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# Mathematical modeling of total flavonoid compounds extraction from conventionally grown soybeans

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**Abstract**

The aim of this study was to investigate the influence of the solvent, the temperature and the extraction time on the extractability of total flavonoid compounds from conventionally grown milled soybeans variety "*Ika*", mean particles size 0.5796 mm. The influence of different solvents (water; 50, 60, 70 and 80% aqueous ethanol solution) was investigated at 50 °C after 60 min, in order to achieve the highest yield of total flavonoids. The most effective solvent (50% aqueous ethanol solution) was used for the kinetics monitoring and the extraction modelling of total flavonoids. The concentration of extracted total flavonoids was monitored at different temperatures (26 °C, 40 °C, 50 °C, 60 °C, 70 °C and 80 °C) for 120 min, at solid-liquid ratio of 20 mL/g. The concentrations of total flavonoids were determined spectrophotometrically. Various mathematical models (Peleg, Page and Logarithmic) were used to describe the extraction kinetics of total flavonoids. The results showed that the used solvent, temperature and extraction time had a significant impact on the kinetics and the extraction yield of total flavonoids. The highest extraction efficiency was achieved at temperature of 80 °C after 120 min (1.417 mg CE/$g_{db}$). The yield of total flavonoids increased with the temperature increase, as well as with the prolongation of the extraction process. The results obtained using mathematical models showed good agreement with the obtained experimental results.

Key words: soybeans, solid-liquid extraction, flavonoids, extraction kinetics, modeling

# Matematičko modeliranje ekstrakcije ukupnih flavonoidnih spojeva iz konvencionalno uzgojenog zrna soje

**Sažetak**

U radu je ispitivan utjecaj otapala, temperature te vremena ekstrakcije na ekstraktibilnost ukupnih flavonoidnih spojeva iz usitnjenog konvencionalno uzgojenog zrna soje sorte "Ika", srednjeg promjera čestica 0,5796 mm. Cilj istraživanja bio je ispitati utjecaj različitih otapala (voda; te 50, 60, 70 i 80%-tna vodena otopina etanola), pri 50 °C nakon 60 minuta, kojim bi se postigao najveći prinos ukupnih flavonoida. Najučinkovitije otapalo (50%-tna vodena otopina etanola) korišteno je u daljnjem radu, a u svrhu praćenja kinetike i modeliranja ekstrakcije ukupnih flavonoida. Koncentracija ekstrahiranih ukupnih flavonoida praćena je pri različitim temperaturama (26 °C, 40 °C, 50 °C, 60 °C, 70 °C i 80 °C) tijekom 120 minuta, uz omjer kruto-tekuće 20 mL/g. Koncentracije ukupnih flavonoida određene su spektrofotometrijskom metodom. Različiti matematički modeli (Peleg, Page i Logaritamski) korišteni su za opisivanje kinetike ekstrakcije ukupnih flavonoida. Utvrđeno je da korištena otapala, temperatura i vrijeme ekstrakcije imaju značajan utjecaj na kinetiku i prinos ekstrakcije ukupnih flavonoida.

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Dobiveni rezultati pokazali su da je najveća učinkovitost ekstrakcije postignuta pri temperaturi od 80 °C nakon 120 minuta (1,147 mg CE/$g_{s.tv.}$) te da prinos ukupnih flavonoida raste povišenjem temperature i produljenjem trajanja procesa ekstrakcije. Rezultati dobiveni upotrebom ispitanih matematičkih modela pokazali su dobra slaganja s eksperimentalno dobivenim rezultatima.

Ključne riječi: soja, ekstrakcija kruto-tekuće, flavonoidi, kinetika ekstrakcije, modeliranje

## Introduction

Soybeans are worldwide leading crop used for many different products, both edible and non-edible, such are oil and proteins for human nutrition, as well as for livestock feeding. It has become an integral part of modern agriculture and food industry practices (Vratarić and Sudarić, 2008). The flavonoid compounds 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 (Malenčić et al. 2007). Flavonoids are a large class of natural products that are widely distributed among the plant kingdom. They are the most represented among polyphenolic compounds and they have important antioxidative and biological properties. Therefore, majority of food safety and nutrition research are focused on this group of compounds. Isoflavones are a subclass of flavonoids that are also described as phytoestrogen compounds, since they exhibit estrogenic activity (Valls et al., 2009). Flavonoids are believed to exhibit series of positive health effects (e.g. anti-cancer, antibacterial, anti-oxidation, immune regulation, etc.). Extraction is an important step in isolation, identification and quantification of phenolic compounds, as well as flavonoid compounds, eventhough there is a lack of standarised extraction methods (Cacace and Mazza, 2003). Solid-liquid extraction is a commonly used isolation method for phenolic (flavonoid) compounds from plant materials (such as soybean). Selection of solvents is one of the most important steps in the extraction process. The most commonly used solvents for the solid-liquid extraction of plant material flavonoid compounds include methanol, ethanol and their liquid mixtures with water and organic solvents (e.g. acetone, ethyl acetate) (Naczk and Shahid, 2004).

The mathematical models are useful engineering tools, which greatly facilitate simulation, optimisation, design and control of processes and contribute to utilization of energy, time, raw material and solvent. The aim of this study was to examine the influence of different solvents (water, and 50, 60, 70, and 80% aqueous ethanol solutions), extraction temperatures (26, 40, 50, 60, 70, and 80 °C), and extraction times (5, 10, 15, 20, 30, 40, 60, 90, and 120 min) on the extractability of total flavonoids from conventionally grown milled soybeans, variety "*Ika*".

## Material and methods

**Material.** The extraction was performed on conventionally grown soybeans, variety "Ika," obtained by the Agricultural Institute of Osijek. The samples were cleaned from impurities (stick, stems, damadge seeds, dirt), milled in a grinder (HR 2860, Philips), and immediately after grinding stored at +4 °C prior to extraction. The soybeans dry matter content was determined by drying the milled soybeans at 105 °C to constant weight. The analysis were done in duplicates and the average dry matter content was noted as percentage. The dry matter content was about 91.9% and was determined in all experimental runs. The total flavonoids concentration was thus expressed on dry basis, which generally provides a more accurate and reliable data comparison. The average particle size (d = 0.5796 mm) was determined using sieve sets (Retsch AS 200, Haan, Germany).

**Extraction.** 1 g of the soybean sample was mixed with 20 ml of solvent in the test tubes. The extraction process was conducted on laboratory scale using a water bath (Julabo SW-23, Germany) for 120 minutes. During the extraction process, the test tubes containing the reaction mixture were incubated in the water bath and shaken for 20 s in 15 min intervals using Vortex (Vibromix 10, Tehtnica, Slovenia). All extraction runs were performed in duplicates. The extracts obtained by the extraction were separated from rough particles by decantation and centrifuged (Sigma 2-16, Germany) at 15000 g for 5 minutes. The supernatant was decanted and filled with distilled water to the defined volume (20 ml). The supernatant was used for the determination of total flavonoids content.

**Solvent influence**. The efficiency of total flavonoids extraction using different solvents (water and 50, 60, 70, and 80% aqueous ethanol solutions) at the temperature of 80 °C was examined. **Influence of extraction temperature**. The extraction was performed at different temperatures (26, 40, 50, 60, 70, and 80 °C) using 50% aqueous ethanol solution which proved to be the most effective solvent for the above listed experimental conditions.

**Extraction time influence**. The extraction was performed at different extraction times (5, 10, 15, 20, 30, 40, 60, 90, and 120 min) at the above mentioned extraction temperatures using 50% aqueous ethanol solution as a solvent.

**Total flavonoid content (TFC).** The concentration of total flavonoid compounds in the extracts was determined by the aluminium chloride colorimetric assay (Marinova et al. 2005) as follows: 1 ml of extract was added to 10 ml volumetric flask containing 4 ml of distilled water and 0.3 ml 5% $NaNO_2$. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6$^{th}$ min, 2 ml of 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 soybeans (mg CE/$g_{db}$).

**Kinetics of solid-liquid-extraction.** The extraction curves (concentration of total flavonoids vs. time) have similar shape as sorption curves (moisture content vs. time), which gives the possibility for using the same mathematical models when describing kinetics.

Therefore, the model proposed by Peleg (1988) was adapted for extraction and used in following form:

$$c(t) = \frac{t}{K_1 + K_2 \cdot t} \quad (1)$$

where $c(t)$ is concentration of total flavonids at time $t$ (mg CE/$g_{db}$), $t$ - extraction time (min), $K_1$ - Peleg's rate constant (min $g_{db}$/mg CE), $K_2$ - Peleg's capacity constant ($g_{db}$/mg CE).

The model proposed by Page was used as follows:

$$c(t) = \exp(-kt^n) \quad (2)$$

where $c(t)$ is concentration of total flavonids at time $t$ (mg CE/$g_{db}$), $t$ - extraction time (min), $k$ and $n$ - constants of Page's model.

Logarithmic model was used as follows:

$$c(t) = a \log t + b \quad (3)$$

where: $c(t)$ is concentration of total flavonids at time $t$ (mg CE/$g_{db}$), $t$ - extraction time (min), $a$ and $b$ - Logarithmic model constants.

**Statistical methods.** Statistica 7.0 (Stat Soft Inc., USA) was used for data analysis. The parameters of modified Peleg's model (constants $K_1$ and $K_2$) were determined from experimental data using non-linear regression (Quasi-Newton method). The concordance between experimental data and calculated value was established by the correlation coefficient (R) and the root mean squared deviation (RMSD) as follows:

$$\mathrm{RMSD} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\mathrm{experimental} - \mathrm{calculated})^2} \quad (4)$$

## Results and discussion

The results showed (Fig. 1) that the highest concentration of total flavonoids was obtained when using 50% aqueous ethanol solution (1.128 mg CE/$g_{db}$) as a solvent, while further increase of the ethanol concentration significantly contributed to a decrease of the extractability of total flavonoids from the soybean samples. The lowest extraction efficiency was obtained when water was used as a solvent (0,195 mg CE/$g_{db}$). 50% aqueous ethanol solution proved to be the most effective solvent (temperature 50 °C, time 60 min) with the obtained

total flavonoids extraction yield 5,781 times higher than the extraction yield obtained when water was used as the extraction solvent.

The highest concentration of total flavonoids from milled soybeans was obtained when using 50% aqueous ethanol as extraction solvent which is in accordance with the results from other authors (Shi et al. 2003; Jokić et al. 2010).

The aqueous ethanol solution (50, 60, 70, or 80%) was selected as the solvent due to its environmental safety, low cost, and lower toxicity compared to other solvents (e.g. methanol). Although the water represents the best solvent solution when it comes to food industry purposes, due to its polarity it extracts other undesirable macromolecules as well (protein, polysaccharide, etc.) especially at higher temperatures and pressures (Rostagno et al. 2003; Tsao and Deng 2004).

Since 50% aqueous ethanol solution proved to be the most effective solvent, it was used as a solvent in further analysis of kinetics of solid-liquid extraction at different temperatures (26, 40, 50, 60, 70, and 80 °C) during 120 minutes.

The extraction curves (concentration of total flavonoids vs. time) have a similar shape as the sorption curves (moisture content vs. time), and can be described using mathematical models of the mass transfer. Therefore, to describe the extraction kinetics, three mathematical models were used: Peleg's, Page's, and the Logarithmic model. The extraction curves (Fig. 2 a, b and c) indicated the experimental increase in the extraction yield with time. A high initial rate of flavonoids extraction can be observed in the extraction curves, followed by a slower extraction rate, and then asymptotically approaching the equilibrium concentration. Similar trends were obtained for modelling solid-liquid extraction of total polyphenols from grape seeds as well as soybeans seeds (Bucić-Kojić et al. 2007; Jokić et al. 2010).

The constant value, correlation coefficient (R) and the root mean squared deviation (RMSD) were calculated for each mathematical model using Statistica 7.0 non-linear methods. The correlation coefficients were high in all experiments (0.934-0.995), and the root mean squared deviations (RMSD) were in the range from 0.023 to 0.095, which implied a good agreement between the experimental and the calculated data.

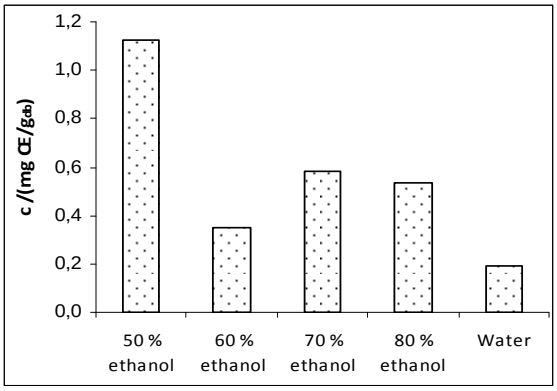


**Fig. 1 The effect of the extraction solvent on the extraction yield of total flavonoids from milled soybeans**

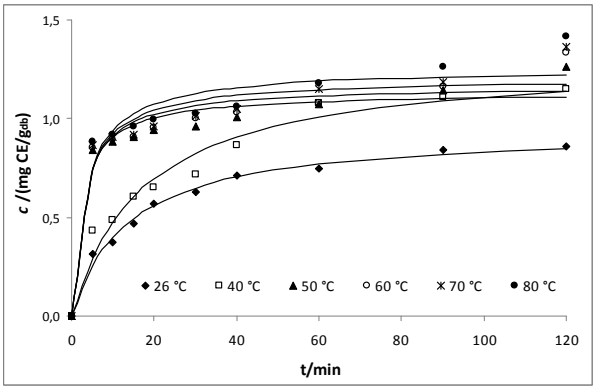


**Fig. 2a The temperature influence on the extraction kinetics of total flavonoids: approximation by Peleg's model**

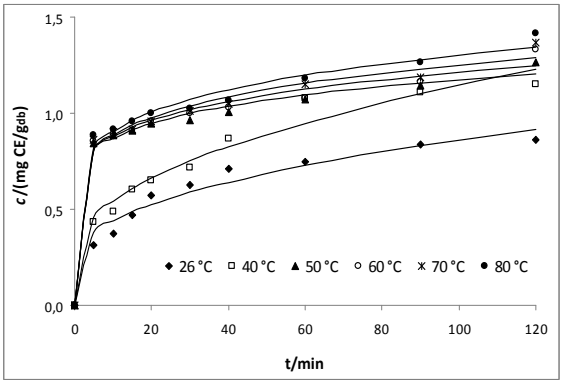


**Fig. 2b The temperature influence on the extraction kinetics of total flavonoids: approximation by Page's model**

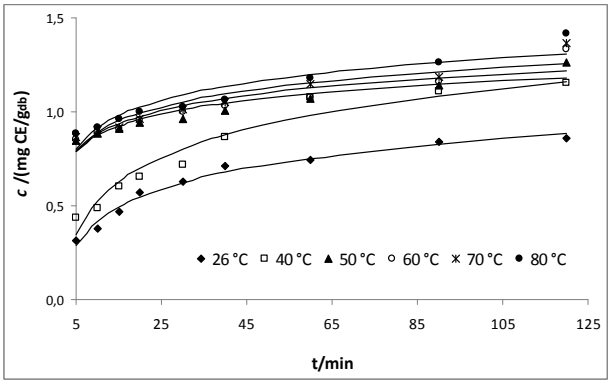


**Fig. 2c The temperature influence on the extraction kinetics of total flavonoids: approximation by Logarithmic model**

## Conclusion

The results showed that the used solvent, temperature and extraction time had a significant impact on the kinetics and the extraction yield of total flavonoids. The yield of total flavonoids increased with the temperature increase, as well as with the prolongation of the extraction process. 50% aqueous ethanol solution proved to be the most effective solvent (temperature 50 °C, time 60 min) with the obtained total flavonoids extraction yield 5,781 times higher than the extraction yield obtained when water was used as the extraction solvent. The highest extraction efficiency was achieved at temperature of 80 °C after 120 min (1.417 mg CE/$g_{db}$). The mathematical models applied showed a good agreement with the experimental results, which allows their application in modelling and optimisation of solid-liquid extraction process for the extraction of total flavonoids from soybeans.

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