Journal of Science and Technology ***55*** *(1) (2017) 15-28*
DOI: 10.15625/0866-708X/55/1/7472

OPTIMIZATION OF ENZYMATIC HYDROLYSIS CONDITIONS FOR INCREASING THE EFFICIENCY OF DRY MATTER EXTRACTED FROM *Limonia acidissima* PULP BY COMBINED CELLULASE -PECTINASE ENZYMES USING RESPONSE SURFACE METHODOLOGY

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Received: 27 November 2015; Accepted for publication: 1 December 2016

ABSTRACT

The *Limonia acidissima (L. acidissima)* fruits are rich in nutrient values and bioactive compounds. The hydrolysis of *L. acidissima* pulp was researched by combined cellulase and pectinase enzymes to increase the yield of dry matter and bioactive compounds. In this study, the hydrolysis conditions by using the combined enzymes for increasing the dry matter recovery were optimized by response surface methodology (RSM). The independent variables were coded as: pH (x_1), incubation temperature (x_2), the total content of combined cellulase-pectinase (x_3) (with the ratio of cellulase/pectinase was 1/1), and hydrolysis time (x_4). The results of the analysis of variance (ANOVA) showed that the variables actively affected the efficiency of extracted dry matter. The optimal conditions of hydrolysis were derived at Z_1 = 4.2, Z_2 = 45 ^oC, Z_3 = 1.6 % (v/dwt), Z_4 = 120 minutes. At the conditions, the efficiency of the prediction model reached 54.76 % and it had no significant difference compared with experimental value (54.69 %)**.** That increased 20.89 % compared with the efficiency from the non-enzymatic extraction. Besides, the recovery efficiency of carbohydrate reached 87.74 %. Further, the content of extracted phenolic, carotenoids and DPPH and ABTS radical scavenging activity highly increased, which reached 106.7 mg GAE, 86.6 mg, 67.1 and 102.1 mg trolox equivalent antioxidant capacity (TEAC) from 100 g *L. acidissima* pulp, respectively.

Keywords: optimization, cellulase, pectinase, *Limonia acidissima* L., DPPH, ABTS radical scavenging activity.

1. INTRODUCTION

Limonia acidissima (syn. *Wood apple, Feronia elephantum, Feronia limonia, Hesperethusa crenulata, Schinus limonia*) is a tropical fruit from the family of Rutaceae. It is a tree yielding

fruit which is popular in India, Sri Lanka, Pakistan, Bangladesh, Burma, Thailand, and most of the Southeast Asian countries [1]. It is one of the important plants that is used for traditional medicine [2]. The fruit is rich in nutrient compared with many other fruits [3]. The nutritional analysis of pulp proved that it contained a potential source of energy, protein and carbohydrate. Total dietary fiber in this fruit included insoluble dietary and soluble dietary fiber (mucilage and pectin). It also contained many vitamins and minerals including vitamin C, vitamin A, thiamine, riboflavin, niacin, Ca, P [4, 5], Na, K, Mg, Zn and Cu, Fe [6]. In addition, it is a medicinal plant, so it has been used widely because of the bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, and other antioxidants which may protect us against chronic diseases. The fruit is available plenty and a cheap source for the exploration of the development of nutraceuticals for diabetes [7, 8]. Positive correlation was observed between polyphenol content and the antioxidant capacities with enormous health benefits. That may be used in food and pharmaceutical applications [9]. Besides, the pulp exhibited good antibacterial activity against gram positive bacteria, antifungal, astringent, anti-inflammatory and insulin secretogouge activities. The antimicrobial activity could be mainly due to the presence of phenolic compounds, thymol. That widely reported to possess high levels of antimicrobial activity [10, 11]. Thymol has been shown to cause disruption of the cellular membrane or inhibition of ATPase activity and release of in tracellular ATP and other constituents [12]. That demonstrates that *L. acidissima* fruits may be used as nutraceuticals for disease prevention and health promoting benefits [4].

The pulp of *L. acidissima* fruits is also eaten raw or is blended with coconut milk and palm-sugar syrup, and drunk as a beverage or nice cream [13]. Especially, the jam and jelly from wood apple are becoming popular in India and Sri Lanka. In India, the fruit was as a “poor man’s food” until processing techniques were developed in the mid-1950s. The using demand of *L. acidissima* fruits has increased remarkably in the last few decades [3].

The fruit cell wall is degraded efficiently by a synergistic action of endo-polygalacturonases and cellulases [14]. In particular, the use of cellulases and pectinases not only increases the recovery from juice but also ensures the quality of the end products [15, 16]. These enzymes help in softening the plant tissue and lead to the release of cell contents. That may be recovered with high yield [17]. Hence, the combination of cellulase and pectinase was used to increase the yield and quality of the extract from *L. acidissima* pulp.

The experimental design by traditional method to find out the optimal values of variables is usually based on individual factors and time-consuming. So it can easily lead to incorrect conclusions as there are many factors having impact at the same time on the objective function. Response surface methodology (RSM), a statistical experimental design which is used in mathematical model, can be effectively applied to optimize biochemical processes. It not only describes the interaction between independent variables on the objective function but also build regression equation expressing the relationship between it. Through which can predict and control the processes. Currently, RSM is being applied to extract bioactive compounds from *Feronia limonia* L. using solvent (alcohol) [10], polysaccharides from *Lycium ruthenicum* fruit using water [18], polysaccharides from *Cornus officinalis* using enzyme [19].

The aim of this study was to optimize the enzymatic hydrolysis conditions (pH, incubation temperature, cellulase and pectinase concentration and hydrolysis time). Furthermore, we studied the effect of hydrolysis to the recovery of phenolic compounds, carotenoids content, DPPH and ABTS radical scavenging activities.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH); 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS); 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) and Potassium persulfate ($K_2S_2O_8$) was purchased from Sigma–Aldrich, USA; BioBasic, Canada; Sigma–Aldrich, USA and China.

2.1.2. Enzymes

Commercial enzymes, Cellulase from *Trichodermareesei* (Declared activity of endoglucanase: 700 EGU/g) and pectinase from *Aspergillusaculeatus* (Declared activity of polygalacturonase: 26000 PG/ml) and, were obtained from Novozymes, Denmark and stored at 4 ^{o}C.

2.1.3. Plant material

Fully ripe *L. acidissima* fruits were collected from TraVinh province, Vietnam during January in 2014. The weight mean per a fruit was about 400 g. The shell, pulp, seed of fruit was about 29.1 %, 64.9 % and 6 % respectively. The fruits were stored at -18 ^{o}C until further use.

2.2. Methods

2.2.1. Experiment procedures

L. acidissima pulp was removed from the fruits and diluted with water in the ratio of 1:1. A sample mass of 100 g, with 50 g the pulp and 50 ml of distilled water, was used for each experiment. Then, pH was adjusted in the range from 3.9 (the natural pH of *L. acidissima* pulp) to 5.1 by adding citrate buffer. Temperature was varied in the range from 40 to 60 ^{o}C. The combined cellulase-pectinase was added in the range from 0.4 to 2 % (v/dwt). The samples were placed in a shaking water bath at a rate of 120 strokes per minute, over a time period of 30 to 150 minutes. After the end of the incubation period, the enzymes were inactivated at 85 ^{o}C for 10 mins. The reaction mixture was filtered by the vacuum filtration method through a Whatman filter paper. The juice was weighed to determine moisture (to calculate the efficiency of dry matter recovery) and diluted directly with distilled water for the following analysis tests, DPPH, ABTS and carotenoids [20]. The central value of each variable was chosen by the statistical analysis ($p < 0.05$) with using Stagraphics centurion XVI. Experiments were repeated three times. Results of the central values of the variables were input data to optimize it as the following experimental design.

2.2.2 Sample preparation to determine the bioactive compounds from the pulp

Duplicate samples (25 g) of *L. acidissima* pulp were dried over night at 45 ^{o}C. The dried power was grinded and mixed with methanol (225 ml) in a becher. Then it was filtrated through a Whatman filter paper. The extracted juice was diluted with distilled water to a suitable concentration for each of the analysis tests and stored at 4 ^{o}C prior to use [9].

2.2.3. Determination of total phenolic content

Total phenolic content (TPC) was determined by using Foline-Ciocalteu [9]. An aliquot of sample extract (0.1 ml) was mixed with distilled water (3 ml). and then 0.5 ml of Foline-Ciocalteu reagent was added. After 3 min, 2 ml of sodium carbonate 20 % was added and mixed thoroughly. The tubes were incubated in a boiling water bath (100 ^{o}C) for exactly 1 min. It was cooled and the absorbance was measured at 650 nm by using spectrophotometer (Genesys 6, Thermo spectroic, USA). The standard curve was linear between 10 and 60 ppm of acid gallic. The results were expressed as milligram (mg) of gallic acid equivalent (GAE) per 100 g of raw material (*L. acidissima pulp)*. The values were done in triplicate.

2.2.4. Determine DPPH radical scavenging activity

DPPH radical scavenging assay was determined by the method developed by Brand-Williams W *et al.* [21]. Here, 0.3 ml of each test sample was mixed with 5.7 ml of a DPPH-methanol solution (A_{515nm} = 1.1±0.02). Then, the mixtures were vortexed vigorously. Then it was put the dark for 20 mins. The absorbance was determined at 515 nm, and the decreased content of DPPH radical scavenging activity in each concentration of sample could be calculated by the formula as shown:

$$\%\ \text{inhibition of the sample} = [1 - \frac{\text{Asample}}{\text{Acontrol}}] * 100 \quad (1)$$

from the equation (1), base on the standard curve: % inhibition = a_1 * trolox concentration + b_1, molecular weight of trolox = 250.29 and the mass of the extracted juice from 100 g of the pulp. That deduced DPPH radical cation by TEAC (mg) of the extracted juice sample from 100 g of the pulp. Where, a_1 and b_1 are the coefficients of the standard curve of DPPH.

The standard curve was linear between 100 and 700 µM trolox by using spectrophotometer (Genesys 6, Thermo spectroic, USA). The results were expressed in mg (TEAC) per 100 g of the pulp. Three replicates of each sample were used for statistical analysis and the final chosen values were reported as mean ± SD.

2.2.5. Determine Free radical-scavenging ability with using a stable ABTS radical cation

Free radical scavenging activity was determined by ABTS radical cation decolourisation assay, described by Re R. *et al*. [22]. ABTS was dissolved in water to a 7 µM concentration. ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS stock solution with 2.45 µM potassium persulfate (final concentration) and kept in the dark at room temperature for 12÷16 hours before use. The radical cation was stable in this form for more than two days in the dark at room temperature. The samples of the $ABTS^{+}$ solution were diluted with redistilled water to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 ^{o}C. After the addition of 3.0 ml of diluted $ABTS^{+}$ solution ($A_{734\ nm}$ = 0.700 ± 0.02) to 30 µL of the extracts, the absorbance was read exactly in 6 min after the initial mixing by using spectrophotometer (Genesys 6, Thermo spectroic, USA). The decreased content of ABTS radical scavenging activity in samples could be calculated by the formula as shown:

$$\%\ \text{inhibition of the sample} = [1 - \frac{\text{Asample}}{\text{Acontrol}}] * 100 \quad (2)$$

From the equation (2), based on the standard curve: % inhibition = a_2 * trolox concentration + b_2, molecular weight of trolox = 250.29 and the mass of the extracted juice from 100 g of the

pulp. That deduced ABTS radical cation by TEAC (mg) of the extracted juice sample from 100 g of the pulp.

Where, a_2 and b_2 are the coefficients of the standard curve of ABTS. The standard curve was linear between 100 and 700 µM trolox. The results were expressed in mg TEAC per 100 g of the pulp. All of determinations were performed in triplicate.

2.2.6. Carotenoids Analysis

Carotenoids content was analyzed by spectrophotometric method (with the UV/VIS spectrophotometer (Genesys 6, Thermo spectroic, USA) at 440 nm [23]. Each homogenized sample (2 g) was placed in a conic retort and 20 ml 96 % ethanol was added. The sample was stirred on magnetic stirrer for 15 minutes then 25 ml of petrol ether was added and continued to stir for one hour. After 3 ÷ 4 hours, both layers were completely divided, the top yellow layer was used for the further analysis of carotenoids at 440 nm. The standard curve was linear between 10 and 60 ppm $K_2Cr_2O_7$. The carotenoids content (mg per 100 g) was calculated by equation:

$$X = \frac{12.5.100 x KE}{36.a} \quad (3)$$

where 12.5 and 36 coefficients for the relationship between $K_2Cr_2O_7$ and carotenoids.

KE – carotenoids equivalent by standard curve.

a– sample weight, g.

2.2.7. Methods of nutrient components analysis

Carbohydrate: by AOAC 986.25 (2011),

Total sugar: by TCVN 4594: 1988,

Reducing sugar: by TCVN 4594: 1988,

Protein: by FAO, 14/7, 1986 page 221,

Lipid: by FAO, 14/7, 1986 page 222,

Calcium: by AOAC 968.08 (2011),

Cellulose: by TCVN 5103:1990,

Pectin: by calcium pectate method.

2.2.8. The dry matter recovery efficiency (Y) from hydrolysis was calculated by equation

$$Y(\%) = \frac{\text{the yield of dry matter in the extracted juice}}{\text{the yield of dry matter in the pulp}} * 100 \quad (4)$$

The dry matter content in the extracted juice (%) = (100- moisture content in the extracted juice) %; The dry matter content in the pulp (%) = (100- moisture content in the pulp) %; The yield of dry matter in the extracted juice = The dry matter content in the extracted juice * the weight of the extracted juice / 100; The yield of dry matter in the pulp = The dry matter content in the pulp * the weight of the pulp / 100.

2.2.9. Experimental design

Response surface methodology (RSM) with star distance of Circumscribed Central Composite (CCC) designs was used to carry out the experiments to optimize the enzymatic hydrolysis conditions, The independent variables were coded pH (Z_1), incubation temperature (Z_2), enzymes concentration (Z_3), incubation time (Z_4). The range and central point value of all the three process variables are shown in Table 1. The variables were coded according to the following equation:

$$x_j = \frac{Z_j - Z_{0_j}}{\Delta Z_j}, j = 1,2,3,...k \quad (5)$$

where, x_j is the dimensionless coded value, Z_j is the actual value of variables, Z_0 is the actual value of variables at the center point, and ΔZ is the step change value. After the conduct of the experiment, the data was fitted with a second-order polynomial equation as follow:

Table 1. The range of variables in Circumscribed Central Composite design.

Independent variables	Unit	Symbol	Code level				
			$-\alpha$	-1	0	1	$+\alpha$
pH		Z_1	3.6	3.9	4.2	4.5	4.8
Incubation temperature	^oC	Z_2	35	40	45	50	55
Enzymes concentration	v/dwt	Z_3	0.4	0.8	1.2	1.6	2.0
Incubation time	min	Z_4	30	60	90	120	150

Table 2. Circumscribed Central Composite design with experimental and predicted values for the efficiency of extracted dry matter.

Run order	Coded variables				Response (Y(%))	
	(x_1) pH	(x_2) temperature	(x_3) Combined enzymes concentration	(x_4) Time	Experimental values	Predicted values from the model
N1	-1	-1	-1	-1	46.87±0.77	46.99
N2	1	-1	-1	-1	49.42±1.78	49.50
N3	-1	1	-1	-1	50.69±1.27	49.82
N4	1	1	-1	-1	50.75±0.52	50.62
N5	-1	-1	1	-1	48.08±0.83	48.50
N6	1	-1	1	-1	50.70±0.66	50.15
N7	-1	1	1	-1	51.15±0.72	50.79
N8	1	1	1	-1	50.38±0.46	50.72

N9	-1	-1	-1	1	48.44±1.33	48.14
N10	1	-1	-1	1	49.82±0.68	50.14
N11	-1	1	-1	1	49.53±0.50	50.04
N12	1	1	-1	1	50.71±0.42	50.32
N13	-1	-1	1	1	50.76±0.04	50.85
N14	1	-1	1	1	51.07±0.37	51.98
N15	-1	1	1	1	52.25±0.50	52.20
N16	1	1	1	1	51.78±0.23	51.62
N17	-2	0	0	0	46.12±0.55	46.34
N18	+2	0	0	0	48.48±0.22	48.27
N19	0	-2	0	0	47.65±1.56	47.11
N20	0	+2	0	0	49.03±0.43	49.58
N21	0	0	-2	0	50.54±0.28	50.87
N22	0	0	+2	0	54.01±0.38	53.68
N23	0	0	0	-2	51.18±0.41	51.65
N24	0	0	0	+2	54.17±0.16	53.70
N25	0	0	0	0	54.08±0.37	54.02
N26	0	0	0	0	54.09±0.10	54.02
N27	0	0	0	0	53.95±0.28	54.02
N28	0	0	0	0	53.99±0.30	54.02
N29	0	0	0	0	54.01±0.38	54.02
N30	0	0	0	0	54.05±0.11	54.02
N31	0	0	0	0	53.99±0.42	54.02

$$Y(\%) = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=1+1}^{4} \beta_{ij} x_i x_j \quad (6)$$

where Y(%) is the predicted response, β_0 is the model constant, β_i, β_{ii} and β_{ij} are model coefficients.

2.2.10. Data analysis

The design of experiments, analysis of the results and prediction of the responses were carried out using Modde 5.0 software. Comparisons of means were performed by one-way ANOVA (analysis of variance) followed by Tukey's test (*p-value* < 0.05).

3. RESULTS AND DISCUSSION

In this study, the relationship between the variables and the response function were identified by four factors inscribed central composite design. Further, the hydrolysis conditions were optimized.

The analysis results of cellulose and pectin contents in the pulp were 1.94 % and 3.87 % respectively. With these obtained results, we conducted a study to find the suitable cellulase/pectinase ratio in the conditions Z_1 = 4.2, Z_2 = 45 ^oC, Z_3 = 0.8 % (v/dwt), Z_4 = 60 min. The control sample conducted with Z_3 = 0 %. Results are shown in Table 3.

Table 3. Effect of the ratio of cellulase/pectinase to the efficiency of dry matter recovery.

cellulase /pectinase	0/0	0/1	1/0	1/1	1/2	2/1	1/3	3/1
Y (%)	31.8±0.9^d	48.3±0.2^c	47.9±0.5^c	51.9±0.3^a	51.6±0.2^{ab}	50.80±0.6^b	51.0±0.2^{ab}	50.83±0.6^b

Note: * Means of triplicate determination ± SD. ^{a}highest significant value; $^{b, c, d}$lower significant value.

Table 3 showed that the efficiency of dry matter recovery achieved the highest value at the ratio of cellulase/pectinase = 1/1 and the lowest value at the control sample. This was explained that enzymes helped to reduce viscosity of the samples rapidly and increase the reaction rate of hydrolysis, resulting in increasing the efficiency of dry matter recovery. We chose this value to optimize the hydrolysis conditions. Table 2 showed that the efficiency of dry matter recovery ranged from 46.12 to 54.17 % on dry weight basis and the maximum efficiency was reached for the 24th run under the experimental conditions of Z_1 = 4.2, Z_2 = 45 ^oC, Z_3 = 1.2 % (v/dwt), Z_4 = 120 min. The lowest efficiency was observed for the 17th run with the following conditions of Z_1 = 3.6, Z_2 = 45 ^oC, Z_3 = 1.2 % (v/dwt), Z_4 = 90 min. Based on these data, the hydrolysis process was optimized for obtaining desirable response at maximum.

3.1 Fitting the model

Table 4. The fitted quadratic model in terms of coded variables for Y(%) responses.

Responses	2nd Order polynomial equation	Regression (p-value)	R^2	R^2 (adjusted)
Y(%)	$54.021 + 0.482195Z_1 + 0.617427Z_2 + 0.703791Z_3 + 0.512901Z_4 - 0.428921Z_1Z_2 + 0.298328Z_3Z_4 - 1.67949Z_1^2 - 1.42019Z_2^2 - 0.436517Z_3^2 - 0.336722Z_4^2$	0.000	0.974	0.952

Table 2 indicated that the results of the predicted and experimental responses for the 31 runs according to the experimental design. Overall, a close relationship between the experimental and predicted values indicated satisfactory of model developed. The predicted quadratic model in terms of coded variables was given in Table 4.

The statistical analysis showed that the proposed model was highly significant (P-value < 0.001) and a very high F-value (F = 43.3917). The coefficient of determination R^2 = 0.974

indicated the compatibility of model. The value of adjusted determination coefficient R^2 (adj) was 0.952, which also confirmed that the model was highly significant [10]. The effects of independent variables and their mutual interaction on the efficiency of the dry matter recovery can be seen on the response surface and contour plots as shown in Fig. 1.

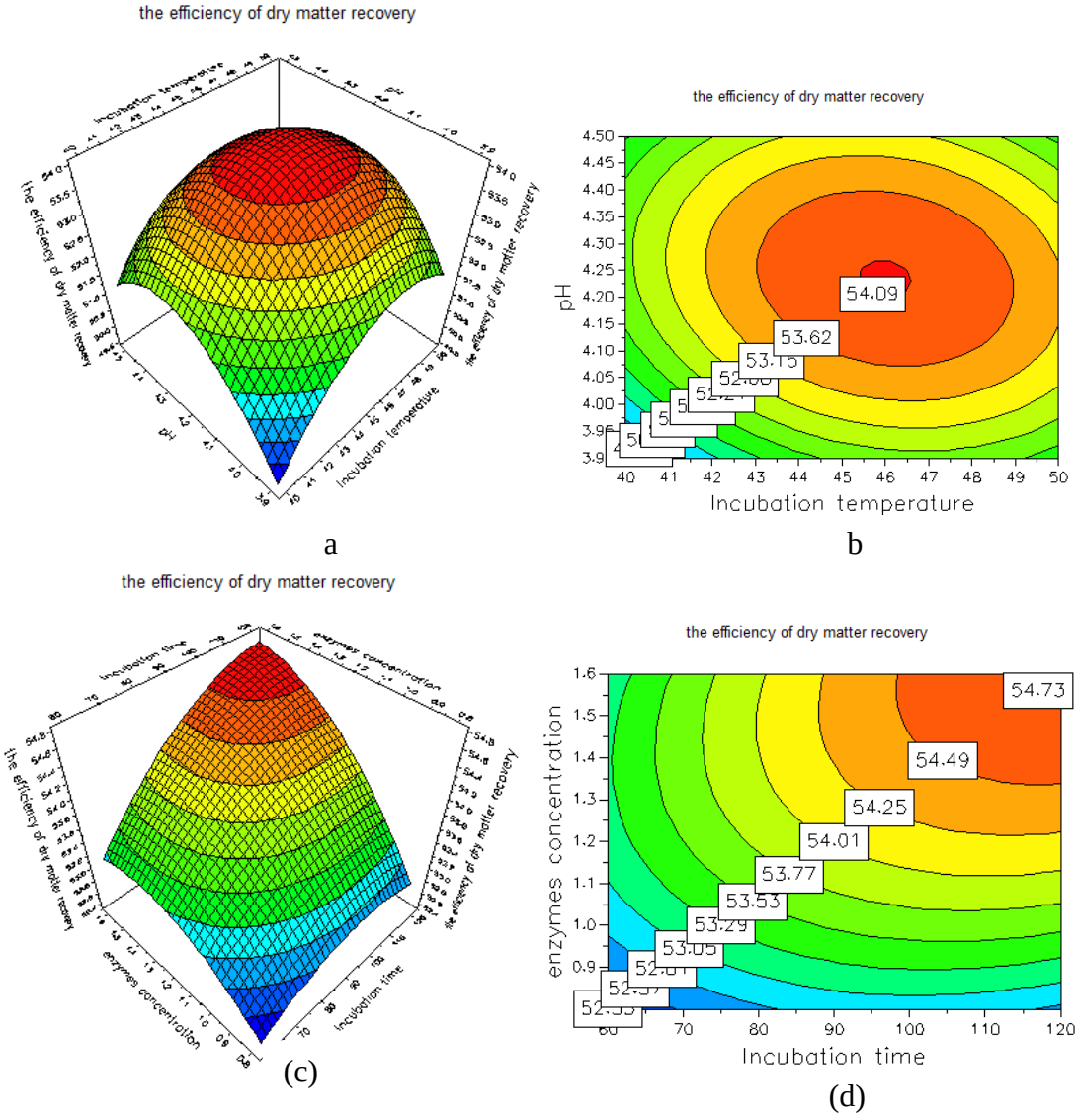

Figure 1. The interactive effect of pH and incubation temperature ((a) and (b)), combined enzymes concentration and incubation time ((c) and (d)) of hydrolysis process on the efficiency of the dry matter recovery from *L. acidissima* pulp.

From Figure 1 and Table 4 it could be deduced that factors such as pH, incubation temperature, combined enzymes concentration and incubation time significantly contributed to affect the dry matter recovery. the optimal conditions for the hydrolysis were derived at pH = 4.2, incubation temperature = 45 ^{o}C, combined enzymes concentration = 1.6 % (v/dwt) and incubation time = 120 minutes, then the efficiency in the prediction of model was 54.76 %. This result was no significant difference in compared with the experimental value (54.69 ± 0.12 %,

$p < 0.05$), that showed a close relationship between the experimental values and the predicted values and indicated the satisfaction of the developed model. The use of the combined enzymes increased the efficiency of the dry matter recovery from *L. acidissima* pulp up to 20.89 % with respect to the efficiency from non-enzymatic extraction (Y = 33.8 ± 2 %) as shown in Table 5. Our results were in accordance with the earlier report by Chadha R *et al*. [24].

Table 5. Response value under optimal conditions.

Responses	Enzymatic extraction		Non-enzymatic extraction*
	Predicted value	Experimental value*	Experimental value*
Y(%)	54.76	54.69±0.12	33.8±2

* Means of triplicate determination ± SD.

3.2. The effect of the optimal extraction conditions on nutrient components recovery

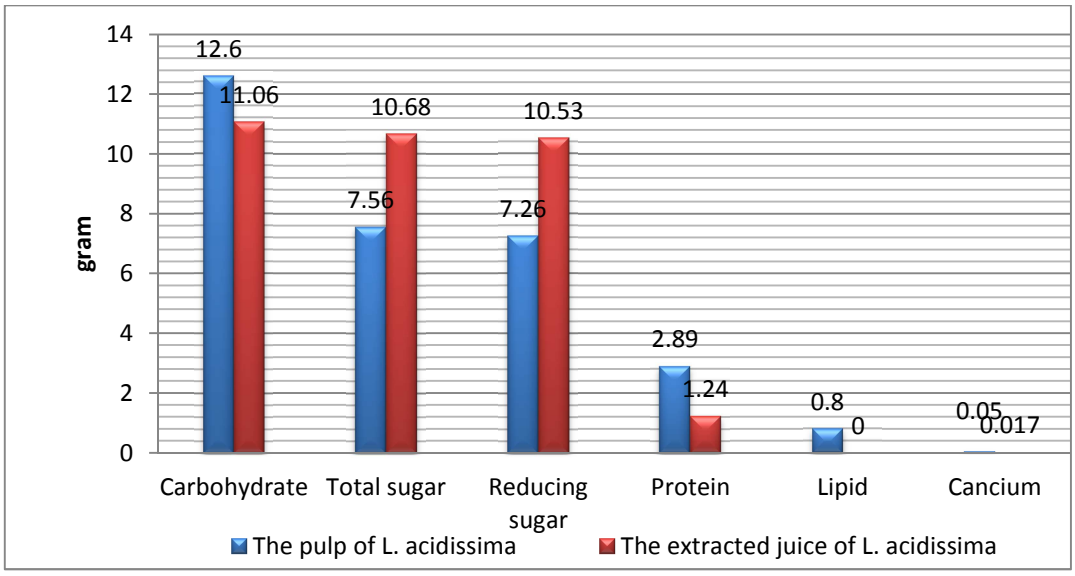

Figure 2. The nutrient components of 100 g *L. acidissima* pulp and extracted juice from 100 g the pulp.

The results in Fig. 2 showed that the carbohydrate, total sugar, reducing sugar, protein and calcium content in the extracted juice reached 11.06, 10.68, 10.53, 1.24, 0.017 g, and the efficiency achieved 87.74 %, 141.29 %, 145.07 %, 42.96 % and 34 %, respectively. The results could be explained that the enzymes broke down complex polysaccharides of plant tissues into simpler molecules like galacturonic acids, glucose, dextrin, maltose [25]. So, the total contents of sugar and reducing sugar of extracted juice were higher than of the pulp of *L. acidissima*. Besides, the efficiency of carbohydrate recovery was high. Otherwise, the combined enzymes treatment caused breaking of carbohydrate-protein complexes, so the positively charged proteins became partially exposed on the particle surface, promoting flocculation, which reduced the efficiency of protein recovery.

3.2. The effect of the optimal conditions of the extraction on total polyphenol, carotenoids content, DPPH and ABTS radical scavenging activities

Figure 3 illustrated that the use of combined cellulase-pectinase not only significantly increased the dry matter recovery but also dramatically increased bioactive compounds. In the optimal conditions of the enzymatic hydrolysis, the total phenolic compounds, antioxidant capacity by DPPH, ABTS method and carotenoids dramatically rose from 53.5 to 106.7 mg, 27.7 to 67.1 mg (TEAC), 31.9 to 102.1 mg (TEAC) and 48 to 86.6 mg. That increased 41.0 %, 30.2 %, 40.3 % and 22.2 % respectively in compared with control sample (without enzyme). That contributed to enrich nutrients in the extracted juice for health benefits from *L. acidissima* pulp. The major antioxidant nutrients of *L. acidissima* fruits are component having strong antioxidant capacity. In general, the TEAC by DPPH method was lower than by ABTS. That results were in good agreement with the earlier report [10].

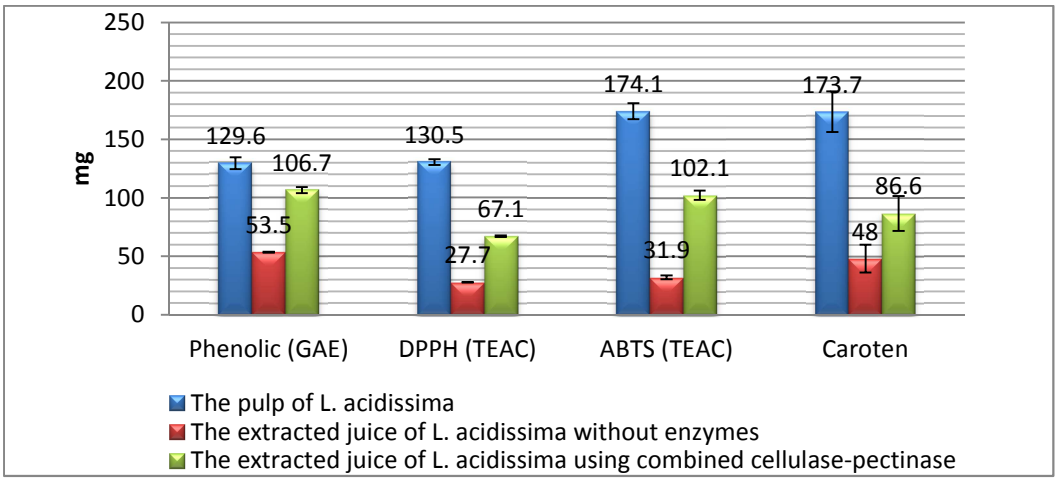

Figure 3. Total phenolic compounds, carotenoids, and antioxidant activities of 100 g of the pulp and the juice from 100 g of the pulp.

4. CONCLUSIONS

This study showed that the use of combined pectinase-cellulase for the hydrolysis (the ratio of 1/1 respectively) at optimal conditions of pH = 4.2, incubation temperature = 45 ^{o}C, the combined enzymes concentration = 1.6 % (v/dwt) and incubation time= 120 minutes caused the significant increase up to 20.89 % in the efficiency of dry matter recovery of the extracted juice from 33.8 to 54.69 %,and there were significant increase in phenolic compounds, carotenoids content and the bioactive compounds by DPPH, ABTS method. That average increased 41.0 %, 30.2 %, 40.3 % and 22.2 % respectively as compared to the control sample. The treatment of combined pectinase and cellulose can achieve high efficiency and can be used to produce many products from *L. acidissima* pulp.

Acknowledgement. This study was funded by TraVinh University, Vietnam, and experiments were conducted in the laboratory of Viet Nam Nat. Uni. Ho Chi Minh City University of Technology. We are thankful for the support and encouragement rendered.

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TÓM TẮT

TỐI ƯU HÓA CÁC ĐIỀU KIỆN THỦY PHÂN BẰNG ENZYME ĐỂ GIA TĂNG HIỆU SUẤT THU HỒI CHẤT KHÔ TỪ THỊT QUẢ TRÁI QUÁCH VỚI SỰ KẾT HỢP CỦA CELLULASE VÀ PECTINASE SỬ DỤNG PHƯƠNG PHÁP BỀ MẶT ĐÁP ỨNG

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Trái quách chứa nhiều dinh dưỡng và các hợp chất có hoạt tính sinh học. Trong nghiên cứu này, quá trình thủy phân thịt quả trái quách đã được khảo sát nhằm làm tăng hiệu suất thu hồi chất khô và các hợp chất có hoạt tính sinh học bằng việc kết hợp cellulase và pectinase. Việc khảo sát các điều kiện thủy phân bằng enzyme đã được tối ưu hóa bằng phương pháp bề mặt đáp ứng. Các biến độc lập được mã hóa như: pH (x_1), nhiệt độ ủ (x_2), tổng nồng độ cellulase và pectinase (x_3) (với tỉ lệ cellulase/pectinasae = 1/1), và thời gian thủy phân (x_4). Các biến mã hóa này tương ứng với các biến thực Z_1, Z_2, Z_3 và Z_4. Kết quả phân tích phương sai thể hiện rằng các điều kiện thủy phân tác động có ý nghĩa đến hiệu suất thu hồi chất khô. Điều kện tối ưu được chọn là Z_1 = 4,2, Z_2 = 45 ^oC, Z_3 = 1,6 % (v/dwt) và Z_4 = 120 phút. Tại điều kiện tối ưu này, hiệu suất thu hồi chất khô theo dự đoán của mô hình đạt 54,76 % và nó không có sự khác biệt ý nghĩa so với hiệu suất từ thực nghiệm (54,59 %). Hiệu suất từ thực nghiệm này tăng 20,89 % so với hiệu suất từ quá trình trích li không sử dụng enzyme. Bên cạnh đó, hiệu suất thu hồi carbohydrat đạt 87,74 %. Hơn nữa, dịch quả trích li chứa tổng hàm lượng phenolic, carotene, hoạt tính chống oxi hóa theo phương pháp DPPH và ABTS đạt ở mức cao, tương ứng là 106,7 mg GAE, 86,6 mg, 67,1 và 102,1 mg tương đương trolox từ 100 g thịt quả ban đầu.

Từ khóa: tối ưu, cellulase, pectinase, trái quách, DPPH, ABTS.