M. Harmanescu, et all. Journal of Agroalimentary Processes and Technologies, Volume XII, No. 2 (2006), 371-376
Full Paper - Food Control Section

CORRELATION BETWEEN TOTAL ANTIOXIDANT CAPACITY AND POLYPHENOLS CONTENT FOR SOME BEER TYPES

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Abstract

The aim of the present work was to determine the total antioxidant capacity and polyphenols content for 10 types of beer from Romanian market. Total antioxidant capacity was analyzed using two methods: FRAP and DPPH. Total polyphenols content was determinated using Folin Ciocalteu reagent. The highest FRAP and DPPH values were identified for Stejar *type beer and the lower values for Bergenbier alcohol free beer. The polyphenols content is in good correlation with values obtained for total antioxidant capacity by FRAP and DPPH methods.*

***Key words**: total antioxidant capacity, FRAP and DPPH methods, total phenols, beer.*

Introduction

Some food components were recognized as protective agents in epidemiological studies in addition to their properties. This is the case of natural antioxidants content in food (Onate-jaén et al, 2005). Antioxidants are closely related with the prevention of degenerative illness, such as cardiovascular, neurological diseases, cancer and oxidative stress dysfunction (Bolck, 1992; Diplock, 1995).

Beer contains compounds with antioxidant properties such as reducing sugars, phenolic compounds, vitamins and Maillard reaction products. It is possible to measure individually these groups of compounds but this methodology may not accurately reflect their

combined action and the measurement of the total antioxidant activity is considered an important food property (Onate-jaén et al, 2005).

The determination of beer antioxidants capacity is not an easy task. Several methods are known to measure the total antioxidant capacity (TAC), but we tried the FRAP and DPPH assays.

Experimental

Reagents and equipment: All chemicals and reagents were analytical grade or purest quality purchased from Sigma, Merck, Aldrich and Fluka. Deionized water was used. Absorption determination for FRAP, DPPH and total polyphenols content was made using SPECORD 205 spectrophotometer by Analytik Jena.

Samples: The samples were obtained from a local store. In the present study were analyzed 10 types of beer: Timişoreana lux, Stejar, Ursus green, Ursus red, Stella Artois, Carlsberg, Heineken, Beck's, Tuborg Gold and Bergenbier alcohol free. All the samples were diluted 1/10 with deionized water.

Evaluation of total antioxidant capacity (TAC) by FRAP method: FRAP method depend upon the reduction of ferric tripyridyltriazine complex to the ferrous tripyridyltriazine by a reductant at low pH. This ferrous tripyridyltriazine complex has an intensive blue color and can be monitored at 593 nm (Benzie & Strain, 1996). Reagents: acetate buffer, 300mM/L, pH 3.6 (3.1g sodium acetate $3H_2O$ and 16 mL conc.; acetic acid per 1L of buffer solution); 10 mM/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM/L HCl; 20mM/L $FeCl_3$6H2O in distilled water. FRAP working solution: 25mL acetate buffer, 2.5mL TPTZ solution and 2.5 mL $FeCl_3$ solution. The working solution must be always freshly prepared. Aqueous solution of known Fe (II) concentration was used for calibration, in a range of 0.1-0.8 mM/L. For the preparation of calibration curve 0.5 mL aliquot of 0.1, 0.2, 0.4, 0.6, 0.8 μM/mL aqueous Fe(II) as Mohr salts solution were mixed with 2.5 mL FRAP working solution. FRAP reagent was used as blank. The absorption was read after 10 min. at 25°C and 593 nm. All determinations were repeated for three times. Total antioxidant capacity in beer was calculated in Fe (II) equivalents. Correlation coefficient (r^2) for calibration curve was 0.9978.

Evaluation of total antioxidant capacity (TAC) by DPPH method: Hydrogen atom – or electron-donation ability of the corresponding beer types was measured from the bleaching of the purple-colored ethanol solution of DPPH. This spectrophotometric assay uses stable 2.2'diphenylpicrylhydrazyl (DPPH) radical as reagent. 0.5 mL of various beer types were added to 2.5 mL of a 1 mM ethanol solution of DPPH. After 10 min. or 40 min. incubation at room temperature the absorbance was read against a blank at 517 nm. TAC as inhibition of DPPH free radical in percent was calculated in following way (Burits & Bucar, 2000; Cuendet et all, 1997):

$$TAC_{DPPH}\ (\%) = (A_{blank} - A_{sample}/A_{blank}) \cdot 100$$

The amount of total polyphenolic compounds: It was used the following reagents: 2.0M Folin-Ciocalteu phenol reagent, gallic acid and anhydrous carbonate. The content of total polyphenolic compounds in beer diluted 1/10 was determined by Folin-Ciocalteu method (1927). For the preparation of calibration curve 0.5 mL aliquot of 0.2, 0.4, 0.6, 0.8, 1.2 μM/mL aqueous gallic acid solution were mixed with 2.5 mL Folin-Ciocalteu reagent (diluted ten-fold) and 2 mL sodium carbonate (7.5%). The absorption was read after 2 h at 20°C, at 750 nm. All determinations were performed in triplicate. Total content of polyphenolic compounds in beer in Gallic acid equivalents (GAE) was calculated. Correlation coefficient (r^2) for calibration curve was 0.9954.

Results and Discussions

The total antioxidant capacity (TAC) by FRAP and DPPH methods (read after 10 min., respectively 40 min.) for analyzed beer samples are presented in Table1. The highest TAC values were identified for Stejar beer: 3.61 mM Fe^{2+}/L (for FRAP method) and 13.11 % (for DPPH read after 10 min.), respectively 17.49 % (for DPPH read after 40 min.). The lower TAC values (FRAP and DPPH methods) were obtained for Bergenbier alcohol free.

Table 1. The total antioxidant capacity (TAC) by FRAP and DPPH methods (read after 10 min., respectively 40 min.) for analyzed beer samples

Samples	TAC_{FRAP} (mM Fe^{2+} /L)	TAC_{DPPH} after 10 min. (%)	TAC_{DPPH} after 40 min. (%)
Timisoreana lux, alc.5%, EP 11.5%	2.43	3.60	5.60
Stejar-TM, alc.7%, EP 14.5%	3.61	13.11	17.49
Ursus green, alc.4.5%, EP 11%	2.80	7.27	10.12
Ursus red, alc.5%, EP 11.6%	3.05	7.50	10.12
Stella Artois, alc.5.2%, EP 11.8%	2.68	2.78	6.17
Carlsberg, alc.5.4%, EP 12%	2.92	6.00	10.12
Heineken, alc. 5%, EP 11.4%	2.49	6.00	10.12
Beck's, alc. 5%, EP 11.2%	2.55	4.61	6.99
Tuborg Gold, alc.5%, EP 11.7%	3.21	11.86	15.74
Bergenbier alcohol free,EP 6.5%	1.46	0.01	0.13

The same order of data was obtained for polyphenols content and is presented in Table 2.

Table 2. The polyphenols content for analyzed beer samples

Samples	Polyphenols (mM gallic acid/L)
Timisoreana lux, alc. 5%, EP 11.5%	2.05
Stejar-TM, alc. 7%, EP 14.5%	2.98
Ursus green, alc. 4.5%, EP 11%	2.24
Ursus red, alc. 5%, EP 11.6%	2.50
Stella Artois alc. 5.2%, EP 11.8%	2.49
Carlsberg, alc. 5,4%, EP 12%	2.60
Heineken, alc 5%, EP 11.4%	2.00
Beck's, alc. 5%, EP 11.2%	2.30
Tuborg Gold, alc.5%, EP 11,7%	2.69
Bergenbier alcohol free, EP 6,5%	1.49

The values obtained for total antioxidant capacity (TAC) using FRAP and DPPH methods are in good correlation. The correlation

coefficient for TAC_{DPPH} read after 40 min. ($r^2 = 0.9307$) is better than TAC_{DPPH} read after 10 min. ($r^2 = 0.90$). The fitting curve of correlation between TAC_{FRAP} and $TAC_{DPPH\ 40\ min.}$ is presented in Figure 1.

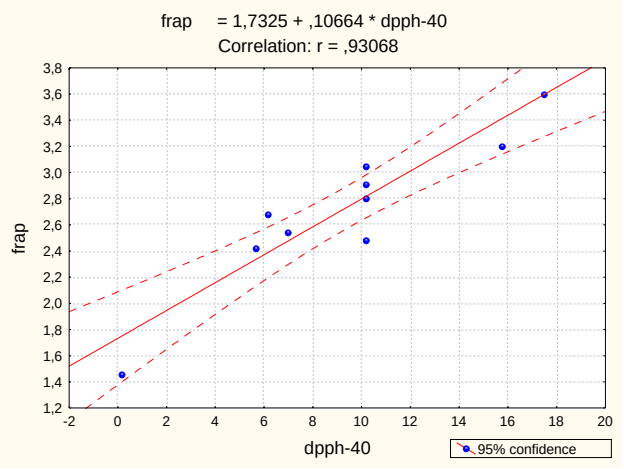

Fig. 1. Correlation between TAC_{FRAP} and $TAC_{DPPH\ 40}$

TAC_{FRAP} is very good correlated with polyphenols content. The fitting curve of this correlation is presented in Figure 2.

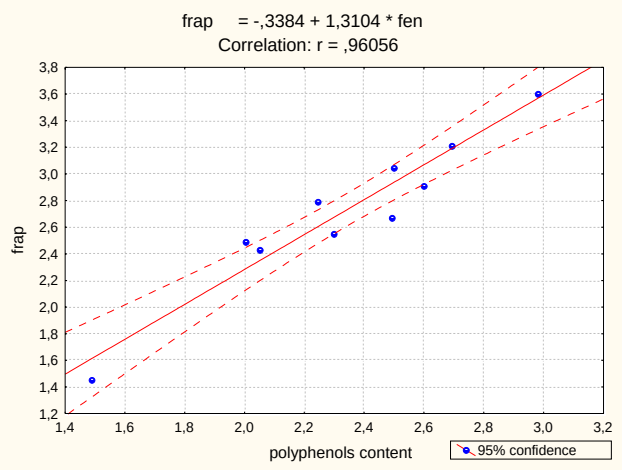

Fig. 2. Correlation between TAC_{FRAP} and polyphenols content

The good correlation between the two methods used for TAC determination (FRAP and DPPH, Figure 1) can be explain by the

similar mechanism of compounds with antioxidant activity present in beer. By FRAP method can be determinated the compounds with reducing activity and by DPPH method can be determinated the compounds with free radical scavenging activity. The polyphenols compounds from beer present both these properties. They are a very good reducing agent and in the same time present a very good free radical scavenging activity [Onate-jaén et all, 2006]. The good correlation obtained between TAC_{FRAP} and polyphenols content (Figure 2) confirms also the previous observation.

Conclusions

For all beer types TAC values (by FRAP or DPPH methods) were in good correlation with polyphenols content. These compounds with antioxidant activity from beer present both reducing and radical scavenging activity. The values for polyphenols, TAC_{FRAP} and TAC_{DPPH} were in the same range. No high difference was observed between the alcoholic beer types. Only alcohol free beer presents lower antioxidant and radical scavenging activities.

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