THE STABILITY OF RUTIN AND CHLOROGENIC ACID DURING
THE PROCESSING OF BLACK ELDER (SAMBUCUS NIGRA)
INFLORESCENCE

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Black elder inflorescence has been traditionally used in Central Europe both in folk and official medicine. This plant material is a rich source of two biologically active components, rutin and chlorogenic acid. Nevertheless, there is a lack of data on the changes of their content during processing.

The stability of rutin and chlorogenic acid during drying and the long-term storage of black elder inflorescence were analysed in this study. The rutin content was determined by capillary electrophoresis using solid-phase extraction. HPLC was used for the determination of chlorogenic acid. The dependence of rutin and chlorogenic acid content on the temperature of drying and storage duration were monitored and statistically evaluated by a two-way ANOVA test. The contents of rutin and chlorogenic acid revealed no statistically significant changes when dried at temperatures of 22 °C and 30 °C. The significant decrease in contents of both studied compounds was found at a drying temperature of 50 °C. The decrease in content of rutin was about 20%, in chlorogenic acid about 12%.

The content of both studied compounds also decreased after long-term storage (at a temperature of 22 °C for one year). The decrease in content of rutin was greater than that of chlorogenic acid.

Keywords: black elder, Sambucus nigra L., rutin, chlorogenic acid, high-performance liquid chromatography, micellar electrokinetic chromatography

Elder (Sambucus) is a genus of species, belonging to the family Adoxaceae. The plants are used as ornamentals (JUDD et al., 2002). The black elder (Sambucus nigra L.) is native to continental Europe, the British Islands and around the Black Sea. It grows also in North Africa (Algeria, Morocco) but is absent in South Spain, North Scotland, North Scandinavia and Iceland. The black elder was brought to Asia, North America, Australia and New Zealand (ATKINSON & ATKINSON, 2002).

The first references of the use of the black elder flowers for medicinal purposes in Europe can be found in the medieval herbaria, according to which the preparations were used in curing colds and swelling. Both flowers (Flos Sambuci) and fruits (Fructus Sambuci) are listed as an official pharmaceutical prepareate in the Czech Codex of Medicaments. They are used in Central European traditional medicine for the treatment of cold and respiratory diseases.

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The black elder inflorescence contains volatile terpenic substances, cholin, flavonoid glycosides, sambunigrin, glycosides of phenolic acids with dominant chlorogenic acid and tannins. Rutin and isoquercitrin have been reported as the main flavonoid glycosides (Piëtta et al., 1992; Dawidowitz et al., 2003). The fruits contain various organic acids, anthocyanins and vitamins A and C (Kaaack & Austed, 1998).

Rutin and chlorogenic acid are the most important compounds in black elder, and have valuable qualities. Quercetin and rutin act as an important component of the anti-oxidative system preventing the formation of peroxiradicals, eliminating free oxygen radicals (Cook & Samman, 1996) and binding some metal cations (Hassing et al., 1999). Chlorogenic acid has diverse biological activities, including antioxidant and anti-carcinogenic effects (Jiang et al., 2000) as well as an inhibitory effect on the mutagenicity of aflatoxin B (Francis et al., 1989).

The black elder is ranked among preferred medical plants (Murkovic et al., 2004). The flowers are also used for the production of refreshing drinks (Jorgensen et al., 2000), and the fruits are used for the preparation of juices, wines and jams.

It is known that the contents of effective substances in wildly growing plants vary with season, growth condition and other factors (Kaaack & Austed, 1998). However, there is an absence of information on the changes of phenolic substances content during commonly used processing techniques (drying, storage) of black elder inflorescence. Thus, the aim of our study was to identify the conditions of drying that produced the highest yields of rutin and chlorogenic acid. The changes in the levels of both substances were monitored during long-term storage of dried material.

1. Materials and methods

1.1. Plant material

The inflorescences with about half of its flowers fully opened were collected from widely growing black elder during a sunny windless day in June 2004. The site was situated at the outskirts of town České Budějovice (Czech Republic), about 500 m away from a motorway. The plant species were identified by Kubát and co-workers (2002) by Dr. Božena Šerá, Academy of Sciences of the Czech Republic, České Budějovice. A specimen of S. nigra L. (No. CB 8457/3) from this region is stored in the herbarium of the South Bohemia Museum in České Budějovice.

1.2. Drying the plant material

Three methods of drying were used. The inflorescences were divided into three parts (about 1000 g each and dried at 22 °C, 30 °C and 50 °C) in a laboratory drier with air circulation (Memmert, Modell 400, Germany). The drying was finished when a constant weight was reached. The dried materials were homogenized by a laboratory mill into fine powder. The samples of three differently dried materials (each about 20 g) were stored at –18 °C until analysis. The fresh material (100 g) was freeze-dried.

1.3. The storage of dried materials

The powdered dried materials were put into closed plastic bottles and stored in a dark room at 22 °C for 12 months. The contents of rutin and chlorogenic acid were determined after 1, 3, 6 and 12 months of storage.
1.4. Chemicals and equipments

The standards (chlorogenic acid ≥95%, rutin hydrate 95%, HPLC grade) were purchased from Sigma-Aldrich (Steinheim, Germany). Solvents and SPE columns RP-18 were obtained from Merck (Darmstadt, Germany). 1-naphthylacetic acid was purchased from Spolana (Brno, Czech Rep.).

The separation and quantification of rutin was performed using micellar electrokinetic capillary chromatography (MECC): SpectraPhoresis 2000 (Thermo Separation Product, Fremont, CA, USA). The equipment consisted of capillary electrophoresis with fused-silica capillary of 70 cm length and 75 μm i.d. (CElest FS75 CE column, Supelco) and a UV-VIS scanning detector.

Separation and quantification of chlorogenic acid was performed with liquid chromatography (HPLC): HP 1050 (Hewlett-Packard, USA), DAD detector (HP 1040, Hewlett-Packard, U.S.A.), Phenomenex Luna C18 (2), 3 μm, 2×150 mm column.

1.5. Determination of rutin by MECC

The method of rutin determination consists of rutin extraction from plant material with 50% methanol and analysis by micellar electrokinetic capillary chromatography (MECC). Rutin is isolated and pre-concentrated during solid phase extraction. This method was used in the other study (KALINOVÁ & DADÁKOVÁ, 2006).

The dried material (0.25 g) was homogenized, then extracted with 12.5 ml methanol and 12.5 ml water containing 80 mg ascorbic acid as an antioxidant. The extraction was performed at 90 °C in a water bath under a reflux cooler for 90 min. After cooling, the material was centrifuged at 1800 g for 15 min. The sediment was re-suspended in 12.5 ml methanol and 25 ml water and centrifuged under the same conditions. The combined supernatants were diluted with water to 500 ml; the pH was adjusted to 3.0 with 1 M HCl. The solution was then filtered through a glass fiber filter (GF/C, Whitman, England). The sorption of diluted substances was performed on solid phase (SPE) columns RP-18 conditioned with 10 ml methanol and 25 ml water and centrifuged under the same conditions. The combined supernatants were diluted with 500 ml methanol and 25 ml water. The adsorbed substances were eluted by 1.4 ml methanol. The internal standard (200 μg 1-naphthylacetic acid) was added to the final eluate.

The samples with the internal standard were analysed by capillary electrophoresis. The running buffer (pH=9.2) contained 10 mM Na-tetraborate, 10 mM boric acid, 20 mM sodium-dodecyl-sulphate (SDS) and 15% (v/v) methanol. The analysis was performed at 25 °C, 20 kV; hydrodynamic injection 2 s, detection of analyte at 270 nm. The analytical response is the ratio of rutin peak area with the internal standard.

1.6. Determination of chlorogenic acid by HPLC

The method for chlorogenic acid determination was carried out according to KAYANO and co-workers (2003).

The homogenized dried material (0.25 g) was extracted with 3 ml 90% methanol under shaking for 30 min. The samples were centrifuged under the same conditions as for the isolation of rutin. The sediment was washed twice with 1 ml 90% methanol. All the supernatants were pooled and their volume was marked. The extracts were analysed by liquid chromatography HP 1050 (Hewlett-Packard, USA), DAD detector (HP 1040, Hewlett-Packard, USA.) Phenomenex Luna C18 (2), 3 μm, 2×150 mm column. The mobile phase A: 5% acetonitrile + 0.15% trifluoroacetic acid. The mobile phase B: 80% acetonitrile + 0.15% trifluoroacetic acid. The gradient of phase B grows from 0% to 53% during 55 min. Chlorogenic acid was detected at 220 nm.
1.7. Method validation

The specificity of both compounds was confirmed by comparison with spectra and retention time of standard compounds and standard addition.

Both methods were validated according to Suchánek (1999). The following parameters were tested: linear working range, limits of detection (LOD) and quantification (LOQ), intra-day and inter-day precision, recovery and measurement uncertainty.

The quantity was determined with the help of a calibration curve. The working ranges (0.01–2 mg/g for rutin, 0.002–0.8 mg/g for chlorogenic acid) were proposed and the linearity of working range was tested. The values for residuals of standard deviation for linear and non-linear calibration function were calculated according to Czech Standards (1994a; 1994b). According to the results of F-test, both proposed working ranges are linear.

The LOD was calculated as triple RSD of ten independent analyses of blank matrix material (dried cauliflower – spiked with rutin or chlorogenic acid), for the assessment of LOQ the RSD was decupled. The intra-day and inter-day precision and measurement uncertainty were estimated from the data obtained by analysis of internal control material (dried black elder inflorescence). Intra-day precision was evaluated by repeated eight analyses during one day. The data for inter-day precision were obtained by determination of analytes content over a 5-day period. The parameters of both methods are presented in Table 1.

Table 1. Parameters of analytical methods

<table>
<thead>
<tr>
<th>Method parameter</th>
<th>LOD (+10–3 mg/g)</th>
<th>LOQ</th>
<th>Precision within-day (%)</th>
<th>Precision day-to-day (%)</th>
<th>Expanded uncertainty (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>2.31</td>
<td>10</td>
<td>2.40</td>
<td>3.98</td>
<td>15</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.40</td>
<td>2</td>
<td>1.91</td>
<td>3.25</td>
<td>10</td>
</tr>
</tbody>
</table>

LOD: limit of determination; LOQ: limit of quantification

The recovery of rutin or chlorogenic acid was determined for each sample by method of standard addition (100 and 200% of analysed amount). Using the above-mentioned methods, we obtained recovery 92–98% for rutin and 95–101% for chlorogenic acid.

The measurement uncertainty was estimated to 10 and 15% for chlorogenic acid and rutin, respectively, and comprises partial uncertainties in all steps including purity of standards (according to Suchánek, 2001). The combined standard uncertainty was 4.5 and 5.8% for chlorogenic acid and rutin, respectively. Expanded uncertainty was 10 and 15% for chlorogenic acid and rutin, respectively (with coverage factor 2 and rounding).

1.8. Statistical methods

The data concerning the effect of different time and temperature storage on rutin and chlorogenic acid contents were computed by a two-way ANOVA test. The contents of investigated compounds were assessed using the mixed model with random factors (temperature, time) without repetition. The a-priori hypothesis that the drying temperature of black elder inflorescence negatively affected the content of investigated compounds was tested by one-way ANOVA with time as the co-variable characteristic. Correlations between storage time and contents of rutin and chlorogenic acid and t-test between various contents of

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both compounds under a temperature regime were also assessed. The statistical analyses and the box and whiskers graphs in this study were computed using the STATISTICA program. The basic statistical tools (MS Office-Excel) were used for confirmation of linearity.

2. Results and discussions

The stability of the two dominant phenolic compounds in the inflorescence of black elder was studied. The inflorescences were dried at 22, 30 and 50 °C and then stored at 22 °C for one year. The plant materials dried at 22 °C and 30 °C were creamy yellow without a brownish shade. The inflorescences dried at 50 °C obtained an intense ochre-brown colour that is an evidence of Maillard browning accompanied with a degradation of some components in the dried material. Their contents were also determined in freeze-dried inflorescence where 19.8 and 16.3 mg/g of rutin and chlorogenic acid, respectively, were detected. The content of these substances in freeze-dried material was comparable with levels obtained in the inflorescences of material dried at 22 °C. The obtained results correspond with the data of DAWIDOWITZ and co-workers (2003) who found 20.5–28.7 mg/g of rutin in dried material. The drying conditions were not mentioned in that article.

![Box and whiskers plot showing the content of rutin in Sambucus nigra inflorescence after drying at three different temperatures.](image)

*Fig. 1. Content of rutin in Sambucus nigra inflorescence after drying at three different temperatures.*

Rutin contents were compared by one-way ANOVA (factor time was covariable, F=25.28, P<8×10⁻⁵).

\[\pm \text{ std. dev.}; \quad \pm \text{ std. err.}; \quad \text{mean}\]
The occurrence of chlorogenic acid in the flowers of black elder is known (HAWRYL et al., 2002) but the exact changes in content have not yet been determined.

The storage time and temperature significantly affected the contents of rutin (ANOVA all effects: $P_{\text{time}}<2\times10^{-5}$, $P_{\text{temperature}}<2\times10^{-6}$) and chlorogenic acid (ANOVA all effects: $P_{\text{time}}<3\times10^{-6}$, $P_{\text{temperature}}<8\times10^{-9}$).

Differences in content of rutin in the inflorescence following three drying temperatures (22 °C, 30 °C, 50 °C) were tested by one-way ANOVA (with time as a covariable). A significant difference: $P<8\times10^{-5}$ (Fig. 1) was found. A comparable result was found in the content of chlorogenic acid in relation to the drying temperature (time as covariable, $P<2\times10^{-9}$) (Fig. 2).

The influence of long-time storage (up to one year) on the contents of rutin and chlorogenic acid was also determined. The plant material was analysed four times during the year (Table 1). The contents of both compounds decreased during storage (Fig. 3). We found a negative correlation between content and time ($P<0.05$ and $P<0.01$ for rutin and chlorogenic acid, respectively).

![Fig. 2. Content of chlorogenic acid in Sambucus nigra inflorescence after drying at three different temperatures. Chlorogenic acid contents were compared by one-way ANOVA (factor time was covariable, $F=216.26$, $P<2\times10^{-9}$).](image-url)
3. Conclusion

Our data showed that drying of black elder inflorescences at 50 °C had a disadvantageous effect. Drying at this temperature caused losses of the studied compounds. Drying at room temperature seemed to be the most suitable method for preservation of plant material both for home use and for commercial purposes. Our results also showed that the dried inflorescences of black elder were a significant source of rutin and chlorogenic acid even after one-year storage.

The content of rutin in the inflorescences of black elder reached about 17 mg/g of dry mass. Black elder, then, is one of the plants with the highest content of rutin.

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References


