

A Paperelectrophoretic Investigation on Milk Serum Proteins

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Investigations of milk serum proteins by the classical Tiselius-electrophoresis¹⁻³ have revealed 5—6 electrophoretically separable components in normal milk serum. The present paper describes a study of milk serum proteins by the newly developed paperelectrophoretic method and a preliminary investigation of the effects of pasteurization and sterilization, as made in the dairy industry, on these proteins.

EXPERIMENTAL

Preparation of the milk samples. Fresh cowmilk was divided into three parts: one part was used as such, one part was pasteurized in a plate pasteurizer for 15 sec. at 72° C **, one part was sterilized for 25 min. at 119° C. These three milk samples were further treated in exactly the same way.

By addition of normal hydrochloric acid the pH was brought to 4.6 under control of the glass electrode. Casein, together with some other (denatured) proteins, was separated by centrifugation and the resulting whey filtered. After an overnight dialysis in cellophane tubes against three times renewed distilled water the clear wheys were lyophilized.

From the dry powders thus obtained samples were analyzed for their moisture and protein contents. The protein content was calculated from the results of micro-Kjeldahl determinations using the factor 6.35.

Preparation of the paper electropherograms. From the lyophilized powders three solutions were made each having a protein concentration of 6 %. These were analyzed by paper electrophoresis using a veronal buffer of pH 8.6 and ionic strength 0.1. The paper electropherograms were made essentially according to Grassmann and Hannig⁴, using 110 V potential during 13 hrs. The application of the sample and the decolorization of the amidoblack-dyed strips were, however, made by a slightly modified procedure⁵. The paper strips (Fig. 1) were measured by a selfrecording strip-photometer according to Miettinen and Moiso⁶.

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** Kindly arranged by Dr. M. Kreula from this institute, at the dairy of Valio in Helsinki.

RESULTS

The results of protein determinations for 100 mg of powders, dried during 3 hrs at 105° C, are given in Table 1.

Table 1. Protein contents of wheys from normal and heat-treated milks.

Whey-powder of	mg protein per 100 mg dry powder
Natural milk	25.7
Pasteurized milk	25.2
Sterilized milk	13.3

It can be easily seen, that in the case of sterilized milk about half of the whey proteins have been co-precipitated with casein. The brown colour, developed by sterilization of whole milk also separated in the casein precipitate.

The results of paper electrophoresis are presented in Table 2 and Figs. 1 and 2.

5 components are clearly separated in the electropherograms of *normal whey*, as can be seen from Fig. 1A and 2A. They have been tentatively identified as components a, b, c, d + e, and f of Smith¹. The location of Smith's component "d" in the paper electropherograms is not quite certain yet, but in a number of densitograms the β -lactoglobulin-peak has been unsymmetrical in a way suggesting the presence of a second component.

The mean relative concentrations of the separated components, calculated by the above mentioned method⁵, as well as the distances of the peaks on the densitogram from the starting line (a kind of "mobility"-value) are compared in Table 2 with the relative concentrations and mobilities obtained by Smith with the classical electrophoresis¹. As electroendosmosis was not measured in our experiments, we have made no effort to calculate the real mobility values.

Table 2. Mobilities and relative concentrations of proteins of normal whey, determined by E. L. Smith¹ with the Tiselius-electrophoresis, compared with the relative concentrations and distances of the peaks from the starting line, determined by paper electrophoresis.

	Immunoglobulines		c	d	e β -lacto-	f
	a eu-	b pseudo-				
Classical electrophoresis, mean values, by E. L. Smith ¹	% u *	% u	% u	% u	% u	% u
	6 -1.7	4 -2.5	18 -3.6	12 -4.5	55 -5.1	5 -6.4
Paper electrophoresis, mean values	% mm	% mm	% mm	% mm		% mm
	7 20	13 26	16 37	60 48		4 60

* u = sq. cm per volt per sec. $\times 10^{-5}$.

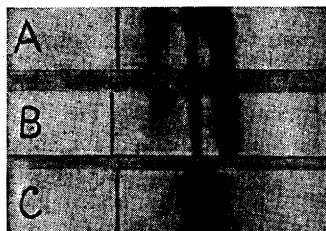


Fig. 1. Whey proteins from normal (A), pasteurized (B) and sterilized (C) milk, separated by paper electrophoresis.

Paper electrophoretic results correspond fairly well to those obtained by Tiselius-electrophoresis, except that values obtained for immunoglobulins by paper electrophoresis are about twice as high. This may be due to a higher affinity of these proteins to amidoblack, as has been suggested by Miettinen and Moisio⁵ to be the case with the γ -globulins of human serum. Another explanation would be a real difference between the milk samples analysed by Smith and the present authors.

The patterns obtained with the whey from *pasteurized milk* (Fig. 1B and 2B) do not differ much from those of normal milk. However, the zone of immunoglobulins is usually less sharp, more material being left between this zone and the starting line. The relative concentration of the immunoglobulin-fraction has also a tendency to be slightly lower than in normal whey. It seems therefore, that even the H.T.S.T.-pasteurization used (15 sec. 72° C) has caused some denaturation of this nutritionally important protein fraction.

Sterilization has a very great effect upon the whey proteins. Proteins, which are not already co-precipitated with casein, give in paper-electrophoresis one diffuse zone (Fig. 1C), which migrates with a mobility between that of component c and β -lactoglobulin (Fig. 2C), being evidently denatured β -lactoglobulin. By sterilization almost all of the whey proteins seem thus to be denatured and substantially changed.

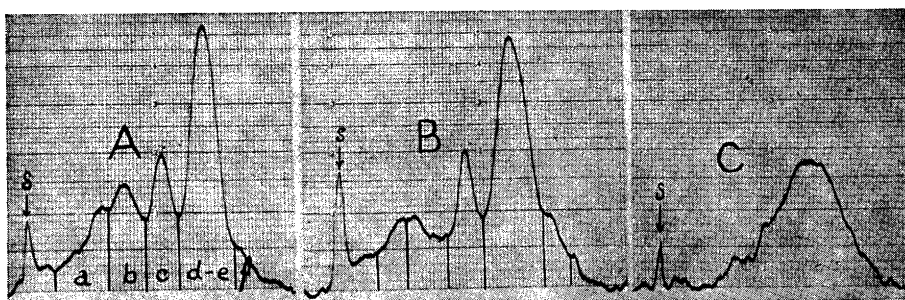


Fig. 2. Densitograms from paper electropherograms, made of whey proteins from normal (A), pasteurized (B) and sterilized (C) milk. S = starting line. Components acc. to Smith¹: a = euglobulins, b = pseudoglobulins, c = component, d = component, e = β -lactoglobulin, f = component.

SUMMARY

Paper electrophoresis of normal whey, prepared by acid-precipitation of casein at pH 4.6, has given results otherwise comparable to those obtained by earlier workers with the Tiselius-electrophoresis, except that values twice as high have been obtained for immunoglobulins by paper electrophoresis.

Whey from the H.T.S.T.-pasteurized (15 sec. 72° C) milk has given approximately similar results, although the appearance of the electropherograms and the relative concentrations calculated from the densitograms suggest a slight denaturation of the immunoglobulins.

The "whey" from sterilized milk has given only one diffuse zone (β -lactoglobulin with decreased mobility), which shows that milk serum proteins are substantially changed in sterilization.

The authors wish to acknowledge their great indebtedness to Professor A. I. Virtanen for his very kind interest and for the many facilities which have been put at their disposal.

We also wish to thank Mr. Tauno Moisio for assistance in the photometric determinations and Miss Vuokko Mickelsson for micro-Kjeldahl determinations.

One of us (J.E.V.) wishes to express his thanks for a scholarship from the Finnish Government.

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Received July 10, 1953.