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RESEARCH ARTICLE

Optimization of flocculation and clotting time of camel milk with camel and goat rennets, and chicken pepsin in comparison with cow milk using response surface method (RSM)

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ABSTRACT

To solve the difficulty of coagulation of camel milk, three animal coagulating enzymes: Camel rennet, goat rennet, and chicken pepsin were used. The study was focused on the optimization of two milk coagulation key parameters: pH and temperature using the response surface methodology. The cow milk is used as reference. Characterization of the coagulating enzymes extract showed that the protein content is higher in chicken pepsin [20 ± 0.00 mg/ml] followed by camel and goat's extracts [15.4 ± 0.00 mg/ml, 8.8 ± 0.00 mg/ml]. However, coagulating and specific activities were more important in camel rennet [111.12 ± 1.23 RU.ml^{-1}, 7.21 ± 0.03 RU.mg^{-1}] than in goat's rennet and chicken pepsin. On the other hand, coagulant strength was for camel rennet 1/4166.67 SU, goat rennet 1/2531.64 SU, and chicken pepsin 1/6153.85 SU. Moreover, results of pH and temperature optimization of camel milk flocculation showed the following pairs: [X1 = 5.35, X2 = 42] for camel rennet, [X1 = 5.48, X2 = 30] for goat rennet and [X1 = 5.49, X2 = 39.45] for chicken pepsin. Also, the coagulation showed the following couples: [X1 = 5.37, X2 = 39.09] for camel rennet, [X1 = 5.36, X2 = 38.84] for goat rennet and [X1 = 5, X2 = 42] for chicken pepsin. The conclusion was flocculation and coagulation optimum points of camel and cow milk with coagulating enzymes studied are different based on surface plot analysis and the relationship between response and variables.

Keywords: Camel milk; Chicken pepsin; Coagulation; Rennet; RSM optimization

INTRODUCTION

In the southern Mediterranean basin countries, camel breeding represents a central activity in the occupation of the steppe and desert pastoral space and the maintenance of agricultural activity of the oasis systems, in the zootechnical development of desert areas, and the control of desertification. In addition, climate changes in this part of the world marked by increased desertification of the Saharan fringes are resulting in a decrease in natural resources and the need for reasoned management of water resources (Faye et al., 2014). In Algeria, camel farming is concentrated in semi-arid and arid areas. Ten dromedary populations were identified in the country out of a total of 26 populations in Africa and 97 in the world (Harek et al., 2017).

Camel milk plays an important role in feeding nomads and the populations of southern Algeria since recent years, it has started to be sold in northern regions. Its position is significantly different, depending on the types of camel routes, season, husbandry systems, parity number, and stage of lactation (Alloui-Lombarkia et al., 2007; Hadef et al., 2018). Camel milk also called white gold of the desert is more similar to human milk than any other milk and differs from other ruminant milk (Young et al., 2017). The content of cholesterol and sugar is lower in camel milk than the milk of other ruminants, while minerals (sodium, potassium, iron, copper, zinc and magnesium), vitamin C, niacin protective proteins (Kula and Tegegne, 2016; Young et al., 2017) and unsaturated fatyy acids (Konuspayeva et al., 2008) are higher. In addition, the dietary and medicinal qualities

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of camel milk are renowned. It is hypoallergenic (Shabo et al., 2005), antidiabetic (Agrawal et al., 2003), and anti-infective (El-Agamy et al., 1992). For that, camel milk is an asset for good diversification of the products offered such as cheese (Boudjenah-Haroun et al., 2011; Konuspayeva and Faye, 2021). Unfortunately, transformation of camel milk in cheese-making after enzymatic coagulation seems difficult. Farah and Ruegg (1989), El-Agamy and Nawar (2000) and Youg et al. (2017) indicate that casein micelles of camel milk are bigger in diameter than the milk of other species.

Failure of coagulum formation is due to non-specific interaction of the protease with camel kappa casein (κ-CN). Camel κ-CN contains a distinctly different cleavage site for aspartic proteases as compared with bovine κ-CN (Kappeler, 1998). In cheese manufacturing, calf rennet is the most common enzyme used worldwide as a milk-clotting agent due to its high milk-coagulating ability and higher yield of cheese. However, with the worldwide increase of cheese yield and supply shortage of calf rennet, researchers have been obliged to study and develop calf rennet substitutes (Lopes et al., 1998).

Thus, various clotting agents are important as substitutes for calf rennet, but these sources are not suitable for quality cheese production since they produce extremely bitter tastes (Neelakanta et al., 1999; Walsh and Li (2000); Kumar et al., 2006) Recombinant chymosin can also be used to produce cheese, but its use may be limited for consumer concern regarding genetically engineered foods (Egito et al., 2007). As animal clotting agents, there are studies that prove the ability to use camel rennet (Boudjenah-Haroun et al., 2011; Boudjenah-Haroun et al., 2014; Hattem et al., 2017), goat rennet (Zhang and Wang, 2007) and chicken pepsin (Benyahia-Krid et al., 2016) for bovine milk coagulation.

In order to coagulate camel milk, very limited studies have been carried out on the capacity of camel rennet to coagulate camel milk. Studies carried out by Wangoh *e*t al. (1993), Elagamy (2000), Sibouker et al. (2005), Boudjenah-Haroun et al. (2011) and Ramet (2011) have shown the possibility of using coagulating enzymes extracted from dromedaries' abomasum. Camel chymosin was also used to investigate factors influencing the gelation and rennetability of camel milk (Hailu et al., 2016) and lately, in white brained soft cheese characterization (Bouazizi et al., 2021). Studying the clotting time of camel milk, Wangoh et al. (1993) have shown that this parameter was significantly reduced when camel rennet was used instead of bovine rennet. Different studies revealed that rennet coagulation of camel milk follows a similar mechanism as that of cow milk. However, the action of rennet on camel milk results in coagulation in flakes form without a firm coagulum (Young et al., 2017; Bittante et al., 2021).

Recently, Felfoul et al. (2022) demonstrated that the induction of acidification promotes dromedary coagulation. However, there are no studies targeting the clotting of camel milk by other animal coagulating enzymes such as chicken pepsin to optimize temperature and milk pH clotting parameters. This study investigates the dromedary milk coagulation exploiting rennet and other available animal coagulant enzymes, such as camel or goat rennets and chicken pepsin, compared to cow milk coagulation tested in the same modalities. The flocculation and the clotting time conditions (milk pH and temperature) with coagulating enzymes are optimized.

MATERIALS AND METHODS

Milk samples

Three raw milk types were used. Dromedary milk samples were collected from El Oued region (southeast of Algeria) from herds of *Camelus dromedarius* of *Sahrawi* population living in semi-extensive breeding in natural ranges. Also, goat milk samples were collected in the same region from herds of *Arbia* goats. Cow's milk was collected from the same region and used for results comparison.

These samples were collected cleanly and sent to laboratory in a cooler containing ice packs, then frozen at -18°C until further use.

Berridge substrate

Skimmed milk powder (12 g) was used for Berridge substrate preparation (100 ml) which is dissolved in 100 ml of $CaCl_2$ solution (0.01 M). The pH of the prepared milk solution was adjusted to 6.4 with HCl or NaOH (1N) solutions.

Animals' abomasum and chicken proventriculus

Abomasum were collected from camels less than one year and from goats of 3 months old. Abomasum were recovered from a slaughterhouse. Proventriculus were collected from chicken less than 50 days old from poultry slaughter in El Oued. Camel or goat abomasum and chicken proventriculus were washed with tap water, degreased, covered in a sterile bag and frozen at -18°C until enzyme extraction.

Physicochemical analysis of camel and cow milks

Assessment of main characteristics

The milk pH measurement was carried out at 25°C. The value was read directly on pH meter after immersing its electrode in milk to be analyzed, which was calibrated beforehand using calibration solutions. The acidity was measured by titrating milk containing phenolphthalein with caustic soda (0.1N NaOH) (AFNOR, 1986).

Density measurement was carried out by introducing a lactodensimeter into a sufficiently large and deep test tube containing milk, then a directly reading the value.

Total dry matter, which is the product resulting from the drying of milk, was obtained by evaporation of water in an oven set at 103±2 ºC for 3 hours (AFNOR, 1980).

Specific nutrient assessment

Nitrogen content was determined by Kjeldahl method (ISO8968-1: 2014) and protein level was calculated by the following relation: $Mp = M_N \times 6.25$ (Mp: protein masse, M_N: nitrogen masse). Fats were determined according to GERBER method (ISO2446: 2008) and lactose content was determined by the BERTRAND method (AFNOR 1986).

Determination of mineral salts

Concentration of different mineral elements in the camel, goat and cow milks was determined by spectroscopic analyses: sodium (flame emission spectrometry); potassium (optical emission spectrometry); magnesium, Copper, Zinc, Iron, Lead (atomic absorption spectrometry) (ISO 6955: 1982) and titrimetric assay for calcium (FIL 36A).

Extraction of coagulating enzymes

Fig 1 summarizes experimental design of clotting enzymes protocol extraction and studied parameters using response surface method for pH and Temperature variation.

Camel and goat rennets

The extraction was carried out according to the protocol used by Wangoh et al. (1993). The goat and dromedary abomasum were cut into slices (1 cm^2), a maceration is made in a 6% NaCl solution (1:10 w/v) containing 2% boric acid. The mixture was then filtered and centrifuged. The pH of the supernatant was then lowered to 4.7 and the extracts were kept at 25°C. The pH was then increased to 5.5, followed by a final centrifugation (Table 1). The enzyme extract was stored at (-18°C) until use.

Chicken pepsin

The pepsin extraction was carried out following the extraction protocol proposed by Bohak (1970). The proventriculi were minced and then macerated in a saline solution for 3 hours. The extract was adjusted to pH 2. The mixture was filtered and the retentate was removed. Finally, the filtrate was centrifuged followed by pH adjustment to 6.4. The enzyme extract was stored at (-18°C) until use.

Characterization of coagulating enzymes

Coagulant activity

Coagulant activity (CAU) or rennet unit RU is defined by the quantity of enzyme contained in 1ml of enzymatic solution which can coagulate 10ml of BERRIDGE substrate (Berridge, 1955) in 200s at 30°C. The technique consists of adding to the milk, 1ml of the enzymatic extract followed by homogenization (3 turns), then noting when visible casein flakes are formed on walls of the test tube (Mahaut et al., 2003).

Clotting time

Clotting time is the point at which first droplets of whey appear on surface of gel, the coagulum becomes rigid and no longer flows on walls of tube. It is carried out directly on 10ml of raw milk maintained at 35°C in a water bath,

Table 1: Factors codes and levels of the experimental design for parameters of pH [from 5 to 6.7] and T° [from 30 to 42°C].

Levels (coded)	Factors (not coded)	
	pH	T (°C)
Min Point (-α)	5	30
Point (-1)	5.25	31.76
Central point (0)	5.85	36
Point (+1)	6.45	40.24
Max Point (+α)	6.7	42

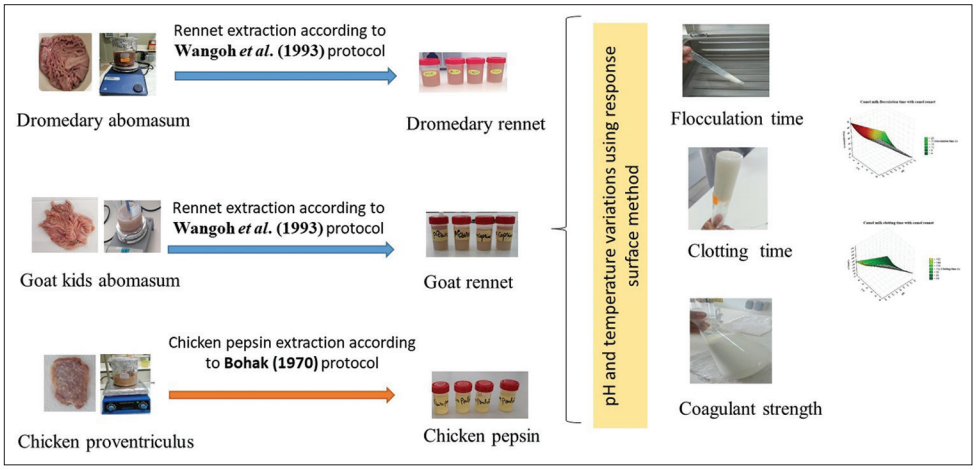

Fig 1. Experimental design of clotting enzymes protocol extraction and studied parameters using response surface method for pH and temperature variation

and then 1ml of enzymatic solution is added. In enzymatic clotting, the setting time is between 25 and 30min (FAO, 1955; Alais, 1974).

Coagulant strength

Coagulant strength of an enzyme extract or a coagulating enzyme represents the volume of coagulated milk per unit of enzyme extract, in 40min, at 35°C and pH 6.4 (Nouani et al., 2009).

Protein determination of enzyme extract

The protein assay method is the same as cited for milk proteins by changing the conversion coefficient (6.25).

Specific activity

Specific activity is calculated directly, it's the number of units of activity per unit of mass (RU/mg) of protein. It indicates the enzyme purity (Nouani et al., 2009).

Optimization of coagulating enzymes activity

Experimental design

The response surface methodology, using a central composite design (CCD) with two factors at five levels ($-\alpha, -1, 0, +1, +\alpha$), was used to optimize effects of pH (X_1) and temperature (X_2) on flocculation time (Y_1) and clotting time (Y_2) of milk samples. The pH and temperature were respectively varied from 5 to 6.7 and from 30°C to 42 C°. The experimental design generated 13 trials. A second-degree equation was determined from the experiments to predict different responses as a function of the studied parameters (pH and temperature) using the following formula (1):

This type of model allows estimation of a response surface to study linear effects, quadratic effects and interaction effects of pH and temperature (T) on flocculation and clotting time.

Table 2: Experiment matrix of orthogonal composite plane centred with two factors.

Test	Coded values		Uncoded values	
	A	B	pH	T
1	0	0	5.85	36
2	1.414	0	6.7	36
3	0	1.414	5.85	42
4	0	-1.414	5.85	30
5	-1.414	0	5	36
6	0	0	5.85	36
7	0	0	5.85	36
8	-1	-1	5.25	31.76
9	0	0	5.85	36
10	1	1	6.45	40.24
11	0	0	5.85	36
12	-1	1	5.25	40.24
13	1	-1	6.45	31.76

$$Y=b_0+ b_1 X_1+ b_2 X_2+ b_{11} X^2_1 +b_{22} X^2_2 +b_{12} X_1 X_2 \ldots\ldots(1)$$

With:

Y: predicted response;

b_0, b_1, b_2, b_{11}, and b_{22}: coefficients of the equation with

b_0: constant;

b_1 and b_2: coefficients of the linear terms;

b_{11} and b_{22}: coefficients of the quadratic terms;

b_{12}: interaction coefficients;

X_1 and X_2: uncoded values of the independent variables (pH and T).

For all experimental design tests, 1ml of enzymatic extract was added to 10ml of camel milk or cow milk. The pH was adjusted with lactic acid. After three reversals then triggering of chronometer, the test tube is either kept in rotary movement (for the flocculation tests), or kept without rotation (for the clotting tests) in a water bath according to the temperature test.

Table 2 shows the experiment matrix of the orthogonal composite plane centered with two factors for the 13 tests.

Statistical analysis of data and graphical representations

Minitab 19 software (Minitab Inc., State College, PA) was used to determine coefficients for each response. The degree of significance of coefficients was determined using the p-value, and the significance level was set. Flocculation and clotting time surface plots for each enzyme type are shown using statistica software (version 10, Statsoft Poland).

Table 3: Physicochemical characterization of milks used (mean value±standard deviation).

Paramater	Camel milk	Cow milk (standard)
pH	6.56±0.34	7.15±0.05
Dornic acidity	18±1.00	13±0.5
Fat (g/l)	37.76±1.54	29±11.00
Lactose (g/l)	31.4±0.2	51.6±0.57
Proteins (g/l)	28.6±0.4	34±09.00
Specific gravity at 20°C (g/cm^3)	1.029±0.001	1.028±0.00
Dry matter (g/l)	106.18±0.9	200±7.5
Ash (g/l)	7.16±1.66	5.15±2.07
Chlorides (g/l)	4.46±0.3	1.9±0.3
Calcium (%)	0.09±0.001	0.7±0.01
Sodium (mg/l)	678±0.8	185±1.00
Potassium (mg/l)	2.29.10^3±0.2	0.7.10^3±0.5
Magnesium (mg/l)	1.5±0.09	1.1±0.02
Copper (mg/l)	0.48±0.04	0.4±0.01
Zinc (mg/l)	4.39±1.00	3.00±0.00
Iron (mg/l)	2.32±0.7	1.9±0.8
Lead (mg/l)	≤ 0.01	≤ 0.01

RESULTS AND DISCUSSION

Physicochemical analysis of milks

The main physicochemical characteristics of camel milk are shown in table 3. The pH value of camel milk is similar to those given by several authors (Kamoun, 1996; Kappeler et al., 1998; Young et al., 2017). In fresh camel milk, pH ranges from 6.55 to 6.7 (Konuspayeva et al., 2009) and is slightly lower than cow milk (Al haj and Al Kanhal, 2010). The variation of pH value could be due to differences in hygiene of the milking practices and the total microbial count of milk and it can be affected by feeding and water availability (Hadef et al., 2018). Acidity of camel milk was higher than cow milk. It agrees with the findings of Hadef et al. (2018).

Specific gravity was respectively around 1.028 for cow milk and1.029 for camel milk. Density depends on dry matter content, fat content, temperature and diet of the animal (Debouz et al., 2014). This density is in agreement with Pak et al. (2019). According to Young et al. (2017), the specific gravity of camel milk is near that of cow milk (1.029 and 1.032 respectively).

For camel milk fat content, our results are similar to that given by Alloui-Lombarkia et al. (2007) (37.44±5.40g/l). As showed by Shuiep et al. (2018) fat content of camel milk is affected by management conditions and seasons. In addition, Konuspayeva et al. (2009) observed that there is a large variation in camel milk fat content across the world. They also showed that camels living in East Africa have a milk composition that contains more fat than milk camels living in Africa and Western Asia. In addition, Abdalla et al. (2015) reported that the low percentage of fat in milk camels reflects poor nutrition under desert conditions.

Lactose content is significantly lower in camel milk compared to cow milk. This result is in agreement with Alloui-Lombarkia et al. (2007) (34.20± 9.04 g/l) and less than that found by Young et al. (2017) (45.6g/l). According to Brezovečki et al (2015) the amount of lactose in camel milk varies from 29.1 g/l to 41.2/l, which is less when compared to 44-58 g/l in cow milk. Large differences in lactose content may be conditioned by animal nutrition which depends on plants type with which the animals are fed (Alloui-Lombarkia et al., 2007).

Camel milk protein content is lower than that of cow milk. This result is in agreement with Debouz et al. (2014) (28.1±0.12g/l). However, it's relatively lower compared to that reported by Attia et al. (2000) and Konuspayeva et al. (2009) of 35 to 45g/l. Milk proteins concentration varies according to the season, stage of lactation and of calving number.

Average dry matter value content in cow milk is 200g/l while for camel milk is 106g/l, this value is almost similar to that given by Ismaili et al. (2019) which is 104.2g/l. Milk dry matter content varies depending on the stage of lactation. Thus, it decreases during the month following calving, then increases following an increase in the rate of fat and nitrogen (Debouz et al., 2014; Ismaili et al., 2019).

According to Pak et al. (2019), all components of camel milk decreased from December to February and had a tendency to grow from June to August. Also, food supply plays an important role and can explain the particular richness of camel milk.

From these results, it appears that the mineral content of camel milk (7.16±1.66g/l) is higher compared to cow milk (5.15±2.07g/l). Camel milk value is in agreement with Brezovečki et al. (2015) (from 6g/l to 9g/l). Ash in camel milk is subject to breed, analytical procedures, nutrition, and water consumption. It is a rich source of chloride because of feed consumed by camels, such as *Atriplex* and locust tree, which usually rich in salt.

Characterization of enzymatic extracts

Characterization results of camel rennet, goat rennet and chicken pepsin are shown in table 4.

Camel rennet characteristics

Enzymatic extract of camel rennet obtained as described by Wangoh et al (1993) protocol has a liquid texture and a light brown color. It is characterized by a protein content of 15.4±0.00 mg/ml. This value is higher than that found by Boudjenah-Haroun (2012) which is (1.54 mg/ml). The camel rennet coagulant activity is 111.12±1.23 RU.ml^{-1}. This result is higher than the values of Siboukeur et al. (2005) (0.155 RU.ml^{-1}), Mahboub (2009) (0.081±0.003 RU.ml^{-1}) and Boudjenah-Haroun (2012) (0.360±0.02 RU.ml^{-1}). Specific activity and coagulant strength are respectively 7.21±0.03 RU.mg^{-1} and 1/4166.67 SU.

Table 4: Main characteristics of enzymatic extracts (mean value±standard deviation).

Enzyme	Protein (mg/ml)	Coagulant activity (RU.ml^{-1})	Specific activity (RU.mg^{-1})	Coagulant strength (SU)	Clotting time (s)		
					Camel milk	Goat milk	Cow milk
Camel rennet	15.4±0.00	111.12±1.23	7.21±0.03	1/4166.67	181±1.00	98.13±0.87	61.27±0.73
Goat rennet	8.8±0.00	1.52±0.01	0.172±0.00	1/2531.64	294.97±5.33	59.87±0.73	59.93±1.27
Chicken pepsin	20±0.00	1.98±0.02	0.1±0.01	1/6153.85	119.4±0.6	105±2.4	88.2±4.2

According to Siboukeur et al. (2005) and Boudjenah-Haroun et al. (2011), coagulating activity of camel enzymatic extracts is influenced by animals'age However, the animal's diet has an influence on the enzymatic content of dromedary abomasum as mentioned by Boudjenah-Haroun et al. (2013). Mahboub et al. (2012) studied the influence of the storage temperature on the coagulant activity of camel enzymatic extracts and shows that freezing at -20°C and refrigeration at +4°C are recommended in cheese production. Regarding the clotting time, it appears that camel rennet reacts better with cow milk (61.27±0.73s) compared to goat and camel milks (98.13±0.87s, 181±1.00 respectively).

Goat rennet characteristics

The liquid extract of goat rennet prepared has a light brown color, with a protein content of 8.8±0.00 mg/ml. This value is higher than compared to that reported by Boumediene (2013) (1.53 mg/ml). It is characterized by a coagulant activity 1.52±0.01 RU.ml^{-1} and a coagulant force 1/2531.64 SU. Goat rennet coagulates cow and goat milks in a shorter time (59.93±1.27s, 59.87±0.73s respectively) than that found for camel milk (294.97±5.33s).

Chicken pepsin characteristics

Chicken pepsin, extracted following the protocol of Bohak (1970), is a yellowish liquid. Protein content is 20±0.00 mg/ml; which is in agreement with Siar (2014) (20.10±0.73 mg/ml); it is higher than those reported by Adoui (2007) and Aïssaoui-Zitoun et al. (2017) (8.77±0.41 mg/ml, 3.20±0.60 mg/ml respectively) and lower than reported by Benyahia-Krid (2013) (35.40±0.40 mg/ml). Coagulant activity is 1.98±0.02 RU.ml^{-1}; it's less than Aïssaoui-Zitoun et al. (2017) which is 10.2±1.10 RU.ml^{-1}. Chicken pepsin has a coagulant strength of 1/6153.85 SU. It reacts better with cow milk

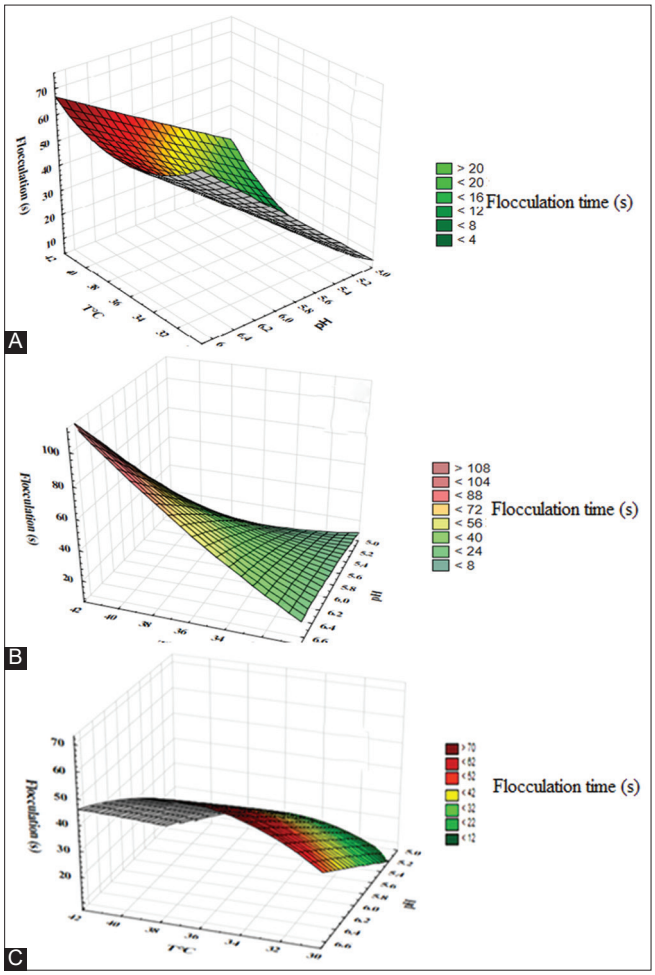

Fig 2. Surface response plot of camel milk flocculation time using camel rennet (A), goat rennet (B) and chicken pepsin (C)

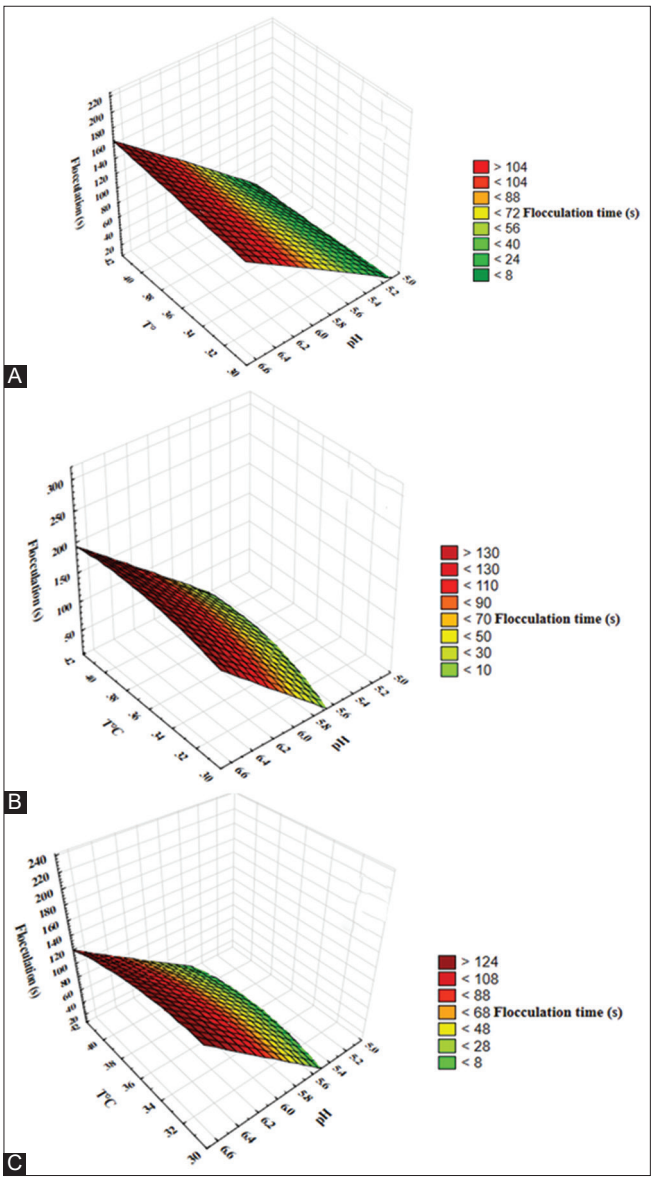

Fig 3. Surface response plot of cow milk flocculation time using camel rennet (A), goat rennet (B) and chicken pepsin (C)

(88.2±4.2s) compared to that of goat or camel milks (105±2.4s, 119.4±0.6s respectively).

Optimization of coagulating enzymes activity

Effect of pH and temperature on camel milk flocculation

Flocculation time responses of camel milk with camel and goat rennets and with chicken pepsin are presented in table 5.

For each enzymatic extract, the camel milk flocculation time changed with pH or milk temperature. At this pair (pH: 5/T: 36°C), the flocculation time of camel milk was minimal with camel rennet (3.6±0.00s), goat rennet (5.4±0.00s) and chicken pepsin (7±0.04s). However, the maximum flocculation times using camel's rennet or chicken pepsin were 76±0.02s and 73±0.04s, also obtained at the same pH (6.7) and milk temperature (36°C). Differently, using goat rennet, the maximum flocculation time of the camel milk was 114±0.00s and at pH 6.45 and 40.24 °C.

As preconized by Ramet (1997) to choose enzymes extract for milk coagulation, flocculation time mustn't exceed 200s. This is verified by results obtained during this study where the flocculation time did not exceed 200s for all proteolytic enzymes tested.

Plot in fig 2 shows that camel milk flocculation is sensitive to the variation in pH (linear effect) ($p<0.05$) with camel rennet and chicken pepsin. On the other hand, flocculation with goat rennet is influenced by pH and with interaction between pH and temperature (linear effect and interactive effect) ($p<0.05$). Based on the surface plot analysis and the relationship between response and variables, a camel milk floccuation time is optimal assuming these pairs of pH and temperature: [X1=5, X2=40.06] for camel rennet, [X1=5, X2=42] for goat rennet and [X1=5, X2=42] for chicken pepsin.

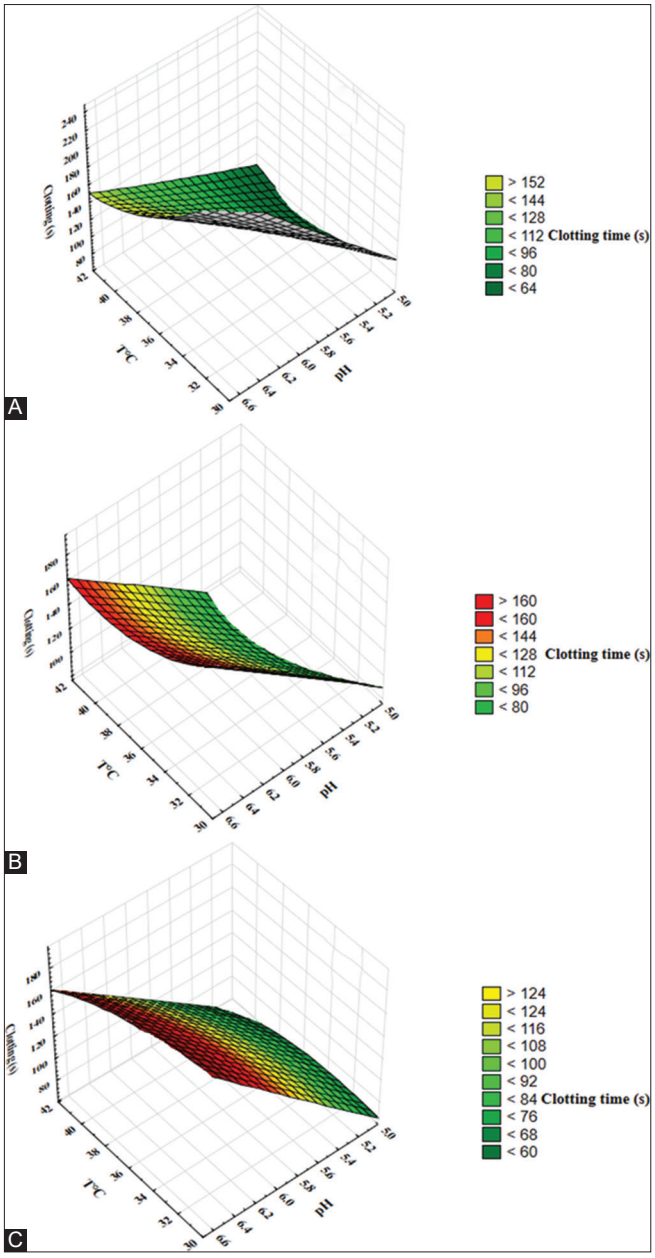

Fig 4. Surface response plot of camel milk clotting time using camel rennet (A), goat rennet (B) and chicken pepsin (C)

Effect of pH and temperature on cow milk flocculation

Table 6 shows that camel and goat rennets have a minimum flocculation time (4±0.02s, 5±0.00s) with (pH: 5/T: 36) and (pH:5.25/T:40.24) respectively. However, chicken pepsin reacts with cow's milk for minimal flocculation time of 7s at (pH: 5.85/T 3 6).

For (pH: 6.7/T: 36) pair, camel rennet, goat rennet and chicken pepsin flocculate cow milk for a long time (225±0.04s), (320±0.02s) and (242±0.00s) respectively.

Table 5: Flocculation time responses of camel milk with camel rennet, goat rennet or chicken pepsin.

Test	Responses (s)				
	Coded values		PCM[a]	PCP[b]	PP[c]
	X_1	X_2	Y_1		
1	0	0	38±0.04	26.4±0.00	25±0.02
2	1.414	0	76±0.02	58±0.004	73±0.04
3	0	1.414	38±0.00	42±0.04	12±0.00
4	0	-1.414	26±0.04	29±0.04	32±0.04
5	-1.414	0	3.6±0.00	5.4±0.00	7±0.04
6	0	0	13±0.04	48±0.00	39±0.03
7	0	0	9±0.04	46±0.02	37±0.04
8	-1	-1	33±0.04	7±0.02	16±0.04
9	0	0	12±0.00	16±0.04	31±0.00
10	1	1	58±0.04	114±0.00	45±0.00
11	0	0	37±0.02	43±0.04	34±0.04
12	-1	1	37±0.02	43±0.04	34±0.04
13	1	-1	57±0.01	27±0.04	20±0.03

a: camel rennet, b: goat rennet, c: chicken pepsin.

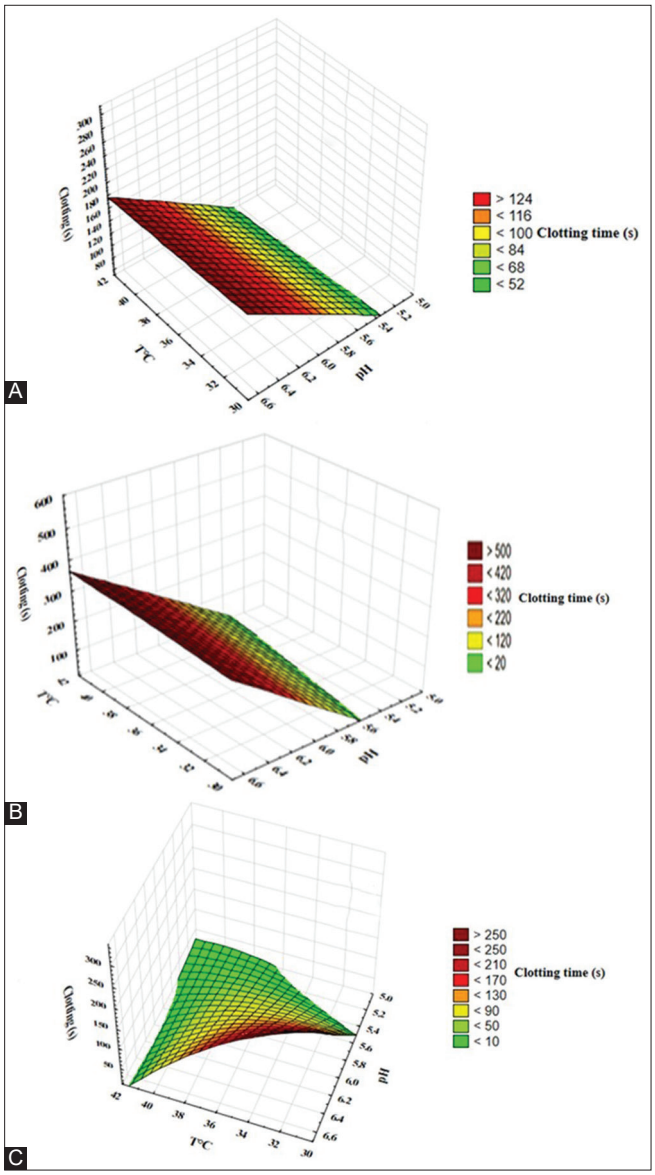

Fig 5. Surface response plot of cow milk clotting time using camel rennet (A), goat rennet (B) and chicken pepsin (C)

The plot in fig 3 shows that cow milk flocculation is sensitive only to pH variation (linear and quadratic effect) with camel rennet and chicken pepsin ($p<0.05$). For goat rennet, flocculation is sensitive at the same time to the variation in pH (linear and quadratic effect) and to the variation in temperature (linear effect) ($p<0.05$). Based on surface plot analysis and the relationship between response and variables, a cow milk flocculation time is optimal assuming these pairs of pH and temperature: [X1=5.35, X2=42] for camel rennet, [X1=5.48, X2=30] for goat rennet and [X1=5.49, X2=39.45] for chicken pepsin.

By comparing our results, camel rennet and chicken pepsin react with camel milk and cow milk with a minimum and maximum time of flocculation for the same temperature

Table 6: Flocculation time responses of cow milk with camel rennet, goat rennet or chicken pepsin.

Test	Responses (s)				
	Coded values		PCM[a]	PCP[b]	PP[c]
	X_1	X_2		Y_1	
1	0	0	39±0.01	10±0.00	17±0.04
2	1.414	0	225±0.04	320±0.02	242±0.00
3	0	1.414	41±0.02	44±0.04	19±0.00
4	0	-1.414	60±0.00	7±0.03	41±0.02
5	-1.414	0	4±0.02	27±0.03	32±0.004
6	0	0	45±0.01	15±0.00	49±0.00
7	0	0	49±0.02	21±0.02	7±0.02
8	-1	-1	56±0.04	6±0.01	12±0.04
9	0	0	50±0.04	10±0.02	7±0.01
10	1	1	92±0.03	190±0.01	127±0.02
11	0	0	52±0.01	8±0.00	7±0.03
12	-1	1	38±0.04	5±0.00	7.8±0.00
13	1	-1	111±0.04	150±0.04	121±0.00

a: camel rennet, b: goat rennet, c: chicken pepsin.

(pH:6.7/T:36) and (pH:5/T:36) respectively. While goat rennet reacts differently.

After optimization of flocculation responses of camel milk and cow milk, our results show that the optimum flocculation points with camel and goat rennets, chicken pepsin are different between the two milk types.

Effect of pH and temperature on camel milk clotting

Clotting time responses of camel milk with camel rennet, goat rennet and chicken pepsin are given in table 7. Results show that camel and goat rennets react with camel milk and give a clotting time of (60±0.02s) and (76±0.02 s) respectively. For chicken pepsin, clotting time (59±0.04s) and flocculation time (7±0.04s) are minimal at the same level of pH and temperature.

Plot in fig 4 shows that camel milk clotting time with camel rennet or goat rennet is influenced by the pH (positive linear and quadratic effect) and also by the temperature (linear and positive effect). The regression coefficient b_1, b_{11} and b_2 have a value of ($p<0.05$). Coagulating enzymes coagulate camel milk for a time not exceeding 15 minutes. However, pH has (a unique significant effect) on the clotting camel milk with chicken pepsin where regression coefficient b_1 has a value of ($p<0.05$). Based on the surface plot analysis and the relationship between response and variables, a camel milk clotting time is optimal assuming these pairs of pH and temperature: [X1=5.37, X2=39.09] for camel rennet, [X1=5.36, X2=38.84] for goat rennet and [X1=5, X2=42] for chicken pepsin. These enzymes technological parameters (pH and temperature) could be useful for camel milk clotting on an industrial scale.

Table 7: Clotting time responses of camel milk with camel rennet, goat rennet or chicken pepsin.

Test	Clotting time responses (s)				
	Coded values		PCM[a]	PCP[b]	PP[c]
	X_1	X_2		Y_2	
1	0	0	159±0.00	115±0.02	112±0.02
2	1.414	0	226±0.02	196±0.04	197±0.04
3	0	1.414	115.8±0.00	100±0.04	85±0.02
4	0	-1.414	168±0.01	124±0.04	115.8±0.00
5	-1.414	0	103.8±0.00	79.8±0.00	59±0.04
6	0	0	73±0.02	86±0.04	143±0.04
7	0	0	88±0.02	94±0.02	107±0.04
8	-1	-1	124±0.02	120±0.00	112±0.02
9	0	0	94±0.02	93±0.03	119±0.02
10	1	1	149±0.04	156±0.00	174±0.00
11	0	0	121.2±0.02	76±0.02	132±0.00
12	-1	1	60±0.01	76±0.02	96±0.00
13	1	-1	248±0.04	186±0.03	198±0.03

a: camel rennet, b: goat rennet, c: chicken pepsin.

Table 8: Clotting time responses of cow milk with camel rennet, goat rennet, or chicken pepsin.

Test	Clotting time responses (s)				
	Coded values		PCM[a]	PCP[b]	PP[c]
	X_1	X_2		Y_2	
1	0	0	60±0.01	12±0.00	51±0.01
2	1.414	0	313±0.02	603±0.01	348±0.01
3	0	1.414	60±0.02	66±0.00	22.8±0.00
4	0	-1.414	72±0.01	9±0.001	54±0.03
5	-1.414	0	72±0.00	66±0.003	99±0.02
6	0	0	64±0.03	21±0.002	54±0.00
7	0	0	59±0.04	42±0.00	9±0.04
8	-1	-1	77±0.04	15±0.01	48±0.00
9	0	0	63±0.00	12±0.02	9±0.03
10	1	1	189±0.01	99±0.00	21±0.01
11	0	0	57±0.00	9±0.003	9±0.02
12	-1	1	51±0.01	11±0.04	24.6±0.04
13	1	-1	120±0.04	289±0.01	194±0.02

a: camel rennet, **b**: goat rennet, **c**: chicken pepsin.

Effect of pH and temperature on cow milk clotting

Clotting time responses of cow milk with camel rennet, goat rennet and chicken pepsin are given in table 8. According to those results, all coagulating enzymes show a maximum coagulation time for (pH: 6.7/T: 36). While chicken pepsin reacts with cow milk with a clotting time of 9s.

The plot in fig 5 shows that pH has (a linear and quadratic significant effect) on the clotting of cow milk with camel rennet, goat rennet and chicken pepsin. Regression coefficient b_1 and b_{11} have a value of ($p<0.05$). Coagulating enzymes coagulate camel milk for a time not exceeding 15 minutes at all levels studied. Based on the surface plot analysis and the relationship between response and variables, a cow milk clotting time is optimal assuming these pairs of pH and temperature: [X1=5.36, X2=42] for camel rennet, [X1=5.39, X2=30] for goat rennet and [X1=5.82, X2=42] for chicken pepsin.

As a comparison, goat rennet reacts with camel milk and cow milk with a minimum and maximum time of clotting for the same level (pH: 5.85/T: 36) and (pH: 6.7/T: 36) respectively, While camel rennet and chicken pepsin react differently. In addition, the optimum clotting points with camel rennet, goat rennet and chicken pepsin are different between the two types of milk.

CONCLUSION

Traditionally, camel milk is known for its inability to rapid coagulation. In this study, we use different proteolytic enzymes (camel rennet, goat rennet and chicken pepsin) with optimization of pH and temperature. Results show that for any successful processing of camel milk, it is therefore possible to use other coagulation enzymes instead of calf rennet by controlling the milk parameters. The optimum combination of pH and temperature for camel milk coagulation with camel rennet is [pH: 5.37, T: 39.09°C], with goat rennet is [pH: 5.36, T: 38.84°C] and with chicken pepsin is [pH: 5, T: 42°C]. Compared with cow milk, the optimum coagulation points are different. This can be justified by the effect of changes in pH and temperature on the affinity of the enzymes studied for the caseins of camel milk and cow milk.

Author contributions

Mrs. Biya Bouras has conceived and designed camel milk coagulation analysis, performed statistical analyses, interpreted results and wrote the paper. Dr Ouarda Aissaoui Zitoun initiated this research and directed the entire experimental study. She also supervised the article redaction and interpretation of the results and edited the manuscript with the first author. Dr Férial-Aziza Benyahia contributed in analysis design and corrected the final paper. Mrs Djeghim Fairouz performed statistical analyses and results with the first author. Prof Mohammed Nassereddine Zidoune supervised the experimental data.

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