STUDY OF OPTIMAL EXTRACTION AND STABILITY OF THE EXTRACTED DYE FROM RED DRAGON FRUIT (*Hylocereus polyrhizus*) PEEL EXTRACTS USING ETHANOL EXTRACTION METHOD

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by

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Fourth Year – Graviton

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APPROVAL SHEET

This research paper herein entitled:

STUDY OF OPTIMAL EXTRACTION AND STABILITY OF THE EXTRACTED DYE FROM RED DRAGON FRUIT (*Hylocereus polyrhizus*) PEEL EXTRACTS USING ETHANOL EXTRACTION METHOD

Prepared and submitted by Jenny Wendy S. Balbuena, Marmelou G. Popes, and Arvin John E. Tejereso in partial fulfillment of the requirements in Science Research 2, has been examined and recommended for acceptance and approval.

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STUDY OF OPTIMAL EXTRACTION AND STABILITY OF THE EXTRACTED DYE FROM RED DRAGON FRUIT (*Hylocereus polyrhizus*) PEEL EXTRACTS USING ETHANOL EXTRACTION METHOD

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ABSTRACT

Industries using Red Dragon Fruit are starting to develop here in the Philippines. Production of the fruit is becoming vital. However, people thought its peel to be useless and thus considering it as a waste. This study aimed to utilize the peel as a natural dye source by finding the optimal condition in extracting the dye from the peel. The samples were blended at varying times (1, 2, 3, 4 and 5 min). The ethanol (80%) was evaporated at varying temperatures (25, 35, 45, 55 and 65°C). Highest betacyanin yield was obtained at 1 minute blending time. For the varying evaporating temperatures, highest yield was obtained at 65°C. Samples were then tested of its physico-chemical properties (light stability, pH and pH stability). The dye experienced an excessive degradation during the third day of light exposure. It reacted with basic environment showing a color change from red to yellow. The pH of the dye showed that the dye is slightly acidic with pH ranging from 4.51 to 4.86. Further studies should be conducted in the optimization of the results and by including other variables in finding the optimal condition. Nonetheless, results showed that utilization of the peel by extracting dye from it using ethanol extraction method is feasible.

Keywords: red dragon fruit, ethanol extraction method, peel, dye, optimal condition, stability

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CHAPTER 1

INTRODUCTION

A. Background of the Study

Dyes are commonly used to color clothes, hair and even food. They are divided into two main groups: synthetic and natural. Synthetic dyes are derived from several chemical compounds like copper sulfate, mercury, lead and sodium benzoate (as a preservative) that at certain amounts can trigger genetic problems and even mental problems (Institute for Agriculture and Trade Policy 2009). They are difficult to make because even a small difference in the suggested ratio of the chemical compounds like carboxylic acids and benzidine can yield to a different color like the Yellow 5 and Yellow 6. They can also trigger cancer at high amounts(Kobylewski and Jacobson 2010).Because of the harm that synthetic dyes possess there is a strong need from consumers for more natural products because it is saferand health beneficial. The rate of replacing synthetic dyes by natural product has been increasing because of the anxiety about proven harmful effects in food producing industries of artificial food dyes (Boyd 1998; Jackman and Smith 1996 as cited by Phebe and others 2009).

Natural dyes on the other hand, are derived from renewable resources. They are divided into two groups the lipid-based and water-based dyes. Lipid-based dyes can be used in clothing and in hair dyes because they can't easily be washed off. Water-based dyes are used in coloring foods because they are highly miscible. Sources for natural dyes are considered to be used because they posses certain coloring pigments like betacyanin, carotene, lycopene, lutein, bixin and many others. Plants are the sources of natural dyes but sometimes insects, algae, fungi and cyanobacteria are also used (Mortensen 2006).

One of thecommonly usedplant sources of natural dyes is the red beet. It is a commercially competitive source of natural dyes due to the presence of betalains which causes the vibrant color of the plant. It is mainly used for food coloring.Betacyanin, the red pigment, is the major component that occupies 95% of the red beet extract.However, these extracts have drawbacks like unfavorable earthy flavor causedby geosmine and pyrazine derivatives and possession of the carcinogenic compound nitrosamine (Esquivel and others 2007 as cited by Harivaindaran and others 2008).

With this situation, Harivaindaran and others (2008) said that there is a demand for an alternative source for betacyanin. Red dragon fruit is a perfect candidate as a substitute for red beet becauseit has a high potential to become as a new source of natural dye. Red dragon fruit dye contains the same betalain-forming enzymes and same array of color pigments like the red beet have. In contrast to red beet, it doesn't have the mentioned drawbacks and negative sensorial impact such as the unpleasant peatiness of the crop and high nitrite concentrations associated with the formation of carcinogenic nitrosamines (Harivaindaran and others 2008).

Red dragon fruit has many uses and many products can be made out of it. Aside from eating the flesh of thefruit, its frozen pulp is widely used in many industries to make ice cream, yogurt, jelly, preserves, marmalade, juice, candy and pastries. The unopened flower buds can also be cooked and eaten as a vegetable and its seeds contain oil that is a mild laxative (Crane and Belerdi 2007). Dacuycuy said a number of products can be made from dragon fruits, among them cupcake from dragon fruits flesh and rind; macaroni from rind; *lumpiang shanghai, empanadita,* and *siomai* from its dried flowers; ice cream and jam from flesh and rind; soap from its stem; and wine from its flesh.The peel of the fruit itself contains a high concentration of betacyanin however; it is often discarded and considered as a waste product in industries and consumers (Jamilah and others 2011).

The dye from the red dragon fruit's peel and flesh is commonly extracted using ethanol extraction method. Phebe and others (2009), Nurliyana and others (2010), Choo and Yong (2011), Lim and others (2007), Kunnika and Pranee (2011) and Wichienchot and others (2009) extracted betacyanin from the red dragon fruit using ethanol. However, the methods used by these researchers varied in terms on the conditions they used in extraction. They used varying blending times of 5 min, 2 min, and 1 min and varying temperatures of 25°C, 30°C, and 40°C.These diverse conditions result to different qualities of dye extracted. The dyes vary in terms of its betacyanin content and color stability. An optimal condition is needed to settle this problem.High quality dyes are what the food industries opt to and this can be attained if an optimal condition is made in extractions.

Thus, in this study, the optimal condition in extracting a dye from the red dragon fruit peel using ethanol extraction method was determined.

B. Statement of the Problem

What is the optimal condition to extract the dye from Red Dragon fruit(*Hylocereus polyrhizus*) peel using ethanol extraction method?

C. Objectives

This study aimed to determine the optimal condition in extracting a dye from red dragon fruit peel using ethanol extraction method. Specifically, this study aimed:

PHASE 1

1. to measure the betacyanin content of dyes extracted at varying blending timesof the peel with the ethanol: 1 min, 2 min, 3 min, 4 min, 5 min.
2. to compare the betacyanin content of dyes extracted at varying time in blending the peel with the ethanol: 1 min, 2 min, 3 min, 4 min, 5 min.

PHASE 2

3. to measure the betacyanin content of dyes extracted at varying temperature in evaporating the solvent: 25°C, 35°C, 45°C, 55°C, 65°C.
4. to compare the betacyanin content of dyes extracted at varying temperature in evaporating the solvent: 25°C, 35°C, 45°C, 55°C, 65°C.

PHASE 3

5. to evaluate the physico-chemical characteristics (light stability, pH stability, and pH level) of the dye extracted using the determined optimal conditions.

D. Significance of the Study

The determination of the optimal conditions of the ethanol extraction method when used in extracting betacyanin from the red dragon fruit peel could give people an idea of flesh and peel being used as a source of dye. This will also open the minds of Filipino businessmen to make use of red dragon fruit as a new venture since they can make use of its peel and flesh.

Finding the optimal conditions could be used to extract a high quality dye from the peel in terms of its betacyanin content and color stability. The determination of this optimal condition can aid dye manufacturers to obtain a high quality and cost efficient dyes. The conditions that yielded from this study can help researchers to study extensively the betacyanin coming from the red dragon fruit peel.

E. Scope and Delimitations

This study focused on finding the optimal conditions in the extraction of betacyanin using ethanol extraction method. Other extraction methods were considered. This study is delimited to extraction of betacyanin from the red dragon fruit peel only. Other plant parts of the fruit and other dye sources were notused for extraction.

The optimal conditions determined only include the blending time and evaporating temperature. Other parameters like ethanol concentration, light, pH and solvent-to-peel ratio were not considered for they are difficult to perform due to lack of equipments and skills needed.

The blending time may either cause increased or decreased betacyanin content. This is for the reason that the increase in blending time increases the area exposed to ethanol thus extracting more betacyanin. But if the cell membranes are severely damaged due to extensive blending, lesser betacyanin will be extracted. This is due to the rigorous blending resulting to the lower betacyanin count. Furthermore, higher blending may implicate higher processing cost.The blending time had five variations only (1, 2, 3, 4, 5 minutes). Each variation was performed in triplicates.

The evaporating temperature may cause a decline in the betacyanin content. An increased evaporating temperature gives a faster extraction time. Furthermore, the temperature affects the betacyanin amount because at certain high temperatures, specifically 70°C, betacyanin are being destroyed. This then results to a lesser betacyanin count.The evaporating temperature also had five variations only (25, 35, 45, 55, 65 degree Celsius). Each variation was performed in triplicates.

F. Definition of Terms

Betacyanin - a group of purple plant pigments found in leaves, flowers, and roots of members of the order Caryophyllales (McGraw-Hill Dictionary of Scientific and Technical Terms Fifth Edition).

In this study, betacyanin is the coloring pigment responsible for the red color of the red dragon fruit.

Betalain - is a water-soluble nitrogen-containing pigment synthesized from the amino acid tyrosine into two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins (Azeredo 2009).

In this study, betalain refers to the red pigment in red dragon fruit which is the betacyanin.

Blending time - is the length of mixing two or more substances having different properties to obtain a final product having characteristics different from those of the starting materials (McGraw-Hill Dictionary of Scientific and Technical Terms Fifth Edition).

In this study, blending time refers to the length of time at which the peel is blended in order to increase the surface area of the peel exposed to ethanol.

Color Stability - is the resistance of materials to change in color that can be caused by light or aging, as of petroleum or whiskey (McGraw-Hill Dictionary of Scientific & Technical Terms 2003).

In this study, color stability would mean the stability of the betacyanin extract measured by exposure to fluorescent light.

Ethanol - A colorless liquid, miscible with water, boiling point 78.32°C; used as a reagent and solvent. This is also known as ethyl alcohol; grain alcohol (McGraw-Hill Dictionary of Scientific and Technical Terms Fifth Edition).

In this study, ethanol is the solvent used to extract betacyanin from the red dragon fruit peel.

Evaporating Temperature - is the temperature at which a liquid is converted to vapour when heated (Britannica.com 2008).

In this study, evaporating temperature is the temperature at which the extract is vaporized inside the rotary evaporator.

Optimal Condition - is the condition at which thedegree or amount of something is the most favorable(The American Heritage Dictionary of the English Language 2009).

In this study, optimal condition is to be determined in terms of blending time and evaporating temperature at which the dye extract contains the highest amount of betacyanin.

Red Dragon Fruit - (*Hylocereus polyrhizus*) is commonly known as pitaya. It is a fruit with red-purple colored flesh with minute black seeds having exotic features, attractive colors, nutritional value and pleasant taste (Harivaindaran and others 2008).

-In this study, red dragon fruit is the natural plant source of the coloring pigment betacyanin.

CHAPTER 2

REVIEW OF RELATED LITERATURE

A. Dye

Dyes are commonly used to color clothes, hair and even food. They are divided into two main groups: synthetic and natural. In scientific terms, dye is a soluble colorant that attaches in molecular form to the fibers, as opposed to a pigment,which exists as much large particles that are attached to the fiber with a binder (Katz 2003).

A.1 Synthetic Dyes

Synthetic dyes are derived from several chemical compounds like copper sulfate, mercury, lead and sodium benzoate (as a preservative) that at certain amounts can trigger genetic problems and even mental problems (Institute for Agriculture and Trade Policy 2009). They are difficult to make because even a small difference in the suggested ratio of the chemical compounds like carboxylic acids and benzidine can yield to a different color like the Yellow 5 and Yellow 6. They can also trigger cancer at high amounts (Kobylewski and Jacobson 2010).

A.2 Natural Dyes

Natural dyes on the other hand, are derived from renewable resources.Dyeing from natural sources is the oldest way of colouring textiles. Natural dyes can give color of almost any shade for yarn and fabric. Thefirst colours used to dye fabrics were obtained from animal and vegetable sources (Natural Dyes Accessed 2012).

A.2.1 Lipid-based

Lipid-based dyes can be used in clothing and in hair dyes because they can’t easily be washed off.

A.2.2 Water-based

Water-based dyes are used in coloring foods because they are highly miscible.

A.2.3 Sources

Sources for natural dyes are considered to be used because they posses certain coloring pigments like betacyanin, carotene, lycopene, lutein, bixin and many others. Plants are the sources of natural dyes but sometimes insects, algae, fungi and cyanobacteria are also used (Mortensen 2006).

B. Red Dragon Fruit

B.1 Economical Value

Red dragon fruit (*Hylocereus polyrhizus*), commonly known as pitahaya is a member of the family *Cactaceae* (Crane and Belerdi 2007). The red dragon fruit is now becoming one of the major products of the north, especially in the Ilocos Region where a dragon fruit festival is held on July (Cruz 2011). In most grocery stores in Metro Manila, fresh dragon fruit costs about Php180-200 per kilo.

The fruit has many uses and many products can be made out of it. Aside from eating the flesh of the fruit, its frozen pulp is widely used in many industries to make ice cream, yogurt, jelly, preserves, marmalade, juice, candy and pastries. The unopened flower buds can also be cooked and eaten as a vegetable and its seeds contain oil that is a mild laxative (Crane and Belerdi 2007). Dacuycuy said a number of products can be made from dragon fruits, among them cupcake from dragon fruits flesh and rind; macaroni from rind; *lumpiang shanghai, empanadita,* and *siomai* from its dried flowers; ice cream and jam from flesh and rind; soap from its stem; and wine from its flesh.

B.2 Nutritional Value

People buying are paying for its abundant nutritional value. The dragon fruit is rich in fibre and vitamin C. Vitamin C from dragon fruit is easily absorbed in the body compared to the synthetic vitamin C that can be bought in drugstores (Silan 2008). Also, Dragon fruit is believed to able to lower cholesterol concentration, to balance blood sugar concentration, to prevent colon cancer, to strengthen kidney function and bone, to strengthen the brain workings, increasing the sharpness of the eyes as well as cosmetic ingredients (Suryono 2006 as cited Rahmawati and Mahajoeno 2009).

B.3 Physico-chemical Characteristics

B.3.1 Peel

Red dragon fruit (*Hylocereus polyrhizus)* peel is purple-red in color and consists 22% of the fruit weight. The peel is often disregarded during processing in beverage production industries (Jamilah and others 2011).

The concentration of betacyanins in the peel of 25, 30 and 35 days after flower anthesis was 0.24, 3.99 and 8.72 mg/mL, respectively.

The red dragon fruit peel contains a moisture content of 92.7%. High amounts of Betacyanin pigment (150.46±2.19 mg/100 g) and pectin (10.8%) are present in the peel. Glucose, maltose and fructose were detected but no trace of sucrose and galactose. The peel contained a high insoluble and soluble dietary fibre with a ratio of 3.8:1.0 (Jamilah and others 2011).

B.3.2 Flesh

Red dragon fruit flesh contains higher betacyanin content compared to its peel (Phebe and others 2009).

The concentration of betacyanins in the flesh of 25, 30 and 35 days after flower anthesis was 2.40, 7.93 and 11.70 mg/mL betacyanins.

The dark-red flesh known to be a rich source of vitamin B1, vitamin B2, vitamin B3, and vitamin C, protein, fat, carbohydrate, crude fiber, flavonoid, thiamin, niacin, pyridoxine, kobalamin, glucose, phenolic, betacyanins, polyphenol, carotene, phosphorous, iron and phytoalbumins (antioxidant properties) (Jaafar and others 2009).

B.3.3 Comparison of Peel and Flesh

The table below derived from the data collected by Jamilah and others (2011) and Jaafar and others (2009) shows the proximate analysis between the peel and flesh.

Table 1. Comparison between the peel and flesh

Nutritional contents	Peel	Flesh
Moisture	92.65±0.10	82.5-83
Protein	0.95±0.15	0.159-0.229
Fat	0.10±0.04	0.21-0.61
Ash	0.10±0.01	-
Carbohydrate	6.20±0.09	-

The tables below of Kunnika and Pranee (2011) further show the comparison between the peel and flesh.

Table 2. Bioactive compounds and antioxidant activity of the various samples of flesh of red dragon fruit

Functional substances / RS	Hydrolysate level			
	F0 (RS = 22.39±0.65 mg glucose/g FM)	F1 (RS = 25.61±0.09 mg glucose/g FM)	F2 (RS = 58.54±0.02 mg glucose/ g FM)	F3 (RS = 70.56±0.19 mg glucose/g FM)
DPPH (EC_{50}: µg FM A / µg DPPH)	3.27±0.05	2.03±0.04	1.33±0.57	1.05±0.33

ABTS (μg TE B /g FM)	332.14±0.21	840.78±0.01	1,001.21±0.13	1,029.60±0.67
Total phenolics (mg GAE[C] /100gFM)	480.47±0.01	693.39±0.04	1,043.33±0.57	1,049.18±0.33
Total flavonoids (mg CE[D] /100g FM)	288.27±0.04	1,094±0.01	1,281.21±0.13	1,310.10±0.67

Each value represents a mean ± standard deviation.

Means,with the different letter in the row are significantly difference ($p<0.05$)

RS = reducing sugars, A FM= fresh mass, B TE=Trolox equivalents , [C]GAE=gallic acid equivalents , [D]CE=catechin equivalents

Table 3. Bioactive compounds and antioxidant activity of the various samples of peels of red dragon fruit

Functional substances / RS	Hydrolysate level			
	P0 (RS = 10.63±0.99 mg glucose/g FM)	P1 (RS = 20.35±0.09 mg glucose/g FM)	P2 (RS = 32.73±0.02 mg glucose/g FM)	P3 (RS = 44.54±0.65 mg glucose/g FM)
DPPH (EC_{50}: μg FM A / μg DPPH)	20.88±0.02	3.11±1.09	2.03±0.19	2.71±0.02
ABTS (μg TE B /g FM)	110.41±0.06	596.70±0.52	754.28±0.19	815.03±0.22
Total phenolics (mg GAE[C] /100gFM)	191.24±0.05	324.63±1.09	379.37±0.19	561.76±0.02
Total flavonoids (mg CE[D] /100g	32.63±0.03	143.70±0.52	175.28±0.19	220.28±0.22

FM)				

Each value represents a mean ± standard deviation.

Means,with the different letter in the row are significantly difference ($p<0.05$)

RS = reducing sugars, A FM= fresh mass, B TE=Trolox equivalents , CGAE=gallic acid equivalents , DCE=catechin equivalents

Table 4. Total dietary fiber of the various samples of flesh and peel of red dragon fruit

Total dietary fiber (g/ 100g FM)	Flesh		Peel	
	F0 (RS = 22.39±0.65 mg glucose/g FM)	F3 (RS = 70.56±0.19 mg glucose/g FM)	P0 (RS = 10.63±0.99 mg glucose/g FM)	P3 (RS = 44.54±0.65 mg glucose/g FM)
Total dietary fiber	2.50 ±0.06 b	2.61 ± 0.03 b	3.62 ±0.01 a	3.72 ± 0.05 a
- Soluble dietary fiber	0.90 ±0.02 d	1.63 ± 0.01 c	1.93 ±0.05 b	3.53 ± 0.03 a
- Insoluble dietary fiber	1.67 ±0.03 a	0.98± 0.01 b	1.69 ±0.07 a	0.19 ± 0.08 c

Each value represents a mean ± standard deviation.

Means,with the different letter in the row are significantly difference ($p<0.05$)

FM = fresh mass

B.4Plant Development, Harvest Season and Storage

Buds of the fruit are contained in aerioles along its three-ribbed stem and emerge in the summer months. Once emerged, the buds then form into branches or flowers. The scented, white, night-blooming flowers attract bats and moths. Bees and other insects visit the flowers before dusk as the petals open and after dawn as the flowers begin to close. Flowers only open for two days, after which, fruit set and development is rapid. Fruit can be harvested approximately 28 days after the flower closes. The fruit must be fully expanded and have 85% pink colour in the skin. Fruit can then be left on the stem from 10 to 15 days at this stage (Luders and McMahon 2006).

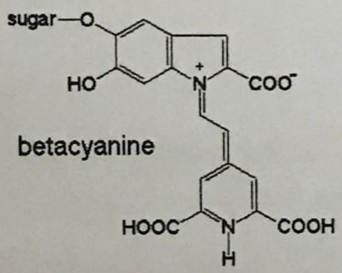
In the Philippines, red dragon fruits are harvested on the months May to November (Adriano 2009).

It is a delicate fruit hence, it needs a proper storage. Red dragon fruit can be stored for two to three months at 7-10°C with a relative humidity of 90-98% (Luders and McMahon 2006).

C. Betacyanin

C.1 Characteristics

Betalains , water-soluble nitrogen-containing pigments, are synthesised from amino acid tyrosine to betacyanins (red-violet) and betaxanthins (yellow-orange). Betacyanin contains a cyclo-3-4-dihydroxyphenylalalanine residue.



The absorption maximum below pH 3.5, shifts toward lower wavelengths, and above pH 7 the change is toward upper ones; out of the pH range 3.5–7.0 the intensity of the visible spectra decreases. Optimal pH range for maximum betanin stability is 5–6 (Huang & von Elbe, 1985, 1987; Castellar et al., 2003; Vaillant et al., 2005 as cited by Azeredo 2006). The stability of the pigment is also exponentially affected by the water activity. Betalains also react to molecular oxygen leading to its degradation. Betalain stability has been reported to be improved by antioxidants or by a nitrogen atmosphere. Also, the stability of the pigment is impaired by light exposure (Von Elbe et al., 1974; Attoe & von Elbe, 1981; Cai et al., 1998 as cited by Azeredo 2006). Furthermore, some metals cations like iron, copper, tin and aluminium are reported to accelerate its degredation (Pasch & von Elbe, 1979; Attoe & von Elbe, 1983; Czapski, 1990; Sobkowska et al., 1991 as cited by Azeredo 2006). Antioxidants have been described to enhance betalain stability. Temperature is considered to be the most important factor on the stability. Studies show that increasing temperatures result to increasing degradation rates (Azeredo 2006).

C.2 Reaction towards Ethanol

With the application of ethanol extraction, betacyanin would leak out via osmosis. Since betacyanin is water soluble and not lipid soluble, it remains in the vacuole when the cells are healthy. If the integrity of a membrane is disrupted, however, the contents of the vacuole will spill out into the surrounding environment (Vernier Software & Technology).Generally, increases in salt, acetone and ethanol concentration are correlated with increases in absorbance of solutions in which beet cells have been immersed, and thus affect membrane permeability (Ding). We used the ethanol extraction method because it is known that ethanol can make the betacyanin leak out from the peel of red dragon fruit through diffusion.

C.3 Storage Conditions

Storage of solution under low oxygen levels results in decreased pigment degradation(Von Elbe et al., 1974; Huang & von Elbe, 1987 as cited by Azeredo 2006). The extracts should be kept for some time under temperature below 10 °C and pH around 5.0. (Huang & von Elbe, 1985, 1987 as cited by Azeredo 2006).

C.4 Betacyanin Determination

Betacyanin can be detected and measured through the usage of UV-VIS Spectrophotometer and High Performance Liquid Chromatography. It can be expressed in units such as mg/100 g of fresh weight.

C.4.1 UV-VIS Spectrophotometer

UV-VIS Spectrophotometer works by measuring the intensity of light transmitted of a narrow bandpass and then scans the wavelength in time in order to collect a spectrum.

Absorption is a ratiometric measurement in a UV-VIS; these instruments generally require the user to measure two spectra, one sample and one blank. The blank should be identical to the sample in every way except that the absorbing species of interest is not present (Greenlief 2004).

The betacyanin content was then calculated in a similar way to that reported by Phebe and others (2009), with some modification by using the following formulas:

Betacyanins content (mg/100 g of fresh weight) = A_{538} (MW) V (DF) x 100/(εLW)

Where A_{538} = absorbance at 538 nm (λ max),

L (path length) = 1.0 cm,

DF = dilution factor,

V = volume extract (mL),

W = fresh weight of extracting material (g).

For betanin, ε (mean molar absorptivity) = 6.5×10^4 L/mol cm in H_2O

and MW= 550.

The usage of spectrophotometer was based on **UNICO® 2100 SERIES** SPECTROPHOTOMETER **USER'S MANUAL.**

C.4.2 High Performance Liquid Chromatographer

All chromatography involves a mobile phase. HPLC was developed based on the principles of Thin Layer Chromatography (TLC) but is fully automated and more quantitative (Center for Synthesis & Chemical Biology 2006).

The HPLC works by injecting a sample into a mobile liquid phase and the sample passes along a stationary phase. Although injection can be done manually, the user may opt to inject samples using a computer. The stationary phase contains a column which is usually stainless and packed with silica particles bonded with alkyl chains. The length of chains depend on the molecule being analysed (Cente for Synthesis & Chemical Biology 2006).

HPLC has the advantage of being rapidly applicable in a quantitative basis. However, HPLC requires a more complex equipment(WHO 2007).

C.5 Betacyanin Stability Measurement

The stability of betacyanin can be measured in terms of high temperature, pH, H_2O_2, and light.

C.5.1 High Temperature

Betacyanin can be measured in terms of high temperature by measuring the content of the betacyanin under varying temperatures. The betacyanin solution extracted is put in water, a bath at 25°C (control), 40, 60, 80 and100°C, after 5, 10, 15 and 20 min, the absorbance at 538nm is used to determine the concentration (Wang and others 2006).

C.5.2 pH

Through manipulation of the pH value, betacyanin stability can also be measured. The betacyanin solution is adjusted to different pH value using HCl and NaOH, then the absorbance at 538 nm were determined 10 min later (Wang and others 2006).

C.5.3 H_2O_2

A concentrated H_2O_2 is added to the betacyanin solution to reach the finalH_2O_2 concentrations of 0 (control), 0.2%, 0.4% and 0.8% H_2O_2 , then the absorbance at 538 nm is determined after 0, 2, 4, 6, 8,10 and 12 h (Wang and others 2006).

C.5.3 Light

Light exposure degrades betacyanin and shows an increased exposure results to a lower stability (Azeredo 2006). Betalain stability was influence greatly by light. Light has been a major factor in deteriorating the color of betalain. Exposure to light causes up to 50% of loss after one week storage in room temperature (Woo and others 2011).

Thebetacyanin solution was put under adaylight lamp with light intensity of 2 000 µmol m -2 s -1and 0.1% betacyanin solution kept in the darkness wasused as control, the temperature was 25°C, then the absorbance at 538 nm is then determined 0, 2, 4, 6, 8, 10 and12 h thereafter (Wang and others 2006).

D. Extraction Methods

D.1 Aqueous Method

The known amount of dyestuff is boiled in 100ml of soft water at 100°C (Chet 2009, p21).

For betacyanin extraction from red dragon fruit peel, ten grams of dragon fruit peel is immersed into 30mL of pH 5 distilled water and the dye is extracted at 100°C for 5 min (Harivaindaran and others 2008).

D.2 Alkaline Method

First, 1% alkaline solution is prepared with addition of 1g of NaOH in 100ml of water. Then the dye material is entered and boiled at 100°C (Chet 2009, p22).

D.3 Acidic Method

Acidic solution of 1% is prepared by adding 1ml of HCl in 10ml of soft water. Then the material is entered and boiled at 100°C (Chet 2009, p22).

D.4 Ethanol Extraction Method

Ethanol extraction method is characterized by adding ethanol and blending the sample for some time which will then be filtered. The filtrate will be centrifuged and evaporated using a rotary evaporator (Phebe and others 2009).

Ethanol, also known as ethyl alcohol and grain alcohol, is a clear colorless liquid. Ethanol, CH_3CH_2OH, melts at –114.1°C, boils at 78.5°C, and has a density of 0.789 g/mL at 20°C. Its low freezing point has made it useful as the fluid in thermometers for temperatures below –40°C, the freezing point of mercury, and for other low temperature purposes, such as for antifreeze in automobile radiators (Sakhashiri 2009).

E. Factors Affecting the Quality of the Dye Extract

Based on the ethanol extraction method used by Phebe and others (2009), parameters that may affect the extract quality are blending time, ethanol concentration, peel to solvent ratio and evaporating temperature.

E.1 Blending Time

Blending the fruit increases the surface area exposed to ethanol. The more surface area exposed to the ethanol, the more it can extract the betacyanin. The increase therefore in blending time increases the betacyanin content of the extract. But if the cell membranes are severely damaged due to extensive blending, lesser betacyanin will be extracted. This is due to the rigorous blending resulting to the lower betacyanin count (Ametajds and others 2003).

E.2 Evaporating Temperature

Increasing temperature results to increasing betacyanin degradation (Azeredo 2006). Therefore, increasing the evaporating temperature may decrease the betacyanin content of the extracted dye. Higher Temperatures above 70 °C could result in thermal degradation of betalains (Ferna´ndez-Lo´ pez and Almela, 2001 as cited by Azeredo and others 2009).

E.3 Light

Betalain stability was reported to be impaired by light exposure (Von Elbe et al., 1974; Attoe & von Elbe, 1981; Cai and others, 1998). Attoe & von Elbe (1981) showed an inverse relationship between betalain stability and light intensity (in the range 2200–4400 lux). UV or visible light absorption excites p electrons of the pigment chromophore to a more energetic state (p*), increasing reactivity or lowering activation energy for the molecule (Jackman & Smith

1996). Betalain light-induced deg-radation is oxygen dependent, because the effects of light exposure are negligible under anaerobic conditions (Attoe & von Elbe 1981; Huang & von Elbe 1986).

E.4 pH

Betalains is relatively stable over the broad pH range from 3 to 7 (Jackman & Smith, 1996 as cited by Azeredo 2006), which allows their application to low acidity foods. Below pH 3.5, the absorption maximum shifts toward lower wavelengths, and above pH 7 the change is toward upper ones; out of the pH range 3.5–7.0 the intensity of the visible spectra decreases. Optimal pH range for maximum betanin stability is 5–6 (Huang & von Elbe, 1985, 1987; Castellar et al., 2003; Vaillant and others, 2005 as cited by Azeredo 2006).

E.5 Concentration of Ethanol

Chew and others (2011) made a study about the effect of concentration of ethanol on the recovery of phenolic compounds from Centella asiatica. They found out that at higher concentrations of ethanol, more phenolic compounds can be recovered.

E.6 Storage Condition

Dye extract for betacyanin determination is kept in a 1 mL Eppendorf tube, wrapped with aluminium foil and stored at dark at -20°C (Phebe and others 2009).

F. Characterization of Dye

F.1 pH Level Measurement

The pH level will be measured using a pH meter.

G. Related Studies

G.1 Study of Optimal Temperature, pH and Stability of Dragon Fruit (*Hylocereus polyrhizus*) Peel for Use as Potential Natural Colorant

The study of Harivaindaran and others (2008) aimed to explore the feasibility of the peel as a natural colorant using simple water extraction method and to test the stability of the pigments. The parameters used were varied temperatures: Room temperature (RT), 50, 80 and 100°C; varied heating time: 1, 2, 3, 4, 5 and 10 min and a series of pH range. Results show that The efficient condition in order to get the highest possible betacyanin content was heating samples at 100°C for 5 min in a pH 5 citric solution. Then stability test was made, the pigments were dried and soaked in distilled water after that the pigments were then exposed to light. Outcomes showed that soaked pigments had high pigment retention and able to stay up to 7 days.

G.2 Red-fleshed pitaya (*Hylocereus polyrhizus*) fruit colour and betacyanin content depend on maturity

The objectives of Phebe and others (2009) were to determine colour using using Minolta CR-300 Chroma Meter, total betacyanin content by using a SECOMAM-PRIM Light-Vab-S/N 1143 spectrophotometer and its separation in the peel and flesh of the fruit harvested at 25, 30 and 35 days after flower anthesis (DAA) through HPLC by gel filtration chromatograph and to inspect the utility of tristimulus colour measurement as predictors of pigment content in red dragon fruits. In pigment extraction, 20 g of peel and flesh was extracted in a blender for 5 min using 40 mL 80% aqueous ethanol. It was filtered using a Whatman No. 1 filter paper. The filtrate was then centrifuged at 12000 rpm under -4°C for 35 min. And for another 35 min, it was evaporated using a rotorary evaporator at 25°C until 6 mL remained. After concentrating, the extract of each sample was then kept in the 1 mL Eppendorf tube, wrapped with aluminum foil and stored at dark at -20°C. There were significant relationships between DAA and colour, and total betacyanin contents of peel and flesh. The concentration of betacyanins in the peel of 25, 30 and 35 DAA was 0.24, 3.99 and 8.72 mg/mL, respectively and flesh which was higher than peel has 2.40, 7.93 and 11.70 mg/mL betacyanins.

G.3 Pigment identification and antioxidant properties of red dragon fruit (Hylocereus polyrhizus)

The goal of the study of Rebecca and others 2010 was to know the pigments present in the red dragon fruit and to investigate more about its antioxidant properties. Pigment was identified using the HPLC method and results show that betanin was present at a retention time of 11.5 min. The antioxidant properties were known using the total polyphenol assay which expresses gallic acid as equivalent and results show that there were 86.10 mg of total polyphenolic compound in 50.0g of dried fruit extract. The antioxidant activity was confirmed using the reducing power assay and the results indicated that the reducing capability increased from 0.18 to 2.37 with the increase in weight of the dry sample from 0.03 to 0.5g. The amount of condensed tannin was measured using the Vanillin-HCl assay and results displayed that the dried sample had an equivalent of 2.30 mg catechin/g. The DPPH· radical scavenging activity determination measured the effective concentration for dragon fruit was 2.90 mM vitamin C equivalents/g dried extract.

G.4 Physico-chemical characteristics of red pitaya (Hylocereus polyrhizus) peel

Jamilah and others (2011) conducted a study to determine the physico- chemical characteristics of red dragon fruit peel in order to know if there is a possibility of recovering from the so-called "wastes" of the fruit, its peel. The methods used are soxhlet extraction method (moisture, ash, and lipid), micro-kjeldhal method (protein), spectrophotometer method (betacyanin content), HPLC method (organic acid and sugar), pectin assay (pectin), enzymatic-gravimetric method (dietary fibre content). Results show that the peel has a moisture content of 92.7%, low in soluble solids, protein, ash and fat content. Betacyanin pigment (150.46 ± 2.19 mg/100g) and pectin (10.8%) were high. Glucose, maltose and fructose were detected but not sucrose and galactose. The peel had also a very high insoluble and soluble dietary fibre and showed a good ratio (3.8:1.0).

G.5 Stability of Betalain Pigment from Red Dragon Fruit (*Hylocereus polyrhizus*)

Woo and others (2011) did investigate on the stability of the red dragon fruit. The fruits were homogenized in ethanol to separate pectic substances. They used the UV/Vis spectrophotometric analysis to detect the presence of betacyanin. The absorbance peaks where at 230 and 537 nm which indicated the presence of betacyanin. For three weeks, changes of betacyanin concentration were observed due to storage condition, light, temperature, pH and additives through UV-Vis spectrophotometer at 537 nm. Results showed that light plays an important role in betalain degradation. Refrigeration storage at 4°C without light can preserve the color of the fruit juice up to 3 weeks.

G.6 Extraction of Betacyanin from Red Beet root (Beta vulgaris L.) and to evaluate its antioxidant potential

Extraction of Betacyanin from Red Beet root (Beta vulgaris L.) and to evaluate its antioxidant potential was a study made by Suganyadevi and others 2010. The study's objectives were to extract and to evaluate the betalain content of red beet root and its antioxidant potential by enzymatic and non enzymatic method. The non-enzymatic method was done by quantification of vitamin C, and DPPH method. Results showed that there was presence of two flavanoid compounds through HPTLC analysis. So it reveals that, the high antioxidant potential may be due to these compounds. Because of this, the production of beet root pigments has to be performed using cell suspensions and transformed roots in bioreactors.

CHAPTER 3

METHODOLOGY

A. Experimental design

This experimental study aimed to determine the optimal condition in extracting the Red Dragon fruit (*Hylocereus polyrhizus*) peels' dye using ethanol extraction method. The blending time and the evaporating temperature were manipulated. The effects on the amount of betacyanin in each dye that was extracted was measured and observed. Treatments for the blending time and the evaporating temperature were 1, 2, 3, 4 and 5 minutes and 25, 35, 45, 55 and 65 degrees centigrade, respectively. The Red Dragon fruits were bought from a local farm in Lezo, Aklan during the months of May to June.

The Red Dragon fruits were randomly assigned to the two manipulations. Extraneous variables are the weather and accuracy of measurements. There were three replicates for all the independent variables and their treatments. Statistical analyses were performed using ANOVA.

B. Sample Time and Place of the Study

The Red Dragon fruits were collected from a local farm in Lezo, Aklan during the months of May to June 2012.

The study was conducted at the Research laboratory, Philippine Science High School Western Visayas Campus, Jaro, Iloilo City, from May to June 2012.

C. Materials and Equipment

- 1 ml of sodium acetate triphosphate at pH 8.7
- Centrifuge
- daylight lamp set-up
- Ethanol
- Filter paper
- Ice chest
- pH meter

- potassium chloride at pH 5.5
- Rotary evaporator
- Salt solution
- Screw-cap test tubes
- UV-VIS spectrophotometer

D. Preparation of Equipment and Reagents

The UV-Vis spectrophotometer, rotary evaporator, centrifuge, screw-cap test tubes, sodium acetate triphosphate at pH 8.7, and potassium chloride at pH 5.5 used came from Philippine Science High School-WVC laboratory. The filter paper used was bought from Josmef Pharmacy. Ethanol used to extract betacyanin was bought from Prince Valiant International Corporation. The blender was borrowed from Tejereso's residence. The ice chest where the samples were contained came from Popes' residence. The daylight lamp set-up was made by a carpenter in Aklan.

E. Collection of Red Dragon Fruits

Red Dragon fruits (*Hylocereus polyrhizus*) were purchased from a local farm in Lezo, Aklan between the months of May. The fruits were prepared for transport to Iloilo by washing the fruit to remove accumulated dirt. The fruits were washed using distilled water. The fruits were then cut equally into four and then placed in a transparent plastic. They were then frozen inside an ice chest that is below freezing point in temperature. After preparation, the preserved fruits were then brought to the PSHS-WVC campus research laboratory where they were tested.

F. Preparation of the peel

The frozen fruits were thawed at room temperature. Its peel was separated from its flesh by gently pulling it off like peeling a banana. The peel that was detached is now ready for extraction.

G. Extracting the dye

Phases 1 and 2 included the determining of the optimal blending time and temperature for betacyanin extraction from Red Dragon fruit peel. This phase was done based on the method used by Phebe and others (2009).

G.1 Phase 1 (Varying blending times)

Five samples of twenty grams of peel were blended with 40 mL of 80% ethanol. The varying blending times that were used are 1, 2, 3, 4 and 5 minutes. The extracts were thenfiltered using a filter paper. The filtrates were then centrifuged using a centrifuge at 1200 rpm for 35 minutes. The final extracts were then evaporated using a rotary evaporator at 25 degrees centigrade for 35 minutes until 6 mL of the initial volume remained.

The betacyanin content (mg per 100g of fresh weight material) was measured using the UV-VIS spectrophotometer and the formula for betacyanin content (SC (mg L^{-1}) = ((A x MW x 1000 x DF) / (molar extinction coefficients x length of cuvette))). Following the betacyanin content determination was the comparison of the amount of betacyanin in each dye extracted using varying blending times. Betacyanin contents were statistically analyzed using ANOVA.

G.2 Phase 2 (Varying evaporating temperatures)

Five samples of twenty grams of peel were blended with 40 mL of the 80% ethanol with the optimal blending time that was made. The extracts were then filtered using a filter paper. The filtrates were then centrifuged using a centrifuge at 1200 rpm for 35 minutes. The final extracts were then evaporated using a rotary evaporator at varying temperatures for 35 minutes until 6 mL of the initial volume remained. The varying temperatures were 25, 35, 45, 55 and 65 degrees centigrade.

The betacyanin content (mg per 100g of fresh weight material) was measured using the UV-VIS spectrophotometer and the formula for betacyanin content (SC (mg L^{-1}) = ((A x MW x 1000 x DF) / (molar extinction coefficients x length of cuvette))). Following the betacyanin content determination was the comparison of the amount of betacyanin in each dye extracted using varying evaporating temperatures. Betacyanin contents were statistically analyzed using ANOVA.

G.3 Phase 3 (Dye Characterization)

Evaluated the physico-chemical characteristics of the dye extracted using the determined optimal conditions.

G.3.1 Color Stability

Phase 3 included the measurement of the color stability of the dye that was extracted from the red dragon fruit's peel using the determined optimal conditions after performing the other phases. It was done using the method adopted from Wang and others (2006). The color stability of the dye was measured by exposing the extract to light from a daylight lamp 15" up 12 hours a day for three days. After exposure each day, the betacyanin content was measured.

Betacyanin content of the dye exposed were compared to the control by statistical analyses using ANOVA.

G.3.2 Stability at Variable pH

The stability of the dye at variable pH was tested by treating about 1 ml of sample with 1 ml of sodium acetate triphosphate at pH 8.7 and with potassium chloride at pH 5.5. The colour change was observed (Strack 1989 as cited by Suganyadevi and others 2010).

G.3.3 pH Level Measurement

The pH level was measured using a pH meter.

H. Safe Handling of Chemical

H.1 Ethanol

Ethanol is kept away from heat, sparks and open flame. Spilling, skin and eye contact are avoided. Ethanol is ventilated well; breathing in the vapours is avoided. Use approved respirator if air contamination is above acceptable level. Do not use contact lenses.

Ethanol is flammable/combustible. It is away from oxidizers, heat and flames. Be reminded that it may attack some plastics, rubber and coatings. It is kept in cool, dry, ventilated storage and closed containers. Ground the container and transfer equipment to eliminate static electric sparks.

Respiratory protection is used if the general level exceeds the Occupational Exposure Level (OEL). Protective gloves made of butyl rubber are used. Wear approved chemical safety goggles where eye exposure is reasonably probable. Contact lenses are worn when working with this chemical!

Use engineering controls to reduce air contamination to permissible exposure level. Provide eyewash station and safety shower. Appropriate clothing to prevent repeated or prolonged skin contact is used. (Ethyl Alcohol MSDS, sciencelab.com, Inc.)

I. Waste Disposal

I.1 Ethanol

Ethanol may run into process drains if greatly diluted with water. It will be removed to open atmosphere for dispellation of vapours and will absorb in vermiculite or dry sand, and then dispose in licensed special waste site. It will dispose of in accordance with Local Authority requirements (Ethyl Alcohol MSDS, sciencelab.com, Inc.).

I.2 Red Dragon Fruit Flesh and Peel

Flesh of the fruit that was used was eaten so that the flesh can be used and not go as waste. The excess peel and the remains of the peel during the extraction process were disposed by putting them inside a plastic and disposing them inside a trash can.

I.3 Red Dragon Fruit Extract

The excess extracts were properly disposed by setting it aside inside a waste container. The extract inside the container was diluted in water then was poured into the sink. The faucet was kept opened while the pouring is done to assure that if there are harmful chemicals present, they will be properly diluted.

J. Data Organization

Figure 1. The absorbance (A_{538}) and concentration of betacyanin leaked from Red Dragon fruit peel (*Hylocereus polyrhizus*) following different blending times.

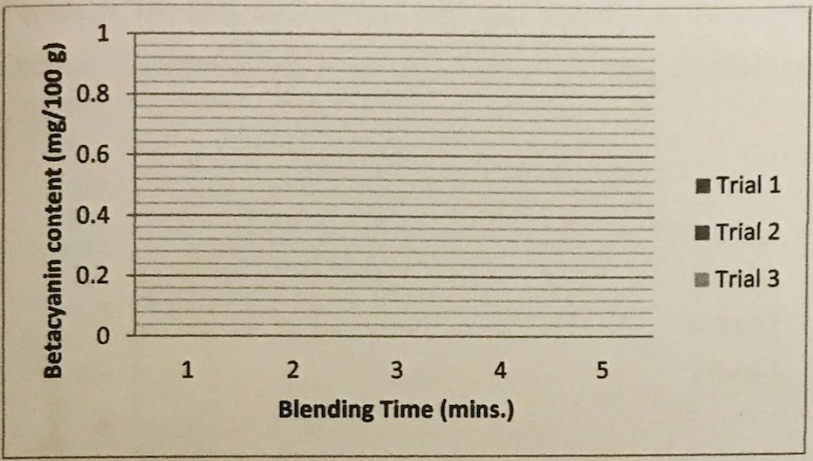


Table.5. The absorbance (A_{538}) and concentration of betacyanin leaked from Red Dragon fruit peel (*Hylocereus polyrhizus*) following different blending times.

Blending Time (mins)	Replicate number	A_{538}	Betacyanin content (mg/100 g)
1	1		
	2		
	3		
2	1		
	2		
	3		
3	1		
	2		
	3		
4	1		
	2		
	3		

5	1		
	2		
	3		

Figure 2. The absorbance (A_{538}) and concentration of betacyanin leaked from Red Dragon fruit peel (*Hylocereus polyrhizus*) following different temperatures.

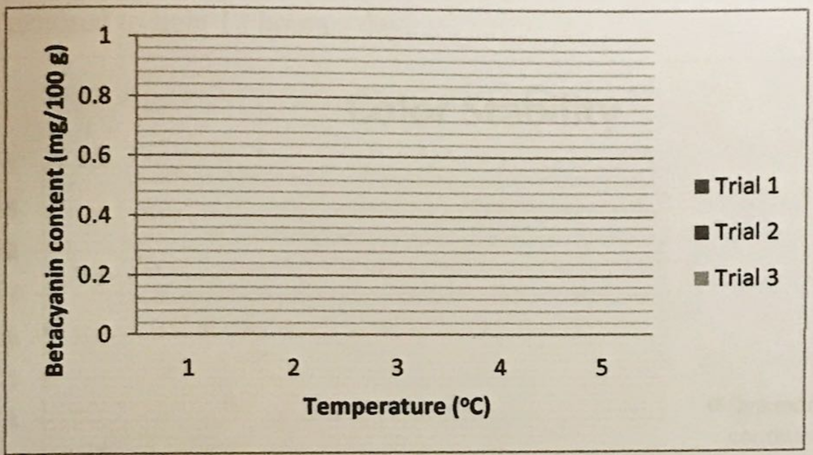
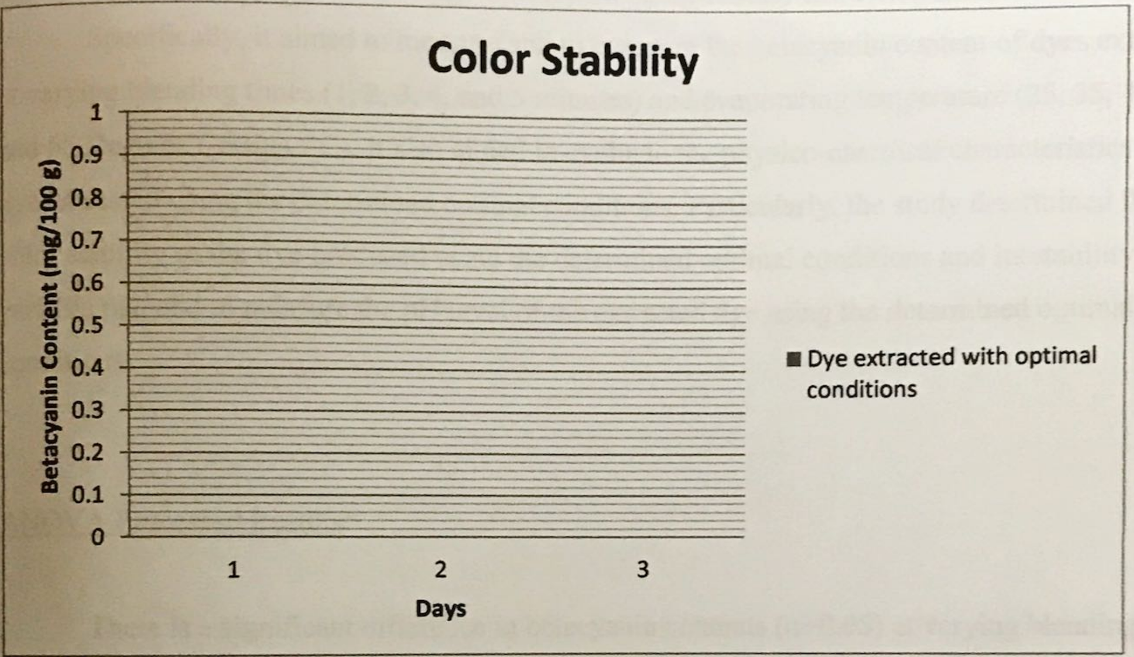


Table.6. The absorbance (A_{538}) and concentration of betacyanin leaked from Red Dragon fruit peel (*Hylocereus polyrhizus*) following different temperatures.

Temperature (°C)	Replicate number	A_{538}	Betacyanin content (mg/100 g)
25	1		
	2		
	3		
35	1		
	2		
	3		
45	1		
	2		
	3		
55	1		
	2		

	3		
65	1		
	2		
	3		

Figure 3. The betacyanin content of the dye extracted with the optimal conditions and the control exposed to light 12 hours a day.



CHAPTER FOUR

RESULTS AND DISCUSSION

The study determined the optimal blending time and evaporating temperature in extracting betacyanin from Red Dragon Fruit peel using ethanol extraction method.

Specifically, it aimed to measure and to compare the betacyanin content of dyes extracted at varying blending times (1, 2, 3, 4, and 5 minutes) and evaporating temperature (25, 35, 45, 55, and 65 Degrees Celsius, °C). It also aimed to evaluate the physico-chemical characteristics of the dye extracted using the determined optimal conditions. Particularly, the study determined the color stability of the dye produced using the determined optimal conditions and its stability at variable pH, and to measure the pH level of the extracted dye using the determined optimal conditions.

ANOVA Processed Results

There is a significant difference in betacyanin contents ($\alpha=0.05$) at varying blending times and varying temperatures. There is also a significant differnce in betacyanin contents ($\alpha=0.05$) before and after a period of light exposure (Table 7).

Table 7. ANOVA Processed Results

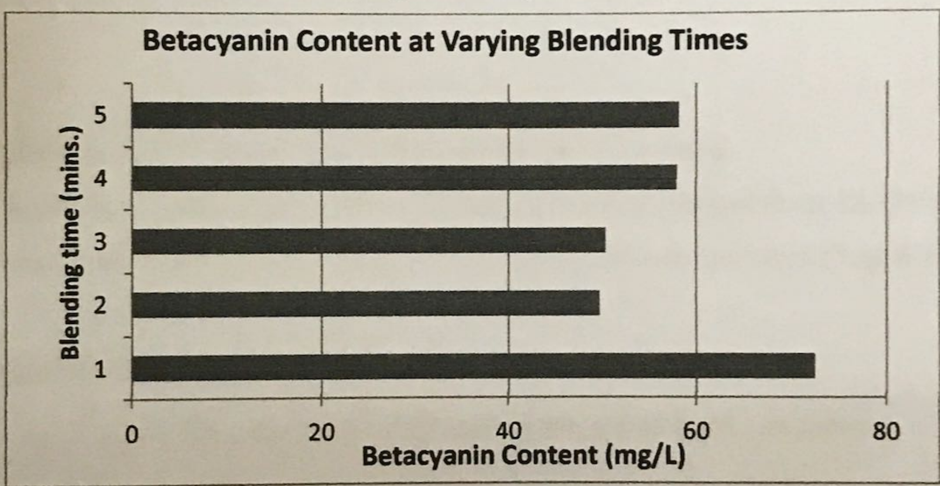
Dependent variables		Sum of Squares	df	Mean Square	F	Significance
Blending Time	Between Groups	223.440	4	55.860	118.204	.000
	Within Groups	4.725	10	.473		
	Total	228.166	14			
Temperature	Between Groups	12349.969	4	3087.492	9.294	.002
	Within Groups	3322.175	10	332.217		
	Total	15672.144	14			
Light Stability	Between Groups	3221.797	3	1073.932	12.895	.002
	Within Groups	666.243	8	83.280		
	Total	3888.040	11			

A. Results

A.1. Betacyanin Content at Varying Blending Times

The betacyanin contents at varying blending times of 1-5 minutes ranges from 32.175 to 44.092 mg/L. The highest betacyanin content was achieved at a blending time of 1 min while the lowest betacyanin content was achieved at a blending time of 2 minutes (Figure 4). One minute blending time was significantly higher than blending times 2, 3, 4 and 5 minutes. While the two minute blending time was significantly lower than blending times 4 and 5 minutes but insignificantly different to the 3 minute blending time. Blending times 4 and 5 showed no significant difference between each other. The results showed a decrease from blending time of 1 to 2 minutes and an increasing trend from 2 to 5 minutes.

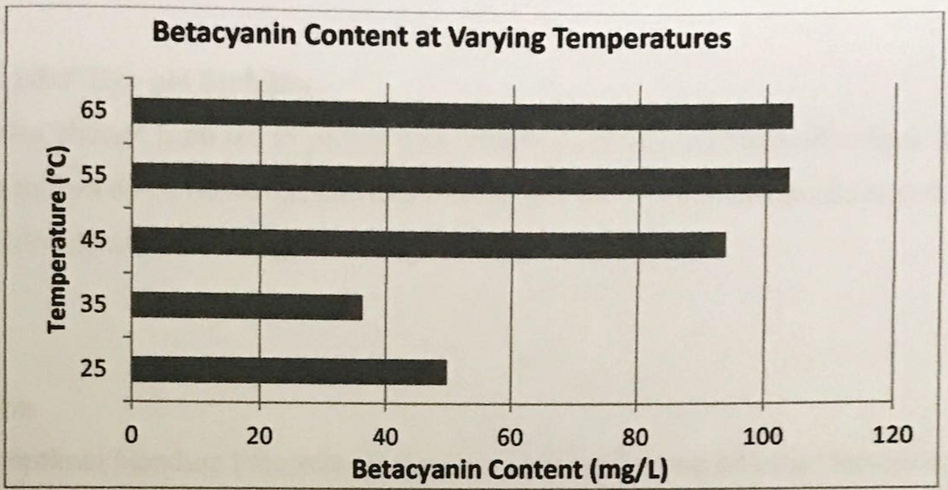
Figure 4. Betacyanin Content at Varying Blending Times



A.2. Betacyanin Content at Varying Evaporating Temperatures

The betacyanin contents at varying evaporating temperatures of 25-65°C ranges from 29.486 to 109.847 mg/L. The highest betacyanin content was achieved at an evaporating temperature of 65°C while the lowest betacyanin content was achieved at an evaporating temperature of 35°C (Figure 5).The evaporating temperature of 25°C was significantly lower than evaporating temperatures of 55 and 65°C. Moreover, the evaporating temperature of 35°C was significantly lower than 45, 55, and 65°C evaporating temperature. The results showed a decrease from an evaporating temperature of 25 to 35 °C and an increasing trend from 35 to 65 °C.

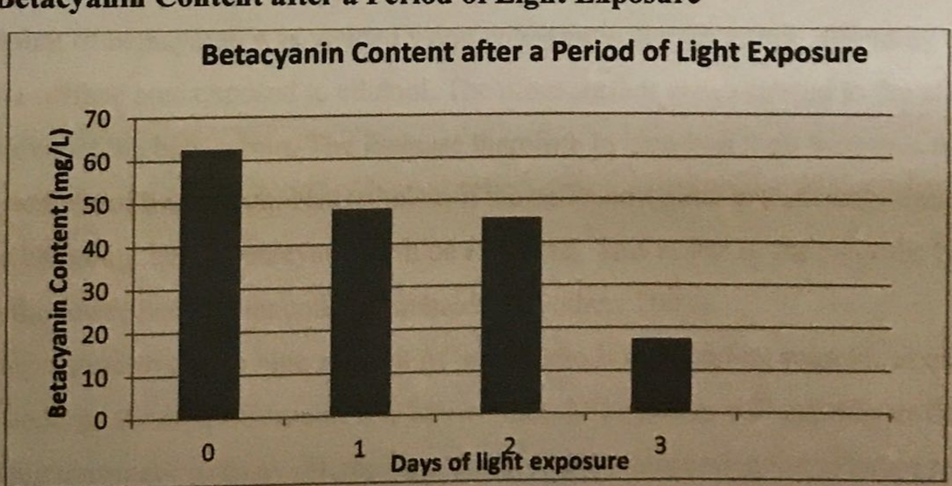
Figure 5. Betacyanin Content at Varying Evaporating Temperatures



A.3. Betacyanin Content after a Period of Light Exposure

The betacyanin contents after light exposure of 0-3 days ranged from 12.375 to 69.819 mg/L. The betacyanin content decreased as days of light exposure increases (Figure 6).

Figure 6. Betacyanin Content after a Period of Light Exposure



A.4. RDF Dye pH Level Measurements

The red dragon fruit dye extracted using the optimal conditions had pH levels ranging from 4.51-4.86. Dye samples 1, 2, and 3 have a pH level of 4.86, 4.51, and 4.86 respectively.

A.5. RDF Dye pH Stability

A color change from red to yellow was observed after the addition of sodium acetate to the red dragon fruit dyes. However, no color change was observed after the addition of potassium chloride to the red dragon fruit dyes.

B. Discussion

The optimal blending time was obtained at 1 minute holding all other factors constant. Highest betacyanin content was yielded at 65 degrees Celsius (°C) keeping all other factors constant and using the determined blending time. The stability test to light exposure of samples showed that betacyanin content decreased upon prolonged exposure to light. Samples reacted towards the Sodium Acetate Triphosphate with pH 8.7, a basic solution. However, samples showed no reaction towards Potassium Chloride (5.5 pH). The dye extracted had the pH of 4.74 showing it to be acidic.

Results showed that increasing the blending time decreases the betacyanin content. Highest amount of betacyanin was yielded using one minute blending time. Blending the fruit increases the surface area exposed to ethanol. The more surface area exposed to the ethanol, the more it can extract the betacyanin. The increase therefore in blending time increases the betacyanin content of the extract. Nevertheless if the cell membranes are severely damaged due to extensive blending, lesser betacyanin will be extracted. This is due to the rigorous blending resulting to the lower betacyanin count (Ametajds and others 2003).

The best temperature to obtain high amount of betacyanin is evaporating samples at 65°C. As betacyanin undergo thermal treatment; it is known that the pigments will experience degredation and fluctuating chromatic activity (Herbal and others, 2006). Increasing temperature results to increasing betacyanin degradation (Azeredo 2006). However, this thermal degredation only makes a signifant effect at temperatures higher than 70°C (Ferna´ndez-Lo´ pez and Almela, 2001 as cited by Azeredo and others 2009). Ethanol evaporates faster when a higher temperature is used. A decrease in ethanol in the solution means a solution which is more concentrated with betacyanin. Furthermore, increasing the temperature shortens the time it takes to rotary evaporate the samples, decreasing the thermal degradation on the samples.

Betacyanin content decreased when exposed more to light. Earlier studies showed that Light exposure degrades betacyanin and shows an increased exposure results to a lower stability (Azeredo 2006). That is why samples experienced degradation of betacyanin amount during 3 days of light exposure. Highest degradation was experienced on the 3rd day.

The extracted dye had the pH level of 4.74 stating that it is acidic. Since it is acidic, samples changed color from red to yellow after exposure to the basic solution Sodium Acetate Triphosphate with pH 8.7. While, on the other hand, no color change had been observed when samples were exposed to the acidic solution Potassium Chloride with pH 5.5.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This study aimed to determine the optimal condition in extracting a dye from Red Dragon fruit (Hylocereus polyrhizus) peel using the ethanol extraction method and to evaluate its physico-chemical characteristics.

It specifically:

1. measured the betacyanin content of dyes extracted at varying blending timesof the peel with the ethanol: 1 min, 2 min, 3 min, 4 min, 5 min;
2. compared the betacyanin content of dyes extracted at varying time in blending the peel with the ethanol: 1 min, 2 min, 3 min, 4 min, 5 min;
3. measured the betacyanin content of dyes extracted at varying temperature in evaporating the solvent: 25°C, 35°C, 45°C, 55°C, 65°C;
4. compared the betacyanin content of dyes extracted at varying temperature in evaporating the solvent: 25°C, 35°C, 45°C, 55°C, 65°C;
5. evaluated the physico-chemical characteristics (light stability, pH stability, and pH level) of the dye extracted using the determined optimal conditions.

A. Summary of Findings

After data gathering, we came with these findings:

1. The betacyanin content of dyes extracted at one to five minutes blending times ranged from 32.175 to 44.092 mg/L.
2. The highest betacyanin content was achieved at a blending time of one minute while the lowest betacyanin content was achieved at a blending time of two minutes.
3. Amount of betacyanin of dyes extracted using 25°C to 65°C ranged from 29.486 to 109.847 mg/L.

4. The highest betacyanin content was achieved at an evaporating temperature of 65°C while the lowest betacyanin content was achieved at an evaporating temperature of 35°C.
5. The betacyanin contents after light exposure of 0-3 days ranged from 12.375 to 69.819 mg/L. Betacyanin decreased as days of light exposure increases. The Red Dragon Fruit dye extracted using the optimal conditions had pH levels ranging from 4.51-4.86. Dye samples 1, 2, and 3 have a pH level of 4.86, 4.51, and 4.86 respectively. A color change from red to yellow was observed after the addition of sodium acetate to the red dragon fruit dyes. However, no color change was observed after the addition of potassium chloride to the red dragon fruit dyes.

B. Conclusion

The study was able to establish the following conclusions:

The optimal blending time for extracting Red Dragon Fruit dye using ethanol extraction method was one minute. Sixty five degrees Celsius (°C) was determined to be the optimal evaporating temperature in the extraction. Betacyanin content of the dyes decreased upon exposure to light. The dye was acidic with a pH level of 4.74 and reacted when exposed to a basic solution (Sodium Acetate Triphosphate, pH 8.7).

C. Recommendations

The researchers recommend the following:

1. Further studies on the optimal conditions other than blending time and temperature in extracting Red Dragon Fruit dyes using ethanol extraction method.
2. Further tests be conducted using more than three replicates.
3. Optimization of the results of this study, this can be done by varying 2 or more factors at the same time and see their relationships.
4. Comparison also between other extraction methods.

LITERATURE CITED

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APPENDIX A

RAW DATA

Blending Time (min.)	Absorbance at 538nm			
	Replicate A	Replicate B	Replicate C	Average
1	0.479	0.481	0.463	0.474
2	0.371	0.351	0.355	0.359
3	0.371	0.356	0.369	0.365
4	0.378	0.381	0.379	0.379
5	0.381	0.382	0.379	0.381

Blending Time (min.)	Betacyanin Content (mg/L)			
	Replicate A	Replicate B	Replicate C	Average ± S.D.
1	43.908	44.092	42.442	43.480 ± 0.903462
2	34.008	32.175	32.542	21.908 ± 0.970109
3	34.008	32.633	33.825	33.489 ± 0.746582
4	34.650	34.925	34.742	34.772 ± 0.140023
5	34.925	35.017	34.742	34.894 ± 0.140023

Temperature (°C)	Absorbance at 538nm			
	Replicate A	Replicate B	Replicate C	Average
25	0.193	0.254	0.531	0.328
35	0.244	0.269	0.200	0.238
45	0.618	0.470	0.754	0.602
55	0.554	0.675	0.806	0.661
65	0.643	0.681	0.719	0.681

Temperature (°C)	Betacyanin Content (mg/L)			
	Replicate A	Replicate B	Replicate C	Average ± S.D.
25	29.486	38.806	81.125	49.806 ± 27.52079
35	37.278	41.097	30.556	36.310 ± 5.337027
45	94.417	71.806	115.194	93.806 ± 21.70091
55	84.639	103.125	123.139	103.634 ± 19.25505
65	98.236	104.042	109.847	104.042 ± 5.805556

Days of Light Exposure	Absorbance at 538nm			
	Sample A	Sample B	Sample C	Average
0	0.322	0.451	0.457	0.410
1	0.323	0.383	0.241	0.316
2	0.292	0.274	0.333	0.300
3	0.171	0.090	0.081	0.114

Days of Light Exposure	Betacyanin Content (mg/L)			
	Sample A	Sample B	Sample C	Average
0	49.194	68.903	69.819	62.639 ± 11.65225
1	49.347	58.861	36.819	48.227 ± 10.89053
2	44.611	41.861	50.875	45.782 ± 4.619686
3	26.125	13.750	12.375	17.317 ± 7.572913

Sample	pH level
1	4.86
2	4.51
3	4.86
Average	4.74

Chemical Used	Reaction Towards Chemical (Color Change)		
	Replicate A	Replicate B	Replicate C
Sodium Acetate Triphosphate (8.7 pH)	Yellow	Yellow	Yellow
Potassium Chloride (5.5 pH)	Red	Red	Red

APPENDIX B

SPSS One-Way ANOVA

1: Varying Blending Time

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	223.440	4	55.860	118.204	.000
Within Groups	4.726	10	.473		
Total	228.166	14			

2: Varying Temperature

ANOVA

VAR00004

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12349.969	4	3087.492	9.294	.002
Within Groups	3322.175	10	332.217		
Total	15672.144	14			

PAST Tukey's Pairwise

Blending Time

	A	B	C	D	E
A		0.0001761	0.0001761	0.0001761	0.0001761
B	26.68		0.8336	0.04742	0.03382
C	25.22	1.465		0.2245	0.1643
D	21.98	4.704	3.24		0.9994
E	21.67	5.013	3.549	0.3087	

Evaporating Temperature

	A	B	C	D	E
A		**0.888**	**0.08395**	**0.03026**	**0.02901**
B	**1.282**		**0.02071**	**0.007699**	**0.007399**
C	**4.181**	**5.463**		**0.9607**	**0.9547**
D	**5.115**	**6.397**	**0.934**		**1**
E	**5.154**	**6.436**	**0.9727**	**0.03871**	

APPENDIX C

PLATES

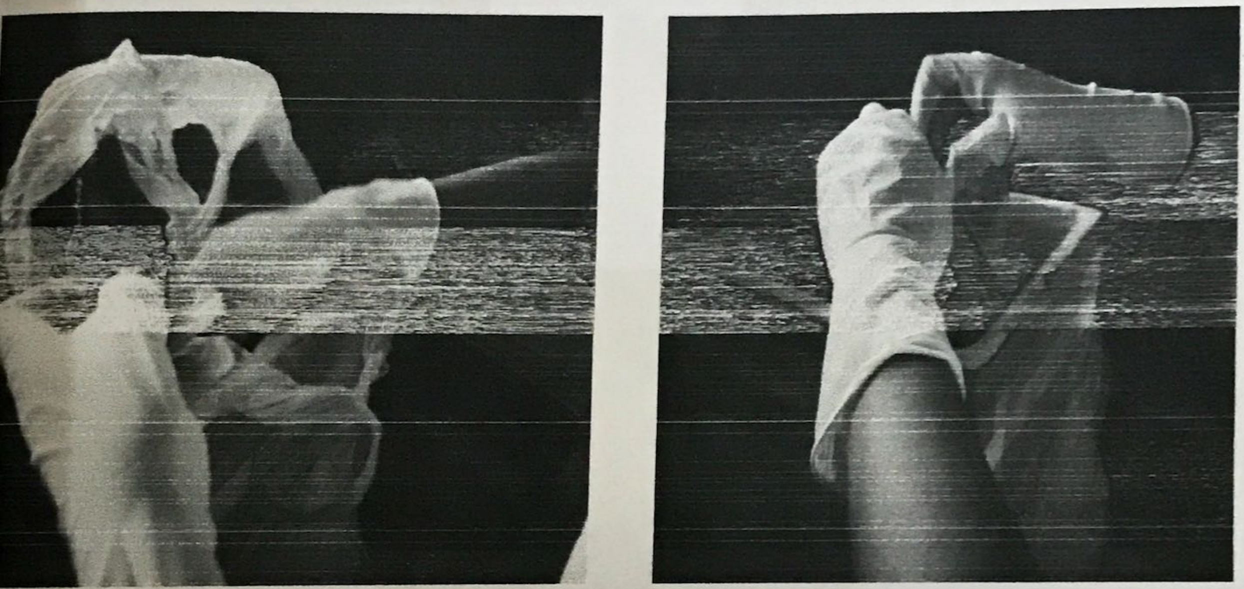

Plate 1. Red Dragon Fruit peel taken out of storage

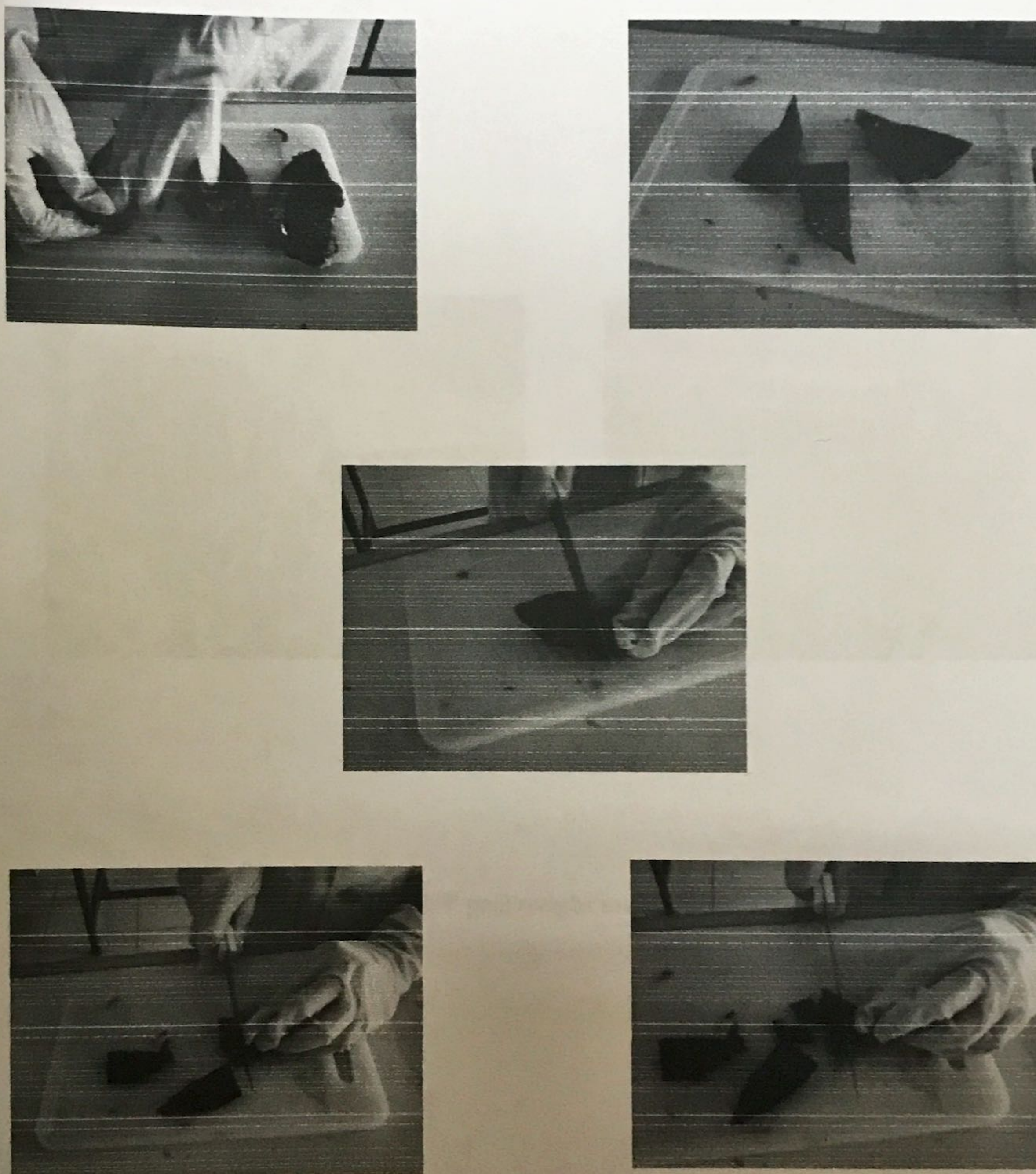

Plate 2. Cutting of RDF peel

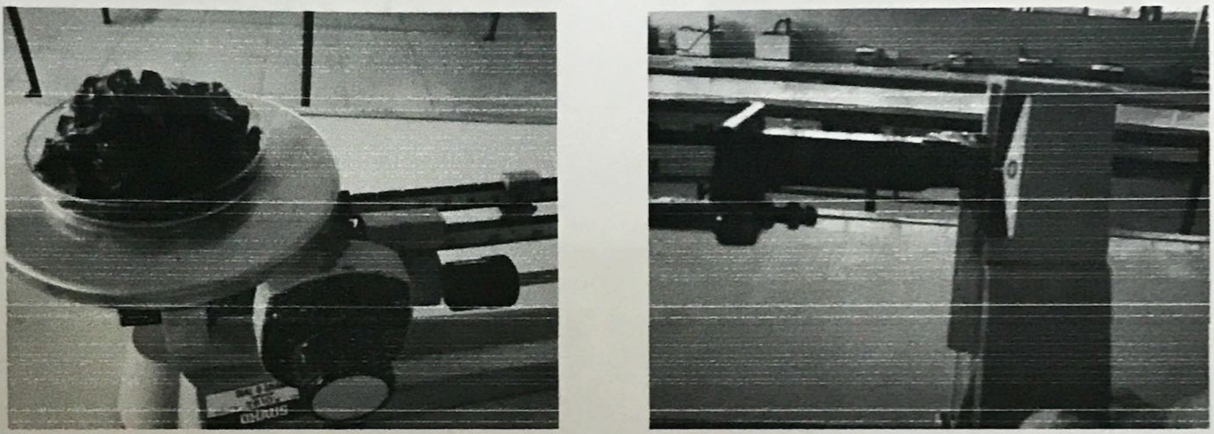

Plate 3. RDF peel weight measurement

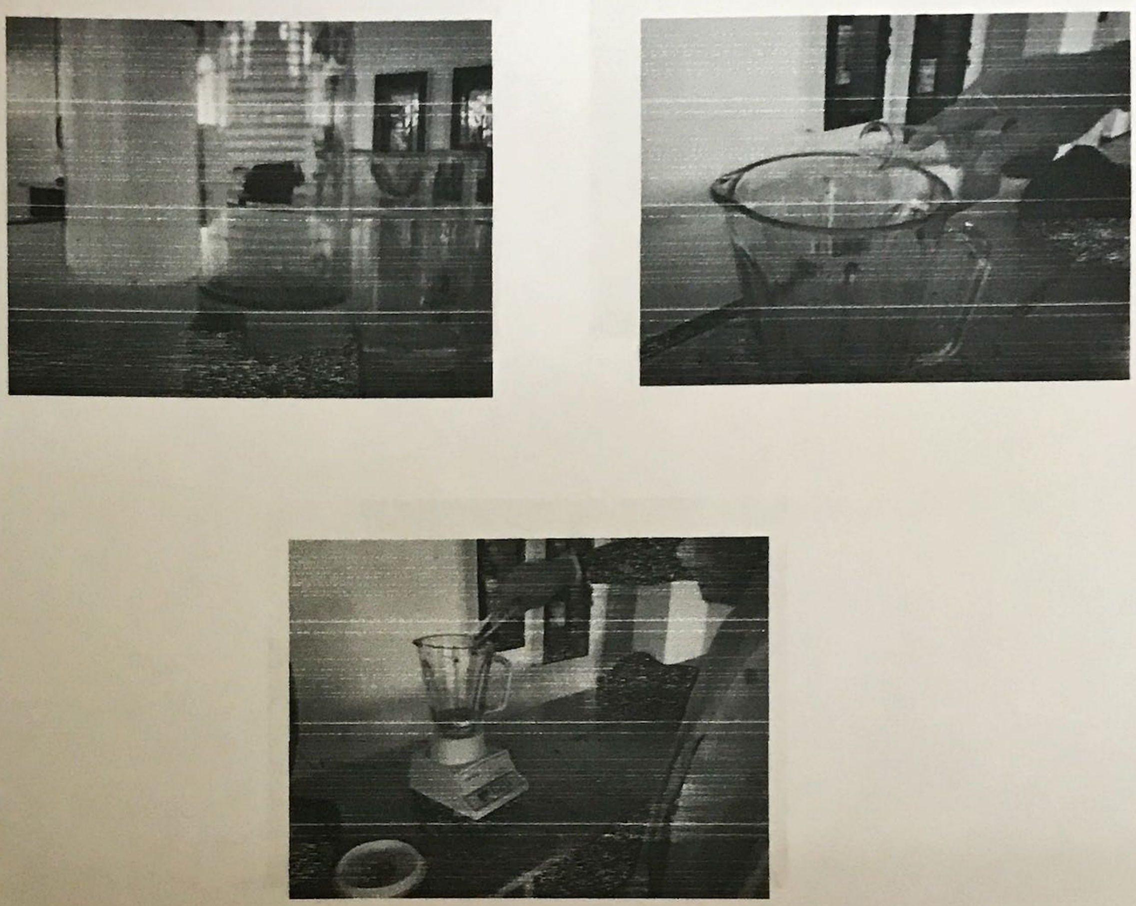

Plate 4. Ethanol Preparation

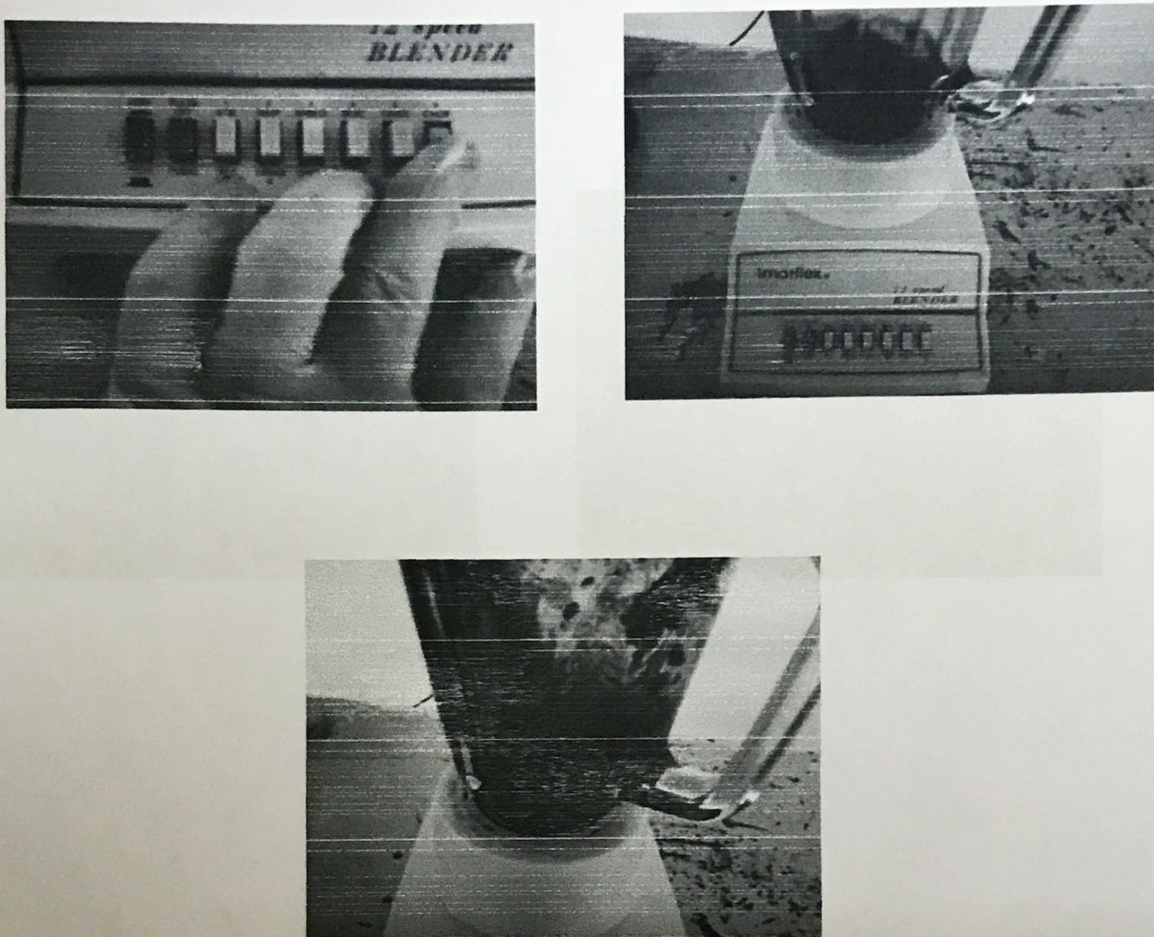

Plate 5. Blending

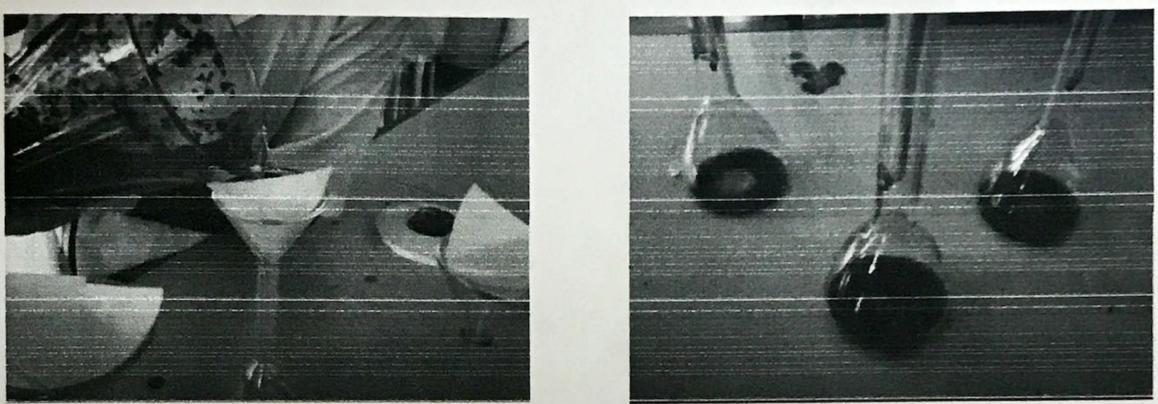

Plate 6. Filtering

Plate 7. RDF extract in rotary evaporator

 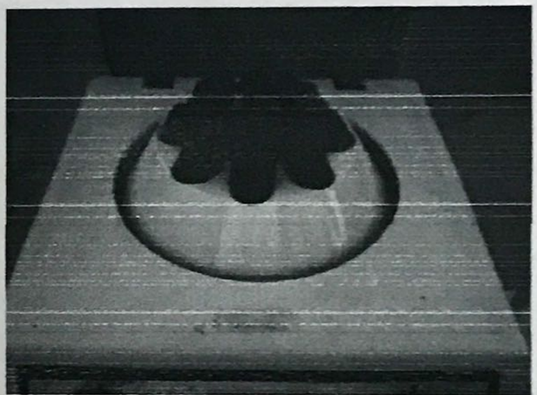

Plate 8. Centrifugation preparation and centrifugation

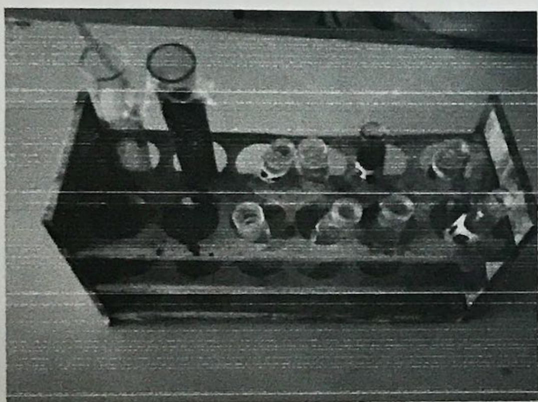 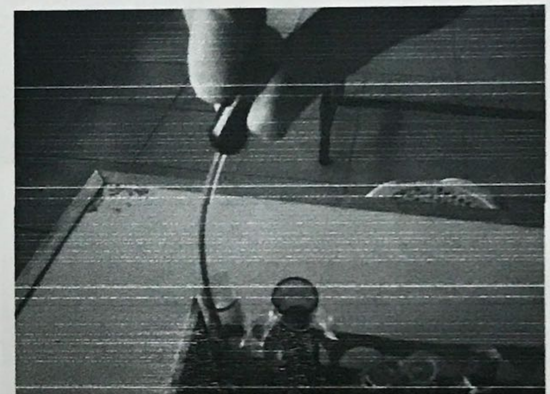

Plate 9. Transfer of RDF extract to other container

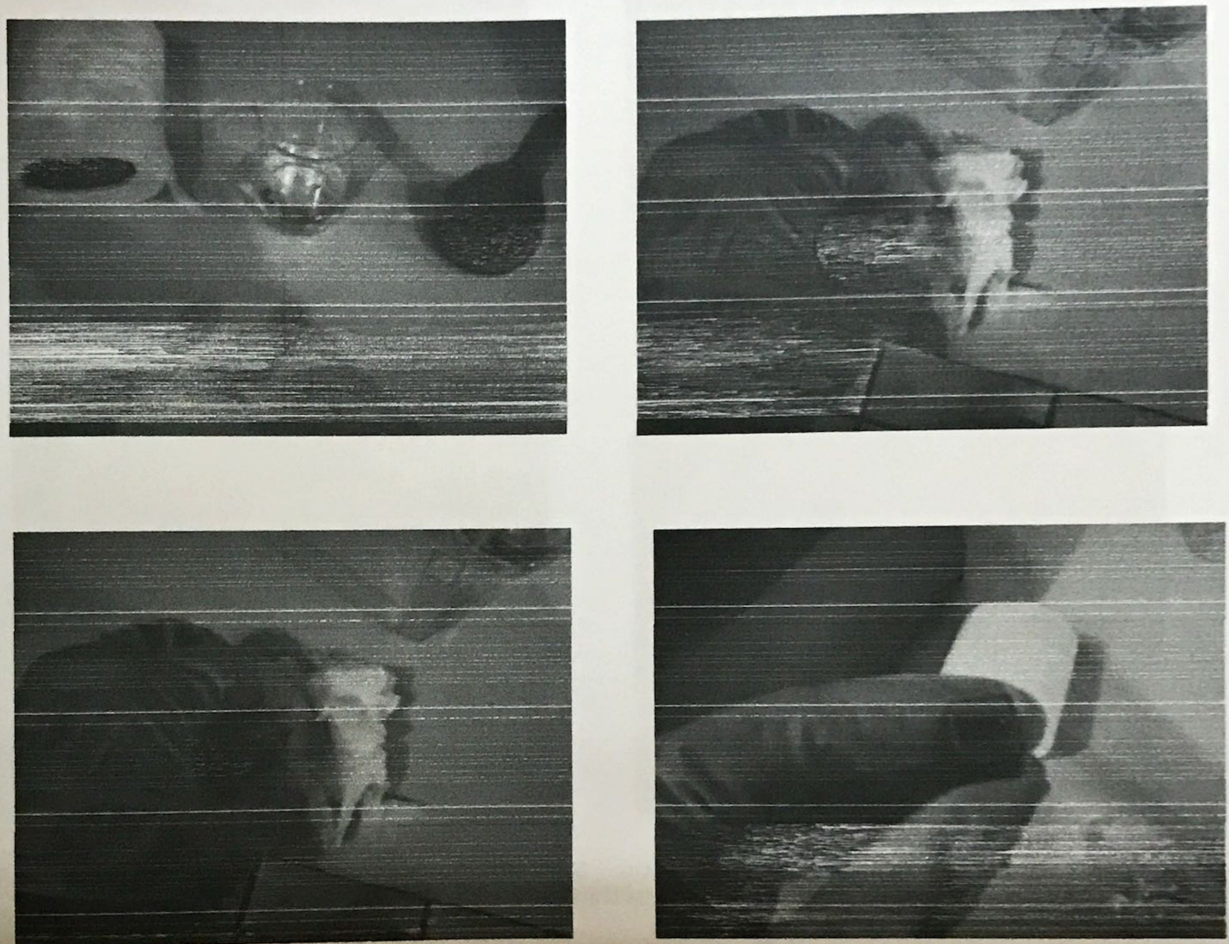

Plate 10. Spectrophotometer analysis preparation

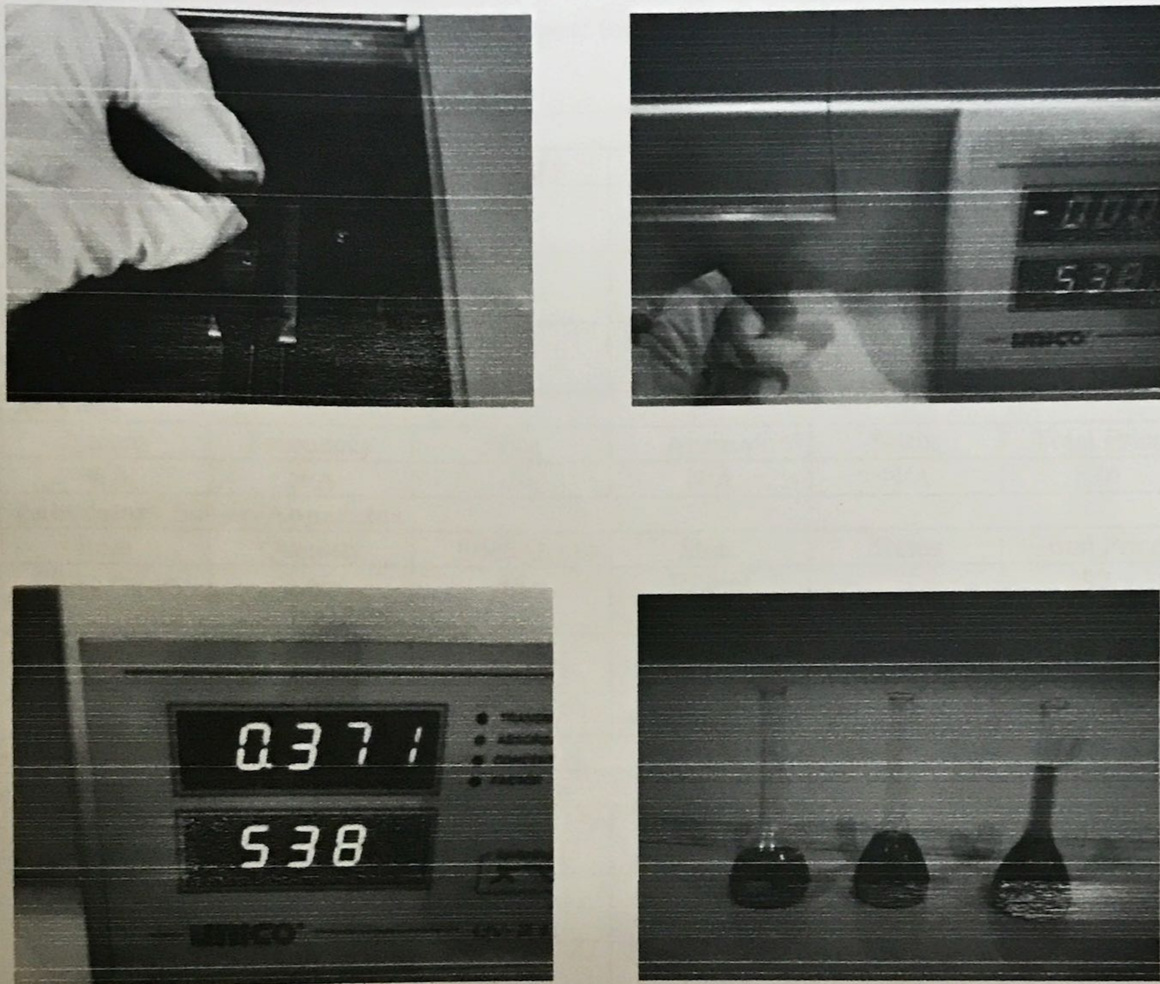

Plate 11. Spectroophotometric analysis of RDF dye

APPENDIX D

Liquidation Report for the Pfizer Grant

Chemicals/Media					
Item	Quantity	Price/Qty	Store	Status	Total Price
Red Dragon Fruit	5 kg	200	Lezo, Aklan		1000
Distilled Water	1-15L Galon	400	Sm Jaro		400
Ethanol	3L	300	Prince Valiant Int'l Corp.		900
Services					
Nature	Frequency	Price	Agency	Status	Total Price
N/A	N/A	N/A	N/A	N/A	N/A
Laboratory Set-up/Apparatus					
Item	Quantity	Price/Qty	Store	Status	Total Price
Filter Paper	2 pcs	46	Josmef Pharmacy		92
Fluorescent Bulb	1	99.75	City Hardware, Kalibo, Aklan		99.75
Transportation					
Item	Quantity	Price/Qty	Store	Status	Total Price
Iloilo-Aklan	1	191	Ceres Bus Line		191
Antique-Aklan	1	182	Ceres Bus Line		182
Aklan-Iloilo	3	191	Ceres Bus Line		573
Other Materials					
Item	Quantity	Price/Qty	Store	Status	Total Price
N/A	N/A	N/A	N/A	N/A	N/A

Total Expenses

	Amount
Chemicals/Media	2300
Services	N/A
Laboratory Set-up/Apparatus	191.75
Transportation	946
Other Materials	N/A
Total	3437.75

RECEIPT

Date May 13 , 20 12

RECEIVED from Irwin John Rejerso

Address Gaub Cabatuan, Iloilo City

the sum of Pesos Nine hundred pesos only ₱ 900.00

as ☑ full / ☐ partial payment for 3L ethanol

Partial ______ Cash ______

Balance ______ Check No. ______

Received by:

PRINCE VALIANT INT'L. CORP

AUTHORIZED SIGNATURE

RECEIPT

Date May 13, 20 12

RECEIVED from Arvin John Tejereso

Address: Gaub Cabatuan, Iloilo City

the sum of Pesos Four Hundred pesos only ₱ 400.00

as ☑ full / ☐ partial payment for 15 L distilled water

Partial ____________ Cash ____________

Balance ____________ Check No. ________

Received by:

AUTHORIZED SIGNATURE

RECEIPT

Date April 28, 20 12

RECEIVED from Marmelda Popar

Address Mabilo, Kalibo, Aklan

the sum of Pesos ninety-nine pesos and seventy centavos only = 99.75

as ☑ full / ☐ partial payment for 1 pc. florescent bulb

Partial ________ Cash ________

Balance ________ Check No. ________

Received by:

City Hardware, Kalibo Aklan

AUTHORIZED SIGNATURE

RECEIPT

Date May 14, 20 12

RECEIVED from Jenny Wendy Balbuena

Address Bitoon, Jaro, I-C.

the sum of Pesos Ninety-two pesos only ₱ 92.00

as ☑ full / ☐ partial payment for 2 pcs. filter paper

Received by:

Partial ______ Cash ______

Balance ______ Check No. ______

JOSHEF PHARMACY

AUTHORIZED SIGNATURE

JIMMY FRUITS STAND

JIMMY O. RUBINO - Prop.
Roxas Avenue Poblacion, Kalibo, Aklan
TIN: 919-664-572 Non-VAT
Tel. # 268-1489

№ 4183

CASH SALES INVOICE

Date April 29, 2012

SOLD to Marmelun G. Popes

Address Philippine Science High School

Bus. Name/Style ______ TIN ______

DESCRIPTION	Qty.	Unit Cost	AMOUNT
5kls. Red Dragon fruit		P200	P1000

Amount Payable .... P 1000

ELITE PRINTING PRESS TIN 114-281-511
Roxas Ave., Kalibo, Aklan Tel.# (036) 268-9315
OCN 2AU00 0355989 07-01-2009
50 Bkts. 50X2 2751 - 5250

Signature

CERES LINER - JARO

TIN 001-890-888-002 - VAT
DUNGON B. JARO, ILOILO CITY
(VAT-Exempt)

BUS NO.					PESOS			cts
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7	7	7	7	7	7	7	7	60
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9	9	9	9	9	9		9	80
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HALF		STUDENT			SR.			00
ORD		A/C			N-STOP			00

№ 785818 AD

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F	DATE		FR. KM.			TOTAL		
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O			7	7		7	7	7
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Please keep ticket for inspection
PASSENGER TICKET

SPEEDEX Printing Center
10,000 Stubs SN 000001AD - 1,000,000AD
OCN 2AU0000686688 03.01.12

CERES LINER - JARO

TIN 001-890-888-002 - VAT
DUNGON B. JARO, ILOILO CITY
(VAT-Exempt)

BUS NO.					PESOS			cts
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SPEEDEX Printing Center
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OCN 2AU0000686688 03.01.12

CERES LINER - JARO

TIN 001-890-888-002 - VAT
DUNGON B. JARO, ILOILO CITY
(VAT-Exempt)

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S			6	6	6	6	6	6
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N			8	8	8	8	8	
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Please keep ticket for inspection
PASSENGER TICKET

SPEEDEX Printing Center
10,000 Stubs SN 000001AD - 1,000,000AD
OCN 2AU0000686688 03.01.12

CERES LINER - JARO

TIN 001-890-888-002 - VAT
DUNGON B. JARO, ILOILO CITY
(VAT-Exempt)

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№ 660825 AC

SPEEDEX Printing Center, 136 Rizal - Lopez Jaena Sts., Iloilo City
10,000 Stubs SN 000001AD - 1,000,000AD
OCN 2AU0000710589 05.17.12

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Please keep ticket for inspection
PASSENGER TICKET

CERES LINER - JARO

TIN 001-890-888-002 - VAT
DUNGON B. JARO, ILOILO CITY
(VAT-Exempt)

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Please keep ticket for inspection
PASSENGER TICKET

SPEEDEX Printing Center
10,000 Stubs SN 000001AD - 1,000,000AD
OCN 2AU0000686688 03.01.12

APPENDIX E

Letter

Republic of the Philippines
Department of Science and Technology
Philipppine Science High School – Western Visayas
Doña Lawaan H. Lopez Campus
Bito-on, Jaro, Iloilo City, Iloilo

May 18, 2012

Engr. Rowen Gelonga
Regional Director of DOST VI
Jaro, Iloilo City, Iloilo

Dear Engr. Gelonga,

Greetings! We are Marmelou G. Popes, Arvin John E. Tejereso and Jenny Wendy S. Balbuena, incoming fourth year students of Philippine Science High School Western Visayas Campus. In our Research II class, we are required to conduct our approved study.

Our study is entitled "Study of Optimal Blending Time, Temperature and Stability of Red Dragon Fruit (*Hylocereus polyrhizus*) Peel using Ethanol Extraction Method". For our study, we will need a rotary evaporator. We would like to seek approval if we can use these devices this coming Monday, May 21, 2012. We are truly sorry for asking and sending this letter in such a short notice.

You can contact us in this number, 09161212673, or at this email, marmz_popes@yahoo.com.

Thank you very much sir. We hope to hear from you soon.

Respectfully yours,

Marmelou Popes   Arvin John Tejereso   Jenny Wendy Balbuena

Noted:

Virna Jane Navarro
CISD chief

Shena Faith Ganela
Director III