Beer Hazes. II. Further Analyses of Basic Components by High Performance Liquid Chromatography[1]

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ABSTRACT

Hazes 1–4 and phenolic concentrates 5–8 were prepared from ale and lager. Lager Hazes 2 and 4 were then treated with acid and alkali buffers to prepare Samples 9–12. Hydrolysates of lager Hazes 1–4 (Samples 13–16) were also prepared to study the behavior of phenolic components. The hazes and their derivatives were subjected to analytical procedures for polyphenols, carbohydrates, and protein. High performance liquid chromatography (HPLC) was performed on a Microbondapak C_{18} column to identify and quantify phenolic monomers and on an Aminex HPX-87 column to determine fermentable and nonfermentable carbohydrates. Quantification was performed with the aid of appropriate reference compounds. Molecular weight distribution of haze components was determined using marker compounds by HPLC on coupled protein columns I-125 and I-250. Significant changes in the composition of fresh beer hazes as compared to those of aged and forced beer hazes were observed. Aging or forcing of beer led to increased tanninogen contents in beer hazes. The total carbohydrate content (by HPLC) increased from fresh to aged to forced beer hazes for both ale and lager. The protein content (by micro-Kjeldahl) dropped severely in forced lager haze as compared to that in fresh and aged lager hazes. Low-molecular weight components were much more abundant in forced lager haze than in fresh or aged lager hazes.

Key words: *Ale hazes, Aminex HPX-87, Buffer extracts, Hydrolysates, Lager hazes, Microbondapak C_{18}, Protein columns I-25 and I-250*

In work reported in an earlier article (5), hazes were isolated from an ale and their phenolic concentrates were prepared. These samples were then subjected to high performance liquid chromatography (HPLC) for the analyses of phenolic monomers (PM) and carbohydrates. In the present study, analogous samples (Samples 1–8) were prepared from both lager and ale. Additionally, acidic and alkaline extracts were prepared from fresh lager haze (Samples 9 and 10) and from forced lager haze (Samples 11 and 12). Hydrolysates of lager Hazes 1–4 (Samples 13–16) were also hazes and their derivatives were then subjected to the following analyses: tanninogens (anthocyanogens and catechins) (4), "reducing sugars" by dinitrosalicylic acid (DNS) method (1), and protein by Kjeldahl (9). PM were determined by HPLC on Microbondapak C_{18} and carbohydrates on an Aminex HPX-97 column as described earlier (5). Molecular weight distribution of nitrogenous components was determined by HPLC on coupled protein columns I-125 and I-250 with the aid of reference marker compounds. This type of HPLC, applied to Samples 9–12, allowed us to determine that acidic proteins were dominant in beer hazes. The HPLC of the hydrolysates, performed under the conditions used for PM determination, showed several strong peaks in the visible spectrum (436 nm), apparently related to catechin-derived products (2). Significant differences found in the composition of fresh beer hazes, as compared to those of aged and forced beer hazes, may help to elucidate the dynamics of beer aging. Analytical results for lager-derived Hazes 1–4 and their derivatives were compared with those for the analogous ale-derived samples, and important differences were found.

EXPERIMENTAL

Equipment

This work involved centrifugation and freeze-drying to prepare the samples and HPLC for their analyses. Our laboratory equipment consisted of a centrifuge (International, model B-20A), a freeze-drier (New Brunswick Scientific), and an HPLC system made of the following units (all Waters): injector U6K; WISP (Waters Intelligent Sample Processor) 710-A; pumps 6000A and M-45; detector, absorbance model 440, and differential refractomonitor R 401. Flow and solvent gradients were done using a System Controller, model 720. Also used were a recorder and integration system data module, model 730; Microbondapak C_{18} (Waters) and Aminex HPX-87 (Bio-rad) column packings; and protein columns I-125 and I-250 (Waters). Reference compounds were of reagent grade and eluents of chromatographic grade. Infrared spectra were recorded on a Perkin-Elmer 521 spectrophotometer.

Preparation of Ale Samples

Ale Hazes (1–4) and their phenolic concentrates (Samples 5–8) were prepared as described (5).

Preparation of Lager Samples

Haze 1. This haze was isolated from fresh lager by centrifugation at 11,000 rpm and processed as described earlier (5) to give a fluffy grayish solid (average yield: 5 mg/L).

Haze 2. The water-soluble portion of Haze 1 was prepared analogously to Haze 2 obtained from ale (5). It gave a fluffy beige solid (average yield: 92%).

Haze 3. This was isolated from a three-month-old lager (stored at room temperature) as described for the analogous ale haze (5). The water-soluble portion was a fluffy beige solid (average yield: 8.5 mg/L).

Haze 4. This was obtained from lager beer forced at 60°C for 72 hr as described for ale (5). The water-soluble portion was a brownish solid (average yield: 32.3 mg/L).

Samples 5–8. These samples, analogous to ale Preparations 5–8 (5), were prepared in the same way from lager Hazes 1–4, respectively (average yield: 25%).

Sample 9. Lager Haze 1 (100 mg) was stirred with 100 ml of citric acid-sodium citrate buffer, pH 3, for 1 hr and centrifuged at 10,000 rpm at room temperature for 15 min. The supernatant was freeze-dried and then dried in a vacuum-desiccator to constant weight. An ignition test was positive, showing the presence of inorganic salts. The preparation was treated with ethyl acetate (3 × 50 ml). The extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure at room temperature. Drying in a vacuum desiccator yielded a sticky yellowish solid (20 mg).

Sample 10. The centrifugate, after the isolation of Sample 9, was stirred with 100 ml of an alkaline buffer (boric acid-borax), pH 8.6, for 1 hr and centrifuged as described. The supernatant was freeze-dried and then dried in a vacuum desiccator to constant weight. An ignition test was strongly positive. Consequently, the product was treated with ethyl acetate (as described) to give a fluffy white solid (22.6 mg).

Sample 11. This was prepared from Haze 4 (100 mg). Preparation was analogous to that of Sample 9. The ignition test was positive and the product was treated with ethyl tate to leave a yellowish sticky solid (76.1 mg).

Sample 12. This was prepared, analogously to Sample 10, from the centrifugate after the isolation of Sample 11. The ignition test was positive and the treatment with ethyl acetate yielded a fluffy white solid (22.7 mg).

Samples 13–16. These were prepared from Hazes 1–4, respectively. Haze (50 mg) was refluxed overnight with 40 ml of

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dilute HCl (1:1). The product was concentrated under reduced pressure at about 60°C until no more HCl evolved. A brownish oil was obtained.

Analyses

Turbidity was determined "as is," as formazin turbidity units (FTU) (1FTU = 0.014 EBC units) and as forced FTU.

Tanninogens (anthocyanogens and catechins) were analyzed according to Dadic (4). Color tests on phenolics (vanillin, Prussian blue, and *p*-toluenesulfonic acid) were conducted as described earlier (3). "Reducing carbohydrates" were analyzed using the DNS method (1). Absorbance was read at 540 nm and the calibration curve was prepared with glucose. The nitrogen content was determined using the micro-Kjeldahl method (9), and the protein content was calculated using a factor of 6.25. Infrared spectra were taken using KBr pellets.

HPLC

Phenolics on Microbondapak C_{18}. Lager and ale samples 1–16 were dissolved in 10% aqueous 2-propanol and clarified by filtration through a Nucleopore filter (1 μm). Concentration of the samples varied from 20 to 85 μg/10 μl.

All samples were then subjected to HPLC with the same reference phenolics as before (5). HPLC conditions were: column, 30 cm × 3.9 mm, linear gradient elution (curve 6); solvent A, H_2O/propionic acid (1,000:10); solvent B, H_2O/ethanol (50:50). The gradient was: initially, 100% solvent A; after 25 min, 50% solvent B in solvent A; after 40 min, 70% solvent B in solvent A; after 50 min, 100% solvent A (flow rate, 2 ml/min; pressure, 2,000 psi). Chart speed was 0.5 cm/min. Detection (absorbance model) was conducted at 254, 280, 313, 340, 365, 405, 436, and 546 nm.

Quantification of the individual PM in hazes and their derivatives was done by determining the response factor for each compound in the calibration mixture of 16 reference phenolics and feeding these factors to the data module memory. The recovery of the injected calibration mixture was 102%.

Carbohydrates on Aminex HPX-87. Hazes 1–4 and lager Samples 9–12 were dissolved in 10% aqueous 2-propanol and the HPLC analyses performed as for ale hazes (5).

Molecular Weight Distribution of Components. Hazes 1–4 and lager Samples 9–12 were dissolved in 10% aqueous 2-propanol and the HPLC analyses performed on two I-125 and two I-250 protein columns, joined in series. Elution was done using a phosphate buffer (0.2*M* monobasic sodium phosphate + 0.2*M* dibasic sodium phosphate) containing 30% acetonitrile, pH 7.1. A decreasing flow gradient of 1 ml/min to 0.5 ml/min over 40 min was applied isocratically. Chart speed was 0.5 cm/min. Detection (absorbance model) was conducted at 280 nm. Reference compounds were chromatographed under the same conditions, serving as molecular weight distribution markers. In decreasing order of molecular weight (the higher molecular weight compounds elute first), they were: ferritin (Sigma Chem. Co., St. Louis, MO), mol wt 540,000, R_t 20.6 min; bovine albumin (J. T. Baker Co., Phillisburg, NJ), mol wt 67,000, R_t 23.4 min; peroxidase (Worthington Chem. Corp., Freehold, NJ), mol wt 40,000, R_t 26.1 min; cytochrome C (National Biochemical Co., Cleveland, OH), mol wt 13,000, R_t 34.2 min; NADH disodium salt (Boehringer Mannheim GmBH, W. Germany), mol wt 745.4, R_t 38.0 min; and tyrosine (Matheson Coleman & Bell, Norwood, OH) mol wt 181, R_t 31.8 min.

TABLE I
Anthocyanogens and Catechins (Tanninogens) in Lager Hazes 1-4

Sample	AV[a] (%)	CV[b] (%)	TV[c] (%)
1	1.7	9.7	11.4
2	1.6	9.6	11.2
3	2.8	18.0	20.8
4	2.3	15.1	17.4

[a]Anthocyanogen value.
[b]Catechin value.
[c]Tanninogen value (= AV + CV).

RESULTS AND DISCUSSION

Lager yielded significantly lower amounts of hazes (Samples 1–4) than ale did in the earlier study (5). This was particularly true for the forced beer haze, which was over five times as abundant for ale as for lager. This corresponded well with lower turbidity in lager than in ale. Only a small portion of the total PM passed from beer into hazes (for both ale and lager). However, anthocyanogens and catechins, proteins (polypeptides), and carbohydrates were found to be major components of the examined beer hazes.

Infrared spectra[2] confirmed the complex structure of hazes and the presence of major components determined by other means and reported in this work.

Phenolics

Tanninogen (anthocyanogen and catechin) analyses of lager Hazes 1–4 are given in Table I. Flavan-3-ol derivatives, shown by the catechin value (CV), are much more abundant than flavan-3,4-diol derivatives, shown by the anthocyanogen value, in all hazes. This is probably due to a higher sensitivity of anthocyanogens as compared with catechins, particularly concerning oxidation. Chill hazes are grayish-white when freshly isolated but turn brown on oxidation. Interestingly, both anthocyanogens and catechins are most abundant in Haze 3, followed by Haze 4, but least abundant in fresh lager hazes (Samples 1 and 2). The heating factor must be considered in comparing the analyses of Hazes 3 and 4. Aging of beer appears to increase tanninogen values (particularly CV) in hazes isolated from it, compared to the values in hazes isolated from fresh beer.

Samples 5–8 did not contain anthocyanogens or catechins, showing that boiling Hazes 1–4 with 1.25*N* sodium hydroxide for 5 min effectively alters their basic tanninogen structure. The presence

TABLE II
Phenolic Monomers in Lager Hazes 1-4 by High Performance Liquid Chromatography on Microbondapak C_{18} (280 nm)

Peak Number[a]	Compound	R_t (min)	Content in Hazes (mg/kg) 1	2	3	4
1	Gallic acid	4.0	2	2	2	3
2	Protocatechuic acid	6.0	8	10	22	29
3	Gentisic acid	7.2	Tr[b]	Tr	Tr	Tr
4	*p*-Hydroxybenzoic acid	8.4	3	5	10	25
5	D-(+)-Catechin	10.8	60	120	158	162
6	Vanillic acid	11.3	24	40	43	50
7	Caffeic acid	12.4	Tr	Tr	Tr	Tr
8	Chlorogenic acid	12.8	Tr	Tr	Tr	Tr
9	Syringic acid	13.7	6	8	10	13
10	L-(−)-Epicatechin	14.9	55	100	120	130
11	*p*-Coumaric acid	16.7	15	27	27	29
12	Ferulic acid	19.1	157	200	220	225
13	Sinapic acid	20.2	10	18	24	32
14	Trans-o-coumaric acid	21.6	Tr	Tr	Tr	Tr
15	Quercetin	33.0	87	90	112	123
16	Kaempferol	37.6	72	101	168	186
Total						
Amount			499	729	916	1,007
Percent			0.05	0.07	0.09	0.10

[a]Numbers correspond to peak numbers in Figs. 1 and 2.
[b]Traces.

[2]Unpublished data.

of phenolic compounds in these preparations was, however, positively shown by Prussian blue and *p*-toluenesulfonic acid color reactions.

Acidic buffer extracts (Samples 9 and 11) and alkaline buffer extracts (Samples 10 and 12) contained only traces of tanninogens. Haze hydrolysates (Samples 13–16) were not analyzed for tanninogens because these sensitive compounds were obviously altered by the harsh reaction conditions employed.

Table II shows the concentrations of PM in lager Hazes 1–4 (in mg/kg), determined by HPLC of Samples 5–8 with detection at 280 nm. Although very minor components of hazes (up to 0.1% of the total haze weight), these compounds may reveal some characteristic data about the formation and composition of hazes. Thus, the concentration of PM clearly increases in lager hazes as beer is aged either at room temperature or by forcing. The same trend was observed for ale hazes (5). The present experiment with ale Hazes 1–4 showed that total PM concentration in ale hazes is significantly higher than that in lager hazes. The most abundant PM were (in decreasing order): 1) ferulic acid, catechin, kaempferol, epicatechin, quercetin, and vanillic acid in Haze 2; 2) ferulic acid, kaempferol, catechin, epicatechin, quercetin, and vanillic acid in Hazes 3 and 4. Thus, the same PM appear as abundant in all types of hazes examined (with an inversion in order of catechin and kaempferol).

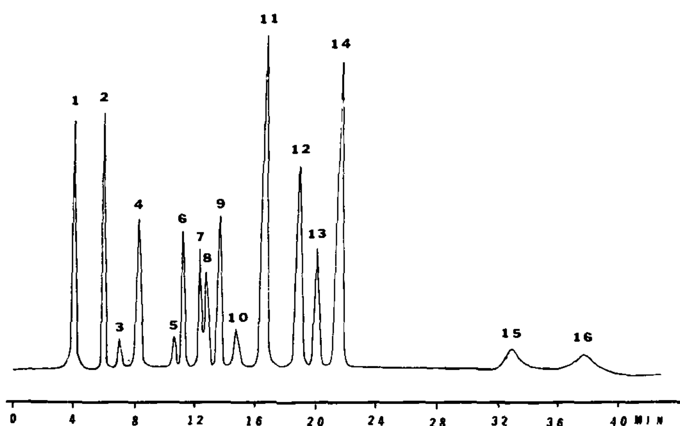

Fig. 1. High performance liquid chromatography (Microbondapak C_{18}) of a cocktail of 16 reference phenolics. Linear gradient elution; detection at 280 nm. Peak numbers are given in Table II.

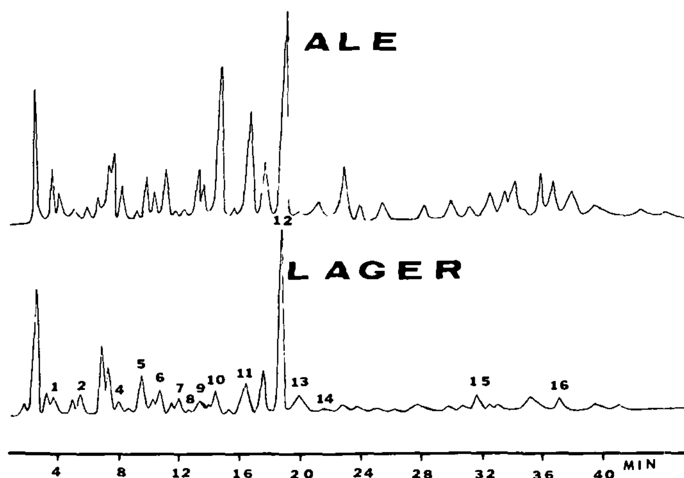

Fig. 2. High performance liquid chromatography (Microbondapak C_{18}) of fresh lager and ale hazes (1 mg and 1.2 mg injected, respectively). Linear gradient elution; detection at 280 nm. Peak numbers are given in Table II.

Sixteen PM were also measured in Samples 9–16, using the same chromatographic procedure. Generally, the concentrations of PM decreased in the following order: phenolic concentrates (Samples 5–8), hydrolysates (Samples 13–16), hazes (Samples 1–4), and buffer extracts (Samples 9–12). The total PM concentrations in hydrolysates were the following: 0.12% (Sample 14), 0.14% (Sample 15), and 0.23% (Sample 16). Thus, the same trend was maintained as for PM concentrations in the original lager hazes. The most abundant PM in the hydrolysates were (in decreasing order): catechin, quercetin, epicatechin, kaempferol, vanillic acid, ferulic acid, and *p*-hydroxybenzoic acid. Consequently, the same PM were important in the hydrolysates as in the original hazes (only the order of abundance was changed).

Figure 1 shows a chromatogram of the calibration mixture of 16 reference phenolics with detection at 280 nm. All compounds were well resolved (peaks 1–16), permitting an accurate determination of the response factors. An ethanolic gradient gives a more stable baseline than the corresponding gradient using methanol (5). Interestingly, flavonols elute significantly later than phenolic acids and catechins.

Figure 2 shows chromatograms of hazes isolated from fresh lager and ale (recorded under the same conditions as for Fig. 1). Although the amount of the ale haze injected (1.2 mg) was only slightly higher than that of the lager haze (1 mg), the PM concentrations are clearly higher in the former haze than in the latter one. The peaks correspond to the phenolics in Fig. 1, which enabled us to quantify their concentrations in hazes. A number of unidentified peaks appeared in the chromatogram of Haze 2 (Fig. 2) as well as in those of other samples (not shown).

Haze hydrolysates (Samples 13–16) absorb both in ultraviolet and in visible spectral regions, whereas the original hazes absorb only in ultraviolet. Thus, Samples 13–16 were chromatographed under the same conditions as the reference phenolics but with detection conducted at various wavelengths (254, 280, 313, 340, 365, 405, 436, and 546 nm). Figure 3 shows a chromatogram of Sample 14 with detection at 436 nm. Similar chromatograms were obtained with D-(+)-catechin and L-(−)-epicatechin subjected to hydrolysis under the conditions used to prepare Samples 13–16. This indicates the chemical similarity between the products of the catechin hydrolysis and the products of the haze hydrolysis. Thus, one can reasonably expect that at least some components of the haze hydrolysates (as shown, for example, in Fig. 3) are related to phlobaphenes, "brown pigments," or the catechin-type products indicated by the CV. The absorbance at 436 nm increased in the lager haze hydrolysates in the following order: fresh (Sample 14), aged (Sample 15), and forced (Sample 16). Out of six major peaks in the visible spectrum, a peak at R_t 15.50 min, which is the most abundant in the haze hydrolysate, increased with the increasing

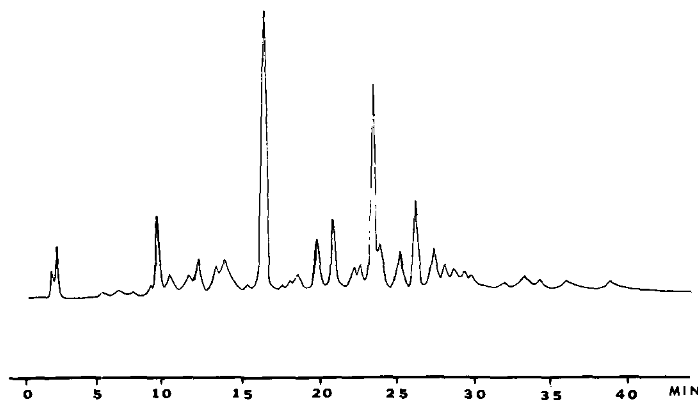

Fig. 3. High performance liquid chromatography (Microbondapak C_{18}) of fresh lager haze hydrolysate (Sample 14). Linear gradient elution; detection at 436 nm.

detection wavelength up to 436 nm. (At 546 nm no absorption occurred.) The intensity of the six major peaks increased in the following order: fresh, aged, and forced.

Carbohydrates

Attempted determination of "reducing sugars" before hydrolysis and of total carbohydrates after hydrolysis (as reducing monosaccharides) in haze and other complex substrates containing polyphenols, using such reagents as DNS, leads to excessively high carbohydrate contents and excessively low polyphenol contents (5). Calculating the degree of polymerization of carbohydrates on this basis is, thus, suspect. Indeed, a recent study (10) confirmed that some phenolics (rutin and quercetin) give a significant "pseudoreducing sugar" response (27 and 6.2%, respectively). The use of DNS reagent in substrates containing (almost) exclusively carbohydrates (such as corn syrup) is valid.

In this work we determined total carbohydrates by HPLC. The "reducing sugars" were determined using the DNS method, and the obtained values were considered only for comparison purposes. The determination of higher polysaccharides (DP_4 +) by HPLC can be improved by using a more accurate standard, eg, a syrup of 42 dextrose equivalent (7).

The percentages of "reducing sugars" in lager Hazes 2–4 were 12.0, 16.3, and 20.0, respectively. Those in buffer-extracted Hazes (Samples 9–12) were 4.7, 5.0, 0.9, and 4.2, respectively. Comparison of the values for the various samples is interesting. The content of "reducing sugars" increased from fresh (Sample 2) to aged (Sample 3) to forced lager haze (Sample 4). This content was severely reduced in buffer extracts of Haze 2 (Samples 9 and 10) and of Haze 4 (Samples 11 and 12). Samples 5–8 did not contain any carbohydrates, as expected.

Table III gives the percentage of total carbohydrates in lager Hazes 2–4 obtained by HPLC on Aminex HPX-87 with refractomonitor detection. The total carbohydrate content increased from fresh to aged to forced lager hazes, following the trend reported for ale hazes (5). The total content and the spectrum of carbohydrates in lager hazes are comparable with those of ale hazes (5), with some obvious differences. The total carbohydrate contents obtained by HPLC were greater than the contents of "reducing sugars."

Molecular Weight Distribution of Components

Table IV gives percent nitrogen and percent protein. The protein content of the forced lager haze (Sample 4) dropped markedly as compared with the protein contents of fresh lager (Samples 1 and 2) and aged lager (Sample 3) hazes. Alkali-acidic treatment of Hazes 1–4 resulted in a marked decrease in protein contents (Samples 5–8).

Figure 4 shows molecular weight distribution for fresh, aged, and forced lager hazes and a chromatogram of a cocktail of molecular weight markers. This distribution, showing three major areas of molecular weights, was obtained by HPLC of Samples 2–4. The same molecular weight distribution for these samples is given in Table V as relative area percentage. The presence of polysaccharides can be excluded because the chromatographic detection was done at 280 nm. Thus, the "low" molecular weight area includes nitrogenous components and phenolic moieties. The "medium" and "high" molecular weight areas are due exclusively to proteins (polypeptides) in the hazes. The exact protein composition of hazes is still unknown, although the major portion of the protein moiety appears to be derived from prolamines (hordeins and glutelins, about two thirds of the total) and from albumins and globulins (about one third) (6,8). Comparison of the overall amino acid composition of hazes with that of barley shows that no specific barley protein is responsible for haze formation (6).

Figure 4 and Table V show the following: 1) lager hazes contain much more of low molecular weight components than of high ones; 2) low molecular weight components are much more abundant in forced lager haze than in fresh or aged lager haze; 3) forced lager

TABLE III
Total Carbohydrates in Lager Hazes 2-4 by High Performance Liquid Chromatography on Aminex HPX-87 with Refractomonitor Detection

Component	R_t (min)	Percent in Haze 2	3	4
Dextrin	8.4	9.4	18.2	29.7
Maltose	10.7	2.7	5.9	5.6
Glucose	12.7	0.5	1.4	0.9
Fructose	14.9	Tr[a]	Tr	Tr
Total		12.6	25.5	36.2

[a] Traces.

TABLE IV
Protein[a] in Lager Hazes 1-4 and Samples 5-8

Sample	N (%)	Protein (%)
1	4.74	29.6
2	5.23	32.7
3	5.46	34.1
4	2.28	14.2
5	1.01	6.3
6	0.36	2.2
7	0.50	3.1
8	0.86	5.4

[a] By the micro-Kjeldahl method (9), N × 6.25.

TABLE V
Molecular Weight Distribution in Lager Hazes 2-4 by High Performance Liquid Chromatography on Protein Columns I-125 and I-250

Molecular Weight[a]	Relative Area Percent in Haze 2	3	4
0–10,000; low	85.7	53.4	96.7
10,000–50,000; medium	5.0	13.0	0.5
Over 50,000; high	9.3	33.6	2.8

[a] Approximate range.

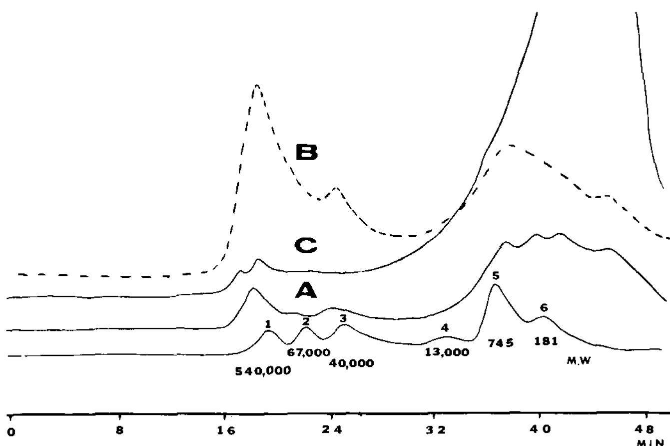

Fig. 4. Molecular weight distribution by high performance liquid chromatography (coupled I-125 and I-250 protein columns) for fresh (**A**), aged (**B**), and forced (**C**) lager hazes (Samples 2–4) and a cocktail of molecular weight (M.W.) markers. Eluent: phosphate buffer, pH 7.1; detection at 280 nm.

haze contains particularly low amounts of medium and high molecular weight components as compared with other hazes; and 4) aged beer haze contains much higher relative amounts of medium and high molecular weight components than the corresponding fresh lager hazes do.

Hazes 1–4 derived from an ale showed a molecular weight distribution very similar to that of the lager hazes. The results for the forced beer (both ale and lager) hazes indicate that heating has a crucial effect on the molecular weight distribution of the haze components. Hydrolytic decomposition of protein material apparently takes place in beer on forcing, which does not happen in beer aged at room temperature. This shows a basic difference between aging beer at room temperature and forcing it by heating. This should be kept in mind when comparing the results of forcing beer tests and rapid tests for shelf life prediction.

The high content of low molecular fraction in Haze 4 relative to those in Hazes 2 and 3 (Table V) and its lower protein content (Table IV) are certainly interrelated. Moreover, these values affect the haze formation by complexation with phenolics in the respective hazes (Samples 2–4). These interactions are obviously very complex and will not be further discussed at this stage.

Molecular weight distribution of buffer-treated hazes (Samples 9–12), obtained under identical HPLC conditions, showed the following interesting features: 1) no high molecular weight components; 2) alkaline buffer extracts (Samples 10 and 12) that showed only traces of material, thus indicating their nonprotein nature; 3) detection of almost all of the material by HPLC of the acidic buffer extracts (Samples 9 and 11), indicating that almost the total protein content of Hazes 2 and 4 was made up of acidic proteins.

The solubility of hazes in buffers and the yield of these products should be taken into account. Fresh lager haze (Sample 2) yielded 20% of the acidic buffer extract (Sample 9) plus 22.6% of the alkaline buffer extract (Sample 10). Forced lager haze (Sample 4), on the other hand, yielded 76.1% of the acidic buffer extract (Sample 11) plus 22.7% of the alkaline buffer extract (Sample 12). This agrees with the fact that 96.7% of the low molecular weight components was present in Haze 4, the precursor of Samples 11 and 12 (Table V). These components were almost completely soluble in the acidic and alkaline buffers (76.1 + 22.6 = 98.7% of the total haze soluble). In Sample 2, the ratio between the acidic and alkaline components is about 1:1, and over a half (57.4%) of the material is not soluble in either buffer, showing that fresh lager haze has a different composition than forced lager haze.

The major components measured in fresh, aged, and forced lager hazes (Samples 2–4) include anthocyanogens and catechins (tanninogens) (Table I), total carbohydrates by HPLC (Table IV), and protein (Table V). When percentages of these components are totaled, the percents of undetermined components for Samples 2, 3, and 4 are 43.5, 19.6, and 32.2, respectively. Calculation of protein content by using the factor 6.25 is only approximate. Bearing this in mind, the percentage of the unknown components in the hazes is accounted for by those components that are not determined by the above-used methods. In addition to minor amounts of PM, determined by HPLC (Table II), the unidentified components in hazes may include the following substances: polyphenol-protein (polypeptide) complexes; flavonol glycosides (such as quercitrin, isoquercitrin, rutin, and astragalin); esters and glycosides of phenolic and hydroxycinnamic acids (such as neochlorogenic acid); methoxylated phenolics related to lignins; coumarins; nonreducing carbohydrates; carbohydrate moiety of glycosides; various nitrogenous components; inorganic constituents, 2–14% (8); inorganic constituents that yield ash on combustion, up to 3% of total haze material (8), etc. The existence of a clear correlation between tanninogen, carbohydrate, and protein contents in hazes is not evident at this stage.

SUMMARY

1. Lager yields significantly lower amounts of haze than ale does, particularly on forcing. This corresponds well with the turbidity data for the two beers.

2. Both aging and forcing of beer lead to increased tanninogen contents (particularly catechin value) in its hazes.

3. The total phenolic monomer concentration in ale hazes is significantly higher than that in lager hazes.

4. A plausible relationship between some components of hydrolyzed hazes and the catechin value was established.

5. The total carbohydrate content (by HPLC) increases from fresh to aged to forced beer hazes (for both ale and lager).

6. The protein content (by micro-Kjeldahl) drops severely in forced beer haze as compared to that in fresh and aged beer hazes.

7. All hazes contain much more of low-molecular weight components than of high ones.

8. Low-molecular weight components are much more abundant in forced beer haze than in fresh or aged beer hazes.

9. Forced beer haze contains very low amounts of medium and high molecular weight components as compared with amounts in fresh and aged beer hazes. This shows a basic difference between forcing beer (at 60° C) and aging it at room temperature.

10. Almost the total protein content of the examined hazes consists of acidic proteins.

11. Tanninogens, total carbohydrates (by HPLC), and proteins (by Kjeldahl) account for most of the known components of beer hazes. The percentage of the undetermined components is the lowest in aged beer haze, followed by that in forced beer haze, and is the highest in fresh beer haze.

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