# THE EFFECT OF SOLVENT AND TEMPERATURE ON EXTRACTION YIELD OF PHENOLIC COMPOUNDS FROM SOYBEANS, ANTIOXIDANT ACTIVITY AND COLOUR OF EXTRACTS

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## ABSTRACT

In this study the influence of solvent concentration (50, 60, 70 and 80% ethanol) and extraction temperature (25, 40, 50, 60, 70 and 80 °C) on the extraction yield of total phenolics and total flavonoids from organic soybeans were investigated. The total phenolic content (TPC) in soybean extract was determined spectrophotometrically by Folin-Ciocalteu micro method at 765 nm and results were expressed as gallic acid equivalents. The content of total flavonoids (TFC) was also measured spectrophotometrically using the aluminium chloride colorimetric assay at 510 nm with (+)-catechin as standard. Antioxidant activity (AA) of soybean extracts was evaluated using $DPPH^{\cdot}$ radical scavenging method. CIE $L^*a^*b^*$ system was used for determination of colour parameters of extracts.

The results showed statistically significant influence of solvent concentration and temperature on extraction of phenolic compounds. The best extraction yield of phenolic compounds was obtained when 50% ethanol was used at 80 °C (TPC: 4.5 $mg_{GAE}/g_{db}$; TFC: 2.56 $mg_{CE}/g_{db}$).

Weak correlations ($r \leq 0.298$) were found between antioxidant activity of extracts and phenolic compounds. Furthermore, the phenolic content and total colour change of extracts were not correlated.

**Key words**: extraction, solvent, temperature, soybeans, total phenolics, total flavonoids, antioxidant activity, colour

## INTRODUCTION

Soybean has received considerable attention the last decade because of its potential in reducing the formation and progression of certain types of cancers and some chronic diseases such are cardiovascular disease, osteoporosis, Alzheimer disease, etc. [1]. The phenolic content should be considered as an important feature of soybeans, besides protein and oil content. Soybeans are widely accepted as a "healthy food" and some of their pharmacological effects could be attributed to the presence of these valuable

constituents [2]. Phenolic acids, flavonoids and isoflavonoids presented in soybean seeds may be partially responsible for these health benefits through their antioxidants activity [3]. Developing food ingredients with enhanced antioxidative activity is desirable for both the food industries and consumers [4]. Over the last few decades, consumers demand for healthier food is focused on environmentally sustainable agricultural systems that have enhanced rapid expansion of organic farming [5].

With the exception of isoflavonoids, a few studies have been carried out on the other phenolic compounds present in soybean, especially in organically grown soybeans. Thus, the aim of this study was to examine the influence of solvent concentration and temperature on the extraction yield of phenolic compounds from organic soybean as well as antioxidant activity and colour of extracts.

## MATERIALS AND METHODS

***Material.*** The extraction was performed on organically grown soybeans variety "Ika" produced in a plantation located in Eastern Slavonia. Sample was cleaned of foreign materials (sticks, stems, leaves, damaged seeds, and dirt). Material was grounded and sieved using sieve sets (Retsch AS 200E) and determination of average particles size were performed. Prepared samples were stored at +4 °C prior to extraction. Dry matter content of soybeans was determined by drying of milled soybeans at 105 °C to constant mass and noted as percentage. The average particles size was 0.5796 mm and dry matter content was 90.5%.

***Extraction.*** In the first set of experiments, the effect of ethanol concentration (50, 60, 70 and 80%) on extractability of phenolic compounds at 80 °C was analyzed. The purpose of second experimental run was to analyze the influence of different temperatures (25, 40, 50, 60, 70 and 80 °C) on extraction of phenolic compounds from organic soybeans using 50% ethanol, which proved to be the most effective solvent in the first experimental set.

In the test flasks, 1 g of milled soybean sample was mixed with 20 ml of solvent. The extraction process was conducted in laboratory scale by using water bath (Julabo SW-23, Deuschland) with shaking (200 rpm) for 120 minutes in two repetitions. Thus obtained suspensions were centrifuged (Sigma 2-16, Deutschland) for 5 minutes at 15000g. Supernatants were decantated and pooled to know volume with extraction solvent which gave soybean extracts for determination total phenolics, total flavonoids, antioxidant activity and colour measurement.

***Total phenolic content (TPC).*** The content of total phenolic compounds in the extracts was determined by Folin-Ciocalteu micro method as follow: 40 $\mu$l of extract was mixed with 3160 $\mu$l of distilled water and 200 $\mu$l of Folin-Ciocalteu reagent in test tube. After 30 seconds to 8 minutes, 600 $\mu$l of 20% of sodium carbonate solution was added then test

tube was shaken for 10 seconds on the Vortex and put to incubation in a water bath at 40 °C. Absorbance was measured after 30 minutes on UV/VIS spectrophotometer (UV-1700 Shimadzu, Japan) at 765 nm against blank sample and obtained values were used to calculate the total phenolic content in soybean extracts using gallic acid as a standard. Blank sample was prepared with water instead of extract. Analyses were carried out in three repetitions and final results were expressed as gallic acid equivalent per a dry basis of soybeans ($mg_{GAE}/g_{db}$).

***Total flavonoid content (TFC).*** The concentration of total flavonoid compounds in the extracts was determined by the aluminium chloride colorimetric assay [6] as follows: 1 ml of extract and 0.3 ml 5% sodium nitrite solution were added to test tube containing 4 ml of distilled water. After 5 min, 0.3 ml 10% aluminum chloride was added. After 6 minutes, 2 ml 1 M sodium hydroxide was added and the total volume was corrected 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 soybeans ($mg_{CE}/g_{db}$).

***Antioxidant activity assay (AA).*** Antioxidant activity of soybean extracts obtained at different extraction regimes was evaluated by spectrophotometric method against stable 2.2-diphenyl-1-picrylhydrazyl radical (DPPH·) [7]. The extract (0.2 ml) reacted with 3.8 ml of 6.6 x $10^{-5}$ mol/l of DPPH solution prepared daily. Absorbance at 515 nm was measured on spectrophotometer after 30 minutes. Blank sample was prepared with extraction solvent instead of extract while ethanol was the blank for DPPH solution. All tests were performed in triplicate and the results were expressed as mass of inhibited DPPH per dry basis of soybean ($mg_{inh.DPPH}/g_{db}$).

***Colour measurement.*** The colour of extracts was measured using Minolta CR-400 Chromameter. Analyses of colour values were in duplicate. Three parameters, $L^*$ (lightness), $a^*$ (redness) and $b^*$ (yellowness), were used to study changes in the colour. The total colour difference ($\Delta E^*$) was calculated as follows [8]:

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

where $L_0^*$, $a_0^*$ and $b_0^*$ indicate colour parameters of 50% ethanol.

***Statistic analysis.*** One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's *post-hoc* test) were used to evaluate the significant difference of the data at $p < 0.05$. Data were expressed as means ± standard deviation (SD).

## RESULTS AND DISCUSSION

This paper examined the impact of solvent concentration (50, 60, 70 and 80% ethanol) and extraction temperature (25, 40, 50, 60, 70 and 80 °C) on the extractability of phenolic substances from soybeans variety "Ika".

Obtained total phenolics content in the extracts using different solvents at the extraction temperature of 80 °C for 120 minutes ranged from 2.46 to 4.50 $mg_{GAE}/g_{db}$ and total flavonoids from 0.60 to 2.56 $mg_{CE}/g_{db}$, where the content of the above compounds in the extracts decreased with increasing concentration of ethanol (Table 1).

**Table 1.** Total phenolics (TPC), total flavonoids (TFC) and antioxidant activity (AA) of soybean extracts (variety "Ika") obtained by using different extraction solvent at 80 °C

| SOLVENT | TPC[A] [$mg_{GAE}/g_{db.}$] | TFC[B] [$mg_{CE}/g_{db}$] | AA [C] [$mg_{inh.DPPH}/g_{d.}$] | TFC/TPC [%] |
|---|---|---|---|---|
| **50% ethanol** | 4.50 ± 0.06 $^{a}$ | 2.56 ± 0.04 $^{a}$ | 2.14 ± 0.10 $^{a}$ | 56.88 |
| **60% ethanol** | 2.75 ± 0.13 $^{b}$ | 1.02 ± 0.04 $^{b}$ | 0.79 ± 0.04 $^{d}$ | 37.28 |
| **70% ethanol** | 2.59 ± 0.08 $^{c}$ | 0.85 ± 0.03 $^{c}$ | 1.21 ± 0.10 $^{c}$ | 32.81 |
| **80% ethanol** | 2.46 ± 0.05 $^{d}$ | 0.60 ± 0.03 $^{d}$ | 1.82 ± 0.01$^{b}$ | 24.39 |

Data are expressed as mean value of replication (n) ± SD (standard deviation); $^{A}n = 6$, $^{B}n = 4$, $^{C}n = 6$
The same letter in the same column indicates no significant differences (Duncan's test, $p < 0.05$)

The ethanol concentration had higher impact on extractability of flavonoid than on other extracted phenolic compound that can be seen from TFC/TPC ratio, which increased with decreasing ethanol concentration (24.39 - 56.88%).

Similar data for the extraction yield of phenolic substances from soybeans have been published by Malenčić et al. [2] where specific differences exist because of different methodologies of experimental work as well as different variety of soybean samples.

According to given results, 50% of aqueous ethanol solution showed the best extraction efficiency for the given experimental conditions, and it was used as a solvent in the further analysis of temperature influence on extractability of phenolic substances. Within the examined temperature interval, it was found that the content of phenolics increased with increasing temperature from 25 °C to 80 °C and that the effect of temperature was

statistically significant (ANOVA, *Duncanov post-hoc test*, $p < 0.05$). Content of total phenolics in soybean extracts after 120 minutes extraction increased from lower to higher extraction temperatures (2.21 - 4.50 $mg_{GAE}/g_{db}$), and total flavonoids ranged from 1.01 to 2.56 $mg_{CE}/g_{db}$ (Table 2).

**Table 2.** Total phenolics (TPC), total flavonoids (TFC) and antioxidant activity (AA) of soybean extracts (variety "Ika") obtained at different extraction temperatures using 50% aqueous ethanol solution

| TEMPERATURE [°C] | TPC[A] [$mg_{GAE}/g_{db}$] | TFC[B] [$mg_{CE}/g_{db}$] | AA[C] [$mg_{inh.DPPH}/g_{db}$] | TFC/TPC [%] |
|---|---|---|---|---|
| **25** | $2.21 \pm 0.19^{e}$ | $1.01 \pm 0.09^{f}$ | $1.12 \pm 0.12^{c}$ | 45.62 |
| **40** | $3.16 \pm 0.06^{d}$ | $1.32 \pm 0.01^{e}$ | $2.31 \pm 0.07^{a}$ | 41.89 |
| **50** | $3.25 \pm 0.04^{d}$ | $1.67 \pm 0.01^{d}$ | $0.43 \pm 0.05^{d}$ | 51.22 |
| **60** | $3.38 \pm 0.07^{c}$ | $1.96 \pm 0.02^{c}$ | $1.30 \pm 0.05^{c}$ | 57.89 |
| **70** | $4.15 \pm 0.09^{b}$ | $2.20 \pm 0.02^{b}$ | $1.31 \pm 0.04^{c}$ | 52.99 |
| **80** | $4.50 \pm 0.06^{a}$ | $2.56 \pm 0.04^{a}$ | $2.14 \pm 0.10^{b}$ | 56.88 |

Data are expressed as mean value of replication (n) ± SD (standard deviation); $^{A}n = 6$, $^{B}n = 4$, $^{C}n = 6$
The same letter in the same column indicates no significant differences (Duncan's test, $p < 0.05$)

Increasing of extraction temperature had more positive effect on extractability of flavonoid than on other extracted phenolic compound that confirmed by increasing TFC/TPC ratio from 41.89 to 56.88%.

Antioxidant activity of obtained soybean extract was estimated spectrophotometrically by DPPH method based on the reduction of DPPH· radical in the presence of antioxidant from extract which is reflecting as change of solution colour from purple to yellow and decreasing of UV-light absorbance at 515 nm. Values of antioxidant activity were in range 0.43 – 2.14 $mg_{inh.DPPH}/g_{db}$ regardless of the extraction conditions (Tables 1 and 2). Results showed that increase of phenolic content in the extracts with increasing temperature and decreasing ethanol concentration was not accompanied by a corresponding increase in antioxidant activities of soybean extracts. It is also confirmed by the weak correlation ($r \leq 0.298$) between antioxidant activity of extracts and total phenolic compounds as well as between content of flavonoids and antioxidant activity ($r \leq 0.160$). These results are comparable with results of other authors [2, 9] who have found weak correlation between content of examined phenolics and antioxidant activity in soybean extracts.

Figure 1 shows total colour changes ($\Delta E^*$) of soybean extract samples at different extraction temperatures with 50% of aqueous ethanol solution as a solvent. $\Delta E^*$ values varied from 3.57 to 5.01. According to ANOVA analysis ($p < 0.05$; Duncan's *post-hoc* test) $\Delta E^*$ of extracts were statistically different but linear correlation with extraction temperature were not observed. Furthermore, results indicate that determined phenolic compounds didn't make significant contribution to development of extract colour because of the week correlations between phenolic compounds ($r \leq 0.275$) and total colour changes of soybean extracts as well as flavonoids ($r \leq 0.353$).

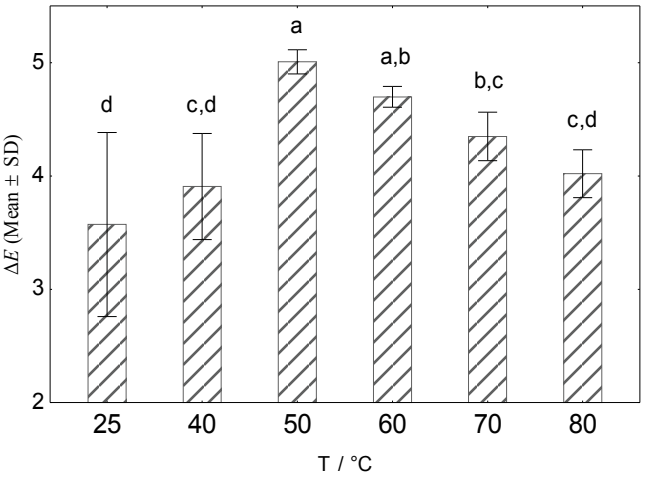


**Figure 1.** Total colour change of extracts at different extraction temperatures (a, b, c, d - groups which differed statistically significant ($p < 0.05$) from one to another according to different extraction temperatures)

## CONCLUSIONS

The results showed statistically positive impact of solvent and temperature on the extraction yield of phenolic compounds. The best extraction yield of phenolic compounds was obtained by 50% aqueous ethanol solution at 80 °C (TPC: 4.5 $mg_{GAE}/g_{db}$; TFC: 2.56 $mg_{CE}/g_{db}$). Weak correlations ($r \leq 0.298$) were found between antioxidant activity of extracts and phenolic compounds. Furthermore, the phenolic content and total colour change of extracts were not correlated.

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