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INFLUENCE OF EXTRACTION TYPE ON THE TOTAL PHENOLICS, TOTAL FLAVONOIDS AND TOTAL COLOUR CHANGE OF DIFFERENT VARIETIES OF FIG EXTRACTS

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Original scientific paper

Summary
The study examined the influence of ultrasound-assisted extraction and solid-liquid extraction with 80% of aqueous ethanol solution on the total phenolics, total flavonoids and total colour change of extracts from different varieties of figs (Bjelica, Termenjača, Crnica, Bružetka bijela and Šaraguja). The total phenolic content was determined by using Folin Ciocalteau assay. The content of total flavonoids was measured spectrophotometrically by using the aluminium chloride colourimetric assay. Colour changes were obtained by chromameter CR-400 (Minolta) in L*a*b* colour model. Ultrasound-assisted extraction showed highest total phenolic content (up to 13.72%) and total flavonoid content (up to 18.55%) compared to classic solid-liquid extraction. According to given results total colour changes of extracts were reduced (up to 32.1%) applying ultrasound. Significant difference was found between the total phenolic and total flavonoid content according to different varieties of fig.

Keywords: fig, extraction, phenolics, flavonoids, colour

Introduction

The cultivated fig (*Ficus carica L.*) belongs to botanical family *Moracea*. Figs are a widespread species commonly grown, especially in warm, dry climates and they are mostly concentrated in the Mediterranean. Figs are an excellent source of minerals, vitamins and dietary fibre, fat and cholesterol-free and contain essential amino acids as well as high content of phenolic substances (Slavin, 2006; Solomon et al., 2006).
Phenolic compounds represent an important component of fruit because of their significantly contribution to the taste, colour and nutritional value of fruits (Veberic et al., 2008), and in recent years have been the subject of scientific research for many researchers because of their positive effects on human health, which is attributed to the antioxidation activities.
Extraction is an important step in isolation and in later identification and quantification of phenolic compounds (Cacace and Mazza, 2003). Solid-liquid extraction is a commonly used method of isolation phenolic compounds from plant materials. Selection of solvents is one of the most important steps in the extraction, and in previous studies the most commonly used solvent for the solid-liquid extraction of phenolic compounds from plant materials are methanol, ethanol and their liquid mixtures with different content of water and other organic solvents such as acetone, ethyl acetate (Naczk and Shahid, 2004).
Ultrasound-assisted extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques. The main benefits of use of ultrasound in solid–liquid extraction include the increase of extraction yield and faster kinetics. Ultrasound can also reduce the operating temperature allowing the extraction of thermolabile compounds (Wang and Weller, 2006).
The phenol content from fig have been recently analysed by Duenas et al. (2008), Solomon et al. (2006) and Veberic et al. (2008), but literature data about the influence of different extraction methods on the extractability of phenolic compounds from figs are scarce. This study examined the influence of ultrasound-assisted extraction and solid-liquid extraction with 80% of aqueous

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ethanol solution on the total phenolics, total flavonoids and total colour change of extracts from five different autochthonous varieties of figs (Bjelica, Termenjača, Crnica, Bružetka bijela and Šaraguja).

Materials and methods

Material

Fruits from five selected commercial fig (*Ficus carica L.*) varieties: Bjelica, Termenjača, Crnica, Bružetka bijela and Šaraguja were harvested in region of Istria at the optimal ripening time (given by experts in agronomy) in year 2008. Fig samples were frozen at -20 °C and than freeze dried in the freeze drying equipment (LIO-10P, Kambic d.o.o., Slovenia). Samples were grounded a blender before the extraction. Dry matter content of fig samples were determined by drying of 5 g milled fig samples at 105 °C to constant mass. Analyses were done in duplicate and the average dry matter content was noted as percentage (%). The content of dry matter was in range from 85-88% depending from fig variety of samples used in this study.

Solid-liquid extraction

In the test tubes, 0.5 g of fig sample was mixed with 20 ml of 80% aqueous ethanol solution. The extraction process was conducted in laboratory scale by using water bath (Julabo SW-23, Germany) with shaking (200 rpm) for 15 minutes at 50 °C. All extraction runs were performed in duplicate. Obtained extracts were separated from rough particles after extraction by decantation and were centrifuged (Sigma 2-16, Germany) for 10 minutes on 10 000g. Supernatant was decanted and updated to the known volume (20 ml). Supernatant was stored at +4 °C until further analysis.

Ultrasound-assisted extraction

Fig samples (0.5 g) were extracted by 80% aqueous ethanol solution (20 ml) as a solvent. The extraction process was carried out using ultrasonic bath (Sonorex Super RK 100 SH) at the 50 °C for 15 minutes in two repetitions.
Ethanol extracts were centrifuged at 10 000 g for 10 minutes and pooled with extraction solvent. Supernatant was stored at +4 °C until further analysis.

Total phenolic content (TPC)

The concentration of total phenolic compounds in the extracts was determined by Folin-Ciocalteau micro-method (Waterhouse, 2009) as follows: 40 µl of extract was mixed with 3160 µl of distilled water and 200 µl of Folin-Ciocalteau reagent. After 30 seconds to 8 minutes, 600 µl of 20% of sodium carbonate solution was added. All test tubes with mixture were shaken for 10 seconds on the Vortex and incubated in a water bath at 40 °C. Absorbance was measured after 15 min on UV/VIS spectrophotometer (UV 1700 Shimadzu, Japan) at 765 nm against blank sample. Blank sample was prepared with water instead of extract. Determination of total phenolic compounds was carried out in a duplicate and calculated from the calibration curve obtained with gallic acid, which was used as a standard and final results were recalculated and expressed as gallic acid equivalent per dry basis of fig samples (mg GAE/gdb).

Total flavonoid content (TFC)

The concentration of total flavonoid compounds in the extracts was determined by the aluminium chloride colourimetric assay (Marinova et al., 2005) as follow: 1 ml of extract was added to 10 ml volumetric flask containing 4 ml of distilled water and 0.3 ml 5% NaNO2. After 5 min, 0.3 ml 10% AlCl3 was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Determination of total flavonoid compounds was carried out in a duplicate and calculated from the calibration curve obtained with (+)-catechin, which was used as a standard and final results were recalculated and expressed as (+)-catechin equivalent per a dry basis of fig samples (mg CE/gdb).

Colour measurement

The colour of samples was measured using Minolta CR-400 Chromameter. The figs were milled in a coffee grinder to obtain fine powder. Analyses of colour values were done twenty times for each sample. Three parameters, L (lightness), a (redness) and b (yellowness), were used to study changes in the colour. L refers to the lightness of the samples and ranges from black = 0 to white = 100. A negative value of a indicates green, while a positive one indicates red-purple colour. Positive b indicates yellow and negative blue colour. The total colour difference (ΔE) was calculated as follows (Hunter, 1975):

$$\ddot{A}E=\sqrt{\left[\left(L-L_0\right)^2+\left(a-a_0\right)^2+\left(b-b_0\right)^2\right]} \quad (1)$$

where L0, ao and b0 indicate colour parameters of fresh fig samples. Fresh fig samples were used as the reference and a higher ΔE represents greater colour change from the reference material.

Statistical analysis

Statistica 7.0 (Stat Soft Inc., USA) was used for data analyzing. One-way analysis of variance (ANOVA) and multiple comparisons (post-hoc LSD) were used to evaluate the significant difference of the data at $p<0.05$. Data were expressed as means ± standard deviation. Experiments were replicated two times for statistical purpose.

Results and discussion

The aim of this study was to examine the influence of ultrasound-assisted extraction and solid-liquid extraction with 80% of aqueous ethanol solution as a solvent on the total phenolics, total flavonoids and total colour change of extracts from five different autochthonous varieties of figs: Bjelica, Termenjača, Crnica, Bružetka bijela and Šaraguja. (Fig. 1).

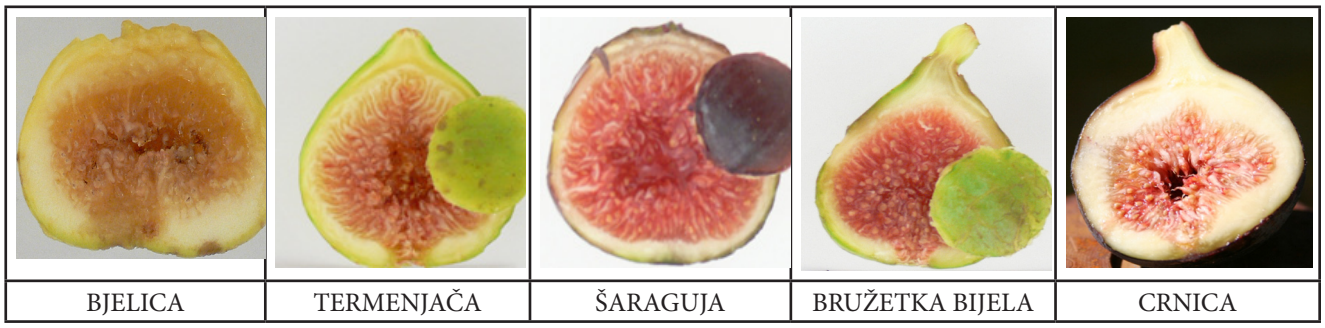

Fig. 1. The autochthonous fig varieties in Istria (Croatia)

Dry matter content in all experimental runs was determined and the results of total phenol and total flavonoid concentrations were expressed on dry basis, which generally provides more accurate and reliable data comparison.

Fig. 2 showed the total colour change (ΔE) of freeze dried fig samples. It can be seen that fig sample variety Crnica showed the smallest total colour changes, while fig sample variety Termenjača showed the highest colour changes according to fresh fig sample. An ANOVA analysis showed the existence of four groups which differed significantly ($p < 0.05$) from one to another according to different fig varieties. Represented results show that there are no statistically significant differences according to colour changes between freeze dried fig samples variety Bjelica and Bružetka bijela.

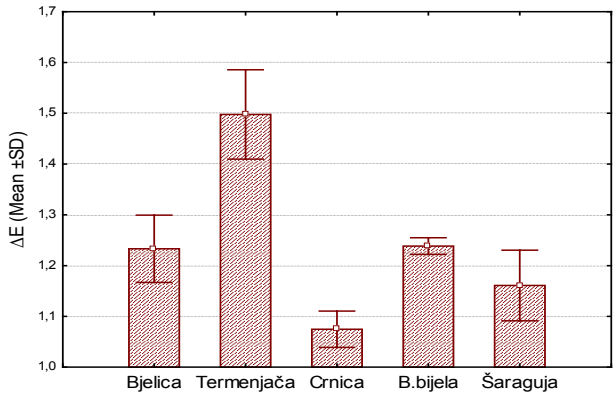

Fig. 2. Total colour change (ΔE) of freeze dried fig samples

Fig. 3 showed the total colour change (ΔE) of extracts obtained by ultrasound-assisted extraction and solid-liquid extraction with 80% aqueous ethanol solution from different fig varieties. It can be seen that total colour changes of extracts were significantly reduced applying ultrasound up to 31.1% in fig variety Bjelica, 6.2% in fig variety Termenjača, 15.2% in fig variety Crnica, 26.6% in fig variety Bružetka bijela and up to 3.1% in fig variety Šaraguja compared to classic solid-liquid extraction.

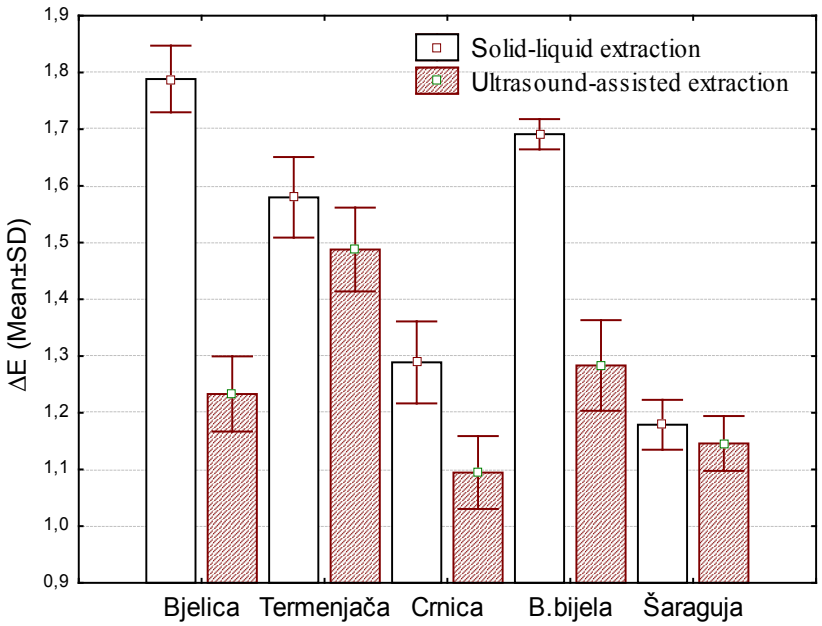

Fig. 3. Total colour change (ΔE) of extracts from different fig varieties

Fig. 4 and Fig. 5 showed the influence of two investigated extraction methods on total phenolic content and total flavonoid content in different fig extracts, respectively. The aqueous ethanol solution was selected as solvent due to environmental safety, low cost and less toxicity, unlike the other solvents (e.g. methanol).

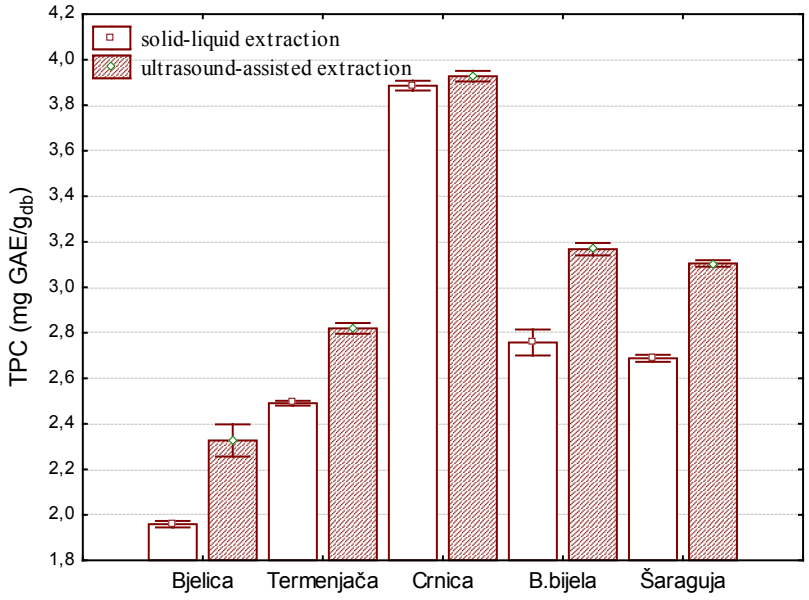

Fig. 4. Effect of extraction type on total phenolic content in different fig extracts

Although for the food purposes water represented the best solvent selection, as a polar solvent (such as the phenolic compounds) water extracted the other undesirable macromolecules as well (protein, polysaccharide, etc.) especially at higher temperatures and pressures (Rostagno et al., 2003; Tsao and Deng, 2004). Spigno et al. (2007) found that a higher content of water in the ethanol/water solution (concentration of aqueous ethanol solution lower than 50%) reduced the extraction of polyphenols. Also, Rostango et al. (2004) found that it is necessary to add a certain amount of water in the extraction solvent in order to improve the extraction of phenolic compounds. The water content higher than 60% resulted in a reduction of the extraction yield the same components. Using pure ethanol as solvent reduced the extraction efficiency since the polyphenols, due to a number of hydroxyl groups (such as flavonoids, glycosides), are hydrophyl, and as such generally more soluble in water-ethanol solutions than in pure alcohol. The reason for low content of phenolic substances in water extracts can also be the increased activity of enzymes (polyphenol oxidase, PPO) that degraded phenolic substances unlike alcoholic media in which the same is inactive (Lapornik et al., 2005).

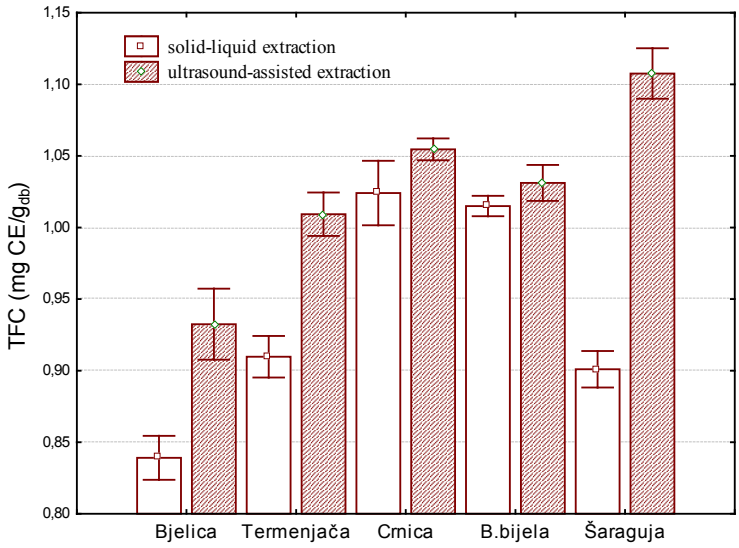

Fig. 5. Effect of extraction type on total flavonoid content in different fig extracts

Represented results (Fig. 4 and Fig. 5) showed that ultrasound-assisted extraction increased the total phenolic content (up to 13.72%) as well as the total flavonoid content (up to 18.55%) in fig samples. The reason for this is the mechanical effects of ultrasound which caused a greater penetration of solvent into cellular materials and improved mass transfer. Ultrasound in extraction can also disrupt biological cell walls, facilitating the release of contents. The efficient cell disruption and effective mass transfer can increased the extraction yield and, furthermore, ultrasound can even improved the quality of extracts (Mason et al., 1996; Wang and Weller, 2006). The highest concentration of total phenols was obtained from fig extracts variety Crnica, while the lowest concentration of total phenols was achieved from fig variety Bjelica. Statistical analysis (ANOVA, post-hoc LSD, $p<0.05$) showed that applied extraction methods had no statistically significant influence only on total phenolic content in the fig samples variety Crnica. The darker varieties of figs resulted in the highest content of phenolics and flavonoids compare to white type of figs such as analyzed variety of fig Bjelica. The similar results have been published by Veberic et al. (2008), where the phenol content in the white variety of fig ("Škofjotka") was significantly lower than in the darker varieties ("Miljska figa" and "Črna petrovka").

Similar data for the extraction yield of phenolic compounds from figs had been published by

other authors, where specific differences exist because of different methodologies of experimental work, sample diversity (climate, variety, etc.) or part of the sample from which extraction was carried out (fruit with or without skin, pulp, leaves) and ways of expression the content of phenolic compounds (expressed on the mass of fresh sample, or on the mass of dry matter) in the sample. Marinova et al. (2005) were determined the phenolic content from freeze-dried fig samples with 80% aqueous methanol solution at room temperature. The extraction yield of total phenolic content from fig sample was expressed as mg gallic acid equivalents GAE/100g fresh weight (TPC = 59.0 mgGAE/100g fresh mass) and total flavonoid content was expressed as mg catechin equivalents CE/100g fresh weight (TFC = mgCE/100g fresh mass).

Conclusions

According to experimental results in this study, ultrasound-assisted extraction showed the highest total phenolic content (up to 13.72%) and total flavonoids content (up to 18.55%) compared to classic solid-liquid extraction. Represented results show that total colour changes of extracts were reduced (up to 32.1%) applying ultrasound. Significant difference was found between total phenolic and total flavonoids content according to different varieties of fig. The darker varieties of figs resulted in the highest content of phenolics and flavonoids compare to white type of figs. Ultrasound can increase the extraction yield and improved the quality of extracts. Additional research is required to investigate the chemical composition of these phenolic compounds in fig samples.

Acknowledgements

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References

Cacace J E, Mazza G (2003) Mass transfer process during extraction of phenolic compounds from milled berries. J Food Eng 59:379-389

Duenas M, Pérez-Alonso J J, Santos-Buelga C, Escribano-Bailón T (2008) Anthocyanin composition in fig (Ficus carica L.). J Food Compos Anal 21:107-115

Hunter RS (1975) Scales for the measurements of colour difference. In: Measurement of Appearance, John Willy & Sons, New York, USA, pp 133–140

Lapornik B, Prošek M, Golc Wondra A (2005) Comparison of extracts prepared from plant by-products using different solvent and extraction time. J Food Eng 71: 214-222

Marinova D, Ribarova F, Atanassova M (2005) Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Chem Technol Metall 40: 255-260

Mason TJ, Paniwnyk L, Lorimer JP (1996) The uses of ultrasound in food technology. Ultrason Sonochem 3: 253–260

Naczk M, Shahidi F (2004) Extraction and analysis of phenolics in food. J Chromatogr A 1054: 95-111

Rostango MA, Palma M, Barroso CG (2004) Pressurized liquid extraction of isoflavones from soybeans. Anal Chim Acta 522: 169-177

Slavin JL (2006) Figs: Past, Present and Future. Nutr Today 41: 180-184

Solomon A, Golubowicz S, Yablowicz Z, Grossman S, Bergman M, Gottlieb HE, Altman A, Kerem Z, Flaishman MA (2006) Antioxidant activities and anthocyanin content of fresh fruits of common fig (Ficus carica L.). J Agric Food Chem 54: 7717-7723

Spigno G, Tramelli L, De Faveri DM (2007) Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J Food Eng 81: 200-208

Tsao R, Deng Z (2004) Separation procedures for naturally occurring antioxidant phytochemicals. J Chromatogr B 812: 85-99

Veberic R, Colaric M, Stampar F (2008) Phenolic acids and flavonoids of fig fruit (Ficus carica L.) in the northern Mediterranean region. Food Chem 106: 153-157

Wang L, Weller CL (2006) Recent advances in extraction of nutraceuticals from plants. Trends Food Sci Techn 17: 300–312

Waterhouse AL (2009) Determination of total phenolics. In: Current Protocols in Food Analytics Chemistry, Wrolstad, R.E. (ed.), : John Wiley & Sons Inc., New York