Journal of Food Engineering 117 (2013) 443–449

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Supercritical fluid extraction of forskolin from *Coleus forskohlii* roots

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ARTICLE INFO

Article history:
Available online 12 December 2012

Keywords:
Coleus forskohlii
Forskolin
Supercritical extraction
Solubility parameter
Entrainer
Pre-treatment

ABSTRACT

Forskolin (FSK), a labdane diterpene compound having high nutraceutical and therapeutic activity has been extracted from dried *Coleus forskohlii* roots using supercritical carbon dioxide (SC-CO_2). The solubility parameter of FSK, CO_2, and entrainer solvents was calculated and validated with experimental results. Theoretically, pressure and temperature had significant effect on extraction of FSK. A maximum of 50.32% recovery of FSK was obtained after SC-CO_2 extraction at 40 °C, 250 bar and extraction time of 60 min. Use of methanol as an entrainer at 20% v/w of dried *C. forskohlii* roots under optimized conditions improved the recovery of FSK to 74.29%. The recovery of FSK further increased marginally to 77.08% when pre-treated with ultrasonication and commercial enzyme preparation of Stargen® 002 and Accellerase® 1500.

1. Introduction

Coleus forskohlii Briq, a herbal plant belonging to the Lamiaceae family, is indigenous to India and is recorded in Ayurvedic *Materia Medica* under the Sanskrit name Makandi and Mayini (Shah, 1996). This plant grows in tropical and subtropical regions of India, Pakistan, Sri Lanka, East Africa and Brazil at 600–1800 m elevation. In India, the crop is cultivated in Gujarat, Maharashtra, Rajasthan, Karnataka and Tamil Nadu in an area of more than 2500 ha for its tuberous roots. With the present annual production of about 100 tons from 700 ha in India, cultivation of *C. forskohlii* is picking up due to its economic potential (Singh et al., 2011). Traditionally, the roots have been used for preparation of pickles and also for medicinal purposes by the ayurvedic school of medicines. This plant has been used for treating abdominal disorders, heart diseases, respiratory disorders, insomnia, epilepsy and angina (Ammon and Muller, 1985). The juice from the roots of the plant is given to children suffering from constipation (Singh et al., 1980).

Nutraceuticals are components of traditional and exotic foods that have the potential to augment human health. In present times, nutraceuticals are appealing to nutritionists and health care professionals because of the growing body of knowledge supporting the claimed health benefits. Plants and their extracts contain bioactive compounds such as lipids, phytochemicals, flavors, fragrances and pigments. These plant extracts are used in food, cosmetic and pharmaceutical industries. Different extraction techniques have been investigated to obtain such valuable natural compounds from plants for commercialization. The roots of the *C. forskohlii* plant are a unique source of forskolin (FSK), a labdane diterpene compound. FSK, a nutraceutical compound, is used as a dietary supplement for reduction of body weight by promoting lean body mass (Badmaev et al., 2002). FSK also increases cyclic adenosine monophosphate (cAMP) via activation of adenylate cyclase (Metzger and Lindner, 1981). FSK is useful in the treatment of asthma, glaucoma, cardiovascular diseases and certain types of cancer (Suryanayanan and Pai, 1998). Its further use in treating mood disorders and its anticancer activities (Agarwal and Parks, 1983) is well known. Although total synthesis of FSK has been reported, it is not commercially viable (Corey et al., 1988). Conventionally, FSK has been isolated from the roots of *C. forskohlii* using Soxhlet apparatus with organic solvents such as hexane, alcohol, chloroform, and acetone (Srivastava et al., 2002). This method requires longer time and also large quantities of solvents which can also cause additional environmental problems (Luque de Castro and Garcia-Ayuso, 1998). Hydrotropic (Mishra and Gaikar, 2009) and microwave assisted extraction (Devendra and Gaikar, 2010) are the other methods reported for extraction of FSK, of which the former results in impure FSK.

The wide scale use of organic solvents by industries represents a serious threat to the environment. Consequently, there is an interest to adopt new sustainable and eco-friendly extraction techniques with reduced consumption of organic solvent and reduced extraction time. One such alternative to the use of organic solvents is the application of supercritical fluid extraction (SFE) protocol (Edward et al., 2009). The extraction of bio-actives using SFE technique covers principles of green technology as it is solvent-free and safe (Wang and Weller, 2006; Sajilata et al., 2008). The low supercritical temperature of CO_2 makes it possible for the extraction of thermolabile compounds (Wang and Weller, 2006). The higher

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http://dx.doi.org/10.1016/j.jfoodeng.2012.12.012

Nomenclature

ΔE_v or ΔE_{vap}	summation of all cohesive energy (cal/mol)	δ_1	solubility parameter of solute at temperature T_1 (cal/cm^3)$^{1/2}$
P_c	critical pressure (bar)	δ_2	solubility parameter of solute at temperature T_2 (cal/cm^3)$^{1/2}$
T_c	critical temperature (°C)	ΔH_{vap}	heat of vaporization of solvent
V	molar volume	R	gas constant = 1.987 cal/mole K, 8.314 J/mole K, 0.08205 L atm/K mole
Δv	summation of all molar volume (cm^3/mol)		
$\rho_{r.SF}$	reduced density of the supercritical fluid (g/cm^3)		
$\rho_{r.L}$	reduced density of liquid state (g/cm^3)		

diffusivity of a supercritical fluid is higher than other liquids permits rapid mass transfer, resulting in an enhanced extraction rate than that obtained by conventional solvent extractions (Roy et al., 1996).

Till date, review of the literature show no reports on the appropriate conditions of SFE for the extraction of FSK from *C. forskohlii* roots, although it is believed to be commercially practiced. Supercritical carbon dioxide (SC-CO_2) extraction process has been used to extract FSK by SAT group, New Delhi (www.co2extract.com). Majeed and Prakash (2003) reported the use of SC-CO_2 extract from *C. forskohlii* roots in their experiments on evaluating antimicrobial activity of the essential oil. The present work reports on determination of solubility parameters for FSK, SC-CO_2/entrainer and developing an efficient protocol for SFE for FSK from *C. forskohlii* root powder.

2. Materials and methods

2.1. Materials

C. forskohlii roots were procured from Salem, Tamilnadu, India. Dried roots were ground in a mill fitted with 18 mesh to a particle size <1 mm and stored in an air tight container for further studies. Standard FSK was a gift sample from Medicinal and Natural Product Research Laboratory, Institute of Chemical Technology, Mumbai, India. All the chemicals and solvents used in the present study were of AR grade and purchased from S.D. Fine Chemicals Limited, Mumbai, India. Enzymes (Stargen® 002 and Accellerase® 1500) were gifts from Genencor International, Mumbai, India. CO_2 cylinders were supplied by Bombay Carbon Dioxide Gas Company, Mumbai, India.

2.2. Estimation of solubility parameters for FSK, CO_2 and entrainer

Estimation of solubility parameter is one of the ways to obtain information on solubility of a solute in supercritical fluid (Kagliwal et al., 2011a; Kim et al., 2008). The solubility parameter estimation provides a semi-quantitative evaluation of experimental conditions to be selected for optimized extraction conditions. The solubility parameter, δ, of a supercritical fluid can be estimated by using the following equation (Castro et al., 1994)

$$\delta(\mathrm{cal/cm^3})^{1/2} = 1.25\sqrt{P_c}\frac{\rho_{r.SF}}{\rho_{r.L}} = 0.47\rho_{r.SF}\sqrt{P_c} \quad (1)$$

where, ρr.SF is the reduced density (g/cm3) of the supercritical fluid, ρr.L is the reduced density of liquid state and P_c is the critical pressure (bar). This equation reflects the variation of the solvent power of the supercritical fluid as a function of density.

The solubility parameter (δ) of a given solute can be estimated by using the Fedors group contribution method (Fedors, 1974) if the molecular structure of the solute is known. Table 1 lists group contribution of FSK and eluicidates the procedure to estimate its solubility parameter by using the Fedors method. The Hildebrand solubility parameter is defined as the square root of the cohesive energy density and calculated by using the following equation (Hansen, 2000)

$$\delta(\mathrm{cal/cm^3})^{1/2} = \sqrt{\frac{\sum_i (\Delta E_v)_i}{\sum_i (\Delta v)_i}} \quad (2)$$

where, $\sum(\Delta Ev)i$ is the summation of cohesive energies (cal/mol) and $\sum(\Delta v)i$ is the summation of molar volumes (cm^3/mol).

Using the above equation, δ_{FSK} for FSK at 25 °C was found to be 12.81 (cal/cm^3)^{½} or 26.215 MPa^{½}. The critical temperature of FSK was calculated using the equation: $T_c = 535\ \log\sum\Delta T_i$, where ΔT_i is the summation of the critical temperatures of the contributing groups.

The Fedors method is known to be useful for estimating the extraction potential of complex molecules using supercritical fluids (Sajilata et al., 2010). The solubility of solute in SC-CO_2 as a function of temperature can be described as follows (Galia et al., 2002)

$$\delta(\mathrm{cal/cm^3})^{1/2} = \delta_1\left(\frac{V_1}{V_2}\right)^{1.13} = \delta_1\left(\frac{\rho_2}{\rho_1}\right)^{1.13} = \delta_1\left(\frac{T_c - T_2}{T_c - T_1}\right)^{0.33} \quad (3)$$

where, T_c is the critical temperature estimated by a group contribution method and δ_2 and δ_1 are the solubility parameter of the solute at temperature T_2 and T_1 (Reid and Sherwood, 1966). Hildebrand (Hildebrand, 1936) proposed the square root of the cohesive energy density as a numerical value indicating the solvency behavior of a specific solvent. The solubility parameter of entrainer solvent, δ, at different temperatures and pressures was calculated using Eq. (4):

$$\delta(\mathrm{cal/cm^3})^{1/2} = \left(\frac{\Delta E_{vap}}{V}\right)^{1/2} = \left(\frac{\Delta H_{vap} - RT}{V}\right)^{1/2} \quad (4)$$

Table 1
Solubility parameter of forskolin using Fedors group contribution.

Group	No.	$\sum\Delta e_i$	$\sum\Delta v_i$	$\sum\Delta_{Ti}$
–CH$_3$	6	6750	201	10.74
–CH$_2$–	3	3540	48.3	4.02
>CH–	4	3280	−4	1.8
>C<	5	1750	−96	−1.1
=CH2	1	1030	28.5	1.59
=CH–	1	1030	13.5	1.4
–CO–O–	1	4300	18	5.32
–O– (aromatic)	1	1650	3.8	1.56
–CO–	1	4150	10.8	5.36
–OH (aromatic)	3	21360	30	28.95
C$_6$ ring	3	750	48	8.04
Heteroatom in ring	1	–	–	0.45
Subs. On C in double bond (non aromatic)	1	–	–	0.58
Total ($\sum$)	–	49590	301.9	68.71