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Extraction of Phenolic Compounds from Grape Seed Using Supercritical CO_2 and Ethanol as a Co-solvent

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초임계 이산화탄소와 에탄올 보조용매를 이용한 포도씨로부터의 페놀성 화합물의 추출

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Abstract

A supercritical fluid extraction was performed for the extraction of phenolics from grape seeds which up to now have been discarded. The optimum condition for extraction process was predicted through response surface methodology using central composit experimental design. The extraction amount of grape seed phenolics was increased by increasing extraction temperature, pressure, and concentration of co-solvent (ethanol). The optimum extraction conditions were 84.83℃, 51.50MPa and 1.27% ethanol. The yield of phenolics using SFE was higher with 3 folds than ethanol and 4 folds than hexane but less than 80% methanol. In the respects of food poisoning, the approved solvents were restricted to ethanol and hexane. So, SFE for extraction of phenolics could be powerful alternative method for solvent extraction.

Key words : Phenolics, supercritical fluid extraction, co-solvent, response surface methodology

Introduction

The term of phenolic compounds embraces a wide range of compounds that possess an aromatic ring bearing a hydroxyl substituent, including their functional derivatives. Phenolic compounds presented in many plants are directly related to the characteristics of foods such as taste, palatability, nutritional value, pharmacological and toxic effects and microbial decomposition. However, nowadays many researchers have been interested in these compounds because of their abilities in recovering or removing ironic ions and their properties of anti-cancer, anti-mutant and inhibitors of bindings between HIV and CD4 proteins(1,2).

Specially, it was found that grape seed had many functional phenolics such as procyanidins, (+)-catechin, and epicatechin(3-5). Grapes are cultivated world wide and consumed as raw or processed foods. However, almost all grape seed are discarded during processing. Extraction of phenolics from a discarded grape seeds, could be useful in producing high value-added products at the same time, reusing otherwise wasted resource. Supercritical fluid extraction method has advantages of selective extraction for a certain material and fast

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extraction rate, because of its characteristics of low viscosity, large diffusion coefficient similar to gas and solubility equivalent liquid due to a high density. Supercritical carbon dioxide extraction is usually conducted at low temperatures. Therefore, it is a useful extraction method for heat sensitive natural materials. Moreover, the extracts have no harmful organic solvent residuals and are easily recovered(6-8). In this study, supercritical carbon dioxide and carbon dioxide mixed with co-solvent (ethanol) were adapted to extracted phenolics from discarded grape seeds. The effects of process variables(temperature, pressure, concentration of co-solvent) were investigated and optimum extraction condition was obtained through response surface methodology. Supercritical fluid extraction(SFE) was compared with traditional solvent extraction methods in terms of yields.

Materials and Methods

Materials

Grape seeds were obtained after extraction juice from grapes which were cultivated at Kyungsan in Kyungpook Province. The grape seeds were washed with tap water five times and air dried in cool and dark room. The dried seeds were ground with pulverizer and sieved 25 0~355㎛. The ground seed contained 10% water.

Supercritical fluid extraction of phenolic compounds

Supercritical fluid extraction unit(ISCO Inc., model SFX2-10, USA) is depicted in Fig. 1. CO_2 and ethanol as a co-solvent were compressed and supplied to the extraction column using a syringe pump(ISCO Inc., model 260D, USA). The system was designed to maintain a constant extraction pressure at any temperature and flow rate. The volume of the extraction column was 10㎖. CO_2 and ethanol were set to flow downward in the column to prevent channeling. The restrictor was a stainless capillary column(O.D. 300㎛). The heating chamber placed after the restrictor in order to avoid dry ice formation resulting from big pressure drop. Four grams of ground grape seed were filled in the extraction vessel. The system was emptied with gaseous CO_2 to remove air at low pressure for 5min. After the extractor reached the set temperature, the system was pressurized. Thirty minutes were allowed for the equilibration of temperature and pressure, and the extraction was performed with the mixed fluid (CO_2+ethanol) flowing through the column and the extracted phenolics was separated through pressure reduction.

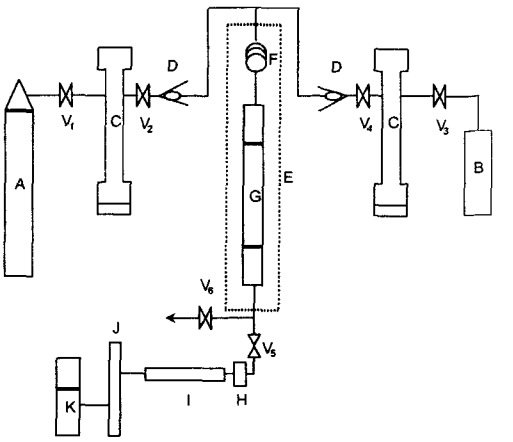

Fig. 1. Schematic diagram of supercritical fluid extraction system.
A: Carbon dioxide cylinder B: Ethanol cylinder C: Syringe pume and controller D: Check valve E: Constant temperature bath F: Pre-heater G: Extraction column H: Filter I: Capillary restrictor J: Heating chamber K: Sovent trap V1~V6: Valves.

Experimental design

Response surface methodology(RSM) was used to optimize the extraction conditions for extraction amount of phenolics. The experimental design was a central composite design with three independent variables of extraction temperature(X_1 : 35~75℃), extraction pressure (X_2 : 20.67~48.23 MPa), and concentration of ethanol as a co-solvent(X_3 : 0~4%) at five levels(9,10). The independent variables were coded to -2, -1, 0, 1, 2. Central composite design is shown in Table 1. The complete design consisted of 15 experimental points. Second order regression mathematical function using SAS(statistical analysis system) program was adapted to the response variable Y(extraction amount of phenolics) in terms of three independent processing factors for supercritical fluid extraction. The second order polynominal equation was as followed.

Table 1. Central composite experimental design at five levels for RSM

Xi	Extraction Conditions	Level				
		-2	-1	0	1	2
X1	Temperature(℃)	35	45	55	65	75
X2	Pressure(MPa)	20.67	27.56	34.45	41.34	48.23
X3	Co-solvent(%, v/v)	0	1	2	3	4

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{11}X_{12}+b_{22}X_{22}+b_{33}X_{32}$$

Additional experiments were carried out to monitor the extracted amount of grape seed phenolics depending on time at fixed extraction condition(34.45 MPa, 55℃ and 2% ethanol, v/v). Grape seed phenolics was extracted during 15min to 75min. At extraction conditions of central point, each variables were modified to monitor the extraction amount of grape seed phenolics while other variables were fixed.

Measurement of phenolic compounds

Total phenolics were determined by the procedure described by Coseteng and Lee(11). The extracts were read with optical density of absorbance at 700 ㎚ in the range of the standards (5-200㎎ tannic acid/㎖). For estimation of total phenolics, 0.5g of the ground seed after SFE and before SFE were extracted with 20 ml of 80% methanol for two hours in shaking incubator at 200 rpm and filtered with filter paper No. 2(Whatman Ltd. England). Three ㎖ of Folin and Ciocalteau Phenol Reagent(Sigma Chemical Co., St. Leuis, MO, USA) was added to 3㎖ of the extract. The sample was mixed. After 3 min, three ㎖ of saturated sodium carbonate solution were added and then shaken. The optical density of the solution was measured after one hour. The amount of total phenolics was calculated from a standard curve of tannic acid prepared.

Extraction yield

Extraction yield by SFE was compared with that of solvent extraction methods. Soxhlet method was used for the solvent extraction. The solvents were methanol, ethanol, acetone, pentane, hexane, diethyl ether and dichloromethane which were all first grade solvent. The yields by each solvent and SFE were compared with 80% methanol extracts which showed the highest amount of phenolics.

Results and Discussion

Effect of temperature

Figure 2 showed effect of temperature during extraction at 34.45MPa and 2% ethanol as a co-solvent. Increasing temperature gradually increased the extraction amount of phenolics. This trend was observed commonly in experimental regions at a fixed pressure and concentration of co-solvent. These findings disagree with Tusda(12) who reported that the extraction yield peaked close to 40℃ and the extracted amount was decreased as further increasing temperatures as comparing with the extraction amount of antioxidants from Tamarind seed coat depending on temperature changes in SFE. Generally, increasing temperatures caused increment of vapor pressure of a solute and decrease of supercritical solvent density(13-17). So, there was a turning point where supercritical fluid density dominated the extraction amount below a certain pressure and temperature and the vapor pressure of a solute dominate it above. This case was very often found in the references of oil or antioxidants extraction using SFE(18,19). The phenolics of grape seeds have generally smaller molecular weight than oils. This means that the material having low boiling point have higher vapor pressure at low temperature. Therefore the turning points of the phenolics would be lower than oils having turning points at 15~20MPa, 40℃. The extraction amount of phenolics was accordingly increased as increasing temperature in this study.

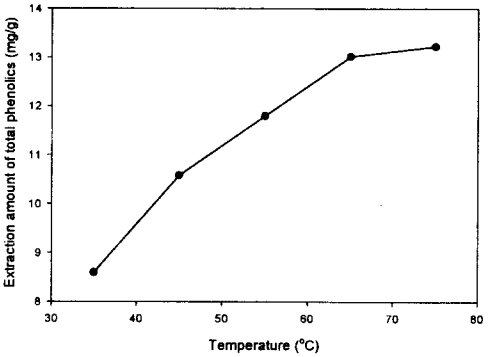

Fig. 2. Extraction amount changes of total phenolics as a function of extraction temperature at 34.45 MPa and 2% ethanol (v/v) as a co-solvent.

Effect of pressure

Figure 3 showed the changes of extraction amount depending on pressure at 55℃ and 2% ethanol as a co-solvent. The extraction amount increased gradually with increasing pressure. This result showed the same trend in the case of oil extraction using SFE. Generally the solubility of supercritical fluid increases as increasing pressure because of density enhancement.

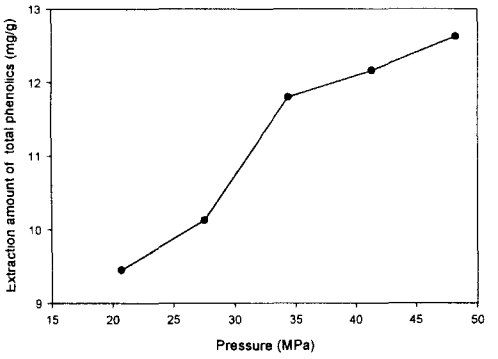

Fig. 3. Extraction amount changes of total phenolics as a function of extraction pressure at 55℃ and 2% ethanol (v/v) as a co-solvent.

Comparing with other researches, Tsuda and Cortesi(20) examined the extraction amount of antioxidants at the range of 10~30MPa and 13~25MPa, respectively and no significant improvement of extraction amount depending on pressure was found. This study examined in the experimental range of 20~48MPa and it was shown in Fig. 3 that the extraction rate was sharply increased between 27.56 and 34.45MPa. This result mean that the higher pressure than Tsuda and Cortesi's experimental pressure region is needed for the extraction of phenolics.

Effect of co-solvent

The extraction curve of phenolics as function of the different concentrations of ethanol as a co-solvent was shown in Fig. 4 at 55℃ and 34.45MPa. According to the extraction curve, the increment of ethanol concentration made rapid increment of extraction amount exponentially. Tsuda reported the use of co-solvent (ethanol) enhance the extraction efficiency in case of extraction for flavan-3-ol polyphenolic compounds such as (-)-epicatechin. Murga(21) reported that proanthocyanidins and epicatechin bearing the same basic structure of flavan-3-ol were extracted more from grape seed in the use with a co-solvent. It was thought that the use of ethanol as a co-solvent induced the increasing of the supercritical fluid's polarity to enhance extraction efficiency.

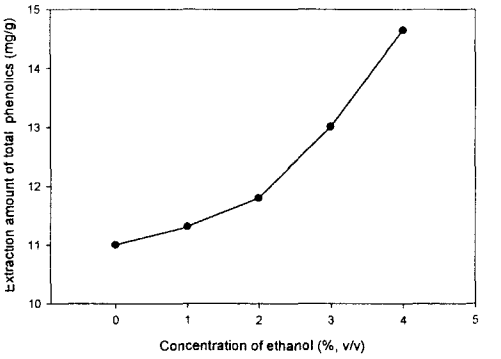

Fig. 4. Extraction amount changes of total phenolics as a function of ethanol concentration (v/v) as a co-solvent at 55℃ and 34.45 MPa.

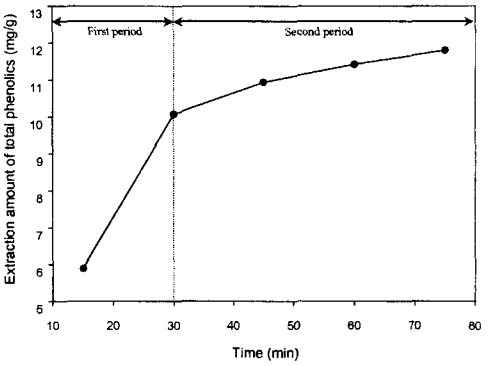

Fig. 5. Extraction amount changes of total phenolics as a function of extraction time at 55℃, 34.45 MPa and 2% ethanol (v/v) as a co-solvent.

Effect of extraction time

Relationship between extraction amount and extraction time was showed in Fig. 5 at 55℃, 34,45MPa and 2% ethanol as co-solvent. The extraction curve showed steep slope for 30 min and increased slowly after 30min. The extraction amount of phenolics was 50.45% during 15min and 76.05% during 30min. A great deal of phenolics were extracted within 30min. This trend is general phenomena of SFE. A lot of extractable materials was on the surface of particles and the aimed materials were extracted more in the first period than second period. The first period could be defined as a direct contact extraction

period between solute phase on surface and solvent phase without molecular diffusion but the second period as the extraction period through molecular diffusion.

Response surface methodology for optimization

RSM was used to examine the extraction amount of phenolics as function of the changes of supercritical fluid extraction variables. Response surface plots showed that the extraction amount of phenolics was almost linearly increased as increasing temperatures and pressures and increased exponentially as concentration of ethanol in Fig. 6. The regression equation of response surface was followed(R2=0.98).

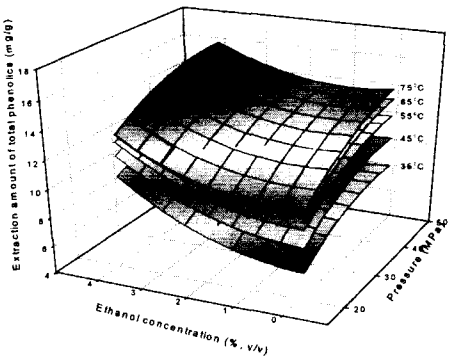

Fig. 6. Response surface plot for the extraction amount of total phenolics using supercritical fluid extraction from grape seed.

y=-9.164049+0.332431X1+0.332714X2+0.730736X3-0.002295X12+0.001321X1X2-0.004164X22-0.008625X1X3-0.012373X2X3+0.249347X32.

The optimum condition of supercritical fluid extraction process for phenolics were 84.83℃, 51.50MPa and 1.27% ethanol concentration as a co-solvent through canonical analysis. Correlation between parameters such as independent variables and dependent variable, was determined by the Pearson analysis(SAS 1990). For extraction amount of phenolics, pressure was 0.399 correlation coefficient and temperature 0.669 and the concentration of ethanol 0.512. Accordingly, the major affecting factors were in the orders of temperature, concentration of co-solvent and pressure.

Comparison of extraction yield by SFE and solvent extraction

The extraction yield of phenolics using SFE was compared with traditional solvent extraction method in Table 2. The extraction yields were decreased in the orders of methanol, ethanol, acetone, ethyl ether, pentane, hexane and dichloromethan. These orders were the degree of polarity and molecular weight. The best solvent was methanol. The extraction yield of phenolics using SFE was 13.97mg/g at the optimum condition. The yields were calculated with 100×extraction amount of phenolics/ extraction amount of phenolics using 80% methanol solvent. The yield of phenolics using SFE was higher with 3 folds than ethanol and 4 folds than hexane but less than 80% methanol. In the respects of food poisoning, the approved solvents were restricted to ethanol and hexane. So, SFE for extraction of phenolics could be powerful alternative method for solvent extraction.

Table 2. Extraction yield comparisons between SFE and traditional solvent extraction

Extraction methods	Extraction amount of phenolics(mg/g)	Extraction yield(%)
Ethanol	5.86	35.11
Acetone	4.79	28.67
Pentane	1.09	24.50
Hexane	3.44	20.61
Dichloromethan	2.69	16.12
Ethyl ether	4.75	28.46
Methanol	12.3	73.69
80%-Methanol	16.69	100.00
*SFE	13.97	83.71

* : at optimum coditions of SFE.

Acknowledgement

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요 약

최근 식품산업에서 주목을 받고 있는 초임계 유체 추출방법을 이용하여 농산 폐자원으로 버려지고 있는 포도씨로부터 페놀성 화합물들의 추출을 시도하였다. 초임계 유체로는 이산화탄소를 이용하였고 보조용매로

는 에탄올을 이용하였다. 초임계 유체상에서 페놀성 화합물의 추출경향은 압력과 온도가 증가함에 따라 추출량이 증가하는 것으로 나타났다. 압력의 증가에 따른 추출량의 증가는 초임계 이산화탄소의 밀도의 상승에 기인한 초임계 용매상의 용해력의 증가에 따른 것이며 온도의 증가에 따른 추출량의 증가는 포도씨에 존재하는 용질의 증기압의 증가에 기인하는 것으로 생각된다. 또한 보조용매의 사용은 추출효율은 높일 수 있었는데 이는 초임계 이산화탄소에 보조용매가 첨가됨으로서 용매상이 극성을 띄게 되어 용해력이 증가된 때문으로 생각된다. 시간의 변화에 따른 추출량은 초기 15분 내에 50.45%, 30분 내에는 76.05%가 추출되어 추출초기에 상당한 량이 추출되는 것으로 나타났다. 이는 분쇄된 포도씨의 표면에 용질상이 많이 분포한 이유로 생각된다. 반응표면분석에 의한 최적추출조건은 84.83 ℃, 51.50 MPa, 1.27 % 에탄올 농도로 나타났으며 이때의 최적조건에서의 추출량은 용매추출법과 비교했을 때 에탄올에 비해서는 3배, 헥산에 비해서는 4배로 나타났다.

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