ELECTROPHORETIC STUDIES OF OXIDIZED AND NORMAL FLAVORS IN MILK

by

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B. S., Mankato State Teachers College
Mankato, Minnesota, 1949

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

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INTRODUCTION

There are a number of investigations reported in the literature on the subject of foreign and oxidized flavor in milk. The majority of these papers treat the flavor irregularities from the standpoint of the diet of the cow or the possible contamination in the handling of the milk. There is little evidence of actual work done on the protein constituents of milk and their effect on the flavor. Roadhouse and Koestler (13) have studied the composition of milk quite extensively in regard to its influence on flavor. However, these investigators contended that flavor was mainly identified with the chloride-lactose relation. The protein content was apparently not of great interest to them. One of their conclusions was that the protein substances present in milk took a subordinate, if not negligible, part in the flavor in milk.

Based upon the above conclusion, it will be the purpose of this investigation to determine whether the protein material in milk can in any manner be related with flavor. The technique of electrophoresis should indicate any change in the protein fractions on comparison of an oxidized flavor pattern with a normal flavor pattern. These whey protein fractions can be characterized by their respective mobilities and isoelectric points.

There are several published methods for the isolation of whey proteins in milk. Harland and Ashworth (7) have outlined a salt-acid method. The usual procedure employed in the preparation of whey proteins for electrophoresis studies involves the precipitation of casein from skim milk by acid or rennet, followed

by the concentration of the casein-free proteins by lyophilization. Smith (14) and Deutsch (6) carried out electrophoresis studies of whey proteins prepared by this method. The proteins to be used in this study were prepared by the Harland and Ashworth method since it is one of the simplest procedures. Stanley et al. (15) have shown that there is no qualitative difference in whey proteins prepared by either the salt and/or rennet-lyophilization methods.

In this thesis, the term "oxidized flavor" denotes a peculiar taste not usually associated with a generally accepted normal milk flavor. The flavor need not be rancid, disagreeable, or otherwise unpleasant. Merely a distinctly different flavor present in a sample of milk, as contrasted to the normal, qualifies that sample for the oxidized category. In terms of monetary value and consumer appeal, oxidized flavor presents a real challenge to investigators.

MATERIALS AND METHODS

The Electrophoresis Apparatus

The electrophoresis apparatus used in this investigation was a modified Tiselius (18) apparatus manufactured by the Klett Manufacturing Company, New York City. A complete procedure on operation and description of this apparatus has been fully outlined in the literature (1, 2, 9, 10, 16) and will not be reiterated in this thesis. The essential parts of the instrument, however, consisted of a mercury vapor lamp to serve as a light source, a constant temperature thermostat bath equipped with a refrigeration unit capable

of maintaining near zero temperatures, and a long focal length camera. These parts were mounted on a channel iron optical bench. The optical bench was secured on three concrete piers set firmly through the laboratory floor and terminating in concrete footings in the basement subsoil. The installation at Kansas State College is shown in Plate I.

The electrophoresis cells were clamped in the brass rack as shown in Plate II. This rack assembly was placed in the constant temperature bath set at 0.5° C. during the course of a run.

Cleaning of the Glassware

After each run, the electrode vessels were thoroughly washed with a warm detergent solution. They were rinsed several times with distilled water and allowed to dry before being used.

The bottom plate, center section, and upper plate of the electrophoresis cell were also washed in a warm detergent solution. The inside surfaces of the three sections were best cleaned by using a pipe cleaner moistened with the detergent solution. Great care was taken to see that the ends of the pipe cleaners were bent over before using to prevent any scratching of the optical surfaces on the center section. The parts were rinsed with distilled water, given an acetone rinse, and left to dry between runs.

Preparation of Whey Proteins

The milk for this investigation was obtained from individual cows at the Kansas State Dairy. The sampling process consisted of getting milk with an oxidized flavor as well as milk with a

normal flavor. The samples were taken at two-week intervals to determine the persistence of the oxidized flavor. The same dairy animals were used each time. Each individual sample was one gallon of whole milk.

The cream was separated from the whole milk by a Sharples Super Centrifuge. Two liters of the skim milk were saturated with 650 g of sodium chloride and placed in a circulating water bath at 40° C. This salted milk stood at this temperature, with an occasional shaking, for 24 hours.

The casein was filtered off using a Buchner funnel fitted with a Whatman No. 2 filter paper. Casein, sufficient to form a mat about one-eighth inch thick, was poured into the funnel. The remainder of the casein was discarded. The non-casein filtrate came out a clear solution after being passed through this filter-casein combination several times. To make sure that all of the casein had been removed, the non-casein filtrate was again filtered. The second filtration was through a one-fourth inch mat of filter paper that had been previously pulped in a Waring blender.

One liter of the non-casein filtrate was adjusted to pH 2.0 by the addition of 10 percent hydrochloric acid with rapid stirring and allowed to stand overnight at room temperature. The precipitated whey proteins were collected on a Whatman No. 2 filter paper and transferred to a cellophane dialysis sack with a minimum of distilled water. These whey proteins were then dialyzed against distilled water at 4° C. until free of chloride ion. The protein solutions were removed from the dialysis sacks, diluted to 125 ml

with distilled water at 4° C., and frozen in the sharp-freeze room.

Prior to making a run, the protein sample was thawed. A 10 ml portion was diluted to 20 ml using buffer solution. The diluted sample was dialyzed in a two-liter bottle at 4° C. for 72 hours against three changes of buffer solution. The final buffer solution, against which the sample was dialyzed, was used as the solvent in the electrophoresis cells to form the boundaries for migration. The various buffers used in this investigation are recorded in Table 1.

Table 1. Buffers used in the investigation.[a]

Buffer	pH	Buffer salt M.	NaCl M.
1. Glycine	2.8	0.10	0.05
	3.8	0.10	0.05
2. Sodium acetate ($3H_2O$)	4.3	0.08	0.02
3. Cacodylic acid	5.8	0.06	0.05
	6.8	0.06	0.03
4. Sodium veronal	7.8	0.06	0.03

[a]Concentrated HCl or NaOH pellets added to produce desired pH.

The samples were run in all the buffers at the pH values indicated. In general, the sodium acetate trihydrate proved to give the best resolution of the protein fractions present. On this basis, it was chosen as the buffer for the major portion of the thesis work.

Boundary Formation and Observation

A cold room, which maintains a temperature of from 5° C. to 10° C. was used for the electrophoresis work. This room was used

to carry out the dialysis of the protein samples and the loading of the cells. Using a cold room of this type reduced the denaturation of the protein samples, and reduced the strain on the glass cells and electrode vessels when the assembled apparatus was immersed in the 0.5° C. thermostat.

The electrophoresis cells, Plate II, used for the investigation were of the type holding about 11 ml of liquid including the bottom plate. The ground glass surfaces of the bottom plate, center section, and the upper plate were greased sparingly with a 1:1 mixture of liquid paraffin and white vaseline as recommended by Tiselius (18). Stern and Reiner (17) found convective disturbances when other lubricants were substituted for the above grease mixture. The three cell sections were placed together making certain that all air bubbles were excluded for a continuous seal. When the seal was formed, the three sections were aligned so that the openings in each coincided exactly. This cell assembly was then placed in the brass support rack and clamped loosely.

Using a 5 ml syringe fitted with a 10 inch hypodermic needle, 5 ml of the protein solution were introduced into the cell assembly via the right channel. The cell assembly was then tipped back and forth to expel all air bubbles that might have accumulated at the junction of the bottom plate and the center section. When the air bubbles were expelled, the center section and top plate were displaced laterally to the right so that the bottom and center channels no longer coincided. The right channel was filled with the protein solution until a slight excess was observed above the upper plate. The protein solution remaining in the left channel was withdrawn and retained.

The left channel was rinsed six times with 5 ml portions of the buffer to be used for the particular run. This channel was filled with buffer to a level slightly above the upper plate. The upper plate was rotated gently to dislodge any air bubbles that might have collected at the junction of the center section and the upper plate. Care was exercised so that the center section was not moved with reference to the bottom plate.

While the center section and bottom plate were held firmly in place, the top plate was moved to the left until it was in alignment with the bottom plate. The cell assembly was then clamped firmly in place in the rack by tightening up the thumb screws snugly. The right half of the upper plate was rinsed with the buffer as prescribed above after the remaining protein had been withdrawn and saved.

The arms on the electrode vessels were connected to the upper plate of the cell assembly by means of gum rubber tubing. The electrode vessels were filled with the buffer to the lower level of the ground glass fittings on the electrode vessel arms. Then the electrodes were put in place in the vessels, and care was taken that most of the bubbles were removed from the inside of the vessels as the electrodes submerged. The buffer level was again brought to the lower level of the ground glass fittings on the electrode vessel arms by withdrawing the necessary portion with the 5 ml syringe. The three tapered fittings were lubricated with the 1:1 grease mixture. The fitting with the three-way stopcock was placed in the right electrode vessel arm; the stopcock was connected between the horizontal opening and the arm.

Approximately 20 ml of a saturated sodium chloride solution was injected, by means of the syringe, through the silver tubing on the electrodes. This was necessary to form a layer of sodium chloride around each electrode. Sufficient buffer was added to the left electrode vessel to bring it to the common level and then covered with the plastic cap. The capillary on the left electrode vessel was "tipped off" with the forefinger to remove any bubbles that might have arisen.

In the foregoing paragraphs the importance of bubble removal in the cell assembly was emphasized. Such bubbles, if left in the cell assembly, might become dislodged and rise during the course of a run with the result that the boundary between the protein solution and buffer would be destroyed.

After cleaning the optical surfaces of the cell with lens paper, the rack and cell assembly were removed from the cold room and placed in the thermostat bath. The assembly was set approximately an inch behind the schlieren lens. While the temperature was becoming equilibrated, the compensator syringe on the side of the thermostat bath was filled with the same buffer used in the electrode vessels. The rubber tube on the syringe was connected to the horizontal opening on the three-way stopcock, and the stopcock turned clockwise to connect the horizontal and vertical openings. The plunger on the manually operated compensator syringe was driven upward to expel any air bubbles in the tube. The stopcock was turned clockwise through 90°. Now the vertical opening was connected with the electrode vessel arm. The long hypodermic needle on the 5 ml syringe was inserted into the

vertical opening of the three-way stopcock and moved downward to remove any bubbles. The stopcock was then rotated clockwise to connect the horizontal opening with the electrode vessel arm. This was the final connection for boundary formation.

When the temperature had become equilibrated, the boundaries were formed between the protein solution and the buffer. First, the electrodes were connected to the current source to avoid any disturbance. At the same time, the stirring motor and the refrigerating motor are turned off to minimize vibrations. The boundary formation was expedited by moving the center section of the cell assembly to the left by means of the rack-and-pinion arrangement incorporated into the brass rack. The boundaries were moved into view by very slowly forcing buffer from the compensator syringe into the electrode vessel. The boundary moving downward in the right channel is designated as the descending boundary, and the boundary rising in the left channel the ascending boundary. The boundaries were in position when the descending boundary was about one-half inch below the wire reference stretched across the rack behind the cell channels. This position was attained by turning the gear wheel on the compensator syringe through six complete turns over a period of approximately seven minutes. This extended period of time is necessary to prevent boundary disturbance.

The cells must be carefully lined up in the thermostat bath to get a full view of the boundaries on the photographic plate. This was done by placing the brass disc with the two adjustable parallel vertical slits into position just in front of the

photographic plate, and with both sides of the schlieren lens mask open, the assembly rack was moved until the center cell section was directly in line with the optical path. This could be checked by the full illumination of the vertical slits.

When the boundaries were in position as outlined above, zero time pictures were taken of both the descending and ascending channels on Kodak 9 by 12 cm metallographic plates. The current and timer were turned on simultaneously. A current of 20 ma was supplied by the direct current regulator, Plate III, as set up by McColloch (12) and modified by Brandt (5). A thermostat temperature of 0.5° was employed in all runs. Additional scanning photographs were taken of the descending and ascending boundaries at two other time intervals depending on the rapidity of the splitting off of the various protein fractions. The current and timer were turned off during the photographic process. During the last series of runs, a total of four photographic plates were used instead of six. This was accomplished by photographing only a small portion of the original zero time boundary pattern on a plate along with the normal first time interval boundary pattern. This was done for the descending and ascending channel patterns. The exposed plates were developed in Kodak X-Ray commercial developer four minutes at 20° C. and fixed ten minutes in commercial X-Ray Fixer at the same temperature. Open trays were used for the developing and fixing.

The Plate Carriage

The arrangement for moving the photographic plate (11)

horizontally, as the schlieren diaphragm is raised vertically, is shown in Plate IV. The holder, H, for 9 by 12 cm plates is shown partially inserted into the plate carriage, C. Although this carriage is provided with flanges that move in the slots X and Y, these function merely as light baffles, the clearance being sufficient to ensure that the horizontal alignment is maintained solely by the bar, B. This bar, B, fits into the groove at L. The carriage is loosely coupled, by means of a thumbscrew, to the bushing, A, and driven by the threaded rod, R. The rod is then connected through gears F and G, which is hidden from view, to a shaft that is parallel to R but behind the plate P. This shaft engages, through a clutch operated by knob, K, the slow-speed shaft of the synchronous motor and reducing unit indicated by M. Also hidden by the plate, P, are the spiral gears that connect shaft G with the rod, T, that drives the schlieren diaphragm.

The mask for the cell images in the scanning procedure consists of a disc, D, that is provided with two vertical slits. The mask fits into a recess in the plate, E, so that its surface is flush with that of the plate. Removal of the mask permits viewing the images on a ground glass that may be substituted for the plate holder, H.

The brass scale, S, located at the top of plate P is graduated in mm. Use of this scale facilitates the placement of more than one boundary pattern on the same plate as previously discussed.

The Shutter Assembly

The aperture selector disc-variable sector disc arrangement

(8) in front of the camera lens is shown in Plate V. The disc, B, is provided with a series of openings. By rotation of the disc any one of these openings may be placed in front of the objective mounted at O. This disc fulfills the same function as the iris diaphragm of an ordinary camera. The shapes of the openings are such, however, that they provide a control of the exposure without restricting the lens aperture vertically. This ensures that an opening will not act as a schlieren diaphragm.

On the same axis with disc B, but rotating independently behind it, is a second disc, C. This disc is provided, in one quadrant, with a circular hole matching the lens aperture. In the opposite quadrant is an adjustable sector shaped opening. Driven through spur gears by a small synchronous clock motor, not visible in the photograph, this disc serves as the camera shutter. When viewing the pattern on the ground glass plate, or photographing it with the scanning device, this shutter is not used. For either of these procedures, the circular hole in the shutter is left directly in front of the lens. In the photography of schlieren bands and cylindrical lens patterns, the sector opening is adjusted to give the proper exposure. The shutter motor is started, and the dark slide of the plate holder removed as an opaque quadrant of the shutter disc moves across the lens. The quadrant containing the open sector then crosses the face of the lens and makes the exposure, after which the dark slide is re-inserted as the second opaque quadrant cuts off the light. On completion of the revolution, the circular opening is again in front of the lens.

Measurement of Conductance

A portion of each sample was retained for a conductivity measurement. An Ostwald conductivity cell, set in the thermostat bath, was used with a Leeds and Northrup No. 4725 Wheatstone Bridge. The balance point was determined by using a Leeds and Northrup 2420-a Galvanometer in conjunction with the bridge.

Method of Plate Evaluation

The pattern areas, due to each component present, were measured with a planimeter from traced enlargements using a magnification factor of 4. The peaks were divided by the method of Tiselius and Kabat (19). In their method, an ordinate was drawn from the lowest point between two adjacent peaks.

The mobilities of the migrating fractions were calculated from a fixed reference line, using the ascending boundary patterns. The distances migrated by a peak were measured using a microprojector with one mechanical stage.

RESULTS AND DISCUSSION

The electrophoretic patterns of whey proteins prepared by the method of Harland and Ashworth were investigated in milk with an oxidized flavor as well as milk with a normal flavor. The experimental work was carried out at a pH of 4.3 in a sodium acetate buffer. The patterns showed three well-defined peaks, 3, 4, and 5, (Plates VI and VII) in relatively the same positions. The original supposition was that the protein material in milk with an oxidized flavor

would have an electrophoresis pattern different from normal flavor milk. This supposition was substantiated by electrophoresis of milk with each of the previously mentioned flavors.

Table 2. Comparison of percentage areas of oxidized flavor and normal flavor milk, ascending patterns.

Oxidized flavor samples				Normal flavor samples			
Sample	Area 3	Area 4	Percent Area 4	Sample	Area 3	Area 4	Percent Area 4
153 I	129	102	44.2	164 I	102	50	32.9
II	96	40	29.2	II	70	35	33.3
III	125	25	16.7	III	96	50	34.2
IV	43	4	8.5	V	86	65	43.0
VI	83	70	45.7	VI	114	114	50.0
VII	94	63	40.1				
336 I	108	98	47.6	394 II	86	85	49.7
II	118	26	18.1	III	71	69	49.3
III	91	19	17.3	V	39	35	47.3
V	40	2	4.8	VI	104	114	52.3
VI	119	91	43.3	VII	93	153	60.8
VII	113	47	29.4				

The areas used as bases for the calculations were measured from ascending patterns. The ascending peaks were better resolved, although the descending patterns showed the same numbers and positions of less sharply divided peaks.

Plate VI consists of some of the typical electrophoretic patterns of the oxidized flavor samples obtained. Each of the figures was a pattern run on the sample at an interval of two weeks. At the outset of the investigation, it was presupposed that the oxidized flavor present would yield an electrophoretically distinct fraction. However, as shown later, this was not the case.

Closer examination of the figures in Plate VI revealed that component 4 characterized the oxidized samples, rather than a separate peak. There was some doubt as to whether this fraction was a lactoglobulin that could be associated with oxidized flavor or an immune lactoglobulin. Post-partum times and mobility measurements precluded the immune lactoglobulin identity.

The problem involved an interpretation of component 4 in such a manner that the change from oxidized flavor to normal flavor could be followed. It was noted that component 3 appeared consistently throughout the series of runs. This component was therefore chosen as a reference from which to compare the relative change in the area of fraction 4. The areas of components 3 and 4 were summed and 4 was determined as a percentage area. For one oxidized flavor sample, 336, (Table 2) the percentage areas were on a decrease from 47.6 to 4.8. The same was true for the other oxidized sample 153. This trend correlated exactly with a decrease in persistence of oxidized flavor through sample 336 V, after which time 336 became normal flavor milk. The percentage areas of 336 VI and 336 VII were more nearly like those of the normal flavor milk, sample 164. The electrophoretic patterns of 336 VI and 336 VII were very similar to those of sample 164, Plate VII.

The electrophoretic patterns presented in Plate VII are of milk with a normal flavor. The patterns are of individual samples collected twice monthly as was previously done for the oxidized flavor samples. The procedure for evaluating the samples was the same as that for the oxidized milk. Again, areas of fractions

3 and 4 were added and fraction 4 was calculated as a percentage of the combined area. The percentage areas for peak 4 in both normal samples, 164 and 394 (Table 2) were constant up to and including 164 III and 394 V. The remainder of the samples in the two sets indicated some area change.

There were some deviations in the percentage area relationships for both the oxidized flavor and the normal flavor toward the ends of the respective sampling periods. These deviations appeared as increases in percentage areas of component 4. A change from dry feed to pasture was assumed to be the reason for the deviations. Despite the percentage area enlargements of peak 4 after the diet switch, all electrophoretic patterns were normal. There were no evidences of oxidized flavors present following this diet change.

Table 3. Comparison of the mobilities x 10^5 of β-lactoglobulin (Peak 5) at various pH values for oxidized and normal milk, ascending patterns.

Sample Number	pH Values					
	2.8	3.8	4.3	5.8	6.8	7.8
Oxidized Flavor						
153 I	+9.57	+6.96	+3.44	-1.92	-3.44	-4.83
336 I	+9.26	+6.14	+3.93	-2.59	-4.35	-5.73
Normal Flavor						
164 I	+10.09	+4.84	+3.77	-2.66	-3.55	-4.96
394 II	+ 9.69	+5.39	+4.04	-2.48	-3.41	-5.41

The pH-mobility curves were plotted from the data in Table 3. The isoelectric point at pH 5.1 was determined from this plot in Fig. 1. There was apparently little difference in the isoelectric points of the oxidized flavor samples as compared to the isoelectric point for the normal flavor samples. The value of 5.1 for the isoelectric point of β-lactoglobulin appears consistent with that of Brandt et al. (4).

Since much of the work done on whey proteins was at pH 7.8 in a sodium veronal buffer, some of the initial runs were made with this pH-buffer combination. The patterns included in Plate VIII are from these runs. A comparison of the mobilities of components 3 and 4 at pH 4.3 in sodium acetate buffer with the mobilities of the same components at pH 7.8 in sodium veronal buffer was desired. The results comprise the data of Table 4.

Table 4. Comparison of mobilities x 10^5 of components 3 and 4 at pH 4.3 and pH 7.8, ascending patterns

Sample Number	pH 4.3		pH 7.8	
	Peak 3	Peak 4	Peak 3	Peak 4
		Oxidized Flavor		
153 I	+2.04	+2.73	-2.01	-2.74
336 I			-2.41	-3.70
336 II	+1.27	+2.10		
		Normal Flavor		
164 I	+1.61	+2.37	-2.10	-3.70
394 I			-1.70	-3.99
394 II	+1.31	+2.07		

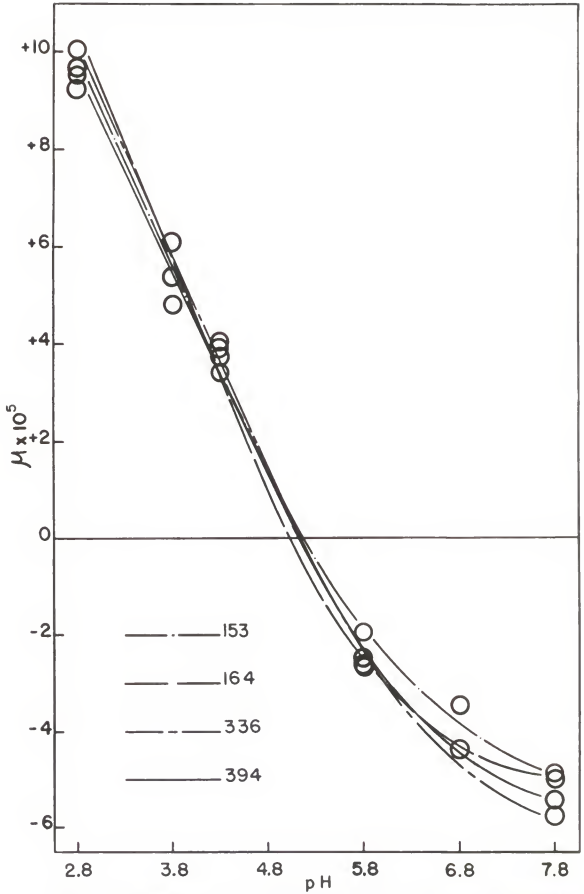

FIG. I. pH-MOBILITY CURVES FOR β-LACTOGLOBULIN

It was further desired to compare the mobility values at pH 7.8 with those of other investigators. The mobilities of the normal lactoglobulins (Components 3 and 4) reported in this thesis were in close agreement with those of Bain and Deutsch (3). Fractions 3 and 4 for the oxidized lactoglobulins had mobilities considerably less. There were no similar mobility values available in the literature for sodium acetate buffer at pH 4.3.

SUMMARY

1. Electrophoretic patterns were obtained for the whey proteins of cows milk having an oxidized flavor and a normal flavor. These proteins were prepared by the salt-acid method.

2. Three electrophoretically distinguishable components were resolved by the electrophoresis of these whey proteins.

3. The persistence of the oxidized flavor present was followed by the percentage area changes of peak 4 in relation to the consistently present peak 3.

4. The normal samples investigated showed a constant percentage area value for the peak 4 with respect to peak 3.

5. Some deviations in the percentage area relationships were attributed to a diet change.

6. The isoelectric points of the whey proteins with oxidized flavor and with normal flavor were very close to each other. The value determined was 5.1.

7. The mobilities of components 3 and 4 in sodium veronal buffer at pH 7.8 agreed closely with those of the literature.

ACKNOWLEDGMENT

The author wishes to express his sincere thanks to Dr. A. C. Andrews, his major professor, for the advice and guidance tendered throughout the investigation. Thanks are also due Dr. C. H. Whitnah for his many suggestions.

Appreciation is expressed to Dr. R. E. Silker for making available chemistry department supplies and equipment.

Special thanks must be given to W. G. Stanley for his help in the operational procedures of the electrophoresis apparatus. His assistance in the photographic work contained in the thesis was most valuable.

Thanks are extended to John Colwell for his many photographic plate measurements and routine calculations.

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APPENDIX

EXPLANATION OF PLATE I

Electrophoresis installation

at Kansas State College

PLATE I

EXPLANATION OF PLATE II

Fig. 1. The electrophoresis cell assembled into its supporting rack.

Fig. 2. The Tiselius electrophoresis cell.

PLATE II

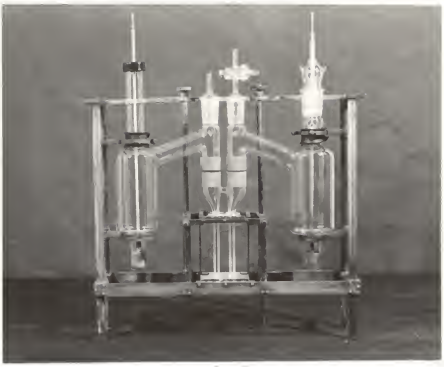


Fig. 1.

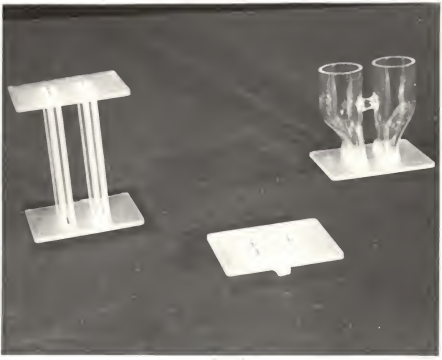


Fig. 2.

EXPLANATION OF PLATE III

Direct current regulator

PLATE III

EXPLANATION OF PLATE IV

The plate carriage

P Supporting plate

K Clutch knob

M Reducing unit

D Mask disc

S Brass scale

H Plate holder

C Carriage

X Y Light baffles

B Alignment bar

L Alignment groove

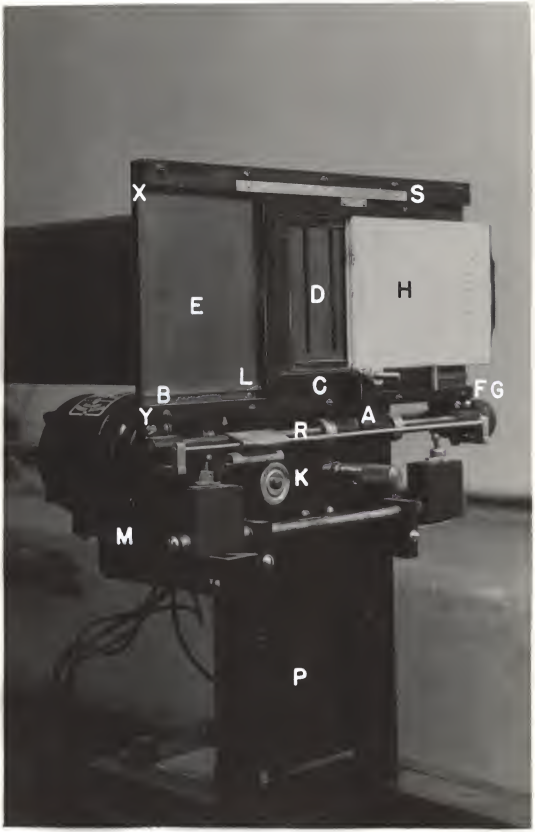
A Coupling bushing

R Threaded rod

F G Driving gears

E Mask plate

PLATE IV



EXPLANATION OF PLATE V

The shutter assembly

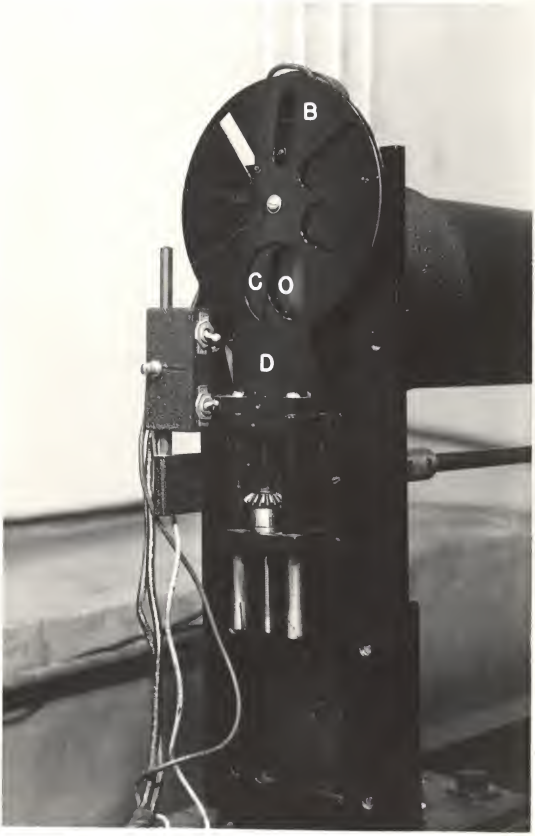
B Aperature selector disc

C Variable sector disc

D Schlieren diaphragm

O Objective

PLATE V



EXPLANATION OF PLATE VI

Typical ascending patterns of
oxidized flavor samples.

Fig. 1. 336 I taken at pH 4.3 after 150 minutes.

Fig. 2. 336 II taken at pH 4.3 after 150 minutes.

Fig. 3. 336 III taken at pH 4.3 after 150 minutes.

Fig. 4. 336 V taken at pH 4.3 after 150 minutes.

Fig. 5. 336 VI taken at pH 4.3 after 150 minutes.

Fig. 6. 336 VII taken at pH 4.3 after 150 minutes.

PLATE VI

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

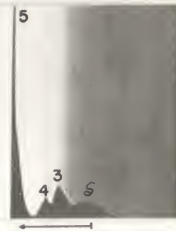


FIG. 6.

EXPLANATION OF PLATE VII

Typical ascending patterns of
normal flavor samples.

Fig. 1. 164 I taken at pH 4.3 after 150 minutes.

Fig. 2. 164 II taken at pH 4.3 after 150 minutes.

Fig. 3. 164 III taken at pH 4.3 after 150 minutes.

Fig. 4. 164 V taken at pH 4.3 after 150 minutes.

Fig. 5. 164 VI taken at pH 4.3 after 150 minutes.

PLATE VII

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

EXPLANATION OF PLATE VIII

Ascending patterns of oxidized and normal flavor samples at pH 7.8.

Fig. 1. 153 I (oxidized) taken after 150 minutes.

Fig. 2. 336 I (oxidized) taken after 150 minutes.

Fig. 3. 164 I (normal) taken after 150 minutes.

Fig. 4. 394 I (normal) taken after 150 minutes.

PLATE VIII

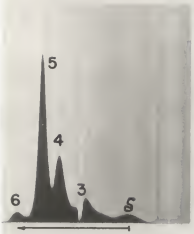


FIG. 1.

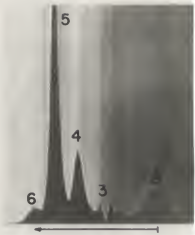


FIG. 2.

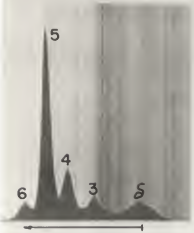


FIG. 3.

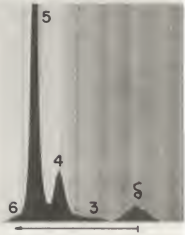


FIG. 4.

ELECTROPHORETIC STUDIES OF OXIDIZED AND NORMAL FLAVORS IN MILK

by

ELLSWORTH BENJAMIN BEETCH

B. S., Mankato State Teachers College
Mankato, Minnesota, 1949

AN ABSTRACT OF

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

Roadhouse and Koestler studied the composition of milk quite extensively in regard to its influence on flavor. The protein content apparently was not of great interest to them. One of their conclusions was that the protein substances present in milk took a subordinate part in the flavor of the milk. In view of this conclusion, it was the purpose of this work to determine whether the protein material in milk could in any manner be related to flavor. Electrophoresis was used in this work with the hope that it would indicate changes in protein fractions upon comparison of oxidized flavor patterns with normal flavor patterns. These whey protein fractions could then be characterized by their respective mobilities and isoelectric points.

Whole milk samples from individual cows were obtained at two-week intervals from the Kansas State College Dairy. The same dairy animals were used each time. These samples consisted of milk with an oxidized flavor as well as milk with a normal flavor. The whey proteins were isolated by the salt-acid method published by Harland and Ashworth. The samples were run in various buffers at several pH values. In general, the sodium acetate trihydrate buffer at pH 4.3 proved to give the best resolution of the protein fractions present. It was therefore chosen as the buffer for the major portion of the thesis work.

From the beginning of the work, it was hoped that the oxidized flavor present would yield an electrophoretically distinct fraction. This was not the case, however. A lactoglobulin

fraction, designated as component 4, characterized the oxidized samples, and not the formation of a separate peak. At the beginning of the study, component 4 showed a large area in the oxidized flavor samples. As the sampling progressed, the tendency for oxidized flavor to be present decreased. The area of component 4 likewise decreased. Another lactoglobulin fraction, namely component 3, appeared consistently throughout the series of runs. This component was chosen as a reference from which to compare the relative changes in the area of fraction 4. The persistence of the oxidized flavor present was followed by calculating the percent change in the area of peak 4 in relation to the area of peak 3. The normal samples investigated showed a constant percentage area value for the peak 4 with respect to peak 3.