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Machalová, Zdeňka
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Student: Ing. Zdeňka Machalová
Supervisor: Ing. Helena Sovová, CSc.
Supervisor–specialist: Ing. Marie Sajfrtová, Ph.D.

Plant material is a rich source of biologically active compounds. Conventional extraction methods, which are still widely used for isolation of these compounds, have some disadvantages such as a long extraction time and use of a large amount of organic solvent. Therefore, interest in high-pressure extraction methods with minimal consumption of organic solvent to isolate these compounds has increased. Pressurized solvent extraction (PSE) and supercritical fluid extraction with CO_2 as a solvent (SFE) represent two of these methods.

The PSE uses elevated temperature and pressure, which facilitate better penetration of solvent to the matrix pores and offers advantages with regard to extraction time, yields and consumption of solvent. In spite of the high temperature above the normal boiling point of solvent, thermo-labile substances are mostly preserved because of the short extraction time (5–20 min). The elevated pressure is mainly used to keep the solvent liquid.

Contrary to PSE, the SFE is carried out at low temperatures and is mostly used for the extraction of nonpolar compounds, but it is also suitable for the extraction of polar compounds by adding a small amount of organic solvent into the system (as modifier). The SFE is more selective and provides extract of higher purity than conventional methods and also allows isolation of thermo-labile compounds due to the use of low temperature.

A combination of these methods can increase the concentration of polar compounds in the final extract. Firstly, SFE removes the nonpolar compounds, and then PSE should provide a more concentrated extract using the same material.

Phytoecdysteroids are polar compounds located in various plant parts. The most famous ecdysteroid is 20-hydroxyecdysone (20-HE), which is the most abundant ecdysteroid in the adaptogenic plant *Leuzea carthamoides* [1]. The 20-HE is known for its anabolic and tonic effects on mammals and is used as dietary supplement. Moreover, 20-HE

is a steroid hormone responsible for insect moulting and metamorphosis. Therefore, it could be applied to control the harmful insect population in agriculture [2].

This work is focused on the extraction of 20-HE from roots of *Leuzea carthamoides* using SFE, PLE, and Soxhlet extraction. The SFE and PSE conditions (pressure, temperature, concentration of solvent and extraction time) were optimized to improve the extraction yields and concentrations of 20-HE in extracts. These methods were compared with a conventional Soxhlet-type extraction. The SFE experiments were carried out at temperature 40 °C, pressure 30 MPa and with addition of 5–20 % of co-solvent (ethanol). PSE was made at temperatures from 80 °C to 120 °C, pressures from 10 to 30 MPa and static extraction time from 5 to 15 min. Soxhlet extraction with ethanol was used to evaluate the total content of 20-HE in roots. The concentration of 20-HE in extracts was determined by HPLC coupled with mass spectrometry.

In case of PSE, the concentration of 20-HE in extract and total yield of extract were significantly influenced by temperature and extraction time. In the contrary to that, the concentration of 20-HE in the CO_2 extracts and yield of the CO_2 extracts were dependent on the concentration of ethanol in CO_2.

References

1. L. Kokoska and D. Janovska, Phytochemistry 2009, 70, 842–855.
2. L. Dinan, Phytochemistry 2001, 57, 325–339.