MINISTRY OF EDUCATION AND TRAINING
DA NANG UNIVERSITY

TA THI TO QUYEN

EXTRACTION AND APPLICATION OF ANTHOCYANINS FROM PURPLE SWEET POTATO IN FOOD PROCESSING

Major: Food Technology

Code: 62.54.01.01

SUMMARY OF TECHNICAL DOCTRINE THESIS

DA NANG – 2018

MINISTRY OF EDUCATION AND TRAINING

DA NANG UNIVERSITY

Supervisors: **1. Prof. Dr.Sc. Dao Hung Cuong**

2. Assoc. Prof. Dr.Sc. Truong Thi Minh Hanh

Reviewer 1: . Prof. Dr.Sc. Nguyen Thi Hien

Reviewer 2: Prof. Dr.Sc. Nguyen Hoang Loc

Reviewer 3: Prof. Dr.Sc. Tran Dinh Thang

The dissertation was protected at the doctoral thesis review meeting held at the University of Da Nang on July 9, 2018

The thesis can be found at:

- Information and Learning Resource Center, University of Da Nang

- Library, University of Da Nang

INTRODUCTION

1. Reasons for choosing the thesis: Nowadays, mostly synthetic pigments used in food are not really safe for consumers. Purple sweet potato (PSP) contains high levels of anthocyanin pigments. Besides of beautiful colors and food safety, anthocyanin is also a compound with many precious biological activities. PSP is currently grown in Vietnam and is mainly PSP HL491. However, there are very few studies on anthocyanins of PSP HL491. For those reasons, the research of the selected thesis was: extracting, determining the content, composition and biological activity of anthocyanins from PSP HL491 variety; Developing the process of extracting anthocyanins from PSP and using in food processing. This research, not only gets a natural pigment with a beautiful color and a lot of valuable biological activities for food, but also enhances the value of PSPHL491 variety in Vietnam.

2. Objectives of the research: Developed the process of extracting anthocyanins from PSP and proposed the standard for anthocyanin-rich pigments from PSP; And applying anthocyanin pigments from PSP to food processing.

3. Research content: Selection, processing PSP raw materials to get anthocyanins; Build the process of extracting anthocyanins from PSP and fermented ethanol PSP residue; Identification characteristics and composition of anthocyanins from PSP HL491 variety; Applied anthocyanin pigments from PSP to food processing.

4. Scientific significance: Introduce the method of selection and treatment of PSP raw for high anthocyanin content; determine the conditions of extracting anthocyanins from PSP for higher content and color level; Determination of color fastness, composition of anthocyanins and biological activities of anthocyanins from PSP

HL491; Develop the process of extracting anthocyanins from PSP to natural pigment using in food technology; Proposed a standard for anthocyanins-rich pigments from PSP as a coloring additive in food technology.

5. Practical meaning: Increasing in value of PSP HL491, grown in Vietnam, contributing to farmers' income, extracting pigment from PSP and applying to food technology. The pigment from PSP replaces artificial colors which is always a potential risk to the health of consumers; Create natural pigment from abundant, cheap materials; Produces anthocyanins-rich foods as a functional food that has anti-aging effects, anti-cancer, etc.

6. The composition of the thesis: The thesis consists of 143 pages, including 38 tables and 68 photographs. Introduction, 04 pages; Conclusions and recommendations, 03 pages; Published works, 01 page; Reference, 13 pages. The main content of the dissertation is divided into three chapters: Chapter 1. Document overview, 29 pages; Chapter 2. Materials and Methods, 18 pages and Chapter 3: Results and Discussion, 75 pages.

CHAPTER 1. DOCUMENT OVERVIEW

1.1. Anthocyanins

Anthocyanins are compounds of anthocyanidin (cation flavylium, or aglycon) (Figure 1.1) with sugar (which may bind to organic acids in the case of acylated anthocyanins).

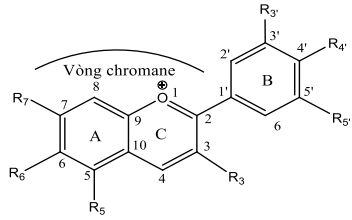


Figure 1.1. Basic structure of aglycon

Difference between anthocyanins is determined by number and position of hydroxyl group in molecule, methylation level of the hydroxyl group, nature and location of sugar groups attached to the molecule, nature and number of acid groups attached to the molecule. Anthocyanins is the largest water-soluble pigments in the plant. Anthocyanins is capable of absorbing light rays in the visible area with maximum absorption at 510 - 540 nm. Color fastness of anthocyanins depends on structure, pH, temperature, oxygen, light, enzyme, copigmentation, etc. Anthocyanins are compounds that had been shown to be biologically active and effective against some diseases such as anti-aging, anti-cancer, risk reduction of cardiovascular disease, etc; However, depending on the type of anthocyanins, biological activities may be expressed in varying degrees. At present, anthocyanins are used in food processing mainly in two areas of functional food production and coloring additives for food.

1.2. Purple sweet potato

PSP with scientific name is *Ipomoea batatas L.*,There are many varieties of PSP in the world. In Vietnam there are currently two popular PSP varieties, HL491 and Murasakimasari.

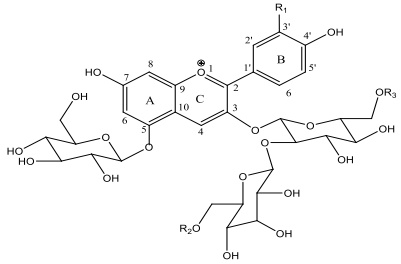


Figure1.9. Anthocyanin structure of PSP

The different anthocyanins of PSP are due to the different

groups R1, R2, R3. The PSP varieties with different composition and content of anthocyanins, PSP has the deeper color, the higher of anthocyanin content.

1.3. Extraction of anthocyanins from plants

The most suitable solvent for extraction of anthocyanins from plants is a mixture of alcohol/water and acidified to increase polarity. The most commonly used anthocyanins purification method is to use a solvent to separate the fraction or extract by column iron..

1.4. Overview of research on anthocyanins from PSP

In the world, studies on anthocyanins from PSP mainly focus on extraction conditions, content, color fastness, structure, antioxidant activity and anti-cancer of anthocyanins from PSP varieties. However, there is no research conducted on development of the extraction of anthocyanins process from PSP and its use in food. In Vietnam, mostly studies of anthocyanins are from mulberry, purple cabbage. Studies of PSP mainly focus on processing products from PSP. Studies on the composition, characteristics of anthocyanins from Vietnam's PSP HL491 had been still deficient.

CHAPTER 2. MATERIALS AND METHODS

2.1. Materials: The main raw material used for the study was PSP, named *Ipomoea batatas L.*, which used PSP HL491 variety. There were also some auxiliary materials such as: Spritase HiTaA 17105 L and Spritase GA 14400 L of Celina Choynowska - Poland, Yeast *S. cerevisiae* RV100 of Angel Group - China; Maltodextrin of Roquette - France; *S. aureus, E. coli, A. niger, S. cerevisiae* of Technical Center for Standards, Metrology and Quality 2.

2.2. Chemicals and research equipment:

2.2.1. Chemicals: The chemicals used in the research were: DPPH,

Acid galic, Folin-Ciocalteu, Vitamin C, $Na_2HPO_4.12H_2O$, Acid citric, HCl, Acid acetic, KCl, Etyl axetat, v.v... were of analytical grade.

2.2.2. Equipments: Equipments used in the study such as: Mini B-290 Spray Dryer, LC-MS LCQ-Duo-Thermo System,BUCHI 200 vacuum rotator, UV-Vis Cary 60 spectrophotometer, MX50 moisture meter, Vortex IKA, Minolta-CR400 color measurement, Vacuum drying oven, etc.

2.3. Methods

- Physical and chemical methods: determination of moisture content by the MS 70 infrared rapid tester, determination of heavy metal content by atomic absorption spectrometry (AAS), determination of reducing sugar and starch content by Bertrand method, determination of protein content by Kjeldahl method, determination of total acid content by titrating method, determination of total phenolic content by mg of gallic acid/100g of raw material, determination of anthocyanin contentby differential pH method; Determination of antioxidant activity by DPPH method, purification of anthocyanins by column chromatography, determination of anthocyanins by LC-MS, etc.

- Biological methods: Determination of antimicrobial activity by agar diffusion method, identification of cancer cell toxicity by SRB method.

- Mathematical methods: Experimental planning for Level II of Box and Hunter, optimization of multi-objective functions by forbid area elimination, solving the problem of maximizing the objective function by the Excell-solver program, processing of empirical data.

- Product sensory evaluation: the sensory evaluation method is used to select the most preferred color.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Selection and processing of PSP raw materials

3.1.1. Select PSP-growing areas: To choose the PSP HL491 for high levels of anthocyanins, conducted a survey anthocyanin content of 3 PSP-growing areas were: Ha Lam, Thang Binh, Quang Nam; Binh Hoa, Krong Ana, Dak Lak and Thanh Dong, Binh Tan, Vinh Long. The results of PSP in Vinh Long had the highest anthocyanin content, followed by Dak Lak, PSP in Quang Nam with the lowest anthocyanin content.

3.1.2. Influence of PSP size

The PSP HL491 of Vinh Long was classified into three sizes: large, medium and small. The anthocyanin content of the three PSP were determined and no significant differences in the anthocyanin content were observed. Thus, the size of PSP did not significantly affect the anthocyanin content.

3.1.3. Some of chemical components of PSP raw material

Table 3.1. Some of chemical components of PSP HL491 in Vinh Long

Component		***Content***
Moisture (% of mass of material)		67,83 ± 0,11
Protein (% of mass of material)		1,01 ± 0,04
Starch (% of mass of material)		26,46 ± 0,14
Reducing sugar (% of mass of material)		1,87 ± 0,08
Cellulose (% of mass of material)		1,17 ± 0,08
Ash (% of mass of material)		1,13
Heavy metal (mg/kg)	As	no detect (<0.05)
	Hg	no detect (<0.05)
	Pb	no detect (<0.05)
	Cd	no detect (<0.05)
Color index	L*	32,13 ± 0,92
	a*	8,69 ± 0,50
	b*	1,01 ± 0,08

3.1.4. Loss of post-harvest PSP: Post-harvest PSP losses were strong. The loss of anthocyanins was greater than of weight and starch. The PSP dumped at room temperature 20-30°C, after 6 weeks the proportions of weight, starch and anthocyanins reduced to 75.74%, 97.74% and 51.67%, respectively; The PSP contained in perforated cartons at room temperature reduced the loss, after 6 weeks the proportions of weight, starch and anthocyanins reduced to 85.67%, 98.01% and 61.65%, respectively;Storage at cold temperatures in perforated PE bags significantly reduced post-harvest PSP losses, after 6 weeks, the proportions of weight, starch and anthocyanins reduced to 96,07%, 99,10% and 90,96%%, respectively.

3.1.5. Processing PSP raw materials to extract anthocyanins

a) Methods of processing PSP raw materials: The PSP was processed in 3 ways: (1) The PSP were grinded before extraction of anthocyanins (M1), (2) The PSP were soaked in solvent to medium small grinded and extraction of anthocyanins (M2), (3) The PSP were cooked and making small into paste (M3) and extraction of anthocyanins. The M3 sample was the highest anthocyanin content, followed by the M2 sample and the M1 sample as the smallest.

b) Production of PSP powder to extract anthocyanins: PSP after being cooked was dried. Drying method and drying temperature were studied. Results of the PSP anthocyanins had high color fastness, after hot air drying at 70°C and sun exposure, the content of anthocyaninswere not significantly different and were lower than vacuum drying only 4%. To investigate the drying temperature, the PSP after being cooked was dried by hot air at 60-100°C, resulting in an suitable temperature for drying of PSP was at 80°C.

3.2. Extraction of anthocyanins from PSP

3.2.1. Extraction of anthocyanins from PSP paste

A) Conditions for extraction of anthocyanins from PSP paste: some

conditions for extraction of anthocyanins from PSP paste such as solvent type, type and concentration of acid for solvent acidification, ratio between solvents, temperature, time and material/solvent ratio were investigated. The appropriate conditions for extraction of anthocyanins from PSP paste were: solvent ethanol/water = 75/25 with 1% (v/v) HCl (36-38%), temperature 80°C, time 180 seconds and material/solvent = 1/16. The extraction of anthocyanins from PSP paste with these conditions reduced the time extraction and the anthocyanin content increased about 11% compared to the extraction method by Bridgers et al. (2010) in 3.1.1.

b) Optimization extraction conditions of anthocyanins from PSP paste: The extraction conditionsof anthocyaninfrom PSP paste were optimized with two targets: high anthocyanin content and high anthocyanin color. The influence range of the selected factors and the experimental conditions of the level II rotation method of Box and Hunter was presented in Table 3.6. Construct the experimental matrix for variables and experiments experiments according to the matrix, resulting in Table 3.7.

Where: y_1 is the content of anthocyanins, mg/100g dry matter and y_2 is the color of anthocyanins.

Constructing the mathematical model for y_1 and y_2, the resulting regression equation for the anthocyanin content and the coloration were as follows:

$y_1=207,186+3,661x_1+2,315x_2-4,838x_3-2,276x_1x_3-2,102x_1^2-1,888x_2^2-7,101x_3^2$

$y_2=4,193+0,196x_1-0,692x_3-0,174x_1^2-0,181x_2^2-0,218x_3^2$

The Excel-Solver program was used to find the optimum for each target function, resulting in:

y_{1max} = 211,283mg/100g dry matter when Z_1 = 81,6°C; Z_2 = 198,4 seconds; Z_3 = 69,7% (v/v)

y_{2max} = 4,797 when Z_1 = 75,6°C; Z_2 = 180 seconds; Z_3 = 59,1% (v/v).

Table 3.6. Experimental conditions of the level II rotation method

Factors	***Levels***					***Variable range (λ)***
	+α	***Upper +***	***Base 0***	***Lower -***	***-α***	
Z_1, °C	86,8	80	70	60	53,2	10
Z_2, seconds	230,5	210	180	150	110,5	30
Z_3, *%v/v*	91,8	85	75	65	58,2	10

Table 3.7. The matrix of the level II rotation method and experimental results

N		x_1	x_2	x_3	y_1	y_2
2^k	1	+	+	+	192,464	4,058
	2	-	+	+	205,038	4,549
	3	+	-	+	197,592	4,081
	4	-	-	+	209,293	4,514
	5	+	+	-	186,826	2,772
	6	-	+	-	189,829	3,076
	7	+	-	-	192,011	2,798
	8	-	-	-	195,074	3,012
2k	9	-α	0	0	195,653	3,353
	10	+α	0	0	207,347	4,086
	11	0	-α	0	198,595	3,739
	12	0	+α	0	205,614	3,661
	13	0	0	-α	194,929	4,757
	14	0	0	+α	179,797	2,434
n_0	15	0	0	0	206,088	4,111
	16	0	0	0	207,534	4,237
	17	0	0	0	208,216	4,140
	18	0	0	0	208,424	4,215
	19	0	0	0	207,638	4,183
	20	0	0	0	206,775	4,295

Utopian point with coordinates Y_{kt} (y_{1max}, y_{2max}) là Y_{kt} (211,283; 4,797). Select the lower limit of $y_1$was 205 (α_1 = 205; Select the lower limit of y_2 is 4,4 (α_2 = 4,4). From y_1 and y_2, the combinational target function was constructed R(X) = [r_1 (y_1) . r_2

$(y_2)]^{1/2} = [(y_1 - 205)/(211,283 - 205) * (y_2 - 4,4)/(4,797 - 4,4)]^{1/2}$. The Excel-Solver program was used to find the optimal function for the target function R (X) to be maximized, with the conditions: - 1,682 $\leq x_1$; x_2; $x_3 \leq 1,682$; $y_1 > 205$ and $y_2 > 4,4$.Result $Z_1 = 76,7°C$; $Z_2 = 192,5$seconds; $Z_3 = 66,2\%$ (v/v). At that time$y_1 = 209,174$ reaching 99,0% of the maximum anthocyanin content and $y_2 = 4,422$ reaching 92,2% of the maximum color. Thus, optimal conditions for the extraction of anthocyanins from PSP paste were: temperature 77°C; time 193 seconds and ethanol content was 66% (v/v).

* *Experimental test:* From the optimal conditions to conduct verification experiment. The extracted anthocyanins content was 208,035mg/100g dry matter and the color was 4.403, nearly equal results compared with calculations.

3.2.2. Extraction of anthocyanins from PSP powder

Some conditions for extraction of anthocyanins from PSP powder such as temperature and solvent may be referred to according to PSP paste. However, it was necessary to investigate the extraction time and material/solvent ratio, as a result of drying of the cell material, so that these two factors can be changed. Results of conditions for extraction of anthocyanins from PSP powder were: temperature 77°C; time: 8 minutes, ethanol: water was 66:34 with 1% HCl and material/solvent ratio (g/ml) was 1/33.

3.2.3. Comparison of anthocyaninextraction process from PSP paste and PSP powder

The anthocyanin extraction time from PSP powder was longer than PSP paste, but the anthocyanin content was not significantly different. The antioxidant activity of anthocyaninextract from PSP paste and PSPpowder was compared. The half-inhibition concentration (IC_{50}) of anthocyanin extract from PSP paste and PSP powderwere not significantly different (IC_{50} of PSPpaste 5.841 μg/ml and PSP powder 5.830 μg/ml). Thus, the extraction of anthocyanins

from PSP paste and PSP powder gave similar anthocyanin levels and the antioxidant activity was also negligible.

3.2.4. Determination of anthocyanin content in PSP: To determined anthocyanin content in PSP, extract several times until colorless residue. Results total anthocyanin contents in PSP HL491 grown in Binh Tan, Vinh Long were 243mg/100g dry matter (73mg/100g raw material), which was relatively high compared to some PSP varieties in other countries.

3.2.5. Purifying anthocyanin extracts from PSP

a) Neutralize the antho extract from PSP

After extracting and concentrating the solvent, neutralize HCl in the extracts with solution Na_2CO_3 20% until the extract from red to violet, received the crude anthocyanin extract (CT). The volume of Na_2CO_3 20% used to neutralize 100 ml of the extract from PSP 30°Bx was 11.2 ml.

b) Purified anthocyanin extracts from PSP

CT extracts were purified by column chromatography to obtain pure anthocyanin extract (LS). Analyze some indicators of CT and LS extract, the results were shown in Table 3.10.

Table 3.10. some indicators of CT and LS extract

Các chỉ tiêu		***CT***	***LS***
Total anthocyanins/100g raw material		$208,04^a \pm 2,03$	$169,66^b \pm 2,43$
Total phenolic, mg AG/100graw material		$923,87^a \pm 14,22$	$315,18^b \pm 10,43$
Total acid content (according to citric acid, g/100ml)		$208,04^a \pm 2,03$	$169,66^b \pm 2,43$
Colors		$11,45^a \pm 0,31$	$8,66^b \pm 0,25$
Percent polymer color, %		$15,30^a \pm 0,13$	$10,46^b \pm 0,76$
Color index	L*	21,98 ± 0,94	25,79 ± 4,70
	a*	1,00 ± 0,48	0,33 ± 0,20
	b*	1,12 ± 0,64	0,71 ± 0,39

LS extract had total phenolic content and percent color of polymers decreased. This proves that the purification process removes many non-anthocyanins from the extract. However, the purification process reduced the anthocyanin efficiency and the color of anthocyanin decreased.

3.3. Characteristics and composition of PSP anthocyanins

3.3.1. Spectral characteristics of PSP anthocyanins

LS extract was further purified by column chromatography and scanned at a wavelength of 260-760nm. Results in the ultraviolet (260-400nm) absorption at pH 1 and 4.5 were almost coincided. However, in the visible region (λ> 400nm) the absorbance at pH 1 and 4.5 was significantly different, the maximum wavelength absorbed at pH 1 was 522nm.

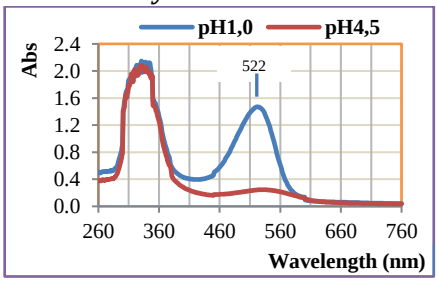


Figure 3.27. UV-Vis Spectrum of PSP anthocyanins in buffer solution pH 1 and pH 4.5

3.3.2. Anthocyanin compositions of PSP

LS extract after purification by column chromatography was analyzed on Agilent-USA HPLC-MS-DAD system.

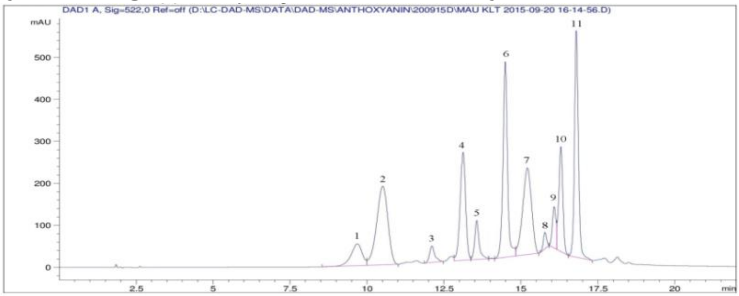


Figure 3.28. HPLC chromatography of anthocyanins from PSP

The results in Figure 3.33 anthocyanin extracts at least 11 peaks had high optical absorption at the maximum absorption wavelength of anthocyanin. To test whether these peaks were anthocyanins or not, the MS analysis of the obtained peaks was performed. Based on mass spectrometry MS with ChemSketch software and reference the published results had identified 11 types of anthocyanins from PSP HL491 (Table 3.12).

Table 3.12. Identification of anthocyanin compounds in PSP

Peak	***retention time(min)***	***m/z***			***Compound identity***	***Area, %***
		MH^+	***aglycon***	***Other fragment ions***		
1	9,686	773	287	449, 611	Cyanidin 3-sophoroside-5-glucoside	4,76
2	10,530	787	301	463, 625	Peonidin 3-sophoroside-5-glucoside	17,18
3	12,059	893	287	449, 731	Cyanidin 3-p-hydroxybenzoylsophoroside-5-glucoside	1,68
4	13,120	907	301	463, 745	Peonidin 3-p-hydroxy benzoylsophoroside-5-glucoside	11,58
5	13,627	949	287	449, 787	Cyanidin 3-(6"-feruloyl sophoroside)-5-glucoside	3,40
6	14,541	963	301	463, 801	Peonidin 3-(6"-feruloyl sophoroside)-5-glucoside	17,09
7	15,242	949	301	463, 787	Peonidin 3-caffeoyl sophoroside-5-glucoside	15,00
8	15,752	1111	287	449, 949	Cyanidin 3-(6"-caffeoyl-66"'-feruloylsophoroside)-5-glucoside	1,03
9	16,043	1111	301	463, 949	Peonidin-dicaffeoylsophoroside-5-glucoside	2,90
10	16,331	1069	301	463, 907	Peonidin 3-caffeoyl-p-hydroxy benzoyl-sophoroside-5-glucoside	8,27
11	16,838	1125	301	463, 963	Peonidin-caffeoyl-feruloyl sophoroside-5-glucoside	17,07

3.3.3. Effect of pH and temperature on color fastness

a) Effect of pH on color fastness of PSP anthocyanins

The results in Figure 3.37 show that at pH = 3, the PSP anthocyanins had a high color fastness, while at pH 5-6, the anthocyanins were less stable. So when applying PSP anthocyanins as a coloring additive in food processing should be used for products about pH 3.

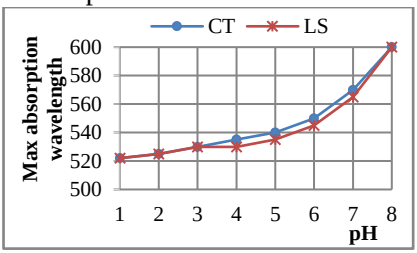


Figure 3.36. Effect of pH on maximum absorption wavelength

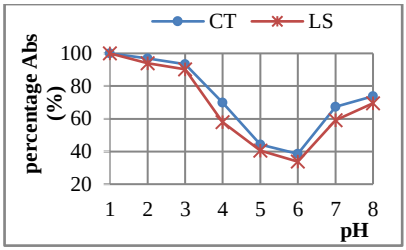


Figure 3.37. Effects of pH on color fastness

b) Effect of temperature on color fastness of PSP anthocyanins

The two temperatures commonly used in storage and food processing were chosen to be 30°C and 90°C, and at pH 3, 5, 7. Results for storage time at 30°C and heat 90°C, anthocyanins from PSP at pH 3 were more stable than pH 5 and 7. At the same time, CT extract had higher color fastness than LS extract.

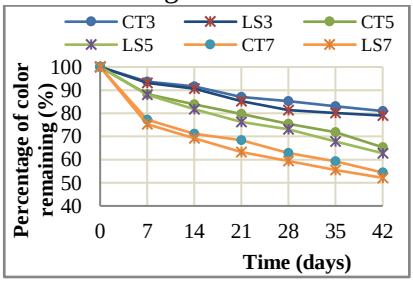


Figure 3.38. Color fastness of anthocyanins over time at 30 ° C

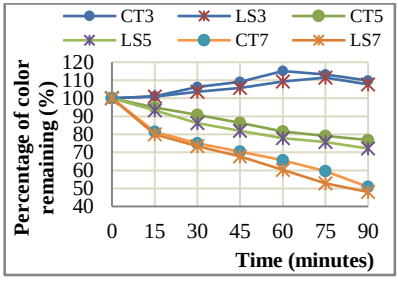


Figure 3.39. Color fastness of anthocyanins over time at 90°C

3.3.4. Some biologically active of PSP anthocyanins

a) Antioxidant activity of PSP anthocyanins

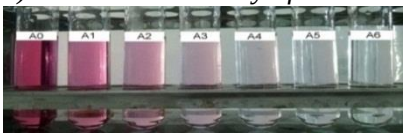


Figure 3.40. Color of CT extract after dilution

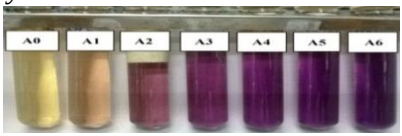


Figure 3.41. Discoloration of DPPH by antho concentration

The IC_{50} value of the CT extract was 5.841 µg/ml, the LS extract was 7.107 µg/ml and the vitamin C was 5.332 µg/ml. Thus, the antioxidant activity of CT extract was higher than that of LSextract and was close to that of vitamin C.

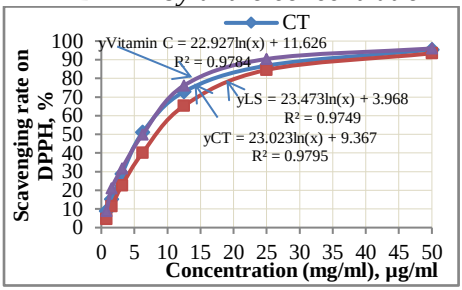


Figure 3.42. DPPH radical-scavenging activity of CT, LS và vitamin C

b) Antimicrobial activity of PSP anthocyanins

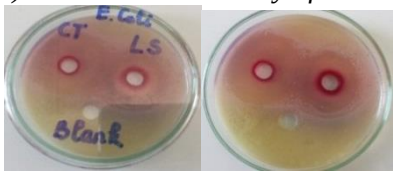


Figure 3.43. Antimicrobial activity of PSP antho to E. coli

Figure 3.44. Antimicrobial activity of PSP anthoto S. aureus

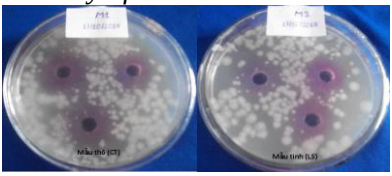


Figure 3.45. Antimicrobial activity of PSP anthoto A. niger

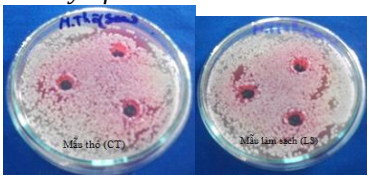


Figure 3.46. Antimicrobial activity of PSP anthoto S. cerevisiae

Both CT and LS extract had strong antimicrobial activity, the strongest being *E. coli*, followed by *S. aureus* and finally *A. niger*. However, no activity against *S. cerevisiae*. Minimal concentrations were capable of inhibiting ≥ 90% (MIC) of the microbial load of CT extract lower than LS extract.

c) Anti-cancer activity of PSP anthocyanins

CT extractwere tested for cytotoxic activity of liver cancer and lung carcinoma. Results of CT extract had toxic activity against these two cancer cell lines in vitro and the cytotoxic activity of lung carcinoma was greater than that of liver cancer.

Table 3.15. Anti-cancer activity of CT extract

Mẫu	***IC_{50} (μg/ml)***	
	Hep-G2 cell line (liver cancer)	***LU-1 cell line (lung carcinoma)***
Control (+)	0,29	0,31
CT	10,61	8,76

3.4. Produced and proposed standard for anthocyanin-rich pigments from PSP

3.4.1. Produced anthocyanin-rich pigments from PSP

CT extracts with color, color fastness, antioxidant activity and antibacterial are higher than LS extracts should be selected to study to create pigments. From CT extracts, we studied two forms of pigments, liquid and powder.

a) Preparation of liquid colorants: The crude anthocyanin extracts from PSP were concentrated at 60°C, 72mmHg until the concentration ≥ 60°Bx, obtaining a liquid color (LĐ).

b) Spray drying mode to obtain color powder:

Some factors affecting the spray drying process were: maltodextrin content as carrier, spray air temperature and injection

rate were surveyed. The results of suitable spray conditions for anthocyanin extracts from PSP were: maltodextrin 30%, temperature 160-180°C, spray flow 20-30ml/min.

c) Develop an extraction of anthocyanins process from PSP

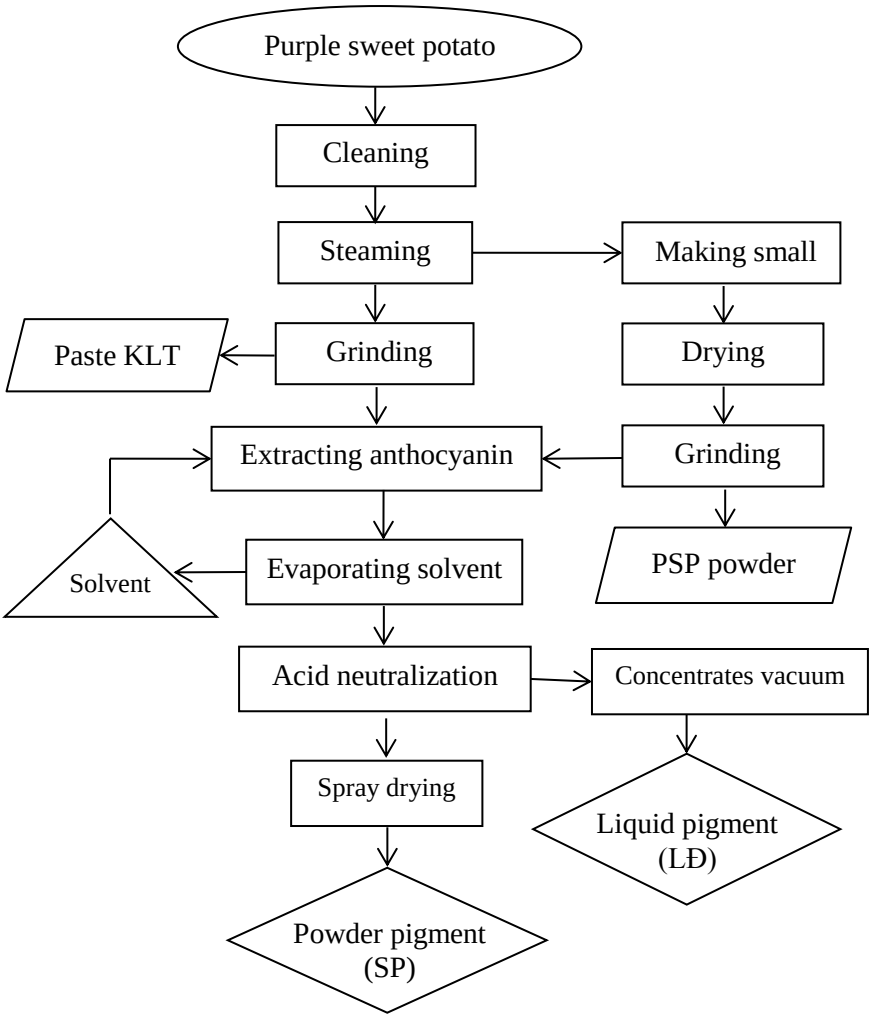


Figure 3.47. Process diagram of anthocyaninextraction from PSP

3.4.2. Ferment ethanol from PSP residue after anthoextraction

a) Identification of some indicators of PSP residue: PSP residue after extraction of anthocyanins was with 66.54% moisture content and 26.23% starch content.

b) PSP starch hydrolysis conditions

* *Water usage:* PSP was hydrolyzed by the enzyme α-amylase (Spritase HiTaA 17105 L) with a PSP residue/water ratio of 1/2, 1/3 and 1/4. As a result, the ratio of PSP residue/water = 1/3 was easy to hydrolyze and a concentration of 10.2°Bx.

* *PSP starch hydrolysis time:* PSP starch was liquefied by enzyme α-amylase, after 40 minutes liquefaction process is finished. Then, PSP starch was glucose-forming by enzyme glucoamylase, after 150 minutes maximum reduction sugar was 8.35%.

c) Ethanol fermentation time fromPSP residue: S. cerevisiae powdered yeast was activated, then added to aqueous solution of 10% (v/v) for fermentation. After 72 hours of fermentation, the maximum ethanol content in ripe vinegar was 6.82% (v/v).

Thus, the time of liquefying, glucose-formingand ethanolfermentation were 40 minutes, 150 minutes and 72 hours, respectively. After fermentation, ripe vinegar was distilled and refined to recover ethanol products.

3.4.3. Test production of anthocyanin pigment and fermented ethanol PSP residue

Two anthocyanin-rich pigments liquid and powders were produced experimentally, the amount of raw materials used for each type was 3kg. PSP residue after extraction of anthocyanins was fermented ethanol. Calculated the cost of consuming raw materials for extraction of anthocyanins process from PSP and fermented

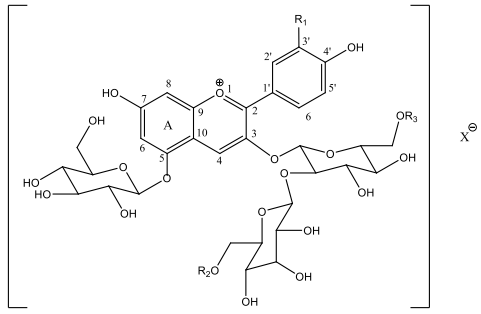
ethanol PSP residue. The result of material price of liquid pigment was 15,216 VND/100ml and powder pigment was 22,270 VND/100g. Some quality indicators of the pigment were determined, resulting in powder pigment (SP) with moisture 5.76%, anthocyanin 478.24mg/100g, total acid 2.82mg/100g, and liquid pigment (LD) with concentration 60.03%, anthocyanins 407,21mg/100g, total acid 2.48mg/100ml. Two pigments were completely soluble in water and heavy metals were not detectable.

3.5. Proposed standard for anthocyanin-rich pigments from PSP

The quality criteria for anthocyanin-rich pigments from PSP were given in Table 3.24, based on: food safety indicators; the results of the analysis of the original quality criteria and after 6 months of preservation; and characteristics, composition of anthocyanins from PSP.

Table 3.24. Technical requirements of anthocyanin-rich pigments

TECHNICAL REQUIREMENTS OF ANTHOCYANIN-RICH PIGMENTS FROM PURPLE SWEET POTATO	
1. Other name, index	Purple sweet potato extract INS 163 (vi) ADI = 0-2,5mg/kg body weight.
2. Define	The pigments were obtained from the cooked PSP, containing the dissolved ingredients of PSP.
Chemical name	The main colorants are anthocyanins, glucosides of the anthocyanidin peonidin and cyanidin.
Chemical formula	Cyanidin 3-sophoroside-5-glucoside: $C_{33}H_{41}O_{21}X$ Peonidin 3-sophoroside-5-glucoside: $C_{34}H_{43}O_{21}X$

	X là H, acid caffeic, acid ferulic, acid p-hydroxybenzoic
Molecular formula	 R_1 = OH: cyanidin, R_1 = OCH_3: peonidin R_2, R_3: H, acid caffeic, acid ferulic, acid p-hydroxybenzoic
3. Sensory	purple, liquid or powder
4. Function	Create color
5. Technical requirements	
5.1. Qualitative	
Solubility	Water soluble
Spectrum	The extract at pH 1 gives a maximum absorption wavelength at 522 nm
Color reaction	Qualified
5.2. Purity	
Alkali colorants	Qualified
Other acid colorants	Qualified
lead	Not more than 2 mg/kg
5.3. Quantitative	
Content of anthocyanins	Not less than 340mg/100ml with liquid pigment and 350mg/100g with powder pigment.
Moisture	For powder color does not exceed 10%
Concentration	For powder colors not less than 60%

We have sent color samples and a standard proposal for anthocyanin rich products from KLT to Warrantek Company. The company's appraisal results are the technical requirements in the basic standards that match the results of the analysis on color samples.

3.6. Application of anthocyanin-rich pigments from PSP in food processing

3.6.1. Application of anthocyanin-rich pigments from PSP were a coloring additive in the production of marshmallows: The pigments were added at the end of marshmallows production process at 0.4%, 0.6%, 0.8% and 1.0%. Marshmallows products were evaluated sensitively on color by taste test on 50 people. The result of marshmallow samples with 0.8% LD pigments were the most selected. Marshmallows with anthocyanin pigments from PSP had a beautiful red color, moisture 11.23% and reducing sugar 36.81%, quality standard TCVN 5908: 2009. Especially, the content of anthocyanins were 2.21mg/100g. Analysis of anthocyanins in marshmallows, results maximum absorbance wavelength (λ_{max}) of anthocyanin extract from marshmallow and from PSP were overlapped. This proves that the marshmallows production process did not change the structure of anthocyanins and anthocyanins retain their biological activity.

3.6.2. Producing anthocyanin-rich-powder drink from PSP as a functional food: The saccharose content added to drinking anthocyanins-rich powder from PSP was 10% and the citric acid content was 1.0g/liter. SP pigments was mixed with citric acid and saccharose to form beverages samples with levels of anthocyanins 10, 15, 20 and 25mg/100ml, then additional 10% saccharose and

citric acid 1,0g/liter. The beverages samples were evaluated sensitively with 50 members. As a result, the sample of anthocyanins 20mg/100ml were chosen by many people (27/50 people). 100g SP pigments with anthocyanin content of 478.24 ± 3,07mg/100g will mixed 2.391ml beverages with anthocyanin content 20mg/100ml. So the mixing ratio: SP pigments 100g; Saccharose sugar 239.1g and citric acid 2.4g. The total weigh of 341.5g will mix 2.391ml of beverages. Expected soluble anthocyanin-rich-powder drink packages when used will be mixed in 150 ml of water. So that the weigh for each package was 21g/package.

An anthocyanin-rich-powder drink production process from PSP was proposed in Figure 3.55.

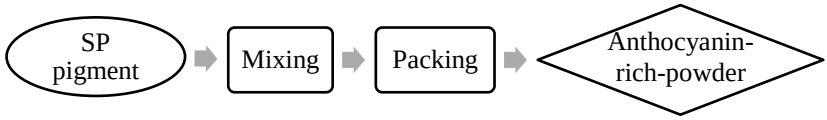


Figure 3.55. Process diagram of anthocyanin-rich-powder drink production from PSP

The anthocyanin-rich-powder drink was defined quality indicators. Sensory results had a quality score of 16.80, achieving good quality.The physical and chemical indicators: moisture 3.52%; Total sugar 73.33%; Total acid 0.92% and anthocyanins 140.91mg/100g. Microbiological criteria meet requirements of QCVN 6-2: 2010/BYT. Similar to marshmallow, anthocyanins in anthocyanin-rich-powder drink from PSP were analyzed. The maximum absorbance wavelength (λ_{max}) of anthocyanin extract from anthocyanin-rich-powder drink and from PSP were overlapped.

CONCLUSIONS AND RECOMMENDATIONS

a. Conclusions

1) The influence of some factors on antho content such as material size, postharvest losses, KLT processing, etc., was determined. Anthocyanin content of HL491 PSP in Vinh Long was 243 mg / 100g dry matter, which was higher than that of some PSP varieties in other countries.

2) Determined the optimal conditions for extracting anthocyanin pigments from PSP for high level of content and color. In other hand, raw pigments give higher level of color, antioxidant activity, microbial resistance than cleaned-form pigments.

3) Determined that there were at least 11 types of anthocyanins in the PSP HL491 variety. And it was confirmed that PSP HL491 was cultivated in Vinh Long with high antioxidant activity, resistance to *E. coli, S. aureus*, anti-cancer of the liver and lung in vitro.

4) Determined the conditions for the formation of two anthocyanin-rich pigments liquid and powders from KLT extracts. At the same time, the price of materials for the production process of liquid pigment and powder pigment.

5) Developed an anthocyanin extraction process and proposed the standard for anthocyanin-rich pigments from PSP.

6) Determination of KLT ethanol fermentation condition after extraction of anthocyanin.

7) Applied the anthocyanin extracted from PSP to the production of marshmallows, which conformed to standard TCVN 5908:2009.

8) Proposed an anthocyanin-rich-powder drink from PSP as a form of functional food with high anthocyanins content (140.91mg/100g).

B. Contribution of the thesis

1) A new method to treat post-harvest PSP for extraction of

anthocyanins was proposed to limit the postharvest losses as forming KLT power or KLT paste.

2) The first study on the HL491 KLT variety was cultivated in Vinh Long - Vietnam and found that the PSP HL491 had relatively high anthocyanin content and 11 types of anthocyanins with high bioactive activity. Especially, this is the first published anthocyanin from PSP that has anti-cancer of the liver and lung in vitro.

3) An anthocyanin extraction process from PSP were developed and a basic standard for anthocyanin-rich pigments from PSP was proposed as a coloring additive that currently not included in the food additives category.

4) Successfully applied anthocyanin-rich pigments from PSP instead of synthetic pigments to the production of marshmallows; At the same time, a new, anthocyanin-rich-powder drink from PSP was developed as a functional food that had anti-aging effects and prevents cancer.

C. Recommendations

1) Production of anthocyanin-rich pigments from PSP and fermented ethanol PSP residue should be researched on a pilot scale.

2) Research on the application of anthocyanin-rich pigments from PSP to the production of marshmallows in candy production facilities.

3) Production and construction of certification records that the anthocyanin-rich-powder drink from PSP was a functional food.

4) Study the solution to limit moisture absorption of anthocyanin-rich pigments and anthocyanin-rich-powder drink from KLT.

PUBLIC SCIENTIFIC ARTICLES

1. Ta Thi To Quyen, Huynh Thi Kim Cuc, Thai Thi Anh Ngoc (2013), "Study on antioxydant capacity of antho from purple sweet potato", Bài báo được trình bày tại hội thảo quốc tế *"Agricultural engineering and post-harvest technology for ASIA sustainability (AEPAS)"*, December 5-6, 2013, Hanoi, Vietnam, *Science technics publishing house*, pp. 436-442.

2. Ta Thi To Quyen, Huynh Thi Kim Cuc, Cu Thi Ngoc Thuy, Dao Hung Cuong (2014), "Extraction of Anthocyanins pigment from Purple sweet potato", *Journal of Science and Technology*, University of Da Nang, 11 (84), vol. 1, pp. 55-59.

3. Huynh Thi Kim Cuc, Tran Thi Thanh Man, Le Thi Thao Tien, Ta Thi To Quyen, (2014), "Laboratory production of anthocyanin-rich juice from purple sweet potato", *Paper presented at National Workshop on Food Science and Technology towards a Sustainable Food Industry and published in the Journal of Science and Technology*, Vietnamese Academy of Sciences and Technology, vol. 52 - No. 5C, pp. 372-378.

4. Ta Thi To Quyen, Huynh Thi Kim Cuc, Truong Thi Minh Hanh, Dao Hung Cuong (2015), "The effects of storage methods to the losses of post-harvest purple sweet potato", *Journal of Science and Technology*, Da Nang University, 7(92), pp. 93-97.

5. Nguyen Thi Hong Ngan, Huynh Thi Kim Cuc, Ta Thi To Quyen (2015), "An experimental study on laboratory-scale production of anthocyanins-rich powders from purple sweet potato" *Journal of Science and Technology*, Ministry of Agriculture and Rural Development, 2 (2), pp. 56-61.

6. Ta Thi To Quyen, Huynh Thi Kim Cuc, Truong Thi Minh Hanh, Dao Hung Cuong (2015), "Optimization for extraction conditions of anthocyanin pigments from purple sweet potato", *Journal of Chemistry*, Vietnamese Academy of Sciences and Technology, 6E1, 253. , pp. 205-209.

7. Ta Thi To Quyen, Huynh Thi Kim Cuc, Nguyen Thi Thu Thuy, Dao Hung Cuong (2016), "A*ntioxidant and antimicrobial activity of anthocyanin pigments from purple sweet potato*", Journal of Chemistry and Applied, 2 (34), pp. 75-79.

8. Ta Thi To Quyen, Huynh Thi Kim Cuc, Dao Hung Cuong (2017), Utility Solution " Method of extracting anthocyanin pigments from purple sweet potato", Property Gazette Industry of the Department of Intellectual Property, 346, A (01.2017), p. 347.