

Optimization of Subcritical Water Hydrolysis of Rutin into Isoquercetin and Quercetin

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ABSTRACT: Maximum production of isoquercetin and quercetin simultaneously from rutin by subcritical water hydrolysis (SWH) was optimized using the response surface methodology. Hydrolysis parameters such as temperature, time, and CO₂ pressure were selected as independent variables, and isoquercetin and quercetin yields were selected as dependent variables. The regression models of the yield of isoquercetin and quercetin were valid due to the high *F*-value and low *P*-value. Furthermore, the high regression coefficient indicated that the polynomial model equation provides a good approximation of experimental results. In maximum production of isoquercetin from rutin, the hydrolysis temperature was the major factor, and the temperature or time can be lower if the CO₂ pressure was increased high enough, thereby preventing the degradation of isoquercetin into quercetin. The yield of quercetin was considerably influenced by temperature instead of time and CO₂ pressure. The optimal condition for maximum production of isoquercetin and quercetin simultaneously was temperature of 171.4°C, time of 10.0 min, and CO₂ pressure of 11.0 MPa, where the predicted maximum yields of isoquercetin and quercetin were 13.7% and 53.3%, respectively. Hydrolysis temperature, time, and CO₂ pressure for maximum production of isoquercetin were lower than those of quercetin. Thermal degradation products such as protocatechuic acid and 2,5-dihydroxyacetophenone were observed due to pyrolysis at high temperature. It was concluded that rutin can be easily converted into isoquercetin and quercetin by SWH under CO₂ pressure, and this result can be applied for SWH of rutin-rich foodstuffs.

Keywords: subcritical water hydrolysis, isoquercetin, quercetin, CO₂ pressure, response surface methodology

INTRODUCTION

Rutin (quercetin-3-*O*-rutinoside) is a well-known flavonoid glycoside in the buckwheat species (1). It has several functional properties such as antioxidant, anti-inflammatory, anticarcinogenic, antithrombotic, cytoprotective, and vasoprotective effects in humans (2). However, its antioxidant activity was about 2.3-fold lower (3,4), and its bioavailability was 2.5-fold lower than that of quercetin in rats by oral administration (5). Even though quercetin has better functional properties than rutin, it shows poor absorption in the small intestine, and the bioavailability of quercetin in humans by oral administration in capsule form was reported to be less than 1% due to the lower solubility of quercetin in water (15 mg/L) (6,7).

Recently, isoquercetin (quercetin-3-*O*-glucoside) has attracted attention as a promising compound because it has higher solubility (95 mg/L) than quercetin in water (6,7). Isoquercetin also shows better bioavailability (8) and more antiproliferative effect than rutin and querce-

tin (9). The antioxidant activity of isoquercetin was a little lower than that of quercetin (10). Therefore, it is desirable to maximize the production of isoquercetin together with quercetin during the hydrolysis of rutin.

Subcritical water (SW) has been used as an environment friendly technique for the hydrolysis of cellulose, polysaccharides, proteins, and other bioactive compounds (11-13). SW has a low dielectric constant and a large ion product due to the destruction of hydrogen bonds between water molecular at higher temperature. Therefore, SW can act not only as an organic solvent, but also as a hydrolysis catalyst (14,15). The catalytic capacity of SW can be increased by the addition of CO₂ due to the high concentration of hydrogen ions from the dissociation of carbonic acid, which is formed once CO₂ is dissolved in water.

There are several reports on subcritical water hydrolysis of biomaterials such as conversion of whey proteins into peptides and free amino acids (11), production of monosaccharides and bio-active compounds from marine

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polysaccharides (12), conversion of rutin into quercetin (13), and hesperidin into hesperitin (14). Subcritical water hydrolysis is promising from an industrial and environmental point of view because subcritical water can potentially replace organic solvents such as methyl alcohol or acetone in certain applications (15).

The Box Behnken design (BBD) in response surface methodology (RSM) can be applied to determine the optimal conditions using multiple regression analysis (16). Ravber et al. (13) optimized SW hydrolysis (SWH) of rutin into quercetin by RSM using three parameters: temperature, time, and rutin concentration. However, they did not include CO₂ pressure as an independent variable because the addition of CO₂ increased the yield of quercetin by only 10% with increasing pressure from 5 to 21.5 MPa. They also established the optimum conditions only for quercetin yield.

In this study, the yield of isoquercetin was optimized together with quercetin because isoquercetin has better physiological functionality than rutin and quercetin. CO₂ pressure was included as an independent variable because the SW temperature can be lower with the increase of CO₂ pressure, thereby preventing the degradation of isoquercetin.

The objective of this study was to optimize maximum production of isoquercetin and quercetin simultaneously from rutin by SWH under CO₂ pressure using BBD as a preliminary study before the application to the hydrolysis of the buckwheat species. Hydrolysis parameters such as temperature, time, and CO₂ pressure were selected as independent variables, and the isoquercetin and quercetin yields were selected as dependent variables.

MATERIALS AND METHODS

Chemicals

Rutin trihydrate was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Isoquercetin, quercetin, and formic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile [high-performance liquid chromatography (HPLC) grade] was purchased from Merck (Darmstadt, Germany) and N,O-bis(trimethylsilyl) trifluoroacetamide was purchased from Supelco (Bellefonte, PA, USA).

Experimental design

SWH were optimized using BBD for investigating the influence of independent variables on dependent responses. The main parameters affecting SWH such as temperature (°C, X₁), time (min, X₂), and CO₂ pressure (MPa, X₃) were selected as independent variables. Dependent variables such as the isoquercetin yield, quercetin yield, and rutin loss were selected. Table 1 shows the coded values

Table 1. Levels of independent variables used in the Box-Behnken design

Independent variable	Symbols	Factor levels		
		-1	0	+1
Temperature (°C)	X ₁	140	160	180
Time (min)	X ₂	10	20	30
CO ₂ pressure (MPa)	X ₃	5	10	15

of experimental factors and their corresponding ranges. The experimental design comprised of 17 experiments including five central points for estimating the experimental errors. Regression analysis between independent variables and dependent responses was performed, and the second-order polynomial model was used for data fitting as shown in the equation (1):

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (1)$$

where Y is a dependent response, X's are independent variables, and β's are coefficients. This model includes linear, quadratic, and interaction terms.

Subcritical water hydrolysis

SWH was carried out using our self-built laboratory-scale system (Fig. 1). SWH was performed in a 300-mL high-pressure stainless steel vessel (Autoclave Engineers Fluid, Erie, PA, USA). A 100 mg of rutin suspension in 100 mL of distilled water was loaded into the vessel. It was purged using nitrogen gas to remove oxygen dissolved in the sample solution, then heated to the desired tem-

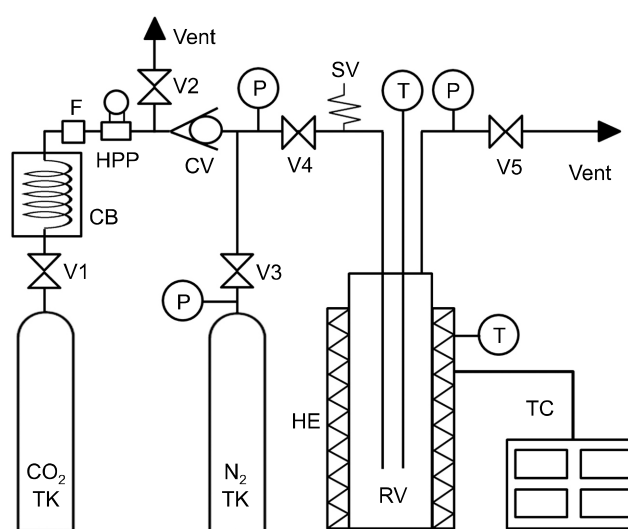


Fig. 1. Schematic diagram of the subcritical water hydrolysis system. HE, heat exchanger; N₂ TK, nitrogen gas tank; CO₂ TK, carbon dioxide tank; CB, cooling bath; F, in-line filter; HPP, high pressure pump; CV, check valve; TC, temperature controller; P, pressure gauge; RV, reaction vessel; SV, safety valve; T, temperature gauge; V, on/off valve.

perature. After that, liquefied CO₂ was pumped into the reactor to the desired pressure. After hydrolysis for the desired time, the vessel was rapidly cooled to ambient temperature by dipping in tap water, and the CO₂ in the vessel was vented. The hydrolyzate was collected, filtered through filter paper (No. 5A, Advantec Toyo Kaisha, Ltd., Tokyo, Japan), and used for HPLC analysis.

HPLC analysis

The contents of rutin, isoquercetin, and quercetin in the hydrolyzate were analyzed using HPLC (Alliance 2965, Waters Corporation, Milford, MA, USA) with a photo diode array detector (Waters 2998, Waters Corporation). Separation was carried out using a XTerra[®] RP C18 column (250 mm×4.6 mm, 5 μm), and the column temperature was set to 30°C. The mobile phase was 0.5% formic acid in water (solvent A) and acetonitrile (solvent B), and the flow rate was 1.0 mL/min. The gradient program of the mobile phase was: 0 min 20% B, 15 min 20% B, 20 min 70% B, 25 min 70% B, and 30 min 20% B. The injection volume was 10 μL, and all compounds were detected at 360 nm.

Calculation of isoquercetin yield, quercetin yield, and rutin loss

The conversion yield (%) of isoquercetin or quercetin was calculated according to the stoichiometry of the reaction using equation (2) as mentioned in Ravber et al. (13):

$$\text{Conversion yield (\%)} = \frac{Q_c \times \frac{MW_R}{MW_C}}{Q_{R0}} \times 100 \quad (2)$$

where Q_c is the quantity of the hydrolyzed product, Q_{R0} is the quantity of initial rutin load, and MW_R and MW_C are molecular weights of rutin and isoquercetin or quercetin, respectively.

The rutin loss (%) during the hydrolysis reaction was calculated using the equation (3):

$$\text{Rutin loss (\%)} = \frac{Q_{R0} - (Q_R + Q_{IQ} + Q_Q)}{Q_{R0}} \quad (3)$$

where Q_{R0} is the quantity of initial rutin load, Q_R is the quantity of the residual rutin after the hydrolysis reaction, and Q_{IQ} and Q_Q are the quantities of isoquercetin and quercetin produced, respectively.

Gas chromatography/mass spectrometry (GC/MS) identification of degradation products

The degradation products during the SWH reaction at high temperature were analyzed using a Agilent series GC 6890N GC with an HP 5973 mass spectrometer and an HP 7683 autosampler (Agilent Technologies, Santa

Clara, CA, USA). The column was an HP-5 MS capillary column (30 m×0.25 mm, 0.25 μm). The gradient program of the column temperature was: 1 min 120°C, 21 min 220°C, 29 min 300°C, and 34 min 300°C. The injector and transfer line temperatures were 280 and 300°C, respectively. The carrier gas was helium, and its flow rate was 0.6 mL/min. The injection volume was 1 μL at a split ratio of 1:20 (17).

Statistical analysis

The experimental results were tested by analysis of variance (ANOVA) using Design Expert 10.0 software trial version (Stat-Ease, Inc., Minneapolis, MN, USA) at the significance level of 0.05. The adequacy of the response surface models was estimated by the P -value, the determination coefficient (R^2), and coefficient of variance (C.V.) (18). The validity between predicted and experimental values was compared by Student's t -test with SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Fitting of the response surface models

The experimental results of isoquercetin yield, quercetin yield, and rutin loss are shown in Table 2. The results of ANOVA of the quadratic polynomial models for the investigated responses and coefficients are shown in Table 3. The model equations of all the responses were reduced by adjusting the P -value below 0.05 in order to fit the

Table 2. Experimental results of isoquercetin yield, quercetin yield, and rutin loss from subcritical water hydrolysis of rutin with different temperature (X_1), time (X_2), and CO₂ pressure (X_3)

Run no.	Independent variables			Responses		
	X_1 (°C)	X_2 (min)	X_3 (MPa)	Isoquercetin yield (%)	Quercetin yield (%)	Rutin loss (%)
1	140	10	10	3.31	6.70	6.45
2	140	30	10	7.33	17.73	9.03
3	180	10	10	11.23	71.34	7.01
4	180	30	10	0.95	81.28	14.23
5	140	20	5	4.37	8.72	6.30
6	140	20	15	5.84	13.26	9.64
7	180	20	5	6.69	75.31	11.11
8	180	20	15	1.82	84.24	10.53
9	160	10	5	11.39	21.52	5.92
10	160	10	15	13.07	31.78	5.12
11	160	30	5	13.40	49.49	5.20
12	160	30	15	10.50	66.94	4.81
13	160	20	10	13.21	42.61	5.78
14	160	20	10	13.60	43.52	5.35
15	160	20	10	13.52	42.33	4.83
16	160	20	10	13.79	45.92	4.91
17	160	20	10	13.65	43.02	6.15

Table 3. Estimated regression coefficients and results of analysis of variance (ANOVA) of the quadratic polynomial models for investigated responses

Source	Yield of isoquercetin			Yield of quercetin			Rutin loss		
	Coefficient	F-value	P-value	Coefficient	F-value	P-value	Coefficient	F-value	P-value
Model		52.470	<0.0001		141.597	<0.0001		15.491	0.0003
β_0	13.46			43.86			5.34		
X_1	-0.020	0.004	0.9511	33.22	377.868	<0.0001	1.43	6.184	0.0261
X_2	-0.85	7.322	0.0268	10.51	37.856	<0.0001	ns	ns	ns
X_3	-0.58	3.364	0.1040	5.15	9.067	0.0100	ns	ns	ns
$X_1 \times X_2$	-3.57	64.332	<0.0001	ns	ns	ns	ns	ns	ns
$X_1 \times X_3$	-1.59	12.709	0.0073	ns	ns	ns	ns	ns	ns
$X_2 \times X_3$	-1.15	6.622	0.0330	ns	ns	ns	ns	ns	ns
X_1^2	-7.64	310.280	<0.0001	ns	ns	ns	3.95	24.797	0.0002
X_2^2	ns ¹⁾	ns	ns	ns	ns	ns	ns	ns	ns
X_3^2	-1.26	8.439	0.0197	ns	ns	ns	ns	ns	ns
Lack of fit		32.945	0.0026		15.874	0.0086		11.214	0.0162
R^2	0.9813			0.9703			0.6888		
Adjusted R^2	0.9626			0.9635			0.6443		
Predicted R^2	0.8183			0.9387			0.4552		
C.V. (%)	9.61			11.02			22.66		
Adequate precision	18.888			37.306			4.853		

β_0 , a constant; X_1 , temperature (°C); X_2 , time (min); X_3 , CO₂ pressure (MPa); R^2 , determination coefficient; C.V., coefficient of variance.
¹⁾ns: no significant level at $P < 0.05$.

models better.

The regression model of the isoquercetin yield was valid due to a high F -value (52.4) and a low P -value (<0.0001). Furthermore, the high regression coefficient ($R^2=0.9813$) indicates that the polynomial model equation provides a good approximation of experimental results. The high-adjusted regression coefficient ($R^2=0.9626$) also indicates good correlation of the experimental results and is in reasonable agreement with the predicted R^2 (0.8183) because their difference is less than 0.2. Relatively low C.V. (9.6%) for the isoquercetin yield indicated good reproducibility of the model, and the adequate precision (signal to noise ratio, 18.8) of the model was higher than 4, therefore indicating an adequate signal. However, lack of fit ($P=0.0026$), relative to pure error, was less than 0.05 due to the over fit of the model. Lack of fit is determined by error of the central point, and an insignificant lack of fit indicates that the model equation is adequate for predicting the responses. However, the model can be overfitted when the central point error is low, and the lack of fit of the model may be significant. Other studies reported that although the lack of fit was significant, optimization was performed because the regression coefficient (R^2), adjusted R^2 , and predicted R^2 of the prediction model showed good fit (19,20).

The regression model of the quercetin yield was greatly reduced by adjusting the P -value below 0.05 because the relationship between independent and dependent variables was nearly linear, and the response surface linear model was adequate for predicting the responses. The ANOVA test showed that the regression model of the

quercetin yield was valid due to the high F -value (141.5), low P -value (<0.0001), high R^2 (0.9703), high adjusted R^2 (0.9635), less difference (0.2) between the adjusted and predicted R^2 , relatively low C.V. (11.0%), and higher adequate precision (37.3). Lack of fit P -value of the quercetin yield (0.0086) was less than 0.05 due to over fit of the model.

The regression model of the rutin loss was greatly reduced by adjusting the P -value below 0.05. Lower R^2 (0.6888), lower predicted R^2 (0.4552), adjusted R^2 (0.6443), and relatively higher C.V. (22.6%) indicated that the model of rutin loss was not appropriate for the response surface model.

Effect of hydrolysis parameters on the yield of isoquercetin

Fig. 2A shows the response surface plots for the effect of hydrolysis parameters on the yield of isoquercetin. The yield of isoquercetin was greatly influenced by temperature instead of time (Fig. 2A-1). The yield of isoquercetin was greatly increased as the increase in temperature from 140°C to 160°C and reached a maximum at 160°C, and then decreased to 180°C. The isoquercetin yield was decreased at a higher temperature and time due to the thermal instability of isoquercetin and the increased thermal degradation to other compounds (14,21). Ko et al. (21) reported that the yield of isoquercetin by SW extraction from *Saururus chinensis* peaked at 150°C/15 min and decreased at high temperature due to thermal degradation. Therefore, in order to enhance the production of isoquercetin using SWH, rutin should be hydrolyzed for a short time at high temperature or for a

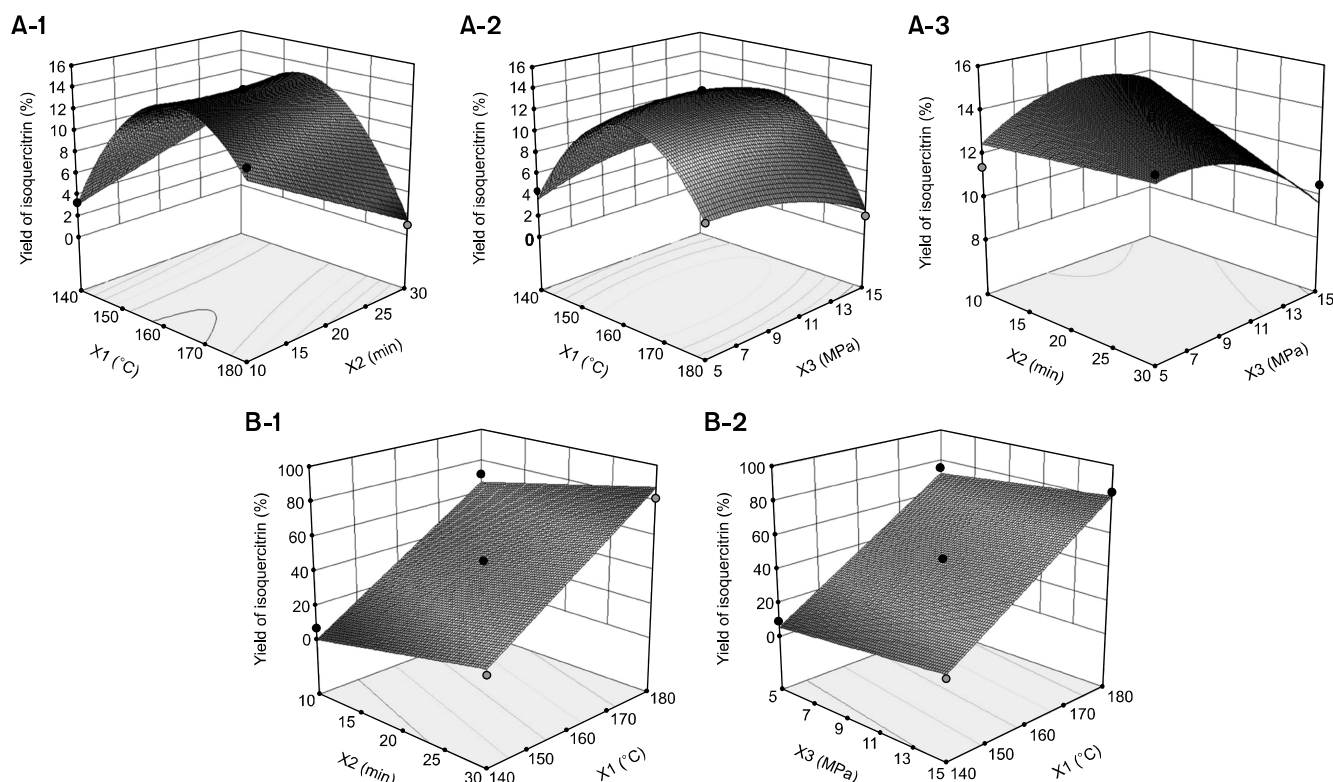


Fig. 2. Response surface plots and contour plots for the effects of hydrolysis temperature (X_1), time (X_2), and CO_2 pressure (X_3) on the yields of isoquercetrin (A) and quercetrin (B).

long time at low temperature.

Fig. 2A-2 shows the relationship between CO_2 pressure and temperature. At 180°C , the yield of isoquercetrin was low regardless of CO_2 pressure due to the increased conversion of isoquercetrin produced from rutin into quercetrin. Ruen-ngam et al. (14) reported that the activation energy (83.8 kJ/mol) required for cleaving the glycosidic bond between rhamnose (rhamnosidic bond) and hesperetin- β -glucoside was higher than that (4.1 kJ/mol) of glucose to produce hesperetin. The yield of isoquercetrin was highest at 160°C regardless of CO_2 pressure due to the decreased degradation of isoquercetrin into quercetrin. However, at 140°C , the yield of isoquercetrin was greatly influenced by CO_2 pressure instead of temperature, and it increased with increasing CO_2 pressure due to the increased conversion of rutin into isoquercetrin at lower pH. Therefore, the SW temperature can be lower with the increase of CO_2 pressure, thereby preventing the degradation of isoquercetrin.

Fig. 2A-3 shows the relationship between CO_2 pressure and hydrolysis time. At 30 min, the yield of isoquercetrin was maintained at the maximum at low CO_2 pressure, and then decreased with the increase of CO_2 pressure. At 10 min, the isoquercetrin yield was greatly influenced by CO_2 pressure instead of hydrolysis time, and it increased with increasing CO_2 pressure.

Therefore, in the SW hydrolysis of rutin into isoquercetrin, temperature was the major factor, and the hydroly-

sis temperature or time can be lower if the CO_2 pressure was increased high enough, and the degradation of isoquercetrin into quercetrin at high temperature and time can be prevented because isoquercetrin is more sensitive to heat than quercetrin (21).

Effect of hydrolysis parameters on the yield of quercetrin

Fig. 2B shows the response surface plots for the effect of hydrolysis parameters on the yield of quercetrin. Fig. 2B-1 shows the relationship between temperature and time at 10 MPa. The yield of quercetrin was considerably increased with increasing temperature from 140°C to 180°C at all hydrolysis times due to the thermal stability of quercetrin compared to isoquercetrin and further production of quercetrin by hydrolysis of isoquercetrin. With increasing the hydrolysis time, the yield of quercetrin was slightly increased at all temperatures. Ravber et al. (13) reported that SW temperature had a significant effect on the hydrolysis of rutin into quercetrin, and the yield of quercetrin was increased up to 205°C .

Fig. 2B-2 shows the relationship between temperature and CO_2 pressure at 20 min. The yield of quercetrin was considerably increased with increasing temperature from 140°C to 180°C at all CO_2 pressures. At the same temperature, the yield of quercetrin was slightly increased with increasing CO_2 pressure. Therefore, the yield of quercetrin was considerably influenced by temperature instead of time and CO_2 pressure.

Table 4. Optimum conditions for maximum yields of isoquercetin and quercetin from subcritical water hydrolysis of rutin

Responses	Temperature (°C)	Time (min)	CO ₂ pressure (MPa)	Predicted yield (%)	
				Isoquercetin	Quercetin
Isoquercetin+quercetin	171.4	10.0	11.0	13.7	53.3
Only isoquercetin	168.0	11.1	8.6	14.2	46.5
Only quercetin	179.8	22.5	14.7	1.3	84.4

Optimization conditions for maximum yields of isoquercetin and quercetin

Optimum conditions of independent variables for maximum yields of isoquercetin and quercetin are shown in Table 4. The objective of this study was to optimize hydrolysis parameters for maximum production of isoquercetin and quercetin simultaneously. In this case, the optimal condition was temperature of 171.4°C, time of 10.0 min, and CO₂ pressure of 11.0 MPa, where the predicted maximum yields of isoquercetin and quercetin were 13.7% and 53.3%, respectively.

Optimum conditions of independent variables for maximum production of isoquercetin or quercetin are also shown in Table 4. The optimal condition for maximum production of only isoquercetin was temperature of 168.0°C, time of 11.1 min, and CO₂ pressure of 8.6 MPa, where the predicted maximum yield of isoquercetin was 14.2%. In case of the maximum production of quercetin, the optimal condition was temperature of 179.8°C, time of 22.5 min, and CO₂ pressure of 14.7 MPa, where the predicted yield of quercetin was 84.4%. Hydrolysis temperature and CO₂ pressure for maximum production of isoquercetin and quercetin simultaneously were higher than those for the maximum production of only isoquercetin and lower than those for maximum production of only quercetin. Hydrolysis temperature, time, and CO₂ pressure for maximum production of isoquercetin were lower than those of quercetin because isoquercetin is more heat labile than quercetin.

Verification of the optimal condition

Verification of the optimal condition for maximum production of isoquercetin and quercetin simultaneously was examined using temperature of 171°C, time of 10

min, and CO₂ pressure of 11 MPa. The comparison of predicted and experimental values for the yields of isoquercetin and quercetin are shown in Table 5. There was a statistically good agreement ($P < 0.05$) between the predicted value (13.7%) and the experimental value ($14.9 \pm 0.8\%$) of the isoquercetin yield. On the other hand, the experimental value ($46.9 \pm 1.0\%$) of the quercetin yield showed a statistical difference to the predicted value (53.3%) ($P < 0.05$). However, the difference (12.0%) between predicted and experimental values was similar to C.V% (11.02) in the regression model of the quercetin yield as shown in Table 3.

GC/MS identification of degradation products

Degradation products during the hydrolysis reaction were identified by GC/MS, and the hydrolysate at 180°C and 10 MPa for 30 min was chosen for sample analysis due to the highest rutin loss (Table 6). Protocatechuic acid and 2,5-dihydroxyacetophenone may be expected to be decomposed from A and B rings of quercetin molecules, respectively. Propanoic acid and 4-hydroxyvaleric acid were expected to be decomposed from C-ring of quercetin molecules. Hydroquinone and trihydroxybenzene were also observed. Buchner et al. (22) reported that quercetin was degraded into fragmentation compounds such as protocatechuic acid and 2,3-dihydroxy-(3',4'-dihydroxyphenyl)-prop-2-en-1-al by heat treatment. In our study, we detected six decomposition compounds. However, we did not detect 2,3-dihydroxy-(3',4'-dihydroxyphenyl)-prop-2-en-1-al.

In conclusion, the response surface methodology was effectively used to optimize the effects of the hydrolysis parameters such as temperature, time, and CO₂ pressure for hydrolysis of rutin into isoquercetin and quercetin.

Table 5. Predicted and experimental values of isoquercetin and quercetin yields obtained under the optimal hydrolysis condition

Hydrolysis variables			Parameter	Predicted values (%)	Experimental values (%)
X ₁	X ₂	X ₃			
171	10	11	Isoquercetin yield	13.7	14.9±0.8
			Quercetin yield	53.3	46.9±1.0*

X₁, temperature (°C); X₂, time (min); X₃, CO₂ pressure (MPa). Data are given as means±SD (n=3).

*The value is significantly different from the predicted value ($P < 0.05$)

Table 6. Gas chromatography/mass spectrometry analysis of degradation products from the subcritical water hydrolysate at 180°C and 10 MPa for 30 min

Retention time (min)	Name	Relative peak area (%)
7.3	Hydroquinone	6.2
9.6	Propanoic acid	9.9
9.8	4-hydroxyvaleric acid	35.1
11.4	1,2,3-trihydroxybenzene	30.0
15.9	Protocatechuic acid	12.5
18.9	2,5-dihydroxyacetophenone	6.3

The optimum condition for maximum production of isoquercetin and quercetin simultaneously was temperature of 171.4°C, time of 10.0 min, and CO₂ pressure of 11.0 MPa, where the predicted maximum yields of isoquercetin and quercetin were 13.7% and 53.3%, respectively. The CO₂ pressure was an important factor for production of isoquercetin at low temperature and time. The quercetin yield was greatly affected by temperature than time and CO₂ pressure. This result can be applied for the subcritical water hydrolysis of rutin-rich foodstuffs.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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