Extraction of camel rennet and its comparison with calf rennet extract

By J. WANGOH[1], Z. FARAH and Z. PUHAN

Laboratorium für Milchwissenschaft, Eidgenössische Technische Hochschule Zürich, ETH Zentrum, CH-8092 Zürich, Switzerland

1. Introduction

Camel milk requires more calf rennet than cow milk to coagulate and the relative amount of rennet needed varies widely (2, 8, 14, 16). Extracts of adult camel abomasa have been used to coagulate cow milk with success (5, 6, 7). However, these enzymes have not been tried on camel milk. Rennet extracts from lamb and cow calves were found to be more effective with the milk of the respective species (12), while pig chymosin and pepsin respectively, were found to have a higher milk clotting activity in pig milk than in cow milk (9). Accordingly, it would not be surprising if camel rennet is more effective on camel milk than calf rennet. This work was therefore aimed at extracting camel rennet and testing its ability to coagulate camel and cow milk compared to calf rennet extract, chymosin and pepsin.

2. Materials and methods

Abomasa. Camel calf abomasa were obtained from Ol Maisor Ranch in Kenya. Milk fed calves were slaughtered at the age of 3-4 weeks and their abomasa removed, dry salted and sun dried. Commercial cow calf abomasa were obtained from Winkler AG (Switzerland).

Camel and cow milk powders. Camel milk was obtained from Ol Maisor Ranch in Kenya, held at 4 °C and transported to the laboratory within 24 h where it was defatted, freeze dried and kept until use. The cow milk used was Extra Low Heat spray dried skim milk powder obtained from Milchpulverfabrik Sulgen (Switzerland).

Enzymes. Microbial chymosin was obtained from Gist Brocades (France) and porcine pepsin from Siegfried, Zofingen (Switzerland).

Preparation of *rennet extracts.* Extraction was done after cutting the dry cow or camel calf abomasa into 1cm^2 slices, soaking them in 6 % NaCl solution (1:10, w/v) containing 2 % boric acid and stirring continuously over 4 days at 5°C. The mixture was then filtered and centrifuged at 1,500 rpm for 15 min. The pH of the supernatant was then lowered from 5.5 to 4.7 with 1 N HCl and the extracts held at 25 °C for 24 h to activate 'the zymogens. The pH was thereafter raised to 5.5 with 1 N NaOH and the mixture centrifuged to obtain the final rennet extract.

Preparation of *milk substrates.* Camel and cow milk powders were reconstituted according to IDF Standard (13).

Determination of *milk clotting activity.* Thromb-elastograph D (Hellige GmbH, FRG) at 32°C using 10ml milk and 200 µl enzyme preparation was used during extraction of enzymes from the abomasa and the subsequent activation. IDF Standard method, Appendix A (13) was used for all the other enzyme activity determinations. To compare the activity of a particular enzyme in different milks, the stock solution of an enzyme was diluted to give a clotting time of 5.5 min in milk, and the activity of the enzyme was calculated for that milk.

Separation of *extract into fractions.* The IDF Standard method (13) was used to separate the rennet extracts into fractions with the modification that the flow rate was set at 1 ml/min and the optical density (OD) of the eluate was continuously monitored at 226 nm and recorded by LKB 2238 Uvicord SII UV Monitor and LKB 2210 Potentiometric Recorder (LKB-Produkter AB, Sweden), respectively. 1-ml Samples were taken at intervals during elution and their clotting activity was determined in both cow and camel milk.

3. Results and discussion

Enzymes extraction and activation

The clotting activity of the extracts from both cow and camel calf abomasa during extraction and before activation is shown in Fig.1.

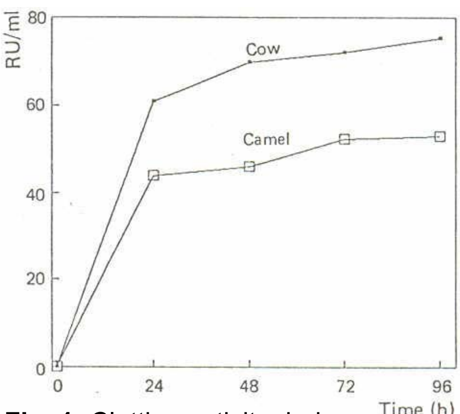

Fig. 1: Clotting activity during extraction of camel and calf rennet

[1] Permanent address: Department of Food Science, Technology and Nutrition, University of Nairobi, Box 29053, Nairobi, Kenya

The main increase in clotting activity occurred during the first 24 h. Under similar experimental conditions, for rennet extracts from lamb and kid abomasa (1) and from cow abomasa (15). maximum clotting activities were obtained between 24-48 h of extraction. Fig. 2 shows the change in clotting activity during activation of the zymogen in the solutions after extraction.

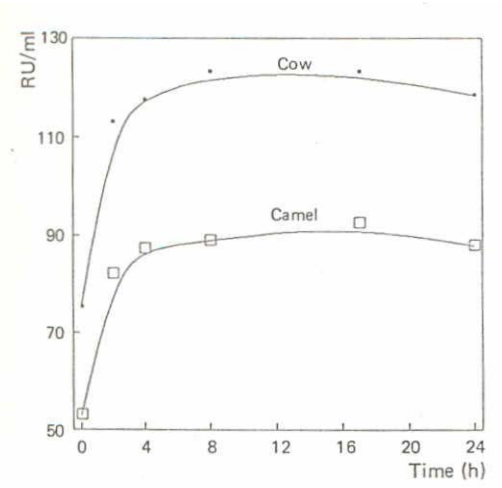

Fig. 2: Clotting activity of the extracts during activation at pH 4.7

The shape of the activation curves was similar for both extracts. The main increase in clotting activity was observed in the first 8 h of activation, 60 % for calf and 73 % for camel rennet extract. In commercial rennet production, activation requires 14-36 h at pH 4.6 and 5°C (15). Under laboratory conditions, however, maximum activation has been achieved already in 4-10 h at pH 4.7 and 25 °C (3,17).

Activity of the rennet extracts and their fractions

Typical absorbance curves together with clotting activity on both cow and camel milk samples as determined during elution of rennet extracts are shown in Figs 3 and 4.

The absorbance patterns for both camel and calf rennet extract were similar. However, the maximum clotting activity of the first fraction from calf rennet extract did not coincide with maximum absorbance at 226 nm, *e.g.* with the highest protein concentration. Similar results have been reported in the literature (4). In contrary, in camel rennet extract the maximum absorbance coincided well with that of the clotting activity. Additionally, the first fraction of camel rennet extract showed two, and the second fraction one active peak, whereas in calf rennet extract only one active peak was detected in each fraction.

The first fraction of calf rennet extract coagulated cow milk readily but caused no coagulation after more than 1 h in camel milk, while the second fraction coagulated camel milk much faster than cow milk (Fig. 3). Both milks responded equally well to the first fraction of camel rennet, but

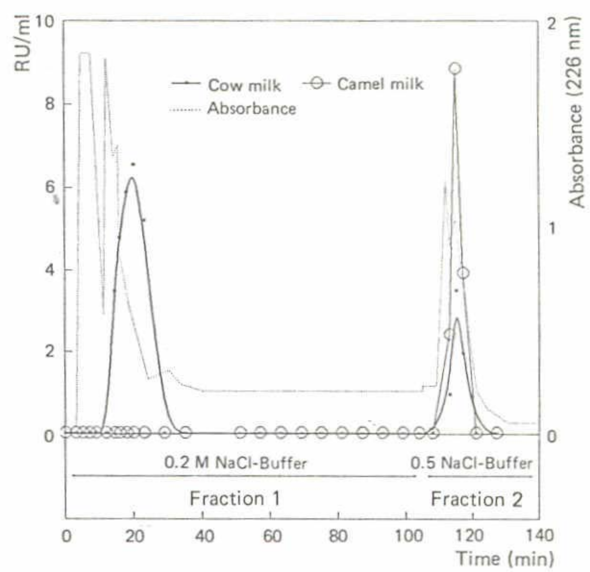

Fig. 3: Clotting activity of calf rennet fractions with cow and camel milk

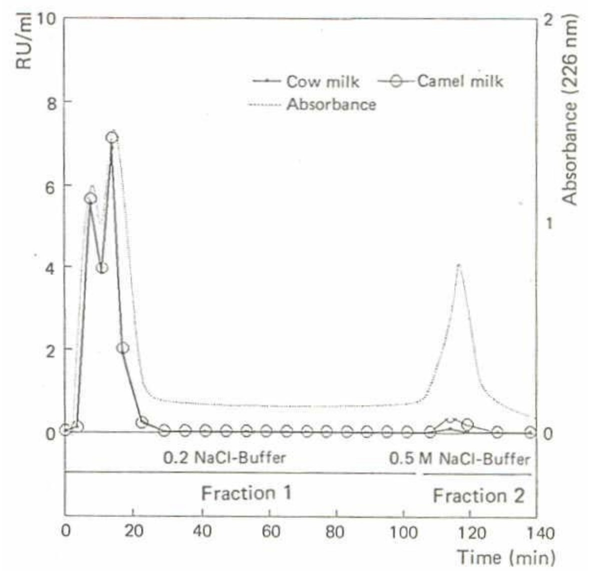

Fig. 4: Clotting activity of camel rennet fractions with cow and camel milk

camel milk responded better to the second fraction though the activity of the fraction was low (Fig. 4).

Since it is known that in calf rennet the first fraction contains chymosin and the second pepsin, pure commercial chymosin and porcine pepsin were tested on their ability to coagulate both cow and camel milk. Porcine pepsin was selected because it is being used for cheese manufacture (11). Furthermore bovine and porcine pepsin were shown to have a similar milk clotting activity per mg protein and to differ only in their general proteolytic activity (10, 11). Camel milk was coagulated 5 times faster by porcine pepsin and 7 times slower by chymosin than cow milk (Table 1).

The ability of the extracts to coagulate both cow and camel milk were then tested. The analysis of variance and multiple range analysis for clotting time of cow and camel milk by calf and camel rennet extracts are shown in Table 2.

Table 1: Estimation of activity of pure chymosin porcine pepsin in cow and camel milk

Enzyme	Chymosin		Pepsin	
Milk	Cow	Camel	Cow	Camel
Activity of stock enzyme, RU/ml	72.5[a]	10.9[b]	4.5[a]	22.1[b]
Stock enzyme	1/250	1/40	1/78	1/15
Activity ratio (a:b)	6.7:1		1 :4.9	
Clotting time, see	345.0	365.7	352.8	330.2
Standard deviation*	4.1	3.7	5.3	4.9

*Three independant determinations

Camel milk was clotted slightly, but not significantly better by camel rennet than cow milk. Camel milk was, however, clotted slower by cow rennet extract. Interactions between milks and rennet extracts were significant. These results can be explained by the fact that the coagulation of camel milk by cow rennet extract was primarily due to the pepsin content of the cow rennet as shown in Fig. 3. The activity of the pepsin fraction compensated in this case the low activity of chymosin fraction in clotting of camel milk as also confirmed in Table 1. The large variations in the ability of cow rennet to coagulate camel milk reported in literature (2, 8, 14, 16) may be explained by differences in the pepsin content of the rennet used. The better coagulation of camel milk by camel rennet (Table 2) cannot be explained at present time. However, it could be the result of better suitability of camel rennet for coagulating camel milk. Similar observations were made with rennet and milk of other species (9, 12).

4. Conclusions

The camel rennet extract coagulated camel milk slightly better than cow milk, while calf rennet extract coagulated camel milk less readily. The chymosin fraction of calf rennet extract had very little activity on camel milk while the pepsin fraction coagulated it much more readily than cow milk. Further tests showed that camel milk was coagulated 5 times faster than cow milk by pepsin, but 7 times slower by chymosin.

It was concluded that the coagulation of camel milk by calf rennet is primarily due to the pepsin content of the calf rennet. Therefore, camel milk should be coagulated with camel rennet or pepsin as it is not coagulated readily by calf chymosin.

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5. References

(1) ANIFANTAKIS, E., GREEN, M.L.: J. Dairy Res. 47221-230 (1980)

(2) BAYOUMI, S.: Kieler Milchwirtschaftliche Forschungsberichte 42 3-8 (1990)

(3) BISCHOFSBERGER, T., PUHAN, Z.: Milchwissenschaft halt 34 (10) 614-617 (1979)

(4) BERANKOVA, E., SAJDOK, J., RAUCH,P., KAS, J.: Neth. Milk Dairy J. 42 337-340 (1988)

(5) EL-ABBASSY, F.: Egyptian J. Dairy Sci. 15 87- 92 (1987)

(6) EL-ABBASSY, F., WAHBA, A.: Egyptian J. Dairy Sci. 14 181-186 (1986)

(7) EL-BATAWY, M.A., AMER, S.N., IBRAHIM, S.A: Egyptian J. Dairy Sci. 15 93-100 (1987)

(8) FARAH, Z., BACHMANN, M.R.: Milchwissenschaft 42 689-692 (1987)

(9) FOLTMANN, B., JENSEN, A.L., LONBLAD, P., SMIDT, E., AXELSEN, N.H.: Comp. Biochem. Physiol. 68B 9-13 (1981)

(10) FOX, P.F.: J. Dairy Sci. 36 427-433 (1969)

(11) GREEN, M.L.: J. Dairy Res. 39 261-273 (1972)

(12) HERIAN, K., KRCAL, Z.: Prumysi-potravin 22 (5) 137-139 (1971)

(13) International Dairy Federation: FIL/IDF Standard 110A (1987)

(14) MOHAMED, M.A., MURSAL, A.I., LARSSON-RAZNIKIEWICZ, M.: Milchwissenschaft 44 278-280 (1989)

(15) PLACEK, C.: Industrial Engineering and Chemistry 52 (1) 2-8 (1960)

(16) RAMET, S.P.: World Animal Review 61 11-26 (1987)

(17) RAND, A.G., ERNSTROM, C.A.: J. Dairy Sci. 47 181-187 (1964)

Table 2: Analysis of variance and multiple range for clotting time of cow and camel milk with calf and rennet extracts

Anova						Multiple range analysis		
Source	SS	df	Mean square	F-ratio	Significance level	Milk	Rennet	CT(min)
Main effect	2.9492	2	1.4746	21.453	0.0000	Camel	Camel	4.17[a]
Milk	0.1367	1	0.1367	1.989	0.1776	Cow	Camel	4.36[ab]
Rennet	2.8125	1	2.8125	40.917	0.0000	Cow	Cow	4.67[b]
Interactions	0.6380	1	0.6380	9.282	0.0077	Camel	Cow	5.21[c]
Residual	1.0998	16	0.6874					
Total	4.6870	19						

CT = Clotting time
[abc] = Means joined by the same letters are not significantly different P (95 %)

6. Summary

WANGOH, J., FARAH, Z., PUHAN, Z.: Extraction of camel rennet and its comparison with calf rennet extract. Milchwissenschaft 48 (6) 322-325 (1993).

86 Camel rennet (extraction)

Camel rennet was extracted from camel calf abomasa by the method used for bovine rennet. The clotting activity was determined during extraction and activation. Both camel and cow abomasa extracts were fractioned and the clotting activity of the fractions compared. Camel rennet coagulated camel milk slightly faster than cow milk, while calf rennet extract coagulated camel milk less readily than cow milk. The chymosin fraction of calf rennet showed weak activity on camel milk while the pepsin fraction coagulated the same much more readily than cow milk. The first fraction of camel rennet coagulated cow and camel milk equally well, whereas the second fraction showed higher clotting activity with camel milk. It is concluded that the coagulation of camel milk by calf rennet is primarily due to the pepsin content of the calf rennet. The reported large variations in the ability of bovine rennet in coagulating camel milk can be explained by the differing pepsin content of the rennet used. Camel milk should therefore be coagulated with camel rennet or pepsin.

WANGOH, J., FARAH, Z., PUHAN, Z.: Gewinnung von Kamellab und der Vergleich mit Kälberlab. Milchwissenschaft 48 (6) 322-325 (1993).

86 Kamellab (Extraktion)

Aus Labmagen von jungen Kamelen wurde Lab extrahiert nach der gleichen Methode, wie sie bei der Herstellung von bovinem Kälberlab angewendet wird. Die Gerinnungsaktivität wurde während der Extraktion und Aktivierung bestimmt. Kamellab zeigte mit Kamelmilch eine kürzere Gerinnungszeit als mit Kuhmilch, wahrend Kälberlab mit Kamelmilch längere Gerinnungszeiten als mit Kuhmilch aufwies. Die Fraktionierung von Kälberlab ergab, dass Chymosin nur eine geringe und das Pepsin eine gute Gerinnungsaktivität mit Kamelmilch aufweist. Die erste Fraktion aus dem Kamellab koagulierte gleich gut mit Kuh- und Kamelmilch, und die zweite Fraktion ergab eine höhere Gerinnungsaktivität mit Kamelmilch. Daraus wurde gefolgert, dass die Gerinnung von Kamelmilch mit Kälberlab weitgehend von dessen Pepsingehalt abhängig ist. Die in der Literatur erschienenen Angaben Ober grosse Unterschiede in der Gerinnung von Kamelmilch mit Kälberlab sind höchstwahrscheinlich auf den unterschiedlichen Pepsingehalt im Kälberlab zurückzuführen. Die Ergebnisse der Untersuchungen lassen den Schluss zu, dass für die Gerinnung von Kamelmilch am besten Kamellab oder Pepsin eingesetzt werden soll.