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Optimization of supercritical CO_2 extraction of oleoresin from black pepper (*Piper nigrum* L.) and antioxidant capacity of the oleoresin

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Abstract

Supercritical fluid extraction (SCFE) of oleoresin from ground black pepper (*Piper nigrum* L.) with carbon dioxide was studied. The aim of this study was to investigate the effect of different operating factors on the extraction yield and the piperine content in the extracts as well as to evaluate the antioxidant capacity of the extract. A Response Surface Methodology (RSM) with a 3-factor Central Composite Orthogonal Design (CCOD) was used to determine the effects of pressure (200-300 bar), temperature (35-55°C), and extraction duration (90-150 min) on the extraction yield. The results indicated that a quadratic model adequately represented the experimental data. The optimum parameters for the extraction of oleoresin obtained by RSM included 266 bar pressure, 47°C temperature and 150 min time, with the predicted extraction yield of 5.33%. The piperine content in the extracted oleoresin varied from 25.74% to 48.32%. Meanwhile, the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging capacity of the extract was a bit lower than that of ascorbic acid, showing 86.6% of inhibition at a concentration of 80 mg/L.

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Introduction

Supercritical fluid extraction (SCFE) technology has been employed for extraction of variety of active compounds from plant materials including spices and herbs for decades (Díaz-Reinoso *et al*., 2006). CO_2, as a solvent, offers a range of advantages over conventional organic solvents for being non-toxic, inert, non-flammable, non-explosive and inexpensive. It is also odorless, colorless, generally accepted as GRAS solvent for pharmaceuticals and food, leaving no solvent residue in the product (Rizvi et al., 1986; Sankar, 1989). With SCFE, better quality products can be achieved. The recovery of high-value and low-volume end products from dilute process streams is typical for many food, pharmaceutical, and biotechnological processes (Pereira and Meireles, 2010).

Black pepper (*Piper nigrum*) is not only an important spice but also a natural medicinal agent used to treat many diseases such as arthritis, nausea, fever, migraine headaches, poor digestion, strep throat and even coma (Zachariah and Parthasarathy, 2008). The therapeutic properties of black pepper includes analgesic, antiseptic, antispasmodic, antitoxic, aphrodisiac, diaphoretic, digestive, diuretic, febrifuge, laxative, rubefacient and tonic (especially of the spleen) (Srinivasan, 2007). Piperine, a major component in black pepper oleoresin, helps to increase aborption and bioavailability of other bioactive compounds (Duangjai *et al*., 2012). The antioxidative action of black pepper has been attributed to pirerine and its isomers (Tipsrisukond *et al*., 1998).

Oleoresin from black pepper has been traditionally produced with organic solvent extraction (Borges and Pino, 1993). It bears both flavor and pungent compounds of pepper fruits and has many applications in food, cosmetics and pharmacy. Attempts were made to extract oleoresin (Sovová *et al*., 1995; Perakis *et al*., 2005) or essential oil (Ferreira *et al*., 1999; Ferreira and Meireles, 2002) from black pepper with SCFE. By using SCFE, fractionation of oleoresin can be made and the fraction with high content in pirerine can be achieved (Nguyen *et al*., 1998).

So far, efforts have been done for development of mathematical models which describe thermodynamic constraints (solubility and selectivity) and kinetic constraints (mass transfer rate) in SCFE of black pepper essential oil or oleoresin (Sovová *et al*., 1995; Ferreira and Meireles, 2002; Perakis *et al*., 2005; Izadifar and Abdolahi, 2006). However, no work has been conducted for process optimization using a Response Surface Methodology (RSM) for black pepper oleoresin extraction.

RSM is an approach to use a matrix of designed experiments to describe an effect of process variables

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on responsive variables. A statistical model is constructed and utilized to predict the level of the process variables for an optimal response. Instead of using natural values, the coded variables are used for construction of experimental design (Myers and Anderson-Cook, 2009). The Central Composite Design is an experimental design for constructing a second order (quadratic) model for the response variable without needing to perform a complete three-level factorial experiment. It can be an effective method for optimizing the conditions of supercritical CO_2 extraction (Jiao *et al*., 2008; Mariod *et al.*, 2010). The aim of this study was to apply SCFE for production of oleoresin from black pepper. The effect of technological parameters for high yield of oleoresin was studied by using a Central Composite Orthogonal Design (CCOD). Moreover, free radical scavenging activity of the oleoresin and the piperine content in the oleoresin samples were examined.

Materials and Methods

Materials

Black peppercorns were collected from a local market in Binh Phuoc Province, Vietnam, packaged in double PE zipper bags and kept in cold conditions. The black pepper samples were tested for moisture content (ISO 939:1980), volatile content (ISO 6571:1984), total ash (ISO 928:1997), crude fiber (ISO 5498: 1981) and bulk density (ISO 959-1,2:1989) (ISO, 1989). The samples were ground to achieve an average particle size of about 1.0 mm.

Experimental apparatus and procedure

The experiments were carried out on the THAR SFE-100 apparatus (Waters, USA), with an extraction vessel of 100 mL and a collection vessel of 500 mL. Samples of 30 gram of ground peppercorns were placed in the extractor, covered by two layers of glass bead and filter paper at the two end of the vessel. Operating variables such as pressure, temperature and extraction duration were controlled automatically by computer, while the flow rate of CO_2 was fixed at 10 g/min.

Experimental design

The CCOD for three variables, namely pressure P, temperature T and extraction duration t, includes eight factorial points, six star points and one center point. The experiment at star and center points was duplicated, making the total number of 22 runs. In RSM, the natural variables were transformed into coded variables, and the upper and lower level was coded as +1 and -1, respectively, while the basic level to zero. The coded values at star point were ± 1.147 (Myers and Anderson-Cook, 2009). Table 1 shows the real and coded values for three independent variables. Regression analysis was performed on the experimental data for determination of coefficients of the second order polynomial model:

$$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Where Y is the response variable (extraction yield), and b_0, b_i, b_{ii}, and b_{ij} are regression coefficients of variables for intercept, linear, quadratic, and interaction terms, respectively and X_1, X_2 and X_3 are independent variables.

Free radical scavenging activity

Antioxidant activity of the extracted oleoresin (the sample with highest yield) was investigated based on radical scavenging capacity, against stable DPPH (1,1-diphenyl-2-picrylhydrazyl hydrate, Sigma-Aldrich, Germany). The procedure was reported elsewhere as follows (Khalaf *et al.*, 2008). A stock solution of the test extract was dissolved in methanol at 5 mg/mL concentration. By using suitable dilution, the working solutions were exactly generated with the concentration 20, 40, 60 and 80 mg/L. Ascorbic acid was used as positive reference and was prepared in the same manner as for the oleoresin. DPPH was prepared in methanol at 20 mg/L and 3 mL of this solution was mixed with 3 mL of the sample solution or reference solution, separately. The blank samples were made by adding 1mL of methanol to 1 mL of 20 mg/L DPPH. The solution mixtures were left stand at dark for 30 minutes. Absorbance was measured at 517 nm and the % inhibition was calculated.

$$\text{Percent (\%) inhibition of DPPH activity} = [(A - B)/A] \times 100$$

where A = optical density of the blank and B = optical density of the sample.

Piperine analysis

Piperine content in the oleoresin extracts was determined by a spectrophotometric method (AOAC, 1987), measuring absorbance at 345 nm and using standard piperine (97%) from Sigma-Alrich.

Statistical analysis

ANOVA was performed using SPSS software version 16.0, for comparing mean values, at $p < 0.05$ and with LSD test. The statistical model for optimization of the extraction parameters was determined by using Design-Expert software version

7.0.3.

Results and Discussion

Composition of the black pepper

The black pepper samples had a moisture content of 11.67% ± 0.07 and a bulk density of about 550 kg/m3. In general, moisture content of plant materials used for supercritical fluid extraction should be less than 14% w/w (Pereira and Meireles, 2010). The volatile oil, total ash and crude fiber content the in black pepper samples were 2.6 ± 0.1%, 3.8 ± 0.3% and 11.3 ± 0.1%, respectively.

Extraction yield and optimization

The extraction yield was defined as a ratio of the weight of extracted oleoresin to the sample weight (in %). The yield of black pepper oleoresin, obtained at different extraction parameters, varied from 2.33 to 5.67 (%). By using multiple regression analysis of the experimental data (Table 1), the relationship between independent variables and the dependent variable was determined. The insignificant terms in the regression equation were excluded and the statistical model is shown as:

$$Y = 4.25 + 0.35X_1 + 0.35X_3 + 0.40X_1X_2 - 0.66X_1^2 - 1.05X_2^2 + 0.42X_3^2$$

Where: Y- the yield of black pepper oleoresin; X_1- pressure; X_2- temperature; X_3- extraction duration (coded).

The experimental and predicted yield under different extraction conditions are shown in Table 1. The model was significant (p = 0.0013) and adequate as the coefficient of determination was satisfactorily high (R^2 = 0.84 and adjusted R^2 = 0.72). The high coefficient of determination R^2 and the high adjusted R^2 are good indicators of the data validity (Mariod *et al*., 2010).

The effect of P, T and t on the extraction yield of black pepper oleoresin was investigated at pressures of 200-300 bar, temperatures of 35-55°C and extraction duration in 90-150 min. The highest extraction yield (5.67%) was acquired at 250 bar, 45°C and 150 min while the lowest yield was 2.33%, achieved at 206 bar, 54°C and 94 min. The results demonstrated that the effect of pressure and temperature on the extraction yield was significant (Mariod *et al*., 2010).

The response surface was constructed in order to predict the relationship between independent variables and the dependent variable and to determine the optimal levels of the independent variables for the extraction yield (Figure 1). Figure 1a shows the effect of pressure and temperature on the extraction yield of black pepper oleoresin. At a certain value of temperature, pressure shows quadratic effect on the extraction yield. At first, when pressure is increased, the CO_2 density is increased, too, hence the solvent power (Tipsrisukond *et al*., 1998). However, at higher pressures (closed to 300 bar), the extraction yield declined with the rise of pressure, probably due to reduction of solute diffusivity in dense pack of solvent molecules at higher pressures (Rizvi *et al*., 1986; Mariod *et al*., 2010). A same pattern is observed for the effect of temperature, in a range of 35-55°C. Increase in temperature could stimulate increase in solute diffusivity, however, at higher temperatures, the solvent density is decreased significantly causing the reduction in solvent power (Jiao *et al*., 2008). The effect of extraction pressure and extraction duration on the oleoresin yield is illustrated in Figure 1b and the effect of temperature and extraction duration in Figure 1c. At a certain value of pressure and temperature, the dependence of the extraction yield on the extraction duration appears linear.

Izadifar and co-worker found that a neural network model is a good tool for predicting effect of

Table 1. Experimental design with actual and predicted yield of extraction

Run	Parameters: Actual (Coded)			Extraction yield (%)	
	Pressure (bar)	Temperature (°C)	Time (min)	Actual	Predicted
1	294 (+1)	54 (+1)	146(+1)	4.16	4.09
2	206 (-1)	54 (+1)	146(+1)	2.46	2.59
3	294 (+1)	36 (-1)	146(+1)	2.43	3.22
4	206 (-1)	36 (-1)	146(+1)	3.13	3.31
5	294 (+1)	54 (+1)	94(-1)	3.06	3.40
6	206 (-1)	54 (+1)	94(-1)	2.33	1.89
7	294 (+1)	36 (-1)	94(-1)	3.23	2.54
8	206 (-1)	36 (-1)	94(-1)	3.27	2.62
9	200(-1.147)	45 (0)	120(0)	2.64 ± 0.24	3.15
10	300(+1.147)	45 (0)	120(0)	3.96 ± 0.11	3.77
11	250 (0)	35(-1.147)	120(0)	2.68 ± 0.15	3.86
12	250 (0)	55(+1.147)	120(0)	2.89 ± 0.27	3.39
13	250 (0)	45 (0)	90(-1.147)	3.79 ± 0.08	3.72
14	250 (0)	45 (0)	150(+1.147)	5.67 ± 0.09	4.65
15	250 (0)	45 (0)	120(0)	4.51 ± 0.22	4.25

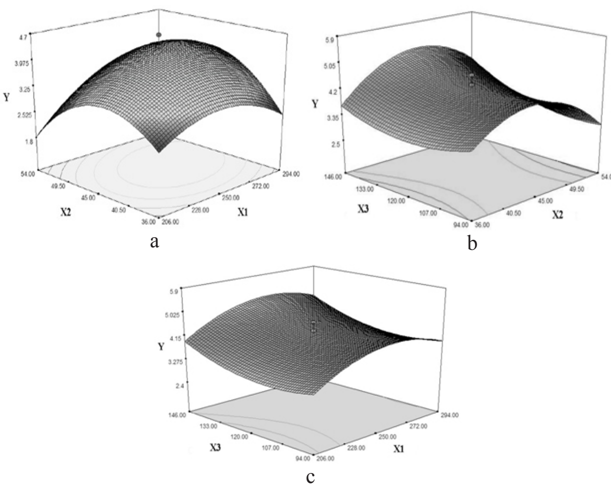


Figure 1. Response surface for Extraction Yield (Y) as three-dimensional plot against:
(a) X_1=Pressure and X_2=Temperature, X_3=0
(b) X_2=Temperature and X_3= Extraction Duration, X_1=0
(c) X_1=Pressure and X_3= Extraction Duration, X_2=0

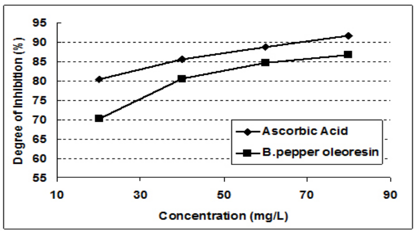


Figure 2. DPPH scavenging activity of black pepper oleoresin and ascorbic acid

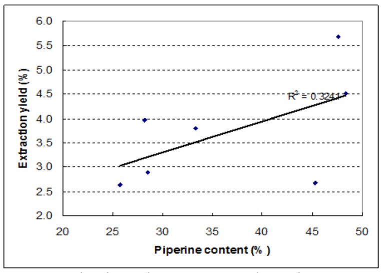


Figure 3. Correlation between piperine content and extraction yield

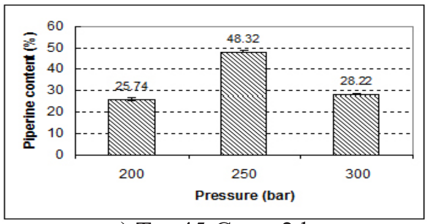
different parameters such as pressure, temperature, residence time, solvent flow rate, etc. on the extraction yield of black pepper essential oil. However, this approach demands a large pool of experimental data involving a wide range of all variables (Izadifar and Abdolahi, 2006). The optimum extraction conditions for maximum response of extraction yield, obtained from the model within the experimental ranges were determined by using the Software Design Expert 7.0.3. The extraction yield reaches maximum value of 5.33% at pressure 266 bar, temperature 47°C and in 150 min of extraction. Other authors obtained the yield of 6.47%w/w (extracted/feed) of black pepper oleoresin by using SCFE at 280 bar, however, the temperature tested was 60°C (Tipsrisukond *et al.*, 1998).

Antioxidant activity

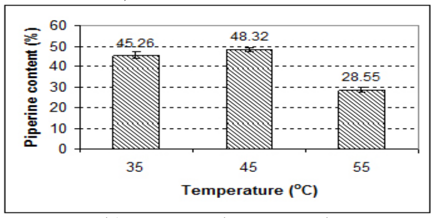
The antioxidant capacity of the black pepper oleoresin sample extracted at the condition of 250 bar, 45°C and 2.5 h was evaluated with the DPPH radical-scavenging assay, in comparison with ascorbic acid as the reference. The DPPH radical-scavenging activity, expressed as % of inhibition of the radical, is shown in Figue 2. The antioxidant activity of the sample was high, nearly 80-90% in comparison with ascorbic acid. At the concentration of 80 mg/L, degree of inhibition of pepper oleoresin reached to 87%, while that of ascorbic acid was 92%.

Piperine content in oleoresin

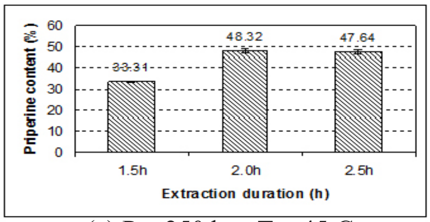
The piperine content in black pepper oleoresin extracted at different pressures, temperatures and durations was in a range from 25.74 to 48.32%, however, it shows no correlation with the extraction



a) T = 45°C, t = 2 h



b) P = 250 bar, t = 2 h



(c) P = 250 bar, T = 45°C

Figure 4. Piperine content in oleoresin at different extraction conditions

yield with R^2 = 0.32 only (Figure 3). The highest piperine value of 48.32% was attained at 250 bar, 45°C and 120 min. At these conditions, the extraction yield was quite high, too (4.5%), however, at conditions of 250 bar, 35°C and 120 min, the piperine content was 45.3% but the yield only 2.68%. At a fixed level of temperature and extraction duration, dependence of the piperine content on pressure appears quadratic (Figure 4a). The same pattern is observed for the dependence of piperine content on temperature at fixed pressure and extraction duration (Figure 4b). As observed in Figure 4c, piperine was extracted fully just after 2 h of extraction.

The piperine content in black pepper oleoresin extracted by maceration and Soxhlet method was in a range of 25 to 33.3 % with the oleoresin yield from 10 to 14 wt% (Borges and Pino, 1993). Tipsrisukond and co-workers extracted black pepper oleoresin by S-CO_2 extraction with the yield from 4.6 to 6.47 wt%, and the piperine content in the oleoresin was from 32.3 to 39.4% (Tipsrisukond *et al.*, 1998). In general, supercritical extraction of black pepper could obtain oleoresin with high content in piperine, and with partial fractionation, it could reach 60% (Nguyen *et al.*, 1998).

Conclusion

Operation parameters, namely pressure, temperature and extraction duration strongly affect the yield of extraction of black pepper oleoresin in the supercritical CO_2 process. The extraction yields varied from 2.33 to 5.67 w% (extract/feed). By using RSM with a CCOD, the optimal conditions for extraction included 266 bar pressure, 47^{o}C temperature and 150 min time, giving the yield of 5.33%. The piperine content of the extracted products was in a range from 25.74 to 48.32%. By manipulating operation parameters, one could obtain a product with high piperine content, up to 50%. Black pepper oleoresin shows high antioxidant capacity. It's DPPH radical scavenging capacity is comparable to that of ascorbic acid, though a bit lower.

References

Association of Official Analytical Chemists (AOAC). 1987. Piperine in Pepper Preparations, Spectrophotometric Method, AOAC 987.07. JAOAC 70, 112.

Borges, P. and Pino, J. 1993. Preparation of black pepper oleoresin by alcohol extraction. Food/Nahrung 37(2): 127-130.

Díaz-Reinoso, B., Moure, A., Domínguez, H. and Parajó, J. C. 2006. Supercritical CO_2 extraction and purification of compounds with antioxidant activity. Journal of Agricultural and Food Chemistry 54(7): 2441-2469.

Duangjai, A., Ingkaninan, K., Praputbut, S. and Limpeanchob, N. 2012. Black pepper and piperine reduce cholesterol uptake and enhance translocation of cholesterol transporter proteins. Journal of Natural Medicines 67(2): 303-310.

Ferreira, S. R. and Meireles, M. A. A. 2002. Modeling the supercritical fluid extraction of black pepper (*Piper nigrum* L.) essential oil. Journal of Food Engineering 54(4): 263-269.

Ferreira, S. R., Nikolov, Z. L., Doraiswamy, L. K., Meireles, M. A. A. and Petenate, A. J. 1999. Supercritical fluid extraction of black pepper (*Piper nigrun* L.) essential oil. The Journal of Supercritical Fluids 14(3): 235-245.

International Standardization Organization (ISO). 1989. ISO 939-1980; ISO 6571-1984; ISO 928-1997; ISO 5498: 1981; ISO 959 (1,2). International Standardization Organization, Geneva.

Izadifar, M. and Abdolahi, F. 2006. Comparison between neural network and mathematical modeling of supercritical CO_2 extraction of black pepper essential oil. The Journal of Supercritical Fluids 38(1): 37-43.

Jiao, S. S., Li, D., Huang, Z. G., Zhang, Z. S., Bhandari, B., Chen, X. D. and Mao, Z. H. 2008. Optimization of supercritical carbon dioxide extraction of flaxseed oil using response surface methodology. International Journal of Food Engineering 4(4).

Khalaf, N. A., Shakya, A. K., Al-Othman, A., El-Agbar, Z. and Farah, H. 2008. Antioxidant activity of some common plants. Turkish Journal of Biology 32(1): 51-55.

Mariod, A. A., Abdelwahab, S. I., Gedi, M. A. and Solati, Z. 2010. Supercritical carbon dioxide extraction of sorghum bug (*Agonoscelis pubescens*) oil using response surface methodology. Journal of the American Oil Chemists' Society 87(8): 849-856.

Myers, R. H. and Anderson-Cook, C. M. 2009. Response surface methodology: process and product optimization using designed experiments (Vol. 705). John Wiley and Sons.

Nguyen, U., Anstee, M. and Evans, D.A. 1998. Extraction and fractionation of spices using supercritical fluid carbon dioxide. Proceedings of the 5th meetings on Supercritical Fluids. Nice, France, p. 523-528.

Perakis, C., Louli, V. and Magoulas, K. 2005. Supercritical fluid extraction of black pepper oil. Journal of Food Engineering 71(4): 386-393.

Pereira, C. G. and Meireles, M. A. A. 2010. Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. Food and Bioprocess Technology 3(3): 340-372.

Rizvi, S. S. H., Daniels, J. A., Benado, A. L. and Zollweg, J. A. 1986. Supercritical fluid extraction: operating principles and food applications. Food Technology, 40.

Sankar, K. U. 1989. Studies on the physicochemical characteristics of volatile oil from pepper (*Piper nigrum*) extracted by supercritical carbon dioxide. Journal of the Science of Food and Agriculture 48(4): 483-493.

Sovová, H., Jez, J., Bártlová, M. and St'astová, J. 1995. Supercritical carbon dioxide extraction of black pepper. The Journal of Supercritical Fluids 8(4): 295-301.

Srinivasan, K. 2007. Black pepper and its pungent principle-piperine: a review of diverse physiological effects. Critical reviews in food science and nutrition 47(8): 735-748.

Tipsrisukond, N., Fernando, L. N. and Clarke, A. D. 1998. Antioxidant effects of essential oil and oleoresin of black pepper from supercritical carbon dioxide extractions in ground pork. Journal of Agricultural and Food Chemistry 46(10): 4329-4333.

Zachariah, T. J. and Parthasarathy, V.A. 2008. Black Pepper. in Parthasarathy, V. A., Chempakam, B. and Zachariah, T. J. (Eds.). Chemistry of Spices, p. 21-40. CABI.