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Changes in the Chemical Composition of Beer During the Brewing Process as a Result of Added Enzymes[1]

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ABSTRACT

The effect of adding enzymes to a mixture of malt and sorghum during the brewing process was studied according to the following criteria: presence of soluble nitrogen-containing compounds and sugars, filterability, physical stability of the finished product, amount of extract yielded by raw materials in the malting process, and taste. Addition of Neutrase to the malt gave a significant increase in the nitrogen content of the wort and the beer. These low molecular weight nitrogen compounds (α-amino nitrogen, amino acids, albumose, and peptides) are decisive in determining the taste, foam, stability, and chill sensitivity qualities of beer. The advantages of adding enzymes such as Neutrase and the carbohydrases Termamyl and Cereflo in the brewing process are seen in the increased amounts of soluble nitrogen-containing compounds and carbohydrates, a decreased viscosity leading to higher filtration rates, an increased yield, and the possibility of using larger amounts of cheaper unmalted adjuncts to obtain a similar product.

Key words: *Added enzymes, Brewing, Composition*

An important index of beer quality is its physical stability, which depends on the mashing process and on the amount of minerals, protein substances, and polyphenols in the beverage (5,10–13,22–24,37).

Beer brewing has always involved the action of enzymes in the mashing process to break down high molecular weight proteins, dextrins, nonstarchy polysaccharides (14,32), glucans (17), and pentosans (30,35) and to increase filterability (31,39,40) and protein stability (15,19,36) of beer. Barley malt was originally the most important raw material in beer processing and was the only source of essential enzymes. Less expensive raw materials such as rice, maize grist, barley, and sorghum have little or no enzyme content, except for the β-amylase of barley; their starch, protein, and glucan have to be digested by other enzymes in the grist. Thus the amount of the cheaper adjuncts that can be added to the mash is restricted by the limited supply of enzymes from the malt itself.

This research studied the possibility of increasing the amount of adjunct (in this case, sorghum) in the mixture of raw materials in the brewing process by using added enzymes. The following factors were examined to determine the optimum conditions for such treatment: 1) comparison of the degree of fermentation and the degree of proteolysis in the wort as a function of the addition of enzymes, and 2) quantitative determination of the various nitrogen-containing compounds in the raw materials and in all stages of the brewing process.

MATERIALS AND METHODS

Materials

All the experiments were conducted on lager beer (10° B) under processing conditions at the National Brewery Ltd., Netanya, Israel. Six thousand kilograms of raw materials, comprised of 65% malt and 35% sorghum in addition to 240 hl of water, were used in the mash. Enzymes (Novo Industrias, Denmark) were used to assist in the utilization of adjuncts (Termamyl and Neutrase) and to supplement the malt quality (Cereflo and Fungamyl).

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Termamyl 60 L. This α-amylase liquifies adjuncts. It has a standardized activity of 60 kilo Novo Amylase units per gram (25). The temperature optimum is 50° C in the pH range 5–7.

Neutrase 1.5 S. This proteinase, which breaks down proteins to peptides, contains 1.5 Anson units per gram (26). At 55° C the optimum pH is in the 6–7 range.

Cereflo 200 L. This enzyme, composed of β-glucanase and α-amylase, splits β-glucan in malt. It has a standardized activity of 200 β-glucanase units per gram (27). At 50° C its optimum pH is 7.5.

Fungamyl 800 L. This α-amylase hydrolyzes starch and dextrins to fermentable sugars. It contains 800 fungal amylase units per gram (28). The temperature optimum is 5–10° C at pH 4.5.

Mash Samples

The National Brewery Ltd. used a mash decoction system. One mash was 100% malt and contained the enzymes Neutrase and Cereflo; the other was comprised of the sorghum and 10% of the total weight of the malt and contained the Termamyl enzyme. Both were mashed at 50° C, and the all-malt mash was retained at this temperature during the liquefaction of the sorghum. The two mashes were then mixed and heated to 76° C after saccharification steps at 60–62° C and at 71° C. Control I was this regular product of National Brewery Ltd., but without the enzymes added. Control II was the all-malt mash, used to obtain comparative data on nitrogen content.

Two test samples were used. Test I contained 65% malt and 35% sorghum plus 0.1% Neutrase 1.5 S based on the weight of the malt, 0.1% Termamyl 60 L based on the weight of the sorghum, and 0.3 g of Fungamyl 800 L per hectoliter of wort. Test II was Test I plus 0.025% Cereflo 200 L based on the weight of the malt.

Procedures

For all samples, the wort was boiled for 1.5 hr with the hop extract added in three portions (110 g/hl). After being boiled, the wort was pumped into a settling tank and cooled by means of a plate heat exchanger to a pitching temperature of 10° C. It was pitched with *Saccharomyces Carlsbergensis* yeast, with a 50% (v/v) solid content of yeast slurry in a proportion of 0.5 L/hl. At this point, the fourth enzyme (Fungamyl) was added to the test wort. The maximum temperature reached during 7–8 days of fermentation was 12° C.

The young beer was transferred at a uniform apparent degree of fermentation of 73%, except for Control I, which was transferred at 69%. Lagering lasted five weeks. Before filtration, Protesal (a chillproof enzyme of Schwarz Services International Ltd., New York) was added (2 g/hl).

The beer from each batch was clarified by diatomite filtration and polished through a sheet filter.

The analyses of the malt, wort, and beer were carried out by EBC methods (9) and by the methods of Bausch (2), Kruger and Bielig (18), and Moll et al (20).

The nitrogen content of samples was determined by the Kjeldahl or Dumas methods (1), (Buchi nitrogen determination system: digestion apparatus, Buchi 425; distillation unit, Buchi 320). For the determination of coagulable and albumose nitrogen, the methods of Kolbach and Wilharm (4) and De-Clerck (7) were used. For the actual nitrogen determination after these procedures, the precipitates and filtrates of these tests were digested and distilled

and their nitrogen contents determined by Kjeldahl.

The total protein was precipitated by 10% trichloroacetic acid (TCA); the nitrogen content, determined by the Kjeldahl method, was multiplied by a factor of 6.25 and reported as crude protein.

Total protein and albumin were determined by spectrophotometric methods from a lyophilized sample, using a concentration of 100 mg/ml for each determination.

Total protein was also determined by the Biuret reaction, based on the interaction between copper and peptide bonds of proteins. This gave a purple color with an absorption maximum at 540 nm (16).

Total albumin was found from the reaction between albumin and Bromcresol green in a suitable buffer to form an Albumin-BCG complex with a blue color and an absorption maximum of 630 nm (8).

The lyophilized samples of Control I and Tests I and II beer contained different amounts of unfermented solids; (100 ml of Control I contained 4.25 g, Test I 3.40 g, and Test II 3.00 g). These weights were therefore used to calculate the results and relate them to the original samples.

Total globulin was calculated as the difference between the total protein (from TCA) and the albumin.

Peptide and free amino nitrogen were determined spectrophotometrically on a UV-VIS Spectrophotometer Varian Techtron, model 635, in the 321–570 nm region (9,29). Elemental C, H, N, Cl and S were determined by microanalysis.

The viscosity was measured with an Ostwald viscosimeter and calculated according to Kruger and Bielig (18) on the basis of one particular concentration of wort and beer. Chill haze (physical stability) was measured with the EBC hazemeter after one day at 40° C, followed by chilling to 0° C for 24 hr.

The foam stability was determined by the modified Carlsberg method as the Sigma value of beer (33,34). The exponential equation, which represents a unimolecular reaction, is applied to the settling of a static beer foam and used to obtain Sigma, the average lifetime of a bubble in the foam.

TABLE I
Analysis of Malt and of Sorghum Grits

Qualitative Indices	Malt	Sorghum
Total protein (%, db)	11.9	10.6
N, Kjeldahl (%, db)	1.80	1.54
N, Dumas (%, db)	1.38	1.53
C (%, db)	41.9	40.0
H (%, db)	6.59	6.82
Cl (%, db)	0.74	0.39
S (%, db)	0.31	0.30
Laboratory wort		
Extract, fgdb (%)	80.3	85.3
Extract, fine/coarse difference (%)	1.0	...
Soluble protein (% db)	5.5	...
Soluble N (mg/100 ml)	98.0	...
Free amino N (mg/100 ml)	20	...
Apparent attenuation, limit of fermentation (%)	71.4	...

RESULTS AND DISCUSSION

The following indices were determined by EBC Methods (9) but because they were within the prescribed limits of Control I, are not presented in the tables: for malt and sorghum—moisture, 1,000-kernel weight, and germination test; for laboratory wort—extract, color before and after boiling, pH, saccharification, filtration time, Hartong VZ 45, and ITT; for technological wort—extract, color, clarity, pH, saccharification, and aroma; for beer—apparent and real extract, original gravity, color, pH, saccharification, alcohol, diacetyl, iron, copper, oxygen, ITT, SO_2, CO_2, air, Asbach, Hartong, bitterness, and turbidity. In addition, individual amino acids were evaluated in the beers, using a Beckmann model 120/3 amino acid analyzer and the two-column procedure (21,38). Here, too, no differences outside the limits of experimental error were found, and the results are not reported in the tables.

Analytical data obtained on the malt and sorghum grits used in the experiments are given in Table I. The sorghum grits had somewhat lower protein content and substantially higher laboratory extract value than did the malt.

Analytical and process data other than for the nitrogenous constituents are shown in Table II for both the worts and resultant

TABLE II
Analyses of Commercial Worts and Beers[a]

	Wort				Beer			
Qualitative Indices	Control I	Control II	Test I	Test II	Control I	Control II	Test I	Test II
C (%, db)	41.07	37.85	38.39	38.68	39.10	39.66	39.97	39.40
H (%, db)	6.41	6.40	6.49	6.41	6.80	6.28	6.63	6.57
Cl (%, db)	0.54	0.35	0.69	0.69	1.20	0.93	1.07	0.97
S (%, db)	0.10	0.12	0.15	0.12	0.60	0.48	0.45	0.56
Viscosity (cP)	1.97	...	1.90	1.71	1.56	...	1.43	1.34
Apparent attenuation limit of fermentation (%)	69.0	...	72.0	72.5	74.0	84.9	85.8	87.0
Extract of sparge water (%)	0.97	...	0.46	0.37	...	...	...	...
Soluble extract of spent grain (%)	5.0	...	8.4	5.1	...	...	...	...
Polyphenols (mg/L)	156	168	161	165	155	161	153	149
Brewhouse yield (%)	69.3	...	70.3	70.5	...	...	...	...
Taste test (number of votes expressed for beer)	...	...	...	...	5	...	0	3
Anthocyanogens (mg/L)	40.0	45.4	42.4	44.6	30.6	30.6	28.8	28.2
Polymerization index	3.9	3.7	3.8	3.7	5.1	5.3	5.3	5.3
Mash filtration (hr)	4.1	...	3.9	3.4	...	...	...	...
Wort volume (hl)	427	...	430	438	...	...	...	...
Chill haze (EBC units)	...	...	...	...	1.1	0.8	0.8	0.7
Foam stability (Sigma)	...	...	...	...	94	122	115	125

[a] All brews are from a malt/sorghum mash, except for Control II, from an all-malt mash.

beers.

Microanalytical results indicated that the carbon and hydrogen values were comparable from wort to beer and similar to those shown for the brewing materials (Table I). However, differences were observed for chlorine and sulfur, which may have some significance.

Viscosity data for the worts of the malt/sorghum brews reflect the influence of enzyme addition during processing. From Test II, which had Cereflo 200 L added to the mash, a low beer viscosity of 1.34 was obtained. This lowered wort viscosity coincided with a substantial reduction in mash filtration time to less than 4 hr.

The apparent attenuation limit of fermentation was improved by the use of enzymes in the mash. This can be seen in Tests I and II worts and most dramatically in the Test I and II beers, where Fungamyl 800 L was present during fermentation and storage. The value of 87% is regarded as somewhat high for lager beer. However, the 74% for the Control I beer is lower than desired. A more restricted level of enzyme addition should provide the desired level of 80–82%.

The quantity of anthocyanogens and polyphenols was somewhat lower in the Test I and II beers than in the Control II beer, no doubt because of the use of sorghum grits and hop extract in the test brews. Values in all samples were lower than would be anticipated in regular beer production, about 220 mg/L (18). The polymerization index for all samples is higher than would be expected from other reports on all-malt beers (3,6).

From an economics standpoint, the enzyme-treated mashes gave a 1% higher brewhouse yield than did Control I. With regard to beer quality, the enzyme-processed brews gave substantially better beer foam stability than did the Control I brew. However, when eight tasters compared the beers from the enzyme brews with beer from Control I for aroma, taste, and intensity and quality of bitterness, three of the tasters preferred the test product and five the control. This single test cannot be considered significant but does emphasize the need for continued attention to this important quality factor.

The nitrogenous content, by type, in the worts and beers is shown in Table III, with reference data given for Control II. Use of enzymes in brewing substantially increased both the wort and beer total nitrogen values over these from Control I but with levels still substantially lower than those found for Control II. Free amino nitrogen was also increased and, in this instance, reached levels similar to that of the all-malt control. On the other hand, coagulable nitrogen was not significantly increased over the Control I wort or beer and remained relatively low compared with Control II.

Further examination of the nitrogenous constituents of the four beers showed that albumose nitrogen and peptide nitrogen fractions were increased by the enzyme treatment; in the case of peptide N, the increase was to values above that of Control II. TCA-precipitable protein, protein found by the Biuret method, albumin, and globulin values were all increased markedly over the Control I beer values by the enzyme treatment. This was particularly apparent for the TCA protein, the Biuret protein, and albumin. Because the Test I and Test II beers and worts are quite similar in nitrogen and protein patterns, the major effect is obviously that of the mash treatment, presumably the activity of the proteinase Neutrase 1.5 S.

SUMMARY

The influence of the enzymes on mashing, on wort fermentation and beer storage processes, and on beer quality itself was examined in the National Brewery Ltd. Addition of the proteolytic enzyme, Neutrase, to the mash, in addition to other enzyme supplements, resulted in the increased nitrogen content of the wort and the beer. The increase in the low molecular weight nitrogenous compounds, such as amino acids (free amino N), albumoses, and peptides may have a decisive role in determining the taste, foam, stability, and chill sensitivity characteristics of beer.

An imbalance in the nitrogenous compounds may also lead to a darker colored beer and to physical, chemical, and taste instability; the production of these compounds must therefore be carefully regulated (23,24).

With the use of enzymes, the amount of fermentable carbohydrates (estimated by the apparent attenuation limit) in the wort and beer also increased greatly.

Taste differences were noticeable in the product, but no statistically significant preference was obtained.

The advantages of enzyme addition in the brewing process are seen in the increased amounts of soluble nitrogen-containing compounds and fermentable carbohydrates, the decreased viscosity leading to higher filtration rates, the 1% increase in yield, and in the possibility of using larger amounts of the cheaper unmalted adjuncts to obtain a similar product.

This has been the first stage in the study of enzyme use in the brewing process. Future studies are planned to consider the effects of an increase in the relative amounts of unmalted adjuncts and variation in the proportions of enzymes obtained from a number of sources.

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TABLE III
Nitrogen Content[a] of Commercial Worts and Beers[b]

	Wort				Beer			
Qualitative Indices	Control I	Control II	Test I	Test II	Control I	Control II	Test I	Test II
N, Kjeldahl (%, db)	1.43	2.03	1.79	1.72	0.74	1.26	0.98	1.02
N, Dumas (%, db)	1.39	1.89	1.65	1.54	0.65	1.06	0.80	0.80
Soluble N	82.4	123.2	92.8	92.1	60.5	83.3	72.3	67.9
Free amino N	20	24	23	23	9	12	12	11
Coagulable N	3.0	12.7	2.8	3.2	0.8	1.8	1.0	0.8
Albumose N	...	...	...	...	4.5	6.1	4.9	5.0
Peptide N	...	...	...	...	16.1	18.5	20.6	20.9
Protein (TCA)	...	...	...	...	28.1	115.0	86.7	87.5
Protein (Biuret)	...	...	...	...	32.4	118.7	88.4	89.0
Albumin	...	...	...	...	11.7	76.8	64.9	66.0
Globulin	...	...	...	...	16.4	38.1	21.8	21.5

[a]All values given are in mg/100 ml, except for the Kjeldahl and Dumas N.
[b]All brews are from a malt/sorghum mash, except for Control II, from an all-malt mash.

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