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Polarographic Investigation of Proteins in the Brewing Process

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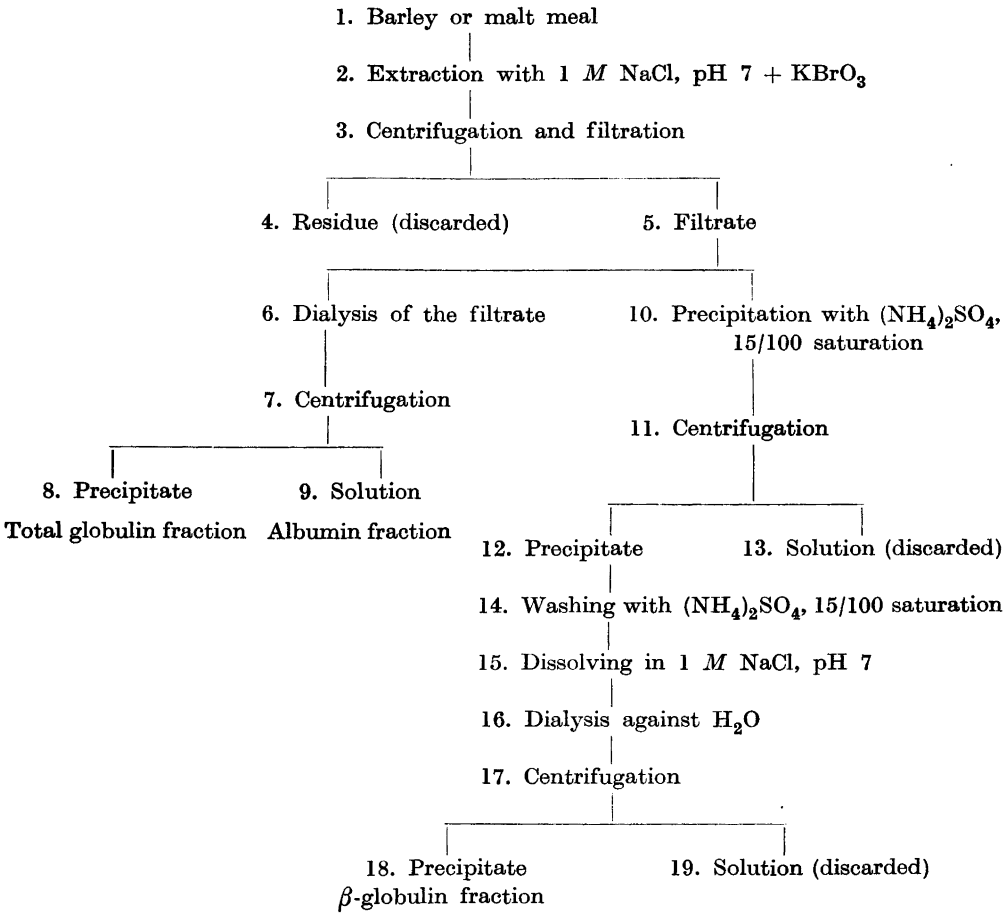
The proteins in beer have a great influence on its chemical stability. Together with tannin, they constitute the hazes formed when beer is chilled or oxidized. Hartong [1] and Helm [2] have pointed out that sulphur plays an important part in the formation of these hazes. We have tried to find a convenient method to study proteins in the brewing process, especially with respect to sulphur in proteins. The polarographic method, applied to the study of proteins by Brdička [3–5], seemed to be suitable for this purpose. Brdicka found that in certain solutions of cobalt ions, proteins give rise to a characteristic polarographic 'double wave', the height of which varies with the concentration of the proteins. He has shown that the effect observed is caused by sulphydryl and disulphidic groups in the protein molecules, and has given an extensive review of protein polarography [6].

The investigations described in this paper have been performed on proteins in barley, malt, wort, and beer. The proteins from barley and malt were divided into three fractions: albumin, total globulins and β-globulin, by methods described below. The different fractions were studied separately. Only albumin fractions were obtained from wort and beer. Two different varieties of malting barleys, Kenia and Heimdal, grown in 1947, were investigated. Some measurements were repeated on barley grown in 1948.

The main objectives of these investigations were to find out if some characteristic differences existed in the polarographic effects of the different protein fractions, and if these effects were influenced by the different stages in the brewing process.

PROTEINS FROM BARLEY

The following scheme of preparation, which is a modification of the schemes given by Quensel [7] and Danielsson and Sandegren [8], has been used to separate the protein fractions from barley. The same scheme has been used for malt.



100 g of barley or malt meal were extracted with 800 ml 1 *M* NaCl, pH 7.0, to which solution had been added 0.1 % of $KBrO_3$ to inactivate the proteolytic enzymes. All dialyses were made at 4° C. The amount of total nitrogen in the different protein fractions was determined by the micro Kjeldahl method. The protein content was obtained from the nitrogen content by multiplying by the factor 6.25. Some of the albumin and β-globulin fractions were in-

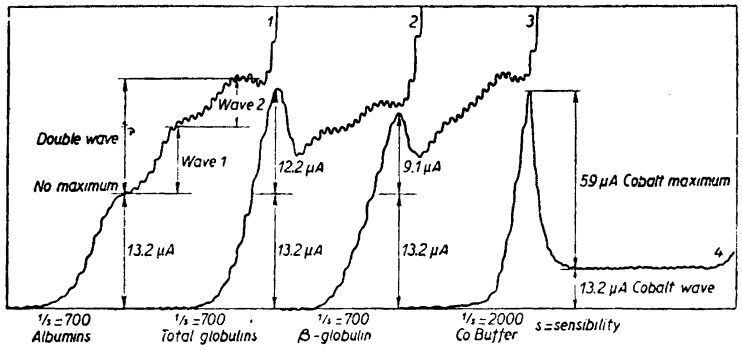


Fig. 1. Polarograms of malt protein fractions. Protein concentration 10 γ/ml.

vestigated by electrophoresis, and it was found that the amount of foreign protein components was very low. The 'total globulin' fraction contained a mixture of four globulin components [cf. 7]. Table 1 shows the approximate percentage distribution of the protein fractions in different barleys. These figures represent, not only the two barley varieties mentioned above, but a greater number of varieties grown in different years and in different places in Sweden.

Table 1. Composition of barley proteins.

Fraction	N, per cent of total N
Albumin	6.0—14.4
Total globulins	2.8—11.0
β-globulin	0.7— 5.0

The polarographic experiments were carried out as follows. The polarograph used was manufactured by Radiometer, Copenhagen, Denmark. The diameter of the cathode capillary was 0.05 mm, and the drop-time 4.1 sec. in distilled water when no e. m. f. was applied. The anode consisted of a saturated calomel electrode. The concentration of the test solution was 4×10^{-3} *N* $CoCl_2$, 0.2 *N* NH_4Cl, 0.2 *N* NH_4OH, and 5—240 γ protein per ml. The total volume of the test solution was 10 ml. The determinations were made at 25° C. The results were generally reproducible within 5 %.

Fig. 1 shows the type of curves obtained. At higher protein concentrations, the waves become less pronounced. At concentrations over 240 γ/ml, it is impossible to distinguish and measure the waves. The albumin fractions give more pronounced waves than the globulins, the curves of which are more

drawn out. When no proteins are present in the test solution, a sharp maximum appears in the cobalt wave before the diffusion current is reached (Fig. 1, curve 4). This maximum can be suppressed by adding small amounts of surface active agents to the solution. The maximum decreases in a given manner, with decreasing surface tension [9]. The results of the measurements of cobalt maxima are given in Table 2. The table shows that the albumin fraction has a considerably greater suppressing effect than the other two fractions. Thus, the albumin is the most surface active of the protein components. The results reported are from the Kenia variety. The Heimdal variety gave essentially the same results.

*Table 2. Suppressing effect of the protein fractions on the cobalt maximum.**

Origin	Concentration of proteins, γ/ml	Height of cobalt maximum, μA					
		Albumin		Total globulins		β-globulin	
Barley	5	0	0	(24)	(19)	(16)	(17)
»	10	0	0	12.0	12.7	9.1	8.8
Malt	5	4.3	4.0	(19)	(19)	(18)	(18)
»	10	0	0	12.2	12.7	9.1	9.6
Wort	5	0	0	—	—	—	—
»	10	0	0	—	—	—	—
Beer	5	0	0	—	—	—	—
»	10	0	0	—	—	—	—

Another important difference between the protein fractions was found when the heights of the polarographic 'double waves' were compared. Each fraction was electrolyzed in concentrations of 5, 10, 20, 40, and 80 γ/ml, and the wave height plotted against the concentration. A typical diagram is given in Fig. 2. The β-globulin always gave a considerably higher wave than the albumin at the same concentration. This is shown in Table 3, which contains the wave heights at a concentration of 80 γ/ml. The activity of the total globulin fraction is somewhat varying. It generally takes an intermediate position between the other two fractions in the range of concentration just mentioned.

* The values in brackets had to be approximately extrapolated, because the cobalt maxima ended outside the polarographic paper. The heights of the cobalt maxima are given in microampéres. From the current corresponding to the top of the maximum is subtracted the cobalt diffusion current obtained from pure cobalt buffer solutions.

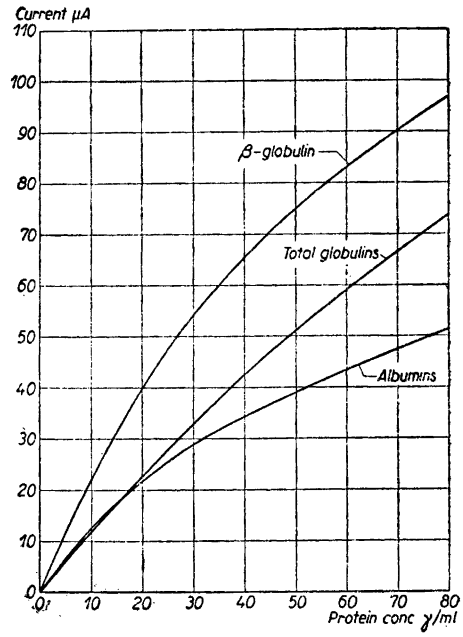


Fig. 2. Variation in the height of the protein 'double wave' with the concentration of proteins from Kenia barley.

*Table 3. Height of protein 'double wave' from different barley protein fractions. Concentration of protein 80 γ/ml.**

Origin	Height of 'double wave', μA		
	Albumin	Total globulins	β-globulin
Kenia, 1947	51.5	73.5	97.0
Heimdal, 1947	48.5	64.5	70.5
Kenia, 1948	46.5	77.0	77.5
Heimdal, 1948	42.0	42.0	53.5

It is evident from the table that the globulins from Kenia barley, especially the β-globulin, are polarographically more active than those of Heimdal barley.

PROTEINS FROM MALT

Part of the barley samples were malted in stockings in malting drums (of the Topf type) at a temperature of 17° C for 7 days. The kilning was

* In the analyses of barley from 1948, 0.1 *N* NH_4Cl and 0.1 *N* NH_4OH have been used in stead of 0.2 *N* (*cf.* page 1029).

performed as for Pilsner malt. The malt proteins were then extracted and fractionated in the same way as the barley proteins.

The surface activity of the malt protein fractions seems to be almost the same as for the barley protein fractions (Table 2).

With respect to polarographic activity, no characteristic differences were observed between the malt protein fractions. Comparison with the proteins of barley, shows that the activity of the albumin is increased about 30 % by malting (Table 4). The activity of the total globulins and the β-globulin is the same as in barley, or in the latter case somewhat lower.

Table 4. Influence of the different stages in the brewing process on the polarographic activity of the albumin fraction. Concentration 80 γ/ml.

Origin	Height of albumin 'double wave', μA			
	Barley	Malt	Wort	Beer
Kenia, 1947	51.5	68.5	56.0	38.5
Heimdal, 1947	48.5	64.0	45.5	35.5

PROTEINS FROM WORT AND BEER

The malt was mashed by the 'Congress method' for extract determinations in malt adopted in Salzburg in 1930. The wort was then boiled for one hour with hops (2.5 g per liter) and filtered. Part of the clear filtrate was dialysed against water. No precipitate appeared, which indicated that no appreciable amount of globulins was present. The dialysed wort, containing only high-molecular water soluble nitrogen compounds, has been called the 'albumin fraction', although the water soluble nitrogen compounds do not consist of the original albumin from malt. They originate from all three malt protein fractions, which are split into less high molecular compounds during mashing and wort boiling. The hopped wort was fermented with bottom fermenting yeast. After filtration, the beer was dialysed. The 'albumin fraction' (water soluble nondialysable nitrogen compounds) was then investigated.

From Table 2 it may be seen that the surface activity of the 'albumin fractions' from wort and beer equals that of barley albumin.

Table 4 shows that mashing, wort boiling, and fermentation decrease the polarographic activity of the 'albumin fraction'. The activity of wort 'albumin' is about the same as that of barley albumin.

DISCUSSION

According to the investigations described above, the albumin of barley is considerably more surface active than the globulin fractions. This agrees with the results obtained by Sandegren and Säverborn [10] during investigations of surface films on water solutions of these substances. Furthermore the albumin fraction retains its surface activity during the brewing process. This has, of course, a great significance for the foam quality of the beer.

It was found that the β-globulin of barley and malt has a higher polarographic activity than the other two fractions. Consequently, it contains greater amounts of reactive sulphydryl and disulphidic groups. It does in fact, contain a higher percentage of sulphur than the other fractions [11]. Thus it seems probable that the degradation products of β-globulin are more rapidly oxidized and precipitated than the other proteins in beer. This conclusion agrees with results from investigations of the chill haze substance. It was found that the protein component of this substance probably originated from the β-globulin of barley [11]. The conclusion is further supported by the fact that proteins in beer, freed from chill haze, have a somewhat lower polarographic activity than proteins in ordinary beer.

The polarographic activity of malt albumin was shown to be about 30 % higher than that of barley albumin. Danielsson and Sandegren [8] have shown that the β-amylase activity of barley and malt is connected with the albumin fraction. They also found that the malt albumin has a considerably higher β-amylase activity than barley albumin. Weill [12] has shown that the β-amylase activity is probably due to prosthetic sulphydryl groups. The fact that the β-amylase activity, as well as the polarographic activity, of the albumin is increased during malting seems to confirm this theory.

SUMMARY

1. The polarographic method has been applied to the investigation of proteins in the brewing process, and found to be very useful in this field.

2. The albumin fraction has the strongest suppressive effect on the cobalt maximum, and thus the highest surface activity of the protein fractions of barley and malt.

3. The β-globulin has the highest polarographic activity of the protein fractions of barley and malt.

4. The polarographic activity of the albumin fraction has been shown to increase during malting and then decrease during mashing, wort boiling, and fermentation.

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