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Physicochemical, antioxidant and microbiological behavior of soursop purees during thermal treatment

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ABSTRACT

In this study, the influence of thermal treatment at temperatures 70, 80, and 90°C for 0 to 45 minutes on the quality of soursop puree was investigated in terms of physicochemical properties and antioxidant activity. Total phenolic content and antioxidant activity of the sample were evaluated using spectrophotometric methods, ascorbic acid was tested using the titration method. The result showed that high temperature and prolonged heat treatment accelerated the degradation of physicochemical properties in soursop puree. The kinetic degradation of polyphenol, ascorbic acid, and antioxidant activity belonging to temperature was performed by Arrhenius equation with the E_a values were 54.42 kJ/mol, 48.83 kJ/mol, and 20.07 kJ/mol, respectively. Total aerobic counts and ΔE values of all samples were less than 1 x 10^2 CFU/g and 2.51, respectively. The soursop puree reached a good quality for all the tested attributes at 80°C for 15 minutes.

1. INTRODUCTION

Soursop (*Annona muricata* L.) is a valuable food resource of bioactive compounds such as alkaloids, flavonoids, phenolic, dietary fiber, and minerals (Badrie & Schauss, 2010; Luzia & Jorge, 2012; Chithra et al., 2016). In Viet Nam, this fruit was planted a lot in Mekong Delta. However, soursop is a climacteric fruit, and its post-harvest life is short. Indeed, studies to find a way to extend the shelf-life of soursop need to be carried out. Thermal processing such as pasteurization, which is a traditional method use in the food industry for the preservation of fruit products. Pasteurization is a mid-heat treatment and the most cost-effective tool for reducing the activity of enzymes and the cells of the vegetative microorganism (Petruzzi et al., 2017). Pasteurized fruit product such as puree product has simple processing, transportation, preservation, and consumption. Additionally, the pasteurized puree is a semi-product, it can use as a material for beverage, consistency, ice cream product, or use directly.

Besides, the function eliminates pathogenic and spoilage microbial; in most cases, thermal treatment can change the nutritional profile and sensory quality of fruit. Several research studies have been found the physicochemical properties, especially, the antioxidant activity of the fruit product can be degraded undergone thermal treatment such as dragon fruit puree (Liaotrakoon et al., 2013), pineapple puree (Chutintrasri & Noomhorm, 2007), and orange juice (Vikram et al., 2005). However, the information about the influence of temperature during thermal treatment on the physicochemical properties in soursop is still limited. Therefore, the main objective of this study is to determine the physicochemical, antioxidant, and microbiological behavior of soursop puree during thermal treatment.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Material

Soursop (*Annona muricata*) was purchased at Thu Duc agricultural wholesale market during March and April 2021, its source from Mekong Delta in Viet Nam. Raw material had characteristics of dark green skin, without defects of physical injures and rottenness.

2.1.2. Chemical

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from TCI-Japan; Folin-Ciocalteus reagent and 2, 6-dichlorophenol indophenol (DCIP) was purchased from Merck-Germany; gallic acid, sodium carbonate anhydrous (Na_2CO_3), L-ascorbic acid ($C_6H_8O_6$), methanol (CH_3OH), phenolphthalein ($C_{20}H_{14}O_4$), sodium hydroxide (NaOH) and oxalic acid ($C_2H_2O_4$) were purchased from Xilong-China.

2.2. Sample preparation

The fruit was washed, peeled, and deseeded. Next, the pulp was soaked in 0.1% $Na_2S_2O_5$ with ratio 1:2 (w/v) for 30 mins for antibrowning. The pulp was ground in a blender and passed through a 1 mm sieve mesh. The collected puree was used on the same day to further study.

2.3. Thermal treatment

Aliquot of 40 g puree (initial temperature 29-31°C, pH 3.4-3.7) was poured into a polypropylene bag (10 x 15 cm, thickness 0.1 mm). The sealed containers were then placed in a thermostatic water bath (WNB 14, Memmert, Germany) at selected temperatures (70, 80, and 90°C). After the sample reached the desired center temperature (testing by Digital thermometer TA278, China), the purees were heated for 0, 15, 30, and 45 mins, respectively. After heating, the samples were instantly taken out and immediately cooled in an ice-water bath to stop the reaction. The unheated puree was used as a control sample. The samples were kept in the fridge to prior further assay. Individual samples were replicated 2 times.

2.4. Analytical method

2.4.1. *Determination color parameters*

The color parameters (L*, a*, and b*) were measured by using a colorimeter (Konica Minolta CR 400, Japan). L* value corresponds to lightness and varies from 0 to 100, a* value measures redness when positive and greenness when negative, b* value means yellowness when positive and blueness when negative. The ΔE is the difference between the L*, a*, and b* of the sample, and the standard (subscript "0" refers to the color reading of soursop puree unheated (Chutintrasri & Noomhorm, 2007).

$$\Delta E = \sqrt{(L_0\text{-}L)^2+(a_0\text{-}a)^2 + (b_0\text{-}b)^2}$$

2.4.2. Tritatable acidity (TA)

The amount of acid is determined by the titration method with 0.1N NaOH solution and phenolphthalein as a color indicator (ISO 750:1998). Ten g of the sample was placed into a 100 mL volumetric flask, then distilled water was added to the mark. The mixture was shaken well and was allowed to stand for 10 minutes before being filtered by quantitative filter paper. Then 10 mL of the filtrate was added 1-2 drops of 1% phenolphthalein and was titrated with 0.1 N NaOH solution until appearing persistent pink color. TA was calculated by the following equation:

$$\text{TA (\%)} = \frac{V \times K \times V_2 \times 100}{V_1 \times m}$$

Where: V: Volume of titrated NaOH 0.1 N (mL), V_1: Volume of titrated aliquot (10 mL), V_2: Volume of diluted aliquot (100 mL), m: weight of sample (10 g), K: corresponding citric acid coefficient (0.0064)

2.4.3. Total soluble solids content (TSS)

Total soluble solid was measured by using handheld refractometer (0-33%, Atago, Japan)

2.4.4. Microbiological properties

The aerobic count was obtained using the pour plate technique on plate count agar, standard method agar (Maturin & Peeler, 2001). Each soursop puree was diluted (10^{-1} to 10^{-3}) with NaCl solution 0.85% and then 1 mL of each dilution was used for total aerobic count assay. The plates were overturned and incubated at 35± 1 °C for 48 h. Duplicates were done for each dilution. The number of microorganisms was expressed as CFU/g.

2.4.5. Extraction bioactive compound

Fruit extracts for total phenolics content and antioxidant activity were measured in methanol extract were according to Xu et al. (2008), with some modifications. One gram of soursop puree was extracted with 9 mL of 80% methanol for 30 mins at room temperature. After vortexing for 10 mins, the supernatant was taken out by filtering through

the qualitative filter paper 102. The collected extracts were stored in the fridge.

2.4.6. Total phenolic content

The Folin–Ciocalteu's reagent was used for total phenol content determination (Lim et al., 2007). The aliquot of 0.3 mL of extract was added to 1.5 mL of Folin–Ciocalteu's reagent (diluted 10 times with distilled water) and 1.2 mL of 7.5% sodium carbonate. After vortexing, the samples were left for 30 mins. The total phenol content was determined by using a spectrophotometer at a wavelength of 765 nm (Jasco, V730, Japan). The standard curve was prepared using solutions of gallic acid (GAE) and then total phenolic content was expressed as mg GAE / 100 g dry matter (dm).

$$\boldsymbol{TPC} = \frac{(\boldsymbol{y-b})\ \boldsymbol{x\ V\ x\ DF\ x\ 100}}{\boldsymbol{a\ x\ m\ x(100\% - moisture\ content\%) \times 1000}}$$

Where y: OD value of sample. a and b are the coefficient in the standard curve. V: volume of extracted solution (mL). DF: dilution factor. m: mass of sample (g). 100/1000: the coefficient converts from µg/g to mg/100g.

2.4.7. Antioxidant activity determinations

The DPPH reagent was used for antioxidant activity determination (Thaipong et al., 2006). The aliquot of 0.2 ml of extract was added to 4 mL of 0.1 mM DPPH solution. After vortexing, the samples were left for 30 min. The antioxidant activity was determined by using a spectrophotometer (Jasco, V730, Japan). at a wavelength of 517 nm. The standard curve was prepared using solutions of ascorbic acid (AA) and then antioxidant activity was expressed as mg AAE/100 g dry matter (dm).

$$DPPH = \frac{(y-b)\ x\ V\ x\ DF\ x\ 100}{a\ x\ m\ x(100\% - moisture\ content\%) \times 1000}$$

Where y: OD value of sample. a and b are the coefficient in the standard curve. V: volume of extracted solution (mL). DF: dilution factor. m: mass of sample (g). 100/1000: the coefficient converts from µg/g to mg/100g.

2.4.8. Ascorbic acid content

Ascorbic acid content was determined using the 2, 6- dichlorophenol-indophenol titration method (Dinesh et al., 2015). Five mL of working standard (0.1 mg/mL) of L- ascorbic acid and 10 mL of 4% oxalic acid were pipetted into a 100 mL conical flask. The content in the flask was titrated against the dye solution (V1) until the appearance of pale pink color persisted for a few minutes. Five mL of the test sample was similarly titrated against the dye solution (V2). The ascorbic acid content in the test samples was determined using the formula:

$$Ascorbic\ acid\ \left(\frac{mg}{100g}\right) = \frac{0.1 \times V_1 \times 10 \times 100}{V_2 \times 1 \times 5}$$

2.4.9. The proportion of bioactive compound

Proportion of total phenolic retention (%) = $\frac{A_1}{A_0}$ x 100, where A_1 is the total phenolic content in the heated sample, A_0 is the total phenolic content in the sample unheated. This equation was not only used for phenolic, but also for ascorbic acid.

Proportion of total phenolic reduction (%): 100% - the proportion of total phenolic retetion. This equation used not only phenolic but also ascorbic acid.

2.4.10. Kinetic degradation

The equation was followed Vikram et al. (2005). The deterioration of phenolic, ascorbic acid as well as antioxidant activity in soursop puree undergone thermal treatment was calculated based on the first-order model was as follows: $\ln A = \ln A_0 - kt$.

Where, A is the parameter at time t (after heating); A_0 is the parameter (before heating); k is the degradation rate constant (min^{-1}) obtained from slope of a graph $\ln(A/A_0)$ over time; t is the thermal treatment time (min).

Activation energy (E_a, kJ/mol) is determined by the equation Arrhenius as follows:

$$k = k_A x\ e^{\frac{-E_a}{R\,T}}$$

Where, k_A is a constant present the Arrhenius equation, which is temperature-dependent. R is the universal gas constant (8.3145 J/ mol K); T is the absolute temperature (K).

3. STATISTICAL ANALYSIS

The results were expressed mean $\pm$ SD. The effect of temperature and time was evaluated by a two-way analysis of variance (ANOVA) using the general linear model procedure of JMP ver 13.0. The effect of any of the factors was significant at $p < 0.05$.

4. RESULT AND DISCUSSION

4.1. Physicochemical properties of soursop fruit

In comparison with the result of Lim et al. (2006), the total phenolic content (mg/100g fresh weight

(FW)) of the selected soursop pulp was higher than mangosteen (54 mg/100g), orange (75 mg/100g), approximate equal guava (138 mg/100g). Table 1 showed the amount of ascorbic acid in soursop pulp was higher than mangosteen (5.8 mg/100g), dragon fruit (8.0 mg/100g), and lower than guava (144 mg/100g), orange (67 mg/100g), papaya (108 mg/100g) (Lim et al., 2006). The pH value of soursop 3.68 was adapted for pasteurization processing (pH<4.5). The total acidity was found in the citrus fruit including mandarin, lemon, sweet orange (from 0.90% to 1.5%) by Xu et al. (2008) was greater than soursop 0.83%.

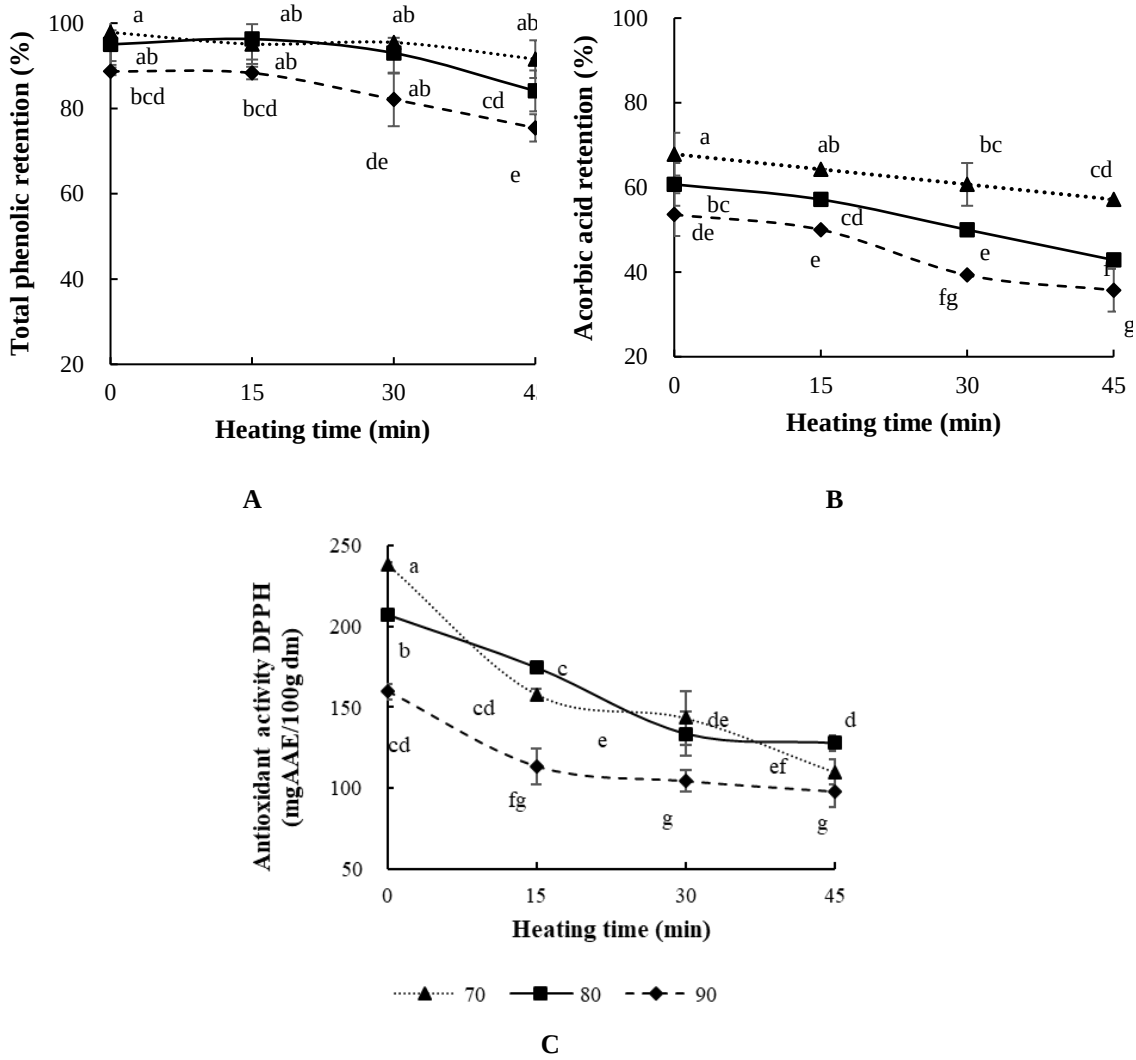
Table 1. The physicochemical properties of soursop fruit, N=9.

Attribute	Result
Weight (g)	1294.44 ± 218.58
L* of peel	51.44 ± 3.25
a* of peel	-6.78 ± 1.97
b* of peel	17.03 ± 4.05
L* of pulp	81.45 ± 2.65
a* of pulp	-1.00 ± 0.24
b* of pulp	13.27 ± 1.78
pH	3.68 ± 0.12
Total acidity (%)	0.83 ± 0.22
Total soluble solid (%)	12.52 ± **1.50**
Total phenolic content (mg GAE/100g FW)	159.60 ± 15.59
Ascorbic acid content (mg/100g FW)	26.07 ± 4.71
DPPH (mg AAE/100g FW)	59.61 ± 13.28

4.2. Effect of thermal treatment on antioxidation activity

According to Figure 1, the retention of bioactive compounds in soursop puree deteriorated from 97.86% to 75.44% (polyphenol), from 67.86% to 35.71% (ascorbic acid), which follow the temperature increased (from 70℃ to 90℃) for 0 to 45 mins. As a consequence, the antioxidant activity of the sample reduced from 238.21 to 97.84 mg/100g dm. The thermal treatment strongly impacted the bioactive compounds of the sample at 90℃; meanwhile, the behavior of antioxidative properties in soursop puree treated 70℃ and 80℃ were not clearly separate, except ascorbic acid. Polyphenol and vitamin C belong to the heat-sensitive substance (Wang et al., 2017). Several previous study results proved that one such as soursop juice (Ampofo-Asiama and Quaye, 2019), dragon fruit (Liaotrakoon et al., 2013), pomegranate juice (Paul & Ghosh, 2012).

The degradation of the bioactive compound and antioxidant activity of soursop puree were found to fit the first-order kinetic (Table 2, Figure 2). Generally, the degradation rate k (min^{-1}) increased in the following order 70, 80, and 90℃; specifically, its value at 90℃ and 80℃ higher than 3 times and 2 times comparable 70℃. In this study, the E_a value of total phenolic (54.42 kJ/mol), ascorbic acid (48.83 kJ/mol), and antioxidant activity (20.07 kJ/mol) of soursop puree during thermal processing were found. Higher activation energy means that a degradation reaction for a specific compound needs a lower temperature. In other words, the E_a value indicated the ascorbic acid-sensitive temperature higher than polyphenol. The E_a values varied for polyphenol and ascorbic acid were determined in the other fruit. For instance, the E_a values of polyphenol were 16.85 kJ/mol for red flesh dragon fruit puree (Liaotrakoon et al., 2013), 18.52 kJ/mol for bael juice (Ipsita & Uma, 2018). The E_a values of ascorbic acid were 39.84 kJ/mol for pomegranate juice (Paul & Ghosh, 2012), 39.84 kJ/mol for orange juice (Vikram et al., 2005). The difference in polyphenol profile and product characteristics may be caused by the difference of E_a values in the previous studies with this study.



The value has different letters superscript show statistically significant differences at the α = 0,05 confidence level.

Figure 1. Effect of thermal treatment on (A) Total phenolic content (B) Ascorbic acid content and (C) Antioxidant activity in soursop puree

Table 2. Kinetic parameter for bioactive compounds and antioxidant activity in soursop puree during thermal treatments

Attribute	Temperature (℃)	k (min^{-1})	R^2	E_a (kJ/mol)
Total phenolic	70	0.0013	0.86	54.42
	80	0.0027	0.71	
	90	0.0037	0.90	
Ascorbic acid	70	0.0038	0.99	48.83
	80	0.0079	0.97	
	90	0.0097	0.95	
Antioxidant activity (DPPH)	70	0.0109	0.95	20.07
	80	0.0114	0.94	
	90	0.0161	0.94	

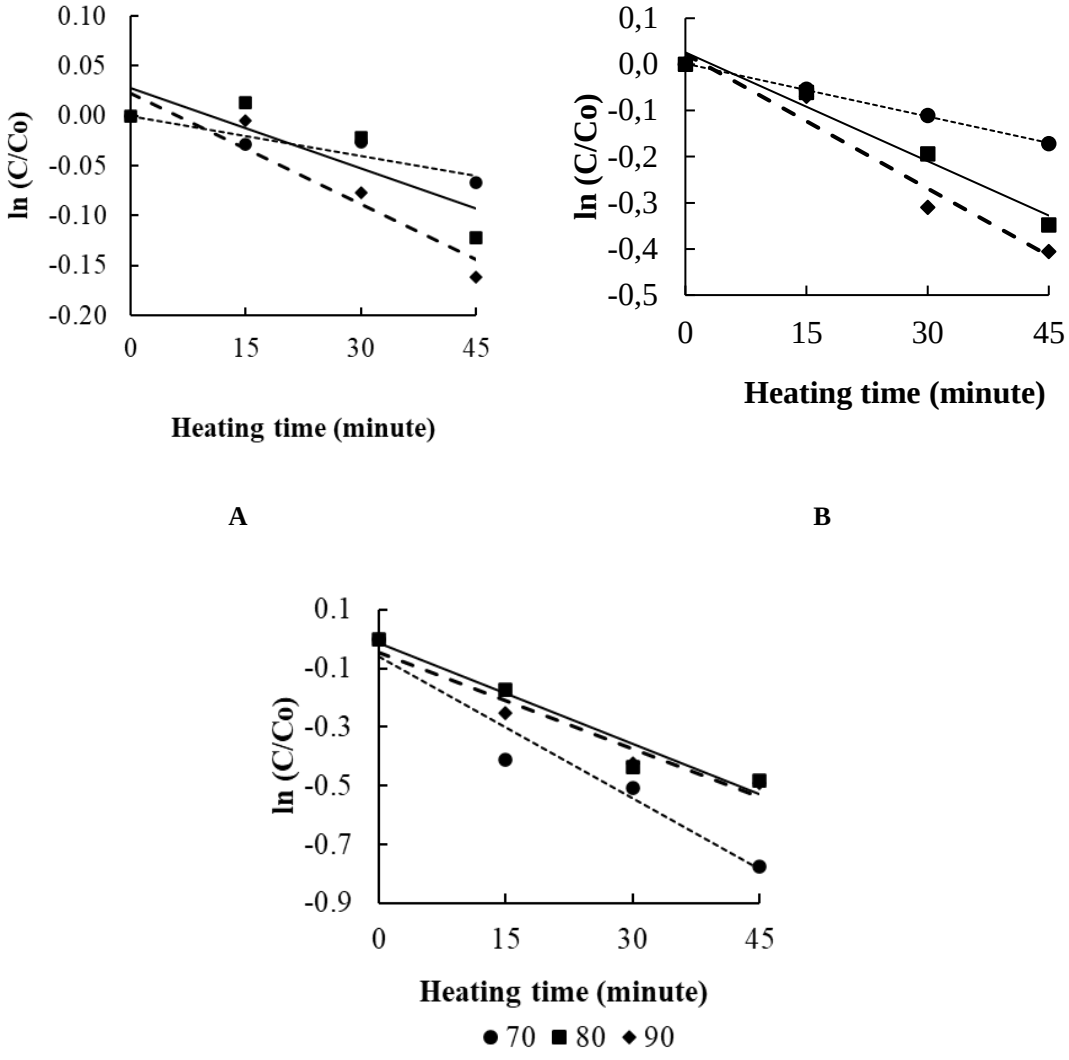


Figure 2. The degradation of (A) phenolic, (B) ascorbic acid, and (C) antioxidant activity in soursop puree during thermal treatment

The change of antioxidative properties in soursop puree depending on the temperature can display following Arrhenius model (Figure 3). Figure 3 also showed between the total phenolic content, ascorbic acid, antioxidant activity, and temperature had a negative relationship.

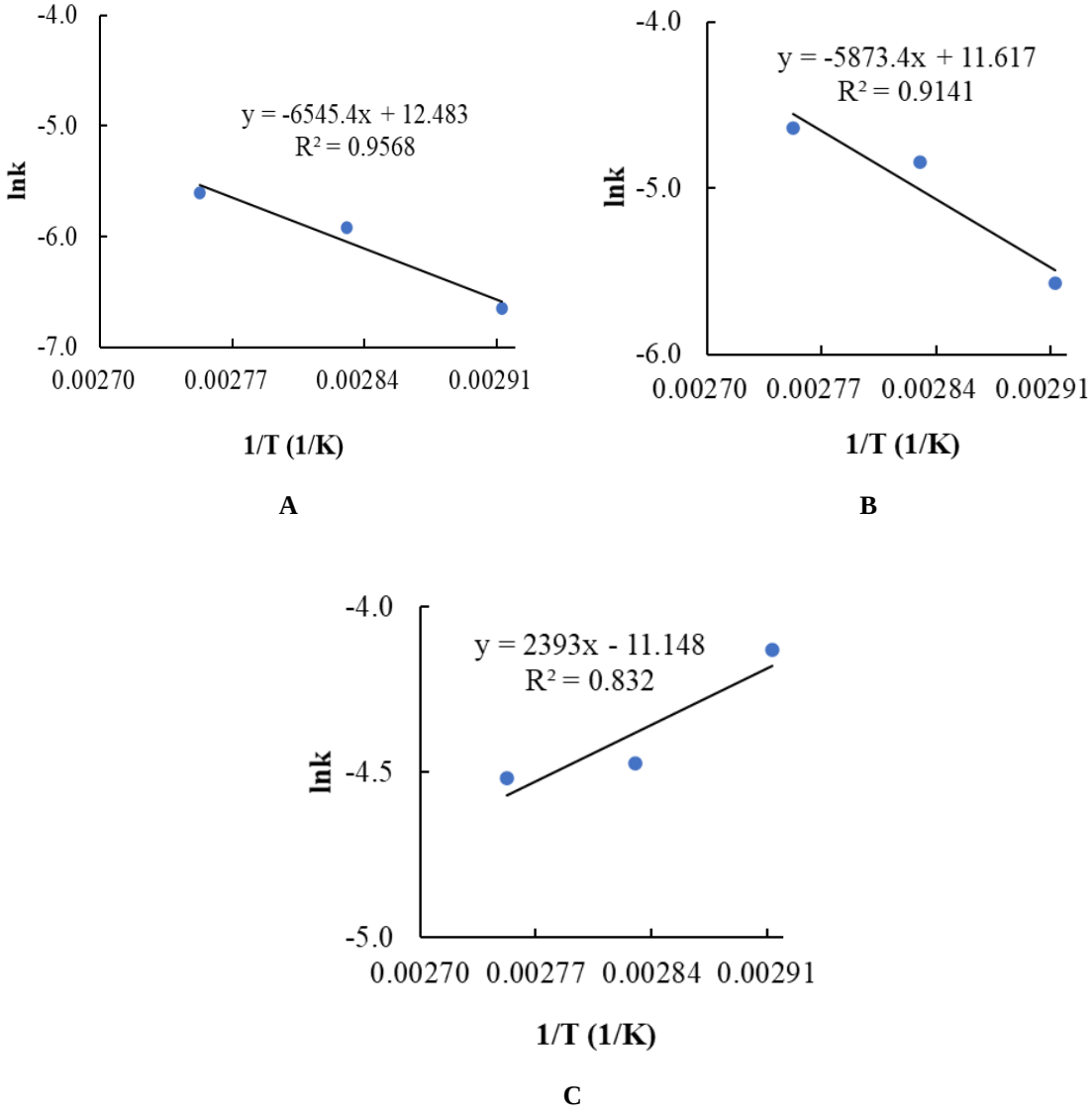


Figure 3. Arrhenius model relationship between temperature and the degradation of (A) total phenolic content, (B) Ascorbic acid content, (C) antioxidant activity (DPPH)

4.3. Effect of thermal treatment on color properties changes

The ΔE value was accelerated by temperature, particularly at high temperatures. For instance, after 45 minutes, the soursop puree treated at 70℃, 80℃, and 90℃ had ΔE values with 2.01, 2.20, and 2.51, respectively (Figure 4). These values were different statistically at $p<0.05$. The ΔE value from 2.0 to 3.5 meant that inexperienced observers also noticed the difference (Mokrzycki and Tatol, 2011). It meant that the color of samples was different after heating 45 mins at all temperatures. However, the vision of all samples is quite similar, its color was white. This result agreed with Shourove et al (2020) and Chutintrasri and Noomhorm (2007), those authors found the ΔE value of pineapple puree and star fruit juice, respectively, increased with time and treatment temperature.

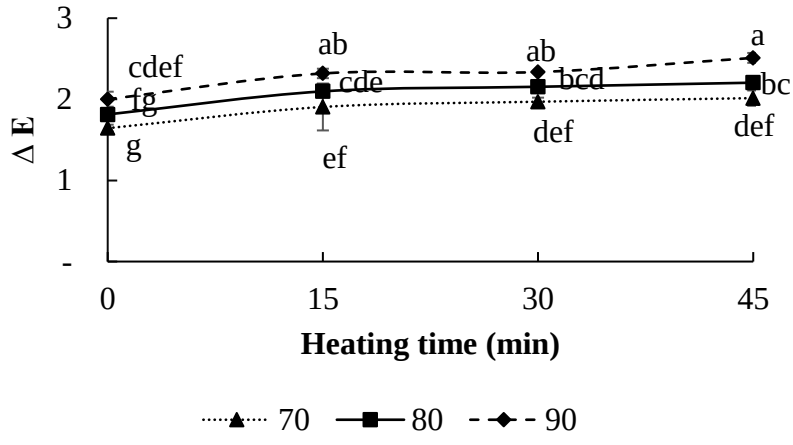


Figure 4. Effect of thermal treatment on color properties changes of soursop puree

The value has different letters superscript show statistically significant differences at the α = 0,05 confidence level.

Table 3. Correlation coefficient for relationships among physicochemical properties in soursop puree undergone thermal treatment

Attribute	TPC	Ascorbic acid	DPPH
Ascorbic acid	0.95**	1.00	0.92**
DPPH	0.76*	0.92*	1.00
Δ E	-0.89**	-0.94**	-0.91**

**: p< 0.01, **p< 0.001*

The correlation between physicochemical properties such as phenolic content, ascorbic acid content, antioxidant activity, ΔE of soursop puree after heat treatment was evaluated (Table 3). The results showed the existing relation between all the mentioned attributes and most of the correlation coefficient R2 over 0.9. The total phenolic content, ascorbic acid content, and antioxidant activity had a positive correlation; in contrast, the ΔE value got a negative correlation with those parameters.

4.4. Effect of thermal treatment on total aerobic count

The high temperature and prolonged time eliminated the total aerobic count in soursop puree, in which, this phenomenon occurred strongly at temperature 90℃ (Figure 5). Generally, the heated soursop puree samples contained less than 0.73 x 10^2 CFU/g, which adapted request of the Vietnamese Ministry of Health for fruit products was less than 1 x 10^2 CFU/g (QĐ/46/2007/QĐ-BYT). This phenomenon could be explained by the denaturation of the protein after heat treatment; as a caused gave the modified function of the membrane, the peptidoglycan cell wall, the nucleoid, and enzymes in microbial cellular structures (Cebrián et al., 2017).

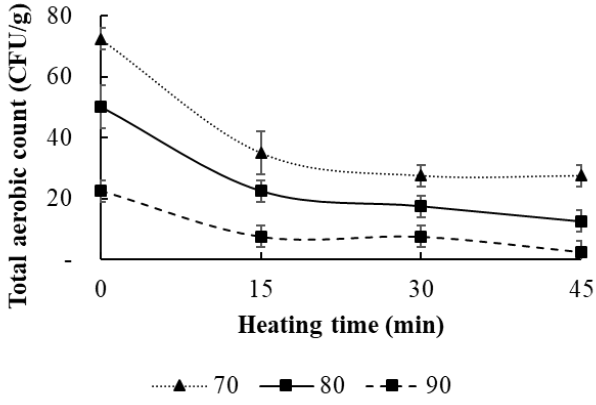


Figure 5. Effect of thermal treatment on total aerobic count in soursop puree

4.5. Effect of heating on physicochemical properties in soursop puree

The most physicochemical properties and antioxidant activity of soursop puree were affected by thermal treatment (Table 4). For instance, after heating, the soursop puree approximately reduced 2% for phenolic, 42.86% for ascorbic acid, and 36.13% for antioxidant activity. The total aerobic count was reduced 2 log CFU/g than the sample unheated. The other attributes such as color properties, pH, total acidity, and the total soluble solids were slightly different. The deterioration of antioxidative properties of fruit products after thermal treatment was found in the previous studies. The ascorbic acid content reduced 3.70% for the acerola pulp (Mercali et al. 2012), 23.23% for the pomegranate juice (Paul & Ghosh, 2012), 7.60% for the blended tropical juice (acerola, acai, pineapple) (Wurlitzer et al., 2019). The total phenolic content was reduced by 9.70% for the pomegranate juice (Paul & Ghosh, 2012), 4.10% for the blended tropical juice (acerola, acai, pineapple) (Wurlitzer et al., 2019).

Table 4. The physicochemical properties between sample with and without thermal treatment

Attribute	Sample unheated	Sample heated at 80℃ /15 mins	p value
L*	65.85 b ± 0,73	67.91 a ± 0,10	< 0.0318
a*	-2.36 a ± 0,04	-2.53 b ± 0,02	< 0.0085
b*	3.77 a ± 0,03	3.73 a ± 0,01	< 0.2155
pH	3.45 b ± 0.01	3.53 a ± 0.01	< 0.0077
Total acidity (%)	0.78 a ± 0.22	0.66 b ± 0.22	< 0.0256
Total soluble solid (%)	9.75 a ± 0.35	8.10 b ± 0.14	< 0.0256
Total phenolic content (mg GAE/100g dm)	898.71a ± 15	880.53 a ± 18.52	< 0.3934
Ascorbic acid content (mg/100g dm)	19.6 a ± 3.96	11.2 b ± 0.00	< 0.0955
DPPH (mg AAE/100g dm)	273.16 a ± 8.46	174.49 b ± 32.46	< 0.0532
Total aerobic count (102 CFU/g)	15.18 a ± 0.32	0.23 b ± 0.04	< 0.0002

In a row, the value has different letters superscript show statistically significant differences at the α = 0,05 confidence level.

5. CONCLUSIONS

This study found the Ea value of polyphenol, ascorbic acid, and antioxidant activity DPPH were 54.42 kJ/mol, 48.83 kJ/mol, and 20.07 kJ/mol, respectively. After heating at temperature 70-90℃ for 15-45 mins, the packed soursop puree in polypropylene bag had a total aerobic count less than 1 x 102 CFU/g, ΔE value from 1.91 to 2.51. Moreover, the value of total soluble solids, pH, total acidity of the sample after heating had slightly different with the sample unheated. The soursop puree was treated at 80℃ for 15 min had a good quality such as bioactive compound, antioxidant activity, total aerobic count. The research needs to be continued to determine the number of microorganisms such Escherichia coli, Staphylococcus aureus, Clostridium perfringens during heat treatment, which ensures product safety. Moreover, the sensory quality of the product needs to evaluate. The heated soursop puree had the potential application in foodstuffs such as jelly, jam, juice, and yogurt.

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