

## Different Forms of Nitrogen and the Stability of Beer

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Nitrogenous compounds present in beer were determined by methods of chromatography, gel filtration, electrophoresis, and spectroscopy. The chill haze formation was shown to consist of  $\alpha$ -hordein and  $\beta$ -globulin fractions to which the stability of the commercial product can be related. Effective means of removal of nitrogenous materials are described. The quality of beer is not determined by the content of total protein, but rather by the relative amounts of nitrogen that is soluble, that can be precipitated by  $MgSO_4$ , that is titratable by formaldehyde, and that is present as amino acid. All of these forms are found in beer after technological processing. With the use of appropriate filtering agents that have been covered in this study, it is possible to modify the nitrogen content in beer and improve the stability and hence the quality of beer. Particularly good adsorbents for this purpose were found to be kieselgel stabifix, diatomite, and bentonite.

The presence of nitrogenous and sulfurous compounds in beer and their chemical compositions have been reported by a number of researchers (Chen et al., 1973; Clapperton, 1971; Clark, 1960; Drawert et al., 1971, 1973; Drawert et al., 1973; Gorinstein, 1973a,b, 1974a; Heath-erbell, 1976; Hebert and Strobbe, 1972; Hejgard and Boeg-Hansen, 1974; Hudson, 1973; Narziss and Roettger, 1973a,b; Phillips, 1972; Savage and Thompson, 1973; Sielicka, 1973; Sommer, 1974; Steiner, 1973; Ten Hoopen, 1973).

The levels of Lundin fraction nitrogen, amino acids, and microelements which are retained in a beer to maintain foam head formation, retention, palate fullness, flavor, and stability were also given (Bulgakov, 1959; Gorinstein, 1973b, 1974c, 1976).

In this study we will be interested in determining (a) the distribution of nitrogenous compounds in beer, (b) their effect on the chill haze stability of beer, and (c) effective means for their removal.

### APPARATUS, REAGENTS, AND PROCEDURES

The present investigation was carried out on "Zhuguli" nonfiltered beer (for details, see Gorinstein, 1974a, b, 1975). For comparison we report results for brews clarified by filtering through cotton filter bed masses: control mass of 34 nephelos units and test mass of 55 nephelos units.

The chill haze was obtained by storing beer for 48 h at 0 °C, or by adding 0.5 mL of ammonium sulfate to 10 mL of beer and then cooling for 1 h (Bulgakov, 1959).

Total nitrogen was determined by the methods of Duma and Kjeldahl (AOAC, 1970; Bulgakov, 1958; Klimova, 1967). Then the nitrogen was multiplied by 6.25 to determine crude protein.

Organic nitrogen was determined using potassium persulfate (Gertner and Grdinic, 1965), and protein nitrogen alone after precipitation of the total protein with cupric hydroxide. Determination of formaldehyde titratable nitrogen was based on the addition of formaldehyde which binds with amino groups.

Amino nitrogen was determined by the ninhydrin method (Lie, 1973; Marinelli, 1975). Soluble and coagulable proteins were investigated according to the methods of Kolbach and Wilharm (Bulgakov, 1959).

The determination of amino acids was carried out on an amino acid analyzer (Arslanbekova et al., 1973; Hampel, 1973; Kurganova et al., 1974; Laszlo and Joth, 1972), or by means of ion-exchange chromatography using single and

double columns (Maendl et al., 1974; Moll et al., 1972; Reiner and Piendl, 1974). This latter technique employed either cationic exchange resin KY-2 or stabifix. For chromatography on KY-2, the column was eluted with  $NH_4OH$ , and on stabifix with 70% formaldehyde solution, followed in both cases by hydrolysis with 6 N HCl. Nitrogen of the amino acids also produced was then determined by the Kjeldahl method. A sufficient quantity of the amino acids was isolated for UV, IR and NMR analyses.

Amino acids were analyzed on a Beckman Model 120/3 by the two-column procedure of Spackman et al. (1958), after hydrolysis of the protein samples with 6 N HCl at 110 °C for 24 h. Infrared spectra were taken on a Unicam SP 200G spectrophotometer in KBr and Nujol. Ultraviolet spectra were recorded on a ultraviolet spectrophotometer in the 200–450 nm region. Nuclear magnetic resonance spectra were measured on a Varian T-60 spectrometer with TSP (sodium 3-trimethylsilylpropionate-2,2,3,3- $d_4$ ,  $(CH_3)_3SiCD_2CD_2CO_2$ ) reference standard and deuterium oxide as solvent.

The nitrogenous substances were also fractionated according to Lundin (Bulgakov, 1959). The solubility of proteins in different solutions was the basis for selecting and utilizing the procedure of fractionation (for details, see Gorinstein, 1974b).

Colloid-protein (chill haze) stability of beer was judged using a limit of precipitation in milliliters of ammonium sulfate (Bulgakov, 1959) Moll and Vinh, 1973; Steiner, 1972). As the limit of precipitation increased, chill haze stability also increased.

Protein fractions were concentrated by the tannin-caffeine method (Gordon et al., 1950; Fertman and Gorinstein, 1968; Gorinstein, 1975). The tannin-caffeine method is based on the protein complex forming property of tannin. These complexes were then dissolved with caffeine. Glutelin and hordein, which are normally insoluble in milk aqueous conditions, were found to be soluble in caffeine.

For horizontal electrophoresis a veronal-medinal buffer of pH 8.6 (Gordon et al., 1950; Fertman and Gorinstein, 1968; Gorinstein, 1975), containing agar gel in 1.5–2% concentration, was used. The sample of beer (400 mL) was treated with (a) 30 mL of 4% solution of tannin, followed by dissolving the precipitate by addition of 0.2 g of dry caffeine, or (b) with 200 mL of 4 M ammonium sulfate in order to separate out the protein fractions. The samples of the concentrated protein (0.04 mL) were placed on the agar gel and electrophoresis was carried out for 5 h at 40–80 V. Then the gels were strained for 2 h in 0.5% amido black in 7.5% acetic acid solution. They were then destained

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