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# Prevention of Enzymatic Browning by Chemical Treatment on *Etlingera elatior* Puree Processing

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# Prevention of Enzymatic Browning by Chemical Treatment on *Etlingera elatior* Puree Processing

**Nor Aini Fatihah Mohamed Anuar, Faridah Kormin, Nurul Alyani Zainol Abidin, Fadzelly Abu Bakar, Siti Fatimah Zahrah Mohd Fuzi, Fatimah Sabran**

Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia

**Abstract.** Simplex-centroid mixture design has been applied to investigate the effectiveness of chemical formulation prior to prevent enzymatic browning on *Etlingera elatior* puree as well as to study its antioxidant properties and consumer acceptance on the product by using a 9-point hedonic scale analysis. Formulation consists of four different food grade chemicals (Ascorbic acid, Ethylenediamine tetraacetic acid (EDTA), Citric acid, and Benzoic acid). Spectrophotometric was used to assay the polyphenol oxidase enzyme (PPO) activity, by measuring the initial rate of quinone formation, as indicated by increase in absorbance value. Antioxidant properties of puree samples were measured through scavenging activity (DPPH), total phenolic content and total flavonoid content. Total color different (ΔE) of processed *Etlingera Elatior* were determined by Hunter L*a*b*. Optimization suggested a chemical formulation containing 50% ascorbic acid and 50% citric acid as the best proportion prior to its ability in enzyme inhibition, antioxidant characteristics and consumer acceptance.

**Keywords:** Simplex-centroid mixture design, food grade chemical formulation, *Etlingera elatior*; polyphenol oxidase

## 1. Introduction

*Etlingera elatior* is a culinary herb, locally known as torch ginger flower which can be processed into puree to prolong its shelf-life [1]. The young flower shoot of *Etlingera elatior* was reported to have antimicrobial, cytotoxic and anti-tumor promoting properties [2]. Browning is one of the most important problems occurred in agriculture products during processing and storage. Previous study by Tripathi and Variyar (2015) and He and Luo, (2007) reported that browning in fresh-cut vegetables mainly involves metabolism of phenolic compound into their oxidized products that can be minimized through application of food grade chemical treatment [3, 4]. According to Food Law and Regulation 1985 (1995), no specification on puree products however, puree can contain permitted nutrients, food conditioners, flavoring, and coloring substances with permitted doses level [5, 6].

Improper processing and storage of *Etlingera elatior* puree reduced overall product quality and marketing value. Browning results in loss of visual appeal and can adversely affect nutritional and sensory properties as well as overall quality expectation of final products [3]. It is crucial to determine the best treatment for the most accepted formulation in terms as the best proportion for its ability in enzyme inhibition, antioxidant properties and consumer acceptance. In this study, the main aim is to

determine the best treatment formulation of permitted food grade chemicals on *Etlingera elatior* puree processing method to minimize the effect of enzymatic browning.

## 2. Materials and Methods

### *2.1. Puree preparation*

Fresh raw material was purchased from the supplier in Batu Pahat, Johor. *Etlingera elatior* used in this study were purchased from one of the biggest torch ginger flower suppliers in Malaysia which was in Bindu, Batu Pahat, Johor. Chemicals used were food grade. Fresh harvested torch ginger flowers were cut and immerged in chemical solution with various formulations for 30 minutes under controlled condition. Treatment solution were then removed, and sample was finely grinded into puree. Grinded puree was then cooked under 72°C, for 15 minutes and packed into sterilized glass jar. This puree was then used for this experiment.

### *2.2. Simplex-centroid mixture design*

Simplex-centroid mixture design was used to formulate and optimise design mixture of selected 4 food grade chemicals to inhibit enzymatic browning in torch ginger flower puree. Twenty different formulations are designed in mixture of four chemicals. Which were used to treat torch ginger flower puree. The independent factors are proportions of different components of blend. The factors of interest are ascorbic acid, EDTA, citric acid and benzoic acid. Design-Expert® 10.0 Software from State-Ease Inc. was employed for experimental design, data analysis and model building [7]. Mixture design of the experiment was shown in Table 1.

**Table 1.** Food grade formulation by Simplex-centroid mixture design and its effect on PPO activity, Color difference and antioxidant property of *Etlingera Elatior* puree

| Std run no | F 1 Ascorbic acid (ppm) | F 2 EDTA (ppm) | F 3 Citric acid (ppm) | F 4 Benzoic acid (ppm) | R 1 PPO Activity (EU/min/ml) | R 2 Color Different | R 3 DPPH (% scavenging activity) | R 4 TPC (ug/ml) | R 5 TFC (ug/ml) | R 6 Sensory |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 600 | 0 | 0 | 0 | 0.00067 | 12.69 | 33.51 | 17.52 | 483.13 | 6.85 |
| 2 | 0 | 600 | 0 | 0 | 0.00170 | 17.61 | 29.60 | 9.97 | 453.53 | 3.85 |
| 3 | 0 | 0 | 600 | 0 | 0.00130 | 14.71 | 32.31 | 9.82 | 303.00 | 5.56 |
| 4 | 0 | 0 | 0 | 600 | 0.00130 | 19.17 | 33.65 | 4.87 | 578.33 | 5.30 |
| 5 | 300 | 300 | 0 | 0 | 0.00170 | 17.38 | 35.40 | 9.40 | 347.27 | 4.58 |
| 6 | 300 | 0 | 300 | 0 | 0.00033 | 12.95 | 35.14 | 10.97 | 343.00 | 5.23 |
| 7 | 300 | 0 | 0 | 300 | 0.00067 | 13.43 | 32.57 | 10.28 | 322.73 | 5.59 |
| 8 | 0 | 300 | 300 | 0 | 0.00033 | 15.60 | 25.93 | 10.43 | 249.67 | 4.37 |
| 9 | 0 | 300 | 0 | 300 | -0.00230 | 15.91 | 33.97 | 12.21 | 305.27 | 5.75 |
| 10 | 0 | 0 | 300 | 300 | -0.00033 | 14.61 | 10.48 | 11.29 | 359.13 | 7.58 |
| 11 | 300 | 100 | 100 | 100 | -0.00033 | 12.73 | 34.56 | 13.72 | 406.47 | 6.21 |
| 12 | 100 | 300 | 100 | 100 | 0.00300 | 14.74 | 31.30 | 9.74 | 338.87 | 7.33 |
| 13 | 100 | 100 | 300 | 100 | -0.00030 | 14.26 | 31.52 | 12.89 | 370.73 | 6.25 |
| 14 | 100 | 100 | 100 | 300 | 0.00033 | 16.57 | 42.02 | 12.01 | 310.60 | 5.53 |
| 15 | 150 | 150 | 150 | 150 | 0.00033 | 17.81 | 40.85 | 9.39 | 293.53 | 5.49 |
| 16 | 600 | 0 | 0 | 0 | 0.00067 | 17.48 | 34.67 | 12.47 | 378.33 | 5.60 |
| 17 | 0 | 600 | 0 | 0 | 0.00130 | 20.30 | 29.74 | 7.69 | 290.73 | 3.85 |
| 18 | 0 | 0 | 600 | 0 | 0.00200 | 13.42 | 32.45 | 8.53 | 294.87 | 5.56 |
| 19 | 0 | 0 | 0 | 600 | 0.00230 | 19.44 | 33.80 | 8.38 | 471.53 | 5.30 |
| 20 | 300 | 300 | 0 | 0 | 0.00067 | 15.68 | 35.55 | 13.05 | 267.67 | 4.58 |
| Fresh | - | - | - | - | 0.00390 | - | 90.17 | 30.96 | 463.80 | 7.33 |

* F is factor or independent variable; R is response or dependent variable.
* PPO unit (EU/min/ml); DPPH unit (% scavenging activity); TPC unit (ug/mL); TFC unit (ug/mL).

*2.3. Preparation of crude enzyme extract*
Crude enzyme extraction was done followed method of Manohan and Wai, (2012) with slight modification. The suspension was centrifuged at 6000 rpm for 30 minutes at 4°C. The supernatant was used as crude PPO extract as well as for PPO calibration curve [8].

*2.4. Polyphenol oxidase enzyme (PPO) assay*
Polyphenol oxidase (PPO) activity was determined spectrophotometrically as described by Manohan and Wai, (2012). Initial rate of o-quinone formation were measured by increased of absorbance value at 30ºC, 410 nm in 15 seconds intervals with catechol as the substrate. PPO activity were determined by reaction mixture of 0.1ml freshly prepared crude enzyme extract, 3.9ml of 100mM phosphate buffer (pH 7.0) and 0.1ml of 50mM catechol. PPO activity were done in triplicate and the results expressed as means. The initial velocity was calculated from the slope of the absorbance versus time curve. One unit (U) of PPO activity was defined as the amount of the enzyme that increased the absorbance by 0.001 minute$^{-1}$ under the favorable assay conditions [8, 9].

*2.5. Antioxidant properties assay*

*2.5.1. DPPH radical scavenging activity* The ability of each formulation constituents to scavenge the stable DPPH free radical was estimated according to procedure described by Miliauskas *et al*., (2004) with slightly modified method of Brand-Williams, Cuvelier and Berset, (1995). The experiment was done in triplicate. Ascorbic acid was used as standard and DPPH percentage free radical scavenging activity was measured followed equation 1 [10, 11].

$$\text{Scavenging activity (\%)} = \left(1 - \frac{(A_{sample} - A_{empty})}{A_{control}}\right) \times 100 \quad (1)$$

*2.5.2. Total phenolics content (TPC)* TPC of puree sample was determined by Folin-Ciocalteu colorimetric asay by Miliauskas *et al*., (2004) with slight modifications. 100 µl of sample were mixed with 2 ml of sodium carbonate (2g in 100ml distilled water), left for 2 minutes at room temperature. Folin-Ciocalteus reagent were added and left for another 30 minutes before absorbance value were taken at 750 nm. The experiment was done in triplicate and standard calibration curve was plotted [10].

*2.5.3. Total flavonoids content (TFC)* TFC were determine followed method of Shehata *et al*., (2014) with slight modification. 1 ml of sample was mixed with 1ml of 2% aluminium trichloride before incubated for 15 minutes. The absorbance was taken at 430 nm and experiment were done in triplicates. Rutin was used as standard and TFC value were calculated by compared the absorbance value against the standard [12].

*2.6. Physical analysis*
Total soluble solids (TSS) have been estimated by refractometer (Atago, Tokyo, Japan). Moisture analyzer was used to identify the moisture content of processed puree. Heating temperature and weight of samples used was standardized into 140°C and 1g respectively. Aqua Lab model water activity meter used to determine puree sample water activity. The pH analyzer was used to determine the puree pH value. Colour spectrophotometer (Hunter Lab 4500L) was used to analyze the L* (light/dark), a* (red/green) and b* (blue/yellow) values of puree sample. Equation below used to determine the total color difference between all three coordinates (ΔE*).

$$\Delta E^* = [(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2]1/2 \quad (2)$$

*2.7. Sensory evaluation*

Evaluation had been done to 25 trained panelists to identify their demand on color, taste and overall acceptance of the puree through simple hedonic test. The panelists evaluated the puree colour by ranking it according to their most preferred puree by ranking. Ranking scale is from 1 (less preferred) until 9 (most preferred) [13, 14].

*2.8. Calculation and statistics*

The statistical analysis was performed using Design-Expert® 10.0 Software from State-Ease Inc. where values are expressed as ANOVA. The statistical significance of each model was determined by variance analysis (ANOVA) at 5%. The smaller the magnitude of P-value, the more significant is the corresponding coefficient [15].

## 3. Results and Discussion

*3.1. PPO enzyme assay*

From table 1, it shows that each treatment gave different effects against the enzymatic reaction. For single chemical as variable, ascorbic acid showed the best enzymatic inhibition effect with 0.00067 EU/min/ml, followed by citric acid (0.0013 EU/minl/ml), benzoic acid (0.0013), and EDTA (0.0017 EU/min/ml). However, combination of more than one chemical gave better enzymatic inhibition effects. Combination of ascorbic acid and citric acid (300ppm + 300ppm) give the lowest enzyme activity and effective treatment formulation to inhibit enzymatic browning compared to the treatment with ascorbic acid only with 0.00033 EU/min/ml.

Table 2 is the ANOVA analysis of PPO assay showing significant results of $\alpha<0.0001$. Lack of fit model is not significant, thus is desirable as we want a fit model. The $R^2$ value is 0.9416, reasonably close to 1, which is acceptable to indicate the good interactions between the variables.

*3.2. Color analysis*

Colour change closely related to samples enzymatic browning due to o-quinone and melanin compound formation of the processed puree. High formation in yellow colour may be caused by the highest formation of o-quinone and melanin, products of polyphenolase activity. However, changes colour to green indicated reduction in pinkish colour of the puree. Some samples detected with highest color difference as the yellow tone presence was highest. In term of treatment solution, benzoic acid need buffer solution to be active and maximize their function in treatment solution, meanwhile ascorbic acid and citric acid have been added together as these two natural food additives act as buffer solution that provide lower pH in treatment solution and triggers benzoic acid activation.

ANOVA analysis from the color study (Table 2) shows there is significance in results $\alpha<0.0001$. The lack of fit for this model is non-significant thus desirable as we want a model that fits. From the ANOVA analysis, the $R^2$ value calculated is 0.4725, reasonably close to 0.5, which is acceptable to indicate the moderate good interactions between the variables.

*3.3. Relationship between DPPH, TPC, TFC and enzymatic browning.*

Based on table 1, processed *E. elatior* showed reduction in TPC value compared to fresh one due to pasteurization process. Nurhuda *et al*., (2013) found that boiling process above 95°C would destroyed the antioxidant components of vegetables. Since pasteurization temperature used was lower than 95°C, TPC would not be destroyed completely but value would be slightly lesser as compared to the fresh's value. However, phenolic compound act as substrate for enzymatic pathway, therefore, lesser compound was good as it retarded further browning reaction [16].

The ANOVA analysis (Table 2) of antioxidant assay for DPPH, TPC, and TFC shows significance in results with $\alpha<0.0001$. As for DPPH, the lack of fit for this model is non-significant thus desirable as we want a model that fits. The $R^2$ value calculated is 0.9681, reasonably close to 1, which is acceptable to indicate the good interactions between the variables. For TPC, the lack of fit for this model is non-

significant thus desirable as we want a model that fits and the $R^2$ value calculated is 0.4390. As for TFC, the lack of fit for this model is non-significant thus desirable as we want a model that fits and the R2 value calculated is 0.3802, reasonably close to 0.5. For both TPC and TFC, the $R^2$ value were closed to 0.5 and acceptable to indicate the moderate good interactions between the variables studied.

*3.4. Sensory analysis*

Combination of ascorbic acid and citric acid showed only moderate consumer acceptance during sensory analysis. This combination has been selected among the best formulation according to its potential benefits based on the rest three factors which are enzymatic activity, color different, and the antioxidant activity.

**Table 2.** Statistical analysis (ANOVA) of PPO Activity, colour different, and antioxidant properties (DPPH, TPC, and TFC), and sensory evaluation

| Response variables | Sum of square | Degree of freedom | Mean square | F value | Prob. | |
|---|---|---|---|---|---|---|
| **PPO Activity-Special cubic** | | | | | | |
| Regression | $2.373\times10^{-5}$ | 13 | $1.825\times10^{-6}$ | 7.44 | 0.0108 | significant |
| Residual | $1.473\times10^{-6}$ | 6 | $2.455\times10^{-7}$ | | | |
| Lack of fit | $1.174\times10^{-6}$ | 1 | $1.174\times10^{-6}$ | 0.43 | 0.5396 | not significant |
| Pure error | $1.355\times10^{-6}$ | 5 | $2.711\times10^{-7}$ | | | |
| Cor total | $2.520\times10^{-5}$ | 19 | | | | |
| $R^2$ | | | | | | 0.9416 |
| Adj $R^2$ | | | | | | 0.8149 |
| Adeq precisior | | | | | | 12.582 |
| **Color-Linear** | | | | | | |
| Regression | 48.55 | 3 | 16.18 | 4.78 | 0.0146 | significant |
| Residual | 54.19 | 16 | 3.39 | | | |
| Lack of fit | 36.79 | 11 | 3.34 | | 0.9600 | not significant |
| Pure error | 17.40 | 5 | 3.48 | | | |
| Cor total | 102.74 | 19 | | | | |
| $R^2$ | | | | | | 0.4725 |
| Adj $R^2$ | | | | | | 0.3736 |
| Adeq precisior | | | | | | 6.0110 |
| **Response variables** | **Sum of square** | **Degree of freedom** | **Mean square** | **F value** | **Prob.** | |
| **DPPH-Special cubic** | | | | | | |
| Regression | 722.81 | 13 | 55.60 | 14 | 0.0020 | significant |
| Residual | 23.84 | 6 | 3.97 | | | |
| Lack of fit | 23.12 | 1 | 23.12 | < 0.0001 | 0.2320 | significant |
| Pure error | 0.71 | 5 | 0.14 | | | |
| Cor total | 746.65 | 19 | | | | |
| $R^2$ | | | | | | 0.9681 |
| Adj $R^2$ | | | | | | 0.8989 |
| Adeq precisior | | | | | | 19.6490 |
| **TPC-Linear** | | | | | | |
| Regression | 58.17 | 3 | 19.39 | 4.17 | 0.0232 | significant |
| Residual | 74.34 | 16 | 4.65 | | | |
| Lack of fit | 45.33 | 11 | 4.12 | 0.71 | 0.7050 | not significant |
| Pure error | 29.00 | 5 | 5.80 | | | |
| Cor total | 132.51 | 19 | | | | |
| $R^2$ | | | | | | 0.4390 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Adj $R^2$ | | | | | | 0.3338 |
| Adeq precisior | | | | | | 6.5370 |
| **TFC-Linear** | | | | | | |
| Regression | 50070.80 | 3 | 16690.27 | 3.27 | 0.0486 | significant |
| Residual | 81633.74 | 16 | 5102.11 | | | |
| Lack of fit | 53986.05 | 11 | 4907.82 | 0.89 | 0.5984 | not significant |
| Pure error | 27647.69 | 5 | 5529.54 | | | |
| Cor total | $1.317\times10^5$ | 19 | | | | |
| $R^2$ | | | | | | 0.3802 |
| Adj $R^2$ | | | | | | 0.2640 |
| Adeq precisior | | | | | | 5.6900 |
| **Sensory-Special cubic** | | | | | | |
| Regression | 17.81 | 13 | 1.37 | 5.85 | 0.0199 | significant |
| Residual | 1.41 | 6 | 0.23 | | | |
| Lack of fit | 0.62 | 1 | 0.62 | 3.99 | 0.1021 | not significant |
| Pure error | 0.78 | 5 | 0.16 | | | |
| Cor total | 19.21 | 19 | | | | |
| $R^2$ | | | | | | 0.9269 |
| Adj $R^2$ | | | | | | 0.7684 |
| Adeq precisior | | | | | | 9.2330 |

## 4. Conclusion

As a conclusion, unwanted enzymatic browning during processing and storage of *E. elatior* puree can be minimized through pre-treatment of food grade chemicals formulation. Combination of ascorbic acid (300ppm) and citric acid (300ppm) showed the lowest color different, moderately high in DPPH, moderately high in antioxidant activity (TPC and TFC). It can be suggested that combination of ascorbic acid and citric acid, or combination of both acids with EDTA and benzoic acid under controlled ratio would enhance the inhibition of polyphenol oxidase activity.

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