Optimization of temperature and pasteurization time of soursop juice (Annona muricata) by response surface methodology in pilot scale

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Abstract. Interest in soursop and its derivatives has increased over time, with many scientific articles reporting its health benefits. Several recent studies reported the presence of bioactive compounds and phytochemicals from soursop juice. However, climatic fruit tends to have different post-harvest handling than others. A series of processes from harvesting to extraction played an important role in its final product. The thermal inactivation of the PPO enzyme, which causes brownish color, can be used to improve the quality of soursop juice. This process can be carried out using the MTLT (Mild Temperature Long Time) pasteurization process using a customized double jacket heater by considering the thermostress properties of bioactive compounds in soursop juice. This study aims to determine the optimal formulation for process parameters also provide the optimal choice for the pasteurization process in pilot scale and database for the transition to industrial production. Response Surface Methodology (RSM) was a method used to optimize the process and formulation of soursop fruit juice. In this study, two factors were used, namely pasteurization temperature (56-80 °C) and heating time (5-15 min) obtained by previous research to determine the most optimal Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Color measurement, Total Dissolve Solid and Viscosity.

1. Introduction
As typical of other climatic fruit characteristics, soursop tends to have different post-harvest handling from fruit in general. A series of processes from harvesting to extraction can play an essential role in the final product having maximum benefits. One of the most common processes for making juice is the pasteurization process. This process aims to kill harmful organisms such as bacteria, molds, yeasts that exist in food ingredients. Although researchers have chosen a certain time/temperature profile to minimize the effects of some foods’ exposure to heat, this process is still a major challenge for the incipient soursop juice processing industry.

Response Surface Methodology (RSM) was a method used to optimize the process and formulation of soursop fruit juice. In the process of optimization and finding the best possible product from the ongoing batches, an experimental design called the central composite design (CCD) concept has emerged [1]. The CCD model is an integral part of response surface mythology. This type of optimization model's biggest advantage is that it is more accurate, and there is no need for a three-level factorial experiment for building a second-order quadratic model [2]. This study was aimed to determine the optimal formulation for process parameters also provide the optimal choice for the pasteurization
process in pilot scale and database for the transition to industrial production. Referring to pilot plant scale research conducted by Wijana [3], research on a pilot plant scale is used to reduce the risks associated with constructing large-scale processes. Wijana's study provides information on making quality baby java orange syrup using equipment in the pilot plant laboratory and determining the effect of processing on a pilot plant scale on the quality of baby java subgrade orange syrup. The pilot plant scale can be used as a reference for manufacturing a full-scale plant capable of producing in much larger capacity.

2. Material and methods

2.1. Preparation of Sample
Soursops were purchased in Blimbing, Malang. Selected fruits were ripened, fresh, unspoiled, and undamaged with a weight of around 6 to 7 kg. Soursops were washed with water, peeled, and get sed removed, then put the pulp into a slow juicer (Sharp Slow Juicer EJ-C20Y-RD). Soursop juice obtained was set Brix value according to SNI and was ready to be pasteurized.

2.2. Chemical
Gallic Acid reagent used as calibration standard was purchased at Nurra Gemilang, Malang, East Jawa Province, Indonesia. Folin-Ciocalteau (FCR) and Na₂CO₃ were sourced from PHY Edu, Malang. Other chemicals such as distilled water, methanol were obtained from Duta Jaya, Malang.

2.3. Experimental Design
We used Design Expert 13 software for optimization. To be specific, we used RSM, Central Composite Design (CCD), to optimize the extraction process of soursop juice by generating a set of experimental trials (14 runs). CCD is the most commonly used fractional factorial design used in the response surface model. This experiment was conducted using two variable factors (temperature and time) and 4 responses (TDS, Viscosity, TPC, and Color). The final set consists of 14 runs with 5 center points as shown below (Figure 1).

| Factor | Name             | Units | Type       | Minimum | Maximum       | Coded Low | Coded High | Mean  | Std. Dev. |
|--------|------------------|-------|------------|----------|---------------|-----------|------------|-------|-----------|
| A      | Temperature      | Celsius | Numeric   | 51.03    | 84.97 -1 → 56.00 | +1 → 80.00 | 68.00     | 9.41  |
| B      | Pasteurization Time | Minutes | Numeric   | 2.93     | 17.07 -1 → 5.00 | +1 → 15.00 | 10.00  | 3.92  |

Figure 1. Matrix of variables (Screenshot of Design Expert 13).

The least-squares multiple regression method is usually used to study the relationship between independent variables and dependent variables. According to the experimental data, the multiple regression equation can be used to fit the quadratic polynomial equation, as shown below.

\[ y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \]  \hspace{1cm} (1)

Where y represents the predicted response, \( \beta_0 \) is the intersection of the model and \( \beta_1, \beta_{11} \), \( \beta_{12} \) and \( \beta_{22} \) are the regression coefficients of the linear, quadratic and interaction effects of the model, respectively. \( X_1 \) and \( X_2 \) are factors [4].

2.4. Parameters determination

2.4.1. Total phenolic content. Determination of the total phenol content was carried out by the Folin-Ciocalteau method with Gallic acid as the standard according to Liu et al.[5]. The results were derived from the calibration curve (10-50 ppm) of gallic acid equivalents. The prepared sample will be reacted with 10% Folin-Ciocalteau reagent and 7.5% Sodium Carbonate (Na₂CO₃) reagent. Then the maximum
absorbance of the mixture was measured at 656.5 nm using a UV-VIS spectrophotometer. This reaction will produce a blue color complex which indicates the sample contains phenolic compounds. Measurement of total phenol content using a UV-Vis spectrophotometer (Shimadzu Type UV-1280).

2.4.2. Total flavonoid content. Flavonoid contents in selected plant extracts were determined using aluminium chloride in a colorimetric method according to Aryal et al. [6]. The results were derived from the calibration curve (10-50 ppm) and in equivalents of quercetin expressed. Then the maximum absorbance of the mixture was measured at 486.9 nm using a UV-VIS spectrophotometer. The sample solution reacted with 10% $\text{AlCl}_3$, 5% $\text{NaNO}_2$ and 0.5 mL distilled water, then incubated for 5 minutes and added 4 ml of NaOH and 2.4 ml of distilled water. Measurement of total flavonoid content using a UV-Vis spectrophotometer (Shimadzu Type UV-1280).

2.4.3. Color measurement. Color measurement was done with a color reader (FRU WF30-8) according to Hutching,1999 [7]. The principle of the color reader tool is the measurement of color differences through the reflection of light by the surface of the reading sample. Turn on the color reader by pressing the power button. Placing the lens on standard porcelain perpendicularly and pressing the “Target” button will display the value on the screen ($L^*$, $a^*$, $b^*$) which is the standardized value.

2.4.4. Total dissolved solid and viscosity. Measurement of total dissolved solids using a refractometer according to SNI 01-3546-2004 [8]. The total dissolved solids content of soursop juice was determined using a digital refractometer (Model: ABBE AMTAST WYA-2S), measured at 25 ºC and calibrated using distilled water, 1-2 samples were inserted into the refractometer prism, and the total dissolved solids content was expressed as °Brix. Viscosity measurements were carried out using Tuning Fork Vibro Viscometer SV-10 [9]. The sample was measured 45 ml, placed in the container provided, and then installed until the tuning fork dipped perfectly. The results were recorded as shown by the monitor with units of MPa s at 25 ºC.

2.5. Equipment
A double jacket pasteurizator was used for obtaining 14 samples of soursop juice. This equipment designed in Argo Jaya Manufaktur workshop.

![Figure 2. Double Jacket Pasteurizator. Impeller motor (1), temperature sensor (2), impeller (3), Water (4) and Control panel box (5).](image)

The outer jacket used in this study was made of 2.0 mm. iron plate with a heating chamber volume of 3 L. The double chamber was used to distribute heat evenly. The experimental schematic is shown in Figure 1, and the specification of the pasteurizator is presented in Table 1.
Table 1. Specifications of the tools and equipment.

| No | List      | Specification                  |
|----|-----------|--------------------------------|
| 1  | Volume    | 3 L                            |
| 2  | Size      | 10 × 54.5 cm                   |
| 3  | Iron Plate| 2.0 mm                         |
| 4  | Thermocouples | Thermocouple Type K         |
| 5  | Temperature range | 30-99 °C              |
| 6  | Impeller type | HE3 Impeller              |
| 7  | Heat Source | 1500 Watts of electric power  |

3. Results and discussion

3.1. Experimental design

Optimal conditions were performed using the curved response methodology (RSM) (9). The central design combined for two independent variables was applied. The independent variables of different temperature levels (A) and pasteurization time (B) affected the release of the total phenol content (TPC), Total Flavonoid Content (TFC), color parameter, viscosity and Total Dissolve Solid. Figure 3 shows the coding of experimental design and the actual level. In this study, a total of 14 experiments were based on two levels of experimental design.

![Figure 3. Factors and levels for RSM (Screenshot of Design Expert 13).](image-url)

The model's compatibility is expressed through the value of the correlation coefficient R2. As shown in Table 2, the calculated highest and lowest R2 coefficient was 0.94 and 0.6562, showing that 94% and 65% of experimental data is compatible with predicted data by model. Seng et al. [10] suggested that R2 values of more than 0.75 were relatively adequate for prediction purposes. So we need further research for color measurement parameters to find out the error by doing data retrieval. Furthermore, the model also showed adequate precision with the adequate precision (AP) value of 5.61-14.35. The adequate precision value is used to direct the design space. If greater than 4.0, this model can be used. A coefficient of variation is a statistical measure of the dispersion of the data point around the mean. CV value (%) of the polyphenol content and color measurement was 1.86-11.52%, so the dispersion of TPC data was quite far from prediction.
Table 2. Compatibility of model.

| Coefficients | Total Phenolic Content (TPC) | Flavonoid | Lightness (*L) | %Brix | Viscosity |
|--------------|------------------------------|-----------|----------------|--------|-----------|
| $\beta_0$    | -0.996371                   | -0.347631 | -1.92348       | +9.75723 | +465.36049 |
| $\beta_1$    | +0.048417                   | +0.017500 | +1.20472       | +0.014657 | +2.17106  |
| $\beta_2$    | +0.041132                   | +0.062463 | +1.09404       | +0.011036 | -1.15637  |
| $\beta_{12}$ | -0.000185                   | -0.000562 | -0.017167      | -0.007510 | +0.007642 |
| $\beta_{11}$ | -0.000351                   | -0.00092  | -0.007510      | +0.007642 | -1.15637  |
| $\beta_{22}$ | -0.001378                   | -0.001084 | +0.007642      | +0.007642 | -1.15637  |
| Lack of fit (P-value) | 0.2877 (Ns) | 0.4275(Ns) | 0.2018(Ns) | 0.2921(Ns) | 0.5642 (Ns) |

Coefficients of variation (CV %) 1.95 2.52 1.86 0.9185 2.87

Adequate Precision (AP) 14.3562 9.8567 5.6149 12.4772 9.5407

R² 0.9462 0.9101 0.7539 0.7319 0.6562

b0, constant; b1, linear coefficient of temperature; b2, linear coefficient of pasteurization time, b12, interaction, b11, quadratic coefficient of temperature, b22, quadratic coefficient of pasteurization time; Ns = Not Significant

3.2. Total Phenolic Content (TPC)

The total phenolic content in soursop conducted in 14 samples can be seen in Figure 2. The variables observed were the temperature and the length of pasteurization time that significantly affected the TPC ($P<0.05$).

$$Y_i = -70.86121 + 2.04804A +2.13216B -0.007075AB -0.014206A^2 -0.075898B^2$$ (2)

The influence of various factors on the content of polyphenols is represented by a three-dimensional surface response graph in Figure 4: the red area indicates the highest polyphenol content (0.849701 mg GAE/g sample), and the blue area indicates the lowest result (0.671054 mg GAE/g sample).

Figure 4. Interaction 3D diagram of TPC.

Increasing temperature increases the total phenol, but a higher temperature causes a lower number of TPC. The low TPC value can be caused by phenol's characteristic, which is easily decomposed by light and high temperature. On the other hand, temperature changes can also increase cell wall permeability to maximize the phenol extraction process. According to Yamaki [11], the author also showed a similar result. The polyphenol compound is an antioxidant, so it is very susceptible to
decomposition under light and temperature conditions because it is a heat-sensitive substance. Therefore, heat is one of the factors that directly affect the decomposition of phenolic compounds. Just as Gonçalves et al. [9] studied the blanching conditions that affect the total polyphenol content in carrots, the author found that the polyphenol content increases when the blanching temperature increases are completely reduced.

3.3. Total flavonoid content (TFC)
Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables, and certain beverages [13]. The total phenolic content in soursop conducted in 14 samples can be seen in Figure 2. The variables observed were the temperature and the length of pasteurization time that significantly affected the TPC (P<0.05).

\[
Y_1 = -0.347631 + 0.017500A + 0.062463B - 0.000562AB - 0.000092A^2 - 0.001084B^2
\]  

(3)

The influence of various factors on the content of polyphenols is represented by a three-dimensional surface response graph in Figure 5: the red area indicates the highest flavonoid content, and the blue area indicates the lowest result.

![Figure 5. Interaction 3D diagram of TFC.](image)

It was observed that the effect of solvents on TFC was similar to that on TPC. The highest TFC was obtained 0.561709 mg QE/g sample, and the lowest was 0.468449 mg QE/g sample, similar to [14]. A similar trend was observed in the amount of TPC. As the pasteurization time or temperature increases, the TFC in the extract decrease with a slight increase at a certain combination of temperature and time. Effect of the temperature on total flavonoid explained by Guo et al. in [15] that high temperature leads to increased extraction efficiency since it accelerates active ingredient diffusion. Meanwhile, solvent viscosity and surface tension decreased with increasing temperature, contributing to sample (*Inula helenium*) wetting and matrix penetration. As soon as the extraction temperature exceeds 60°C, the extraction performance decreases slightly.

3.4. Color (Lightness)
The color characteristic is an important indicator of quality; it reflects the sensory appeal and quality of the juice produced during the pasteurization process. Also, there is a significant relationship between the temperature and the lightness of the juice since the model did show significant effects on quadratic regressions.

\[
Y_2 = -1.92348 + 1.20472A + 1.09404B - 0.017167AB - 0.007510A^2 + 0.007642B^2
\]  

(4)
The influence of various factors on the color (lightness) is represented by a three-dimensional surface response graph in Figure 6.

![Figure 6. Interaction 3D diagram of Lightness (*L).](image)

The color change at high temperatures may be due to the sensitivity of the Polyphenol oxidase (PPO) enzyme to heat. Another reason may be the non-enzymatic browning of the monosaccharides present in the juice, according to [10]. Another paper stated that the reducing sugars present in peach puree, mainly glucose and fructose, participate directly in the non-enzymatic browning reaction. Some disaccharides, such as sucrose, are also hydrolyzed during heat treatment, leading to glucose and fructose formation. Therefore, the evolution of the sugar content can be used as an indicator of non-enzymatic browning changes, and some authors have carried out studies [11].

3.5. **Total dissolved solid and viscosity**
TDS Viscosity was significantly increased with higher temperature, as observed in Figure 5. This is in accordance with the research of Permatasari et al. [18] which states that the total dissolved solids of material are influenced by the presence of temperature and heating time. This is due to the breaking of chemical bonds in carbohydrates to produce sugars that have high hygroscopic properties.

![Figure 7. Interaction 3D diagram of (a) TDS and (b) Viscosity.](image)

Figure 7 shows that temperature and time affect the results of fluid viscosity. Based on research conducted with Perawati et al. [19] the viscosity of calamansi orange marmalade increases when the temperature and heating time are higher. Evaporation occurs when the water phase becomes a gas phase during an increase in temperature resulting in a product with a higher viscosity.

3.6. **Optimization of mixing parameters**
The desired product characteristics were considered as follows: high total phenolic content (TPC), high Total Flavonoid Content (TFC), in range Total Dissolved Solid (TDS), in range Viscosity, and high
value of color (lightness). The desirability function was used for numerical optimization to determine the optimum pasteurization process conditions with desirable response goals (Figure 8). As a result, the predicted optimum temperature and pasteurization time were 63.826°C and 12.884 minutes, respectively. To check the adequacy of the response surface model, three experiments were carried out under the recommended optimal conditions. Compare the experimental value with the predicted value. Furthermore, theoretical verification of the experimental data and the fitted data using a two-sample t-test showed that there is no significant difference between these values (P>0.05).

![Figure 8. Response surface plots showing the effects temperature and pasteurization time on the overall response desirability of soursop juice.](image)

The optimal properties of soursop juice obtained from this experimental study were 0.814 of TPC, 0.558 of TFC, 45.622 of TDS 589.032 of viscosity and 10.835 of lightness with the value of desirability of 0.837. Overall, the result for this study for TPC parameter indicated good quality and high number of regression but still need improvement for color measurement (lightness) parameter with a low value of regression. Also the verification process need to be done to ensure that the data obtained were accurate.

4. Conclusions
The results of the study on the interaction of temperature and time in the pasteurization of soursop juice shown that the model is suitable for test data with high correlation coefficients. At a temperature of 63.826°C and 12.884 minutes, the maximum content of TPC, TFC, TDS, Viscosity and lightness are 11.7 mg GAE / g, 0.558 mg QE/g, 10.835%brix, 589.032 MPa s, and 45.4 * L. The significant interaction between these parameters was considered on a pilot scale. Research can be used as data for expansion of the scale of production. However, this study needs more research to reduce the number of noises especially for lightness parameter.

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