

## COMPARATIVE CONTENTS OF SOME PHENOLICS IN BEER, RED AND WHITE WINES

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### ABSTRACT

It is generally assumed that the higher the total polyphenols content of a beverage, the greater is its antioxidant activity. Our previous experiments on laboratory animals and clinical investigations showed that the content of total polyphenols is higher in white wine than in beer, but beer possessed a higher antioxidant activity. In order to find the sources, which determine the degree of the antioxidant activity the comparative content of some important phenolics in beer, red and white wines was examined. Total polyphenols, procyanidins, epicatechin, quercetin, ferulic, *p*-coumaric and gallic acids were determined in these beverages. The content of total polyphenols was significantly higher in red wine than in white wine and beer ( $p < 0.0025$  in both cases). Similar relationship was found for procyanidins, epicatechin, and quercetin, ferulic, *p*-coumaric and gallic acids ( $p < 0.0005$  in most cases). The contents of total polyphenols and quercetin were significantly higher in white wine than in beer ( $p < 0.0125$  and  $0.01$ , respectively). But the contents of procyanidins, epicatechin and ferulic acid were statistically significant higher in beer than in white wine ( $p < 0.005$ ,  $p < 0.05$  and  $p < 0.0025$  respectively). The higher contents of procyanidins, epicatechin and ferulic acid in beer is a possible explanation of the marked antioxidant activity of diets supplemented with this beverage rather than with white wine in our experiments on laboratory animals.

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### INTRODUCTION

Coronary artery disease (CAD) is the most dangerous disease of the second half of the XX century. The scientific community had proposed some measures for reducing the high level of morbidity and mortality from CAD. In the last two decades the mortality from this disease was

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declining in USA at a rate of 2% per year. But even now CAD is responsible for one of every three deaths in men as well as in women (1,2). Therefore it is not a surprise that efforts to control this disease are continuing. In past the main targets of this struggle were the major risk factors of atherosclerosis, and first of all the high level of cholesterol in blood, which is the anatomic basis of CAD. The fight against the high levels of lipids and especially cholesterol-rich low density lipoprotein cholesterol (LDL-C) and other major risk factors led to a success. The intensive multiple risk factor reduction has a beneficial effect on coronary atherosclerosis in men and women. It was shown that this disease regression is twice as frequent in the risk reduction group as in control group (3). But now the scientists are looking beyond cholesterol (4,5). Recent studies suggest that one of the most important mechanisms predisposing to development of atherosclerosis is the oxidation of LDL-C particles (6,7). There are evidences that oxidation of LDL-C enhances its atherogenicity and facilitates the penetration of lipids into arterial wall (4,5). It was established that phenolic substances inhibit the oxidation of LDL-C (8-10). It is known that polyphenols are a large family of natural compounds, which are from chemical point of view characterized by presence of one or more benzene-type ring (11). They are directly related to some characteristics of foods inter alia such as taste, palatability and nutritional value (11). Recent studies *in vitro* and *in vivo* show that only some polyphenols possess antioxidant properties (8,9,12-14). Among them are some phenolic acids and flavonoids such as *p*-coumaric and quercetin (14). A unique group of phenolic metabolites is tannin (11). The biologically most active among tannins is epicatechin. These phenols with relatively high molecular weight have antioxidant ability, which is 20 times stronger than vitamin E (15). As was mentioned, LDL-C, the "building material" for the atherosclerotic plaques, penetrates the arterial wall only after peroxidation. In order to prevent peroxidation or in other words to prevent the development of the future atherosclerotic plaques some authors in the last years propose diets rich in nutritional antioxidants (16-18). In most western countries alcohol beverages are an integral part of diet (19) and consist about 4 to 6% of the average energy intake (20). It is generally assumed that the higher the total polyphenols content of a beverage, the greater is its antioxidant activity (21). Our previous experiments on laboratory animals and clinical investigations did not support these claims (22-25). We conducted an experiment on 60 male Wistar rats with standard weight of 120 g each. All rats were divided into 4 equal in number groups: three experimental (EG1, EG2 and EG3) and one control (CG), each of 15 animals. The rats were fed basal diet (BD) consisting of 70.5% starch, 18% ovalbumin, 5% salt-mix, 5% sunflower oil, 1% cod liver oil, 0.3% choline chloride and 0.2% vitamins. All groups of rats during 4 weeks of the experiment were fed BD supplemented with dry red wine (EG1), beer (EG2) and dry white wine (EG3). The rats of the CG were fed BD only. Before and after completion of the experiment we performed a wide range of laboratory tests. These tests have included inter alia lipid peroxides (LP) as an indicator of the status of the antioxidant activity. Before the experiment there were no significant differences in the levels of LP in the EG1, EG2, EG3 and CG (1.21-1.22 nmol/L). After the experiment we noted a significant decrease in the levels of LP only in the EG1, EG2, EG3 (0.80; 0.81 and 1.01 nmol/L for red wine, beer and white wine respectively;  $p$  for EG1 and EG2 < 0.005; for EG3 < 0.05). The decrease in the level of LP in the EG2 fed diet supplemented with beer was significant greater than in EG3 fed diet supplemented with white wine ( $p$  < 0.01). And we registered these results in spite of the fact that total polyphenol content was significant higher in white wine than in beer (436.2 and 345.1 mg/L respectively;  $p$  < 0.0125). Therefore not only the quantitative content of total polyphenols must be taken in consideration. It is important to study the content of various phenolics in alcoholic beverages. Among the phenolic acids found in alcoholic beverages the most significant is the ferulic acid (26,27). But also procyanidins, epicatechin, quercetin, *p*-coumaric, and gallic acids are important constituents of total polyphenols. In order to find out the source of higher antioxidant activity of beer versus white wine it was decided to determine the contents not only of total polyphenols, but also of the above mentioned phenolics.

As far as we know there are no studies, which compare the influence of various phenolics in beer, red and white wines on the antioxidant activity of laboratory animals.

### MATERIALS AND METHODS

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

Sample Preparation. Tempo Beer Industries produced Maccabee beer samples, used in this study. This beer was prepared (cycles lasted between 21 and 28 days) using a standard industrial technological process. 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 metabisulfide (2 g/hl) and protosal (5 g/hl) were added to the aging beer. Filtration of beer was done as follow: in the first stage of filtration kieselgurh (100 g/hl) and in the second stage special polishing sheets were used. During filtration the beer was carbonated additionally to the standard level 5.0 - 5.5 g/l of CO<sub>2</sub>.

The wine samples of Chardonnay (dry white wine, alcohol 11.5%) and of Cabernet Sauvignon (dry red wine, alcohol 10.5%) were tested. Varieties of grapes were selected from different growing areas as the basic grapes for different technological treatments. All technological processes (crushing, fermentation, racking and fining) were carried out under industrial conditions using grapes for these wines (Carmel Wine Corporation at Rishon-Le-Zion, Israel).

Analytical procedures. The total polyphenols were determined colorimetrically using Folin-Ciocalteu method (28). Samples of wines and beer (100µL) filtered through a 0.45µm Millipore (type HA) were mixed with 900µL of distilled water and 5 ml of 0.2 N Folin-Ciocalteu reagent. Four milliliters of saturated sodium carbonate (75 g/L) were added to the mixture and then shaken. The absorbance of the solution at 765 nm was measured after 2 h with a Unikon 930 spectrophotometer. Quantitation was based on the standard curve of 100, 200, 300 and 500 mg/L gallic acid prepared at the same time.

Determination of phenolic acids was done spectrophotometrically and fluorometrically (29, 30). P-coumaric, ferulic and gallic acids were chosen as standards for fluorometric analysis. The standards were dissolved in 0.01 M ethanol. Then aqueous working solutions for calibration graphs. Wine and beer (degassed samples) were purified with n-hexane, extracted with ethyl acetate, evaporated to dryness and dissolved in 25 mL of water. Fluorescence emission spectra were measured using Model FP-770, Jasco-Spectrofluorometer. For each of the phenolic acids the following conditions were used: a) p-coumaric acid at wavelengths of excitation 330 nm and emission 443 nm; pH of the sample was adjusted to 10.7; b) ferulic acid at wavelengths of excitation 340 nm and of emission 460 nm; pH of the sample was 11.2; c) gallic acid at wavelengths of excitation 260 nm and of emission 357 nm; pH of the sample was 4.63. Procyanidins were isolated by Sephadex LH 20 from 10 mL of each sample. The procyanidins were eluted from the column with 15 mL of methanol. Epicatechin, catechin, quercetin and procyanidins were measured using Kontron UV spectrophotometer at scan range of 250-350 nm. Samples were dissolved in ethanol solutions. All procyanidins were expressed as catechin.

Statistical analysis. To verify the statistical significance of all parameters we calculated the values of means and standard deviation (M±SD) and 95% confidence intervals (CI) of means. Where it

was appropriate, data were tested by 2-way ANOVA. The  $p$  values of  $< 0.05$  were adopted as statistically significant. All following data are means of three measurements.

## RESULTS

The mean values of total polyphenols  $\pm$  SD for white wine, red wine and beer were  $436.2 \pm 16.2$ ,  $831.2 \pm 36.1$ ,  $345.1 \pm 12.1$  and their CI - 348.7-487.7, 716.3-946.1 and 306.6-383.6 mg/L respectively. The total polyphenols content in red wine was statistically significant higher than in white wine and in beer ( $p < 0.0025$  for both cases). The concentration of total polyphenols in white wine was significantly higher than in beer ( $p < 0.0125$ ).

The mean values of epicatechin for white wine, red wine and beer were 56.1, 195.1, 65.5 and their CI - 50.1-62.1, 175.4-214.8 and 58.8-72.2 mg/L respectively. The mean values of quercetin for white wine, red wine and beer were 1.29, 8.11, 0.95 and their CI - 1.13-1.45, 7.17-9.09 and 0.82-1.08 mg/L, respectively. The contents of epicatechin (epicat) and of quercetin (querc) are graphically shown in Fig 1.

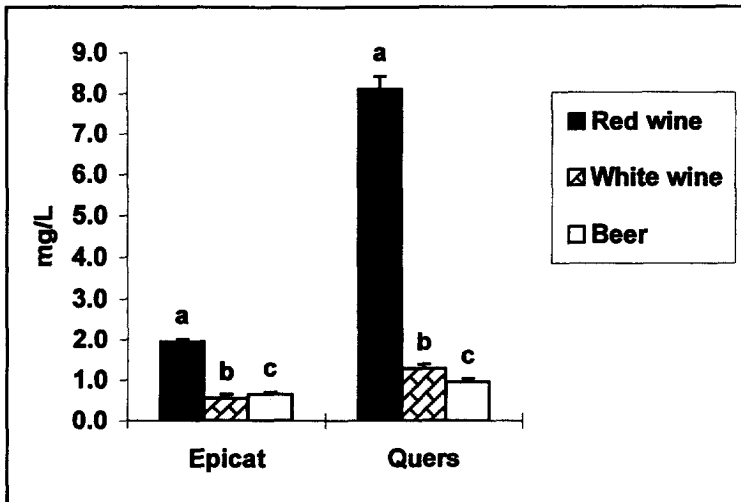


Figure 1.  $M \pm SD$  (vertical lines). Bars with different letters are significant different ( $p < 0.05$ ). Epicatechin content in red wine is significant higher than in white wine and in beer. The content of this phenolic is significant higher in beer than in white wine. The content of quercetin in red wine is significant higher than in white wine and in beer. The content of quercetin in white wine is significant higher than in beer.

The mean values of  $p$ -coumaric acid for white wine, red wine and beer were 2.2, 4.1, 2.1 and their CI - 1.85-2.25, 3.56-4.64 and 1.75-2.45 mg/L respectively.

The mean values of gallic acid for white wine, red wine and beer were 3.1, 5.6, 2.9 and their CI - 2.79-3.41, 5.09-6.19 and 2.59-3.21 mg/L, respectively.

The contents of p-coumaric and of gallic acids are graphically shown in Fig 2.

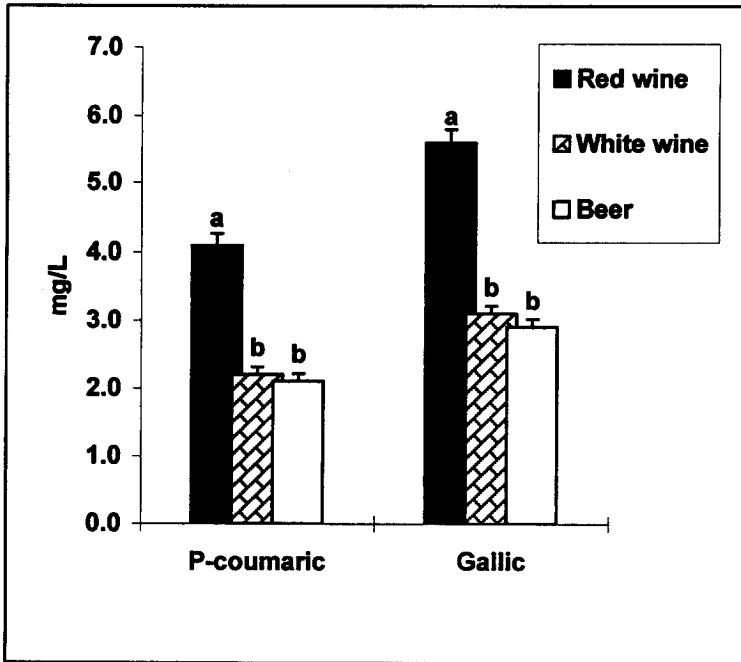


Figure 2.  $M \pm SD$  (vertical lines). Bars with different letters are significant different ( $p < 0.05$ ). P-coumaric acid content in red wine is significant higher than in white wine and in beer. The content of p-coumaric acid in white wine is higher than in beer but statistically not significant ( $p < 0.3$ ). The content of gallic acid is significant higher in red wine than in white wine and in beer. The content of this phenolic acid in white wine is higher than in beer but not significant ( $p < 0.35$ ).

The mean values of ferulic acid for white wine, red wine and beer were 3.9, 7.2, 6.8 and their CI - 3.39-4.41, 6.31-8.09 and 6.1-7.5 mg/L, respectively.

The mean values of procyanidins (Proanth) for white wine, red wine and beer were 49.9, 70.2, 62.3 and their CI - 44.8-55.0, 63.5-76.9 and 56.3-68.3 mg/L respectively. The contents of ferulic acid and procyanidins are graphically shown in Fig 3.

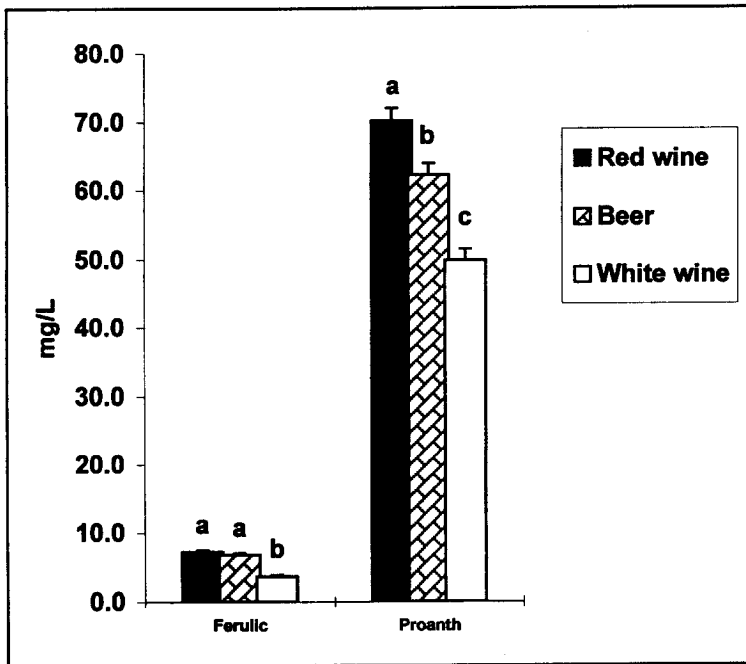


Figure 3.  $M \pm SD$  (vertical lines). Bars with different letters are significant different ( $p < 0.05$ ). The content of ferulic acid is significant higher in red wine than in white wine ( $p < 0.0025$ ) and statistically not significant higher than in beer ( $p < 0.1$ ). The content of this phenolic acid in beer is significant higher than in white wine. The content of the procyanidins is significant higher in red wine than in white wine and in beer. The content of the procyanidins in beer is significant higher than in white wine.

### DISCUSSION

It is well known that CAD is one of the most dangerous diseases in the Western industrialized countries. In the last couples of years a major emphasis was made on the positive influence of nutritional antioxidants, which prevent the peroxidation of the LDL-C and in this way hinder the penetration of this lipoprotein in arterial walls (10). Nutritional antioxidants are usually found in vegetables, fruits and alcoholic beverages (8,18). It was shown that there is a strong positive association between antioxidant intake and CAD mortality (16,17). It may be suggested that so-called French paradox be connected to the polyphenol content of wine rather than to alcohol concentration (22,23,29,31). And even more. According to some authors an increased susceptibility of LDL-C to oxidation was observed during high alcoholic consumption (21,32). It is generally assumed that the higher the total polyphenolic content of a beverage, the greater is its antioxidant activity (21). Therefore we supposed that the influence of diet supplemented with white wine on the antioxidant activity of rats would be higher than of diet supplemented with beer. But we found in our experiments on laboratory animals and in clinical investigations that in spite of higher

content of total polyphenols in white wine, a beer supplemented diet led to a marked antioxidant activity (22-25). This fact indicated that the antioxidant activity was connected not only to the concentration of total polyphenols. In order to reveal the possible reason of this finding we decided to study some important components of total polyphenols in beer and compare them with the contents of these substances in red and white wines. It is known that the content of the studied compounds is influenced by several conditions, which include inter alia the kind of grapes and barley, region, climate conditions, ripeness and some others. Therefore, in the investigation of phenolics the same wine and beer samples were used like in our experiment on laboratory animals. Only a few of phenolic compounds are considered to be biologically active (11). Therefore the contents only of total polyphenols, procyanidins, epicatechin, quercetin, ferulic, *p*-coumaric and gallic acids were studied. The results of this investigation show that the contents of procyanidins, epicatechin and ferulic acid were statistically significant higher in beer than in white wine. Therefore it can be supposed that the higher contents of procyanidins, epicatechin and ferulic acid in beer was the source of the marked antioxidant activity of diets supplemented with this beverage rather than with white wine in our experiments on laboratory animals and in clinical investigations.

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