OPTIMIZATION OF FACTORS AFFECTING SYRUP PRODUCTION FROM "SIM" FRUIT (*Rhodomyrtus tomentosa*) FOR HIGH ANTHOCYANIN CONCENTRATION AND GOOD QUALITY

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ABSTRACT

Rhodomyrtus tomentosa or Rose Myrtle is a wild plant native to Southeast Asia. Its berry or fruit is sweet, edible and medicinally used as a folk remedy for various diseases. The fruit contains high concentration of anthocyanin, a natural polyphenol with powerful antioxidant activity. In this study, Sim fruits harvested from Mang-Den, a highland area in Komtum, were pretreated with pectinase to maximize yield, transmittance (clarify) and anthocyanin in the filtrate. After taste adjustment with sugar and citric acid, the juice was pasteurized for preservation. The extraction by pectinase enzymes was optimized using response surface methodology. The results showed that the extraction condition with 0.1% pectinase at 40^{o}C in 60 min was optimal for maximum yield of fruit juice (62.93%), clarity (T=38.3%) and amount of anthocyanin (68.52 mg/L). Pasteurization with $PU_{85}^{8,3}$ = 9.18 minutes at 85^{o}C for 4 minutes yielded syrup with good safety and high anthocyanin concentration.

Keywords: Anthocyanin, pasteurization, pectinase, *Rhodomyrtus tomentosa* fruit, syrup.

Tối ưu hóa các yếu tố ảnh hưởng đến quá trình sản xuất sirô sim (*Rhodomyrtus tomentosa*) để có hàm lượng anthocyanin cao

TÓM TẮT

Trái "Sim" là loại trái mọng nước phân bố nhiều ở vùng Đông Nam Á. Trái Sim rừng có thể ăn được và chứa nhiều dược chất trị nhiều bệnh. Trái Sim chứa hàm lượng anthocyanin cao. Anthocyanin là hợp chất polyphenol có khả năng chống ôxy hóa rất tốt. Trong nghiên cứu này, Sim từ Mang Đen, Kontum được xử lý với enzyme pectinase để tối ưu hóa hiệu suất thu hồi, độ trong và hàm lượng anthocyanin. Sau khi phối chế với đường và acid, dịch Sim được vô chai và thanh trùng ở nhiệt độ và thời gian khác nhau. Quá trình trích ly dịch Sim bằng enzyme pectinase được tối ưu hóa bằng phương pháp bề mặt đáp ứng (Response surface methodology). Kết quả cho thấy rằng điều kiện trích ly tối ưu là 0,1% pectinase ở nhiệt độ 40^{o}C trong 60 phút để có được hiệu suất thu hồi (62.93%), độ trong (T=38.3%) và hàm lượng anthocyanin (68.52 mg/L) cao nhất. Để đạt được chất lượng cao về an toàn vệ sinh và hàm lượng anthocyanin cao, sirô Sim được thanh trùng với giá trị $PU_{85}^{8,3}$ = 9.18 (phút) ở điều kiện 85^{o}C trong 4 phút.

Từ khóa: Anthocyanin, pectinase, sim, sirô, thanh trùng.

1. INTRODUCTION

Rhodomyrtus tomentosa fruit or "Sim" fruit is a wild berry mainly distributed in highland and mountains in Vietnam, especially in Phu Quoc, Kien Giang and Mang Den, Kontum. Sim fruit has been recognized as an excellent source of anthocyanins, with the anthocyanin content of its skin being approximately 4.358 g/kg dry weight, indicating that the fruit has great potential as an ingredient for functional beverages (Liu et al., 2012). Anthocyanins are the principal water-soluble pigments responsible for the red, blue, and purple colors. Anthocyanins are commonly present in plants and non-toxic (Nabae et al., 2008). Anthocyanins are particularly attractive as natural substitutes for synthetic pigments and antioxidants (He and Giusti, 2010). In addition,

an increasing number of studies have demonstrated that anthocyanins have the ability to prevent chronic and degenerative diseases including type 2 diabetes, cardiovascular disease and cancer (Felgines et al., 2006; Ghosh and Konishi 2007; Wu et al., 2006).

Efficient extraction of Sim juice is one of the most important steps for syrup production from Sim crudes. However, Sim crudes are usually too pulpy and pectinacious to yield juices. One of the most effective methods is the enzymatic liquefaction technique. Anthocyanins degrade easily and discolor to form undesirable brown pigments in products such as fruit juices and syrups. Discoloration makes consumers perceive loss of the product quality (Torskangerpoll and Andersen, 2005). Anthocyanin stability is affected by several factors including pH, temperature, light, oxygen, enzymes, ascorbic acid, sugars, sulfur dioxide and metal ions (Francis and Markakis, 1989; Mazza and Brouillard, 1987). Thermal treatments (pasteurization and concentration) adverse strongly on the stability of anthocyanins in fruit juices such as blueberry, strawberry and blood orange. There have not been many studies about optimization of effects of enzymatic extraction and pasteurization on change of anthocyanins in sim syrup. The aim of this study was to optimize the temperature/time/enzyme concentration for extraction of anthocyanin from Sim, and to optimize pasteurization for good quality of Sim syrup. High sugar content in the sim syrup is usepful to enhance the shelf-life of the product and inhibit degradation of anthocyanin. The sim syrup can be diluted and served as fruit juice drink with high contents of vitamin and anthocyanin.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Fruits

Sim fruits were collected from Mang Den-DakLong, Kon Tum from February to April, 2013. They were cleaned and then frozen at -20°C for a week in Mang Den. The frozen Sim fruits were transported by airplane or trucks to Can Tho, and futher stored at -20°C in the freezers until use for experiments in Food Technology Department, Can Tho University.

2.1.2. Enzyme source

Pectinex Ultra SP-L (Denmark) was used in the food industry for fruit juice processing to reduce viscosity and juice extraction. Pectinex Ultra SP-L is a commercial pectinase enzyme from *Aspergillus aculeatus*. It contains different pectinolytic and cellulolytic enzymes [endo-polygalacturonase (EC 3.2.1.15; C.A.S. No. 9032-75-1), endopectinylase (EC 4.2.2.10; C.A.S. No. 9033-35-6) and pectin esterase (EC 3.1.1.11; C.A.S. No. 9025-98-3)], and other activities. It is recommended that the optimum enzyme reaction conditions are pH 3.5–6.0 and temperature range below 50°C

2.2. Processing line

Sim fruit → Cleaning & washing → Freezing (-20°C) → Transporting → Storing (-20°C) → Washing → Grinding → Adding water (2.5kg water with 5kg sim crude) and Pectinex → Hydrolyzing → Filling into the cotton bag → Filter pressing (100-120kg/cm^2) → Blending (with sugar and citric acid)→ Filling in glass → Sealing → Pasteurizing → Sim syrup

If 5kg sim crude was added with 2.5kg water, the sim filtrate would be 5.6kg after extracting with Pectinex. Sugar (sucrose) and citric acid were blended with the Sim filtrate to have 50 brix and pH=3.7 for good sensory attributes of taste and colour (study was not shown in this paper).

2.3. Experimental design

2.3.1. Optimization of concentration of pectinase, temperature and time for extraction of Sim juice

Three levels of each of three factors, pectinase concentration, temperature and time for extraction of Sim juice were studied:

Pectinase (%) x temperature (°C) x time (min) = [0.05, 0.1, 0.15] x [40, 60, 80] x [35, 40, 45] = 27 experiments

Each experiment was done with 3 replicates.

2.3.2. Effects of pasteurization on quality of syrup and loss of anthocyanin

Two factors, temperature and time for pasteurization of Sim syrup were studied follow:

Temperature (°C) x time (min) = [85, 90, 95] x [2, 4, 6] = 9 experiments.

Each experiment was done with 3 replicates.

One thermal sensor was put in the middle of the center glasses (220mL of syrup/bottle) in the retort to record the temperature of the product with time. The other was put outside of the glasses to record and monitor the temperature of the retort. The temperature profiles were recorded on line for every minute on the computer to calculate the thermal processing values as shown in section 2.4.4. The retort (∅= 40cm, h=60cm) was heated with the steam supplied by the generator with the vapor pressure of 4 kg/cm^2.

2.4. Methods

2.4.1. Juice yield determination

$$y = \frac{m_J - m_w}{m_F} * 100\ \% \qquad [1]$$

where, y (%) is the yield of fruit juice, m_J(g) *is* the weight of juice, m_w (g) is the weight of water added, m_F is the weigth of sim fruit.

2.4.2. Transmittance (clarity) determination

The transmittance (T) was determined by a UV-Vis spectrophotometer model U-2800 (Simadzu, Japan). (Sin et al., 2006):

$$A = \log\left(\frac{I_o}{I}\right) x\lambda \qquad [2]$$

Where, A is the absorbance, I_o and I are the light intensity before and after transmission through the cuvet, λ is the wave length of the light (660nm). The transmittance (T) can be calculated as:

$$T = \left(\frac{I}{I_o}\right) x100\% \qquad [3]$$

2.4.3. Total anthocyanin measurement

The total anthocyanin content was determined according to the spectrophotometric pH-differential method (Lee et al., 2005). Briefly, an aliquot (1 mL) of the extract was mixed with 0.025 M potassium chloride buffer (pH 1.0, 4 mL) and 0.4 M sodium acetate buffer (pH 4.5, 4 mL). The absorbance of the mixture was measured at 510 and 700 nm using a UV-Vis spectrophotometer model U-2800 (Simadzu, Japan). The absorbance was calculated as A = [(A510 − A700) at pH 1.0] − [(A510 − A700) at pH 4.5] with a molar extinction coefficient of 26,900 for anthocyanin. The total anthocyanin content was calculated as cyanidin-3-glucoside equivalents as the following eaquation:

$$C = \frac{A \times M \times DF \times V \times 10^3}{\varepsilon \times L \times m} (mg/L) \qquad [4]$$

where A is the absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2 Da), DF is the dilution factor, V is the final volume (mL), 10^3 is the factor for conversion from g to mg, ε is the cyanindin-3-glucoside molar absorbance (26,900), L is the cell path length (1 cm), and m is sample weight (g).

2.4.4. Total microbial count determination

Colonies grown in petri dishes by spreading 1 mL of the sample on the medium of Plate Count Aga were used to determine the count of viable microorganisms. The samples may be diluted to enable counting visually. The total microbial count could be calculated as the following equation:

$$X = \frac{N}{\left(n_1 + 10^{-1}.n_2 + 10^{-2}.n_3 + ... + 10^{(i-1)}.n_i\right).d} \qquad [5]$$

Where, N is the total counts on the dishes, n_1 is the number of count on the dish with the 1st dilution, n_2 is the number of count on the dish with the 2nd dilution, n_3 is the number of the count on the dish with the 3rd dilution, n_i is the number of count on the dish with the i dilution, d is the dilution for the first count and X is the total microbial count /1mL.

2.4.5. Total acid and sugar contents

Total acid was determined by neutralization with NaOH 0.1N using color indicator of phenolphthalein (Pham Van So and Bui Thi Nhu Thuan, 1991).

Sugar content was determined according to Bertrand method using Fehling A and B (Pham Van So and Bui Thi Nhu Thuan, 1991).

2.4.6. Pectin content

Pectin content was determined by measurement of pectate calcium (Pham Van So and Bui Thi Nhu Thuan, 1991). 20 g of sample was added and mixed with 100 mL NaOH 0.1 N for hydrolyzing at 28°C in 7 hours. Then, 50 mL of acetic acid 0.1 N was added, mixed and incubated at 28°C for 5 min, and precipitated with 50 mL of $CaCl_2$ 1.0 N at 28°C for 1 hour. After boiling for 5 min, the precipitant (pectat calcium) was filtered and dried on the filter paper. The precipitant was washed with the boiling water until no remain of Cl^- by testing the drain water with $AgNO_3$ 1.0%. After washing, the precipitate on the filter paper was dried until the weight remained unchanged.

The content of pectin was calculated as the following equation:

$$pectin = \frac{m * 100 * 0.92}{m_s} \qquad [6]$$

Where, m is the weight (g) of pectate calcium (precipitant), 0.92 is conversion factor from pectat calcium to pectin, m_s is the weight (g) of sample.

2.4.7. Pasteurization value calculation

Product has pH much less than 4.5, so-called acidic products, hence, food poisoning organisms of the type *Clostridium botulinum* do not germinate. Consequently, it is only necessary to inactivate molds and yeasts. This can be done at much lower temperatures, with the result that the F_0-values are very low, since the lethal rate at a temperature of 80◦C is 7.76 × 10^{-5} min^{-1}. A more practical unit for quantifying the lethal effect of this type of process is the pasteurization unit *PU* (Holdsworth and Simpson 2007) given by

$$PU\ {}^{z}_{T_{ref}} = \int_0^t 10^{\frac{(T - T_{ref})}{z}} dt \qquad [7]$$

Where t is the time, T is temperature of the product, T_{ref} is the reference temperature, z is the thermal destruction rate analogous. In this study, with the pH = 3, the Sim syrup has to achieve the *PU*-value higher than 5 min using the T_{ref} = 85°C and z = 8.3°C (Ly Nguyen Binh and Nguyen Nhat Minh Phuong, 2011; Weemaes, 1997).

2.5. Statistical analysis

Response surface methodology (RSM) is an effective statistical method based on a multivariate non-linear model, and has been widely used for optimizing complex process variables (Mundra et al., 2007). Using Statgraphics 15, RSM was used to describe and optimize the extraction of anthocyanins from Sim crudes.

3. RESULTS AND DISCUSSION

3.1. Composition of Sim fruit

In this study, the sugar content (27.23%), the total acid (0.76%) and pectin (2.76%) of whole sim fruit from Mang Den, Kom Tum was higher those from Phu Quoc, Kien Giang (Nguyen Thi Ngoc Ngan, 2009). The contents were different due to effect of growing conditions. However, the anthocyanin concent (75.46mg/100g) in whole sim fruit from Mang Den, Kom Tum was lower than that (160mg/100g) from Thai Nguyen and Hai Duong (Lai Thi Ngoc Ha et al., 2013). Beside of growing conditions, the method analysis might contribute to the difference of anthocynin concentration.

Table 1. Composition (/100g dry weight) of Sim fruit

Composition	Content
Sugar (g)	27.23 ± 0.25
Total acid (g)	0.76 ± 0.01
Pectin (g)	2.76 ± 0.07
Anthocyanin (mg)	75.46 ± 0.73

3.2. Optimization of concentration of pectinase, temperature and time for extraction of Sim juice.

Extraction is an important step to gain high yield of juice containing high concentration of soluble solid concentration and high concentration of anthocyanins. However, Sim crudes with high concentration of pectin are too turbid and viscous which is difficult to filter and collect juice. Using pectinase to break down pectin in the cell wall of fruit, the filtrate would have more yield (Nadeem, 2009), high concentrations of soluble solid and anthocyanins.

Optimization of pectinase concentration, temperature and time for yield in the filtrate

The surface response shows effects of temperature, time and pectinase enzyme on the yield of the filtrate (Figure 1).

There was significant difference of the filtrate yields between different pectinase concentration, temperature and time. When the incubation temperature increased upto 40°C, the filtrate yield increased. Then the yield went down when the temperature was higher 40°C. This could be explained that the pectinase enzyme hydrolyzed pectin of the fruit cell wall to release more juice and reduced the viscous of the crudes to improve filterability (Nguyen Trong Can et al., 1998; Viquez et al., 1981). It is also reported that pectinase enzyme breaks down the link between pectin and cellulose of the cells and tissues to release the soluble substrates (sugar, acid, vitamin and anthocyanin) resulting increase of the yield. It was found that the hydrolysis of pectin could increase the extraction yield 10% more than the control (Wolfbrother, 2011).

The response surface could be fitted and described by the model with $R^2=0.97$ as shown below:

$$Yield = H (\%) = - 105.90 + 7.16X + 0.54Y + 171.85Z - 0{,}09X^2 - 0.01XY - 0.01Y^2 - 717.04Z^2 \quad [8]$$

Where, X is temperature (°C), Y is time (min), Z is pectinase concentration (%).

The optimal extraction conditions for the filtrate yield (62.3%) was pectinase enzyme of 0.1% at temperature of 40°C for 60 minutes. Nguyen Thi Ngoc Ngan (2009) reported the highest filtrate yield of sim crude from Phu Quoc was obtained when treated with pectinase concentration (0.8%) for 5 hours while the filtrate yield of was only 59.17% when sim crudes was treated with pectinase concentrate (0.6%) for 60 minutes.

Chauhan and Gupta (2004), and Le Viet Man et al. (2010) have emphasized the acceptance of any model with $R^2 > 0.75$. Therefore, the R^2 of this model and the following models were higher than 0.75 which was acceptable. Shahadan and Abdullah (1995) found that use of 0.04% pectinase enzyme (Pectinex Ultra SP-L, Novozymes A/S, Denmark) at 30°C with pH 3.4 was effective to reduce viscosity and improve filterability in the preparation of clarified banana juice.

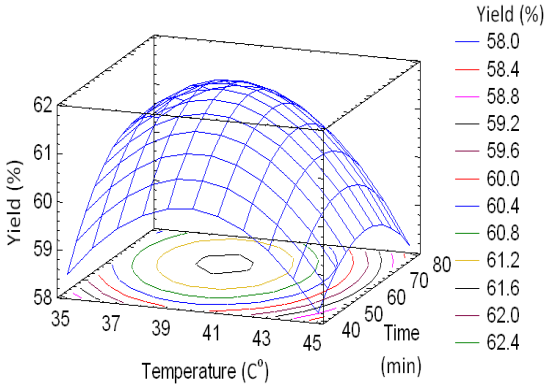

Figure 1. Response surface plots of the yield of the filtrate affected by incubation temperature and time

Using the Eq.[8], the values of yield were predicted from pectinase concentration, temperature and time. Figure 2 shows that the predicted yield and actual yield had high correlation coefficient of 0.95. It means that the model (Eq.[8]) could be used to describe the yield as a function of pectinase concentration, temperature and time in the extraction process.

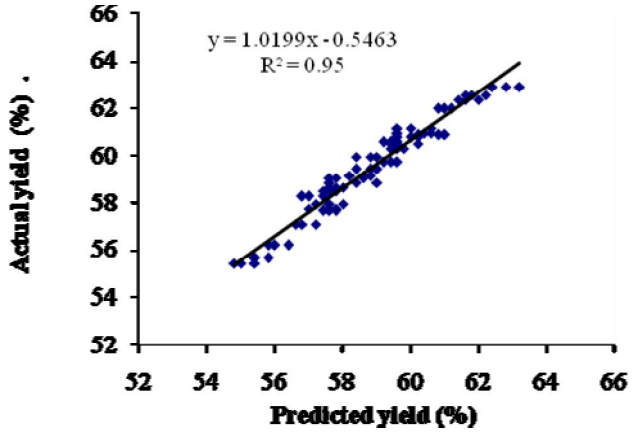

Figure 2. Relationship between the actual and predicted yields

Optimization of pectinase concentration, temperature and time for transmittance of the filtrate

The surface response shows effect of temperature, time and pectinase enzyme on the transmittance of the filtrate (Figure 3).

It is known that fruit juice contains a lot of substrates including pectins and protein which cause viscosity and stupidity of juice. The Pectinex can have pectinase and protease which break down the pectin and protein molecules to decrease viscosity and stupidity in fruit juice (Hoang Kim Anh, 2007). The filtration of fruit juice will be efficient, if the juice is pretreated with pectinase (Le Ngoc Tu, 2003)

There were significant differences of the transmittance of filtrate between different pectinase concentration, temperature and time. When the incubation temperature increased upto 40°C, the transmittance of the filtrate increased. Fruit juices contain colloids that are mainly polysaccharides (pectin, cellulose, hemicellulose, lignin and starch), protein, tannin and metals (Vaillant et al., 2001). The major problem is that the presence of pectin causes cloudiness during the preparation of fruit juices. The pectinase hydrolyses pectin and separate the complexes of pectin–protein resulting in flocculation of pectin and protein. Many studies reported that pectinase enzyme

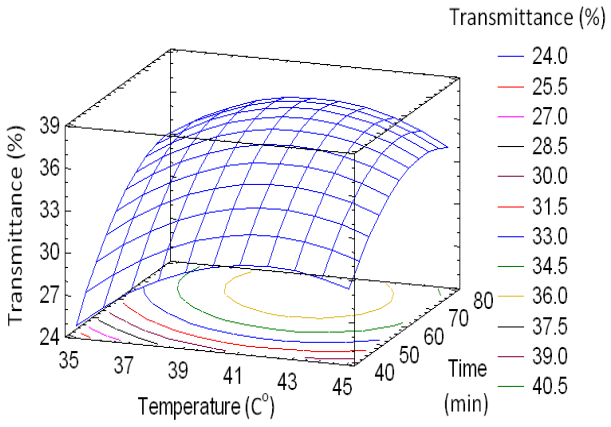

Figure 3. Response surface plots of the transmittance of the filtrate affected by incubation temperature and time

was used for clarification of fruit juices (Kashyap et al., 2001; Lee et al., 2001).

The response surface could be fitted and described by the model with $R^2=0.78$ as shown below:

Transmittance = -230.26 + 9.47X + 1.07Y + 612.65Z – $0.12X^2$ – $0.01Y^2$ – 1.01YZ – $2382.96Z^2$ [9]

Where, X is temperature (°C), Y is time (min), Z is pectinase concentration (%).

The optimal extraction conditions for the transmittance (38.3%) of the filtrate was pectinase enzyme of 0.1% at temperature of 40°C for 60 or 80 minutes.

Optimization of pectinase concentration, temperature and time for anthocyanin concentration in the filtrate

The surface response shows effect of temperature, time and pectinase enzyme on anthocyanin concentration in the filtrate (Figure 4).

There were significant differences of the anthocyanin concentrations of filtrate between different pectinase concentration, temperature and time. When the incubation temperature increased upto 40°C, the anthocyanin concentrations of the filtrate increased.

The concentration of anthocyanin increased with concentration of pectinase enzyme. It is known that pectinase can be helpful to extract colorants (e.g., anthocyanin), tannin and other soluble solids (sugar and acid) to enhance the quality of juice (Le Ngoc Tu, 2003; Hoang Kim Anh, 2007; Tadakittisarn et al., 2007; Liu et al., 2012).

The response surface could be fitted and described by the model with $R^2=0.81$ as shown below:

Anthocyanin = -313.06 + 15.25X + 1.25Y + 503.07Z - $0,19X^2$ - $0,01Y^2$ – $1971.41Z^2$ [10]

Where, X is temperature (°C), Y is time (min), Z is pectinase concentration (%).

The optimal conditions for anthocyanin concentration (68.52 mg/L) in the filtrate extracted from the whole sim fruit was pectinase enzyme of 0.1% at temperature of 40°C for 60 minutes. Liu et al. (2012) found that the optimal conditions for extracting anthocyanins from the fruit skin of downy rose-myrtle (sim fruit) were 64.38 °C, 116.88 min, 15.7:1 liquid-solid ratio, with the corresponding anthocyanin content = 4.345 mg/g. The reasons can be that they studied the skin of sim fruit which contains higher content of anthocyanin.

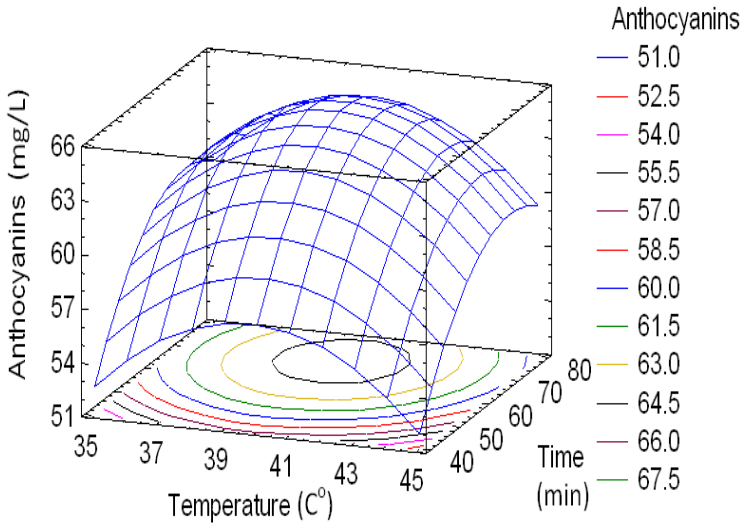

Figure 4. Response surface plots of the anthocyanin concentration of the filtrate affected by incubation temperature and time

3.3. Effects of pasteurization on quality of syrup and loss of anthocyanin

3.3.1. Effects of pasteurization on safety

Food in the cans or bottles has to be sterilized or pasteurized to inactivate enzymes and microorganisms for safety and preservation (Nguyen Trong Can and Nguyen Thi Le Ha, 2009). The sim syrup with the pH of 3.6 was treated thermally with the T_{ref} = 85°C and z = 8.3°C (Ly Nguyen Binh and Nguyen Nhat Minh Phuong, 2011; Weemaes, 1997). The temperature profiles of Sim syrup heated at 85°C shown on Figure 5 are representative for pasteurization of all samples in this study. These temperature profiles of Sim syrup of the same heating temperature (85°C) were heated at different holding times.

The temperature profiles at 80, 85 and 90°C were used to calculate PU-values of pasteurization process (PU = $PU_{coming\ up}$ + $PU_{holding}$ + $PU_{cooling}$) using [Eq.7]. The PU-values and total microbial counts of the pasteurized Sim syrup are shown in Table 2.

The longer holding times were, the higher PU-values and the lower total counts were. If the Sim syrups were pasteurized at 85 90°C for 2 6, the PU-values would be 7.8 40 higher PU-value = 5 (Ly Nguyen Binh and Nguyen Nhat Minh Phuong, 2011; Weemaes, 1997) and the sim syrups would be safe with the total microbial count = 0. However, the higher PU-values were the more loss of anthocyanin and the lower sensory values.

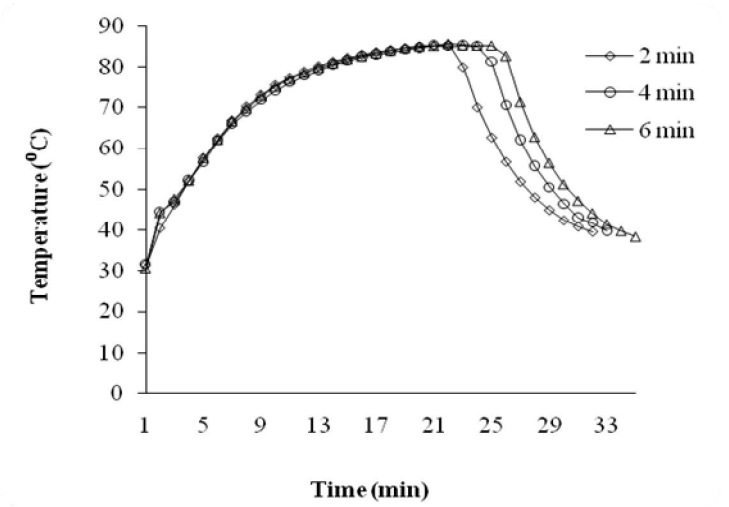

Figure 5. Temperature profiles of Sim syrup pasteurized at heating temperature of 85°C with holding times for 2, 4 and 6 minutes

Table 2. Effects of pasteurization on PU-values with z = 8.3 & T_{ref} = 85°C and total microbial counts

Product temperatures (°C)	Holding times (min)					
	2		4		6	
	PU-value	CFU/g	PU-value	CFU/g	PU-value	CFU/g
80	1.86	$8.2x10^1$	2.21	$9.4x10^2$	3.15	$5.0x10^1$
85	7.82	$5.7x10^1$	9.18	-	11.07	-
90	20.98	-	33.06	-	40.33	-

Note: '-', no microbial counts.

3.3.2. Effects of pasteurization on loss of anthocyanin

Pasteurization improves the safety and the shelf life of Sim syrup product. However, anthocyanin is degradable due to heat treatment during pasteurization. Anthocyanins degrade easily to form unacceptable browning compounds during thermal process (Torskangerpoll and Andersen, 2005; Liu et al., 2013).

The thermal process for Sim syrup was applied at 85°C for 4 min to obtain $PU_{85}^{8.3}$ = 9 min, no total microbial counts and high sensory values. The $PU_{85}^{8.3}$ = 9.18 min for sim syrup with pH = 3.5 meets requirement for the juice product (Holdsworth and Simpson, 2007; Weemaes, 1997). If the product is heated with lower $PU_{85}^{8.3}$ = 9.18 min, the product will not be safe. If the product is heated with higher $PU_{85}^{8.3}$ = 9.18 min, the overcooking will cause high loss of anthocyanin and high waste of electricity and time.

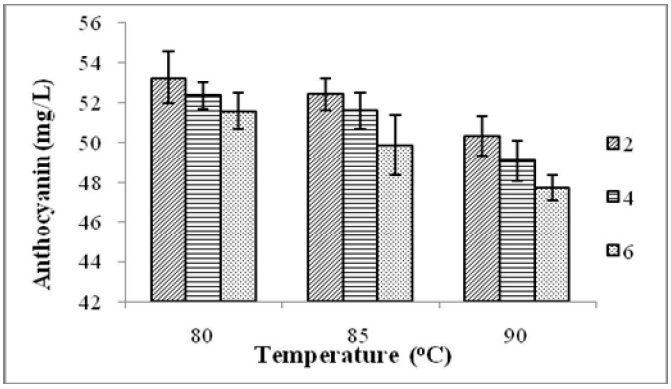

Figure 6. Change of anthocyanin concentration with temperature and time during pasteurization

4. CONCLUSION

Pretreatment of Sim crudes by pectinase could be described by models for yield, transmittance and anthocyanin concentration in the filtrate as a function of pectinase concentration, temperature and time. They could be optimized by using pectinase enzyme 0.1 % at temperature 40°C for 60 minutes to have the highest yield (62.93%), clarity (38.3%, T) and anthocyanin concentration (68.52 mg/L) in the Sim extract. Sim syrup was pasteurized at temperature 85°C with holding time of 4 min to have PU-value = 9.18 min, high safety and high anthocyanin concentration retained in the Sim fruit syrup. This product is a natural and nutritious fruit drink containing high energy, vitamins, and anthocyanin which is able to prevent chronic, and diabetes, cardiovascular disease and cancer. Production of sim syrup utilizeingthe wild fruit for new food product development is helpful to increase income for famers living in the highlands.

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