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PROPERTIES OF SOME ANIMAL DERIVED  
MILK COAGULATING ENZYMES

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Neil Harvey Clarke

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## ABSTRACT

Extracts of milk coagulating enzymes were obtained, adult bovine from the abomasums of pasture fed cattle and lamb rennet from the abomasums of partially milk fed lambs slaughtered between three and six months of age. Both rennets were, in the presence of sodium chloride, most stable at 10°C or lower at pH 4.7 for adult bovine and pH 4.1 for lamb. Heat treatment in a sodium chloride solution (200 mg/ml) at 68°C for 60 minutes destroyed less than 35% of the activity of each rennet. Under the same conditions calf rennet was completely inactivated.

The cheesemaking properties of adult bovine and lamb rennets were compared with calf rennet. Adult bovine and calf rennets responded similarly to changes of pH however lamb rennet appeared less active than calf rennet at higher pH's when measured in caseinate solution but appeared more active than calf rennet when measured in milk by a curd tension method. The optimum temperature for milk coagulating activity was 40°C for adult bovine and calf rennets but only 30°C for lamb rennet when measured in caseinate solution. The curd tension of milk coagulated with the three rennets increased with time but both adult bovine and lamb rennets appeared more sensitive to milk calcium levels than calf rennet. Whole casein,  $\alpha_s$ -,  $\beta$ - and k-caseins were hydrolysed in a similar manner by the three rennets. Adult bovine rennet was the most proteolytic on whole and  $\beta$ - caseins while calf rennet hydrolysed  $\alpha_s$ - and k-caseins more rapidly than the other rennets.

Two pepsins, of similar amino acid compositions, were isolated, one from adult bovine rennet and one from lamb rennet which also contained a rennin. All three enzymes were purified so that lamb rennin and pepsin each produced only a single band on polyacrylamide gel

electrophoresis but the adult bovine pepsin produced two bands and appeared to be heterogeneous.

Cheese made with adult bovine, lamb or a 50/50 mixture of calf-adult bovine rennets were compared with cheese made with calf rennet and found to be similar in manufacturing characteristics, flavour and body after three and six months storage at 6 or 13°C. Polyacrylamide gel electrophoretograms of these cheese after one to twelve months storage at 6°C showed that the milk coagulant had no effect on the casein degradation products produced in the cheese although the rate of degradation varied slightly.

Perchloric acid was found to be a good protein precipitant for quenching casein-rennet reactions and was utilized in a method which was developed for assaying milk coagulating activity. Rennet was added to sodium caseinate solution and one minute later the reaction quenched with perchloric acid. The quantity of peptides hydrolysed from the casein was measured at 217 mμ and was directly proportional to rennet activity over a limited range of activity.

A method for removing mucoproteins, the major impurity in rennet, was developed and a commercial scale plant commissioned. A diethylaminoethyl cellulose based resin, equilibrated with a citrate buffer, retained the rennet enzymes while the mucoproteins passed through uninhibited. The enzymes were eluted with the same buffer made 1.0 M with sodium chloride. The eluent was more active than the original rennet, was crystal clear in appearance and remained so during twelve months storage when normally mucoproteins would have precipitated out of solution to form a cloud in the rennet.

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# TABLE OF CONTENTS

	page
ACKNOWLEDGEMENTS . . . . .	iv
LIST OF FIGURES . . . . .	viii
LIST OF TABLES . . . . .	x
 <u>CHAPTER I</u> INTRODUCTION . . . . .	 11
 <u>CHAPTER II</u> RENNET ASSAYS . . . . .	 14
REVIEW OF LITERATURE . . . . .	14
The coagulation of milk . . . . .	14
Rennet Assays . . . . .	19
METHODS . . . . .	22
Rennet source . . . . .	22
Precipitants for casein-rennet reaction mixtures . . . . .	22
Assay . . . . .	24
RESULTS . . . . .	25
Precipitants for casein-rennet reaction mixtures . . . . .	25
Assay . . . . .	25
DISCUSSION . . . . .	30
 <u>CHAPTER III</u> ENZYME PURIFICATION . . . . .	 32
REVIEW OF LITERATURE . . . . .	32
Enzyme purification . . . . .	32
METHODS . . . . .	35
Ion exchange chromatography . . . . .	35
Gel chromatography . . . . .	35
Enzyme purification . . . . .	36
Electrophoresis of purified enzymes . . . . .	37
Commercial rennet purification . . . . .	37
RESULTS . . . . .	39
Rennet enzymes purification . . . . .	39
Enzyme purity and homogeneity . . . . .	43
Commercial rennet purification . . . . .	43
DISCUSSION . . . . .	49

<u>CHAPTER IV</u>	ENZYME PROPERTIES	51
REVIEW OF LITERATURE		51
Enzyme properties		51
Hydrolysis of casein fractions		54
METHODS		58
Effect of pH on rennet stability		58
Effect of heat on rennet stability		58
Variations of rennet activity with pH		59
Effect of temperature on rennet activity		60
Curd tension measurements		60
Proteolysis of whole casein		61
Casein fractionation		62
Hydrolysis of casein fractions		63
Molecular weight determination		64
Amino acid analysis		65
RESULTS		66
Effect of pH on rennet stability		66
Effect of heat on rennet stability		66
Variations of rennet activity with pH		67
Effect of temperature on rennet activity		67
Effect of time on curd tension		72
Effect of pH on curd tension		72
Effect of calcium concentration on curd tension		75
Proteolysis of whole casein		75
Casein fractionation		78
Hydrolysis of casein fractions		78
Molecular weight determinations		85
Amino acid composition		87
DISCUSSION		90
<u>CHAPTER V</u>	CHEESE MANUFACTURE	95
REVIEW OF LITERATURE		95
Cheese manufacture in New Zealand		95





# LIST OF FIGURES

Figure	Page
1. The comparison of 0.30-M perchloric acid (○), 0.12-M (●) and 0.73-M (Δ) trichloroacetic acids as precipitants for casein-rennet reaction mixtures using adult bovine (A), calf (B) and lamb (C) rennets. . . . .	26
2. The comparison of $A^{217}$ and RU/ml for adult bovine (A), calf (B) and lamb (C) rennets. . . . .	28
3. Elution patterns of adult bovine (A) and lamb (B) rennets from ion exchange chromatography on DEAE cellulose. . . . .	40
4. Elution patterns of adult bovine (A), lamb 1 (B) and lamb 2 (C) enzymes from gel chromatography on Sephadex G-100 gel. . . . .	41
5. Electrophoretic pattern of adult bovine (1), Lamb 1 (2) and lamb 2 (3) purified enzyme solutions	44
6. The elution pattern of calf rennet from a commercial DEAE ion exchange column. . . . .	46
7. Elution patterns of traditional (A) and purified (B) calf rennets from ion exchange chromatography on DEAE cellulose. . . . .	48
8. The effect of pH on the stability of adult bovine (A), calf (B) and lamb (C) rennets at ionic strengths of 0.04 (3, (○), 20, (●) and 30, (Δ) °C) and 0.85 (30, (▲) °C). . . . .	68
9. The effect of heat on the stability of adult bovine (○), calf (●) and lamb (Δ) rennets in 200 mg/ml (A) and 17 mg/ml (B) sodium chloride solutions. . . . .	69
10. The variation of milk coagulating activity with pH for adult bovine (○), calf (●) and lamb (Δ) rennets. . . . .	70
11. The effect of temperature on the milk coagulating activity of adult bovine (○), calf (●) and lamb (Δ) rennets. . . . .	71
12. The effect of time on the curd tension of milk coagulated by adult bovine (○), calf (●) and lamb (Δ) rennets. . . . .	73

13. The effect of pH on the curd tension of milk coagulated by adult bovine (O), calf (●) and lamb (Δ) rennets. . . . . 74
14. The effect of calcium concentration on the curd tension of milk coagulated by adult bovine (O), calf (●) and lamb (Δ) rennets. . . . . 76
15. The proteolysis of whole casein by adult bovine (O), calf (●) and lamb (Δ) rennets. . . . . 77
16. Elution pattern of whole acid casein from ion exchange chromatography on DEAE cellulose. . . . . 79
17. Elution pattern of whole acid casein from gel chromatography on Sephadex G-100 gel. . . . . 80
18. Alkaline polyacrylamide gel electrophoresis of  $\alpha_s$ -casein hydrolysed for 0, 1, 4, 15, 30, 60, 120 and 240 minutes at pH 6.5 by adult bovine (A), calf (B) and lamb (C) rennets. . . . . 81
19. Alkaline polyacrylamide gel electrophoretograms of  $\beta$ -casein hydrolysed for 0, 1, 4, 15, 30, 60, 120 and 240 minutes at pH 6.5 by adult bovine (A), calf (B) and lamb (C) rennets. . . . . 83
20. Acid polyacrylamide gel electrophoretograms of k-casein hydrolysed for 0, 15, 30 and 60 minutes by adult bovine (A), calf (B) and lamb (C) rennets. 84
21. The comparison between the logarithm of the molecular weights and the elution volumes from a Sephadex G-100 gel column for bovine serum albumin (O), ovalbumin (●), myoglobin (Δ) and cytochrome C (▲). . . . . 86
22. Alkaline polyacrylamide gel electrophoretograms of cheese, after one, three, six, nine and twelve months storage at 60°C, manufactured with adult bovine (A), calf (B), lamb (C) and mixture (D) rennets. . . . . 106

LIST OF TABLES

Table	Page
I    A regression equation and standard deviation for the comparison of A <sup>217</sup> and RU/ml between 0.01 and 0.09 RU/ml for adult bovine, calf and lamb rennets. . . . .	27
II   The amino acid composition of bovine pepsinogen, lamb rennins A and B and calf prorennin.	53
III   Molecular weight estimations for adult bovine, lamb 1 and lamb 2 enzymes from gel chromatography measurements. . . . .	85
IV   Amino acid composition of adult bovine, lamb 1 and lamb 2 enzymes. . . . .	88
V    The frequency of the difference between flavour scores of cheese made with adult bovine (B), lamb (L) or mixture (M) rennets compared with the corresponding cheese made with calf (C) rennet. . . . .	101
VI   Normal variable values testing the hypotheses that cheese made with calf rennet was preferred in half of the comparisons with cheese made with adult bovine, lamb or mixture rennets.	102
VII   The average scores of flavour characteristics for cheese made with adult bovine, calf, lamb or mixture rennets. . . . .	103
VIII   The percentage of judgements allotted to each body score of cheese manufactured with adult bovine, calf, lamb or mixture rennets. . . . .	104