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RESEARCH ARTICLE

Optimum Conditions for the Enzymatic Hydrolysis of Citron Waste Juice Using Response Surface Methodology (RSM)

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Abstract This study investigated the optimum conditions, including pH, temperature, incubation time, and enzyme concentration, for the enzymatic manufacture of citron waste juice by pectinase and cellulase using response surface methodology (RSM). A central composite design was used as an experimental design for the allocation of treatment combination. The highest yield was 71.1% at pH 5.00, 51.15°C, 53.74 min, and 0.44%, whereas the lowest turbidity was 0.20 at pH 4.87, 50.21°C, 49.99 min, and 0.44%. The highest hunter's L (lightness), a (redness), and b- (yellowness) values were 47.39 (pH 4.86, 50.72°C, 51.60 min, and 0.44%), -1.34 (pH 5.48, 49.36°C, 46.29 min, and 0.21%), and 13.18 (pH 5.49, 50.05°C, 43.31 min, and 0.23%), respectively. The highest electron donating activity was 68.36% at pH 4.51, 50.62°C, 47.00 min, and 0.23%. The highest limonin and nomilin contents were 3.49 mg/100 g (pH 4.51, 50.30°C, 48.34 min, and 0.21%) and 1.56 mg/100 g (pH 4.59, 50.08°C, 66.07 min, and 0.30%).

Keywords: citron waste, enzymatic treatment, yield, response surface methodology

Introduction

Citron (*Citrus junos* Sieb. ex TANAKA) is a citrus fruit native to northeast Asia, including Korea, China, and Japan. The fruit originated from China but was introduced to Korea and Japan during the Tang dynasty. Citron fruit looks like a very small grape fruit with uneven skin, and can be either yellow or green depending on the degree of ripeness. Citron is processed into juice and is often preferred to vinegar as an ingredient in sauces and salad dressings due to its special flavor. Particularly in Korea, it is commonly used as a raw material for beverages and herbal medicines due to its unique flavor and effectiveness against colds (1-3).

During citron processing, about half of the fruit is converted into juice while the rest ends up as waste comprising peels, seeds, segment membranes, and fruit pulp, which is usually dumped at great expense. Therefore, the processing of food waste has been become a very serious environmental issue as well as the subject of much research. Such a study was conducted on the use of waste from citron (4). Citrus peel is rich in polyphenolic compounds, which are have antioxidant (5), anti-cancer (6), and anti-microbial activities (7). Citrus peel extract is of potential interest to the food industry as it retards oxidative changes in food, thus improving quality, and nutritional value.

The use of enzymes in fruit juice processing can improve quality, yield, and processing efficiency (8). One of the main applications of enzymatic processing is in the production of apple juice, using polygalacturonase (PG) and pectinesterase (PE) for juice clarification. These depectinising enzymes are also used to improve the yield and pressability of apple pulp. In recent years, degradative enzymes containing pectinase, cellulase, and hemicellulase activities have been introduced for the complete breakdown of fruit cell walls. These enzymatic processes produce high yields without the need for pressing (9). Mouri and Kayama (10) suggested a method known as 'total

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liquefaction' for the production of orange juice. However, technical details of the method and its effect on product quality are not yet available.

Therefore, this study aimed to investigate the effects of pH, temperature, incubation time, and enzyme concentration on the yield, turbidity, color, electron donating ability (EDA), limonin, and nomilin content of citron juice subjected to enzymatic hydrolysis using response surface methodology (RSM).

Materials and Methods

Sample preparation Citron processing residues (peel, attached membranes, and seeds) after manufacture of citron tea were obtained from a local food processing company (Metome, Ochang, Korea) and stored at −20°C. Pectinase (Pectinex 5XL, 5,000 FDU/mL) and cellulase (Celluclast 1.5L, 700 EGU) were obtained from Novozymes A/S (Bagsvaerd, Denmark).

Samples of citron waste were used after thawing. The moisture content of obtained citron waste was 8.1%. The citron waste (50 g) was placed into a sample bottle with water. A waste:water ratio (w/w) of 2:3 was used for extraction.

For each experiment, about 125 g of waste/water slurry was subjected to various enzymatic treatment conditions as shown in Table 1. Following enzymatic treatment, enzyme inactivation was performed by heating the suspension at 100°C for 10 min in an autoclave. The treated juices were then centrifuged at 3,000×g for 10 min (Supra 22K; Hanil, Seoul, Korea), and the supernatants were filtered through Whatman fiter paper No. 2. Raw citron waste was subjected to the same process as treated juices, except for incubation time. The citron juice was kept at −20°C until analysis.

Experimental design for RSM RSM was applied in order to determine the optimum conditions for producing citron waste juice. As shown in Table 1, the experimental design for processing conditions was based on a central composite design. Independent variables such as pH (X_1: 4.5-5.5), temperature (X_2: 45-55°C), incubation time (X_3: 20-80 min), and enzyme concentration (X_4: 0.05-0.45%) were assigned as numbers (-1, 0, 1). The total number of experiments was 20, including 16 at factorial points and 4 replications at the centre point (11). The dependent variables (Yn), such as yield (Y_1), turbidity (Y_2), lightness (Y_3), redness (Y_4), yellowness (Y_5), EDA (Y_6), limonin content (Y_7), and nomilin content (Y_8), were calculated 3 times, and their average values were used for regression analysis. SAS software (SAS Institute, Cary, NC, USA) was used for analysis of variance (ANOVA) and Duncan's multiple range tests (at $p<0.05$).

Physicochemical analysis The yield of total soluble solids (%) was determined by calculating the dry weight of supernatant obtained from the reactant/dry weight of reactant (20 g) as a percentage (13). Turbidity was determined by measuring the absorbance at 660 nm using a ultraviolet (UV)-Vis spectrophotometer (Cary-100 Bio; Varian, Sydney, Australia) (12). Distilled water was used as a reference. The Hunter's color L (lightness), a (redness), and b-(yellowness) values were determined using a colorimeter (CR-300; Minolta, Osaka, Japan). Samples (15 mL) were put into a glass cell and measured. All measurements were taken at random, and the averages were reported. The L-, a-, and b-values of the standard plate were 96.29, 0.14, and 1.96, respectively.

DPPH radical scavenging activity The scavenging activity for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was evaluated using a modified method of Blois (14) involving the measurement of absorbance at a wavelength of 525 nm using a ultraviolet (UV)/VIS spectrophotometer. The percentage inhibition of activity was calculated as $(A_0- A_1)/A_0\times 100$, where A_0 is the absorbance without the sample and A_1 is the absorbance with the sample.

High performance liquid chromatography (HPLC) analysis of limonoids Limonoid analysis was performed using the method reported by Jeong *et al.* (15). HPLC analysis of limonoids from treated citron waste juices was performed using an Agilent 1100 series instrument (Waldbronn, Germany) equipped with a pump, autosampler, and degasser. Treated citron waste juices were filtered through a 0.45-μm membrane and injected at ambient temperature into an Eclipse XDB-C18 (4.6×250 mm, Agilent Technologies, Palo Alto, CA, USA). The isocratic mobile phases (acetonitrile: 10 mM acetic acid, 40:60) were used for limonoid analysis at a flow rate of 1.0 mL/min followed by detection at 210 nm. Two limonoids (limonin and nomilin) were confirmed by comparing their retention times in LC with their standards (Sigma-Aldrich, St. Louis, MO, USA). The amounts of these 2 in the extracts were calculated based on the calibration curve of each standard.

Statistical analysis All data were expressed as mean± standard deviatiom (SD). ANOVA and Duncan's multiple range tests (at $p<0.05$) were performed using the SAS software (SAS Institute).

Results and Discussion

Yield Pectolytic enzymes are used for increasing the yield of juice from stone fruits such as peaches, plums, and

Table 1. Experimental data for yield, turbidity, and color of citron waste juice by central composite design using response surface analysis

Independent variables				Dependent variables				
pH	Temp. (℃)	Time (min)	Enzyme conc. (%)	Yield (%)	Turbidity	Hunter's color		
						L	a	b
5.5 (1)	55 (1)	80 (1)	0.45 (1)	67.80±0.86$^{k1)}$	0.59±0.04^{g}	43.25±1.66^{f}	-1.79±0.14^{cd}	11.66±0.43^{ab}
5.5 (1)	55 (1)	80 (1)	0.05 (-1)	58.65±1.99^{ef}	0.84±0.03^{j}	41.81±0.87^{def}	-1.35±0.13^{e}	13.17±1.26^{def}
5.5 (1)	55 (1)	20 (-1)	0.45 (1)	67.12±1.86^{jk}	0.42±0.01^{bcd}	45.83±0.45^{j}	-1.67±0.01^{cd}	14.59±0.44^{g}
5.5 (1)	55 (1)	20 (-1)	0.05 (-1)	51.91±0.69^{c}	0.84±0.05^{j}	40.73±1.23^{cde}	-1.23±0.04^{e}	13.12±0.54^{def}
5.5 (1)	45 (-1)	80 (1)	0.45 (1)	57.66±2.21^{e}	0.48±0.01^{cde}	42.11±1.73^{def}	-1.58±0.10^{d}	12.30±0.50^{bcd}
5.5 (1)	45 (-1)	80 (1)	0.05 (-1)	44.93±0.85^{a}	0.75±0.02^{i}	40.15±1.34^{bcd}	-1.22±0.08^{e}	13.10±0.72^{def}
5.5 (1)	45 (-1)	20 (-1)	0.45 (1)	58.89±0.38^{ef}	0.44±0.04^{cde}	41.38±1.61^{cdef}	-1.32±0.09^{e}	12.84±0.64^{cde}
5.5 (1)	45 (-1)	20 (-1)	0.05 (-1)	53.94±0.09^{d}	0.91±0.07^{j}	39.48±0.32^{bc}	-0.90±0.14^{f}	14.05±0.23^{fg}
4.5 (-1)	55 (1)	80 (1)	0.45 (1)	63.38±0.83^{gh}	0.20±0.07^{a}	46.00±0.32^{j}	-1.78±0.01^{cd}	11.74±0.35^{ab}
4.5 (-1)	55 (1)	80 (1)	0.05 (-1)	47.83±0.62^{b}	0.65±0.02^{gh}	41.82±1.42^{g}	-1.36±0.08^{e}	12.37±0.17^{bcd}
4.5 (-1)	55 (1)	20 (-1)	0.45 (1)	62.19±0.89^{g}	0.12±0.02^{a}	47.93±0.64^{k}	-2.07±0.11^{b}	12.82±1.16^{cde}
4.5 (-1)	55 (1)	20 (-1)	0.05 (-1)	47.27±0.52^{b}	1.11±0.08^{k}	38.45±0.41^{b}	-1.03±0.06^{f}	12.92±0.22^{cde}
4.5 (-1)	45 (-1)	80 (1)	0.45 (1)	68.50±0.32^{k}	0.16±0.05^{a}	49.17±0.34^{k}	-2.40±0.06^{a}	11.24±0.43^{a}
4.5 (-1)	45 (-1)	80 (1)	0.05 (-1)	60.18±1.15^{f}	0.69±0.04^{hi}	44.07±1.18^{hij}	-1.78±0.17^{cd}	11.81±0.24^{ab}
4.5 (-1)	45 (-1)	20 (-1)	0.45 (1)	64.88±0.65^{hi}	0.35±0.07^{b}	45.06±1.39^{ij}	-2.03±0.17^{b}	11.96±0.03^{abc}
4.5 (-1)	45 (-1)	20 (-1)	0.05 (-1)	59.76±0.24^{f}	0.73±0.03^{hi}	42.62±2.06^{gh}	-1.75±0.18^{cd}	13.30±0.37^{def}
5.0 (0)	50 (0)	50 (0)	0.25 (0)	62.02±0.40^{g}	0.39±0.07^{bc}	45.58±0.55^{j}	-2.07±0.12^{b}	12.35±0.27^{bcd}
5.0 (0)	50 (0)	50 (0)	0.25 (0)	65.46±0.17^{ij}	0.41±0.00^{bc}	45.81±1.11^{j}	-2.06±0.09^{b}	11.71±0.75^{ab}
5.0 (0)	50 (0)	50 (0)	0.25 (0)	66.74±1.85^{ijk}	0.51±0.12^{def}	45.09±1.67^{ij}	-1.83±0.20^{c}	11.39±0.09^{ab}
5.0 (0)	50 (0)	50 (0)	0.25 (0)	67.91±1.20^{k}	0.52±0.05^{ef}	43.85±1.13^{ghij}	-1.73±0.10^{cd}	11.41±0.42^{ab}
Control				48.99±1.31^{b}	1.47±0.00^{l}	34.42±0.13^{a}	-0.96±0.03^{f}	13.63±0.05^{ef}

[1)]Each value is mean±SD (n=3); Any means is the same column followed by the same letter are not significantly (p<0.05) different by Duncan's multiple range test.

apricots (16). The yield of citron waste juice prepared using various experimental conditions is shown in Table 1. The average yield of raw citron waste juice was 48.99%, ranging from 44.93 to 68.50% according to the treatment conditions. The highest yield (68.50%) was observed at pH 4.5, a temperature of 45°C, an incubation time of 80 min, and an enzyme concentration of 0.45%. In contrast, the lowest yield (44.93%) was observed at the same temperature and incubation time, but at a different pH (5.5) and enzyme concentration (0.05%). The optimum conditions for increasing yield were pH 5.00, 51.15, 53.74 min, and 0.44% (71.10%). As treatment time and enzyme concentration were increased, so was the total yield (Fig. 1). This is due to the increased degradation of polymeric cell wall saccharides, such as cellulose and pectin, into smaller saccharides at high enzyme concentrations. Suad *et al.* (17) reported that yield of soluble solids according to enzyme concentration was increased gradually to 1% for *Safri* date fruits. The whole F-value was affected by pH, temperature, and enzyme concentration more so than by incubation time (p<0.01, Table 2). R^2 for the yield was 0.9249 (Table 3) and the model (lack of fit) was suitable (p>0.34).

Turbidity Fresh fruit juices are usually cloudy and have colloidal suspensions. In the case of oranges and tomatos, this cloudiness is actually a desirable and acceptable (18). The turbidities of citron waste juices prepared under various experimental conditions are shown in Table 1. The turbidity of raw citron waste juice was as high as 1.47 depending on the treatment conditions and ranged from 0.12 to 1.11. The lowest turbidity (0.12) was observed at pH 4.5, a temperature of 55°C, an incubation time of 20 min, and an enzyme concentration of 0.45%. In contrast, the highest turbidity (1.11) was observed with at the same pH, temperature, and incubation time, but with a lower enzyme concentration (0.05%). Therefore, turbidity was mainly a function of enzyme concentration. To be explicit, an increase in enzyme concentration drastically decreased turbidity. Furthermore, increased pH reduced the turbidity. This is due the dessolution of pectin and colloidal suspensions in citron waste by enzyme treatment. Sohn *et al.* (19) reported that the turbidity of orange juice was decreased at an enzyme concentration of 0.03% at 40°C and 60 min. During the clarification process, the amount of pectin in the juice was decreased, therefore reducing the turbidity (20). However, Jeong *et al.* (12) observed that the turbidity of persimmon vinegar depended on incubation time. The whole F-value was affected by enzyme

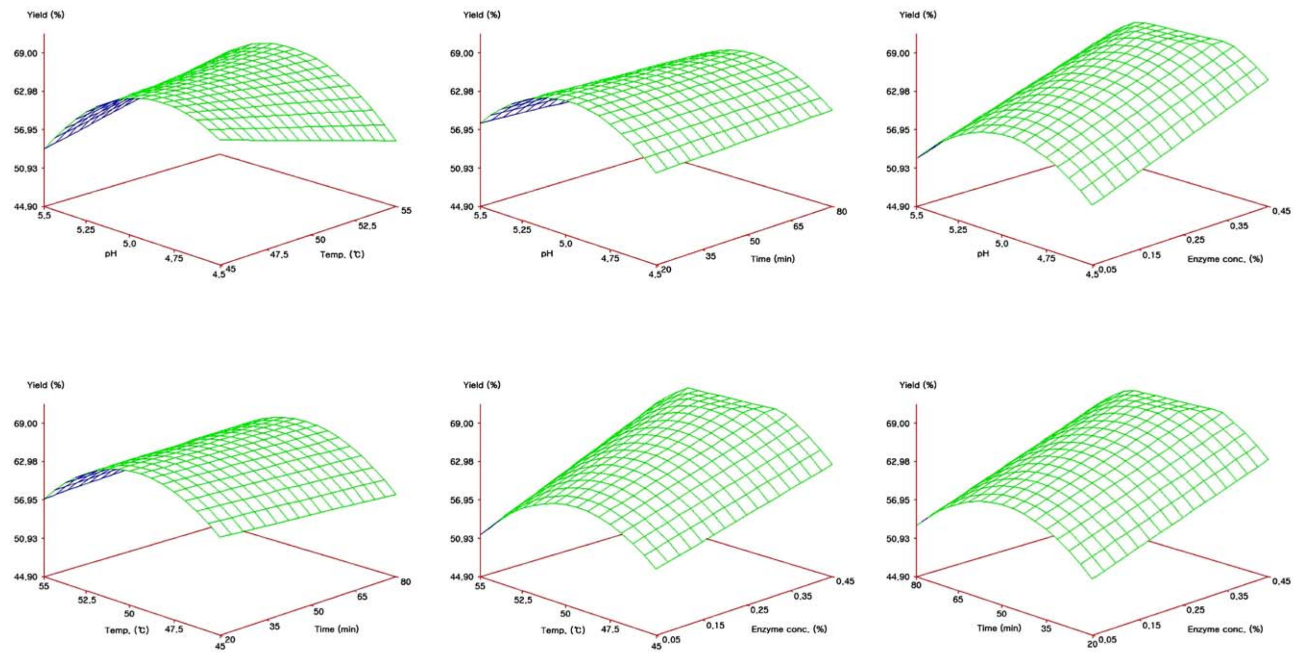

Fig. 1. Effect of pH, incubation temperature, incubation time, and enzyme concentration on the yield of citron waste juice.

concentration more so than by pH, temperature, or incubation time (p<0.001, Table 2). R^2 for the turbidity was 0.9166 (Table 3) and the model was suitable (p>0.14).

Color characteristics The quality and clarity of juice is judged first on its characteristics of appearance such as color. Color is therefore a very important sensory attribute (21). The color of citron waste juice treated under various conditions is shown in Table 1. The L, a, and b-values in raw citron waste juice were 34.42, -0.96, and 13.63, respectively. Both the highest L-value (49.17) and lowest a-value (-2.40) occurred at pH 4.5, a temperature of 55°C, an incubation time of 20 min, and an enzyme concentration of 0.45%. The highest b-value (14.59) occurred at the same temperature, time, and enzyme concentration, but at a different pH (5.5). As pH and enzyme concentration were increased, the color value also tended to increase. Abdullah *et al*. (5) reported that the L-value was increased with increasing enzyme concentration in carambola fruit juice. As a whole, the F-value of L along with the a-value were affected by pH and enzyme concentration more so than by temperature and incubation time (p<0.01, Table 2). However, the b-value was not found to have a significant effect. R^2 for the L, a, and b-value were 0.9157, 0.9265, and 0.8083, respectively (Table 3).

DPPH radical scavenging activity Antioxidants decrease the absorbance of DPPH radical by using hydrogen donation to scavenge radicals, producing a visible change from purple to colorless (22). The DPPH radical scavenging activity of citron waste juice treated under various conditions is shown in Table 4. Raw citron waste juice exhibited its lowest radical scavenging activity at a concentration of 50 mg/mL. Treatment of citron waste juice (50 mg/mL) produced the highest scavenging activity (75.33%) at pH 4.5, a temperature of 55°C, an incubation time of 20 min, and an enzyme concentration of 0.05%. The optimum conditions for DPPH radical scavenging

Table 2. Regression analysis of the physicochemical properties of citron waste juice

Variable	F-value							
	Yield	Turbidity	Color			EDA[1)]	Limonin content	Nomilin content
			L	a	b			
pH	8.89**[1)]	3.40	7.90**	11.25**	3.14	6.46*	11.07**	4.94*
Temperature	7.78**	0.35	3.53	5.20*	0.65	9.59**	2.03	3.45
Time	0.57	1.56	1.82	1.38	3.05	3.42	0.99	7.20**
Enzyme conc.	13.12**	18.28***	12.00**	9.92**	1.57	0.33	1.20	5.90

[1)]Electron donating ability
[2)]*p<0.05, **p<0.01, ***p<0.001

Table 3. Regression coefficients of the second order polynomials for yield, turbidity, color, electron donating ability (EDA), limonin, and nomilin contents under various treatment conditions

Parameter[1)]	Estimates							
	Yield	Turbidity	Color			EDA	Limonin content	Nomilin content
			L	a	b			
Intercept	-225.11	13.17	-65.06	14.03	98.17*	755.69**	28.45*	5.39
X_1	206.15*[2)]	-4.98	60.26	-10.07*	-36.48*	-217.73*	-7.90	-2.14
X_2	-8.56***	0.01	-1.87*	0.35**	0.15	-4.61*	-0.14	0.03
X_3	-0.15	-0.02	0.32	-0.01	0.02	-1.38**	-0.02	0.06*
X_4	-44.28	-3.07	19.38	-1.55	-12.00	-35.24	-4.97	-3.22
$X_1 \times X_1$	-28.41**	0.49	-7.87*	1.39**	3.90*	15.78	0.52	0.27
$X_2 \times X_1$	1.57***	-0.00	0.38*	-0.07**	-0.03	0.92*	0.03	-0.01
$X_3 \times X_1$	-0.04	0.00	-0.03	-0.00	-0.00	0.12	0.00	-0.01*
$X_3 \times X_2$	0.01	0.00	-0.00	0.00	-0.00	0.02*	-0.00	-0.00
$X_4 \times X_1$	-1.18	0.59	-6.76	0.44	0.36	7.14	0.78	-0.72
$X_4 \times X_2$	1.48	-0.03	0.55	-0.04	0.20	-0.02	0.00	0.11*
$X_4 \times X_3$	0.06	0.01	-0.07	0.00	-0.02	-0.04	0.01	0.02*
R^2	0.9249	0.9166	0.9157	0.9265	0.8083	0.8882	0.8886	0.8764
Lack of fit	$p>0.34$	$p>0.14$	$p>0.22$	$p>0.56$	$p>0.25$	$p>0.18$	$p>0.18$	$p>0.19$

[1)]X_1, pH; X_2, temperature (°C); X_3, time (min); X_4, enzyme concentration (%)
[2)]*$p<0.05$, **$p<0.01$, ***$p<0.001$

Table 4. Experimental data on electron donating ability (EDA), limonin, and nomilin contents of citron waste juice by central composite design using response surface analysis

Independent variables				Dependent variables		
PH	Temp. (°C)	Time (min)	Enzyme conc. (%)	EDA (%)	Limonin content (mg/100 g)	Nomilin content (mg/100 g)
5.5 (1)	55 (1)	80 (1)	0.45 (1)	74.40±0.37[jk1)]	2.95±0.12[bc]	1.22±0.13[bc]
5.5 (1)	55 (1)	80 (1)	0.05 (-1)	70.79±4.52[hijk]	3.09±0.15[bcd]	1.14±0.07[bc]
5.5 (1)	55 (1)	20 (-1)	0.45 (1)	61.76±1.18[cdef]	2.97±0.22[bc]	1.16±0.10[bc]
5.5 (1)	55 (1)	20 (-1)	0.05 (-1)	65.64±3.18[efgh]	2.97±0.14[bc]	1.30±0.06[cd]
5.5 (1)	45(-1)	80 (1)	0.45 (1)	57.50±3.74[bc]	3.04±0.20[bc]	1.32±0.04[cd]
5.5 (1)	45(-1)	80 (1)	0.05 (-1)	54.03±3.03[b]	2.54±0.14[a]	1.19±0.10[bc]
5.5 (1)	45(-1)	20 (-1)	0.45 (1)	54.89±3.60[b]	2.39±0.22[a]	0.73±0.02[a]
5.5 (1)	45(-1)	20 (-1)	0.05 (-1)	56.89±2.89[bc]	2.45±0.24[a]	1.63±0.26[efg]
4.5 (-1)	55 (1)	80 (1)	0.45 (1)	66.07±2.41[efghi]	3.59±0.25[gh]	1.85±0.07[g]
4.5 (-1)	55 (1)	80 (1)	0.05 (-1)	75.33±1.16[k]	3.54±0.05[gh]	1.32±0.26[cd]
4.5 (-1)	55 (1)	20 (-1)	0.45 (1)	71.07±4.39[hijk]	3.37±0.21[defg]	1.53±0.02[def]
4.5 (-1)	55 (1)	20 (-1)	0.05 (-1)	66.23±4.93[fghi]	3.55±0.17[fgh]	1.35±0.30[cd]
4.5 (-1)	45(-1)	80 (1)	0.45 (1)	60.52±7.05[cde]	3.22±0.02[cde]	1.77±0..34[fg]
4.5 (-1)	45(-1)	80 (1)	0.05 (-1)	64.74±2.07[efg]	3.46±0.11[efg]	1.81±0.14[g]
4.5 (-1)	45(-1)	20 (-1)	0.45 (1)	69.75±1.15[ghij]	3.25±0.12[cdef]	0.96±0.07[ab]
4.5 (-1)	45(-1)	20 (-1)	0.05 (-1)	71.23±0.67[ijk]	3.83±0.23[h]	1.31±0.11[cd]
5.0 (0)	50 (0)	50 (0)	0.25 (0)	62.33±0.69[cdef]	2.84±0.15[b]	1.20±0.16[bc]
5.0 (0)	50 (0)	50 (0)	0.25 (0)	63.19±0.62[def]	3.10±0.16[bcd]	1.33±0.10[cd]
5.0 (0)	50 (0)	50 (0)	0.25 (0)	58.45±1.92[bcd]	3.11±0.04[bcd]	1.20±0.21[bc]
5.0 (0)	50 (0)	50 (0)	0.25 (0)	60.44±0.25[cde]	2.98±0.16[bc]	1.40±0.05[cde]
Control				29.01±0.85[a]	3.08±0.42[a]	0.87±0.06[a]

[1)]Each value is mean±SD (n=3); Any mean that is the same column followed by the same letter are not significantly ($p<0.05$) different by Duncan's multiple range test.

activity (68.36%) were precisely pH 4.51, a temperature of 50.62°C, an incubation time of 47.00 min, and an enzyme concentration of 0.23%. Woo *et al.* (23) reported that DPPH radical scavenging activity in citron seed (56.02%)

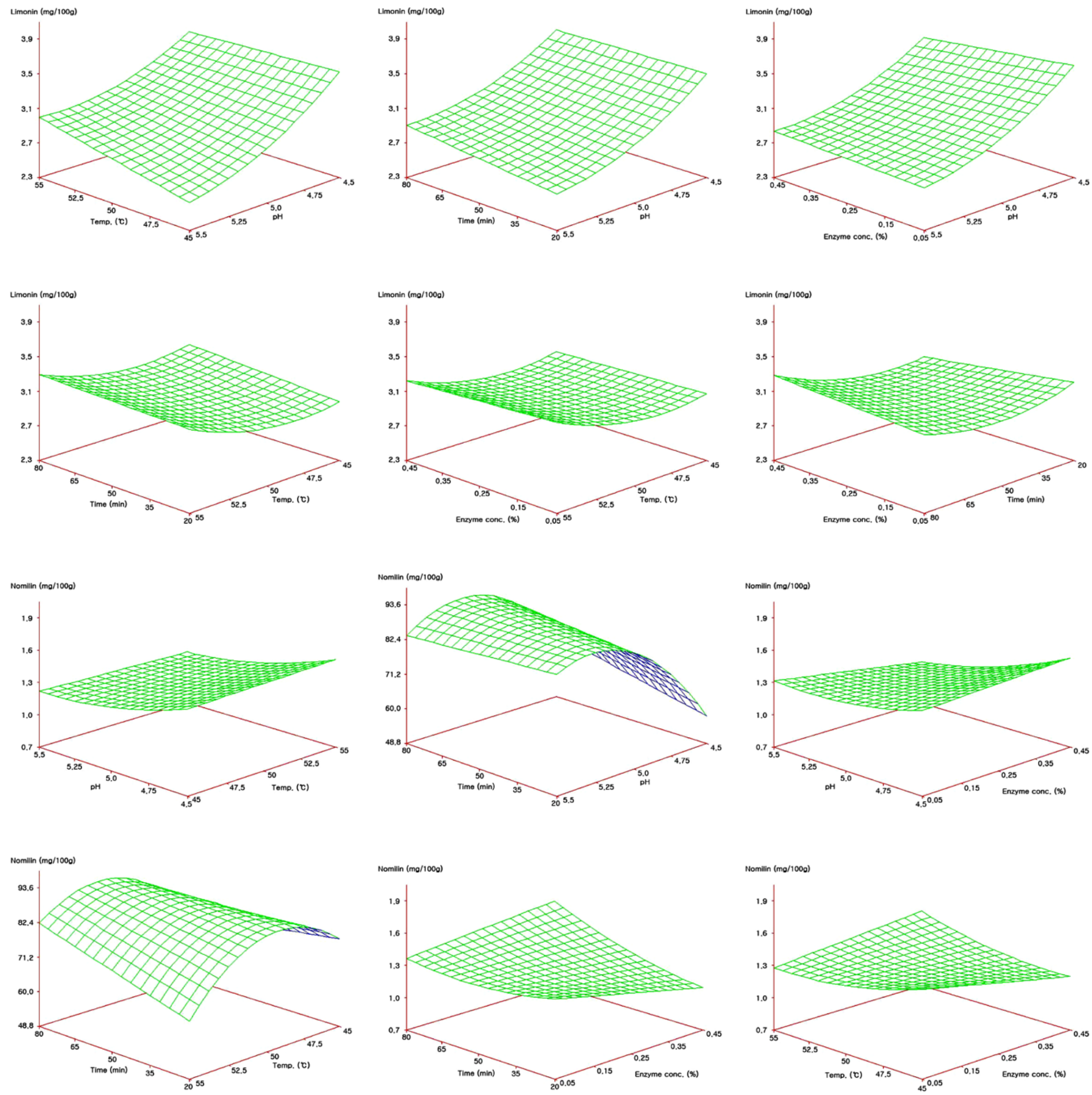

Fig. 2. Effect of pH, incubation temperature, incubation time, and enzyme concentration on the limonin and nomilin contents of citron waste juice.

at 60°C and 300 rpm for 2 hr. Lee *et al*. (24) reported that free radical scavenging is 2 times higher in citrus peel hydrolysate compared with citrus peel. The DPPH radical scavenging activity of treated citron juice was increased significantly upon increased treatment temperature. As a whole, the F-value was affected by pH and temperature more so than by time and enzyme concentration (p<0.05, Table 2). R^2 for DPPH radical scavenging activity was 0.8882 (Table 3) and the model was suitable (p>0.18).

Limonin and nomilin contents HPLC is a powerful method for the analysis of limonoids in citrus fruit due to its ease of operation and versatility (25). The amounts of limonin and nomilin in citron waste juice treated under various conditions are shown in Table 4. The concentrations of limonin and nomilin content in raw citron waste juice were 3.08 and 0.87 mg/100 g, respectively. The limonin content ranged from 2.39 to 3.83 mg/ 100 g depending on treatment conditions, whereas the nomilin content ranged from 0.73 to 1.85 mg/100 g. The highest limonin content (3.83 mg/100 g) occurred at pH 4.5, a temperature of 55°C, an incubation time of 20 min, and an enzyme concentration

Table 5. Comparison between the predicted and observed response variables of the optimum conditions

Responses		pH (X_1)	Temp. (°C, X_2)	Time (min, X_3)	Enzyme conc. (%, X_4)	Predicted value	Experimental value
Yield (%)		5.00	51.15	53.74	0.44	71.10	70.30
Turbidity		4.87	50.21	49.99	0.44	0.20	1.07
	L	4.86	50.72	51.60	0.44	47.39	40.24
Color	a	5.48	49.36	46.29	0.21	-1.34	-0.80
	b	5.49	50.05	43.31	0.23	13.18	13.16
EDA[1)] (%)		4.51	50.62	47.00	0.23	68.36	64.52
Limonin content (mg/100 g)		4.51	50.30	48.34	0.21	3.49	3.34
Nomilin content (mg/100 g)		4.59	50.08	66.07	0.30	1.56	1.14

[1)]Electron donating ability

of 0.05%, whereas the highest nomilin content (1.85 mg/100 g) occurred at the same pH, temperature, and time, but at a different enzyme concentration (0.45%). The optimal conditions for the production of limonin and nomilin were at pH 4.51, 50.30°C, 48.34 min, and 0.21% (3.49 mg/100 g) and pH 4.59, 50.08°C, 66.07 min, and 0.30% (3.49 mg/100 g), respectively. Boo *et al*. (26) reported that the amounts of limonin and nomilin were 55.8 and 46.1 μg/g, respectively, in citron juice sac. Jeong *et al*. (15) reported that the limonin and nomilin contents affecting by the extraction temperature more than extraction time. The limonin and nomilin contents of citron waste juices increased significantly with decreased pH (Fig. 2). As a whole, the F-value was affected more by pH than by temperature, time, or enzyme concentration in limonin content (p<0.05, Table 2). But, in nomilin content, the F-value was affected more by time than by pH, temperature, or enzyme concentration (p<0.01, Table 2). R^2 for the limonin content was 0.8886 (Table 3) and the model was suitable (p>0.18), and R^2 for the nomilin content was 0.8764 (Table 3) and the model was suitable (p>0.19).

Predicted and observed values of citron waste juice Table 5 compares the predicted and observed response variables of the optimum conditions for citron waste juice. The optimum conditions for yield, turbidity, color (L, a, and b-value), EDA, limonin, and nomilin content were pH 5.00, 51.15°C, 53.74 min, and 0.44%; pH 4.87, 50.21°C, 49.99 min, and 0.44%; pH 4.86, 50.72°C, 51.60 min, and 0.44%; pH 5.00, 51.15°C, 53,74 min, and 0.44%; pH 5.48, 49.36°C, 46.29 min, and 0.21%; pH 5.49, 50.05°C, 43.31 min, and 0.23%; pH 4.51, 50.62°C, 47.00 min, and 0.23%; pH 4.51, 50.30°C, 48.34 min, and 0.21%; and pH 4.59, 50.08°C, 66.07 min, and 0.30%, respectively. At each optimum range, the yield, turbidity, color (L, a, and b-value), EDA, limonin, and nomilin content were predicted as 71.10%, 0.20, 47.39, -1.34, 13.18, 68.36%, 3.49, and 1.56 mg/100 g, respectively. Under the same conditions, experimental values were 70.30%, 1.07, 40.24, -0.80, 13.16, 64.52%, 3.34, and 1.14 mg/100 g, respectively.

In conclusion, the different conditions for enzyme treatment showed that pH, temperature, and enzyme concentration significantly affected the yield, turbidity, color (L, a, and b-value), EDA, limonin, and nomilin content of citron waste juice. However, The optimum conditions for yield, turbidity, color (L, a, and b-value), EDA, limonin, and nomilin contents showed different results. The recommended enzyme treatment for citron waste juice production were pH 5.00, 51.15°C, 53.74 min, and 0.44% enzyme concentration.

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