

# Changes in Nitrogen, Polyphenol and Fatty Content in Beer Following Enzymatic Action

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## ABSTRACT

Changes in the nitrogen and polyphenol content in beer samples were determined by spectroscopic techniques after the addition of varying amounts of enzymes.

The low molecular weight nitrogen compounds are decisive in determining the functional changes in proteins such as solubility and coagulability which influence the quality characteristics of the beer related to taste and stability.

The attenuation limit of fermentation can be used to detect some functional changes in starch as to the quality and quantity of carbohydrates.

An acetone-soluble fraction of beer samples has been examined by gas chromatography-mass-spectrometry using the trimethylsilylation in order to increase the volatility of the compounds.

Mass spectrometry was used to determine the structure of this fraction which was identified as mainly glycerol.

## INTRODUCTION

In beer the change in nitrogenous, polyphenol and fatty substances during the fermentation process is very important because of its effect on the colloidal system of the product. (Bellmer, 1976; Dadic, 1976; Daiber, 1975; Gorinstein, 1971 and 1978; Gorinstein, et al., 1978; Narziss and Bellmer, 1976; Vrteleva and Dolesalova, 1976).

Such change results from the action of enzymes, either endogenous or supplementary (Hebert and Scriban, 1976; Knoepfel and Pfenninger, 1976; Krauss and Eifer, 1976; Sfat, 1975).

The acetone or methanol extractable fraction was the subject of several studies which reported in polyphenols and fatty acids (Drost et al., 1971; Gracey and Barker, 1976; Jacobsen, et al., 1974; Mac Pherson and Buckee, 1974; Sandra, et al., 1975).

Our research aimed at studying the changes in nitrogen-polyphenol content and identifying the structure of acetone extractable fraction of beer samples.

## MATERIALS, APPARATUS AND PROCEDURES

All the experiments were carried out on Lager Israeli Beer under processing conditions. Enzymes (NOVO Industries, Denmark) were employed for different biochemical reactions: Termamyl 80L for liquefaction of adjuncts;

## SINTESIS

Se determinaron por medio de técnicas espectroscópicas los cambios en el contenido de nitrógeno y polifenol en muestras de cerveza después de la adición de diferentes cantidades de enzimas.

Los compuestos de nitrógeno de bajo peso molecular son decisivos para determinar los cambios funcionales en proteínas como la solubilidad y la coagulabilidad que influyen las características de calidad de la cerveza relacionadas con el gusto y la estabilidad.

El límite de atenuación de la fermentación puede usarse para detectar algunos cambios funcionales en el almidón como son la calidad y cantidad de carbohidratos.

Se ha examinado una fracción soluble en acetona de las muestras de cerveza por cromatografía y espectrometría de masas usando la trimetilación con objeto de aumentar la volatilidad de los compuestos.

Se usó espectrometría de masas para asignar la estructura de esta fracción que se identificó principalmente como glicerol.

Neutrase 1.5 S for breakdown of proteins to peptides; Cereflo 200L for splitting  $\beta$ -glucan in malt; Fungamyl 800L for hydrolysis of starch and dextrin to fermentable sugars.

The following are the compositions of the samples employed in this study:

Control I—60% malt + 40% sorghum;

Test I—Control I + Neutrase + Cereflo + Termamyl;

Test II—Test I + Fungamyl

Test III—50% malt + 50% sorghum + Neutrase + Cereflo + Termamyl;

Test IV—Test III + Fungamyl;

Control II—100% malt, this sample was introduced for obtaining comparative data on nitrogen content.

The analyses of the malt, wort, and beer were carried out by the EBC method (European Brewery Convention, Analytica-EBC, 1975) and by other methods (American Society of Brewing Chemists, 1977; Kruger and Bielig, 1976).

Coagulable and albumose nitrogen was treated according to the methods of Koblach, Wilharm and De-Clerck (De-Clerck, 1964 and 1965) and then as for total nitrogen content, the nitrogen content of those samples was determined by the Kjeldahl or Dumas methods (Buchi Nitrogen Determination System: Digestion Apparatus, Buchi, 425, Distillation Unit, Buchi 320).

Both total protein and albumin were determined by direct spectrophotometric methods from the lyophilized sample. For each determination the concentration of 100 mg/ml of lyophilized sample was used.

Total protein was determined by the precipitation with

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TABLE I.  
Composition and Properties of Wort and Beer Samples

	Wort						Beer					
	1 Control I	2 Control II	3 Test I	4 Test II	5 Test III	6 Test IV	7 Control I	8 Control II	9 Test I	10 Test II	11 Test III	12 Test IV
Qualitative Indices												
Apparent attenuation limit fermentation, %	78.9		77.9	78.1	72.1	74.6	79.3		79.7	81.5	78.2	80.6
Polyphenols, mg/l	150	168	160	158	154	140	130	161	139	144	134	125
Anthocyanogens, mg/l	31.2	45.4	35.8	34.8	32.8	30.0	24.2	30.6	26.8	27.6	25.2	23.8
P.I.	4.8	3.7	4.5	4.5	4.7	4.6	5.4	5.3	5.2	5.2	5.3	5.3
Forms of nitrogen, (mg/100 ml)												
Total soluble	65.4	123.2	71.4	76.3	76.3	71.0	37.1	83.3	46.2	49.0	49.0	47.6
α-amino	16.6	18.0	18.6	18.9	18.1	17.9	5.4	12	6.8	6.6	6.4	5.9
Coagulable	3.4	12.7	2.4	3.6	2.2	3.1	2.3	1.8	1.6	2.3	2.0	2.3
Protein (TCA)							48.1	65.6	70.0	83.1	65.6	56.9
Albumin							17.4	30.9	49.0	60.0	45.4	37.6
Globulin							30.7	34.7	21.0	23.1	20.2	19.3
Chill haze, EBC units							1.1	0.8	0.8	0.8	0.8	0.7
Glycerol*, mg/100 ml							9.0	6.0	15.0	14.0	19.0	20.0

\*approx. amounts

10% trichloroacetic acid. The nitrogen content of the precipitate, determined by the Kjeldahl method, was multiplied by 6.25 and reported as crude protein.

Total albumin was determined by the reaction between albumin and bromocresol green in a suitable buffer to form albumin-BCC complex with a blue colour at an absorption maximum of 630 nm (Doumas et al., 1971).

Total globulin was calculated as a difference between the total protein and the albumin.

α-amino nitrogen was determined spectrophotometrically on a UV-VIS Spectrophotometer Varian Techtron, Model 635 in the 570 nm region (Analytica EBC, 1975).

Chill haze (protein stability) was measured with the EBC hazemeter after one day at 40°C, followed by chilling to 0°C for 24 hours.

The polyphenols and anthocyanogens were determined according to EBC and ASBC.

The amount of glycerol was estimated by weight after extraction with acetone from lyophilized sample of beer.

The mass spectra of the crude oils were measured with a Varian MAT CH-5 DF mass spectrometer, using direct inlet, 70 eV and 240°C ion source.

The gas chromatography mass spectra were measured with a LKB-2091 GC mass spectrometer, using a 2 mm x 2 m glass SE 30 CC column with a 10 ml/min carrier gas. The temperature of the column was increased from 100 - 160°C, 10°/min in order to obtain sharp peaks, whereas the gas-separator was 260°C, and the ion source 240°C at 70 eV.

The TMS derivatization was carried out by allowing to stand overnight, about 10 mg crude oil with 0.3 ml BSA (Bis-Trimethyl-silyl Acetamide). 0.5 µl of the mixture was injected to GCMS.

### RESULTS AND DISCUSSION

From the analytical data obtained in Table I for Control and Test samples of wort and beer in this study the following observations can be made:

1. The maximum attenuation limit of beer fermentation with addition of Fungamyl was about 80.6 - 81.5% (Tests II and IV, in columns 10,12) compare to Control I (column 7).

2. The quantity of polyphenols, and hence that of reducing substances, was highest in all malt sample Control II (columns 2 and 8) and decreased with increasing use of sorghum as raw material (Control I - Test IV in columns 7 and 9-12). The polymerization index (P.I.) as determined by the ratio of anthocyanogens to polyphenols attained similar values for all samples after technological processing. (It has been suggested that the polyphenol level can be regulated by the choice of raw materials. This in turn could be used to adjust the proportion of polyphenols to protein content and affect to some extent the colloidal stability of the product.)
3. The use of enzymes tended toward increases in all nitrogen-containing substances. Addition of Neutrase during mashing increased the amount of α-amino nitrogen, thus increasing the ratio of α-amino nitrogen to total nitrogen and the amount of soluble components. This indicates that the extent of proteolysis in mash containing enzymes (columns 3-6) is different from that in Control I mashes (column 1).
4. The difference between the amount of α-amino nitrogen in wort and in beer can be used as an index for the extent of fermentation within the samples. This difference is the largest for Test II mashes (column 4), and indeed, the apparent attenuation limit of fermentation in these samples is the highest (column 10). The availability of assimilable nitrogen is required for the fermentation and storage processes.
5. Although the highest soluble nitrogen content is found in the all malt sample (Control II, in column 2), the degree of proteolysis as seen in the α-amino nitrogen content is larger in the mixed mashes where enzymes have been added (Tests I - IV, in columns 3-6). Since α-amino nitrogen is a nutrient for yeast, the degree of fermentation will be increased by enzymatic reactions.
6. Soluble nitrogen content decreased when the proportion of sorghum was increased compared to Control II (columns 2 and 8), though this content was still larger with added enzymes (Test I - IV in columns 3-6 and 9-12), than in Control I.

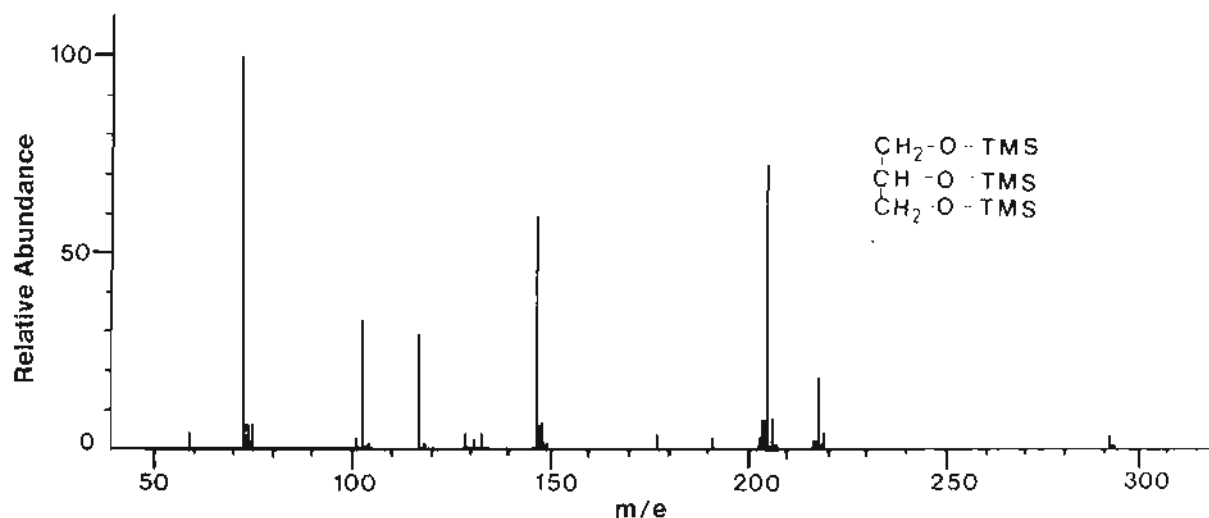


Figure 1. The Mass Spectrum of tris-trimethylsilyl found in the acetone extractable fraction of beer.

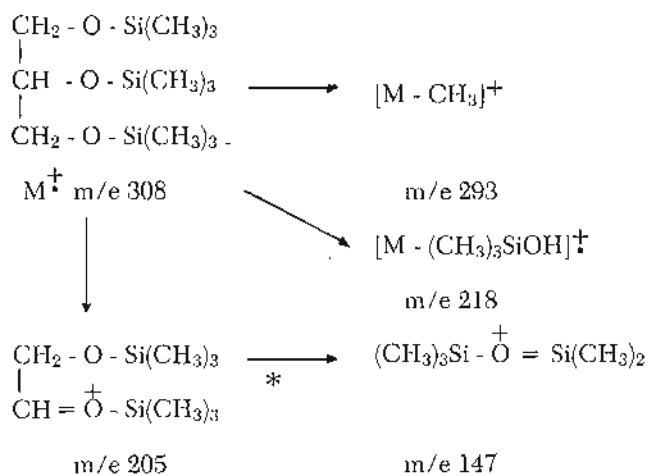
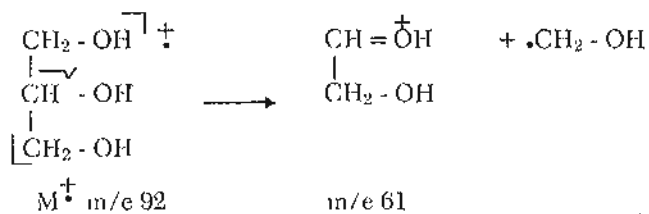
7. There were no dramatic effects on coagulable nitrogen by enzyme addition. The decrease in coagulable nitrogen in the all malt sample (Control II in columns 2 and 8) from wort to beer is the largest single factor in its loss of total soluble nitrogen content.
8. The content of proteins of different molecular weights was different in each sample but there was a tendency towards increases in albumin and reductions in globulin content both in wort and beer where enzymes were added (columns 9-12). As a result of the increased albumin compared to globulin these beer samples do have higher stability (chill haze in columns 9-12) than the Control samples (column 7).
9. Different samples of beer after lyophilization to dryness yield a powder. Further extraction of the powder with acetone or alcohol gives an oily product (6-20 mg/100 ml).

IR measurements of this oil showed a large peak corresponding to hydroxyl group and only small absorption peaks at the rest of the IR spectrum. Mass spectral measurements by direct injection of this oil show a prominent peak at  $m/e$  61 suggesting together with the IR spectrum an alcohol type compound. The GC-MS of the silylated oil showed that about 90% of the silylated material is in one peak at the gas chromatogram. The Mass spectrum of the compound represented by their peak is shown in Figure 1.

The peak with the highest mass ratio is at  $m/e$  293 and no molecular ion was observed, a characteristic of polyglycols due to loss of a methyl radical (Diekman, et al., 1968). This observation led to a silylated polyglycol with a molecular weight of 308, corresponding to tris-trimethyl silylated glycerol. The fragmentation pattern corresponds to that of synthetic silylated glycerol found in the literature (Stenhagen et al., 1974).

The fragmentation mechanism is shown in the scheme 1.

In the light of above results, the ion represented by  $m/e$  61 in the mass spectrum of the crude oil is formed by a carbon-carbon bond cleavage of the glycerol as is shown below:



Scheme 1.

The amount of glycerol in beer is similar for each test sample (columns 9-12), but is slightly less in Controls I and II (Columns 7 and 8). We believe that this glycerol is a product of hydrolysis of lipids and glycoproteins during the enzymatic action and might be a criterion for the estimation of these compounds.

#### ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Industry, Trade and Tourism (Office of the Chief Scientist), Canadian Brewery Labatt's and National Brewery Ltd., Tel Aviv, and personally to Mr. Murray Goldman (Toronto), who supported this work by contract.

Many thanks are due to Dr. Victor (The Hebrew University) for her help in the organization of this manuscript, and to S. Feldman, C. Krupnik, S. Melzer (National Brewery Limited), A. Conway (The Hebrew University) for technical assistance in obtaining results.

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