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Antioxidant Capacity of a Turkish Traditional Alcoholic Drink, Raki

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Raki is an aniseed flavoured traditional Turkish alcoholic drink. Antioxidant capacity of raki samples from different commercial brands were evaluated by CUPRAC, DPPH, TEAC and ORAC assays and correlations between these assays and total phenolic content were also investigated. Additionally, the one-way ANOVA and Bonferroni tests were performed to compare differences between values of the samples. Results indicated that different raki samples exhibited different antioxidant capacity and total phenolic content. The mean antioxidant capacity values of samples were in the order of: ORAC>TEAC>CUPRAC>DPPH. The correlations of total phenolic content of samples with their CUPRAC, TEAC and ORAC results were found statistically significant, while DPPH assay showed no significant correlation.

INTRODUCTION

Raki is an aniseed flavoured distilled spirit that is widely consumed in Turkey. It is often served with seafoods or meze. Raki is described as a spirit that is produced by double distillation of suma or suma mixed with agricultural based ethanol and flavouring it with aniseed (*Pimpinella anisum*) in the Turkish Food Codex [Anli & Bayram, 2010]. The main raw material of raki is suma, which is a distillate with a maximum 94.5% ethanol content. Raisins, molasses and/or grape must are used for suma production [Yucesoy & Ozen, 2013]. In the raki production process, traditional copper alembics with a maximum capacity of 5000 L are used for distillation of suma. After the distillation, distillate is diluted to 45% alcohol. Finally, sugar is added to end product in order to sweeten raki and it is stored for ageing at least one month before bottling [Anli & Bayram, 2010].

Many *in vitro* assays have been conducted to evaluate the antioxidant capacity of food products and drinks [Pellegrini *et al*., 2003; Li *et al*., 2005; Zulueta *et al*., 2007, 2009a; Schwarz *et al*., 2009]. However, determination of antioxidant capacity of a particular sample cannot be performed accurately by any single assay [Ozyurek *et al*., 2011; Bernaert *et al*., 2012]. Consequently, at least two assays should be used in order to assess antioxidant capacity accurately [Li *et al*., 2011; Meng *et al*., 2011].

Plants are an important source of natural antioxidants. It was reported that aniseed had strong antioxidant activity [Gülçın *et al*., 2003]. Fu *et al*. [2011] demonstrated antioxidant capacity of grape samples. The use of grapes and/or grape based material, aniseed to produce raki may contribute to the ingestion of naturally occurring antioxidant compounds. However, the antioxidant properties of raki have not been reported elsewhere. Therefore, antioxidant capacity of raki samples from different commercial brands were analysed in this research and four different assays were tested for antioxidant capacity determination to provide a more reliable investigation. In addition, total phenolic content was also analysed to evaluate correlations between total phenolic content and antioxidant capacity.

MATERIAL AND METHODS

Samples

Seven different commonly consumed raki samples of different brands were purchased from local supermarkets and stored in their original bottles at 4°C. Samples were classified and coded according to their raw materials used in the production: Raki samples produced from dried type grapes (A, B, E, F), fresh type grapes (C, G) and fresh-dried type grapes (D, which is a combination of fresh and dried type grapes).

Antioxidant capacity assays

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity was determined using the method described by Tafulo *et al*. [2010] with some modifications. Added amount of sample and DPPH reagent, reaction time modifications were applied to the method. Briefly, 0.25 mL of samples were mixed with 2.75 mL of 0.1 mmol/L DPPH. The absorbance at 517 nm was determined after reaction time of 30 min. The standard curve was constructed using Trolox (0.02–0.10 mmol/L) and the results were expressed as μmol/L Trolox equivalent (TE).

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CUPRAC (cupric reducing antioxidant capacity) assay was carried out by using method described by Apak *et al*. [2008] with slight modification. Added amounts of reagents were modified in the method. In brief, 0.75 mL copper (II) chloride (10 mmol/L), 0.75 mL neocuproine (7.5 mmol/L), 0.75 mL ammonium acetate buffer (1 mol/L, pH=7.0), 0.75 mL water and 0.1 mL sample were mixed in a cell. After 30 min, the absorbance at 450 nm was measured. Trolox (0.05–0.50 mmol/L) was used as a reference standard. Results were expressed as μmol/L TE.

For TEAC (trolox equivalent antioxidant capacity) assay, method described by Re *et al*. [1999] was used with some modifications. Added amount of sample and ABTS reagent, reaction time modifications were applied to the method. ABTS radical cation ($ABTS^{\bullet+}$) stock solution was prepared from 7 mmol/L ABTS and 2.45 mmol/L potassium persulphate solutions. The working solution was prepared by diluting the stock solution with phosphate buffer solution (75 mmol/L, pH=7.2) to an absorbance of 0.700±0.003 at 734 nm. Then, 2 mL of $ABTS^{\bullet+}$ working solution were mixed with 0.1 mL sample. The absorbance was read at 734 nm, 15 min after the reaction started. A standard curve was obtained by using Trolox (0.005–0.05 mmol/L) and results were expressed as μmol/L TE.

ORAC (oxygen radical absorbance capacity) assay was conducted as described by Zulueta *et al*. [2009b] with minor modification which was modification of value of C_{Trolox} used in the following equation. Briefly, 0.25 mL of samples were mixed with 1.5 mL of 4 nmol/L fluorescein. This solution mixture was incubated at 37°C for 15 min. After this period, 0.25 mL of 160 mmol/L AAPH (2,2'-azobis(2-methylpropionamidine)) was added to start reaction and fluorescence intensity was read immediately until the fluorescence intensity became less than 5% of the value of the initial reading. Subsequent readings were made every 2 min at the wavelengths of excitation and emission of 493 and 515 nm, respectively. ORAC values were calculated by applying the following equation and the results were expressed as μmol/L TE.

$$ORAC = [C_{Trolox} \times (AUC_{Sample} - AUC_{Blank}) \times k] / / (AUC_{Trolox} - AUC_{Blank})$$

where C_{Trolox} is the Trolox concentration (0.3 μmol/L), k is the sample dilution factor, and AUC is the area under the fluorescence decay curve. The area under the curve (AUC) was calculated according to the following equation:

$$AUC = (f_0 - f_{n+2}) + 2(f_2 + f_4 + f_6 + \ldots\ldots\ldots + f_{n+2})$$

where f_0 is the initial relative fluorescence intensity at 0 min and f_2, f_4, $f_{6, \ldots\ldots}$ f_{n+2} are the relative fluorescence intensities at times, 2, 4, 6, and n+2 min.

Determination of total phenolic content

Total phenolic contents (TPC) were determined according to the Fu *et al*. [2011] with slight modification which was modification of added amount of Folin-Ciocalteu reagent. Briefly, 0.5 mL of the sample was added into 1.0 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 1.0 mL of saturated sodium carbonate solution (about 75 g/L) was added. This solution mixture was then incubated for 2 h at room temperature. After incubation, the absorbance was measured at 760 nm. A standard curve was obtained by using gallic acid (0.02––0.40 mmol/L) and results were expressed as milligram of gallic acid equivalent per one liter of raki sample (mg GAE/L).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were evaluated on the standard deviation of the response of the blank and the slope using the ratio 3.3σ/S and 10σ/S, respectively, where σ is the standard deviation of the response of 10 blank samples and S is the slope of the calibration curve of the analyte.

Statistical analysis

All values were analysed by GraphPad Prism 5 and Microsoft Excel Software. Three independent aliquots of the sample were measured and all measurements were replicated three times for each sample. The data were expressed as means±standard deviation (SD). The one-way ANOVA and Bonferroni tests were used to determine differences among means and the differences were considered as significant with $p<0.05$. Pearson's correlation analysis was performed between the antioxidant capacity and TPC.

RESULTS AND DISCUSSION

The antioxidant capacity of raki samples which was determined by CUPRAC, DPPH, TEAC and ORAC assays are shown in Table 1. The results pointed out that all raki samples displayed antioxidant properties. The highest antioxidant capacity values were obtained from sample F (dried type grapes), followed by E (dried type grapes), in CUPRAC, TEAC and ORAC assays. For the DPPH assay, E showed the highest antioxidant capacity, followed by A (dried type grapes). Sample G (fresh type grapes) exhibited the lowest radical scavenging activity in DPPH and TEAC assays. In terms of the ORAC and CUPRAC assays, B (dried type grapes) and C (fresh type grapes) displayed the lowest results, respectively. To the best of our knowledge, there has been no study that evaluated antioxidant capacity of raki. However, Pellegrini *et al*. [2003] investigated some alcoholic drinks TEAC values. They reported 1290, 40 and 1040 μmol/L TE for cognac, rum and beer, respectively. Mitić *et al*. [2013] reported the DPPH and TEAC values of beers ranged from 260–830 μmol/L TE and 140–350 μmol/L TE, respectively. The results obtained from this study are in agreement with data found for the other alcoholic drinks.

Studies have indicated that the major constituents of aniseed are anethole, estragole, eugenol, umbelliferon, coumarins [Gülçın *et al*., 2003]. Grapes are rich in phenolic compounds such as gallic acid, caffeic acid, chlorogenic acid, kaempferol, quercetin [dos Lima *et al*., 2014]. These phenolic compounds have been considered to have high antioxidant activity. Antioxidant properties of raki samples may be attributed to these useful natural compounds.

TPC of raki samples were evaluated by using Folin Ciocalteu reagent. In general, TPC results of samples showed

TABLE 1. Antioxidant capacities (μmol/L TE) and TPC (mg GAE/L) of raki samples.

Samples	Methods				
	CUPRAC	DPPH	TEAC	ORAC	TPC
A	100.43±5.17	59.00±3.61	121.35±9.46	207.34±6.52	8.31±0.40
B	97.20±3.11	55.37±4.51	98.51±4.43	105.96±5.68	5.83±0.30
C	87.33±2.29	46.13±4.50	107.66±5.98	176.80±9.08	5.42±0.35
D	122.62±2.36	49.30±5.11	114.86±7.45	177.48±4.47	7.42±0.21
E	132.79±4.03	61.70±1.13	142.34±9.60	211.12±11.19	8.48±0.12
F	141.84±9.18	58.62±2.63	152.97±7.37	232.99±8.92	8.82±0.24
G	109.60±8.91	43.52±1.96	95.95±6.84	181.82±9.03	6.93±0.50

Data are expressed as means ± SD. $p<0.05$. Dried type grapes (A, B, E, F), fresh type grapes (C, G), fresh-dried type grapes (D).

similar trend with antioxidant capacity assays in this study, where sample F (dried type grapes) exhibited the highest TPC followed by E, A, D, G, B, (A, B, E; dried type grapes, D; fresh-dried, G; fresh) while sample C (fresh type grapes) demonstrated the lowest TPC (Table 1).

Antioxidant capacity and TPC values of samples were found statistically different by ANOVA-One way test for all assays ($p<0.05$). In general, raki samples made from dried type grapes revealed higher antioxidant capacity and TPC than those made from fresh type grapes in this study. Bonferroni test was performed to evaluate differences between values of samples. Results are seen in Table 2. Based on this post-hoc test results, while statistical differences between four different sample pairs were found significant, two different sample pairs showed no statistical differences for all assays. Generally, this test showed significant differences between raki samples made from dried type grapes and those made from fresh type grapes. This confirmed the higher antioxidant capacity values of raki samples made from dried type grapes.

The influence of the method on antioxidant capacity was evaluated and the corresponding descriptive statistic data are presented in Table 3. The mean antioxidant capacity values of raki samples were in the order of: ORAC>TEAC>CUPRAC>DPPH. Tafulo *et al*. [2010] investigated the antioxidant capacity of different beer samples with the aid of TRAP (total radical trapping antioxidant parameter), TEAC, DPPH, FRAP (ferric-ion reducing antioxidant parameter), CUPRAC and ORAC assays. Like results found in the present study, they obtained the highest antioxidant capacity value by ORAC assay, while DPPH assay displayed the lowest value. As can be seen from Table 3, the mean values of CUPRAC and TEAC methods are very close to each other, 113.12 and 119.09 μmol/L TE, respectively. This could be due to the fact that CUPRAC and TEAC are similar antioxidant capacity assays with close reduction potentials.

The correlations between antioxidant capacity and TPC of samples are evaluated in the present study. The correlation of TPC of samples with their CUPRAC, TEAC and ORAC (0.8071, 0.8196 and 0.7998, respectively) antioxidant capacities were found statistically significant. These significant correlations indicated that phenolic compounds could be one of the main components responsible for free radical scavenging ability of raki samples. However, considering the results for DPPH, a significant correlations between TPC and DPPH (0.6643) could not be found. The DPPH assay is technically simple, but some disadvantages limit its applications.

TABLE 2. Bonferroni post-hoc test results.

Samples	CUPRAC	DPPH	TEAC	ORAC	TPC
A *vs* B	ns	ns	*	*	*
A *vs* C	ns	*	ns	*	*
A *vs* D	*	ns	ns	*	ns
A *vs* E	*	ns	ns	ns	ns
A *vs* F	*	ns	*	*	ns
A *vs* G	ns	*	*	*	*
B *vs* C	ns	ns	ns	*	ns
B *vs* D	*	ns	ns	*	*
B *vs* E	*	ns	*	*	*
B *vs* F	*	ns	*	*	*
B *vs* G	ns	*	ns	*	*
C *vs* D	*	ns	ns	ns	*
C *vs* E	*	*	*	*	*
C *vs* F	*	*	*	*	*
C *vs* G	*	ns	ns	ns	*
D *vs* E	ns	*	*	*	*
D *vs* F	*	ns	*	*	*
D *vs* G	ns	ns	ns	ns	ns
E *vs* F	ns	ns	ns	ns	ns
E *vs* G	*	*	*	*	*
F *vs* G	*	*	*	*	*

*Indicates significant differences at $p<0.05$. ns; not significant. Dried type grapes (A, B, E, F), fresh type grapes (C, G), fresh-dried type grapes (D).

TABLE 3. Descriptive statistics of antioxidant capacity (μmol/L TE) and TPC (mg GAE/L) assays.

Method	Number of samples	Mean	SD	Confidence interval 95%		Max.	Min.	LOD	LOQ
				Upper limit	Lower limit				
CUPRAC	7	113.12	19.97	127.94	98.30	141.84	87.33	5.52	16.73
DPPH	7	53.38	7.06	58.62	48.14	61.70	43.52	5.48	16.61
TEAC	7	119.09	21.61	135.13	103.06	152.97	95.95	1.33	4.05
ORAC	7	184.79	40.55	214.89	154.69	232.99	105.96	0.03	0.09
TPC	7	7.32	1.33	8.30	6.34	8.82	6.34	0.63	1.92

DPPH is a long lived nitrogen radical. Many antioxidants may react slowly or may even be inert to this radical [Huang *et al*., 2005; Bernaert *et al*., 2012]. This could explain why results obtained by DPPH assay were found non-significant with TPC results. Many studies have shown significant correlations between the results after analysing antioxidant capacity and TPC of various food samples. Bernaert *et al*. [2012] examined antioxidant capacity of leek extracts. They also reported significant correlation coefficient between ORAC and TPC, but in the same study they also found no statistically significant correlation between the results of DPPH and TPC assays. Lu *et al*. [2011] and Song *et al*. [2010] detected significant correlation between TPC and TEAC. Apak *et al*. [2006] investigated the antioxidant capacity of different medicinal and food herbs. They showed that the TPC results of samples correlated significant with those of CUPRAC and TEAC.

CONCLUSIONS

The finding of this study indicated that raki has antioxidant properties. However, antioxidant capacity values of raki samples showed variations based on method used in this investigation. The highest results were obtained by using ORAC and the lowest results were obtained by using DPPH assay. Raki samples from different brands also showed different antioxidant capacity and TPC values. In general, correlations between antioxidant capacity assays and TPC were found statistically significant.

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