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Effect of Extraction Conditions on the Extractability of Phenolic Compounds from Lyophilised Fig Fruits (*Ficus Carica* L.)

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In this work the influence of 50–80% (v/v) aqueous ethanol and the temperature of extraction (25–80°C) on the extractability of total phenolics, total flavonoids and total proanthocyanidins from different fig fruits was investigated. The best extraction conditions (80%, v/v aqueous ethanol, 80°C) obtained in the experiments with lyophilised *Ficus carica* L. cv. Šaraguja were used while performing the extraction of phenolic compounds from other fig varieties (Bružetka bijela, Crnica, Bjelica and Termenjača). The antioxidant capacity was measured in all fig fruits as well.

It has been shown that the temperature of extraction and ethanol to water ratio have a statistically significant influence on the extraction of phenolic compounds from fig fruits variety Šaraguja. The highest content of phenolic compounds was found in fig variety Crnica while the lowest one in fig variety Bjelica. According to the results obtained in this study, fig fruits can be considered as a natural source of phenolic compounds with good antioxidant capacity.

INTRODUCTION

Fig is one of the oldest domesticated fruit species that usually grows in hot and dry climate areas. Fruits of fig trees are widely used in fresh or dried form. They are an excellent source of minerals, vitamins, amino acids, crude fibers as well as phenolic compounds [Lianju *et al.*, 2003; Solomon *et al.*, 2006]. Phenolic compounds are important constituents of fruits quality because of their contribution to the taste and colour of the fruit [Veberic *et al.*, 2008]. In recent years, phenolic compounds from natural sources have been the subject of interests in many scientific researches due to their positive effects on human health, attributed mainly to their antioxidant activities. In the review paper of Tsao & Deng [2004], phenolic compounds and carotenoids were reported to be the most important groups of natural antioxidants. The two main groups of phenolic compounds in fig fruits are phenolic acids (*e.g.* gallic acid, chlorogenic acid, syringic acid) and flavonoids (*e.g.* (+)-catechin, (-) epicatechin, anthocyanin) [Duenas *et al*., 2008; Veberic *et al.*, 2008].

Extraction is an important step in the isolation and later in the identification and quantification of phenolic compounds [Cacace & Mazza, 2003]. Since the phenolic compounds of different plants differ structurally, it is very difficult to develop a standardised extraction method that would simultaneously extract all inherent phenolic compounds [Naczk & Shahidi, 2006].

The extractability of the phenolic compounds depends on the type of the solvent, nature and preparation of material to be extracted, chemical structure of phenolic compounds, temperature, extraction time, solid-liquid ratio, extraction method employed and possible presence of interfering substances. Solvent extraction, *i.e*. solid-liquid extraction, is commonly used for the isolation of phenolic compounds from plant material.

The selection of the solvent is one of the most important steps in the process of extraction. Methanol, ethanol or propanol and their mixtures in water, as well as the acetone, ethyl acetate and dimethylformamide are so far the most commonly used solvents in the extraction of phenolic compounds from the plant materials [Escribano-Bailon & Santos-Buelga, 2003; Naczk & Shahidi, 2006].

Despite the complete phenolic profile of the fig published by some authors [Duenas *et al*., 2008; Solomon *et al.*, 2006; Veberic *et al.*, 2008], there are no studies on the effect of extraction conditions on the extraction of phenolic compounds from fig fruits. Given the above, the aim of this study was: a) to examine the influence of extraction conditions and variety on extractability of phenolic compounds from lyophilised fig fruits, and b) to measure the antioxidant capacity of resultant extracts.

MATERIALS AND METHODS

Materials

Different varieties (Šaraguja, Bružetka bijela, Crnica, Bjelica and Termenjača) of fresh fig fruits (*Ficus carica* L.) were collected from a fig plantation in Istrian region of Croa-

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tia. The fig fruits were harvested at the optimal ripening time in the year 2008. Before extraction fig fruits were halved and lyophilised. Lyophilisation of the samples was performed in LIO-10P (Kambič d.o.o., Slovenia) by freezing at –35°C and dehydration at 0.5 mbar (primary drying period) and 0.03 mbar (secondary drying period). The temperature was continuously increasing from -5°C in the primary drying period up to +25°C in the secondary drying period.

Dry matter content of the lyophilised samples was determined by drying at 105°C to constant mass.

Extractions of phenolic compounds

Two different experiments were performed. The aim of the first experiment was to investigate the effect of volume ratio of the solvents on the extractability of phenolic compounds at 80°C. The solvents were prepared by mixing 96% ethanol and water to obtain the following solutions: 50, 60, 70 and 80% (v/v) aqueous ethanol. The aim of the second experiment was to investigate the effect of temperature on the extractability of phenolic compounds. These experiments were performed at different temperatures: 25, 40, 50, 60, 70 and 80°C using the previously estimated ethanol/water ratio as a solvent for the extraction.

In all experiments, 0.5 g of milled lyophilised fig fruit was extracted with 20 mL of the solvent. Extractions were conducted in sealed flasks which were placed in a water bath (Julabo, SW - 23, Germany) and shaken at 200 rpm for 120 min. After extraction, the suspension was centrifuged at 15,000 × g for 5 min (Sigma 2–16, Germany). The supernatants were separated and pooled with the solvent up to 20 mL. Thus obtained fig fruit extracts were determined for total phenolics content, total flavonoids content, total proanthocyanidins content and antioxidant capacity. All experiments were performed in duplicate to check the reproducibility.

Determination of total phenolics content (TP)

The TP of fig fruit extracts was determined using Folin-Ciocalteu micro method [Waterhouse, 2009]. The reaction mixture contained 40 μL of extract, 3160 μL of pure water, 200 μL of the Folin-Ciocalteu reagent and 600 μL of 20% sodium carbonate solution. After 30 min of incubation at 40°C, the absorbance was read at 765 nm (UV-1700 Shimadzu, Japan). The TP are expressed as gallic acid equivalent per a dry basis of fig fruit (mg_{GAE}/g_{db}). The data presented in tables are mean values of six determinations.

Determination of total flavonoid content (TF)

The TF was measured using the colorimetric method with aluminium chloride [Marinova *et al*., 2005]. Determination of TF in each extract was conducted in duplicate and the TF was expressed as (+)-catechin equivalent per a dry basis of fig fruit (mg_{CE}/g_{db}). The results are expressed as a mean value of four measurements ±SD.

Determination of total proanthocyanidin content (TPA)

The TPA was determined by UV spectrophotometry method according to Škerget *et al*. [2005]. The analysis was performed in duplicate for each extract and TPA was calculated according to the following equation:

$$TPA\ (g/L) = (A \times MW \times DF)/(\varepsilon \times l) \quad (1)$$

where A denotes absorbance of the extract, MW molar weight of cyanidin (287 g/mol), DF dilution factor (ratio of total volume of the reaction mixture to volume of the extract), ε molar extinction coefficient of cyanidin (34700 L/(mol·cm)), and l - pathlength (cm).

The final TPA in extracts was expressed on a dry basis of fig fruit (mg/g_{db}) and data were shown as a mean value of four measurements ±SD.

Determination of Antioxidant Capacity (AC)

The AC of extracts was measured spectrophotometrically according to Sánchez-Moreno *et al.* [1998] with some modifications. In brief, 0.2 mL of the extract was added to 3.8 mL of daily prepared 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) solution dissolved in 96% ethanol (0.026 mg/mL). After 30 min of incubation, the absorbance was read at 515 nm. Results of AC were expressed as vitamin C equivalents (mg_{VCE}/mL) using the following equation:

$$AC\ (mg_{VCE}/mL) = 0.0011 \times I\ (\%) + 0.0015 \quad (2)$$

where $I\ (\%) = [(A_{DPPH}\text{-}A_{ext})/(A_{DPPH})]\cdot 100$ and denotes percentage inhibition of DPPH• radical. All measurements were performed in triplicate to check the reproducibility, which gave a total of six data of AC from both extractions. The mean values of results were expressed as vitamin C equivalents per a dry basis of fig fruit (mg_{VCE}/g_{db}) ±SD.

Statistical analysis

All statistical analyses were performed using Statistica 8.0 software (StatSoft, Inc., USA). Results were analysed using the ANOVA and Duncan *post-hoc* test ($p<0.05$) and expressed as the mean±standard deviation (SD).

RESULTS AND DISCUSSION

It is well known from literature data that extraction conditions and characteristics of the sample can affect the efficiency of the extraction, independently or interactively [Liyana-Pathirana & Shahidi, 2005]. Regarding the plant materials, the solvent and the temperature are the process parameters that generally have the greatest impact on the efficiency of extraction of bioactive compounds from the plant material.

It is generally known that alcohol/water solutions exert a better influence on the extractability of phenolic compounds in comparison to the mono-component solvents [Pinelo *et al.*, 2005; Spigno *et al.*, 2007]. Moreover, ethanol and water are from the toxicological point of view much safer and therefore more suitable for the food industry than the other organic solvents [Huh *et al.*, 2004]. The amounts of extracted phenolic compounds obtained in this study at 80°C after 120 min by different solvents were as follows: total phenols in the range of 2.4–3.7 mg_{GAE}/g_{db}, total flavonoids 0.44–2.5 mg_{CE}/g_{db} and total proanthocyanidins 0.68–0.87 mg/g_{db}. The content of these phenolic compounds were observed to increase with an ethanol volume increasing in the solvent from 50 to 80% as shown in Table 1.

TABLE 1. Total phenolics content (TP), total flavonoids content (TF), total proanthocyanidins content (TPA) and antioxidant capacity (AC) of fig fruits (variety Šaraguja) extracted by using different solvent composition at 80°C.

Solvent	TP[a] (mg_{GAE}/g_{db})	TF[b] (mg_{CE}/g_{db})	TPA[c] (mg/g_{db})	TF/TP	TPA/TP	AC[d] (mg_{VCE}/g_{db})
50% ethanol	2.4±0.10[a]	0.44±0.04[a]	0.68±0.01[a]	0.18	0.28	0.46±0.05[a]
60% ethanol	2.6±0.06[b]	0.56±0.04[b]	0.70±0.01[ab]	0.22	0.27	0.91±0.09[b]
70% ethanol	2.7±0.06[b]	0.71± 0.02[c]	0.73±0.01[b]	0.26	0.27	0.92±0.08[b]
80% ethanol	3.7±0.09[c]	2.5±0.06[d]	0.87±0.01[c]	0.68	0.24	1.1±0.02[c]

Data are expressed as a mean value of replication (n)±SD (standard deviation); [a]n = 6, [b]n = 4, [c]n = 4, [d]n = 6.

TABLE 2. Total phenolics content (TP), total flavonoids content (TF), total proanthocyanidins content (TPA) and antioxidant capacity (AC) of fig fruits (variety Šaraguja) extracted by using 80% (v/v) ethanol at different extraction temperatures.

Temperature (°C)	TP[a] (mg_{GAE}/g_{db})	TF[b] (mg_{CE}/g_{db})	TPA[c] (mg/g_{db})	TF/TP	TPA/TP	AC[d] (mg_{VCE}/g_{db})
25	2.5±0.05[a]	0.68±0.04[a]	0.67±0.01[a]	0.27	0.27	1.5±0.6[c]
40	2.9±0.20[b]	1.0±0.01[b]	0.72±0.01[ab]	0.35	0.25	1.1±0.11[b]
50	3.1±0.09[b]	1.7±0.09[c]	0.77±0.01[b]	0.55	0.25	1.5±0.08[c]
60	3.4±0.22[c]	2.3±0.23[d]	0.86±0.04[c]	0.68	0.25	1.0±0.12[a]
70	3.6±0.12[cd]	2.3±0.02[d]	0.87±0.02[c]	0.64	0.24	1.0±0.03[a]
80	3.7±0.09[d]	2.5±0.06[e]	0.87±0.01[c]	0.68	0.24	1.1±0.02[b]

Data are expressed as a mean value of replication (n)±SD (standard deviation); [a]n = 6, [b]n = 4, [c]n = 4, [d]n = 6.

TABLE 3. Total phenolics content (TP), total flavonoids content (TF), total proanthocyanidins content (TPA) and antioxidant capacity (AC) of different fig fruits extracted by using 80% (v/v) ethanol at 80°C.

Fig variety	TP[a] (mg_{GAE}/g_{db})	TF[b] (mg_{CE}/g_{db})	TPA[c] (mg/g_{db})	TF/TP	TPA/TP	AC[d] (mg_{VCE}/g_{db})
Bjelica	2.6±0.17[a]	2.1±0.05[a]	0.73±0.01[a]	0.81	0.28	0.82±0.05[a]
Termenjača	3.1±0.06[b]	2.2±0.10[a]	0.86±0.01[b]	0.71	0.28	1.4±0.03[c]
Šaraguja	3.7±0.09[c]	2.5±0.06[c]	0.87±0.01[b]	0.68	0.24	1.1±0.02[b]
Brežutka bijela	3.9±0.10[d]	2.3±0.07[b]	0.89±0.00[b]	0.59	0.23	1.3±0.13[c]
Crnica	4.7±0.09[e]	2.5±0.05[c]	1.2±0.02[c]	0.53	0.26	2.0±0.05[d]

Data are expressed as a mean value of replication (n)±SD (standard deviation); [a]n = 6, [b]n = 4, [c]n = 4, [d]n = 6.

Since the best extraction efficiency was obtained with 80% (v/v) aqueous ethanol, this solvent was used in further experiments where: a) the influence of temperature on the extractability of phenolic compounds was investigated, and b) phenolic compounds were extracted from different fig varieties.

Within the investigated temperature interval (25–80°C), the extractability of phenolic compounds was increasing along with increasing temperature. The influence of temperature was shown to be statistically significant (ANOVA, Duncan *post-hoc* test, $p<0.05$) (Table 2). The total phenolic compounds content in fig fruit after 120 min of extraction varied in the range of 2.5–3.7 $mg_{GAE}/g_{db,}$ while the content of total flavonoids was 0.68–2.5 mg_{CE}/g_{db} and total proanthocyanidins 0.67–0.87 mg/g_{db} from lower to higher temperatures. A statistically significant difference in the content of phenolic compounds, confirmed by ANOVA and Duncan *post-hoc* test ($p<0.05$), was found in fruits of other fig varieties (Bjelica, Termenjača, Šaraguja, Brežutka bijela, Crnica). The highest content of total phenolic compounds was found in variety Crnica (4.7 mg_{GAE}/g_{db}), followed by varieties Brežutka bijela (3.9 mg_{GAE}/g_{db}), Šaraguja (3.7 mg_{GAE}/g_{db}) Termenjača (3.1 mg_{GAE}/g_{db}) and Bjelica (2.6 mg_{GAE}/g_{db}) (Table 3). Other authors [Solomon *et al.,* 2006; Marinova *et al*., 2005] extracted phenolic compounds from figs too, but it is difficult to compare their findings with our results due to differences in extraction method applied, part of fig subjected to extraction and the mode of expression of results (on dry or fresh basis of figs). For instance, Solomon *et al.* [2006] extracted phenolic compounds from fresh peeled figs using acidified ethanol at a room temperature. They expressed the extraction yield per mass of the fresh sample (TP=0.49–2.8 mg_{GAE}/g, TF=0.02–0.22 mg_{CE}/g). In turn, Marinova *et al.* [2005] extracted phenolic compounds from lyophilised figs with 80% methanol at a room temperature, and expressed results per mass of the lyophilised samples (TP=0.59 mg_{GAE}/g, TF=0.20 mg_{CE}/g).

Flavonoids are a significant subgroup of phenolic compounds in fig fruits, which was confirmed with the value of the TF/TP ratio in fig fruits. In general, the TF/TP ratio increased from 0.18 to 0.68 with increasing ethanol content in the extraction solvent (Table 1). The TF/TP ratio also increased from 0.27 to 0.68 with the increasing of the temperature (Table 2). Fruits of all tested fig varieties had a high TF/TP ratio which was in the range from 0.53 to 0.81 (Table 3). The role of solvent composition, extraction temperature and fig variety had a lesser influence on TPA/TP ratios in fig fruits, which were in the range from 0.23 to 0.28 (Table 1–3). However, the lower temperature (25°C) and the lower content

of ethanol in the solvent (50%, v/v) enabled achieving slightly higher TPA/TP ratios in fig fruits than in the experiments with other extraction conditions. According to these results, it is clear that even when the TP and TPA content increased with temperature and ethanol content in the solvent, the greater effect was directed to the TP, not to the TPA. A similar trend was reported in the case of extraction of total phenolic compounds and proanthocyanidins from grape seeds [Huh *et al.*, 2004].

Antioxidant activity of phenolic compounds derives from the high redox potential and the ability of donating electrons or hydrogen atoms to reactive radicals, which leads to stopping undesirable chain-reaction that cause oxidative stress [Tsao & Deng, 2004]. The antioxidant capacity of fig fruits varied from 0.46 to 2.0 mg_{VCE}/g_{db} in all performed experiments (Table 1-3) and was determined by measuring the inhibition of DPPH• radical. These results can be considered indicative of a good antioxidant capacity. Solomon *et al*. [2006] determined the total antioxidant capacity of common fig fruits extracts by ABTS•$^+$ test and results were expressed as Trolox equivalent (0.21–19.87 μmolTE/g). These data are not comparable with AC of fig fruits obtained in our study because of different assays applied. Du Toit *et al*. [2001] reported AC for a mixture of different fruits (apple, pear, banana and orange) to account for 0.79 mg_{VCE}/g_{fruit}.

The influence of the solvents used (Table 1) on the antioxidant capacity of fig fruits was similar to their influence on phenolic compounds content. The antioxidant capacities were observed to increase with ethanol content increasing in the solvent from 50 to 80% (v/v). The antioxidant capacity of fig fruits extracted with 60 and 70% (v/v) aqueous ethanol was not statistically different. Furthermore, the higher content of phenolic compounds in the fig fruits caused by extraction temperature increase was not accompanied by the expected increase in the antioxidant capacity (Table 2). The highest antioxidant capacity was measured at 25 and 50°C, while the lowest one was observed at 60 and 70°C. The statistical analysis confirmed that the tested fruits of different fig varieties (Table 3) displayed different antioxidant capacities. The highest antioxidant capacity was exhibited by variety Crnica and the lowest on by variety Bjelica. The difference in the antioxidant capacity of fig varieties Termenjača and Brežutka bijela was not statistically significant.

The correlation coefficients (r) calculated between the antioxidant capacity of fig fruits and corresponding TP (r=0.191), TF (r=0.451) and TPA (r=0.343) were weak. These results imply that phenolic compounds were not the major contributors to the antioxidant capacity of the investigated fig fruits.

According to the available literature, there is no general conclusion about the correlation between the content of phenolic compounds from plants and antioxidant capacity. Thus, some authors have found a high correlation [Makris *et al.*, 2007; Pinelo *et al.*, 2005; Turkmen *et al.*, 2006] while others did not find any correlation between phenolics content and antioxidant capacity [Ruberto *et al.*, 2007; Yu *et al.*, 2002]. The likely reason for this may be the difference in methods of extract preparation, the diversity of methods used to determine antioxidant capacity, results interpretation, the difference in the evaluation of the effect of interfering substances (ascorbic acid, saccharides and/or possibly carotenoids), *etc.* [Stratil *et al.*, 2007].

CONCLUSIONS

The study addressing the effect of extraction conditions on the extractability of phenolic compounds from lyophilised fig fruits (*Ficus carica* L.) revealed that the fig variety and extraction process conditions had a significant effect on the content of phenolic compounds in the fruits. A higher ethanol to water ratio and a higher extraction temperature had a positive influence on the extractability of phenolic compounds.

The highest content of total phenolic compounds (TP=4.7 mg_{GAE}/g_{db}) was exhibited by fig variety Crnica and the lowest content by fig variety Bjelica (TP=2.6 mg_{GAE}/g_{db}) using 80% (v/v) aqueous ethanol at 80°C.

Taking into account that phenolic compounds contribute to fig colour, the results obtained somehow justify the names of fig varieties. Translated from Croatian language, Crnica means “black fig” and Bjelica means “white fig”.

Furthermore, the results obtained in this study indicate that fig fruits can be considered as a natural source of phenolic compounds with good antioxidant capacity.

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