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Optimization of hydrolysis of rutin in subcritical water using response surface methodology

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ABSTRACT

In this study, subcritical water has been used as a medium for hydrolyzing the glycoside bonded antioxidant quercetin-3-O-rutinoside (rutin) into its free aglycone form. Effect of temperature, treatment time, rutin concentration and the atmosphere used for establishing the pressure in the reactor was studied and the optimal combination of reaction parameters was established using response surface methodology in a 3×3 Box-Behnken design. Optimal reaction conditions were found to be a temperature of 196 °C, time of 12.35 min, rutin concentration of 1.08 mg/mL and CO_2 atmosphere at pressure of 215 bar. Since high-temperature was applied, possibility of formation of hydrothermal degradation products existed, therefore total amounts of degraded quercetin and formed 5-hydroxymethylfurfural were determined. Results show that near the optimal conditions, practically all rutin is hydrolyzed. Quercetin degradation is unavoidable, although the effect can be minimized by increasing the CO_2 pressure, but simultaneously that benefits to the formation of 5-hydroxymethylfurfural.

1. Introduction

Recently, many studies of the application of subcritical water as a green and environment friendly extraction medium have been published [1–11]. Subcritical water has many beneficial properties compared to classic organic solvents, such as giving higher yields, higher purities of extract and low solvent and recycling costs. Compared to ambient water, subcritical water represents water heated above its boiling point while held under a high pressure in order to stay in its liquid form. With heating of the water above its normal boiling point many physical and chemical properties begin to change. At these high temperatures, the thermal motion of water molecules increases to such an extent that much fewer hydrogen bonds between them can be formed [12]. This consequently causes a decrease in polarity, viscosity and surface tension, which makes subcritical water more similar to organic solvents such as methanol or ethanol [12]. Subcritical water is therefore emerging as an alternative greener solvent for applications that normally require "organic solvent-like" properties. Nevertheless, in the subcritical region another property of water changes drastically, namely the ionic product. Compared to the normally low ionic product of water at ambient conditions, the ionic product of subcritical water is much higher, which means that at these conditions subcritical water acts as an aggressive acid/base catalyst, capable of producing far greater number of H^+ and OH^- ions than ambient water [13]. Simply stated, subcritical water is highly reactive and in many cases is capable of degrading (hydrolyzing) many organic compounds, such as biopolymers (carbohydrates and proteins), to their simpler constituents (sugars, amino acids etc.) but simultaneously many other side reactions can occur. For example sugars can degrade to various products such as furfurals, organic acids, ketones etc. [13], whereas amino acids can be degraded to amines or even ammonia [14].

In the case of bioactive compounds such as polyphenolic antioxidants, often these are not present in their free aglycone form but are in many combinations bonded to sugars, forming so called glycosides [3,15]. In order to obtain free aglycones, after the extraction, these compounds need to be hydrolyzed, what is usually performed by acid catalyzed or enzyme catalyzed hydrolysis [16]. With the application of subcritical water as an extraction medium this can be done simultaneously, without the use of a catalyst, which means that no further downstream processing is needed. However, the high temperatures and reactive medium applied in subcritical water extraction can oppositely decrease the yield of the thermally labile bioactive compounds. It is therefore important to find and optimize the conditions at which the glycoside compounds are hydrolyzed to their aglycone form most efficiently and at which the further degradation is minimal. Knowledge about the limitations of the hydrolytic effect of subcritical water on glycoside bonded antioxidants is very limited. In many cases, authors who

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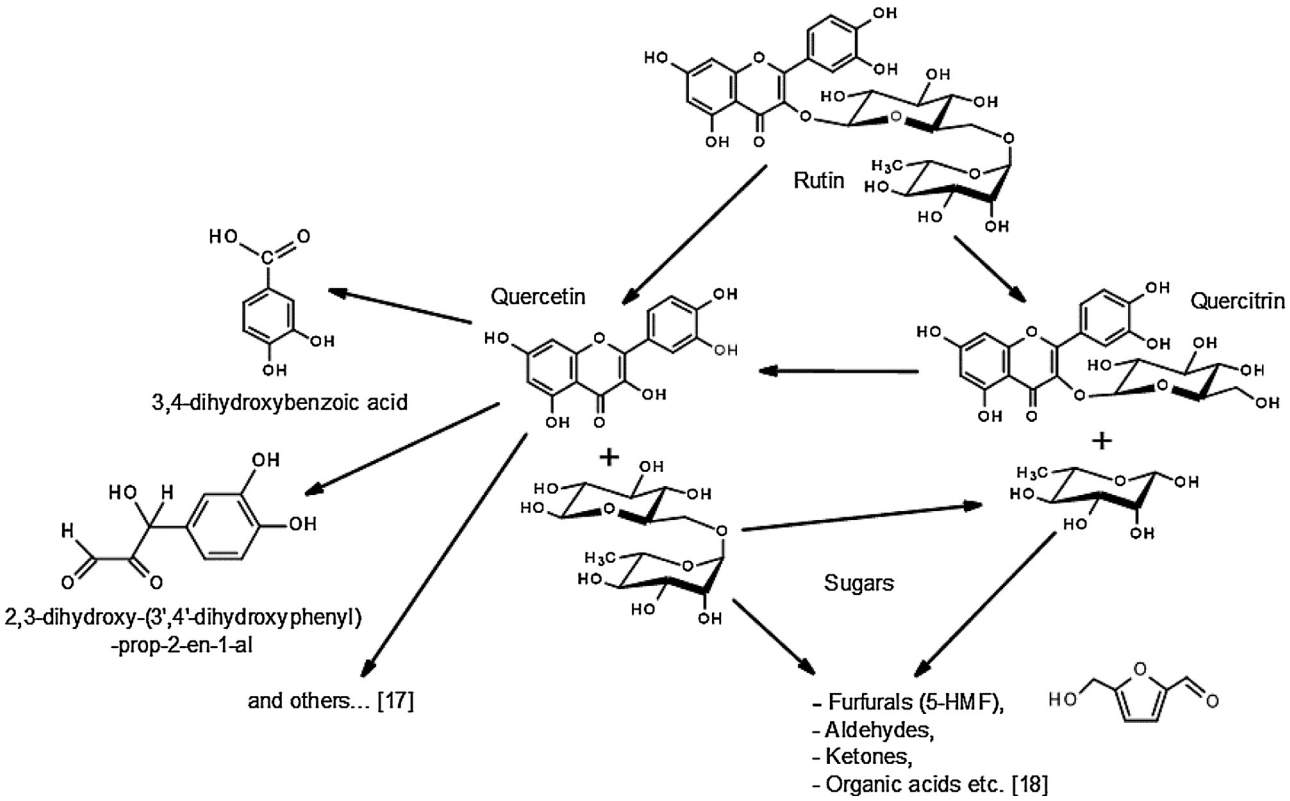

Fig. 1. Possible hydrothermal degradation products of rutin in subcritical water.

have reported a hydrolytic effect of the subcritical water during the extraction process, did not account the degradation that occurred at the same time and have in general only reported an increase of free aglycone or total phenolic content in their extracts [1,2,5,10]. Although some authors did notice a degradation of their products, they did not quantify the amount of degraded products [6,11].

In the present study, subcritical water is used for hydrolyzing the glycoside bonded quercetin-3-O-rutinoside (rutin), an antioxidant commonly found in many plant extracts, into its free aglycone form, quercetin. Reaction parameters which influence the aglycone (quercetin) formation, namely temperature (T), treatment time (t), rutin concentration (c) and pressure of applied gas (N_2 or CO_2) for establishment of pressure in the reactor (p) are studied and preliminary ranges of these parameters are determined. A Box-Behnken experimental design is performed in order to optimize the reaction parameters that give the maximum yield of quercetin. Since high-temperature is applied for the reaction, the possibility of formation of many other products of hydrothermal degradation exist what is in a simplified way presented in Fig. 1 [17,18]. One, of the more common degradation product, 5-hydroxymethylfurfural was observed and quantified. Total amounts of degraded quercetin were also determined.

2. Materials and methods

2.1. Materials

All standards and solvents were of analytical grade and were used as provided without further purification. Rutin trihydrate (≥95%) and 5-hydroxymethylfurfural (5-HMF, ≥98%) were purchased from Acros Organics (Belgium). Quercetin (≥98%) was purchased from Sigma–Aldrich (Germany). Methanol and acetic acid were purchased from Merck (Germany).

2.2. Subcritical water hydrolysis

Subcritical water hydrolysis of rutin was performed in a 75 mL high-temperature high-pressure batch autoclave (series 4740 stainless steel, Parr Instruments, Moline, IL, USA), designed for maximum operating temperature of 350 °C at 550 bar.

For a single experiment 10 mL of freshly prepared aqueous suspension of rutin was introduced into the autoclave. Before treatment, the autoclave was purged three times with inert nitrogen in order to remove present atmospheric oxygen, which could cause oxidation side reactions of the standard solution. The autoclave was heated electrically and stirring of the media was performed by using a magnetic stirrer.

After treatment under controlled conditions the autoclave was quickly cooled in an ice bath. A sample aliquot of 0.5 mL was collected and diluted with methanol to a concentration that allowed its analysis. The prepared solution was filtered through a 0.2 μm teflon membrane filter and immediately injected into the high performance liquid chromatograph (HPLC).

2.3. HPLC analysis

Suspensions obtained after treatment with subcritical water were analyzed for rutin and its hydrolysis product quercetin. The dehydration product of hexose sugars, 5-hydroxymethylfurfural (5-HMF), was also quantified.

The Agilent 1100HPLC system consisted of a binary pump, an autosampler, a column heater and a variable wavelength detector (VWD). Separation of the above mentioned compounds was performed on an Agilent Zorbax SB-C18 analytical column (150 mm × 4.6 mm, i.d., 1.8-μm particle size). The column temperature was set to 35 °C and the flow rate of mobile phase was equal to 0.55 mL/min. The mobile phase consisted of two solvents, namely 2% acetic acid in methanol (elution A) and 2% acetic acid in water