Journal of Medicinal Plants Research Vol. 6(7), pp. 1130-1139, 23 February, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.756
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Directionally enzymatic hydrolysis of rutin for biosynthesis of quercetin

Jun Wang[1], Meng Wang[1], Fu-An Wu[1,2]*, Zhong-li Chen[3,4] and Shi-ming Cui[3,4]

[1]School of Biotechnology and Chemical Engineering, Jiangsu University of Science and Technology, Zhenjiang 212018, P.R. China.
[2]Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212018, P.R. China.
[3]National Mulberry Silk Industrial Engineering Technology Research Center, Nantong 226600, P.R. China.
[4]Xinyuan Cocoon Silk Group Co. Ltd., Nantong 226600, P.R. China.

Accepted 21 December, 2010

Quercetin, an aglycon of rutin, is widely applied as an important material in food and pharmaceutical industries. In the present study, in order to investigate the optimal reaction conditions for directional biosynthesis of quercetin by enzymatic hydrolysis of rutin catalyzed by snailase, the influences of temperature, substrate concentration and pH value on enzymatic hydrolysis of rutin were investigated by the conversion yield of quercetin from rutin with single-factor test. The results indicated that the crude snailase had good biocatalytic ability for selective hydrolysis of one rutinose from rutin. When the hydrolysis reaction conditions catalyzed by snailase were as follows: temperature 34°C, pH 6.2, and substrate concentration 0.0242 mg/ml, the highest conversion yield of quercetin was 98.18%. Moreover, selected metal ions (Cu^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, K^{+} and Na^{+}) all had depressant effect on the conversation of quercetin from rutin with different strength. The hydrolysis product was purified by solvent extraction and crystallization, in which the chemical structure was identified as quercetin by ^{1}H-NMR and ^{13}C-NMR. In conclusion, enzymatic conversion process of quercetin from rutin is simple, practical and can provide a reference in the trial production.

Key words: Rutin, quercetin, enzymatic hydrolysis, directional biosynthesis, snailase.

INTRODUCTION

In plants, flavonol aglycones are most commonly present conjugated at the 3-position of the unsaturated C-ring with a sugar moiety, forming O-b-glycosides such as quercitrin or rutin (Boots et al., 2008). Quercetin is a naturally-occurring flavonol that has a long history of consumption as part of the normal human diet and one of important active compounds in traditional Chinese medicine (TCM) (Thangasamy et al., 2009). Because a number of biological properties of quercetin can be beneficial to human health, interest in the addition of this flavonol to various traditional food and pharmaceutical products has been increasing (Chen et al., 2010). Recent pharmacological studies have shown that quercitrin possess a wide range of biological activities, including anti-oxidant (Huebbe et al., 2010), metal cations chelating (Sun et al., 2008), anti-carcinogenic (Murakami et al., 2008), cardioprotective (Khoo et al., 2010), plaque-stabilizing (Saunders et al., 2009), and anti-inflammatory properties (Rogerio et al., 2010). The antioxidant activity of this molecule is higher than well-known antioxidant molecules ascorbyl, trolox and rutin. This is due to the number and position of the free hydroxyl groups and the catechol-type B-ring in the chemical structure of quercetin (Kumari et al., 2010). Therefore, quercetin has been widely used in oxidative stress related researches, and lots of natural products are rich in quercetin and their extracts are being used as functional ingredients and TCM for several industrial products (Erlund, 2004: Wang et al., 2008; Wach et al., 2007). Several years ago, the use of quercetin in pharmaceutical field is limited, because its instability and

*Corresponding author. E-mail: fuword@163.com Tel: 86-511-8561-6571.

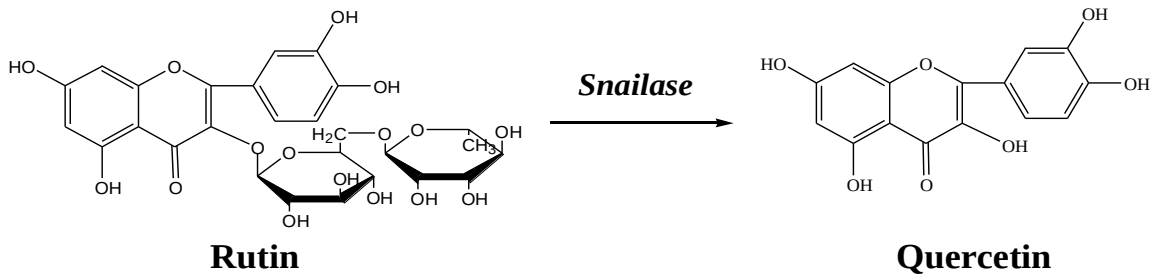

Figure 1. Directional biosynthesis of quercetin by enzymatic hydrolysis of rutin with snailase.

low solubility in aqueous media both *in vitro* and *in vivo*, which result in poor bioavailability, poor permeability, instability and extensive first pass metabolism before reaching the systemic circulation (Thangasamy et al., 2009). Notwithstanding, now the application of quercetin in pharmaceutical industry is sharply increasing by two basic ways to circumvent these problems, including entrap/adsorb it into biodegradable polymeric nanoparticles and chemically modifiy it for improving its solubility (Kumari et al., 2010; Li et al., 2009; Wu et al., 2008; Hossion et al., 2010; Mochizuki et al., 2009). So, establishing the conventional process of quercetin production on a large scale is the basic demand of its high additional value application in food and pharmaceutical industry.

Nowadays, acetylation (De Oliveira et al., 2009) and hydrolysis reaction are two basic ways to improve biological activities of flavonoids. In fact, flavonoids' acylation can be effectively accomplished by enzymatic processes (De Oliveira et al., 2010; Lue et al., 2010; Mellou et al., 2006). As flavonoids show a very large structural diversity (i. e. number and positions of glycoside units and hydroxyl groups, degree of oxidation of the Cring), they have different sizes, flexibilities, charge distributions, hydrophilic–hydrophobic balance properties, and are likely to yield different interaction modes with the lipase (De Oliveira et al., 2009). Moreover, a solution to improve the hydrophobic nature of flavonoids consists in their hydrolysis, which can lead to the cleavage of some sugar groups that are directly implicated in the beneficial properties of these molecules (Mauludin et al., 2009).

Quercetin is generally accumulated in plants (flowers, fruits and leaves) as glycosides such as glucosides, rutinosides and xylosides, so it can be obtained from plants via extraction of the quercetin glycosides followed by hydrolysis to release the aglycone and subsequent purification (Mauludin et al., 2009). Rutin is widely distributed in many plants in the world (Wang et al., 2010), such as *Alabastra sophorae japonicae* with concentration range of 10 to 28% in China (Mauludin et al., 2009). It is suggested that rutin is the ideal material for producing quercetin by hydrolysis method. There are two basic hydrolysis method of rutin: acidic hydrolysis and enzymatic hydrolysis. However, besides common process problem encountered in the acidic hydrolysis such as low yield and difficulties in purification of product, the use of corrosive and toxic acids were the major drawback in these chemical hydrolysis methods. Furthermore, the production of quercetin could be unstable due to its low aqueous solubility and instability in acidic aqueous solutions. Therefore, synthesizing quercetin by acidic hydrolysis methods of rutin is laborious and present several drawbacks in a practical point of view.

Contrarily, enzymes exhibit excellent region- and stereoselectivity towards the synthesis of heat sensitive and regioisomerically pure aglycons (Bojarov and Kren, 2009). The use of enzyme as biocatalysts for production of highly value quercetin have many potential benefits such as ability of glycosidase to catalyze cleavage of glycosyl groups from flavonoid glycosides under relatively mild reaction conditions and reduction of environmental pollution (Souza et al., 2010). In recent years, it has been reported that the use of enzymatic hydrolysis method offers some advantages, such as the increase in the selectivity and speed of hydrolysis reaction, and easy separation of product from substrate as well as the improvement of product stability (Do et al., 2009). Based on this, several trials have been conducted to obtain quercetin with different conversion yield by enzymatic hydrolysis of rutin with different glycosidase. However, no report has been published on the use of snailase for production of quercetin from rutin by enzymatic hydrolysis method. As shown in Figure 1, the snailase-catalyzed cleavage of the rutin sugar moiety leads to the release of rutinose and quercetin. In order to obtain highly purified quercetrin, we need to choose a suitable hydrolysis process to synthesising quercetrin from hydrolysates of rutin for the further pharmacological investigation and application research as natural antioxidant. Unfortunately, less attention has been paid to the directional hydrolysis of rutin catalyzed by snailase.

Snails have long been recognized as an excellent source of many major cell-wall degradation enzymes, including complex cellulases (endoglucanase, exoglucanase, and β-glucosidase) and β-galactosidase (Yoon et al., 2005). Many literatures reported that the

digestive juice of the snail, *Helix pomatina*, contained cellulases, chitinase, and β-glucosidase (Hu et al., 2009; Hu et al., 2007). Indeed, to our knowledge, no study reporting the biosynthesis of quercetin from the direct enzymatic hydrolysis of rutin by snailase is available in the literature.

The effects of various experimental conditions, such as pH value, temperature, substrate concentration,enzymatic reaction time, and metal ion on the conversion yield of quercetin are studied in order to determine the optimum hydrolysis conditions. Thenrecrystallization was successfully applied to purify the quercetin obtained from the hydrolysates of rutin. Its structure was characterized by ^{1}H NMR and ^{13}C NMR.

MATERIALS AND METHODS

Chemicals and reagents

Standards of rutin and quercetin were purchased from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). Snailase was purchased from Sanland chemical Co., LTD (Xiamen, China). All reagents used were of analytical grade except methanol and acetonitrile, which were of HPLC grade purchased from TEDIA Co. (Fairfield, OH, USA). Water was purified using an Elga Purelab Option-Q purification system (Elga Labwater, High Wycombe, Bucks, UK) and had a resistivity of not less than 18.0 MΩ cm. This water was used for cleaning procedures and in the preparation of all buffer solutions. All water solutions were prepared with ultrapure water filtered through a 0.45 μm membrane filter.

HPLC and NMR analysis

High-performance liquid chromatography-ultraviolet (HPLC-UV) was performed using HITACHI Pump L-7100 with a UV-VIS Detector L-7420 (Techcomp Ltd., Shanghai, China) and N-2000 workstation (Hangzhou Mingtong S&T Ltd., Hangzhou, China). Separation and determination of rutin and quercetin by using HPLC-UV methods was at a flow rate of 1.0 mL/min, detected at 360 nm, and on an Alltima C_{18} column (250 × 4.6 mm; i.d. 5 μm) with a mobile phase consisting acetonitrile: 0.02% phosphoric acid solution (20:80, v/v), and acetonitrile: pH 4 ammonium acetate buffer (27:73, v/v), respectively (Wang et al., 2009).

Concentrations of rutin and quercetin were calculated from peak areas using calibration curves. All solutions were filtered through a 0.45 μm filter before injection. All samples were determined in triplicate.^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d6 using a Bruker 300 Hz spectrometer.

Enzymatic hydrolysis of rutin catalyzed by snailase

Conversion yield of quercetin was measured by mixing 10 mg crude snailase with 20 mL saturated solution of rutin and 20 mL water. The mixtures were incubated for different time at various substrate concentrations, temperatures, and pH value while fixing the other conditions in a temperature controlled heating water bath. The reaction was stopped by adding 5 mL methanol. And then, the crude hydrolysis products of rutin were centrifuged at 10000 rpm for 10 min, the supernatants were filtered through a 0.45 μm filter before injection. All samples were determined in triplicate. The conversion yield of CA was calculated as expressed in Equation (1).

$$Conversion\ yield\ of\ quercetin\ (\%) = \frac{molar\ concentration\ of\ quercetin\ (mmol/L)}{initial\ molar\ concentration\ of\ rutin\ (mmol/L)} \times 100\% \quad (1)$$

The crude product of quercetin was purified by extraction and silica gel column chromatography with n-hexane-ethyl acetate (50:50, v/v) as eluent and recrystallized in mixed solvent methanol-water (70:30, v/v).

Effect of various metal ions on the conversion yield of quercetin

The effect of different metal ions on the conversion yield of quercetin catalyzed by crude snailase was determined by the addition of corresponding ion at a final concentration of 1.0 mM to the reaction mixture, and assayed under the previous conditions. The enzymatic hydrolysis of rutin were carried out in the presence of $CuSO_4$, $CaCl_2$, $MgSO_4$, $FeSO_4$, KCl and NaCl, respectively.

RESULTS AND DISCUSSION

The influence of pH value on conversion yield of quercetin

Traditionally, flavonoid aglycon could be formed by hydrolysis of flavonoid glycoside (Takahama et al., 2009), so the first step in enzymatic hydrolysis of rutin is to optimize the operating conditions (pH value, enzymatic reaction time, substrate concentration, temperature, metal ion, etc.) to obtain an efficient biosynthesis of quercetin. In the present study, optimization of the reaction conditions in enzymatic hydrolysis method was carried out by using a single-factor method. Hydrolysis mechanism of glycosides is always thought to proceed primarily through an A1 carbonium ion reaction (Laopaiboon et al., 2010), so the pH value of enzymatic reaction system is very important for biosynthesis of quercetin via directionally hydrolysis of rutin. The effects of pH on conversion yield of quercetin were investigated by varying the pH of 0.02 mg/mL aqueous rutin solution in the range of 3.0 to 8.0, which reactions were incubated at 35°C and stirred for 2 h at 120 rpm. The influence of pH value on conversion yield of quercetin by enzymatic hydrolysis of rutin is shown in Figure 2.

From (Figure 2) we can see that snailase could effectively catalyze the cleavage of a terminal rutinoside group from rutin to quercetin and rutinose. Essentially, the cleavage of terminal rutinoside group from rutin catalyzed by snailase is contributed to β-glucosidase (EC 3.2.1.21). The reason is that snailase is a crude glycosidase containing glucosidase, cellulase, amylase, and so on (Han et al., 2007). β-glucosidase family, as well as most other GH families, catalyze glycoside hydrolysis via a double displacement mechanism involving two carboxylic acid-containing side chains in the active site. One of these groups, a carboxylate, functions as a nucleophile, leading

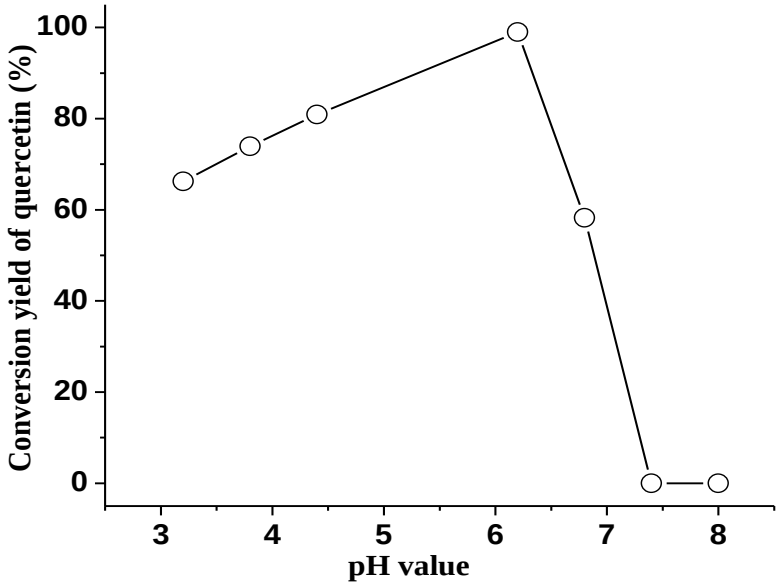

Figure 2. The influence of pH value on conversion yield of quercetin by enzymatic hydrolysis of rutin.

to a glucosyl-enzyme intermediate (an acylal). The other, a carboxylic acid, acts as a general acid catalyst in the formation of this intermediate, and a general base catalyst in its breakdown (Bowers et al., 2007). The pH value of enzymatic reaction system was found to be the most important determinant of the conversion yield of quercetin. The highest conversion yield of quercetin was found to be at pH 6.2 using snailase as catalyst. These results are in accordance with some literature reports showing optima pH of 5 to 7 for partial purified β-glucosidase from *Trichoderma reesei* (Gautam and Simon, 2006), *Aspergillus niger* (Seidle et al., 2005), *Cladosporium fulvum* (Gao et al., 2010), and *Humicola insolens* (Souza et al., 2010). It also indicated that the snailase was very sensitive to pH and lost its activity entirely when pH is beyond 7.4. This could be explained that amino acid side chains in the active site may act as weak acids and poses with critical functions that depend on their ionizing state, and elsewhere in the enzyme, ionized side chains may play an essential role in the interactions that maintain protein structure (Lu et al., 2006). Therefore, optimum pH value is around 6.2, which is essential to obtain maximum conversion yield of quercetin by directionally enzymatic hydrolysis of rutin.

The influence of temperature on conversion yield of quercetin

Previous studies had shown that temperature influences the biosynthesis yield of quercetin by enzymatic hydrolysis of rutin with snailase, since it has an effect on enzyme and product stability, and on substrate solubility. In the present study, the effects of temperature on conversion yield of quercetin were investigated by varying the temperature of 0.02 mg/ ml aqueous rutin solution in the range of 28 to 40°C, which reactions were incubated in aqueous rutin solution with pH 6.2 and stirred for 2 h at 120 rpm. So, the influence of temperature on conversion yield of quercetin was investigated by enzymatic hydrolysis reaction under different temperature (Figure 3). As can be seen from Figure 3, range of reaction temperature from 28 to 37°C was good for transformation of quercetin from rutin, and the highest conversion rate of quercetin was found when the reaction was at 34°C. When temperature was lower than 28°C, only some quercetin were found be growing with low enzyme activity. It is also not suitable for biosynthesis of quercetin by enzymatic reaction when temperature was up to 28°C. This occurs because as the temperature changes this supplies enough energy to break some of the intramolecular attractions between polar groups and the hydrophobic forces between non-polar groups within the enzyme structure (Lu et al., 2008). Therefore, the optimum enzymatic reaction temperature was set at 34°C.

The influence of substrate concentration on conversion yield of quercetin

To clarify the third factor, a test was performed in which rutin as substrate was added to reactor for quercetin

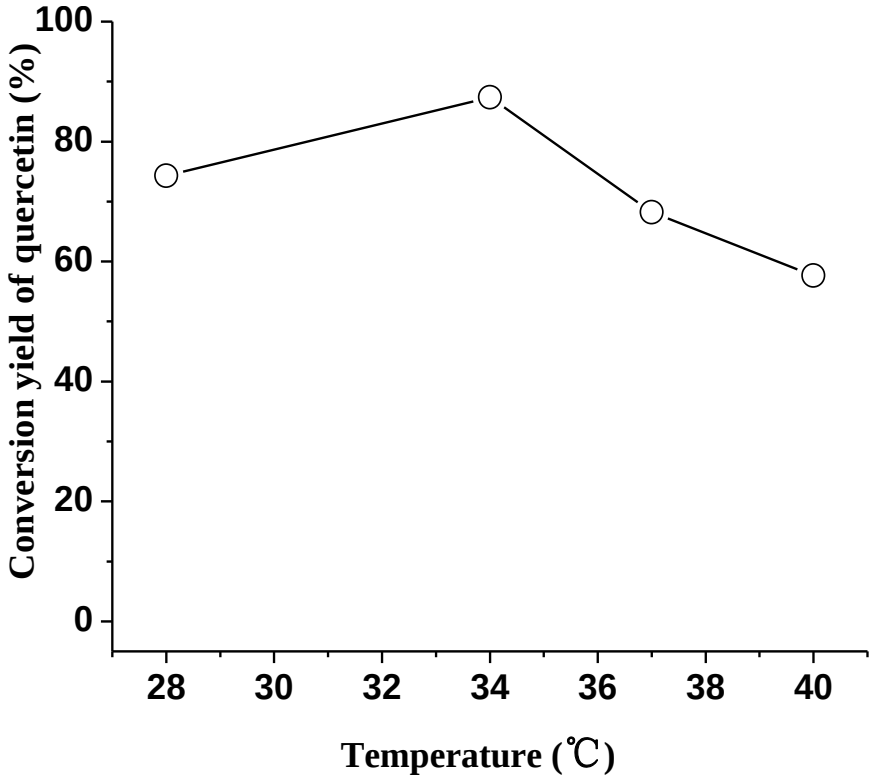

Figure 3. The influence of temperature on conversion yield of quercetin by enzymatic hydrolysis of rutin.

production. In the present study, the effects of substrate concentration on conversion yield of quercetin were investigated by varying the substrate concentration of the aqueous rutin solution in the range of 0.02 to 0.08 mg/mL, which reactions were incubated in aqueous rutin solution with pH 6.2 at 34°C and stirred for 2 h at 120 rpm. Therefore, the influence of substrate concentration on the snailase-catalyzed hydrolysis of rutin was shown in Figure 4. As can be seen from Figure 4, when the concentration of rutin was increased from 0.02 to 0.08 mg/ml, the conversion yield of quercetin was sharply decreased from near 98.2 to 0%. It is indicated that when initial concentration was above 0.02 mg/mL, the hydrolysis rate of rutin started to slow down remarkably, this phenomenon demonstrated that substrate concentration is playing an important part in biosynthesis of quercetin. For lower substrate concentrations, enzymatic reaction equilibrium is not reached. For higher concentrations, poor solubility of rutin leading to diffusion problems may occur. Therefore, substrate inhibition and product degradation are the possible reasons for the decrease in conversion yield when the rutin concentration was augmented (Buque-Taboada et al., 2005), and the optimal concentration of rutin for quercetin production by snailase was considered to be 0.02 mg/ml.

The influence of reaction time on conversion yield of quercetin

In the present study, the effects of reaction time on conversion yield of quercetin were investigated by varying the reaction time in the range of 0.5 to 3.5 h, which reactions were incubated of 0.02 mg/mL aqueous rutin solution with pH 6.2 at 34°C and stirred at 120 rpm. The enzymatic hydrolysis time (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 h) influence the conversion yield of quercetin from rutin (Figure 5). Figure 5 showed the conversion yield curve of quercetin hydrolyzed by rutin, which reflect the biosynthesis ability and stability of quercetin in this enzymatic reaction system. When the enzymatic reaction time was 2 h, the maximal yields of quercetin were obtained. However, after 2 h, with the increase of enzymatic reaction time, the yields of quercetin rapidly decreased. It is indicated that quercetin as enzymatic hydrolysis product was unstable in this reaction system with the presenting of snailase.

Therefore, the enzymic hydrolysis of rutin is carried out with various enzymes which possibly act sequentially according to two steps: firstly, β-glucosidase, make the cleavage of the terminal rutinose, and the corresponding aglycon (quercetrin) is released; subsequently, quercetin

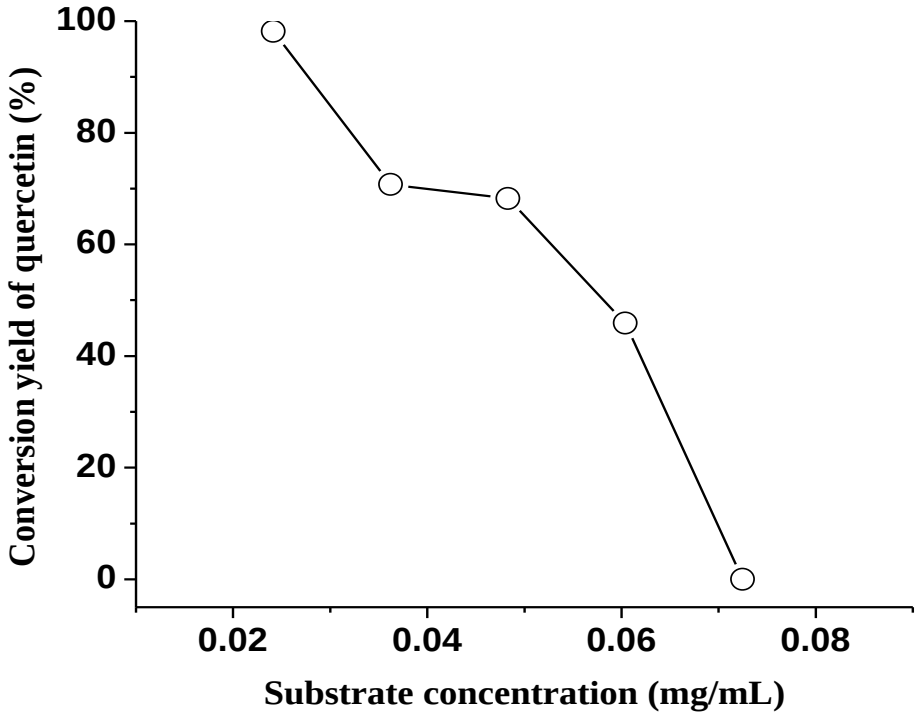

Figure 4. The influence of substrate concentration on conversion yield of quercetin by enzymatic hydrolysis of rutin.

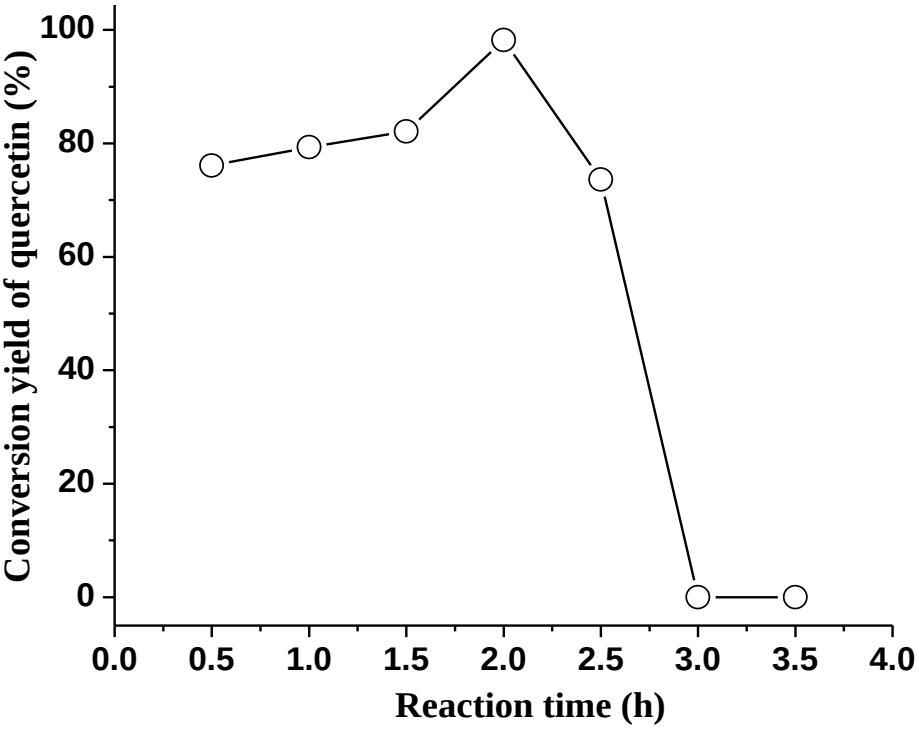

Figure 5. The influence of reaction time on conversion yield of quercetin by enzymatic hydrolysis of rutin.

Table 1. Effect of various metal ions on conversion yield of quercetin by enzymatic hydrolysis of rutin.

Metal ions (1.0 mM)	Conversion yield of quercetin (%)
Control	98.8
$CuSO_4$	0
$CaCl_2$	51.0
$MgSO_4$	50.7
$FeSO_4$	0
KCl	53.2
NaCl	0

maybe continuously degrading to other products catalyzed by other enzymes in the crude snailase at the same time. Therefore, the optimum enzymatic reaction time was 2 h. When snailase is catalyzed by hydrolysis reaction conditions of rutin was as follows: temperature 34°C, pH6.2, and substrate concentration 0.0242 mg/mL, the optimum conversion yield of quercetin was 98. 18%.The result indicated that the crude snailase had good biocatalytic ability for selective hydrolysis of one rutinose from rutin.

The influence of metal ions on conversion yield of quercetin

Most of the metal ions tested have a slight stimulatory influence or an inhibitory influence on enzyme activity and conversion yield of product. In the present study, the enzymatic hydrolysis of rutin was carried out in the presence of $CuSO_4$, $CaCl_2$, $MgSO_4$, $FeSO_4$, KCl and NaCl, respectively (Table 1). Table 1 clearly indicated that selected metal ions all inhibit the enzyme activity of snailase result in decreasing the conversion yield of quercetin by enzymatic hydrolysis of rutin. The activity of the crude sailase was completely depleted in the presence of Ca^{2+}, Mg^{2+} or K^+, but residual activities of 51.0, 50.7 and 53.2%, respectively, were observed in the presence of the same ions at 1 mM concentration. In contrast, Cu^{2+}, Fe^{2+} or Na^+ totally abolished the activity at 1 mM concentrations. Therefore, selected metal ions all had depressant effect on the conversation of quercetin from rutin with different strength.

^{1}H NMR and ^{13}C NMR spectra of quercetin

The quercetin was purified by solvent extraction and crystallization, which purity was about 98% analyzed by HPLC. ^{1}H NMR and ^{13}C NMR spectra of quercetin were recorded in DMSO-d_6 using a Bruker 300 Hz spectrometer (Figure 6). As can be seen from Figure 6, for quercetin, ^{1}H-NMR (300 MHz, DMSO-d_6) (ppm): δ6.18 (1H, d, J = 1.8 Hz, H-6), 6.40 (1H, d, J = 2.1 Hz, H-8), 6.89 (1H, d, J = 8.4 Hz, H- 5'), 7.54 (1H, dd, J = 2.1 and 8.7 Hz, H-6'), 7.67 (1H, d, J = 2.1 Hz, H- 1'), 12.49 (1H, s, 5-OH). ^{13}C-NMR (300 MHz, DMSO-d_6) (ppm): δ 156.1 (C-2), 135.7 (C-3), 175.8 (C-4), 160.7 (C-5), 98.1 (C-6), 163.8 (C-7), 93.3 (C-8), 156.1 (C-9), 102.9 (C-10), 121.9 (C-1'), 115.0 (C-2'), 145.0 (C-3'), 147.6 (C-4'), 115.5 (C-5'), 119.9 (C-6'). From the data of ^{1}H-NMR and ^{13}C-NMR (Williams and Wender, 1953; Aderogba et al., 2006), it could be concluded that the hydrolysate was quercetin. That is, quercetin can be effectively produced by selectively hydrolyzing one rutinose from rutin.

Conclusions

In the present study, a crude snailase was used to produce quercetin by directionally enzymatic hydrolysis of rutin and the reaction conditions were optimized. The results indicated that the crude snailase had good biocatalytic ability for selective hydrolysis of one rutinose from rutin. When the hydrolysis reaction conditions of rutin catalyzed by snailase were as follows: temperature 34°C, pH 6.2, and substrate concentration 0.0242 mg/mL, the optimum conversion yield of quercetin was 98.18%. Moreover, metal ions (Cu^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, K^+ and Na^+) all had depressant effect on the conversation of quercetin from rutin with different strength. The hydrolysis product was purified by solvent extraction and crystallization, which chemical structure was identified as quercetin by ^{1}H-NMR and ^{13}C-NMR. In conclusion, enzymatic conversion process of quercetin from rutin is simple, practical and can provide a reference in the trial production.

ACKNOWLEDGEMENTS

This work was supported by College Natural Science Research Project of Jiangsu Province (08KJB530002), Science and Technology Support Program of Jiangsu Province (BE2010419), Qing Lan Project of Jiangsu

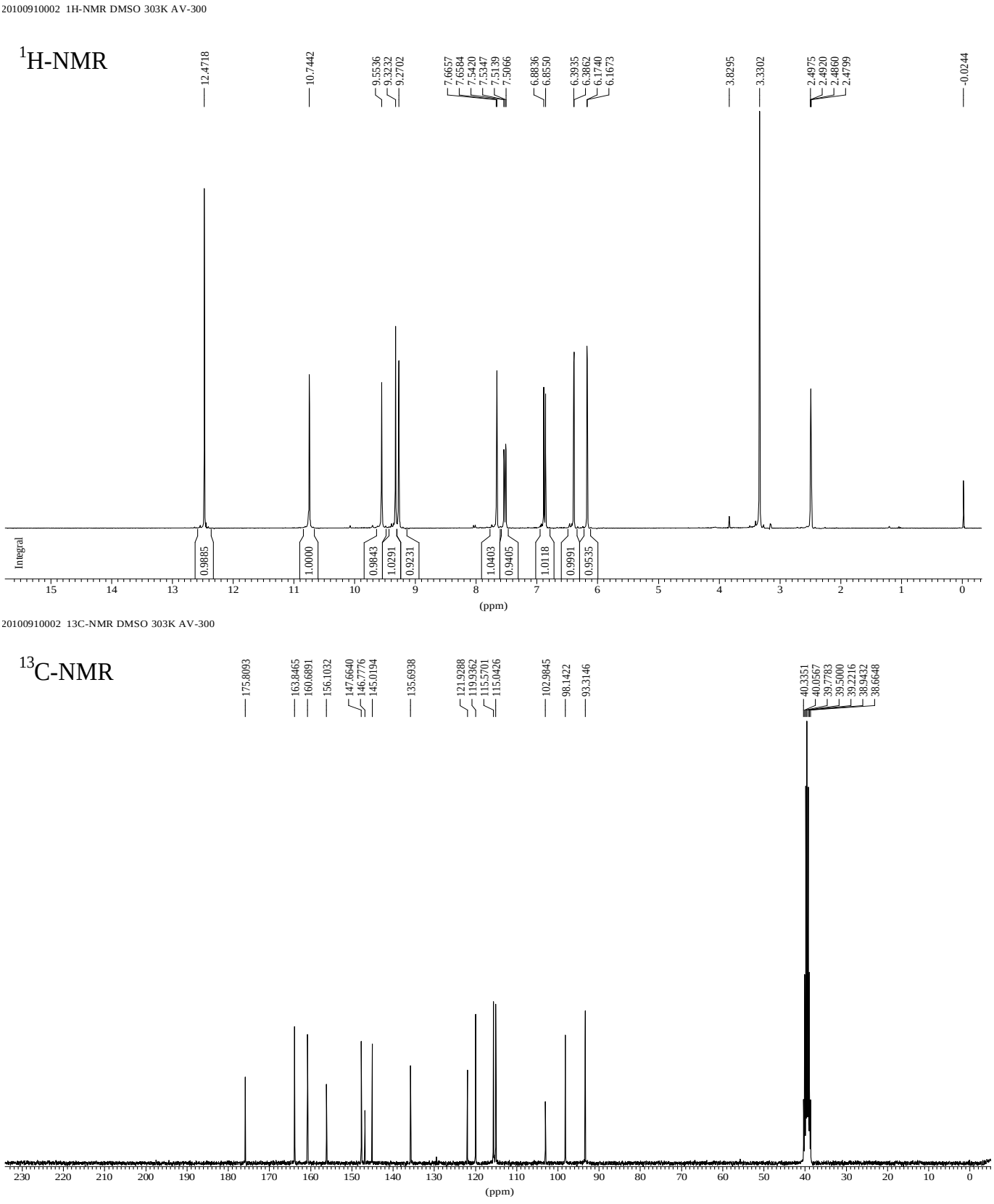

Figure 6. ^{1}H NMR and ^{13}C NMR spectra of quercetin were recorded by a Bruker 300 Hz spectrometer.

Province, Natural Science Foundation of Jiangsu Province (BK2009213), Research Projects of Jiangsu University of Science and Technology (35211002, 33201002), and Modern Agro-industry Technology Research System of China (CARS-22).

REFERENCES

Aderogba MA, Ogundaini AO, Eloff JN (2006). Isolation of two flavonoids from bauhinia monandra (kurz) leaves and their antioxidative effects. Afr. J. Tradit. Complemt., 3: 59-65.

Bojarov P, Kren V (2009). Glycosidases: a key to tailored carbohydrates. Trends Biotechnol., 27 : 199-209.

Boots AW, Haenen GRMM, Bast A (2008). Health effects of quercetin: From antioxidant to nutraceutical. Eur. J. Pharmacol., 585 : 325-337.

Bowers EM, Ragland LO, Byers LD (2007). Salt effects on [beta]-glucosidase: pH-profile narrowing. Biochim. Biophys. Acta, Proteins Proteomics, 1774 : 1500-1507.

Buque-Taboada EM, Straathof AJJ, Heijnen JJ, van der Wielen LAM (2005). Substrate inhibition and product degradation during the reduction of 4-oxoisophorone by Saccharomyces cerevisiae. Enzyme Microb. Tech., 37 : 625-633.

Chen C, Zhou J, Ji C (2010). Quercetin: A potential drug to reverse multidrug resistance. Life Sci., 87: 333-338.

De Oliveira EB, Humeau C, Chebil L, Maia ER, Dehez F, Maigret B, Ghoul M, Engasser J (2009). A molecular modelling study to rationalize the regioselectivity in acylation of flavonoid glycosides catalyzed by Candida antarctica lipase B. J. Mol. Catal. B: Enzym., 59: 96-105.

De Oliveira EB, Humeau C, Maia ER, Chebil L, Ronat E, Monard G, Ruiz-Lopez MF, Ghoul M, Engasser J (2010). An approach based on Density Functional Theory (DFT) calculations to assess the Candida antarctica lipase B selectivity in rutin, isoquercitrin and quercetin acetylation. J. Mol. Catal. B: Enzym., 66: 325-331.

Do Y, Kim J, Chang S, Hwang J, Kim W (2009). Enhancement of polyphenol bio-activities by enzyme reaction. J. Mol. Catal. B: Enzym., 56: 173-178.

Erlund I (2004). Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. Nutr. Res., 24: 851-874.

Gao J, Zhao X, Liu H, Fan Y, Cheng H, Liang F, Chen X, Wang N, Zhou Y, Tai G (2010). A highly selective ginsenoside Rb1-hydrolyzing [beta]-d-glucosidase from Cladosporium fulvum. Process Biochem., 45 : 897-903.

Gautam S, Simon L (2006). Partitioning of [beta]-glucosidase from *Trichoderma reesei* in poly(ethylene glycol) and potassium phosphate aqueous two-phase systems: Influence of pH and temperature. Biochem. Eng. J., 30: 104-108.

Han Y, Sun BS, Hu XM, Zhang H (2007). Transformation of Bioactive Compounds by Fusarium sacchari Fungus Isolated from the Soil-Cultivated Ginseng. J. Agric. Food Chem., 55: 9373-9379.

Hossion AML, Otsuka N, Kandahary RK, Tsuchiya T, Ogawa W, Iwado A, Zamami Y, Sasaki K (2010). Design, synthesis, and biological evaluation of a novel series of quercetin diacylglucosides as potent anti-MRSA and anti-VRE agents. Bioorg. Med. Chem. Lett., 20: 5349-5352.

Huebbe P, Wagner AE, Boesch-Saadatmandi C, Sellmer F, Wolffram S, Rimbach G (2010). Effect of dietary quercetin on brain quercetin levels and the expression of antioxidant and Alzheimer's disease relevant genes in mice. Pharmacol. Res., 61: 242-246.

Khoo NKH, White CR, Pozzo-Miller L, Zhou F, Constance C, Inoue T, Patel RP, Parks DA (2010). Dietary flavonoid quercetin stimulates vasorelaxation in aortic vessels. Free Radical Biol. Med., 49: 339-347.

Kumari A, Yadav SK, Pakade YB, Singh B, Yadav SC (2010). Development of biodegradable nanoparticles for delivery of quercetin. Colloid Surface B, 80: 184-192.

Hu Y, Luan H, Ge G, Liu H, Zhang Y, Zhou K, Liu Y, Yang L (2009). Deoxynojirimycin enhanced the transglycosylation activity of a glycosidase from the China white jade snail. J. Biotechnol, 139: 229-235.

Hu Y, Luan H, Hao D, Xiao H, Yang S, Yang L (2007). Purification and characterization of a novel ginsenoside-hydrolyzing [beta]-d-glucosidase from the China white jade snail (*Achatina fulica*). Enzyme Microb. Tech., 40: 1358-1366.

Laopaiboon P, Thani A, Leelavatcharamas V, Laopaiboon L (2010). Acid hydrolysis of sugarcane bagasse for lactic acid production. Bioresour. Technol., 101: 1036-1043.

Li H, Zhao X, Ma Y, Zhai G, Li L, Lou H (2009). Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. J. Control Release, 133: 238-244.

Lu DQ, Li H, Dai Y, Ouyang PK (2006). Biocatalytic properties of a novel crude glycyrrhizin hydrolase from the liver of the domestic duck. J. Mol. Catal. B: Enzym., 43: 148-152.

Lu DQ, Zhang SM, Li H, Dai Y (2008). Separation of glycyrrhetic acid monoglucuronide from glycyrrhizin hydrolysates by macroporous resins. J. Biotechnol., 136: S472-S473.

Lue B, Guo Z, Xu X (2010). Effect of room temperature ionic liquid structure on the enzymatic acylation of flavonoids. Process Biochem., 45: 1375-1382.

Mauludin R, Müller RH, Keck CM (2009). Development of an oral rutin nanocrystal formulation. Int. J. Pharm., 370: 202-209.

Mellou F, Loutrari H, Stamatis H, Roussos C, Kolisis FN (2006). Enzymatic esterification of flavonoids with unsaturated fatty acids: Effect of the novel esters on vascular endothelial growth factor release from K562 cells. Process Biochem., 41: 2029-2034.

Mochizuki K, Ishima Y, Hayano K (2009). Synthesis and structures of zinc complexes with new binucleating ligands containing alkoxide bridges, and their activities in the hydrolysis of tris(p-nitrophenyl)phosphate. Inorg. Chim. Acta, 362: 2722-2727.

Murakami A, Ashida H, Terao J (2008). Multitargeted cancer prevention by quercetin. Cancer Lett., 269: 315-325.

Rogerio AP, Dora CL, Andrade EL, Chaves JS, Silva LFC, Lemos-Senna E, Calixto JB (2010). Anti-inflammatory effect of quercetin-loaded microemulsion in the airways allergic inflammatory model in mice. Pharmacol. Res., 61: 288-297.

Saunders F, Oommen M, Lapjitkusol R, Amankwatia E, Kaminski L, Swanson A, Mitchell A, Wallace HM (2009). Potentiation of the cytotoxic effects of natural chemopreventative agents, ellagic acid and quercetin. Toxicology, 262: 21.

Seidle HF, Huber RE (2005). Transglucosidic reactions of the Aspergillus niger Family 3 [beta]-glucosidase: Qualitative and quantitative analyses and evidence that the transglucosidic rate is independent of pH. Arch. Biochem. Biophys., 436: 254-264.

Souza FHM, Nascimento CV, Rosa JC, Masui DC, Leone FA, Jorge JA, Furriel RPM (2010). Purification and biochemical characterization of a mycelial glucose- and xylose-stimulated [beta]-glucosidase from the thermophilic fungus Humicola insolens. Process Biochem., 45: 272-278.

Sun S, Chen W, Cao W, Zhang F, Song J, Tian C (2008). Research on the chelation between quercetin and Cr(III) ion by Density Functional Theory (DFT) method. Journal of Molecular Structure: THEOCHEM, 860: 40-44.

Takahama U, Tanaka M, Hirota S, Yamauchi R (2009). Formation of an oxathiolone compound from rutin in acidic mixture of saliva and buckwheat dough: Possibility of its occurrence in the stomach. Food Chem., 116: 214-219.

Thangasamy T, Sittadjody S, Burd R (2009). Quercetin: A Potential Complementary and Alternative Cancer Ther., 563-584.

Wach A, Pyrzynska K, Biesaga M (2007). Quercetin content in some food and herbal samples. Food Chem., 100: 699-704.

Wang J, Lu DQ, Zhao H, Wang JL, Jiang B, Ling XQ, Chai H, Ouyang PK (2010). Discrimination and classification tobacco wastes by identification and quantitation of polyphenols with LC-MS/MS. J. Serb. Chem. Soc., 75: 875-891.

Wang J, Wu FA, Zhao H, Liu L, Wu QS (2008). Isolation for total flavonoids from mulberry (Morus alba L.) leaves with macroporous resins. Afr. J. Biotechnol., 7: 2147-2155.

Wang J, Wu FA, Zhao LL, Wu T, Yang Q, Liang Y, Fang SQ, Jiang X, Wang M (2009). HPLC in reversed phase mode: tool for

investigation of isoquercitrin from enzymatic biosynthesis products of rutin. In: Proceedings of International Conference of Natural Products and Traditional Medicine (Ed. ZHU K X). Berlin-Herdelberg: Springer Press, 548-552.
Williams BL, Wender SH (1953). Isolation and identification of quercetin and isoquercitrin from apricots (*Prunus armeniaca*). Arch. Biochem. Biophys., 43: 319-323.
Wu T, Yen F, Lin L, Tsai T, Lin C, Cham T (2008). Preparation, physicochemical characterization, and antioxidant effects of quercetin nanoparticles. Int. J. Pharm., 346: 160-168.
Yoon KY, Cha M, Shin SR, Kim KS (2005). Enzymatic production of a soluble-fibre hydrolyzate from carrot pomace and its sugar composition. Food Chem., 92: 151-157.