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Proteins of beer affect lipid levels in rats

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Abstract

Consumption of dry matter of alcoholic beverages leads to improved lipid metabolism and increased antioxidant activity in experiments on rats. Proteins and amino acids are part of the dry matter. Are proteins and amino acids playing a role in these changes? Amino acid analysis, electrophoretic separation and Fourier transform-infrared spectra (FT-IR) were used to determine and characterize proteins and amino acids in beer and white wine. The contents of total proteins, albumin and of most studied amino acids in beer were significantly higher than in white wine ($P < 0.05–0.0005$). Thirty-six rats were divided in 3 groups, each 12. The rats of the Control group were fed basal diet (BD) only and the BD of the two experimental groups (B and WW) was supplemented with lyophilized, polyphenol-free beer and white wine, respectively. Before and after completion of the 4 weeks feeding period, total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol, triglycerides (TG) and lipid peroxides (LP) were examined. Only in the group of rats (B) fed diet, supplemented with beer a significant decrease in the level of TC, LDL-C and TG was observed ($P < 0.05$, 0.05 and 0.005, respectively). No differences in the level of LP in all 3 groups were found. Therefore, only diet supplemented with lyophilized, polyphenol-free beer, which has significantly higher concentration of proteins and essential amino acids than white wine does affect the level of

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plasma lipids in rats.

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1. Introduction

In most Western countries alcohol beverages are an integral part of diets [1]. They consist about 4 to 6% of the average energy intake [2]. In the last years some authors proposed special diets in order to prevent development of atherosclerosis, one of the most dangerous diseases of our time [3,4]. Epidemiological, experimental and clinical investigations show that such diets, supplemented with various kinds of alcoholic beverages have a positive influence on prevention of atherosclerosis [5–7]. It was demonstrated that consumption of moderate quantities of alcoholic beverages leads to improved lipid metabolism and to increased antioxidant activity, which is mainly attributed to polyphenols of their dry matter [8–11]. However the dry matter of alcoholic beverages contains not only polyphenols, but also proteins and amino acids. It is known that proteins and essential amino acids of foods are supplying the required building blocks for protein biosynthesis of human [12]. But some authors claim that these substances have much wider biological functions [13,14]. A well documented experiment shows that casein or whey protein fed to piglets during the suckling period affects blood lipid levels, HMG CoA reductase activity, glucagon, cortisol, and weight gain [15]. Therefore, it cannot be excluded that proteins and essential amino acids of the dry matter of alcoholic beverages are biologically active. In our early experiments with Maccabee beer and white wine we did not take into consideration proteins of alcoholic beverages [8,9]. Are they playing a certain role in improving lipid metabolism and antioxidant activity? In order to answer this question Goldstar beer with a high content of proteins and white wine were chosen. These two beverages affect plasma lipids and antioxidant activity in rats [8,9]. Quantitative content and qualitative characteristics of proteins and essential amino acids in beer and white wine were studied and compared for their influence on the plasma lipids and lipid peroxides in rats. It was shown that phenolics of dry matter of beer and white wine affect plasma lipids and lipid peroxides in rats [8,9]. In order to exclude the influence of alcohol and phenolics we used lyophilized, polyphenol-free beer and lyophilized polyphenol-free white wine. As far as we know there are no such studies.

2. Materials and methods

2.1. Chemicals

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

2.2. Beer and white wine samples

The Goldstar beer samples were produced by Tempo Beer Industries, Natania, Israel. The samples of Savignon Blanc white wine of Carmel Wine Corporation were used. Original beer and white wine samples were rich in total polyphenols (345 and 436 mg/L respectively) and essential phenolics: ferulic, gallic and *p*-coumaric acids, procyanidins, epicatechin and quercetin [16]. However, lyophilized, polyphenol-free beer and lyophilized polyphenol-free white wine were used.

2.3. Protein extraction

High molecular weight fraction of beer and white wine samples were prepared according to Dale and Young [17]. Samples were placed in dialysis tubing and dialyzed against several changes of distilled water for 72 h. The material retained by the dialysis membrane was freeze-dried. In addition, beer and white wine proteins were extracted with acetone (sample ratio 5:1, v/w). The precipitate was freeze-dried and extracted with a solvent [55% 2-isopropanol (2-ProOH) and 5% 2-mercapthoethanol (2-ME): sample ratio 6:1, v/w].

2.4. The protein content

Proteins were determined according to Lowry et al. [18] and Bradford [19], using Uvikon 930 spectrophotometer. Albumin concentrations were measured colorimetrically at 628nm or 600 nm using Sigma diagnostic kits 631–2 and 625–2, respectively.

2.5. Amino acid content

Amino acid composition in bound form was done by the procedure of Spackman et al. [20]. Freeze-dried samples were hydrolyzed for 22–44-66 hr at 110^{o}C with 6M HCl in evacuated tubes with and without previous oxidation by performic acid. The vacuum-dried material was analyzed and applied on a Beckman 120C automatic amino acid analyzer. For tryptophan determination, samples were hydrolyzed with 4N LiOH for 20–24-28–36-40 hr at 110^{o}C following by treatment with 6M HCl for 22 hr at the same temperature.

2.6. Electrophoretic separation

The sample was dissolved in lysine buffer containing 9 M urea, 70mM DTT and 2% ampholyte (pI 3–10) and applied for isoelectric focusing (IEF) in concentration of 33μg/μl [16]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done according to Laemmli [21] on homemade acrylamide mini gels with gradient of 10–20%. The samples of 20 μg/μl protein were put on the gels. M_r markers (Sigma) were from 14 to 66 kDa. The sample buffer contained 10 mM Tris-HCl, pH 8.0, 2.5% (w/v) SDS, 1 mM EDTA, 0.01% bromophenolblue, and in the case of reduced peptides, 5% (w/v) 2-mercaptoethanol (2-ME).

Extracted proteins from beer and wine 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 and destained, as reported by Van-Seuningen and Davril [22].

2.7. FT-IR spectra

A Perkins 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 15s.

2.8. Animals and diets

36 male Wistar rats with standard weight of 120 g were used in this experiment. All rats were divided into 3 groups: 2 experimental (B and WW) and one control (CG), each of 12 animals. The rats were housed individually in stainless steel metabolic cages. All 3 groups were fed basal diet (BD), which included wheat starch, casein, soybean oil, and mineral and vitamin mixtures. The rats of CG were fed BD only. The dry matter of the studied alcoholic beverages was properly prepared and added daily to the BD of the B and the WW in quantity corresponding to 6.0 ml of beer and 2.0 ml of wine, respectively. Duration of experiment was 4 weeks [8,9].

As was mentioned, in order to test the influence of proteins and amino acids only lyophilized polyphenol-free beer and lyophilized polyphenol-free white wine were used. Polyphenols of these beverages were extracted with ethyl alcohol.

The diets were served once a day at 10 a. m. ad libitum together with the dry matter of the beverages introduced with distilled water by stomach intubation.

The energy of the BD supplemented with beverages for rats of the B and WW groups (397.3 to 401.7 kcal/100g of diet) and the energy of the BD for rats of CG (393.7 kcal/100g of diet) did not differ. The food intake was monitored daily and body gains – on a weekly basis.

Before and after completion of the four weeks feeding period, blood samples from the tail vein were drawn. After the centrifugation, plasma was removed and a wide range of laboratory tests was performed. These tests included total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and lipid peroxides (LP). TC, HDL-C and TG were determined enzymatically. TC and TG were measured as described by Trinder and Webster [22] with kits (PAP 100, # 6.122.4 and 6.123.6, respectively); HDL-C was determined by the same enzymatic methods after the precipitation of LDL-C and VLDL-cholesterol (VLDL-C) fractions with phosphotungstic acid in the presence of magnesium ions with kit (# 6.159.1) from Bio Merieux (Marcy l'Etoile, France). LP was determined colorimetrically [24] in direct reaction between methylene blue derivative MCDP, 10-N-Methyscarbamoyl-3,7-dimethylamino-10H-phenothiazine) catalyzed by hemoglobin using kit (9#CC-004) from Kamiya Biomedical Company. LDL-C was calculated according to the Friedewald formula [25].

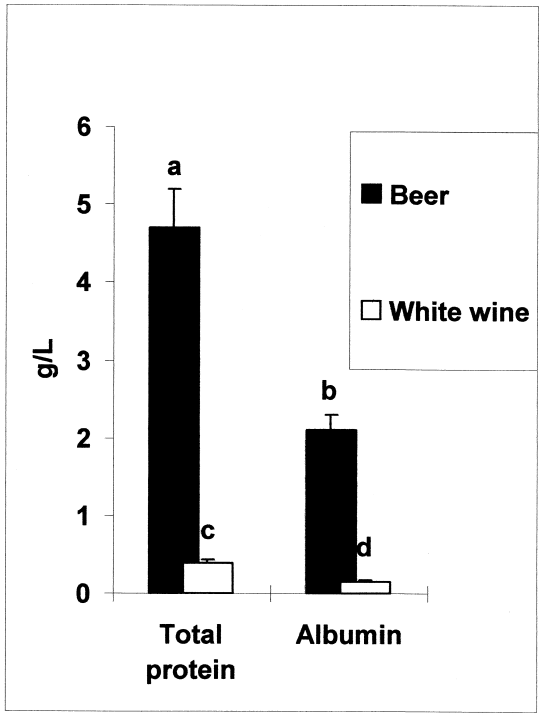

Fig. 1. Total proteins and albumin in beer and white wine. Means±SD (vertical lines). Bars with different letters are significantly different ($P < 0.05$).

2.9. Statistical analysis

To verify the statistical significance of all parameters the values of means, standard deviation ($M \pm SD$) and 95% CI of means were calculated. Where it was appropriate, data by 2-way ANOVA were tested. The P values of less than 0.05 were adopted as statistically significant. All following data are means of 5 measurements.

3. Results

The results of the investigation of proteins in Goldstar beer and in Savignon Blanc white wine are shown in Fig. 1. According to the Fig. 1, the contents of total proteins and albumin were significantly higher in beer than in white wine ($p < 0.0005$ in both cases).

The results of essential amino acid contents in Goldstar beer and in Savignon Blanc white wine are summarized in the Table 1. According to this Table, the contents of all studied amino acids (Table 1) were higher in beer than in white wine. But the differences were significant only for valine, methionine, isoleucine, leucine, phenylalanine, and histidine ($p < 0.05$; $p < 0.025$; $p < 0.005$; $p < 0.01$; $p < 0.01$ and $p < 0.05$, respectively).

The results of the qualitative studies of proteins in Goldstar beer and in Savignon Blanc

Table 1
Essential amino acids in white wine and beer (μmol/L)

Amino acids	White wine	Beer	P
Valine	136.5 ± 13.1 [102.9–170.1]	192.8 ± 20.5 [142.7–242.9]	<0.05
Methionine	26.7 ± 2.4 [20.5–32.9]	56.1 ± 5.6 [41.7–70.5]	<0.0025
Isoleucine	198.1 ± 17.1 [104.2–192.0]	269.7 ± 21.7 [213.9–325.5]	<0.005
Leucine	152.5 ± 15.2 [113.4–191.6]	264.8 ± 26.6 [206.7–322.9]	<0.01
Phenylalanine	66.6 ± 6.7 [49.4–83.8]	114.9 ± 11.6 [106.9–44.7]	<0.01
Histidine	85.7 ± 8.8 [63.1–108.3]	115.7 ± 9.6 [91.0–140.4]	<0.05
Lysine	198.4 ± 18.1 [151.9–244.9]	247.9 ± 20.9 [194.2–301.6]	<0.1
Arginine	217.9 ± 70.9 [164.2–271.6]	231.6 ± 22.1 [174.8–288.4]	<0.35
Tryptophan	5.1 ± 1.4 [1.5–8.7]	5.2 ± 1.4 [1.6–8.8]	<0.4875
Threonine	257.3 ± 26.6 [188.9–325.7]	261.1 ± 26.7 [192.5–329.7]	<0.475

Mean values, 95% CI of means and ± SD of 5 measurements.

white wine are shown in the Fig. 2 and Fig. 3. As can be seen (Fig. 2-I), the strongest protein bands were located in the acidic range about pH 4.0–5.0 or 3.75–4.55. Some proteins are present at the extreme acid end of the gel (pH 2.0–2.5), but most of the isoelectric points (pI) were in the range of 3.5–6.5. The results of the analytical isoelectric focusing correspond to the findings of other authors [17,26,27].

SDS-PAGE demonstrated that white wine proteins were separated with 4 fractions (Fig. 2-II, lane 3) with weak bands 28, 36 and 45 and average ones with 34 and 68 kDa [28].

Infrared spectra of proteins of beer (Fig. 3-II) and of white wine proteins (Fig. 3-I) show similar bands at 3373 cm^{-1} (amino acid peak) and at 2936 cm^{-1} (-CH_2 stretching vibrations). Peaks at 3373 cm^{-1} 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 cm^{-1}, 1530 cm^{-1} and 1300–1250 cm^{-1}) are typical for a protein spectrum [29].

The results of the growth of the rats of all 3 groups are summarized in the Table 2. According to Table 2, addition to BD of B and WW groups of lyophilized polyphenol-free beer and lyophilized polyphenol-free white wine respectively did not cause significant changes in diet intake, body gains of the animals and efficiency of diets.

In order to find out if proteins of the dry matter of beer and white wine influence the plasma lipid levels of the laboratory animals, a wide range of laboratory tests before and after completion of the trial were performed. Among these tests were determination of the plasma lipids and lipid peroxides.

At baseline the three groups did not differ from one another in the plasma lipid concentration and the level of LP (data not shown).

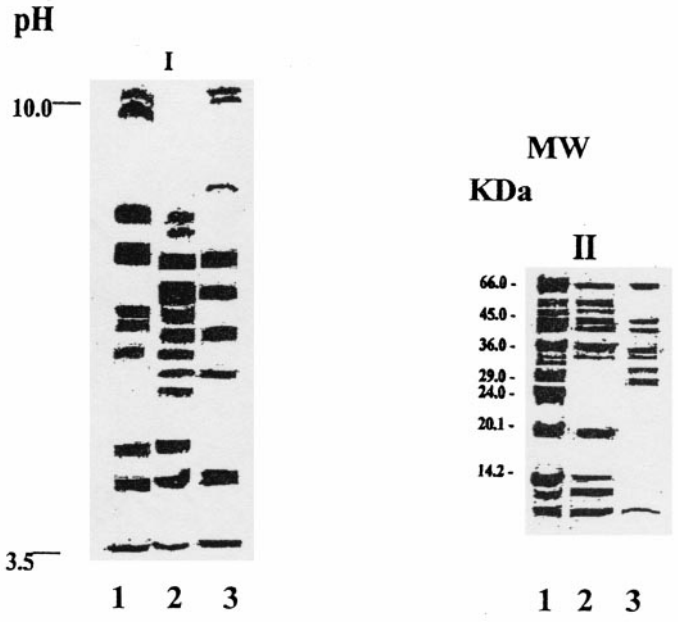

Fig. 2. Electrophoretic analyses of beer and white wine proteins. I – electrofocusing: lane 1, molecular marker; lane 2, 3 – proteins precipitated with acetone from beer and white wine. M_{Ip} markers had the following pH range: 3.5–10. II – SDS-PAGE separation on homemade mini gradient gels of 10–20%: lane 1 –molecular marker; lanes 2,3 - proteins precipitated with acetone from beer and white wine. M_w markers had the following sizes: 14.2; 20.1; 24; 29; 36; 45 and 66 kDa.

The results of the TC, LDL-C, HDL-C, TG and LP tests after completion of the investigation are summarized in Table 3.

After 4 weeks feeding period only in the groups of rats fed diet, supplemented with beer (B) a significant decrease in the level of TC, LDL-C and TG was registered ($P < 0.05$, 0.05 and 0.005, respectively). No significant changes in the level of lipids in WW and CG were observed. The level of lipid peroxides in all three groups remained unchanged.

4. Discussion

In the last years some authors propose diets in order to prevent development of atherosclerosis [3,4]. Epidemiological, experimental and clinical investigations indicate that such diets, supplemented with various kinds of alcoholic beverages have a positive influence on patients with coronary atherosclerosis [5–7,30].

It was shown that consumption of moderate quantities of beer and wine leads to a significant improvement in lipid metabolism, antioxidant and anticoagulant activities [1,6, 7,31].

Initially some authors suggested that the alcohol component of beverages is responsible for this phenomenon [2,32]. It was shown that biological activity of the alcoholic beverages

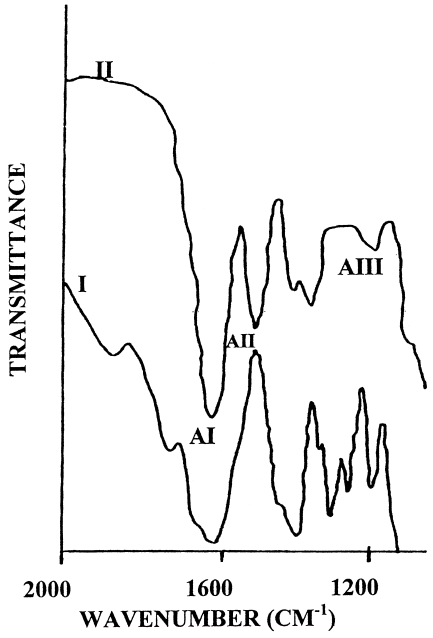

Fig. 3. FT-IR spectra of proteins from: (II), beer; (I), wine. A I, A II and A III - amide I, II and III bands, respectively.

is mainly connected to their dry matter rather than to the alcohol content [8–11]. Content of some phenolics rather than the quantity of total polyphenols determines biological activity of alcoholic beverages [9]. Lyophilized beverages exert the same positive influence like alcohol containing beverages [9,10].

The dry matter of alcoholic beverages contains not only polyphenols. Amino acids, peptides and proteins are important constituents of food in general and of alcoholic beverages in particular. It is common knowledge that amino acids of food supply the required building blocks for protein biosynthesis of human [12]. However some authors claim that these

Table 2
Diet intake, body gains and diet efficiency ratio

Groups	Av. intake of diet (g/4 weeks)	Av. body gain (g/4 weeks)	Diet efficiency ratio
B	373.1 ± 49.5	92.8 ± 29.1	0.248 ± 0.05
	[265.2–481.0]	[29.4–156.2]	[0.138–0.358]
WW	370.2 ± 52.4	91.4 ± 28.9	0.247 ± 0.05
	[256.0–484.4]	[28.4–154.4]	[0.137–0.357]
CG	369.3 ± 50.9	90.9 ± 29.2	0.246 ± 0.05
	[258.9–479.7]	[27.3–154.5]	[0.136–0.356]

Mean values, 95% CI of means and ±SD.

Abbreviations used: B, experimental group fed beer supplemented diet; CG, control group, WW, experimental group fed white wine supplemented diet.

Table 3
Total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides (mmol/L) and lipid peroxides (μmol/L)

	TC	LDL-C	HDL-C	TG	LP
Control	2.79 ± 0.11^a [2.55–3.03]	1.20 ± 0.08^a [1.03–1.37]	1.55 ± 0.09^a [1.35–1.75]	0.65 ± 0.04^a [0.56–0.74]	1.21 ± 0.09^a [1.01–1.41]
B	2.53 ± 0.10^b [2.31–2.75]	0.97 ± 0.08^b [0.80–1.14]	1.55 ± 0.09^b [1.35–1.75]	0.45 ± 0.04^b [0.36–0.54]	1.19 ± 0.08^a [1.02–1.37]
WW	2.79 ± 0.11^a [2.55–3.03]	1.19 ± 0.09^a [0.99–1.39]	1.56 ± 0.09^a [1.36–1.76]	0.64 ± 0.04^a [0.55–0.73]	1.20 ± 0.09^a [1.00–1.40]
ANOVA (P value)					
B	<0.05	<0.05	NS	<0.05	NS
WW	NS	NS	NS	NS	NS

Mean values, 95% CI of means and $\pm$ SD ($n = 12$).

Abbreviations used: B, experimental group fed beer supplemented diet; CG, control group; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; LP, lipid peroxides; NS, not significant; TC, total cholesterol; TG, triglycerides; WW, experimental group fed white wine supplemented diet. Means in a column not sharing the same superscripts are significantly different ($p \leq 0.05$).

compounds have much wider biological functions [13,14]. Recently it was shown in a well documented experiment that casein or whey protein fed to piglets during the suckling period affect blood lipid levels, HMG CoA reductase activity, glucagon, cortisol, and weight gain [15].

As was mentioned, in our early experiments with Maccabee beer we did not take into consideration proteins of this alcoholic beverage [8,9]. It is possible that proteins of the dry matter of alcoholic beverages do affect plasma lipids and lipid peroxides. In order to find an answer to this question, total proteins, and its water-soluble fraction - albumin and essential amino acids were investigated in Goldstar beer and white wine, which have a different degree of biological activity [8,9]. To exclude the influence of alcohol and phenolics lyophilized, polyphenol-free beer and lyophilized polyphenol-free white wine were used.

It is known that content of the studied compounds is influenced by some conditions, which include inter alia region, climate conditions, ripeness and some others. Therefore we knew that the results of our investigation would be slightly different of the results of other authors [12]. It was found that the contents of total proteins and albumin in Goldstar beer were significantly higher than in Savignon Blanc white wine. Also the contents of most of the essential amino acids (valine, methionine, isoleucine, leucine, phenylalanine, and histidine) in beer were significantly higher than in white wine.

As was mentioned in the Materials and Methods section, before feeding laboratory animals alcohol and polyphenols from beer and wine were removed. Therefore the influence of above-mentioned substances on lipid metabolism and antioxidant activity of laboratory animals was excluded.

After completion of the 4 weeks feeding period the lipid spectrum and lipid peroxides of the rats of experimental and control groups were examined. Only in the groups of rats fed diet, supplemented with beer (B) a significant decrease in the level of TC, LDL-C and TG was registered ($P < 0.05$, 0.05 and 0.005, respectively). No significant differences in the contents of lipid peroxides in experimental and control groups were observed.

As was mentioned, Larson et al., 1996 [15] have found that proteins affect lipid metabolism in laboratory animals. Our investigation does confirm the findings of Larson et al., 1996 [15], but only when the diet was supplemented with Goldstar beer, containing significantly higher level of proteins and essential amino acids than white wine.

In the last years some authors claim that proteins possess antioxidant properties [33,34]. They demonstrated that protein insufficiency aggravates the enhanced lipid peroxidation and reduced activities of antioxidative enzymes in rats fed diets high in polyunsaturated fat [33]. Protein effect on the antioxidant activity of phenolic compounds in a lecithin-liposome oxidation system was shown [34]. Our experiments did not confirm that proteins of alcoholic beverages possess antioxidant properties. Therefore, it is a need for further investigations.

In conclusion: 1. The contents of total proteins and albumin in beer are significantly higher than in white wine. 2. The contents of the most of the essential amino acids in beer are significantly higher than in white wine. 3. Only diet, supplemented with lyophilized, polyphenol free beer, which has a higher concentration of proteins and essential amino acids than white wine does affect the level of plasma lipids and leads to significant decrease in TC, LDL-C and TG in rats. 4. No differences in the level of LP in all 3 groups were found. 5. It can be suggested that only polyphenols of the dry matter of alcoholic beverages are able to improve the plasma antioxidant activity in rats.

References

[1] Renaud S, Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 1992;339:1523–6.

[2] Christiansen C, Thomsen C, Rasmussen D, Hauerslev C, Balle M, Hansen C, Hermansen, K. Effect of alcohol on glucose, insulin free fatty acids and triacylglycerol responses to a light meal in non insulin dependent diabetic subjects. Br J Nutr 1994;71:449–54.

[3] Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993;342:1007–11.

[4] Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaur I, Guidollet J, Touboul P, Delaye J. Mediterranean alpha-linolic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454–9.

[5] Thun MJ, Peto R, Lopez AD, Monaco JH, Henley SJ, Heath CW, Jr, Doll R. Alcohol consumption, and mortality among middle-aged, and elderly U. S. adults. New Engl J Med 1997;337:1763–4.

[6] Gorinstein S, Zemser M, Lichman I, Berebi A, Kleipfish A, Libman I, Trakhtenberg S, Caspi A. Moderate beer consumption and some positive biochemical changes in patients with coronary atherosclerosis. J Intern Med 1997;241:47–51.

[7] Gorinstein, S., Zemser, M., Berliner M, Goldstein R., Libman I, Trakhtenberg S, Caspi A. Moderate beer consumption and the blood coagulation in patients with coronary atherosclerosis. J Intern Med 1997;242: 219–24.

[8] Gorinstein S, Zemser M, Weisz M, Haruenkit R, Trakhtenberg S. The influence of dry matter of different alcoholic beverages on lipids, proteins, and antioxidant activity in serum of rats. J Nutr Biochem 1998;9: 131–5.

[9] Gorinstein S, Zemser M, Weisz M, Halevy SH, Martin-Belloso O, Trakhtenberg S. The influence of alcohol-containing and alcohol-free beverages on lipid levels and lipid peroxides in serum of rats. J Nutr Biochem 1988;9:682–6.

[10] Serafini M, Maiani G, Ferro-Luzzi A. Alcohol free red wine enhances plasma antioxidant capacity in humans. J Nutr 1998;128:1003–7.

[11] Mosinger B. Polyphenolics but not alcohol in beer and wine protect serum low-density lipoprotein against atherogenic modification. Cor Vasa 1994;4:171–4.
[12] Belitz HD, Grosch W. Amino acids, peptides, proteins. Food Chemistry. Belitz H. D., Grosch W., 2nd ed. Berlin Heidelberg New York London Paris Tokyo: Springer Verlag, 1999:8–88.
[13] Niazi AHK, Chaudhary MI, Malik MA. Effect of amino acids on the production of α-amylase by a submerged culture of Aspergillus oryzae. Pak J Biochem 1979;1:24–8.
[14] Morgan B. Protein and your body. Nutr Health 1986;8:1–6.
[15] Larson MR, Donovan S, Potter S. Effects of dietary protein source on cholesterol metabolism in neonatal pigs. Nutr Res 1996;16:1563–74.
[16] Gorinstein S, Caspi A, Zemser M, Trakhtenberg S. Comparative contents of some phenolics in beer, red and white wines. Nutr Res 2000;1:131–9.
[17] Dale CJ, Young TW. Fractionation of high molecular weight polypeptides from beer using two dimensional gel electrophoresis. J Inst Brew 1988;94:28–32.
[18] Lowry OH, Rosenbrough NJ, Farr, AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–70.
[19] Bradford MM. A rapid, and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[20] Spackman DH, Stein WH, Moore S. Automatic recording apparatus for the use in chromatography of amino acids. Anal Chem 1958;30:1190–206.
[21] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
[22] Van-Seuningen I, Davril M. A rapid periodic acid-schiff staining procedure for the detection of glycoproteins using *the* Phast System. Electrophoresis 1992;13:97–9.
[23] Trinder P, Webster D. Determination of HDL-cholesterol using 2, 4, 6- tribroma-3-hydroxybenzoic acid with a commercial CHOD-PAP reagent. Ann Clin Biochem 1984;21:430–3.
[24] Tateishi T, Yoshimine N, Kuzuya F. Serum lipid peroxide assayed by a new colorimetric method. Exp Gerontol 1887;22:103–11.
[25] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[26] Yokoi S, Tsugita A. Characterization of major proteins and peptides in beer. J Amer Soc Brew Chem 1988;46:99–103.
[27] Gorinstein S, Moshe R, Wolfe FH, Berliner M, Rotenstreich A, Tilis K. Characterization of stabilized and unstabilized beers. J Food Biochem 1990;14:161–72.
[28] Mesrob B, Gorinova N, Tsakov D. Characterization of the electrical properties and molecular weights of the proteins in white wines. Nahrung 1983;27:727–33.
[29] Kaiden K, Matsui T, Tanaka S. A study of the amide III band by FT-IR spectrometry of the secondary structure of albumin, myoglobin, and α-globulin. Appl Spectrosc 1987;42:180–4.
[30] Friedman LA, Kinball AW. Coronary artery disease and alcohol consumption in Framingham. Am J Epidemiol 1986;124:481–9.
[31] Frimpong NA, Lapp JA. Effects of moderate alcohol intake in fixed or variable amounts on concentration of serum lipids and liver enzymes in healthy young men. Am J Clin Nutr 1989;50:987–91.
[32] Croft KD, Puddey IB, Rakic V, Abu-Amsha R, Dimmit SB, Beilin LJ. Oxidative Susceptibility of Low-Density Lipoproteins - Influence of Regular Alcohol Use. Alcohol Clin Exp Res 1996;20:980–4.
[33] Huang CJ, Fwu ML. Protein insufficiency aggravates the enhanced lipid peroxidation and reduced activities of antioxidative enzymes in rats fed diets high in polyunsaturated fat. J Nutr 1992;122:1182–9.
[34] Heinonen M, Rein D, Satue-Gracia MT, Huang SW, German, JB, Frankel EN. Effect of protein on the antioxidant activity of phenolic compounds in a lecithin-liposome oxidation system. J Agric Food Chem 1998;46:917–22.