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TG-DTG AS AN EFFECTIVE METHOD FOR THE CHARACTERIZATION OF RUTIN EXTRACTED FROM THE BUDS OF *SOPHORA JAPONICA* L.

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The thermal behavior of rutin extracted from the buds of *Sophora japonica* L. by different methods and conditions have been investigated using TG and DTG. The results showed that every sample had different mass loss, curve shape, and peak location related to varied extraction technology. The TG-DTG characteristics of the rutin sample extracted by alkali-dissolution and acid-sedimentation with the solution adjusted to pH 9 and simply borax as stabilizer were highly similar to that of standard rutin, with the maximal purity determined by spectrophotometry. Therefore, the TG-DTG patterns could be served to characterize rutin extracted from the buds of *Sophora japonica* L.

Keywords: *buds of Sophora japonica L., rutin, TG-DTG*

Introduction

Buds of *Sophora japonica* L., locally known as 'Huaimi', have been used as Chinese folk remedies for over 2000 years. It exhibits excellent pharmaceutical properties, such as stopping bleeding and relieving excess fire in the liver, which are mainly attributed to its abundant flavonoids constituents [1]. Flavonoids are products of secondary metabolism in plants and are of interest to the pharmaceutical and food industries because of their reported wide range of biological effects [2, 3]. In the buds of *Sophora japonica* L., flavonoids are presented in the main form of rutin, which is a flavonol glycoside comprised of the flavonol backbone and the glycon rutinose. Modern researches have proved that rutin have the efficacies of strengthening fragile capillaries, reducing cholesterol, improving blood circulation and resisting inflammation, etc. [4]. It is often used in the treatment of increased capillary fragility associated with hypertension in humans. Typically, rutin have been extracted from the buds of *Sophora japonica* L. using alkali-dissolution and acid-sedimentation, ethanol reflux and ultrasonic-assisted extraction methods [5, 6]. However, the action of the extraction process can cause alterations at the original chemical composition in that part of the plant that is being studied, therefore influence the physical and chemical characteristic of the compounds presented. Accordingly, the quality control of rutin extracted from the buds of *Sophora japonica* L. employing different methods and conditions has become a necessary undertaking.

Although HPLC has always been used for the analysis of rutin flavonoids from the buds of *Sophora japonica* L. [7], the use of HPLC to analyze traditional Chinese medicine has several shortcomings such as long analysis time, low resolution, and short column lifetime, owing to easily contamination [8]. Nowadays, thermal analysis (TA) is increasingly recognized as an important analytical technique because of its simple, high-efficiency, ultra-small sample volume, and ease of sample preparation as well as curve analysis [9, 10]. Thus, TA has been widely applied in the drug analyzing fields such as chemical identification, determination of the physical and chemical constants, the investigation of active compounds of traditional Chinese medicine and quality controlling of herb medicine [11–13]. However, to the best of our knowledge, few researches have been published on the application of TA technique in the quality controlling of rutin extracted from the buds of *Sophora japonica* L. In this work, rutin extracted via different methods and conditions were studied by means of TG-DTG curves in order to establish a simple and effective method to determine these products.

Experimental

Materials and chemicals

The buds of *Sophora japonica* L. (Tianjin Pharmaceutical Holdings, Ltd.) were powdered to pass through 250 μm screen and dried in a convection oven at 60°C until mass constancy. Standard rutin

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were purchased from Tianjin Codex and Standard Instrument Corporation. All the chemicals used including calcium oxide, borax, sodium metabisulfite, ethanol, ether and acetone were of analytical grade.

Sample preparation

The alkali-dissolution and acid-sedimentation, ethanol reflux and ultrasound-assisted extraction methods employed are summarized as follows, and all experiments were performed in replicate.

Alkali-dissolution and acid-sedimentation

10 g of dried buds power were extracted with 200 mL of limewater (pH=9). Sodium metabisulfite and borax were added into the system in order to prevent hydroxyl from being oxidized. The residue was separated by vacuum filtration and extracted again under the same conditions. Subsequently, the filtrate of the twice-extraction was mixed and acidified (pH 2–3) using 1 M HCl to precipitate the rutin and left to stand overnight. The acid-insoluble rutin was washed with distilled water and dried in a vacuum drying oven at 65°C.

The pH value of extracting solution was adjusted to 8, 9 and 10 using limewater in order to prepare rutin samples extracted with different alkaline conditions.

The usage of stabilizer was designed as adding both borax and sodium metabisulfite, simply borax and without stabilizer, in order to prepare rutin samples extracted with different stabilizer conditions.

Ethanol reflux

10 g of dried buds power were extracted with 70% *v*/*v* aqueous ethanol (100 mL) at 65±1°C for 2 h, and repeated the above operation once. The filtrate of the twice-extraction were mixed and concentrated in a rotary evaporator at 50°C under reduced pressure to remove half of the ethanol, and then deposited overnight at 4°C. Subsequently, the crystal rutin was filtered and washed with 5 mL ether followed by 5 mL acetone. The resulting washing extract was collected and dried in vacuum at 65°C.

Ultrasound-assisted extraction

An ultrasound cleaning bath KQ3200DE (China Kunshan ultrasound instrument Co., Ltd), which has a frequency of 40 kHz and a maximum peak power of 120 W, was used for extracting rutin. The ultrasound-assisted extraction was performed under the same conditions as alkali-dissolution and acid-sedimentation but with ultrasonic treatment in the ultrasonic cleaning bath at ultrasonic power of 120 W and bath temperature at 30±1°C for 30 min.

Methods

Quantitative analysis of the rutin

The measurement of rutin followed the spectrophotometry described in [14]. The method was based on a color reaction of rutin with $Al(NO_3)_3$ in alkaline environment. The concentration of rutin was determined with a spectrophotometer (SP-2000UV, China) at 510 nm against a calibration curve established with standard rutin. The results were reported as averages each of two replications. The content value of rutin extracts were estimated as the ratio of the mass of rutin to the mass of dried extracts.

Thermogravimetric analysis

Thermogravimetric data (TG and DTG) of standard rutin and different samples were determined by a Pyris/Diamond TG analyzer (produced by Perkin-Elmer). All TG–DTG tests were performed under a dynamic atmosphere of dry nitrogen at a flow rate of 150 mL min^{-1} over a temperature interval of 25.0–800.0°C at a heating rate of 10°C min^{-1}. Platinum crucibles were used to hold 5.0±0.5 mg samples for analyses.

Results and discussion

TG-DTG characteristic of standard rutin

TG-DTG curves of standard rutin showed that the thermal decomposition of it occurred in four stages. The first stage showed a weak mass loss step, associated with the loss of the loosely absorbed water on the particle surface, which ended at 134°C. Between 134 and 333°C, rutin lost two water molecules to become anhydrous, then decomposed with effervescence [15]. Further mass loss of the complexes occurred in the range of 333–498°C, when the evaporation of rutin took place. Finally, the compound was gradually decomposed into the final product.

TG-DTG characteristic of rutin samples extracted by different methods

TG-DTG curves of rutin samples obtained by different extraction methods were represented in Fig. 1. As shown, compared with sample C and D, the TG-DTG curves of sample B were more similar with that of standard rutin. However, the peak temperature at the first dehydration of the DTG curve was much higher for C than for standard rutin, which could be explained by the co-extracted impurity, such as betulin and sophoradiol, which were easily released during the ethanol reflux. As for D, the shape of its DTG main peak was quite distinct when compared with standard rutin, indicating that the quality of sample D was significantly affected under the ultrasonic conditions given in ‘Ultrasound-assisted extraction’. The content of rutin samples extracted with different methods were presented in Table 1. As can be seen, the rutin content of B extracted by alkali-dissolution and acid-sedimentation was 82.5%, as the highest among all the samples. Whereas, the observed reduction of the rutin content of the extracts isolated by ethanol reflux and ultrasonic-assisted extraction was due to the by-products and the decomposition of effective ingredients during ultrasonic treatment, respectively.

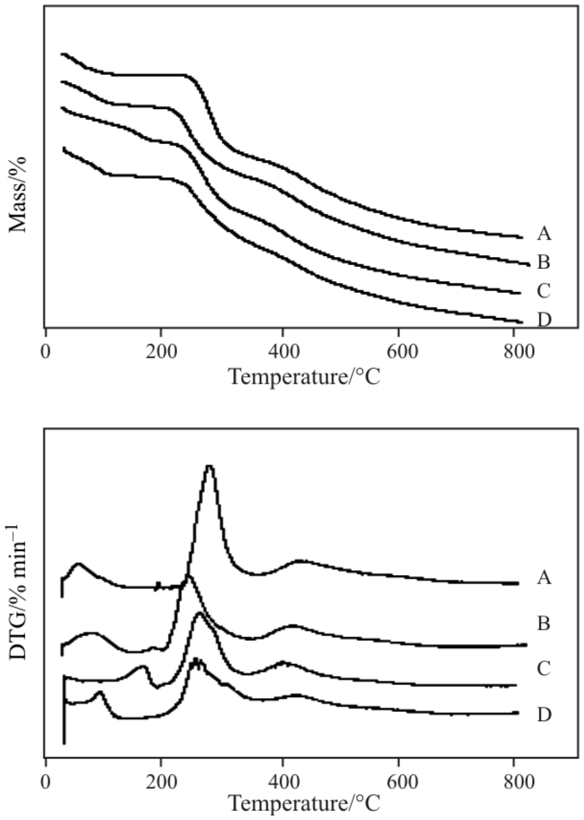

Fig. 1 TG-DTG curves of the samples extracted by different methods; A – standard rutin, B – extract by alkali-dissolving and acid-sedimentation, C – extract by ethanol reflux, D – extract by ultrasound-assisted method

Furthermore, with standard rutin as reference, the analysis results about the initial-end mass loss temperatures and the mass loss rate of each rutin sample were given in Table 1. Firstly, the mass loss below 134°C of sample D was notably higher than the observed in standard rutin during the first dehydration stage. This increase was related with an increase of the amount of the absorbed water, due to the increase in the specific surface area caused by ultrasonic treatment. In the second stage, the TG curves of all the samples showed sharp thermal mass loss stages, as predicted by their DTG curves. The mass loss of sample D was 23.64%, which was much lower than 29.71% of the standard rutin. As for DTG curves, the highest temperature of peak 2 in sample B and D moved to the lower side with the peak shapes a bit flattened. Especially, DTG curve of D exhibited multistep loss processes, which might reflected the lower content of the ultrasonic extracting sample. In the third and fourth stages, the mass loss of each sample was close to that of standard rutin. By analyzing the parameters of mass loss rate, combined with peak patterns, sample B showed the most similar TG-DTG characteristic with standard rutin. Therefore, the alkali-dissolution and acid-sedimentation method was proved to be the optimal.

TG-DTG characteristic of rutin samples extracted with different pH

The TG-DTG curves of rutin samples extracted with different pH values of solution were represented in Fig. 2. The TG-DTG curves of F were mainly in accordance with that of standard rutin. However, sample E behaved differently than the other two samples. Obviously, its second peak given in DTG curve moved to the lower side and decreased in intensity, followed with an additional flattened peak originated at 288°C, which was not observed at standard rutin. This might be an indication of other chemical components coexisted with rutin in sample E. The rutin con-

Table 1 Rutin content and thermogravimetric characteristics of B, C and D extracted by different methods

Entry[*]	Rutin content/%	Mass loss/% in each decomposition stage				Total mass loss/%
		First 24–134°C	Second 134–333°C	Third 333–498°C	Fourth 498–800°C	
A	99.72	7.74	29.71	16.29	14.55	68.26
B	82.53	8.98	26.21	17.19	15.37	67.76
C	67.82	7.77	31.61	17.40	12.46	69.24
D	59.01	9.37	23.64	17.13	13.73	63.87

[*] Entry A–D were as same as note of Fig. 1

tent of the samples extracted with different pH values (Table 2) revealed that the desirable extracting pH was 9 for its highest content of rutin. Whereas, the lowest purity of sample E extracted by weak alkali (pH 8) may be attributed to the simultaneous dissolution of rutin along with some neutral impurity during the extraction process. Besides, strong alkali (pH 10) was disadvantage to the extraction of rutin either, because the flavonol backbone was easily being destroyed when exposed to strong base environment during heating process.

Furthermore, with standard rutin as reference, the analysis results about the initial-end mass loss temperatures and the mass loss rate of each rutin sample were given in Table 2. In the first stage, the mass loss of all the samples E, F and G were somewhat higher than that of the standard rutin with sample E prominently. In the second stage, sharp thermal mass loss stages appeared both in samples F and G, as observed in standard rutin. Whereas the mass loss of F reached 29.43%, which was better closed to the standard one. However, a change in slope on the TG curve of sample E leaded to a lower mass loss of 25.32% in this stage. In the third decomposition stage, the mass loss of sample E overrun furthest, with the corresponding DTG curve sharper than the standard rutin. In the last stage, the samples decomposed gradually with the mass loss of sample E the lowest. Moreover, the total mass loss of F was very alike to standard rutin, compared with the other two samples. Taking the whole consideration, it can be seen that the TG-DTG characteristic appeared was strongly influenced by the alkaline condition of the extraction solution, with sample F of higher similarity with standard rutin. Hence, the best pH of extracting solution was selected as 9.

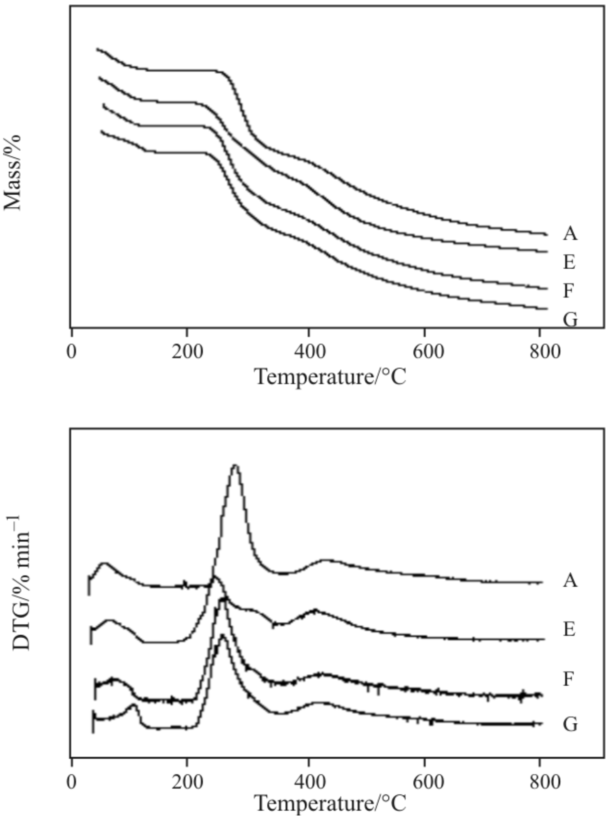

Fig. 2 TG-DTG curves of the samples extracted at different pH values; A – standard rutin, E – extract at pH=8, F – extract at pH=9, G – extract at pH=10

TG-DTG characteristic of rutin samples extracted with different stabilizer

Stabilizer was always used in the extraction of flavonoids in order to protect its flavonol backbone against degradation. Single stabilizer (borax) and compound stabilizer (borax and sodium metabisulfite) were the most commonly used ones in the extraction of rutin. TG-DTG curves of rutin samples extracted with different stabilizer were represented in Fig. 3. Both TG and DTG curves of sample I had a relatively similar shape with that of standard rutin indicating their related chemical composition. However, TG-DTG curves of sample H were not suitable, as the peak 2 of its DTG curve split into a double-overlapping peak and shifted toward lower temperature, which may be regarded as the degradation of flavonols backbone without the protection of stabilizer during extraction process. As for sample J, two slightly shoulder peaks appeared in its DTG curve at 217 and 250°C, respectively. The rutin content of

Table 2 Rutin content and thermogravimetric characteristics of E, F and G extracted with different pH values

Entry*	Rutin content/%	Mass loss/% in each decomposition stage				Total mass loss/%
		First 24–134°C	Second 134–333°C	Third 333–498°C	Fourth 498–800°C	
A	99.72	7.74	29.71	16.29	14.55	68.26
E	50.51	9.25	25.32	19.95	10.19	64.71
F	80.61	8.40	29.43	17.24	13.23	68.30
G	54.64	7.87	28.90	16.51	12.49	65.77

*Entry A, E–G were as same as note of Fig. 2

Table 3 Rutin content and thermogravimetric characteristics of H, I and J extracted with different stabilizer

Entry*	Rutin content/%	Mass loss/% in each decomposition stage				Total mass loss/%
		First 24–134°C	Second 134–333°C	Third 333–498°C	Fourth 498–800°C	
A	99.72	7.74	29.71	16.29	14.55	68.26
H	54.60	9.23	24.54	16.72	13.83	64.31
I	97.23	8.34	29.93	16.78	15.47	70.51
J	81.22	8.33	27.94	15.06	13.13	64.45

*Entry A, H–J were as same as note of Fig. 3

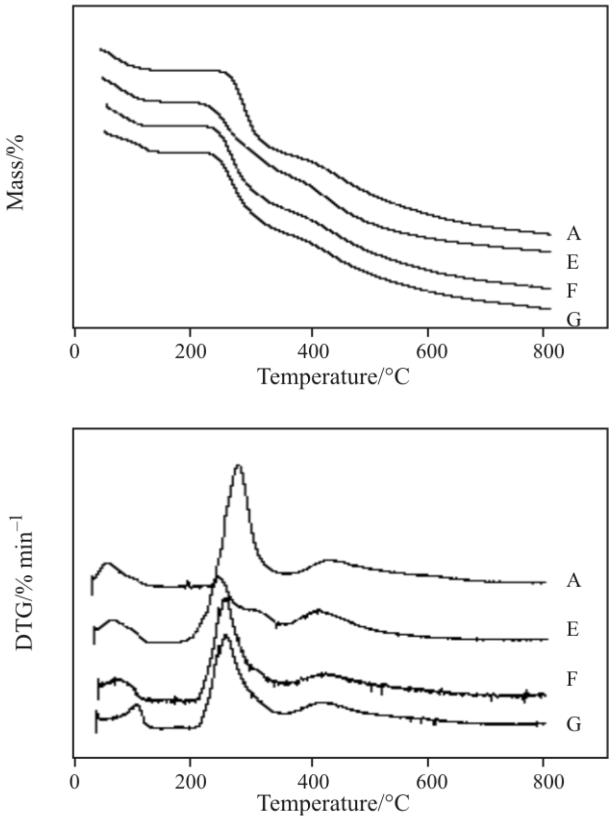

Fig. 3 TG-DTG curves of the samples extracted by different stabilizer; A – standard rutin; H – extracted without stabilizer, I – extract with borax as stabilizer, J – extract with borax and sodium metabisulfite as stabilizer

samples H, I and J listed in Table 3 further indicated that the purity of sample I extracted using single stabilizer borax was the highest among all the extracts.

Furthermore, with standard rutin as reference, the analysis results about the initial–end mass loss temperatures and the mass loss rate of each rutin sample were given in Table 3. In the first stage, a limited increase in the mass loss of samples H, I and J was detected. In the second stage, all the samples decomposed quickly, with the mass loss of sample I (29.93%) mostly closing to that of the standard rutin (29.71%). From the second mass loss step of sample H, we had observed a mild change in the slope of its TG curve, which was confirmed by its lower mass loss of 24.54%. In the third stage, the mass loss of samples H and I were in accordance with the standard rutin while that of sample J decreased to 15.06%. Further decomposition of these samples processed almost in the similar process. By comprehensive consideration, the thermal data in each decomposition stage, as well as the peak shape of sample I, was better closed to that of standard rutin than H and J. According to the above results, we considered that it was necessary to add stabilizers in the extracting of rutin, with borax as the best choice.

Conclusions

It was demonstrated that the thermal decomposition processes of rutin obtained from the buds of *Sophora japonica* L. were remarkably affected by the extraction methods and conditions studied in the present work. The TG-DTG characteristics of the products extracted by alkali-dissolution and acid-sedimentation with the solution adjusted to pH=9 and simply borax as stabilizer were similar to that of standard rutin, and the results were in good agreement with quantitative spectrophotometry. From the results we can recommend that the TG-DTG analysis was an appropriate method as a tool for quality control of different rutin samples extracted from the buds of *Sophora japonica* L.

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