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Proteins and amino acids in beers, their contents and relationships with other analytical data

S. Gorinstein [a,*,1], M. Zemser [a], F. Vargas-Albores [b], J-L. Ochoa [b], O. Paredes-Lopez [c], Ch. Scheler [d], J. Salnikow [e], O. Martin-Belloso [f], S. Trakhtenberg [g]

[a]*Department of Pharmaceutical Chemistry, School of Pharmacy, The Hebrew University-Hadassah Medical School, POB 12065, Jerusalem 91120, Israel*
[b]*Division de Biologia Experimental, Centro de Investigaciones Biologicas del Noroeste SC, PO Box 128, La Paz, Baja California Sur, 23000 Mexico*
[c]*Departamento de Biotecnologia y Bioquimica, Centro de Investigacion y de Estudios Avanzados del IPN, Unidad Irapuato, Apdo. Postal 629, 36500, Irapuato, Gto, Mexico*
[d]*Wittman Institute of Technology and Analysis of Biomolecules, Teltow, Germany*
[e]*Institut fur Biochemie und Molekulare Biologie, Technische Universitat Berlin, Berlin, Germany*
[f]*Department of Food Technology, University of Lleida, Alcalde Rovira Roure 177, 25198 Lleida, Spain*
[g]*Kaplan Medical Center, Rehovot, Israel*

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Abstract

Fluorometry, ion-exchange chromatography, electrophoretic separations and Fourier transform–infrared (FT–IR) spectra were used to determine and characterize amino acids and proteins in 15 different beer samples. Proteins precipitated by ammonium sulfate yielded complex electrophoretic patterns. The major bands corresponded to 45–40 kDa as determined by a two-dimensional gel electrophoresis (2-DE). Proteins and some amino acids are partially responsible for nutritional value and stability of beer. Therefore, electrophoretic analysis revealed that protein characterization of beer during all technological stages might be useful in its quality. FT–IR protein spectra showed the presence of I, II and III amide bands. Protein distribution and amino acid composition of beer differ significantly, depending on the raw materials and enzymatic reactions used in beer technology. Concentrations of histamine (3.02–3.23 mg/l), proline (1.60–3.13 mg/l) and tyramine (3.61–7.4 mg/l) increased during beer fermentation. Statistically significant change was registered in the protein content of the final product, which was less than that in wort ($p < 0.005$). Levels of tyramine and proline, which were higher than in wort ($p < 0.025$) showed significant changes. This investigation shows that, in Israeli, Mexican and Brazilian beers, the contents of protein and amino acids are in accordance with the international standards.
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Keywords: Beer; Fluorescence; Amino acids; Protein extraction; Electrophoretic separation

1. Introduction

Nutritional value and stability are the most important qualities of alcoholic beverages. Many different compounds affect the properties of beer (Asano, Shinagawa, & Hashimoto, 1983; Cerutti, Finoli, Peluzzi, & Vecchio, 1985; Outtrup, 1989), but first and foremost are the alcohol and protein contents. It has been shown that the stability of beer and its organoleptic characteristics depend on the interaction between proteins, amino acids and polyphenols (Gorinstein et al., 1990; McMurrough, Hennigan, & Cleary, 1985; Outtrup, 1989; Yokoi & Tsugita, 1988). The amounts of proteins, amino acids and zinc in beer are related to the technological process of its preparation, especially during fermentation. Proline and lysine are the most important amino acids in beer. It is known that proline residues are responsible for the affinity towards proanthocyanidins (Outtrup, 1989) and take part in the production of aromatic compounds and, in this way, influence beer quality. Histamine and tyramine are biogenic amines found in various foods and beverages. It was observed that high histamine and tyramine contents in foods lead to toxicological problems known as "histamine intoxication"

* Corresponding author. Tel.: +972-2675-8690; fax: +972-2641-0740.
E-mail address: gorin@cc..huji.ac.il (S. Gorinstein)
[1] Author is affiliated with the David R. Bloom Center for Pharmacy.

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and can cause "food migraines" and hypertensive crises (Crook, 1981; Ramantanis, Fabbender, & Wenzer, 1984; Taylor, 1985; Youdim, Bonham, Sandler, Hanington, & Wilkinson, 1971; Zee, Simard, & Desmarias, 1981). Also, hypertensive crises may occur in patients under treatment with mono-amino-oxidase-inhibiting drugs (MAOI) after ingesting foods with high contents of histamine (Blackwell, 1963; Youdim et al.).

As far as is known, there are no studies on the changes in amino acid and protein contents of Israeli beers during technological stages (wort, fermented wort, green beer and final product) of beer making. Therefore it was decided to study the dynamics of the changes in amino acid and protein contents and see whether these contents are in the accordance with agreed international standards. Beers brewed in Israel, and trade beer samples from Mexico and Brazil, have been studied and compared. Beer composition was evaluated at all technological stages, and in different batches, in order to determine whether or not there was a uniformity in the contents of these substances.

2. Materials and methods

2.1. Materials

All reagents were of analytical grade. Deionized and distilled water was used throughout. All chemicals were purchased from Sigma Chemical Co.

2.2. Sample preparation

Fifteen beer samples collected at different stages of technological preparation were investigated in this study. The Maccabee and Goldstar beer samples were produced by Tempo Beer Industries. Mexican and Brazilian commercial beers were also studied and compared with Israeli ones. The beers were prepared using a standard industrial technological process with variations including different raw materials (Gorinstein, 1986; Gorinstein et al., 1990). Mashing was done by decoction. After boiling, the wort was transferred to vertical fermenters for 8 days fermentation. After fermentation, the green beer was removed to storage tanks. During 14 days of storage, potassium metabisulfite (2 g/hl) and protesal (5 g/hl) were added to the aging beer. In the first stage of filtration, kieselguhr (100 g/hl), and in the second stage, special polishing sheets were used. During filtration, the beer was carbonated additionally to the standard level of 5.0–5.5 g/l of CO_2. In addition to conventional methods, analytical analysis, including protein isolation and electrophoretic separation, spectrophotometry and fluorometry were applied for determination of proline, histamine and tyramine. Determination of the level of zinc present in the wort and beers was done by extrapolation of the standard after reading the zinc concentration by atomic absorption spectrophotometry at a wavelength of 213.9 nm (ASBC, 1974).

2.3. Protein content

The protein content of the samples was determined by the Lowry, Rosenbrough, Farr, and Randall (1951) and Bradford (1976) methods, using a Uvikon 930 spectrophotometer.

2.4. Fluorescence

Fluorescence measurements were done using a Model FP-770 Jasco spectrofluorometer. The temperature of the samples was maintained at 30°C with a thermostatically controlled circulating water bath. The lysine content was determined fluorimetrically according to Hesse, Römer, Miosga, and Wachtel (1987). A fluorescent complex between lysine and orthophthalaldehyde was formed. A spectrofluorometric reading was made at 340 nm of excitation and 442 nm of emission. Lysine HCl was used as a standard. Determinations of histamine and tyramine were carried out by the Vidal-Carou, Izquierdo-Pulido, and Marine-Font (1989) and Rivas-Gonsalo, Garcia-Moreno, Gomez-Cerro, and Marine-Font (1979) methods. Histamine was extracted from beer samples with *n*-butanol in an alkaline medium and transferred to 0.1 N hydrochloric acid. A fluorescent complex with *o*-phthalaldehyde solution was formed and fluorescence was measured at wavelengths of excitation 340 nm and of emission 430 nm. The standard solutions were prepared from histamine dihydrochloride. The extraction of tyramine with ethyl acetate in an alkaline medium from beer samples followed the transferring to 0.2 N HCl, and interaction with α-nitroso-β-naphthol and nitric acid containing 2% $NaNO_2$. Then fluorescence was read at wavelengths of excitation 465 nm and of emission 540 nm. Tyramine hydrochloride was used for calibration solutions.

Determination of proline was done according to Yokotsuka (1988), which included its oxidation with sodium hypochlorite and hydrogen peroxide before the reaction with *o*-phthalaldehyde in the presence of 2-mercaptoethanol and a high concentration of Brij 35 for fluorophore formation. Then the mixture was centrifuged at $1000\times \mathbf{g}$ for 5 min to remove a yellow precipitate. The fluorescence of the supernatant was measured at wavelengths of excitation 360 nm and emission 450 nm. Proline was used as a standard.

2.5. Protein extraction

High molecular weight fractions of beer samples were prepared according to Dale and Young (1988). Samples

of beer were placed in dialysis tubing and dialyzed against several changes of distilled water for 72 h. Then the material retained by the dialysis membrane was freeze-dried. In addition, beer proteins were extracted with a ratio of acetone to sample of 5:1 (v/w). The precipitate was freeze-dried and extracted with a solvent (55% 2-ProOH and 5% 2-ME): sample ratio 6:1 (w/v).

Proteins were also extracted using solid ammonium sulfate which was added to beer to obtain 80% relative saturation. The precipitate formed during 48 h at room temperature was collected by centrifugation (20 min at $7000 \times \boldsymbol{g}$), dialyzed, lyophilized before storage at 4°C until use. This fraction was designated as Ammonium Sulfate Protein Precipitate (ASPP).

2.6. Ion exchange chromatography

One and a half g of dialyzed and lyophilized crude beer samples were dissolved in 5 ml of THA (10 mM Tris-HCl + sodium azide 0.02%; pH 8.0) and dialyzed against 500 ml of the same buffer (3 times, 12–16 h, 10°C). The sample was put on the DEAE-Sepharose (Pharmacia Fine Chem.) column (53 ml) equilibrated with THA, and washed with the same buffer until the absorbance (280 nm) was near zero. The absorbed proteins were eluted with NaCl–THA (0.5 M NaCl dissolved in THA) by washing the column until the absorbance was near zero. The 2-ml fractions were collected and the absorbance at 280 nm was measured.

2.7. Electrophoretic separation

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was done as by Laemmli (1970) on acrylamide gradients of:

a. On precast commercial gels of 10–15% (PhastGel high density, Pharmacia) using the PhastSystem (Pharmacia) electrophoresis apparatus. The samples (400 ng) in 10 μl of sample buffer were put on the gels.

b. On homemade mini gels of 15%. The samples of 20 μg/μl protein were put on the gels.

M_r markers (Sigma) were from 14 to 70 kDa. The sample buffer contained 10 mM Tris-HCl, pH 8.0, 2.5% (w/v) SDS, 1 mM EDTA, 0.01% bromophenol blue, and in the case of reduced peptides, 5% (w/v) 2-mercaptoethanol (2-ME). Lyophilized crude extracts and extracted proteins from all beer samples were dissolved in this sample buffer. Then all prepared samples were put on gels. The run was done for 4 h; the gel was fixed and stained with Coomassie Brilliant Blue R-250 or silver-stained. The destaining was done as reported in the instructions in Pharmacia LKB (1985) and Van-Seuningen and Davril (1992).

c. 2-D electrophoresis was done following the procedure of Dale and Young (1988) and Otto et al. (1996). The sample was dissolved in lysine buffer containing 9 M urea, 70 mM DTT and 2% ampholyte (Servalyte 2-4) and applied in the first dimension for isoelectric focusing (IEF) in concentration of 33 μg/μl. Then SDS–PAGE in the second dimension was done.

2.8. FT–IR spectra

A Perkin–Elmer 2000 FT–IR spectrometer was used to record IR spectra. Lyophilized material was mixed with KBr and the pellet was pressed at 10,000 kg/cm^2 for 15 s.

2.9. Statistics

The results of this investigation are mean values of triplicates. In order to verify the statistical significance of the studied parameters the Student *tp* test was used. Mean (M), their confidence intervals (CI) and ± standard deviation (m) were calculated. Then according to the formula, $M_2 - M_1/\sqrt{m_1^2 + m_2^2}$, the value of t was defined. The table "Significance Limits of the Student Distribution" gave significance of p. The p values of < 0.05 were adopted as statistically significant.

3. Results and discussion

Conventional and fluorometric methods of analysis gave results for beers Maccabee, Goldstar, Tecate and Kaizer (Table 1). All these data were in the range of standard beers and corresponded with data of Delcour, Vanhamel, Moerman, and Vancraenenbroeck (1987).

The content of protein [mean values (M), standard deviations (m) and CI of means] was 9.16 ± 0.35, 8.06–10.26; 8.55 ± 0.27, 7.69–9.44; 8.5 ± 0.26, 7.67–9.33; and 6.37 ± 0.23, 5.64–7.10 g/l for wort, fermented wort, green beer and final product, respectively. The results of the changes in the main indices of beer which, are based on the $M \pm m$ values, are shown in Figs. 1, 3, 4, 6 and 7. The steady decline in the protein content during the process of beer preparation is presented in Fig. 1. The protein content in the final product was statistically lower than that during all stages of the technological process ($p < 0.005$ for wort, fermented wort and green beer).

3.1. Fluorescence

One of the many calibration curves, which have been used for the determination of the content of amino acids, is shown in Fig. 2. The content of histamine ($M \pm m$ and CI of M) was 3.04 ± 0.38, 1.83–4.25; 3.20 ± 0.35, 2.09–4.31; 3.63 ± 0.39, 2.39–4.87 and 3.23 ± 0.30, 2.28–4.18 mg/l for wort, fermented wort, green beer and final product, respectively. During all

Table 1
Changes in chemical composition of beer during technological process

Technological stages	Amino acids (mg/l)					
	Protein (g/l)	Histamine	Tyramine	Proline	Lysine	Zn (mg/l)
Wort[a]	9.16 ± 0.35	3.04 ± 0.38	4.97 ± 0.12	1.60 ± 0.45	0.167 ± 0.10	0.120 ± 1.80
Wort[b]	11.4 ± 0.42	5.58 ± 0.41	5.83 ± 0.19	5.10 ± 0.39	0.188 ± 0.12	0.138 ± 1.40
F. Wort[ad]	8.55 ± 0.15	3.20 ± 0.35	9.40 ± 0.39	2.76 ± 0.25	0.171 ± 0.08	0.109 ± 0.29
F. Wort[bd]	9.32 ± 0.26	5.44 ± 0.27	10.6 ± 0.51	5.81 ± 0.28	0.198 ± 0.15	0.033 ± 0.41
Green Beer[a]	8.50 ± 0.20	3.63 ± 0.50	9.20 ± 0.28	4.00 ± 0.34	0.150 ± 0.32	0.182 ± 0.32
Green Beer[b]	8.12 ± 0.33	5.69 ± 0.15	10.15 ± 0.47	6.36 ± 0.42	0.160 ± 0.28	0.199 ± 0.94
Beer M.[ac]	6.37 ± 0.59	3.23 ± 0.30	6.34 ± 0.21	3.13 ± 0.47	0.180 ± 0.21	0.103 ± 0.24
Beer M.[be]	5.45 ± 0.45	5.25 ± 0.47	6.87 ± 0.38	4.44 ± 0.38	0.193 ± 0.17	0.168 ± 0.82
Beer G.[af]	5.62 ± 0.48	3.50 ± 0.45	6.27 ± 0.25	3.80 ± 0.30	0.161 ± 0.09	0.151 ± 0.28
Beer G.[bf]	6.36 ± 0.39	5.75 ± 0.38	6.64 ± 0.29	4.54 ± 0.36	0.175 ± 0.24	0.286 ± 0.72
Control[g]	4.94 ± 0.42	2.88 ± 0.37	4.70 ± 0.83	1.43 ± 0.30	0.180 ± 0.07	0.124 ± 0.36
Control A[h]	4.73 ± 0.45	2.13 ± 0.29	4.20 ± 0.26	1.24 ± 0.27	0.150 ± 0.04	0.112 ± 0.48
Control B[i]	4.34 ± 0.20	3.13 ± 0.40	5.10 ± 0.38	1.16 ± 0.36	0.1855 ± 0.07	0.122 ± 0.18
Control C.[j]	4.07 ± 0.35	5.50 ± 0.43	8.44 ± 0.64	1.31 ± 0.40	0.154 ± 0.31	0.221 ± 0.13
Control C[k]	4.18 ± 0.22	4.01 ± 0.31	6.07 ± 0.23	1.81 ± 0.31	0.137 ± 0.21	0.142 ± 0.37

[a] Beer Maccabee, prepared under normal industrial conditions in 1992–1993.
[b] Beer Maccabee prepared under normal industrial conditions in 1993–1994.
[c] Mean ± standard deviation (eight measurements).
[d] Fermented wort.
[e] Beer Maccabee (filtration).
[f] Beer Goldstar (filtration).
[g] Beer Maccabee, prepared under normal industrial conditions in 1987, Control[g].
[h] Control A = Control[g] + horizontal fermentation.
[i] Control B = ControlA + PVPP.
[j] Control C = Mexican trade beer Tecate.
[k] Control C = Brazilian trade beer Kaizer.

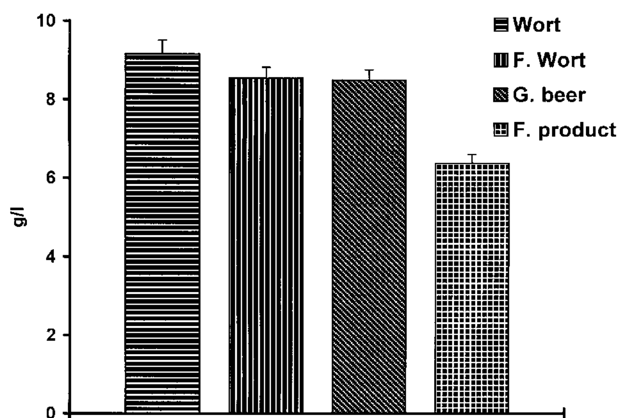

Fig. 1. Protein content in wort, fermented wort, green beer and beer (final product). Mean ± standard deviation (vertical lines).

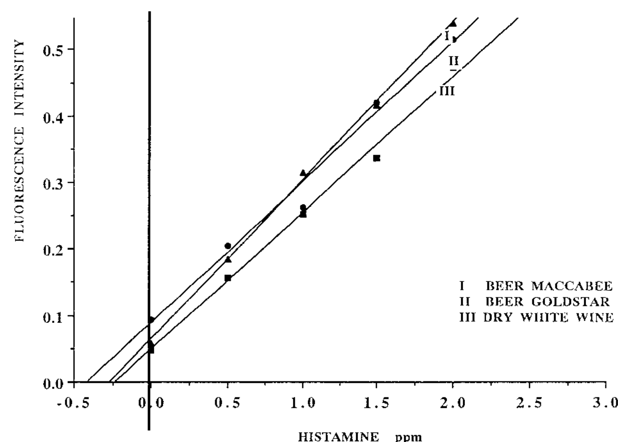

Fig. 2. Calibration curve for determination of histamine content: I, beer Maccabee; II, beer Goldstar; III, dry white wine.

stages of beer preparation there was not a statistically significant change in the histamine content (Fig. 3). The final product, in comparison to the histamine content in all three studied stages, gave the following results: $p < 0.4$, 0.4875 and 0.25 for wort, fermented wort and green beer, respectively.

The content of tyramine ($M \pm m$ and CI of M) was 4.97 ± 0.32, 3.95–599; 9.40 ± 0.39, 8.16–10.64; 9.20 ± 0.38, 7.99–10.41 and 6.34 ± 0.31, 5.36–7.32 mg/l for wort, fermented wort, green beer and final product, respectively. The changes in tyramine content in all stages of beer preparation are shown in Fig. 4. The tyramine content, which was increased in two stages (fermented wort and green beer) in comparison to the wort, declined in the final product but remained significantly higher than in wort ($p < 0.025$).

The content of proline ($M \pm m$ and CI of M) was 1.6 ± 0.21, 0.93–2.27; 2.76 ± 0.25, 2.03–3.49; 4.00 ± 0.34, 2.92–5.08 and 3.13 ± 0.3, 2.18–4.08 mg/l for wort, fermented wort, green beer and final product, respectively.

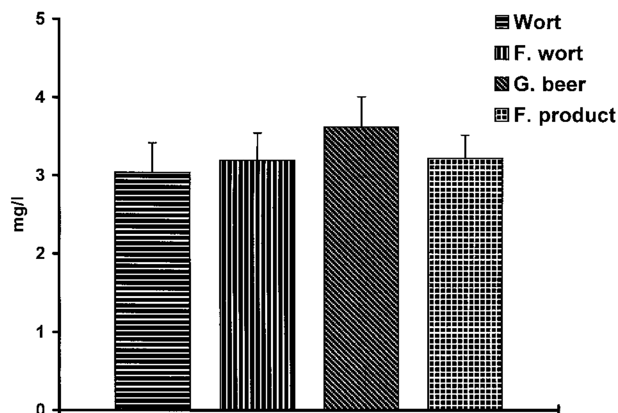

Fig. 3. Histamine content in wort, fermented wort, green beer and beer (final product). Mean ± standard deviation (vertical lines).

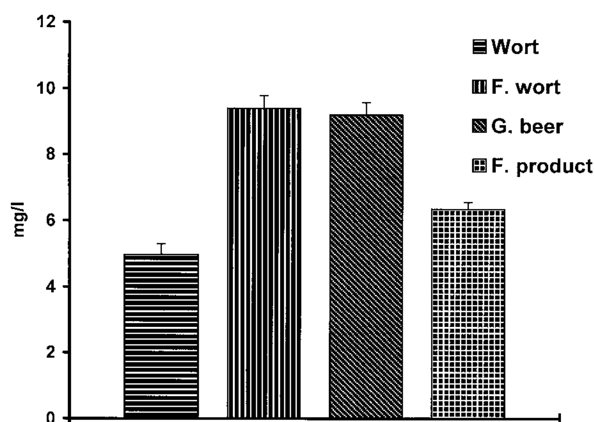

Fig. 4. Tyramine content in wort, fermented wort, green beer and beer (final product). Mean ± standard deviation (vertical lines).

Changes in proline content of beer during the technological process (Fig. 5) confirm that its maximum corresponds to the fermentation and storage stages. Filtration decreases its quantity. There are no data for proline content in the beer literature. Obtained values of proline concentration are one order of magnitude less than for wine (Yokotsuka, 1988). Proline content increased at the second and third stages of manufacture (Fig. 6). The content of proline in the final product, in comparison to the wort, decreased but remained significantly higher than in wort ($p < 0.025$).

The content of lysine ($M \pm m$ and CI of M) was 0.167 ± 0.03, 0.077–0.257; 0.171 ± 0.04, 0.041–0.3; 0.150 ± 0.03, 0.06–0.24 and 0.18 ± 0.04, 0.05–0.31 mg/l for wort, fermented wort, green beer and final product, respectively. Fig. 7 illustrates the changes in lysine content during all stages of beer preparation. There was a small increase of lysine content in the final product in comparison to the wort, but this increase was statistically not significant ($p < 0.4875$).

As stated, the quality and stability of beer are related to the quantity of protein and certain amino acids. It has been established that the amount of protein and

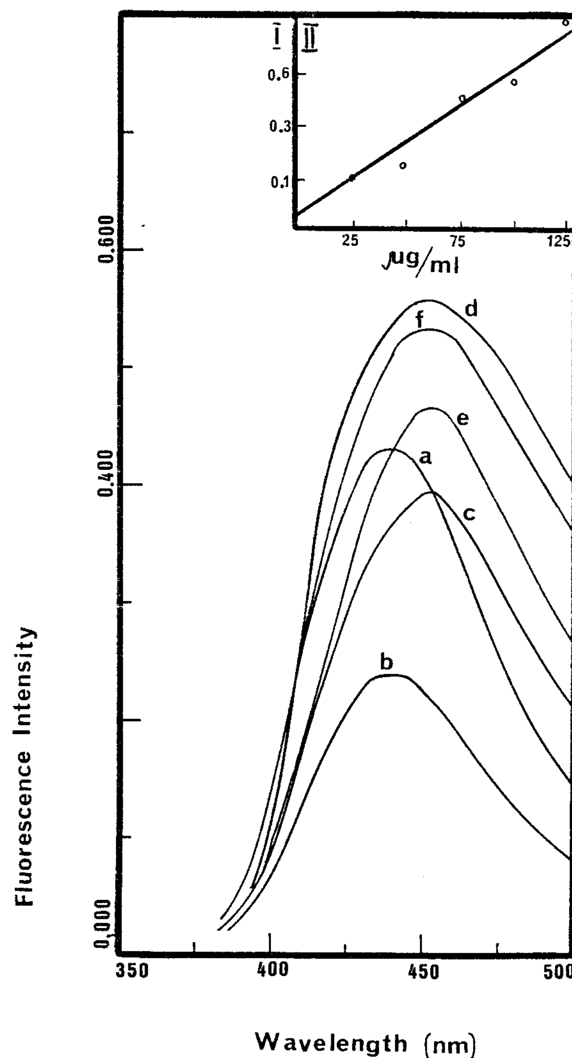

Fig. 5. Fluorescence emission spectra of the changes in the proline content of beer during the brewing process. Excitation, 360 nm. I: a, refers to the standard sample of proline with concentration of 150 mg/ml; b, c, d, e and f, correspond to the proline concentrations in wort, fermentation, storage and filtration of Maccabee and Goldstar, respectively. Dilutions of product 1:20 were examined. II: Insert: calibration curve of proline.

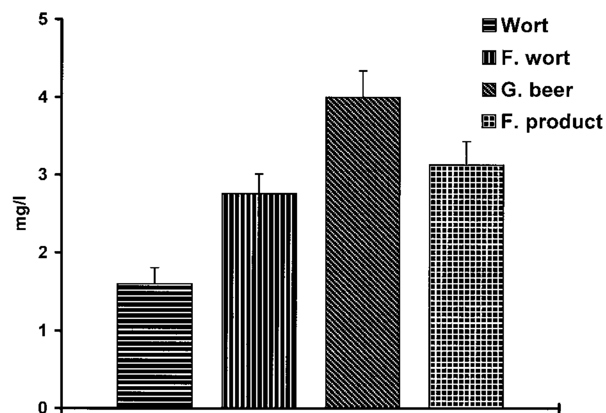

Fig. 6. Proline content in wort, fermented wort, green beer and beer (final product). Mean ± standard deviation (vertical lines).

amino acids in beer depends on the technological process of beer making (Asano et al., 1983; Belleau & Dadic, 1981). However, up to now there are no comprehensive studies of the quantities of protein and important amino acids during the stages of beer making or in the final product. Therefore, we investigated the amount of protein, histamine, tyramine, proline and lysine in wort, fermented wort, green beer and final product. The contents of protein, histamine, tyramine, proline and lysine changed throughout the stages of the technological process of beer making. The changes of the contents of protein, tyramine and proline are statistically significant, and the contents of all studied substances in the final product are in accordance with the accepted international standards. The main increase, in tyramine content, was observed during fermentation and storage (Vidal-Carou, Codony-Salcedo, & Marine-Font, 1990). This could be related to yeast activity during fermentation and to nonspecific microbial contaminations (Lactobacillus and Pediococcus) during beer storage. Tyramine is known to induce amine release from platelets. Beer contains 0.63–0.74 μg/ml, which is unlikely to account for the different [14C]-hydroxytryptamine (5HT)-releasing properties of these beverages. The final filtration decreased the level of amino acids in comparison to reports by other authors (Izquierdo-Pulido, Vidal-Carou, & Marine-Font, 1991; Thalacker, 1982). The histamine values found in Israeli beers, Maccabee and Goldstar were 3.50 and 5.75 mg/l (Chen & Van Gheluve, 1979). These data are in agreement with all known levels. Levels were too low to produce toxicological effects. At the present time no maximum tolerable limits have been set for histamine and tyramine in beers. But some authors have indicated the level of 10 mg/l histamine as a "maximum tolerable level" which may cause toxical effects (Izquierdo-Pulido et al.; Thalacker, 1982; Zee et al., 1981). In all samples of Spanish beer, histamine contents were less than 5 mg/l. Comparative data from different European beers are similar except "Pilsen"-types, which were about 17.0 mg/l.

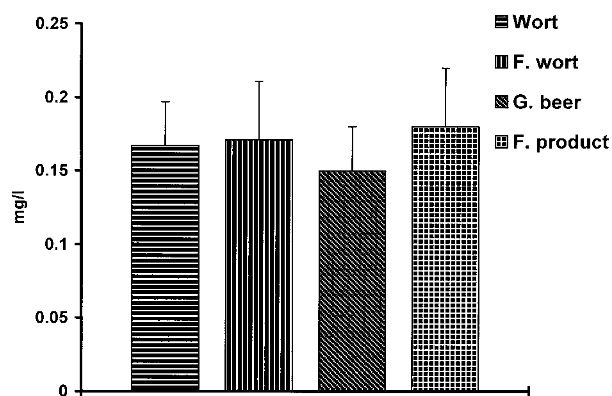

Fig. 7. Lysine content in wort, fermented wort, green beer and beer (final product). Mean ± standard deviation (vertical lines).

Considerable decreases in the protein content occur during filtration as summarized in Table 1. It may be concluded that results fit in the acceptable range, but it is not only superfluity of protein that provokes instability of beer. Colloidal turbidity results from interaction between polyphenols and proteins (Asano et al., 1983; Belleau & Dadic, 1981; Delcour et al., 1987; Outtrup, 1989). The same amount of protein in the presence of a small quantity of polyphenols may give a stable product (Belleau & Dadic, 1981; Gorinstein et al., 1990).

3.2. Ion exchange chromatography

The results of chromatography on DEAE-Sepharose for Israeli and Mexican beers (Fig. 8) showed that part of the beer crude extract is not absorbed to the column (Peak A). The main protein content is retained and could be eluted with NaCl (Peak B), and the yield was about 91.3% (Table 2). Peaks A and B contain the color of the crude extract, but peak A was more intensive in color than peak B. Peak C, which was eluted from the column by 0.1 M HCl, was colorless. The two patterns, which are shown in the Fig. 8, are different. Much more color was found in peak A1 in comparison with A. This can be explained by the differences in the raw materials used. All ion-exchange chromatography patterns of beer

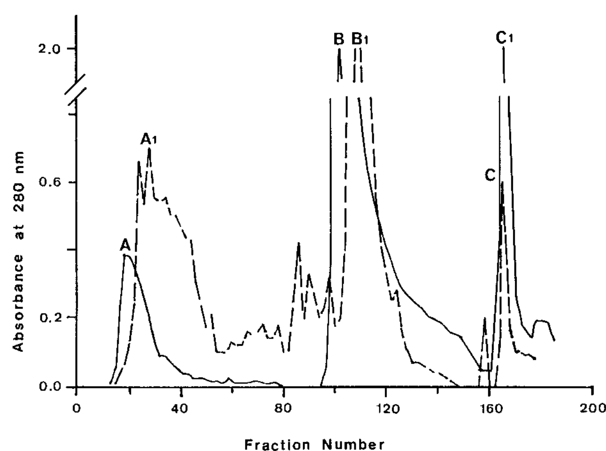

Fig. 8. DEAE-sepharose chromatographic patterns of crude beer extracts from (——), Israeli Maccabee; (- - -), Mexican Tecate. Peaks of the contents of protein recovery are: A, B, C from Israeli Maccabee; A_1, B_1, C_1 from Mexican Tecate.

Table 2
Protein content and percentage of recovery by DEAE-sepharose chromatography

	Volume (ml)	Total protein (mg)	Yield (%)
Crude extract	5	26.00	100
Peak A	19	1.69	6.5
Peak B	25	23.75	91.3
Peak C	17	< 0.005[a]	0.0

[a] According to sensitivity of the Bradford method.

crude extracts investigated in this study were different from those, shown in Fig. 8. The variations were found in the intensities of colors in peaks A and B (data are not shown).

3.3. Electrophoretic separation

Peaks A and B and the proteins from crude beer samples (Israeli, Mexican and Brazilian) were applied to SDS–PAGE before and after reduction with 2-ME. The Maccabee peak B (Fig. 8, peak B), Maccabee beer sample, Tecate peak B_1 (Fig. 8, peak B_1); Tecate beer and Kaizer beer showed similar bands. Reduced beer samples migrated in several bands with an apparent molecular weight of about 10–14, 20, 24, 35 and (the main band) at 40–42 kDa. The unreduced samples showed that the main protein material remained in one band of 14 kDa. Detection of proteins was very low because only 1.69 mg of protein was recovered (peak A, Table 2). The peaks B and B_1 showed three bands: two at 10–16 and the main one at 40 kDa. Some of the bands were diffused. There was a similarity between ASPP and acetone patterns. All the patterns had two bands in common, of 14 kDa and 40 kDa. The protein spots of peak B spots were concentrated mostly in the mass of 40–45, 30–37 and 10–15 kDa. Some proteins are present at the extreme acid end of the gel (pH 2–2.5). But most of isoelectric points (pI) were in the range of 3.50–5.0. The results showed that proteins from beer samples were totally consistent with one another (as for the main fraction of 40–45, 30–37 and 10–15 kDa). These results are in accordance with our previous work (Gorinstein et al., 1990) and with some other authors (Dale & Young, 1988; Yokoi and Tsugita, 1988). The electrophoretic analysis of beer proteins produced at different periods (1987 and 1992–1994) showed similar patterns. Protein composition in SDS–PAGE patterns did not differ by the year of beer production.

3.4. FT–IR spectra

Infrared spectra of beer samples: ASPP from (A) Maccabee beer and (B) the crude sample are shown in Fig. 9. The samples show similar bands at 3373 cm^{-1} (amino acid peak) and at 2936 cm^{-1} (-CH_2 stretching vibrations). Peaks at 3373 and 3320 cm^{-1} are similar in the shown two samples. Amide I (A I), Amide II (A II) and Amide III (A III) bands (in the range of 1650, 1530, and 1300–1250 cm^{-1}) are typical for ASPP (Kaiden, Matsui, & Tanaka, 1987). The peaks at 2936 and 2934, 1658, 1653 cm^{-1} were found in two samples (Fig. 9, positions A and B). Notable is the absence in the crude sample (Fig. 9, position B) of the bands at 1541 and 1243 cm^{-1}, corresponding to Amides II and III type bonds, respectively. Absence of the

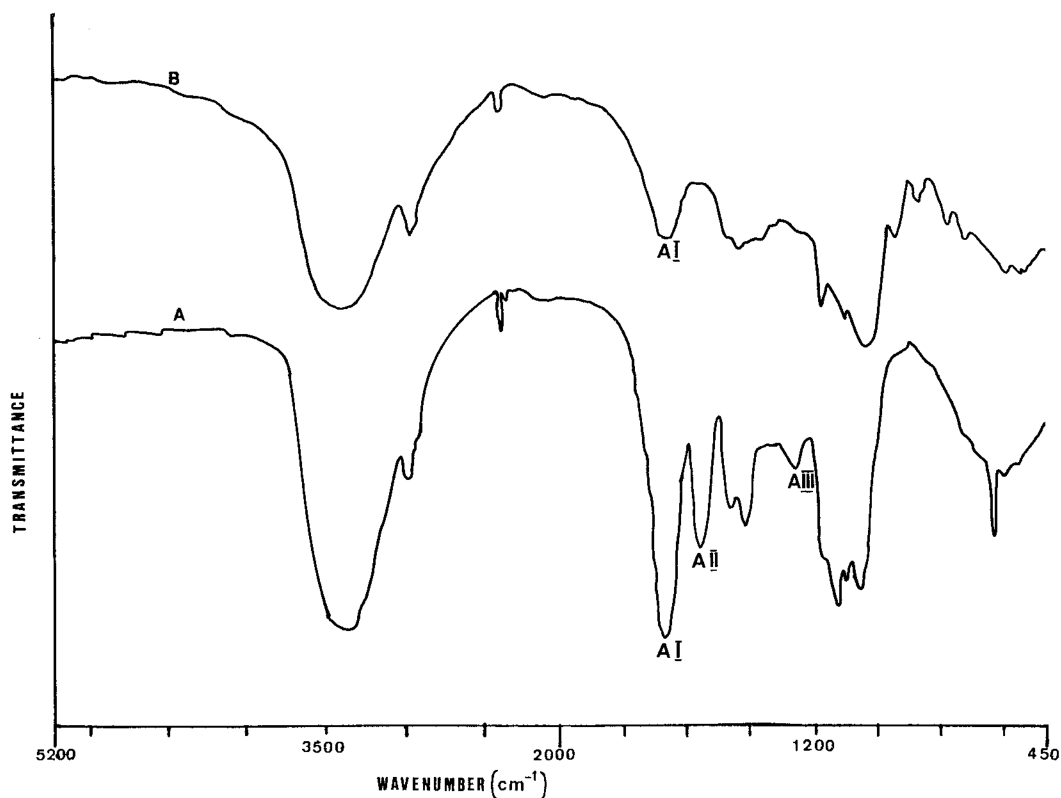

Fig. 9. FT–IR spectra of beer: (A) ammonium sulfate-precipitable proteins; (B) crude dialyzed and lyophilized sample. A I, A II and A III, amide I, II and III bands, respectively.

Amide II and Amide III bands can be seen in the crude sample but the displacement of band positions also appeared in some other beer samples.

4. Conclusion

Fluorometric parameters, FT–IR spectra and electrophoretic mobility were measured to elucidate the technological changes in beer samples. Proteins showed major bands corresponding to 45–40 kDa. The amounts of proteins and amino acids depend on the materials and technology used for beer preparation. These substances are partially responsible for nutritional value and stability of beer. Significant changes were registered in the protein content of the final product, which was less than in wort, and in the levels of tyramine and proline, which were higher than in wort. The comparative study of different beers showed that the amount of protein and some acids were within the limits and corresponded with other investigations. FT–IR confirmed the typical protein spectra in beer samples with the presence of amide I, II and III bands.

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