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Enzymatic Clarification of Soursop Juice by Pectinase/Cellulase Enzymes Ratio

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Abstract: Soursop juice clarification from enzymatic treatment of pectinase (*Aspergillus aculeatus* / Pectinex Ultra Clear®) and cellulase (*Trichoderma reesei*) ratio enzymes were studied. The enzymes were mixed in different percentage ratio of pectinase (0 – 2.0% v/w) and cellulase (0 – 2.0% v/w) formulated from simplex lattice mixture design using Design expert v.6.0.4. The absorbance of soursop juices were measured by spectrometric method. Extraction yield and viscosity of the treated juices were determined. The physicochemical properties including pH, total soluble solid (TSS) and total reducing sugar of the juice were determined using Lane-Eynon method. The results show that absorbance (0.77 – 0.35) and viscosity (0.09 Pa.s – 0.02 Pa.s) were significantly decreased when the enzyme mixture of 1.0:1.0 (pectinase:cellulase) was applied. The extraction yield increased up to 60% from 30.42% (v/v) to 75.00% (v/v) for the enzyme mixture of 0.5:1.5 (pectinase:cellulase). The decreases in pH were not significant for 1.5:0.5 (pectinase:cellulase) enzyme mixture while TSS (16.17 ºBrix - 18.70 ºBrix) and total reducing sugar (7.44% - 10.55%) increases significantly for enzyme ratio of 0.5:1.5 (pectinase:cellulase). The best enzyme mixture was found to be 0.5:1.5 (pectinase:cellulase). Based on multiple linear regression analysis, quadratic model was more significant compared to other models. This study has shown that enzymatic treatment improves the quality and yield of soursop juice while maintaining the nutritional contents compared to untreated soursop juice.

Keywords: Soursop Juice, Enzymatic Clarification, Pectinase, Cellulase, Absorbance, Yield, Viscosity

1. Introduction

Different commercial enzymes like pectinases, amylases and cellulases were used to obtain maximum clarified juice. Soursop fruit is a very pulpy fruit with mushy white flesh and have a lot of fibres. Thus, the juice produced from the pulp has high viscosity. It is known that most of the juice extraction processes are not producing satisfactory quantity and quality. For instance, the conventional extraction method able to produce recovery rate between 62 and 82.5% of soursop pulp depending on the type of equipment, methods of extraction, cultivar and cultural practices, and number of fruit seeds

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while an extraction of soursop by a single enzyme namely pectinase able to increase the juice production by 41% [1], [2]. This study investigated the hypothesis that the enzymatic treatment improved the clarification of soursop juice. The objectives of this study are to determine the pectinase/cellulase enzyme ratio for maximum clarification of soursop juice and to analyse the absorbance, extraction yield, viscosity and physicochemical properties (pH, total soluble solid and total reducing sugar) in untreated and treated soursop juice.

2. Materials and Methods

2.1 Materials

The mature green stage soursop fruits were bought from a vendor at R&R area, Pagoh, Johor. The fruits were first allowed to ripen at room temperature before the soursop pulp were separated from skin and de-seeded by hand and frozen at -20 ℃ for further treatment. Two enzymes, pectinase from *Aspergillus aculeatus* / Pectinex Ultra Clear® and cellulase from *Trichoderma reesei* from Sigma Aldrich were purchased and kept in -80 ℃. All chemicals used in this study were either general, analytical or food grade depending on the analysis.

2.2 Soursop Pulp Preparation

Various ratio of pectinase and cellulase enzyme were formulated by commercial software (Design Expert v.6.0.4). Next, all the mixture formulations were incubated at 50 ℃ for 2 hours. Then, the mixtures were heated up to 90 ℃ for 5 minutes for enzyme inactivation. The mixtures were then filtered using muslin cloth and the filtrates were collected for further analysis of clarity, extraction yield, viscosity and physicochemical properties. Figure 1 shows the overall research flow chart for this study.

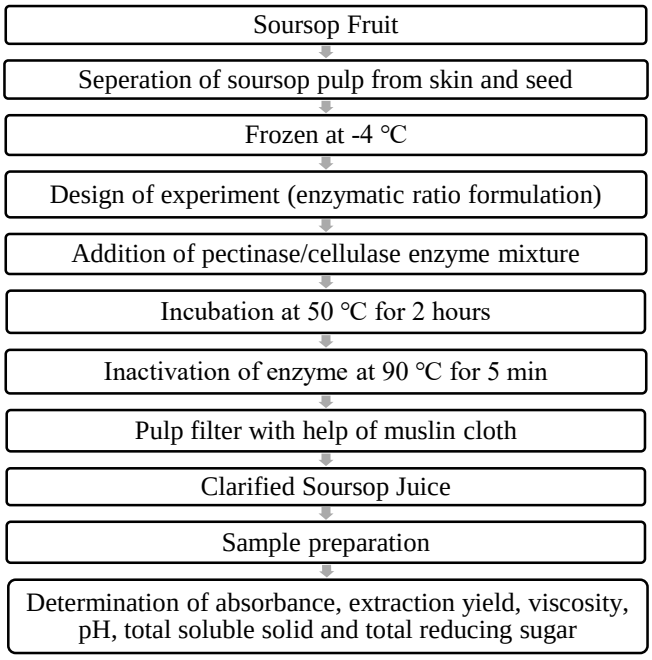

Figure 1: Overall flowchart designed for this study

2.3 Design of Experiment

The soursop juices were treated with various enzymes ratios formulated from simplex lattice mixture design (Design Expert®) with a total of five runs of experiments as presented in Table 1. Two different enzymes, namely pectinase and cellulase were selected based on literature as independent variables in the mixture design, and pH was selected as response to determine the best enzymes ratio. Table 2 shows the summary of the design.

Table 1: Simplex lattice mixture design experimental layout

Experiment	Pectinase (%v/w)	Cellulase (%v/w)
1	2.00	0.00
2	1.50	0.50
3	1.00	1.00
4	0.50	1.50
5	0.00	2.00

Table 2: Design summary used for enzymatic clarification of soursop juice

Study Type	:	Mixture		Experiments	:	5	
Initial Design	:	Simplex Lattice		Blocks	:	No blocks	
Design Model	:	Quadratic					
Component	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A	Pectinase	%v/w	Mixture	0.000	2.00	0.000	1.000
B	Cellulase	%v/w	Mixture	0.000	2.00	0.000	1.000
				Total =	2.00		

2.4 Enzymatic Treatment of Soursop Juice

For each experiment, according to Karim *et al*., (2018) [3], 100 g of thawed soursop pulp was subjected to different enzymatic treatment conditions. The enzyme was added to the soursop pulp and stirred with glass rod. The independent process variable for the enzymatic treatment process were pectinase enzyme concentration (0.5 – 2.0 %v/w), cellulase enzyme concentration (0.5 – 2.0 %v/w) at incubation temperature of 50 °C and holding time of 120 min. The temperature of enzymatic treatment was adjusted to the desired temperature using a water bath (WNE 29, Memmert, Germany). Post treated soursop juice was conducted by heating up the juice at 90 °C for a 5 min for enzyme inactivation before being filtered using muslin cloth and the filtrate were collected for further analysis. The sample without enzymatic treatment used as control sample.

2.5 Absorbance of juice

Pulpy materials were separated from soursop juice (40mL) by centrifugation (5804 R, Eppendorf, Germany) at 3000 rpm for 15 min at 4 °C. The juices were then filtered through a muslin cloth. The filtrates were filled into respective cuvettes and the clarity of the juices were measured at 660 nm using UV–vis spectrophotometer (T60 U, PG Instruments, UK). Distilled water was used as reference.

2.6 Yield of juice

The filtered juices from previous analysis were measured for their weight and volume. The volume of juice before and after filtration from each samples were measured in a 100 mL measuring cylinder. Then, the weight of juices recorded using mass balance. Clarified juices yield (%) were be calculated using calculation as shown in Eq. 1 [4]:

$$Yield\ of\ juice = \frac{Volume\ of\ clear\ juice}{Volume\ of\ sample} \times 100\% \quad Eq.1$$

2.7 Determination of viscosity

The viscosity measurement was conducted using a rheometer (Discovery HR-1, TA Instruments, US). Undiluted juice (1.5 mL) was poured on the surface of measuring bob (60 mm, 2.006º cone plate, Peltier plate Steel) until it was fully covered. It was then spun at sheer rate range between 0.5 to 1001 s^{-1} at 30 ℃ and the reading at 100 s^{-1} for the juice viscosity. The unit of viscosity was expressed as Pa.s.

2.8 Determination of physicochemical properties

Undiluted juice (1.5 mL) was used to measure pH value using a digital pH meter (pH700, Eutech Instruments, UK). Buffer solutions of pH 4.0, 7.0 and 10.0 were used as reference. Total soluble solids content of undiluted juice was determined using a digital hand-held refractometer (PAL-1, Pocket, Japan) and data reported as ºBrix.

Total reducing sugar was determined using Lane & Eynon method [5]. For the preliminary titration, 1.0, 2.0 and 3.0 g of control juice samples were diluted with distilled water in a 100 mL volumetric flask. The burette was then filled with first juice sample. Each Fehling A and B solutions (5 mL) were pipetted into 250 mL conical flask. The juice sample was invert titrated and the solution was heated to boil on hotplate (Isotemp, Fisherbrand, Sweden). Methylene blue indicator (4 drops) were added to the conical flask. The addition of juice sample was continued until the blue colour turned into a brick-red end point. The same procedure was repeated for all juice samples where volume of the sample solution (titre) should be between 15 to 50 mL maintaining the total boiling time of 3 min. The titre value, V_1 was recorded and 3.0 g, as value of W in the formula, of juice sample was chosen. Then, 5 mL from each Fehling A and B solutions were pipetted into a 250 mL conical flask. 3.0 g from each formulation were diluted with distilled water in a 100 mL volumetric flask, V_2. The sample solution was then added into burette. The flask containing Fehling solutions was heated until boiling and 4 drops of methylene blue solution was added. The titration was completed within 1 min by adding 2 to 3 drops of sample at a time, until the indicator decolourized and end point reached (solution changed to brick-red). The titre value, V_3 was recorded.

The percentage of total reducing sugar was calculated using the following Eq. 2:

$$Total\ reducing\ sugars = \frac{0.0025 \times V_1 \times V_2}{V_3 \times W} \times 100\% \quad Eq.2$$

3. Results and Discussion

3.1 Clarity of Soursop Juice

Clarity is a main index of commercially viable juices [6]. The presence of pectic substances in fresh juices induces turbidity. In addition, pectin molecules show high water holding capacities, which create a coherent network structure and viscous juice. Based on the Figure 2, the absorbance shows significant increase ($P \leq 0.05$) in the clarity of juice samples for juice with enzyme ratio of 1.0:1.0 (pectinase:cellulase) and observable increase with 1.5:0.5 (pectinase:cellulase). They produced the highest clarity (lowest absorbance value) with the percentage reduction up to 50%. While the clarity for 2.0 pectinase was very low. The higher the value of absorbance caused more light absorbed by the sample which indicates the cloudiness of the solution.

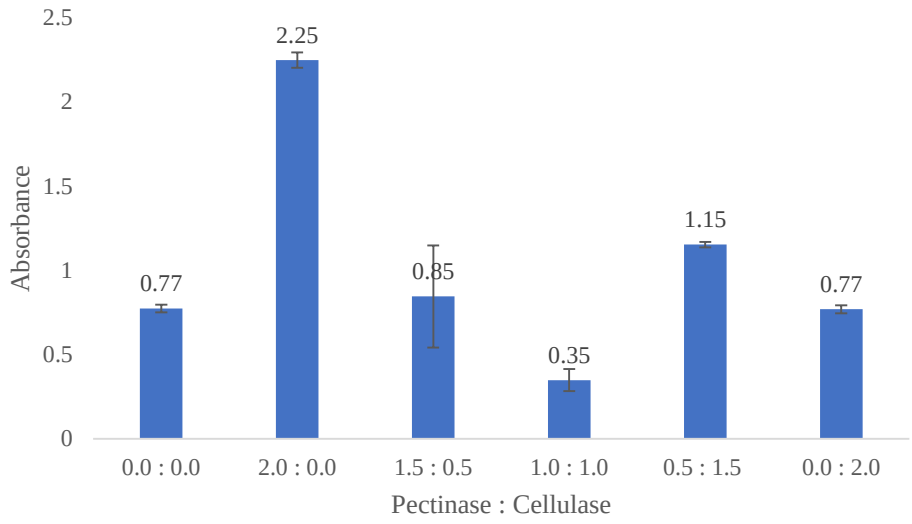

Figure 2: Absorbance of soursop juice after treated by pectinase/cellulase enzyme

As the temperature of enzyme treatment (50 °C) was below the denaturation temperature (40 – 60 °C) of pectinase and cellulase enzymes, it may have helped in the enzymatic treatment. By exposed positive part of the protein underneath when using enzyme mixture, the clarification rate can be improved by reducing electrostatic repulsions of the cloud particles caused them to accumulate into larger particles and settle down [7]. Previous research reported that the clarifying rate may improve by the amount of the enzyme used as a result of repulsions between positive amino acids and other components leads to a larger cloud particle formation that are eventually settled [6].

3.2 Extraction Yield of Soursop Juice

Juice extraction can be carried out by various mechanical procedures but, as opposed to enzymatic juice treatment, the yield of juice in mechanical processes is low. The extraction yield of treated soursop juice in Table 3 shows an increment up to 60% (significantly ($P \leq 0.05$)) when enzyme mixture of 0.5:1.5 (pectinase:cellulase) applied. From 40 mL of sample, the maximum juice obtained was 30 mL while the untreated juice produced only 12.17 mL. Enzyme ratio of 1.5:0.5 (pectinase:cellulase) and 2.0 cellulase also had relatively high extraction yield.

Table 3: Extraction yield (% v/v) of enzymatic treated soursop juice

Formulation	Pectinase (%v/w)	Cellulase (%v/w)	Weight (g)	Volume (mL)	Yield (%v/v)
Control	0	0	10.77 ± 0.62	12.17 ± 0.76	30.42 ± 1.91
1	2.0	0	23.97 ± 0.64	23.60 ± 0.69	59.00 ± 1.73
2	1.5	0.5	26.14 ± 0.85	28.13 ± 0.81	70.33 ± 2.02
3	1.0	1.0	18.29 ± 1.41	19.43 ± 1.62	48.58 ± 4.05
4	0.5	1.5	29.24 ± 0.25	30.00 ± 0.20	75.00 ± 0.50
5	0	2.0	24.45 ± 1.58	27.00 ± 1.00	67.50 ± 2.50

In fruit processing, the use of pectic enzymes is important to increase the yield of juice, boost the filtration rate and generate high quality juices for the concentration process. The increase in the yield

of juice is mostly related to an increase in juice soluble sugars. For fruit juice that undergone enzymatic treatment, the substance is degraded by exogene enzymes to the middle lamella and cell wall pectin as it is converted into soluble materials including acid and neutral sugar [7].

3.3 Viscosity of Soursop Juice

Results of viscosity of soursop juice samples are shown in Figure 3. Significant decrease ($P \leq 0.05$) was achieved when the soursop juice was treated with enzymes. When the soursop juice was treated with pectinase and 1.0:1.0 (pectinase:cellulase) shows the lowest viscosity value with up to 77% reduction than untreated soursop juice. This is proportional with the clarity result where lower viscosity is linked to better clarity of the juice [8].

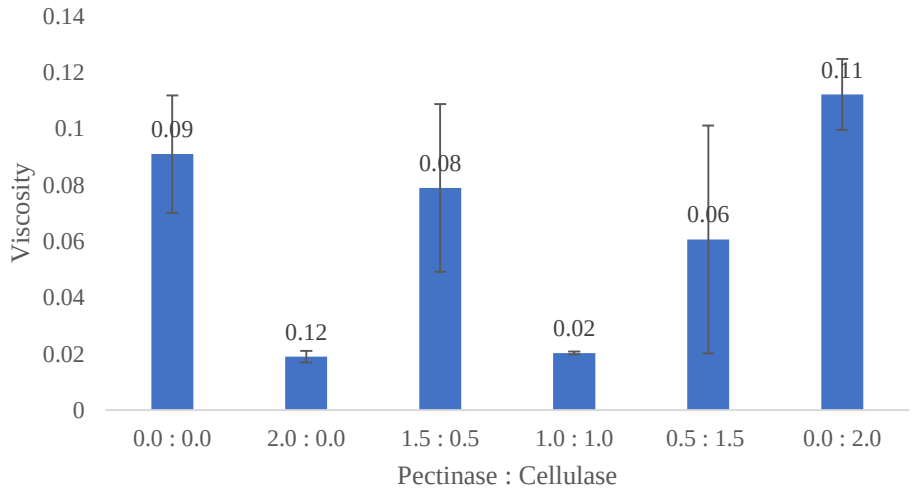

Figure 3: Viscosity (Pa.s) of soursop juice after treated by pectinase/cellulase enzyme

Furthermore, the turbidity and viscosity are caused primarily by hemicelluloses, starch, pectin, cellulosic and bound lignin [9], which explains little changes of consistencies took place when soursop pulp was added with cellulase as the sample for 2.0 cellulase enzyme ratio cause the highest viscosity. This implies that the combination of pectinase and cellulase has allowed the soursop juice to improve concentration where 1.0:1.0 (pectinase:cellulase) gave the lowest viscosity value.

3.4 Physicochemical Properties of Soursop Juice

The pH is insignificantly decrease ($P > 0.05$) (Figure 4) with the lowest value by 1.5:0.5 (pectinase:cellulase) enzyme ratio possibly due to the increase of pectin degradation in galacturonic acid concentration because the pectinase enzyme concentration used in the mixture is higher than cellulase [3], [7]. For pectinase treatment on soursop juice, the pH decreased from 3.7 to 3.58, hence it was assumed that the quality is unchanged [2]. In addition, the study of enzyme mixture on soursop puree also reported that the increased of cellulase concentration with pectinase enzyme did not significantly impact the pH of puree ($P > 0.05$) [3]. It also explains that the control, the sample treated with only cellulase and the samples with low volume of pectinase had higher pH value than other enzyme mixtures. The previous study also stated that the effect of pectinase was the main the reason to any pH changes in both pectinase and cellulase mixtures.

Next, the TSS shows significant increase ($P \leq 0.05$) from control sample where the highest reading of ^{o}Brix was recorded for 0.5:1.5 (pectinase:cellulase) mixture as shown in Figure 5. This is probably because cellulase breaks down the cell wall and releases cell material, such as soluble sugars including glucose and fructose, in the solution. The synergistic effects between pectinase and cellulase improved soluble solid extraction in fruits [3]. The increase in TSS also may partially caused by increasing soluble

sugar, resulting from conversion of insoluble pectin by pectinolytic enzymes and from the action of cellulase in the production of soluble sugars from cellulose [7]. Measuring the total solid will therefore evaluate the clarification phase [10], [11] where clarified juice supposed to contain less total solid.

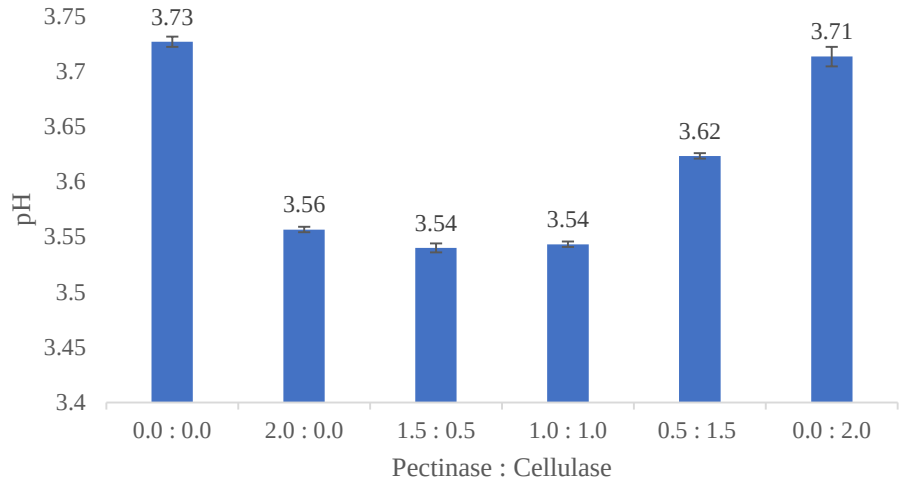

Figure 4: pH of Soursop Juice after treated with pectinase/cellulase enzyme

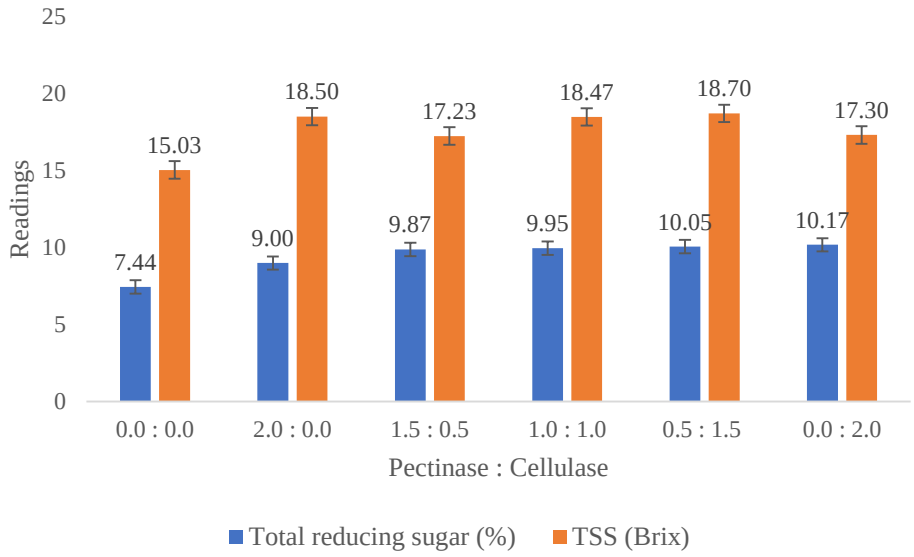

Figure 5: TSS (°Brix) and total reducing sugar (%) of soursop juice after treated with pectinase/cellulase enzyme

For the total reducing sugar, 0.5:1.5 (pectinase:cellulase) enzyme ratio also shows the highest reading due to higher concentration of cellulase than pectinase used in the treatment might have a degrading effect on the cell wall, resulting in low molecular sugars released [3]. Thus, from the results obtained, it is proven that the increase of total reducing sugar are proportional to the increase of TSS, thus, from this study, 0.5:1.5 (pectinase:cellulase) produced highest reading for both parameters. Furthermore, the increase in total reducing sugar decreased the absorbance reading of high pectinase containing sample, which leads to increased amount of pectin decomposed [12]. However, the trend of total reducing sugar is increasing as the volume of cellulase enzyme increased. Increased natural sugar levels in juices due to partial degradation the sucrose by enzyme treatment [7].

3.5 Simplex Lattice Mixture Analysis

Experimental design data were analysed further by using multiple-linear regression method and pH value was used as response. For an appropriate model range based on the highest F-statistics significance suitable for clarification of soursop juice, sequential F-tests were performed on linear to cubic solutions. Table 4 shows the findings of ANOVA for all three models. The quadratic model demonstrated high F-value (95.65) and low p-value (0.0103).

Table 4: ANOVA for significance of regression model

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	R-Squared
Linear	0.016	1	0.016	7.57	0.0706	0.7162
Quadratic	0.022	2	0.011	95.65	0.0103	0.9897
Cubic	0.022	3	0.007	33.37	0.1264	0.9901

The R-squared value analysis reveals that the quadratic model is more suitable to be chosen for optimisation (R-squared quadratic 0.9897) compared to cubic model as the differences are insignificant while the F-value is significantly different. These data showed that the most significant is the quadratic model. The R-squared value of the quadratic model is stated at 0.9897 and indicates that two components in the mixture together will explain about 98.97% of the variability in the response leaving just 1.03% of the variability unexplained. The significance of F-value is where the probability of model's null hypothesis not to be rejected, thus, the higher F-value is needed to conclude that the regression coefficients are exactly zero. Therefore, subsequent data analysis was analysed using a quadratic model only. The simplex lattice design for a two-component system using predicted and actual pH value represented by a line segment as shown in Figure 6.

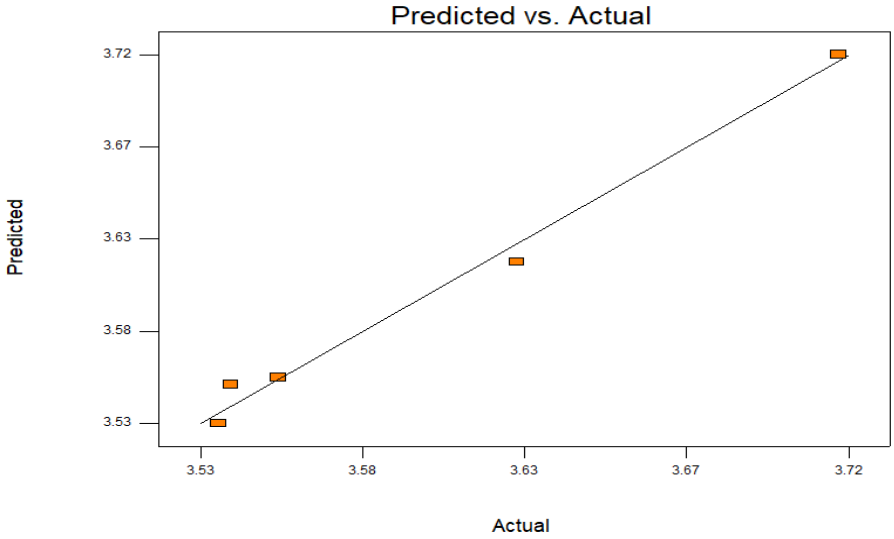

Figure 6: Comparison between actual and predicted pH values

The pH revealed a good linear correlation between actual and predicted value [13]. For each model, as shown in Table 4.6, a fair agreement was obtained between the predicted R-squared and the adjusted R-squared. The 0.9798 of adjusted R-squared is proportional with the 0.9398 of adjusted R-squared where the difference is even below 0.1. Therefore, further study can be conducted to optimize the pH of the enzymatic treated soursop juice with optimum process parameters to improve the clarification rate of the juice.

Table 5: R-squared data of different regression models

Source	R-Squared	Adjusted R-Square	Predicted R-Squared
Linear	0.7162	0.6216	-0.1236
Quadratic	0.9897	0.9793	0.9398
Cubic	0.9901	0.9604	-0.4906

4. Conclusion

The usage of pectinase and cellulase enzymes in clarification of soursop juice able to increase the juice extraction yield, total soluble and total reducing sugar while decreasing the absorbance of juice, viscosity and pH. Different ratio of enzyme produced different results. The optimum enzymes ratio obtained was 0.5:1.5 (pectinase:cellulase). This study demonstrated that the enzyme mixtures able to increase the clarity of the treated juice, reduced its viscosity and produced higher yield compared to untreated juice. Other than that, addition of enzymes mixture improved the physicochemical properties of the juice. Interestingly, the juice produced are free from separation and are properly homogenized.

Acknowledgement

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