RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 42 Issue 1: 14-19 (March 2023)

Camel Milk Curd Properties: Application of a Kiwi Juice as a Coagulant

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10.18805/ajdfr.DRF-275

ABSTRACT

Background: The transformation of camel milk into cheese is an operation considered very delicate because of several difficulties encountered in achieving coagulation. The present study aims to improve coagulation abilities of camel milk using enzyme extracts from kiwi juice.

Methods: The optimum characteristics of extract were determined (pH, temperature and $CaCl_2$ concentration), a fresh cheese was manufactured and the physicochemical and organoleptic qualities were determined.

Result: Our results concerning the characterization of the enzymatic extract showed an extraction yield as 63.97%±5.22. The optimum coagulation conditions were: pH=6.6, temperature= 40°C and a volume of used enzymatic extract as 1.5 ml. A fresh cheese was made from camel milk with a particular nutritional quality and consistency. The kiwi proteases displayed chymosin-like properties and thus hold the best potential for use as a milk coagulant in cheese production.

Key words: Camel milk, Coagulant activity, Proteolytic activity, Vegetable enzymes.

INTRODUCTION

Camels (*Camelus dromedarius*) are of particular interest in arid and desert regions. Their unique adaptability makes this species ideal for exploitation facing the challenges of climate and perfect allies for food security under changing climate. Camel milk is mainly consumed raw just after milking or fermented but rarely processed into cheese (Konuspayeva and Faye, 2016) due to a low amount of κ-casein (Farah, 1993). Hence, several studies focused on the functional and in spite of the above difficulties, satisfactory cheese can be made when cheese-making procedures are adapted to camel milk's particular characteristics (Ramet, 2001). Proteases from plant sources offer a high potential as nonanimal rennet alternative in production of cheese, food (*e.g.*, production of novel dairy products, meat tenderizers and protein hydrolyzates production) and medicine (*e.g.*, digestive and anti-inflammatory agents) (Katsaros *et al.,* 2010; Huang *et al.,* 2011). Furthermore, the use of plant proteases in cheese manufacturing promotes the greater acceptability by vegetarians, some religious groups and exigency of banning genetically engineered food (Grozdanovic *et al.,* 2013). For years, plant extracts have been used as milk coagulants in traditional cheeses which are mainly produced in Mediterranean countries, West Africa and Asia (Roseiro *et al.,* 2003). Indeed, dried cardoon flowers of *Cynara cardunculus* L. and *Cynara humillis* L. have been used for centuries in the Iberian Peninsula to prepare certain varieties of cheese with a creamy soft-texture and exquisite flavor. The common plant proteases papain and ficin (from papaya and fig, respectively) have a low milk-clotting activity/proteolytic activity (MCA/PA) ratio and have often been mentioned as the principal obstacle to their utilization in cheese making (Aworh *et al.,* 1987). Besides texture, the flavor of cheeses may also be affected by the type of plant extract used which leads to bitterness in the final product (Grozdanovic *et al.,* 2013). The use of fruit extracts such as kiwi (*Actinidia L*) extract resulted in less off-flavor notes which are attributed to the production of short peptides that are responsible for bitterness in dairy products when ficin (fig or papaya protease) is used (Su *et al.,* 2009).

Hence, the objective of the present study was to evaluate the camel milk-clotting activities of kiwi extracts compared to commercial rennet.

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How to cite this article: Fguiri, I., Sboui, A., Atigui, M., Arroum, S., Ayeb, N., Dbara, M., Hammadi, M. and Khorchani, T. (2023). Camel Milk Curd Properties: Application of a Kiwi Juice as a Coagulant. Asian Journal of Dairy and Food Research. 42(1): 14-19. doi: 10.18805/ajdfr.DRF-275.

Submitted: 08-03-2022 **Accepted:** 10-11-2022 **Online:** 18-11-2022

MATERIALS AND METHODS

Samples

Fresh kiwi (*Actinidia deliciosa*) were obtained from a local market (Medenine, Tunisia). Camel chymosin FAR-M was obtained from CHYMAX® M. 1000 International Milk Clotting

Units (IMCU) ml/l, Chr. Hansen A/S, Hørsholm, Denmark. Fresh camel milk was collected from she-camels (*Camelus dromadarius)* belonging to the Arid Land Institute (IRA Medenine, Tunisia). Samples were brought to the laboratory in an isotherm container and were analyzed and processed upon arrival.

Preparation of extracts

Extracts were prepared as described by Mazorra-Manzano *et al.* (2013). Briefly, kiwi was peeled and sliced. Then, water extracts were prepared by adding one equal part (w/v) of 20 mM sodium phosphate buffer (pH 7.2). Water extracts were homogenized and then centrifuged at 5000 g for 30 min at 4°C (Thermo Electron LED GmbH Am Kalkberg, Germany) and filtered through double cheesecloth to remove suspended particles. Fresh extracts were stored at 4°C and were either used the same day for protein and milk-clotting activity determination or frozen at -20°C for further use.

pH and dry matter of enzymatic extract were determined using International standard methods (AFNOR, 1993). The protein content was determined according to the Bradford method (1976), using bovine serum albumin (BSA) as standard.

Optimization of pH, temperature and $CaCl_2$ concentrations

Optimum pH and temperature were determined according to the method of Kunitz (1947). To determine the optimal pH for the enzyme activity; 1 ml of plant extract was added to 10 ml of camel milk at different pH values (5;5.6;6.2;6.6;7.5 and 8.5) at 30°C and flocculation time (Tc) were measured. In order to determine the optimum temperature, the purified enzyme solution was incubated with casein solution at various temperatures ranging from 30 to 60°C for 10 min in a controlled temperature water bath and the flocculation time was noted. The optimal $CaCl_2$ concentration was prepared by dissolving 12 g of skimmed milk powder in 100 ml of $CaCl_2$ solution with a calcium ion concentration range of 0.01 to 0.09 M, added in tubes containing 10 ml of milk at pH= 6.6 and at T= 30°C, the flocculation time was then determined.

Milk-clotting activity

The milk-clotting activity (MCA) of each extract was determined according to the Berridge method (1952) modified by Collin *et al.* (1977) with some modifications. One unit of enzymatic activity or rennet unit (RU) corresponds to the number of units of weight or volume of milk that can be coagulated with 1 ml of coagulant preparation in 100 seconds and at optimal pH and temperature for each plant extract or commercial rennet. The time needed for curd formation was recorded (Tc). MCA was expressed as RU units and calculated as follows:

$$RU = (10 * V) / (Tc * Q)$$

- RU: Unit of coagulating activity or rennet unit.
- V: Volume of substrate (ml).
- Q: Volume of coagulant extract (ml).
- Tc: Flocculation time (sec).

Proteolytic activity

Proteolytic activity was determined by the method of Bergere and Lenoir (1997) using BSA as a substrate. Briefly, 1 ml of 1% protein substrate solution (0.1 M Phosphate buffer, pH 7.0) was mixed with 1 ml of coagulant and incubated for 60 min at 35°C. After incubation, the reaction was stopped by the addition of 1 ml of 12% (w/v) trichloroacetic acid. The mixture was vortexed vigorously. The absorbance of the clear filtrate was measured at 280 nm. One unit of the enzymatic activity (U) was defined as the amount of protein that gave an increase of one unit in the absorbance at 280 nm under the described conditions.

Cheese making process

Fresh camel milk was pasteurized at 65°C for 30 minutes then cooled to 40°C. The starter culture (*Lactococcus lactis*) isolated from artisanal fermented milk was then added to decrease pH at 5.5. After about one hour, the enzymatic preparation was then added at the rate of 10% of milk and mixed thoroughly. The mixture was incubated for 24 hours at 37°C. After coagulation the whey was drained to obtain a fresh cheese and kept at 4°C for further analysis.

Cheeses characterization

The physicochemical characteristics were determined using International standard methods (AFNOR, 1996). The protein content was determined according to the Bradford method (1976), using (BSA) as standard. The moisture content (Hm) was calculated according to the following formula:

$$Hm = 100\text{-}DMC$$

Where:

Hm: Humidity (%).

DMC: Dry matter content.

As microbiological analysis, the total viable counts were determined on a plate count agar (Oxoid Ltd., Basingstoke, UK) at 30°C for 72 h, total coliforms on violet red bile agar (Oxoid) at 30°C for 24 h, mesophilic and thermophilic lactobacilli on MRS agar (Oxoid) at 30°C and 45°C for 48 h under anaerobiosis, respectively, lactococci on M17 agar (Oxoid) at 30°C for 48 h, yeasts and molds on Worth agar (Oxoid) at 30°C for 72 h. Results were expressed as log colony-forming units per ml of milk or gram of cheese.

The fat-soluble and water-soluble vitamins were determined by LC-MS chromatography according to Albala-Hurtado (1997).

Minerals in milk and cheese were quantified using Flame Atomic Absorption Spectrometer (ICE 3000 series AA Spectrometer).

Texture profile analysis

Texture properties (TPS) of the cheese samples were determined by a Texture Analyzer Brookfield (model CT-3, MA, USA). Cheeses were carefully cut into pieces (15 × 15 mm) with a cheese slicer, compressed to eliminate the air for 1 hour and the values have been read directly. 2 parameters

were set (penetration speed: 2 m/s; the distance of penetration: 10 mm).

Sensory analysis

Samples of cheese were subjected to sensory evaluation by 42 untrained panelists. Cheese samples were assessed for their taste, color, flavor, acidity and texture. Panellists received a set of four samples per session, representing cheeses made with kiwifruit extracts and the control made with chymosin. Each sample was evaluated in duplicate. The panellists were asked to drink plain water at the beginning of the sensory evaluation and between samples to try to make the palate conditions similar for each sample. Cheese traits are rated on the basis of 10 cm unstructured lines with 10 points scale (0 = lower intensity, 10 for higher intensity). Scores were the distances (cm) from the left anchor point. At the end of sensory evaluation, panellists were asked to rank overall cheese acceptability.

Statistical analysis

The experiments were conducted in triplicate and data were presented as the mean±standard deviation. Data related to physicochemical characteristics, TPS and sensory analysis were subjected to analysis of variance (ANOVA) using the SPSS 20. Duncan's multiple range tests were used to test differences between means with type of rennet as the main factor.

RESULTS AND DISCUSSION

Characterization of enzymatic extract

The physicochemical characteristics of the enzymatic extracts obtained were showed in Table 1. The pH of kiwifruit extract was 3.46±0.01. The dry matter content was 88.45±1.55 g/l and had significantly higher protein content (74.08±1.25 g/l).

Optimum conditions of the enzymatic extract clotting activity

As shown in Fig 1, the temperature for optimal clotting activity of kiwifruit extract studied in this assay was at 40°C. The effect of temperature proceeds mainly on the secondary phase of coagulation which corresponds to the aggregation

Table 1: Physicochemical characterization of the three enzymatic extract.

Extract	pH	Dry matter (g/l)	Proteins (g/l)
Kiwi	3.46±0.01^{c}	88.45^{b}±1.55	80.58^{a}±7.42

^{a,b} Means with the same superscript letter in same column are not significantly different (P>0.05).

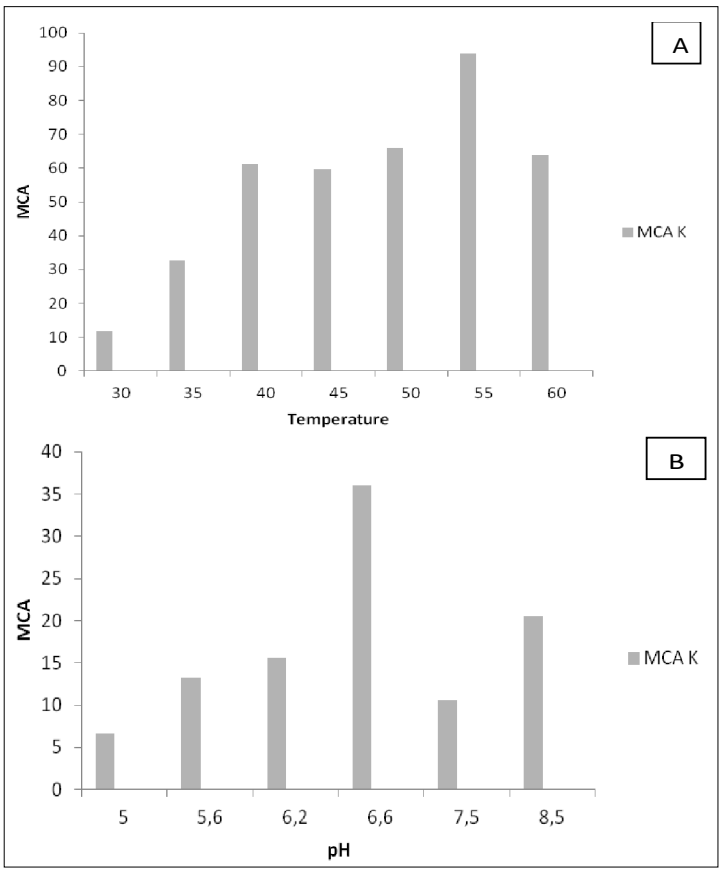


Fig 1: Optimum condition of clotting activity of enzymatic enzyme: A: Optimum temperature; B: Optimum pH; MCA K: Milk clotting activity of Kiwi.

step. This is due to the importance of hydrophobic interactions in the aggregation of hydrolysed micelles (Boudjenah, 2012).

The optimal clotting activity was at pH 6.6 for kiwifruit. Ramet, (1989) reported that all enzymes used in cheese manufacture are acidic proteases, their activity are generally optimal at pH values close to 5.5. Few studies on milk-clotting substitutes for calf rennet applied to camel milk are available. Grozdanovic *et al.* (2013) reported that kiwifruit extract prepared at pH 5.0 showed patterns of coagulum and whey proteins comparable with those obtained by chymosin using bovine milk. $CaCl_2$ concentration has no significant effect on clotting activity. Similar results were mentioned by Castillo *et al.* (2002) who consider that the main effect of $CaCl_2$ is important on aggregation and firming. Thus, it is usually added as a texturing agent. Indeed, the presence of ionized calcium is essential for the achievement of the secondary phase of milk coagulation since Ca^{2+} ions neutralize negative casein micelle residues to form a firm curd in the second phase of the coagulation process (Pires *et al.,* 1998).

Proteolytic activity

Fig 2 showed that camel milk cheese with kiwifruit have the lowest proteolytic activity compared to chymosin.

In the cheese industry, it is sought that the coagulating enzymes used have high coagulant activity and low proteolytic activity (Ramet, 1997).

The proteolytic activity of the extracts depends on several factors, such as the plant source, concentration and type of protease (Silvestre *et al.,* 2012).

Cheese curd yield

Kiwifruit showed the highest curd yield (20.71%, Fig 3) when compared to chymosin.

This can be explained by the fact that cheese yield also depends on other factors such as milk quality and composition, heat treatment of milk, type of cheese and the processing methods used (Mazorra-Manzano *et al.,* 2013).

Plant coagulants have long been considered as possible substitutes for chymosin in the cheese making process, but their potential for such use depends on their catalytic properties, stability and specificity, as these factors can affect cheese yield and sensory properties (Jacob *et al.,* 2011). In the cheese industry, the choice of the coagulating enzyme is a very determining factor. The most appropriate enzyme is the one with the highest clotting activity. The clotting activity is very changeable because it is strongly

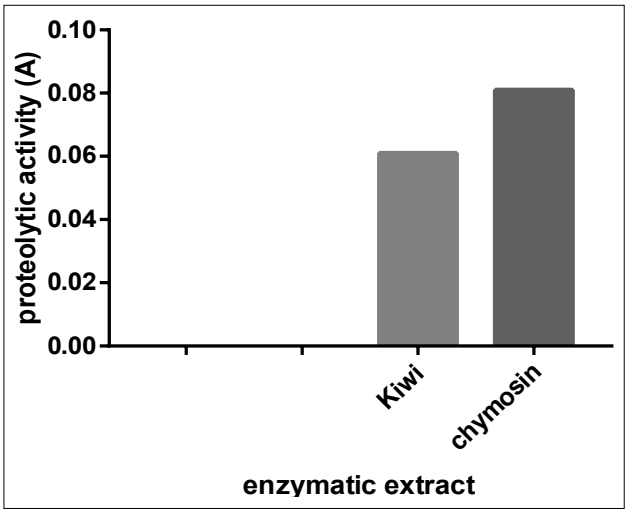


Fig 2: Proteolytic activity of enzymatic extract.

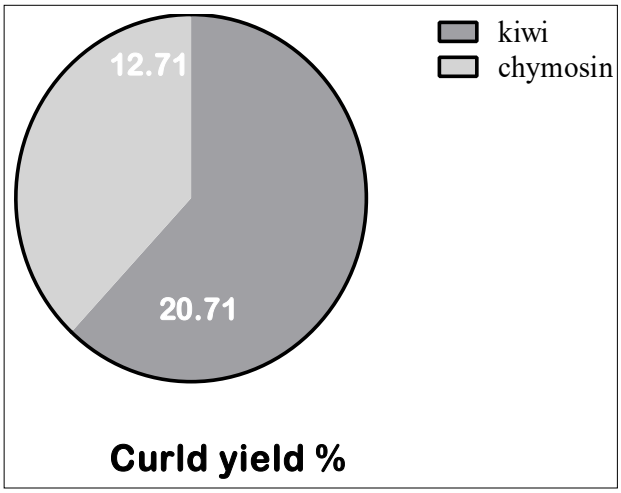


Fig 3: Curd yield (%) with different enzymatic extract (Chymosin, kiwi).

influenced by the state of maturity of the plant and by the conditions of collection and storage (Veisseyre, 1979).

Physicochemical characteristics of curd cheese

The physicochemical characteristics of camel cheese with kiwifruit are shown in Table 2.

A significant difference was revealed on ash and protein content and this is depending on the type of extract. The curd obtained with kiwifruit extract showed the highest proteins content. This result is similar with that found by Fguiri *et al.* (2021).

Table 2: Physicochemical characteristics of camel's cheese.

Type of cheese	pH	Dry matter (%)	Ash (%)	Humidity (%)	Proteins (g/l)
CMC K	6.01±0.02[a]	35.15±0.74[a]	5.19±0.32[a]	64.85±0.74[a]	31.25±0.47[a]
CMC Chy	6.32±0.02[a]	42.25±0.32[b]	6.31±0.54[a]	72.12±0.13[a]	27.25±0.22[b]

[a,b] Means in the same line followed by the same letter are not statistically different P>0.05; CMC K: Camel milk cheese with kiwi; CMC Chy: Camel milk cheese with chymosin.

Table 3: Fat-soluble and water-soluble vitamins of cheese with different enzymatic extract.

Water soluble vitamins	CMC K	CMC Chy	Fat-soluble vitamins	CMC K	CMC chy
B5 (ppm)	0.115	0.108	K (ppm)	0.003	0.379
B7 (ppm)	375.034	37.784			
B12 (ppm)	3.338	1.895	E (ppm)	0.114	0.419

CMC K: Camel milk cheese with kiwi; CMC Chy: Camel milk cheese with chymosin.

Table 4: Mineral composition of camel cheese with different enzymatic extract.

	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Fe (ppm)
CMC K	605.2	15.08	63.11	6.9	0.256	0.381
CMC chy	9.03	17.3	61.21	1.69	0.22	0.188

CMC K: Camel milk cheese with kiwi; CMC Chy: Camel milk cheese with chymosin.

Vitamins and mineral content of cheese

The fat-soluble vitamins analyzed were: K and E. The water-soluble vitamins were: B5, B7 and B12. The result was showed in Table 3.

Camel cheese with kiwifruit showed higher levels of water-soluble vitamins B7 (375,034 ppm), B12 and Vit B5 compared to camel cheese with chymosin. The main fat-soluble vitamins detected in camel cheese was vit E.

As shown in Table 4 the most predominant mineral element in camel cheese with different enzymatic extract were Na, K, Ca, Mg, Zn and Fe. Mineral content of camel cheese with kiwifruit was higher than other extract especially in Na (605.2 ppm) and Ca (63.11 ppm) content.

Texture profile analysis (TPA) of cheese curds

The results of TPA analysis of fresh cheese curds produced from the different plant coagulants are shown in Table 5.

Cheese curd texture varied greatly as a function of the different treatments, ranging from 1.15 to 4.33 mj ($P<0.05$). The highest values ($P>0.05$) were obtained for curds made with chymosin and kiwi (4.21 mj and 4.33 mj respectively). These results on curd deformation agree with the proteolytic activities of the extracts lower for the control (chymosin) and kiwi extract (Fig 2). This result was similar to that published by Mazorra-Manzano *et al.* (2013) who found highest curd hardness in kiwi fruit than melon and ginger in cow cheese.

Sensory evaluation of curd cheese

The sensory quality of cheeses varies according to the manufacturing technology and the chemical and microbiological characteristics of the raw material used. The result of sensory evaluation is shown in Table 6.

The addition of enzymatic extract have a significant effect on taste, texture, smell, acidity and color of camel cheese. However, camel milk cheese with kiwifruit showed the best texture. The cheeses obtained are characterized by a slightly bitter taste whatever the extract used. Benani, (2017) stated that the coagulant agent is characterized by a high proteolytic activity which confers a bitter taste on cheese.

Table 5: Effect of type of coagulant on curd texture profile analysis (TPA).

Camel milk cheese	Texture (mj)
CMC K	4.33 ± 0.20^{a}
CMC Chy	4.21 ± 0.32^{a}

Values with different superscript letters within the same column are different ($P<0.05$). Values are the mean±standard deviation, CMC K: Camel milk cheese with kiwi; CMC Chy: Camel milk cheese with chymosin.

Table 6: Sensory evaluation of camel curd cheese with different extract.

	Taste	Texture	Odor	Acidity	Colour
CMC K	3.85^{b} ±2.76	6.38^{a} ±2.57	6.38^{b} ±2.01	6.12^{b} ±2.42	5.81^{c} ±2.15
CMC Chy	1.70^{c} ±1.49	4.64^{b} ±2.92	2.54^{b} ±2.11	2.91^{ab} ±2.87	4.38 ±3.34

Values with different superscript letters within the same column are different ($P<0.05$). Values are the mean±standard deviation.

CONCLUSION

The kiwi enzymatic extract produced a curd with similar characteristics to those obtained when using commercial chymosin.

The textural properties of fresh curds obtained with kiwi and pineapple extracts were similar to those produced using chymosin. The differences observed between plant coagulants and chymosin may have some effect on the texture and flavor of cheeses, opening the possibility for the production of new cheese varieties with additional plant flavors.

Conflict of interest: None.

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