วิธีการสกัดเพียงบางส่วนสำหรับการวิเคราะห์ปริมาณกรดเฟอรูลิกในรำข้าว

Partial extraction method for quantifying ferulic acid in rice bran

ชลันธร เศวตวงศ์ [1] สุภัทรา ลิลิตชาญ[1] พัชราณี ภวัตกุล[1] กรณ์กนก อายุสุข[2] และคณิต กฤษณังกูร[2]

Chaluntorn Sawetavong[1], Supathra Lilitchan[1], Patcharanee Pavadhgul[1] Kornkanok Ayusuk[2] and Kanit Krisnangkura[2]

บทคัดย่อ

โดยทั่วไปการวิเคราะห์กรดเฟอรูลิกในรำข้าวนิยมใช้วิธีการสกัดด้วยตัวทำละลายอินทรีย์ หรือวิธี Soxhlet extraction แต่วิธีนี้ใช้เวลานาน และมีความยุ่งยากซับซ้อน ดังนั้นงานวิจัยนี้จึงสนใจพัฒนาวิธีวิเคราะห์กรดเฟอรูลิกในรำข้าว โดยอาศัยหลักการสกัดด้วยตัวทำละลายเพียงบางส่วนร่วมกับการวิเคราะห์สารด้วยเทคนิค HPLC จากผลการวิเคราะห์ปริมาณกรดเฟอรูลิกในรำข้าวสายพันธุ์ กข 5 ด้วยวิธีการสกัดเพียงบางส่วนด้วยตัวทำละลายเมทานอลมี 0.38 มิลลิกรัม/รำข้าว 1 กรัม (น้ำหนักแห้ง) ซึ่งมีค่าใกล้เคียงกับการหาปริมาณกรดเฟอรูลิกด้วยวิธี Soxhlet extraction ซึ่งมีค่าเท่ากับ 0.33 มิลลิกรัม/รำข้าว 1 กรัม (น้ำหนักแห้ง) โดยวิธีใหม่ที่นำเสนอนี้สามารถลดปริมาตรของตัวทำละลาย ลดเวลา และไม่ต้องใช้เครื่องมือเฉพาะสำหรับสกัดสาร นอกจากนี้ในงานวิจัยนี้ได้วิเคราะห์หาปริมาณกรดเฟอรูลิกโดยวิธีการสกัดเพียงบางส่วนด้วยตัวทำละลายเมทานอลในรำข้าวสายพันธุ์ต่างๆ 5 สายพันธุ์ พบว่ารำข้าวสายพันธุ์ปราจีนบุรี 7 มีปริมาณกรดเฟอรูลิกสูงสุด ในขณะที่รำข้าวสายพันธุ์ กข 5 มีปริมาณกรดเฟอรูลิกต่ำสุด โดยค่า K_d ของกรดเฟอรูลิกในรำข้าวที่อุณหภูมิ 30 องศาเซลเซียส มีค่าอยู่ระหว่าง 1.73-3.36

ABSTRACT

Ferulic acid in rice bran is usually quantified using separation techniques such as solvent extraction or Soxhlet extraction method but these methods are time-consuming and complicated. Therefore, the objective of this study is to develop a method for determination of ferulic acid content in the rice bran using partial extraction and HPLC. The determination of ferulic acid in RD 5 rice bran variety by using methanol partial extraction was 0.38 mg/g (dry weight basis), similar to that determined using Soxhlet extraction method (0.33 mg/g). The proposed method consumes less hazardous organic solvent, takes shorter extraction time and requires no special extraction apparatus. Ferulic acid in 5 rice bran varieties was analyzed by the developed technique. Prachin Buri 7 variety had the highest ferulic acid while the lowest was found in RD 5. The K_d value of the ferulic acid between methanol and bran at 30 °C are between 1.73-3.36.

Key Words: Ferulic acid, Rice bran, Adsorption coefficient, Partial extraction

C. Sawetavong: chalunt@yahoo.com

[1] ภาควิชาโภชนวิทยา คณะสาธารณสุขศาสตร์ มหาวิทยาลัยมหิดล

Department of Nutrition, Faculty of Public Health, Mahidol University.

[2] สายวิชาเทคโนโลยีชีวเคมี คณะทรัพยากรชีวภาพและเทคโนโลยี มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี

Biochemical Technology Division, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi.

INTRODUCTION

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is a phenolic compound commonly found in fruits, vegetables and grains (Herrmann K, 1989; Graf, 1992). Ferulic acid have beneficial physiological effects such as antioxidant, anti-microbial, anti-inflammatory, anti-thrombosis anti-cancer, anti-diabetic, anti-ageing and anti-atherogenic activities (Ou and Kwok, 2004). It is widely used in food, pharmaceutical and cosmetic industries (Graf, 1992).

The extraction method of ferulic acid has many methods. Most extraction method is solvent extraction. The major disadvantage of solvent extraction technique is the use of large volumes, long extraction time, expensive, toxic and high-purity organic solvent. Recently, Lilitchan et al. (2008) developed method for the determination of the total lipids and γ-oryzanol contents in rice bran by partial extraction method. The results have shown that the partial extraction method can be used in the analytical laboratory for determination of total lipids and γ-oryzanol in rice bran with accuracy comparable to that determined by classical solvent extraction methods. This proposed method is simple, rapid, economical and requires no special extraction apparatus. Thus, the objective of this study interest to extend the partial extraction method of Lilitchan et al. (2008) for the determination of ferulic acid in the bran from different rice varieties using partial extraction with the aid of adsorption coefficient (K_d) and HPLC.

Theory

The ferulic acid content in each extract obtained from partial extraction and its liquid/solid adsorption coefficient (K_d) are determined by solving two simultaneous equations as described below.

According to the definition of K_d:

$$K_d = \frac{C_m}{A_s} \quad (1)$$

$$K_d = \left(\frac{M_m}{V_m}\right)\left(\frac{g_s}{M_s}\right) \quad (2)$$

when K_d is the adsorption coefficient

C_m is the concentration of the solute in organic solvent (g/l)

A_s is the amount of the solute (M_s) being adsorbed by the adsorbent = M_s/g_s

M_m is the amount of the solute in organic solvent (g)

M_s is the amount of the solute in solid phase (g)

V_m is the volume of organic solvent (l)

g_s is the weight of solid phase (g)

Obtaining of the K_d value in Eq.1 is essential for determination of the amount of substance in the sample and it can be solved by two simultaneous mathematic equations.

In the case of two samples of same weight (1.0 g), the volumes of the solvents are double in one sample.

If defining, Y = the total mass of the solute in the original sample

X_1 = the mass of the solute in supernatant of the first sample

X_2 = the mass of the solute in supernatant of the second sample vial (the volume of the solvent is double)

Substitution these values into Eq.(2)

$$K_1 = \left(\frac{X_1}{V_1}\right)\left(\frac{1}{Y - X_1}\right) \quad (3)$$

and

$$K_2 = \left(\frac{X_2}{V_2}\right)\left(\frac{1}{Y - X_2}\right) \quad (4)$$

but $K_1 = K_2$ and $V_2 = 2V_1$ therefore,

$$\left(\frac{X_1}{V_1(Y - X_1)}\right) = \left(\frac{X_2}{V_2(Y - X_2)}\right) \quad (5)$$

rearranging Eq.(5),

$$Y = \frac{X_1 X_2}{2X_1 - X_2} \quad (6)$$

Therefore, The Y value can be calculated from the Eq.(6). Substitution the Y value into Eq. (3) or (4), K_d is obtained.

MATERIALS AND METHODS

1. Materials

Rice bran samples (RD 5, Hom Mali , Hom Nin, Khao Dawk Mali 105, Prachin Buri 7) were obtained from Center of Excellence on Rice Molecular Breeding and Product Development, Kasetsart University Kamphangsaen Campus and local rice mill from Nakhonnayok and Amnatcharoen. Rice bran samples were passed through sieve screen and stored in polyethylene bags at 4 °C in a refrigerator. Ferulic acid was purchased from Fluka Chemical (Buchs, Switerland). Solvents (analytical

and HPLC grade) were products of Merck (Darmstadt, Germany). Moisture was determined by drying at 105 °C until constant weight was obtained.

2. Extraction of ferulic acid in rice bran

2.1 Soxhlet extraction

Ferulic acid content of rice bran extract was carried out according to Renuka Devi and Arumughan (2007).

2.2 Partial extraction

The amount of ferulic acid in rice bran was using partial extraction method following a reported method of Lilitchan et al (2008). Rice bran (1.0 g) were weighed into two identical vials and extracted with methanol in 4 and 8 ml. Vortex mixing for 1 min at room temperature and removed in water bath for 3 min at 30°C. The samples were centrifuged for 5 min at 2,500 rpm. The rice bran extract was filtered through a 0.45 µm pore size syringe filter before injection into the HPLC column.

The ferulic acid determination was calculated in the equation (6) for determined Y value and The calculation of the K value was obtained from Y value was added in equation (3) or (4).

2.3 Analysis of ferulic acid by HPLC

The RP-HPLC consisted of Waters 510 HPLC pump (Waters, USA), 7125 model Rheodyne injector (Cotati, California, USA) with a 20 µL sample loop, Pinnacle DB C18 (5 µm 250×4.6 mm), Waters 2487 UV spectrophotometer (Waters, USA) with mobile phase of methanol/acetic acid (99.5:0.5 v/v), flow rate 1.2 ml/min and UV detector at 326 nm. Ferulic acid in the extracts was quantified against the standard curve.

RESULTS AND DISCUSSION

1. Linearity and calibration curve

The maximum adsorption of ferulic acid in methanol was located at 326 nm. The calibration curve is linear between 0.001-0.1 mg/ml for ferulic acid (Figure 1). A correlation coefficient (R^2) of greater than 0.999 was obtained.

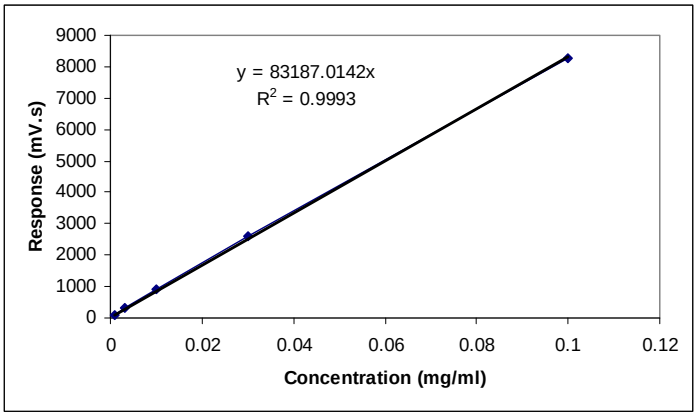

Fig 1. Standard calibration curve

2. Ferulic acid content

The ferulic acid content in rice bran obtained by Soxhlet extraction and partial extraction are listed in Table 1.

Table 1. Comparison of the ferulic acid content in rice bran using Soxhlet extraction and partial extraction with methanol

Extraction method	Ferulic acid	
	Content (mg/g)	K_d
1. Soxhlet extraction	0.39 ± 0.009	-
2. Partial extraction	0.38 ± 0.001	3.36 ± 0.26

Data represented mean ± standard deviation (n = 3).

These results showed that the content of ferulic acid by using partial extraction was approximately the same as the Soxhlet extraction. There were no significant differences between the Soxhlet extraction and partial extraction. Thus, it was concluded that the partial extraction method was reliably extract and quantify ferulic acid in rice bran.

The amount of ferulic acid of rice bran obtained by partial extraction agree well with those reported by Renuka Devi and Arumughan (2007) was 0.23-0.26 mg/g and Butsat and Siriamornpun (2010) was 0.21-0.33 mg/g.

The proposed method consumes less hazardous organic solvent, takes shorter extraction time and requires no special extraction apparatus.

In addition, the K_d value of ferulic acid between rice bran and methanol can be also determined by this method. So this method was applied to the analysis of the ferulic acid content in five rice bran varieties.

3. Quantification of rice bran samples

The moisture content of rice bran is in the range 10-13 % (w/w). The amount of ferulic acid in five rice bran varieties was reported in Table 2. Ferulic acid content was in ranged of 0.38-0.59 mg/g (dry weight basis). The highest ferulic acid content was observed for Prachin Buri 7 while the lowest found in RD 5. The amount of ferulic acid from different rice varieties are slightly higer than those studies of Renuka Devi and Arumughan (2007) and Butsat and Siriamornpun (2010). Potential reasons for these variations include differences in genotypes and the milling techniques (Malekian et al, 2000).

Table 2. Quantitative determination of ferulic acid content from different rice bran varieties using the partial extraction with methanol

Rice bran sample	Moisture (% w/w)	Ferulic acid	
		Content (mg/g)	K_d
1. RD 5	12.42 ± 0.31	0.38 ± 0.001	3.36 ± 0.26
2. Hom Mali	10.43 ± 0.15	0.55 ± 0.002	1.98 ± 0.10
3. Hom Nin	10.28 ± 0.17	0.56 ± 0.011	1.73 ± 0.19
4. Khao Dawk Mali 105	11.55 ± 0.03	0.48 ± 0.003	2.20 ± 0.15
5. Prachin Buri 7	13.83 ± 0.13	0.59 ± 0.056	2.58 ± 0.06

Data represented mean ± standard deviation (n = 3).

The ferulic acid adsorption coefficient from different varieties are slightly varied. The sources of these variations are not studied. However, the proposed partial extraction method provides a rapid and accurate method for determination of the amounts of ferulic acid in rice bran and it may be used to differentiate rice varieties.

In addition, knowledge of the K_d value can be used to forecast the amount of substance in the sample.

Table 3. Comparision of experimental value and calculated value of ferulic acid content from different rice bran

Rice bran Sample	Content of ferulic acid (mg/g)		% Deviation
	Experimental value	Calculated value *	
1. RD 5	0.38 ± 0.001	0.38 ± 0.006	0.00
2. Hom Mali	0.55 ± 0.002	0.57 ± 0.017	3.64
3. Hom Nin	0.56 ± 0.011	0.58 ± 0.016	3.57
4. Khao Dawk Mali 105	0.48 ± 0.003	0.47 ± 0.017	-2.08
5. Prachin Buri 7	0.59 ± 0.056	0.58 ± 0.007	-1.69

* Calculated value was obtain from equation (3) or (4) by using K_d value.

Data represented mean ± standard deviation (n = 3).

The amount of ferulic acid in rice bran are calculated and listed in Table 3. All the calculated values are in good agreement with the experimental values. The highest deviation is only 3.64 %

Besides, ferulic acid was estimated from all the rice varieties suggesting its potential uses for material in the functional food and nutracuetical industries.

CONCLUSION

This study has shown that partial extraction can be used in the analytical laboratory for determination of ferulic acid in rice bran with accuracy equal to Soxhlet extraction method. This method is rapid and economical.

Prachin Buri 7 variety had the highest ferulic acid while the lowest was found in RD 5. The K_d value of the ferulic acid between methanol and bran at 30 $^{\circ}$C are between 1.73-3.36. Then, All rice brans are a good source for ferulic acid.

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