



Supercritical carbon dioxide extraction of *Trigonella foenum-graecum* L. seeds: Process optimization using response surface methodology



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ABSTRACT

Pretreatment of fenugreek (*Trigonella foenum-graecum* L., Fabaceae) seeds was achieved using the step of defatting and acid hydrolysis. The obtained hydro-isolate (HI) was used as initial feed for diosgenin isolation by supercritical CO₂ extraction (SC-CO₂). Preliminary tests performed at conditions resulting in SC-CO₂ density ranging from 800 to 920 kg/m³ indicated that at some pressure, temperature as well as consumption of supercritical fluids the optimal working conditions for diosgenin isolation could be determined. For this purpose, the following range of working conditions of SC-CO₂ were tested by using Central Composite Rotatable Design (CCRD) and Response Surface Methodology (RSM): pressure from 16.6 to 33.4 MPa, temperature from 30 to 50 °C and consumption of SC-CO₂ from 11.6 to 28.4 g_{CO2}/g_{dm}. The result of this investigation indicated that maximum yield of 0.81 mg diosgenin from 1 g of fenugreek seeds on dry basis (about 1.3% of total extract) at 24.6 MPa, 43.5 °C, and 20.87 g_{CO2}/g_{dm} could be obtained.

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1. Introduction

Diosgenin (Fig. 1) is a biologically active compound, well known as one of the most significant steroid precursors in synthesis of steroidal drugs such as estrogen, progesterone, contraceptive agents, corticosteroids, etc. with wide therapeutic use and commercial value in the pharmaceutical industry [1,2]. As principal steroidal sapogenin, its activity was also found to be hypercholesterolemic, cardiostonic, anticancer, antidiabetic and virostatic. Therefore its usefulness in maintenance of health is widespread [3–5].

The traditional source of diosgenin is usually genus *Dioscorea* known for the high-yield isolation of this steroidal sapogenin, whose amount was determined to be from 1 to 8% on a dry weight basis, besides belonging to genus *Dioscorea* the plant known for high content are *T. foenum-graecum* L., *Tribulus terrestris* L., *Zygophyllaceae*, *Smilax china* L., *Smilacaceae* [6–10]. Quantitative analysis of fenugreek (*Trigonella foenum-graecum* L.) extracts showed levels of diosgenin from 0.5 to 2% [6,11], leading to the conclusion that optimized extraction of diosgenin from fenugreek might be economically competitive to the extraction from genus *Dioscorea*.

Advantage of easier cultivation of fenugreek and its rapid growth with matured seeds 3–4 months after semination established the fenugreek as appropriate good starting material for diosgenin production [12,13].

Fenugreek seeds showed decisive hypolipidemic activity as defatted fraction of seeds decrease the serum's total cholesterol, LDL, VLDL cholesterol and triglyceride levels in plasma [14,15]. Seeds contain 1–2% steroidal sapogenins, mainly diosgenin contained in the plant as steroidal saponin glycoside. Generally, the extraction methods of diosgenin from fenugreek seeds require pretreatment of the plant including defatting as the first step, followed by acid hydrolysis with aim to prepare corresponding hydro-isolate [15–18].

In this study, the supercritical CO₂ (SC-CO₂) extraction was applied in order to extract diosgenin rich fraction from prepared hydro-isolate from fenugreek. The aim of this study was to analyze the yield of total extract and content of diosgenin which might be obtained at different conditions of SC-CO₂ extraction. The first part of performed investigation was related to determination of different SC-CO₂ density influence on total extract yield. On the basis of obtained preliminary data, more tests at different pressures, temperatures and consumptions of supercritical fluids were realized in order to find the optimal working condition for SC-CO₂ diosgenin isolation. The response surface methodology (RSM) and central composite design (CCD) were applied as recently reported in the

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Nomenclature

m_{CO_2}/m_s	amount of CO ₂ spent per plant used for experiment (g _{CO₂} /g _{dm})
P	pressure, MPa
t	temperature, °C
x_1	coded variable of pressure
x_2	coded variable of temperature
x_3	coded variable of consumed CO ₂
Y	yield of diosgenin, mg _{diosgenin} /g _{dm} , or % (g _{diosgenin} /100 g _{dm})
r	coefficient of correlation, line in Fig. 5
R^2	coefficient of determination
R^2_{adj}	adjusted coefficient of determination
β	regression coefficient

Abbreviations

dm	dry material
HI	hydro-isolate
EX	extract
LDL	low-density lipoprotein cholesterol
VLDL	very-low-density lipoprotein cholesterol
HPLC	high-performance liquid chromatography
DAD	diode array detector
RSM	response surface methodology
CCRD	central composite rotatable design
SFE	supercritical fluid extraction
SC-CO ₂	supercritical CO ₂ extraction

literature [9,12,19,20]. The efficiency of defined conditions of SC-CO₂, expressed through diosgenin content from fenugreek seeds was also compared to the yield of diosgenin determined by Soxhlet extraction.

Many techniques of diosgenin isolation from fenugreek were reported in literature [4,6,12,21,22], but not one related to the application of SC-CO₂. The advantages of supercritical fluid extraction (SFE) compared to traditional techniques are already well known; supercritical extract is solvent-free, the SFE extraction could be realized much faster and finally, there is no need for further steps of supercritical extracts purification.

Furthermore, studies related to SFE of diosgenin from other plants did not cover a wide range of pressure, temperature and SC-CO₂ consumed for extraction and did not analyze the effects of process parameters and their interactions on diosgenin yield. Moreover, reported studies did not give information related to the significance of pretreatment process (defatting and hydrolysis of seeds) which is very important for realization of diosgenin isolation using SC-CO₂. It was proved that selectivity of CO₂ extraction of diosgenin can be changed by using various temperature and

pressure (i.e. density of SC-CO₂), thus tuning the solvent power of SC-CO₂ which is directly related to density.

Applied pretreatment methods of different seeds include subsequent hydrolysis as already shown in the case of diosgenin extraction from *T. terrestris* [9]. Such method of pretreatment was also applied in this study but combined with the defatting process as the first step of hydro-isolate preparation from the fenugreek seeds. As mentioned before the acid hydrolysis of steroidal saponins is a necessary treatment of seeds which leads to release of diosgenin from sugar chains while the defatting step enables obtaining a purer SC-CO₂ extract.

2. Experimental

2.1. Plant material

The seeds of *Trigonella foenum-graecum* L., Fabaceae (fenugreek) were collected in July 2014 in the province of Vojvodina, the northern part of Serbia. The voucher specimen is deposited at the Institute for medicinal and plant research “Josif Pancic”, number 11550412. Two hundred grams of seeds were air-dried and milled in a blender for 60 s just before defatting and acid hydrolysis.

2.2. Pretreatment of plant seeds

2.2.1. Defatting

Approximately 200 g of milled fenugreek seeds were defatted by refluxing with 625 mL hexane in a Soxhlet apparatus for 4 h. After that, the hexane was evaporated to dryness and yield of defatted oil from seeds was calculated. The yield of collected fat was in range of $0.78 \pm 0.2\%$.

2.2.2. Acid hydrolysis

Defatted air-dried seeds were hydrolyzed with 2M HCl for 3 h at 100 °C (plant material:HCl = 1:4) in a vessel with a magnetic stirrer. After hydrolysis the mixture was cooled and filtered under vacuum by the Buchner apparatus. Neutralization of filtered sediment was achieved by adding 5% Na₂CO₃ and water, sequentially washed and filtered through a filter paper until neutral pH value was achieved. Neutralized and filtered sediment was dried in vacuum at 80 °C to constant moisture content less than 5%.

2.3. Supercritical CO₂ extraction-SFE

Extraction was carried out in a semi-batch Autoclave Engineers Screening System previously described in detail [23].

The extractor was charged with approximately 40 g of defatted and hydrolyzed seeds (HI) of fenugreek, previously dried to constant moisture content. Extraction of prepared HI at the selected operating conditions of pressure, temperature, and time was performed. The obtained extract was collected into a sample container and the total extract yield was calculated. Extracts were kept in a refrigerator (at –5 °C) for diosgenin analysis by high-performance liquid chromatography (HPLC).

2.4. Soxhlet extraction

Defatted and acid hydrolyzed seeds of fenugreek were extracted for 4 h using traditional Soxhlet extraction with hexane. Diosgenin yield was calculated to be 1.90 mg/g dry fenugreek seeds which was held as 100% for comparison with yield obtained by SC-CO₂ extraction in the form of recovery. The yield of extract after pretreatment of seeds (defatting and hydrolysis) was 27%.

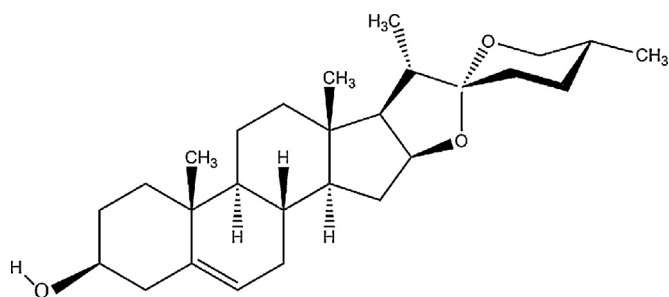


Fig. 1. Chemical structure of diosgenin.

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