ABILITY OF GASTRIC ENZYME EXTRACT OF ADULT CAMEL TO CLOT BOVINE MILK

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Abstract Algeria is experiencing significant development of the dairy sector, where consumption of milk and milk products increased by 2.7 million tons in 2008 to 4,400,000 tons in 2013, and cheese production has reached 1640 tons in the year 2014 with average consumption of 0.7 kg / person / year. Although rennet is still the most used coagulating enzyme in cheese, its production has been growing worldwide shortage. This shortage is primarily due to a growing increase in the production and consumption of cheese, and the inability to increase in parallel the production of rennet. This shortage has caused very large fluctuations in its price). To overcome these obstacles, much research has been undertaken to find effective and competitive substitutes used industrially. For this, the selection of a local production of rennet substitute is desirable. It would allow a permanent supply with limited dependence on imports and price fluctuations. Investigations conducted by our research team showed that extracts coagulants from the stomachs of older camels are characterized by a coagulating power than those from younger camels. The objective of this work is to study the possibility of substituting commercial rennet coagulant by gastric enzymes from adult camels for coagulation bovine milk. Excerpts from the raw camel coagulants obtained are characterized through their teneures proteins and clotting and proteolytic activities. Milk clotting conditions by the action of these extracts were optimized. Milk clotting time all treated with enzyme preparations and under different conditions was calculated. Bovine rennet has been used for comparison. The results show that crude extracts from gastric adult camel can be good substituting bovine rennet.

Keywords: Camel, Milk, Cheese, Gastric Extracts, Coagulation, Algeria

Introduction

Processing milk into cheese is a milk preservation method widely used in the industrial and artisanal plan. Its development is often achieved through the use of coagulating enzymes whose tonnage and diversity are constantly changing. Different proteases capable of hydrolyzing the Kapa casein may cause coagulation of the milk and allow the manufacture of derivates products.

However, this condition is not always sufficient for widespread use in milk processing industry because the ratio of clotting activity and the proteolytic activity are decisive for the good choice of the enzyme . Rennet is an enzyme preparation extracted from young calves fed milk curd (and sacrificed before weaning) is the agent most used for milk coagulation (Desmazeaud 1997). Although this coagulating enzyme is still the most used in cheese, its production has been growing worldwide shortage. This shortage is primarily due to a growing increase in the production and consumption of cheese, and the inability to increase in parallel the production of rennet. This shortage has caused very large fluctuations in its price (Cuvellier 1993). Today its use is facing the major constraint sacrifice of young calves and therefore much getting increasingly important enzyme likely to meet growing needs.

Indeed, the increase in world milk production and the overall increase in cheese consumption have accentuated this handicap and have promoted a particular research effort to explore alternative ways of rennet by other coagulating enzymes of original animal, plant and microbial.

Algeria, which knows a total dependence of our dairies in manufacturing ingredients, must participate in this effort especially since domestic production of raw milk and consumption of dairy produce are experiencing a substantial increase in recent years.

In this regard, we proposed to see how the isolated enzyme extracted from adult dromedaries could be a substitute of bovine rennet to coagulat the bovine milk

MATERIALS AND METHODS

Materials

Abomasal tissues

The camel abomasal tissues were obtained from camel slaughterhouse of Ouargla, Algeria. The abomasums were obtained from adult camels. The abomasal tissues were cleaned with running water, defatted, cut in slices, packaged in plastic bags and frozen at -18°C.

Commercial enzymes

Bovine pepsin in powder form and bovine rennet containing 80% chymosin and 20% pepsin were purchased from Texel-Poulenc (France).

Milk samples

The milk samples were collected in sterile bottles and delivered in a cooler with ice to the laboratory.

Methods

Extraction of gastric enzymes from camel abomasal tissues

The method of gastric enzymes extraction from bovine abomasal tissue as described by Valles and Furet (1977) was used with minor modifications. The steps involved were: 1) soaking of a known weight of sliced abomasal tissue in 1.25 volume of 0.2 M HCL at 42°C temperature for 60 minutes and filtration through a paper filter , 2) clarification: of the extract using 1% (V/V) of 1M solution of Al_2SO_4 and 5% of a 1M solution of Na_2SO_4 (1M) heated to 42°C; , After filtration a yellowish clarified solution was obtained; 3) concentration: a double solution of saturated NaCl containing 1% (w/w) of concentrated HCl was added to the known weight of the abomasal tissue. After mixing, the mixture was put to rest for one hour, centrifuged at 2100 g for 20 minutes, the supernatant was discarded and the wet weight was recorded followed up by adding 10% (w/v) of distilled water. The pH of the concentrated filtrated was adjusted to 5.5 with Na_2HPO_4 at 42°C. The extracted camels' gastric enzymes obtained were assigned the label DCE (Dromedary 's Coagulant Extract. The fresh DCE analyzed, and some samples were stored at 4°C with the addition of 10% (v/v) of thymol and 10% NaCl for preservation purpose.

Protein analysis of the DCE

The method of Lowry et al. (1951) was used to determine the protein content of the gastric enzyme extracts of camels. The amount of proteins (g/ml) was obtained using a standard curve based on bovine serum albumin (BSA).

Clotting activities of the DCE

The method of Berridge (1952) was used. The main steps were the following.

The standard substrate was the "low heat" milk powder at 10% (w/v) solution in $CaCl_2$ (0.01M) solution, and the pH was adjusted to 6.5 with 0.1 N NaOH. The DCE was added at 1ml/10ml of standard substrate and mixed manually and incubated in a water bath at 30°C. After thoroughly mixing three times, the clotting time zero started. The clotting activity equation as reported by Berridge (1952) in rennet units (RU) was used:

$$RU = 10 \times V/Tc \times Q$$

RU: rennet unit;

V: volume of standard substrate (ml);

Q: volume of DCE (ml);

Tc: time of clotting (sec).

Clotting strength

The clotting activity of the DCEwas also reported in clotting strength of Soxhlet (F) based on the equation of Bourdier and Luquet, (1981).

$$F = RU/0.0045$$

F: Clotting strength of Soxhlet.

Proteolytic activity

The method of Bergere and Lenoir (1997) for the proteolytic activity of the DCE was used. In addition the clotting activity was optimized by using the method of Shamet et al. (1992).

Bovine milk coagulation was carried out by using the method of Ramet (1997). However, the

flocculation time was measured visually by the method of Lenoir et al. (1997) at different pHs and temperatures.The flocculation time is the time between the addition of coagulating enzyme of the appearance of flakes visible to the naked eye. This method consists in the introduction of 10 ml of milk in a test tube with a fixed concentration of CaCl2 solution and incubated at the desired temperature after the addition of a coagulant enzyme concentration. The concentration of the enzyme is such that the flocculation of milk to pH = 6.3 (a concentration of 0.01 M CaCl2 and 30 ° C) occurs after about 15 minutes.

Optimization of the flocculation time

The flocculation time (ft) of bovine milk was optimized at four different pHs (5.5, 6.0, 6.3 and 6.6) and at three different temperatures (30, 37 and 39°C). The mixture is adjusted to the desired pH with 0.1 M HCl, incubated at the desired temperature (Lenoir et al. 1997).

Statistical analysis

All experiments were performed with three replicates each. All data are reported as means with standard deviations. An analysis of variance (ANOVA) was applied to assess differences among the "Dromedary's Coagulant Extract" (DCE) and the commercial enzymes by using SPSS (Statistical Package for the Social Sciences) software version 18.0.

RESULTS AND DISCUSSION

Characterization of gastric extract enzymes

The protein average in g/l of the DCE was 1.44.

Clotting and proteolytic activities

Table 1 : Proteolytic activity of the enzymatic preparations

Enzymatic preparations	**Clotting activity (RU)**	**Proteolytic activity**
DCE	$0.410^{a} \pm 0.020$	$0.84^{a} \pm 0.015$
Pepsin bovine (Pb)	$0.123^{b} \pm 0.002$	$0.84^{a} \pm 0.020$
Rennet bovine (Rb)	$0.164^{c} \pm 0.002$	$1.34^{b} \pm 0.015$

The clotting activity was the highest for the DCE compared to the two commercial enzymes, rennet and pepsin bovine. They were significantly different at $P \leq 0.05$ (Table 2).

DCE showed a lower proteolytic activity than Rennet bovine (Rb) (1.34 ± 0.015). However its proteolytic activity was close to that of the bovine pepsin (0.84±0.015) .

An increase in absorption at this wavelength (280 nm) could be fully attributed to the formation of unspecific cleavage products. Kappeler et al., (2006) observed that the proteolytic activity of enzymes increased with higher incubation temperatures and also when the pH of the assays was decreased.

In cheese production, the desired enzyme should have high clotting activity and low

proteolytic activity (Ramet, 1997). DCE could be considered as a good source of enzyme for cheese making. The high clotting activity and low proteolityc activity as shown by DCE A are a pre-requisite for an acceptable rennet substitute (Fox, 1969; El-Agamy, 2000a) for making cheese.

Except for the bovine pepsine (Figure 1), the time required to reach the flocculation of bovine milk was short. These results are in agreement with those of Kappeler et al., (2006)

the flocculation time of DCE was shorter for bovine milk than pepsin bovine(P-b) but close to rennet bovine (Pr-b). This finding is in agreement with other researchers (Ramet, 1994, Siboukeur et al., 2005). In order to define the affinity of the enzyme preparations to the substrate (cow milk), the the flocculation time of bovine milk was determined. Figure 1 shows that the DCE demonstrated an affinity for this milk, indicating that this enzyme would be suitable for bovine milk coagulation.

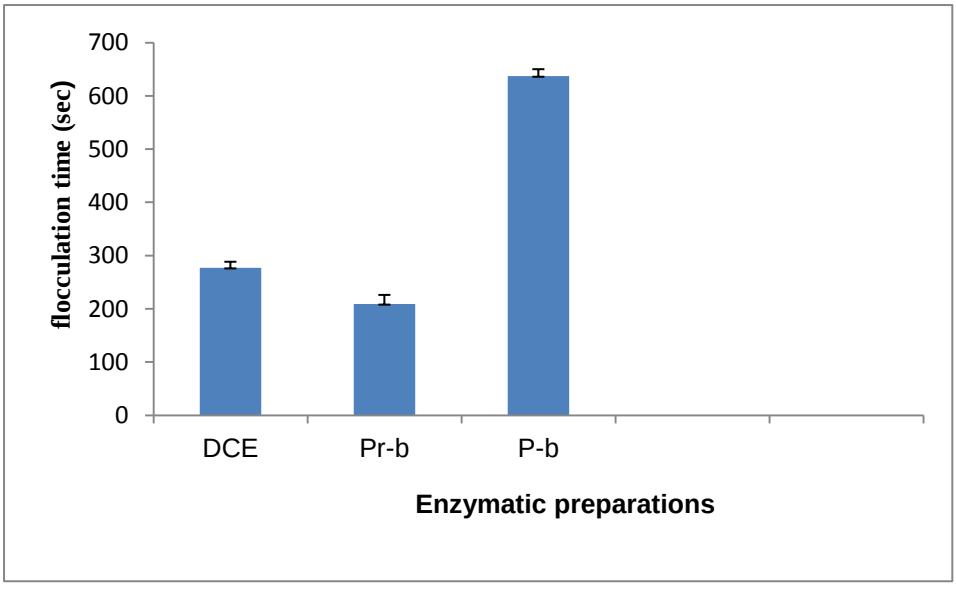

Figure 1: Effect of the enzymatic preparations on the flocculation time of bovine milk

Effect of pH on the flocculation time

Milk clotting activity was influenced by the pH of the milk at the stage. All enzyme preparations exhibited almost a linear curve with an increased pH from 5.5 to 6.6. The optimum pH for clotting cow milk by the DCE was at 5.5. Also it appeared that DCE was less affected by the increased pH (Figure 2).

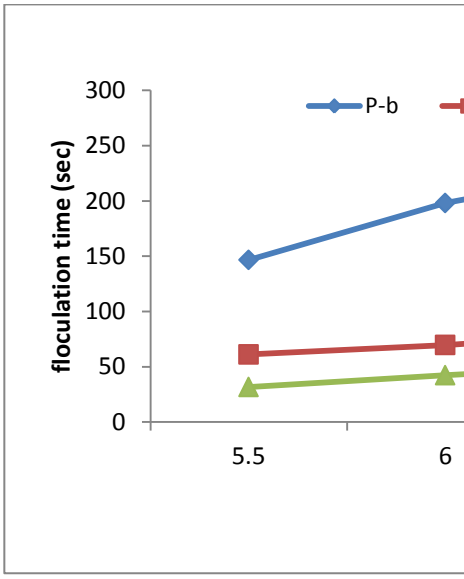

Figure2: Effect of renneting pH of cow milk on the

flocculation time.

The pH of the milk for rapid flocculation is very important during cheese making since the acidification by the lactic acid bacteria helps the enzyme activity in which the enzyme is a protease having an optimum activity around pH 5.5. This contributes to the destabilization of the casein micelles (Ramet, 1994). In fact, Lenoir et al. (1997) and Chazarra et al. (2007) found that the effect of pH of milk on flocculation was very sensitive and apparent, thus the flocculation time is further reduced if the renneting pH is far below the normal pH of milk. This is in agreement with the findings of Ramet, 1985, 1993, that all clotting cheese enzymes are acid protease, that their activity is optimum at pH 5.5 and that the kappa casein presents stability at pH 5.6. This is not the case for camel milk since the slow pH drop in camel milk is not conducive to the clotting activity (Ramet, 1985, 2001).

The effect of temperature on the flocculation time of the enzymes on cow milk is shown in figure 3.

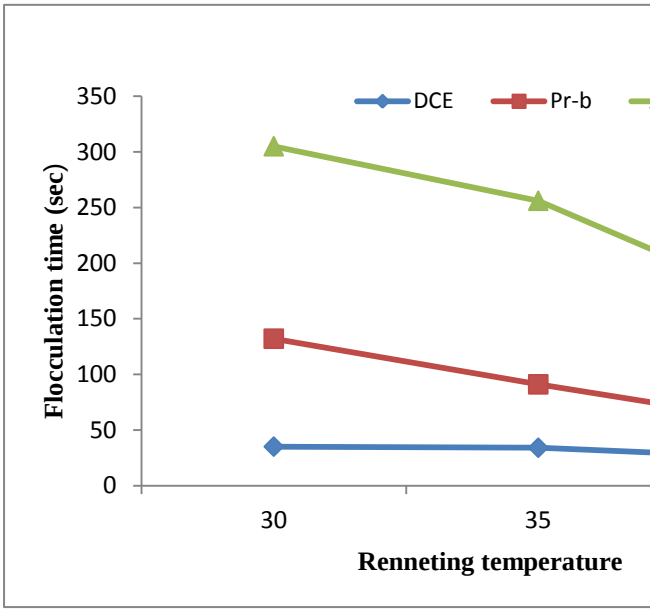

Figure 3: Effect of temperature of bovine milk on the flocculation time

Temperatures (30, 37 and 39°C) led to a decrease in flocculation time by all these enzymes. However, the shortest flocculation time was obtained with DCE , indicating that DCE could be the more stable enzyme but all the enzymes showed an optimum activity and flocculation time at 39C (Figure 3). This is in line with the data reported by Ramet (1985, 1993), that the optimum temperature of most clotting enzymes were around 40-50°C, but beyond these values there was a progressive denaturation of the enzyme and at 65°C there was no activity. Similarly, Mohanty et al., (2003) reported that the proteolytic activity of buffalo chymosin treated at different temperatures exhibited a relatively stable proteolytic activity curve up to a temperature of 55°C after which there was a decline of the activity. Measuring the flocculation time at different temperature suggested which enzyme would be more suitable for milkcow clotting in a short period as well as the manufacture of various type of cheeses, such as soft, semi-hard and hard cheese.

Overall, based on the data reported and in line with other researchers' reports (Farah and Bachman, 1987; Mehaia, 1992; Ramet, 1993; Desmazeaud, 1990; Thouvenod, 1997), this study proposes an optimum temperature at 39°C and a pH of 5.5 for an optimum clotting activity and flocculation time using gastric enzymes extracted from camels. From the results obtained it can be concluded that the crude enzymes extracted from old camels

coagulate cow milk. Elsewhere the results of the study undertaken by Bansal et al., (2009), suggest that camel chymosin can be used successfully to make Cheddar cheese with lower levels of proteolysis but with good flavor.

This study focused primarily on the coagulation step that represents the key step in making cheese but additional studies are necessary on the performance and characteristics of the cheese obtained with these enzymes

Conclusion

The non-purified enzyme preparations (DCE) obtained from the older camels showed a good coagulation activity on cow milk. Flocculation time data showed that the DCE and bovine rennet (Pr-b) had good specificity towards bovine casein. In addition, the short flocculation time obtained for DCE of older camels at an optimum temperature at 39°C and a pH of 5.5 was encouraging since older camels are more available for slaughter in Algeria. Therefore the production of DCE from older camels could be an excellent substitute for the commercial chymosin for cheese making . It was recommended that additional research be conducted to purify the extract, to characterize the extract using electrophoreses.

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