Formulation for a Soursop (Annona muricata L.) Nectar Supplement using Response Surface Methodology for Optimization of Food Thickener

Nhi Yen Thi Tran1,2,3*, Dao Tan Phat1,2, Van Thinh Pham1,2,3, Nguyen Nhan Quyen1, Huynh Ngoc Thanh Tam4, Tran Thanh Truc5,

1Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
2NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
3Graduate University of Science and Technology, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
4Faculty of Environmental and Food Engineering, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
5Biotechnology Research and Development Institute, Can Tho University, Can Tho City, Vietnam
6College of Agriculture, Can Tho University, Can Tho City, Vietnam

*Corresponding author: ttynhi@ntt.edu.vn, tttruc@ctu.edu.vn

Abstract. Soursop, due to its nutritional properties, is gaining attention in the food and beverage industry. Soursop nectar, as a beverage, provides vitamins, antioxidants, and energy. Studies on how additives could alter sensory properties of the product might satisfy the needs of consumers for products with better visual qualities. The mixing of Pectin (PT), carboxymethyl cellulose (CMC), and xanthan gum (XG) directly affects the viscosity in the nectar. Optimization by response surface methodology showed that PT-CMC and XG-CMC interactions were significant at <0.05. The coefficient of determination of the model was high (R² = 0.9847). The lowest viscosity of the soursop nectar was 70 mPa.s, achieved by following contents of additives: 0.01378% of PT, 0.0071963% of CMC and 0.0051443% of XG.

1. Introduction
Natural compounds have been the interest of food processing technology to manufacture products with better health benefits and visual attractions [1-14]. Soursop (Annona muricata L.) is a plant that confers various health benefits such as anti-cancer, arthritis prevention and liver support. Soursop is also known for its abundance in antioxidants. The plant is native to the Americas and the Caribbean, with the genus Annona, of the Annonaceae family [15-17]. Other functionalities of soursop include enhancement of skin elasticity, hair health and reduction of symptoms of rheumatism by providing vitamin C and magnesium. Soursop extract has been shown to contain active compounds such as serotonin that naturally present in the human brain. As a result, fruit processing of soursop fruit has been getting attention and attempted, prompting soursop juice and jam production activities. In a previous report, a soursop yogurt formulation has been developed, containing 10-15% of soursop nectar added to soursop.
yogurts blend [18]. However, in large scale manufacture food products, especially juice, the durability of the product is crucial as the deposition of particles and their distribution position in the fluid system are uneven and less stable than in the fruit material [19]. Often times, the addition of structural stabilizers might resolve the issue. In particular, Pectin (PT), Carbonxymethyl cellulose (CMC), and Xanthan Gum (XG) are common stabilizers that have been conventionally used in the food industry to alter physical properties and rheology of the nectar products. For apple juice, CMC and XG have been demonstrated to be effective stabilizing agent [20]. In a previous study, XG has been shown to be able to keep zinc in the diet [21]. For orange juice, XG also may act as a stabilizing agent when being used at the concentration of 0.02-0.14%. Pectin is also another agent that was shown to be influential in determining viscosity of mango nectar under ultrasound [22]. These properties are mostly due to the presence of a large number of hydroxyl groups (-OH), which significantly enhance affinity for water molecules to be linked and form hydrophilic groups.

In this study, we determined optimal parameters for mixing additives in a soursop nectar formulation. A response surface methodology (RSM) procedure was employed in combination with a Central composite design (CCD) to establish the response function. In the past, this method has been applied extensively in studies involving extraction of essential oils and anthocyanins from plants [23-27], addition of colloidal additives in cloudy green asparagus juice [28], and optimal ultrasound conditions affecting polyphenol oxidase in soursop [29]. Viscosity of the soursop nectar was selected as the desired outcome for optimization. The results are expected to aid in developing soursop related consumer products.

2. Materials and method

2.1. Preparations of sample
Soursop leaves are purchased in Tan Phu Dong area, Tien Giang province, Vietnam. Fruit was intact and unripe. After pretreatment, shell, seed and core of the fruit were removed. The pulp was pulverized with water at the ratio of 1:4 using a grinder (model SHD5322, Sunhouse) for 1 minute. Soursop puree is adjusted to Brix of 12 using 60°Brix sugar syrup. Additives were then added and assimilated using ultrasound instrument (GT22227QTS model, GuangDong GT Ultrasonic Co., Ltd, China) with a continuous wave design of 15 minutes.

2.2. Chemicals
The 60°Brix syrup was purchased from Bien Hoa Sugar Company, Dong Nai Province, Vietnam. Pectin and Carboxymethyl cellulose and Xanthan Gum were purchased from Unionchem (Unionchem, Co. Ltd, China).

2.3. Experimental design
To optimize the addition of additives, first a set of experimental trials was generated by using CCD in the Design Expert 11 software. Three variables, which are content of PT, CMC, and XG were selected as independent variable and viscosity was selected as the response. The set consisting parameters for 20 experiments was shown as in Table 1 and Table 2. Of which 8 experiments were factorial, 6 experiments were axial points and 6 experiments were center points.

| Code | Independent factors          | Units | Level  |
|------|------------------------------|-------|--------|
| A    | Pectin                       | %     | -α     | -1    | 0     | +1    | +α    |
| B    | Carboxymethyl cellulose      | %     | 0.0159104 | 0.05  | 0.1   | 0.15  | 0.18409   |
| C    | Xanthan Gum                  | %     | 0.0163641 | 0.03  | 0.05  | 0.07  | 0.0836359 |
Table 2. Experimental design for 3 factors

| Std. order | Runs order | A  | B  | C  |
|------------|------------|----|----|----|
| 1          | 18         | 0.05 | 0.03 | 0.03 |
| 2          | 8          | 0.15 | 0.03 | 0.03 |
| 3          | 10         | 0.05 | 0.07 | 0.03 |
| 4          | 3          | 0.15 | 0.07 | 0.03 |
| 5          | 20         | 0.05 | 0.03 | 0.07 |
| 6          | 9          | 0.15 | 0.03 | 0.07 |
| 7          | 11         | 0.05 | 0.07 | 0.07 |
| 8          | 12         | 0.15 | 0.07 | 0.07 |
| 9          | 15         | 0.0159104 | 0.05 | 0.05 |
| 10         | 6          | 0.18409 | 0.05 | 0.05 |
| 11         | 14         | 0.1 | 0.0163641 | 0.05 |
| 12         | 17         | 0.1 | 0.0836359 | 0.05 |
| 13         | 5          | 0.1 | 0.05 | 0.0163641 |
| 14         | 1          | 0.1 | 0.05 | 0.0836359 |
| 15         | 7          | 0.1 | 0.05 | 0.05 |
| 16         | 19         | 0.1 | 0.05 | 0.05 |
| 17         | 4          | 0.1 | 0.05 | 0.05 |
| 18         | 16         | 0.1 | 0.05 | 0.05 |
| 19         | 13         | 0.1 | 0.05 | 0.05 |
| 20         | 2          | 0.1 | 0.05 | 0.05 |

2.4. Viscosity measurement
Viscosity was measured according to Ibrahim et al. (2011) [20]. To be specific, the mixture was placed in a 250ml beaker and thermally cooled by a bath at 28°C. An NDJ-THER 5S viscometer is used with a rotation speed of 60 rpm. All sample was measured with identical time and rotation speed. The result was expressed in the form of mPa.s

2.5. Statistical analysis
Each experiment was performed in triplicate. Microsoft Excel software (Microsoft Inc., Redmond, WA, USA) was used to input data and perform average calculation. ANOVA was carried out by Design-Expert statistical software version 11 (DE11). The optimal concentration parameter of additive in sour sop nectar mixture is predicted with significance level below 5%.

3. Results and discussion
The viscosity results in the predicted model are shown in Table 3. In particular, the prediction model has a correlation coefficient R²=0.9847 indicating compatibility with experimental data. Estimation results showed that the Predicted R² was 0.8831, which is lower than the Adjusted R² of 0.9709. However, Adeq Precision, which measures the signal to noise ratio, is 31.967, which is greater than 4. This indicates an adequate signal, suggesting that this model can be used to navigate the design space. From the established equation, a response could be calculated from any given set of independent variables. In addition, magnitude of coefficients also represents relative impact of corresponding factors. Clearly, all three additives showed significant effects for the colloidal system in the nectar mixture (p<0.05). On the other hand, the second order interaction with positive coefficients of 41.71 and 13.44 indicate that pectin concentration exert greatest impact on viscosity, followed by CMC and XG.
Table 3. Equation and regression analysis of modeling for mixing process

\[
\text{Vis(mPa.s)} = 69.95 + 26.51A + 23.97B + 13.23C + 8.95AB + 4.02AC + 15.82BC + 41.71A^2 + 13.44B^2 - 5.51C^2
\]

| Std. Dev | Mean | C.V. % | R²    | Adjusted R² | Predicted R² | Adeq Precision |
|----------|------|--------|-------|-------------|--------------|----------------|
| 8.87     | 103.85 | 8.54   | 0.9847 | 0.9709      | 0.8831       | 31.9667        |

Significance of interactions terms signifies that the colloidal system is a mixture of solutions having viscosity linearly proportional to the concentration of additives. Potential antagonistic interactions when mixing between PT and XG (p > 0.05) are shown in Table 4. Most likely, the homogeneous and disordering effect of ultrasound to the large structure branch of xanthan gum causes a decrease in viscosity [30].

Table 4. Analysis of variables

| Source | Sum of Squares | df | Mean Square | F-value | p-value | Adeq Precision |
|--------|----------------|----|-------------|---------|---------|----------------|
| Model  | 50570.80       | 9  | 5618.98     | 71.46   | <0.0001 | significant    |
| A-PT   | 9599.00        | 1  | 9599.00     | 122.08  | <0.0001 | significant    |
| B-CMC  | 7846.72        | 1  | 7846.72     | 99.79   | <0.0001 | significant    |
| C-XG   | 2388.73        | 1  | 2388.73     | 30.38   | 0.0003  | significant    |
| AB     | 640.22         | 1  | 640.22      | 8.14    | 0.0171  | significant    |
| AC     | 129.12         | 1  | 129.12      | 1.64    | 0.2289  |                |
| BC     | 2003.23        | 1  | 2003.23     | 25.48   | 0.0005  | significant    |
| A²     | 25088.52       | 1  | 25088.52    | 319.07  | <0.0001 | significant    |
| B²     | 2603.75        | 1  | 2603.75     | 33.11   | 0.0002  | significant    |
| C²     | 438.11         | 1  | 438.11      | 5.57    | 0.0399  | significant    |
| Residual | 786.31       | 10 | 78.63       |         |         |                |
| Lack of Fit | 782.50     | 5  | 156.50     | 205.38  | <0.0001 |                |
| Pure Error | 3.81         | 5  | 0.7620     |         |         |                |
| Cor Total | 51357.11     | 19 |            |         |         |                |

High significant p < 0.0001, significant p < 0.05, not significant p > 0.05

Comparison between the actual viscosity and the predicted viscosity of the model is shown in Figure 1. The concentration values were situated nearby or on the 45 degree line of the plot (Figure 1.A), suggesting that the predicted level is highly accurate. The absence 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 1.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.
Optimization of mixing parameters: Figure 2 showed the surface reaction of viscosity when changing the concentration of additives in soursop nectar. The results indicated that increasing PT concentration from 0.05-0.15% tended to cause viscosity to decrease slightly at low concentrations. Further increasing additives caused the viscosity to improve. The contour plots showed that the lowest viscosity was achieved at 0.1% PT, 0.05% CMC, and the α value of XG of 43.33 mPa.s. Potentially at low concentrations, the hydrocolloids whose focus is on the CMC (molecular structure consisting of both hydrophilic equatorial sides and a hydrophobic axial plane) have produced adsorption that reduces the surface tension between water and lipid phase in the mixture, resulting in a slight decrease in viscosity [31].

In Figure 2.B, the inverse interaction with the significant level (p> 0.05) between XG and PT was observed, indicating that the viscosity of the nectar is highly dependent on the increased PT concentration (0.05-0.15%). On the other hand, in the range of 0.05% < PT <0.125%, the viscosity level fluctuates slightly, and the changes eventually became negligible when the XG concentration increased from 0.03 to 0.7%. Interactions between XG and other additives were negative, suggesting that the addition of XG in the mixture should be considered. The interaction effects of variables on nectar viscosity could be explained through the linear correlation (p=0.0005) of XG and CMC in Figure 2C. When the concentration of both variables increased from 0.03-0.07%, the viscosity increased. The free movement of hydroxyl groups in the solution creates an increase in their affinity for water molecules to form a hydrophilic compound, which leads to swelling and increased viscosity [28]. The negligible difference in the mPa.s value at 0.05% of XG and CMC has been reported previously [32].

In general, the use of PT has been observed to have a better effect on stability as well as viscosity in solution. Protein residue is a plausible explanation for the ability to improve viscosity in the aqueous phase [31], [33]. On the other hand, interactions from CMC and XG are referenced to a consistent concentration in the process. The estimated statistical model brings the optimal parameters for nectar products that meet the target of about 70 mPa.s. This optimal viscosity corresponded to following parameters: pectin = 0.113708%; carboxymethyl cellulose = 0.0428037%, and Xanthan Gum = 0.0448557% with desirability equal 1.
Fig. 2. 2D and 3D interaction diagram of additive to viscosity (A) pectin and carboxymethyl cellulose, (B) Pectin and xanthan gum, and (C) xanthan gum and carboxymethyl cellolose

4. Conclusions
From the results on the interaction of PT, CMC, and XG on the viscosity in soursop’s nectar, the established model has shown adequacy and suitability in predicting viscosity of the product given varying concentrations of additives. The optimal viscosity determined from the model was 70mPa.s. Significant interactions between PT-CMC and CMC-XG were found while non-significant interactions between PT-XG were reported. This study serves as the precursor for possible production scale ups of soursop products. However, it is worth noting that this study only focuses on viscosity. Therefore, attention should be paid to the ability to maintain the colloidal system (stability), turbidity and sensory properties of the product in further research.
Acknowledgements
This work was supported by grants from Science and Technology Department of Tien Giang Province, Viet Nam.

References
[1] Hien T T, Ha K L, Chinh D N, Phat T D, L Nhan H T L, Hai D N, Trinh D N, Viet D N V, Truc T T and Giang L B 2019, Vietnam Processes 7 56
[2] Linh T V N, Nhi Y T T, Duc T L, Chinh D N, Giang L B 2019 Food Sci. Nutr. 00 1–7
[3] Cang H M, Le T T T, Diep T T, Nhan H T L, Trinh D N, and Giang L B 2018 Asian J. of Chem. 30 293–297.
[4] Hien T T, Nhan T P N, Trinh D N, Van T T H, Giang L B 2018 Solid State Phenome. 279 217–221.
[5] Nhan T P N, Hien T T, Nhan H T L, Anh Q N P, Huy T L, Trinh C T N, Trinh D N, Giang L B 2018 Solid State Phenome. 279 235–239.
[6] Minh P N, Thu T M, Tham H N, Giang L B 2018 J. of Global Pharma Technol. 10 186 – 192
[7] Phan A N Q, Giang L B, Trinh D N, Nhan H T L 2019. 19 974-978
[8] Linh V T N, Duyen M N, Chinh D N, Giang L B and Duc T L 2019 Processes 7 21.
[9] Minh P N, Giang L B, Chau M H, Loan Y L, Tram V T B, Truyen T V 2019. J. of Pharma. Sci. and Res. 11 279-283
[10] Cang H M, Nguyen T S V, Nhan H T L, Chinh D N and Giang L B 2019 Processes 7 90.
[11] Toan Q T, Le T T T, Pham M Q, Do T L, Vu M C, Chinh D N, Giang L B, Minh L B and Pham Q L 2019 Molecules 24 895
[12] Nhi Y T T, Nhan T P N, Thanh T V, Chinh D N, Duc T L, Vu D N, Vy A T, Thinh V P, Truc T T 2020 IOP Conf. Ser. Mater. Sci. Eng. 736 022065.
[13] Hien T T, Nhan T P N, Van T H H, Nhan H T L, Giang L B, Trinh D N, IOP Conf. Ser. Mater. Sci. Eng. Pap. 479 012015.
[14] N.P. Minh, T.H.P. Trang, N.T.T. Trang, and L.G. Bach 2019 Research on Crops 20 180-186 (2019)
[15] Coria-Téllez A V, Montalvo-González E, Yahia E M, Obledo-Vázquez E N 2018 Arabian J. of Chem. 11 662-691
[16] Indrawati L, Pramono S, and P. Ascobat 2018 J. Glob. Pharma Technol. 9 35–40
[17] Nhi Y T T, Nhan T P N, Thanh T V, Vu D N, Thin V P, Vuy A T, Duc T L 2020 IOP Conf. Ser. Mater. Sci. Eng. 736 022064.
[18] Badrie N and Schauss A G 2010 Elsevier 2010 621–643
[19] Monteiro E, Gutierrez R, Souza B A and Guzman M A U 2017 Food Hydrocoll 62 158–164
[20] Ibrahim G E et. al. 2011 Food Hydrocoll. 25 91–97
[21] Ebrahiminezhad A, Moeeni F, and Taghizadeh S 2019 Foods 8 1–10
[22] Huang B, Zhao K, Zhang Z, Liu H, Hu H, and S. Pan 2018 LWT - Food Sci. Technol. 91 414–422
[23] T.P. Dao, T.H. Tran, D.T. Nguyen, D.C. Nguyen, D.H. Nguyen, N.T. Hong Le, D.T. Si, N.T.T. Huong, B.L. Minh 2019 Asian J. of Chem., 311639–1642, 2019.
[24] Phat T D, Hien T T, Trinh D N, Chinh D N, Hai D N, Nhan H T L, Si T D, Huong T T N, Minh L B 2019 Asian J. of Chem. 31 1639–42
[25] Nhut T P, Tran B P, Hien T T, Chinh D N, Nhan T P N, Nguyen T Q, Viet D N V, Tien X L, Trinh D N, Giang L B 2019 IOP Conf. Ser. Mater. Sci. Eng. Pap. 479 012012
[26] Phat T D, Hien T T, Trinh D N, Chinh D N, Nhan T P N, Nhan H T L, Tien X L, Hai D N, Viet D N V, Giang L B 2019 Asian J. of Chem. 31 977–981.
[27] Phat T D, Quyen C T N, Hien T T, Thin V P, Long Q P, Toan Q T, Nguyen H N, Vo D H M, Tien X L, Trung Y N L, Nguyen T T, Nhi Y T T, Truc T T and Muoi V N 2020 Asian J. Chem. 32 237–243
[28] Linh N T V, Mai N T V, Nhi Y T T and Duc T L 2019 Asian J. Chem. 31 2345–2350
[29] R. Cordeiro et al. 2015 LWT - Food Sci. Technol. 62 883–889
[30] Tiwari P J B K, Muthukumarappan K and C C 2010 *Int. J. Food Prop.* **13** 223–233
[31] Mirhosseini H, Ping C, Aghlara A, Hamid N S A, Yusof S, and Huey B 2008 *Carbohydr. Polym.* **73** 83–91
[32] EYaseen E I, Herald T J, Aramouni F M, and Alavi S 2005 *Food Res. Int.* **38** 111–119
[33] Mirhosseini H, Tan C P, and Naghshineh M 2010 *J. Food, Agric. Environ.* **8** 134–139.