Journal of Food and Dairy Sciences

Journal homepage & Available online at: www.jfds.journals.ekb.eg

Improving the Efficiency of the Traditional Method of Camel Chymosin Extraction Using Ultrasound and a Freeze-Drying Process

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ABSTRACT

Many problems are associated with the traditional extracting methods of camel chymosin; they are costly, time-consuming, and have low concentrations of chymosin. This study aimed to increase the efficiency of the traditional methods of camel chymosin production. Two experiments were carried out; the first experiment was implemented using dried camel abomasum as a powder and strips (4-7mm), and exposed to ultrasound exposure time at (20, 40, and 80 min). The chymosin extracted using the dried powder with ultrasound exposure time at 80 min treatments caused a higher significant increase in milk clotting activity and lower proteolytic activity compared to the strips and control treatments. The second experiment was implemented using the freeze-dried process for both chymosin extracted from the strips and powder treatments, which was selected as a higher in total clotting activity and chymosin yield. Upon comparison to the traditional samples, the freeze-dried treatments caused a higher significant increase in specific milk-clotting time and MCA/PA ratio by approximately 4.2 fold and 24.0 fold, respectively. Moreover, the freeze-dried process caused a significant increase in chymosin yield, curd viscosity, firmness, and reduced the syneresis of milk curd. Furthermore, the results confirmed that the viscosimetric measurement can be used as a new indicator to predict the cutting time of camel milk curd. The use of ultrasound with the freeze-dried process can be recommended as an appropriate treatment for the extraction of camel chymosin.

Keywords: Camel Chymosin, Ultrasound, Freeze-drying, Clotting activity, Proteolytic activity.

INTRODUCTION

Camels (*Camelus dromedaries*) serve as the best livestock multi-purpose animals adapted to the harsh environmental condition. They have a greater potential as dairy animals in arid and semi-arid ecosystems than other domesticated animals. Camel milk can be considered the mainstay of the human diet, even under extremely hostile conditions can survive and produce good quality milk for a long time of (12-18 months).

During the last decade, there has been great progress in the intensive dairy management and machine milking of dromedary camels in several countries around the world, modern milking machines were introduced to the milking practices of dromedaries in large-scale camel dairy farms in the United Arab Emirates, Saudi Arabia, as well as in small-scale farms in Tunisia, Sudan, Australia, Europe, and USA (Nagy & Juhasz, 2016).

Making cheese from camel milk is a way to create a trade and value-addition for camel keepers which can help to improve their economic condition (Khan *et al*., 2004). Enzymatic coagulation plays an important role in cheese yield and quality. Rennet is the fourth stomach (abomasum) obtained from sucking calves and contains mainly two main acid proteolytic enzymes, the chymosin (EC 3.4.23.4) and pepsin A (EC 3.4.23.1), the chymosin being is principally responsible for clotting milk and the pepsin for proteolysis. The rennet from the young calf is rich in chymosin, while, the rennet from the older calf is rich in pepsin, and these fractions depend on the age of the animals and fed when slaughtered (Broome & Limsowtin, 1998).

Reports on the coagulation of camel milk are often conflicting and the gelation of camel milk was difficult under the same conditions used for other milk of dairy animals. Several studies on camel milk gelation have used bovine rennet enzymes, camel gastric enzyme extracts, or plant enzyme mixtures (El Zubeir & Jabreel, 2008; Hailu, *et al*., 2014 and Mehaia, 1993). The majority of attempts to make cheese from camel milk using traditional methods have encountered certain challenges. These challenges are associated with longer coagulation time, with the same amount of calf rennet the coagulation time of camel milk is two to three folds longer than in cow milk. Moreover, the action of rennet on camel milk leads to poor coagulation with no firm coagulation, and the cheese curd was soft and weak. (Farah & Bachmann, 1987; Farah & Rüegg, 1989 and Mohamed, 1990).

Due to the technical difficulties of camel's milk coagulation, many studies were focused on the coagulation properties of camel's milk to find a solution and improve the technological aptitudes of camel milk for cheese production. These studies can be divided into two groups: previous preliminary studies, make camel cheese using bovine rennet; the initial attempts to coagulation camel milk increased the bovine rennet concentration compared with that usually used for clotting cow's milk to 50- times as a possible means to accelerate camel milk coagulation, but the coagulum obtained showed a fragile, weak coagulum and poor cheese yield (Abd-El Salam & Benkerroum 2006 and Larsson-Raźnikiewicz & Mohamed 1986).

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DOI: 10.21608/jfds.2022.174356.1079

Similar views were expressed by (Al-haj & Al-Kanhal, 2010 and Kappeler *et al*., 2006) where they mentioned that camel milk alone cannot be coagulated with bovine rennet and the milk coagulation was a very limited ability enzymatically due to limited access to the protease to the k–casein substrate. The limited access of bovine chymosin to the catalytic site in camel κ- casein was attributed to camel milk κ- casein having a different site for hydrolysis by chymosin compared with bovine milk κ-casein. Bovine chymosin hydrolyzed bovine κ- casein at the Phe^{105}-Met^{106} bond; whereas camel chymosin hydrolysis site on camel milk κ- casein is Phe^{97}-Ile^{98}.

The second group of studies used traditional methods for the extraction of camel calf rennet. The work carried out by (El-Abbassy & Wahba, 1986; El-Batawy *et al*., 1987 and El-Batawy, 1991[a,b]) shows that the crude gastric enzymes produced from the old camel's stomach had Pepsin-rich enzyme. They estimated the effectiveness of camel rennet extracted through coagulating the reconstituted dried skim cow's milk, but not camel milk. Most research that used the traditional methods for the extraction of camel rennet reported some drawbacks, including the longer time required for the extraction process and the lower clotting activity of chymosin. The poor ability of camel milk to be coagulated by camel rennet enzymes is probably associated with the lower activity and concentration of camel chymosin enzyme extracted using traditional methods.

Recently, very limited laboratory studies not applied to a commercial scale have been carried out on the effects of recombinant or pure gastric chymosin enzymes extracted on the camel milk coagulate, those studies reported that enzymes have provided good milk clotting activity and limited proteolytic activity toward camel milk casein (Isselnane *et al*., 2016; Kappeler *et al*., 2006 and Haroun *et al*., 2012).

Although the above-mentioned traditional techniques of camel chymosin extraction are regarded as a reference, they are costly, time-consuming, and low in clotting activity for camel milk. One possible way to compensate for the camel rennet deficit is developing the traditional method of extraction of camel chymosin for the complete extraction of biologically active enzymes from abomasa, preservation of their activity, and increased quality.

The use of ultrasound for enzyme extraction, and freeze-dried is a new process that has recently been investigated in food processing. The amount of chymosin extracted from an animal's tissue by ultrasound depends on its content and the shape of the tissues, as well as the simultaneous selective action of ultrasound on animal tissues which destroys specific tissues without affecting others, the proteins, minerals, and other elements, move easily to the extraction medium from the torn and broken tissues (Zayas, 1971 and Zayas & Smolski, 1970).

Moreover, Freeze-drying has been widely used for stabilizing biological and preservation of various types of food and biological materials (enzymes, proteins, vitamins, etc.), because this procedure is believed to be effective to maintain the biological activities of the material over a long period time (Adams, 1991 and Ibrahim & Khalifa, 2015b).

In recent decades, several continuous and non-visual and non-destructive rheological methods were carried for estimated the actual clotting time and the curd cutting time such as (Penetrometer, Rheometer, and Viscometer). This technique allows continuous measurement of the rheological properties of the milk gel during the whole coagulation process (Castillo *et al*., 2000; Castillo *et al*., 2002 and Castillo, 2006). Due to the importance of the curd cutting time on the final cheese quality, the viscosimetric measurement can be used to evaluate milk clotting activity and cutting time of camel chymosin curd as a useful tool to monitor camel milk coagulation and predict gelation and cutting time.

Nevertheless, no information exists about the evaluation of used ultrasound and freeze-dried process for camel rennin extraction as a new development for traditional methods. Therefore, the purpose of this study was aimed: (i) To establish the influences of the cutting and grinding dry abomasum, the ultrasound exposure time, and the freeze-drying process on the clotting, proteolytic activity of the camel curd, as well as the effects on yield of camel chymosin and rheological properties of curd. (ii) To evaluate the viscosimetric measurements as a predicted measurement of camel milk-clotting activity and optimum curd-cutting time.

MATERIALS AND METHODS

Camel milk

Fresh whole camel milk was collected in sterile bottles from a camel herd breed Magrabi (*Camelus dromedarius*) owned by nomads located at Sidi-Barani areas, Matrouh Governorate, North West Coast, Egypt and delivered in an ice cooler to the laboratory. The milk samples were analyzed by using a Lactoscan (Model Lactoscan SL, Milkotronic Ltd, Bulgaria) calibrated for camel milk. The gross composition of raw camel milk was: 12.12±0.11 % total solids, 3.32±0.02 % total protein, 3.35±0.03 % fat, 4.60±0.12 % lactose, 0.17±0.01 % titratable acidity, 6.64±0.01 pH, respectively. The somatic cell count was (< 470,000/ml) in camel milk samples.

A commercial dry coarse fine food grade salt (sodium chloride) was purchased from El-Nasr Saline's Company, Egypt. Boric acid, Sodium benzoate, and calcium chloride were purchased from El-Gomhouria Company, Cairo, Egypt. All chemicals used were analytical grade and obtained from Sigma-Aldrich (Munich, Germany). Double distilled water was used for enzyme dilution and Milli-Q water was used for eluent preparation.

Preparation of camel calf abomasum

A total of 15 camel calves' fourth stomachs (Abomasum's) tissues, 6-9 months old, were obtained from a local slaughter house in Belbeis city, Al Sharqia Governorate, Egypt. Among 15 camel abomasum samples (80 %) abomasum were found to be filled with liquefied curdled material (ingesta), which indicated that young camels had only been fed milk. While (20%) abomasa seemed to be filled with a mixture of liquefied ground grass and curdled material (ingesta and digesta), which were not suitable for rennet production. The abomasum (average weight of fresh stomach was 825.5g) was defatted, the internal parts were cleaned by removing a slight superficial layer from the top of the tissues and washed with cold water. The camel stomach tissues were blown air through the lips and salted with 12% NaCl salt, then dried at room temperature, which varied from 20-25 ºC for 35-39 days. The average weights of the dried vells were 247.5g with a drying ratio of (33.35%). The dried abomasum was shredded into small strips of 4-7 mm and mixed carefully,

weighed in 100g portions in sealed polyethylene bags, and stored frozen at -15 °C.

The traditional method of extraction camel rennet

The traditional process of camel chymosin extraction was performed according to the method of El-Batawy *et al*., (1987) with minor modifications. The dried abomasum was cut into strips of 4-7 mm and was added at the rate of 20% (w\v) to the extraction solution, containing the 10% NaCl and 4% boric acid. The solution was adjusted to pH about 5.5 with 1N HCl for seven days with daily stirring for the mixture. At the end of the extraction period, the remaining abomasum was stained off in a suitable piece of cloth and the abomasum-filtered extract was centrifuged at 2300 g for 10 minutes. The pH of the extracted supernatant was lowered from 5.5 to 4.7 with 1N HCl and the extracts were held at 25 °C for 24 hr. to activate the zymogens. After the maximum activity was obtained and no further increase in activity occurred in (24 hr.), excess acid was neutralized to pH 5.7 by adding a stoichiometric amount of a solution of sodium bicarbonate. Next, 0.01% sodium benzoate was added to preserve enzyme activity. The extract solution of caramel-like color is stored in the refrigerator at about 5ºC.

Preparation of camel abomasum powder

The camel abomasum (~ 93%) relative humidity was dried as the same in the traditional method for at least 35 days, after that the dried abomasum was ground in a commercial grinder (meat grinder) with 2-mm holes and mixed with 10 % (w\w) with salt and ground again to obtain a powder. The abomasum powder samples were stored at 5 °C in opaque-covered containers until used.

Ultrasound extraction treatments

The ultrasound extraction methods of camel chymosin were carried out according to (Kim & Zayas, 1991 and Zayas, 1987). Before ultrasound extraction, the dried camel abomasum strips or powder tissue samples were added at the rate of 20% (w\v) to the extraction solution containing the 10% NaCl (pH 5.3-5.5) and allowed to swell for 60 min at 20°C in shaking incubator. All experimental swelling samples were treated with a Fisher brand™ scientific sonic (Fisher-Scientific-Model 505, Sonic-Dismembrator). The extraction temperature was controlled at a cooling coil. The extractions were carried out at the frequency of ultrasound generated at 20 kHz (cycles/second), and the specific intensity was 36 W/cm. The concentration of NaCl in the extracted solution was 1:20 of the abomasum to NaCl solution. The effects of ultrasound treatments for 20, 40, and 80 min. were studied at 20°C. After the ultrasound treatments were achieved, the other extraction steps were carried out in the same of the control method.

Freeze-drying of extracted camel chymosin

The extracted camel chymosin samples that gave the lowest coagulation time and the greatest chymosin yield were selected for the freeze-drying process. The freeze-drying process of the ultrasound treatments and control camel chymosin samples was performed in a freeze dryer (Thermo-Electron-Corporation-Heto power dry LL300 Freeze Dryer, Czech Republic). The freeze-dryer system was operated for the initial freezing at -45°C for 2 h, followed by two steps to evaporate the water under the reduced pressure. The first step, drying at 30°C at 5×10^{-3} mbar pressure for 48 h, while the secondary drying at 5°C for 3 h at the same pressure. After the end of the process, the lyophilized samples were sealed in polyethylene plastic bags labeled under vacuum and stored at 5°C. To reconstitute the freeze-dried chymosin, 1 mg was dissolved in 10 ml of 0.1 M acetate buffer (pH 4.4-5.5).

Enzymatic activities

Determination of milk clotting activity

The milk clotting activity of camel chymosin samples was determined according to the method of (IDF-FIL157/1992) with a slight modification in solutions of a standard milk substrate, where the dried nonfat cow's milk was replaced using nonfat camel's milk as a substrate. The milk substrate was prepared by dispersing (12 g of skimmed camel milk powder, Camelicious co., Al Ain Dairy, Dubai-UAE) in 100 ml water containing 0.01 M anhydrous $CaCl_2$ with pH 6.6, and the reaction mixture was shaken for 5 min. The reconstituted camel milk was left for 1 h. before carrying out the activity test. One milliliter of camel chymosin was added to 10 ml milk, mixed thoroughly, and incubated at 35 °C. A stopwatch is activated and the time required for the first appearance of milk breaks into visible particles on the wall of a rotating test tube was recorded as the milk clotting time (MCT). One Soxhlet Unit (SU) of milk clotting activity was defined as the amount of enzyme that clots 10 ml of milk substrate within 40 min (2400 sec) at 37°C (Lucey, 2002). Milk clotting activity (MCA) is expressed in terms of Soxhlet unit (SU) by using the following equation: Soxhlet Unit (U/ml) = (2400/ clotting time (sec)) x (dilution factor)

Determination of specific clotting activity

Specific activities were expressed as units of activities per mg protein from the equation formula:

Specific clotting Activity A_{sp} = W x MCA / mg protein content (Units/mg)

Where, MCA is the milk clotting activity of rennin and W is the weight of rennin after drying (g).

Protein contents

The total protein content of the substrate was determined by the dye-binding method as described by (Bradford, 1976) based on protein interaction with the Coomassie Brilliant Blue G-250 reagent and measuring the absorbance at 595 nm.

Determination of general proteolytic activity

Preparation of camel milk casein:

Whole camel milk casein was used as a proteolytic substrate and prepared as described by Trujillo *et al*., (2000). The whole casein was precipitated from skimmed camel milk by isoelectric at pH 4.6 with 1 M HCl. The precipitated casein was separated from the whey protein by centrifugation at 2500×g for 30 min. The precipitated casein was washed three times with distilled water. Finally, the whole camel milk casein was solubilized at pH 7.0 with 1 M NaOH, then dialyzed against pure water at 4 °C and stored lyophilized at -20 °C.

Proteolytic Activity (PA)

The general proteolytic activity of camel chymosin was determined by the casein digestion method as described by Chopra and Mathur, (1983). In brief, the reaction mixture contained 1.1 ml of (2 g/100 ml whole camel casein solution in 0.1 mol/l sodium phosphate buffer (pH 6.5) in the presence of 5 mM cysteine and 2 mM ethylenediaminetetraacetate (EDTA)) and 0.1 ml of camel chymosin solution (1/10 dilution). The mixture was incubated at 32 °C for 2 h. The reaction was stopped by the addition of 2 ml of 4 g/100 ml trichloroacetic acid (TCA). The amount of precipitated nitrogen compounds

was recovered by centrifuging at 4000×g for 30 min. after the addition of 1 ml of three times diluted Folin-Ciocalteu reagent in 1 ml of the filtrate, which specifically reacts with the amino acids tyrosine and tryptophan. The absorbance of colour developed was detected spectrophotometrically at A_{280} nm as a proteolytic activity enzyme unit. One unit (PU) of enzyme activity is represented as the micrograms (µg) of tyrosine released from casein per one minute by 1 ml of enzyme solution (using tyrosine for the standard curve). The Proteolytic Activity (PA) was obtained from the formula:

Proteolytic Activity PA (U/ml) = Δ Abs $_{280}$ nm x 10 x D / V x T

Where: PA= Proteolytic Activity; Abs_{280} nm is the variation of absorbance between assay and control; D is dilution factor, V is the volume of purified extract solution and T is the reaction time.

Specific proteolytic Activity

The specific proteolytic activities were calculated by the formula:

SPA= U proteolytic activity/mg protein

Chymosin yield:

The rennet yield was defined from the formula:

Chymosin yield (Y/g) =C x A/ W

Where: C=chymosin activity Soxhlet unit (SU) units/ml; A= amount of chymosin solution extracted (ml); W=weight of dry abomasum, (g).

Also, the adjusted yield of rennin was determined as the percentage of the changeover control.

Rheological analysis of camel rennet gel

Gel dynamic viscosity

The Dynamic rheological measurements of renneted camel milk gel were recorded continuously using the rotational viscometer (Brookfield viscometer, Model LDV II+ Pro with Rheocalc software, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) at a constant shearing speed. Immediately after the addition of one ml of diluted camel chymosin to 100 ml of the milk, the mixtures were stirred for 30 s and kept in a thermostatically controlled water bath at 37°C throughout the process. The spindle (LV-SC4-18) was used and allowed to rotate in the samples at 10 rpm and at a shear rate of 0 and 300 s^{-1}. after the 40s of rennet addition the measurements were started and each step of the flow behaviour curve was obtained every 10 s for 20 min. at 37°C. All the rheology parameters were converted using the Rheocalc software in relationships between dynamic viscosity/time curves (mPas) and plotted on linear graph paper. By using a simple method developed by (Csanádi, *et al*., 2010) for observing milk clotting based on a commercial vibration viscometer. The flocculation point or coagulation time (the first linkage among casein micelles begins) of the renneting camel milk was determined through the continuous monitoring of viscosity and defined graphically from the flow curves. The flocculation point or coagulation time was evident from the sudden decrease of the curve phase angle of 90° (for viscous fluid -i.e. milk) to around 15°- 40° at the flow curves of the milk gel. The peak curves at maximum viscosity were defined graphically from the flow curves and recorded as a point of cutting time of camel curd.

Gel firmness

The firmness of the camel chymosin curd samples (volume 100 ml) was determined using a Texture Analyzer (TA-XT2 - Stable Micro Systems, Haslemore, England) as a maximum resistance force after 1h. of coagulation. To avoid variations in camel milk gel surface structure, all samples were kept and measured at 20 °C in closed cylindrical containers. The instrument was adjusted to the following conditions: a cylindrical acrylic probe with a diameter of 35 mm in height, a penetration maximum depth of 15 mm. and a test speed of 1 mm/s^{-1} was used. The measuring run at initial force was 3.0g and the load cell of 5.0 kg (Sandoval-Castilla *et al*., 2004). The maximum force measured was used as a measure of camel chymosin gel firmness.

Syneresis index

The gel syneresis index of camel chymosin gel was estimated according to the method of by the method reported by Ibrahim & Khalifa, (2015a). This involved a 100 g of curd samples which were placed in the funnel fitted with Whatman filter paper no. 1. After 6 h of drainage, the amount of free whey was expressed in (milliliters whey/100 g curd) and syneresis was calculated by using the following equation:

Syneresis of whey (%) = (F/W) X100

Where F= filtered whey collected after drainage ml,
W = weight of the sample

Statistical analysis

All the determinations were made in triplicate and the experimental data were analyzed statistically using the analysis of variance (ANOVA) as a completely randomized design. The data were carried out according to the SPSS package for Windows®, SPSS Statistics v. 26.0, 2019 (IBM, SPSS package program, SPSS Inc., Chicago, USA), and the obtained results were considered significant at ($p<0.05$).

RESULTS AND DISCUSSION

The first experiments using the ultrasound treatments for camel chymosin extraction were performed in three series for each type of dried abomasum. The use of ultrasound treatments resulted in a significant reduction of operation extraction time of both powder and strips tissue by approximately 5 hr. in comparison to control experiments about 8 days.

As shown in Table 1. the changes in particle size through grinding abomasum to powder form significantly ($p<0.05$) increased the total clotting activity, MCA/PA ratio, and decreased proteolytic activities than a strip form or traditional control treatments. The grinding of abomasum tissues to powder allowed greater chymosin extraction than shredding strips. Zayas, (1986) attributed the effectiveness of chymosin extraction to the dependence on the size and shapes of the abomasum tissue particles, the small particle size of the abomasum increased the degree of chymosin extraction and enzymatic activity of extracts.

The data presented in Table 1 showed that the clotting activities of the extracted chymosin increased with increasing the ultrasound exposure time. The prolonged ultrasound exposure at 80 min caused a higher significant ($p<0.05$) increase in the total clotting activities and decreased its proteolytic activities compared to other ultrasound exposure 20 or 40 min treatments.

Zayas, (1986) noted that the duration of ultrasonic time was correlated with the concentration of chymosin and the highest yield and activity of chymosin enzyme were achieved after 60 min of ultrasonic exposure time. Similarly, (Kim & Zayas, 1991) reported that the ultrasound treatments destroyed the cellular structure through cavitation effects, which caused enhancement in the penetration and increment the permeability and accelerated releasing chymosin enzymes from cells to the medium.

Table 1. Effect of ultrasonic exposure time and the particle size of dried abomasum on milk-clotting and proteolytic activities.

Method of extraction	Dried abomasum particle size	Ultrasound exposure time (min)	Protein (mg/ mL)	Milk Clotting activities (MCA)			Proteolytic activities U/ mL		MCA/PA ratio
				Soxhlet Unit (U/ml)	Milk Clotting time in(sec)	Specific clotting activity(U/mg)	Proteolytic activity (µg tyrosine /min./ml)	Specific Proteolytic activity(U/mg)	
Control		Non	1.26a±0.12	27.71f±0.46	866.33a±14.19	21.46d±1.79	0.95a±0.05	0.76a±0.05	0.29g±0.01
Ultrasound	Strips	20	1.33a±0.15	46.63e±0.26	514.67b±2.89	35.27c±3.91	0.83ab±0.05	0.63ab±0.10	0.56f±0.04
		40	1.37a±0.06	49.71e±1.18	483.00c±11.36	36.44c±2.46	0.80bc±0.06	0.59bc±0.07	0.62df±0.03
		80	1.40a±0.17	54.53d±1.49	440.33d±12.06	39.32c±4.60	0.73bcd±0.05	0.52bc±0.06	0.75cd±0.08
	Powder	20	1.33a±0.06	63.36c±2.93	379.33e±18.01	47.64b±4.15	0.69cd±0.07	0.52bc±0.06	0.92bc±0.09
		40	1.43a±0.06	70.18b±2.74	342.33f±13.20	48.97b±0.65	0.66d±0.11	0.47c±0.09	1.08ab±0.22
		80	1.33a±0.12	77.07a±4.00	312.00g±16.64	58.17a±6.95	0.62d±0.07	0.47c±0.09	1.24a±0.09
Total mean effect									
Control			1.26a±0.12	27.71c±0.46	866.33a±14.19	21.46 c±0.12	0.95a±0.05	0.76±a0.05	0.29c±0.01
Dried Strips			1.37a±0.12	50.29b±3.58	479.33b±33.38	37.01b±0.12	0.79b±0.07	0.58b±0.08	0.65b±0.10
Dried Powder			1.37a±0.09	70.20a±6.58	344.56c±32.35	51.59a±0.09	0.66c±0.08	0.49b±0.08	1.08a±0.19

[abcd] **Means values within the same column and having different superscripts are significantly different (p< 0.05).**

It appeared that the ultrasound exposure time treatment at 80 min with abomasum powder (UP_{80}) had significantly higher effectiveness to camel chymosin extraction than the ultrasound exposure time 80 with strips form abomasum (US_{80}) and the control treatment.

A significantly higher increase in Soxhlet unit of chymosin enzyme concentration was obtained using the UP_{80} treatments; a Soxhlet unit was significantly increased from (27.7 ±0.5 for control to 77.1 ±4.0 U/mL-1 for (UP_{80}) treatments. Despite that the significant decrease in the time of initiation of gel formation caused in the case of both UP_{80} and US_{80} treatments, a significant decrease in (MCT) was observed from (866.3±14.2 sec. for control to 312.0±16.7 sec. for UP_{80}) treatments. For the traditional (control) clotting enzyme, camel milk was clotted slightly and took a long time to curd aggregation and even become coagulated the curd was a weaker gel. Sbodio & Revelli, (2012) found that the lower clotting time was related to the concentration of chymosin enzyme interacting with casein micelles. Furthermore, the specific clotting activity significantly increased from (21.5±1.8 for control to 58.2±7.0 U/mg for UP_{80}) treatments. The observed data showed that the significantly increased in the specific clotting activity to approximately 2.71 times in coagulation time was primarily due to the higher chymosin enzyme concentration and activity compared to the traditional treatment as shown in (Table 1).

The data from Table 1 showed a significant decrease in proteolytic activity (PA) for the UP_{80} treatments 0.62 ±0.07 than that in the traditional control treatment 0.95 ±0.05 µg tyrosine/min./ml. This finding is in agreement with Kim & Zayas, (1991) who reported that the chymosin treated by ultrasound might lost proteolytic activity as the ultrasound exposure time increased more easily than that extracted by the traditional method. The rennet with excessive PA enzymes is not used in cheese-making due to the higher proteolytic activity in the cheese. It causes defects in the texture and flavor of ripened and stored cheeses Ben Amira *et al*., (2017).

Furthermore, the MCA/PA ratio is a useful indicator of the chymosin efficiency to be used as a coagulant for cheese making. The UP_{80} treatments significantly increased the MCA/PA ratio, the MCA/PA ratio was increased from 0.29 ±0.01 for the control to 0.62 ±0.07 for the UP_{80} treatments. The increase in the MCA/PA ratio is considered to be the ideal milk clotting enzyme.

The yield of the chymosin extracted by ultrasound and traditional methods was compared (Table 2). Results revealed that the yield of the chymosin enzyme increased with increasing grinding size of dried abomasum tissues. As a result of lowering particle size to powder, the abomasum cells absorbed more water and the permeability of abomasum tissues increased more than strips treatment. This would cause an increase in chymosin migration from the abomasum tissue to the solution and increases the yield.

Table 2. Effect of ultrasound exposure time and particle size of dried abomasum tissues on chymosin yield.

Method of extraction	Particle size of dried abomasum	Ultrasound exposure time (min)	Chymosin yield (g)	Adjusted yield (%)
Control		non	140.86f±3.84	100.00f±0.01
Ultrasound	Strips	20	238.76e±1.41	169.50e±1.00
		40	253.66e±7.31	180.08e±5.19
		80	276.89d±10.44	196.57d±7.41
	Powder	20	324.17c±15.75	230.13c±11.18
		40	358.07b±13.35	254.20b±9.48
		80	388.25a±15.80	275.63a±11.21
Total mean effect				
Control			140.86c±3.84	100.00c±0.01
Dried Strips			256.44b±17.83	182.05b±12.66
Dried Powder			356.83a±30.66	253.32a±21.76

[abcd] **Means values within the same column and having different superscripts are significantly different (p< 0.05).**

Moreover, the yield of chymosin extracted depends on the ultrasound exposure time. As the ultrasound exposure time was increased to 80 min, the yield of the chymosin enzyme and the adjusted yield increased. The UP_{80} treatments showed the highest significance ($p<0.05$) increase in the yield from 140.9±3.9g for control to 388.3±15.8g for UP_{80} treatments. The use of ultrasound treatment showed more yield due to the higher level of chymosin enzyme extracted and the absence of chymosin inactivation as the result of short-term holding of the enzyme in the salt solution. Zayas, (1986) explained that the ultrasound treatment increases the vapor pressure inside the cell tissue which, in turn, increases the diffusion rate leading to the rupture of the cellular structure, giving correspondingly higher activity and yield of chymosin.

It is of practical importance to have a higher yield and activity of camel chymosin. The adjusted yield was calculated as a % of the changeover control, the UP_{80} treatment was a higher increase in chymosin yield 275.6±11.2 % than all abomasum strips 196.6±7.4 %.

The results confirmed that the use of UP_{80} treatment for camel chymosin extraction was the best effective treatment increasing the total milk clotting activity and yield followed by US_{80} treatment, as well as a significant decrease in the extraction time. Therefore, the second experiment suggested that the freeze-drying process to improve this chymosin extracted and their shelf-life via the concentration and study the effects on the yield, milk clotting activities, proteolytic activity rheological parameter (Table 3).

Table 3. Effect of freeze-drying of chymosin extracted on the milk-clotting and proteolytic activities.

Method of extraction	Freeze-dried of chymosin extracted	Soxhlet Unit (U/ml)	Protein (mg/g)	Milk Clotting activities		Proteolytic activities		MCA/PA ratio
				Visual Milk Clotting time (sec)	Specific Clotting activity (U/mg)	Proteolytic activity (µg tyrosine /min./mg)	Specific proteolytic activity(U/mg)	
Control		56.95c±0.98	1.13a±0.07	421.67a±7.26	50.52c±2.35	1.03a±0.02	0.92a±0.11	4.10c±0.02
Ultrasound	Strips	88.46b±2.25	1.27a±0.07	271.67b±6.94	70.20b±3.95	0.55b±0.01	0.43b±0.02	4.99ab±0.03
	Powder	110.23a±4.05	1.23a±0.03	218.33c±8.33	89.68a±5.56	0.33c±0.01	0.27c±0.01	6.96a±0. 50

[abcd] **Means values within the same column and having different superscripts are significantly different (p < 0.05).**

The result shows clearly that, a marked increase in total clotting activity and a decrease in proteolytic activity were observed in both ultrasound freeze-dried powder (UFP) and ultrasound freeze-dried strips (UFS) enzymes extracted samples compared to the control treatment (Table 3). The changes in milk clotting activities were directly proportional to enzyme concentration. As a result freeze-dried process, any water in the camel chymosin extracted is changed directly from ice to water vapor without first changing into the water and the solids were concentrated. Izutsu *et al.* (1991) and Ken-ichi *et al*., (1993) evaluated the effects of freeze-dried on the *β*-galactosidase enzyme and indicated that the freeze-drying process can improve its long-term stability and activity. Similarly, the related report by De Jesus & Maciel Filho, (2014) and Biazus *et al*., (2009) found that α-amylase, obtained from the freeze-dried process showed higher enzymatic activity and low water activity.

The ultrasound freeze-drying process significantly ($p<0.05$) increased the milk-clotting activity as a Soxhlet unit, and the treatment of (UFP) was higher in Soxhlet units (110.2±4.1 U/mg) followed by (UFS) treatment (88.46±2.3 U/mg), while the traditional freeze-dried control was the lowest it was (57.0±0.98 U/mg).

Moreover, the use of ultrasound with freeze-drying treatments was significantly accelerating the overall clotting activity. Interestingly, increasing the chymosin concentrations resulted in faster gels because of the effect on the aggregation stage of the reaction.

In comparison to the traditional control samples without the freeze-dried (Tables 1 and 3), the milk clotting time was reduced by approximately 3.97 and 3.19 fold for treatments (UFP) and (UFS) extracted samples, respectively. A similar observation has been reported previously (Yonas *et al*., 2016) where they found that normally gel development of camel milk can be improved by using a higher concentration of camel chymosin. Increasing the chymosin concentration resulted in higher clotting activity and better accessibility of κ - casein for the action of camel chymosin.

In addition, the proteolytic activity and specific proteolytic activity were lower for (UFP) and (UFS) treatments than that in the same samples without the freeze-drying (Tables 1 and 3); as a result, a significant increase in the MCA/PA ratio was found.

Compared to the traditional control samples without the freeze-dried, the (UFP) treatments showed the highest increase in specific milk-clotting activity 89.7±5.6 compared to the control 21.5±1.8. The (UFP) treatments showed also a significantly higher MCA/PA ratio 7.0±0.5 comparison to control 0.3±0.01. An ideal milk coagulant should possess the highest possible MCA/PA ratio with the lowest possible PA. The higher proteolytic leads to products lost in whey and the cheese yield is decreased. Our results were in agreement with those of Liburdia *et al*., (2014) they stated that the MCA/PA ratio has been considered crucial for quality cheese production ranging from 0.68 to 9.58.

With general strong clotting activity and low proteolytic activity of freeze-drying powder (UFP) can make a strong camel milk curd form, leading to high cheese yields. Thereby the concentration method of camel chymosin was a great adequate technique to provide milk clotting, making them suitable for large-scale studies. These results confirmed that the ultrasound and freeze-drying method was considered to be the most appropriate for producing camel chymosin for coagulating camel milk with shorter clotting times.

Table 4. Effect of freeze-drying of chymosin extracted on the chymosin yield

Method of extraction	Freeze-dried of chymosin extracted	Chymosin yield (g)	Adjusted yield (%)
Untreated	Control	289.42c±2.66	100.00c±0.00
Ultrasound	Strips	452.92b±11.79	283.02b±8.41
	Powder	562.97a±26.42	361.89a±26.32

[abcd] **Means values within the same column and having different superscripts are significantly different (p < 0.05).**

Table 4. summarizes the results obtained from the effect of using ultrasound and freeze-drying process on camel chymosin yield and adjusted yield. These results indicated a significant ($p<0.05$) increase in yield and adjusted yield of camel chymosin for (UFP), and (UFS) treatments than control treatments (Tables 2 and 4). It appeared that the (UFP) treatments had a higher significant ($p<0.05$) chymosin yield of 563.0±26.4 g than in the (UFS) 452.9±11.8 g or freeze-dried control 289.4±2.7 g, respectively.

In addition, the results indicated the use of the freeze-drying process caused a significant ($p<0.05$) increase in the adjusted yield as a percentage of control, the (UFP) 361.9±26.3% compared to (UFS) 283.0±8.4%, respectively. The increase in the yield and adjusted yield of the freeze-dried camel chymosin may be due to the higher level of chymosin extracted with a fewer foreign proteins in the rennet, as well as the absence of chymosin enzyme inactivation as the result of the short-term process of the enzyme in extraction solution, unlike the prolonged traditional method.

Monitoring the rheological properties is one of the means for measuring and evaluating the gel formation during coagulation. The viscosimetric measurement was evaluated to monitor camel milk coagulation and predict gelation and cutting time. To estimate cutting times, we followed a similar method

reported by (Castillo *et al*., 2000 and Castillo *et al*., 2003), by determining the relationship between cutting time and the maximum viscosity on the viscosity curves.

The linear curves of viscosity vs. time were shown in Fig. 1, the viscosity curves closely resembles the above previous reported literature as shown in (Fig. 2) in terms of both shape and trend.

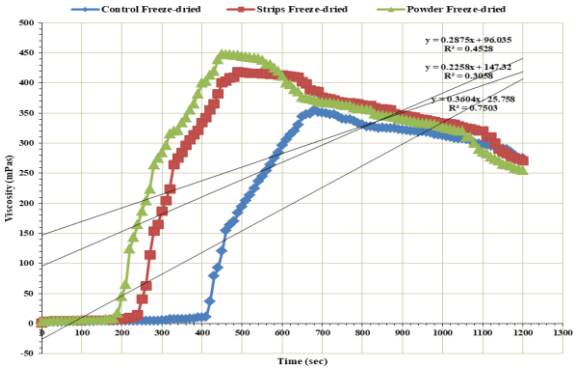

Fig. 1. The changes of viscosity profile of camel milk gel

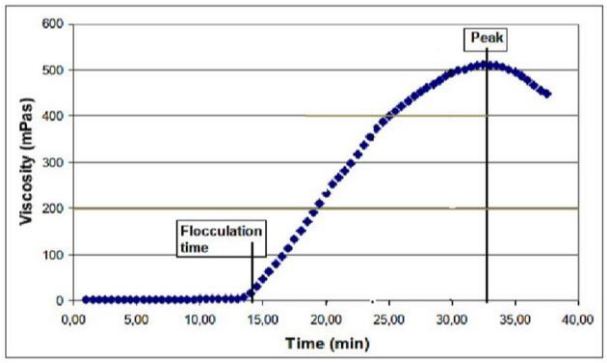

Fig. 2. Typical curves recorded by vibrating viscometer as described by (Castillo *et al.* 2000, Castillo *et al.* 2002).

The viscosity curves show a steady line at the beginning of milk coagulation which refers to the flocculation point. The flocculation point was correlated with milk viscosity. We found the flocculation point was affected by ultrasound and freeze-dried treatments and caused changes in the viscosity curve trend. After adding the camel chymosin the casein micelles begin to the linkage and the viscosity curves were observed to be linear fit sheet (slop). After starting coagulation the curd viscosity gradually increased up to a critical point or the maximum peak time which was used as non-visual cutting time Fig. 1, then the viscosity peak time gradually slowed down. This decrease in viscosity may be due to the partial hydrolysis in a hairy layer of κ-casein particles.

As can be seen from Fig. 1 the viscosity represents an increasing trend, but the ultrasound and freeze-drying treatments have a markedly greater effect on the flocculation point and cutting time than the control treatment because it contains higher chymosin enzyme concentration, which caused an increased the camel milk curd viscosity. These results agreed with the data reported by Ustunol *et al*., (1993) who observed a significant increase in curd firmness up to a critical point besides the cutting time decrease as enzyme concentration increases.

The rheological measurements of camel milk curd are presented in Table 5. The results clearly show that the ultrasound freeze-drying treatments had a positive effect on shortening the flocculation time of camel milk curd and on speeding up the clotting formation process than control treatments. The (UFP) treatments caused a significantly reduced flocculation time of 203.4±2.3 sec than the (UFS) treatment of 266.7±1.4 sec, while the control treatment had the highest flocculation time 408.7±2.3 sec. Fox *et al*., (2017) observed a similar trend and reported that the flocculation time was inversely proportional to the enzyme concentration. The chymosin enzymes directly affected the interaction and aggregation of casein micelles in the primary phase of coagulation. The lower chymosin concentration leads to longer coagulation times and a weaker or finer gel matrix structure.

The cutting time was calculated using the maximum viscosity of camel milk-curdling from Fig. 1, and the main values of cutting time were placed in Table 5. The cutting time for (UFP) treatment was significantly lower (450.0±0.1 sec at maximum viscosity, 448.0a±0.01 mPas) than for (UFS) treatment (490.0±0.1 sec at maximum viscosity 419.0±0.01 mPas), while the control treatment was the highest in cutting time 680.0±0.1 sec at maximum viscosity 355.0±0.01 mPas.

Table 5. Effect of freeze-drying of chymosin extracted on the rheological characteristics of camel milk curd.

Method of extraction	Freeze-dried chymosin extracted	Viscosimetric measurements			Gel firmness (g)	Syneresis (%)
		Coagulation time (sec)	Maximum viscosity (mPas)	Cutting time (sec)		
Untreated	Control	408.67a±2.33	355.0c±0.01	680.01a±0.06	36.19c±0.50	25.90a±0.60
Ultrasound	Strips	266.67b±1.43	419.0b±0.02	490.02b±0.09	41.43b±0.64	20.97b±0.77
	Powder	203.37c±2.31	448.0a±0.01	450.01bc±0.10	46.47a±0.68	18.30c±0.49

[abcd] **Means values within the same column and having different superscripts are significantly different (p < 0.05).**

These results can be explained by the fact that the raises in curd viscosity may occur because of the increases in the number of camel casein particles interacting with chymosin. The particles assemble in lines resulting in many chains elevating viscosity. Similar results have been reported in the literature of Lóopez *et al*., (1999) they noticed that with an increase of rennet concentrations the viscosity rate increases during coagulation.

Furthermore, the coagulation times measured using viscosimetric measurement method data were not different from the corresponding results obtained from the Berridge visual milk clotting time data. Comparing the mean values of the viscosimetric measurement and visual milk clotting time method indicates that they are comparable. Thus, the viscosimetric method was valid according to the determination of milk coagulation time. These results suggest that the vibration viscometer is useable as an objective indicator of the clotting time of the gelation of camel milk and also can be used to determine the total milk-clotting activity of camel chymosin by continuous monitoring of milk viscosity.

To predict the gel cutting time, it is not enough to have a curd viscosity that only determines the gel cutting time, but the gel must be cut when it has reached optimal firmness Castillo, (2006). So, the curd firmness of the camel chymosin curd was determined at the maximum viscosity.

The firmness has an important impact on the optimum gel firmness at the cutting time of milk curd. The results of curd firmness were shown in Table 5. A higher significant (p< 0.05)

increase in curd firmness was recorded for the (UFP) treatment (46.5±0.67). Slightly below these values was the (UFS) treatment (41.4±0.6). The maximum decrease in firmness was observed for control treatment (36.2±0.5). Additionally, the higher curd firmness may be related to the higher extensively cleavage of camel caseins through the higher concentration of chymosin enzyme which increase their stickiness and leading to longer chains in the network. Similar results were reported by Van Vliet *et al*., (1991) who found that the decrease in flocculation time and increase in gel firmness rate were directly ascribed to the positive effect on the enzymatic phase of chymosin coagulation. Also, the lower concentration of chymosin is associated with cheese softening and loss of curd firmness.

Moreover, the data indicated that the use of ultrasound with freeze-drying treatments caused a significant decrease ($p<0.05$) in syneresis (%) than in control treatment (Table 5). The (UFP) treatment gave the lower syneresis (18.30±0.5%) compared to the (UFS) treatment (20.97±0.8%), whereas the control treatment showed the highest syneresis 25.90±0.6%. This increase in firmness and lower syneresis for the ultrasound freeze-drying treatments could be due to an increase in the rate of casein aggregation as a result of an increase in the action of rennet concentration as compared with control (Mehaia & El-Khadragy 1998).

The ultrasound and freeze-drying process techniques are efficient technology for the development of the conventional camel chymosin method extraction. We have further improved the traditional camel chymosin extraction method and developed curd measurement parameters, to represent useful steps for further trials that could be improved the camel cheese processing in industrial scale.

REFERENCES

Abd-El Salam, M., & Benkerroum, N. 2006. North African brined cheeses. Brine Cheese, 139-187.

Adams, G. D. J. 1991. Freeze-drying of biological materials. Drying technology, 9(4), 891-925.

Al-haj, O.A. & Al-Kanhal H.A. 2010. Compositional, technological and nutritional aspects of dromedary camel milk. International Dairy Journal, 20: 811–821.

Ben Amira, A., Besbes, S., Attia, H., & Blecker, C. 2017. Milk-clotting properties of plant rennets and their enzymatic, rheological, and sensory role in cheese making: A review. International Journal of Food Properties, 20 (sup1), S76-S93.

Bergkvist, R. 1963. The proteolytic enzymes of Aspergillus oryzae. I. Methods for the estimation and isolation of the proteolytic enzymes. Acta Chem. Scand., 17, 1521.

Biazus, J. P. M., Souza, R. R. D., Márquez, J. E., Franco, T. T., Santana, J. C. C., & Tambourgi, E. B. 2009. Production and characterization of amylases from Zea mays malt. Brazilian archives of Biology and technology, 52, 991-1000.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2), 248-254.

Broome, M.C., & Limsowtin, G.K.Y. 1998. Milk coagulants. Australian journal of dairy technology, 53(3), 188.

Castillo M., Payne F.A., Hicks C.L. & Lopez M.B. 2000 Predicting cutting and clotting time of coagulating goat's milk using diffuse reflectance: effect of pH, temperature and enzyme concentration. Int. Dairy J., 10:551-562.

Castillo, M., Payne, F. A., Hicks, C. L., Laencina, J., & López, M. B. 2002. Effect of calcium and enzyme in cutting time prediction of coagulating goats' milk using a light scattering sensor. International Dairy Journal, 12(12), 1019-1023.

Castillo, M., Payne F.A., Hicks, C.L., Laencina, J.S. & Lopez, M.B. 2003. Modeling casein aggregation and curd firming in goats' milk from backscatter of infrared light. J. Dairy Res., 70, 335–348.

Castillo, M. 2006. Cutting time prediction methods in cheese making. Encyclopedia of Agricultural, Food, and Biological Engineering, 1(1-7).

Csanádi J., Hovorkáné Horváth, M. Z., & Kiss A. 2010. A simple method for observing rennet coagulation in goat milk. Review of faculty of engineering analecta technica szegedinensia, 2010(1), 22-30.

Chopra, A.K., Mathur, D.K., 1983. Factors affecting protease production by *Bacillus stearothermophilus* RM-67. J. Food Protect. 46, 1020–1025.

De Jesus, S. S., & Maciel Filho, R. 2014. Drying of α-amylase by spray drying and freeze-drying-a comparative study. Brazilian Journal of Chemical Engineering, 31, 625-631.

El Zubeir, I.E., & Jabreel, S.O. 2008. Fresh cheese from camel milk coagulated with Camifloc. International Journal of Dairy Technology, 61(1), 90-95.

El-Abbassy, F. & Wahba, A. 1986. Studies on camel pepsin. I. Effect of some additives. Egyptian Journal of Dairy Science, 14, 181–186.

El-Batawy, M. A., Amer, S. N., & Ibrahim, S. A. 1987. Camel abomasum as a source of rennet substitute. Egypt. J. Dairy Sci, 15, 93-100.

El-Batawy, M.A.1991[a]. Some factors affecting the clotting activity of adult camel rennet. Egyptian J. Dairy Sci.,19: 1, 117-130.

El-Batawy, M.A.1991[b]. Purification and characterization of milk clotting enzymes from adult camel rennet. Egyptian J. Dairy Sci., 19: 2, 315-325.

Farah, Z., & Bachmann, M.R. 1987. Rennet coagulation properties of camel milk. Milchwissenschaft, 42, 689–692.

Farah, Z., & Rüegg, M. W. 1989. The size distribution of casein micelles in camelmilk. Food Microstructure, 8, 211-216.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. 2017. Enzymatic coagulation of milk. In Fundamentals of cheese science (pp. 185-229). Springer, Boston, MA.

Hailu, Y., Seifu, E., & Yilma, Z. 2014. Clotting activity of camel milk using crude extracts of ginger (Zingiber officinale) rhizome. African Journal of Food Science Technology, 5(3), 90-95.

Haroun, B., Laleye, S., Chahra, L. C. S., Farida, M. M., Saliha, S. A., & Abderrahmane, M. 2012. Coagulation of camel milk using dromedary gastric enzymes as a substitute of the commercial rennet. American Journal of Food Technology, 7(7), 409-419.

Ibrahim A. H., & Khalifa S. A. 2015a. The effects of various stabilizers on physiochemical properties of camel's milk yoghurt. Journal of American Science, 11(1), 15-24.

Ibrahim, A. H., & Khalifa, S. A. 2015b. Effect of freeze-drying on camel's milk nutritional properties. International Food Research Journal, 22(4).

IDF., 1992. Bovine rennets: Determination of total milk-clotting activity. IDF Standard, Vol. 157, International DairyFederation, Brussels, Belgium.

Isselnane, S., Boudjenah-Haroun, S., Nouani, A., Ahmed-Zennia, S. S., Bouazza, B., Yabrir, B., & Abderrahmane, T. I. 2016. Molecular characterization of crude enzymatic extract from Algerian camel abomasum (Camelus dromedarius). Emirates Journal of Food and Agriculture, 28 (3), 217-223.

Izutsu K., Yoshioka S., and Takeda Y. 1991.The effects of additives on the stability of freeze-dried β-galactosidase stored at elevated temperature. Int. J. Pharm. 71:137–146.

Kappeler, S. R., Rahbek-Nielsen, H., Farah, Z., Puhan, Z., Hansen, E. B., & Johansen, E. 2006. Characterization of recombinant camel chymosin reveals superior properties for the coagulation of bovine and camel milk. Biochemical and Biophysical Research Communications, 342(2), 647-654.

Ken-ichi, I., Sumie, Y., & Tadao, T. 1993. Stabilization of β-galactosidase by amphiphilic additives during freeze-drying. International journal of pharmaceutics, 90(3), 187-194.

Khan H., Athar I.H., &Aslam M. 2004. Evaluation of cheese prepared by processing camel milk. Pak. J. Zool. 36:323-326.

Kim, S. M., & Zayas, J. F. 1991. Comparative quality characteristics of chymosin extracts obtained by ultrasound treatment. Journal of food science, 56(2), 406-410.

Larsson-Raźnikiewicz, M., & Mohamed, M. A. 1986. Why is it difficult to make cheese from camel milk?. In MILK the vital force (pp. 113-113). Springer, Dordrecht.

Liburdia, K., Benuccia, I., Zeppab, G., & Estia, M. 2014. Native plant proteases as novel coagulants for milk clotting. In Italian Forum on Industrial Biotechnology and Bioeconomy-IFIB (pp. 1-1).

Lóopez, M., Jordan, M. J., Granados, M. V., Fernandez, J. C., Castillo, M., & Laencina, J. 1999. Viscosity changes during rennet coagulation of Murciano-Granadina goat milk. International journal of dairy technology, 52(3), 102-106.

Lucey, J. A. 2002. Formation and physical properties of milk protein gels. *Journal of dairy science*, *85*(2), 281-294

Mehaia, M. A. 1993. Fresh soft white cheese (Domiati-Type) from camel milk: composition, yield, and sensory evaluation. J. Dairy Sci. 76:2845-2855.

Mehaia, M. A., & El-Khadragy, S. M. 1998. Physicochemical characteristics and rennet coagulation time of ultrafiltered goat milk. Food chemistry, 62(3), 257-263.

Mohamed, M.A.1990. Camel milk: chemical composition; characterization of casein and preliminary trial of cheese-making properties. Thesis, svertiges Lantbruks University, Uppsala, Sweden.C.F. DSA (1990), 05206160.

Nagy, P., & Juhasz, J. 2016. Review of present knowledge on machine milking and intensive milk production in dromedary camels and future challenges. Trop Anim Health Prod., 48, 915–926.

Sandoval-Castilla, O., Lobato-Calleros, C., Aguirre-Mandujano, E., & Vernon-Carter, E. J. 2004. Microstructure and texture of yogurt as influenced by fat replacers. Int. Dairy J., 14(2), 151-159.

Sbodio, O. & Revelli, G. 2012. Milk's coagulation. Development of a device for online "monitoring" of the process. Progress made in Argentina, RIA, 38, 1-13.

Trujillo, A. J., Guamis, B., Laencina, J., & López, M. B. 2000. Proteolytic activities of some milk clotting enzymes on ovine casein. Food Chemistry, 71(4), 449-457.

Ustunol, Z., C. L. Hicks, F. A. Payne, and K. Milton. 1993. Effect of pH, temperature and enzyme concentration on diffuse reflectance profiles of coagulating milk. Italian J. of Food Sci., 2: 107-114.

Van Vliet, T., Van Dijk, H. J. M., Zoon, P., & Walstra, P. 1991. Relation between syneresis and rheological properties of particle gels. Colloid and Polymer Science, 269(6), 620-627.

Yonas H., Ipsen, R., Hansen, E. B., Eyasu Seifu., & Mitiku Eshetu. 2016. Factors influencing gelation and rennetability of camel milk using camel chymosin. International Dairy Journal, 60: 62-69.

Zayas J. F. 1986. Effect of ultrasonic treatment on the extraction of chymosin. Journal of Dairy Science, 69(7), 1767-1775.

Zayas J. F. 1987. Properties and quality characteristics of rennin extracted by ultrasound. Biotechnology and bioengineering, 29(8), 969-975.

Zayas J. F & Smolski N.T. 1970.Changes in ultrastructure of muscular tissue resulting from ultrasonic tenderization. Page 1141 in XVI Eur. Congr. Meat Res. Workers, Sofia, Bulgaria.

Zayas J. F. 1971. Investigation of the application of ultrasonic vibrations in technological processes of meat industry. Proc. XVII Eur. Congr. Meat Res. Workers, Bristol, Eng.

تحسين كفاءة طريقة الاستخلاص التقليدية لكيموسين الإبل باستخدام الموجات فوق الصوتية وعملية التجفيف بالتجميد

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الملخص

ترتبط العديد من المشكلات بطرق الاستخراج التقليدية لكيموسين الإبل. فهي مكلفة وتستغرق وقتا طويلا وتنتج تركيزات منخفضة من الكيموسين. لهذا هدفت هذه الدراسة إلى زيادة كفاءة الطريقة التقليدية لإنتاج كيموسين الإبل. حيث أجريت تجربتين؛ نفذت التجربة الأولى باستخدام منفحة الإبل في صورة مسحوق مجفف او شرائح (4-7 مم) ، ثم عوملت بجهاز الموجات فوق الصوتية وفى اوقات تعرض مختلفة (20 ، 40 ، 80 دقيقة). اظهرت المنفحة المستخلصة والمعاملة باستخدام المسحوق المجفف مع وقت التعرض بالموجات فوق الصوتية عند 80 دقيقة زيادة معنوية في نشاط تجبن حليب الابل وانخفاض النشاط التحللى مقارنة بالشرائح والكونترول . تم تنفيذ التجربة الثانية باستخدام عملية التجفيف بالتجميد لأفضل معاملات في المحصول والكفاءة التجبنيه لكيموسين المستخلص من الانفحة في شكل الشرائح و المسحوق. بالمقارنة مع العينات المعاملات التقليدية أظهرت النتائج بان عملية التجفيد بالتجميد ادت الى زيادة معنوية لكل من النسبة التجبنيه الى التحلليه وكذلك معامل التجبن الكلى المتخصص بحوالي 4.2 و 24.0 ضعفًا ، على التوالي. علاوة على ذلك ، أدت عملية التجفيف بالتجميد الى زيادة كبيرة في محصول الكيموسين ، ولزوجة الخثرة ، والصلابة ، وانخفاض فصل الشرش من الخثرة. ايضا أكدت النتائج أنه يمكن استخدام مقياس اللزوجة كمؤشر جديد للتنبؤ بوقت قطع خثرة حليب الإبل. يمكن التوصية باستخدام الموجات فوق الصوتية مع عملية التجفيف بالتجميد كطريقة مناسبة لاستخلاص كيموسين الإبل