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EVALUATION OF RENNET SUBSTITUTE FROM ARTICHOKE (*Cynara scolymus* L.) FLOWERS EXTRACTS: STUDY THE FACTORS AFFECTING THE ACTIVITY OF MILK CLOTTING

Heba-Allah H. Abdel-Raouf, A.A. El-Neshwy, A.M. Rabie and S.A. Khalifa*

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

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ABSTRACT: Factors affecting milk clotting activity of rennet substitute extracted from green bracts of artichoke (*Cynara scolymus*) flowers in different buffer solutions were evaluated. These factors include extraction pH, clotting temperature, extract concentrations, addition of $CaCl_2$, NaCl and glucono delta-lactone (GDL) at different concentrations. The proteolytic activity of different extract solutions and rheological properties of produced curd was studied as well. The obtained results indicated that the optimum clotting activity of artichoke crude extracts improved water holding capacity and susceptibility to syneresis obtained at pH value of 5.0-6.0, temperature of 65-70°C and 3%, 0.04-0.05, 3%, 0.5% of crude extract, $CaCl_2$, NaCl and GDL concentrations, respectively. The optimum clotting activity of artichoke crude extracts indicated that the sodium acetate buffer solution T4 (5% NaCl in sodium acetate buffer, pH 5.0) and sodium phosphate buffer, T5 (5% NaCl in sodium phosphate buffer, pH 5.5), T6 (5% NaCl in sodium phosphate buffer, pH 6.5), and T7 (5% NaCl in sodium phosphate buffer, pH 7). Results also indicated that artichoke crude extracted in sodium phosphate buffer solutions and sodium acetate buffer (T4) had higher proteolytic activity than other buffers. The best rheological properties of resultant curds were noticed in sodium phosphate buffers (T5, T6, T7) and sodium acetate buffer (T4).

Key words: Artichoke, *Cynara scolymus*, milk clotting activity, crude protein extracts, proteolytic activity, rheological analysis.

INTRODUCTION

Calf rennet is the most ancient known milk clotting enzyme and still the most widely used as biocatalyst in cheese making. The coagulating properties of calf rennet are related to the chymosin, an aspartic protease which considered the best coagulating agent thanks of its high specificity in cleaving the bond of κ-casein phenylalanine 105- methionine 106 bonds (Ahmed *et al*., 2009).

The worldwide increase in cheese production and consumption in the line with increasing the price of calf rennet led to search and investigate new sources of milk coagulating enzymes replacing calf rennet in cheese making (Guiama *et al.,* 2010). Plant extracts, and various new vegetable sources of enzymes for milk clotting have been studied in recent years (Hashim *et al.,* 2011).

The majority of these vegetable milk clotting enzymes belongs to the aspartic protease family (Simoes and Faro, 2004). *Ficus carica* (El-Shibiny *et al*., 1973), *Calm viscera* (Gupta and Eskin, 1977), *Ananas comosus* (Cattaneo *et al*., 1994), and *Carica papaya* (Cabezas, 1981) were used as plant sources for the milk clotting enzyme. However, these enzymes are undesirable in cheese making because of their high proteolytic activity and bitterness of produced cheese (Ahmed *et al*. 2009).

Artichokes (*Cynara scolymus* L.) are perennial, frost sensitive, thistle-like plants with edible part known as flower buds, which sprout from the terminal part of the major stem and on lateral stems. Each unopened flower bud

* **Corresponding author: Tel. :** +201229931517
E-mail address: salahkhalifa2006@yahoo.com

resembles a deep green pine cone, 7–10 cm in diameter, round, but slightly elongated. Several pointed, leathery green bracts fold around a purple-blue flower. Cynarases enzymes have been identified from artichoke (Verissimo *et al.* 1998), but because the flowers of this plant are usually consumed as a vegetable, the properties and possible applications of these cynarases are less known.

The use of flower extracts from *Cynara* genus and, in particular, of *Cynara scolymus* and *Cynara cardunculus*, is limited because of the multiplicity of aspartic protease molecular forms (Liorente *et al.*, 2014).

Shah *et al.* (2014) used plant proteases in cheese making in the form of crude or purified extract for many centuries.

In general, the enzymes used as milk coagulant have aspartic proteases as an essential enzyme, while cysteine and serine proteases have been reported as well, thanks to their ability of milk clotting under specific conditions.

Proteases are required by plants in all aspects of their life cycle. Proteases have been classified into many groups based on their catalytic mechanism used during the hydrolytic process. The principle catalytic types are aspartate, serine, cysteine, and metalloproteases (Bah *et al.*, 2006). Aspartic proteases have two main aspartic residues at their catalytic site. They are most active at acidic pH and show high preferential specificity for cleavage the peptide bonds between hydrophobic amino acid residues which are important for the catalytic activity (Domingos *et al.*, 2000). It produces cardosins, cyprosins and aspartic proteases which found to be accumulated in only mature flowers (petals and pistils) (Cordeiro *et al.*, 1998).

Cynarase isolated from artichoke (*Cynara scolymus*) has been reported in many previous studies for milk clotting activity as an aspartic protease with 2 aspartic acid residues at its catalytic site (Llorente *et al.*, 1997; 2004; Sidrach *et al.*, 2005; Chazarra *et al.*, 2007).

The extract of *C. scolymus* contains three proteases (cynarases A, B and C), three cynarases are glycoproteins in addition to their milk clotting activity (Sidrach *et al.*, 2005). The enzymes of coagulant activity may be decreased by the purification of plant extract compared to the crude extraction of cynarases A and C, however cynarase B increase its activity (Chazarra *et al*., 2007).

Therefore, the aim of the present study was to investigate the optimum extraction conditions of rennet substitute from the bracts of *Cynara scolymus* flowers and evaluating their clotting and proteolytic activity.

MATERIALS AND METHODS

Materials

Skim milk powder

Skim milk powder imported from USA extra grade, supplied by Egyptian Company for Dairy Products and Food Additives "EGY- DAIRY" 10th of Ramadan city, Sharkia Governorate, Egypt, was used for the preparation of reconstituted skim milk that used for evaluating the activity of crude enzyme extract in milk clotting and proteolysis.

Plant rennet

Leathery green bracts fold around flowers of globe artichoke (*Cynara scolymus* L.) byproduct is the residue from fresh-handling and industrial processing of artichoke hearts was obtained from El Marwa for preserving and frozen vegetables and fruits El-Obour City, Egypt. These industries produce the type of artichoke byproducts: vegetable stuff composed of outer bracts. Fresh green bracts of artichoke flowers were dried at room temperature and hand crushed to obtain the powder.

Methods

Protein extraction

Plant crude protein extracts were obtained using homogenization under different conditions. About 100g of artichoke powder was reconstituted in 200 ml of different buffers (Mohan, 2006) and homogenized by stirring at 4°C. The used buffers were defined as follows: T1=Distilled water, T2=5% NaCl in distilled water, T3=5% NaCl in sodium acetate buffer (pH 3.8), T4=5% NaCl in sodium acetate buffer (pH 5.0), T5=5% NaCl in sodium phosphate buffer (pH 5.5), T6=5% NaCl in sodium phosphate buffer (pH 6.5), T7=5% NaCl in sodium phosphate buffer (pH 7), T8=5% NaCl

in Tris-HCl buffer (pH 8.0). Subsequently, the extract of each buffer was filtrated through cheesecloth and centrifuged at 1000 rpm for 45 min at 4°C (Nouani, *et al.,* 2009). The supernatant was collected and dialysed at 4°C against 0.1 M sodium acetate buffer (pH 5.0) overnight (Ahmed *et al.,* 2009).

Evaluation of milk clotting activity

Milk clotting activity was determined according to the method described by Ahmed *et al.* (2009). The substrate (10% reconstituted skim milk in 0.01 M $CaCl_2$) was prepared and the pH was adjusted to 6.5. The substrate (10 ml) was preincubated at different temperatures for 5 min, the temperature was adjusted to 50°C, 55°C, 60°C, 65°C and 70°C using a thermostatically controlled water bath. Briefly, 1, 2, 3, 4 and 5% by volume of the crude extract was added into 10ml of the milk samples and unit of milk clotting activity (MCU) was determined by rotating the test tube at regular interval times and checking for visible clot formation on the wall of the test tubes. The following formula was used for calculating the milk clotting unit (MCU) (Nouani *et al.,* 2009).

Unit of milk clotting activity (U/ml) = (2400/t) × (S/E), where t is the time required for clot formation, S is the volume of skim milk and E is the volume of crude enzyme extract. The result was expressed as MCU/ml.

The optimum conditions

The optimum coagulation activity of enzymatic extracts was determined according to observing the required time for milk coagulation by varying the studied parameters. It is expressed as relative activity (%) (Nouani *et al.,* 2009).

The optimum pH

The optimum pH for milk coagulation using 3% of the crude enzyme extract was determined at different pH values 5-6.5 incubated at 65°C. The pH value at which the milk coagulated in the shortest time was recorded as the pH optimum value required for milk coagulation using the plant extract.

The optimum temperature

A temperature range of 50 - 70°C was used to determine the optimum temperature for crude enzymatic activity using 3% of crude enzyme extract.

The optimum concentration of $CaCl_2$

The optimum concentration of $CaC1_2$ was determined by observing the milk coagulation time watered down with $CaC1_2$, at concentrations between 0.01 to 0.05% in a test tube incubated at 65°C with added 3% crude enzyme extract.

The optimum concentration of NaCl

The optimum concentration of NaCl was determined by observing the milk coagulation time watered down with NaCl, at concentrations between 1 to 10% in a test tube incubated at 65°C with added 3% crude enzyme extract.

The optimum concentration of GDL

The optimal concentration of Glucono-Delta-Lactone (GDL) was determined by observing the milk coagulation time watered down with GDL, at concentrations between 0.1and 0.5% in a test tube incubated at 65°C with added 3% crude enzyme extract.

The optimum concentration of crude enzyme extract

The volumes of crude enzymatic preparations added to the milk vary according to the suitable dilution factor of 1, 2, 3, 4 and 5% of crude extract in 10 ml of milk in a test tube incubated at 65°C.

Measurement of proteolytic activity

The proteolytic activity of coagulant extracts allows for the evaluation of the rate of the degradation rate of casein (Sigma, Biochemical, and reagents) during the primary reaction. It consists of measuring, the increase of non-protein nitrogen (NPN) in trichloracetic acid (TCA) at 12% of the final mixture (Nouani *et al.,* 2009). Briefly, 3 ml of crude enzyme extract was added to 10 ml of 3% degradation of casein (pH 6.5) in a test tube and incubated at 65°C for 1, 2, 3, 4, 5 and 6 hours.

Rheological analysis

Susceptibility to syneresis (STS) and water holding capacity (WHC) was evaluated according to Isanga and Zhang (2009). The substrate (10% reconstituted skim milk in 0.03% $CaCl_2$, 0.03% GDL), and 1, 2, 3, 4 and 5% of the crude enzyme extract were added. The substrate was subsequently incubated at 65°C and the formation of curd was observed. The

coagulation end point was recorded when discrete particles were discernible. The experiment was carried out in triplicate.

Water holding capacity

The water holding capacity (WHC) was determined according to the following formula:

$$WHC\,(\%) = (1 - \frac{W_1}{W_2}) \times 100$$

Where:

W_1= Weight of whey after centrifugation.

W_2= Initial sample weight.

Coagulated milk was centrifuged for 30 min at 1000 rpm at 4°C.

Susceptibility to syneresis

The sample of coagulated milk susceptibility to syneresis (STS) was measured by placing a 100 ml of the sample on a filter paper placed on the top of a funnel. After 6 hr., of drainage, the volume of the whey was collected and measured. The index of syneresis is calculated as follows:

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

Where:

V_1 = Volume whey after drainage.

V_2 = Initial volume sample.

Statistical Analysis

The data were analyzed by ANOVA according to the appropriate experimental designs and reported as means (± standard deviations), which were separated by Duncan's Multiple Range Test at $p \leq 0.05$ (Cochran and Cox, 1992) and least significant difference (LSD) test using SPSS computer program, version 20 (SPSS Inc., Chicago, IL, USA). Triplicate measurements were performed for each analysis.

RESULTS AND DISCUSSION

Extraction Conditions

Effect of pH on clotting activity

Results presented in Table 1 show the effect of different pH values at 65°C on the clotting activity of crude enzyme extracts from artichoke (*Cynara scolymus)*. Results indicated that reducing pH value resulted in a great reduction of milk clotting time. Results indicated also that the optimum pH value was found to be 5.5 to 5 which resulted in short clotting time. These results are in agreement with those reported by Nouani *et al.* (2009). However, Sidrach *et al.* (2005) reported that the optimum pH for artichoke proteases was 5.0, while Chen *et al.* (2003) found the optimum pH value was as much as 6.0 for artichoke protease.

Significant decrease in the activity (by about 80-90%) in pH value close to the neutrality for the protease of *Cynara cardunculus* was observed by (Heimgartner *et al.*, 1990; Chazarra *et al.*, 2007).

Nouani *et al*. (2009) reported that the fig tree and artichoke extracts were stable at the pH range from 3-7 and 70-100% of the initial activity was preserved after 24 hr., of incubation at 4°C and beyond this pH, the loss of activity was begun, fast for the fig tree extract and slow for the artichoke extract. Similar results are reported by Sidrach *et al.* (2005) after 60 hr., of incubation at laboratory temperature.

Clotting temperature

Table 2 shows the effect of temperature on clotting time (sec.) of artichoke proteinase. Results indicated that clotting time was decreased as the clotting temperature raised from 50-70°C. Moreover, the best clotting activity was noticed at 65-70°C which resulted in shorter clotting time. These results are in concordance with those reported by Sidrach *et al.* (2005) and Nouani *et al.* (2009).

The thermophilic nature of plant proteases was reported by Sidrach *et al.* (2005) and Chazarra *et al.* (2007) on cynarase (70°C), Raposo and Domingos (2008) on the protease from *Centaurea calcitrapa* (52°C), Lo Piero *et al.* (2002) on the lettucine from *Lactuca sativa* (50°C). The study of thermal stability indicated that all the enzymes studied are sensitive to high temperatures. They lose their activity depending on incubation time and the temperature of the reaction medium. At 45°C, the loss of activity is very rapid during 8 hr., of incubation. It is about for rennet and ficine and 30% for cynarase. At 55°C, they are totally inactive. The

Table 1. Effect of pH on milk clotting activity using artichoke crude extracts

Treatment	pH values				Mean effect
	6.5	6.0	5.5	5.0	
			Clotting time (Sec.)		
T1	2286.6^{b}	271.80^{r}	190.20^{u}	133.80^{yz}	720.6±838^{a}
T2	2190.6^{c}	307.20^{q}	134.40^{yz}	85.80^{z}	679.5±811^{c}
T3	1153.2^{h}	187.20^{v}	144.00^{w}	70.20^{z}	388.65±428^{f}
T4	1268.4^{g}	139.80^{x}	88.80^{C}	27.60^{z}	381.15±480^{g}
T5	1292.4^{f}	255.00^{s}	132.60^{z}	61.80^{z}	435.45±463^{f}
T6	1578.0^{e}	321.60^{p}	138.60^{x}	66.60^{z}	526.2±569^{e}
T7	1874.4^{d}	324.00^{o}	135.60^{y}	69.00^{z}	600.75±686^{d}
T8	2347.2^{a}	215.40^{t}	126.60^{z}	93.00^{z}	695.55±883^{b}
Mean effect	1748.85±47^{A}	252.75±64^{C}	136.35±26^{D}	75.98±29^{E}	553.48
			U/ml (unit of milk-clotting activity)		
T1	10.50^{y}	88.30^{q}	126.18^{n}	179.37^{j}	101.09±64.0^{g}
T2	10.96^{y}	78.13^{r}	178.57^{jk}	279.72^{f}	136.85±102^{f}
T3	20.81^{w}	128.21^{n}	166.67^{m}	341.88^{e}	164.39±119^{c}
T4	18.92^{wx}	171.67^{lm}	270.27^{g}	869.57^{a}	332.61±321^{a}
T5	18.57^{wx}	94.12^{q}	181.00^{j}	388.35^{b}	170.51±137^{b}
T6	15.21^{xy}	74.63^{r}	173.16^{kl}	360.36^{c}	155.84±130^{d}
T7	12.80^{y}	74.07^{r}	176.99^{jkl}	347.83^{d}	152.92±125^{d}
T8	10.22^{y}	111.42^{o}	189.57^{i}	258.06^{h}	142.32±96.0^{e}
Mean effect	14.75±4.09^{E}	102.57±32.27^{C}	182.80±38.41^{B}	378.14±200.45^{A}	169.57
			RA% (Relative activity)		
T1	56.52^{lmn}	93.82^{efg}	69.72^{hij}	46.19^{n}	66.56±16.61^{d}
T2	59.00^{klm}	83.01^{fgh}	98.66^{efg}	72.03^{ghi}	78.18±13.63^{cd}
T3	112.07^{def}	136.22^{cd}	92.08^{efg}	88.03^{efg}	107.10±55.87^{b}
T4	101.89^{defg}	182.40^{b}	149.32^{bc}	223.91^{a}	164.38±51.44^{a}
T5	100.00^{efg}	100.00^{efg}	100.00^{efg}	100.00^{efg}	100.00±1.47^{b}
T6	81.90^{fgh}	79.29^{fgh}	95.67^{efg}	92.79^{efg}	87.41±6.94^{bc}
T7	68.95^{hij}	78.70^{fgh}	97.79^{efg}	89.57^{efg}	83.75±10.69^{bc}
T8	55.06^{mn}	118.38^{cde}	104.74^{defg}	66.45^{ijk}	86.16±24.78^{c}
Mean effect	79.42±21.88^{B}	108.98±52.54^{A}	101.00±21.28^{A}	97.37±51.60^{A}	96.69

Mean (±SE). Values with small letters in the same column and values with capital letters in the row having different superscripts differ significantly ($p \leq 0.05$).

RA: Relative activity: Was calculated as 100% at pH and increase or decrease according to clotting time (sec.).

T1=Distilled water, T2=5% NaCl in distilled water, T3=5% NaCl in sodium acetate buffer (pH 3.8), T4=5% NaCl in sodium acetate buffer (pH 5.0), T5=5% NaCl in sodium phosphate buffer (pH 5.5), T6=5% NaCl in sodium phosphate buffer (pH 6.5), T7=5% NaCl in sodium phosphate buffer (pH 7), T8=5% NaCl in Tris-HCl buffer (pH 8.0).

Table 2. Effect of temperature on milk clotting activity using artichoke crude extracts

Treatment	Temperature					Mean effect
	50 °C	55 °C	60 °C	65 °C	70 °C	
			Clotting time (Sec.)			
T1	2231^{a}	628.80^{fgh}	1189.80^{b}	798.00^{de}	563.40^{hij}	1082.20±671^{a}
T2	793.2^{de}	621.60^{fg}h	550.80^{hij}	607.20^{ghi}	493.20^{jkl}	613.20±104^{cd}
T3	985.2^{c}	870.60^{cd}	658.80^{efg}	685.20^{efg}	378.60^{mn}	715.68±214^{b}
T4	753^{def}	614.40^{fgh}	561.00^{hij}	548.40^{hij}	271.20^{no}	549.60±162de
T5	753.6^{def}	573.60^{hij}	469.80^{lm}	497.40^{ijk}	186.60^{o}	496.20±190^{e}
T6	734.4^{def}	624.60^{fgh}	633.00^{fgh}	606.00^{ghi}	315.00^{no}	582.60±146^{cd}
T7	684.6^{efg}	612.00^{fgh}	477.00^{klm}	547.20^{hij}	196.80^{o}	503.52±173^{e}
T8	852.6^{cd}	753.00^{def}	642.00^{fgh}	667.20^{efg}	257.40^{no}	634.44±209^{c}
Mean effect	973.45±493.37^{A}	662.33±191.98^{B}	647.78±220.26^{B}	619.58±91.57^{B}	332.78±130.56^{C}	647.18
			U/ml (unit of milk-clotting activity)			
T1	10.76^{w}	38.17^{lm}	20.17^{v}	30.08^{s}	42.60^{jk}	28.36±12.07^{g}
T2	30.26^{rs}	38.61^{lm}	43.57^{j}	39.53^{l}	48.66^{hi}	40.13±6.31^{e}
T3	24.36^{y}	27.57^{t}	36.43^{nop}	35.03^{p}	63.39^{f}	37.36±14.27^{f}
T4	31.87^{qr}	39.06^{lm}	42.78^{jk}	43.76^{j}	88.50^{d}	49.19±20.81^{c}
T5	31.85^{qr}	41.84^{k}	51.09^{g}	48.25^{i}	128.60^{a}	60.33±35.99^{a}
T6	32.68^{q}	38.42^{lm}	37.91^{lmn}	39.60^{l}	76.19^{e}	44.96±16.73^{d}
T7	35.06^{p}	39.22^{l}	50.31^{gh}	43.86^{j}	122.00^{b}	58.09±33.55^{b}
T8	28.15^{t}	31.87^{qr}	37.38^{mno}	35.97^{op}	93.24^{c}	45.32±25.03^{d}
Mean effect	28.12±7.40^{D}	36.85±4.51^{C}	39.96±9.35^{B}	39.51±5.56^{B}	82.90±30.21^{A}	45.47
			RA% (Relative activity)			
T1	33.78^{w}	91.22^{g}	39.49^{v}	62.33^{s}	33.12^{w}	51.99±23.11^{h}
T2	95.01^{d}	92.28^{efg}	85.29^{i}	81.92^{k}	37.83^{v}	78.47±21.59^{e}
T3	76.49^{l}	65.89^{r}	71.31^{p}	72.59o^{p}	49.29^{u}	67.11±9.88^{g}
T4	100.08^{c}	93.36^{def}	83.74^{ij}	90.7^{g}	68.81^{q}	87.34±11.03^{c}
T5	100.00^{c}	100.00^{c}	100.00^{c}	100.00^{c}	100.00^{c}	100.00±1.53^{a}
T6	102.61^{b}	91.83^{fg}	74.22^{no}	82.08^{jk}	59.24^{t}	82.00±15.38^{d}
T7	110.08^{a}	93.73^{de}	98.49^{c}	90.9^{g}	94.82^{d}	97.60±7.18^{b}
T8	88.39^{h}	76.18^{lm}	73.18^{no}	74.55^{mn}	72.49^{op}	76.96±6.12^{f}
Mean effect	88.31±23.18^{A}	88.06±10.81^{A}	78.22±18.26^{C}	81.88±11.51^{B}	64.45±23.47^{D}	80.18

Mean (±SE). Values with small letters in the same column and values with capital letters in the row having different superscripts differ significantly ($p \leq 0.05$). RA: Relative activity: Was calculated as 100% at a temperature and increase or decrease according to clotting time (sec.).

T1=Distilled water, T2=5% NaCl in distilled water, T3=5% NaCl in sodium acetate buffer (pH 3.8), T4=5% NaCl in sodium acetate buffer (pH 5.0), T5=5% NaCl in sodium phosphate buffer (pH 5.5), T6=5% NaCl in sodium phosphate buffer (pH 6.5), T7=5% NaCl in sodium phosphate buffer (pH 7), T8=5% NaCl in Tris-HCl buffer (pH 8.0).

thermostability seems to express a varietal nature. On the other hand, the *Centaurea calcitrapa* protease retains 100% of its initial activity at 70°C after 6 hr., of incubation (Raposo and Domingos, 2008).

Huang *et al*. (2011) showed that ginger protease had an optimum proteolytic activity at a temperature ranged from 40 to 60°C with maximum activity detected at 70°C. Moreover, 70% of milk clotting activity was maintained as the temperature was increased to 65°C with high specificity for κ-casein with temperature increasing.

The concentration of crude enzyme extract

The general rule stating that the time of coagulation (T) in inversely proportion to the amount of the enzyme (E) as expressed by equation (E. × T.=K) holds only true over a narrow range of the enzyme concentration from 1 to 5/10 ml of substrate. The results in Table 3 show that the equation (E. × T.=K) is only theoretical, but on the practice, it was found that (K) is nearly constant in crude enzyme extracts of artichoke (*Cynara scolymus*) from 1 to 5 (ml/ 10 ml of milk). The results also indicated that the crude enzyme extracts of artichoke were more significantly influenced by enzyme concentration. Results indicated that increasing the extract concentration resulted in decreasing the clotting time. Best clotting activity and acceptable curd properties were obtained at an extract concentration of 3-5%. It could be also noticed that increasing the enzyme concentration significantly reduced the clotting time (El-Abbassy, 1977; Magdoub *et al*., 1984 ; Abd El-Gelil and El- Zawahary, 2004). These results are in agreement with data reported by Ahmed *et al.* (2009).

Addition of $CaCl_2$

The effect of adding $CaCl_2$ at the different concentrations on clotting activity of artichoke protease is shown in Table 4. Results indicated that addition of $CaCl_2$ greatly enhanced the clotting activity of crude enzyme extracts of artichoke and increasing the concentration of $CaCl_2$ greatly reduced the clotting time. The optimum concentration was found to be 0.03-0.05%, which resulted in short clotting time. Nouani *et al*. (2009) investigated that during the time of the $CaC1_2$ concentration of milk is used in cheese-making (0-20 mM, the per cent composition by mass of the $CaCl_2$ solution is 0.22%) the coagulant activity increases progressively (parabolic velocity) depending on $CaC1_2$ concentration. It is fast for commercial rennet (85-90%) and slows for plant extracts (from 50-80%), for the fig tree sap coagulase and from 12-50% for the coagulase from artichoke flowers. The coagulation of milk by the enzymes planned is slow at concentrations <10 mM of $CaC1_2$ (10 mM $CaCl_2$ solution is 0.11%). The artichoke enzyme is the most sensitive. The effect of Ca^{++} ions in the action of enzymatic coagulation of milk (Bencini, 2002; Najera *et al*., 2003). Lagaude *et al.* (2004), Chazarra *et al*. (2007) and Lo Piero *et al.* (2002), reported that the addition of $CaC1_2$ does not affect catylatic activity of lettucine on whole casein.

Addition of NaCl

Results presented in Table 5 show the effect of NaCl addition at different levels on clotting activity of artichoke protease. Results indicated that increasing the level of NaCl greatly reduced the clotting activity of the different crude extracts of artichoke, showing longer clotting time. Meanwhile, the addition of NaCl at the level of 3% could be recommended. Fuquay *et al*. (2011) reported that the salt or ionic strength may affect the rennet coagulation. The presence of NaCl in the milk may reduce the milk pH, so its pH has to be constant during its coagulation because many changes are pH depending. The addition of NaCl (>0.01 mol l^{-1} NaCl solution is 5×10^{-4}%) reduces the hydrolysis reaction, presumably by inhibiting the electrostatic interactions involved in the formation of the chymosin–κ-casein complex at the active site. And may increases the rennet coagulation time (RCT) throughout reducing the initial rate of aggregation even the pH value maintained constant. So, if high levels of rennet were used to obtain similar RCT, the adding of NaCl more than 100-200 mmol l^{-1} NaCl (100-200 mmol l^{-1} NaCl solution is 0.58-1.16% NaCl) at a constant milk pH produces rennet gels with the high storage module. These results highlight the important role of electrostatic interactions in the rennet coagulation step. Besides to that, adding of NaCl may result in some solubilization of colloidal calcium phosphate (CCP) as well and it may due to substitution/exchange of Na^{+} for Ca^{2+}.

Table 3. Effect of enzyme concentration on milk clotting activity using artichoke crude extracts

Treatment	Concentration of crude extract					Mean effect
	1%	2%	3%	4%	5%	
			Clotting time (Sec.)			
T1	846.6 ^{a}	553.2 ^{h}	377.4^{m}	262.2^{t}	75.0 ^{E}	422±272^{a}
T2	672.6^{e}	367.2 ^{o}	253.2^{v}	198.6 ^{A}	66.6 ^{F}	311±211 ^{e}
T3	739.2^{b}	439.2^{j}	313.2 ^{q}	208.2^{x}	60.0^{G}	351±238^{b}
T4	621.6 ^{f}	373.8^{n}	271.2 ^{r}	153^{B}	33.0^{I}	290±208^{f}
T5	549.0^{i}	373.2^{n}	269.4 ^{s}	147^{D}	30.6^{J}	273±18^{g}5
T6	681.6^{d}	421.8 ^{l}	252.6 ^{v}	199.8^{z}	34.8^{H}	318±227^{d}
T7	607.8 ^{g}	318.0 ^{p}	250.8^{w}	150.6^{C}	32.4^{I}	271±200^{h}
T8	738.0^{c}	427.8 ^{k}	257.4 ^{u}	202.8^{y}	33.0 ^{I}	331±247^{c}
Mean effect	682.05±88^{A}	409.28±67^{B}	280.65±42^{C}	190.28±37^{D}	45.68±17^{E}	321.58
			E.×T.=K			
T1	846.6^{p}	1659.6^{c}	1887.0^{a}	1835.4^{b}	675.0^{r}	1380.72±533^{a}
T2	672.6^{r}	1101.6^{l}	1266.0^{jk}	1390.2^{g}	599.4^{s}	1005.96±327^{c}
T3	739.2^{q}	1317.0^{i}	1566.0^{d}	1457.4^{e}	540.0^{t}	1124.04±4.24^{b}
T4	621.6^{s}	1121.4^{l}	1356.0^{h}	1071.0^{m}	297.0^{uv}	893.40±394^{e}
T5	549.0^{t}	1119.6^{l}	1347.0^{h}	1029.0^{n}	275.4^{v}	864.00±406^{f}
T6	681.6^{r}	1265.4^{jk}	1263.0^{jk}	1398.6^{fg}	313.2^{u}	984.36±431^{d}
T7	607.8^{s}	954.0^{o}	1254.0^{k}	1054.2^{mn}	291.6^{uv}	832.32±353^{g}
T8	738.0^{q}	1283.4^{j}	1287.0^{j}	1419.6^{f}	297.0^{uv}	1005.00±439^{c}
Mean effect	682.05±88.79^{D}	1227.83±204^{C}	1403.25±210^{A}	1331.93±261^{B}	411.08±157.46^{E}	1011.23
			1/t x10^{3}			
T1	1.18^{u}	1.81.0^{r}	2.65^{p}	3.81^{lmn}	13.33^{g}	4.56±4.63^{g}
T2	1.49st	2.72.0^{p}	3.95^{l}	5.04^{j}	15.02^{f}	5.64±5.07^{f}
T3	1.35^{tu}	2.28.0^{q}	3.19^{o}	4.80^{k}	16.67^{e}	5.66±5.81^{f}
T4	1.61^{s}	2.68.0^{p}	3.69^{n}	6.54^{i}	30.30^{c}	8.96±11.17^{c}
T5	1.82^{r}	2.68.0^{p}	3.71^{mn}	6.80^{h}	32.68^{a}	9.54±12.10^{a}
T6	1.47st	2.37.0^{q}	3.96^{l}	5.01^{j}	28.74^{d}	8.31±10.65^{e}
T7	1.65^{rs}	3.14.0^{o}	3.99^{l}	6.64^{hi}	30.86^{b}	9.26±11.30^{b}
T8	1.36^{tu}	2.34.0^{q}	3.89^{lm}	4.93^{jk}	30.30^{c}	8.56±11.32^{d}
Mean effect	1.49±0.20^{E}	2.50±0.39^{D}	3.63±0.45^{C}	5.45±1.03^{B}	24.74±7.81^{A}	7.56

Mean (±SE). Values with small letters in the same column and values with capital letters in the row having different superscripts differ significantly ($p \leq 0.05$).

E.: enzyme concentration

T1=Distilled water, T2=5% NaCl in distilled water, T3=5% NaCl in sodium acetate buffer (pH 3.8), T4=5% NaCl in sodium acetate buffer (pH 5.0), T5=5% NaCl in sodium phosphate buffer (pH 5.5), T6=5% NaCl in sodium phosphate buffer (pH 6.5), T7=5% NaCl in sodium phosphate buffer (pH 7), T8=5% NaCl in Tris-HCl buffer (pH 8.0).

Table 4. Effect of $CaCl_2$ concentration on milk clotting activity using artichoke crude extracts

Treatment	$CaCl_2$ concentration						Mean effect
	0.0%	**0.01%**	**0.02%**	**0.03%**	0.04%	0.05%	
				Clotting time (Sec.)			
T1	1273.8 ^{a}	1024.2 ^{g}	669.6 ^{p}	456.6 ^{u}	317.4^{z}	150.6^{z}	648.70±404^{a}
T2	1092.6 ^{d}	813.6 ^{m}	444.6 ^{w}	306.6 ^{D}	240.6^{z}	140.4^{z}	506.40±348^{d}
T3	1164.6 ^{b}	894.6 ^{j}	531.6 ^{r}	379.2 ^{y}	252.0^{z}	132.6^{z}	559.10±373^{b}
T4	972.6 ^{h}	752.4 ^{n}	452.4 ^{v}	328.2 ^{z}	185.4^{z}	82.2^{z}	462.20±320^{f}
T5	932.4 ^{i}	664.2 ^{q}	451.8 ^{v}	325.8^{z}	177.6^{z}	73.2^{z}	437.50±299^{h}
T6	1027.2 ^{f}	825.0 ^{l}	510.6 ^{t}	305.4^{z}	241.8^{z}	94.2^{z}	500.70±339^{e}
T7	1041.0 ^{e}	735.6 ^{o}	384.6 ^{x}	303.6^{z}	182.4^{z}	90.6^{z}	456.30±340^{g}
T8	1112.4 ^{c}	892.8 ^{k}	517.8 ^{s}	311.4^{z}	245.4^{z}	142.2^{z}	537.00±363^{c}
Mean effect	1077.08±104^{A}	825.30±107^{B}	495.38±81^{C}	339.60±50^{D}	230.33±45^{E}	113.25±29^{F}	513.49
				U/ml (unit of milk-clotting activity)			
T1	18.84^{z}	23.43 ^{BC}	35.84^{w}	52.56^{t}	75.61^{q}	159.36^{h}	60.94±49^{h}
T2	21.97^{z}	29.5 ^{y}	53.98^{t}	78.28^{op}	99.75^{l}	170.94^{f}	75.74±51^{e}
T3	20.61^{z}	26.83 ^{z}	45.15^{v}	63.29 ^{s}	95.24^{n}	181.00 ^{e}	72.02±56^{g}
T4	24.68^{z}	31.9.0 ^{x}	53.05^{t}	73.13 ^{r}	129.45^{k}	291.97^{b}	100.70±94^{b}
T5	25.74^{z}	36.13^{w}	53.12^{t}	73.66 ^{r}	135.14^{i}	327.87^{a}	108.61±107^{a}
T6	23.36^{z}	29.09 ^{y}	47.00 ^{u}	78.59^{op}	99.26 ^{lm}	254.78^{d}	88.68±81^{d}
T7	23.05^{z}	32.63 ^{x}	62.40 ^{s}	79.05^{o}	131.58 ^{j}	264.90^{c}	98.94±84^{c}
T8	21.57^{z}	26.88 ^{z}	46.35^{uv}	77.07^{pq}	97.80 ^{m}	168.78^{g}	73.08±51^{f}
Mean effect	22.48±2^{F}	29.55±3^{E}	49.61±7^{D}	71.95±8^{C}	107.98±8 ^{B}	227.45±62^{A}	84.33
				RA% (Relative activity)			
T1	100.00^{s}	124.37 ^{N}	190.23 ^{F}	278.98 ^{A}	401.32 ^{t}	845.82^{h}	323.45±361^{h}
T2	116.58 ^{P}	156.56 ^{I}	286.50 ^{y}	415.46 ^{r}	529.43 ^{l}	907.26^{f}	401.97±274^{e}
T3	109.38 ^{R}	142.39 ^{K}	239.62 ^{D}	335.92 ^{w}	505.48^{o}	960.63^{e}	382.24±298^{g}
T4	130.97^{M}	169.30 ^{H}	281.56 ^{z}	388.12 ^{v}	687.06 ^{k}	1549.64^{b}	534.44±503^{b}
T5	136.62 ^{L}	191.78 ^{E}	281.94 ^{z}	390.98 ^{u}	717.23 ^{i}	1740.16 ^{a}	576.45±569^{a}
T6	124.01 ^{N}	154.40 ^{J}	249.47 ^{B}	417.09 ^{q}	526.80^{m}	1352.23^{d}	470.67±430^{d}
T7	122.36 ^{O}	173.16 ^{G}	331.20 ^{x}	419.57 ^{p}	698.36 ^{j}	1405.96^{c}	525.10±448^{c}
T8	114.51 ^{Q}	142.67 ^{K}	246.00^{c}	409.06 ^{s}	519.07 ^{n}	895.78^{g}	387.85±275^{f}
Mean effect	119.30±11^{F}	156.83±20^{E}	263.32±39^{D}	381.90±47^{C}	573.09±108^{B}	1207.19±331^{A}	450.27

Mean (±SE). Values with small letters in the same column and values with capital letters in the row having different superscripts differ significantly ($p \leq 0.05$).

RA: Relative activity: Was calculated as 100% at without added $CaCl_2$ and increase or decrease according to clotting time (sec.).

T1=Distilled water, T2=5% NaCl in distilled water, T3=5% NaCl in sodium acetate buffer (pH 3.8), T4=5% NaCl in sodium acetate buffer (pH 5.0), T5=5% NaCl in sodium phosphate buffer (pH 5.5), T6=5% NaCl in sodium phosphate buffer (pH 6.5), T7=5% NaCl in sodium phosphate buffer (pH 7), T8=5% NaCl in Tris-HCl buffer (pH 8.0).

Table 5. Effect of NaCl concentration on milk clotting activity using artichoke crude extracts

Treatments	NaCl concentration						Mean effect
	0.0%	1%	3%	5%	7%	10%	
				Clotting time (Sec.)			
T1	1209.0 ^{lm}	1034.4 ^{p}	1753.2 ^{e}	2400.0^{a}	1164.6 ^{n}	1620.0^{g}	1530.20±478^{b}
T2	1026.6 ^{p}	852.6 ^{t}	974.4^{q}	1091.4 ^{o}	1158.6 ^{n}	1620.0^{g}	1120.60±249^{f}
T3	1173.0^{mn}	984.6 ^{q}	1467.0^{j}	2100.0^{b}	2400.0^{a}	2400.0^{a}	1754.10±589^{a}
T4	930.6 ^{r}	727.2 ^{v}	795.0^{u}	936.0 ^{r}	1273.8 ^{k}	1980.0 ^{c}	1107.10±438^{f}
T5	873.6st	806.4 ^{u}	846.6 ^{t}	918.0^{r}	1475.4^{ij}	1509.6^{i}	1071.60±308^{g}
T6	979.2 ^{q}	858.6 ^{t}	907.8^{rs}	1043.4 ^{p}	1577.4 ^{h}	1591.2^{gh}	1159.60±314^{e}
T7	987.0^{q}	924.6 ^{r}	974.4 ^{q}	1054.2 ^{op}	1896.0^{d}	1230.6 ^{l}	1177.80±349^{d}
T8	1030.8 ^{p}	980.4 ^{q}	1152.6 ^{n}	1269.0 ^{k}	1686.0 ^{f}	1053.6 ^{op}	1195.40±245^{c}
Mean effect	1026.23±119^{E}	896.10±98.74^{F}	1108.88±320^{D}	1351.50±545^{C}	1578.98±401^{B}	1625.63±399^{A}	1264.55
				U/ml (unit of milk-clotting activity)			
T1	19.85 ^{r}	23.20^{no}	821.36 ^{g}	10.00 ^{z}	20.61 ^{q}	14.81 ^{u}	151.64±308^{h}
T2	23.38 ^{n}	28.15 ^{j}	1477.83 ^{d}	21.99 ^{p}	20.71 ^{q}	14.81 ^{u}	264.48±558^{d}
T3	20.46 ^{q}	24.38 ^{m}	981.60^{f}	11.43 ^{y}	10.00 ^{z}	10.00 ^{z}	176.31±370^{g}
T4	25.79 ^{l}	33.00 ^{h}	1811.32 ^{a}	25.64 ^{l}	18.84 ^{s}	12.12 ^{x}	321.12±685^{a}
T5	27.47 ^{k}	29.76^{i}	1700.92 ^{b}	26.14 ^{l}	16.27 ^{t}	15.90 ^{t}	302.74±643^{b}
T6	24.51^{m}	27.95^{jk}	1586.25 ^{c}	23.00^{no}	15.21 ^{u}	15.08 ^{u}	282.00±600^{c}
T7	24.32 ^{m}	25.96 ^{l}	1477.83 ^{d}	22.77 ^{o}	12.66 ^{w}	19.50 ^{r}	263.84±558^{e}
T8	23.28 ^{no}	24.48 ^{m}	1249.35 ^{e}	18.91 ^{s}	14.23 ^{v}	22.78 ^{o}	225.51±471^{f}
Mean effect	23.63±2.43^{C}	27.11±3.12^{B}	1388.31±330^{A}	19.99±5.88^{D}	16.07±3.66^{E}	15.63±3.84^{F}	248.45
				RA% (Relative activity)			
T1	100.00^{m}	116.88^{hij}	68.96 ^{r}	50.38 ^{v}	103.81 ^{l}	74.63 ^{p}	85.78±23^{g}
T2	117.77 ^{h}	141.8 ^{c}	124.08 ^{g}	110.78 ^{k}	104.35 ^{l}	74.63 ^{p}	112.24±21 ^{c}
T3	103.07 ^{l}	122.79 ^{g}	82.41 o	57.57 ^{u}	50.38 ^{v}	50.38 ^{v}	77.77±28^{h}
T4	129.92 ^{f}	166.25 ^{a}	152.08 ^{b}	129.17 ^{f}	94.91 ^{n}	61.06 ^{t}	122.23±36^{a}
T5	138.39 ^{d}	149.93 ^{b}	142.81^{c}	131.7^{ef}	81.94 ^{o}	80.09 ^{o}	120.81±29^{b}
T6	123.47 ^{g}	140.81c^{d}	133.18 ^{e}	115.87^{hij}	76.65 ^{p}	75.98 ^{p}	110.99±26^{d}
T7	122.49 ^{g}	130.76^{ef}	124.08 ^{g}	114.68 ^{j}	63.77 ^{s}	98.24 ^{m}	109.00±23^{e}
T8	117.29 ^{hi}	123.32 ^{g}	104.89 ^{l}	95.27 ^{n}	71.71 ^{q}	114.75^{ij}	104.54±17^{f}
Mean effect	119.05±12^{B}	136.57±15^{A}	116.56±27^{C}	100.68±29^{D}	80.94±18^{E}	78.72±19^{F}	105.41

Mean (±SE). Values with small letters in the same column and values with capital letters in the row having different superscripts differ significantly ($p \leq 0.05$).

RA: Relative activity: Was calculated as 100% at without added NaCl and increase or decrease according to clotting time (sec.).

T1=Distilled water, T2=5% NaCl in distilled water, T3=5% NaCl in sodium acetate buffer (pH 3.8), T4=5% NaCl in sodium acetate buffer (pH 5.0), T5=5% NaCl in sodium phosphate buffer (pH 5.5), T6=5% NaCl in sodium phosphate buffer (pH 6.5), T7=5% NaCl in sodium phosphate buffer (pH 7), T8=5% NaCl in Tris-HCl buffer (pH 8.0).

Zhao and Corredig (2016) reported the same observation concerning the presence of NaCl where its high levels inhibition the gelation and increasing the rennet coagulation time and decreasing the gel stiffness as well compared to control. Also, reported that the final level of NaCl was about 280 and 260 mmol/l (280 and 260 mmol/l solution is 1.5-1.6% NaCl) and the presence of 300 mM NaCl (300 mM NaCl solution is 1.7% NaCl) led to solubilization of CCP and decreasing both the pH and the negative charges on the surface of casein micelles.

Addition of GDL

Results presented in Table 6 show that addition of Glucono-Delta-Lactone (GDL) greatly reduced the clotting time of artichoke protease up to 0.30%. However, increasing the level of GDL more than 0.3% increased again the clotting time. So, it could be noticed that the optimum concentration of GDL was 0.2 - 0.4%. However, preheating the skimmed milk to 90°C followed by acidification at 30°C using slows hydrolysis of GDL shifting the coagulation pH to a value higher than 5.5, and reduces the coagulation time (Horne and Davidson, 1993). These results could be explained on the basis that clotting time of the artichoke was faster with higher acidity and lower pH value (Esposito *et al.,* 2016).

Proteolytic activity

The photolytic activity of crude extracts of artichoke in different buffers is shown in Table 7. Results indicated that artichoke extracted in sodium phosphate buffer (T5, T7 and T6) had higher photolytic activity than other buffers. These results are in agreement with those reported by Liorente *et al.* (2014) as they reported that artichoke protease showed higher photolytic activity at pH 5.0-6.0 but the highest proteolytic activity was noticed at pH 5.0.

Nunez *et al.* (1991) reported that proteolysis of La Serena cheese was found to be at a higher rate when it was made using vegetable rennet than that made using calf rennet. Abd El-Gelil and El-Zawahary (2004) also reported that the enzyme extract from *Solanum dobium* plant showed strong proteolytic activity than calf rennet. The breaking off of the Phe l05-Met l06 bond from κ-casein by aspartyl proteases such as cynarase is similar to that of chymosin and the other proteases of fungal and bacterial (Sidrach *et al.,* 2005). Moreover, the proteolytic effect was the same of *Cynara cardunculus,* which largely used in cheese making (Campos *et al.,* 1990; Silva and Malcata, 1999; Silva *et al.,* 2002). In another comparative study of Heimgartner *et al.* (1990) and Cordeiro *et al.* (1992), they showed an excessive proteolytic activity in crude extracts containing cyranase compared to chymosin. This may be due to the non-specific action of the proteases towards the other milk caseins (αs, and (β-caseins). The bovine α-, β- and κ-casein can be hydrolyzed by the three types of cynarases (A, B and C) while only κ-casein can be hydrolyzed by the crude extract from artichoke at the same peptide band similar to the calf rennet. Moreover, the electrophoretic patterns resulted from α and β-casein were depending on the type of cynarases (A, B and C). These patterns were similar in case of cynarases A and C and different from those of calf rennet, only the patterns resulted from using cynarases B was similar to those of calf rennet (Chazarra *et al.,* 2007)

Coagulation activity strongly depends on the pH and temperature of milk (Chazarra *et al.,* 2007). The optimum proteolytic activity of *Cynaracran dunculuson* on bovine casein occurred at pH range of 5.1 to 6.0 (Garcia *et al.,* 2011). Llorente *et al.* (1997) also showed that crude extract of the (violet) part of mature flowers of *Cynara scolymus* L. illustrated an optimum clotting activity at acid pH ranged from 3.5 to 5.0 for bovine milk and low thermal stability at temperatures above 45°C. *Cynara scolymus* protease exhibited maximum clotting activity at 70°C of cow milk (Sidrach *et al.,* 2005). Rodrigues *et al.* (2009) also reported a maximum of milk clotting activity (MCA) of crude and purified extracts from *Jacaratia corumbensis* at 55°C using cow milk, while the optimum pH for crude and partially purified extracts was found to be 6.5 and 7.0, respectively.

Rheological properties of curd

Table 8 shows the water holding capacity (WHC %) and susceptibility to syneresis (STS %) of curd resulted from the artichoke protease of different extracts. Results showed different variations in both WHC (%) and STS (%) due to the different extraction buffer solutions. The best WHC (%) and STS (%) was observed in curd prepared using sodium phosphate buffer.

Table 6. Effect of Glucono-Delta-Lactone (GDL) concentration on milk clotting activity using artichoke crude extracts

Treatment	GDL concentration						Mean effect
	0.0%	0.1%	0.2%	0.3%	0.4%	0.5%	
Clotting time (Sec.)							
T1	1353.0 ^{a}	391.8 ^{cd}	308.4^{cde}	210.0^{cde}	252.6^{cde}	330.0^{cde}	474.30±419^{a}
T2	1165.8 ^{ab}	384.0 ^{cd}	209.4^{cde}	197.4^{cde}	196.8^{cde}	194.4^{cde}	391.30±363^{abc}
T3	1237.8 ^{ab}	432.6 ^{c}	249.0^{cde}	307.8^{cde}	324.6^{cde}	257.4^{cde}	468.20±359^{ab}
T4	1045.8 ^{b}	250.2^{cde}	228.0^{cde}	201.0^{cde}	192.0^{cde}	187.2^{de}	350.70±320^{c}
T5	1005.6 ^{b}	306.0^{cde}	189.6^{de}	195.6^{cde}	206.4^{cde}	197.4^{cde}	350.10±304^{c}
T6	1100.4 ^{b}	251.4^{cde}	248.4^{cde}	200.4^{cde}	215.4^{cde}	205.2^{cde}	370.20±336^{bc}
T7	1114.2 ^{ab}	312.6^{cde}	138.6^{e}	191.4^{cde}	189.0^{de}	153.0^{de}	349.80±494^{c}
T8	1185.6 ^{ab}	260.4^{cde}	198.0^{cde}	192.0^{cde}	187.8 ^{de}	202.2^{cde}	371.00±375^{bc}
Mean effect	1151.03±313^{A}	323.63±67.82^{B}	221.18±95.05^{C}	211.95±37.43^{C}	220.58±45.01^{C}	215.85±51.87^{C}	390.70
U/ml (unit of milk-clotting activity)							
T1	17.74 ^{J}	61.26 ^{C}	77.82 ^{x}	114.29 ^{n}	95.01 ^{t}	72.73 ^{A}	73.14±30^{g}
T2	20.59 ^{H}	62.50 ^{B}	114.61 ^{n}	121.58 ^{hi}	121.95 ^{h}	123.46 ^{f}	94.12±40^{e}
T3	19.39 ^{I}	55.48 ^{D}	96.39^{qr}	77.97^{wx}	73.94 ^{z}	93.24 ^{u}	69.40±26^{h}
T4	22.95 ^{F}	95.92^{rs}	105.26 ^{p}	119.40 ^{j}	125.00 ^{e}	128.21 ^{c}	99.46±37^{c}
T5	23.87 ^{E}	78.43 ^{w}	126.58 ^{d}	122.70 ^{g}	116.28 ^{m}	121.58 ^{hi}	98.24±38^{d}
T6	21.81 ^{G}	95.47st	96.62 ^{q}	119.76 ^{j}	111.42 ^{o}	116.96 ^{l}	93.67±34
T7	21.54 ^{G}	76.78 ^{y}	173.16 ^{a}	125.39 ^{e}	126.98 ^{d}	156.86 ^{b}	113.45±52^{a}
T8	20.24 ^{H}	92.17 ^{v}	121.21^{i}	125.00 ^{e}	127.80 ^{c}	118.69 ^{k}	100.85±38^{b}
Mean effect	21.02±1.88^{F}	77.25±15.54^{E}	113.96±27.29^{C}	115.76±14.97^{B}	112.30±18.05^{D}	116.47±23.68^{A}	92.79
RA% (Relative activity)							
T1	100.00^{A}	345.33 ^{u}	438.72 ^{s}	644.29 ^{n}	535.63 ^{q}	410.0 ^{t}	412.33±174^{f}
T2	116.06^{yz}	352.34 ^{u}	646.13^{mn}	685.41 ^{h}	687.5^{gh}	695.99^{fg}	530.57±227^{e}
T3	109.31^{zA}	312.76 ^{v}	543.37 ^{q}	439.57 ^{s}	416.82 ^{t}	525.64 ^{r}	391.25±151^{g}
T4	129.37^{wx}	540.77 ^{q}	593.42 ^{p}	673.13 ^{j}	704.69^{ef}	722.76 ^{c}	560.69±208^{c}
T5	134.55 ^{w}	442.16 ^{s}	713.61^{cde}	691.72^{gh}	655.52 ^{lm}	685.41 ^{h}	553.83±214^{d}
T6	122.96^{xy}	538.19 ^{q}	544.69 ^{q}	675.15^{ij}	628.13 ^{o}	659.36 ^{kl}	528.08±194^{e}
T7	121.43^{xy}	432.82 ^{s}	976.19 ^{a}	706.90^{de}	715.87 ^{cd}	884.31 ^{b}	639.59±295^{a}
T8	114.12^{yz}	519.59 ^{r}	683.33 ^{hi}	704.69^{ef}	720.45 ^{c}	669.14^{jk}	568.55±220^{b}
Mean effect	118.48±10.57^{F}	435.50±88.03^{E}	642.43±153^{C}	652.61±84.43^{B}	633.08±101^{D}	656.58±133^{A}	523.11

Mean (±SE). Values with small letters in the same column and values with capital letters in the row having different superscripts differ significantly ($p \leq 0.05$).

RA: Relative activity: Was calculated as 100% at without added GDL and increase or decrease according to clotting time (sec.).

T1=Distilled water, T2=5% NaCl in distilled water, T3=5% NaCl in sodium acetate buffer (pH 3.8), T4=5% NaCl in sodium acetate buffer (pH 5.0), T5=5% NaCl in sodium phosphate buffer (pH 5.5), T6=5% NaCl in sodium phosphate buffer (pH 6.5), T7=5% NaCl in sodium phosphate buffer (pH 7), T8=5% NaCl in Tris-HCl buffer (pH 8.0).

Table 7. Proteolytic activity of crude extracts of artichoke during incubation time

Treatment	Incubation time (hours)						Mean effect
	1	2	3	4	5	6	
				Nonprotein nitrogen (NPN%)			
T1	0.04 ^{m}	0.07 ^{lm}	0.13 ^{k}	0.17^{hij}	0.19^{fghi}	0.2^{efgh}	0.13±0.06^{d}
T2	0.05 ^{lm}	0.07 ^{lm}	0.14^{jk}	0.18^{ghi}	0.2^{efgh}	0.22^{def}	0.14±0.07^{cd}
T3	0.05 ^{lm}	0.07 ^{lm}	0.18^{ghi}	0.22^{def}	0.25^{cd}	0.28^{bc}	0.18±0.09^{b}
T4	0.05 ^{lm}	0.08 ^{l}	0.19^{fghi}	0.21^{efg}	0.27^{bc}	0.29^{ab}	0.18±0.09^{ab}
T5	0.05 ^{lm}	0.08 ^{l}	0.19^{fghi}	0.23 ^{de}	0.28^{bc}	0.32^{a}	0.19±0.10^{a}
T6	0.05 ^{lm}	0.08 ^{l}	0.18^{ghi}	0.21^{efg}	0.25^{cd}	0.28^{bc}	0.17±0.09^{b}
T7	0.05 ^{lm}	0.08 ^{l}	0.19^{fghi}	0.23 ^{de}	0.29^{ab}	0.32 ^{a}	0.19±0.10^{a}
T8	0.04 ^{m}	0.07 ^{lm}	0.16^{ijk}	0.19^{fghi}	0.21^{efg}	0.25 ^{cd}	0.15±0.08^{c}
Mean effect	0.05±0.1^{F}	0.07±0.02^{E}	0.17±0.03^{D}	0.21±0.02^{C}	0.24±0.04^{B}	0.27±0.04^{A}	0.17
				(Non protein nitrogen/ Total nitrogen %) NPN/TN (%)			
T1	7.55^{t}	11.45^{r}	16.25^{q}	18.68^{p}	20.21^{mno}	20.62^{lmno}	15.79±4.95^{f}
T2	9.43^{s}	11.77^{r}	17.28 ^{q}	19.78^{nop}	21.28^{klm}	22.68^{ij}	17.04±5.04^{e}
T3	9.43 ^{s}	11.77^{r}	21.69^{jkl}	24.18^{gh}	26.60^{e}	28.87 ^{cd}	20.42±7.53^{c}
T4	9.43 ^{s}	12.1^{r}	22.89^{i}	23.08 ^{hi}	28.72^{d}	29.90^{bc}	21.02±8.01^{b}
T5	9.43 ^{s}	12.1^{r}	22.89^{i}	25.27^{fg}	29.79^{bcd}	32.99^{a}	22.08±8.93^{a}
T6	9.43 ^{s}	12.1^{r}	21.69^{jkl}	23.08^{hi}	26.60^{e}	28.87^{cd}	20.30±7.39^{c}
T7	9.43 ^{s}	12.1^{r}	22.89i	25.27^{fg}	30.85 ^{b}	32.99^{a}	22.26±9.10^{a}
T8	7.55 ^{t}	11.45 ^{r}	19.75^{op}	20.88^{lmn}	22.34^{ijk}	25.77^{ef}	17.96±6.55^{d}
Mean effect	8.96±0.88^{F}	11.86±0.62^{E}	20.67±2.55^{D}	22.53±2.41^{C}	25.80±3.89^{B}	27.84±4.43^{A}	19.61

Mean (±SE). Values with small letters in the same column and values with capital letters in the row having different superscripts differ significantly ($p \leq 0.05$).

T1=Distilled water, T2=5% NaCl in distilled water, T3=5% NaCl in sodium acetate buffer (pH 3.8), T4=5% NaCl in sodium acetate buffer (pH 5.0), T5=5% NaCl in sodium phosphate buffer (pH 5.5), T6=5% NaCl in sodium phosphate buffer (pH 6.5), T7=5% NaCl in sodium phosphate buffer (pH 7), T8=5% NaCl in Tris-HCl buffer (pH 8.0).

Table 8. Effect of artichoke crude extracts on water holding capacity (WHC%) and susceptibility to syneresis (STS %)

Enzyme con. ml/100ml milk	Treatment								Mean effect
	T1	T2	T3	T4	T5	T6	T7	T8	
					Water holding capacity (WHC %)				
1	30.21^{c}	35.25^{b}	27.24^{c}	49.24^{a}	50.32^{a}	49.25^{a}	49.14^{a}	47.56^{a}	42.28±9.54^{D}
2	34.23^{c}	40.45^{bc}	30.33^{c}	51.23^{a}	54.56^{a}	52.25^{a}	53.11^{a}	50.45^{ab}	45.82±9.70^{C}
3	44.23^{b}	48.34^{b}	36.61^{c}	63.26^{a}	67.41^{a}	63.5^{a}	64.12^{a}	63.35^{a}	56.35±11.24^{A}
4	43.23b^{c}	47.04^{b}	37.13^{c}	58.37^{a}	63.34^{a}	59.48^{a}	58.46^{a}	57.51^{a}	53.51±9.36^{B}
5	42.08^{c}	46.68^{bc}	34.01^{d}	50.81^{ab}	55.87^{a}	52.70^{ab}	51.81^{ab}	50.73^{ab}	48.09±7.23^{C}
Mean effect	38.79±6.16^{D}	43.55±6.15^{C}	33.06±4.84^{E}	54.58±6.21^{B}	58.30±7.3^{A}	55.43±6.03^{AB}	55.32±6.16^{AB}	53.92±6.36^{B}	49.12±10.62
					Susceptibility to syneresis (STS %)				
1	45.5^{a}	42.22^{a}	47.25^{a}	45.24^{a}	41.24^{a}	43.25^{a}	42.11^{a}	43.52^{a}	43.79±3.63^{A}
2	42.26^{a}	40.25^{a}	46.25^{a}	42.25^{a}	39.21^{a}	40.25^{a}	41.02^{a}	41.24^{a}	41.59±3.19^{AB}
3	38.25^{a}	37.25^{a}	40.15^{a}	37.14^{a}	31.41^{a}	33.24^{a}	35.15^{a}	37.5^{a}	36.26±6.65^{C}
4	41.11^{ab}	40.14^{abc}	42.55^{a}	39.25^{abc}	34.11^{c}	35.47^{bc}	39.12^{abc}	40.12^{abc}	38.98±3.39^{BC}
5	45.12^{a}	44.42^{a}	46.32^{a}	45.23^{a}	39.24^{a}	40.33^{a}	43.24^{a}	44.57^{a}	43.56±3.63^{A}
Mean effect	42.44±8.34^{AB}	40.85±3.17^{ABC}	44.50±3.88A	41.82±4.22AB	37.04±4.82^{C}	38.50±4.63^{BC}	40.13±3.60^{ABC}	41.39±3.42^{ABC}	40.84±5.11

Mean (±SE). Values with small letters in the same column and values with capital letters in the row having different superscripts differ significantly ($p \leq 0.05$).

T1=Distilled water, T2=5% NaCl in distilled water, T3=5% NaCl in sodium acetate buffer (pH 3.8), T4=5% NaCl in sodium acetate buffer (pH 5.0), T5=5% NaCl in sodium phosphate buffer (pH 5.5), T6=5% NaCl in sodium phosphate buffer (pH 6.5), T7=5% NaCl in sodium phosphate buffer (pH 7), T8=5% NaCl in Tris-HCl buffer (pH 8.0).

The water holding capacity (WHC %) of crude extracts of artichoke was significantly higher up to 3%, then a slight reduction occurred when the concentration enzyme valued 4-5%. Samples showed a higher level of water holding capacity and lower syneresis. These results confirmed by Fox *et al.* (2000), who reported a direct relationship between moisture and cheese firmness. Galán *et al.* (2008) reported that in relation to the attributes hardness and creaminess, cheeses produced with vegetable coagulant were significantly softer and more buttery than those using calf rennet. A negative correlation was found between cheeses hardness and soluble nitrogen and non protein nitrogen values in cheese made using vegetable coagulant. This may due to the higher proteolytic activity of casein breakdown. Moreover, the first degradations products may contribute to the softer and creamier texture compared to those produced by calf rennet.

The hydrophilic interactions reflecting the water holding capacity occurred between water and proteins play very important role in food systems influencing their flow and texture. Some of the intrinsic parameters could affect the water holding capacity of proteins influencing protein conformation, the chemical composition of amino acids and the polarity/ hydrophobicity of the proteins surface (Barbut, 1999). In addition, crude extracts of artichoke increasing the level of water holding capacity which resulted in the reduction of susceptibility to syneresis (STS%). The lower STS (%) may be explained by the high proteolytic activity (Table 7).

The use of the crude extracts of artichoke was found to be necessary to prevent serum separation, it could be noticed that increasing enzyme concentration decreased whey synersis up to 3%, but increasing enzyme concentration up to 4-5%, increased whey synersis. Syneresis, an undesirable property in crude extracts of artichoke, is the effect of liquid separating from the curd (Wu *et al.*, 2001; Abd El-Gelil and El-Zawahary, 2004).

Conclusion

Crude enzymatic extracts of bracts artichoke (*Cynara scolymus*) flower could be used as rennet substitute. The present study allowed evaluating the main characteristics of crude enzyme extracts, their coagulant and proteolytic activities and the different factors and coagulation parameters affecting these activities. A better understanding of extracted proteases properties was established for a better and future application in milk clotting T4 (5% NaCl in sodium acetate buffer, pH 5.0) and T5 (5% NaCl in sodium phosphate buffer, pH 5.5), T6 (5% NaCl in sodium phosphate buffer, pH 6.5), and T7 (5% NaCl in sodium phosphate buffer, pH 7), respectively. Milk coagulation could be achieved using crude extracts of artichoke improving coagulation milk could be obtained at pH of 5.0-6.0, and 65-70°C as temperature with a concentration of 3%, 0.4-0.05, 3%, 0.5% of crude extract, $CaCl_2$, NaCl, and GDL, respectively. The whey synersis decreased by using crude enzyme concentration of >3%. Moreover, the best values of WHC (%) and STS (%) was observed in sodium phosphate buffer solutions (T5, T6, T7) and sodium phosphate buffer (T4).

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تقييم مستخلصات بديل المنفحة المستخلص من أزهار الخرشوف (*Cynara scolymus*): دراسة العوامل المؤثرة على القدرة التجبنية للبن

هبة الله حلمي عبد الرؤوف - أحمد علاء الدين النشوي - عبد الحميد محمد ربيع - صلاح أحمد خليفة

قسم علوم الأغذية - كلية الزراعة - جامعة الزقازيق - مصر

تم تقييم العوامل المؤثرة على القدرة التجبنية لبديل المنفحة المستخلص من قنابات أزهار الخرشوف (*Cynara scolymus*) في محاليل استخلاص مختلفة، وشملت تلك العوامل pH، درجة حرارة التجبن وتركيز المستخلص، كما تم دراسة تأثير اضافة كل من كلوريد الكالسيوم وكلوريد الصوديوم والجلوكونو دلتا لاكتون بتركيزات مختلفة على القدرة التجبنية للمستخلصات المختلفة، كما تم تقييم القدرة التحللية للبروتين للمستخلصات المختلفة والخواص الريولوجية للخثرة الناتجة (قدرة الاحتفاظ بالماء ومعدل انفصال الشرش)، وتشير نتائج الدراسة ان القدرة التجبنية المثلي للمستخلصات أمكن الوصول اليها عند درجة pH قدرها ٥ – ٦ ودرجة حرارة تجبن قدرها ٦٥-٧٠°م وتركيز مستخلص قدره ٣%، كلوريد الكالسيوم بتركيز ٠.٠٤-٠.٠٥% ، كلوريد الصوديوم بتركيز ٣%، الجلوكونودلتا لاكتون بتركيز ٠.٥%، وتشير نتائج الدراسة أن القدرة التجبنية المثلى تم الحصول عليها من مستخلصات محاليل T4 (٥% كلوريد صوديوم في محلول منظم خلات صوديوم عند pH 0.5) ومستخلصات محاليل منظمه من فوسفات الصوديوم T5 (٥% كلوريد صوديوم في محلول منظم فوسفات صوديوم عندpH ٥.٥) و T6 (٥% كلوريد صوديوم في محلول منظم فوسفات صوديوم عند pH 6.5) و T7 (٥% كلوريد صوديوم في محلول منظم فوسفات صوديوم عند pH ٧.٠)، كما أظهرت نتائج الدراسة ان القدرة التحللية للبروتين كانت اعلي في المستخلص الناتج في محلول منظم لفوسفات الصوديوم ومحلول استخلاص خلات صوديوم (T4) عن باقي المستخلصات، كما تشير النتائج ان أفضل خواص ريولوجية للخثرة يمكن الحصول عليها عند استخدام مستخلصات محاليل منظمه من فوسفات الصوديوم (T5, T6, T7) ومحلول استخلاص خلات الصوديوم (T4).

المحكمـون:

١- أ.د. رزق عـــزب عـــواد أستاذ الألبان – كلية الزراعة – جامعة عين شمس.

٢- أ.د. محمد مجدي زكي العباسي أستاذ الألبان المتفرغ – كلية الزراعة – جامعة الزقازيق.