Quantitative Analysis of Rutin in Buckwheat \textit{(Fagopyrum sp.)} by High Performance Liquid Chromatography

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Summary. For the quantitative analysis of rutin in common buckwheat \textit{(Fagopyrum esculentum)} and tartary buckwheat \textit{(F. tataricum)}, the operating condition of high performance liquid chromatography (HPLC) and methods of sample preparation and extraction were investigated. Reliable analysis method with less than 5 g of sample was established as follows; (1) drying samples for 24 hours at 70°C by using forced air flow oven, (2) grinding 5 g of seed and 2 g of leaf samples into powder for 30 seconds, (3) extracting rutin from 1.0 g of common buckwheat powder with 10 ml of methyl alcohol and from 0.5 mg of tartary buckwheat seed powder and common buckwheat leaf powder with 20 ml of methyl alcohol at 70°C for 30 min. by using Soxhelt extraction apparatus and water bath, and (4) injecting 20 μl of supernatant fluid obtained by centrifugation into chromatograph. The method has high reliability with about 5% of coefficient of variation in rutin content. Establishment of quantitative analysis method of rutin with small amount of seed sample less than 5 g by using HPLC will contribute to the progress of breeding for high rutin content buckwheat varieties.

Keywords: \textit{Fagopyrum esculentum, Fagopyrum tataricum}, buckwheat, rutin content, HPLC analysis

Introduction

Rutin is a kind of flavonol glycoside compound. The main biological property of rutin is to antagonize the increase of capillary fragility associated with some hemorrhagic diseases or hypertension in man\textsuperscript{1234}. Though rutin is contained broadly in plant species\textsuperscript{56}, buckwheat is only one edible source of rutin. Therefore, breeding of high rutin content varieties is one of the valuable breeding objectives of buckwheat. To achieve this breeding objective, a convenient and reliable quantitative analysis method of rutin must be established.

This paper describes a method for the quantitative analysis of rutin in seed and leaf of common buckwheat \textit{(Fagopyrum esculentum)} and tartary buckwheat \textit{(F.tataricum)} by high performance liquid chromatography (HPLC).
evaluation of reproducibility of HPLC analysis.

2. Sample preparation and rutin extraction

**Plant materials**: Common buckwheat and tartary buckwheat were cultivated with a conventional method in the Experimental Farm of Faculty of Agriculture, Shinshu University, in 1992. Fresh leaves 30 days after sowing and full ripe seeds were collected and stored immediately at 30°C for the determination of rutin content. Thereafter, these samples were dried and ground into powder, from which rutin was extracted. The change in rutin content during these processes was checked.

**Drying time**: Changes in weight of sample and rutin content with drying time were measured. Of the frozen sample of common buckwheat, 15 g of seeds (cv. Shinshu-ohsoba) and leaves (cv. Shinano No.1) were sampled and dried in a forced air flow oven (WFO-600ND, Tokyo Rika) at 70°C. From 3 to 24 hours, the weight of sample was measured 6 times and then the rutin content of these samples was analyzed to evaluate the effect of heat drying on rutin content. For the control, the freeze-dried sample was used.

**Sample weight and grinding time**: Seeds and leaves of common buckwheat (cv. Shinshu-ohsoba) and seeds of tartary buckwheat (Nepal local) were dried at 70°C for 24 hours. One to 5 g of these samples were ground into powder, respectively, for 30 seconds by the electric mill (MK-52 M, National), and similarly 5 g of seeds and 2 g of leaves from the same samples were ground into powder for 10, 30, 60 and 120 seconds, respectively. Then their rutin contents were analyzed to make clear the effect of sample weight and grinding time on rutin content.

**Extraction time**: Five grams of seeds and 2 g of leaves were ground for 30 seconds. Rutin was extracted from 1.0 g of the powder with 10 ml of methyl alcohol for the common buckwheat seeds and 0.5 g of the powder with 20 ml of methyl alcohol for the tartary buckwheat seeds and common buckwheat leaves. The powder was decocted for 5 to 60 minutes at 70°C, by using Soxhelt extraction apparatus and water bath (LB-260, TOYO), and then the rutin content of the extract was measured and compared with that of the control in which the extraction was carried out for 120 minutes at room temperature (20°C).

After centrifugation of the extract by CFM-100 (IWAKI), supernatant fluid was used as sample for HPLC analysis.

3. Estimation of experimental error

To evaluate the reliability of the sample preparation method, 2 and 5 g of leaves and seeds were sampled 6 to 24 times from the same samples and ground. Their rutin contents were analyzed and the coefficients of variation in rutin content were calculated.

**Results and Discussion**

1. Reproducibility of HPLC analysis of rutin content

Fig.1 shows the elution pattern of rutin. Rutin was eluted 12 minutes after injection. A linear relationship was obtained between the rutin content and the peak area \( r = 0.998 \) \( p < 0.001 \). Fig.2. The coefficient of variation in the values of peak area obtained by repeated standard rutin injection was calculated.

![Fig. 1. Elution pattern of standard preparation of rutin.](image)

![Fig. 2. Calibration graph for rutin.](image)
tions was only 0.97% and the rutin contents of all samples of seeds and leaves analyzed were included within this calibration range. Therefore, HPLC equipment and operating conditions described above was realized to have high reproducibility and availability.

2. Sample preparation

Drying time: As shown in Fig.3A, the weight of sample became constant after 6 hours drying in seeds and 13 hours in leaves. Rutin content decreased 1.6 and 2.0% in seeds and 6.7 and 7.0% in leaves by 13 and 24 hours drying, respectively (Fig.3B). Because the differences in rutin content were small between 13 and 24 hours drying, the latter was adopted for convenience of daily routine work.

Sample weight and grinding time: Both the seed and leaf samples showed the highest rutin content when 5 g of sample was used and the grinding time was 30 seconds (Fig.4A,B). The effects of sample weight and grinding time on the rutin content was a little in common buckwheat leaf and tartary buckwheat seed. On the other hand, a remarkable change in rutin content was observed in common buckwheat seed samples. This might be due to very low rutin content in common buckwheat seeds compared with those in tartary buckwheat seeds and common buckwheat leaves (Table 1). From these results, grinding 5 g of sample for 30 seconds is thought to be the best for both seed and leaf samples. In spite of this conclusion, 2 g of sample weight was adopted for leaf, because collecting more than 100 fresh leaves for a 5 g of dried leaf sample was so laborious and difference in the rutin content was a little between 2 and 5 g samples.

Extraction time: Rutin contents were the highest at 20 to 30 minutes extraction in all samples (Fig.4C). Compared with the extraction at 70°C, 25~35% and 70% of rutin were extracted in seed and leaf, respectively, at room temperature.

Fig. 3. Changes in weight of sample (A) and rutin content (B) of common buckwheat with drying at 70°C in forced air flow oven.

1) Percentage to the rutin content of freeze-dried sample.

Fig. 4. Relationships of rutin extraction efficiency with sample weight (A), grinding time (B) and extraction time (C) in buckwheat.

1) Percentage to the maximal value obtained in the same sample.
Table 1. The experimental error in HPLC analysis of rutin content.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample weight (g)</th>
<th>No. of replications</th>
<th>Mean (mg/100gDW)</th>
<th>S.D.¹ (mg/100gDW)</th>
<th>C.V.² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>leaf</td>
<td>seed</td>
<td></td>
<td>seed</td>
<td></td>
</tr>
<tr>
<td>Common buckwheat</td>
<td>2</td>
<td>6</td>
<td>3229</td>
<td>107</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>seed</td>
<td>11</td>
<td>13.6</td>
<td>1.4</td>
<td>10.8</td>
</tr>
<tr>
<td>Tartary buckwheat</td>
<td>5</td>
<td>24</td>
<td>15.4</td>
<td>0.9</td>
<td>6.1</td>
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<tr>
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<td>24</td>
<td>5</td>
<td>1523</td>
<td>38</td>
<td>2.5</td>
</tr>
</tbody>
</table>

¹) Standard deviation  ²) Coefficient of variation

From these results, rutin extraction was made at 70°C for 30 minutes.

3. Reliability of the analysis

Coefficients of variation of rutin content of the same samples were about 5% except for the 2 g sample of common buckwheat seed (Table 1). This indicates that the analysis method used in this experiment has high reliability. Establishment of the quantitative analysis method of rutin content with a small amount of seed sample less than 5 g by using HPLC will contribute to the progress of breeding for high rutin content buckwheat varieties.

Literature Cited

高速液体クロマトグラフィーによるソバ (Fagopyrum sp.) 中のルチンの定量分析

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要約
ソバのルチン含量を高速液体クロマトグラフィー（HPLC）で定量分析するための分析条件、試料の調製法および抽出法を検討し、5fg以下の試料で信頼性のある分析法を確立した。手順は以下の通りである。①試料を70℃で24時間乾燥する，②種子では5g，葉では2gの乾燥試料を30秒間粉砕する，③普通ソバ種子では1.0gの粉末試料を10mlのメタノールで，ダッタンソバの種子および両種の葉では0.5gの粉末試料を20mlのメタノールで，70℃，30分間抽出する，④遠心分離後の上澄み液を実証溶液とし，20μlをHPLCに注入，分析する。この方法により変動係数5%前後の測定が可能となった。5g以下の試料での分析法の確立は個体選抜を可能にし，高ルチン含量ソバ品種の育成に寄与すると考えられる。

キーワード：Fagopyrum esculentum，Fagopyrum tataricum，ソバ，ルチン含量，HPLC分析