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Spectroscopic Determination of Glycerol, Polyphenols, and Nitrogenous Compounds in Beer and Wine

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ABSTRACT

The Infrared and Ultraviolet systems were used for the determination of glycerols and polyphenols in beer and wine. Comparison is made with the standard methods (Soxhlet, acetone weight extractable, and carboxymethylcellulose), and results for all methods are presented.

Results from the Infrared and Ultraviolet methods are lower due to the presence of only glycerols or phenols.

Quantitative differences in these components that are dependent on the amounts of raw materials and enzymes in beer have been found. Glycerol, polyphenols, and low molecular weight nitrogen compounds were reported in relation to the physical, chemical, and organoleptic properties, and with respect to the foam stability, viscosity, and chill-proofness of the final product.

INTRODUCTION

The use of enzymic preparations such as α-amylase, protease, and glycanase as hydrolyzing agents of unmalted cereal compounds has been the subject of several studies.[1,2] The sequence of these enzymatic reactions begins with the breakdown of phospholipids of glycerides in beer and leads to the formation of free fatty acids.[3,4] The fatty acids and glycerides destroy the colloidal system, and this factor decreases the foam stability of the final product.[5,6] The enzymatic reactions taking place during beer preparation change the amount of nitrogenous and polyphenolic substances and also glycerol which influence the properties of beer.[7-9]

Thus, it is important to determine the various constituents of beer, especially glycerol and polyphenols. The mass spectral identification of glycerol in beer has been reported.[10] Gas chromatography[11,12], distillation and colorimetry[13,14], acetone and carbon tetrachloride weight extractable methods[15,16], spectrophotometry CMC/EDTA[16], 4-aminoantipyrine[17] have been used for the determination of glycerol and polyphenols. But these methods are very complicated.

Infrared spectroscopy has not been used extensively for quantitative glycerol determination.

In this study a simple method for glycerol and polyphenol determination in the acetone-extractable beer fraction by infrared (IR) and ultraviolet (UV) spectroscopy is reported. This method has also been applied to wine musts. In comparison to other methods this procedure is fairly rapid.

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SINTESIS

Se han usado los sistemas infrarojo y ultravioleta para la determinación de gliceroles y polifenole en cerveza y vino. Se hace una comparación con los métodos estandar (Soxhlet, peso extraible con acetona y carboximetilcelulosa), y se presentan los resultados para todos los métodos.

Los resultados con los métodos infrarrojo y Ultravioleta son más bajos debido a la presencia solamente de gliceroles y fenoles.

Se han encontrado diferencias cuantitativas en estos componeintes que dependen de las cantidades de materias primas y enzimas en la cerveza. Se reportaron glicerol, polifenoles y compuestos nitrogenados de bajo peso molecular en relación a las propiedades físicas, químicas y organolépticas y con respecto a la estabilidad de la espuma, la viscosidad y la estabilidad al enfriamiento del producto final.

METHOD

Apparatus and Reagents

The enzymes (Novo-Industria/s, Copenhagen, Denmark) employed for different biochemical reactions were: Termamyl 60L—for the liquefaction of adjuncts; Neutrase 1.5S—for the breakdown of proteins to peptides; Cereflo 200L—for splitting the β-glucan in malt and barley; and Fungamyl 800L—for the hydrolysis of starch and dextrin to fermentable sugars.

All the experiments were carried out on Lager type Israeli beer made under normal processing conditions using the following materials:

Control 1— 60% malt + 40% sorghum;
Control 2— 100 malt. This sample was introduced for obtaining comparative data on nitrogen content;

Test 1— 65% malt + 35% sorghum with 4 enzymes;
Test 2— 60% malt + 40% sorghum with 4 enzymes;
Test 3— 50% malt + 50% sorghum with 4 enzymes.

The investigation also included some Israeli Semillon musts and wines and other trade beer samples.

Standard solutions of glycerol in acetone were prepared thus: 2,5,10,15,20,25 & 30mg glycerol/ml acetone.

Standard solutions of polyphenols in methanol were prepared 0.01, 0.02, 0.03, and 0.04mg resorcinol/ml methanol.

The glycerol and polyphenol compounds were extracted from beer and wine lyophilizates with acetone, and extracts assayed by IR and UV spectrometry. IR measurements were made on a Perkin-Elmer Model 257 infrared spectrophotometer. UV measurements were carried out with a spectrophotometer Varian Techtron, Model 635.

The analyses of total polyphenols in beer has been described.[16] The viscosity of the samples (a property which

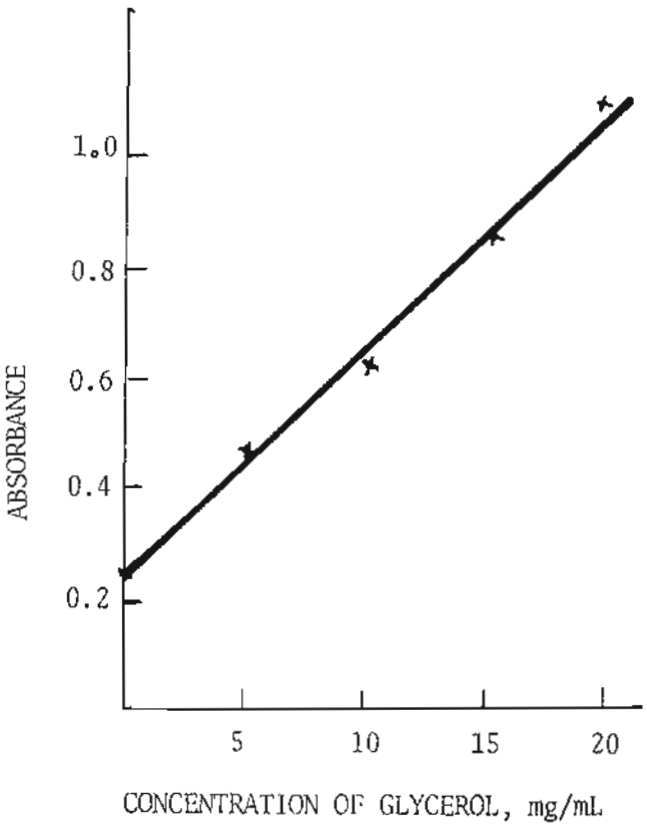

Fig. 1. Calibration curve for glycerol.

determined filterability) was measured with an Ostwald viscosimeter[16,18] and calculated on the basis of one particular concentration of wort and beer.

Albumose was determined by the reported method based on $ZnSO_4$ precipitation.[19] The nitrogen content was determined in parallel samples before and after precipitation by Kjeldahl analysis[19,20] (Buchi Nitrogen Determination System: Digestion Apparatus, Buchi 425; Distillation Unit, Buchi 320).

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

The foam stability was determined by modified Carlsberg method as sigma value of beer.[21]

The determination of peptide nitrogen was carried out by the glyoxal or methylglyoxal method at 321 or 335 nm, respectively.[22]

Preparation of Samples

Initially the beer sample was degassed by shaking and clarified by centrifugation. The 100 ml of each beer or wine samples were lyophilized. The beer or wine lyophylizates were extracted with three 100 ml portions of distilled acetone. The solvents were then evaporated to dryness. Each of the samples was prepared by dissolving the residue in 1 ml.

IR and UV determination

The determination of the glycerol content in beer and wine was made by comparison of the IR absorption spectra of the sample with that of glycerol standard solutions at 1040 cm^{-1}. The glycerol was quantified by comparison of the IR stretching bands at 1040 cm^{-1} based on the experimental data.[23] This band is reported according to the characteristics to primary and secondary alcohols (isopropyl alcohol, cyclohexanol, and t-Buthyl alcohol). Substituents shift the band to a higher frequencies.[23]

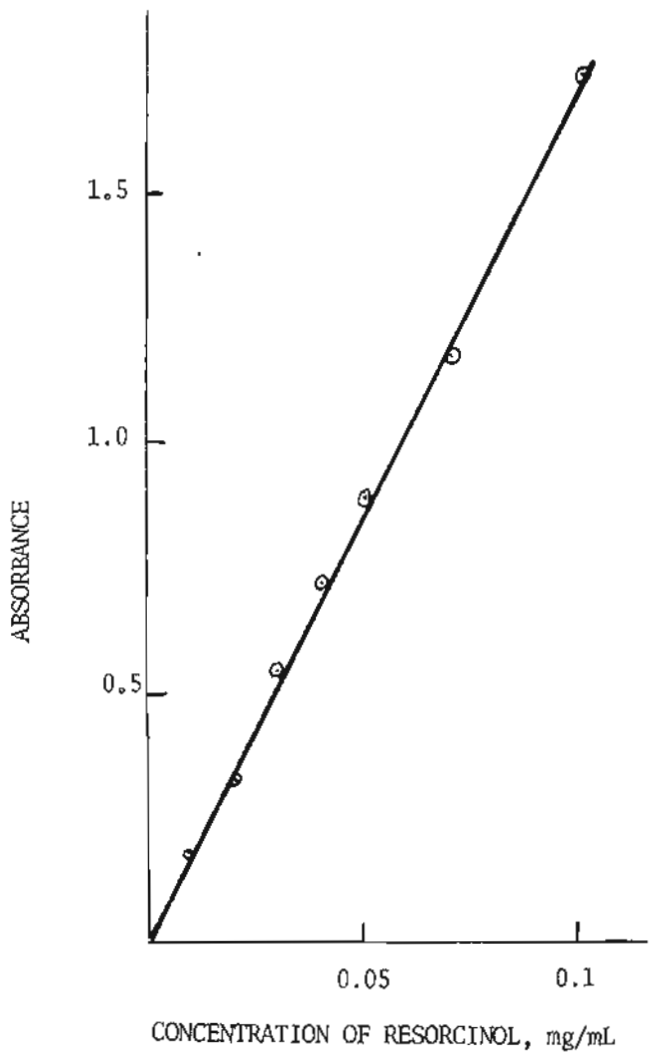

Fig. 2. Calibration curve for resorcinol.

For polyphenol determination the samples and standards were dissolved in methanol, and the spectra were recorded against methanol at 275nm-λ_{max} for phenols.[24]

Calibration curves for glycerol and resorcinol are shown in Figures 1 and 2.

RESULTS AND DISCUSSION

The spectral data of beer sample, glycerol, polyphenols and acetone are shown in Figure 3.

Acetone extractable beer and wine fractions give distinctive absorption patterns similar to that of glycerol. These bands did not change in all beer and wine samples with regard to standards. The broad band at 3600-3200 cm^{-1} is assumed to be the result of absorption of hydroxylic group, including water, therefore this region cannot be used for a quantitative glycerol determination. C-O absorption is seen at 1040 cm^{-1} in all beer and wine samples, in standard glycerol, but was not detected in acetone when used as a solvent. This was the region used for quantitative glycerol determination.

Spectrum of a beer sample showed the only glycerol

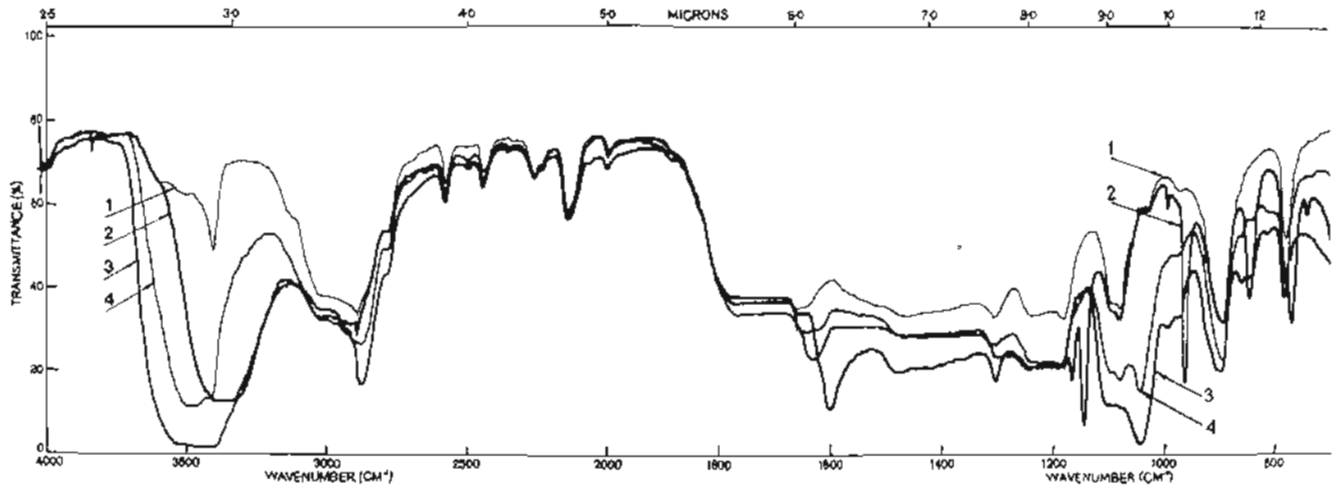

Figure 3.

TABLE 1.
COMPOSITION AND PROPERTIES OF INVESTIGATED SAMPLES

Qualitative Indices	Control 1	Control 2	Wort: Test 1	Test 2	Test 3	Beer: Control 1	Control 2	Test 1	Test 2	Test 3	Maccabee	Amstel	OK	Semillon Must	Semillon Wine
Lyophilized weight, g/100 ml						3.3	3.7	3.4	3.5	3.4	3.8	2.9	4.1	2.3	1.6
Acetone fraction weight, mg/100 ml						19.2	14.1	9.8	22.0	23.2	15.2	14.7	29.8	40.5	240.0
Glycerol, mg/100 ml						9.3	6.0	5.2	19.0	14.2	7.1	11.2	24.4	27.5	130.0
Polyphenols, mg/100 ml						13.0	15.1	14.9	14.4	13.4	15.4				
Viscosity, cP	1.79		1.71	1.64	1.60	1.60		1.34	1.54	1.52	1.61	1.68	1.71		
Mash filtration, hrs	4.1		3.4	3.2											
Albumose, mg/100 ml						4.5	6.1	5.0	8.7	8.6					
Peptide, mg/100 ml				14.0	13.0	16.1	18.5	20.9	22.0	20.5					
Chill haze EBC units						1.1	0.8	0.7	0.8	0.8					
Foam Stability (sigma)						97	122	125	100	114	121	102	82		

characteristic bands and no absorption peaks for polyphenols. The limiting factor in this case was the low concentration of polyphenols in the acetone extractable fraction.

The results of the chemical analyses are summarized in Table 1. Several samples of beers and wines during processing were measured. All the samples except wine gave the same amount of lyophilizate. These ranged from 2.9 to 4.1 g/100 ml. The acetone fractions varied from 10 to 30 mg/100 ml although wine gave a very high content (240 mg/100 ml). Considering that we started with the same amounts of lyophylizate in Control and Test samples, we saw that the increase in the amount of acetone extractable fraction had an increased glycerol content. In Tests 2 and 3 beers more glycerol than in Control 1 beer was found, but the amount of glycerol in Test 1 was low compared to Control 1. Enzymatic reactions led to more glycerol in beer (Tests 2 and 3) because the hydrolysis took place during processing stage. Higher proportions of adjuncts in Test 2 beer showed an increase in glycerol as compared to Test 1, but with increase of sorghum content up to 50% (Test 3) had similar effect as 40% sorghum (Test 2). The higher glycerol content in Tests takes from the acetone extractable fraction are reflected in higher levels of viscosity of the corresponding beers, but this factor slightly decreased the foam stability. However, the viscosity of wort and beer Tests was lower than in Control 1, and the mash filtration time was also reduced.

Further examination of the Table 1 showed only small differences in polyphenols between the Control and Test samples.

Comparison of the commercial beers (Maccabee, OK, and Amstel) showed an increase in the glycerol content (Table 1), and it was proportional to the viscosity and foam stability.

Precision data for glycerol at different concentration levels are summarized in Table 2. It shows the results of twenty determinations at each of four different concentrations.

TABLE 2.
Precision of measurement at Different Concentration Levels for IR Spectroscopic Method

Glycerol in acetone, mg/100ml	Mean absorbance for 20 measurements	±	Std. Dev.	Coefficient of Variation, n=20; %
5	0.46	±	0.012	2.67
10	0.62	±	0.02	3.30
15	0.87	±	0.03	3.54
20	1.1	±	0.07	4.66

TABLE 3.
Comparison of Standard Soxhlet method for fatty substances, acetone extractable method, and IR spectroscopic method for glycerol.

	Soxhlet method for fatty substances		Acetone extractable method with mass spectra		IR spectroscopic method	
Sample	mean concn. mg/100ml	CV,%	mean concn. glycerol mg/100ml	CV,%	mean concn. glycerol mg/100ml	CV,%
Control 1	17.9± 0.6	3	19.2 ± 0.6	3	9.3 ± 0.3	3
Control 2	12.7 ± 0.4	3	14.1 ± 0.4	3	6.0 ± 0.2	3
Test 1	8.3 ± 0.2	3	9.8 ± 0.3	3	5.2 ± 0.2	3
Test 2	20.8 ± 0.6	3	22.0 ± 0.6	3	19.0 ± 0.8	4
Test 3	22.3 ± 0.6	3	23.2 ± 0.6	3	14.2 ± 0.5	4
Maccabee	14.8 ± 0.5	3	15.2 ± 0.4	3	7.1 ± 0.2	3
Amstel	13.6 ± 0.4	3	14.7 ± 0.4	3	11.2 ± 0.3	3
OK	28.8 ± 0.7	3	29.8 ± 0.8	3	25.4 ± 0.6	5

The coefficient of variation for all ten analyses with IR spectroscopic method compare favourably with the acetone and Soxhlet extractable methods as shown in Table 3.

Table 3 shows the results of comparison analyses for some real samples of beers between the Soxhlet method, weight extractable method, and IR spectroscopic method for glycerol. Ten analyses were carried out on each of the 8 samples of beers shown at Table 3.

As expected, results with the IR spectroscopic method are lower than the results with weight extractable method and Soxhlet method because the acetone and carbon tetrachloride extractable fractions contain not only glycerol but also phenols and other fatty substances, such as glycerides, etc.

Addition of enzymes in the mashing process gave a significant increase in low molecular weight nitrogen compounds in albumoses and peptides in Test samples as compared to Control I. This data determined the changes in protein properties such as foamability and viscosity which influence the quality characteristics of the beer and also relate to its taste, foam and chillproofness. Such changes result from the action of enzymes and the amounts of adjuncts. An acetone extractable fraction of beverages was characterized by spectroscopic methods. Comparison of the spectra revealed mainly quantitative differences in glycerol and polyphenol content. Beer samples vary widely in their content of these compounds. Beer prepared with supplemental enzymes was observed to contain more glycerol in the acetone extractable fraction than in beer without enzymes. The amount of glycerol in the acetone extractable fraction could be used to detect the degree of enzymatic hydrolysis of fatty compounds in beer production and to predict beer characteristics such as viscosity and foam.

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