Optimization of Supercritical Carbon Dioxide Extraction of Rosmarinic Acid from Clary Sage

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1. Introduction

Clary sage (*Salvia sclarea* L.) is one of the most popular species of the genus Salvia. It is well-known to be natural source rich in antioxidants which gives it many medicinal properties. Rosmarinic acid (RA), one of the most abundant phenolic compounds, is common to many Salvia species. It is known for its antioxidant properties which make it able to neutralize free radicals in the human body, thus preventing cells from harmful oxidizing action. For this reason, preparations with RA are extensively utilized in food supplements, pharmaceutical and cosmetic industries [1].

Conventional extraction methods of phenolic compounds, including maceration, percolation and reflux extraction, usually use organic solvents in large volumes and long extraction time. Carbon dioxide as an extraction solvent has gained increasing attention because of the advantages of being non-toxic, non-flammable, available, cost-effective and easily removed from the extracted materials[2].

Although the extraction with supercritical carbon dioxide (SC-CO_2) is considered as a promising alternative for sustainable and safe extraction, the polar nature of many bioactive molecules of interest such as phenolic compounds and anthocyanins requires the addition of a co-solvent to increase the affinity and solubility of these compounds. Water and ethanol have been widely used as co-solvents due to their low cost, besides being green solvents, with the possibility of direct use in foods and pharmaceuticals. Furthermore, the use of water-ethanol mixtures as co-solvents has been shown to be more effective in the extraction of phenolic compounds, resulting in higher yields [3].

To the best of our knowledge, this is the first study dealing with the SC-CO_2 extraction of RA from clary sage. In this work, SC-CO_2 extraction of RA, one of the main bioactive constituents of clary sage was investigated by adding a water/ethanol mixture as a co-solvent. The extraction was performed on the sage residue (plant material) obtained after distillation. The extraction conditions namely, temperature, pressure and co-solvent composition, were determined to optimize the extraction yield of RA, using the response surface methodology.

2. Materials and Methods

Extractions were carried out on the aerial parts of clary sage (*Salvia sclarea* L.) obtained after distillation, using supercritical fluid extraction system (SFE Process, Nancy, France) equipped with 250 mL to 1 L extraction vessels and three 0.2 L separators in series. 25 g of clary sage was placed into a 500 mL sample cartridge. The separator temperature was set at 60 °C. The SC-CO_2 have a downward flow in the extraction vessel and the flow rate was kept constant at 60 g/min during the experiments. The pressure and temperature ranged between 100 and 600 bar, 40 and 100 °C respectively. RA is a polar compound and its extraction with only is not efficient enough due to the non-polar structure of CO_2. Thus, extraction was studied in the presence of the mixture of two polar modifiers, ethanol (polarity = 5.2) and water (polarity = 9), added as co-solvents to the supercritical fluid at 10% (m co-solvent/ m CO_2). Ethanol was selected because it is a food grade modifier and also on the basis of its good performances for extraction[4]. The percentage of ethanol in water (v/v), ranging from 0 to 100%, represents the factor co-solvent composition.

The response surface methodology, through a Box-Behnken experimental design, was employed to evaluate the effect of 3 independent variables (pressure, temperature, co-solvent composition) and to search for the optimal operating conditions to maximize the response (RA yield). The Box-Behnken design was generated using the “MODDE” software (Version 12.0.1, Sartorius, Sweden).

3. Results and discussion

Significant variations of RA yields were found among the experiments of the Box-Behnken experimental design. It ranges from 0 to 7.57 mg/g $_{\text{Dry Matter}}$. A second-order polynomial model was used to describe the response and to investigate the effects of the factors and their interaction. All the terms of the model are significant ($p<0.05$) and this was validated with a determination coefficient of 0.942. A 4D contour plot of the response is shown in Figure 1.

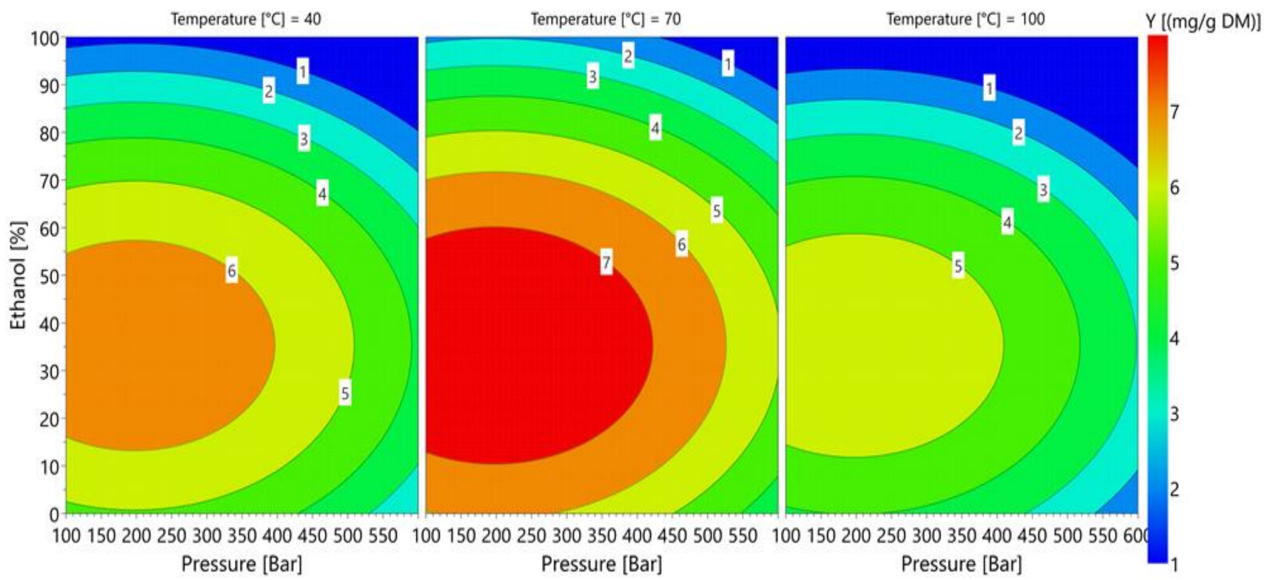

Figure 1. Response 4D Contour Plot showing the combined effects of temperature, pressure and ethanol percentage in co-solvent on the yield (Y) of extraction of RA.

The 4D contour plot presented in figure 1 shows an increase in the RA yield in the extract at mild conditions of temperature and pressure. In fact, high pressure (> 420 bar) and temperature (> 85 °C) conditions are not appropriate to extract RA. As reported by Angelov et al., (2011) a higher pressure does not affect significantly the solvent capacity to extract RA from lemon balm. As a consequence, considering the energy consumption, the author's suggestion to operate at moderate pressure (120–200 bar) seems relevant[4].
The lowest yields of RA were found at high ethanol percentages (> 55%). Thus, using the co-solvent at 100% of ethanol is not favorable for the extraction of RA. Based on the optimization study, the best operating pressure, temperature and co-solvent composition are 196 bar, 65 °C and 35% ethanol, respectively, with a predicted RA yield of 7.9 mg/g $_{\text{DM}}$.

4. Conclusions

The second-order polynomial model was found to fit the experimental data of rosmarinic acid extraction using SC-CO_2. The optimum parameters were found to be 196 bar, 65 °C and 35% ethanol with 7.9 mg/g DM of rosmarinic acid extracted. The presence of water and ethanol is essential to obtain a phenolic enriched extract. The findings have shown that supercritical fluid extraction can be considered as an efficient and cleaner method for the extraction of phenolic compounds compared to other non-conventional techniques.

References

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