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Research Article

Optimisation of enzymatic treatment of avocado (*Persea americana* Mill) juice using response surface methodology (RSM)

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Abstract

The high viscosity and pectin content of tropical fruits have been a persistent issue in the fruit juice industry. Enzymatic treatment using pectinolytic enzymes has emerged as an effective method to improve juice yield and enhance the functional attributes. This study aimed to optimise the enzymatic treatment of avocado pulp to improve juice yield, antioxidant activity, and total phenolic content (TPC). A second-order central composite design using Response Surface Methodology (RSM) was employed to evaluate the effects of varying enzyme concentrations (1.3 - 1.7 % v.w⁻¹), incubation times (82 - 98 min), and avocado pulp-to-water ratios (1:2.16 - 1:3.8) at a constant temperature of 50°C. The models developed for all responses demonstrated a high coefficient of determination ($R^2 > 0.95$), indicating good model reliability. Statistical analysis revealed significant ($p < 0.05$) effects of all independent variables on the response. The optimal conditions for enzymatic treatment of avocado pulp were 1.45 % v.w⁻¹ enzyme concentration, 94.01 min incubation time, and 1:3.5 avocado pulp-to-water ratio. Under these optimal conditions, the predicted values for juice yield, antioxidant activity, and TPC were 93.47 %, 84.38 %, and 53.79 mg GAE.mL⁻¹, respectively. These findings support the effectiveness of pectinolytic enzymes to improve the physicochemical and functional properties of avocado juice.

Keywords

avocado juice, enzymatic treatment, juice yield, antioxidant activity, total phenolic content

Abbreviations

CCD – central composite design; ETAJ – enzyme-treated avocado juice; ETAP – enzyme-treated avocado puree; GAE – Gallic acid equivalent; RSM – response surface methodology; TPC – Total phenolic content

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Introduction

Avocado (*Persea americana* Mill) is one of the most nutrient-dense subtropical fruits, originating mainly from Mexico and Central America (Bhuyan et al. 2019; Jimenez et al. 2021; Zafar and Sidhu 2018). This study utilised locally grown avocados, with Sabah, the top producer in Malaysia, accounting for 282.30 metric tons in 2022 (Malaysian Department of Agriculture 2022). The avocados from Sabah are generally larger than imported ones, typically weighing about 400 to 600 g per fruit. Avocado is known for its various nutritional values, with 67 to 78 % moisture, 13.5 to 24 % lipids, 0.8 to 4.8 % carbohydrates, 1 to 3 % protein, 1.4 to 3 % dietary fibre, and 0.8 to 1.5 % mineral content (Jimenez et al. 2021; Yahia and Woolf 2011). The edible pulp also contains vitamins and bioactive substances and is high in monounsaturated fatty acids (MUFA) (Jimenez et al. 2021). Avocado is also recommended for individuals with diabetic and lipid disorders owing to its low sugar content, making it a favourable dietary choice (Henríquez Arias et al. 2012).

Avocado is also a highly perishable fruit due to its high moisture and fat content (Chuacharoen et al. 2021) and high deterioration rate at room temperature (Henríquez Arias et al. 2012). Research has been conducted to enhance the quality and shelf-life of fresh avocados, which are highly perishable. This research involves various treatments and methods in processing avocado pulp, including avocado puree (guacamole), drinks, packaged slices and chunks, and dehydrated avocado (Jimenez et al. 2021). Avocado-based products, such as smoothies, guacamole, and avocado oil, are widely popular and readily available. According to Pinto et al. (2022), consumers opt for nutritious beverages that suit their fast-paced lifestyles due to the growing interest in health and wellness. This shift in consumer preferences has sparked a demand for natural beverages over artificially flavoured ones (Rajauria and Tiwari 2018).

The primary challenge in fruit juice processing is achieving high juice yield due to the high pectin and other polysaccharides. These inconsistencies are attributed to complex cell wall components, including cellulose, hemicellulose, and pectin, that hinder the release of juice and nutrients during processing (Phing and Kalam 2023). Previous

studies have highlighted that fruit juice extraction and filtration are difficult and time-consuming, mainly when using conventional extraction methods (Sharma et al. 2014; Vivek et al. 2019). Vivek et al. (2019) also reported that conventional extraction processes lead to high maintenance costs, require large amounts of energy, and pose the risk of cross-contamination and high initial capital investment. Consequently, an alternative method is recommended to improve juice yield production while preserving nutritional quality. Enzymatic treatment demonstrated superior effectiveness in enhancing juice recovery compared with alternative methods such as cold and hot extraction (Vivek et al. 2019). Applying such treatment helps tackling the issues relating to the pectin and other polysaccharides that hugely contribute to the low juice yield obtained, high viscosity, and difficulties in filtration process (Nur'Aalia et al. 2011; Phing and Kalam 2023). Enzymatic treatment helps directly target these compounds, where enzyme like pectinase effectively help the breakdown of such complex compounds into smaller chains, reducing its water-binding capacity. Compared with other treatments such as chemical and traditional methods, concerns also include high production costs, product safety, and environmental impact (Ramadan and Moersel 2007). Enzymatic treatment itself has been recognised as one of the green technologies, using food-grade enzymes in fruit juice production.

Enzymatic treatments using cell wall degrading enzymes such as pectinases, cellulases, and hemicellulases are frequently employed in the pre-treatment of the fruit juice process (Huang et al. 2009; Singh et al. 2019). These enzymes play a crucial role in breaking down complex cell wall components, facilitating nutrient release and increasing the overall juice extraction yield (Singh et al. 2019). Additionally, enzymatic treatment reduced the viscosity of the juice, facilitating filtration and centrifugation to improve overall processing efficiency (Sharma et al. 2017). Nevertheless, several variables, such as enzyme concentration, temperature, incubation time, and type of enzyme used, affect the effectiveness of enzymatic treatment. Therefore, optimising these parameters is essential to maximise juice yield and its functional properties. Several studies have shown that optimised conditions for enzymatic

treatment using pectinase enzyme increased juice recovery from sapota (Mamabolo and Tabit 2023) and watermelon (Baskar and Hemalatha 2021). Hence, this research aimed to establish the optimal process conditions (enzyme concentration, incubation time and avocado pulp-to-water ratio) of avocado fruit juice using Response Surface Methodology.

Materials and Methods

Materials. Avocados were purchased from a local market in Kota Kinabalu, Sabah. Avocados at maturity stage 4 with dark green to blackish color and slightly soft texture (Ledger et al. 2011), free from physical damage and 6 to 7 Brix° were chosen in this study. The avocados were peeled, deseeded, cut into small pulp pieces and homogenised in a Waring blender (National, model Kenwood Chef, Malaysia) for 1 min to obtain a homogenous fruit pulp which was used for the enzymatic treatment.

Chemicals. A commercial pectinolytic enzyme, Pectinex Ultra SP-L (with an activity of 3880 PGNU.g⁻¹, optimum pH 4 to 9 and temperature below 50°C) was acquired from Modernist Pantry, LLC (Eliot, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Merck (Darmstadt, Germany), Folin-Ciocalteu and gallic acid from Sigma-Aldrich (Darmstadt, Germany), sodium carbonate from R&M Chemicals (Essex, UK), and methanol from System ChemAR. All chemicals and reagents used in this study were of analytical grade (99%).

Methods. For each experiment, 35 g of avocado pulp was subjected to different pectinase enzyme treatment conditions at a constant temperature of 50°C, as shown in Table 2. After the treatment, the enzymes in the homogenous avocado puree were deactivated by heating to 95 °C for 5 min, followed by rapid cooling to room temperature. The enzyme-

treated avocado puree (ETAP) was then filtered through a fine mesh nylon cloth, and the enzyme-treated avocado juice (ETAJ) was collected and stored in a refrigerator at 4 °C for further analysis. A sample of avocado juice was prepared from the same batch of fresh avocado pulp by manual extraction, without enzymatic treatment. This sample was referred to as the control sample throughout the research. This method of fruit juice production was modified from Vivek et al. (2019).

Enzymatic design and Response Surface Methodology (RSM). Enzyme concentrations (ranging from 1.3 to 1.7 % v. w⁻¹) (X_1), incubation times (ranging from 81.59 to 98 min) (X_2), and avocado pulp-to-water ratio (ranging from 1:2.16 to 1:3.84) (X_3) were used as independent variables for the enzymatic treatment. Table 1 below shows the independent variables and their coded levels, employed into the experimental design. These variable ranges were selected based on the preliminary studies conducted prior to the actual experiment to set the upper and lower values of the independent variables. Response Surface Methodology (RSM) was used in this study to determine the optimum enzymatic treatment of avocado puree. The experimental design, statistical analysis and regression model were performed using Design Expert software (Version 13) (Statease Inc.). The Central Composite Design (CCD) with a quadratic model was employed. A total of 17 runs of the combined effect of the three independent variables, each including three replicates at the center point were generated. The dependent variables (Y) measured were juice yield (Y_1), antioxidant activity (Y_2) and total phenolic content (Y_3). The response surface model for predicting response variables as a function of factor variables was determined using a second-degree polynomial equation, as shown in Eqn. (1).

Table 1. Factors and its coded levels in response surface methodology (RSM)

Factor	Symbol	Low (-1)	Centre (0)	High (+1)	Unit
Enzyme concentration	X_1	1.40	1.50	1.60	% v.w ⁻¹
Incubation time	X_2	85	90	95	min
Pulp-to-water ratio	X_3	1 : 3.0	1 : 3.0	1 : 3.5	-

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

In this equation, Y represents the predicted response and X_1 , X_2 , and X_3 are the independent variables. The coefficients of the polynomial were represented by β_1 , β_2 and β_3 (linear coefficients), β_{11} , β_{22} and β_{33} (quadratic coefficients), and β_{12} , β_{13} and β_{23} (interaction coefficient). The significance and validation of each modelled equation were analysed in terms of coefficient of determination (R^2), adjusted R^2 , predicted R^2 , and analysis of variance (ANOVA), according to Sonawane et al. (2020).

Juice yield. Juice yield in percentage was determined on the mass basis of the filtered juice to the initial raw material, calculated based on the following Eqn. (2) by Makebe et al. (2020).

$$\text{Juice yield} = \frac{\text{filtered juice, g}}{\text{avocado pulp, g} + \text{water added, g}} \times 100, \% \quad (2)$$

1,1-diphenyl-2-picrylhydrazyl scavenging ability assay. DPPH scavenging activity was measured spectrophotometrically according to Vivek et al. (2019). Briefly, the enzyme-treated avocado juice (ETAJ) was added to 0.1 mM methanolic DPPH solution at a ratio of 1:1. The mixtures were then incubated for 30 min in the dark at room temperature. The absorbance of the samples and the blank sample were measured using a UV-Vis spectrophotometer at 517 nm. An inhibition percentage was measured following Eqn. (3).

$$\text{Inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100, \% \quad (3)$$

Total phenolic content. The total phenolic content was carried out using a slightly modified Folin-Ciocalteu method from Wu et al. (2021). The ETAJ sample was allowed to stand in methanol at 1:1 ratio for 15 min prior to analysis. A homogenised diluted sample (1 mL) was added to 1 mL Folin-Ciocalteu reagent (1 : 3 with distilled water) and 3 mL of 7 % sodium carbonate. The mixtures were incubated for 2 h in the dark at room temperature. The absorbance of the sample was measured at 765 nm using a UV-Vis spectrophotometer. The calibration curve for gallic acid was developed with 20 to 100 ppm standard solution ($R^2 = 0.9795$). The total phenolic

content of samples was expressed as gallic acid equivalent (GAE) mg.mL⁻¹.

Statistical analysis. All experiments were carried out in triplicate. A Statistical Package for Social Science (SPSS) was used to analyse the data. Data analysis was carried out using a one-way analysis of variance (ANOVA) at a 95 % significance level and p-values below 0.5.

Results and Discussion

Response surface optimisation of enzymatic treatment. Table 2 presents the experimental values for the response variables, juice yield, antioxidant activity and total phenolic content under different enzymatic treatment conditions generated using the response surface methodology (RSM). A central composite design was used to establish the coefficients of all equations. Table 3 presents the regression coefficients for second-order polynomial equations, as well as the results of linear, interaction, and quadratic terms for each response. Analysis of variance also confirmed that the quadratic models were statistically significant for all response variables as shown in Table 4. The quadratic models for each response were statistically significant ($p < 0.05$) with a non-significant lack of fit ($p > 0.05$) as presented in Table 5. All models were analysed using F-value at a probability of 0.05. The coefficients of determination (R^2 , Adjusted R^2 , and Predicted R^2) obtained for all responses are closer to 1.0, indicating a higher accuracy of the quadratic models (Romeo 2020).

Effect of processing conditions on juice yield.

Table 2 presents the maximum juice yield obtained at an enzyme concentration of 1.4% v.w⁻¹, with 85 min of incubation for a 1 : 3.5 avocado pulp-to-water ratio. The predicted juice yield was determined from the second-degree polynomial equation generated by the software, as shown in Eqn. (4).

$$Y = 93.10 + 1.14X_1 - 0.9958X_2 + 2.22X_3 + 1.12X_1^2 - 0.4012X_2^2 - 0.6441X_3^2 + 0.4009X_1X_2 - 1.02X_1X_3 + 0.5284X_2X_3 \quad (4)$$

The regression model (Table 3) demonstrated that the juice yield was significantly ($p < 0.05$) affected by the positive linear effects of enzyme concentration (β_1) and the avocado pulp-to-water ratio (β_3). The increase of both enzyme concentration and avocado pulp-to-water individually can result in a higher juice yield. The pectinase used in this process breaks down pectin and its complex cell wall matrix, reducing the water holding capacity, and enabling the release of water-soluble pectin (Chen et al. 2023). Hence,

optimal enzyme concentrations will facilitate the more effective breakdown of cell walls in the fruit mash, resulting in easier juice extraction and a higher yield (Phing and Kalam 2023). These findings align with previous studies, demonstrating the positive influence of enzyme concentration on juice yield in bananas (Kumar et al. 2023), guava (Vani et al. 2021), mango (Kunkulol et al. 2018), sapodilla (Singh et al. 2022), and sohiong (Vivek et al. 2019) fruit.

Table 2. Central composite design and response values

Experiment no.	X ₁	X ₂	X ₃	Juice yield, %	Antioxidant activity, %	Total phenolic content, mg GAE.mL ⁻¹
1	1.50	90.00	3.0	93.53	81.85	55.98
2	1.50	90.00	3.0	91.97	81.63	55.80
3	1.60	95.00	2.5	89.62	77.91	60.95
4	1.50	90.00	3.0	93.91	81.84	56.36
5	1.50	98.41	3.0	90.28	81.73	57.00
6	1.40	95.00	3.5	92.24	84.69	53.36
7	1.50	90.00	2.16	87.65	77.80	61.94
8	1.50	90.00	3.84	94.28	85.29	46.90
9	1.40	85.00	2.5	88.47	78.64	48.03
10	1.67	90.00	3.0	91.36	79.83	52.34
11	1.40	85.00	3.5	94.48	83.46	44.83
12	1.60	95.00	3.5	93.67	81.67	49.47
13	1.50	81.59	3.0	93.02	81.11	45.66
14	1.40	95.00	2.5	84.61	80.93	54.71
15	1.60	85.00	3.5	93.81	82.43	44.41
16	1.60	85.00	2.5	92.36	78.22	58.33
17	1.33	90.00	3.0	87.88	82.57	49.81

X₁ represents enzyme concentration, % v.w⁻¹, X₂ represents incubation time, min, X₃ represents liquid ratio.

Additionally, the positive linear relationship between the avocado pulp-to-water ratio and juice yield, with a coefficient of 2.22 ($p < 0.05$), also indicates that increasing this variable substantially enhances the juice yield. As more water was added to the pulp, it helped to decrease the viscosity, making it easier for more juice to be released. Water added to the process also helps to break down the fruit pulp into smaller pieces, increasing the contact area for enzyme interaction (Phing and Kalam 2023). Pradhan et al. (2020) stated that a balanced ratio between the fruit sample and the water added is specifically crucial in enzymatic treatment, to ensure that the enzymes are well dispersed and can act on the larger surface area of the pulp. With the increase in water or juice released from the matrix, water molecules help to reduce the viscosity of fruit

juice by increasing the permeability of the cell walls (Pradhan et al. 2020). The water facilitates the extraction process, allowing the enzyme to act more efficiently on the pulp (Pradhan et al. 2020).

In contrast, the selected experimental value of incubation time showed a negative linear relationship, as indicated by a coefficient of -1.0 ($p < 0.05$). According to Singh et al. (2022), pectinase hydrolyses the pectin cell wall in fruit, resulting in higher juice yield with shorter processing times. With the interconnected factors used, enzyme concentration and the avocado pulp-to-water ratio help maximise the efficiency of pectin hydrolyses for a relatively shorter period of time. In other cases, enzyme denaturation may also occur over time, resulting in the loss of enzyme effectiveness (Singh et al. 2022).

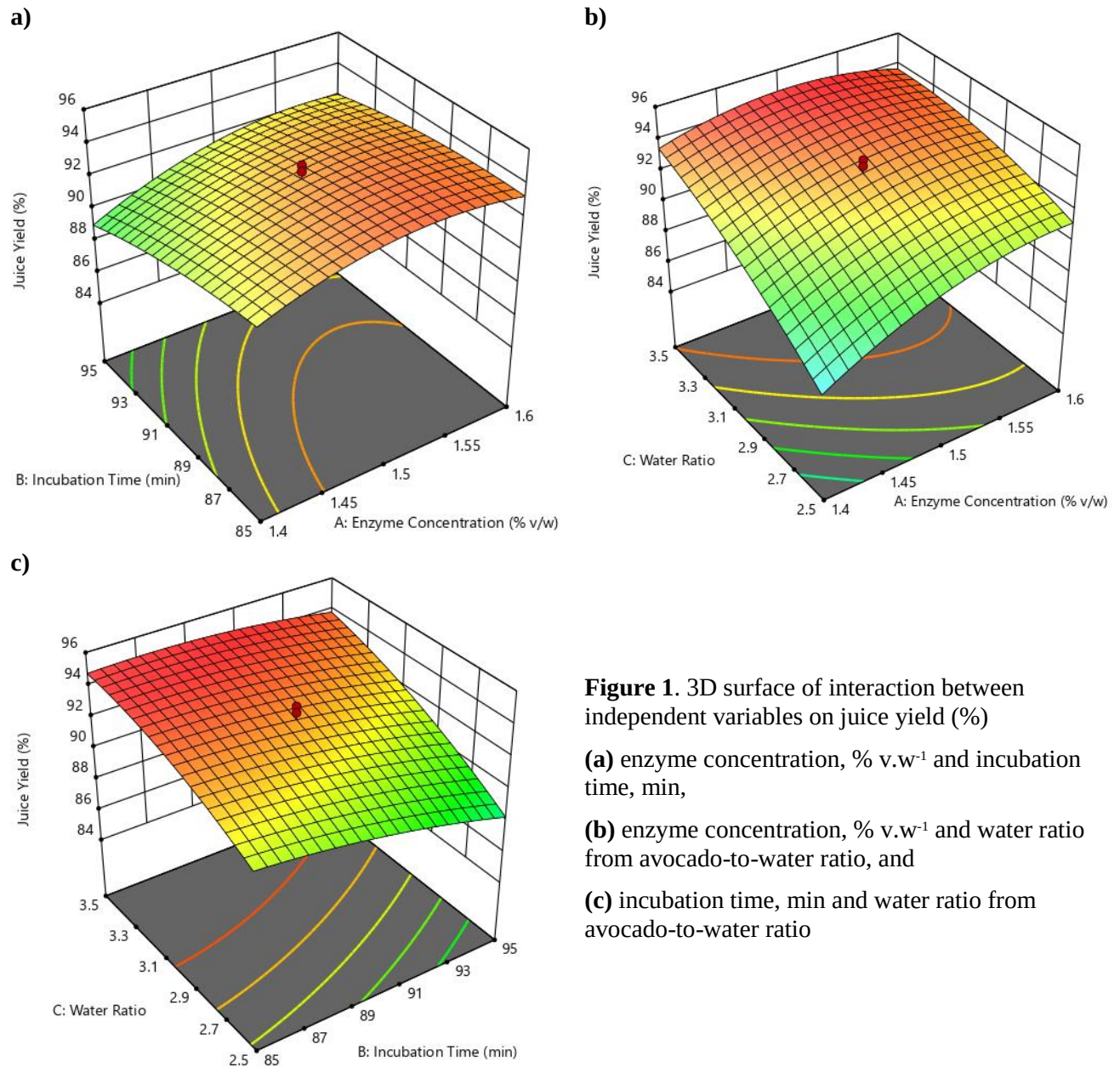


Figure 1. 3D surface of interaction between independent variables on juice yield (%)

(a) enzyme concentration, % v.w⁻¹ and incubation time, min,

(b) enzyme concentration, % v.w⁻¹ and water ratio from avocado-to-water ratio, and

(c) incubation time, min and water ratio from avocado-to-water ratio

Fig. 1 illustrates the interaction of two independent variables simultaneously, with the third variable fixed at the center point. It was found that the interaction between enzyme concentration and avocado pulp-to-water ratio significantly influences juice yield. It is due to the combined effect of both variables, which reduces the response. Therefore, it showed the importance of balancing both experimental values for optimisation. It should be noted that the main changes in avocado juice yield were highly attributed from their individual factors rather than its combined effects. In quadratic terms, enzyme concentration and avocado pulp-to-water ratio negatively affected the

quadratic term of juice yield. This finding shows that the increase of juice yield was initially due to the increase of enzyme concentration and avocado pulp-to-water ratio, individually, to a certain optimal level before it started to show a decline trend beyond its limits. These quadratic terms reveal the parabolic effect of both independent variables, suggesting that beyond a certain point, further increases in enzyme concentration and avocado pulp-to-water ratio may not significantly enhance the yield and could potentially lead to a decrease in yield due to enzyme inhibition or over-treatment (Guo 2024; Tapre and Jain 2014).

Table 3. Regression coefficients for each response

Regression coefficient	Juice yield (Y ₁)				Antioxidant activity (Y ₂)				Total phenolic content (Y ₃)			
	Coefficient Estimate	Std. Error	F-value	p-value	Coefficient Estimate	Std. Error	F-value	p-value	Coefficient Estimate	Std. Error	F-value	p-value
Intercept (β_0)	93.10	0.4474	-	-	81.80	0.1819	-	-	56.06	0.4896	-	-
Linear term												
β_1	1.14*	0.2101	29.24	0.0010	-0.89*	0.0854	107.52	<0.0001	1.21*	0.2299	27.61	0.0012
β_2	-1.0*	0.2101	22.46	0.0021	0.26*	0.0854	8.96	0.0201	3.07*	0.2299	178.47	<0.0001
β_3	2.22*	0.2101	111.46	<0.0001	2.13*	0.0854	624.09	<0.0001	-4.05*	0.2299	309.73	<0.0001
Interaction term												
$\beta_1\beta_2$	0.40	0.2745	2.13	0.1875	-0.57*	0.1116	26.42	0.0013	-0.94*	0.3004	9.84	0.0165
$\beta_1\beta_3$	-1.02*	0.2745	13.76	0.0076	-0.08	0.1116	0.4667	0.5165	-2.61*	0.3004	75.24	<0.0001
$\beta_2\beta_3$	0.53	0.2745	3.71	0.0956	-0.19	0.1116	2.86	0.1347	0.54	0.3004	3.18	0.1177
Quadratic term												
β_1^2	-1.12*	0.2312	23.46	0.0019	-0.29*	0.0940	9.19	0.0191	-1.82*	0.2531	51.80	0.0002
β_2^2	-0.40	0.2312	3.01	0.1263	-0.21	0.0940	4.86	0.0633	-1.73*	0.2531	46.76	0.0002
β_3^2	-0.64*	0.2312	7.76	0.0271	-0.16	0.0940	3.01	0.1263	-0.64*	0.2531	6.35	0.0398

*Level of significance at $p < 0.05$ (β_1 : regression coefficient of X_1 ; β_2 : regression coefficient of X_2 ; β_3 : regression coefficient of X_3).

Intercept was not subjected to any statistical significance testing.

Student's t-test was reported as F-values in Design Expert (RSM) where $F = t^2$

Table 4. Analysis of variance (ANOVA) for response surface quadratic model of juice yield, antioxidant activity, and total phenolic content

Source	df	Juice Yield			Antioxidant Activity			Total Phenolic Content		
		Sum of Squares	Mean Square	F-value	Sum of Squares	Mean Square	F-value	Sum of Squares	Mean Square	F-value
Model	9	125.52	13.95	23.14	77.92	8.66	86.86	491.51	54.61	75.64
Residual	7	4.22	0.60		0.6977	0.0997		5.05	0.7220	
Lack of Fit	5	2.11	0.42	0.40	0.6668	0.1334	8.64	4.89	0.9788	12.22
Pure Error	2	2.11	1.06		0.0309	0.0154		0.1602	0.0801	
Cor Total	16	129.74			78.62			496.56		
R ²		0.9675			0.9911			0.9898		
Adj. R ²		0.9257			0.9797			0.9767		
Pred. R ²		0.8391			0.9340			0.9242		
Adeq. R ²		17.4167			29.6467			26.9507		

Effect of processing conditions on antioxidant activity. The linear terms for enzyme concentration and avocado pulp-to-water ratio showed a significant ($p < 0.05$) effect on antioxidant activity as shown in Table 3. The increase in the avocado pulp-to-water ratio was also increased the antioxidant activity of the juice. A study on apple pomace by Candrawinata et al. (2014) demonstrated that a higher water-to-fruit ratio significantly increases the antioxidant compounds in the solids, which was helped by the water extraction of phenolic compounds. However, while the avocado pulp-to-water ratio ($\beta_3 = 2.13$, $p < 0.05$) shows a positive linear relationship with antioxidant activity, enzyme concentration, on the other hand, shows a negative linear relationship ($\beta_1 = -0.89$, $p < 0.05$). This indicates that as enzyme concentration increases, the antioxidant activity of ETAJ decreases, possibly because the high enzyme concentration slows down the reaction kinetics of antioxidant activity by limiting the free movement of ions (Wang et al. 2017).

The effectiveness of enzymatic treatment in the DPPH assay depends on the enzyme concentration, given by the optimal concentration will enhance the antioxidant activity through maximising the extraction as well as its release in the juice without possible degradation (Wang et al. 2023). Wang et al. (2023) also reported a close association between changes in antioxidant capacity and antioxidant ingredient concentrations, suggesting the release of more bioactive compounds during pectin breakdown. Amobonye et al. (2022) reported a similar negative trend in enzyme concentrations on the antioxidant activity of fruit juice. Regardless of this, the use of enzymes in producing avocado juice still results in higher antioxidant activity than the control sample.

On the other hand, a longer incubation time allows for a more efficient interaction between the enzyme and the substrate to break down the complex matrix of cell walls. This facilitates the release of additional phenolic compounds that can contribute to antioxidant activity. These observations are

similar with studies conducted by Neifar et al. (2011) for pomegranate juice and Sagu et al. (2014) for banana juice. Addition of water also assists the rapid release of antioxidants, attributed from the increase surface contact area for the enzyme activity interaction with the raw samples (Candrawinata et al. 2014).

$$Y = 81.80 - 0.8859X_1 + 0.2557X_2 + 2.13X_3 - 0.2851X_1^2 - 0.2073X_2^2 - 0.1631X_3^2 - 0.5738X_1X_2 - 0.0762X_1X_3 - 0.1888X_2X_3 \quad (5)$$

The interaction between the two parameters is shown on Fig. 2. All interactions showed a negative effect on the antioxidant activity with the interaction between enzyme concentration and incubation time having a significant ($p < 0.05$) effect on the response. For the quadratic terms, only enzyme concentration ($\beta_1^2 = -0.29$) had a significantly negative effect on the antioxidant activity, indicating a parabolic relationship in which, at a certain enzyme concentration, the antioxidant activity no longer shows any changes. Meanwhile, the quadratic terms for incubation time ($\beta_2^2 = -0.21$) and avocado pulp-to-water ratio ($\beta_3^2 = -0.16$) were insignificant, suggesting no substantial parabolic effects on the antioxidant activity of the avocado juice.

Effect of processing conditions on total phenolic content. The effect of three independent variables (enzyme concentration, incubation time, and avocado pulp-to-water ratio) on the total phenolic content was found to be significant ($p < 0.05$). In linear terms, enzyme concentrations ($\beta_1 = 1.21$, $p < 0.05$) and incubation time ($\beta_2 = -3.07$) showed a positive relationship with total phenolic content, where an increase in these variables led to an increase in total phenolic content. The breakdown of pectin releases more phenolic compounds into the juice, thus increasing its concentration. It is due to the solubility of phenolic compounds during the maceration of fruit mash (Oszmiański et al. 2009). However, the avocado pulp-to-water ratio exhibited a negative linear relationship with total phenolic content, indicating that total phenolic content decreases as this variable increases. Consequently, choosing a suitable water ratio was found to be crucial for maximising phenolic extraction, as excessive dilution can eventually hinder the

extraction efficiency and solubility of the compounds (Candrawinata et al. 2014).

As reported by Ribas-Agustí et al. (2018), interactions between phenolic compounds and polysaccharides have a significant impact on the bioaccessibility and bioavailability of phenolic compounds. During juicing, some phenolic compounds might remain bound to the insoluble pulp fraction, resulting in low phenolic yields. The use of commercial pectinase primarily targets the α -1,4 galacturonan backbone of the pectin and its side chains. The breakdown of both the pectin wall and its side chains facilitates the release of bound phenolics into the juice phase (Rocchetti et al. 2022). In practice, proper incubation time ensures an efficient enzymatic treatment. Ultimately, Ngadze et al. (2018) showed that the increase in phenolic content in fruit juice was attributed to the enzymatic treatment compared with the untreated control sample. Without the help of enzymatic treatment, phenolics are often remain entrapped and are eliminated within the pulp.

$$Y = 56.06 + 1.21X_1 + 3.07X_2 - 4.05X_3 - 1.82X_1^2 - 1.73X_2^2 - 0.6379X_3^2 - 0.9423X_1X_2 - 2.61X_1X_3 + 0.5358X_2X_3 \quad (5)$$

Based on the interaction between two independent variables, the total phenolic content obtained indicates that the enzyme concentration-incubation time interaction exhibits a statistically significant relationship towards the response, while the interaction of incubation time and avocado pulp-to-water ratio does not. A negative interaction was found in the first two interactions of the variables, where the increase in both enzyme concentration-incubation time interaction, incubation time, and avocado pulp-to-water ratio decreased the phenolic content in the ETAJ. Nevertheless, the quadratic terms of each independent variables ($\beta_1^2 = -1.82$, $\beta_2^2 = -1.73$, $\beta_3^2 = -0.64$) showed a significant negative coefficient of the compound. Thus, these results signify an optimal condition for each variable, leading to the desired high total phenolic content. An interesting finding was also noted on the negative interaction effect of enzyme concentration and incubation time on both the antioxidant activity and the total phenolic content of ETAJ.

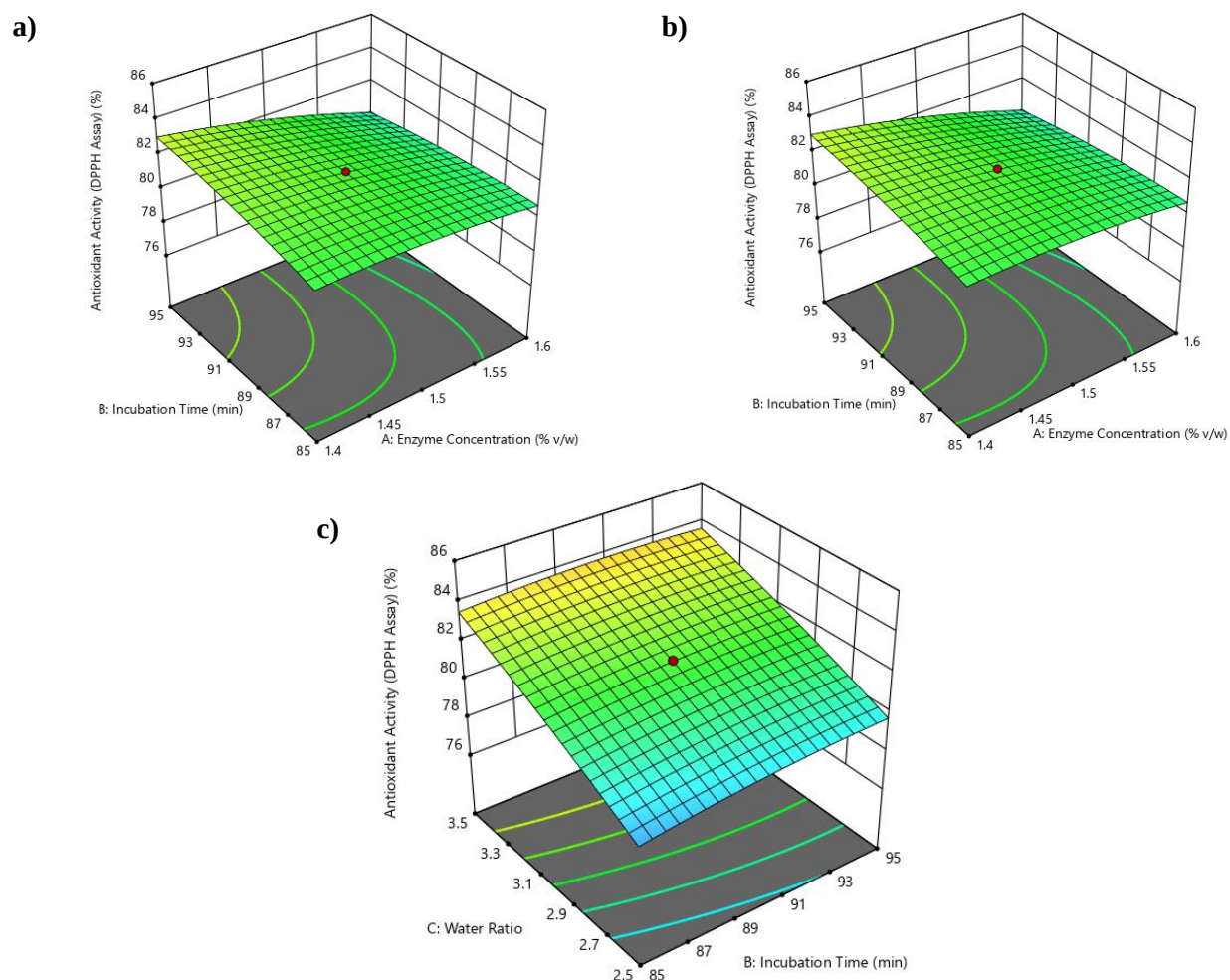


Figure 2. 3D surface of interaction between independent variables on antioxidant activity (%) (a) enzyme concentration, % v.w⁻¹ and incubation time, min, (b) enzyme concentration, % v.w⁻¹ and water ratio from avocado-to-water ratio, and (c) incubation time, min and water ratio from avocado-to-water ratio

This outcome indicates that the paired parameters are not synergistic in increasing these responses. On the other hand, enzyme concentration and incubation time, individually promote better release of phenolics which results in the enhancement of antioxidant capacity of ETAJ. Studies by Wang et al. (2023) demonstrated that high enzyme concentration and prolonged incubation time may lead to over hydrolysis of beneficial molecules that later reduces the phenolic release and, hence, also affects the antioxidant capacity. In this regard, the use of enzymes to destabilise the integrity of the fruit cell walls was needed to reduce interactions

that could potentially hinder the bioavailability of phenolics, thereby increasing the extractability of the compounds (Ribas-Agustí et al. 2018). In addition, a longer incubation time would provide more opportunities for enzymes to hydrolyse the pectin and other structural polysaccharides. Converting the phenolics bound into free forms will increase their availability for extraction. With sufficient incubation time, the enzymatic reactions can reach equilibrium, thereby improving the extraction of phenolics before they start to degrade (Radziejewska-Kubzdela, 2023).

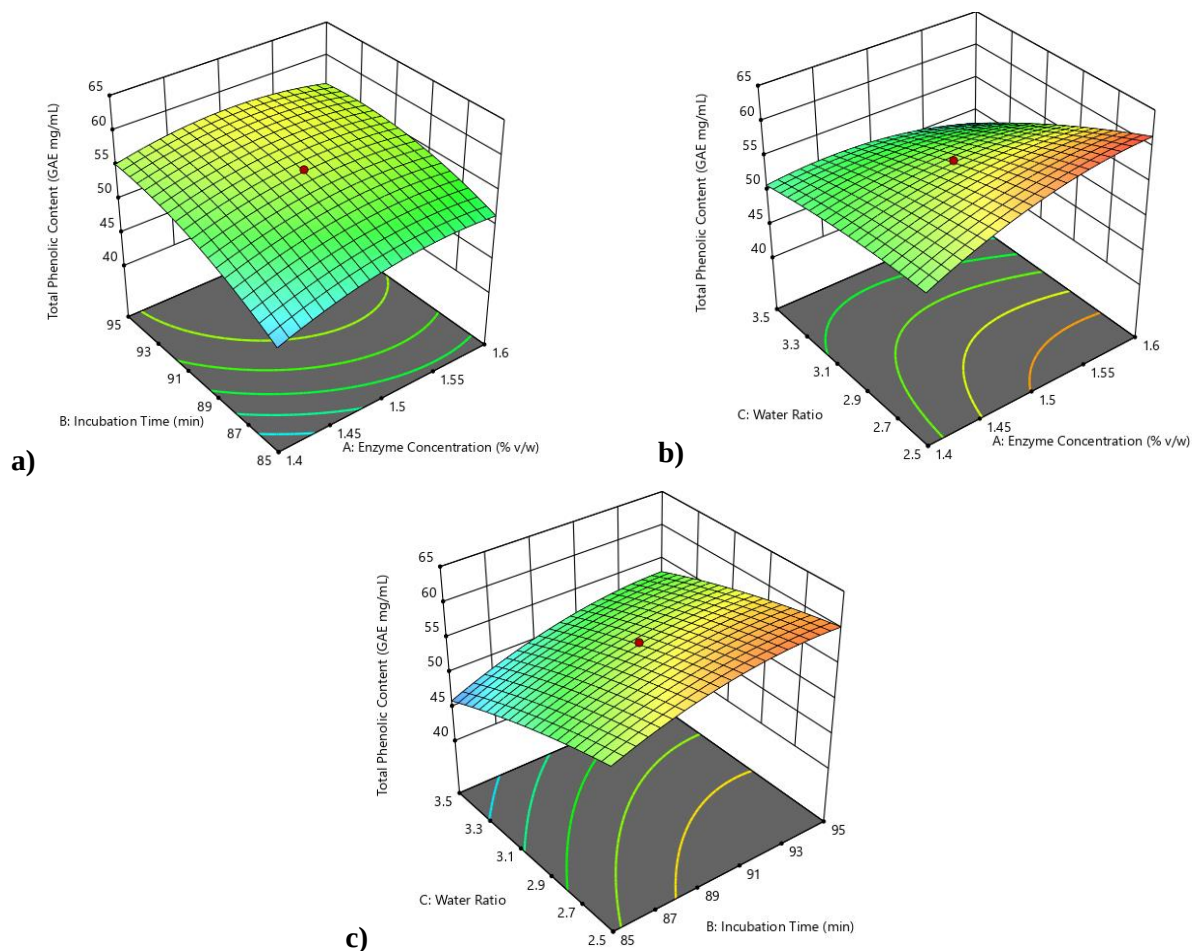


Figure 3. 3D surface of interaction between independent variables on total phenolic content, mg GAE.mL⁻¹ (a) enzyme concentration, % v.w⁻¹ and incubation time, min, (b) enzyme concentration, % v.w⁻¹ and water ratio from avocado-to-water ratio, and (c) incubation time, min

Optimised conditions of enzymatic treatment of avocado juice. A numerical optimisation method was employed to determine the optimal values of variables (enzyme concentration, incubation time and avocado pulp-to-water ratio) for maximising

juice yield, antioxidant activity and total phenolic content. Each response variable (juice yield, antioxidant activity, and total phenolic content) was assigned a goal of “3” based on its importance in the experiment, as detailed in Table 5.

Table 5. Constraints for optimised conditions and responses

Variables	Goal	Lower limit	Upper limit	Importance
Enzyme concentration, % v.w ⁻¹	In range	1.40	1.60	3
Incubation time, min	In range	85.00	95.00	3
Liquid ratio	In range	2.50	3.50	3
Juice yield, %	Maximise	84.61	94.48	3
Antioxidant activity, %	Maximise	77.80	85.29	3
TPC, mg GAE.mL ⁻¹	Maximise	44.41	61.94	3

The results of the numerical optimisation indicated that optimising the avocado juice with an enzyme concentration of 1.45 % v. w⁻¹, an incubation time of 94 min, and an avocado pulp-to-water ratio of 1:3.5, would lead to the highest desirability of 0.750. Under these optimal conditions, the experimental values for juice yield, antioxidant activity, and total phenolic content were 93.47 %, 84.38 %, and 53.79 mg GAE. mL⁻¹, respectively

(Table 5). The enzyme treatment of avocado juice increased by 46.85 %, 25.97 %, and 31.22 mg GAE. mL⁻¹ for juice yield, antioxidant activity, and total phenolic content, respectively, as shown in Table 6. The experimental values obtained were closely matched with the predicted values for the responses, with differences of less than 10 %, suggesting that the model is valid (Swanson 2015).

Table 6. Experimental validation of predicted responses and comparison with control avocado juice at an optimal condition

Responses	Predicted value	Experimental value	Control	Error Difference	Percentage Increase
Juice yield, %	93.47	91.23±1.04	44.38±0.69	2.40	46.85
Antioxidant activity, %	84.38	87.14±1.34	61.17±0.59	3.17	25.97
Total phenolic content, mg GAE.mL ⁻¹	53.79	54.81±0.85	23.59±1.48	1.86	31.22

Control; untreated sample. Values of experimental samples and control are indicated as mean ± SD.

From Table 6, it clearly shows the significant influence of enzymatic treatment of avocado juice towards its juice yield, antioxidant activity, and total phenolic content. The use of pectinolytic enzymes such as pectinase, disrupted the cell wall matrix and significantly benefited the final outcome of this juice production process. Enzymatic processes help hydrolyse polysaccharide structures that hold the water-binding capacity and bound phenolic compounds, releasing them into the juice phase (Phing and Kalam 2023). Although this research does not focus on the lipid yield as one of its responses, studies also show that enzymatic maceration of high-fat fruits in the juicing process significantly modifies the lipid structures. Pectinase does not directly hydrolyse lipids but in fact the treatment may disrupt the cellular structure to release the lipid trapped within. According to Qin and Zhong (2016), the avocado mesocarp, which in our case was used in the production of avocado juice, contains triacylglycerol that were kept within the oil-body inclusions. These inclusions are encompassed by cells walls that are rich in pectin, cellulose, and phospholipids membrane. The use of pectinolytic enzyme helps hydrolyse the cell wall and oligosaccharide chains, releasing the oil body

into the juice phase, hence, leading to more effective lipid release (Vovk et al. 2023).

Conclusions

A central composite design was employed for statistical analysis using a response surface methodology software (Design Expert). The study revealed that enzymatic treatment has significantly improved the juice yield, antioxidant activity, and total phenolic content in avocado juice, with enzyme concentration, incubation time, and the avocado pulp-to-water ratio being the critical factors of the experiment. The optimal enzymatic conditions identified include a higher enzyme concentration (1.45 % v.w⁻¹), a shorter incubation time (94 min), and a higher avocado pulp-to-water ratio (1 : 3.5), achieving a desirability function of 0.750 for avocado juice. Using these optimal conditions, the experimental values obtained for avocado juice yield, antioxidant activity, and total phenolic content were 91.23 %, 87.14 %, and 54.81 mg GAE.mL⁻¹, respectively. These numbers are slightly higher than its predicted values generated by the experimental design, with 93.47 % of juice yield, 84.38 % of antioxidant activity, and 53.79

GAE.mL⁻¹ of total phenolic content. The findings in this paper provide valuable insights, especially in the industrial and commercial aspects of avocado juice production. In industrial and commercial, the use of enzymatic treatment can be integrated into the already existing method of fruit juice production, improving the efficiency of process, reducing the operational cost, enhancing the overall quality, as well as minimising waste produced. With the increase of efficiency, both cost and labor can be reduced, resulting in a more cost-effective procedure. Further studies are needed to focus on the browning and shelf-life of the optimised enzyme-treated avocado juice.

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Author Contributions

Conceptualization, H.M.H., S.F.M.A., and M.A.A.; methodology, H.M.H., and S.F.M.A.; analysis H.M.H.; investigation, H.M.H., A.H.A.; resources, S.F.M.A., M.A.A., A.A.P., A.A.J.; data curation, H.M.H., S.F.M.A., and M.A.A.; writing-original draft preparation, H.M.H.; writing, H.M.H.; review and editing, H.M.H., S.F.M.A., and M.A.A.; visualization, S.F.M.A.; supervision, S.F.M.A., and M.A.A. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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