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One-pot preparation of quercetin using natural deep eutectic solvents

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ABSTRACT

In this study, we have established a green and efficient preparation method of quercetin. Rutin was first extracted from *Sophora japonica* using natural deep eutectic solvents (NADESs), then hydrolyzed into quercetin by rutin degrading enzyme (RDE) obtained from germinated tartary buckwheat in situ. Rutin solubility tests showed that most of the 11 NADESs increased the solubility of rutin by 67-3116 times compared to water. Thus, NADESs could be prior candidate to extract rutin. Extraction efficiency of rutin varied with different NADESs, and a maximum of 291.57 mg g^{-1} was achieved in NADES ChGly, which was prepared by mixing choline chloride and glycerol at a molar ratio of 1:1. After that hydrolysis was performed directly in extraction system by adding RDE with degradation rate of up to 8.36 mg min^{-1}·L^{-1}. Our findings suggest that preparation of quercetin using NADESs was simple and feasible to operate, environmentally friendly, efficient, and inspired the preparation method of bioactive components from a new perspective.

1. Introduction

Deep eutectic solvents (DESs), emerged recently as a novel class of designer solvents, are attracting more and more attention [1]. They overcome several limitations of traditional organic solvents and ionic solvents that are high toxic, difficult to recycle, high cost, volatile and so on. DESs are defined as eutectic mixtures composed of hydrogen bond donors and acceptors. When the compositions are primary metabolites, the formed solvents are also called natural deep eutectic solvents (NADESs) [2]. NADESs have been widely studied in electrochemistry, separation processes and reaction processes due to their unique physicochemical properties [3–7].

Quercetin (Scheme S1), 3,3′,4′,5,7-pentahydroxylflavone, is a flavonoid with many biological activities, and it is widely distributed in the flowers, leaves and fruits of many plants, mostly in the form of glycosides and aglycones [8]. Studies have shown that quercetin has a variety of pharmacological effects, e.g. anti-oxidation [9], anti-cancer, anti-inflammation [10], and anti-virus [11].

In modern food and pharmaceutical industry, quercetin is usually prepared by hydrolysis of rutin extracted from *Sophora japonica* because of its high content [8]. Organic solvents or their combinations with water are commonly used in the extraction due to the extremely low solubility of rutin in water, which leads to a complicated, environmentally harmful and low output process [12–16]. Concentrated sulfuric acid or hydrochloric acid is often used for subsequent conversion process [17], which results in an intense reaction with long time and low yield. Biocatalyst was considered to address these challenges. In recent study, it was reported that rutin degrading enzyme (RDE) prepared from tartary buckwheat can specifically hydrolyze rutin to quercetin. Its catalytic properties were then determined [18–20], but its application in preparation of quercetin with NADESs has not been discussed and known well.

An environmentally friendly preparation method of quercetin was established in this study, in which NADESs were novelly applied to the preparation of natural bioactive ingredients. The extraction of rutin and its conversion to quercetin were completed in one solvent system. NADESs overcoming the limitations of organic extractions are safe and non-toxic as extraction solvents. Bio-catalyst instead of hydrochloric acid or sulfuric acid could get high yield of target product and low by-products because of its specificity. In addition, the process was milder and the time required was shortened.

2. Materials and methods

2.1. Chemical and biological materials

The tartary buckwheat was purchased from local market in Sichuan, China. *Sophora japonica* was obtained from local pharmacy. Rutin

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standard was obtained from Beijing Bailingwei Technology Co., Ltd. NADESs component: alanine (99%), threonine (≧99%), lysine (≧98%), proline (≧99%), and choline chloride (98%) were purchased from Aladdin Chemical Reagent Co., Ltd.(Shanghai, China). 1,2-propanediol (≧99%), xylitol (98%), triethylene glycol (98%), glycerol (≧99%), 1,2-butanediol (98%), and ethylene glycol (≧99%) were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China).

2.2. Preparation and determination of physicochemical properties of NADESs

Eleven NADESs were prepared by mixing hydrogen bond donors and acceptors at a defined molar ratio as shown in Table S1. The mixtures were heated at an elevated temperature (85–100 °C) under constant stirring until a homogeneous liquid was formed. During process the air was well insulated by adding laboratory film to the vessel to prevent absorption of moisture from the environment.

The viscosity was determined by a rheometer at 40 °C; Nile red was used to estimate the polarity, expressed as E_{NR} (molar transition energy); the pH was measured using a pH meter; and rutin solubility was determined by saturating NADESs with excess rutin followed by ultrasounding at 40 °C for 45 min [21].

2.3. Extraction of rutin from Sophora japonica

Sophora japonica powder (40 mg) was dissolved in 1.5 mL of designated NADES. The solution was sonicated in an ultrasonicator (Model: NP-B-100-15; New Power Co., Ltd., Kunshan, China) at 20 Hz, 200 W, for 45 min. Then the solutions were centrifuged at 7000 *g* for 30 min. Rutin concentrations in the supernatant were determined by HPLC as described below. The extraction efficiency was assessed using the following equation: extracted amount ($mg{\cdot}g^{-1}$) = mass of rutin (mg)/mass of *Sophora japonica* powder (g).

2.4. Effect of water content in NADESs on the extraction efficiency of rutin

NADESs with high extraction efficiency were selected and water was added by 0%, 10%, 20%, 30%, and 40% (v/v) to determine the effect of water in NADESs on the rutin extraction efficiency.

2.5. HPLC analysis of rutin contents in extractions

The amounts of rutin in the extraction solutions were determined by reversed phase high performance liquid chromatography (RP-HPLC) on a 4.6 mm × 150 mm (5 μm) Zorbax Eclipse XDB-C18 column (Agilent Technologies, Santa Clara, CA - USA). The HPLC was equipped with an autosampler (Model: SIL-20A), a quaternary pump (Model: LC-20D) and a diode array detector (Model: SPD-M20A) set at 360 nm (Fig. S1), a degasser (Model: CBM-20A), and column thermostat (Model: CTO-20A). The mobile phase was a mixture of methanol and water (50/50, v/v) at flow rate of 0.6 $mL{\cdot}min^{-1}$ with isocratic system. The samples were diluted 5 times with methanol and filtered before HPLC analysis.

2.6. Extraction and catalytic activity determination of RDE from germinated tartary buckwheat

2.6.1. Extraction of RDE from germinated tartary buckwheat

Ripened and uniform tartary buckwheat seeds were selected, weighed to 5 g, and soaked with 1% sodium hypochlorite for 10 min. The seeds were then washed with sterilized deionized water, soaked with water at room temperature for 4 h, and then put in a plat with sterilized gauze. The seeds were germinated in the dark at 15 °C, 20 °C, 25 °C, and 30 °C, respectively for 4 days with 100% humidity. The samples were taken and the soaking water was changed every 24 h.

Each sample was ground to a suspension and then dissolved in 20 mL of 0.02 M acetic acid buffer solution (pH = 5.0) which was stirred at 4 °C for 24 h. The supernatant was collected after centrifugation at 8000 *g* for 20 min. Before storage at 4 °C, the volume was dialysis with distilled water.

2.6.2. Enzyme activity determination of RDE from germinated tartary buckwheat

RDE solution (100 μL) was added to 700 μL of 0.02 M Tris-HCl buffer (pH = 7.0), followed by 200 μL of ethanol containing rutin (0.75 $mg\ mL^{-1}$). The mixture was incubated at 37 °C for 15 min. The reaction was stopped by adding 1 mL of methanol. The degraded rutin amount was determined by HPLC as described above (Figs. S2, S3). One unit of RDE activity was defined as the amount of enzyme required to catalyze 1 μg rutin per minute under the condition above.

2.7. Effect of germination condition, NADESs and water content on the enzyme activity of RDE

2.7.1. Effect of germination condition on the enzyme activity of RDE

Tartary buckwheat seeds germinated at 15 °C, 20 °C, 25 °C, and 30 °C for different time were collected, RDE in each sample were extracted followed by enzyme activity determination.

2.7.2. Effect of NADESs and water content on the enzyme activity of RDE

Different kinds of NADESs were prepared, then diluted by adding deionized water to final concentrations of 20%, 40%, 60%, and 80%, (v/v). Enzyme activities of RDE in these systems were assayed by the method in 2.6.2, respectively.

2.8. Optimization of hydrolysis system of RDE

2.8.1. Single factor test

The single factor test was carried out with 5 factors and 5 levels, namely temperature (25, 30, 37, 40, and 45 °C), reaction time (10, 15, 20, 25, and 30 min), pH (4, 5, 6, 7, and 8), substrate concentration (0.25, 0.5, 0.75, 1, and 1.25 $mg\ mL^{-1}$) and RDE amount (15, 20, 25, 30, and 35 μL), each time only one factor was tested, calculated the rutin degradation rate by the method described in 2.6.2.

2.8.2. Orthogonal analysis and hydrolysis optimization

An orthogonal $L_9\ (3)^3$ test design was used to investigate the optimal hydrolysis condition of RDE. As seen from Table S2, the hydrolysis experiment was carried out with 3 factors and 3 levels, namely temperature (30, 35, and 40 °C), substrate concentration (0.75, 1, and 1.25 $mg\ mL^{-1}$) and RDE amounts (45, 50, and 55 μL). The rutin degradation rate was the dependent variable.

3. Results and discussion

3.1. Preparation, physicochemical properties tests of NADESs

In recent years, NADESs have emerged as a new generation of designer solvents. They can be simply prepared by mixing initial components and stirring at a certain temperature; their properties could also be tailored easily by changing their components [1].

Physical properties of 11 NADESs such as viscosity, polarity, pH, and solubility, were investigated systematically. As shown in Table 1, most NADESs tested were relatively viscous. The highest viscosity (3.18 Pa·s) was observed in GlyLys, 318 times as high as that of in methanol. The polarity of the tested NADESs was similar to that of methanol. This means NADESs could be classified as polar solvents. The pH of NADESs ranged from 5.61 to 7.68, except for GlyLys (10.49), indicating that they were in weak acid to weakly alkaline solvents.

The results of this study indicated that the solubility of rutin in 11 NADESs increased by 67-3116 times compared to that of in water (Table 1), which varied significantly with the type of NADESs. The highest solubility was achieved in ChTEG (280.4 ± 2.7 $mg{\cdot}mL^{-1}$),

Table 1
Viscosity and polarity of NADESs, and solubility of rutin in 80% NADESs.

NADESs Abbreviation	Viscosity[a] (40 °C) (Pa·s)	E_{NR}[b] (kcal·moL^{-1})	pH[c]	Solubility of rutin[d] (mg·mL^{-1})
ProAla	0.09	53.24-54.98	6.68	76.4 ± 5.1[e]
EGAla	0.01	52.95-55.41	7.20	107.4 ± 4.7
GlyThr	0.22	50.01	6.95	59 ± 3.9
GlyAla	0.29	49.82	7.19	165.0 ± 5.0
GlyLys	0.05	49.04-51.70	10.49	79.2 ± 4.1
ChGly	3.18	50.91	5.67	6 ± 2.3
ButThr	0.02	52.95-54.46	6.92	25.0 ± 4.5
ChEG	0.07	49.98-51.61	5.61	146.1 ± 5.2
ChTEG	0.02	55.09-56.06	6.95	280.4 ± 2.7
GlyXyl	0.07	48.87-50.07	7.45	31.6 ± 4.3
GlyPro	0.87	49.55	7.68	178.2 ± 3.3
Methanol	0.01	51.80	6.8	97 ± 4
Water	—[f]	—	7.00	0.09 ± 0.01

[a] Detection at shear rate 0.268 [1·s^{-1}].
[b] Polarity: Nile red concentration, ~ 0.1 mM; $E_{NR} = hcN_A/\lambda_{max}$.
[c] Measured by pH meter.
[d] The solubility of rutin in each solvents was determined by UV analysis of saturated solutions at 40 °C.
[e] Mean ± SD (n = 3).
[f] Not detected.

which has the highest polarity. And the lowest was obtained in ChGly (6 ± 2.3), resulting from its high viscosity.

3.2. Research on extraction of rutin in NADESs

Traditionally organic solvents are used to prepare quercetin to solve the problem of the poor solubility of rutin in water, which could pollute the environment [22]. NADESs could be an alternative to organic solvents for extraction due to high solubility of rutin in such solvents. However, high viscosity of NADESs could be a barrier in applications. Based on the previous study, the high viscosity of NADESs could be reduced by adding a certain amount of water [19]. In this study, 80% NADESs were used to extract rutin from *Sophora japonica* the traditional extraction 80% methanol was selected as a reference solvent.

Fig. 1 shows a significant improvement of rutin extraction by using NADESs as solvents compared to traditional methanol extraction. The amounts of extracted rutin varied with NADES types. The highest yield of rutin (284.81 mg g^{-1}) was obtained in ChGly, followed by EGAla (272.08 mg g^{-1}). GlyXyl only yielded rutin 4.67 mg g^{-1}. Based on rutin yields, four NADESs solvents (PGAla, EGAla, ChGly, and ChTEG) were clearly superior to methanol (149.75 mg g^{-1}). NADESs have similar polarity and pH to the methanol as seen in Table 1, but the rutin solubility is higher (except for ChGly), it may because of the H-bound between rutin and NADESs [2]. To improve the extraction efficiency, water was added to lower its viscosity and increase rutin solubility.

As a promising green solvent, NADESs could be implemented in various applications due to its unique properties, e.g. superior solubility and adjustable physicochemical. The results of this study provide foundation of using NADESs as solvents to extract various water-insoluble biologically active substances.

3.3. Effect of water content in NADESs on extraction efficiency of rutin

Water is an important factor affecting the viscosity of NADESs. In this study different water contents (0–40%) in NADESs system were evaluated for the extraction of rutin. Based on the rutin yields (Fig. 1), ChGly and EGAla were selected for this study. Fig. 2 shows that increasing amounts of water in ChGly up to 20% significantly improved the efficiency of the solvent in extraction of rutin. On the other hand, addition of water into EGAla tended to decrease the efficiency of the solvent. The maximum of ChGly appeared when the water content was 20% (291.57 mg g^{-1}), and for EGAla, it is 10% (275.64 mg g^{-1}).

The addition of appropriate amount of water could lower the viscosity and enhanced the extraction efficiency. Beyond the optimum level (20% for ChGly, 10% for EGAla), the excess amounts of water might interfere chemical interaction between the NADESs and their components, resulting in decreased extraction efficiency [21].

On the other hand, as it was reported in former study, the polarity of water is 48.21 [23], which is similar to most of the NADESs (48.87–56.06, Table 1), so the addition of water will not cause significant changes in polarity.

3.4. Effect of germination condition, NADESs and water content on the enzyme activity of RDE

3.4.1. Effect of germination condition on the enzyme activity of RDE

It has been studied that tartary buckwheat contains high active RDE, which have strong pH and thermal stabilities [24]. Studies on its substrate specificity indicate that the RDE has a strict substrate specificity and only catalyzes rutin to quercetin [19]. RDE has not been available commercially. Most of the researchers obtain RDE from plant materials such as tartary buckwheat by ammonium sulfate precipitation or hydrophobic chromatography [19,24,25]. The catalytic activity of RDE could be improved during the germination of tartary buckwheat. However, some have reported that series of metabolic reactions occurred during the germination process and RDE also underwent several changes in this period [26]. Hence, germination temperature and time were discussed to determine the optimum germination conditions.

Germination temperature and time have a great influence on the catalytic activity of RDE. RDE extracted from the tartary buckwheat which is germinated at 25 °C has the highest catalytic efficiency (Fig.

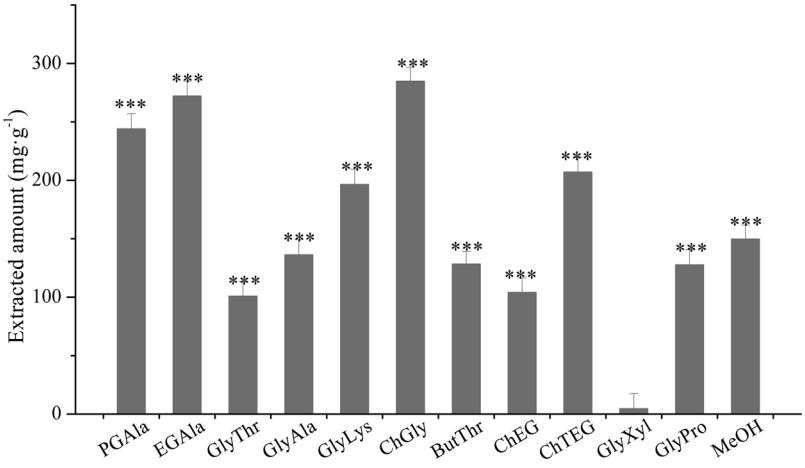

Fig. 1. Extraction yields (mg of rutin per g of *Sophora japonica* powder) for different solvents. Extraction conditions: 40 mg *Sophora japonica* powder, 1.5 mL 80% solvent, 50 °C, 1 h, Ultrasound-assisted extraction (UAE) power 200 W. Extraction efficiencies that were significantly higher in comparison with that of 80% GlyXyl are indicated with *p < 0.05, **p < 0.01 and ***p < 0.001.

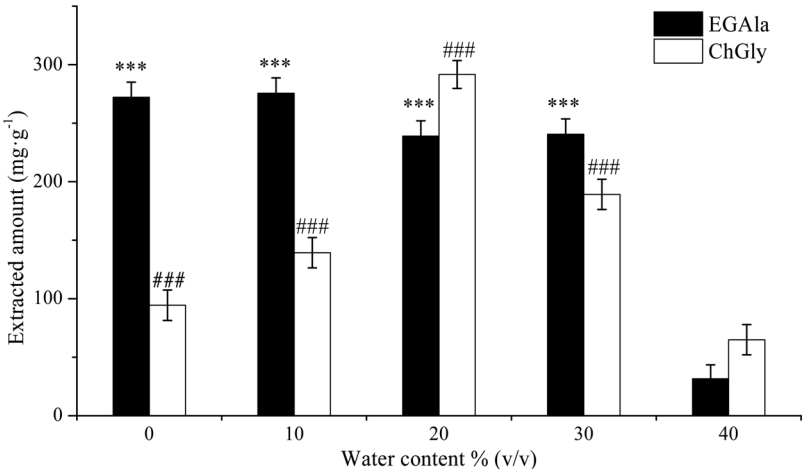

Fig. 2. Effect of natural deep eutectic solvents (NADESs) water content on the extraction efficiency of rutin. Extraction conditions: 40 mg *Sophora japonica* powder, 1.5 mL solvent, 50 °C, 1 h, UAE power 200 W. Extraction efficiencies that were significantly higher in comparison with that of 40% EGAla and 40% ChGly are indicated with $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ of EGAla or $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ of ChGly.

S4). The degradation rate of RDE decreased with time. This result is in line with the previous study. They theorized that the decreased activity of RDE during germination might be due to the absorption and conversion of nutrients [27]. Therefore tartary buckwheat was germinated at 25 °C for 1d to extract RDE in the followed experiments.

3.4.2. Effect of NADESs and water content on the enzyme activity of RDE

In this study, NADESs provided a non-aqueous environment instead of organic solvents, and the water content was optimized with rutin degradation as an indicator.

Fig. 3 shows that rutin degradation rate and the optimum water contents varied with NADESs. In water/NADESs ratios of 20:80-80:20 (v/v), the catalytic activity of RDE in most NADESs systems increased with the proportion of NADESs in the system. The maximum activity occurred at 20% water, except for EGAla at 40% water. Pure NADESs (0% water) with considerable high viscosity (Table 1) might cause difficulties of substrate to bind to enzyme catalytic active center and resulted in low degradation rate. The water activity of NADESs with different water content was also determined, ChGly was taken as an example (Fig. S5), which indicated that the Aw value has the similar trend with water content.

It has been proved that several enzymes are more active in non-aqueous or trace water-containing systems [28] RDE in NADESs with up to 20% water of this study showed better catalytic activities. This increased catalytic activity was due to several factors. The presence of appropriate amounts of water in the non-aqueous system could maintain the active structure of the enzyme, improve the stability of the enzymes [28], increase solubility of the substrate, inhibit side reactions, and make it easier to recover the enzymes [29,30].

3.5. Optimization of hydrolysis system of RDE

The enzyme reaction system is characterized by mild reaction conditions, specific substrate conversion and high conversion efficiency. Generally, reaction time, pH, substrate concentration, amounts of enzyme, and temperature affect the catalytic efficiency of enzymes. In this study, five factors were optimized by orthogonal test to improve the enzymatic activity of RDE to obtain higher reaction efficiency.

The enzymatic activity of RDE increased with the reaction time and it would level off after 15 min (Fig. 4A). This might be due to the substrate that had been reacted or used up. The pH affects the dissociation state of the active center of the enzyme. The optimum pH of RDE suitable for the binding to the substrate was at pH 7.0 (Fig. 4B). At too acidic or too alkaline pH, the enzyme structure would change, and its activity would decrease. Reaction rate increased with substrate concentrations up to 1.0 mg mL^{-1} (Fig. 4C). With the increase of the substrate concentration, the reaction rate increased when the substrate concentration was low and RDE would not be fully bounded, after the enzyme binding reached saturation, the reaction rate would no longer increase.

Rutin degradation shows a rapid rise from 15 μL to 25 μL, and a slight rise from 25 μL to 55 μL, then keep in steady (Fig. 4D). It may

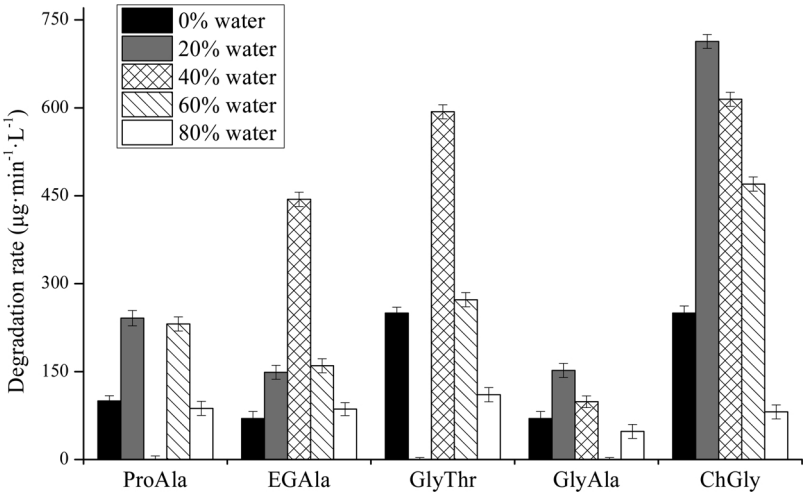

Fig. 3. Effect of NADESs species and water content on the degradation efficiency of rutin degrading enzyme (RDE). Degradation conditions: 100 μL crude RDE, 900 μL co-solvent with different water content (0%, 20%, 40%, 60%, and 80%, v/v) containing 150 μg rutin, 37 °C.

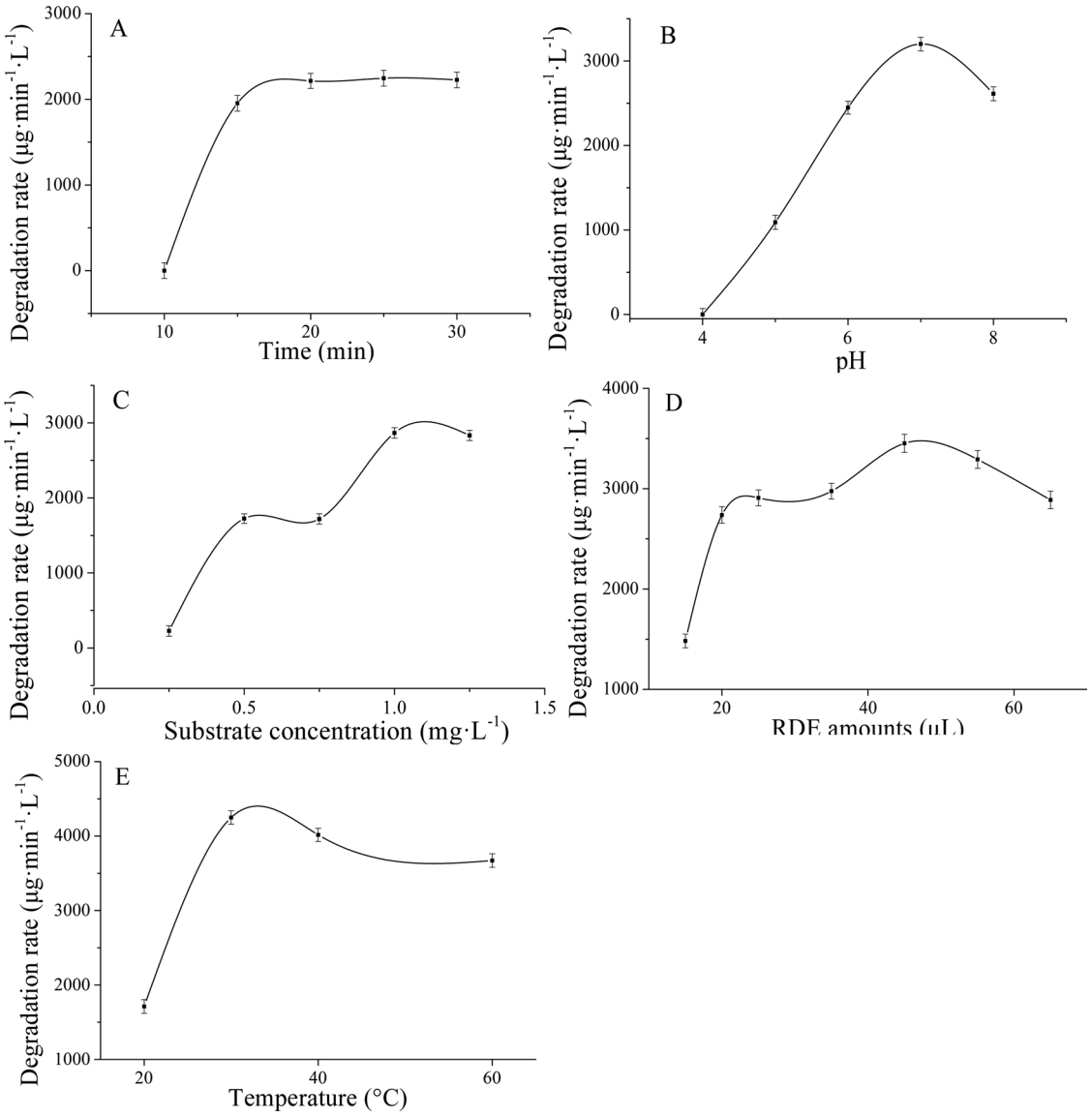

Fig. 4. Single factor experiment on the effect of time (Fig. 4A), pH (Fig. 4B), substrate-concentration (Fig. 4C), RDE amount (Fig. 4D) and temperature (Fig. 4E) on the degradation efficiency of RDE. Standard degradation conditions: 100 μL crude RDE 900 μL 80% ChGly containing 150 μg rutin, 37 °C.

because the substrate was fully bounded. Higher temperature promotes mass transport, as it was reported in former study [31], the viscosity decreases as the temperature rises, but after reaching the optimum temperature of the enzyme, a further increase of temperature caused destruction of molecular structure of the enzyme and irreversible degeneration, resulting in a decrease in enzyme activity. As shown in Fig. 4E, the maximum degradation rate was detected at 30 °C.

Orthogonal test table was designed based on single factor test results described above. The experimental results of orthogonal test are presented in Table 2. Rj1 > Rj2 > Rj3 indicated that the factors affecting hydrolysis from large to small are temperature > substrate concentration > enzyme amounts. The optimum level of each factor was determined by the value of K. The most optimum degradation conditions were as follows: heating in a 35 °C water bath with a substrate concentration of 1 mg mL^{-1} and an enzyme loading of 55 μL. This optimum conditions resulted in a degradation rate of 8.07 mg $min^{-1}·L^{-1}$ (Table 2). Three parallel validation experiments were carried out according to the hydrolysis conditions above, obtained a rutin degradation rate of 8.36 mg $min^{-1}·L^{-1}$, After optimization, the conversion rate was increased to 12.5%. While traditional process could reach a yield of 50% [17]. However traditional ways pose a harsh reaction condition

Table 2
Design and results of L_9 (3^4) orthogonal test[a].

Exp. number	T (°C)	Substrate concentrate (mg·mL^{-1})	RDE amount (μL)	Degradation rate (mg·$min^{-1}·L^{-1}$)
1	30	0.75	45	0.00
2	30	1	50	2.63
3	30	1.25	55	0.26
4	35	0.75	50	0.72
5	35	1	55	8.07
6	35	1.25	45	5.19
7	40	0.75	55	2.17
8	40	1	45	0.00
9	40	1.25	50	0.53
$\overline{K_1}$	1.07	1.06	2.03	–
$\overline{K_2}$	4.66	3.77	1.29	–
$\overline{K_3}$	1.10	1.99	3.50	–
R_j	3.59	2.70	2.21	–

[a] Standard degradation conditions:100 μL crude RDE, 900 μL solvent (80% ChGly) containing 150 μg rutin, 37 °C.

with a lot by-product, and environmentally harmful reagents were needed. In our study, the solvents were environmentally friendly and the enzyme was extracted from plant.

4. Conclusions

In this paper, we have developed a rapid, efficient and environmentally safe method to extract rutin and convert rutin to quercetin by mixing NADESs and water in a co-solvent system. After optimizing both steps, rutin degradation rate was up to 8.36 mg $min^{-1}·L^{-1}$. This method replaces the traditional strong acid, alkali and the organic solvent, which is biodegradable, environmentally friendly and non-polluting. The method described here can be applied to the extraction and preparation of a variety of natural active substances, which have broad application prospects in the pharmaceutical, food and related industries.

Declaration of Competing Interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.procbio.2019.10.019.

References

[1] M. Francisco, A. van den Bruinhorst, M.C. Kroon, Low-Transition-Temperature Mixtures (LTTMs): a new generation of designer solvents, Angew. Chem. Int. Ed. English 52 (2013) 3074–3085.
[2] A. Paiva, R. Craveiro, I. Aroso, M. Martins, R.L. Reis, A.R.C. Duarte, Natural deep eutectic solvents-solvents for the 21st century, ACS Sustain. Chem. Eng. 5 (2014) 1063–1071.
[3] J. Nadia, K. Shahbaz, M. Ismail, M.M. Farid, An approach for polygodial extraction from Pseudowintera colorata (horopito) leaves using deep eutectic solvents, ACS Sustain. Chem. Eng. 6 (2018) 862–871.
[4] L. Duan, L.L. Dou, L. Guo, P. Li, E.H. Liu, Comprehensive evaluation of deep eutectic solventsin extraction of bioactive natural products, ACS Sustain. Chem. Eng. 4 (2016) 2405–2411.
[5] A.P. Abbott, K.E. Ttaib, G. Frisch, K.S. Ryder, D. Weston, The electrodeposition of silver composites using deep eutectic solvents, Phys. Chem. Chem. Phys. 14 (2012) 2443–2449.
[6] F. Xu, J. Sun, M. Wehrs, K.H. Kim, S.S. Rau, A.M. Chan, B.A. Simmons, A. Mukhopadhyay, S. Singh, Biocompatible choline-based deep eutectic solvents enable one-pot production of cellulosic ethanol, ACS Sustain. Chem. Eng. 6 (2018) 8914–8919.
[7] F.S.G. Bagh, K. Shahbaz, F.S. Mjalli, M.A. Hashim, I.M. Alnashef, Zinc (II) chloride-based deep eutectic solvents for application as electrolytes: preparation and characterization, J. Mol. Liq. 204 (2015) 76–83.
[8] A. Wach, K. Pyrzynska, M. Biesaga, Quercetin content in some food and herbal samples, Food Chem. 100 (2007) 699–704.
[9] M.R. de Oliveira, S.M. Nabavi, N. Braidy, W.N. Setzer, T. Ahmed, S.F. Nabavi, Quercetin and the mitochondria: a mechanistic view, Biotechnol. Adv. 34 (2016) 532–549.
[10] F. Li, X.Y. Sun, X.W. Li, T. Yang, L.W. Qi, Enrichment and separation of quercetin-3-O-β-d-glucuronide from lotus leaves (nelumbo nucifera gaertn.) and evaluation of its anti-inflammatory effect, J. Chromatogr. B 1040 (2017) 186–191.
[11] M. Biesaga, A. Stafiej, K. Pyrzynska, Extraction and hydrolysis parameters for determination of quercetin in hypericumperforatum, Chromatographia 65 (2007) 701–770.
[12] B.Y. Zhao, P. Xu, F.X. Yang, H. Wu, M.H. Zong, Biocompatible deep eutectic solvents based on choline choride: characterization and application to the extraction of rutin from *Sophora japonica*, ACS Sustain. Chem. Eng. 3 (2015) 2746–2755.
[13] Q. Xu, Y. Shen, H. Wang, N. Zhang, S. Xu, L. Zhang, Application of response surface methodology to optimise extraction of flavonoids from fructus sophorae, Food Chem. 138 (2013) 2122–2129.
[14] M.G.L. Hertog, P.C.H. Hollman, M.B. Katan, Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands, J. Agric. Food Chem. 40 (1992) 2379–2383.
[15] W. Xiao, L. Han, B. Shi, Microwave-assisted extraction of flavonoids from Radix astragali, Sep. Purif. Technol. 62 (2008) 614–618.
[16] I. Molnár-Perl, Z. Füzfai, Chromatographic, capillary electrophoretic and capillary electrochromatographic techniques in the analysis of flavonoids, J. Chromatogr. A 1073 (2005) 201–227.
[17] G. Zhai, P. Zhu, N. Liu, S. Zhang, R. Chen, X. Ding, J. Zhao, Comparison study on the experimental condition for preparing quercetin by hydrolytic decomposition of rutin, Chem. Adhesion 31 (2009) 33–36.
[18] K. Taeko, The activity of rutin degrading enzyme from flower buds of japanese pagoda tree, J. Food Technol. (2013) 238–241.
[19] T. Yasuda, H. Nakagawa, Purification and characterization of the rutin-degrading enzymes in tartary buckwheat seeds, Phytochemistry 37 (1994) 24145–24154.
[20] X.D. Cui, Z.H. Wang, Preparation and properties of rutin-hydrolyzing enzyme from tartary buckwheat seeds, Food Chem. 132 (2012) 60–66.
[21] Y. Huang, F. Feng, J. Jiang, Y. Qiao, T. Wu, Green and efficient extraction of rutin from tartary buckwheat hull by using natural deep eutectic solvents, Food Chem. 221 (2016) 1400.
[22] C. Turner, P. Turner, G. Jacobson, K. Almgren, M. Waldebäck, P. Sjoberg, E.N. Karlsson, K.E. Markides, Subcritical water extraction and β-glucosidase-catalyzed hydrolysis of quercetin glycosides in onion waste, Green Chem. 8 (2006) 949–959.
[23] Y. Dai, J. Spronsen, G.J. Witkamp, R. Verpoorte, Y.H. Choi, Natural deep eutectic solvents as new potential media for green technology, Anal. Chim. Acta 766 (2013) 61–66.
[24] Z.F. Hou, The optimized purification method's of rutin-degrading enzyme in tartary buckwheat and its metabolic profiles during seed germination process, J. Northwest A&F Univ. 19 (2010) 48–52.
[25] Y.W. Zhang, J. Li, Y. Yuan, J. Gu, P. Chen, Purification, characterization and partial primary structure analysis of rutin-degrading enzyme in tartary buckwheat seeds, Chinese J. Biotechnol. 33 (2017) 796–807.
[26] T. Suzuki, Y. Honda, W. Funatsuki, K. Nakatsuka, Purification and characterization of flavonol 3-glucosidase, and its activity during ripening in tartary buckwheat seeds, Plant Sci. 163 (2002) 417–423.
[27] P. Chen, Z.F. Hou, Metabolic profiles of rutin-degrading enzyme during seed germination process of tartary buckwheat, Acta Agr. Bor-Occid. Sin. 19 (2010) 48–52.
[28] A.M. Klibanov, Enzymes that work in organic solvents, Chemtechnology 16 (1986) 354–359.
[29] E. Durand, L. Jérôme, B. Barea, G. Piombo, E. Dubreucq, P. Villeneuve, Evaluation of deep eutectic solvents as new media for Candida antarctica B lipase catalyzed reactions, Process Biochem. 47 (2012) 2081–2089.
[30] B. Kleiner, P. Fleischer, S. Ulrich, Biocatalytic synthesis of biodiesel utilizing deep eutectic solvents: a two-step-one-pot approach with free lipases suitable for acidic and used oil processing, Process Biochem. 51 (2016) 1808–1816.
[31] Y. Dai, J. Spronsen, G.J. Witkamp, R. Verpoorte, Y.H. Choi, Natural deep eutectic solvents as new potential media for green technology, Anal. Chim. Acta 766 (2013) 61–66.