

**STUDIES ON ENZYMES OF COW'S
AND BUFFALOE'S MILK**

**III.—Proteases and Other Enzymes
Protease, Amylase, Carbonic Anhydrase and Urease**

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Both milks contain no urease enzyme. Buffalo and cow milk have approximately similar activity of carbonic anhydrase, being 0.82 and 0.77 ml CO₂/ml milk respectively. On the other hand buffalo and cow have approximately similar activity of amylase, being 64.9 and 57.7 unit/ml. Buffalo milk has higher protease activity than cow milk, being 149.9 and 103.9 mg nitrogen/ml milk.

Protease, Amylase and urease were quantitatively determined in the samples. As regards the enzyme carbonic anhydrase however, its presence was only detected, no attempt was made to determine it quantitatively.

Experimental and Methods

All samples obtained and treated as previously described in 1.

1. Protease was determined by the method of Warner and Polis (1945), using casein dissolved in borax solution, milk as source of enzyme, and the soluble nitrogen determined after the end of reaction time by semi-micro Kjeldahl method as described by Ling (1956). A blank determination was carried out to determine the soluble nitrogen before the action of the enzyme.

2. Amylase determination in milk was carried out as described by Hale and Rawlins (1951), using calcium acetate-acetic acid buffer, pH 5.2, Starch solution 2% as substrate (B.D.H), Iodine solution, 0.0035 M dissolved in 0.25 M potassium iodide and the reaction was stopped by 2 N sulfuric acid. The transmission was measured at 660 mu wavelength in a Jena Spectrocolorimeter with 1 cm glass cell. The results was calculated according to :

$$\text{Units/100 ml milk} = \frac{100 \times 15 \times \text{transmission}}{4 \times 0.05 \times 50}$$

3. Carbonic anhydrase was detected according to Sumner and Myrbock (1951), using carbonated buffer, pH 10.5 and the change of the pH through 2 h at zero °C. was measured by Beckman Zeromatic pH meter, with glass electrode. The amount of CO₂ liberated per ml milk was measured at calibration curve made by saturated solution of CO₂.

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4. Urease was determined according to Sumner and Hand (1928). The method was made by using urea phosphate buffer, pH 7.0, milk and the reaction was stopped by 1 N HCl. The ammonia present were determined by Nessler's reagent. Calibration curve was made by using pure enzyme.

Results and Discussion

Results in table (1), show the maximum, minimum, means, standard deviations and standard errors of protease, amylase, carbonic anhydrase and urease respectively.

TABLE 1.—MAXIMUM, MINIMUM, MEANS, STANDARD DEVIATIONS AND STANDARD ERRORS OF PROTEASE, AMYLASE, CARBONIC ANHYDRASE, AND UREASE ACTIVITY IN BUFFALO AND COW MILK

Enzyme	Max.	Min.	Mean	S.E.	S.D.
1. Protease					
Buffalo	336	8.96	149.9	12.2	66.9
Cow	249.2	19.6	103.9	13.3	72.9
2. Amylase					
Buffalo	125	28	64.9	9.3	54.6
Cow	66.5	47.5	57.7	2.1	11.7
3. Carbonic anhydrase					
Buffalo	2.1	0	0.82	0.11	0.62
Cow	2.7	0	0.77	0.11	0.62
4. Urease					
Buffalo	0	0	0	0	0
Cow	0	0	0	0	0

mg nitrogen/ml milk. Unit/100 ml milk. ml CO₂/milk.

In Buffalo milk protease ranged from 8.96 to 336 mg nitrogen/ml milk with an average of 149.9 mg nitrogen/ml milk, while in cow milk, the range was from 19.6 to 249.2 mg nitrogen/ml with an average of 103.9 mg nitrogen/ml milk. The difference between the two averages was significant, as shown in table (2).

TABLE 2.—SIGNIFICANCE OF DIFFERENCE BETWEEN THE AVERAGE PROTEASE ACTIVITY OF BUFFALO AND COW MILK

Source of Variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	149.9	46.0	66.9	12.2	2.042	2.548	+
Cow	103.9		72.9	13.3			

The amylase activity in buffalo milk ranged from 21 to 125 unit/100 ml milk with an average of 64.9 unit/100 ml milk.

While in cow milk it ranged from 47.5 to 66.5 unit/100 ml milk, with a mean value of 57.7 unit/100 ml milk. The difference between the two averages as shown in table (3) was insignificant.

TABLE 3.—SIGNIFICANCE OF DIFFERENCE BETWEEN THE AVERAGE AMYLASE ACTIVITY OF BUFFALO AND COW MILK

Source of Variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	64.9	7.2	54.6	9.3	2.042	0.756	—
Cow	57.7 #		11.6	2.1			

Heyndrickx and Peeters (1958), found that the mean value of amylase content in cow milk was 180 mg starch/100 ml, which was more than two folds of the average activity in the local cow milk. Carbonic anhydrase activity of buffalo milk, which ranged from nil to 2.1 ml CO₂/milk with an average of 0.82 ml CO₂/ml milk. While in cow milk it ranged from nil to 2.7ml CO₂/ml milk with an average of 0.77 ml CO₂/ml milk. Its average in both milks was almost the same since the slight difference was insignificant, as shown in table (4).

Alfonso and Bertran (1953), found that the average values for this enzyme in cow milk and goat milk were 14 and 16.5 unit per 100 ml respectively.

Urease was not found in both buffalo and cow milk.

TABLE 4.—SIGNIFICANCE OF DIFFERENCE BETWEEN THE AVERAGE CARBONIC ANHYDRASE ACTIVITY ON BUFFALO AND COW MILK

Source of Variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	0.82	0.05	0.62	0.11	2.042	0.296	—
Cow	0.77		0.62	0.11			

REFERENCES

- ALFONSO, G. C. AND BERTRAN, C. E. (1953). Composition of milk. Carbonic anhydrase, Rhodanase, and Alkaline Phospho in the Milk of Cows, Goats, and Sheep. *Lait*, **33**, 386.
- HALE, W. S. AND RAWLINS, L. C. (1951). Determination of Amylase Activity. *Cereal Chem.*, **28**, 49.
- HEYNDRICKX, G. V. AND PEETERS, G. (1958). Investigations on the Enzymes in Udder Lymph, Plasma, and Milk of Cattle. *Enzymologia Acta, Biocatalytica*, **20**, 161.
- LING, E. R. (1956). "A Text Book Of Dairy Chemistry". Determination of Non-Protein Nitrogen. P. 85. Vol. II. Chapman and Hall, Ltd. London.
- SUMNER, J. B. AND HAND, D. B. (1928). Determination of Urease Activity. *J. Biol. Chem.* **76**, 150.
- SUMNER, J. B. AND MYRBOCK, K. (1951). "The Enzyme Chemistry and Mechanism of Action". Detection of Carbonic Anhydrase. Vol. I. Part. II. Academic Press, New York.
- WARNER, R. C. AND POLIS, E. (1945). Determination of Protease. *J. Amer. Chem. Soc.*, **67**: 529.

أنزيمات اللبن البقرى والجاموسى ٣ - البروتيز والأنزيمات الأخرى

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الملخص

وجد أن كلا من اللبن البقرى والجاموسى لا يوجد فيهما أنزيم اليوربيز كذلك وجد أن اللبن البقرى والجاموسى يحتوى على كمية واحدة تقريبا من أنزيم الكربونيك انهيدريز وهى ٧٧ ، ٨٢ مليلتر ك ٢١ لكل مليلتر من اللبن على التوالى كذلك وجد أن اللبن البقرى والجاموسى يحتويان على كميات متماثلة من أنزيم الأميليز وهى ٥٧٧ ، ٦٤٩ وحدة لكل ١٠٠ مللم من اللبن على التوالى . أما أنزيم البروتين فى اللبن الجاموسى أعلى منه فى اللبن البقرى فكان فى الجاموسى ١٤٩٩ والبقرى ١٠٣٩ مليجرام نيتروجين لكل ملم لبن .

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