



DETERMINATION OF ENZYMATIC HYDROLYSIS CONDITION FOR ENHANCED JUICE RECOVERY FROM PURPLE PASSION FRUIT USING RESPONSE SURFACE METHODOLOGY

(Penentuan Keadaan Optimum bagi Hidrolisis Enzimatik untuk Peningkatan Hasil Jus Markisa Ungu Menggunakan Kaedah Gerak Balas Permukaan)

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Abstract

Passion fruit (*Passiflora edulis*) is a tropical fruit that is only available during specific seasons. It is a member of the *Passifloraceae* family and has a small, egg-shaped appearance. Its juice is high in fibre, polysaccharides, pectin, gum, lignin, cellulose, glucose, protein and other vital elements. This combination makes fruit juice exceedingly turbid and viscous. Turbid juice, distinguished by cloudiness, is classified as lightly processed. Raw fruit juice often appears cloudy and viscous. The enzymatic method offers numerous benefits compared to the mechanical-thermal process of breaking down fruit pulps. The current process of fruit juice production heavily depends on the use of enzymes to enhance operational efficiency. The purpose of this study is to assess the optimal conditions for enzymatic hydrolysis in the clarification of passion fruit juice using pectinase as well as to analyse its physicochemical qualities. The process of numerical optimisation was carried out using Response Surface Methodology (RSM) by utilisation of Central Composite Design (CCD) to determine the optimal conditions for enzyme hydrolysis to achieve the highest yield of juice extract. The optimisation condition of passion fruit juice was investigated by varying independent factors, including enzyme concentration (50 – 250 ppm), incubation temperature (40 – 55°C) and incubation time (30 – 60 minutes). The data obtained from the experiment were examined using the Response Surface Methodology (RSM) of MINITAB Software (Version 19) to determine the optimal conditions, which were then verified. The recommended parameters were specified as 94.79 ppm, 41.19°C and 35.19 minutes. Given these conditions, the juice yield is 91.83%, the clarity is 0.228 abs, and the pulp content is 8.18%. The physicochemical properties (clarity, pulp, colour b^* , fructose, total sugar) of both treated and untreated passion fruit juice were shown to be significantly influenced ($p < 0.05$) by their respective physicochemical qualities.

Keywords: Passion fruit, clarity, enzymatic hydrolysis, response surface methodology

Abstrak

Buah markisa (*Passiflora edulis*) adalah buah tropika yang hanya terdapat pada musim tertentu. Ia berasal dari keluarga *Passifloraceae* dan mempunyai bentuk kecil seperti telur. Jus buah-buahan mempunyai serat yang tinggi, polisakarida, pektin, gam, lignin, selulosa, glukosa, protein dan unsur-unsur lain. Penggabungan ini menjadikan jus buah sangat keruh dan likat. Kekeluhan jus diklasifikasikan sebagai pemprosesan yang tidak lengkap. Jus buah mentah sering kelihatan keruh dan likat. Kaedah enzimatik menawarkan banyak kebaikan berbanding proses mekanikal-terma untuk memecahkan pulpa buah. Proses penghasilan jus buah-buahan sekarang sangat bergantung kepada penggunaan enzim untuk meningkatkan kecekapan operasi. Penyelidikan ini bertujuan untuk menentukan keadaan optimum untuk hidrolisis enzim dalam penjernihan jus buah markisa menggunakan pektinase serta

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menganalisis kualiti fizikokimianya. Proses pengoptimuman telah dijalankan menggunakan kaedah gerak balas permukaan dengan penggunaan reka bentuk komposit pusat untuk menentukan keadaan optimum hidrolisis enzim untuk mencapai hasil ekstrak jus yang tinggi. Keadaan optimum jus buah markisa telah dikaji pada faktor yang tidak bergantung termasuk kepekatan enzim (50 – 250 ppm), suhu inkubasi (40 – 55°C) dan masa inkubasi (30 – 60 minit). Data yang diperolehi daripada kajian ini telah dinilai menggunakan perisian kaedah gerak balas permukaan MINITAB (Versi 19) untuk menentukan keadaan optimum dan kemudian disahkan. Parameter yang disyorkan telah ditetapkan pada 94.79 ppm, 41.19°C dan 35.19 minit. Pada keadaan ini, hasil jus adalah 91.83%, kejernihan jus adalah 0.228 abs dan kandungan pulpa adalah 8.18%. Sifat fizikokimia (kejernihan, pulpa, warna b*, fruktosa jumlah gula) untuk jus buah markisa yang dirawat dan tidak dirawat sangat dipengaruhi pada ($p < 0.05$) oleh kualiti fizikokimia masing-masing.

Kata kunci: Buah markisa, kejernihan, hidrolisis enzim, kaedah gerak balas permukaan

Introduction

Passion fruit (*Passiflora edulis*) is a seasonal tropical fruit with a tiny egg-like form. It belongs to the Passifloraceae family. Passion fruit is a South American native that has long been used in traditional medicine to cure a variety of ailments, including insomnia, bronchitis, asthma and urinary infections [1]. In numerous tropical countries, passion fruit is cultivated as a popular commodity. It is ingested both in its natural and processed forms. Brazil is the world's largest producer and consumer of fresh and processed passion fruit, accounting for 50 – 60% of global production. Domestic consumption in Brazil is predominantly in the form of processed fruit drinks [2]. Currently, this fruit is cultivated all over the world and used in the food sector as an edible fruit. The most common varieties are the purple passion fruit (*Passiflora edulis* Sims), also known as the granadilla, the gulupa (*Passiflora edulis* Sims. *fo edulis*) and the yellow passion fruit (*Passiflora edulis* var. *flavicarpa Degener*) [2]. Unripe passion fruits are green, whereas ripe fruits are round to oval, cream to yellow to dark purple with thin and soft and firm skin, sweet, aromatic and juicy pulp and small seeds covered with mucilage. Ripe fruits are used to make juice or are eaten straight up with a little sugar. However, passion fruit has numerous commercial uses and is employed as a flavoring ingredient in the creation of desserts, ice cream, jam, smoothies and other baked goods. Passion fruits are a good source of dietary fibre, vitamin C and lycopene [3]. According to Wijeratnam et al. [2], purple fruit accounts for most of the fresh market trade while yellow fruit accounts for over 95% of production for juice extraction.

Fruit juices are a great source of fibre, polysaccharides, pectin, gum, lignin, cellulose, carbohydrates, protein and a variety of other nutrients. Fruit juice becomes

extremely turbid and viscous due to the presence of such composition [4]. According to Wilczyński et al. [5], cloudy (turbid) juice is defined as products that have minimal processing. This type of juice is unclarified or pressed juice that has not undergone any clarification procedures. Turbidity, which is measured for fruit juice, is the degree of cloudiness or haziness brought on by the dispersed matter, which is mostly created by the cellular tissues broken down during fruit processing. Therefore, greater turbidity indicates a greater concentration of tissue particles [6]. For business owners, the occurrence of turbidity and sedimentation in juices and concentrates poses a challenging situation. Additionally, consumers do not prefer cloudy juices since they are more interested in the transparency and clarity of the juice [7]. The general instability and propensity for rapid sedimentation of coarse particles can be reduced to increase cloud stability. Cloud stability is greatly influenced by pectin [8].

The enzymatic method has a variety of benefits over mechanical-thermal comminution of various fruit pulps. Enzymes play a crucial role and are ideal for optimizing processes in the production of fruit juices. Their primary goals are to maximize juice extraction from raw materials, improve processing efficiency (pressing, solid settling or removal) and produce a clear, aesthetically pleasing finished product. In comparison to other extraction methods, enzymatic treatment prior to mechanical extraction dramatically increases juice recovery. Enzymatic hydrolysis of cell walls improves extraction yield, reduces sugars and increases soluble dry matter content, galacturonic acid content and titrability of the products. Enzymatic degradation of the biomaterial is dependent on the type of enzyme, incubation duration, temperature, enzyme concentration, agitation, pH and utilization of various enzyme

combinations [9]. Pectin methylesterase (PME), also known as pectinesterase (PE), is an enzyme produced by the fungus *Aspergillus nigeris* found in citrus juices like orange, lemon and tropical fruits like guava, papaya and mango. PME can hydrolyze the pectin present in juices. The activity of PME causes cloud disappearance and the separation of fruit juices into two phases which are a clear liquid and one that contains solid calcium pectate complex precipitates [10].

The objective of this study is to determine the optimum condition of enzymatic hydrolysis on clarification of passion fruit juice using pectinase by Response Surface Methodology (RSM). Additionally, the physicochemical characteristics of the clarified passion fruit juice are determined.

Materials and Methods

Chemical and raw materials

All chemicals used were purchased from Sigma Chemical Co. (St Louis, Missouri). Pectinase enzymes from the source of *Aspergillus spp.* were obtained from Friedemann Schmidt Chemical, Germany. Fully ripe purple passion fruit (*Passiflora edulis*) was obtained from a passion fruit farm in Bukit Kepong, Muar, Johor, Malaysia. Fruits without any visual blemishes were chosen as fruit samples.

Extraction process

A ripe purple passion fruit (75% purple color) with an average weight of 500 – 600 g was rinsed with tap water and dried to remove dirt and residues. Subsequently, the passion fruit was sliced in half. Then, the pulp was scooped from its skin by using a small spoon and filtered using muslin cloth until cloudy juice was obtained.

Optimization of enzymatic hydrolysis condition

Response Surface Methodology (RSM) was used to determine the optimum conditions for the enzymatic treatment of passion fruit juice. The experimental design and statistical analysis were performed using MINITAB statistical software version 19. A central composite design (CCD) was used to study the combined effect of three independent variables which are enzyme concentration (X_1), incubation temperature (X_2) and incubation time (X_3). According to Phung et al. [11], these three variables are enzyme concentration (50 – 250 ppm), incubation temperature (40 – 55°C) and incubation time (30 – 60 min). Table 1 shows the range of three selected test variables that were applied to MINITAB software version 19 to obtain the experimental design. The dependent factors included in this analysis were yield (Y_1), clarity (Y_2) and pulp (Y_3)

Table 1. The coded and uncoded values used in the optimization condition

	- α	-1	0	+1	+ α
Enzyme concentration (ppm)	50	100	150	200	250
Incubation temperature (°C)	40	43.75	47.5	51.25	55
Incubation time (min)	30	37.5	45	52.5	60

For each experiment, about 50 mL of passion fruit juice was subjected to different enzymatic treatment conditions. The temperature was controlled using an incubation shaker. At the end of the enzymatic treatment, the enzyme in the sample was inactivated, and the suspension was placed in the oven at 90°C for 5 minutes. The treated passion fruit juice was centrifuged at 3000 x g for 10 minutes, and the supernatant was collected. Lastly, the juices were filtered through filter paper (Whatman No.1, Whatman International Ltd., Kent, England) using a vacuum pump.

Juice yield

Based on Bora et al. [12], passion fruit juice yield was calculated using the following expression:

$$\text{Juice yield (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100 \quad (1)$$

Where, W_1 = weight of sample before centrifuge, and W_2 = weight of pulp after centrifuge

Clarity

The clarity of the passion fruit juice was determined by using a UV-Vis spectrophotometer (Lambda 35 UV/Vis Spectrometer Perkin Elmer, USA) at 660 nm wavelength, and distilled water was used as blank

according to the method by Sharma et al. [9]. The clarity was expressed as absorbance value (abs).

Pulp volume

The pulp volume of the passion fruit juice was determined using the centrifugal method [13] with slight modification. A centrifuge tube containing 50 mL of passion fruit juice was centrifuged (Eppendorf centrifuge, Model 5804, Japan) at 3000 x g for 10 min at room temperature. Pulp volume was measured as the volume of precipitate, which was directly read from the graduated centrifuge tube and expressed as a percentage of the total passion fruit juice volume using the following expression:

$$\% \text{ Pulp} = \frac{\text{Pulp volume}}{\text{Volume of extract}} \times 100 \quad (2)$$

Titrateable acidity (TA)

The total titrateable acidity (TA) of untreated and enzyme-treated juice was determined by the titration method following literature [14]. The burette was filled with 0.1 M NaOH solution. Then, 10 mL of juice was pipetted into a conical flask. After that, 250 mL of deionised water was added into the conical flask, followed by 0.75 mL of phenolphthalein solution. The mixture was mixed thoroughly. Then, the mixture was titrated with 0.1 M NaOH solution until it showed the faintest discernible pink colour, persisting for 30 seconds. The final burette reading was recorded. The difference between the initial and final readings was calculated. The volume of 0.1 N sodium hydroxide used was recorded. The TA can be calculated using a formula and expressed as the concentration of citric acid (g/mL). The percentage of citric acid was calculated according to the following expression:

$$\% \text{ Acid} = \frac{\text{Volume of 0.1 N NaOH (mL)} \times N \text{ NaOH} \times 6.4}{10 (\text{sample})} \quad (3)$$

Total soluble solid (TSS)

The total soluble solid (TSS) of untreated and enzyme-treated juice was determined using an Abbe refractometer (Digital ABBE Refractometer, Kruss, Optronic, Germany) [15]. A drop of the juice was placed on its prism, and the percentage of TSS was obtained from a direct reading of the refractometer. The results were reported as °Brix.

Sugar content

Sugar content in the untreated and enzyme-treated juice was determined using an analytical High-Performance Liquid Chromatography (HPLC). Waters model 600 instrument with a Refractive Index detector model 2414. Analytical grade acetonitrile was purchased from Merck Sdn Bhd, while Standard fructose, glucose and sucrose were purchased from Sigma Technologies Sdn Bhd. Acetonitrile and deionised water (90:10; v/v) were used as the mobile phase. Juices were filtered through a Millipore 0.45 µm membrane filter. The filtrate was used for the analysis of sugar. A series of standard solutions of 1%, 3%, 6%, 9% and 12% (w/v) of glucose, fructose and sucrose was prepared for developing standard curves of sugar. All the standard solutions were dissolved in distilled water. Then, it was filtered through a Millipore 0.45 µm membrane filter. The amount of glucose, fructose and sucrose in samples was quantified by comparing the peak areas of the samples with those of the sugar standard curve. The chromatography was run using a Carbohydrate High Performance 4 µm (4.6 mm x 250 mm cartridge) column at 22°C, flow rate of 1.3 mL/min and injection volume of 20 µL. The duration of the analysis was 15 minutes [16].

Color measurement

The untreated and enzyme-treated juice color was measured by the L*, a*, and b* color space by a Chroma meter (CR-400, Minolta, Japan). Approximately 2 mL of sample was pipetted into a petri dish and the reflectance was measured directly from the juice sample according to method [17]. The expression of color was characterized as L* (lightness) and a*, b* (chromaticity coordinates) [18].

Results and Discussion

Optimization of enzymatic hydrolysis of passion fruit juice

A central composite design was performed to study the combined effect of three factors (enzyme concentration, incubation temperature and incubation time) on the responses of yield (%), clarity and pulp (%). The predicted and experimental results of the 3-factor, 5-level central composite design are presented in Table 2.

Table 2. Factor and comparison between actual (Y) and predicted (FITS) responses

Run No.	Factor			Responses					
	X ₁	X ₂	X ₃	Y ₁	FITS 1	Y ₂	FITS 2	Y ₃	FITS 3
1	100.0	43.75	37.5	91.39	91.265	0.178	0.180	8.62	8.740
2	200.0	43.75	37.5	91.06	91.152	0.151	0.144	8.94	8.847
3	100.0	51.25	37.5	91.47	91.481	0.196	0.201	8.53	8.518
4	200.0	51.25	37.5	90.27	90.367	0.189	0.197	9.73	9.630
5	100.0	43.75	52.5	90.39	90.311	0.168	0.158	9.61	9.691
6	200.0	43.75	52.5	91.32	91.328	0.169	0.161	8.68	8.672
7	100.0	51.25	52.5	91.92	91.846	0.155	0.159	8.08	8.153
8	200.0	51.25	52.5	91.72	91.863	0.199	0.194	8.28	8.140
9	65.9	47.50	45.0	90.95	91.116	0.164	0.161	9.05	8.883
10	234.1	47.50	45.0	91.23	91.035	0.155	0.160	8.77	8.962
11	150.0	41.19	45.0	90.97	91.040	0.167	0.178	9.03	8.960
12	150.0	53.80	45.0	91.77	91.672	0.233	0.223	8.23	8.325
13	150.0	47.50	32.4	91.02	90.983	0.175	0.168	8.98	9.019
14	150.0	47.50	57.6	91.43	91.439	0.138	0.147	8.58	8.566
15	150.0	47.50	45.0	91.27	91.424	0.139	0.141	8.37	8.567
16	150.0	47.50	45.0	91.43	91.424	0.150	0.141	8.73	8.567
17	150.0	47.50	45.0	91.27	91.424	0.140	0.141	8.57	8.567
18	150.0	47.50	45.0	91.57	91.424	0.153	0.141	8.44	8.567
19	150.0	47.50	45.0	91.43	91.424	0.135	0.141	8.57	8.567
20	150.0	47.50	45.0	91.57	91.424	0.135	0.141	8.73	8.567

X₁ = Enzyme concentration (ppm), X₂ = Incubation temperature (°C), X₃ = Incubation time (min), Y₁ = Yield (%), Y₂ = Clarity (abs), Y₃ = Pulp (%)

As shown in Table 2, the highest actual and predicted yields were 91.92% and 91.86% respectively, under predetermined factors, whereby an enzyme concentration of 100.0 ppm was used, incubation temperature was set at 51.25°C and incubation time of 52.5 mins was decided upon. Meanwhile, the highest predicted yield was found under predetermined factors whereby enzyme concentrations of 200.0 ppm, incubation temperature at 51.25°C and incubation time of 52.5 mins. On the other hand, the lowest actual and predicted yield values were at run number 4 (90.27%) and 5 (90.31%), respectively.

The highest values for actual and predicted clarity were recorded at run number 12 (150 ppm, 53.80°C, 45.0 mins), whereby the values obtained were 0.233 and 0.224, respectively. On the other hand, the lowest value

was recorded at run number 19 (150 ppm, 47.50 °C, 45.0 mins) with 0.135 and 0.142, respectively. For response pulp, the highest actual and predicted values were 9.73% and 9.69%, respectively, observed at run number 4 (200.0 ppm, 51.25°C, 37.5 mins) and run number 5 (100.0 ppm, 43.75°C, 52.5 mins) for actual and predicted respectively. The lowest values were observed at run number 7 (100.0 ppm, 51.25°C, 52.5 mins) and run number 8 (200.0 ppm, 51.25°C, 52.5 mins) with the values of 8.08% and 8.14% respectively.

A regression analysis was carried out to fit mathematical models to the experimental data, aiming at an optimal region for the response studied. By applying multiple regression analysis, the empirical relationship between the input variables and the response variable can be

expressed in the following quadratic, second-order polynomial equation in terms of uncoded values:

Analysis of variance (ANOVA)

The goodness of fit of the regression model was defined by determining the coefficient, R^2 and adjusted R^2 , which provide a measure of how much variability in the observed response value can be explained by the experimental factors and their interactions [19]. In reference to Table 3, the results showed that the R^2 value for yield is 92.72%, which means that the model can explain 92.72% of the variability in the observed

response values and only 7.28% of the variability in the observed response values cannot be explained by the model obtained. The remaining 7.28% of the total variation could be due to other factors which are not included in the model. According to Valchanov et al. [20], the suggested range for R^2 values to have a good fit model is between 70% and 99%. In this light, the closer the R^2 value to 100%, the better the fitting of the empirical model with the actual data and the better the correlation between actual and predicted value.

$$\text{Yield} = 91.4241 - 0.0405 X_1 + 0.3159 X_2 + 0.2278 X_3 - 0.348 X_1 X_1 - 0.068 X_2 X_2 - 0.213 X_3 X_3 - 0.707 X_1 X_2 + 0.799 X_1 X_3 + 0.933 X_2 X_3 \quad (1)$$

$$\text{Clarity} = 0.14194 - 0.00051 X_1 + 0.02266 X_2 - 0.01050 X_3 + 0.01858 X_1 X_1 + 0.05908 X_2 X_2 + 0.01558 X_3 X_3 + 0.0223 X_1 X_2 + 0.0279 X_1 X_3 - 0.0138 X_2 X_3 \quad (2)$$

$$\text{Pulp} = 8.5676 + 0.0393 X_1 - 0.3172 X_2 - 0.2269 X_3 + 0.355 X_1 X_1 + 0.075 X_2 X_2 + 0.225 X_3 X_3 + 0.711 X_1 X_2 - 0.795 X_1 X_3 - 0.930 X_2 X_3 \quad (3)$$

Table 3. ANOVA of multiple regression models for the response variables

Source	DF	Adj SS	Adj MS	F	P	Status
Yield ($R^2= 92.72\%$, Adjusted $R^2= 86.17\%$)						
Regression	9	3.02598	0.336220	14.15	0.000	Significant
Linear	3	0.74033	0.246776	10.39	0.002	Significant
X_1	1	0.00793	0.007930	0.33	0.576	Not significant
X_2	1	0.48192	0.481916	20.28	0.001	Significant
X_3	1	0.25048	0.250481	10.54	0.009	Significant
Square	3	0.27600	0.092000	3.87	0.045	Significant
$X_1 X_1$	1	0.21773	0.217733	9.16	0.013	Significant
$X_2 X_2$	1	0.00825	0.008247	0.35	0.569	Not significant
$X_3 X_3$	1	0.08147	0.081468	3.43	0.094	Not significant
Interaction	3	2.00965	0.669883	28.19	0.000	Significant
$X_1 X_2$	1	0.50000	0.500000	21.04	0.001	Significant
$X_1 X_3$	1	0.63845	0.638450	26.87	0.000	Significant
$X_2 X_3$	1	0.87120	0.871200	36.67	0.000	Significant
Error	10	0.23760	0.023760			
Lack of fit	5	0.14746	0.029493	1.64	0.301	Not significant
Pure Error	5	0.09013	0.018027			
Total	19	3.26358				
Clarity ($R^2= 91.72\%$, Adjusted $R^2= 84.26\%$)						
Regression	9	0.011194	0.001244	12.31	0.000	Significant
Linear	3	0.003012	0.001004	9.93	0.002	Significant
X_1	1	0.000001	0.000001	0.01	0.914	Not significant

Source	DF	Adj SS	Adj MS	F	P	Status
X ₂	1	0.002479	0.002479	24.53	0.001	Significant
X ₃	1	0.000532	0.000532	5.26	0.045	Significant
Square	3	0.006716	0.002239	22.15	0.000	Significant
X ₁ X ₁	1	0.000622	0.000622	6.15	0.033	Significant
X ₂ X ₂	1	0.006287	0.006287	62.20	0.000	Significant
X ₃ X ₃	1	0.000437	0.000437	4.32	0.064	Not significant
Interaction	3	0.001466	0.000489	4.84	0.025	Significant
X ₁ X ₂	1	0.000496	0.000496	4.91	0.051	Not significant
X ₁ X ₃	1	0.000780	0.000780	7.72	0.020	Significant
X ₂ X ₃	1	0.000190	0.000190	1.88	0.200	Not significant
Error	10	0.001011	0.000101			
Lack of fit	5	0.000715	0.000143	2.41	0.178	Not significant
Pure Error	5	0.000296	0.000059			
Total	19	0.012205				
Pulp (R ² = 92.31%, Adjusted R ² = 85.38%)						
Regression	9	3.03843	0.337604	13.33	0.000	Significant
Linear	3	0.74177	0.247258	9.76	0.003	Significant
X ₁	1	0.00746	0.007456	0.29	0.599	Not significant
X ₂	1	0.48568	0.485680	19.18	0.001	Significant
X ₃	1	0.24864	0.248638	9.82	0.011	Significant
Square	3	0.29422	0.098074	3.87	0.045	Significant
X ₁ X ₁	1	0.22751	0.227514	8.98	0.013	Significant
X ₂ X ₂	1	0.01024	0.010237	0.40	0.539	Not significant
X ₃ X ₃	1	0.09151	0.091508	3.61	0.086	Not significant
Interaction	3	2.00244	0.667479	26.36	0.000	Significant
X ₁ X ₂	1	0.50501	0.505012	19.94	0.001	Significant
X ₁ X ₃	1	0.63281	0.632813	24.99	0.001	Significant
X ₂ X ₃	1	0.86461	0.864613	34.14	0.000	Significant
Error	10	0.25325	0.025325			
Lack of fit	5	0.14516	0.029033	1.34	0.377	Not significant
Pure Error	5	0.10808	0.021617			
Total	19	3.29168				

The results in Table 3 showed that the R² value is 91.72% and 92.31% for clarity and pulp, respectively, which means that 91.72% and 92.31% of the variability in the observed response values can be explained by the model and only 8.28% and 7.69% of the variability in the observed response values cannot be explained by the model obtained. The remaining 8.28% and 7.69% of the total variation could be due to other factors which are not included in the model.

The adjusted R² was a corrected value for R² after the elimination of unnecessary model terms. If there are significant terms included in the model, the adjusted R² could be smaller than R². In this present study, it is found that the adjusted R² is close to R². Respective values for adjusted R² were 86.17%, 84.26% and 85.38% for yield, clarity and pulp.

The effects of experimental variables on the linear, quadratic and interaction terms were tested for adequacy and fitness by analysis of variance (ANOVA), and the

results obtained are summarized in Table 3. At the confidence level of 95%, the analysis of variance (ANOVA) indicates that the empirical model for yield, clarity and pulp of purple passion fruit juice provides accurate prediction. The p-value was used to evaluate the significance of each term at a confidence level of 95%. The p-value indicates the probability that the factors have a negligible or insignificant effect on the response variable [21]. The F-value is a statistical measure that contrasts the expected experimental variation derived from replicated design points with the variation of the differences in the average responses at the design points and the corresponding estimated responses using the linear model. The large F value indicates that the RSM model fits the experimental data better. The regression model has a high significance level when it has a high F value and a low p-value. However, for the model to be considered statistically significant, the p-value needs to be less than 0.05 [21].

By using lack-of-fit and coefficient determination (R^2), the adequacy of the model can be revealed. The lack-of-fit test measures the variation of data regarding the fitted model and is one of the important aspects of a reduced model. If the model does not fit the data well, the lack of fit will be significant. A model should be rejected if the results show any significance in the lack-of-fit test [21]. It is preferable to have an insignificant lack-of-fit, as a significant lack-of-fit would suggest that the model fails to accurately describe data in the experimental domain, particularly at points that are not included in the regression [22]. In this study, the selected model showed a non-significant result ($p > 0.05$) with a p-value of

0.301, 0.178 and 0.377 (yield, clarity, pulp), respectively. Thus, an insignificant p-value indicates that the model is good and fits well with the experimental data.

Table 4 shows that the optimum condition for the target goals with an enzyme concentration of 94.79 ppm, incubation temperature of 41.19°C and incubation time of 35.19 mins was feasible; on the other hand, the optimum conditions for the maximum goals with an enzyme concentration of 130.46 ppm, incubation temperature of 53.81°C and incubation time of 36.72 mins, as well as for the minimum goals with an enzyme concentration of 82.90 ppm, incubation temperature of 49.09°C and incubation time of 57.10 mins were not feasible.

Contour and surface plots

Contour and surface plots for the yield, clarity and pulp of passion fruit juice treated with enzyme (pectinase) at a feasible optimum condition are shown in Figures 1, 2 and 3, respectively. The shape of the contour plot (circular or elliptical) indicates whether the mutual interaction between test variables is significant or not. A circular contour plot indicates that the interaction between related test variables is negligible. In contrast, an elliptical contour plot indicates that the interaction between related test variables is significant [23]. This study shows an elliptical contour plot, which indicates a significant interaction effect between enzyme concentration and incubation temperature on the hydrolysis of passion fruit juice.

Table 4. Comparison Values of Target and Predicted Responses for Different Optimum Conditions and Experiment Feasibilities

Goal	Lowest	Target	Upper	Optimum condition			Responses			F/N F
				X ₁	X ₂	X ₃	Y ₁	Y ₂	Y ₃	
Target	Yield	90.27	91.91	91.92						
	FITS	90.3112	91.863	91.863						
	1	6	75	76						
	Clarit y	0.135	0.232	0.233	94.789	41.193	35.189	91.281	0.222	8.737
	FITS	0.1419	0.2236	0.2236	6	3	5	2	5	5
	2	42	77	78						
	Pulp	8.08	9.72	9.73						
	FITS	8.1406	9.69111	9.69111						
	3	79	1	2						
Maximum	Yield	90.27	91.92	91.92						
	FITS	90.3112	91.863	91.863						
	1	6	76	76						
	Clarit y	0.135	0.233	0.233	130.46	53.806	36.718	91.095	0.246	8.906
	FITS	0.1419	0.2236	0.2236	40	7	4	1	5	1
	2	42	78	78						
	Pulp	8.08	9.73	9.73						
	FITS	8.1406	9.69111	9.69111						
	3	79	2	2						
Minimum	Yield	90.27	90.27	91.92						
	FITS	90.3112	90.3112	91.863						
	1	6	6	76						
	Clarit y	0.135	0.135	0.233	82.898	49.092	57.103	91.090	0.138	8.917
	FITS	0.1419	0.1419	0.2236	2	6	8	1	7	6
	2	42	42	78						
	Pulp	8.08	8.08	9.73						
	FITS	8.1406	8.1406	9.69111						
	3	79	79	2						

X₁ = Enzyme concentration (ppm), X₂ = Incubation temperature (°C), X₃ = Incubation time (min), Y₁ = Yield (%), Y₂ = Clarity (abs), Y₃ = Pulp (%), FITS = Predicted response, F = feasible, NF = not feasible

Azlani & Ab Mutalib: DETERMINATION OF ENZYMATIC HYDROLYSIS CONDITION FOR ENHANCED JUICE RECOVERY FROM PURPLE PASSION FRUIT USING RESPONSE SURFACE METHODOLOGY

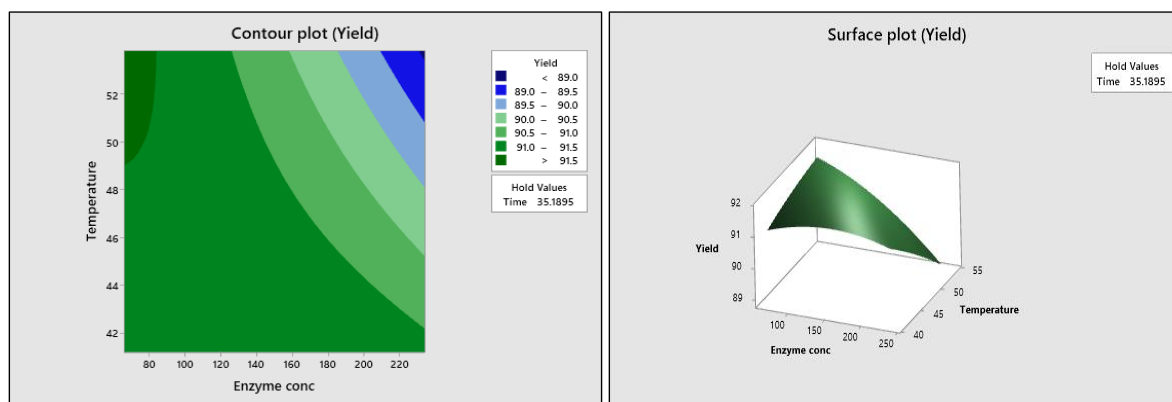


Figure 1. Contour plot and surface plot for the effect of enzyme concentration, incubation temperature and incubation time on the yield of passion fruit juice

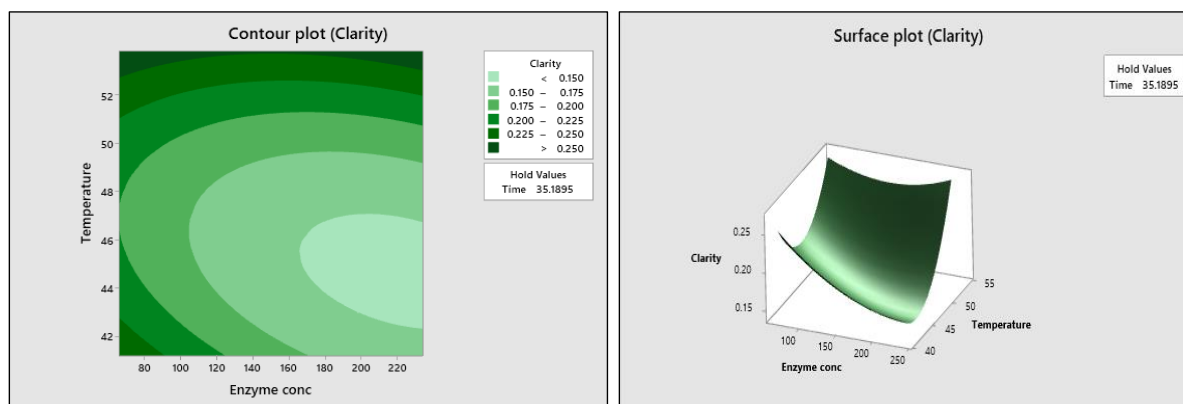


Figure 2. Contour plot and surface plot for the effect of enzyme concentration, incubation temperature and incubation time on the clarity of passion fruit juice

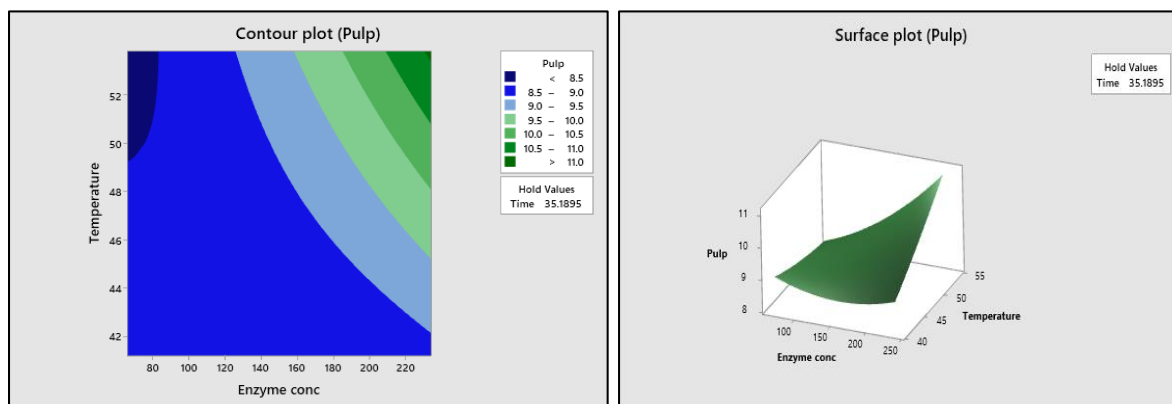


Figure 3. Contour plot and surface plot for the effect of enzyme concentration, incubation temperature and incubation time on the pulp of passion fruit juice

Validations for the optimum condition of enzymatic hydrolysis of passion fruit juice treated with pectinase enzyme were performed. The suitability of the model

equation for predicting the optimum response value was evaluated for the optimum condition under conditions where the enzyme concentration is 94.79 ppm;

incubation temperature is 41.19°C and incubation time is 35.19 mins optimization using actual experimental values for tested using the t-test. The experimental findings of yield, clarity and pulp with the following values: 91.83%, 0.228, and 8.18%. Comparatively, the model-predicted results were 91.29%, 0.223 and 8.75%, respectively. The fact that there was not a significant difference ($p > 0.05$) between the experimental and predicted values for any of the response variables. Therefore, it confirms that the regression model that was created is accurate. Hence, it shows that the models were competent to predict the response variables, which validated the validity and appropriateness of the final reduced model that was fitted by RSM. Furthermore, it was discovered that the models were adequate for forecasting the response variables.

Physicochemical properties of passion fruit juice

The physicochemical properties of treated and untreated passion fruit juice are summarized in Table 5. From the result obtained, the treated and untreated passion fruit juice has a significant effect on the physicochemical properties (clarity, pulp, color b*, fructose, total sugar). The result shows that the yield of passion fruit juice was not significantly different between treated and untreated juice, with 91.83% and 91.01%, respectively. Moreover, it was found that the clarity of passion fruit juice was significantly higher in untreated (0.342) compared to treated juice (0.228). This finding could be due to the presence of the enzyme pectinesterase in the passion fruit juice, which plays an important role in precipitating the passion fruit juice extract. In the juice industry, pectinesterase is responsible for the quality defect of juice cloud loss [24].

Table 5. Physicochemical properties of passion fruit juice

Analysis	Passion Fruit Juice	
	Treated	Untreated
Yield (%)	91.83 ± 0.23 ^a	91.01 ± 0.48 ^a
Clarity (abs)	0.228 ± 0.02 ^b	0.342 ± 0.02 ^a
Pulp (%)	8.18 ± 0.23 ^b	8.99 ± 0.48 ^a
Total soluble solid (°Brix)	14.17 ± 0.00 ^a	13.97 ± 0.09 ^a
Titrateable acidity (%)	3.51 ± 0.02 ^a	3.49 ± 0.02 ^a
TSS/TA ratio	4.04 ^a	4.00 ^a
Colour		
L*	56.67 ± 0.57 ^a	54.95 ± 1.18 ^a
a*	-0.80 ± 0.12 ^a	-0.84 ± 0.04 ^a
b*	6.66 ± 0.04 ^b	7.17 ± 0.06 ^a
Sugar Content		
Fructose (%)	3.56 ± 0.00 ^b	3.82 ± 0.00 ^a
Glucose (%)	0.71 ± 0.13 ^a	0.81 ± 0.04 ^a
Sucrose (%)	3.95 ± 0.00 ^a	4.40 ± 0.30 ^a
Total sugar	8.22 ^b	9.03 ^a

Means within each row with different superscript are significantly different at $p < 0.05$; lower case letters indicate the effect of treated and untreated purple passion fruit juice on its physicochemical properties.

Pectinesterase can be present in the pulp of passion fruit extract. The higher the pulp content, the more pectinesterase will be present, which leads to more cloud clarity. From the result obtained, it was found that untreated passion fruit juice content had higher pulp content (8.99%) compared to treated passion fruit juice (8.18%). Therefore, the clarity of passion fruit juice for untreated showed significantly higher clarity compared

to treated juice. Hence, untreated passion fruit juice contains significantly higher pectinesterase enzyme. Pectinesterase activity leads to the formation of clouds and the separation of juices into two distinct phases, which are a clear liquid and another phase containing solid precipitates from calcium pectate complexes [10]. It was found that the TSS of treated and untreated juice was not significantly different. This finding agreed with

studies done by Phung et al. [11] reported that there was no significant difference in TSS between treated and untreated enzymatic fruit juice. According to Wang et al. [25], the increase in total soluble solids (TSS) in passion fruit juice may be attributed to the increase in soluble sugars resulting from the breakdown of insoluble pectin by pectinolytic enzymes. In addition, the rise in TSS is also associated with a higher level of tissue degradation, resulting in the release of additional substances like sugars that contribute to soluble solids [9].

Titrateable acidity indicates the sourness of the fruit. Acidity in passion fruit juice is reported as citric acid. The acidity of treated and untreated passion fruit juice was not significantly different, where the acidity was 3.51% and 3.49%, respectively. According to Navarrete-Solis et al. [26], an increase in titrateable acidity is associated with the liberation of carboxyl groups from the pectin molecules. A higher amount of TSS and TA can increase the TSS/TA ratio. This result showed that the TSS/TA ratio for passion fruit juice was not significantly different between treated and untreated passion fruit juice, which are 4.04 and 4.00, respectively. A high brix value implies a high concentration of sugar, organic acids and other water-soluble components. Similarly, a high titrateable acidity indicates a high concentration of organic acids [27].

This study found that the color of passion fruit juice or L^* value of treated and untreated juice was 56.67 and 54.95, respectively. The L^* value is an indicator of lightness, and it is desirable to have a high value for clarified juices. Consumers are typically less attracted to products with a dark color since it may suggest that the product has deteriorated. In general, the L^* value follows the same pattern as the clarity of the juice samples, with lighter-colored juice corresponding to greater clarity. An increase in L^* values is most likely caused by the lack of enzymatic browning [17]. Besides that, there is no significant difference between treated and untreated juice in the color a^* with the values of -0.80 (treated) and -0.84 (untreated). The a^* represents the red or green axis where positive and negative values of a^* correspond to red and green values. In this regard, the b^* represents the yellow or blue axis, where positive and negative values correspond to yellow and blue [18]. The treated and untreated juice shows a significant difference in the color b^* , where the b^* values of treated

and untreated are 6.66 and 7.17, respectively. The darkened hue can be linked to nonenzymatic reactions resulting in the creation of pigments with a caramel color [28].

HPLC detected the presence of fructose, glucose and sucrose sugars in passion fruit juice detected. From the result obtained, it was found that glucose and sucrose contents in passion fruit juice are not significantly different in treated and untreated juice. Furthermore, the glucose content in treated and untreated juice was 0.71% and 0.81%, respectively, and the sucrose content in treated juice was 3.95% and 4.0% for untreated juice. Fructose content in untreated juice is significantly higher at 3.82% compared to treated juice at 3.56%. From this study, it was found that sucrose is the main sugar present in passion fruit juice. This finding is in line with [29], which found that the concentration of sucrose is noticeably higher than glucose and fructose. Hence, there is a significant difference between untreated and treated juice in terms of total sugar, with 9.03% in untreated juice and 8.22% in treated juice. The overall sugar concentration is strongly correlated with the soluble-solid content, as most of the soluble solids consist of sugars [30].

Conclusion

Enzymatic hydrolysis of passion fruit juice using pectinase enzyme to produce optimum yield, clarity and pulp was achieved at an optimum condition of enzyme concentration of 94.79 ppm, at an incubation temperature of 41.19°C and in an incubation time of 35.19 mins. In this study, the enzymatic hydrolysis of passion fruit juice produced juice with desirable physicochemical properties, as evident in the significant difference in terms of clarity and pulp. This finding can provide beneficial input to the beverage industry and consumers.

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