# SUPERCRITICAL FLUID EXTRACTION OF THE SEED OF COIX LACHRYMA-JOBI L.

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In this work, the oil and fat in a mixture of the hull and the testa of the seed of Coix Lachryma-Jobi L. is extracted by supercritical carbon dioxide. The investigated temperature and pressure ranges from 35 to 50 $^{o}$C and from 20.7 to 48.3 MPa. It is found that the weight fraction of extractable oil and fat in the mixture depends on the pressure and temperature and can be correlated to $CO_2$ density. The calculated solubility parameter of the oil and fat, 17.03 $J^{1/2}/cm^{3/2}$, implies that supercritical carbon dioxide has the maximum solvent power when it's density reaches 0.973, which is also used to explain the abnormal behavior of the extraction curves. Some oil and fat samples are also measured for its antioxidation activity by observing the reduction of the UV absorption intensity of DPPH. It is found that little of oxidation activity can be obtained if the oil and fat is collected after 2 wt% of the mixture has been recovered. It is recommended to operate the extraction at medium pressure for the purpose of antioxidation activity. However, the calculated solubility parameter for the potential active compounds shows that the extraction should conduct at high pressure such that active compounds can be extracted and entrained with the oil and fat.

## INTRODUCTION

The seed of *Coix Lacryma-jobi L.* is one of the cereal after dehulled or polished, which has been used in treatment of diuretic, stomachic, nourishment, anodyne and antispasmodic in Chinese traditional prescription [1]. The dehulled process produces the hull and testa, and the polished process deprives the bran of the seed. The hull, testa, and bran are treated as waste for a polished plant. Researches showed that the methanol and/or water extract of the waste contains various antioxidants and have anti-tumor and anti-inflammatory activity [1 - 4]. It was also patented that the active compounds in the seed, such as coixole, can be extracted by supercritical carbon dioxide with cosolvent at pressure below 10 MPa [5]. It is interested to know whether the bioactivity of the $CO_2$ extract can be enhanced without

cosolvent. The weight percent of the oil and fat in each part of the seed is listed in Table 1. Huang has analyzed the composition of the free fatty acid of the oil and fat and the major free fatty acid is listed in Table 2 [6]. The most abundant free fatty acid in the oil and fat is C18:1 and C18:2. Other than the oil and fat, several compounds in methanol extract of the waste were identified to have contribution of antioxidation activity including vanillin, *p*-hydroxybenzaldehyde, syringaldehyde, sinapaldehyde, conifery alcohol, ω-hydroxypropioguaiacone, trans-*p*-courmaric, syringic acid, ferulic acid, syringaresinol, dehydrodiconifery alcohol, tricin, naringenin, and etc. In order to recover the useful compounds for human's consumption, supercritical carbon dioxide is applied to extract the oil and fat and other active compounds.

**Table 1** The crude oil and fat content in each part of the seed

| | Hull | Testa | Bran | Dehulled Adlay | PolishedAdlay |
|---|---|---|---|---|---|
| Oil and fat Content(wt%) | 2.0 | 2.9 | 24.4 | 8.3 | 2.9 |

**Table 2** The free fatty acid composition of the oil and fat of the seed

| Free fatty acid | wt% |
|---|---|
| C16:0 | 14.03~14.66 |
| C16:1 | 0.22~0.27 |
| C18:0 | 1.62~1.64 |
| C18:1 | 47.84~49.87 |
| C18:2 | 28.82~28.79 |
| C18:3 | 0.35 |
| C20:0 | 0.35 |
| C29:1 | 0.4 |

Supercritical carbon dioxide possesses high solvent power, high diffusivity, low viscosity, low surface tension, and adjustable physical properties by pressure and temperature. It is widely and maturely applied in natural products extraction. The feasibility study in a bench-scale apparatus is the primary study providing the optimal extraction condition and the extraction strategy. In this work, the feasibility of recovery the oil and fat from the wastes of the dehulled process is conducted, and the operating parameters are optimized. Also, the antioxidation activity of the extracted oil and fat is investigated by the observing the reduction of UV absorption intensity of 1, 1-diphenyl-2-picrylhydrazyl (DPPH).

## EXPERIMENTS AND MATERIALS

The mixture of hull and testa of the seed from the dehulled process is ground by a miller to below 0.1 mm. About 3 grams of the fine particles are loaded into the

ISCO 220 supercritical fluid extraction system. The oil and fat is collected in a sample vial, and methanol is used as the trapping agent. The methanol containing oil and fat is dried in a vacuum decanter for overnight. The residual oil and fat is weighted and recorded and 3 ml of methanol is added again for antioxidation study. The methanol solution is further diluted to different concentration and mixed with the pre-prepared DPPH-methanol solution, which is purchased from Aldrich (> 95%). The absorption intensity of the UV light at 515 nm of the mixing solution is recorded. The reduction of the absorption intensity due to the oil and fat is calculated and correlated with the ratio of the weight of the fats and oils to that of DPPH. Linearity between the reduction of absorption intensity and the weight ratio is found. The slope of the regression line is defined as the antioxidation activity in this work.

The recovery, *E*, is defined as the weight ratio of the oil and fat to that of the loaded sample, and the solvent to feed ratio, *q*, is defined as that of used $CO_2$ to the loaded sample. Both *E* and *q* are recorded for every experiment. For each extraction condition the plot of *E* vs. *q* is prepared to obtain the extraction curve. From the extraction curves and the study of antioxidation activity, the strategy of the optimum extraction condition can be determined.

**RESULTS AND DISCUSSION**

The extraction curves for different extraction conditions are illustrated in Figures 1 - 3. Normally, an extraction curve is combined by the solubility-determined step and the diffusion-determined step. It is observed that the solubility-determined extraction dominates the extraction roughly as *E* below 0.02. Among the six extraction curves, two curves show dramatic increase of the *E* value at near *E* = 0.02 as the *q* value continuously increases. This can be explained by the solubility parameter between the supercritical carbon dioxide and the oil and fat. According to Huang's study, the free fatty acid of the oil and fat of the seed is fully illustrated. Therefore, the solubility parameter of the oil and fat can be calculated as 17.03 $J^{1/2}/cm^{3/2}$ according to the method proposed by Ma [8], who has applied the method to calculate the wheat germ oil as 17.663 $J^{1/2}/cm^{3/2}$. It is assumed that the solubility of the oil and fat in supercritical carbon dioxide reaches maximum as the solubility parameters are equal for both. Since the solubility parameter of supercritical carbon dioxide can be calculated as $d = \frac{8.04 P_C^{1/2} r}{2.66 r_C}$ [9], the oil and fat will totally dissolve into the supercritical carbon dioxide as the density of carbon dioxide reaches 0.973. It is found that two extraction conditions in Figures 1 – 3 have the $CO_2$ density higher than 0.973, which are also the extraction curves that show dramatic increase of *E* value as *E* near 0.02. The maximum recovery for each extraction curve in Figure 1 –

3 can also be correlated to the $CO_2$ density as shown in Figure 4. It is obviously observed that higher $CO_2$ density will result in higher recovery of the oil and fat and lower its extraction time.

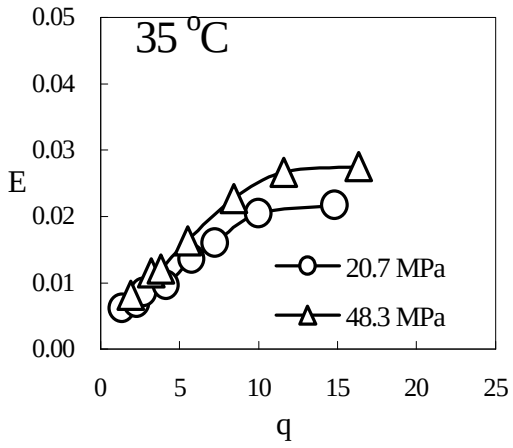


**Figure 1** The extraction curves at 35 °C.

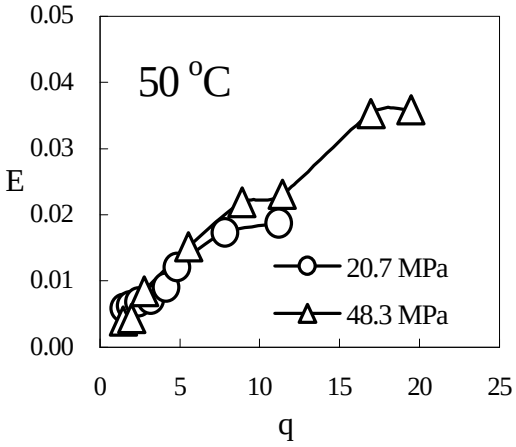


**Figure 3** The extraction curves at 50 °C.

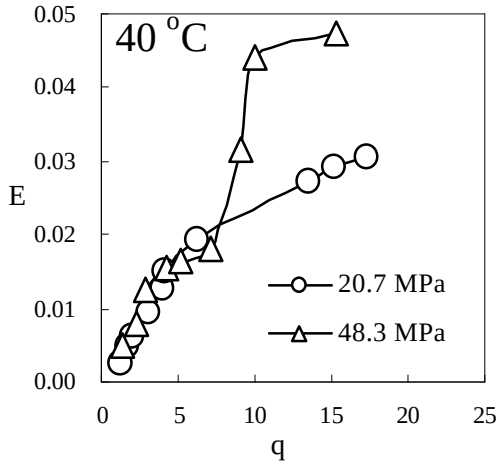


**Figure 2** The extraction curves at 35 °C.

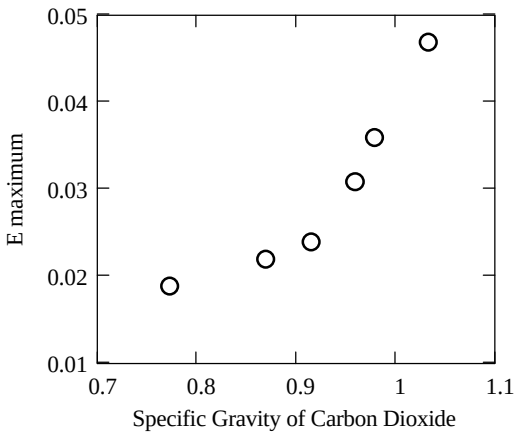


**Figure 4** The correlation between E and $CO_2$ density.

The antioxidation activity of the oil and fat varies with the *E* value. Figure 5 shows the reduction of UV absorption intensity linearly varies the weight ratio of the oil and fat to that of DPPH when extracted at 35 °C and 20.7 MPa. Each line in Figure 5 represents different oil and fat sample collected at different *E* value. The symbol of ? represents that for $E = 0.001991$, the ? is that for $E = 0.004792$, and ? is that for $E = 0.006367$. It is found that the slope of the regression line, which is defined as antioxidation activity in this work, decreases with the *E* value. Further studies on different extraction conditions will depict the correlation. Figure 6

collects all samples of the oil and fat that have been studied for their antioxidation activity. It is found that the correlation is independent on the extraction conditions. The antioxidation activity can be well correlated by the empirical equation, $\text{antioxidation activity} = \exp(-135E - 7.1)$. From Figure 6, it obviously concludes that little of antioxidation activity can be obtained after the E values is larger than 0.02. Therefore, for the purpose of antioxidation it is recommended that the extraction should end up at $E \tilde{}\ 0.02$.

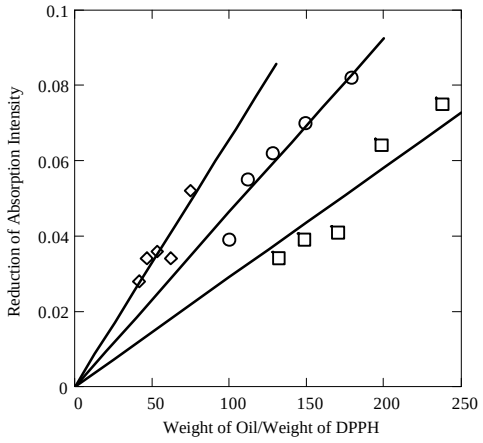


**Figure 5** The reduction of absorption intensity of DPPH with respect to the weight ratio of the oil and fat to DPPH.

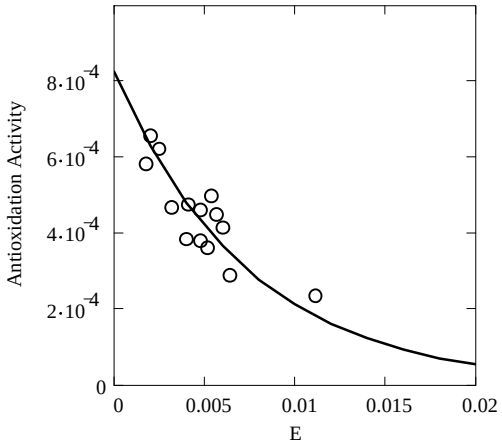


**Figure 6** The correlation between the antioxidation activity and the *E* value.

**Table 3** The solubility parameter of the oil and fat and active compounds

| active compounds | solubility parameter |
|---|---|
| oil and fat | 17.03 |
| coixenolide | 18.56 |
| coixol | 26.72 |
| feruloyl stigmastanol | 18.20-18.23 |
| coixinden A, B | 23.17-23.36 |
| α-monolinolein | 21.16 |

Although the oil and fat collected after *E* larger than 0.02 shows little of the antioxidation activity and the *q* value for different extraction condition is narrowly ranged from 7 ~ 10, the anti-tumor and the anti-inflammatory compounds that have been isolated and identified are not likely extractable at low $CO_2$ density. Table 3 lists the solubility parameter for the oil and fat and some potential compounds that has been identified as the active compounds. It is observed that most antioxidants have normally lower solubility parameter and other active compounds have higher one. Therefore, for the purpose of other medical treatment it is recommended that the extraction should conduct at higher $CO_2$ density. If a multiple stage of

depressurization is considered, the bioactive compounds can also be concentrated after partial depressurized.

## CONCLUSION

In this work, the waste from the dehulled process of the seed of Coix Lachryma-Jobi L. is extracted for its oil and fat by supercritical carbon dioxide. The effects of extraction temperature and pressure are investigated, and the antioxidation activity of the oil and fat is quantitatively measured through the observation of the reduction of UV absorption intensity of DPPH. The solubility parameter of the oil and fat and potential active compounds are also calculated to explain the extraction and to set up the extraction strategy. For the purpose of antioxidation activity, it is recommended that operating at medium pressure is enough. However, it is recommended to extract at higher pressure if the activity of anti-tumor and anti-inflammatory are demanded. A multiple-stage of depressurization is also recommended to concentrate the potential active compounds in the seed.

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