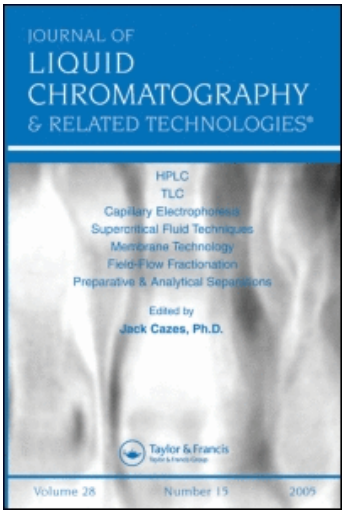

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Optimization of ASE Conditions for the HPLC Determination of Rutin and Isoquercitrin in *Sambucus nigra* L.

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ABSTRACT

The accelerated solvent extraction (ASE) procedure has been examined as a sample preparation method for the HPLC analysis of rutin and isoquercitrin in *Sambucus nigra* L. The experimental extraction parameters (solvent composition, temperature, operation pressure, and static extraction time) have been optimized in order to receive the best recovery of both analytes from the flowers, leaves, and berries of black elder. The ASE recoveries obtained under the optimal extraction conditions were compared with analogous ones obtained by means of the maceration technique.

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Key Words: ASE; Extraction optimization; Flavonols; *Sambucus nigra* L.

INTRODUCTION

Sambucus nigra L. (commonly known as black elder, common elder, and sambucus) is a wide-branched shrubby tree growing up to 10 m in height with a trunk diameter up to 30 cm.[1] It grows in hedgerows, woods, coppices, and waste places throughout Europe, Asia, North Africa, and the United States. All parts of the tree [flowers, berries, and leaves (sometimes also inner bark)] have been famous for their medicinal properties from the days of Hippocrates. For this reason, the tree has long enjoyed a high reputation in domestic medicine and has been called "the medicinal chest of common people." *Sambucus nigra* L. is recommended in catarrhal inflammations of the upper respiratory track. The flowers have relaxing properties and are used to reduce agitation and restlessness. An infusion made from them is a soothing remedy for inflamed eyes and can be used as a gargle for mouth ulcers and tonsillitis. The berries and the leaves have a great diuretic activity. The berries have a long-established effect of regulating bowel activity and moderating extremes of diarrhoea. A syrup made from the berries can be taken as a prophylactic against winter colds. The leaves are used topically to treat bruises, sprains, wounds, burns, and chilblains, and may also be used as a mouthwash.[2–4]

The medicinal properties of black elderberry results, from among other things, the presence of pharmacologically active bioflavonoids. Rutin and isoquercitrin are the main flavonol glycosides of *S. nigra* L.[5] These substances have the capacity for acting as a potent radical scavenger,[6–8] inhibiting a variety of enzymes,[9] and have an antihemorrhagic activity by tightening blood vessels.[10] The importance of their analysis has been recognized by researches who, so far, have most frequently used HPLC for this purpose.[11,12]

The first step in the analysis of medicinal plant constituents is the separation of compounds to be analyzed from the cellular matrix. A broad spectrum of extraction procedures, such as Soxhlet extraction, percolation, maceration, digestion, extraction under reflux, and steam extraction, are currently used.[13–16] Most of them are relatively simple, however, their disadvantages are: long extraction time, labor intensive manual procedures, relatively high solvent consumption, or unsatisfactory reproducibility.[17] A recently developed extraction technique, accelerated solvent extraction (ASE) could, in principle, eliminate some of the drawbacks of other extraction methods.[18–20]

Accelerated solvent extraction allows us to use an extrahent at elevated pressure and, hence, at a temperature above their normal boiling point, and in

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consequence to remove the analytes quickly and efficiently from various matrices.

Accelerated solvent extraction was first applied for the extraction of environmental pollutants (PAHs, PCBs, pesticides, and herbicides) from soils.[21–23] Accelerated solvent extraction in food analysis is used for the extraction of pesticides and mycotoxin residues from fruits and vegetables,[24,25] for fat determination,[26] and for the extraction of vitamins.[27] However, ASE is still very uncommon in the extraction of plant constituents. Most frequently the maceration of herbs in extraction solvent is used.

The aim of this paper is to optimize the ASE process for the HPLC analysis of rutin and isoquercitrin in the flowers, berries, and leaves of *S. nigra* L. The effects of solvent composition, temperature, pressure, and static extraction time were studied in order to find the highest extraction efficiency. The analytical results were compared with the results obtained using the maceration procedure.

EXPERIMENTAL

Plant Materials

Dried flowers of *S. nigra* L. were purchased from a local pharmacy (Herbapol-Lublin, Lublin, Poland). The berries and leaves of the plant were collected in the Botanical Garden of our University and air-dried immediately, under controlled temperature and humidity. A sufficiently large representative samples of the plant materials (ca 500 g) were ground with a Braun cutting mill to obtain particle size of 0.2–0.4 mm and exactly weighted portions of the samples were used for the extraction.

Materials and Reagents

Methanol (analytical-reagent grade), acetonitrile (HPLC grade), glacial acetic acid (analytical-reagent grade), and *n*-hexane (analytical-reagent grade) were purchased from the Polish Factory of Chemicals POCh (Gliwice, Poland). Water was purified on a Milli-Q system from Millipore (Millipore, Bedford, MA). Neutral glass (fraction 0.4–0.6 mm) was used as a dispersing agent in the ASE extraction cell. C_{18} sorbent (Supelclean LC-18) from Supelco Park (Bellafonte, PA) was employed for the SPE of extracts. The standard of rutin (quercetin-3-rutinoside) with the exactly known amount of isoquercitrin (quercetin-3-glucoside) was purchased from Merck (Germany). The chemical structures of the examined compounds are presented in Fig. 1.

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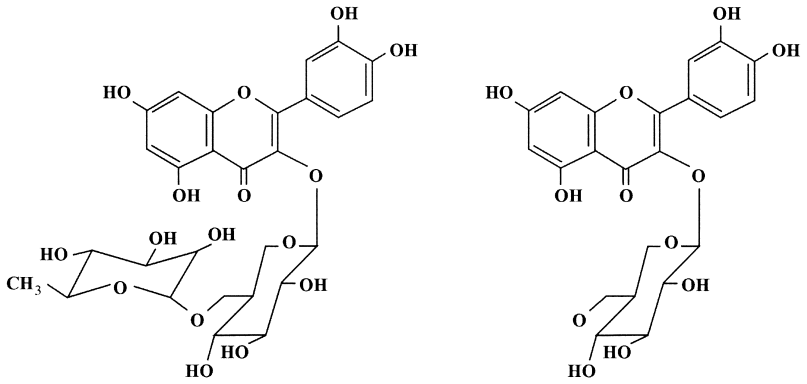

Figure 1. Chemical structures of rutin and isoquercitrin.

Accelerated Solvent Extraction

Accelerated solvent extraction was performed with a Dionex ASE200 instrument (Dionex Corp., Sunnyvale, CA). The plant material (1 g of flowers, berries, or leaves) was mixed with the inert material (neutral glass) and placed into a 22-mL stainless steel extraction cell. The employment of a dispersion agent, such as neutral glass, is recommended in order to reduce the volume of the solvent used for the extraction.[28] The content of the cell was extracted under conditions described in the Discussion below.

The parameters under study were the percentage of methanol in the extracting mixture, and the temperature, pressure, and time of the extraction process. The pre-set default procedure was as follows:

- One extraction cycle.
- The flush of the extracted sample with the same extrahent in the amount equal to 60% of the extraction cell volume.
- The purge of the sample for 120 s applying pressurized nitrogen (150 psi) and the collection of the extract in 60 mL glass vials with teflon-coated rubber caps.

The volume of collected extract was between 25 and 31 mL, depending on the packing density of the extraction cells. Each matrix was extracted three times in the same conditions. Between the runs, the system was washed with an extraction solvent.

Maceration Procedure

A 5 g portion of *S. nigra* L. flowers or leaves or berries was macerated according to the following procedure. Each sample was immersed in 80% (v/v) methanol solution (50 mL) for 24 h at 22°C, and then the mixture was heated for 3 h under reflux at the boiling temperature of the solvent. After cooling, the extract was removed and a new portion of the fresh extrahent was added to the remaining material. The process was repeated three times and all the extracts were pooled together before the following procedure.

Sample Preparation

Each of the obtained extracts was transferred to a 100 mL volumetric flask, which was filled up to its volume with an appropriate extraction solvent. In the case of the maceration procedure, the extracts were filled up to the volume of 250 mL. Because of the presence of different interfering matrix compounds, e.g., chlorophyll in leaves; all diluted extracts were submitted to the same SPE purification procedure. For this purpose, 1 mL of each diluted extract was introduced into the 500 mg C_{18} SPE cartridge and the target analytes were eluted with the aqueous solution of MeOH (60%, v/v), up to the volume of 10 mL. The cartridge was conditioned with 10 mL of acetone (to remove hydrophobic impurities), then with 10 mL of pure MeOH, and finally, with 10 mL of the 60% (v/v) MeOH solution. The SPE recoveries of the examined substances were greater than 98.6%. The resulting solution was subjected to the HPLC analysis ($n = 3$). Before injection samples were filtered through the membrane filtration cartridge Millex-FGS 0.2 μm Filter Unit (Millipore, Bedford, MA).

HPLC Analysis of Extracts

HPLC measurements were performed on a Dionex liquid chromatograph (Dionex Corp., Sunnyvale, CA) consisting of a chromatography enclosure (LC20) containing a PEEK automated injection valve equipped with a 25 μL sample loop, a gradient pump (GP50), an absorbance detector (AD25), and a photodiode array detector (PDA100). The whole chromatographic system was under the control of the PeakNet6 data acquisition system. Chromatographic separations were carried out at 25°C using a Prodigy ODS-2 column (5 μm, 250 × 4.6 mm I.D.) (Phenomenex, Torrance, CA) and a security guard column of the same material. The mixture of aqueous CH_3COOH solution (containing 50 mL of 85% acetic acid per liter) with CH_3CN (75 : 25%, v/v) was used as mobile phase (flow rate 1 mL min^{-1}). The detection wavelength in the applied

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AD-25 was set at 350 nm. During the course of each run, the absorbance spectra from PDA100 (in the range 190–750 nm) were collected continuously.

The identification of the rutin and isoquercitrin peaks was carried out by comparing the retention time of the peaks and their UV–Vis spectra with that of the reference standards. The concentrations of the flavonols in the resulting extracts were calculated from calibration curves.

RESULTS AND DISCUSSION

Identification and Quantitation of Rutin and Isoquercitrin

The optimization of the ASE process was performed using the HPLC analysis of the obtained extracts. The HPLC system used for this purpose gave a relatively quick and satisfactory separation of rutin and isoquercitrin from other constituents of black elder flowers or fruits or leaves extracts. Figure 2 presents exemplary chromatograms corresponding to the three investigated extract types and to the mixture of the standard. The retention times of rutin and isoquercitrin were 3.8 and 4.5, respectively. Table 1 presents the characteristic parameters of the calibration curves, where a and b are coefficients of equation regression $y = ax + b$, and r is the correlation coefficient of the equation. Because, in the examined matrices, rutin exists on two and isoquercitrin on three different concentration levels, the table contains a few equations. As results from the collected data, the rutin and isoquercitrin calibration curves showed good linearity in all the examined concentration ranges ($r \approx 0.999$).

Optimization of Extraction Solvent Composition

According to literature,[5,29] the classic extraction process of flavonols from plant material is usually carried out using alcohols or their mixtures with water, methanol being employed most often.[5,30] Because ASE is generally carried out under high pressure and at an elevated temperature, which can influence the penetration properties of the applied solvent and the partition coefficient of a target analyte, the experiments began with the attempt at finding an optimal solvent composition for the extraction of rutin and isoquercitrin from black elder. As the initial conditions of ASE process, the default method conditions of Dionex Corporation (i.e., temperature 100°C, pressure 100 bar) were adopted. The extractions were performed for 10 min. The results of these experiments are plotted in Fig. 3 (only the flower data are shown for clarity). The presented relationship shows that the amount of

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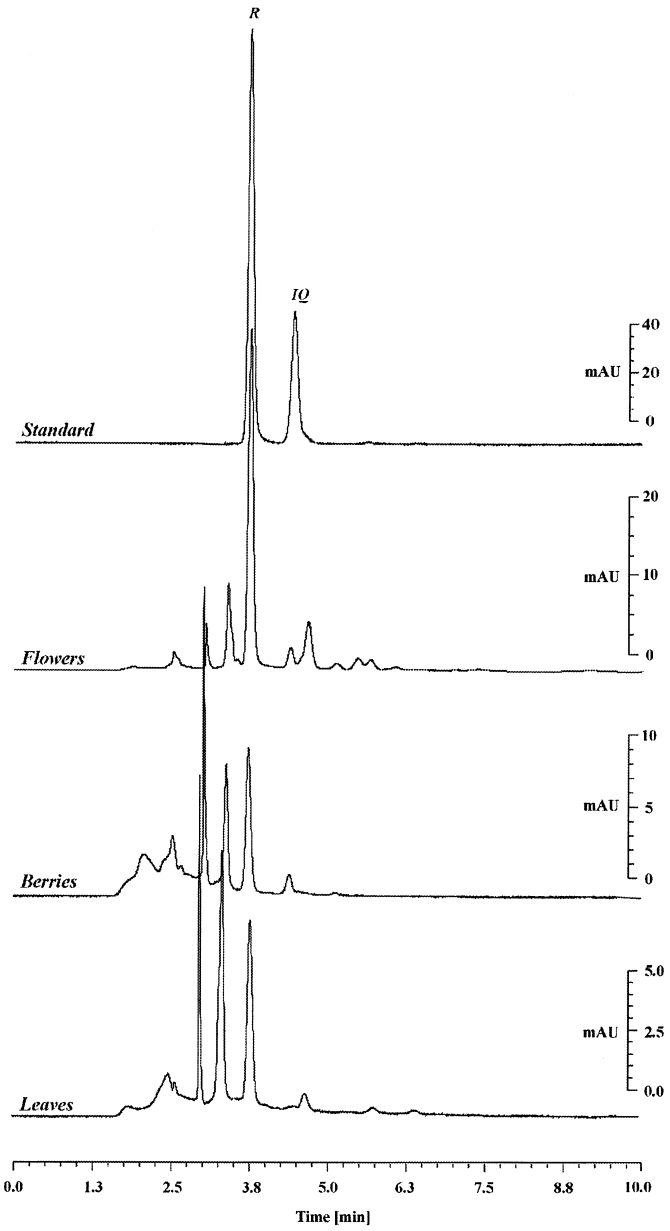

Figure 2. Typical HPLC chromatograms of the methanolic extract of *S. nigra* L. flowers, berries, and leaves, and chromatogram of analyzed standards mixtures (rutin-R; isoquercitrin-IQ). Detection-350 nm.

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Table 1. Analytical characteristic of the calibration curves for rutin and isoquercitrin.

Type of matrix	Analyte	Slope a	Intercept b	r	Detection limit ($\mu g\,mL^{-1}$)	
					Rutin	Isoquercitrin
Flowers	Rutin	611.5143	0.0679	0.9999	0.0623	0.0462
	Isoquercitrin	922.6524	0.0284	0.9998		
Berries	Rutin	567.2690	0.0085	0.9998		
	Isoquercitrin	915.7183	0.0262	0.9986		
Leaves	Rutin	567.2690	0.0085	0.9998		
	Isoquercitrin	816.7493	0.0020	0.9990		

rutin (black diamonds) and isoquercitrin (white circles) increases with the percentage of methanol in the extraction solvent in the range of MeOH 20–80% (v/v). The same trend is seen for the berries and the leaves. The extraction yield of both analytes in pure alcohol is slightly smaller. Probably, it results from the fact that hydrophilic flavonols generally have higher solubility in hydroalcoholic mixtures than in pure alcohol solvent.[31] The investigation revealed that the 80% MeOH solution is the best for the ASE process of rutin and isoquercitrin in the applied conditions. Consequently, it was chosen as the extraction solvent in further study.

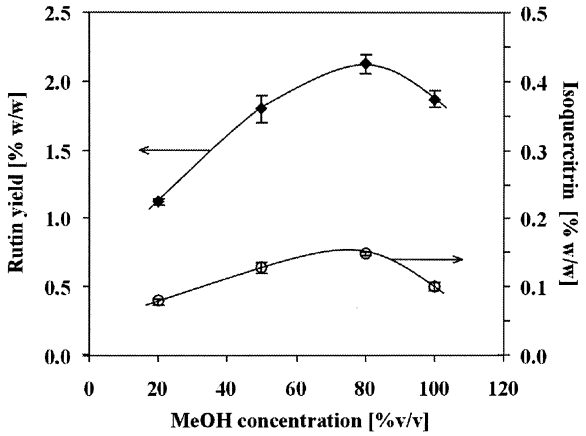

Figure 3. Extraction yield (in weight percentage per gram of dried sample) of rutin (◆) and isoquercitrin (○) as a function of MeOH–H_2O mixture composition, used as the extraction solvent for ASE process.

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Optimization of Extraction Temperature

The influence of temperature on the ASE extraction of rutin and isoquercitrin from the flowers, berries, and leaves of *S. nigra* L. at 100 bar for 10 min. was investigated. The results of this series of investigations are presented in Fig. 4. As shown in Fig. 4(A), the temperature increase up to

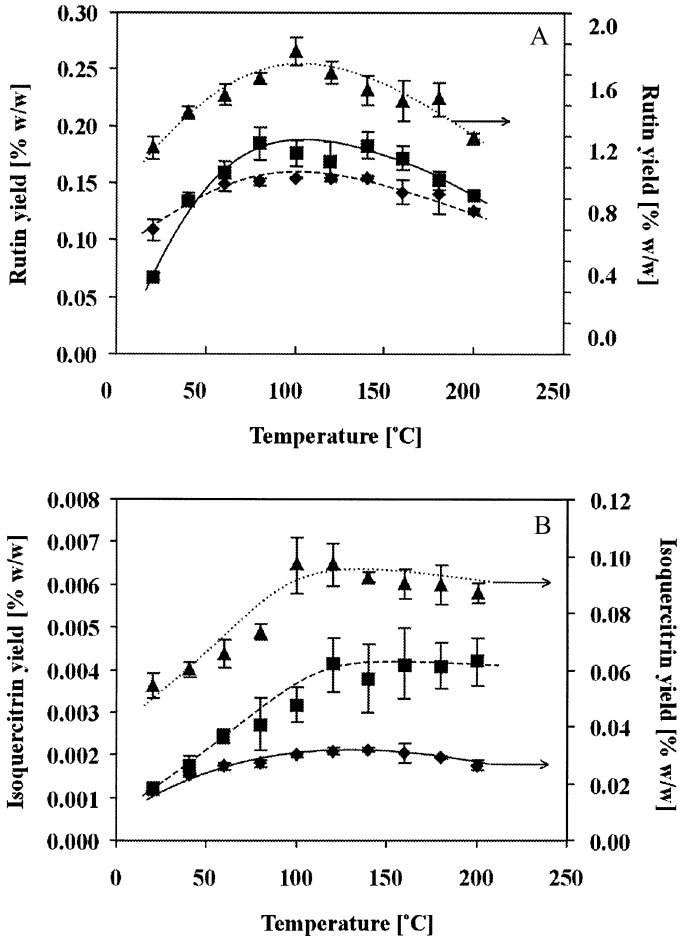

Figure 4. Influence of extraction temperature on the rutin yield from *S. nigra* L. (A) Flowers (···▲···), leaves (—■—), or berries (– – ◆– –). (B) The results for isoquercitrin. For ASE conditions, see text.

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100°C leads to the growth of rutin extraction efficiency. Above 100°C the yield of rutin begins to decrease, though the amounts of the target analyte found at 100°C or 120°C do not differ significantly. The same trend is observed for all the investigated matrices with the greatest changes of extraction efficiency vs. temperature observed for the leaves (solid line with black squares), and the lowest for the berries (dashed line with black diamonds). The obtained results prove that the optimal temperature in which rutin should be extracted is 100–120°C by means of ASE. The decrease in the rutin extraction yield can be explained by the degradation process of the flavonol molecules caused by high temperatures, such as:

- Hydrolysis of the glycosidic form of flavonoid molecule to aglycone and free glycoside (rather unlikely with the increase of the peak corresponding to quercetin, which is the aglycone of both flavonols).
- Their decomposition to other compounds.
- Internal redox or polymerization reactions.[17]
- By changes in the plant matrices (of denaturation type) leading to the recovery loss.

The dependences between the extraction efficiency and operation temperature for isoquercitrin are very similar to those for rutin [see Fig. 4(B)], however, the optimal extraction temperature for this analyte seems to be shifted up to the range of 120–150°C. Further increase in temperature, ranging from 120°C (or 150°C) to 200°C, results in an insignificant diminishing in the isoquercitrin yield. Among all types of the investigated matrices, the leaves give the highest RSD values for the yield of the analyte. This is probably connected with very low isoquercitrin concentration in the leaves tissue (see the appropriate scales). The reasons of the isoquercitrin recovery decrease above 120°C can be the same as those described for rutin. According to the literature,[32] isoquercitrin is more resistant to temperature than rutin. This can explain the shift in the position of the isoquercitrin recovery maximum in the direction of higher extraction temperatures. Despite a slight difference in the optimal extraction temperatures between rutin and isoquercitrin, the following facts determined our decision to perform further experiments at 100°C:

- The rutin concentration level in all the investigated parts of *S. nigra* L. is about ten times greater than that for isoquercitrin.
- At 120°C, cloudy extracts were obtained for the leaves (the same effect was observed for extracts from flowers and berries received in the temperature range of about 140–150°C). The presence of sediment

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may cause undesirable effects, e.g., destruction of SPE columns, loss of analyte as a result of adsorption, etc.

Optimization of Extraction Pressure and Extraction Time

The results of the optimal pressure investigation in the ASE process of rutin and isoquercitrin are illustrated in Fig. 5. The presented data were obtained in the following conditions: sample of investigated matrix—1 g; the extraction solvent—80% (v/v) MeOH solution; extraction temperature—100°C, and static extraction time—10 min. Figure 5(B) presents only the

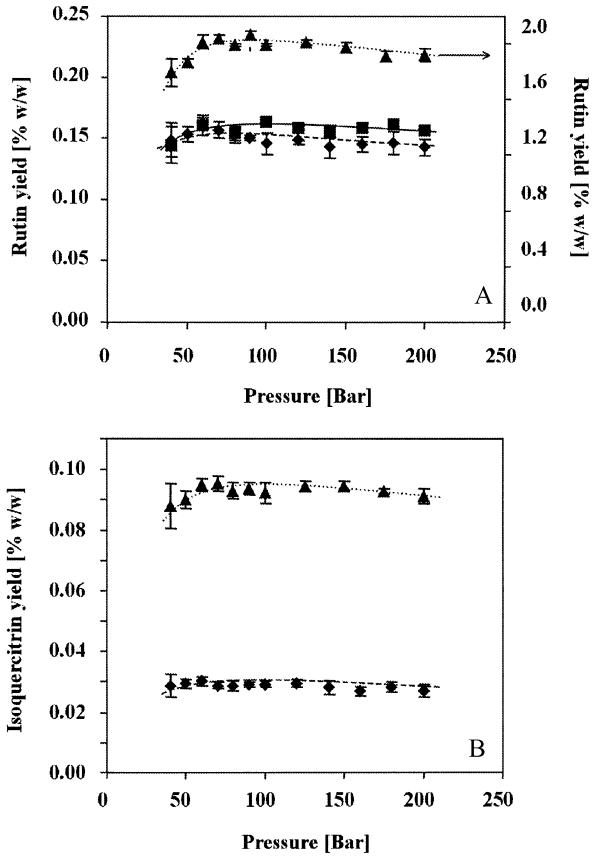

Figure 5. (A) Effect of extraction pressure on the rutin and (B) isoquercitrin yield obtained from the investigated *S. nigra* L. matrices. For symbols, see Fig. 4.

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data for the berries and the flowers, but it should be stressed that the same behavior was observed for the leaves as well. As appears from the data, the effect of extraction pressure on the recovery of both analytes is generally small, although a small increase in each considered case can be seen in the range up to 60 bar. Above this pressure value, no significant effect on the extraction efficiency was observed. However, the plot of the investigated relationship for rutin yield from the berries has a slightly different shape, with a small maximum positioned at about 60 bar.

Figure 6 shows the rutin and isoquercitrin recovery from the investigated matrices vs. static extraction time. The presented results were obtained applying a 80% (v/v) MeOH solution and performing the extraction at 100°C and 60 bar. The plots show that the change in the extraction time from 5 to 30 min is accompanied by very small variations in the extraction efficiency of both analytes. However, the analysis of the presented dependences reveals that in the case of rutin extracted from the flowers and the leaves, 10 min extraction is long enough to reach the extraction equilibrium. The extraction of the same substance from the berries should last up to 20 min.

In all the investigated biological matrices, isoquercitrin demonstrated a longer stabilization time than rutin. It can be attributed to its worse solubility, or to its localization in the plant cells making it more difficult to extract, or to its lower concentration in the plant material, or to all of these facts together.

Comparison of Analytes Recovery by Accelerated Solvent Extraction and by Maceration

It is obvious, that in the case of matrices with the unknown amount of analyte (like plants, herbs etc.), the best are the sample preparation methods giving the highest yield of the analyte. As mentioned in the Introduction, the maceration procedure is one of the sample preparation methods most frequently used for examining flavonols in plant material. Table 2 compares ASE and maceration techniques in rutin and isoquercitrin analysis, both in terms of the analytical results and of their economies. The ASE results listed in the table were obtained by the multi-step extraction of the same sample (until the concentrations of the analytes were below the detection limits—usually 3–5 cycles) in the following conditions: extrahent—80% (v/v) MeOH; temperature—100°C; pressure—60 bar, and time—20 min. The extraction time was established in relation to isoquercitrin, i.e., the substance more difficult to extract.

The presented data indicate that ASE is more economical and gives greater yields of the flavonols. Moreover, the results obtained employing the muti-step ASE procedure in optimal conditions are very close to those presented in[2] for *S. nigra* L.

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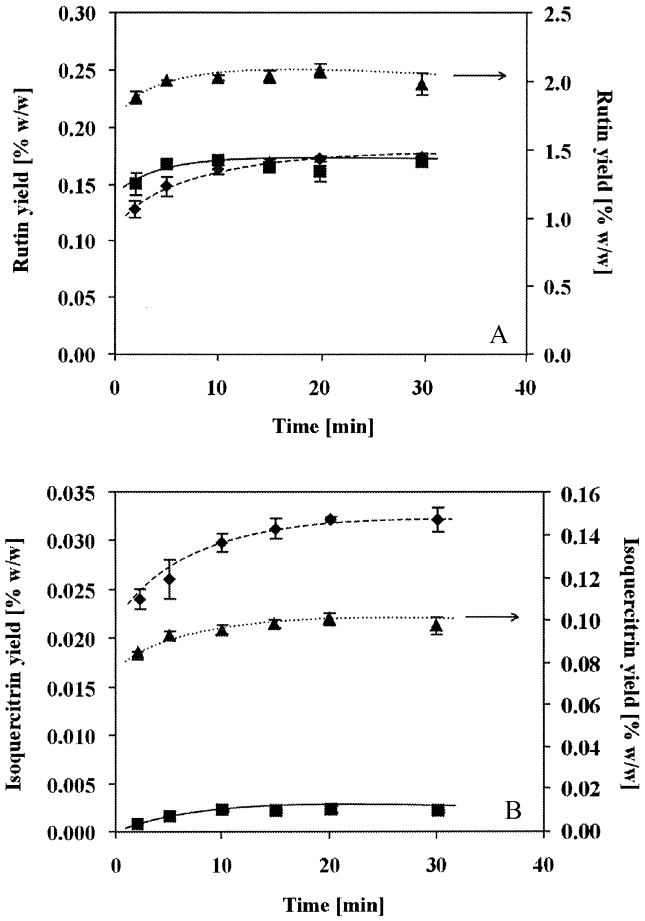

Figure 6. (A) Dependence between the rutin or (B) isoquercitrin yield and static extraction time. For symbols, see Fig. 4.

CONCLUSIONS

The presented results show that ASE should be more strongly recommended for the analysis of flavonols in *S. nigra* L. than maceration. The application of ASE allows extraction of greater amounts of flavonols in each part of the examined plant, as well as to carry out the whole analytical procedure in a shorter time using a smaller amount of solvents. The obtained

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Table 2. Comparison of ASE and maceration procedures in rutin and isoquercitrin analysis in different parts of *S. nigra* L.

	Extraction method	
	Maceration	Exhaustive ASE
Concentration of rutin and isoquercitrin (% w/w)		
Flowers		
Rutin	2.0510 (±0.0240; 1.17)	2.8750 (±0.0300; 1.04)
Isoquercitrin	0.1140 (±0.0040; 3.51)	0.1140 (±0.0300; 2.63)
Berries		
Rutin	0.1612 (±0.0046; 2.85)	0.1890 (±0.0029; 1.53)
Isoquercitrin	0.0186 (±0.0007; 3.76)	0.0346 (±0.0008; 2.31)
Leaves		
Rutin	0.1445 (±0.0082; 5.67)	0.2020 (±0.0059; 2.92)
Isoquercitrin	0.0032 (±0.0002; 6.25)	0.0051 (±0.0002; 3.92)
Total extraction time	81 h (27 h × 3)	60 min[a] (20 min × 3)
Total extrahent volume[b] (mL)	450 (150 × 3)	90 (30 × 3)

Note: Mean values (±SD; RSD).
[a]Optimal data for isoquercitrin in berries.
[b]Data for single experimental point.

results are consistent with the general opinion about ASE usefulness, but they also prove that, for each compound belonging to the same group of substances, the optimal conditions of its pressurized extraction vary a little. The latter result is worth stressing because authors of papers usually assume identical optimal conditions of ASE extraction of whole groups of substances. The data obtained in this study indicates that the quantitative composition of the $MeOH/H_2O$ mixture and temperature are the most important factors in the process of rutin and isoquercitrin extraction, while the extraction time is less important. Above 60 bars, there are no essential differences in the recovery of both examined compounds.

REFERENCES

1. Szafer, W.; Kulczynski, S.; Pawlowski, B. Roś liny Polskie, PWN, Warszawa, 1986.
2. Bartram, T. *Encyclopedia of Herbal Medicine*, 1st Ed.; Grace Publishers: Bournemouth, 1995.
3. Chevalier, A. *The Encyclopedia of Medicinal Plants*; Dorling Kindersley: London, 1996.
4. Kohlmünzer, S. Farmakognozja. Podręcznik dla studentów farmacji. Wydawnictwo Lekarskie PZWL, Warszawa, 1998, 163–176.
5. Pietta, P.; Bruno, A. Separation of flavonol-2-O-glycosides from *Calendula officinalis* and *Sambucus nigra* by high-performance liquid and micellar electrokinetic capillary chromatography. J. Chromatogr. **1992**, *593*, 165–170.
6. Rice-Evans, C.A.; Miller, N.J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol. & Med. **1996**, *20* (7), 933–956.
7. Shahidi, F.; Wannasundra, P.K. Phenolic antioxidants. Crit. Rev. Food. Sci. Nutr. **1992**, *32* (1), 67–103.
8. Vinson, J.A.; Dabbagh, Y.A.; Serry, M.M.; Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. J. Agric. Food Chem. **1995**, *43*, 2800–2802.
9. Formica, J.V.; Regelson, W. Review of the biology of quercetin and related bioflavonoids. Food and Chem. Toxic. **1995**, *33* (12), 1061–1080.
10. Marby, T.J.; Markham, K.R.; Thomas, M.B. *The Systematic Identification of Flavonoids*; Springer Verlag: Berlin–Heidelberg–New York, 1970.
11. Jeffrey, B.; Harborne, F.R.S.; Herbert, B. *The Handbook of Natural Flavonoids I*; Wiley, **1999**, 166 pp.
12. Negre-Salvayre, A.; Affany, A.; Hariton, C.; Salvayre, R. Additional antilipoperoxidant activities of alpha-tocopherol and ascorbic acid on membrane-like systems are potentiated by rutin. Pharmacology **1991**, *42* (5), 262–272.
13. Benthin, B.; Danz, H.; Hamburger, M. Pressurized liquid extraction of medicinal plants. J. Chromatogr. A **1999**, *837*, 211–219.
14. Guyot, S.; Marnet, N.; Laraba, D.; Sanoner, P.; Moutounet, M.; Drilleau, J.F. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a french cider apple variety (*Malus domestica* var. Kermerrien). J. Agric. Food Chem. **1998**, *46* (5), 1698–1705.
15. Guyot, S.; Doco, T.; Souquet, J.M.; Moutounet M.; Drilleau, J.F. Characterization of highly polymerized procyanidins in cider apple (Malus sylvestris var. Kermerrien) skin and pulp. Phytochemistry **1997**, *44* (2), 351–357.

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16. Johnson, E.L.; Schmidt, W.F.; Cooper, D. Flavonoids as chemotaxonomic markers for cultivated Amazonian Coca. Plant Physiol. Biochem. **2002**, *40* (1), 89–95.
17. Alonso-Salces, R.M.; Korta, E.; Barranco, A.; Berrueta, L.A.; Gallo, B.; Vicente, F. Pressurized liquid extraction for the determination of polyphenols in apple. J. Chromatography A **2001**, *933*, 37–43.
18. Richter, B.E.; Jones, B.A.; Ezzell, J.L.; Porter, N.L.; Avdalovic, N.; Pohl, C. Accelerated solvent extraction: a technique for sample preparation. Anal. Chem. **1996**, *68* (6), 1033–1039.
19. Lundstedt, S.; Van Baval, B.; Haglund, P.; Tysklind, M.; Öberg, L. Pressurised liquid extraction of polycyclic aromatic hydrocarbons from contaminated soils. J. Chromatogr. A **2000**, *883*, 151–162.
20. Buldini, P.L.; Cvalli, S.; Trifiro, A. State-of-the-art ion chromatographic determination of inorganic ions in food. J. Chromatogr. A **1997**, *789*, 529–548.
21. Björklund, E.; Nilsson, T.; Bowadt, S. Pressurised liquid extraction of persistent organic pollutants in environmental analysis. Trac-Trends in Anal. Chem. **2000**, *19* (7), 434–445.
22. Saim, N.; Dean, J.R.; Abdullaf, M.P.; Zakaria, Z. Extraction of polycyclic aromatic hydrocarbons from contaminated soil using soxhlet extraction, pressurised and atmospheric microwave-assisted extraction, supercritical fluid extraction and accelerated solvent extraction. J. Chromatogr. A **1997**, *791*, 361–366.
23. Conte, E.; Milani, R.; Morali, G.; Abballe, F. Comparison between accelerated solvent extraction and traditional extraction methods for the analysis of the herbicide diflufenican in soil. J. Chromatogr. A **1997**, *765*, 121–125.
24. Lehotay, S.J.; Lee, Ch. Evaluation of a fibrous cellulose drying agent in supercritical fluid extraction and pressurized liquid extraction of diverse pesticides. J. Chromatogr. A **1997**, *785*, 313–327.
25. Adou, K.; Bontoyan, W.R.; Sweeney, P.J. Multiresidue method for the analysis of pesticide residues in fruits and vegetables by accelerated solvent extraction and capillary gas chromatography. J. Agr. Food Chem. **2001**, *49* (9), 4153–4160.
26. Schäfer, K. Accelerated solvent extraction of lipids for determining the fatty acid composition of biological material. Anal. Chim. Acta **1998**, *358* (1), 69–77.
27. Chase, G.W.; Thompson, B. Accelerated solvent extraction of vitamin K-1 in medicinal foods in conjunction with matrix solid-phase dispersion. J. AOAC INT. **2000**, *83* (2), 407–410.
28. ASE 200 Accelerated Solvent Extractor Operator's Manual, Document No. 031149, Revision 01, Dionex, Sunnyvale, CA, 1995, Sect. 3–5.

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29. Strzelecka, H.; Kamińska, J.; Kowalski, J.; Malinowski, J.; Walewska, E. PZWZ, Warszawa, **1987**, 84.
30. Huck, Ch.W.; Huber, Ch.G.; Ongania, K.; Bonn, G.K. Isolation and characterization of methoxylated flavones in the flowers of Primula Veris by liquid chromatography and mass spectrometry. J. Chromatogr. A **2000**, *870*, 453–462.
31. *Techniques of Flavonoids Identification*; Markham, K.R., Ed.; Academic Press Inc.: London, 1982; 15 pp.
32. *The Merck Chemical Reagents Catalogue*; Darmstadt: Germany, 2002; 1091 pp., 1103 pp.

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