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Unconventional techniques for the extraction of bioactive compounds from various plants

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Abstract

The researches aimed to accomplish the extraction with supercritical carbon dioxide of sulphur compounds from local plants of the *Allium* genus. The thin layer chromatography enabled the qualitative identification of thiosulfinates (with R_f = 0.201 – 0.486), diallyl thiosulfinate (with R_f = 0.458 on the *Allium ursinum* L. extracts) and dipropyl thiosulfinate (with R_f = 0.486 on the *Allium cepa* var. "Rubiniu" extracts, highlighted through a light brown spot).

Keywords: supercritical fluids, *Allium spp,* sulphur compounds

1. Introduction

The supercritical fluid extraction is a high-pressure non-destructive separation process which is based on the solvation power of fluids at a temperature and pressure above their critical point. The supercritical fluids that are used as solvents are referred to as "green" solvents being nontoxic as compared with the organic ones.The supercritical fluids have the characteristics of gases, being able to rapidly diffuse throughout the solid matrix, and liquid like properties that determine them to dissolve the components in the material submitted to analysis. Furthermore, the solvation power and the density of the supercritical fluids vary when pressure and temperature are modified [1]. Consequently, the solvation power of supercritical fluids can be adjusted by choosing optimal values of pressure and temperature. Moreover, the density of the fluids can be modulated for the solubilisation of certain types of bioactive compounds [2-4]. The extracted products can be separated by decreasing the pressure of the fluids. As extraction solvent, the carbon dioxide is the most commonly used supercritical fluid since it has the following advantages: it is inert, non-flammable, nontoxic, available in pure form (99.995% vol.) and inexpensive.

Although the supercritical carbon dioxide (SC-CO_2), along with other supercritical fluids, is a greenhouse gas, its use is beneficial due to the possibility of replacing the organic liquid solvents. The supercritical carbon dioxide has low critical temperature (31.1 °C) thus being fit for the extraction of biological components and thermosensitive materials, having also an easy to reach critical pressure (73.8 bar = 7.38 MPa). These aspects contributed to the increasing interest in the use of supercritical fluids for the extraction of bioactive compounds from dry natural materials or biological products and also for their use in the pharmaceutical industry [5-7]. Plants of the *Allium* genus and the essential oils are mostly used during spring and winter, their quality depending on origin, variety and production conditions. The concentration and the nature of the sulphur compounds from plants of the *Allium* genus are strongly influenced by the breed conditions of plants and by the production conditions of extracts. Usually, the flavour characteristics of onion, garlic and ramson are associated with thiosulfinates formed enzymatically from precursors of amino acids when the plants are cut or shredded. Saengcharoenrat and Guyer claim that the obtaining of onion extracts with supercritical fluids is an alternative solution to vapour distillation and solvent

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extraction [8]. In the pharmaceutical industry, the garlic extracts obtained in mild conditions are preferred for their high content of bioactive compounds. Del Valle *et al.* studied the yield of oleoresins and the selectivity of allicin extraction in supercritical conditions, using a homogenous mixture of lyophilized garlic [9]. Thus, it was concluded that the yield of oleoresins increases slowly as the extraction pressure increases from 150 to 450 bar. Sass-Kiss *et al*. optimized the extraction conditions of garlic powder compounds on laboratory and pilot scale, establishing that, on a limited pressure range (300 – 400 bar), the temperature augmentation determines the increase of the quantity of sulphur rich components [10,11]. On pilot scale, Simandi *et al*. determined that the step by step fractionation allows the selective recovery of sulphur compounds in a second separator, for extracts obtained at 65 °C and 450 bar [12].

2. Materials and Methods

Plants. Bulbs of the hybrid species of red onion *Allium cepa* var. "Rubiniu" were provided by the Research and Development Society for Vegetable Farming, Buzau, Romania. After peeling and chopping by means of a food processor, the samples were frozen at -70 °C (Angelantoni Industrie ultrafreezer) and lyophilized (Martin Christ Alpha 1-4 LD-Plus lyophilizer). The ramson leaves (*Allium ursinum* L.) were harvested from the natural range of forests from Bacau County during March – April 2011. All plants were intact, selected and dried at room temperature and dark.

Reagents and Materials. The vanillin and the silica gel chromatography plates were provided by Merck (Darmstadt, Germany). The toluene, methanol and glacial acetic acid were furnished by Lach-Ner s.r.o (Brno, Czech Republic). The ethanol was supplied by Tunic (Bucharest, Romania) and was used for the closed circuit washing of the extraction plant. The supercritical fluid extraction was carried out with carbon dioxide provided by Technic Gaz S.A. (Buzau, Romania) at a purity of 99.92%. The CO_2, used for supplying liquid solvent to the extraction plant, was delivered in syphon type gas cylinders.

Humidity measurement in raw material. The humidity of raw materials was measured according to the method recommended in AOAC [13].

Supercritical fluid extraction. A supercritical CO_2 extraction plant, designed and furnished by Natex Prozesstechnologie GembH (Ternitz, Austria), was employed to obtain extracts from dried ramson leaves and lyophilized red onion bulbs. The plant is provided with a co-solvent pump and an extractor with a total capacity of 3.5 litres which can function at a maximum pressure of 550 bar and a maximum temperature of 120 °C. The extractor is secured with a fast closing system on its upper side. The separation of the bioactive components can be carried out with the help of two separators: *S1* (1.5 litres capacity, 250 bar max. pressure, 120 °C max. temperature, fast closing system, internal serpentine and jacket) and *S2* (designed for 1.5 litres max. volume, 100 bar max. pressure, 120°C max. temperature and provided with jacket). The control of the extraction parameters (temperature, pressure and CO_2 mass flow) is automatic.

Thin layer chromatography for identification of sulphur compounds. The thin layer chromatography was correlated with the extraction method. The technique proposed by Wagner and Bladt was adapted for the present work and consequently the solid extracts obtained in the first separator were dissolved in methanol (0.05 g la 30 mL methanol) and spotted on a silica gel chromatography plate 60 F254, [14]. After spotting, the toluene – ethyl acetate developing system (10:3) was used, the chromatography plates being dried at 105 °C and immersed in the detecting system (1 g vanillin at 100 mL glacial acetic acid). The sulphur compounds were identified according to the spots colour and the retention factor (R_f) values.

3. Results and discussion

Measuring the humidity content of the solid material to be extracted. The availability of components in the raw material matrix, subjected to extraction with supercritical fluids, is influenced by the humidity of the solid material and by the type of solvent involved in the process. After lyophilisation (red onion bulbs) and drying (ramson leaves), the raw materials were kept in conditions of controlled temperature and humidity (18 – 20 °C) up to their use in the extraction process. The humidity was determined by maintaining the samples (app. 5 g) at 105 °C for 24 hours up to constant weight (Fig. 1).

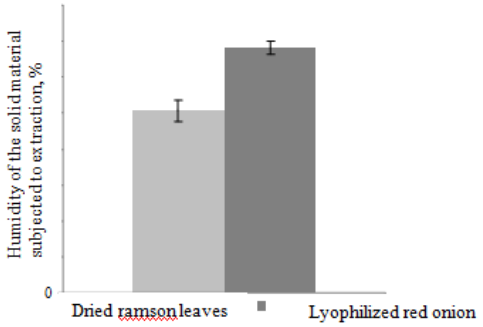

Fig. 1. The humidity of dried raw materials subjected to extraction (The values are averages of two successive determinations ± standard deviation)

In the extraction process, the water in the plant matrix acts in conjunction with the solute in order to interact with the solvent, which leads to the extraction yield decrease. It can be observed that the humidity is below 4%, a value that is close to the recommendations given by the technical literature [15]. The red onion bulbs presented 25.79% more humidity than the dried ramson leaves. Usually, the matrix subjected to extraction must contain up to 14% water. It is considered that the presence of water has the role to facilitate the interaction between solvent and solute and also to facilitate the action of the solvent. However, the presence of a high water quantity can lead to the following negative effects: higher probability of blockage occurrence in separator due to the Joule-Thomson effect, ionisation and hydrolysis of the extracted compounds, shorter life period of extract and foam accumulation as a result of saponins co-extraction [16].

Chromatographic characterization of biologic active sulphur compounds from extracts obtained through unconventional methods. The thin layer chromatography was carried out following the method described by Wagner and Bladt [14]. After spotting the extracts on a silica gel chromatography plate 60 F254 (Merck, Darmstadt, Germany) that was immersed in toluene : acetate 10 : 3 solvent system (Fig. 2) the compounds were qualitatively evaluated as follows: thiosulfinates (yellow – dark yellow spots with R_f from 0.201 to 0.486) and diallyl thiosulfinates (with R_f = 0.458 for the ramson leaves extract). Both extracts displayed 1 – 2 dark yellow spots for R_f = 0.66 – 0.76. The dipropyl thiosulfinate, highlighted through light brown spot (found probably in low concentration – dash line) with R_f = 0.486, is characteristic for onion extracts.

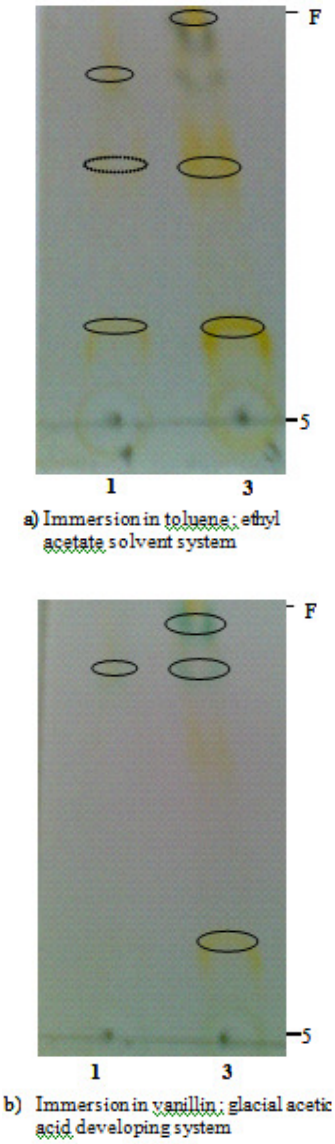

Fig. 2. The thin layer chromatograms of extracts obtained with supercritical carbon dioxide
(1 – Allium cepa var. "Rubiniu"3 – Allium ursinum L.)

After the treatment with developing solution (1 g vanillin at 100 mL glacial acetic acid) less concentrated yellow zones were highlighted for the ramson leaves extract, which are characteristic for thiosulfinates with R_f = 0.208. Blue – grey zones with R_f = 0.76 – 0.82 that are characteristic for sulphides (Fig. 2b) were also highlighted. For the red onion bulb extract a faded grey spot with R_f = 0.67 was identified.

4. Conclusion

Researches regarding the supercritical fluids extraction were carried out on a continuously operating pilot plant. The humidity content of the solid material subjected to extraction was determined, registering for the red onion bulbs a value of 3.409%, 25.79% higher than the humidity of ramson dried leaves.The thin layer chromatography enabled the qualitative identification of sulphur compounds: thiosulfinates (with R_f between 0.201 and 0.486), diallyl thiosulfinate (with R_f = 0.458 for *Allium ursinum* L. extracts) and dipropyl thiosulfinate (with R_f = 0.486 for *Allium cepa* var. "Rubiniu", highlighted through a light brown spot which shows that it is found probably in low concentration).

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Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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