

Optimization of the Solid-Liquid Extraction Process of Phenolic Compounds from Mulberry Fruit

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Abstract

The study was designed to examine the influence of solvent concentration (ethanol/water 20-80% by volume), extraction time (15-240 min) and extraction technique on the extraction yield of phenolic compounds, flavonoids and antioxidant activity from *Morus nigra* L., *Morus rubra* L. and *Morus alba* L. fruits. The optimum conditions for the extraction of total phenols, flavonoids and monomeric anthocyanins by the maceration and ultrasonic extraction processes were: 213.6 min with 80% ethanol, and 182.1 min and 71.2% ethanol, respectively (black mulberry), and 216.5 min with 78.3% ethanol and 198.7 min and 70.6% ethanol, respectively (red mulberry). In case of white mulberry for the extraction of total phenols and flavonoids optimum extraction conditions (maceration and ultrasonic) were 232.7 min and 80% ethanol, and 187.2 min and 68.7% ethanol, respectively (white mulberry). Obviously, ultrasonic extraction was less time consuming, and it requires for all performed extractions solvent with less percentage of ethanol.

Keywords: anthocyanins; flavonoids; maceration; phenolics; response surface methodology; ultrasonic extraction

Introduction

Genus *Morus* is widespread in Asia, Europe, North and South America and Africa. Mulberry is growing in the temperate and sub-tropical regions of the northern hemisphere (Özgen *et al.*, 2009). Black and white mulberry are quite widespread in the area of Serbia (Kostić *et al.*, 2013). The black and red mulberries are found to be especially rich in anthocyanins, flavonoids and phenol compounds (Özgen *et al.*, 2009). The total content of these compounds depends on the geographic location, soil on which the mulberry tree grows, type of extraction, length of extraction, type of solvent. Mulberry gives the uniquely delicious fruit of sour and refreshing taste. Therefore, it is not surprising that it has been used as a folk remedy to treat oral and dental diseases, diabetes, hypertension, arthritis and anemia (Mahesh *et al.*, 2017). The bright black mulberry fruits, which have a very pleasant taste when eaten fresh, are also used in jams, juices, liquors, natural dyes as well as in the cosmetic industry (Gerasopoulos and Stavroulakis, 1997).

The chemical compositions of white, red and black mulberries were determined. It was found that the highest total phenolic and flavonoid yields were observed in black mulberry (Ercisli and Orhan, 2007). To the best of our

knowledge, there are few reports on the optimization of mulberry leaf extraction (Teng and Lee 2013; Tchabo *et al.*, 2015).

There are no available literature data on the optimization of the extraction of mulberry fruit by different ethanol-water solution, nor of the extraction efficiency and the impact of various extraction techniques on the yield and the phenolic composition. The mulberry and its extracts can be used as a good source of natural plant pigment and antioxidant agents. This study will be of considerable interest to the commercial growers of mulberry trees or pharmaceutical industry for potential mulberry supplement production.

The aim of the study is the determination of the effects of the several parameters (extraction time, solvent concentration, extraction method) on the yield of phenolic compounds, flavonoids, and anthocyanins from black, red and white mulberry, and obtaining of optimum conditions computationally using a response surface model. In earlier studies of the extraction kinetics using different techniques, it was found that higher yields are achieved using circulation techniques comparing to the maceration. The operating conditions have an impact on the extraction yield and the kinetics of extraction (Stanojevic *et al.*, 2007; Grujic *et al.*, 2012).

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Materials and Methods

The plant material—fruits of *Morus nigra* L., *Morus rubra* L. and *Morus alba* L. were collected in South-East Serbia in early July 2017.

Apparatus and reagents

An Agilent 8453 UV-vis spectrophotometer (USA) was used for the absorbance measurements and spectra recording, using an optical or quartz cuvettes of 1 cm optical path. The pH measurements were made with Hanna Instruments pH-meter (USA) equipped with the glass electrode.

The Folin–Ciocalteu phenol reagent and sodium carbonate were purchased from Merck Chemical Suppliers (Darmstadt, Germany). Sodium chlorate buffer (pH 1.0) and acetate buffer (pH 4.5) were purchased from the same producer. The other used chemicals including solvents were of analytical grade.

Maceration

Triturated and homogenized fresh mulberry fruit (10 g) was soaked into the mixture of the previously prepared solvent: ethanol-water-HCl (20:79:1 v/v/v), ethanol-water-HCl (50:39:1 v/v/v) and ethanol-water-HCl (80:19:1 v/v/v) at a ratio of 1:10 w/v. Maceration with a solvent system was performed at 15, 127.5 or 240 min at 25 °C. The suspension was then filtered through a Buchner funnel and Whatman No.1 filter paper. The extracts were stored in the refrigerator and in the dark to their further use for the determination of phenolic compounds (Dean, 2009).

Ultrasonic extraction

Milled plant material (10 g) was extracted with the previously mentioned solvent systems in the thermostatic ultrasonic bath (Sonic, Niš, Serbia) with the nominal power: 3×50 W; dimensions of bathrooms: 30×15×20 cm, and at a frequency of 40 kHz. The kinetics of the extraction of phenolic compounds were collected at the indicated time intervals (15-240 minutes). The extracts were separated from the plant material on Buchner's funnel with a weak vacuum and further treated according to the procedure for the determination of total phenols (Vinatoru, 2015).

Determination of total phenolics

Total phenol contents of the extracts were found by the modified Folin-Ciocalteu method. An aliquot of the extracts (1 mL) was mixed with 0.5 mL Folin-Ciocalteu reagent and 2 mL of sodium carbonate (20%). The absorbance was recorded after 10 min of the incubation at

room temperature at 760 nm. The total phenolic content was expressed as mg/100 g gallic acid equivalent (GAE). The result of each assay was obtained from 3 parallel determinations (Singleton and Rossi, 1965).

Determination of total flavonoid content

Total flavonoid content was determined using a spectrophotometric method based on the formation of flavonoid complex with aluminum. Total flavonoid content was calculated as catechin (mg CE/100g) using the equation based on the calibration curve (Stojanovic *et al.*, 2016).

Determination of the total monomeric anthocyanins

The total monomeric anthocyanin content in the plant extracts was determined using the pH-differential method previously described. The result, taken as the monomeric anthocyanin pigment (MAP), was shown as mg of cyanidin-3-*O*-glucoside/L (Giusti and Wrolstad, 2001).

Response surface methodology and optimum conditions for the extraction

Response surface design and the finding of the optimum conditions for the optimization of maceration and ultrasonic extractions were achieved using the software JMP 14.0.1 (SAS Institute Inc., Cary, USA) (Mietlowski, 2008). Two factors were selected: time (15 min, 127.5 min, and 240 min) and % ethanol (20%, 50%, and 80%), and three responses: total phenols, flavonoids and monomeric anthocyanins in case of black and red mulberries, and two responses (total phenols and flavonoids) for white mulberry, and the Central Composite Design with 2 central points. The script for the model was run, and the results were displayed with all statistical data. The optimum conditions regarding time and the solvent system were found using the option of Prediction profiler and the selection of Maximize Desirability. The average value of the optimum time and the solvent system was found from three measurements.

Results and Discussion

In Table 1 it is shown the dependence of the contents of total phenols in ethanol-aqueous extracts of mulberry fruit on time with different concentrations of ethanol (20, 50 and 80%) of maceration process and ultrasonic extraction. The process lasted 15, 127.5 and 240 min. Total phenol content was shown as mg gallic acid equivalents (GAE) per 100 g of fruit.

The content of total phenolics in the tested extracts of black mulberry ranges for maceration and ultrasonic extractions from 60.04 to 150.13 mg and 69.11 to 142.18 GAE/100g fresh fruit, respectively.

Table 1. Average of total phenolic content in different extracts of mulberry fruits for five repetitions (mg GAE/100 g f.w.)

| T | 15 min | | | | | | 127.5 min | | | | | | 240 min | | | | | |
|---------|--------|--------|-------|--------|-------|-------|-----------|--------|--------|--------|--------|--------|---------|--------|--------|--------|--------|--------|
| | Black | | Red | | White | | Black | | Red | | White | | Black | | Red | | White | |
| Ethanol | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. |
| 20% | 60.04 | 69.11 | 50.05 | 62.20 | 45.10 | 55.06 | 100.12 | 101.02 | 92.04 | 92.08 | 88.02 | 86.05 | 118.00 | 100.11 | 112.14 | 88.15 | 107.12 | 81.04 |
| 50% | 77.12 | 93.04 | 70.11 | 84.12 | 63.21 | 77.14 | 120.13 | 120.06 | 105.07 | 110.11 | 99.06 | 98.13 | 128.12 | 120.15 | 120.17 | 112.13 | 113.09 | 103.09 |
| 80% | 90.07 | 110.10 | 80.09 | 100.03 | 74.14 | 92.09 | 137.08 | 140.15 | 122.13 | 125.05 | 111.04 | 101.75 | 150.13 | 142.18 | 138.18 | 129.07 | 130.05 | 121.07 |

In the period of the rapid extraction, washing, and dissolving of the extractive matter from the surface of the destroyed cells of plant material proceeded with more than 90% of total phenols. It shows that the fragmentation of the plant material used for the tests is relatively high with a high degree of the destruction of cells.

The extraction process with ultrasonic mixing is much more efficient than extraction by maceration. The most likely mechanism of action of ultrasonic extraction is intensifying mass transfer and easier penetration of the solvent into the cells of the plant material. In the classical maceration normal mechanism of diffusion through the cell walls is occurred, and therefore this process requires a much longer extraction time (Milenovic *et al.*, 2002).

In Table 2 it is shown the dependence of the contents of flavonoids in the extracts on time with different contents of ethanol obtained by maceration and ultrasonic extraction. The flavonoid content is expressed as mg catechin equivalents (CE) per 100 g of fruit.

The flavonoid content of the extract of black mulberry obtained by maceration and ultrasonic extraction is ranged from 60.02 to 82.08 and from 53.13 to 108.16 mg CE/100g, respectively. Red and white mulberry have less content of flavonoids in all investigated extracts. After the extraction of 120 min by the maceration, an increase in the yield of flavonoids to 240 min is slower. The application of the ultrasonic extraction increases the yield of flavonoids in the tested extracts at the same conditions.

In Table 3 it is shown the dependence of the content of the monomeric anthocyanins with maceration time and ultrasonic extraction for different compositions of the mixture. The content of anthocyanin is expressed as mg of cyanidin-3-*O*-glucoside per 100 g of the fruit.

Anthocyanin content of the extracts obtained by the process of maceration ranges from 52.03 to 137.06 for maceration and from 64.08 to 137.06 mg Cy-3-*O*-glu/100 g for ultrasonic extraction for black mulberry.

The fragmentation of the plant material used for testing is relatively high. A higher degree of the destruction of cells increases the surface area from which in a rapid period

extractive matter was washed down quickly and thus provides a high level of the extraction in this period.

In a period of a rapid extraction (120 min), by maceration, it was extracted 78.60% of total extractive matter, 86.89% of total phenolic compounds, 85.22% of flavonoids and 75.51% of anthocyanins. The period of rapid extraction by ultrasonic extraction was 120 min for dry residue and flavonoids (85.33 and 86.23%, respectively) and 60 min for total phenolic (81.95%) and anthocyanins (86.07%).

The computational modeling-response surface design and subsequent optimization give more easily optimized conditions for both maceration, and ultrasonic extraction (Fig. 1).

The optimum conditions for the extraction of total phenols, flavonoids and monomeric anthocyanins by the maceration process were 213.6 min with 80% ethanol (black mulberry), and 216.5 min with 78.3% ethanol (red mulberry), and 232.7 min and 80% ethanol (white mulberry) for the extraction of total phenols and flavonoids. In case of ultrasound extraction, the extraction of total phenols, flavonoids, and total anthocyanins was the most effective with 182.1 min and 71.2% ethanol (black mulberry), and 198.7 min and 70.6% ethanol (red mulberry), and the extraction of total phenols and flavonoids with 187.2 min and 68.7% ethanol for white mulberry.

Optimizations of ultrasonic-assisted extractions were performed before for the black mulberry (Zou *et al.*, 2011; Espada-Bellido *et al.*, 2017), but different factors were chosen comparing to our analysis.

Solid-liquid extractions were optimized considering the antioxidants and saccharides from black mulberry fruit (Radojkovic *et al.*, 2013; Fang *et al.*, 2014). Mulberry leaves were also the subject of the investigation of the optimal conditions for the extraction (Radojkovic *et al.*, 2012a, b; Tchabo *et al.*, 2018). The response surface methodology was used not only for ultrasonic extractions but also for microwave-assisted extractions of mulberries (Teng and Lee, 2013; Zou *et al.*, 2012).

Table 2. Average of flavonoid content in different extracts of mulberry fruits for five repetitions (mg CE/100 g f.w.)

| T | 15 min | | | | | | 127.5 min | | | | | | 240 min | | | | | |
|-----|--------|-------|-------|-------|-------|-------|-----------|--------|-------|--------|-------|-------|---------|--------|-------|--------|-------|-------|
| | Black | | Red | | White | | Black | | Red | | White | | Black | | Red | | White | |
| | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. |
| 20% | 60.02 | 53.13 | 52.14 | 50.10 | 45.06 | 44.16 | 71.13 | 84.00 | 62.03 | 77.08 | 56.16 | 71.07 | 72.14 | 86.16 | 63.03 | 78.12 | 58.02 | 73.06 |
| 50% | 67.12 | 70.08 | 56.09 | 66.08 | 50.04 | 61.12 | 76.06 | 107.11 | 71.05 | 102.15 | 64.13 | 98.09 | 78.19 | 108.16 | 71.06 | 102.06 | 63.06 | 96.08 |
| 80% | 72.05 | 75.11 | 60.03 | 70.01 | 56.10 | 63.06 | 80.04 | 96.15 | 76.10 | 89.05 | 59.04 | 83.12 | 82.08 | 96.12 | 75.10 | 89.09 | 66.09 | 82.10 |

Table 3. Average of total anthocyanin content in different extracts of mulberry fruits for five repetitions (mg cyanidin-3-*O*-glucoside /100 g f.w.)

| T | 15 min | | | | | | 127.5 min | | | | | | 240 min | | | | | |
|-----|--------|-------|-------|-------|-------|------|-----------|--------|--------|-------|-------|------|---------|--------|--------|--------|-------|------|
| | Black | | Red | | White | | Black | | Red | | White | | Black | | Red | | White | |
| | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. | mac. | u.s. |
| 20% | 52.03 | 64.08 | 41.06 | 52.12 | 0.01 | 0.01 | 96.02 | 92.09 | 81.05 | 74.12 | 0.01 | 0.01 | 110.01 | 94.11 | 105.02 | 75.04 | 0.01 | 0.02 |
| 50% | 68.09 | 86.05 | 56.03 | 65.15 | 0.02 | 0.01 | 125.12 | 110.10 | 110.07 | 90.06 | 0.01 | 0.02 | 137.06 | 118.05 | 126.12 | 101.08 | 0.01 | 0.01 |
| 80% | 72.12 | 86.16 | 61.11 | 66.18 | 0.01 | 0.02 | 118.13 | 110.13 | 102.01 | 88.13 | 0.01 | 0.01 | 123.10 | 110.03 | 108.18 | 100.10 | 0.01 | 0.02 |

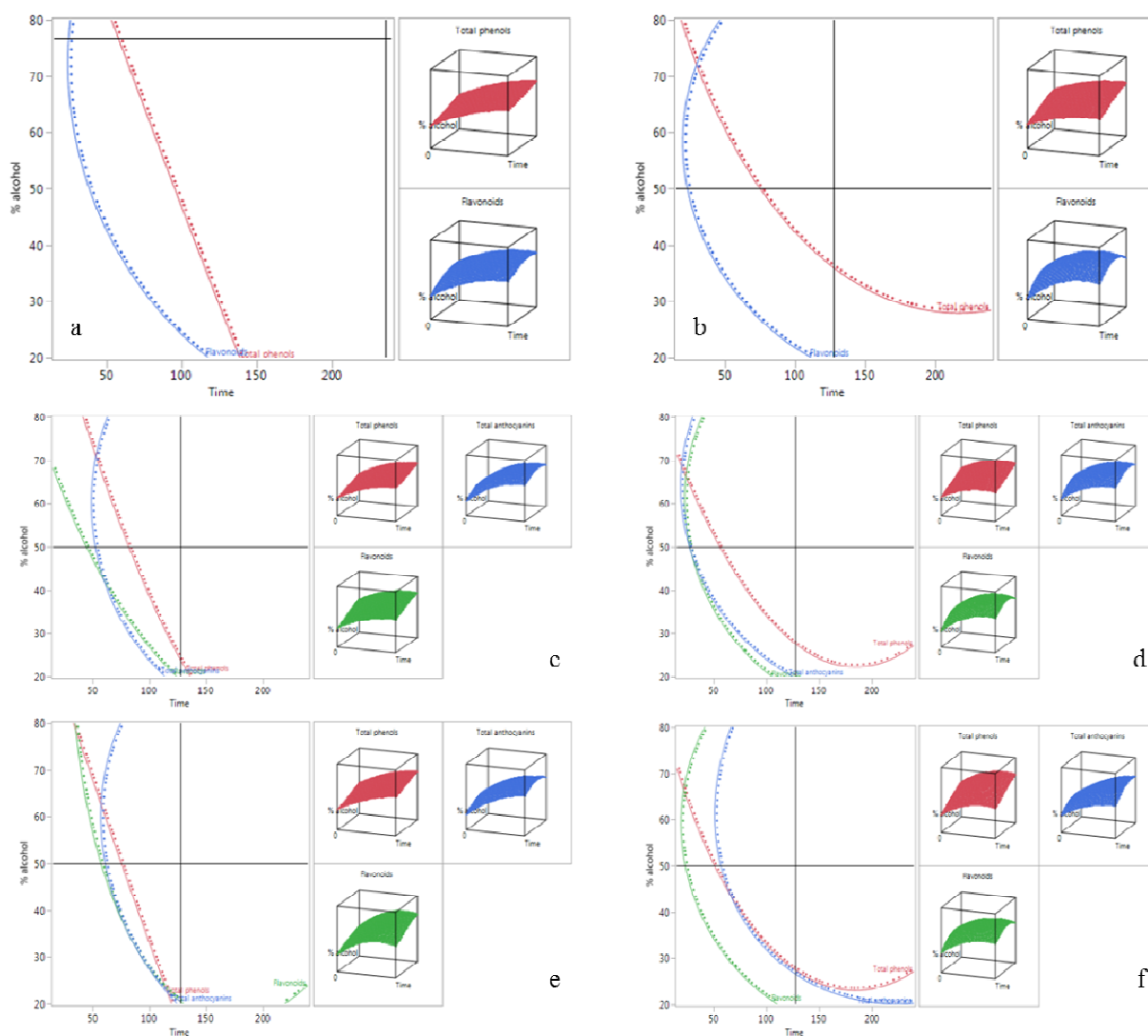


Fig. 1. Contour profilers of the extraction processes: a) white mulberry, maceration; b) white mulberry, ultrasound; c) black mulberry, maceration; d) black mulberry, ultrasound; e) red mulberry, maceration; f) red mulberry, ultrasound

Conclusions

The differences in the content of total phenols, flavonoids and total anthocyanins for the extractions are the results of different polarities of the applied solvent systems, differences in the *Morus* species, extraction technique and time. The computational modeling-response surface design and subsequent optimization give more easily optimized conditions for both maceration and ultrasonic extraction. The obtained results show that the ultrasonic extraction was less time consuming and requires a solvent system with less percentage of ethanol. The results indicate a high content of phenolic compounds, flavonoids, and anthocyanins of black mulberry located in Southeast Serbia, which confirm its nutritional and pharmacological potential.

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