The Adsorption Coefficient (K) for Determination of Total Lipid and Gamma Oryzanol Content in Rice Bran Varieties from the Northern of Thailand

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***Abstract*—This research has studied the extraction and analyzation of gamma- oryzanol and total lipid in new rice bran glutinous varieties which were analyzed by the developed technique. The adsorption coefficient (K) of a solute between a solid phase and a solvent phase were studied in the solid-liquid extraction which is defined by the solid-liquid equilibrium condition used to determine the oil extracted in short time so the operation of extraction time been considered. The percentage of oil extracted (% dry weight) is highly increase when time of extraction at 10 minute (30°C) and then it shown slowly increasing rate. From the K values, it was estimate that about 16.52-41.98 mg/g dry basis of the lipid and 1.15-2.96 mg/g dry basis of the gamma oryzanol were extract by heaxane. Sanphatong 1 had highest total lipid and gamma oryzanol while the lowest content was found in Maejo 2.**

***Index Terms*—adsorption coefficient, rice bran, gamma oryzanol, total lipid**

I. Introduction

Rice (*Oryza sativa* Linn.) production is a significant crop in Thailand and Asian countries. Rice bran, a by-product of rice processing, contains 16-22% lipids, which makes the extraction of rice bran oil (RBO) profitable [1]. RBO is a valuable cooking oil, consumed specially in east [2]. RBO presents unique health benefits, it present a large amount of nutraceutical compounds are gamma oryzanol, a complex mixture of ferrulate esters with sterols and triterprene alcohols and tocopherols/ tocotrienols [3], as well as other compounds which found at lower centration, such as lecithin, carotenoild, long chain alcohol and squalene [4]. The commercial production of RBO was estimated to be about 783 thousand tons, being usually extracted with hexane [2].

Extraction and pressing are the two commonly used methods for separation of oil from raw materials. Many researchers have investigated on the use of super critical extraction and enzyme for enhancing oil extractability [5]. The requirement for a special apparatus is drawback for super critical but it is not required for direct solvent extraction which the advantages of the method and it is not time-consuming but if the extraction solvent capacity is lower, this method must use a large volume of solvent.

Recently, the use of direct solvent extraction has been reported for determination of RBO and gamma oryzanol contents in rice bran [6]. Hexane has been used as the solvent for rice bran extraction by many researchers and industrialists due to the capability, high oil extractability (98%) and easy operation [5].

Solid - liquid extraction is an alternative extraction which uses the rapid equilibrium extraction principle or to be defined as solid – liquid equilibrium (SLE). SLE is described by the distribution or adsorption coefficient (K) of a solute between solid and solvent phase. The adsorption coefficient is the proportion of solute concentration in liquid phase and solid phase at equilibrium state [4] as shown in equation 1,where C_m is concentration of solute in solvent phase and A_s is amount of solute adsorbed by one gram of the adsorbent (rice bran).

$$K = \frac{C_m}{A_s} \tag{1}$$

In the term of C_m can be expanded to ratio of the amount of solute in solvent phase (M_m) with the volume of the solvent (V_m) and the ratio of the amount of solute in solid phase (M_s) with weight of rice bran (g_s) is definition of A_s. The adsorption coefficient can be rewrite in equation 2.

$$K = \left(\frac{M_m}{V_m}\right)\left(\frac{G_s}{M_s}\right) \tag{2}$$

Considering the equation found that if K, M_m, V_m and G_s are known, Eq. (2) can be used to determination of the target term M_s which is the amount of solute in solid phase (rice bran).

In the principle of the adsorption coefficient, it is defined by the solid-liquid extraction in equilibrium condition. Therefore, determination of equilibrium time in the extraction is an important. In this work, the solid - liquid extraction via the adsorption coefficient (K) by solving two simultaneous equations was used to quantify total lipid and γ-oryzanol from the rice bran of new

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glutinous rice varieties from the northern of Thailand and then the qualitative of γ-oryzanol has been done by HPLC.

II. Materials and Methods

A. Materials

Rice brans has been collected and stored in the same conditions. The moisture content of the bran was determined by drying at 105 °c until the weight constant. All samples were analyzed in duplicate

Gamma oryzanol were supplied by the Vegetable Oil Refinery (Bangkok, Thailand). Petroleum ether, 2-propanol, 2-butanol, *n*-hexane and Ethyl acetate in analytical grade were purchase from LabScan (Bangkok, Thailand). Acetronitrile and Methanol in HPLC grade were purchase from Fisher Chemicals (UK).

B. Study of Equilibrium Time

The primary optimum operation in rice bran oil extraction was studied. The weight of dried rice bran is 1 g. used to extract with 5 ml of hexane in screw cap test tube. The reactions were mixed all the time at 30°C and 60°C and taking the sampling reaction to analysis on time.

C. Determination of Total Lipid and γ-Oryzanol by Solid - Liquid Extraction Via the Adsorption Coefficient (K)

Exactly 1.0 g dried bran was weight into two screw cap test tube and extracted with the same type of solvent in 4 and 8 ml for the first and second tube, respectively. Extraction was done by mixing the substance on vortex mixer for 5 min. at room temperature. Centrifugation was used to separate the bran from miscella for 10 min at 4,000 rpm. The two of supernatants was collected and measured the absorbance U-100 UV-VIS spectrophotometer (HITACHI, Japan). Quantification of lipid and γ-oryzanol in the extracts were determined by standard curve. The total lipid and γ-oryzanol were calculated by the adsorption coefficient which was described by solving two simultaneous equations. Expanding Eq.(2)

$$K = \left(\frac{x}{V}\right)\left(\frac{w}{y-x}\right) \quad (3)$$

where x is the amount (g) of lipid or γ-oryzanol in the extract, y is the total amount (g) of lipid or γ-oryzanol in the bran. V is the volume (ml) of the extraction solvent and w is the weight of the extraction bran.

The terms K and y in Eq. (3) are unknowns, thus the two equations are necessary for salvation equation. It was done by double the volume (ml) of the extraction solvent in the second extraction ($V_2 = 2V_1$) and x_1, x_2 are the amount of solute in the two extraction. Equation (4) and (5) are the result of substituting these values into Eq. (3).

$$K_1 = \left(\frac{X_1}{V_1}\right)\left(\frac{w}{y-x_1}\right) \quad (4)$$

and

$$K_2 = \left(\frac{X_2}{V_2}\right)\left(\frac{w}{y-x_2}\right) \quad (5)$$

In this work, assuming the different amount of extraction solvent does not affect in changing the K value resulting in $K_1 = K_2$ (Eq. (6)) and rearranging it the Eq.(7) is obtained.

$$\left(\frac{x_1}{V_1(y-x_1)}\right) = \left(\frac{x_2}{V_2(y-x_2)}\right) \quad (6)$$

$$y = \frac{x_1 x_2}{2x_1 - x_2} \quad (7)$$

Finally, Equation 7 used to calculate the total amount of lipid or γ-oryzanol in the bran and the K value is gained when the y value was substituted in Eq.(4) and (5).

D. HPLC Analysis

The composition of γ-oryzanol was determined by HPLC using acetronitrile/methanol (90:10) solvent system as mobile phase and C18 (Hewlett Packard) HPLC column (150 mm x 4.0 mm i.d.).

III. Result and Disscussion

A. Study of Equilibrium Time

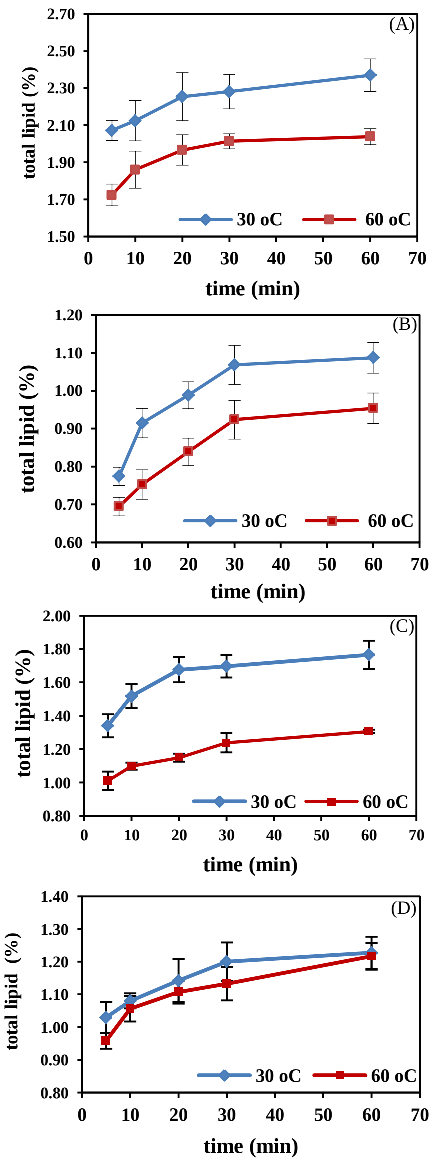

Figure 1. Illustration of total lipid quantity extracted from different rice bran varieties at anytime. (A) Sanphatong 1, (B) Maejo 2, (C) Maejo 4 and (D) Maejo 6.

Due to the study of solid-liquid extraction by adsorption coefficient must be educated in equilibrium condition system. The extraction time for equilibrium condition is very necessary. The percentage of total lipid extracted was determined experimentally by extracting the dried bran in 3 times in anytime of hexane as organic solvent at 30°C and 60°C (the moisture content of rice bran show in range 9.34-10.29%). The amounts of lipid in each extraction time and temperature measured by UV spectrophotometers at 210 nm have been showed in Fig. 1. The percentage of oil extracted (% dry weight) is highly increase when time of extraction at 10 minute and then it shown slowly increasing rate. Therefore, The study of adsorption coefficient extraction technique in equilibrium state system will use extraction time at 10 min which is equilibrium time of the extraction system.

B. Total Lipid and Gamma Oryzanol

For the simultaneous analysis the amount of the total lipids and gamma oryzanol are studied by adsorption coefficient technique which been investigated in equilibrium condition (10 minutes). The total lipids and γ-oryzanol obtained from extraction of rice bran via hexane (% dried weight basis) were quantified by UV spectrophotometric method at 210 nm for total lipid and 314 nm for gamma oryzanol which the data to show in Table I. The external standard calibration curves are linear between 0-150 ppm for total lipids and 0-20 ppm for gamma oryzanol. The regression coefficients are more than 0.999. Total lipid contents were in range 16.52-41.98 mg/g dry basis which agreement with other report [7], [8]. The highest total lipid and gamma oryzanol content was found in Sanphatong 1 while the lowest was observed in Maejo 2. The differences were probably due to the difference in rice varieties including to growth period, the milling techniques and stabilization techniques [9], [10].

The data in Table I indicate that the high values of gamma oryzanol in dry rice bran to establish at the high value of total lipid too but it doesn't relate to the K value. It doesn't found the relationship between Gamma oryzanol and lipid content with the K value due to in each system had been the unity equilibrium.

TABLE I. QUANTITATIVE DETERMINATION OF TOTAL LIPIDS AND GAMMA ORYZANOL IN DIFFERENT RICE BRAN VARIETIES USING THE ADSORPTION COEFFICIENT WITH HEXANE

Rice bran varieties	Moisture (%w/w)	Total lipid (mg/g dry basis)	Gamma oryzanol (mg/g dry basis)	K
Sanphatong 1	10.29	41.98	2.96	3.18
Maejo 2	9.32	16.52	1.15	14.19
Maejo 4	9.34	23.15	2.32	7.70
Maejo 6	9.64	23.46	1.88	0.92

C. The Composition of γ-Oryzanol

In order to identify the gamma oryzanol components of crude RBO extracted by hexane using the adsorption coefficient were analyzed by UV-Vis detector. A typical chromatogram of gamma oryzanol in a crude RBO sample is shown in Fig. 2. Retention times and identification of the components are shown in Table II. The composition shows that the 24-methylene Cycloartenyl ferulate, Δ^7-Campestenyl ferulate, Campestenyl ferulate and Δ^7-Sitostenyl ferulate. The patterns of chromatogram were in good agreement with reported in literature [1], [11], [12,].

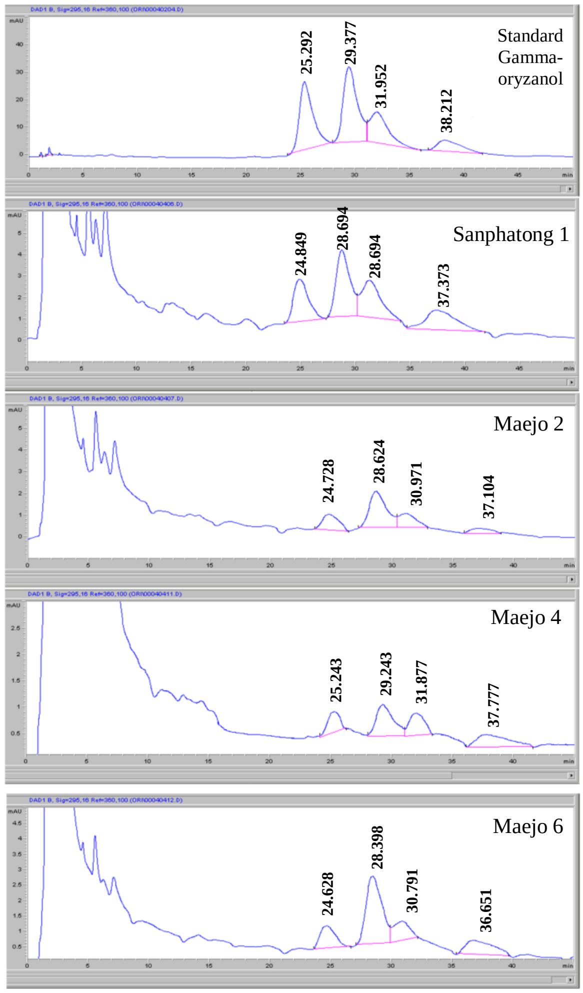

Figure 2. Chromatograms of HPLC of the standard Gamma-oyzanol and hexane extracts of the typical rice bran verities

TABLE II. ANALYSIS OF GAMMA ORYZANOL COMPOUND IN RBO EXTRACTED

Compound	Rice bran varieties /Retention time (min)				
	Standard Oryzanol	Sanpha-tong 1	Maejo2	Maejo4	Maejo6
24-methylene -Cycloartenyl ferulate	25.292	24.849	24.728	25.243	24.628
Δ^7-Campestenyl ferulate	29.377	28.694	28.624	29.243	28.398
Campestenyl ferulate	31.952	31.233	30.971	31.877	30.791
Δ^7- Sitostenyl ferulate	38.212	37.373	37.104	37.777	36.651

IV. CONCLUSIONS

The solid – liquid extraction by adsorption coefficient method can be used for determination of total lipid and γ-oryzanol in rice bran. The method is simple, quickly and the consumption of solvents was largely reduced. It was found that the optimization of equilibrium time is important for these extract technique to accuracy and save time consuming. For the determination and analyzation of gamma oryzanol and total lipid in rice bran using extraction by adsorption coefficient, the amount of the both extracted and gamma oryzanol composition are closely with previous reported.

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