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A Thermogravimetric Study of the Stability under Heat of Iron–Protein Complexes

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Iron–protein complexes were investigated by methods of differential gravimetric and differential thermal analyses. Studying the thermal stability has shown that, under heating, these complexes undergo two basic stages of thermal dissociation: dehydration (180–240°) and decomposition of the dehydrated complex (300–700°). Differences in heat dissociation of metal–protein complexes show the varying stabilities of their chemical bonds. It was also found that an additional introduction of ferric ion (Fe^{3+}) decreases the stability of iron–protein complexes.

One of the prime indicators of beer quality is its colloid-protein stability (Badgley, 1972; Stage, 1972; Steiner, 1972; Schildbach, 1971; Narziss and Roettger, 1973). This depends on the amount of metal–protein complexes in aqueous ethanol medium. Information on the concentration of metal-protein complexes in ethanol media is scarce in the scientific literature (Clapperton, 1971; Stone, 1972; Lundin, 1963; Djurtoft, 1962). In previous works (Fertman and Gorinstein, 1970; Gorinstein, 1973a, b), it was proven that the strength of the bonds between microelements and proteins is measured by the ratio of their quantity in protein fractions to their total content in beer. The data have shown that the most common complexing agents are elements of the eighth group (iron) of the Periodic Table of the Elements (Bagger, 1969; Davies *et al.*, 1969; Gorinstein, 1973a).

In order to study the differences in composition between complexes with and without Fe^{3+}, we added Fe^{3+} at a concentration of 3.5×10^{-3} mg/l. The deposition limit of beer (*i.e.*, its stability) sharply decreases as the Fe^{3+} concentration increases (Gorinstein, 1973b). By using the above concentration, a sediment was formed in the beer.

In this study, we have undertaken to isolate the iron-protein complexes, establish their change in composition by heat dissociation, and study their thermal stabilities.

MATERIALS AND METHODS

The investigation was carried out on "Zhiguli" nonfiltered beer, produced at Lvov Brewery Firm "Kolos," 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. The Kineshma mass is of 34 nephelos units, and the Evlakh of 55 nephelos units. The two are distinguished by their filtering abilities.)

The stability of iron-protein complexes was determined thermochemically (Paulik *et al.*, 1958; Belcher *et al.*, 1960; Keattch, 1967; Gorinstein, 1974). Proteins were concentrated by tannin–caffeine and ammonium sulfate (for details see Fertman and Gorinstein, 1968). The sediment was dried at 30°. Their thermal stability was studied by the thermogravimetric method using the Paulik-Paulik-Erdey derivatograph (Paulik *et al.*, 1958). Four curves were recorded simultaneously on the derivatograph and are presented in Figures 1-4. Curve 1 on all the figures in positions A and C is the curve of differential thermogravimetric analysis, DTG; curve 2 is the curve of differential thermal analysis, DTA; curve 3 is the curve of temperature, *T*; curve 4 on positions B and D is the curve of integral thermogravimetric analysis, TG. Points a-h are the sites of the endothermic effects of substance weight loss at varying temperatures. The investigated substance and the standard—aluminum oxide repeatedly heated—were heated in a platinum crucible. The conditions of the experiment are as follows: weight of substance, 100 mg; thermopair, Pt-Pt/RH; resistance of electric circuit, DTA, 0.1; DTG, 0.1 megohm; rate of heating, 10°/min; range of error of temperature, ±5°. Twelve samples of iron–protein complexes in beer were investigated.

RESULTS AND DISCUSSION

Investigation of iron–protein complexes by the thermogravimetric method has shown that in the temperature interval of 180–240°, one or two endothermic effects of dehydration take place on the DTG and TG curves of all the samples of beer (see Figures 1-3). The nature of the complex is dependent on the radius and the electrical charge of the heavy metals (Fe, Cu, etc.), and dependent on them, in turn, is the temperature of hydration (Kapitonova *et al.*, 1971; Caldin, 1972; Krestov and Kurakina, 1973).

The first endo effect exists in each of the derivatograms presented in Figures 1-3. In the test beer, however, a higher temperature is found than in the other samples. A quantitative interpretation of the different thermoanalysis curves is presented in Table I.

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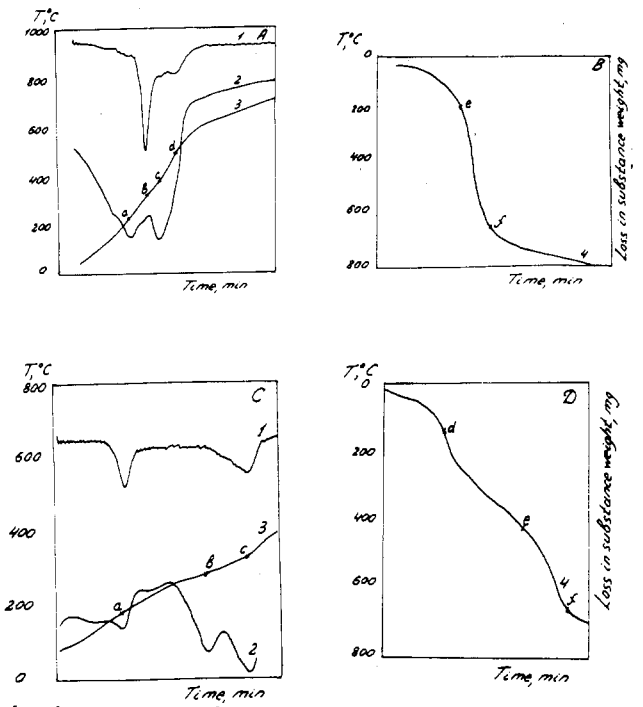

Figure 1. Derivatograms of metal–protein complexes of samples of nonfiltered beer (A,B) without added Fe^{3+} and (C,D) with added Fe^{3+}. The curves of heating: (1) DTG; (2) DTA; (3) T; and (4) TG.

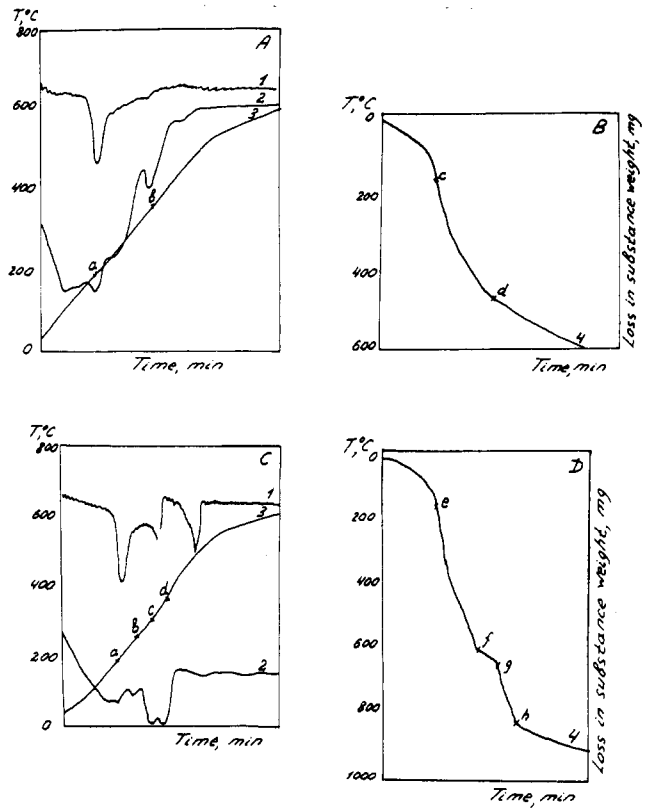

Figure 2. Derivatograms of metal–protein complexes of samples of control beer (A,B) without added Fe^{3+} and (C,D) with added Fe^{3+}.

The loss of mass of the complex in the TG curve of all figures is attributed to the removal of water. In the beginning, loss of mass from 9.7 to 13.3% occurs in the different samples, corresponding to the removal of weakly bound water. Following this, there occurs a sharp decrease in mass (27.6–45.1%) and then a smaller one, the latter indicating that complete decomposition had not previously occurred. 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.

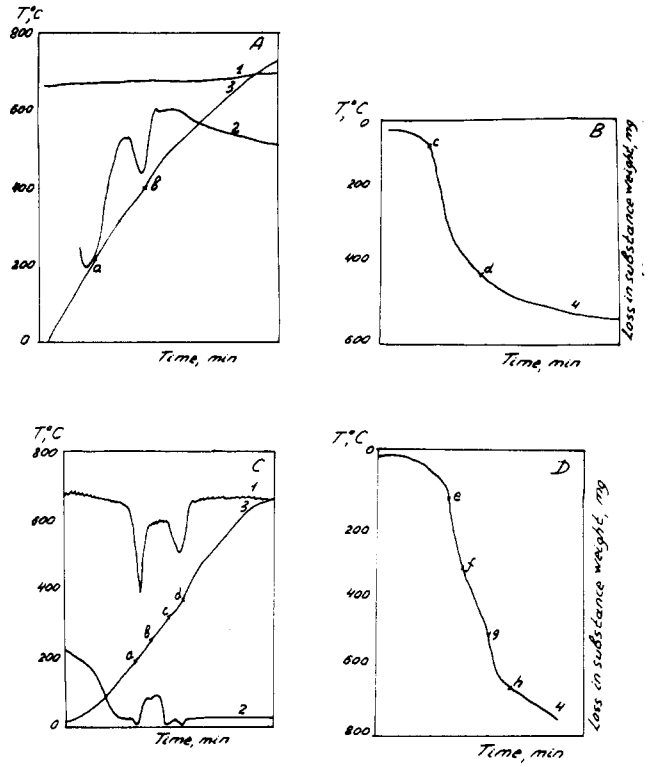

Figure 3. Derivatograms of metal–protein complexes of samples of test beer (A,B) without added Fe^{3+} and (C,D) with added Fe^{3+}.

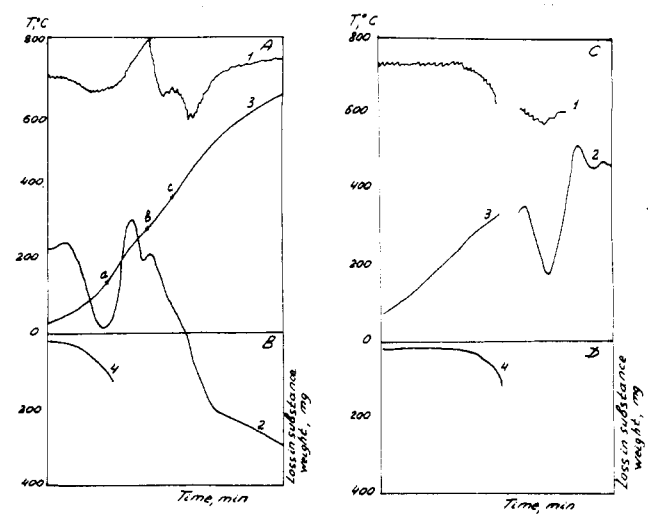

Figure 4. Derivatogram of a sample of test beer with tannin–caffeine (A,B) and derivatogram of ammonium sulfate (C,D).

The decomposition of anhydrous complexes is accompanied by an endo effect in the temperature interval 300–700°. The nature of this effect is somewhat complicated. It may be explained by the decomposition of anhydrous complexes, the oxidation of volatile substances produced as a result of the composition of the complex, or by the endothermic conformational change. As heat is applied, the complexes begin to decompose and a gradual destruction of the remaining molecules takes place upon further application of heat. This destruction is devoid of any special energetic effects. The weak endo effect occurring at

Table I. Results of Thermal and Thermogravimetric Analyses

Samples of beer	Thermal anal. (T effect,°C)	Thermogravimetric anal. % loss of mass	Thermogravimetric anal. T,°C
Nonfiltered	160–180 (–)	13.3	180
	300–320 (–)	45.1	320
Control	224–240 (–)	17.9	240
	400–410 (–)	38.3	410
Test	207–230 (–)	9.7	230
	432–440 (–)	43.3	440
Nonfiltered with Fe^{3+}	203–210 (–)	11.9	210
	328–340 (–)	27.6	340
	365–380 (–)	24.9	380
Control with Fe^{3+}	223–230 (–)	12.3	230
	280–290 (–)	36.0	290
	345–350 (–)	4.0	350
	401–410 (–)	14.7	410
Test with Fe^{3+}	237–240 (–)	12.9	240
	275–290 (–)	36.0	290
	348–360 (–)	4.9	360
	405–410 (–)	14.7	410
Test with tannin	80–100 (–)		
	330–350 (+)		
	230–250 (–)		

[a] A minus sign indicates an endo effect, and a plus sign an exo effect.

the beginning of the isolation of the ligand of the complex coincides with the sharp endo effect taking place during the breakdown of the bond between the complex ligand and the metal ion. Where the addition of Fe^{3+} has been made, the complex decomposes following dehydration. According to the endo effects shown in Figures 1–3, positions C and D, there is a change in composition of the metal-protein complexes due, perhaps, to oxidation of the organic ligand of amino acids. 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 (or nothing at all happening, because of stability).

Data on the derivatograms of nonfiltered, control, and test beer reveal a sharp distinction between their qualitative characteristics and their endo effects (for nonfiltered, control, and test beer without added Fe^{3+} and precipitated by ammonium sulfate, and for the same samples precipitated by tannin). The introduction of Fe^{3+} ions into the nonfiltered, control, and test beer samples alters the character of the derivatograms (for nonfiltered, control, and test beer with Fe^{3+} added and precipitated by ammonium sulfate and the same samples precipitated by tannin). A comparison of positions A,B and C,D in the figures reveals the differences in behavior of these systems.

The second endo effect takes place at a higher temperature than the first. With the introduction of Fe^{3+}, the number of endo effects increases to 3 or 4.

In derivatograms of samples of nonfiltered, control, and test beer with added Fe^{3+}, and precipitated by ammonium sulfate and nonfiltered beer without added Fe^{3+} precipitated by tannin (Figure 4), the substance containing the metal-protein complexes expands and ignites at a temperature near 330°. This behavior can be understood in terms of the nature of the substance. The exothermic effect of the derivatograms in this figure is accompanied by separation of the volatile substances (Haukeli *et al.*, 1973; Postel *et al.*, 1972). Under oxidation of volatile organic products, a small exothermic effect occurs at temperatures of 120, 350, and 630°, which are shown in Figures 3 and 4. The break which exists in the curves of samples of ammonium sulfate, tannin, and iron(III) chloride could not be investigated because of the evaporation which occurred under heating. The derivatograms reveal the breakage in these curves (Figure 4C,D).

CONCLUSION

By thermogravimetric analysis, a comparison of the stabilities of metal-protein complexes with and without added Fe^{3+} was made. It was shown that an additional introduction of Fe^{3+} decreases the colloidal stability in ethanol media, and that the process of thermal dissociation is determined by the nature and behavior of the iron-protein complexes. From our experiments it may be assumed that heat dissociation in isolated iron-protein complexes occurs in two stages: dehydration and gradual decomposition of the dehydrated complexes, followed by separation of the volatile substances. The investigation made use of a simple and reliable method from which knowledge can be gained concerning the structure and composition of metal-protein complexes.

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