

Spirit drinks: a source of dietary polyphenols

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original scientific paper

Summary

There is a long tradition in the production of spirit drinks and using them in the human diet, especially in the Southeast European and Mediterranean regions. The objective of this study was to evaluate whether and which spirits can serve, and to what extent, as a source of biologically active compounds in the human diet. Polyphenolic compounds are biologically active compounds of fruits, vegetables and derived beverages, which have been implicated in their antioxidant activity. Therefore, the total polyphenol content (TPC) and antioxidative properties of 46 spirit drinks and liqueurs produced in Croatia were examined. The total polyphenol content and antioxidant activity were estimated using spectrophotometric methods (Folin-Ciocalteu, DPPH and FRAP), while certain phenols were detected by the HPLC. It was established that spirit drinks aged in wooden casks, such as wine or plum brandy, contain polyphenols ranging from 40-90 mg GAE/L (gallic acid equivalents), whereas walnut or sour cherry liquors contain much more polyphenols ranging from 680-3360 mg GAE/L. The antioxidant activity of analyzed spirit drinks was in correlation with TPC. Walnut and sour cherry liqueur samples had very high antioxidant activity, within the range of those obtained with 1.26 mM Trolox-DPPH assay and 9.5 mM Trolox-FRAP assay.

Keywords: brandy, spirit drinks, liqueur, polyphenols, antioxidant activity

Introduction

Spirit drinks, as a special group of food, have traditionally been a part of the human diet. The beginning of the production of distillates dates back to the 9th century in monasteries and pharmacies, when aromatic and medicinal plants were distilled for the medicinal purposes only. The improvement of these procedures led to the industrial production of spirit drinks. Today, there are numerous types of distilled spirits, which are produced by different technologies.

Depending on the type and production method spirit drinks can be divided into several groups (Regulation on spirit drinks, 2009). Pure distillates, often called brandies, are produced by distillation of different fermented fruit or grain row material. They are characterized by complex aroma compounds originating from the raw material used, alcoholic fermentation and distillation process. Some pure distillates can age in wooden barrels (Pecic et al., 2012). Throughout the ageing, many physical and chemical interactions take place between the barrel, the surrounding atmosphere and the spirit (Pecic et al., 2012; Alanon et al., 2011; Schwarz et al., 2009; Canas et al., 2008; Aoshima et al., 2004). For instance, hydrolyzable tannins and lignins as well as

low molecular mass phenolic compounds such as coumarins and certain phenolic acids are extracted from the wood contributing to spirit aroma, taste and color (Tanaka et al., 2010; Canas et al., 2008). Hence the type of wood as well as the time required for ageing process is among the most important factors that determine the value of the finished product (Alanon et al., 2011).

On the other hand, bitters and liqueurs are produced by maceration of fruits or herbs in alcohol base (spirit, wine or grape marc brandy) with or without sugar addition. Herbs and fruits are rich in specific flavor compounds and essential oils as well as polyphenol compounds. During the maceration process these compounds are extracted and pass into the alcohol base enriching it (Wojdylo et al., 2007; Katalinic et al., 2006; Lu and Foo, 2001). This is a relatively simple method in which the concentration of the alcohol base (60-80%) and the maceration time (approximately 30 days) play the major role in mass exchange rate and yield (Karlin et al., 2011a; Karlin et al., 2011b). Thus, polyphenol compounds in spirit drinks originate from herbs and fruits, or from the wooden barrels.

The publicity surrounding the consumption of alcohol has been predominantly negative. However, it can also have beneficial effects, depending on the

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consumer's physical and physiological characteristics as well as the amount consumed. Epidemiological studies have shown that moderate consumption of alcoholic beverages (moderate alcohol consumption is defined as up to 1 drink per day for women and up to 2 drinks per day for men, Dietary Guidelines for Americans, 2010) has a positive impact on the prevention of coronary artery diseases, it improves lipid metabolism, increases antioxidant activity and reduces mortality from coronary heart diseases and colon cancer (Carusio et al., 2008; Knežević et al., 2011). Until recently, it was thought that the decrease in risk of cardiovascular diseases is due to the ethanol content (Rimm et al., 1991). However, according to the recent studies some beverages such as red wine or cognac might impact mortality more than other alcoholic beverages due to the presence of polyphenol compounds (Carusio et al., 2008). Phenolic compounds have no known nutritional function, but they may be important for human health.

The cultivar and the cultivation region, raw material production year, transport and storage have the major impact on polyphenol composition and concentration

in raw material and derived beverages (Mitic et al., 2012; Pedisic et al., 2010). Therefore, the aim of this study was to evaluate whether the spirit drinks produced in Croatia can serve as source of dietary polyphenols. The differences in concentrations of polyphenols between spirit drinks groups (pure distillates, distillates with ageing, bitters, bitters and fruits liqueurs) as well as differences between samples within the same product group were examined.

Materials and methods

Samples

A total of 46 samples were purchased from local supermarkets. Six different spirit drink groups were analyzed: pure distillates (8 samples), aged distillates (11 samples), „domestic“ rums (4 samples), bitters (6 samples), bitter liqueurs (9 samples), and fruit liqueurs (8 samples). Sample list is presented in Table 1.

Table 1. Analyzed samples list and description

Groups	Subgroups	Samples number
Pure distillates	Williams pears brandy	21,26
	Sour cherry brandy (<i>Maraschino</i>)	23, 24
	Grape marc brandy	31, 32, 33
	Vodka	34
Distillates with ageing	Grape brandy	11, 12, 13, 14
	Plum brandy (<i>Šljivovica</i>)	25, 27, 28
	„Homemade“ grape brandy	51, 52, 53, 54
Homemade rums	Homemade rum	71, 72, 73, 74
Bitters	Bitters (unknown herbs)	41, 42, 43, 44, 45, 46
Bitter liqueurs	<i>G. radix</i> , <i>S. herba</i> , <i>C. herba</i>	61, 62, 63
	<i>A. absinthium</i>	81, 82, 83, 84, 85
	<i>V. album</i> (<i>Biska</i>)	55
Fruit liqueurs	Sour cherry liqueurs	91, 93, 94
	Walnut liqueurs	92, 95, 96
	Cowberry liqueur	97
	Blueberry liqueur	98

Chemicals

Folin-Ciocalteu reagent was obtained from Kemika (Zagreb, Croatia), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-Tris(2-pyridyl)-s-tirazine (TPTZ) from Fluka (Buchs, Switzerland). Gallic, vanillic and *trans*-cinnamic acids, syringaldehyde and vanillin, as well as Trolox were from Sigma-Aldrich (Steinheim, Germany). Syringic and ellagic acid were obtained also from Fluka (Buchs, Switzerland). HPLC grade

methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). The bidistilled water used in sample preparation, solutions and analyses.

Determination of total polyphenol content

Determination of the total polyphenol content (TPC) in samples has been conducted by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, 500 µl of Folin-Ciocalteu's solution was added to the 300 µl

sample (samples 91-98 were diluted 10 times with 96% (v/v) ethanol) and 6 mL distilled water, agitated to homogenize and left to stand in the dark for 5 min in order to perform reaction. 1.5 mL of Na₂CO₃ solution (200 g/L) and distilled water were added to make up the total volume of 10 mL. The samples were left for 2 h in the dark and then absorbance was measured at 760 nm. All spectrophotometric measurements were performed by UV-VIS spectrophotometer UV-Vis Unicam. The calibration curve was prepared with gallic acid solution (GA) ranging from 0 to 500 mg/L, and the results were expressed as gallic acid equivalents (mg GAE/L). All the measurements were performed in triplicate.

Determination of antioxidant activity

Methods used for determining antioxidant activity are based on the study of a reaction in which a free radical are generated and inhibited by the addition of the sample whose antioxidant power is being measured. In this work, for the determination of antioxidative characteristics of spirit drinks, the DPPH radical scavenging activity and ferric ion reducing antioxidant power (FRAP) were used.

DPPH assay

DPPH Radical scavenging activity (1,1-Diphenyl-2-picrylhydrazyl) was determined by adapted method of Brand-Williams et al., (1995). Spirit drink samples (200 µl) were added to the ethanol (2 mL) and 0.1 mM DPPH working solution (2 mL). Samples 91-98 were diluted 10 times with 96% (v/v) ethanol. The absorbance at 525 nm was measured after 30 min of incubation in the dark. DPPH reagent and ethanol were used as a blank reference. Samples percentage of DPPH radical inhibition was compared with 0.063 mM, 0.126 mM and 1.26 mM Trolox. The percentage of DPPH inhibition was calculated by the following equation: % of DPPH radical scavenging activity = $100 \times [A_0 - A_s]/A_0$, where A_0 is the absorbance of DPPH solution with ethanol, while A_s is the absorbance of a DPPH solution with the sample. All experiments were performed in triplicate.

FRAP assay

The FRAP assay was performed as previously described by Benzie and Strain (1999) with some modifications. FRAP reagent solution was made of the mixture of acetate buffering agent (300 mM, pH = 3.6), TPTZ (10 mM solution TPTZ in 40 mM HCl) and FeCl₃ · 6H₂O (20 mM) in volume ratio 10:1:1, respectively). The working FRAP reagent

was prepared fresh on the day of the analysis. All samples, standards and reagents were pre-incubated at 37 °C. The examined sample (80 µL) was mixed with FRAP reagent (2080 µL) and distilled water (240 µL). After the reaction at 37 °C for 5 min the absorbance at 593 nm was measured. The standard curve was constructed by using serial dilution (0.1-2.0 mM) of Trolox stock solution. The final results were expressed as mM Trolox equivalents.

Isolation of polyphenol fractions

The isolation of distilled spirit polyphenol fractions was performed by liquid-liquid extraction according to adapted method of Canas et al., (2008). Samples (10mL) were dealcoholized by means of rotary evaporator at 30 °C and diluted to the original concentration by distilled water. Then, the sample pH was adjusted to pH = 2 with 2M HCl and extracted three times with ethyl acetate (5 mL). The obtained three ethyl acetate extracts were combined and then evaporated to dryness in a rotary evaporator. The dry residue was dissolved in 1.5 mL of methanol and this solution was used for HPLC analyses. This method was appropriate for the extraction of phenolic acids fractions, but for the extraction of other phenolic substances, such as flavonoids from sour cherry liqueur, another solvent such as acidic methanol should be used (Levaj et al., 2010). Extractions of samples were performed in duplicate.

HPLC analyses

The analytical HPLC system was ProStar Varian equipped with a Varian Pro Star 330 photodiode array detector (Varian, Walnut Creek, CA, USA). The HPLC column was Nucleosil 5u C18 100A (Phenomenex, Torrance, CA, USA). The solvents for gradient elution were: A-0.2% o-phosphoric acid, B-metanol, C-acetonitril. The following gradient was used: 96% A, 2% B, 2% C. The flow rate was 1.5 mL min⁻¹. Operating conditions were as follows: column temperature 30 °C and injection volume 20 µL. Chromatograms were recorded at 280 nm. The identification of the compounds was achieved by comparing UV spectra and the retention times of the separated peaks with the retention times of the standards. Quantification was made by the external standard method using calibration of standards as a reference and was based on peak area from HPLC analyses and mass concentration of compound. Caramel was identified by comparing UV spectra of the separated peaks with literature data (Bento, 1995). It was not quantified.

Results and Discussion

Total phenolics content and antioxidant capacity

The six categories of distilled spirits were used to determine the total polyphenol content and antioxidant activities. Due to the specific production of each group of spirit drinks, the values of polyphenol compounds in examined samples were different. Pure distillates as vodka, fermented grape marc brandy, Williams pears brandy as well as Maraschino (distillated marasca cherry), had low total polyphenol content, ranging from 0-22 mg GAE/L (Fig. 1). High temperatures used in the distillation process may lead to the depletion of

polyphenol compounds present in the raw materials these spirits were manufactured from (Goldberg et al., 1999). Furthermore, the volatility of phenolic compounds is lower than that of ethanol and specific aroma compounds, which is why during the distillation process phenolic compounds do not accumulate in spirit drinks. Also, the antioxidant activities of pure distillates, determined by DPPH and FRAP assays, were very low. Values obtained by DPPH method were negative due the ethanol pro-oxidant activity and enhanced free radical formation (data not shown). FRAP values ranged from 0.04-0.25 mM Trolox, except the sample 33 which exhibited higher FRAP value.

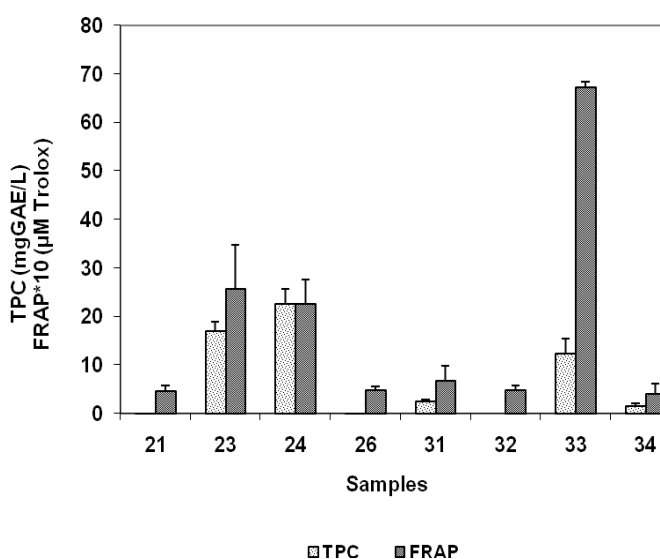


Fig. 1. Total polyphenols (TPC) and antioxidant activity (FRAP) of pure distillate

Spirit rich in polyphenol compounds deriving from wood and ageing are grape brandy and plum brandy called *Šljivovica* (*Slivovitz* or *Slivovitsa*), the traditional brandy in Slavic region of Europe. According to the legislation, there is no obligation regarding the ageing process of plum brandy, but its market value increases if it matures in oak barrels and is of yellow colour (Pecic et al., 2012). The total polyphenol content in these spirits ranged from 40-95 mg GAE/L and 10-40 mg GAE/L in grape and plum brandies, respectively. The antioxidative activities were high, in the range of those obtained by 0,126 mM Trolox (DPPH assay) and 0,1-1,1 mM Trolox (FRAP assay) (Fig. 2). Gallic, vanillic, ellagic and syringic acid, as well as syringaldehyde and vanillin, are the most abundant phenolic compounds in spirit aged in wooden barrels (Goldber et al., 1999; Canas

et al., 2008). These phenolic compounds are also important as wood ageing markers, especially benzoic and cinnamic aldehydes (Canas et al., 2004). The content level of extractable components in brandy depends on numerous factors such as the variety of oak used in the assembling of the barrel, the treatment of the wood during the barrel assembling process, the barrel size and the ageing characteristics, e.g. conditions in the cellar, alcohol content, length of the ageing process etc. (Mosedale and Puech., 1998). Samples 51-54 were samples of „homemade“ grape brandy produced out of 30% of grape brandy blended with ethanol of agricultural origin. Fig. 2 shows these samples had lower phenol content and antioxidative activity, except for the sample 52.

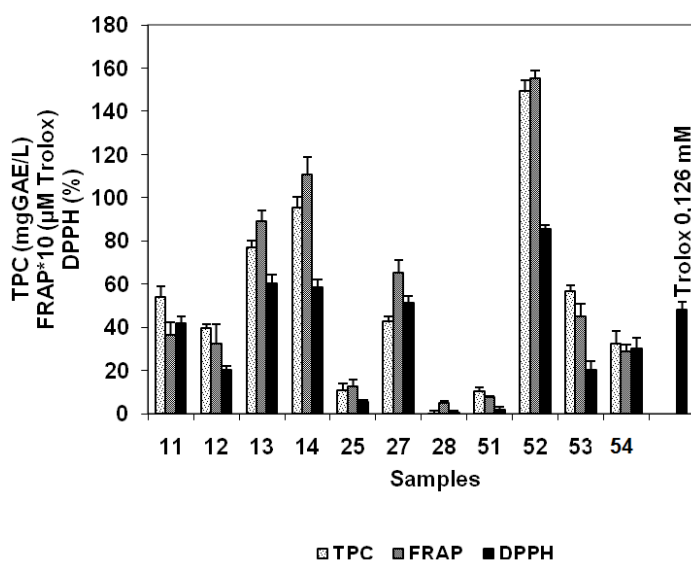


Fig. 2. Total polyphenols (TPC) and antioxidant activity (FRAP, DPPH) of wine brandies and aged plum brandies

Special group of spirit drink is „homemade rum“. This Croatian homemade rum is not to be confused with rum as it is known worldwide. Namely, according to the Croatia’s legislation regarding distilled spirits (Regulation on spirit drinks, 2009) rum is produced by distilling either fermented molasses or sugar cane syrup from sugar manufacture and is aged in wooden barrels. But, due to economic

reasons and according to the legislation, it is allowed to produce and market the homemade rum, a spirit drink produced by flavouring ethyl alcohol of agricultural origin. The tested samples were actually varieties of homemade rum and their total polyphenol content ranged from 50-100 mg GAE/L, except for the sample 73 where the concentration was about 350 mg GAE/L (Fig. 3).

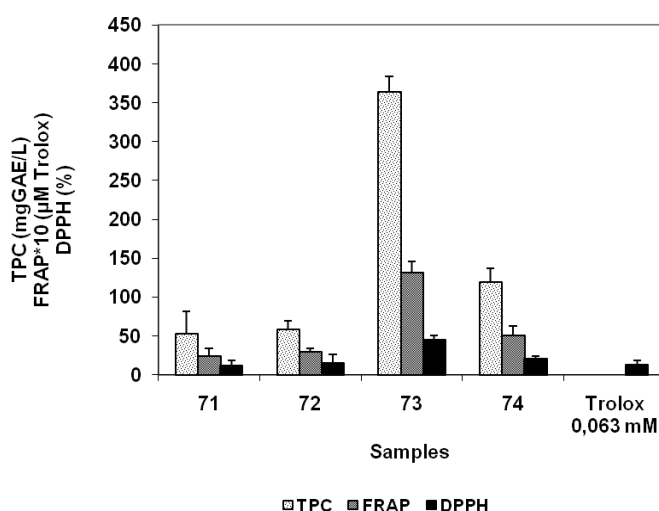


Fig. 3. Total polyphenols (TPC) and antioxidant activity (FRAP, DPPH) of „domestic“ rum

Bitters and bitter liqueurs are very popular in Croatia, especially in the Adriatic (Mediterranean) region, due to the increased knowledge about the medicinal

properties of various herbs. Bitters are produced by maceration of herbs in spirits, usually grape marc brandy, while bitter liqueurs contain added sugar as

well. Bitter-liqueur production can also include co-distillation of macerated herbs and mixing the essences of herb materials with a spirit or wine. Anise (*Pimpinella anisum*), mint (*Menthae piperitae*), gentianian (*Gentianalutea*) and angelica (*Angelica sp*) are frequently used in the production of bitters (samples 41-46). Dominant herbs in bitter liqueurs (samples 61-63) are gentianian (*Gentiane radix*), salvia (*Salvia herba*) and centaury (*Centaurei herba*), while wormwood (*Artemisia absinthium*) is dominant herb in samples 81-85. Sample 55, called *Biska*, is the traditional spirit drink in Istria, one of Croatia's Adriatic regions. It is produced by maceration of mistletoe (*Viscum album*) in fermented grape marc brandy. The results presented in Fig. 4 show that bitters contained from 10-25 mg GAE/L,

while in bitter liqueurs polyphenol compounds ranged from 90-350 mg GAE/L (Fig. 5). The exception was sample 55 which had about 640 mg GAE/L, due to rich polyphenol composition of mistletoe. The antioxidative activity of *Biska* was also high, within the range of those obtained by 0.126 mM Trolox (DPPH assay) and about 4.0 mM Trolox (FRAP assay). Vicas et al., (2011) identified and quantified 17 compounds in mistletoe, including betulinic acid, 12 phenolic acids (gallic, protocatechuic, gentisic, chlorogenic, *p*-OH benzoic, caffeic, syringic, salicylic, *p*-coumaric, ferulic, sinapic, and *trans*-cinamic acid) and 4 polyphenols (naringenin, quercetin, kaempferol and rosmarinic acid) in concentration of about 108 µg/g dw.

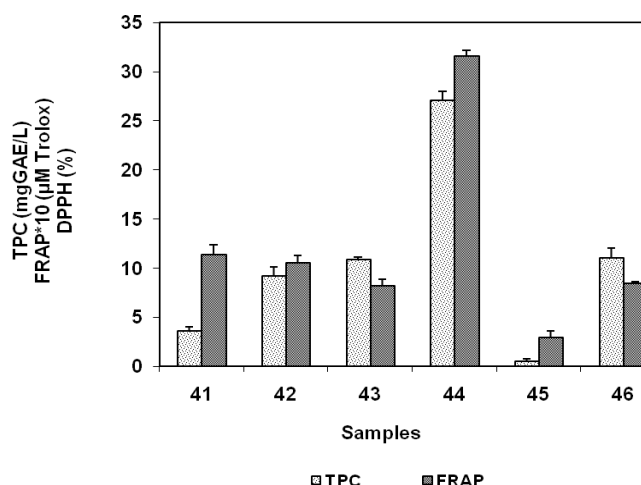


Fig. 4. Total polyphenols (TPC) and antioxidant activity (FRAP) of bitters

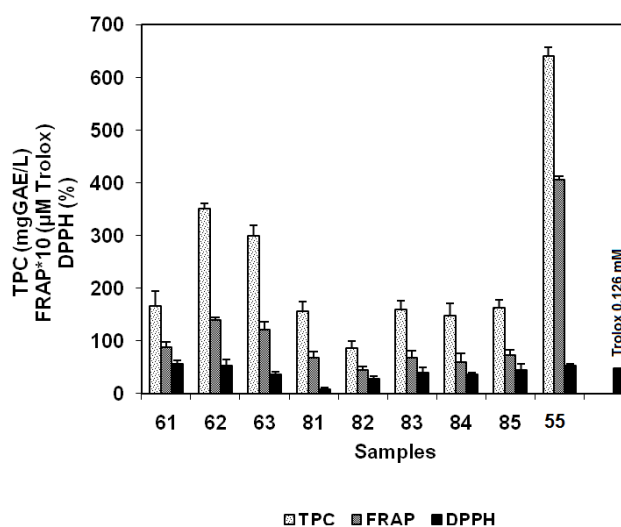


Fig. 5. Total polyphenols (TPC) and antioxidant activity (FRAP, DPPH) of bitter liqueurs

The most important group of spirit drinks regarding content of the dietary polyphenols is the group of fruit liqueurs, such as walnut liqueur (Jakopic et al., 2007; Alampase et al., 2005; Alampase and Pompei, 2005) or sour cherry liqueur, which are produced by maceration of fruits in ethanol or grape marc brandy. The dark brown walnut liqueur is a bitter beverage made from walnuts with green husk, while in production of sour cherry liqueur ripened fruits are used. In the examined samples, the total polyphenol contents were 500-3500 mg GAE/L (Fig. 6). The antioxidative activities were very high, within the range of those obtained with 1.26 mM Trolox DPPH assay. Sample 94 had the highest concentration of total polyphenols due the rich phenolic composition of sour cherries. Sour cherries are considered to be one of the richest sources of phenolic compounds (Pedisic et al., 2007; Levaj et al., 2010; Mitic et al., 2012). They contain significant levels of antocyanins that have been proven to possess strong antioxidant

activities. The major anthocyanins are cyanidin 3-glucosyl rutinoside (about 60 mg/100 g) and cyanidin 3-rutinoside. Sour cherries commonly contain neochlorogenic acid, p-coumaric acid derivative, p-coumaric acid and ferulic acid. Neochlorogenic acid was found to be the major hydroxycinnamic acid in sour cherries, about 10 mg/100g (Mitic et al., 2012). Although many papers on the polyphenol profile of cherries have been published, the polyphenol profile of sour cherry liqueur is not yet available. Stampar et al., (2006) identified thirteen phenolic compounds in walnut husks: chlorogenic, caffeic, ferulic, sinapic, gallic, ellagic, protocatechuic, syringic and vanillic acid, as catechin, epicatechin, myricetin, and juglone. The total polyphenol content in walnuts was about 1500 mg/100 g d.w., while the concentration in the liqueur was about 20 mg/100 mL. Juglone was the most abundant compound in the walnut fruit, while gallic acid was the most abundant in the walnut liqueur.

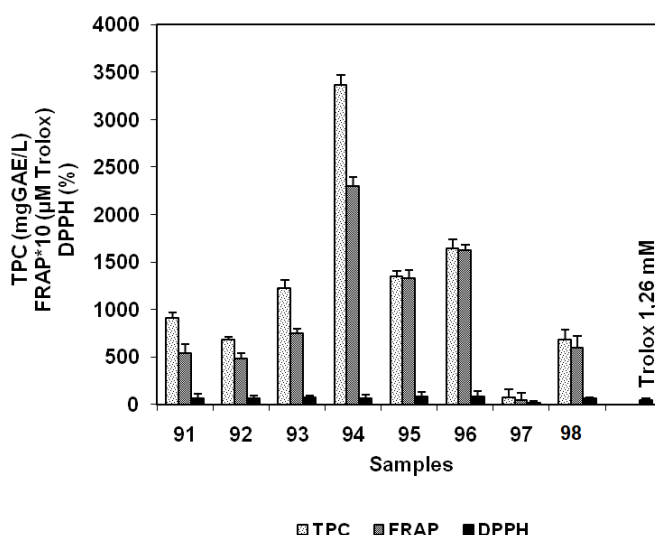


Fig. 6. Total polyphenols (TPC) and antioxidant activity (FRAP, DPPH) of fruit liqueurs

HPLC analysis of polyphenols

The previous results showed difference in the polyphenols concentration among the six categories of spirits. Polyphenol composition of samples was analyzed by HPLC.

HPLC analysis showed that samples 33 and 73 contained caramel, which reacts with Folin-Ciocalteu and FRAP reagents (Fig. 7). Sample 73 and 52 also contained subsequently added vanillin (1.65 mg/100 mL and 0.057 mg/100 mL), much more than other homemade rums and homemade brandies samples (data not shown). Vanillin, tannins and caramel are

color and flavor enhancers and ageing agents, which are added to spirits to shorten the ageing in wooden barrels or to imitate the ageing process (Schwarz et al., 2009; Canas et al., 2004). This kind of imitation, which is actually a fraud intended to mislead consumers, was also noticed in plum brandy sample 25 (Fig. 7). This sample contains caramel added to imitate the ageing process, while sample 27 represents a genuinely mature plum brandy. Gallic (11 µg/100 mL), vanillic (8 µg/100 mL), syringic (20 µg/100 mL) and ellagic (32 µg/100 mL) acid as well as vanillin (32 µg/100 mL) and syringaldehyde (10 µg/100 mL) were detected. Hence sample 27 had

high antioxidative activity, measured by both methods (Fig. 2). The third plum brandy sample (sample 28) had low polyphenol and antioxidant activity values, but there was no attempt to mislead consumers regarding the ageing period since it was colourless. In the homemade brandy (sample 52) along with caramel and vanillin, gallic (70 µg/100 mL) and ellagic (91 µg/100 mL) acid derived from grape

brandy were detected. Gallic (230 µg/100 mL) and ellagic (1130 µg/100 mL) acid are more abundant phenolic acids in „cognac“ (www.phenol-explorer.eu). Therefore, these acids were detected in homemade brandy (30% of original brandy) while the other compounds present in grape brandy in lower concentration (vanillic acid, syringic acid and syringaldehyde) were below the detection limit.

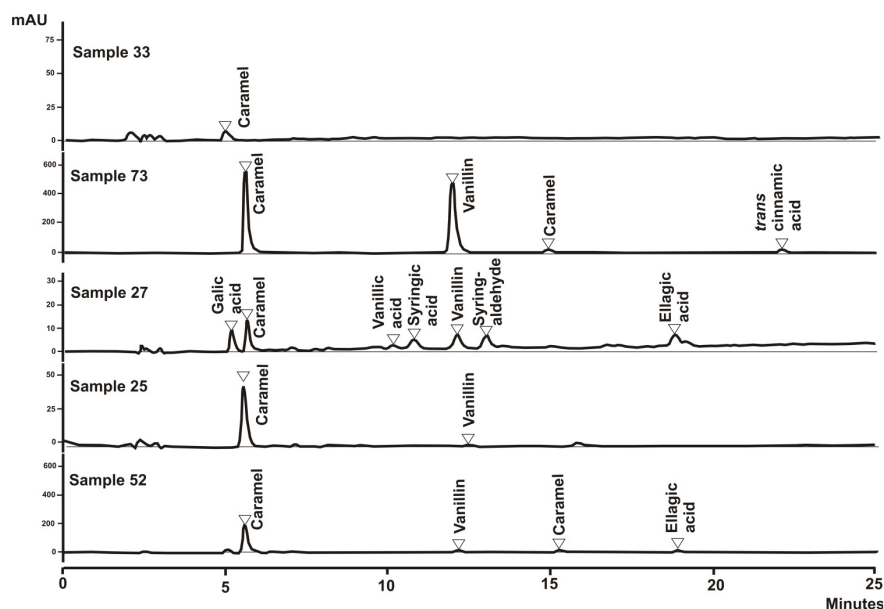


Fig. 7. HPLC chromatograms of polyphenols fraction and ageing agents (vanillin and caramel) in spirit drink tested samples

Correlation between the total polyphenol content and antioxidative activity

Due to the low concentrations of polyphenols in some examined groups of spirits and ethanol prooxidant activity, results of antioxidant activity obtained by DPPH method were negative and results are incomplete. Therefore, the correlation between the total polyphenol content and antioxidative activity was calculated only for the results obtained by FRAP method.

The correlation analysis was made for all spirits, and for each group of samples separately. The polyphenol content and antioxidant power determined by FRAP assay of all samples were very closely correlated (Table 2). Considering each group separately, the homemade rum and aged distillates were best correlated, followed by bitter liqueurs, fruit liqueurs and bitters. These findings indicate that the antioxidant power of spirit drinks is highly influenced by their polyphenol content.

Table 2. TPC and FRAP average values for the examined groups of spirits

Categories of spirits	TPC mg GAE/L	FRAP mM Trolox	FRAP correlation coefficient
Pure distillates	6.32	0.10	R ² -0.9223
Aged distillates	51.72	0.53	R ² -0.9409
Homemade rum	148.35	0,58	R ² -0.9712
Bitters	10.37	0.12	R ² -0.8328
Bitter liqueurs	240.33	1.18	R ² -0.9351
Fruit liqueurs	1238.00	9.54	R ² -0.8777
All samples	-	-	R ² -0.9543

Comparison to other foods rich in polyphenols

To answer the question which alcoholic beverages, and to what extent, can serve as a source of biologically active compounds in the diet, spirit drinks were compared to other polyphenol rich food and drinks. As can be seen from Table 3, the best sources of polyphenol compounds are tea and red

wine, followed by walnut and sour cherry liqueurs. A moderate consumption of alcoholic beverages could be recommended. It is worth noticing that 100 mL of red wine, which is considered to be the best source of antioxidants and consumption of which is often cited as healthy and desirable, contains about the same amount of alcohol as 50 mL of liqueur.

Table 3. Total polyphenols Content (TPC) in different sources

Sources	TPC	Serving size	TPC (mg/serving)	References
Black tea	150 mg/g	Cup (1,5 g)	225	Yashin et al., 2010
Green tea	150mg/g	Cup (1,5 g)	225	Yashin et al., 2010
Red wine	200 mg/100 mL	100 mL	200	Yashin et al., 2010
Walnut liqueur	350 mg/100 mL	50 mL	175	Alamprese and Pompei, 2005
Sour cherry liqueur	336 mg/100 ml	50 mL	168	This work
Coffee	25 mg/g	Cup (5 g)	125	Yashin et al., 2010
Walnut liqueur	164 mg/100 ml	50 mL	82	This work
Dark chocolate	135 mg/100g	40 g	54	Yashin et al., 2010
White wine	50 mg/100 mL	100 mL	50	Yashin et al., 2010
Aged wine brandy	96 mg/100 ml	50 mL	48	This work
Aged wine brandy	62 mg/100	50 mL	31	Canas et al., 2008
Aged plum brandy	42 mg/100 ml	50 mL	21	This work
Walnut liqueur	30 mg/100 ml	50 mL	15	Stampar et al., 2007
Aged plum brandy	30 mg/100 mL	50 mL	15	Pecić et al., 2012

Conclusion

The results here show a difference in the polyphenol concentration among the examined spirits categories. Pure distillates and bitters have low concentration of polyphenols and low antioxidant activity. In the case of brandies, the products that have been in contact with wood, a significant amount of polyphenols is present (it is taken up from the wood), with a corresponding important contribution to the antioxidant activity. Fruit liqueurs as well as bitter liqueurs have the highest content in polyphenols and high antioxidant activity. HPLC analysis showed that the Folin–Ciocalteu and FRAP method can serve only as an informative methods for the total polyphenols concentration and antioxidative activity determination in examined samples. Namely, most of them contain caramel and vanillin, color and flavor enhancers, which react with Folin–Ciocalteu and FRAP reagents. The value of the antioxidant capacity depends on the polyphenolic contents, as well as on flavor and color additives. The results can be used to stimulate the moderate consumption of spirit drinks rich in polyphenols, which are already recognized as drinks that reduce overall mortality due lowering the risk of coronary heart disease.

Acknowledgements

This work has been funded in part with National funds from a grant from the Ministry of Science, Education and Sports of the Republic of Croatia (058-0583444-3483).

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Received: November 29, 2012

Accepted: February 18, 2013