

대두 잔기로부터 유기용매에 의한 이소플라본 추출 열역학적 메카니즘 연구

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Study of Thermodynamic Mechanism for Using Organic Solvent to Extract Isoflavone from Soybean Residuals

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요 약. 대두 잔기로부터 유기용매를 사용하여 이소플라본을 추출하는 열역학적 메카니즘을 조사하였다. 대두 잔기로부터 유기용매를 사용한 이소플라본 추출과정에서의 분배 계수를 결정하기 위한 간단한 모델을 설정하고 K , ΔH^0 , ΔS^0 and ΔG^0 간에 열역학적 함수를 계산하였다. 그 결과 대두 이소플라본 추출은 흡열과정이며 엔트로피 증가과정임을 발견하였다. 온도가 증가할수록 ΔG^0 가 감소하였다.

주제어: 대두 이소플라본, 유기용매, 추출, 열역학적 메카니즘, 대두 잔기

ABSTRACT. The thermodynamical mechanism of the extraction of soybean isoflavones from soybean residuals using organic solvent method has been studied. On the basis of experiments, a simple model for determining the distribution coefficients in organic solvent extraction was employed to calculate the thermodynamical functions between K , ΔH^0 , ΔS^0 and ΔG^0 in the soybean isoflavones extraction process. The results show that the soybean isoflavones extraction is an endothermic and an entropy-increasing process: the ΔG^0 decreases when the temperature arises.

Keywords: Soybean isoflavones, Organic solvent, Extraction, Thermodynamic mechanism, Soybean residuals

INTRODUCTION

Isoflavones are phytoestrogens that are present in soybeans. Researches have found that soy isoflavones demonstrate many physiological functions upon human bodies. Apart from its anti-oxidization capabilities, it can also be used as substitute for treating postmenopausal symptoms. The benefits of soybean isoflavones discovered also include lowering cholesterol levels, preventing both prostate and breast cancers, attenuating bone loss in postmenopausal women, and alleviating menopausal symptoms.¹ Isoflavones are the best drug materials

used in treatment of cardiovascular diseases so far.^{2,3}

The soybean residuals are important byproducts produced during the soybean oil processing and have been proved to be a rich source of isoflavones. However most of these residuals have been used as animal feeds or fertilizer, not only polluting the environment but also wasting resources. Therefore it will be of great significance if these residuals could be made full use of and major bioactive substance could be extracted.^{4,5,6}

In previous studies,⁴ the kinetic mechanism of the soybean isoflavones extraction process by organic

solvent extraction based on Fick's first law of diffusion had been researched, but no researches on the thermodynamics mechanism of the extraction process of soybean isoflavones had been reported so far. Therefore in this study, the thermodynamic mechanism of the extraction process of soybean isoflavones was researched based on the organic solvent extraction,⁴ which gives a valuable theory basis for the comprehensive utilization and extraction of active constituents from the waste residue of the soybeans.

EXPERIMENTAL

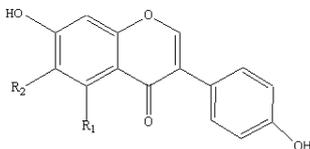
Materials and analysis method

All reagents including ethanol, cyclohexane, acetic acid, methanol, acetone were of AR grade and they were obtained from Shanghai Chemical Reagent Co. with purities of 0.995 in mass fraction. Samples were analyzed with UV spectrophotometer.

Selection of standards

Isoflavones is a compound. There are about twelve isoflavones discovered in soybeans, which are classified into two categories: Aglycon and Glucoside. Aglycon accounts for 2~3% including genistein, daidzein and Glycitein; whereas Glucoside accounts for 97~98%, mainly existing in forms of malonylgenistin, malonyldaidzin, genistin and daidzin. Isoflavones has been shown to be present in four chemical forms: daidzein, daidzin, genistein and genistin respectively.

In this article, genistein was selected as the isoflavone standards and was purchased from the Sigma-Aldrich Group. Its structure is as follows.



The treatment of raw materials

The soybean residuals were first washed to remove dirt, and then soaked for 24 h under room

temperature in cyclohexane to remove lipid. Afterwards these residuals were dried, and then filtered through a 36-mesh nylon filter-sack. Finally, these residuals were stored under room temperature until used.

Experimental procedures

Extractions were carried out at temperature 352.7 K, 348.2 K, 343.2 K, 337.7 K for 3 hours by mixing 2 g of sample powder with 40 mL of 80% ethanol. In order to ensure the extraction equilibrium, a mixing device was placed in the extractor to reduce the thickness of liquid film on the solid surface during the course of extraction, and to accelerate the speed to reach the extraction equilibrium. After equilibrium state was reached, the extracted solution was filtrated, and then the flask was filled up to its volume capacity. Ultraviolet spectrophotometer was used to measure the content of isoflavone in each set of extracts.⁷

The thermodynamic theory for the extraction process

In the process of extraction of natural products, the organic solvent firstly penetrates into the cells; whereas the cellular elements diffuses from the inside to the outside, and then dissolved into solvents. Because the chemical potential of the cellular elements was greater than the chemical potential of the solvent, i.e. $\mu_B(s) > \mu_B(l)$, the free energy of the cellular elements decreased while the free energy of solvent ingredients increased during the process of mass transfer. The sum of free energy change in the two phases was the free energy change of the extraction system. ΔG^0 of the extraction process of mass transfer would change with the concentration of solid-liquid two-phase ingredients.^{8,9}

$$\mu_B(s) = \mu_B^0(s) + RT \ln c_B(s) \quad (1)$$

$$\mu_B(l) = \mu_B^0(l) + RT \ln c_B(l) \quad (2)$$

where, $\mu_B(s)$, $\mu_B^0(s)$, $\mu_B(l)$, $\mu_B^0(l)$, $c_B(s)$ and $c_B(l)$ are the chemical potential of solid phase, the standard chemical potential of solid phase, the chemical po-

tential of liquid phase, the standard chemical potential of liquid phase at the experimental extraction temperature, the concentrations of ingredients in the solid phase and liquid phase, respectively. When the equilibrium was reached, $\mu_B(s) = \mu_B(l)$, then

$$K = \frac{c_B(l)}{c_B(s)} = \exp\left[\frac{\mu_B^0(l) - \mu_B^0(s)}{RT}\right] \quad (3)$$

Where, K is distribution constant of ingredients. It is the thermodynamic constant related to temperature and pressure. As it is difficult to directly determinate the solid-phase concentration in the process of extracting isoflavones, therefore the distribution constant is usually calculated in the following formula,

$$K = \frac{c_B(l)}{c_B(s)} = \frac{c_B(l)}{c_T - c_B(l)} = \frac{c_B(l)}{\sum c_{B,i}(l) - c_B(l)} \quad (4)$$

Where c_T is the total concentration of ingredients in two phases, $c_{B,i}(l)$ is the concentration of extracted solution for i times. When the equilibrium was reached, the concentration $c_B(l)$ was analyzed directly with UV spectrophotometer. The concentration of ingredients in solid-phase was equal to the difference between c_T and $c_B(l)$, theoretically, after an unlimited number of extraction, $c_T \approx \sum c_{B,i}(l)$. In our experiments, when the concentration of ingredients could not be detected in the extracted solution after the 7th extraction, the total concentration of ingredients was approximately equivalent to the sum of extracted concentrations, thus the distribution coefficient could be calculated.

In the case of sealed solvent extraction process under constant temperature and constant pressure, ΔG^0 was calculated by using the following equation.

$$\Delta G^0 = -RT \ln K \quad (5)$$

When the extraction equilibrium was reached in a certain temperature range,

ΔH^0 and ΔS^0 was calculated by using the van't

Hoff equation:

$$\ln K = -(\Delta H^0/RT) + (\Delta S^0/R) \quad (6)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (7)$$

If the $\ln K$ and $1/T$ diagram is made according to eq 6, the slope of the curve equals to $-\Delta H^0/R$; whereas the intercept equals to $\Delta S^0/R$. ΔH^0 , ΔS^0 and ΔG^0 of extraction process could be calculated by using eq 5-7.

RESULTS AND DISCUSSION

The effect of extraction temperature and extraction time

In the solvent extraction process, the extraction temperature and extraction time are the most important factors in thermodynamical activities, and temperature and extraction time have a direct effect on the extraction efficiency of isoflavones. In an extraction experiment,⁴ when 80% ethanol was used as extraction solvent and extraction time was 0.5 ~ 5 hours in the temperature ranges of 323.2 ~ 353.2 K, the experimental results are shown in Table 1 and Figure 1, Figure 2.

During the process of extracting isoflavones, the time periods reached equilibrium for ingredients are different under different extraction temperatures and in different extraction time. Raising the extraction temperature and extending extraction time will enable the system to absorb more energy and pro-

Table 1. The results of experiment in the 352.7~337.7 K temperature range

| T/K | 352.7 | 348.2 | 343.2 | 337.7 |
|-------------------------|----------------------|----------------------|----------------------|----------------------|
| Number of extraction, i | $C_{B,i}(l)$ (mL/mL) | $C_{B,i}(l)$ (mL/mL) | $C_{B,i}(l)$ (mL/mL) | $C_{B,i}(l)$ (mL/mL) |
| 1 | 0.04294 | 0.03902 | 0.03453 | 0.02934 |
| 2 | 0.03321 | 0.02989 | 0.02830 | 0.02294 |
| 3 | 0.01565 | 0.01753 | 0.01647 | 0.01708 |
| 4 | 0.01098 | 0.01167 | 0.01244 | 0.01146 |
| 5 | 0.00684 | 0.00695 | 0.00602 | 0.00698 |
| 6 | 0.00536 | 0.00576 | 0.00541 | 0.00443 |
| 7 | 0.00438 | 0.00448 | 0.00427 | 0.00401 |

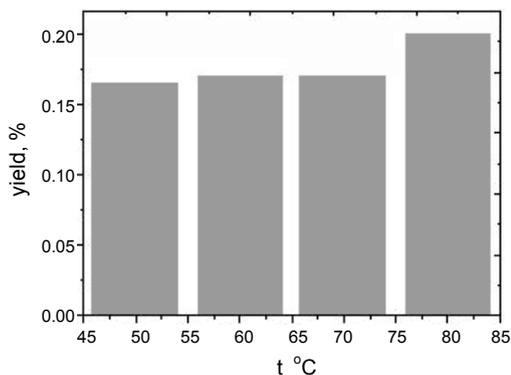


Fig. 1. The effects of extraction temperature on the yields.

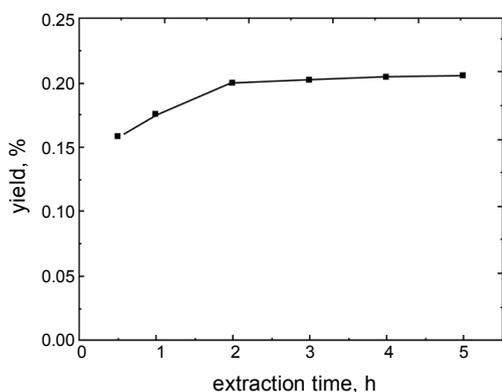


Fig. 2. The effects of extraction time on the yields.

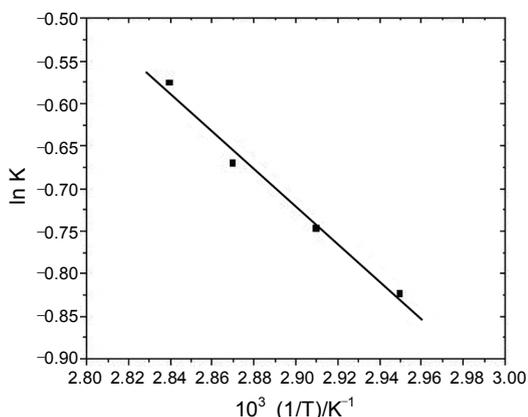


Fig. 3. The relationship of $\ln K$ against $1/T$.

mote the proliferation and mass transfer of ingredients to reach extraction equilibrium. The extraction yields will increase with the increase of the

extraction temperature and extraction time when the extraction temperature is lower than 353.2 K. The extraction yield of soy isoflavones will remain basically unchanged when the extraction time is longer than two hours. So in order to ensure balance, the extraction temperature selected was 353.2 K, and the extraction time was 3 hours.

Enthalpy change ΔH^0 and entropy change ΔS^0 of the extraction process

The equilibrium data was measured by using the above-mentioned method; the experimental results are listed in Table 1.

The distribution coefficient of ingredients at the different extraction temperatures can be calculated by using eq 4. The curve of $\ln K - (1/T)$ was drawn according to the van't Hoff equation and is shown in Figure 3. Enthalpy change ΔH^0 and entropy change ΔS^0 of the extraction process can be calculated from Figure 3 respectively.

The regression equation according to the van't Hoff equation is as follows,

$\ln K = -2.1154/T + 5.4123$, $R^2 = 0.9879$; therefore $\Delta H^0/R = 2.1154$, and $\Delta S^0/R = 5.4123$. So we can obtain, $\Delta H^0 = 17.59$ J/mol, $\Delta S^0 = 45.00$ J/mol. It can be seen from the result that ΔH^0 and ΔS^0 are both positive. It suggests that the extraction process of isoflavones is a process of endothermic and entropy increase. Since the change of the pressure p and volume V in the process of extraction is very small, ΔH^0 is approximately equivalent to the free energy of extraction of isoflavones. i.e.,

$\Delta H^0 = \Delta E^0 + \Delta(pV) \cong \Delta E^0$. In the soy isoflavone extraction system, the extracted energy comes from the unidirectional heat transfer from outside to inside, with most energy being transferred from external solvents via heat convection, therefore less energy is required; and the free energy of extraction is also less.

The free energy change ΔG^0 in the extraction process

ΔG^0 may describe whether the process is spontaneous. The calculated results of Gibbs energy change ΔG^0 in the extraction process is listed in Table 2.

Table 2. The calculated results of Gibbs energy change (ΔG^0 /(J·mol⁻¹))

| T/K | 352.7 | 348.2 | 343.2 | 337.7 |
|--------------|-----------|----------|----------|----------|
| ΔG^0 | -2877.667 | -2840.72 | -2807.88 | -2762.73 |

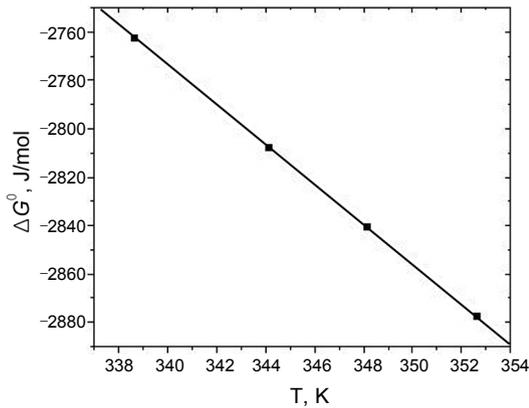


Fig. 4. The effects of temperature on Gibbs energy change of the constituents extracted in processes.

It can be seen from Table 2 that ΔG^0 decreases with the increase of temperature, which suggests that the increase of entropy change ΔS^0 is more significant by comparison with that of the energy required ΔH^0 in the extraction process. The negative values of Gibbs free energy indicate that the extraction process is a spontaneous process.

The temperature effect on Gibbs energy change of the ingredients is shown in Figure 4. The curve of $\Delta G^0 - T$ shows a linear relationship: The higher the temperature, the smaller the ΔG^0 . However, the temperature cannot be raised unlimitedly; the upper limit being the boiling point of the solvent.

CONCLUSION

Through the study of thermodynamic mechanism it is concluded that the extraction of isoflavones is an endothermic and entropy increasing process; ΔG^0 decreases with the increase of temperature, which suggests that the increase of entropy change ΔS^0 is more significant than that of the energy required (ΔH^0) in the extraction process. Therefore the driving force of the extraction process can be improved by raising the temperature within a certain temperature range.

The abundant soybean resources in China provide good natural conditions for the development and utilization of active ingredients in its residuals and byproducts. The study of thermodynamic mechanism can provide a valuable theoretical basis for the comprehensive utilization of soy bean dregs and for the extraction of active ingredients from residuals.

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