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METAL PROTEIN COMPLEXES IN ETHANOL MEDIA

INTRODUCTION

ONE OF THE prime indicators of beer quality is its colloidal stability (Gorinstein, 1968; Fertman and Gorinstein, 1968, 1970). This stability depends on the amounts of minerals in proteins in an aqueous medium (Gorinstein, 1971; Raible, 1967). The main task facing us was to discover the nature of the bonds between the mineral and protein components of beer and their effect on the colloidal stability of the product.

EXPERIMENTAL

THE INVESTIGATION was carried out on "Zhuguli" nonfiltered beer (a type of light beer of 2.8% mas. alcohol), produced at Lvov by Kolos Breweries by use of the double-decoction method, from 60% light malt and 40% nonmalted adjuncts. Standards of comparison for beer were the brews clarified by cotton filtering masses "Kineshma" (control) and "Evlakh" (test). ["Kineshma" and "Evlakh" are the Russian names of samples of cotton fibers. "Kineshma" mass is of 34 nephelos units and the "Evlakh" of 55 nephelos units; the two are distinguished by their filtering ability. For the manufacture of these filtering masses, raw materials such as cotton and cellulose were utilized.] "Kineshma" (control) was processed according to a well-known procedure while "Evlakh" (test) was treated in a different manner (Gorinstein, 1970). Because of this, "Kineshma" was selected as the control.

It is known that four main groups of proteins in beer are distinguishable according to their solubility in different solvents: (a) proteins soluble in water and in dilute salt solutions, as represented by albumin; (b) proteins soluble in dilute salt solutions but dissoluble in water, as represented by globulin; (c) proteins soluble in 60–75% ethyl alcohol, as represented by prolamine and hordein; and (d) proteins soluble in weak alkali solutions, as represented by glutelin.

The solubility of proteins in these solutions was the basis for selecting and utilizing the procedure of fractionation. In this procedure, the proteins were extracted directly from the beer samples with 5% potassium sulfate, 0.2% sodium hydroxide and 70% ethyl alcohol. The extracts were filtered and the proteins precipitated out by 10% trichloroacetic acid, in filtrates of every fraction. The protein sediments were then washed with 1% trichloroacetic acid. The filtrates remaining after precipitation were also investigated.

Iron, copper, zinc, cobalt and manganese in the sediments and the filtrates were determined spectroscopically. Calcium was investigated titrimetrically according to Frey (1968) and by flame photometry; oxalates were examined both gravimetrically and titrimetrically (Koch and Strong, 1965). The stability of metal-protein complexes was determined thermochemically using the Paulik-Paulik-Erdey derivatograph (Paulik et al., 1958). To perform a thermochemical analysis, it is not necessary to first fractionate the proteins. Instead of this, the proteins were precipitated by tannin-caffeine and by ammonium sulfate. The tannin-caffeine method is based on the protein complex-forming property of tannin. These complexes were then dissolved in caffeine (Fertman and Gorinstein, 1968, 1970). The sediments were dried at 30°C.

Four curves were recorded simultaneously on the derivatograph and are presented in Figure 1, a–d: (1a) Curve of differential thermo-

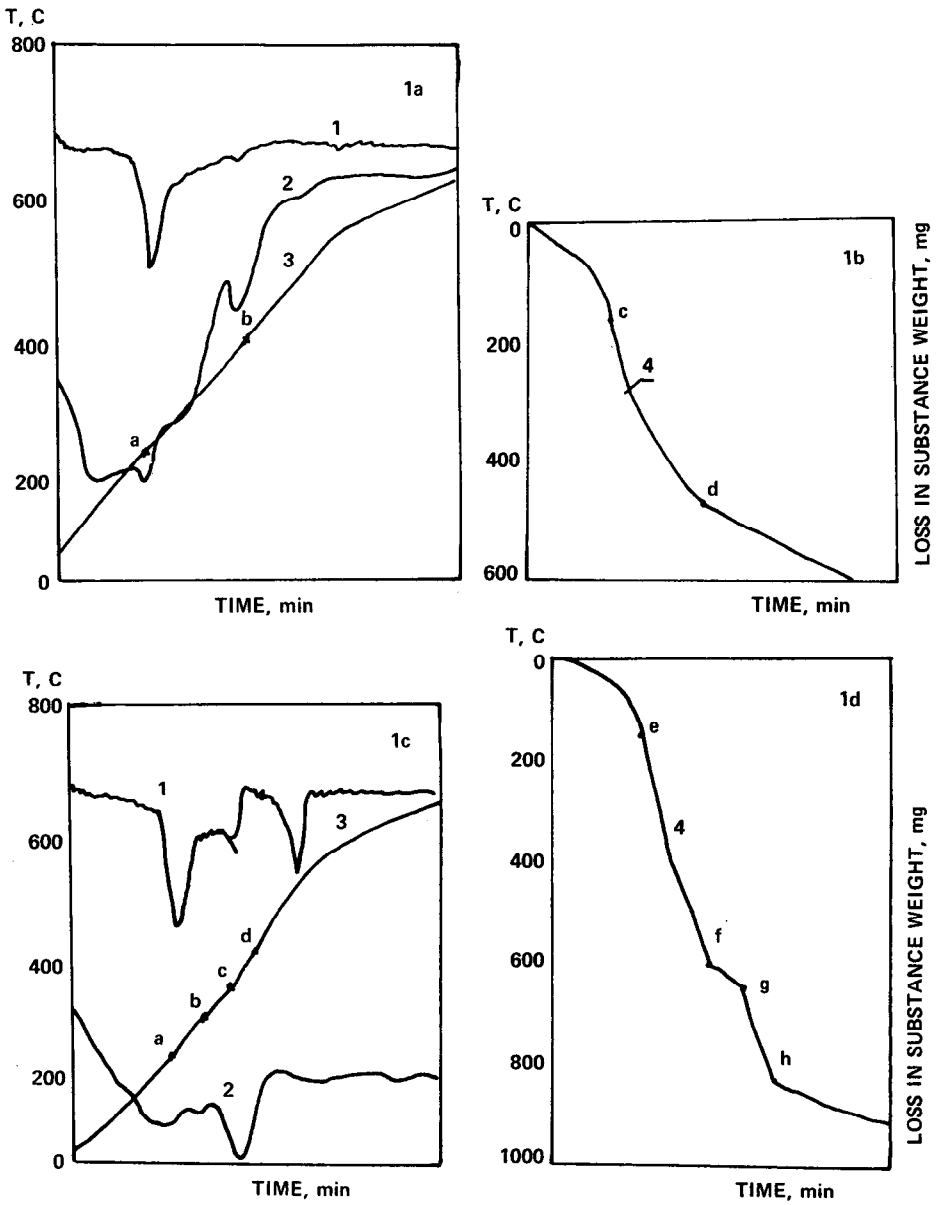


Fig. 1—Derivatograms of metal-protein complexes of samples of control beer (a, b) without added Fe^{3+} and (c, d) with added Fe^{3+}. The curves of heating: (1) DTG; (2) DTA; (3) T; and (4) TG.

gravimetric analysis, DTG; (1b) Curve of differential thermal analysis, ETA; (1c) Curve of temperature, T; and (1d) Curve of integral thermogravimetric analysis, TG. Points a, b, c, d, e, f and h are the sites of the respective endothermic effects of loss in substance weight at varying temperatures. The conditions of the experiment are shown in Table 1.

The colloidal stability of beer was determined using the limit of precipitation of ammonium sulfate (Fertman and Gorinstein, 1968; Gorinstein, 1971, 1973). As the limit of precipitation decreased, the colloidal stability was found to increase.

In the series of experiments, standard solutions of cations of heavy metals were introduced into the samples of control beer. The results of the experiments express the dependence of the elements on colloidal stability.

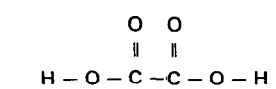
RESULTS & DISCUSSION

THE BOND STRENGTHS between the trace elements and proteins were determined by the ratio of their quantities in protein fractions to the total amount in beer. The data obtained show that in nonfiltered beer, iron and copper are found in complexes with proteins of all fractions. The various protein fractions can be classified according to their iron content in descending order as follows: alkali soluble (glutelin), alcohol soluble (prolamine-hordeine), salt soluble (globulin) and water soluble (albumin).

Zinc, nickel, manganese and cobalt are found mainly in the filtrates after protein precipitation, suggesting that those metals form unstable bonds with proteins. These data are found in Tables 2 and 3. The complexing abilities of the investigated metals, determined by taking a sum total of metal percentages in the protein fractions (aqueous + salt + alkaline + alcoholic), are also shown in Table 3.

The most common complexing agents are, as seen in the Tables, the transition elements copper and iron (Davies et al., 1969; Ritsma et al., 1969; Bagger, 1969; McKenzie, 1969). The considerable complexing ability of copper results from its being contained in beer mainly in the oxidized form (Cu^{2+}) (Suchov and Mitsuya, 1967; Michailidis and Martin, 1969; Nancollas and Poulton, 1969). The same property of iron is explained by the prevalence of the oxidized form over the reduced one.

The complexing ability of the oxalate-ion results from its being a bidentate ligand, which together with amino groups of beer, forms mixed ligands (Fig. 2).



As stated above, the majority of trace elements, especially copper and iron, combine with proteins of all fractions, their amino acids being their ligands (see Tables 2 and 3). Thus, complexes of metals with proteins are formed (Makinen et al., 1969; Nakao et al., 1967; Hamada et al., 1969). It is clear that in these complexes, a metal is bonded not only with a carboxyl group, but with nitrogen as well, by secondary covalent bonds (Fig. 3) when the metal ion (Me^{2+}) is Co^{3+}, Co^{2+}, Fe^{3+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Cu^{+}, Mn^{2+} or Zn^{2+}. Copper (III) and Fe (III) cause another carboxyl group to be picked up.

Thermochemical investigations were carried out to determine the position of the metals in the metal-protein complexes and their bond strengths with the ligand.

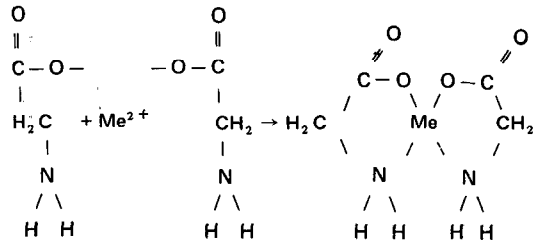


Fig. 3—

Table 1—Experiment conditions

Weight of substance	100 mg
Thermopair	Pt-Pt/RH
Resistance of electric circuit	DTA, 1/10; DTG, 1/10 megohms
Rate of heating	10 deg/min
Range of error of temperature	±5°C

Table 2—Mineral components in filtrate fractions of proteins in nonfiltered beer

Trace elements			Filtrate fractions			
			Aqueous	Salt	Alkaline	Alcoholic
Iron	Total amt ($X10^{-3}$ mg/ℓ)	16.0	1.04	4.37	1.16	1.18
	% of total	–	0.66	2.63	0.74	0.75
Copper	Total amt ($X10^{-3}$ mg/ℓ)	39.21	0.77	2.04	1.15	–
	% of total	–	1.97	5.19	2.97	–
Zinc	Total amt ($X10^{-3}$ mg/ℓ)	24.83	11.34	–	12.74	–
	% of total	–	45.62	–	51.38	–
Nickel	Total amt ($X10^{-3}$ mg/ℓ)	15.94	12.88	–	1.91	–
	% of total	–	80.66	–	12.09	–
Cobalt	Total amt ($X10^{-3}$ mg/ℓ)	9.92	7.39	–	2.13	–
	% of total	–	74.36	–	21.49	–
Manganese	Total amt ($X10^{-3}$ mg/ℓ)	8.47	1.50	–	6.78	–
	% of total	–	17.71	–	80.05	–
Calcium	Total amt (mg/ℓ)	26.00	13.83	–	11.58	–
	% of total	–	53.18	–	44.56	–
Oxalate-ion	Total amt (mg/ℓ)	24.91	–	19.78	–	3.88
	% of total	–	–	79.45	–	15.55

Metal-protein complexes of nonfiltered, test and control beers were studied. The same samples were treated with the ferric ion in the amount of 3.5×10^{-3} mg/ℓ, causing some protein precipitation. In samples both with and without Fe^{3+}, proteins were precipitated by tannin-caffeine and ammonium sulfate. Thermogram data were obtained under the conditions described in Tables 4 and 5 (see also Fig. 1).

Investigations of metal-protein complexes by the thermogravimetric method have shown that in the temperature interval of 180–240°C, one or two endothermic effects of dehydration take place on the DTG and TG curves (see Fig. 1). The nature of the complex as well as that of the external sphere of the heavy metals (Fe, Cu, etc.) depends on the temperature of dehydration. The first effect exists in each of the derivatograms presented.

The loss of mass of the complex in the TG curve is attributed to the removal of water. In the beginning, loss of mass ranging from 9.7–17.9% occurs in the different samples, corresponding to the removal of molecules of water. Following this, there occurs a sharp decrease in mass (27.6–45.1%) and then a smaller one indicating insufficient decomposition of the complex. The loss of mass in complexes of mixed composition (with added Fe^{3+}) begins only under conditions of higher than usual temperature. Complexes are more stable when a smaller loss of mass occurs. The decomposition of anhydrous complexes is accompanied by an endoeffect in the temperature interval 300–700°C. The nature of this effect is somewhat complicated. It may be explained by the decomposition of anhydrous complexes, the oxidation of volatile products produced as a result of the composition of the complex, or by the property of the precipitate of protein substances. Where the addition of Fe^{3+} has been made, the complex decomposes following dehydration due to oxidation of the organic ligand. By examining the TG curve (see Fig. 1d) it can be seen that heating is accompanied by a gradual loss of mass in the sample. In some cases, no change in mass occurs at all, which can be explained by internal rearrangement and reconstruction of the complex. Data on the derivatograms of nonfiltered, control and test beers reveal a sharp distinction between their qualitative characteristics and their endoeffects (Tables 4, 5).

The introduction of Fe^{3+} ions into the nonfiltered, control and test beer samples alters the character of the derivatograms. A comparison of positions 1b and 1d in the figure reveals the difference in behavior of these systems. The second endoeffect takes place at a higher temperature than the first. With the introduction of Fe^{3+}, the number of endoeffects increases to 3 or 4.

According to Gorinstein (1973), beer stability depends mainly on the content of the cation forms of iron, copper and other microelements capable of forming compounds with different protein fractions. Increasing the concentration of these microelements negatively affects beer stability (Gorinstein, 1973). In a

Table 3—Mineral components in protein fractions of nonfiltered beer

Trace elements			Protein fractions				Complexing ability (%)
			Aqueous	Salt	Alkaline	Alcoholic	
Iron	Total amt ($X10^{-3}$ mg/ℓ)	16.0	4.16	15.46	105.49	28.14	–
	% of total	–	2.53	9.62	65.68	17.49	95.22
Copper	Total amt ($X10^{-3}$ mg/ℓ)	39.21	7.08	1.05	27.16	–	–
	% of total	–	18.04	2.54	69.29	–	89.87
Zinc	Total amt ($X10^{-3}$ mg/ℓ)	24.83	0.75	–	–	–	–
	% of total	–	3.00	–	–	–	3.0
Nickel	Total amt ($X10^{-3}$ mg/ℓ)	15.94	1.15	–	–	–	–
	% of total	–	7.25				7.25
Cobalt	Total amt ($X10^{-3}$ mg/ℓ)	9.92	0.40	–	–	–	–
	% of total	–	4.15	–	–	–	4.15
Manganese	Total amt ($X10^{-3}$ mg/ℓ)	8.47	0.19	–	–	–	–
	% of total	–	2.24	–	–	–	2.24
Calcium	Total amt (mg/ℓ)	26.00	0.59	–	–	–	–
	% of total	–	2.26	–	–	–	2.26
Oxalate-ion	Total amt (mg/ℓ)	24.91	–	0.75	–	0.50	–
	% of total	–	–	3.00	–	2.00	5.0

Table 4—Result of the differential thermal analysis

Indices	Samples of beer					
	Non-filtered	Test	Control	Non-filtered with Fe^{3+}	Control with Fe^{3+}	Test with Fe^{3+}
Endoeffects (°C)	180	230	240	210	230	240
	320	440	410	340	290	290
				380	350	360
					410	410

Table 5—Loss in substance weight of metal-protein complexes by thermogravimetric analysis

Indices	Samples of beer					
	Non-filtered	Test	Control	Non-filtered with Fe^{3+}	Control with Fe^{3+}	Test with Fe^{3+}
Loss in substance weight (%)	13.3	9.7	17.9	11.9	12.3	12.9
	45.1	43.3	38.3	27.6	36.0	36.0
				24.9	4.0	4.9
					14.7	14.7

series of experiments, standard solutions of cations of bivalent copper, iron, zinc, nickel, cobalt, manganese, trivalent iron, calcium and oxalate-ion, with C = 10^{-3} mg/100 ml, were introduced into samples of control beer. The dependence of the colloidal stability of this beer on some of the heavy metals contained in it was investigated. Upon introduction of copper ions in the amount of $(0.5-1.0) \times 10^{-3}$ beer. It reflects the dehydration process mg/100 ml, the deposition limit of beer increased. This is because copper cations in small amounts are stabilizers. A further increase of copper concentration from $(1.0-7.0) \times 10^{-3}$ mg/100 ml sharply decreased the deposition limit, because of the breakdown in stability of the colloidal systems. The addition of bivalent iron, cobalt, nickel, zinc and manganese did not greatly affect the colloidal stability of beer, while trivalent iron in concentrations of $(3.5-7.0) \times 10^{-3}$ mg/100 ml sharply decreased it. This confirms the supposition that free Fe^{3+} ions are less active than those of Fe^{2+}. When calcium and the oxalate-ion were added in concentrations of 1.5×10^{-3} and 10.0×10^{-3} mg/100 ml respectively, the colloidal stability decreased.

CONCLUSION

THE PRESENT investigation of clarified samples of beer has shown that the minor inorganic components of beer (iron, copper, zinc, nickel, cobalt and manganese) are bonded with the same protein fractions as are its major inorganic components (calcium and oxalate-ion), even after filtration. It was also found that increasing the concentrations of oxidized iron, copper, oxalate-ion and calcium, decreases the colloidal stability of beer.

The first endoeffect noted in the derivatograms from the test beer occurs at higher temperatures than with nonfiltered taking place in the isolated metal-protein complexes. The second endoeffect takes place at an even higher temperature. With the introduction of Fe^{3+}, the number of endoeffects increases to 3 or 4.

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