends on the capability to hydrolyze for the enzyme specifically on κ-casein [12]. Lots of proteases obtained from plants are able to hydrolyze κ-casein, and whey proteins. Consequently, only a few protease enzymes are obtained from plants that are suitable for the manufacture of cheese. Cheese is one of the best foods in dairy products and most fermented milk-based foods and indicated by its various, textures and flavors. Also maturing is very important stage essential industrial in cheese production, which leads to establishing a cascade of biochemical produced, resulted by a varied array of normal flora of microbial that allow to produced sensory characteristics [13]. It is adding protease [14], proteinase [15] and peptidase enzymes [16] to hard cheese like Ras cheese leads to reducing the cost, accelerating of ripening time, and speeding up the capital cycle. Ras cheese is an authentic Egyptian cheese that is made from a mixture of buffalo and cow milk in equal proportions. It is also called by other names such as Turkish or Romy cheese and is similar to Greek cheese “kefalotyri” [17]. This type of cheese requires a long storage period to develop its aroma, taste and texture as a result of the decomposition of proteins and fats [18]. Ras cheese is very popular in Egypt among consumers mainly due to its quality and nutritional value [19]. Protein and fat in the cheese curd are affected by their proteolytic by protease and lipase enzymes [20].

So the aim of this study is to isolate and purify proteases from S. nigrum and pineapple plants that proteolysis of proteins for use in accelerating the ripening of hard cheese

Material and Methods

Fresh cow's milk were obtained from agriculture research center, El-Serw Station, having T.S 12.41 %, fat 4.10%, protein 3.32 % and ash 0.63 %. Rennet Chy-Max from (Chr Hansen., Holding A/S, Boege, 2970 Hoersholm, Danmark). Cheese starter culture of Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris were obtained from National Research Centre, Egypt. Commercial fine grade salt (sodium chloride and calcium chloride) was obtained from El-Nasser Company, Alexandria, Egypt, and was prepared CaCl2 as a solution before use and added to milk in calculated amount required to give final concentration of 0.02 % (w/v). S. nigrum plant used in this study was collected from Egypt. North Sinai. Rafah city in the month of May when the fruits were fully ripe. The whole fruits of S. nigrum were dried at 30 ○C for 6 days, followed by cleaned, then the fruits were powdered. The fruits powder (16 g/30 ml) was macerated with buffers [21]. The pineapple (Ananas comosus) was collected from local market in Cairo Governorate and the outer shell of the fruits was removed, then they were using the same method.

Preparation of crude enzyme extracts with different buffers

Three extraction buffers (0.1 M Tris buffer pH 7.0, 0.1 M phosphate buffer pH 7.0 and 0.1 M citrate phosphate buffer pH 7.0) and dist. water pH 7.0, to select the one with a high activity of proteases from the S. nigrum fruit and pineapple. All the buffers used according to [22].

Extraction of crude enzyme extracts with 0.1 M phosphate buffer pH 7.0

Sixteen grams of powder from S. nigrum for 24 hr at 4 ºC were soaked in a conical flask 100 ml with 0.1 M phosphate pH 7.0 with frequent shaking, followed by centrifuged at 8000/g for 20 mints at 4 ºC, then filtered by filter paper (Whatman No.1). The aqueous filtrate was used for determination of protease activity [23]. Extraction of bromelain as a protease from Ananas comosus fruit by method as described by [24] with some modifications. Purely ripe pineapple fruits were cleaned and cut into slices and were homogenized and filtered to obtain a crude extract, which was used as the source of determination protease activity.

Protease activity

Protease activity was determined by of [25]. One ml of casein (1 %) as a substrate at 37 °C for 20 min in the same buffer used, and then it was added 1.0 ml of enzyme. The reaction was at the same incubation temperature and time. adding 2.0 ml of trichloroacetic acid (TCA) (0.4 M) to stop the reaction, then filtrated and it was added 1 ml of the filtrate, 5.0 ml of Na2Co3 (0.4 M) was added followed by the folins reagent (1.0 ml) and incubated at the same temperature and time for color development and reading absorbance on the spectrophotometer at 660 nm. For the blank, add the TCA with the substrate before adding the enzyme and then treated as the method described above. A standard curve was prepared using tyrosine (Sigma-Aldrich, St. Louis, MO) at a range from 0.05–0.5 mM. The results were expressed as units of µg

tyrosine per milligram of protein. Protein content was determined calorimetrically at 595 nm by the method of [26] using bovine serum albumin and Coomassie brilliant blue G-250.

Purification of crude enzyme extracts

According to the method by [27] the Crude enzyme was precipitated by concentrations of AS from 10 to 100 % saturation. Suitable concentration of AS solid was added to the supernatant, and then centrifuged under cooling for 20 min at 8000 g. The precipitate was collected with a minimum quantity (5 ml) in 0.1 M phosphate buffer (pH 7.0). The precipitate fraction was dialyzed in the same buffer using dialysis bag and kept in the refrigerator for 48 hr. After the step of the dialysis bag the enzyme extracts were purified by method reported by [28] 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.

Characterization of purified protease enzyme

Optimum pH

The pure enzyme activity was determined of the optimum pH by replacing 0.1 M citrate phosphate buffer (pH 5- 6), 0.1 M phosphate buffer (pH 7- 8), 0.1 M Tris-HCl buffer (pH 9) and glycin-NaOH buffer (pH 10), according to [22]

Optimum temperature

The pure enzyme activity was determined of the optimum temperature by incubating the reaction mixture of the protease assay at different temperatures ranging from 10 to 100 °C for 10 min.

Effect of calcium chloride and sodium chloride concentrations

The effect of the presence of various concentrations of CaCl2 which ranged from (0 -50 mM) and NaCl which ranged from (0 -16 %) on purified protease activity was studied. The activity was measured under standard assay condition and the relative activity was calculated as the percentage of activity remaining after incubation with various concentrations of CaCl2 and NaCl.

Ras cheese manufacture and analysis

Ras cheese was manufactured out as illustrated by [29] with some modifications from the prepared milk. Cheese milk was heated 63±2 °C for 30 minutes, and cooled to 32 °C, then divided into five portions: the first portion was acted as a control (without enzyme). Two enzymes were applied first extracted from bromelain that was used in proportions 0.06 and 0.08 (T1 and T2) respectively, while the second one from S. nigram which also used in proportions 0.06 and 0.08 (T3 and T4) respectively. The other steps of Ras cheese were followed. Storage of cheese was carried out for 120 days at room temperature and cheese was analyzed when fresh, and after 60 and 90 and 120 days of storage period.

Cow's milk was analyzed for fat, protein, and total solid by milk Oscan (Model 133B; N. Foss Electric, Hillerød, Denmark). The same components were analyzed in Ras cheese samples such as TN, SN and ash contents, according to according to [30]. The pH values were estimated using a pH meter (Digital pH meter M41150) equipped with a combined glass electrode. The tiratable acidity was estimated as lactic acid % according to [31]. Salt content was estimated using the Volhard method, according to [32]. TVFA were determined by the distillation method described by [33]. The hardness, cohesiveness, Springiness, gumminess and Chewiness of fresh Ras cheese were tested using Instron Universal Testing Machine (Model 4302, Instron Corporation, Canton, MA) according to the procedure of [34], and load cell of 100 N. Total bacterial count (TC), and molds and yeasts (M&Y) were measured as recommended in Standard Methods for the Examination of Dairy Product (1993) [35]. Ten panelists evaluated the resultant Ras cheese for appearance, body and texture, and flavor, according to [36]. The results were statistically analyzed using [37]. However, the significant differences among means were tested using Duncan's Multiple Range Test.

Results and Discussion

Activity of plant extracted enzyme under different buffering conditions

For the extraction of the protease, fruit of *S. nigrum* and Pineapple plants in distilled water pH 7.0 and three extractions buffers (Tris pH 7.0, phosphate pH 7.0 and citrate phosphate pH 7.0 (0.1 M)), to select the most one with a high activity reasonably . The results of the effect of extraction methods on the protease activity are presented in (Table 1). Extraction of fruits of *S.nigrum* and Pineapple plants with 0.1 M phosphate pH 7.0 gave the highest protease activity (2.61 and 0.86 U/ml) , and Specific activity (12.42 and 4.30) respectively, compared to the other buffers. The dist. water, acetate and citrate gave the highest specific activity were 5.94, 7.92 and 7.34 to protease from *S. nigrum* and 2.92, 4.29 and 3.77 to protease from Pineapple respectively. The results obtained agree with [38] reported that the extraction of bromelain enzyme activity underwent ultrafiltration from pineapple juice, All assays were carried out at pH 7, and results gave the best relative activity of bromelain between 85 and 87 % under operating condition.

Purification step of protease enzymes

Ammonium sulfate Precipitation

A preliminary ammonium sulfate saturation fractionation on protease enzyme activity from *S.*

nigrum and Pineapple fruit. Ammonium sulfate fractionation was used in this study, a first one step to purification the enzymes, where extract the tow crude enzymes of the same buffer that was selected from the previous step was using different concentrations from ammonium sulfate (0–90 % saturation). The results showed that, the highest protease activity at 60 % and 70 % saturation from *S. nigrum* and pineapple, respectively, specific activity, total activity, Sp. protease activity, yield (%) and rate purification (Table 2 and 3) although. Where it was found the saturation of AS greatly affected protease activity (PA), specific activity, total activity, Sp. PA, yield (%) and RP. The results showed that 60 % saturation in (Table 2) gave the high PA (8.11 U/ml), specific activity (15.30), total activity (81.10U/ml), yield (62.14 %) and RP (1.23) for protease from *S. nigrum*. On the other hand, In (Table 3) the range of 70% was selected for potential purification of the protease enzyme from pineapple plant, where it was given the highest protease activity (2.61 U/ml), specific activity (5.43), total activity (26.10 U/ml), yield (60.69 %) and PA (1.07).These results are in conformity with the findings of [38-39] who found the best protease activity at saturation of 40-60 %. Also, [40-42] reported the highest protease activity when the enzyme was precipitated by ammonium sulfate with 50-70 % saturation.

Gel filtration

In other step, the purified protease enzyme from *S, nigrum* and pineapple fruit by Gel filtration in a column chromatographed of Sephadex G-100, and the purification showed at fraction 20 and 30 only one peak, respectively, as shown in (Table 2 & 3 and Fig. 1 & 2) and which was obtained when the collection fraction from dialyzed was loaded on to Sephadex column equilibrated with the same buffer. Purification of the enzyme from *S.nigrum* and pineapple fruit using different purification means resulted in 7.28 and 4.80 rate purification with a yield of 17.35 and 19.76 %, while specific activity of 90.60 and 24.28 respectively.

A simple purification to enzymes procedure was developed in this study to give a high active and stable enzyme from *S, nigrum* and pineapple fruit. Results are in agreement with [39] reported that the protease from *Bacillus licheniformis* gave 33 rate purification with a yield of 20 %. Also [42] reported the bromelain from pineapple gave rate purification 5.74 and yield 85 %. [19] Found that the protease from pineapple fruit to rate purification 1.98 and yield 114.03 %. [43] Reported several proteolytic bands and one diffuse proteolytic band from in *Albizia seed* and sunflower seed extract, respectively. However, the findings of [44], who concluded that two peaks with proteolytic activity, were eluted from purification of *Jacaratia corumbensis* and *Papaya* (Carica papaya). The variation in peak number is mainly due to protein content of different values or separation conditions.

Characterization of Partial purified protease enzymes

Optimum pH

The activity of enzymes is always affected significant by pH, from the results it was found that, the proteolytic activity of enzymes retains its activity in the pH range of 5.0 to 10, and highest purified protease activity from *S. nigrum* and pineapple fruit were observed at pH 7 and 8 respectively (Fig. 3). The results of this study agreed with observed [45] mention that, slight reduction in bromelain activity at pH 7, Similar behavior of optimum pH was reported for protease enzyme from *Bacillus subtilis* MTCC 10422; the enzyme optimal activity at pH 6.0. Also, the proteolytic enzyme retains its activity towards denatured casein in range of (pH 6.0 - 9.0), and highest proteolytic activity at pH 8 [46 – 48][38 & 39] found that the bromelain activity underwent ultrafiltration from pineapple juice. All assays were carried out at pH 7 and 7.5. Also [49] showed the pH optima of 6.0, 6.2 and 8.0, respectively. Proteolytic activity similar behavior of optimum pH also the optimum activity is observed at pH 9.5 and enzyme retains its activity towards denatured casein in the pH range of 6.0 to 10.0, was reported by [50].

Optimum temperature

The results obtained in (Fig. 4) showed the different temperatures of range from 10 to 100 °C and effect on protease activity from *S. nigrum* and pineapple fruit. The protease enzyme activity increased as the increased of temperature from 20 to 50 and 60 °C respectively. The optimal of temperature for the protease activity was also investigated. The highest protease activity of purified was at 50 and 60 °C for both enzymes respectively. The rapidly decreased increased for activity with increased of temperature higher than 60 °C due to effect of thermal denaturation of the protein. These results to protease activity are in agreement with the protease which showed the maxim activity at 55°C and produced by Nocardiopsis sp., [46, 47 and 49] reported that, the optimum activity is observed at temperature 50 ^{o}C, and proteolytic activity increased as the reaction temperature increased from 10 to 50 °C. The maximum protease was recorded at 65 °C for *Rhizomucor miehei* [51]. [38 and 45] reported that the bromelain enzyme obtained was pH 7, and between 30 and 40 °C were the best conditions. The optimum temperature for protease enzyme using in manufacturing of cheese is 40 °C as at this enzyme can retain its high activity, and the optimum temperature to proteolytic activity is 50 ^{o}C, which is also an optimum temperature for most of the thermophilic of lactic acid bacteria. Therefore, in this aspect this enzyme is superior over many other protease enzymes whose optimum activity is at a

high temperature, which lead to speed of curd stiffening and makes cutting of curd difficult and the occurrence of defects in cheese during ripening, this results was in agreement with that observed with [47] Where it was found the optimum temperature of protease ranged from 40 to 50 °C.

Effect of $CaCl_2$ concentrations

Calcium chloride is an important factor in cheese making. 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, concentrations of $CaCl_2$ may be added to study this effect, and $CaCl_2$ was added in different concentrations from 1 to 50 mM on partially purified protease activity of *S. nigrum* and pineapple fruit. As shown in (Fig. 5) indicated that increasing the concentration of calcium chloride with increasing proteolytic activity even 10 and 20 mM calcium chloride, respectively, then the enzyme activity decreases gradually with increasing the concentration of calcium chloride up to 25 mM and then a sharp decline occurred in the activity of the enzyme up to 50 mM. These results show similar behavior with [46] indicated that increasing the concentration of calcium chloride with increasing proteolytic activity even 20 mM calcium chloride, then the enzyme activity decreases gradually with increasing the concentration of calcium chloride up to 40 mM. Also [44] reported that, the protease activity increased with increasing calcium chloride concentration. Protease activity decreased at 35 mM of concentration, probably due to the saturation of negative residues of micelles at increasing Ca^{+2} concentrations or the increase of ionic force [52].

Effect of Sodium chloride concentrations

Usually using the Sodium chloride during the making of cheese and ripening in Egypt because it is used as a preservative to protect milk from spoilage by various microbial. Different concentrations of sodium chloride from (0-16 % NaCl) were used in this study on partially purified protease activity of *S. nigrum* and pineapple fruit. Results showed The enzyme activity remained constant until the concentrations of 3 and 2 %, respectively, As shown in (Fig. 6) the results indicated that increasing the concentration of sodium chloride with increasing protease activity even 3 and 2 % sodium chloride, respectively, then the enzyme activity decreases gradually with increasing the concentration of sodium chloride and then a sharp decline in the activity of the enzyme up to 16 % occurred. The results agreement with the results obtained by [45-46] indicated that increasing the concentration of sodium chloride with increasing proteolytic activity even 2 % sodium chloride, then the enzyme activity decreases gradually with increasing the concentration of sodium chloride and then a sharp decline in the activity of the enzyme up to 16 % occurred. [14] Indicated that with increasing sodium chloride concentration the protease activity decreased. [53] Found that addition of NaCl to milk resulted in a marked high decrease in protease activity. Sodium chloride was reported to maybe decrease the rate of the enzymatic reaction and also the coagulation of renneted micelles [54].

Effect of enzyme concentration

Enzyme activity is affected by increasing its concentration, the effect of the enzyme activity by different concentrations namely from 0.1 to 1.1 (ml enzyme / 2 ml casein) on the substrate was studied. From the results (Fig. 7) it can be deduced that there was a parallel relationship existed between the enzyme concentration and proteolytic activity, that is, the higher the concentration of the enzyme, the greater the activity. There was a change in the proteolytic parameters with the concentration of the enzyme [46 and 55]. The protease activity protease enzymes from *S. nigrum* and pineapple fruit enzyme was highly increased with increase in enzyme concentration.

Chemical composition of Ras cheese

The composition of cheeses samples determined during ripening period is presented in (Table 4). All treatments retained more moisture than control except Ras cheese supplemented with 0.08 % of bromelain enzyme (T2). It was also reported that the moisture content of the cheese treatments decreased with the increase in the percentage of the enzymes. The moisture in all cheese treatments decreased during repining. In all cases, control cheese had more pH and on the contrary less acidity values as compared to Ras cheese supplemented with bromelain or *S. nigrum* enzymes (T1-T4), respectively. The pH values gradually decreased under the action of lactic acid starter activity in converting lactase sugars into lactic acid during ripening. Total nitrogen content of all cheese treatments increased by supplemented with 0.08 % of bromelain enzyme, 0.06 and 0.08 % of *S. nigrum* but decreased with 0.06 % of bromelain enzyme, and the lowest total nitrogen content was in the control sample. On the other hand, the equivalent total nitrogen 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 (4) mention that, the level of SN and TVFA in the control sample were significantly lower than those of the respective other treated cheeses but were increased during ripening period of cheese in all treatments. 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 a result of ripening the increase in due to the different in total solid content of the cheese. Fresh and ripened cheese contained (4.39 / 4.72), (4.48/4.69), (4.52/ 4.79),

(4.56/ 4.81) and (4.62 and 4.85) cheese for control, T1, T2, T3and T4 treatments respectively, these results are similar to what was mentioned [56] where indicated that the level of soluble nitrogen, non-protein nitrogen and TVFA in the control sample were lower than the other treatments. The soluble nitrogen (SN) is considered very an important index for cheese ripening as it reveals the rate of proteolysis of protein [57]. The proteolysis of protein is directly related to the texture, test and flavor during the storage and ripening of cheese .The partially purified protease enzyme using in manufactured of Ras cheese had higher SN content from the control treatment in both fresh time and after the ripening of cheese for 120-day. This might be due to the high rate of degree proteolysis caused by purified proteases. It was observed that in both cheese treatments, the SN content was significantly increased ($P \leq 0.05$) during the ripening time. This might be due to increasing the degree of proteolysis rate throughout the storage period. Similar observations were reported by other authors [57]. The rate of TVFA accumulation increased with increasing the time of ripening in both control and treatments with the partially purified protease. Other authors also noticed a gradual increase in TVFA contents during the ripening time which might be due to increasing the lipolysis degree [58]. And this could be due to the high lipolytic in cheese during ripening time. This was consistent with the results of other authors [59 and 60].

Textural profile analysis

Table 5, shows the calculated rheological parameters of Ras cheese treatments which contain different concentrations from plant protease enzyme compared to those of control cheese when fresh and after 120 days old Ras 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 ripening period. Fresh Ras cheese supplemented with 0.06 % of bromelain enzyme had lower hardness, springiness, gumminess, and chewiness and higher cohesiveness than the control. This was not correlated with the moisture content, but correlated with type and percentage of enzyme. In general, during the first 120 days of ripening, values for the parameters tended to increase gradually at rates that were influenced by moisture loss. These results agree with that reviewed by [56] [61-63], and are partially confirmed by those results of [64-66]. Also [67] found that the low moisture content during ripening lade to increase a consequence hardness significantly.

Microbiological analysis

The results of microbial (log cfu/ml) counts (total count, total coliform group, and yeasts and molds) of Ras cheese treated with different protease concentrations during storage at room temperature for 120 days are presented in (Table 6). The data shows the mean values of some microbiological properties of Ras cheese treated with different protease concentrations. The results indicated that in fresh storage, the total counts were 6.13, 6.14, 6.29, 6.38, and 6.14 log cfu/g and then decreased for all treatments at the end of the ripening period: 5.86, 5.86, 5.72, 5.80, and 5.77 log cfu/g for control, T1, T2, T3, and T4, respectively, as a result of the Ras cheese's ageing and dry salting. [68] Found that the total counts decrease in the cheese samples that have been matured for 3 months ($2.05 \times 10^5 \pm 1.0^3 \times 10^5$). According to [69], LAB was found to be 8×10^7 in fresh of enzyme modified Ras cheese slurry. In other enzyme modified Ras cheese slurry, LAB count ranged from 9.6×10^5 to 2×10^8.

On the other hand, in all treatments of Ras cheese treated with different protease concentrations, molds and yeasts were not detected in the fresh product or after 30 days at temperature 14 ℃, whereas they appeared after 60 days and increased up to 120 days of storage at temperature 14 ℃ in all treatments. Additionally, their counts at the end of the storage period were 5.08, 5.10, 4.62, 5.36, and 4.60 log cfu/g for control, T1, T2, T3, and T4, respectively. Also, [69], who indicated that by examination of enzyme modified Ras 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 and spore forming were not found in all treatments of Ras cheese treated with different protease concentrations during the storage period at room temperature, this is due to the hygienic conditions set during the process, according to [70].

Sensory evaluation of Ras cheese

The sensory attributes of Ras 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 persons from dairy department. National research centre, as panelists representing Product judging panel.

In (Table 7). Appearance the first parameter that the consumer experience when tasted the products and the final score is 10 points. Indicates that during the whole storage period (120 days) all treatments of Ras cheese got the acceptable score significantly ($p \leq 0.05$). The corresponding scores ranged between 7.82 for T2 with 0.08 % protease from Pineapple to 8.72 for T1 with 0.06 % at the end of the storage period, 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 120 days, T1 sample with got the high score significantly ($p \leq 0.05$) comparison to other samples.

In this parameter the whole score is 40 points. The Body &Texture of Ras cheese is very important parameter that makes the consumer prefer it. (Table 7) indicate that these parameters that the texture of all samples was acceptable significantly ($p \leq 0.05$) at the fresh and the end of the ripening period. However, we observed significant differences ($p \leq 0.05$) in texture among the treatments, where the T1 with concentration 0.06% protease from Pineapple most acceptable (37.42), followed by samples with concentration 0.08 % got a score (35.09) at their end ripening period. The reason may be due to the concentration of purified protease from Pineapple extract in the samples with a loss of a little moisture, which leads to an increase in the smoothness of texture.

In this parameter regarding flavor, the whole score is 50 points in (Table 7). Indicates that cheese samples with all concentrations at the zero were acceptable significantly ($p \leq 0.05$) comparison control treatment and T1 got high score at fresh time comparison with other treatments. On the other hand, preference increases in flavor for all cheese samples during ripening period until the end of storage.

Over all, putting the three evaluations (appearance, texture and flavor) all together in (Table.7) 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 ripening period, and after storage for 120 days, the T1 sample maintained the preferred status (91.61) followed by the score 89.83, 86.42, 85.16 and 84.99 for T3, T2, T4 and control sample, respectively. There were significant differences ($p \leq 0.05$) between all samples during the storage period. These results were in agreement with [71].

Conclusion

Generally the results obtained lead to the conclusion that, the protease enzyme from a fruit of *Solanum nigrum* and Pineapple can be considered, to be a promising enzyme source for accelerating cheese ripening. However, the production of these enzymes should be increased. The better method to add protease is adding 0.06% to cheese curd; Because It reduces the period required for Ras cheese ripening to 90 days instead of 120 days without any defects in cheese properties. Ample scope remains for increasing the proteolysis enzymes production by using the Roots, stems, leaves and fruits of plants producing these enzymes, by purification methods to this enzymes, Since it does not give the bitter taste in the cheese manufactured, and This reduces manufacturing costs for both producers and consumers and would be economical.

Competing interests

The authors declare that they have no competing interests

Funding statement

No funding received.

Ethics approval

The topic of the research doesn't require ethic

Consent for publication

Not applicable

Consent to participate

All authors of the manuscript are aware from submission of this manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

TABLE 1. proteases enzyme activity of plant extracts under different buffer conditions.

Type of buffers (0.1 M)	pH	*S.nigrum* (U/ml)	Pineapple (U/ml)	PC (mg/ml) S. nigrum	PC (mg/ml) Pineapple	Specific S. nigrum	Specific Pineapple
distilled water	7.0	1.13	0.41	0.19	0.14	5.940	2.92
Tris maleate	7.0	2.06	0.73	0.26	0.17	7.921	4.29
Phosphate	7.0	2.61	0.86	0.21	0.20	12.42	4.30
Citratec Phosphate	7.0	2.13	0.68	0.29	0.18	7.344	3.77

PC. Protein content

TABLE 2.Purification steps of protease enzyme from *Solanum nigrum* fruit using ammonium sulfate and gel filtration

Purification steps	Volume (ml)	Protease (U/ml)	PC (mg/ml)	Total Activity	Total PC	Sp. Protease	Yield (%)	Rate purification
Crude enzyme Homogenate	50	2.61	0. 21	130.5	10.5	12.43	100.0	1.00
Ammonium sulfate saturation (60%)	10	8.11	0.53	81.10`	5.30	15.30	62.14	1.23
Sephadex G -100	5	4.53	0.05	22.65	0.25	90.60	17.35	7.28

PC. Protein content. **Sp**. Specific activity

TABLE 3. Purification steps of protease enzyme from pineapple fruit using ammonium sulfate and gel filtration

Purification Steps	Volume (ml)	Protease (U/ml)	PC (mg/ml)	Total Activity	Total PC	Sp. Protease	Yield (%)	Rate purification
Crude enzyme Homogenate	50	0.86	0.17	43.00	8.50	5.051	100.0	1.00
ammonium sulfate saturation (70%)	10	2.61	0.48	26.10	4.80	5.437	60.69	1.07
Sephadex G -100	5	1.70	0.07	8.50	0.35	24.28	19.76	4.80

PC. Protein content. **Sp**. Specific activity

TABLE 4. Changes of chemical composition of Ras cheese treated with different proteases concentrations

Parameter	Storage period (Days)	Treatments				
		Control	T1	T2	T3	T4
Moisture (%)	Fresh	$42.38^{a}_{\pm0.08}$	$43.34^{a}_{\pm0.47}$	$41.18^{a}_{\pm0.74}$	$44.05^{a}_{\pm0.62}$	$42.71^{a}_{\pm0.62}$
	30	$41.86^{ab}_{\pm0.27}$	$42.15^{b}_{\pm0.57}$	$39.97^{b}_{\pm0.24}$	$42.19^{b}_{\pm0.27}$	$41.98^{b}_{\pm0.27}$
	60	$40.56^{b}_{\pm0.82}$	$39.34^{c}_{\pm0.33}$	$39.13.^{c}_{\pm0.19}$	$41.92^{c}_{\pm0.22}$	$41.58^{c}_{\pm0.22}$
	90	$39.46^{c}_{\pm0.56}$	$38.75^{d}_{\pm0.52}$	$38.17^{d}_{\pm0.48}$	$40.45^{d}_{\pm0.56}$	$40.28^{d}_{\pm0.56}$
	120	$37.55^{d}_{\pm0.49}$	$38.26^{d}_{\pm0.04}$	$36.47^{e}_{\pm0.67}$	$38.51^{e}_{\pm0.77}$	$39.17^{e}_{\pm0.77}$
SE		0.29	0.25	0.29	0.30	0.30
pH	Fresh	$5.85^{a}_{\pm0.03}$	$5.79^{a}_{\pm0.01}$	$5.81^{a}_{\pm0.01}$	$5.75^{a}_{\pm0.01}$	$5.69^{a}_{\pm0.01}$
	30	$5.50^{b}_{\pm0.04}$	$5.43^{b}_{\pm0.04}$	$5.39^{b}_{\pm0.01}$	$5.42^{b}_{\pm0.55}$	$5.45^{b}_{\pm0.55}$
	60	$5.09^{c}_{\pm0.04}$	$5.24^{c}_{\pm0.10}$	$5.23^{c}_{\pm0.12}$	$5.06^{bc}_{\pm0.02}$	$5.09^{bc}_{\pm0.02}$
	90	$4.89^{d}_{\pm0.10}$	$4.65^{d}_{\pm0.10}$	$4.73^{d}_{\pm0.07}$	$4..65^{bc}_{\pm0.03}$	$4..69^{bc}_{\pm0.03}$
	120	$4.51^{e}_{\pm0.08}$	$4.47^{e}_{\pm0.02}$	$4.42^{e}_{\pm0.01}$	$4.36^{c}_{\pm0.01}$	$4.04^{c}_{\pm0.01}$
SE		0.039	0.06	0.035	0.059	0.059
Titratable acidity (%)	Fresh	$0.90^{e}_{\pm0.01}$	$0.86^{e}_{\pm0.03}$	$0.87^{e}_{\pm0.01}$	$0.82^{e}_{\pm0.01}$	$0.85^{e}_{\pm0.01}$
	30	$1.10^{d}_{\pm0.04}$	$1.14^{d}_{\pm0.09}$	$1.24^{d}_{\pm0.04}$	$1.14^{d}_{\pm0.10}$	$1.28^{d}_{\pm0.10}$
	60	$1.43^{c}_{\pm0.15}$	$1.62^{c}_{\pm0.20}$	$1.60^{c}_{\pm0.04}$	$1.75^{c}_{\pm0.20}$	$1.82^{c}_{\pm0.20}$
	90	$1.72^{b}_{\pm0.13}$	$1.91^{b}_{\pm0.02}$	$1.86^{b}_{\pm0.10}$	$1.91^{b}_{\pm0.02}$	$202^{b}_{\pm0.02}$
	120	$2.15^{a}_{\pm0.07}$	$2.27^{a}_{\pm0.06}$	$2.18^{a}_{\pm0.05}$	$2.32^{a}_{\pm0.05}$	$2.26^{a}_{\pm0.05}$
SE		0.057	0.039	0.038	0.14	0.14
TN (%)	Fresh	$3.52^{e}_{\pm0.07}$	$3.44^{d}_{\pm0.19}$	$3.81^{d}_{\pm0.10}$	$3.55^{e}_{\pm0.11}$	$3.77^{e}_{\pm0.11}$
	30	$3.88^{d}_{\pm0.08}$	$3.86^{c}_{\pm0.18}$	$3.94^{c}_{\pm0.06}$	$3.82^{d}_{\pm0.03}$	$3.93^{d}_{\pm0.03}$
	60	$4.03^{c}_{\pm0.03}$	$3.95^{bc}_{\pm0.07}$	$4.03^{c}_{\pm0.04}$	$3.99^{c}_{\pm0.06}$	$3.96^{c}_{\pm0.06}$
	90	$4.11^{b}_{\pm0.03}$	$4.13^{b}_{\pm0.11}$	$4.21^{b}_{\pm0.04}$	$4.21^{b}_{\pm0.06}$	$4.17^{b}_{\pm0.06}$
	120	$4.15^{a}_{\pm0.05}$	$4.28^{a}_{\pm0.04}$	$4.38^{a}_{\pm0.13}$	$4.32^{a}_{\pm0.04}$	$4.45^{a}_{\pm0.04}$
SE		0.034	0.078	0.048	0.039	0.039
SN (%)	Fresh	$0.24^{e}_{\pm0.02}$	$0.34^{e}_{\pm0.02}$	$0.39^{d}_{\pm0.05}$	$0.47^{d}_{\pm0.05}$	$0.57^{d}_{\pm0.05}$
	30	$0.33^{d}_{\pm0.02}$	$0.37^{d}_{\pm0.08}$	$0.49^{d}_{\pm0.05}$	$0.61^{c}_{\pm0.01}$	$0.60^{c}_{\pm0.01}$
	60	$0.42^{c}_{\pm0.01}$	$0.68^{c}_{\pm0.05}$	$0.68^{c}_{\pm0.06}$	$0.77^{c}_{\pm0.06}$	$0.75^{c}_{\pm0.06}$
	90	$0.65^{b}_{\pm0.04}$	$0.85^{b}_{\pm0.06}$	$0.97^{b}_{\pm0.07}$	$1.023^{b}_{\pm0.05}$	$1.10^{b}_{\pm0.05}$
	120	$0.84^{a}_{\pm0.03}$	$0.92^{a}_{\pm0.03}$	$1.18^{a}_{\pm0.03}$	$1.22^{a}_{\pm0.05}$	$1.28^{a}_{\pm0.05}$
SE		0.013	0.033	0.033	0.027	0.027
TVFA	Fresh	$14.00^{e}_{\pm2.0}$	$16.66^{e}_{\pm1.5}$	$16.11^{d}_{\pm3.1}$	$17.45^{d}_{\pm3.1}$	$16.67^{d}_{\pm3.1}$

Parameter	Storage period (Days)	Treatments				
		Control	T1	T2	T3	T4
(0.1 ml NaOH/ 100g)	30	$15.33^{d}_{\pm2.5}$	$20.33^{d}_{\pm2.5}$	$21.00^{c}_{\pm2.7}$	$22.02^{c}_{\pm1.6}$	$22.47^{c}_{\pm1.6}$
	60	$20.34^{c}_{\pm2.5}$	$24.00^{c}_{\pm2.0}$	$21.66^{b}_{\pm8.3}$	$23.10^{b}_{\pm2.2}$	$27.00^{b}_{\pm2.2}$
	90	$22.00^{b}_{\pm2.0}$	$25.34^{b}_{\pm6.4}$	$24.33^{b}_{\pm5.6}$	$25.69^{a}_{\pm2.9}$	$28.33^{a}_{\pm2.9}$
	120	$23.00^{a}_{\pm3.6}$	$26.33^{a}_{\pm3.0}$	$26.00^{a}_{\pm2.3}$	$29.40^{a}_{\pm2.3}$	$32.00^{a}_{\pm2.3}$
SE		1.49	2.04	2.87	3.44	3.44
Ash (%)	Fresh	$4.39^{d}_{\pm0.02}$	$4.48^{d}_{\pm0.02}$	$4.52^{d}_{\pm0.02}$	$4.56^{d}_{\pm0.03}$	$4.62^{d}_{\pm0.03}$
	30	$4.46^{c}_{\pm0.03}$	$4.49^{d}_{\pm0.02}$	$4.59^{c}_{\pm0.04}$	$4.59^{c}_{\pm0.04}$	$4.63^{c}_{\pm0.04}$
	60	$4.62^{b}_{\pm0.03}$	$4.56^{c}_{\pm0.02}$	$4.64^{c}_{\pm0.03}$	$4.66^{b}_{\pm0.03}$	$4.72^{b}_{\pm0.03}$
	90	$4.63^{a}_{\pm0.03}$	$4.64^{b}_{\pm0.04}$	$4..69^{b}_{\pm0.04}$	$4.68^{a}_{\pm0.07}$	$4.72^{a}_{\pm0.07}$
	120	$4.72^{a}_{\pm0.2}$	$4.69^{a}_{\pm0.03}$	$4.79^{a}_{\pm0.07}$	$4.81^{a}_{\pm0.02}$	$4.85^{a}_{\pm0.02}$
SE		0.016	0.017	0.026	0.025	0.025

Control, Ras cheese without protease enzyme, Ras cheese supplemented with 0.06, 0.08% for each of the protease enzyme from the bromelain (T1 and T2) and *Solanum nigrum* (T3 and T4), respectively. SE, standard error

TABLE 5. Textural profile analysis of Ras cheese treated with different proteases concentration.

Textural properties	Ripening Period (day)	Treatments				
		Control	T1	T2	T3	T4
Hardness N	**fresh**	$6.46^{c}_{\pm0.03}$	$5.87^{c}_{\pm0.073}$	$6.53^{c}_{\pm0.01}$	$6.89^{c}_{\pm0.08}$	$6.85^{c}_{\pm0.02}$
	60	$11.82^{b}_{\pm0.08}$	$12.46^{b}_{\pm0.08}$	$10.85^{b}_{\pm0.02}$	$11.06^{b}_{\pm0.05}$	$10.09^{b}_{\pm0.08}$
	120	$15.31^{a}_{\pm0.25}$	$14.29^{a}_{\pm0.09}$	$13.56^{a}_{\pm0.07}$	$13.35^{a}_{\pm0.02}$	$12.68^{a}_{\pm0.01}$
SE		0.014	0.09	0.082	0.27	0.066
Cohesiveness A area)/(B	**fresh**	$0.436^{b}_{\pm0.013}$	$0.460^{b}_{\pm0.015}$	$0.429^{b}_{\pm0.027}$	$0.432^{c}_{\pm0.02}$	$0.419^{b}_{\pm0.01}$
	60	$0.601^{a}_{\pm0.016}$	$0.531^{a}_{\pm0.021}$	$0.525^{a}_{\pm0.018}$	$0.570^{b}_{\pm0.011}$	$0.538^{a}_{\pm0.02}$
	120	$0.676^{a}_{\pm0.02}$	$0.595^{a}_{\pm0.023}$	$0.586^{a}_{\pm0.019}$	$0.669^{a}_{\pm0.017}$	$0.592^{a}_{\pm0.023}$
SE		0.014	0.037	0.21	0.11	0.018
Springiness Mm	**fresh**	$0.822^{b}_{\pm0.018}$	$0.733^{b}_{\pm0.033}$	$0.672^{b}_{\pm0.02}$	$0.730^{c}_{\pm0.41}$	$0.772^{c}_{\pm0.021}$
	60	$0.882^{b}_{\pm0.02}$	$0.768^{b}_{\pm0.031}$	$0.707^{a}_{\pm0.02}$	$0.832^{b}_{\pm0.53}$	$0.814^{b}_{\pm0.072}$
	120	$1.042^{a}_{\pm0.021}$	$0.821^{a}_{\pm0.025}$	$0.759^{a}_{\pm0.02}$	$0.907^{a}_{\pm0.051}$	$0.916^{a}_{\pm0.033}$
SE		0.12	0.013	0.012	0.014	0.017
Gumminess N	**fresh**	$2.83^{c}_{\pm0.022}$	$2.68^{c}_{\pm0.041}$	$2.80^{c}_{\pm0.027}$	$2.98^{c}_{\pm0.072}$	$2.87^{c}_{\pm0.033}$
	60	$7.06^{b}_{\pm0.027}$	$6.62^{b}_{\pm0.029}$	$5.33^{b}_{\pm0.021}$	$6.30^{b}_{\pm0.063}$	$5.44^{b}_{\pm0.028}$
	120	$10.36^{a}_{\pm0.034}$	$8.54^{a}_{\pm0.035}$	$8.02^{a}_{\pm0.023}$	$8.95^{a}_{\pm0.071}$	$7.35^{a}_{\pm0.021}$
SE		0.011	0.088	0.09	0.12	0.11
Chewiness mm/N	**fresh**	$2.31^{c}_{\pm0.021}$	$1.96^{c}_{\pm0.08}$	$1.88^{c}_{\pm0.033}$	$2.18^{c}_{\pm0.021}$	$2.22^{c}_{\pm0.043}$
	60	$6.22^{b}_{\pm0.029}$	$5.08^{b}_{\pm0.06}$	$3.77^{b}_{\pm0.048}$	$5.24^{b}_{\pm0.028}$	$4.43^{b}_{\pm0.034}$
	120	$10.69^{a}_{\pm0.032}$	$7.01^{a}_{\pm0.03}$	$6.09^{a}_{\pm0.09}$	$8.12^{a}_{\pm0.029}$	$6.72^{a}_{\pm0.052}$
SE		0.078	0.043	0.024	0.55	0.061

Control, Ras cheese without protease enzyme, Ras cheese supplemented with 0.06, 0.08% for each of the protease enzyme from the bromelain (T1 and T2) and *Solanum nigrum* (T3 and T4), respectively. SE, standard error

TABLE 6. Microbial counts of Ras cheese treated with different proteases concentration during storage for 120 days.

Microbial (log cfu/g)	Treatments	Storage period (day)					
		0	30	60	90	120	SE
Total count	**Control**	$6.13^{a}_{\pm0.04}$	$6.08^{a}_{\pm0.02}$	$6.01^{a}_{\pm0.09}$	$5.91^{a}_{\pm0.02}$	$5.86^{b}_{\pm0.04}$	0.34
	T1	$6.14^{a}_{\pm0.12}$	$6.09^{a}_{\pm0.14}$	$6.02^{a}_{\pm0.11}$	$5.94^{b}_{\pm0.11}$	$5.86^{b}_{\pm0.13}$	0.49
	T2	$6.29^{a}_{\pm0.13}$	$6.23^{a}_{\pm0.10}$	$6.15^{b}_{\pm0.09}$	$5.91^{b}_{\pm0.12}$	$5.72^{c}_{\pm0.11}$	0.43
	T3	$6.38^{a}_{\pm0.10}$	$6.22^{a}_{\pm0.09}$	$6.01^{b}_{\pm0.07}$	$5.92^{b}_{\pm0.08}$	$5.80^{b}_{\pm0.10}$	0.31
	T4	$6.14^{a}_{\pm0.11}$	$6.09^{a}_{\pm0.13}$	$6.00^{a}_{\pm0.09}$	$5.89^{b}_{\pm0.08}$	$5.77^{b}_{\pm0.11}$	0.30
Mold & yeast	**Control**	ND	ND	$4.86^{b}_{\pm0.09}$	$4.94^{b}_{\pm0.07}$	$5.08^{a}_{\pm0.09}$	0.40
	T1	ND	ND	$4.88^{b}_{\pm0.08}$	$4.99^{a}_{\pm0.07}$	$5.10^{a}_{\pm0.09}$	0.42
	T2	ND	ND	$4.45^{b}_{\pm0.08}$	$4.54^{a}_{\pm0.11}$	$4.62^{a}_{\pm0.09}$	0.40
	T3	ND	ND	$4.77^{c}_{\pm0.05}$	$5.17^{b}_{\pm0.07}$	$5.36^{a}_{\pm0.08}$	0.40
	T4	ND	ND	$4.52^{a}_{\pm0.11}$	$4.48^{a}_{\pm0.10}$	$4.60^{a}_{\pm0.09}$	0.41

Control, Ras cheese without protease enzyme, Ras cheese supplemented with 0.06, 0.08% for each of the protease enzyme from the bromelain (T1 and T2) and *Solanum nigrum* (T3 and T4), respectively. SE, standard error

TABLE 7. Sensory evaluation of Ras cheese treated with different proteases concentration

Storage	Treatments

Characteristics	period (days)	Control	T1	T2	T3	T4
Appearance (10 points)	Fresh	$6.49^{c}_{\pm0.43}$	$7.06^{d}_{\pm0.10}$	$6.86^{d}_{\pm0.24}$	$6.79^{d}_{\pm0.26}$	$6.46^{d}_{\pm0.26}$
	30	$6.87^{bc}_{\pm0.16}$	$7.47^{c}_{\pm0.15}$	$7.41^{c}_{\pm0.20}$	$7.28^{c}_{\pm0.15}$	$6.94^{c}_{\pm0.15}$
	60	$7.29^{b}_{\pm0.30}$	$7.67^{b}_{\pm0.35}$	$7.67^{c}_{\pm0.08}$	$7.60^{b}_{\pm0.09}$	$7.26^{b}_{\pm0.09}$
	90	$7.84^{a}_{\pm0.12}$	$8.45^{a}_{\pm0.06}$	$7.72^{b}_{\pm0.23}$	$7.78^{a}_{\pm0.04}$	$7.45^{a}_{\pm0.04}$
	120	$8.23^{a}_{\pm0.10}$	$8.72^{a}_{\pm0.08}$	$7.82^{a}_{\pm0.09}$	$8.60^{a}_{\pm0.04}$	$7.93^{a}_{\pm0.04}$
SE		0.15	0.10	0.10	0.084	0.084
Body and texture (40 points)	Fresh	$31.68^{b}_{\pm01.10}$	$34.55^{d}_{\pm0.74}$	$33.56^{b}_{\pm0.74}$	$32.55^{b}_{\pm1.26}$	$32.21^{b}_{\pm1.26}$
	30	$32.16^{b}_{\pm1.23}$	$34.89^{cd}_{\pm0.45}$	$33.89^{b}_{\pm0.81}$	$32.89^{b}_{\pm1.23}$	$32.22^{b}_{\pm1.23}$
	60	$33.44^{ab}_{\pm0.64}$	$35.61^{bc}_{\pm0.48}$	$34.28^{b}_{\pm0.24}$	$33.94^{a}_{\pm0.82}$	$33.28^{a}_{\pm0.82}$
	90	$34.26^{a}_{\pm0.02}$	$36.36^{ab}_{\pm1.14}$	$35.36^{a}_{\pm0.02}$	$34.36^{a}_{\pm0.02}$	$34.03^{a}_{\pm0.02}$
	120	$34.29^{a}_{\pm0.55}$	$37.42^{a}_{\pm0.09}$	$35.09^{a}_{\pm0.09}$	$34.75^{a}_{\pm0.49}$	$34.42^{a}_{\pm0.49}$
SE		0.48	0.39	0.29	0.51	0.51
Flavor (50 points)	Fresh	$39.34^{c}_{\pm0.69}$	$41.67^{d}_{\pm0.78}$	$40.67^{c}_{\pm0.62}$	$41.01^{b}_{\pm0.43}$	$40.01^{b}_{\pm0.43}$
	30	$40.84^{b}_{\pm0.16}$	$43.17^{c}_{\pm0.72}$	$42.17^{b}_{\pm0.46}$	$42.17^{b}_{\pm0.72}$	$40.51^{b}_{\pm0.72}$
	60	$42.04^{a}_{\pm0.98}$	$44.38^{bc}_{\pm0.54}$	$42.71^{ab}_{\pm0.48}$	$44.71^{a}_{\pm0.84}$	$41.51^{a}_{\pm0.84}$
	90	$42.06^{a}_{\pm0.45}$	$45.04^{b}_{\pm0.95}$	$43.71^{a}_{\pm0.86}$	$45.38^{a}_{\pm0.95}$	$42.38^{a}_{\pm0.95}$
	120	$42.47^{a}_{\pm0.48}$	$45.47^{a}_{\pm0.58}$	$43.81^{a}_{\pm0.67}$	$46.47^{a}_{\pm0.11}$	$42.81^{a}_{\pm0.11}$
SE		0.35	0.42	0.34	0.39	0.39
Collective total score (100 points)	Fresh	$77.51^{c}_{\pm0.71}$	$83.28^{c}_{\pm1.39}$	$81.09^{c}_{\pm0.28}$	$80.35^{b}_{\pm0.81}$	$78.68^{b}_{\pm0.81}$
	30	$79.87^{c}_{\pm1.22}$	$85.83^{c}_{\pm1.12}$	$83.47^{b}_{\pm1.38}$	$82.34^{b}_{\pm1.55}$	$79.67^{b}_{\pm1.55}$
	60	$82.77^{b}_{\pm1.83}$	$87.66^{b}_{\pm0.76}$	$84.66^{b}_{\pm0.62}$	$86.25^{a}_{\pm1.68}$	$82.05^{a}_{\pm1.68}$
	90	$84.16^{ab}_{\pm0.34}$	$89.85^{ab}_{\pm2.09}$	$86.79^{a}_{\pm1.05}$	$87.53^{a}_{\pm0.89}$	$83.86^{a}_{\pm0.89}$
	120	$84.99^{a}_{\pm0.82}$	$91.61^{a}_{\pm0.59}$	$86.72^{a}_{\pm0.82}$	$89.83^{a}_{\pm0.38}$	$85.16^{a}_{\pm0.38}$
SE		0.64	0.75	0.52	0.67	0.67

Control, Ras cheese without protease enzyme, Ras cheese supplemented with 0.06, 0.08% for each of the protease enzyme from the bromelain (T1 and T2) and *Solanum nigrum* (T3 and T4), respectively. SE, standard error

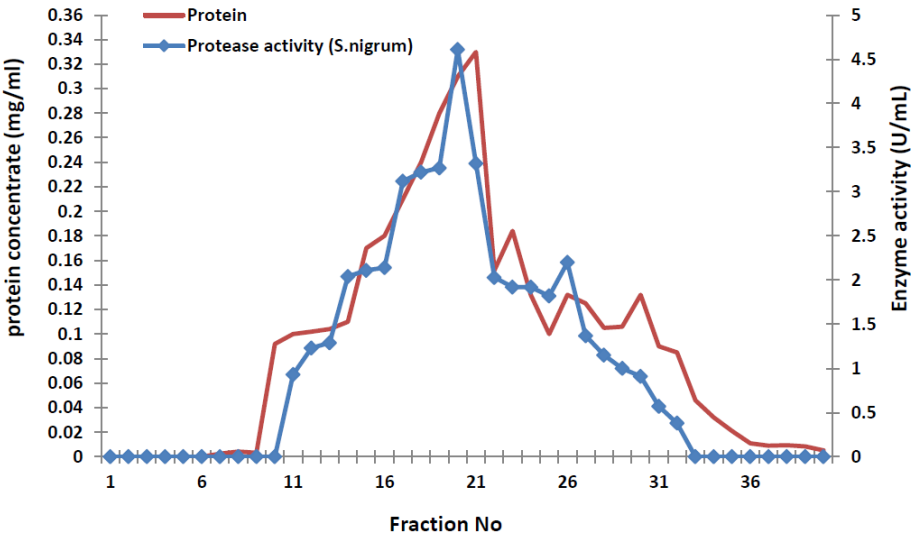

Fig. 1. Gel filtration for the chromatography of protease activity from *S. nigrum* on a Sephadex G-100.

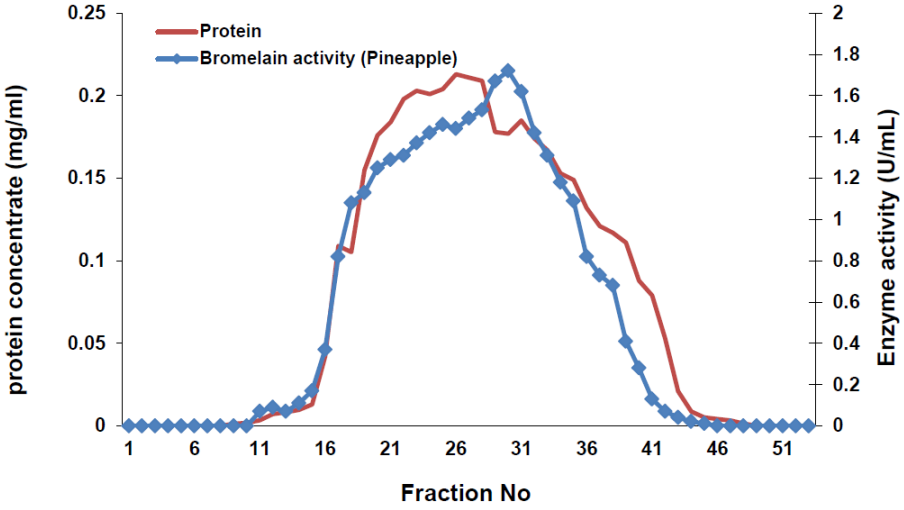

Fig. 2. Gel filtration for the chromatography of bromelain activity from pineapple on a Sephadex G-100.

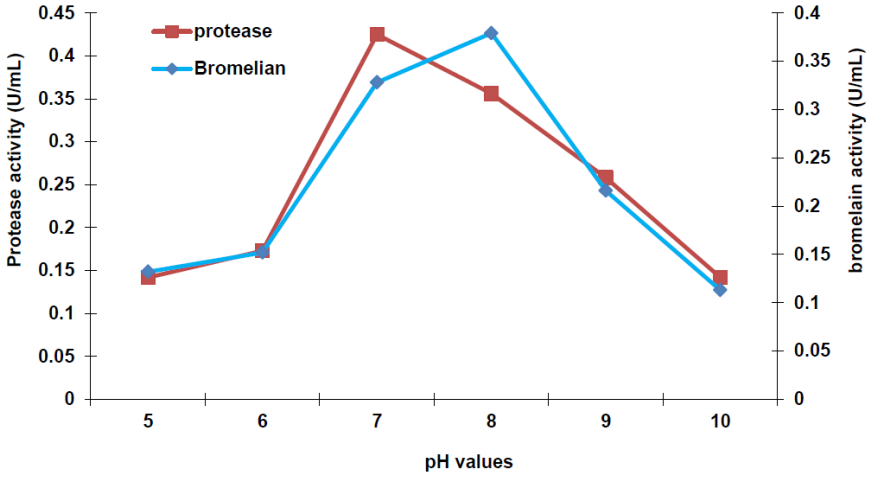

Fig.3. Effect of pH values on purified protease and bromelain enzymes

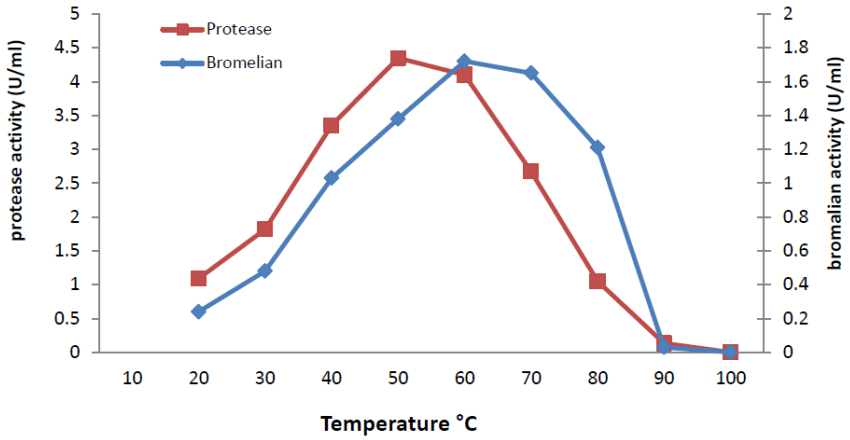

Fig. 4. Effect of temperatures on purified protease and bromelain enzymes

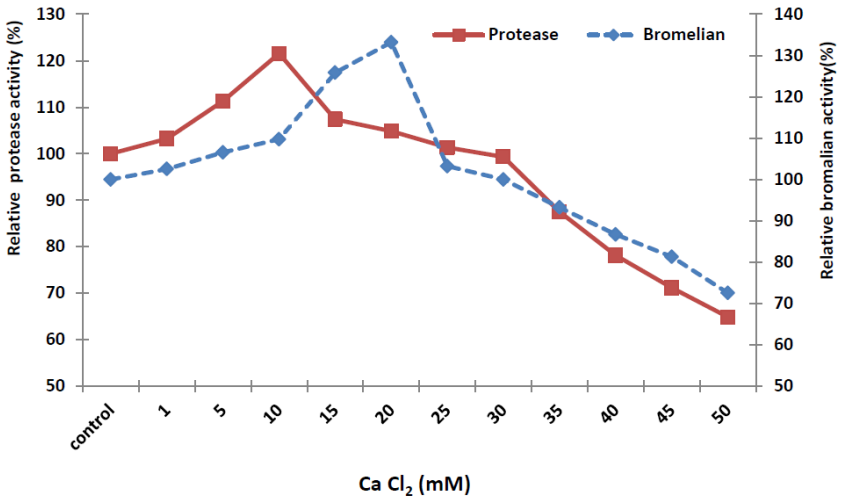

Fig. 5. Effect of Ca Cl_2 concentrations on the purified protease and bromelain enzymes

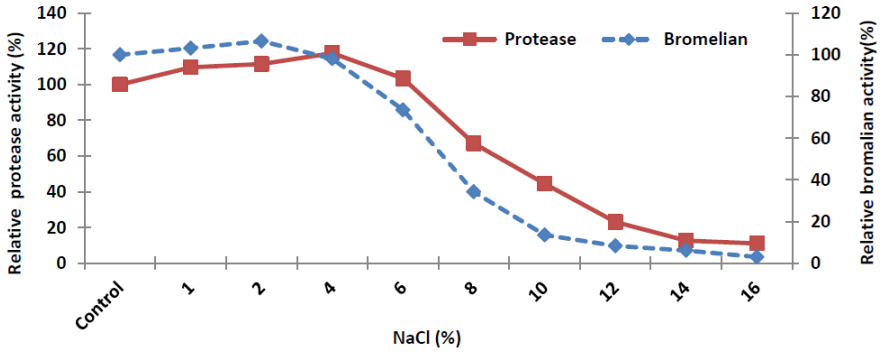

Fig. 6. Effect of NaCl concentrations on the purified protease and bromelain enzymes

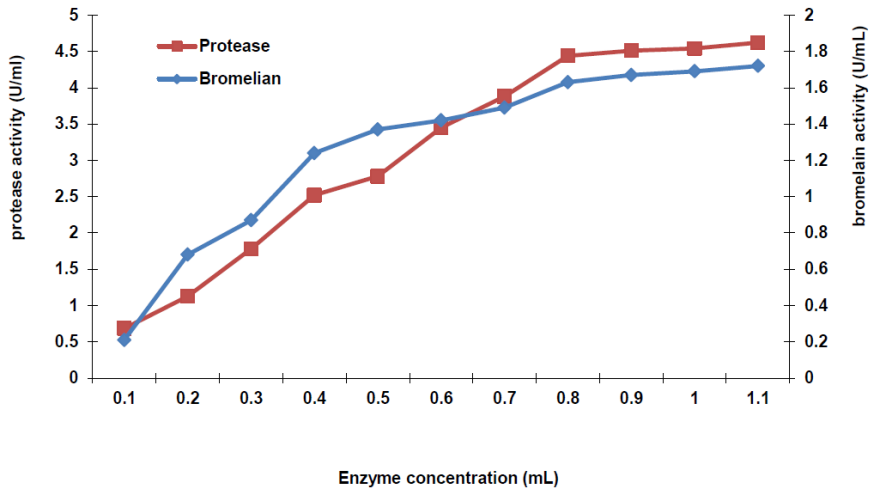

Fig. 7. Effect of different concentration enzymes on the purified extract of protease and bromelain enzyme

References

1. Gonzalez-Rabade, N., Badillo-Corona, J.A., Aranda-Barradas, J.S and del Carmen Oliver- Salvador, M. Production of plant proteases in vivo and in vitro - A review. *Biotechnology Advances*, **29**(6), 983 (2011).
2. Komansilan, S., Djalal, R., Lilik, E and Radiati dan Purwadi. The Effect of Ananas comucus Extracted Bromelain Enzyme Addition Under Different PH On The Physicochemical Properties Of Cottage Cheese. *Jurnal Ilmu dan Teknologi Hasil Ternak.* **15(**1),38– 45 (2020).
3. Turk, B. Targeting proteases: successes, failures and future prospects. *Nat. Rev. Drug Discovery*, **5**, 785 (2006).
4. Ward, O.P. Proteases," in Comprehensive Biotechnology, M.-Y. Murray, Ed., pp. 571–582, Academic Press, Burlington, ass, USA, 2nd edition, (2011)
5. Maurer, H.R. Bromelain: biochemistry, pharmacology and medical use. *Cellular and Molecular Life Sciences*, **58**(9), 1234–1245(2001).
6. Silvestre, M.P.C., Carreira, R.L., Silva, M.R., Corgosinho, F.C., Monteiro, M.R.P and Morais, H.A. Effect of pH and temperature on the activity of enzymatic extracts from pineapple peel. *Food Bioprocess Tech.,* **5**, 1824-1831(2012). https://doi. org/10.1007/s11947-011-0616-5
7. Gil, L.S and Maupoey, P.F. An integrated approach for pineapple waste valorisation. Bioethanol production and bromelain extraction from pineapple residues. *Journal of Cleaner Production*, **172**, 1224-1231. (2018). https//: doi.org/10.1016/j.jclepro.2017.10.284,
8. Chaurasiya, R.S and Hebbar, H.U. Extraction of bromelain from pineapple core and purification by RME and precipitation methods. *Separation and Purification Technolog.* pp 97-90 ،(2013). https://doi.org/10.1016/j. seppur.2013.03.029,
9. Tap, F.M., Majid, F.A.A and Khairudin, N.B.A. Structure prediction of Stem Bromelain from pineapples) Ananas Comosus) using procaricain enzyme as a modelling template. *International Journal of Applied Engineering Research*, **9**, 6109-6111(2016).
10. Kholif, A.M.M., El-Metwally, M.M and Mohamed, D.M. 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. *The Arab Journal of Agricultural Sciences*. **7** (24), 231 -270 (2024).
11. Abbas, S., Shanbhag, T and Kothare, A, Applications of bromelain from pineapple waste towards acne. *Saudi Journal of Biological Sciences,* **28**, 1001–1009 (2021).
12. Jacob, M. and Jarosand Rohm, H. Recent advances in milk clotting enzyme. International *Journal of Dairy Technology,* **64**,14-33(2011).
13. Khattab, A.R., Guirguis, H.A., Tawfik. S.M. and Farag, M.A. Cheese ripening: a review on modern technologies towards flavor enhancement, process acceleration and improved quality assessment. *Trends in Food Science & Technology,* **88**,343–360 (2019).
14. Law, B.A. Accelerated ripening of cheese. In Advances in the microbiology and biochemistry of cheese and fermented milk. *Elsevier Appl. Sci. Pub., London*, 209-228, (1984).
15. Alkhalaf, W., Piard, J., El soda, M., Gripon, J., Desmazeaud, M. and Vassal, L. Liposomes as proteinase carriers for the accelerated ripening of Saint-Paulin type cheese. *Journal of Food Science*., **53**, 1674-1679 (1988).
16. Law, B.A. and Wigmore, A.S., Accelerated ripening of Cheddar cheese with a commercial proteinase and intracellular enzymes from starter streptococci. *Journal of Dairy Research*, **50**, 519-525(1983).
17. Phelan, J.A., Renaud, J. and Fox, P.F. Some non-European cheese varieties. *In Cheese: Chemistry, physics and microbiology* (Vol. 2 Major Cheese Groups, pp. 421–465, (1993).
18. El-Hofi, M.A., Ismail, A.A., AbdRabo, F.H., El-Dieb, S.M. and Ibrahim, O.A. Studies on acceleration of Ras cheese ripening by aminopeptidase enzyme from buffaloes' pancreas. II-Utilization of buffaloes' pancreas aminopeptidase in acceleration of Ras cheese ripening. *Journal of American Science*, **6**(9), 575–581(2010).
19. El‑Sayed, S.M., Kholif, A.M.M., El‑Sayed, H.S and Youssef, A,M, Augmenting the Quality and Shelf Life of Ras Cheese by Adding Microencapsulated Allspice Berry Extract Nanoemulsion. *Food and Bioprocess Technology*. Pp 1-17. https://doi.org/10.1007/s11947-024-03473-2, (2024)
20. Crow, V.L., Coolbear, T., Holland, R., Pritchard, G.G. & Martley, F.G. Starters as finishers: Starter properties relevant to cheese ripening. *International Dairy Journal*, **3**, 423–460(1993).
21. Cheded, M.A. Effect of vegetable rennet on some characters of cheese, M.Sc, *Facurty of Agricurture, Ain-Shams Univ*, (1975)
22. Gomori, G. Preparation of Buffers for Use in Enzyme Studies, *Handbook of Biochemistry and Molecular Biology*, (1955)
23. Abdalla, M.O.M., Kheir, S.E.O., and El Owni, OAO. Effect of Extraction Method, Ammonium Sulfate Concentration, Temperature and pH on Milk-Clotting Activity of *Solanum dubium* Fruit Extract. *Advance Journal of Food Science and Technology,* **3**(1), 40-44(2011).

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

25. Chopra, A.K and Mathur, D.K. Factors affecting protease production by Bacillus stearothermophilus RM-67. *Journal of Food Protection,* **116**, 1020-1025 (1983).

26. Bradford, M.M., 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(1976).

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

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

29. Hofi, A.A., Youssef, E.H., Ghoneim, M.A. and Tawab, G.A. Ripening changes in cephalotyre "RAS" cheese manufactured from raw and pasteurized milk with special reference to flavor. *Journal of Dairy Science*, **53**, 1207-1211(1970).

30. Ling, E.R., A Text Book of Dairy Chemistry 3rd Ed. Vol. 2. practical, pp. 76-98.Chapman and Hall. Ltd London, UK. (1963).

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

32. Richardson, G.H. Standard Methods for the Examination of Dairy Products. 15th Edition, *American Public Health Association*, Washington DC, p. 327, (1985).

33. Kosikowski, F.V. Cheddar Cheese from Water Reconstituted Retentates. *Dairy Science,* **63**,1975-1980(1978).

34. Bourne, M.C. Texture Profile Analysis. *Food Technology*, **32**, 62-66, 72 (1978).

35. Standard Methods for the Examination of Dairy Products Paperback – Import, *American Public Health Association*; 16th edition

36. Bodyfelt, F.W. Dairy Product Score Cards: Are They Consistent with Principles of Sensory Evaluation. *Journal of Dairy Science,* **64**, 2303-2308(1981).

37. SAS Institute, SAS User's Guid / STAT Ver. 6.044th ed. SAS Inst. Inc. Cary, NC, (1990).

38. Gamarra, F.M.C., Santana, J.C.C., Llanos, S.A.V., Pérez, J.A.H., Flausino, F.R., Quispe, A.P.B., Mendoza, P.C., Vanalle, R.M.، Carreño-Farfan, C., Berssaneti, F.T., de Souza, R.R. and Tambourgi, E.B. High Retention and Purification of Bromelain Enzyme (Ananas comosus L. Merrill) from Pineapple Juice Using Plain and Hollow Polymeric Membranes Techniques. *Polymers*, **14**, 264(2022). https://doi.org/10.3390/polym14020264,

39. Sangeetha, R., Geetha, A. and Arulpandi, I. Concomitant production of protease and lipase by bacillus licheniformis vsg1: production, purification and characterization. *Brazilian Journal of Microbiology*, **41**, 179-185(2010).

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

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

42. Otani, H., Matsumori, M and Hosono, A. Purification and some properties of a milk-clotting protease from the young seeds of Albizia julibeissin. *Animal Science and Technology*., (Japan), **62**(5), 424-432 (1991).

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

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

45. Abdeldaiem, A.M., El-Bagoury, E.H., Abbas, F. and Faisal, M.A. Effect of some Factors on the Proteolytic Activities of Bromelain, Cichorium and Papain Extracts. Ismailia *Journal of Dairy Science & Technology*; **6** (1), 1-7(2019).

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

47. Naidu, K.S. Characterization and Purification of Protease enzyme. *Journal of Applied Pharmaceutical Science,* 0**1** (03), 107-112(2011).

48. Kumari Narwal, R., Bharat, B., Ajay, P., Anil, P. and Sarla, M. Purification, physico-chemico-kinetic characterization and thermal inactivation thermodynamics of milk clotting enzyme from Bacillus subtilis MTCC 10422. LWT - *Food Science and Technology,* **65**, 652-660(2016).

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

50. Yadav, R.P., Patel, A.K. and Jagannadham, M.V. Neriifolin S, a dimeric serine protease from Euphorbia neriifolia Linn.: Purification and biochemical characterization. *Food Chemistry*, **132**, 1296–1304(2012).

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

52. Vairo Cavalli, S., Silva, S.V., Cimino, C., Malcata, F.X. and Priolo, N. Extraction and partial characterization of a coagulant preparationfrom Silybum marianum flowers. Its action on bovine caseinate. *Journal of Dairy Research*, **72**(3), 271-275 (2005).

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

54. Zoon, P., Van Vliet, T. and Walstra, P. Rheological properties of rennet-induced skim milk gels. *Neth Milk Dairy J.,* **43**, 17-34(1989).

55. Najera, A.I., De-Renobales, M. and Barron, L.J.R. Effect of pH, temperature, CaCl2 and enzyme concentrations on the rennet-clotting properties of milk: a multifactorial study. *Food Chemistry*, **80**, 345-352 (2003).

56. Abou-zeid, N.A. The use of plant enzymes for ripening acceleration of Ras cheese. *OALIb Journal*; **6**(2), 15–21 (2015).

57. El-Sayed, S.M., Ibrahim, O.A. and Kholif, A.M.M. Characterization of novel Ras cheese supplemented with Jalapeno red pepper. *Journal of Food Process Preserve,* 00, e14535(2020). https://doi.org/10.1111/jfpp.14535.

58. Basiony, M. and Hassabo, R. Composition and quality of low-fat halloumi cheese made using modified starch as a fat replacer. *Starch,* **74**, 2100211 (2021).

59. Meng, F., Chen, R., Zhu, X., Lu, Y., Nie, T., Lu, F. and Lu, Z.A. Newly effective milkclotting enzyme from Bacillus subtilis and its application in cheese-making. *Journal of Agricultural and Food Chemistry,* **66**, 6162–6169 (2018).

60. Abou-zeid, A.N. the use of plant enzymes for ripening acceleration of Ras cheese. *IIOAB Journal* **6**, 15–21(2021).

61. Katsiari, M.C., Voutsinas, L.P. and Kondyli, E. Improvement of sensory quality of low-fat Kefalograviera-type cheese with commercial adjunct culltues. *International Dairy Journal,* 12, 757–764 (2002).

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

63. Bryant A.Z. Ustunol and Steffe, J. Texture of Cheddar cheese as influenced by fat reduction. *Journal of Food Science*, **60**, 1216-1219(1995).

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

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

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

67. El-Shibiny, S., Abd El-Gawad, M.A., Assem, F.M and El-Sayed, S.M. 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 (2018).

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

69. El Nena, M., Awed, S.A., Abbas, M. and Attia, I.A. Production of Enzyme Modified Ras Cheese. *Alexandria Science Exchange Journal*, **34**(2), 249–254 (2013).

70. Saad, M.A., Abd-Rabiu, H.S., Elk tab, E., Rayan, A.M., Abedin, A., Abdelkader, A., Ibrahim, S.F. and Hussein, H. Occurrence of Toxic Biogenic Amines in Various Types of Soft and Hard Cheeses and Their Control by Bacillus polymyxin D05-1. *Fermentation*, **8**(7),327 (2022). https://doi.org/10.3390/fermentation8070327.

71. Alhelli, A.M., Mohammed, N.K., Khalil, E.S. and Hussin, A.S.M. Optimizing the acceleration of Cheddar cheese ripening using response surface methodology by microbial protease without altering its quality features. *AMB Express*, **11**, 45(2021). https://doi.org/10.1186/s13568-021-01205-9

إسراع تسوية الجبن الرأس باستخدام بعض المستخلصات النباتية

عادل محمود محمد خليف*[1] ،علا محمد عادل كامل شلبى[2]، منيره محمود بسيونى[3] ومحمد محمد المتولى[3]

[1]قسم علوم الألبان – المركز القومى للبحوث – الدقى - جيزه - مصر.
[2]قسم الألبان- كلية الزراعة – جامعة المنصورة - مصر.
[3]قسم تكنولوجيا الألبان – معهد بحوث الإنتاج الحيوانى - مركز البحوث الزراعية- الدقى - مصر.

الملخص

تم الحصول على إنزيم البروتييز من ثمار نبات Solanum nigrum ونبات الأناناس، والذي يمتلك القدرة على التحلل البروتينى لاستخدامه فى إسراع تسوية الجبن الرأس. حيث تمت تنقية الانزيم عن طريق الترسيب الجزئي بواسطة كبريتات الأمونيوم للجزء الأكثر نشاطًا بنسبة 60 و70 ٪ على التوالي، متبوعًا باستخدام تقنية الاعمدة الكروماتوغرافية المتسلسلة بواسطة sephadex G 100 للجزء الأكثر نشاطًا، وأشارت النتائج إلى معدل التنقية كان 7.28 و4.80 ضعفًا مع محصول 17.35 و19.76 ٪ لانزيم البروتييز من نباتات Solanum nigrum والأناناس، على التوالي. ووجد أن درجة الحرارة المثلى للإنزيمين هي 50 و 60 درجة مئوية وكان نشاط الإنزيمين مستقرًا عند 20 إلى 80 درجة مئوية. وأظهر الإنزيمين درجة الـ pH المثلى عند 7 و 8، وكانت مستقرة تمامًا في نطاق واسع من الـ pH بين 6.0 إلى 9. وأعطى تأثير كلوريد الكالسيوم بتركيز 10 و 20 مليمول أعلى نشاط نسبي للإنزيمين من البروتييز المنقى حوالي 121.54 و 133.21 على التوالى. كما أعطى كلوريد الصوديوم بتركيز 3 و 2 ٪ أعلى نشاط نسبيل للانزيمين من البروتييز المنقى على التوالى. ومن دراسة تأثير تركيز الإنزيم يمكن استنتاج وجود علاقة موازية بين نشاط البروتييز وتركيز الإنزيمين على التوالي. وتم تصنيع الجبن الرأس وذلك بإضافة الإنزيمين المذكورين أعلاه بتركيزات مختلفة، وقد وجد أن الجبن الراس المعامل بالإنزيمات النباتية محل الدراسة قد طورت بروتينًا قابلًا للذوبان أعلى، وساهم إنزيم البروتييز النباتى المضاف في تحلل الكازين وكان النيتروجين الكلى والنيتروجين الذائب والأحماض الدهنية الطيارة مرتفعًا في الجبن المعامل بالانزيمين من البروتييز، وأظهرت الجبن المعامل بالانزيمات نكهة أفضل وقبولًا أكبر من الجبن الكنترول. وكان لزيادة معدل التحلل البروتيني في الجبن المعامل بالإنزيمات علاقة مباشرة بإسراع تسوية الجبن الرأس

الكلمات الدالة: نشاط إنزيم البروتييز، التنقية، Solanum nigrum، نبات الأناناس، إسراع التسوية.