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# A STUDY ON THE a-CASEIN OF BUFFALO MILK

*By*

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## SUMMARY

a-Casein was separated from casein and fractionated into two electrophoretic components by paper electrophoresis in acid media at pH 2.8 containing 30% urea, using 300 V for 10-12 hrs at room temperature. The $a_1$-fraction (Ca–insoluble) and $a_2$-fraction (Ca-soluble) were precipitated by Ca $Cl_2$ from a-casein.

The average values of N, P, S, tyrosine and tryptophan in $a_1$-casein were 15.08%, 1.32%, 0.67, 9.32% and 2.32% respectively. $a_2$-Casein contained averages of 14.85% N, 0.43% P, 0.39% S, 6.70% tyrosine and 1.20% tryprophan. The differences between the two components in values of their previous constituents were all significant except nitrogen contents which were insignificant.

## INTRODUCTION

a-Casein is one of the fractions found in casein. It is considered a protective colloid and easily attacked by rennin. Recently, a fraction of a-casein known as k-casein was found to be responsible for the enzymatic stability of the casein micelle Waugh and Hippel (1956). A comperhensive study on this fraction will be of a great help in the manufacturing of many dairy products which depends mainly on rennin coagulation in their processing. Moreover, buffalo milk a-casein studies are lagging far behind of cows" which enitself is not complete. Due to all these factors, this study is concerned with the fractionation of a- casein of buffalo milk, the relative distribution of these fractions and their composition.

## MATERIALS AND METHODS

*Preparation of pure electrophoretic a-casein fraction.*

Buffalo milk samples were obtained from the Faculty of Agriculture, Cairo University, and were defated by a milk *seperator*. Casein was precipitated from the skimmilk by I N HCI at pH 4.6 and centrifuged. The casein precipitate was thoroughly washed with distilled water, dried with alcohol and ether.

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Pure electrophoretic component of a-casein was prepared by urea fractionation method of Hipp et al (1952). Crude a-casein was precipitated at 4.63 M urea solution and further purified by repeat dissolving and precipitation in urea and NaCl solutions. Finally it was washed with distilled water, dried with alcohol and ether. Its purity was further checked by electrophoretic separation in a borate buffer of pH 9.2 and 0.125 ionic strength containing 10% urea. The electrophoresis was run for 20 hrs. at 4°C with electrical potential of 150 V and 0.5 mA current per inch of the width of the filter paper strip. Staining was done by azocarmine B reagent after drying at 110°C Abd El-Salam et al (1964).

*Separation of a-casein components.*

The separation was carried out as described by McMeekin et al (1959). Pure a-casein which gave one electrophoretic component in the alkali media was fractionated into two fractions : Ca-insoluble, and Ca-soluble a-caseins by the use of 0.2 M $CaCl_2$ solution.

*Electrophoretic separation of a-casein fractions in an acid medium.*

a- casein was separated by paper electrophoresis using lactic-propionic acid buffer , pH 2.8 Geller et al (1960), containing 30% urea and azocarmine B reagent as the staining solution Abd El-Salam et al (1964). Electric current potential used was 300 V with 1 mA current per 1 inch of the width of the filter paper strip. The separation was run for 12 hrs at room temperature. The bands were quantitatively evaluated, by elution and measuring their optical densities spectrophotometrically Abd El-Salam et al (1964).

*Analytical methods.*

Tyrosine and tryptophan were determined spectrophotometrically according to the method of Godwin and Morton (1946). Nitrogen determination was carried out by the micro Kjeldahl method Ling (1956). Phosphorus was estimated by the Sodium Molybda Stannus chloride method according to Snell and Snell (1949). Sulphur content was determined be the method of Frear (1950).

## RESULTS AND DISCUSSION

Fig. 1 showed two bands in the electropherogram of a-casein of buffalo milk which were designated $a_1$–, and $a_2$ fractions according to their relative mobilitg. $a_1$–casein was the fastest while $a_2$–casein was the slowest. These findings confirmed the results of Geller et al (1960). MacMeekin et al McMeekin et al (1959), however, reported a third band in the free boundary electrophoresis which they designated $a_3$–casein.

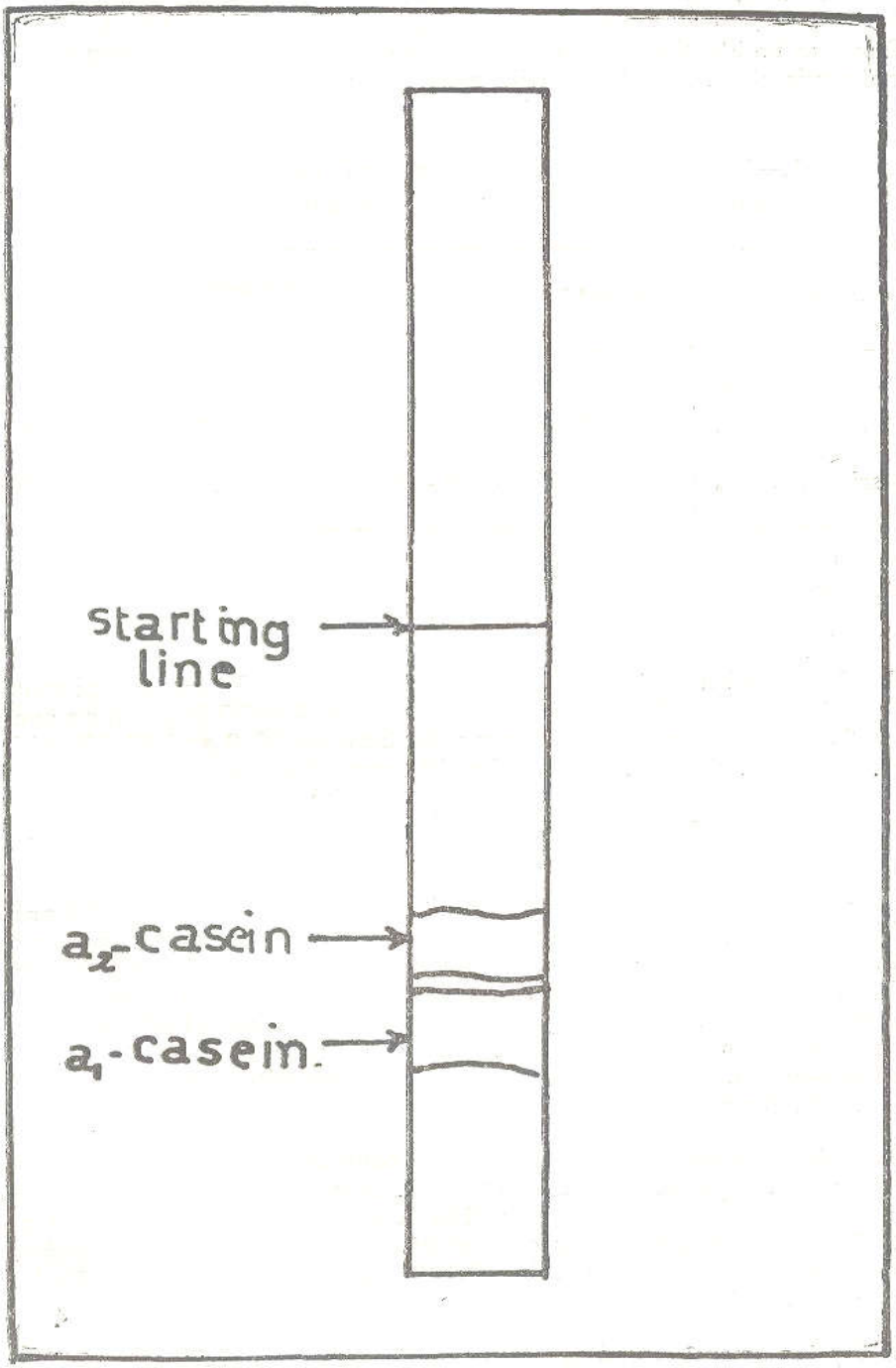


FIG. 1.—Paper electrophoresis of a-casein in acid media.

When a-casein was fractionated by CaCl2 precipitation method, it gave two fraction known as Ca-soluble and Ca-insoluble. These two fractions were found to be identical to the $a_1$– and $a_2$– caseins respectively using paper electrophoresis of a-casein in an acid media as shown in fig 1.

TABLE 1.—**The relative distribution of the electrophoretic components of buffalo milk a-casein***

| Constituent | mean ** | range | Standard deviation | Standard error |
|---|---|---|---|---|
| $a_1$–Casein . . . . | 42.4 | 37.3 - 50.0 | 4.01 | 1.21 |
| $a_2$–Casein . . . . | 57.6 | 50.0 - 62.7 | 4.27 | 1.29 |

* All values are expressed in per cent.

** Average of 11 samples.

Table 1, showed the relative distribution of the electrophoretic components of a-casein in buffalo milk $a_1$–Casein varied from 37.3 to 50.0%with an average of 42.4% ; while $a_2$–casein values were between 50.0 and 62.7% with higher average of 57.6%. From the literature McMeekin et al (1959), Libby and Ashworth (1961), Payenes (1961), and Waugh et al (1960) the amount of $a_2$–casein of buffalo milk was higher than that of cows', while $a_1$–cssein showed reversed tendency.

The nitrogen content of the two fractions $a_1$–, and $a_2$–, were 15.08 and 14.95% respectively, but the difference between them was found insignificant, table 2. However, the average nitrogen content of $a_1$–casein, was higher than that quoted in the literature ; namely 14.1% McMeekin et al (1959), and Hipp and McMeekin (1961). Hipp and McMeekin (1961) reported 14.6% nitrogen for $a_2$–casein which was considered to be identical to Ca-soluble a-casein. This finding was in accordance with the present study being 14.85% for $a_2$–casein.

$a_1$–Casein had an average phosphorus content of 1.32% which was higher than that reported in the literature for the corresponding fraction ; namely 1.18% Libbey and Ashworth (1961), and 0.85% McMeekin et al (1959). The average phosphorus content of $a_2$–casein was slightly higher than that reported by others, being 0.35% Hipp and McMeekin (1961), and Payenes (1961).

The N/P ratio of $a_1$–, and $a_2$–caseins had averages of 11.40 and 36.18% respectively, and the difference between these two averages was significant.

TABLE 2.—**Elemental analyses, tyrosine and tryptophan content of $a_1$- and $a_2$-casein of buffalo milk.**

| Constituent | | Mean | Range | Difference between means | Standard deviation | Standord error | Significance of difference |
|---|---|---|---|---|---|---|---|
| Nitrogen . . . | $a_1$-casein | 15.08 | 14.74 - 15.39 | 0.23 | 0.309 | 0.138 | — |
| | $a_2$-casein | 14.85 | 14.60 - 15.33 | | 0.320 | 0.143 | |
| Phosphorus . . | $a_1$-casein | 1.32 | 1.31 - 1.34 | 0.89 | 0.016 | 0.007 | + |
| | $a_2$-casein | 0.43 | 0.31 - 0.55 | | 0.073 | 0.033 | |
| NLP ratio . . . | $a_1$-casein | 11.40 | 11.11 - 11.71 | 24.78 | 0.50 | 0.22 | + |
| | $a_2$-casein | 36.18 | 26.33 - 47.25 | | 9.04 | 4.03 | |
| Sulphur . . . . | $a_1$-casein | 0.67 | 0.60 - 0.72 | 0.28 | 0.063 | 0.028 | + |
| | $a_2$-casein | 0.39 | 0.35 - 0.45 | | 0.045 | 0.020 | |
| Tyrosine . . . . | $a_1$-casein | 9.32 | 9.25 - 9.41 | 2.62 | 0.061 | 0.027 | + |
| | $a_2$-casein | 6.70 | 6.60 - 6.80 | | 0.071 | 0.032 | |
| Tryptophan . . | $a_1$-casein | 2.36 | 2.20 - 2.50 | 1.16 | 0.117 | 0.052 | + |
| | $a_2$-casein | 1.20 | 1.10 - 1.30 | | 0.082 | 0.037 | |

— Insignificant. + Significant.

The sulphur content of $a_1$–casein was higher than that of $a_2$–casein. However, the average reported for the sulphur content for $a_1$-casein as 1.10 % by McMeekin et al (1959), was higher than that found in the present study ; namely 0.67 %. The same was found on comparing the sulphur content of $a_2$–casein with that reported by other workers. In the present study the average sulphur content of $a_2$–casein was 0.39,% which was less than that which was less than that found by Hipp, Groves, and Mckeekin (1961) for the same fraction, being 0.62 %.

The tyrosine content of $a_1$– and $a_2$–casein differed from that reported for $a_1$– and $a_3$–casein Hipp, Basch, and Gordon (1961) It was found that $a_1$–casein contained higher tyrosine content, 9.32%, than $a_2$–casein, 6.70%. Tose reported by Hipp Basch, and Gordon (1961) (14), were 7.11 and 9.80 % for $a_1$– and $a_3$–casein respectively.

The average tryptophan content of $a_1$–casein (2.32 %) was in accordance with that reported in the literature being 2.13 % (14,15), Gordon and Basch (1961), and Hipp, Basch and Gordon (1961). For $a_2$–casein the average tryptophan content was 1.20 %. Higher results were reported in the literature, being 1.82 % (14, 15). Gordon and Basch (1961), and Hipp, Basch, and Gordon (1961).

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## دراسة الفـا كازين فى اللبن الجاموسى

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### الملخص

فصل ألفـا كازين من بقية مكونات الكازين ثم فصل الى مكونين الفا ١ ، ألفا ٢ بواسطة الحمل الكهربائى فى محلول منظم من حمض اللاكتيك والبرونيونك يحتوى على ٣٠٪ يوريا مع استعمال تيار شديد ٣٠٠ فولت لمدة ١٠ ـ ١٢ ساعة على درجة ٤ مئوية .

كذلك فصل ألفا ١ كازين ( غير ذائب فى وجود الكالسيوم ) وألفـا ٢ كازين ( ذائب فى وجود الكالسيوم ) بواسـطة طريقـة الترسيب فى وجود أيونات الكالسيوم .

وبتحليـل الفـا ١ كازين وجـد أنه يحتوى على ١٥ر٠٨٪ نيتروجـين ، ١ر٣٢٪ فسفور ، ٦٧ر٠٪ كبريت ، ٣٢ر٩٪ تيروزين ، ٣٢ر٢٪ تربتوفان .

كذلك وجد أن ألفـا ٢ كازين يحتوى على ١٤ر٨٥٪ نيتروجين ، ٤٣ر٠٪ فسفور ، ٣٩ر٠٪ كبريت ، ٧٠ر٦٪ تيروزين ، ٢٠ر١٪ تربتوفان .