Optimization of Pomelo juice *Citrus maxima* (Burm.Merr.) Vacuum Concentration by Response Surface Methodology in Pilot scale

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**Abstract.** Because of its healthful nutritional properties, pomelo is gaining traction in the food and beverage industry. Pomelo juice concentration as a beverage provides vitamins, antioxidants, and energy. The Design Expert 11 calculation software is used to optimize the concentration process parameters, the temperature range is determined to include ± α values converted as axial. The results showed a positive correlation between the two factor variables and the obtained target function (TPC), the optimal value for the selected procedure was based on 79 °C with a time of more than 1.78 hours. At this point, TPC retention is 71.121%.

1. **Introduction**

The natural compounds are concerned, leading to the development of food processing technology to serve the needs and health benefits of consumers and solve the problems of supply and demand in the market [1], [2]. Pomelo is a fruit tree of the citrus family, with scientific name *Citrus maxima* (Burm.Merr.) belonging to the Citrus group in the Rutaceae family. Originated in Southeast Asia (most in Thailand and Malaysia) [3]. Health-promoting compounds, typically total polyphenol content (TPC), zeaxanthin, β-cryptoxanthin, and lycopene are mentioned in the pomelo pulp [4]. Vacuum concentrating is used to increase the retention of these nutritional compounds without processing. However, publication in pilot sale on pomelo juice concentration (PJC) is still limited.

At the same time, the RSM method optimally handles the parameters affecting the concentrate process according to Central composite design (CCD) were selected. In the past, this method has been published extensively in studies of extracting essential oils and anthocyanins in plants [5]–[9], show reliability and effectiveness. The result obtained from the algorithm processing of this method is the optimal constant variable for the response function. Therefore, the goal of the research is to interact with concentration temperature (temp) and time in retening TPC for pomelo juice using RSM to make the optimal choice for the vacuum concentrate process pilot scale, to provide a database for the transition to industrial production.
2. Materials and method

2.1. Preparations of sample
Pomelo were purchased in Ben Tre province, Vietnam. Selected fruits were ripened, fresh, unspoiled, and undamaged with the weight of around 1 to 2 kg. Fruits were pre-treated by washing with water. After pre-treatment, the peel and seed were removed. The pulp was squeezed with equipment (model MJ-68MWRA, Panasonic, Malaysia) in 1 minute. Pomelo juice is adjusted to Brix of 20 from 60°Brix sugar syrup up three litters.

2.2. Chemicals
The 60°Brix syrup is prepared from making crystal sugar purchased from Bien Hoa Sugar Company, Dong Nai Province, Vietnam. Folin-Ciocalteu (FCR), Gallic Acid reagent was purchased at Sigma-Aldrich and Chemie, Co Ltd (USA). Other chemicals such as distilled water (pH from 6.5 to 8), methanol (purity 99.5%), Na2CO3 (purity 99.5%), NaHCO3 (purity 99.5%) were sourced from China.

2.3. Experimental design
Response surface methodology, in conjunction with CCD, was employed to optimize the extraction process by generating a set of experimental trials. A calculation of experimental trials and optimum yield was performed using Design Expert 11. A central composite design approach was adopted incorporating two variables factor (time and temperature concentration) and one response (TPC). The final set consists of 13 with 5 center points as shown in Table-1.

Table 1. Matrix for variables

| Code | Independent factors         | Units | Level |
|------|-----------------------------|-------|-------|
| A    | Concentration temperature   | °C    | -1    |
|      |                             |       | 0     |
|      |                             |       | +1    |
| B    | Concentration time          | min   | 105   |
|      |                             |       | 80    |
|      |                             |       | 85    |

2.4. Determin of TPC
The treatment sample is then filtered through Whatman No.1 paper and determine TPC by the Folin–Ciocalteu method (Waterhouse, 2002 [10], adjusted by Silva et al [11]. Extracts (100µl-dilution ratio 1:4) were mixed with 500µl of Folin–Ciocalteu reagent, 400µl of 7.5% (w/v) sodium carbonate solution. Absorbance at 760nm was measured after 1h, using a spectrophotometer. Results were expressed as gram of gallic acid retention in sample (%).

2.5. Equipments
The equipment was designed in Gold Quality CO.,Ltd.

Figure 1. Single cycle vacuum Concentrator (1) Feed valve; (2) Tank; (3) Stirring motor; (4) Cooling system; (5) Condenser water tank; (6) Vacuum pump; (7) Thermostatic tank; (8) Control panel; (9) Exhaust valve.
2.6. Statistical analysis
Each experiment was triplicated. MS software (Microsoft Inc., Redmond, WA, USA) software support
and average calculation. Combined ANOVA processing by Design-Expert statistical software version
11 (DE11). The optimal concentrate parameter of pomelo juice predicted with significance level below
5%.

3. Results and discussion

3.1. Experimental design
Two main factors affecting the concentration process of pomelo juice have been determined from the
single-factor experiment results, namely concentration temperature and concentration time. Next, the
RSM surface response method is applied to optimize the total polyphenol content recovered from the
process. Based on the central complex design (CCD), the quadratic model representing the relationship
between the designed inputs with three levels as shown in Table 1.

The polyphenol content is closely dependent on the concentration temperature factor. Experimental
values from DE 11 design are presented in Table 2. Polyphenol content received is the most 72.134 (%) (std 1)
and the lowest 40.0111 (%) (std 6).

Table 2. Experimental design for 2 factors

| Std order | Runs order | A: Concentration Temperature (°C) | B: Concentration Time (min) | TPC (%) |
|-----------|------------|----------------------------------|----------------------------|---------|
| 1         | 8          | 75                               | 105                        | 72.1342 |
| 2         | 7          | 85                               | 105                        | 50.7863 |
| 3         | 5          | 75                               | 135                        | 57.5436 |
| 4         | 3          | 85                               | 135                        | 41.1324 |
| 5         | 12         | 75                               | 120                        | 60.3456 |
| 6         | 2          | 85                               | 120                        | 40.0111 |
| 7         | 1          | 80                               | 105                        | 71.0213 |
| 8         | 13         | 80                               | 135                        | 56.1876 |
| 9         | 4          | 80                               | 120                        | 70.8145 |
| 10        | 9          | 80                               | 120                        | 64.2367 |
| 11        | 11         | 80                               | 120                        | 72.1576 |
| 12        | 6          | 80                               | 120                        | 63.178 |
| 13        | 10         | 80                               | 120                        | 62.8376 |

To determine the importance of each coefficient, the value of F-value and "Prob.> F" (p-value) was
calculated through the ANOVA data processing software. Table 3 presents the ANOVA results of the
model with the statistical results of each factor.

Table 3. Analysis of variables

| Source     | Sum of Squares | df | Mean Square | F-value | p-value |
|------------|----------------|----|-------------|---------|---------|
| Model      | 1299.94        | 5  | 259.99      | 13.97   | 0.0016  | significant |
| A-Temperature | 562.48        | 1  | 562.48      | 30.21   | 0.0009  | significant |
| B-Time     | 254.52         | 1  | 254.52      | 13.67   | 0.0077  | significant |
| AB         | 6.09           | 1  | 6.09        | 0.3273  | 0.5852  | not significant |
| A²         | 430.05         | 1  | 430.05      | 23.10   | 0.0020  | significant |
| B²         | 2.48           | 1  | 2.48        | 0.1333  | 0.7259  | not significant |
| Residual   | 130.31         | 7  | 18.62       |         |         | not significant |
| Lack of Fit| 50.22          | 3  | 16.74       | 0.8361  | 0.5402  | not significant |
| Pure Error | 80.09          | 4  | 20.02       |         |         |                  |
| Cor Total  | 1430.25        | 12 |             |         |         |                  |

Significant p < 0.05, not significant p > 0.05
From Table 3 ANOVA, the F-value is 13.97 which shows the design model is statistically significant. Of which, with the p-value 0.0016, the model shows that only a 0.16% chance of the F-value can occur due to noise.

The p value <0.05 shows the factor has a statistically significant influence on the results of the model. If the model has many factors with p value > 10%, the accuracy can be reduced. When designing, it is necessary to remove the factors with p value > 10% to improve the statistical significance of the model. Specifically, variables A, B, and A2 have p value <5%, so in the process of concentration, changing these factors have a statistically significant effect on the obtained total Polyphenol content.

Table 4. Equation and regression analysis of modeling for mixing process

| Std. Dev | Mean  | C.V. % | R²    | Adjusted R² | Predicted R² | Adeq Precision |
|----------|-------|--------|-------|-------------|--------------|---------------|
| 4.31     | 60.18 | 7.17   | 0.9089| 0.8438      | 0.6715       | 11.5830       |

The model’s compatibility is expressed through the value of the correlation coefficient R2. As shown in Table 4, calculated R2 coefficient is 0.9089, showing that 90.89% of experimental data is compatible with predicted data by model. In addition, the model also demonstrated adequate precision with the adequate precision (AP) value of 11.5830. 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 in a data series against the mean. CV value (%) of the polyphenol content recovered during vacuum concentration in 13 experiments was 7.17% <10%, so the dispersion of this RSM result is acceptable.

In addition, a number of other factors are also used to evaluate whether the model is completely compatible with the experimental results based on experimental charts and predicted and actual value plots and plot of internally studentized Residuals versus the actual run. Comparing the actual viscosity and the predicted viscosity of the model is shown in Figure 2. The concentration values lie near or on the diagonal of the tissue (Figure 2.A), which means that the predicted level is high accurate. The non-display of the noise variable on the graph indicates that the predicted and actual viscosity results are consistent. The random distribution of viscosity values is shown in Figure 2.B. The dispersion points are almost equal in the upper and lower x-axis. This confirms the clarity of the model with the assumption of constant variance.

Figure 2. Estimation of Model Precision (A) Comparison between Actual Values and Predicted Values and (B) Plot of Internally Studentized Residuals versus the Actual Run

3.2. Optimization of mixing parameters

The relationship between the polyphenol content and the independent variables A, B from Design – Expert 11 software are indicated in the actual equation (Final Equation in Terms of Actual Factors).
This equation can be used to predict the total polyphenol content in the vacuum condensation process of grapefruit juice at the conditions A, B are determined:

\[
\text{TPC} (\%) = 65.51 - 9.68A - 6.51B + 1.23AB - 12.48A^2 + 0.9477B^2 \quad (1)
\]

Where A is the concentration temperature (°C), and B is the concentration time.

When the effect level has a positive value, the investigating factor and the polyphenol content correlate positively and vice versa. From the above equation, we have positive correlation factors with polyphenol content AB and B2. When increasing the value of the survey, the polyphenol content received increased. At the same time, when increasing values of temperature (A), time (B), and temperature squared (A2), the polyphenol content tends to decrease. Specifically, the range of influence values of the factors will be modeled in Figure 3.

![Figure 3. 2D (a) and 3D (b) interaction diagram of TPC](image)

The effect of factors on polyphenol content is depicted by a two-dimensional and three-dimensional surface response diagram: the red area denotes the highest polyphenol content, while the blue area indicates lower results.

Diagram (A) in Figure 2 shows the interaction between temperature and time on the ability to obtain polyphenols during pomelo juice concentration. Based on the variation of the color scale in the chart, when the values of these two factors are changed, the Polyphenol content is also changed. First, we consider the effect of concentration on polyphenol content. At the time of 105 minutes, when the temperature increases from 75 to 81°C, the polpheyenol content tends to be maximized, however, if the temperature increases, the content tends to decrease. Specifically, the maximum amount of polyphenol content 70 % and temperature up to 85°C is obtained, the polyphenol content remains 50 %. Similarly, at 75°C, time tends to tend to temperature. At 107 minutes, the retained polyphenol content was 70 %, if the maximum time was increased (135 minutes), the obtained content was 60 %. We see that when the temperature and time increase, the amount of recovered Polyphenol tends to decrease. Especially the larger temperature change interval, which means that, it affects the large amount of stored polyphenols.

The reason for this, Polyphenol compound is known as 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 affects the decomposition of phenolic compounds. As in the study of blanching conditions affecting total polyphenol content in carrots by Gonçalves et al. (2010) [12], the author obtained when blanching temperature increases, polyphenol content total reduction. In the research on the pasteurization and sterilization process of pineapple juice by Zheng (2011) [13] also showed similar results that the pasteurization temperature increased, along with the increase in storage time, the total polyphenol content reduction. Based on graph (A), total polyphenol
content is predicted to reach the highest recovery value when the concentration temperature is between 75 and 81°C and the time is between 105 and 108 minutes.

### 3.3. Model verification
The probability of obtaining the expected results from the recommended optimal points is 100% or "desirability" = 1.000. From the data shown in figure 2 and applying the Design expert 11 optimization software. The optimum score including temperature and time is listed in Table 4. According to the prediction model, the Polyphenol content of 73.73 (%) is obtained under the optimal conditions. However, to match the actual conditions, the model was verified with the corrected values of the following factors: 79°C was concentrated in 107 minutes. Compared with the model's prediction, the actual polyphenol content obtained is 71.121% with the error 3.3539%, the Polyphenol difference is calculated by the formula as percentage difference of predicted Polyphenol content and actually divided. With an error of less than 5% and an expected level (desirability) of 1.000, it proves that the condition of the model is consistent with the experimental values.

| Table 5. Polyphenol content was obtained at optimal conditions on Design expert 11 |
|---------------------------------|-----------------|-----------------|
| Temperature (°C) | Time (min) | Polyphenol content (%) |
| Predict | 78.65 | 107.215 | 73.73 |
| Run 1 | 79 | 107 | 71.142 |
| Run 2 | 79 | 107 | 68.786 |
| Run 3 | 79 | 107 | 73.436 |
| Medium | 79 | 107 | 71.121 |
| Desirability = 1.000 | Error = 3.539% |

Applying the RSM surface response method and ANOVA analysis of Design expert 11 software, the research has determined the optimal parameters of the vacuum condensing process of pomelo juice to recover the total polyphenols at a temperature of 79°C, time of 107 minutes with a polyphenol content of 71.121%.

### 4. Conclusion
From the results of the study on the interaction of temp and time on the TPC in pomelo juice, it shows the model's suitability for testing data with high correlation coefficients. The maximum amount of total polyphenol recovered was 71.121% at a concentration of 79°C for 107 minutes. Significant interactions between these parameters are considered at pilot scale. This study serves as the data for production scale ups. However, the limitation of this study is that it only focuses on one response. Instead, attention should be paid to the uniformity and scalability of the concentrating system, and at the same time the retention of typical pomelo juice values as ascorbic acid.

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