GEL ELECTROPHORESIS OF THE PROTEINS OF POWDERED MILK

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One of the most important aspects of the evaluation of the quality of powdered milk is to establish the degree of solubility. Though the value of this characteristic is no doubt influenced by many factors, nevertheless, among these the state of protein is one of the most important. The state of milk proteins is affected in addition to the quality of the original milk fundamentally by thermal effects upon milk proteins in the course of pasteurization, pre-condensation and drying.

On the general properties of milk proteins, and within them on their sensitivity to heat, several survey works gave a good orientation. Among these, primarily the books of MCKENZIE [1], ZAJKOVSZKIJ [2], WEBB and JOHNSON [3] and KETTING [4] should be mentioned. On the changes produced in milk and milk products by thermal action, ADRIAN [5] gave recently a comprehensive survey. The investigations by SWEETSUR and WHITE [6, 7] on the thermal stability of milk proteins may furnish fundamental data for the qualitative evaluation of certain heat-treated milk products. From the point of view of the qualification of milk powders, an interesting paper by PIJANOWSKI et al. [8] emphasizes that the rate and nature of the heat treatment of milk powders can be readily followed by the determination of the quantity of non-denaturated whey proteins. ZAVARIN et al. [9] discuss also the thermal changes of whey proteins in conjunction with the manufacture of sweetened, condensed milk. According to their findings, primarily immunoglobulin and α-lactalbumin fractions undergo changes. FURUKAWA and YAMANAKA [10] investigated the insoluble proteins of lean milk powders by electrophoresis. They established that the quantity of the $\varkappa$-fraction decreased, while that of the α_s-β-fraction increased during storage. The quantity of sugars and amino sugars, contained in the insoluble fraction, also increased in the course of storage.

From the aspect of methodology, the disc gel electrophoresis technique is the most efficient. On the likely methods of gel-electrophoretic investigation

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of milk proteins, among others, SWAISGOOD [11] gives a comprehensive survey.

Therefore, it is expected that the various technologies, storage conditions and possibly the quality of milk will be reflected by the gel-electrophoretic properties of total milk proteins. Thus, this investigation might furnish fundamental data for possible use in qualification. These considerations prompted our investigations.

Materials and methods

In the course of our work, primarily powdered milk was investigated, and it was attempted to select milk powders as far differentiated as possible with respect to manufacturing technology and composition. Our study has been complemented by the investigation of a few milk and condensed milk samples. The milk powders studied are seen in Table 1.

To follow possible changes during storage, storage experiments were carried out on the samples produced in Mosonmagyaróvár. The samples were stored at 32 °C and 70% and 90% r. h. for a period of more than a month.

Table 1

Milk powders investigated

No.	Denomination of milk powder	Place of manufacture	Remark
1	Skimmed milk powder	Nyíregyháza	instant
2	Skimmed milk powder	GDR	instant
3	Skimmed milk podwer	Czechoslovakia	instant
4	Skimmed milk powder	Gyula	—
5	Skimmed milk powder	Dombóvár	drum-dried
6	Whole milk powder	Berettyóújfalu	—
7	Whole milk powder	Nyíregyháza	—
8	Whole milk powder	Mosonmagyaróvár	pilot plant
9	Whole milk powder	Mosonmagyaróvár	pilot plant
10	Whole milk powder	Mosonmagyaróvár	pilot plant
11	Whole milk powder	Yugoslavia	—
12	Whole milk powder	GDR	—
13	Whole milk powder	Czechoslovakia	—
14	Whole milk powder	France	instant
15	Whole milk powder	Central Laboratory of Cold-storage Industry	lyophilized

In accordance with the aim of our research, investigations concerned the gel electrophoresis of the proteins of milk powders. Among the possible methods and apparatuses, polyacrylamide gel electrophoresis was chosen, which proved successful both according to the literature and to our own experience. Buffers containing urea were used, because due to the strong interaction between the milk proteins, only in this case could elphograms of satisfactory resolution be obtained. Owing to its relative simplicity and ease of evaluation, separation on gel sticks and an apparatus manufactured by Labor MIM have been used.

Preparation of the milk powders for electrophoresis

0.3 g of the test milk powder sample was dissolved by shaking with 30 ml of buffer solution for 2 hours. The buffer used was TRIS–glycine buffer of H 8.6, containing 4.5 M of urea.

Electrophoresis

7% polyacrylamide gel sticks were used for the gel electrophoresis. The transfer of the samples was made in the sample gel: 1/3 vol. of sample solution +2/3 vol. of gel solution. Electrophoresis was carried out at 8.6 pH, with TRIS–glycine buffer containing 4.5 M urea, for 30 minutes at a current strength of 2 mA/tube, then 5 mA/tube. The time of separation was 60 minutes. Progress of the process was indicated by bromophenol blue indicator.

Development of the protein fractions contained in the gel

Two kinds of processes were used:

— With Amido Black: 1% solution, 30 min soaking, washing with 7% acetic acid solution for 48 hours.
— With Coomassie Brillant Blue R 250: development with a 1 : 19 mixture of a 1% aqueous solution and a 12.5% solution in thrichloroacetic acid.

Gels containing urea must first be fixed in a 5% sulfosalycilic acid—5% trichloroacetic acid solution. In this process, washing can be omitted, because only the proteines become coloured, but the gel background not.

Quantitative evaluation

For quantitative evaluation a densitometer of Joyce Loebl, Model "Chromoscan" was used.

Test results and their evaluation

Some general considerations

There are of least 30 to 40 different proteins in whole milk, including genetic variants and specific proteins present in smaller quantities, but excluding enzyme proteins. It is obvious, therefore, that a relatively large number of

bands occur, and no complete separation of the single components can be achieved. A further fractionation and a subsequent gel electrophoresis of the single sub-fractions would give a more exact separation of the single protein components. However, in view of the immediate aims of the present research, we did not attempt a separation of this kind, a purification of the phases, but studied whether informations can be obtained on the basis of relatively simple preparation and short investigation, likely of use in the qualification of milk powder.

Though the number of the kinds of proteins present in milk is high, the character of the elphogram is determined fundamentally by the casein fractions. With the electrophoresis technique used also by us, the distribution of the fractions is usually the following:

α_s— casein and its genetic variants (A, B, C, D)

β — casein and its genetic variants (A, B, C)

$\varkappa$ — casein and its variants

Among the other important proteins in the mildly alkaline medium used, β-lactoglobulin has a mobility between those of α_s- and β-caseine. Lactalbumine appears in the vicinity of α_{s-1}-casein. In alkaline medium $\varkappa$-casein is among the proteins of low mobility.

In accordance with the abovesaid, the high number of bands, with a few characteristically larger bands is characteristic of the elphograms obtained in our investigations.

Differences between the milk powders manufactured by different technologies

In formulating our research objects, heat treatment of different rates applied in the manufacturing technology of milk powder, has been assumed to be reflected by the state of the proteins of milk powder. An inspection of the manufacturing process shows that milk powders in make are subject to enhanced thermal action in three manufacturing phases, such as pasteurization, heat treatment during condensation, and finally, the thermal effect of drying. As concerns the direction of expected changes, these are determined, in addition to the general effect of denaturation, by the interaction of milk proteins with one another and with the other components of milk. The interactions of milk proteins may result on the one hand in the formation of the quaternary aggregates of certain fractions, and on the other hand in the aggregation of casein, primarily with β-lactoglobulin. The latter is accompanied according to certain assumptions, by the formation of new disulfide bonds. Among the non-protein components, primarily lactose comes into consideration as interacting component. The carbohydrate–protein complexes formed may strongly influence both the state of protein and its dietetic value.

In consideration of the aforesaid, it is expected that proteins and protein complexes, fractionating by interaction, will appear in a broader spectrum,

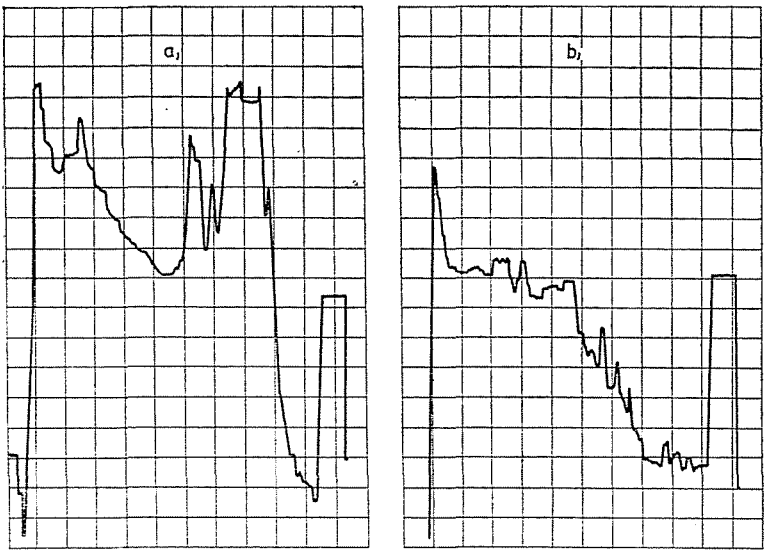


Fig. 1. Densitogram of Protein pherograms of non-fat powdered milk produced a) by spray drying; b) by drum drying

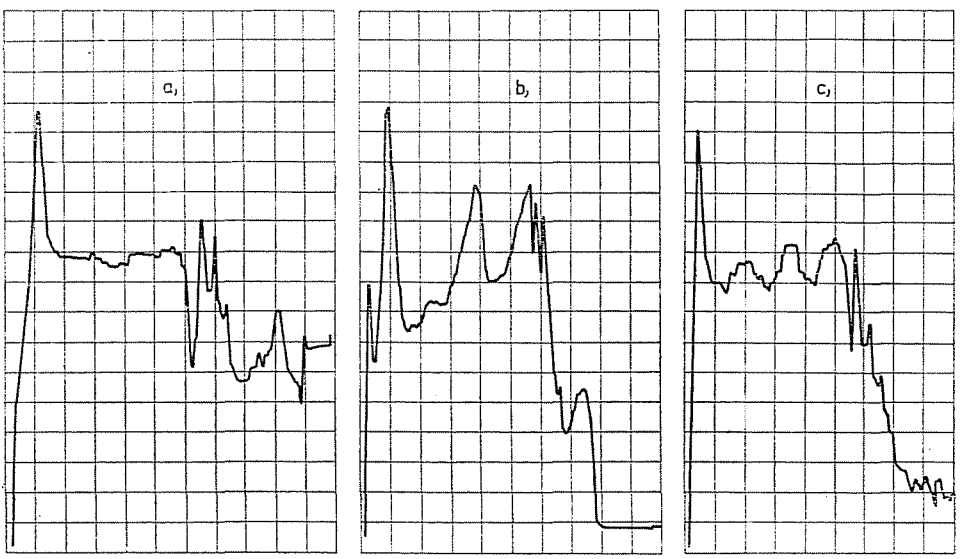
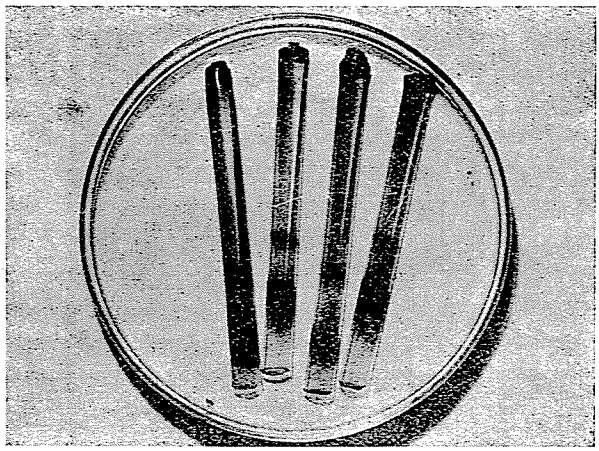
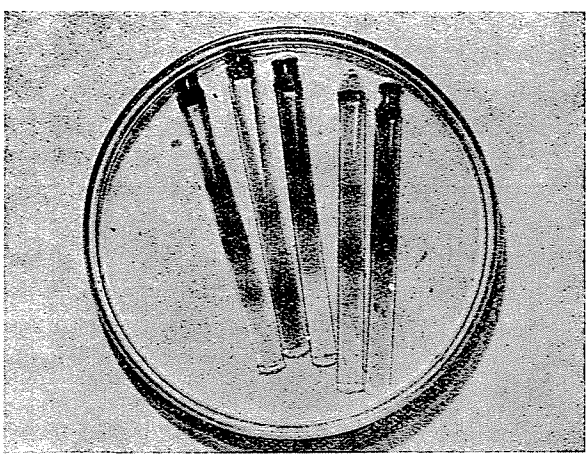


Fig. 2. Densitogram of Protein pherograms of whole milk powders (spray-dried) produced in a) Berettyóújfalu (Hungary); b) Yugoslavia; c) Czechoslovakia

and gel-electrophoretic bands will be exhibited less distinctively, in a more contracted pattern. This assumption is well-supported by a comparison of the elphograms of milk powder samples, prepared by spray drying and drum drying, respectively (see Figs 1a and 1b). The electrophoretic properties of



a)



b)

Fig. 3. Characteristic disc electrophoresis protein pherograms. a) 1 — condensed milk, 2 — milk, 3 — skimmed milk powder, 4 — milk; b) 1 — whole milk powder (GDR), 2 — instant non-fat milk powder (GDR), 3 — whole milk powder (ČSSR), 4 — instant non-fat milk powder (ČSSR), 5 — liophilized milk powder

the proteins of a few unskimmed milk powder samples, manufactured at different places, are shown in Figs 2a, b, c on the basis of the densitograms, prepared from the elphograms. It is clearly seen that the character of the protein state does not differ fundamentally. Differences are due presumably primarily to differences in the raw material (milk). Remarkably, the gel-electrophoretic properties of lyophilized milk powder are also of similar character. Some characteristic photos are shown in Figs 3a and b.

The effect of storage conditions of the proteins of milk powder

As mentioned, storage experiments have also been made on one milk powder sample prepared by spray drying. Partly to shorten the time of experiments, and partly to stress possible changes, the samples were stored at 32 °C at three different relative atmospheric humidities (70%, 80% and 90%). After a longer storage period, certain changes are observed on samples stored at 90% relative humidity and 32 °C (Fig. 4). The character of the change in the elphogram is indicative of protein–protein of protein–lactose interaction.

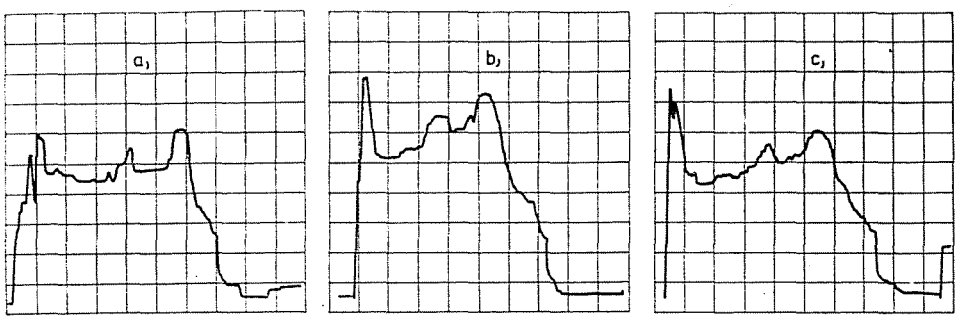


Fig. 4. Densitograms of protein pherograms of whole milk powders stored for a) 10 days, 80% RH, 32 °C, b) 20 days, 80% RH, 32 °C, c) 30 days, 80% RH, 32 °C

Summary

The proteins of powdered milks prepared by different drying technologies have been investigated. Also the effect of storage conditions and composition of milk powders has been studied. It is stated that the changes caused by extensive heating or prolonged storage at higher relative humidities and temperatures may be observed by disc electrophoresis of milk proteins.

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