A Simple Way for the Preparation of Natural Antioxidant Quercetin from Rutin by Subcritical Water

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Abstract

Context: Quercetin (QR) is a natural strong antioxidant of the plant origins. It is used for treating conditions of heart and blood vessels including “hardening of arteries” (atherosclerosis), high cholesterol, heart disease, circulation problems, preventing cancer, for treating chronic infections of the prostate, etc., Aims: The aim of this work was to development and study of an acid-free technique for the preparation of QR from rutin (RT) that requires no use of acids and toxic organic solvents. Materials and Methods: For the first time, the subcritical water that serves as a reactant and a solvent were used to obtain QR in good yields starting from RT. High-performance liquid chromatography combined with mass spectrometry was used to determine the quantitative and qualitative compositions of the obtained products. Conclusions: For the first time, a new acid-free technique was used for the synthesis of natural antioxidant QR from an RT by subcritical water. That way requires no use of acids and/or toxic organic solvents. It has been shown that variation of only one parameter of the process (temperature of subcritical water) allows alteration to the composition of the hydrolysis products. The new method developed for the production of QR in subcritical water is environmentally friendly and faster than conventional hydrolysis methods that use acidic or enzymatic hydrolysis. The proposed technique has a potential for the future development of inexpensive and environmentally friendly technologies for the production of new pharmaceutical plant-based substances.

Keywords: High-performance liquid chromatography–mass spectrometry, hydrolysis, quercetin, rutin, subcritical water

INTRODUCTION

Quercetin (QR) [3,3,4,5,7-pentahydroxyflavone] is one of several naturally-occurring dietary flavonol compounds belonging to a broad group of polyphenolic flavonoid substances [Figure 1]. Flavonol QR exhibits strong antioxidant properties and numerous biological and pharmacological effects, including antioxidant, chelation, anticarcinogenic, cardioprotective, bacteriostatic, and secretory properties.^{1,2} QR is a naturally occurring flavonol that has a long history of consumption as part of the normal human diet. Because a number of biological properties of QR may be beneficial to human health, interest in the addition of QR to various traditional food products has been increasing. Prospects for the widespread use of QR as a dietary supplement makes it necessary to search for low-cost and environmentally friendly production technologies of QR. Nowadays, there are two basic steps for preparation of QR as pharmacological substances or dietary additions: QR can be obtained from plants via extraction of the QR glycosides followed by acidic hydrolysis to release the aglycone and subsequent purification. For conventional synthesis of QR by chemical hydrolysis from its glycosides is usually applied mineral acids (acidic hydrolysis of rutin (RT) by phosphate acid, sulfate and hydrochloride acids). RT (quercetin-3-rhamnol glucoside) is a natural flavonoid (Vitamin P), which possesses an antioxidant activity with broad protective potential.^{3,4} It is low molecular weight polyphenolic compound that is widely distributed in vegetables and fruits. Also for production, QR enzymatic hydrolysis of RT (reaction time was 12–24 h)^{5} can be used. It should be noted that conventional acid hydrolysis of RT requires 2–3 h. Thus,
conventional methods of hydrolysis and enzymatic hydrolysis of RT require significant time costs. Along with this, purification is needed of the hydrolysis products. The alternative to the conventional methods of using acids to catalyze hydrolysis is to perform the reaction in subcritical water.[6] In recent years, subcritical water has been used as a cheap, environmentally friendly solvent for extraction, synthetic transformations and the recycling of different organic wastes as, for example, agricultural production process in wastes.[7]

The hydrothermal reaction has been attracting much attention because of the fascinating physical and chemical characteristics of water near its critical point. In these conditions, water exhibits a much lower dielectric constant and a much larger ion product. The ion product or dissociation constant is about three orders of magnitude higher near the critical point (in the temperature range from 220°C to 270°C) than it is for ambient liquid water. Under these conditions, there is a high H₂O⁺ and OH⁻ ion concentration. As such, some acid-catalyzed organic reactions can be carried out without the addition of acids. However, the ion product decreases greatly above the critical point. This fact makes subcritical water an ideal reaction medium for the hydrolysis of organic compounds. In this study, it is for the first time proposed to perform hydrolysis of the RT in a medium of subcritical water for the production of QR. The aim of this work was to study the suggested acid-free technique to obtain QR in good yields starting from RT.

**Materials and Methods**

Acetonitrile, methanol high-performance liquid chromatography (HPLC grade), water for HPLC/mass spectrometry (MS) spectrometry and RT (C₂₇H₃₀O₁₄, Mw 610.25 ≥ 96.6%) was purchased from Merck (Germany). Hydrochloric acid was purchased from JSC “Vekton” (Russia). QR (C₁₅H₁₀O₄, Mw 302.10, ≥98.2%) was purchased from JSC “Diaem” (Russia). HPLC analysis was performed using Agilent 1200 LC system (ZORBAX SB C₁₈, 150 mm × 2.1 mm, 3.5 μm, mobile phase: 0.01 N H₂SO₄; CH₃CN – 79:21% (v/v) (0-25 min); flow rate, 0.13 mL/min; t = 25°C; UV-detector wavelengths, 205 nm and 254 nm. Qualitative analysis of the compositions of hydrolysis products was performed by MS with electrospray ionization (Bruker Daltonics MicrOTOF-Q MS-spectrometer).

**Conventional hydrolysis of rutin with hydrochloric acid**

A sample of 0.10 g of RT (content of RT 96.6%) is dissolved in 1.5 ml of hot methanol, and 0.25 ml of concentrated hydrochloric acid (density 1.179) are added. The hydrolysis is carried out for 3.0 h at 100°C. At the end of the hydrolysis, the precipitate is filtered through paper and washed with distilled water to a neutral pH. The washed precipitate of QR is dried at 80°C for 2–3 h; the weight of the precipitate is 0.042 g.

**Hydrolysis of rutin by subcritical water**

The hydrolysis procedure in the subcritical water was performed using self-made reactor (autoclave). The reactor has inner volume 10 mL (D inner = 12 mm). The original substance RT (0.061 g) was put into self-made stainless steel reactor. Into a reactor was filled with 7 mL of distilled water. The reactor was hermetically closed and put into a drying oven, where it was kept at a certain temperature (accuracy ± 1°C) for 1 h. After that, the reactor was cooled down to room temperature (15 min) in a tank filled with cold water. Its content was quantitatively transferred to a paper filter, filtered, and washed with MeOH: CH₃CN (1:1) until the color disappears. The aliquots were diluted to the concentration required for analysis by HPLC/MS.

**Results and Discussion**

In accordance with the tasks of this work, the hydrolysis products of RT were obtained using different hydrolysis schemes to analyze the quantitative and qualitative compositions of the targeted products of hydrolysis. The hydrolysis products of RT were obtained using different hydrolysis schemes to analyze quantitatively and qualitatively the compositions of the targeted products of hydrolysis. Our previous studies showed that the transformations of biologically active compounds in subcritical water were most complete in the temperatures range of 100°C–280°C [Figure 2]. Therefore, here we studied in detail the temperature dependence of the yield of hydrolysis products in this temperature range and the composition of the products obtained. The effect of the temperature of the subcritical water on the yield of QR was studied in the range of 100°C–280°C. The amount of QR in the dry hydrolysate obtained by subcritical water at different temperatures is represented in Figure 2.

By increasing the temperature of subcritical water from 100°C to 200°C, the amount of QR in the hydrolysate was increased from 0.6–27.3 mg in the sample. Further increase in temperature led to a decrease in the amount of QR. Analysis of the data obtained showed that at 200°C, the yield of QR was the highest, but still lower than the theoretical yield (if the stoichiometry of hydrolysis is taken into account in this case). At a temperature of 230°C RT was not found by HPLC in either the precipitate or solution; hence, hydrolysis had occurred completely.

After treatment of QR (without any additions) by subcritical water, the content of QR was 81%, 77%, and 74% at 200°C,
Previously, the study of the thermal treatment of QR and RT in an aqueous model system (cooking) was carried out in detail in the work of Buchner et al. [4]. QR and RT were heated (100°C) and their degradation was studied by HPLC. The influence of pH and the involvement of oxygen in the degradation were studied. Strong degradation of the model substances took place under weak basic and oxidative conditions. QR showed the most intense degradation. In comparison with QR, the QR glycoside, RT, showed higher stability towards oxidation. This behavior results from the prevention of the formation of the carbanion by the glycosidation of the 3-hydroxyl group. For this reason, the yield of QR in the subcritical water was the highest, but still lower than the theoretical yield. The more detailed study of the products of degradation of QR in subcritical water (temperature 100°C–280°C) is planned to be done in the future.

Variation of only one parameter of the process (temperature) altered the composition of the hydrolysis products. For example, at 190°C, one of the substances of the hydrolysis composition is Mono-QR, whereas, at 200°C, the major substance of the composition (3) is the targeted products of hydrolysis-QR. The product of hydrolysis in subcritical water at 200°C, corresponding to the peak with a retention time of 19.61 min, has been identified as QR by HPLC, and MS data.

The comparison of the efficiencies of the different schemes for hydrolysis of RT is given in Table 1. The data of Table 1 show that the QR content in the product obtained in the medium of subcritical water is comparable with its yield obtained by the traditional method. However, the process is three times faster. Moreover, this method does not require the use of expensive and toxic organic solvents.

**Conclusions**

For the first time, a new acid-free technique was used for the synthesis of natural antioxidant QR from an RT by subcritical water. That way requires no use of acids and/or toxic organic solvents. It has been shown that variation of only one parameter of the process (temperature of subcritical water) allows alteration to the composition of the hydrolysis products. The new method developed for the production of QR in subcritical water is environmentally friendly and faster than conventional hydrolysis methods that use acidic or enzymatic hydrolysis.

The proposed technique has a potential for the future development of inexpensive and environmentally friendly technologies for the production of new pharmaceutical plant-based substances. Compared to the previously reported methods, this protocol has advantages of operational simplicity, chromatography-free separation, high overall yield, inexpensive, and common reagents as well as less waste pollutants, rendering it an alternative suitable for industrial production.

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**Figure 2:** Dependence of the amount of quercetin in the hydrolysate obtained in subcritical water at the temperature of the medium (the size of the figure at the discretion of the editor).

**Table 1:** Comparison of the efficiencies of the different schemes for hydrolysis of the rutin (the original substance rutin 61 mg)

| Hydrolysis (time, min) | Temperature (°C) | Yield of QR (mg in sample) | Yield of QR (%) |
|------------------------|-----------------|-----------------------------|-----------------|
| Acidic hydrolysis (HCl) | 180             | 100                         | 25.6            |
| Hydrolysis by subcritical water | 60             | 200                         | 27.3            |

QR: Quercetin

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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