Electrophoretic Method for Studying Proteins in Beer

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The proteins in beer were concentrated by the tannin-caffeine method, followed by electrophoresis on agar gel to separate 7 protein fractions: albumin, glutelin-1, glutelin-2, β-globulin, and α-, β-, and γ-hordein. Emission spectroscopy and spectrophotometry of electrophoretically separated proteins revealed ferro- and cuprometal-protein complexes. Analysis of the data shows that beer clarification decreases the content of the trace elements (iron, copper, calcium, and oxalate ion) and that of total protein and protein fractions (albumin, β-globulin and α-, β-, and γ-hordein).

An important index of the quality of beer is its colloidal protein stability which depends on the amount of minerals, protein substances, and polyphenols present in the beverage (1–5). Polyphenols (tannins) which remain in a soluble state after boiling combine with proteins to form complexes with minerals; their precipitation as "breaks" imparts to beer its colloidal stability (6–13).

In the process of mashing, boiling partially coagulates the protein substances. Three of the 4 globulin fractions found in the mash are precipitated, partly or completely, as the temperature rises to 70°C with only the β-globulin fraction, although turbid, remaining in solution. This fraction consists of both soluble and insoluble proteins, polyphenols, and carbohydrates.

The precipitate of beer at chill haze also contains calcium, chromium, cobalt, copper, iron, lead, magnesium, nickel, and silicon (14). Data on these components are limited. The interrelation of trace elements and proteins in beer and methods for concentration of the proteins have also not been adequately investigated. Therefore the present study was undertaken; its aim is to determine qualitative and quantitative characteristics of proteins and trace metals in beer (by electrophoresis and emission spectroscopy), their interaction with each other, and their influence on the stability of the medium. Techniques similar to those presented here may be generally applied to alcoholic beverages (15–17).

Experimental

The investigation was carried out on Zhuguli unfiltered beer (a type of light beer from 60% malt and 40% unmalted adjuncts produced by a double decoction method, Kolos Breweries, Lvov, U.S.S.R.). Standards of comparison were brews clarified by cotton filtering masses: Kineshma (control) and Evlakh[1] (test). Kineshma was selected as the control because it was processed by a more standard procedure.

Metal ions in alcoholic beverages were investigated by emission spectroscopy (18–25). Samples (50 ml each) were treated in the following manner: the samples were dried and ashed, and the ash residues were then mixed with standards and a buffer. The resulting mixtures were burnt in an electric arc of alternating current on a Spectrograph I SP-28 (19, 23, 26). Iron, cobalt, and nickel were also determined by the method of Vondenhof and Beindorf (20). The quantitative determination of these elements in the presence of each other is based on the formation of complexes with PAN (a polyamide synthetic fiber).

Oxalate ion abundance was determined by precipitation of the protein fraction with added calcium acetate (27). Calcium was determined by complexing with ethylenediaminetetraacetic acid in the presence of the metallochromic indicator calcein and by flame photometry (28).

The protein concentrates were obtained by the tannin-caffeine method, with ammonium sulfate, and with acrylex and molselect jellies (29–33). The jellies differ in their degree of swelling (15 ml/g for acrylex and 5 ml/g for molselect) and in the size of the molecules remaining. The concentrated protein was dialyzed by electrophoresis on agar and polyacrylamide gels (34–39). Colloid protein stability (degree of precipitation) and biological

[1] Kineshma and Evlakh are the Russian names of samples of cotton fibers. Kineshma mass is 34 nephelos units and Evlakh mass is 55 nephelos units, one differing from the other in filtering ability.

stability were determined by conventional methods. For horizontal electrophoresis, an EFA-1 apparatus and a Veronal-Medinal buffer of pH 8.6 containing agar gel in 1.5–2.0% concentration were used. Prior to horizontal electrophoresis the beer sample (400 ml) was treated with one of the following in order to separate the protein fractions: (1) 30 ml 4% tannin solution followed by dissolving the precipitate in 0.2 g caffeine; (2) 200 ml 4*M* ammonium sulfate; (3) 40 g acrylex; or (4) 112 g molselect. The concentrated protein fractions (0.04 ml) were placed on agar gel and electrophoresis was carried out for 5 hr at 40–80 v.

For vertical plate gel electrophoresis, a UIP-1 apparatus was used with tris-glycine buffer (pH 8.3) on polyacrylamide gel (8%). The dry sample (10 g) was dissolved in the buffer, and 0.4 ml of this solution was placed on the gel. Electrophoresis was then carried out for 3 hr at 100–300 v.

Total nitrogen and trace elements were determined in fractions of proteins concentrated by the tannin-caffeine method. Electrophoresis on agar gel plates gave a clean separation into albumin fractions. Globulin fractions were separated less well. Dried electrophorograms were analyzed by a recording microphotometer, Nauchno-Issled. Labor. Yorki Model MF-4. Correlation between the separated protein fractions was made according to the peaks in the electrophorogram. Blood serum was utilized for comparison of the fractions of proteins obtained (31).

In all fractions, unknown substances were analyzed by the Kjeldahl method, and trace elements were analyzed spectroscopically (19, 20, 23, 26).

Results and Discussion

The electrophoretic patterns on agar gel are represented by the 3 curves in Fig. 1. The area

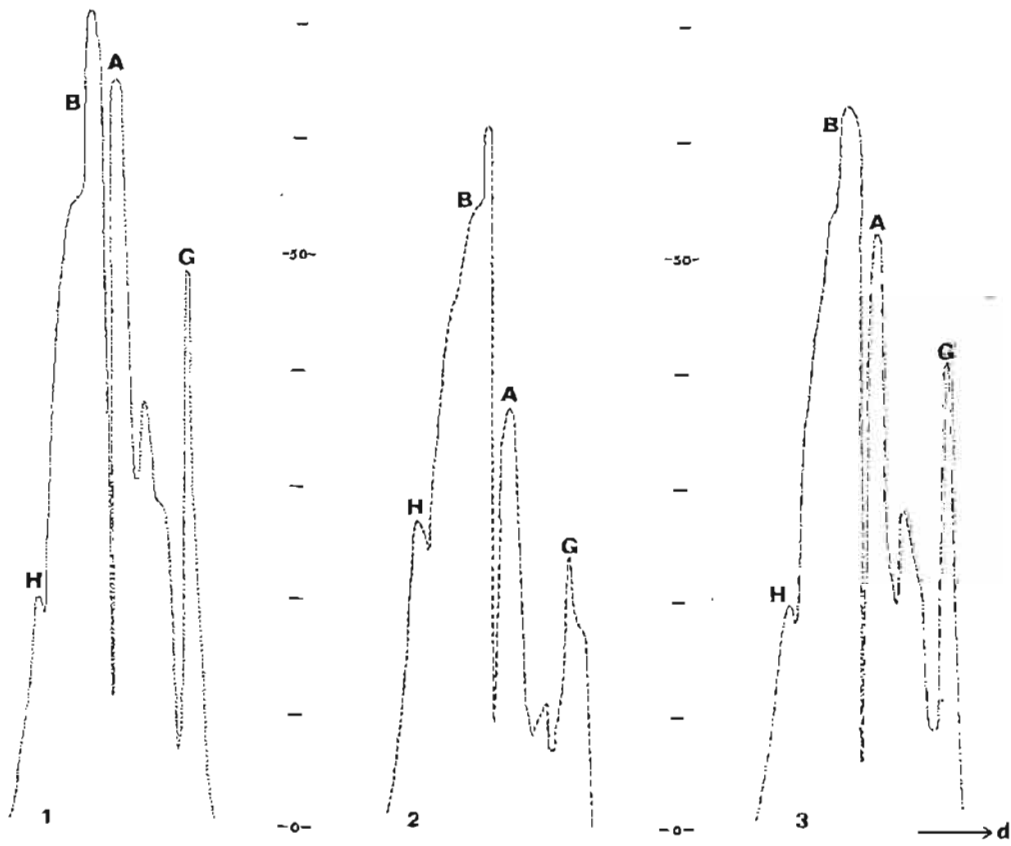

FIG. 1—Electrophoretic patterns of proteins precipitated by tannin-caffeine in unfiltered (1), test (2), and control (3) samples: A, albumin; B, globulin; G, glutelin; H, hordein.

between each of the curves and the abscissa characterizes the content of the protein fractions in beer filtered through different absorbents. The largest area is between curve 1 and the abscissa, and the least between curve 2 and the abscissa.

The decrease of protein maxima on the electrophorogram of the sample of test beer is explained by the partial decrease of high molecular weight proteins upon filtration. Electrophoretic separation of proteins gave 4–7 fractions, depending on the method of concentration. Concentration by the tannin-caffeine method gave the largest number of fractions, including albumin, 2 fractions of glutelin, β-globulin, and α-, β- and γ-hordein. Concentrating proteins with ammonium sulfate and acrylex gave albumin, glutelin-1, β-globulin, and α- and β-hordein; molselect gave albumin, glutelin-1, β-globulin, and α-hordein (Table 1). Comparison of the protein results obtained by the Kjeldahl analysis with data obtained by other means indicates that the tannin-caffeine method is preferable, as can be seen from electrophorograms of proteins of unfiltered beer on agar (Fig. 2) and polyacrylamide gels (Fig. 3).

Electrophoretic fractionation of proteins divides them into 2 groups: the albumin-glutelin group and the globulin-hordein group. Both groups of proteins were analyzed for trace elements. According to the analyses (Table 2) all the beer samples contained a higher content of albumin-glutelin than globulin-hordein. All cobalt, nickel, and calcium ions and most of the iron and copper ions were in the albumin-glutelin fractions. The oxalate ion and smaller amounts of iron and copper are found in the globulin-hordein fractions. A certain percentage of iron and copper with oxalate is negatively charged, suggesting that these metals are in complex ions.

Table 3 shows that clarification of the beer decreases the content of trace elements: iron (total content and especially in the globulin and hordein fractions), copper (total content and especially in the globulin fractions), calcium, and oxalate, all of which affect the colloid-protein stability of beer. This was determined by using the limit of precipitation in ammonium sulfate. As the limit of precipitation decreases, colloid-protein stability increases.

Electrophoresis has been used to separate and characterize proteins in beer. This information is of value in the study of haze in beer.

Table 1. Comparison of fractions of proteins (mg/100 ml) obtained by electrophoresis

Protein	Tannin-caffeine			Ammonium sulfate			Acrylex			Molselect		
	Unfiltered	Control[a]	Test[a]	Unfiltered	Control	Test	Unfiltered	Control	Test	Unfiltered	Control	Test
Albumin	82.75	81.11	80.05	41.23	32.73	28.70	46.79	44.39	40.76	47.98	42.48	33.94
Glutelin-1	82.01	81.77	80.65	65.71	100.47	151.57	84.75	83.91	83.75	77.31	76.17	75.42
Glutelin-2	21.26	20.61	20.00	100.74	61.55	—	—	—	—	—	—	—
β-Globulin	57.39	58.60	54.20	64.65	57.48	55.49	69.66	68.30	60.66	64.58	63.55	60.05
α-Hordein	28.79	28.32	—	48.51	42.17	38.88	52.75	50.16	50.16	110.30	108.36	90.00
β-Hordein	19.51	11.58	11.00	—	—	—	38.06	35.85	30.05	—	—	—
γ-Hordein	38.85	37.99	34.91	—	—	—	—	—	—	—	—	—
Total	340.57	319.98	280.81	320.84	301.40	274.64	292.01	283.61	265.38	300.17	281.06	259.41
Total (Kjeldahl method)	358.75	341.88	297.50	358.75	341.88	297.50	358.75	341.88	297.50	358.89	341.88	297.50

[a] Filtering samples of Zhuguli beer, using Kineshma (control) and Evlakh (test) as filtering masses.

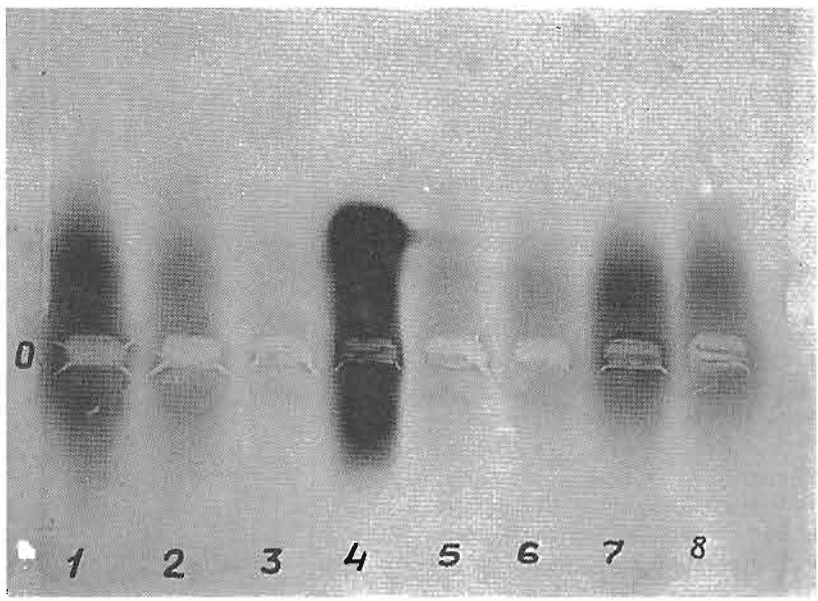

FIG. 2—Electrophoretic patterns of beer on agar gel. Samples precipitated by tannin-caffeine: 1, unfiltered; 2, control; 3, test. Samples precipitated by ammonium sulfate: 4, blood serum; 5 and 6, test; 7, unfiltered; 8, control.

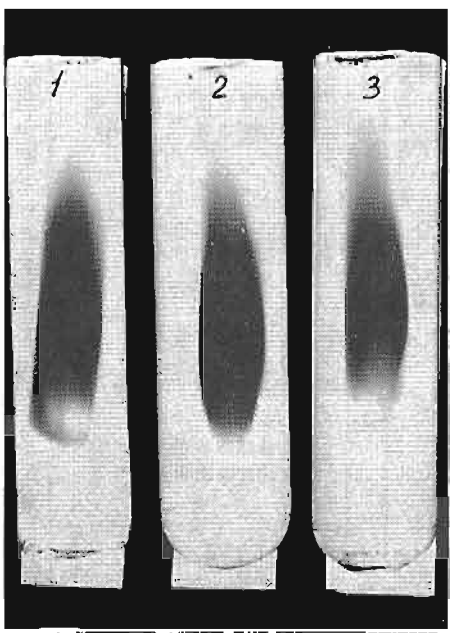

FIG. 3—Electrophoretic patterns of unfiltered beer on polyacrylamide gel. Samples concentrated by tannin-caffeine: 1, unfiltered; 2, control; 3, test.

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References

(1) Gorinstein, S. (1973) *Food Eng.* 45(8), 74
(2) Sogawa, H. (1972) *Rep. Res. Lab. Kirin Brew. Co.* 15, 15–24
(3) Gorinstein, S. (1974) *Brew. Dig.* 49(3), 46–48
(4) Djurtoft, R. (1965) *J. Inst. Brew. London,* 71, 305–315
5) Narziss, L., & Roettger, W. (1973) *Brauwissenschaft* 26(7), 217–220
(6) Drawert, F., Radola, B. J., Mueller, W., & Goerg, A. (1971) *Eur. Brew. Conv. Proc. Congr.* 14, 479–488
(7) Patel, G. B., & Ingledew, W. M. (1973) *J. Inst. Brew. London* 79, 392–396

Table 2. Composition of protein fractions and trace elements in ethanol media

Samples	Albumin + glutelin, mg/100 ml	Glutelin + albumin[a]					Globulin + hordein, mg/100 ml	Hordein + globulin[b]			
		10^{-6} g/L				mg/L		10^{-6} g/L		mg/L	
		Iron	Copper	Cobalt	Nickel	Calcium		Iron	Copper	Oxalate	
Unfiltered	185.02	109.50	34.20	0.40	1.10	0.58	154.55	43.54	1.00	1.20	
Control	183.40	71.00	32.60	0.24	0.80	0.52	136.20	28.29	0.94	0.90	
Test	163.20	33.50	31.52	0.03	0.50	0.31	117.10	13.35	0.67	0.60	

[a] Oxalate was not detected in this fraction.
[b] Calcium, cobalt, and nickel were not detected in this fraction.

Table 3. Effect of various filtration techniques on metals, protein-metal complexes, and protein fractions of beer

Indices	Unfiltered	Control	Test
10^{-6} g/L			
Iron	161.00	105.00	49.30
Iron-β-globulin	15.46	10.03	4.74
Iron-hordein	28.14	18.26	8.61
Copper	39.25	37.48	30.26
Copper-β-globulin	1.05	0.94	0.67
mg/L			
Calcium	26.00	24.00	16.00
Oxalate	24.90	20.90	12.70
mg/100 ml			
Protein, total	358.75	341.88	297.50
β-globulin	67.39	58.60	54.20
α-hordein	28.79	28.32	—
β-hordein	19.51	11.58	11.00
γ-hordein	38.86	37.99	34.91
Biological stability, days	—	8.0	9.0
Limit of precipitation, ammonium sulfate, ml/100 ml	—	8.0	11.0

(8) Narziss, L., & Roehtger, W. (1973) *Brauwissenschaft* 26(9), 261–267
(9) Herrman, K., & Mosel, H. (1973) *Brauwissenschaft* 26(9), 267–272
(10) Guenther, K. R., & Stutler, J. R. (1965) *Amer. Soc. Brew. Chem. Proc.* 30–33
(11) Lundin, H. (1963) *Wallerstein Lab. Commun.* 90, 75–77
(12) Meredith, W. O. S., & Trachuk, K. (1964) *J. Inst. Brew. London* 70, 410–415
(13) Sandegren, E. (1957) *Eur. Brew. Conv. Proc. Congr.* 123–126
(14) Chapon, L. (1965) *J. Inst. Brew. London* 71, 299–304
(15) Fertman, G. I., Gorinstein, S. B., Andryushechenko, A. V., Makstelene, Z., & Sablinskas, P. (1970) *Kharchova Prom.* 5, 32–33; thru (1971) *Chem. Abstr.* 74, 41114m
(16) Gorinstein, S. (1974) *J. Food Sci.* 39, 953–956
(17) Gorinstein, S. B. (1971) *Izv. Vyssh. Uchebn. Zaved. Pishch. Tekhnol.* 6, 98–100; thru (1972) *Chem. Abstr.* 76, 125367a
(18) Heckman, M. (1970) *JAOAC* 53, 923–925
(19) Steiner, R. L., & Oliver, R. T. (1963) *Amer. Soc. Brew. Chem. Proc.* 111–113
(20) Vondenhof, T., & Beindorf, M. (1968) *Monatsschr. Brau.* 21(6), 156–157
(21) Wright, F. C., & Riner, J. C. (1972) *JAOAC* 55, 662–664
(22) Schoeneman, R. L., Dyer, H. R., & Earl, M. E. (1971) *JAOAC* 54, 1247–1261
(23) Meredith, M. K., Baldwin, S., & Andreasen, A. A. (1970) *JAOAC* 53, 12–16

(24) Stone, I. (1972) *JAOAC* 55, 547–548
(25) Burke, K. E., & Albright, C. H. (1970) *JAOAC* 53, 531–533
(26) Viske, V. B., & Feaster, J. F. (1964) *Amer. Soc. Brew. Chem. Proc.* 189–193
(27) Koch, G. H., & Strong, F. M. (1965) *Anal. Chem.* 37, 1092–1095
(28) Frey, S. W. (1968) *Brew. Dig.* 43(3), 78–82
(29) Doty, W. H., Munson, A. W., Wood, D. E., & Schneider, E. L. (1970) *JAOAC* 53, 801–803
(30) Bernfeld, P., & Misselbaum, J. (1956) *J. Biol. Chem.* 220, 851–860
(31) Fertman, G. I., & Gorinstein, S. B. (1968) *Ferment. Spirit. Prom.* 34, 15–18; thru (1968) *Chem. Abstr.* 69, 75576a
(32) Gordon, A. (1960) *Biochim. Biophys. Acta* 23, 42–45
(33) Gordon, A., Keil, B., Sebesta, K., Knessl, O., & Sorm, F. (1950) *Coll. Czech. Chem. Commun.* 15, 23–28
(34) Brown, D., & Boyd, W. J. R. (1966) *J. Inst. Brew. London* 72, 541–546
(35) Gordon, A., Gardell, S., & Aquist, S. (1950) *Acta Chem. Scand.* 4, 907–913
(36) Grabar, P., & Williams, C. (1955) *Biochim. Biophys. Acta* 17, 167–175
(37) Meredith, W. O. S. (1963) *Amer. Soc. Brew. Chem. Proc.* 5–11
(38) Coduri, R. J., & Rand, A. G. (1972) *JAOAC* 55, 461–463
(39) Coduri, R. J., & Rand, A. G. (1972) *JAOAC* 55, 464–466

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