EFFECTOF ENZYME CONCENTRATION AND TEMPERATURE ON VISCOSITYAND BETACYANIN CONTENT FROM PITAYA WASTE EXTRACT

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SUPERVISOR'S DECLARATION

I hereby declare that I have checked this project and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The project has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Special dedication to my supervisor, my family members,
my friends, my fellow colleague and all faculty members
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NOMENCLATURE

˚C	Degree Celsius
%	Percentage
MPa	Mega Pascal
USD	United States Dollar
RM	Ringgit Malaysia
Tons	Tonnes
$	Dollar
pH	Power of Hydrogen
˚F	Degree Fahrenheit
g/cm^3	Gram per centimeter cube
µm	Micro meter
mm	Millimeter
M	Molarity
V	Volume
mL	Milli-Liter
g	Gram
h	Hour
cP	Centipoises
wt.	Weight
s	Second

KESAN KEPEKATAN ENZIM DAN SUHU TERHADAP KELIKATAN DAN KANDUNGAN BETASIANIN DARIPADA EKSTRAK KULIT BUAH PITAYA

ABSTRAK

Oleh kerana pencelup buatan menunjukkan kesan negatif dalam bidang makanan dan tekstil, pewarna semulajadi telah mendapat keutamaan terutamanya dalam bidang tekstil. Kulit buah naga digunakan sebagai sumber untuk pewarna semulajadi dalam eksperimen ini. Terdapat satu kekangan yang menghalang pewarna semulajadi yang dihasilkan untuk melekat pada kain kerana sifat fizikalnya yang sangat likat yang mana dipercayai disebabkan oleh kandungan pektin. Kajian ini bertujuan untuk mengurangkan kelikatan pewarna semulajadi dengan menggunakan enzim yang bernama pektinase dan dijalankan dalam skala yang kecil. Eksperimen ini juga bertujuan untuk mengkaji kesan kepekatan enzim dan suhu terhadap pengurangan kelikatan dan kandungan pigmen betasianin dalam pewarna semulajadi. Eksperimen ini menggunakan kaedah kimia dan biologi dimana kaedah kimia merujuk kepada pengekstrakan pewarna semulajadi daripada kulit pitaya meggunakan air sebagai pelarut. Manakala, kaedah biologi pula merujuk kepada tindakbalas enzim yang digunakan untuk menggurangkan kelikatan pewarna semulajadi. Dua faktor yang dikaji dalam eksperimen ini iaitu kesan kepekatan enzim dan suhu telah menunjukkan kepekatan enzim dan suhu yang optimum terhadap pengurangan kelikatan pewarna semulajadi. Pada kepekatan enzim 2.5% dan suhu 50^{o}C, pengurangan kelikatan yang amat besar dan pegurangan pigmen betasianin yang sedikit dalam pewarna semulajadi dapat diperhatikan. Daripada kajian ini, adalah dicadangkan bahawa ujikaji lanjutan boleh dilaksanakan terhadap pH dan kepekatan pewarna semulajadi bagi mendapatkan kelikatan yang rendah dan kandunag betasianin yang tinggi.

EFFECT OF ENZYME CONCENTRATION AND TEMPERATURE ON VISCOSITY AND BETACYANIN CONTENT FROM PITAYA WASTE EXTRACT

ABSTRACT

As synthetic dye has shown up few hazards in contributing in food and textile industries, natural dye has gain its priority in those fields especially in textile sector. In this experiment, pitaya's waste was selected as a source for natural dye. Thus, there is one obstacle that prevents the natural dye to fulfill the requirements needed in textile industries as the physical properties of the natural dye which is high in viscosity causing it not fasting on cloth which is believed, due to pectin content. In order to come over this problem, this research aim to reduce the viscosity of the natural dye using commercialize pectinase, in a small scale and study the effect of the enzyme concentration and temperature on the reduction of viscosity of the natural dye and also observe the difference in betacyanin content. This experiment was carried out by chemical and also by biological mechanism. Chemical mechanism refers to solvent extraction using water to extract the dye from the fruit, whereas, biological manner refers to usage of enzyme to reduce the viscosity of the natural dye. When enzyme concentration varies form 0.1 % to 5%, the viscosity reduced gradually until the enzyme concentration is 2.5% then the reduction is insignificant. Whereas, the temperature shown a similar result. The highest reduction in viscosity is when the reaction temperature is set at 50^{o}C. It is because, when temperature increases, the rate of reaction will increase and at one point, the rate of reaction will decrease. It is because the enzymes will be denatured at high temperatures. From this research, it is recommended that further studies can be done to the pH and concentration of natural dye in order to obtain low viscosity and high betacyanin content.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Colours play an important role in enhancing the aesthetic appeal for food and clothing products. In ancient times, natural dye which is obtained from plants pigment through biological manner was commercialized. Then, as technology and exploration in chemical industries enhanced, synthetic dye was introduced and undeniable it gained a positive response from food and textile manufacturer as it is cheap, easier to apply, more colorfast, and could be produced in a wider and brighter range of colors. Slowly, when hazards of synthetic dye come to the public attention, its position as a dye is threatened. Again, natural dye has gained its popularity back due to its nature characteristics.

As a consequence, extraction colour from plant has gained much attention from many researches. Precisely, colour properties from pitaya waste can be alternative way to replace synthetic chemical in many fields of industries. Production of natural dye from pitaya waste can be optimized since there is a growing interest in

the use of natural pigments for food colouring because natural products are associated with quality and health promotion whereas synthetic pigments are critically assessed by consumers (Downham and Collins, 2000).

1.2 Problem Statement

In order to reduce the usage of synthetic chemical as a conventional dye, there are alternative ways by using natural resources such as pitaya waste to produce natural dye. It is undeniable that pitaya fruit has all the creditability to produce a natural dye, there are still crisis encountered in the application of the dye obtained from the pitaya waste. Hence, using waste to transform to beneficial product will reduce many environmental issues. Other than that, natural dye will be more safer and environmental friendly to use in textile industries. Although the natural dye has all its advantages, the problem occurred when the dyes are harder applied onto the fabrics. Concisely, the colour not fasting on the fabric and it is easily removed from the fabric. It has been determined that the physical property of the dye such as high viscosity, resulting in the mentioned scenario. Due to its features, it is deduced that one of the possible component that caused the dye to show up such characteristic is pectin.

1.3 Research Objectives

This study is carried out:

i) To investigate the effect of enzyme concentration and temperature on viscosity of the natural dye.

ii) To investigate the effect of enzyme concentration and temperature on betacyanin content of the natural dye.

iii) To compare the fastness of the natural dye before and after the enzymatic reacton.

1.4 Scope of the Study

The scopes of study are:

i. Red pitaya waste is used which was bought in Taman Tas.

ii. The dye extracted from the fruit using water extraction by applying ratio 1:3 of 1g of raw material to 3 mL of water.

iii. Sedimentation through centrifugation is carried out for half an hour under $4^{o}C$ at 8000 revolution per minute (rpm) and filtration through stainless steel filter fabric (0.3 mm mesh) was used to separate the insoluble residue (Harivaindaran et al., 2008).

iv. Pectinase enzyme was used to reduce the viscosity of the natural dye in pH of 5.5 and at $50^{o}C$.

v. The effect of enzyme concentration (0.1 % to 5%) and temperature (30^{o}C to 100^{o}C) on reduction of viscosity and changes in betacyanin content will be studied.

1.5 Significance of the Study

This study is conducted in order to reduce the viscosity from the dye extracted from pitaya peel. The reduction in viscosity is extremely beneficial for the dye as it increases the dye's properties in term of stability. This accent will make the dye to last long onto the fabrics and suitable used as a conventional dye in textile industries.

In this study, commercialize pectinase enzyme will be used to break down the pectin content which is believed to cause the high viscosity in the dye. The effect of enzyme concentration and temperature will be studied on reduction of viscosity and betacyanin content in the natural dye. The finding from this study hopefully will be useful for the industrial especially textile industries and economic purpose.

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CHAPTER 2

LITERATURE REVIEW

2.1 Natural Dye

Natural dyes are dyes or colorants derived from plants, invertebrates, or minerals. The majority of natural dyes are vegetable dyes from plant sources – roots, berries, bark, leaves, wood and other organic sources such as fungi and lichens. The earliest surviving evidence of textile dyeing was found at the large Neolithic settlement at Çatalhöyük in southern Anatolia, where traces of red dyes, possible from ochre (iron oxide pigments from clay), were found. Polychrome or multicolored fabrics seem to have been developed in the 3rd or 2nd millennium BCE (Barber, 1991). Textiles with a "red-brown warp and an ochre-yellow weft" were discovered in Egyptian pyramids of the Sixth Dynasty (2345-2180 BCE). There is a growing interest in the use of natural pigments for textile coloring because natural products are associated with quality and health promotion whereas synthetic pigments are critically assessed by consumers (Downham and Collins, 2000). Natural colorants from plant sources are receiving growing interest from both textile manufacturers and consumers in the continuing replacement of synthetic dyes (Duhard et al., 1997;

Stintzing and Carle, 2004). In the early 21st century, the market for natural dyes in the fashion industry is experiencing a resurgence (Calderin and Jay, 2009) Western consumers have become more concerned about the health and environmental impact of synthetic dyes in manufacturing and there is a growing demand for products that use natural dyes. The European Union, for example, has encouraged Indonesian batik cloth producers to switch to natural dyes to improve their export market in Europe.

2.1.1 Classification of Natural Dye

Natural dyes can be classified (Gulrajani and Gupta, 1992) in a number of ways. The earliest classification was according to alphabetical order or according to the botanical names. Later, it was classified in various ways, e.g. on the basis of hue, chemical constitution, application class etc. In the colour index the natural dyes are classified according to the hue (Predominating colour). The number of dyes in each hue is as follows in table 2.1:

Table 2.1 The number of natural dyes in each hue as per the colour index.(Source: Gulrajani and Gupta, 1992)

CI Natural	No. of Dyes	Percent
Yellow	28	30.4
Orange	6	6.5
Red	32	34.8
Blue	3	3.3
Green	5	5.5
Brown	12	13
Black	6	6.5

On the basis hues, natural dyes can be classified as follows (Dedhia, 1998)

i) Red colour dyes: most red dyes are hidden in roots or barks of plants or camouflaged in the bodies of dull grey insects. They are almost invariably based on anthraquinone and its derivatives. These dyes are stable to light and washing.

ii) Yellow colour dyes: Yellow is the liveliest and perhaps the most abundant of all hues in nature. About 90% of the yellow dyes are flavonoids. Generally, they produce pale shade with quicker fading except turmeric, which produce dull deep shade but considered to be susceptible to light as they emit fluorescence. Wash fastness rating of natural yellow dyes ranges from fair to excellent, e.g., tesu, turmeric, kapila.

iii) Blue colour dyes are indigo and woad, give excellent fastness to light and washing.

iv) Black colour dyes: Black shades, generally obtained from tannin rich plant natural dyes and appreciably substantive towards cellulosic and protein fibre, imparts good overall fastness properties. Examples – logwood, harda, custard apple etc.

Natural dyes can also be classified on the basis of their chemical constitution (Dedhia,1998).

i) Indigoid dyes: Indigo and tyrian purple are the most common examples of this class. Another blue dye, woad also possesses indigo as the main dyeing component.

ii) Anthraquinone dyes: Almost all the red natural dyes are based on the anthraquinoid structure having both plant and mineral origin. Madder, lacs, kermes, cochineal are some of the dyes possess this type of structure. These are generally mordant dyes.

iii) Alphanaphthoquinones: Typical example of this class is lawsone (henna), cultivated mainly in India and Egypt. Another similar dye is juglone, obtained from the shells of unripe walnuts. These dyes are generally disperse dyes and give shades of orange.

iv) Flavonoids, which yield yellow dyes can be classified under flavones, isoflavones, aurones and chalcones. Flavones are colourless organic compounds. Most of the natural yellows are derivatives of hydroxyl and methoxy substituted flavones and isoflavones. Common example is weld (containing luteolin pigment) giving brilliant and fast colours on both wool and silk.

v) Betalains actually comprised of two groups of pigments: the red–purple betacyanidins and the yellow betaxanthins both of which are water-soluble. Betacyanidins are conjugates of cyclo-DOPA and betalamic acid, and betaxanthins are conjugates of amino acids or amines and betalamic acid.

vi) Anthocyanidins: The naturally occurring member of this class includes carajurin, a direct orange dye for wool and cotton. It is obtained from the leaves of bignonia chica.

vii) Carotenoids: The class name carotene is derived from the orange pigment found in carrots. In these, the colour is due to the presence of long conjugated double bonds.

2.2 Source of Natural Dye

Natural dye are derived from naturally occurring sources such as plants (e.g., indigo and saffron); insects (e.g., cochineal beetles and lac scale insects); animals (e.g., some species of mollusks or shellfish); and minerals (e.g., ferrous sulfate, ochre, and clay) without any chemical treatment (Samantha et al., 2009).

2.2.1 Natural Dye from Animal

A good example is cochineal, which is a brilliant red dye produced from insects living on cactus plants. The properties of the cochineal bug were discovered by pre-Columbian Indians who would dry the females in the sun, and then ground the dried bodies to produce a rich, rich red powder (Vankar, 2000). When mixed with water, the powder produced a deep, vibrant red coloring. Cochineal is still harvested today on the Canary Islands. In fact, most cherries today are given their bright red appearance through the artificial color "carmine", which comes from the cochineal insect. Natural dyes are derived from animals are summarized below:

i) Cochineal insect (Red)

ii) Cow Urine (Indian Yellow)

iii) Lac insect (Red, Violet)

iv) Murex Snail (Purple)

v) Octopus (Sepia Brown)

In this research, natural dye from animal source did not consider, because mostly, in order to extract the dye from animal, the animals has to be dead. So, if massive application of natural dye is extracted from animal or insects, it may harm the population of the indicated lives. Hence, the extraction of dye from animal requires a more tedious and involves chemical substances which may cause harm to the users.

2.2.2 Natural Dye from Plant

Many plants have been identified as potentially rich in natural dye contents, and some of them have been used for natural dyeing for quite some time. Various parts of plants like roots, stems barks, leaves, fruits and seeds may contain colouring matter which can be exploited. Normally, natural dyes are extracted from the roots, stems, leaves, flowers, fruits and vegetables (Vankar, 2000). Some plants may have more than one colour depending upon which part of the plant one uses. The shade of the colour a plant is picked, how it was grown, soil conditions, etc. the minerals in the water used in a dye bath can also alter the colour. Some natural dyes contain natural mordants. About 500 plant origin dyes, colouring matter derived from root, leaf, trunk, and others are shown in the Table 2.2 below.

Table 2.2 Common natural dyestuff obtained from different vegetable origin. (Source Ashis and Adwaita, 2002)

Part of the plants	Dyestuff
Root	Turmeric, Madder (Manjistha), Onions, Beet-root
Bark/ Branches	Purple bark, Sappan wood, Shillicorai, Khair, Red, Sandalwood
Leaf	Indigo, Henna, Eucalyptus, Tea, Cardamon, Coral Jasmine, Lemon Grass
Flowers (Petals)	Marigold, Dahlia, Tesu, Kusum
Fruits/Seeds	Latkan, Pomegranate rind, Beetle nut, Myrobolan (Harda)

Natural dye can be extracted from plant easily without any chemical substances mostly,by water extraction. In addition, extraction of dye from plant can be extracted mostly from waste such as from peel or small portion of plant which will not give effect to the plant. In Malaysia, the society generates abundant agricultural wastes with the volume of approximately 5 million tones annually and is expected to double by the year 2010 (Park et al.,2007). Some of these wastes include oil palm trunks and fronds, palm kernel cake, sugar cane baggase, rice husk, rice straws, coconut fibers and meal, cocoa pods, rubber wood dusts, fruit peels and many other wastes materials.

The management of these wastes effectively and economically must be given utmost priority in the country in ensuring not only in reducing the detrimental impact of the wastes to the environment, but most importantly in the transformation of these wastes into useful raw materials for the production of added value commodities of industrially commercial potentials. Annually, roughly 2534.2 ton dragon fruits produced in Malaysia (Cheah and Zulkarnain 2008). The peels are mostly waste material resulting from the dragon fruit juice processing industry and normally discarded. These discarded peels, as mentioned above, may cause severe

problem especially to environment particularly water pollution. Since, pitaya peel has a high content in betacyanin pigment, it has all the creditability to be a sustainable source for natural dye production in Malaysia.

Betalains are a class of red and yellow indole-derived pigments found in plants of the Caryophyllales, where they replace anthocyanin pigments (Clement and Mabry, 1996). Betalains also occur in some higher order fungi. They are most often noticeable in the petals of flowers, but may color the fruits, leaves, stems, and roots of plants that contain them. They include powerful antioxidant pigments such as those found in beets. Betalains are the nitrogenous vacuolar pigments of 13 families within the plant kingdom also accumulating in some members of the Basidiomycetes (Gill and Steglich, 1987; Gill, 1994). The name "betalain" comes from the Latin name of the common beet (*Beta vulgaris*), from which betalains were first extracted. The deep red color of beets, bougainvillea, amaranth, and many cacti results from the presence of betalain pigments. The particular shades of red to purple are distinctive and unlike that of anthocyanin pigments found in most plants. There are two categories of betalains:

- Betacyanins include the reddish to violet betalain pigments.
- Betaxanthins are those betalain pigments which appear yellow to orange.

Among the betaxanthins present in plants include vulgaxanthin, miraxanthin and portulaxanthin, and indicaxanthin. Plant physiologists are uncertain of the function that betalains serve in those plants which possess them, but there is some preliminary

evidence that they may have fungicidal properties. The structure of betacyanin is shown in the Figure 2.1 below.

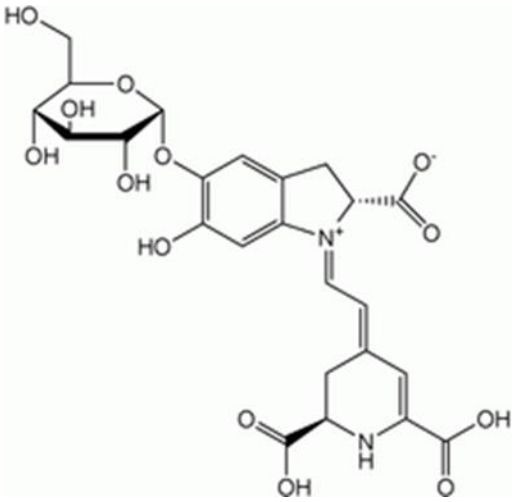

Figure 2.1 Betacyanin structure

Natural dye that contain betacyanin pigment was chosen to be extracted in this research because it exhibit a strong red colour which is has a strong attraction and made the studies to examine its property easier and clearer. Other than that, betacyanin pigment can be obtain in the products that easily available such as dragon fruit, and beet root. Hence, the betacyanin is also can be obtained from waste such as pitaya peel which has been selected in this research.

2.2.3 Natural Dye from Mineral

Mineral origin colourant are derived from specific mineral natural source or so-called mineral colours are produced from inorganic compounds. Some of the important mineral colourant are chrome-yellow, iron-buff, narkin-yellow, Prussian-blue and manganese brown (Ashis and Adwaita, 2002). Ocher is a dye obtained from an impure earthy ore of iron or a ferruginous clay, usually red (hematite) or yellow (limonite). In addition to being the principal ore of iron, hematite is a constituent of a number of abrasives and pigments. Natural dye which is derived from mineral is not considered in this research because the source is not available abundantly as other resources.

2.3 Types of Natural Dye

2.3.1 Lac Dye

It is extracted from lacifer lacca insect. It is used for dyeing of wool, silk and cotton fibers (Mortensen, 2006). It gives reddish with tin mordant and purplish with copper mordant. This type of natural dye is not considered in this research because it involves animal sources, a typical insect which the source is limited.

2.3.2 Annatto

It is prepared from the seed of annatto. It is used in the dyeing of silk and wool. It gives orange and peach colour. Its botanical name is bixin (Agarwal and Gupta, 1992). This type of dye is also not considered in this research because the source of the natural dye that is the seed of annatto is not abundantly available in our country. Annatto is commonly used in Latin America and Carribean regions.

2.3.3 Harda

It is prepared from fruits of Harda and it yields yellow and gray colours with aluminum and ferrous mordant respectively. It can be used in coloration of wool and silk (Bhattacharya and Shah, 2000). In this research, the dyes from Harda fruit is not considered because it is derived from a fruit and not waste. If dye is extracted from fruit, then competition for the fruit will be higher.

2.3.4 Himalayan Rhubard

It is manufactured from Himalayan herb. The root of this plant is used for the manufacture of dye stuff. It gives yellow and oranges. It can be used directly and with Alum Mordant on wool or silk. This type of dyes is also not considered in this research as the source for the dye is not available easily and abundantly in our country.

2.3.5 Indigo Blue

It is a fermented dyes of leaves of indigo ferra tinctoria. It gives blue colour. It can dye cotton. This type of dyes is not considered in this research because in order to produce this dye, the leaves have to be soaked in water and fermented in order to convert the glycoside indican naturally present in the plant to the blue dye indigotin (Kamal et al., 1993). The fermentation is quite particular and long extraction time, this is why this type of dye is not considered in this research.

2.3.6 Kamala Dye

It is prepared from the deposits on flowers of Kamala tree. It gives yellow colour on wool and silk. It can be used directly of with mordant as well (Bhattacharya and Shah, 2000). This type of the dyes is also not used in this research, because it uses the flowers of the tree which is not waste. So the competition may be higher to obtain this flower as this flower has been used for herbal remedies. Hence, this flower is also not available in our country.

2.3.7 Manju Phal

It is manufactured from the nut galls of Manju Phal tree. It is used for dyeing of silk, and wool, both directly or with mordant (Ashis and Adwaita, 2002). It gives

cream and grey colours with alum and iron mordant. This type of dye is also not covered in this research because in order to use it and test it, the usage of mordant as stated is required. Extra cost will be generated by using mordant.

2.3.8 Gum Arabic

It is manufactured from the bark of Indian Gum Arabic tree. It is used for dyeing of cotton with mordant. It yields brown shade having very good fastness. Gum Arabic has various uses and applications due to its property (Paliwal, 2001). Gum Arabic is not usually use for dyeing textile or cloth. This type of dye normally use for watercolor painting.

2.3.9 Trigonella Foenum Graecum

It is prepared from the fenugreek seeds. It is used in the dyeing of cotton fabrics. It gives yellow shades with metallic mordents like copper sulphate and ferreous sulphate (Paliwal 2001). The function of this fenugreek seed in producing natural dye is insignificant since this seeds is widely used for other applications. That is why this type of dye is not chosen for this research.

2.4 Uses of Natural Dye

2.4.1 Textile

Natural dye has a big creditability to be implemented in textile industries. These dyes can be used for coloration of textile material at different stages such as on the yarn, on the fabric and even can be applied on the apparels. This research has focused or narrowed down its scope of natural dye application, the problem encountered and the solution for the natural dye to be applicable in the textile industries based on several reasons. Firstly, the application of dye is majorly used in textile field compared to other application field of natural dye. So, this research will be focusing more the problem and solution for the natural dye application in the textile field.

2.4.2 Cosmetics

The natural dyes from dolu and rind of pomegranate are used in coloration of lipsticks and other cosmetics (Dominique, 2010). They are manufactured by water extraction method. The coloring matter can be extracted by water extraction method. The coloring matter can be extracted with Super Critical Fluid Extraction Method.

2.4.3 Edible Dyes

Most common dye, which can be used for coloration of the edible items, are annatto seeds. The water soluble extract can be used for coloration of butter and oil soluble extract can be used for coloration of ghee and ice cream (Gulrajani, 1992). They are simple extracts of annatto seeds, which gives 55 to 60% yields when their extracts are prepared. Lac dye is also derivative of lac and is similar to cochineal and has been in use for colouring food besides fabrics since ancient times.

2.4.4 Food Items

This is the sector where natural dyes can be consumed in appreciable amount. This sector consumes 10 to 20 ton of natural dyes in a year (Gulrajani, 1992). Moreover, the consumption, of natural dye in beverages sector can be up to 20 to 40 ton per year.

2.4.5 Leather

Leather industry is already using natural tannin for tanning of leather sole. However, this use is confined to cottage and small-scale leather units (Vankar, 2000). The large manufacturers are using Chrome Tanning. At present, none of the leather units are using natural dyes for coloration of their products. If sufficient and

offensive efforts are done, the natural dyes can be captured in leather sector in a big way.

2.5 Extraction of Natural Dye

The extraction of natural dye can be comprehensively divided into extraction method and extraction technology.

2.5.1 Method of Extraction

The extraction of natural dye is basically depend on medium in which the dye is extracted. There are mainly four methods used in extraction of natural dyes which they are aqueous method, alkaline method, acidic method and alcoholic method (Ashis and Adwaita, 2002).

2.5.1.1 Aqueous Method

The known amount of dyestuff is boiled in 100 ml of soft water at 100^{o}C. Then, the dye solution is filtered and finally the optical density is recorded. The aqueous extraction is where the solvent used to extract the desired component in the interested substances. It is preferable in most of the research especially involving plants or fruits for medication or consuming purposes simply because this method is

100% safe for human being to consume as no other chemical solvent or substances are introduced in this process. Table below shows the research that involving fruits or vegetables extraction using water.

Table 2.3 Examples of research using aqueous extraction

No.	Description	Reference
1.	Extraction of Mucilage from whole white mustard seed.	(David et al., 2000)
2.	Extraction of Jordanian plant species to determine its antioxidant and total phenolic content.	(Tawaha et al., 2007)
3.	Direct Extraction of oil from sunflower seeds by twin-screw extruder.	(Evon et al., 2007)
4.	Extraction of *Bryophllum Pinnatum* Leaf for determination of neuropharmacological effects.	(Salahdeen et al., 2006)
5.	Determination of aqueous extracts of galls of *Querus infectoria* as antibacterial agents.	(Basri et al., 2004)
6.	Extraction of medicinal plants.	(Nair et al., 2006)
7.	Rosemary extracts added in gelatins from tuna-skin and bovine-hide gelatin.	(Gomez-Estaca et al.,2008)
8.	Extraction of Labiateae, Vitaceae and Cyperaceae for antimicrobial activity against bacteria.	(Parekh et al., 2006)
9.	Production of anti-inflammatory activity.	(Falodum et al., 2006)
10.	Extraction of *Harpagophytum procumbens* Roots for African traditional medicine for treatment.	(Mahomed et al., 2006)
11.	Extraction of Nigerian savannah plants for comparative in *vitro* trypanocidal activities.	(Atawodi., 2004)

This method is preferable in this research because the aqueous solvent used is water which has no harm to the natural dye.

2.5.1.2 Alkaline Method

First, 1% alkaline solution is prepared with addition of 1 g of NaOH in 100 ml of water. Then the dye material is entered and boiled at 100^{o}C (Verma and Gupta, 1995). Finally, the dye solution is filtered and the optical density is recorded. This type of extraction is not applied in this research due to its working pH which is in alkaline state. This will alter the natural dye constituents or affect the betacyanin structure.

2.5.1.3 Acidic Method

Acidic solution of 1 % is prepared by adding 1 ml of HCL in 10 ml of soft water (Deo and Paul, 2000). Then the material is entered and boiled at 100^{o}C. Finally, the dye solution is filtered and the optical density is recorded. This method is also not used in this research as it is conducted in acidic environment. The betacyanin which is sensitive to pH change will definitely undergo some reaction and the structure will be affected.

2.5.1.4 Alcoholic Method

Firstly, in this extraction method, 50 ml of alcohol is added to equal amount of water (Nanda et al., 2001). Then the dye material is poured in the diluted alcoholic

medium, boiled and finally the dye solution is filtered. This method is also not used in this research because alcoholic compound will alter the extraction pH thus will cause a small deviation in physical or chemical properties of the natural dye obtained for this research.

2.5.2 Extraction Technology

Conventionally, extraction can be carry out in the stated method previously, in aqueous, acidic or alkaline medium. At present, small scale producers and manufacturers are using extraction technology method. Even the local dyers using more crude method for extraction using metallic flax and crude process in refined way using blender condenser, distillation plant and drier and crystallization unit with the capacity of 300 ton per year (Dominic, 2010). The latest modern techniques of extraction are carried out with the use of extraction plant, reverse osmosis process and the latest is supercritical fluid extraction method. This method is very common in developed countries.

2.5.2.1 Solvent Extraction

This technique was developed just before the dawn of twentieth century. Now it has been commercialized in recent years. This technology has been improved to

reduce the waste generation and eco-effectiveness of extraction technology (Harivaindaran, 2008). Ultrasonic extraction followed by micro-wave extraction of solid finds extensive use mainly on organic solvents extraction. Solvent extraction is applied in this research because it uses water as a solvent to extract the natural dye. Water as a solvent, eventually reduce the harm and did not affect the natural dye structure.

2.5.2.2 Supercritical Fluid Extraction

This is a further advancement making significant step over the use of conventional solvent extraction technology. It uses CO_2 as extraction media. This technique is used for the extraction of natural products in foods, pharmaceuticals and chemical industries too.

It makes it possible to work at moderate temperature without affecting the organoleptic qualities and the active ingredients of the extracts obtained. Moreover, it makes it possible to obtain 100% natural extracts, completely free from extraction solvent residues. At the end of the extraction, an expansion phase which is achieved by reducing pressure causes the CO_2 to change from the supercritical state to the gaseous state which enables it to be removed completely from the CO_2 extract obtained (Vankar et al., 2001).

2.5.2.3 Microwave Assisted Extraction Technology

This is a high speed method used to selectively target compounds from various raw materials (Raja and Kala, 2005). The technology uses a microwave applicator as the energy source during solvent extraction. The advantage of this technology faster the processing, produce better yields, improve quality, lower energy consumption, reduce solvent level and low capital investments. This method is not considered in this research because this extraction technology is most efficient in extracting organic compound from solid samples such as soil, animal tissue and plants by using less organic solvent (Linling et al., 2003) . An organic solvent have to be added in order to extract the natural dye. This will cause extra cost for the research.

2.5.2.4 Continuous Steam Distillation Process

The Continuous Steam Distillation Process is a separation process using steam as a media but instead of batch type, this process is continuous (Tiwari et al., 2010). The process consists of a totally insulated pneumatic conveying system using super-heated steams as a carrier gas. This technology is also not considered in this research because it is suitable for continuous and large scale production. In this research, the natural is extracted in lab scale and by batch.

2.6 Pitaya waste

Reports on pitaya flesh's pigment, antioxidant properties and their stabilities are numerous (Wybraniec et al., 2001; Stintzing et al., 2002; Wybraniec and Mizrahi, 2002; Stintzing et al., 2003; Herbach et al., 2004; Moßhammer et al., 2005; Wu et al., 2006; Mahattanatawee et al., 2006; Esquivel et al., 2006; Herbach et al., 2006; Esquivel et al., 2007a; Phebe et al., 2009, Khalili et al., 2009).

Both the red pitaya flesh and peel were rich in polyphenols and antioxidants with the peel exhibiting higher antioxidant activities (Wu et al., 2006). Stintzing et al. (2002) suggested that pitaya peel may posses the same set of betalain forming enzymes as the flesh had, since the betacyanin pattern was found to be similar. To date, literature search indicated that the work on the pitaya peel is still scanty. Pitaya peels are often discarded during processing, especially in the beverage production industries. Phebe et al. (2009) and Harivaindaram et al. (2008) had suggested their potentials as natural colorants and thickening agent or as a moisturizer in cosmetic products (Stintzing et al.,2002).

Therefore, the present study was undertaken to determine more intensively the physico-chemical composition of the peel from pitaya fruit, so as to provide information on the possibilities of recovering value added ingredients from the pitaya peel for various commercial applications.

Table 2.4 Proportion and proximate composition of the pitaya peel. (Source: Jamilah et al.,2011)

Parameter		Value
a) Propotion		
i)	Skin thickness (cm)	0.46 ± 0.007
ii)	Flesh (g/100g)	64.50 ± 1.68
iii)	Peel (g/100g)	21.98 ± 1.04
b) Peel Composition (means ± standard error (%), n =3)		
i)	Moisture	92.65 ± 0.10
ii)	Protein	0.95 ± 0.15
iii)	Fat	0.10 ± 0.04
iv)	Ash	0.10 ± 0.01
v)	Carbohydrate	6.20 ± 0.09

Table 2.5 Physico-chemical properties of pitaya peel. (Source: Jamilah et al., 2011)

Properties		Values
a) pH		5.06 ± 0.01
b) Brix (TSS)		6.00 ± 0.00
c) Titratable acidity (TA) (gL^{-1})		0.19 ± 0.04
d) Hunter Lab Colour		L = 16.65 ± 0.06
		a = 23.89 ± 0.23
		b = 4.61 ± 0.07
e) Betacyanin content (mg/100 g DM)		150.46 ± 2.19
f) Organic Acids Concentration (%)		
i)	Oxalic	0.80 ± 0.01
ii)	Citric	0.08 ± 0.00
iii)	Malic	0.64 ± 0.00
iv)	Succinic	0.19 ± 0.00
v)	Fumaric	0.01 ± 0.00
	Total acid	1.72

Table 2.6 Carbohydrate components of pitaya peel (Source: Jamilah et al., 2011)

Carbohydrate components		Percentage (%)
1) Pectin		10.79 ± 0.01
2) Starch		11.07 ± 0.03
3) Cellulose		9.25 ± 1.33
4) Lignin		37.18 ± 1.02
5) Sugars		
i)	Glucose	4.15 ± 0.03
ii)	Maltose	3.37 ± 0.01
iii)	Fructose	0.86 ± 0.02
iv)	Sucrose	ND
v)	Galactose	ND
	Total	8.38
6) Total dietary fiber		69.30 ± 0.53
i)	Insoluble	56.50 ± 0.20
ii)	Soluble	14.82 ± 0.42
iii)	Ratio of IDF : SDF	3.8: 1.0

2.7 Pectin

Pectin is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It was first isolated and described in 1825 by Henri Braconnot (Berk, 1996). It is produced commercially as a white to light brown powder, mainly extracted from citrus fruits, and is used in food as a gelling agent particularly in jams and jellies. It is also used in fillings, medicines, sweets, as a stabilizer in fruit juices and milk drinks, and as a source of dietary fiber (Maria et al., 2005).

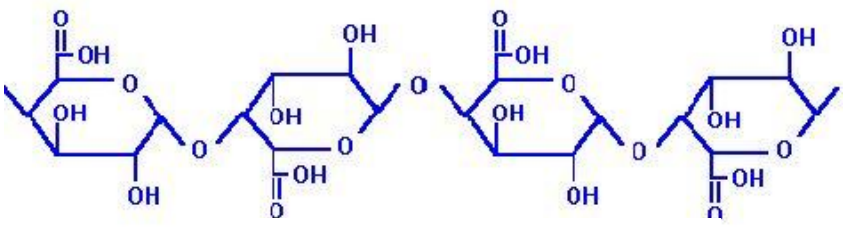

Figure 2.2 Pectin Structure

Pectins, also known as pectic polysaccharides, are rich in galacturonic acid. Several distinct polysaccharides have been identified and characterised within the pectic group. Homogalacturonans are linear chains of α-(1–4)-linked D-galacturonic acid. Substituted galacturonans are characterized by the presence of saccharide appendant residues (such as D-xylose or D-apiose in the respective cases of xylogalacturonan and apiogalacturonan) branching from a backbone of D-galacturonic acid residues. Rhamnogalacturonan I pectins (RG-I) contain a backbone of the repeating disaccharide: 4-α-D-galacturonic acid-(1,2)-α-L-rhamnose-(1. From many of the rhamnose residues, sidechains of various neutral sugars branch off. The neutral sugars are mainly D-galactose, L-arabinose and D-xylose, with the types and proportions of neutral sugars varying with the origin of pectin. Another structural type of pectin is rhamnogalacturonan II (RG-II), which is a less frequent complex, highly branched polysaccharide.Rhamnogalacturonan II is classified by some authors within the group of substituted galacturonans since the rhamnogalacturonan II backbone is made exclusively of D-galacturonic acid units (Buchanan et al., 2000).

Isolated pectin has a molecular weight of typically 60–130,000 g/mol, varying with origin and extraction conditions. In nature, around 80 percent of carboxyl groups of galacturonic acid are esterified with methanol. This proportion is decreased to a varying degree during pectin extraction. The ratio of esterified to non-esterified galacturonic acid determines the behavior of pectin in food applications. This is why pectins are classified as high- vs. low-ester pectins (short HM vs. LM-pectins), with more or less than half of all the galacturonic acid esterified. The non-esterified galacturonic acid units can be either free acids (carboxyl groups) or salts with sodium, potassium, or calcium. The salts of partially

esterified pectins are called pectinates, if the degree of esterification is below 5 percent the salts are called pectates, the insoluble acid form, pectic acid (CPKelco, 2002). Table 2.7 below shows the research that has been done regarding pectin.

Table 2.7 Examples of research using Pectin

No	Description	Reference
1.	Pectins from Dragon Fruit (*Hylocereus polyrhizus*) Peel	(Nazaruddin et al., 2011)
2.	Extractions and Characterization of Pectin from Dragon Fruit using Various Extraction Conditions.	(Norazelina et al.,2012)
3.	Characterization of pectin extracted from banana peels under different conditions using an experimental design.	(Happi et al., 2008)
4.	Effect of acid extraction and alcohol precipitation conditions on the yield and purity soy hull pectin.	(Kalapathy et al., 2008)
5.	Physicochemical properties of pectin from ambarella peels *(Spondias cytherea)* obtained using different extraction conditions.	(Koubala et al., 2008)
6.	Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design	(Levigne et.,2006)
7.	Water-based extraction of pectin from flavedo and albedo of orange peels	(Liu et al.,2006)
8.	A comparative study on functional properties of beet and citrus pectins in food systems	(Mesbahi et al.,2005)
9.	Extraction and physicochemical characterization of pectins from sunflower head residue	(Miyamoto et al.,2010)
10.	Extraction and characterization of pectins from cocoa husks	(Mollea et al.,2008)

Pectin content in the natural dye has believed increase the viscosity of the natural dye which causes the natural dye which obtained from pitaya waste have less fastness to cloth.

2.8 Pectinase

Pectinase is a general term for enzymes, such as pectolyase, pectozyme and polygalacturonase, commonly referred to in brewing as pectic enzymes. These break down pectin, a polysaccharide substrate that is found in the cell walls of plants. One of the most studied and widely used commercial pectinases is polygalacturonase. It is useful because pectin is the jelly-like matrix which helps cement plant cells together and in which other cell wall components, such as cellulose fibrils, are embedded. Therefore pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples and sapota. Pectinases have also been used in wine production since the 1960s. The function of Pectinase in brewing is twofold, first it helps breakdown the plant (typically fruit) material and so helps the extraction of flavours from the mash. Secondly the presence of pectin in finished wine causes a haze or slight cloudiness, Pectinase is used to break this down and so clear the wine (Semenova et al., 2006).

They can be extracted from fungi such as Aspergillus niger. The fungus produces these enzymes to break down the middle lamella in plants so that it can extract nutrients from the plant tissues and insert fungal hyphae. If pectinase is boiled it is denatured (unfolded) making it harder to connect with the pectin at the active site, and produce as much juice. Pectinases are also used for retting. Addition of chelating agents or pretreatment of the plant material with acid enhance the effect of the enzyme. As they are enzymes, pectinases have an optimum temperature and pH

at which they are most active. For example, a commercial pectinase might typically be activated at 45 to 55 °C and work well at a pH of 4.5 to 5.5. Pectinase is commonly used in fruit industries to speed up fruit juice extraction (Danielle et al., 2009).

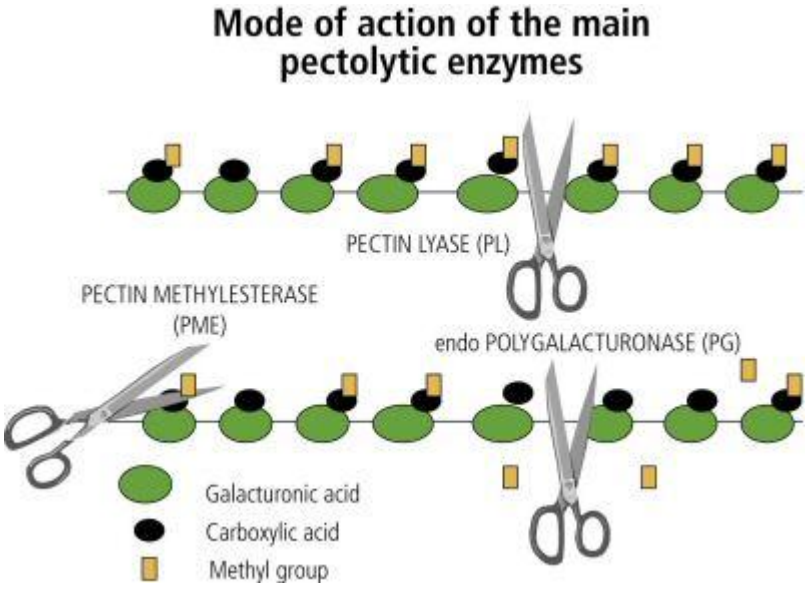

Figure 2.3 Mechanism of pectinase enzyme (Source: Danielle et al., 2009)

2.9 Other application of Pectinase

Over the years, pectinases have been used in several conventional industrial processes, such as textile, plant fiber processing, tea, coffee, oil extraction, treatment of industrial wastewater, containing pectinacious material, etc. They have also been reported to work on purification of viruses and in making of paper (Danielle et al.,2009). They are yet to be commercialized.

2.9.1 Fruit Juice Extraction

The largest industrial application of pectinases is in fruit juice extraction and clarification. Pectins contribute to fruit juice viscosity and turbidity. A mixture of pectinases and amylases is used to clarify fruit juices. It decreases filtration time up to 50%. Treatment of fruit pulps with pectinases also showed an increase in fruit juice volume from banana, grapes and apples. Pectinases in combination with other enzymes, e.g., cellulases, arabinases and xylanases, have been used to increase the pressing efficiency of the fruits for juice extraction (Alkorta et al., 1998). Vacuum infusion of pectinases has a commercial application to soften the peel of citrus fruits for removal. This technique may expand in future to replace hand cutting for the production of canned segments. Infusion of free stone peaches with pectinmethylesterase and calcium results in four times firmer fruits.

2.9.2 Textile processing and bioscouring of cotton fibers

Pectinases have been used in conjunction with amylases, lipases, cellulases and hemicellulases to remove sizing agents from cotton in a safe and ecofriendly manner, replacing toxic caustic soda used for the purpose earlier. Bioscouring is a novel process for removal of noncellulosic impurities from the fiber with specific enzymes. Pectinase have been used for this purpose without any negative side effects on cellulose degradation (Emre, 2004).

2.9.3 Degumming of Plant Bast Fibers

Bast fibers are the soft fibers formed in groups outside the xylem, phloem or pericycle. The fibers contain gum, which must be removed before its use for textile making (Zhang et al., 2000). The chemical degumming treatment is polluting, toxic and non-biodegradable. Biotechnological degumming using pectinases in combination with xylanases presents an eco-friendly and economic alternative to the above problem.

2.9.4 Waste water Treatment

Vegetable food processing industries release pectin, containing wastewaters as by-product. Pretreatment of these wastewaters with pectinolytic enzymes facilitates removal of pectinaceous material and renders it suitable for decomposition by activated sludge treatment (Kashyap et al., 2001).

2.9.5 Coffee and Tea Fermentation

Pectinase treatment accelerates tea fermentation and also destroys the foam forming property of instant tea powders by destroying pectins. They are also used in coffee fermentation to remove mucilaginous coat from coffee beans (Alkorta et al., 1998).

2.9.6 Animal Feed

Pectinases are used in the enzyme cocktail, used for the production of animal feeds. This reduces the feed viscosity, which increases absorption of nutrients, liberates nutrients, either by hydrolysis of non-biodegradable fibers or by liberating nutrients blocked by these fibers, and reduces the amount of faeces (Molina et al.,2001).

2.9.7 Oil Extraction

Citrus oils such as lemon oil can be extracted with pectinases. They destroy the emulsifying properties of pectin, which interferes with the collection of oils from citrus peel extracts (Kashyap et al., 2009).

2.9.8 Improvement of Chromaticity and Stability of Red Wines

Pectinolytic enzymes added to macerated fruits before the addition of wine yeast in the process of producing red wine resulted in improved visual characteristics (colour and turbidity) as compared to the untreated wines. Enzymatically treated red wines presented chromatic characteristics, which are considered better than the control wines. These wines also showed greater stability as compared to the control (Alkorta et al., 1998).

2.9.9 Papermaking

During papermaking, alkaline peroxide bleaching of mechanical pulps solubilizes acidic polysaccharides which are troublesome interfering substances. Some of these acidic polysaccharides are pectins, or polygalacturonic acids. The ability of polygalacturonic acids to complex cationic polymers (cationic demand) depends strongly on their degree of polymerization, so monomers, dimers, and trimers of galacturonic acid did not cause measurable cationic demand, but hexamers and longer chains had high cationic demand. Pectinases can depolymerize polymers of galacturonic acid, and consequently lower the cationic demand of pectin solutions and the filtrates from peroxide bleaching. (Reid et al., 2000)

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter 3, it mainly focuses on the approach of extraction of natural dye from Pitaya peel and followed by enzymatic reaction. The extraction technology implemented in this research is solvent extraction by using water as a solvent because of several reasons. Firstly, it is because the nature of water itself. Basically, water does not bring detrimental effect to human and environmental friendly. The method that has been used in this research is based on the study of which the title is characterization of ß-cyclodextrin complexes with natural dye applying low-technology methods for natural dye extraction by Bechtold *et* al., (2003).

3.1 Materials Used In This Study

The Pitaya fruit was obtained from a fruit stall in Taman Tas. The raw material is then peeled to get its waste, then the waste was blend and stored at -20^{o}C not more than 2 days (48 hours). This is because to make sure that the blend material is kept fresh and to avoid the colour from fading caused by an oxidation process.

3.2 Methodology of Flow Chart

Methodology flow chart is used as guideline for the experiment. As illustrated in Figure 3.1, the process begins with the first pretreatment stage and end with analysis by using Ultra Violet visible spectrometer and viscometer.

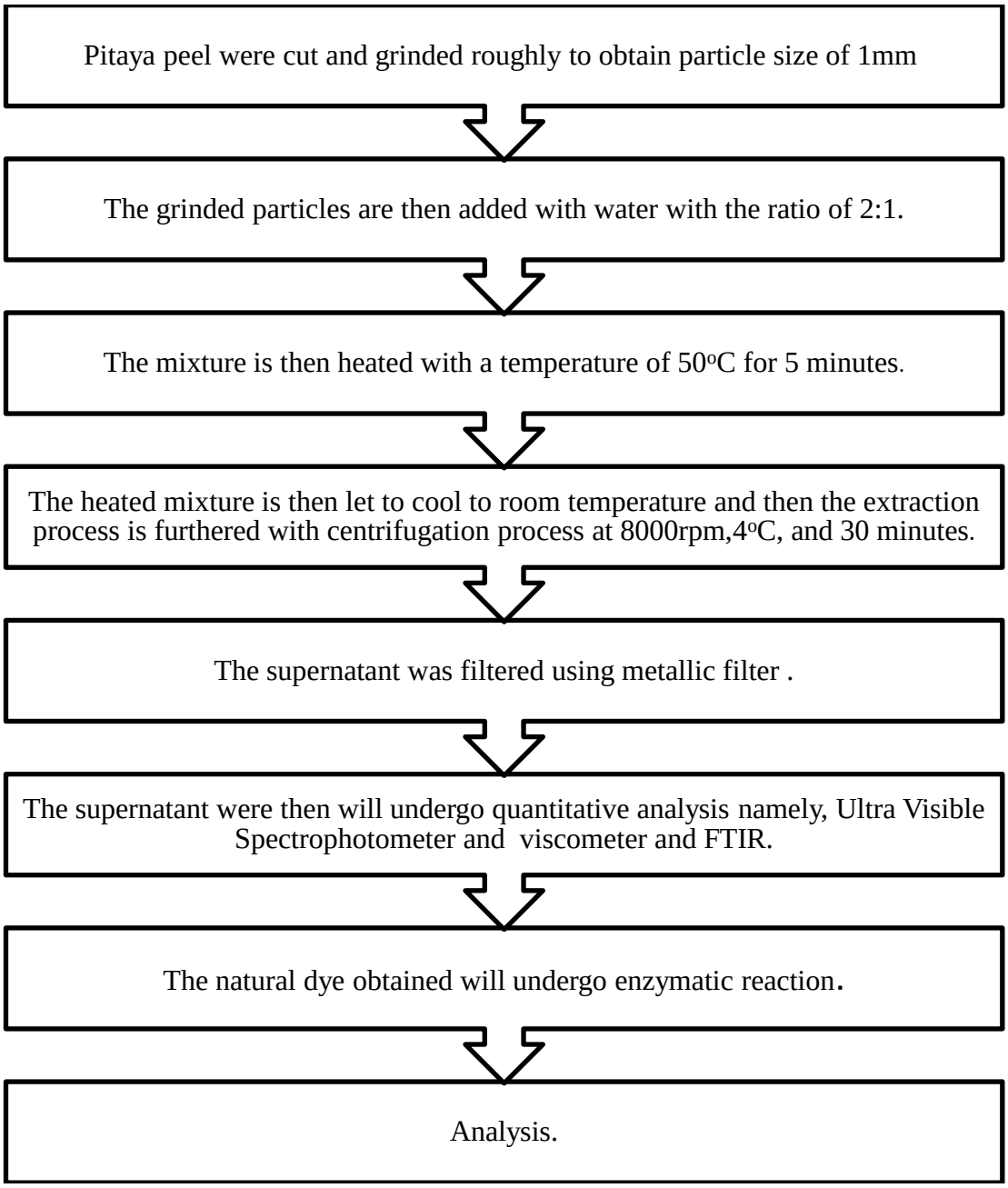

Figure 3.1 The Flow Chart of Extraction of natural dye from Water.

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Figure 3.2 illustrate the extraction step of the natural dye from pitaya waste

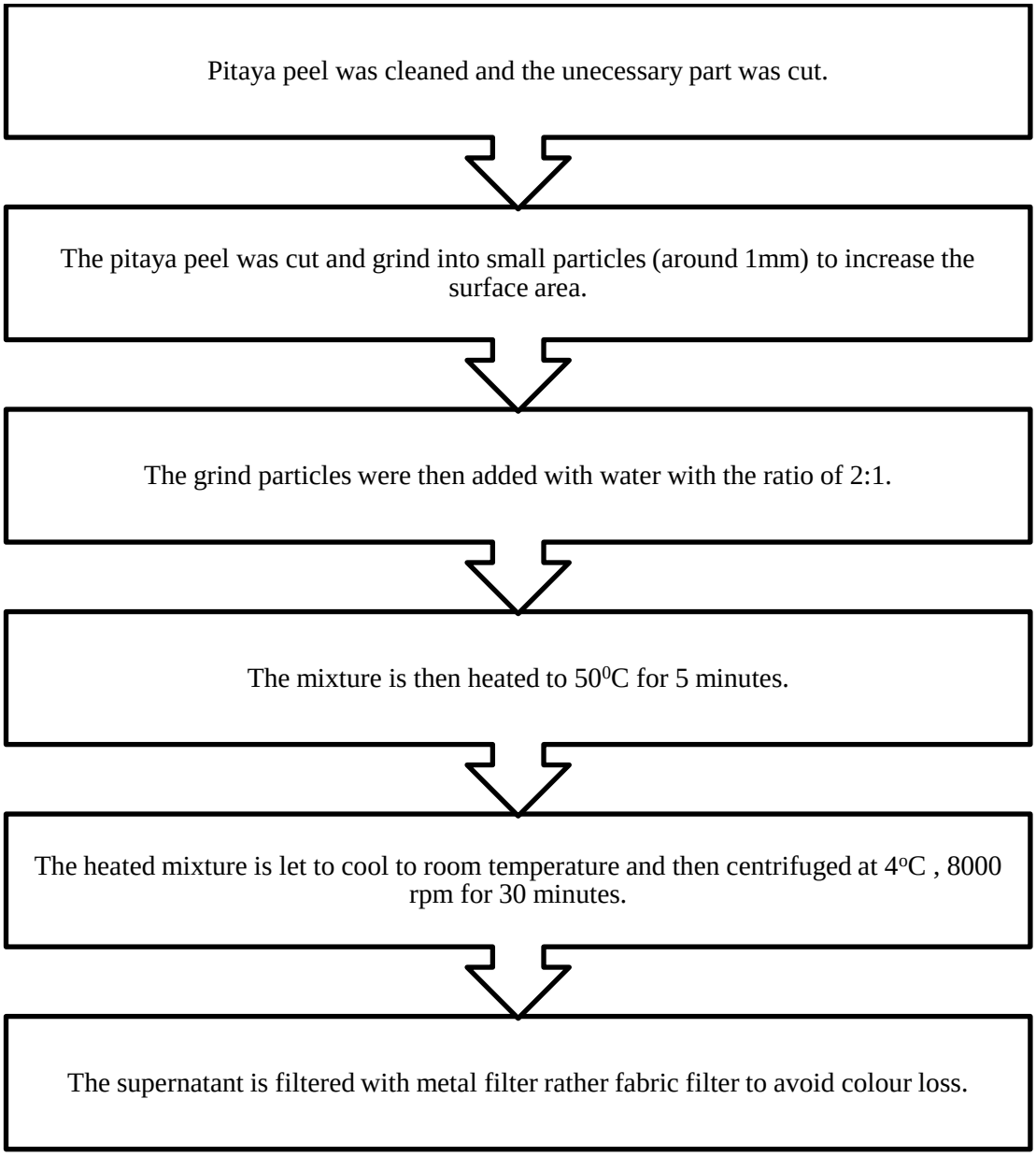

Figure 3.2 Procedure of Extraction of Natural Dye using water.

Figure 3.3 explain the steps involved in determining the betacyanin content in the natural dye extracted from pitaya waste.

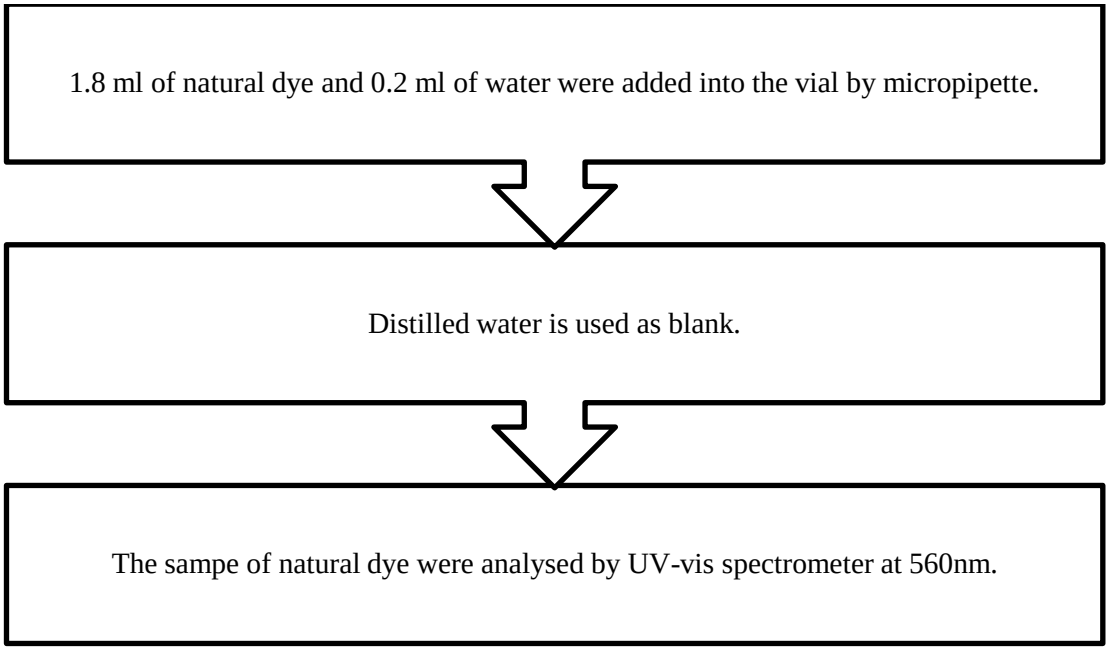

Figure 3.3 Procedure of Total Betacyanin content in Natural Dye

Figure 3.4 shows the step involves in determining the viscosity of the natural dye extracted from pitaya waste

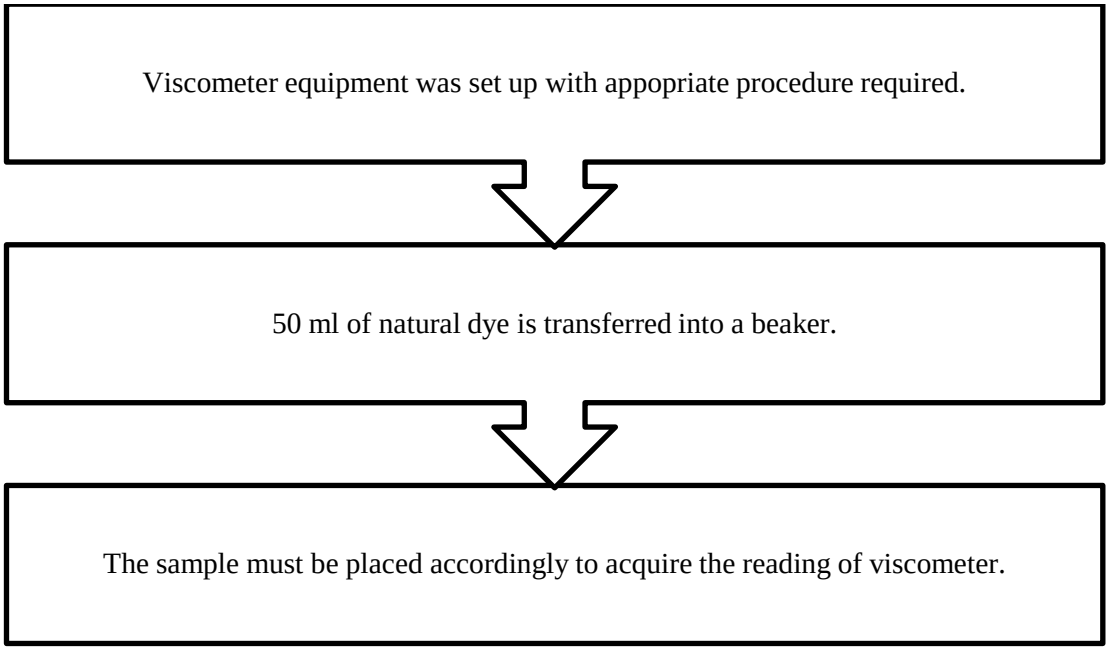

Figure 3.4 Procedure for viscosity reading for natural dye

Figure 3.5 shows the step involves in enzymatic reaction which test the enzymatic concentration varies from 0.1% to 5% on the reduction of viscosity of the natural dye and change the content of betacyanin content.

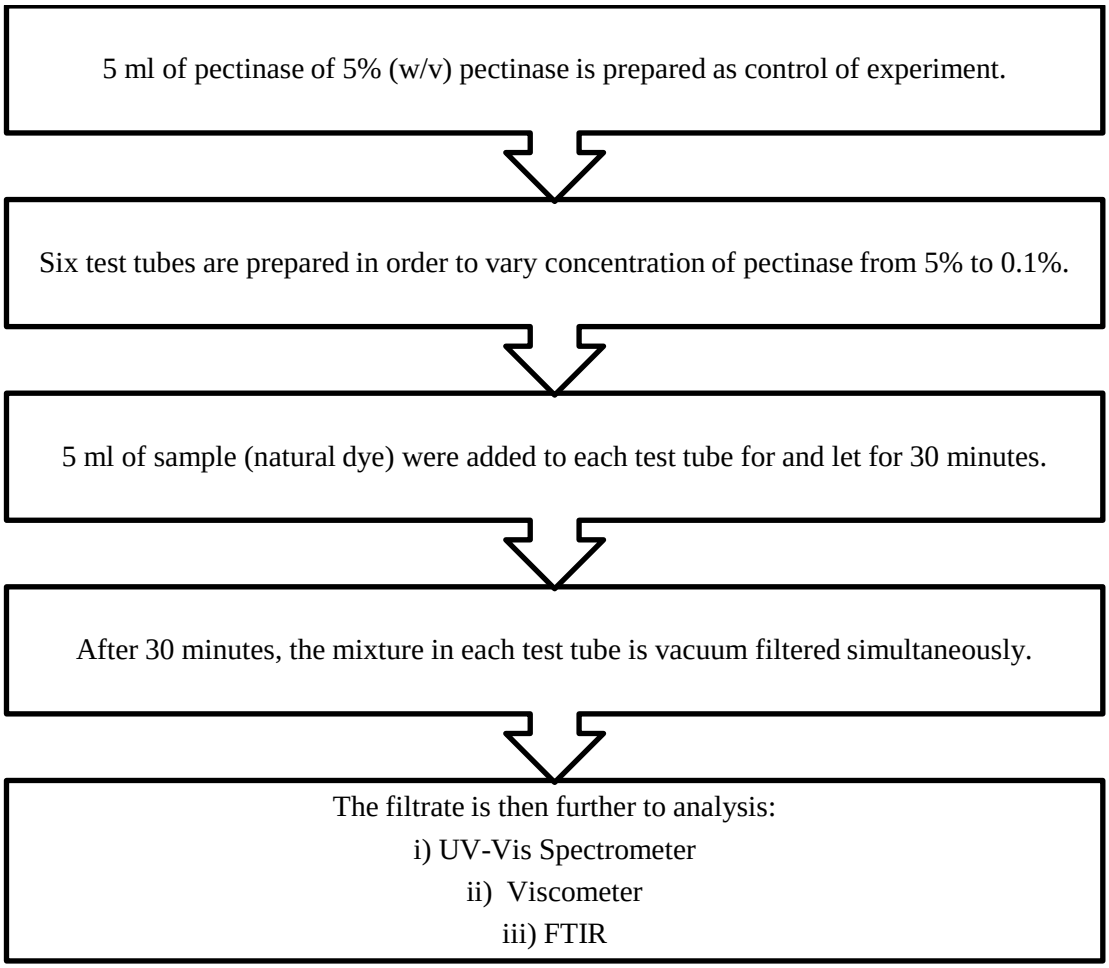

Figure 3.5 Enzymatic reaction for natural dye (Enzyme concentration from 5% to 0.1%)

Figure 3.6 illustrates the steps involves in the enzymatic reaction which is held on the temperature range from 30°C to 100°C.

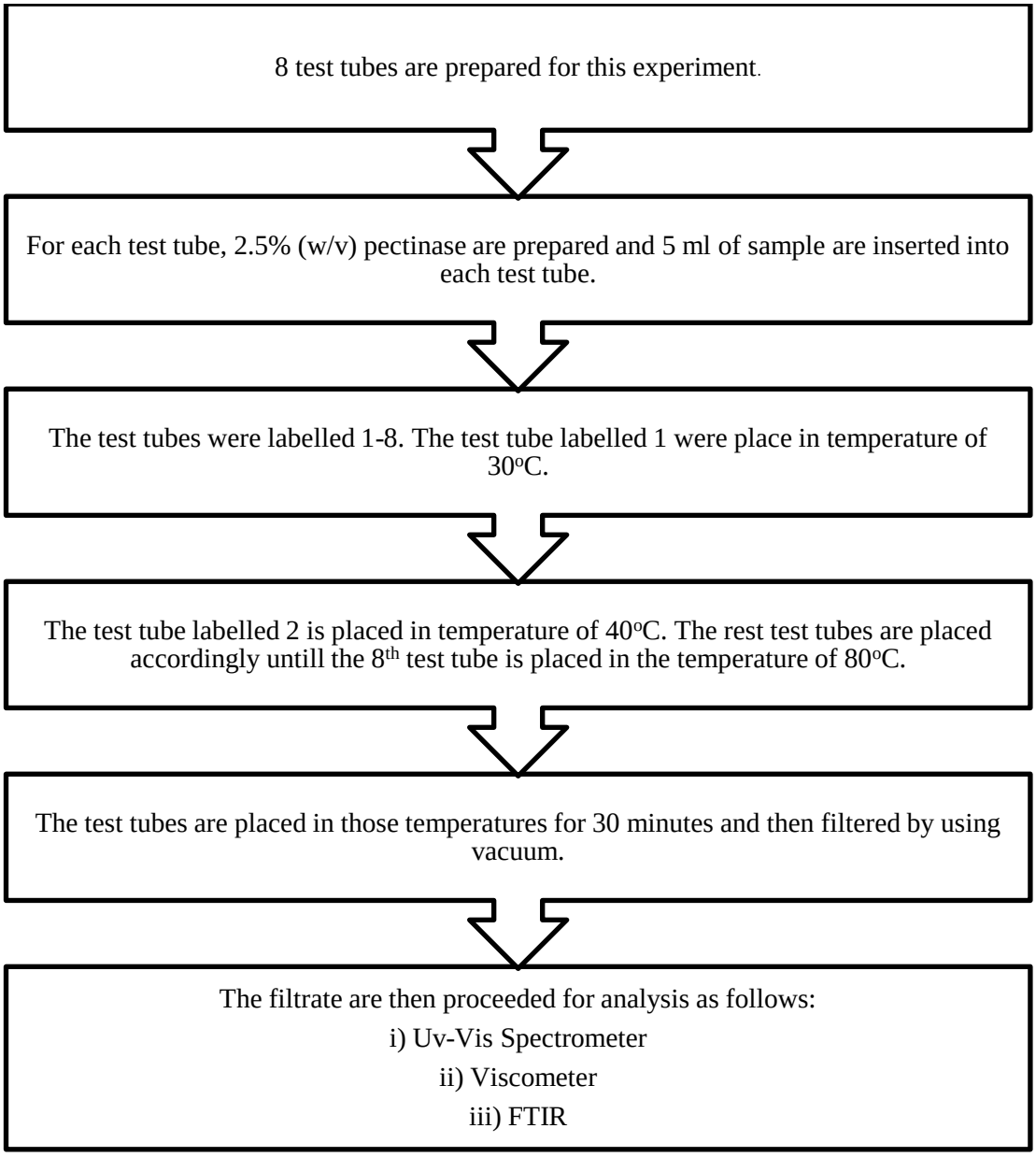

Figure 3.6 Procedure of enzymatic reaction of natural dye (temperature range from 30-100°C)

3.3 Preparation of Sample

Fresh pitaya was bought from the farm located at Kuantan Road for 5 kg. All the pitaya fruits that have been bought from the farm were then being peeled at the same time to ensure the condition of the properties is same for every single piece of the peels. After that, the peels were cut into smaller pieces. Then the smaller pieces were grinded in blender. The process of cutting and grinding the pitaya peel using the stainless steel blender is shown in Figure 3.7, Figure 3.8 and Figure 3.9.3.3

Figure 3.7 Sample of Pitaya Peels in Large Pieces

Figure 3.8 Process of cutting larger Pitaya peel into smaller Peel.

Figure 3.9 Grinding of Pitaya Peel

3.4 Extraction of Pitaya Peel

The blended Pitaya Peel were then diluted with water with the ratio of 2:1. The mixture is then heated in temperature of 50^{o}C for 5 minutes. Then the mixture is let to be cooled to room temperature as shown in Figure 3.10.

Figure 3.10 Diluted blended Pitaya Peel

3.5 Centrifugation

After the extraction of natural dye, separating of the heated mixture will proceed. The mixture will then centrifuge at 8000 rpm, 4^{o}C and for 30 minutes (Harivaindran et al., 2008). The mixture has to be filled in the 50 ml container with tightly closed the lid. When placing the container in the Centrifuge, balancing is

important as imbalance of the placement of the containers in the Centrifuge would affect the spinning of the machine which will resulted in incomplete solid liquid separation. Figure 3.11 shows that the container being place in the Centrifuge. Whereas, Figure 3.12 and figure 3.13 shows the mixture before and after the centrifugation process.in figure 3.13 a clear separation between liquid phase (supernatant) and solid phase can be viewed.

Figure 3.11 Placing the natural dye extracted in Centrifuge

Figure 3.12 Sample of the Natural dye extracted before centrifugation

Figure 3.13 Sample of the natural Dye extracted after centrifugation.

3.6 Filtration of the Supernatant

After the extraction process were completely done for the two stages heating and centrifuge, the supernatant obtained from the centrifugation process were then filtered by using the metal filter.

3.7 Analysis of Total Betacyanin Content and Viscosity of Natural Dye

3.7.1 Analysis of Total Betacyanin Content by UV-Vis Spectrometer

Distilled water used as a blank in this analysis. Then for the sample optical density reading, 1.8ml of sample and 0.2 ml of distilled water are added in the vial by micropipette. Then the mixture is allowed to mix by shaking the vial upside down. The sample is then ready for the analysis. Figure 3.13 shows the natural dye that is used for this analysis.

Figure 3.14 Natural Dye that has been used for UV-Vis analysis.

After obtain the optical density reading for the natural, the value has to be introduced in a equation mentioned below to calculate the total betacyanin content for the natural extracted (Herbach et al., 2007).

$$BC\ (mg\ L^{-1}) = \frac{\mathrm{A} \times \mathrm{MW} \times 1000 \times \mathrm{DF}}{\epsilon \times \mathrm{l}}$$

Where:

A = Absorbance

DF = Dilution Factor

MW = Molecular Weight of beta cyanin

= 550 $gmol^{-1}$

ϵ = Molar Extinction Coefficient

= 60, 000 L/mol cm in H_2O

l = Path length of cuvette

= 1cm

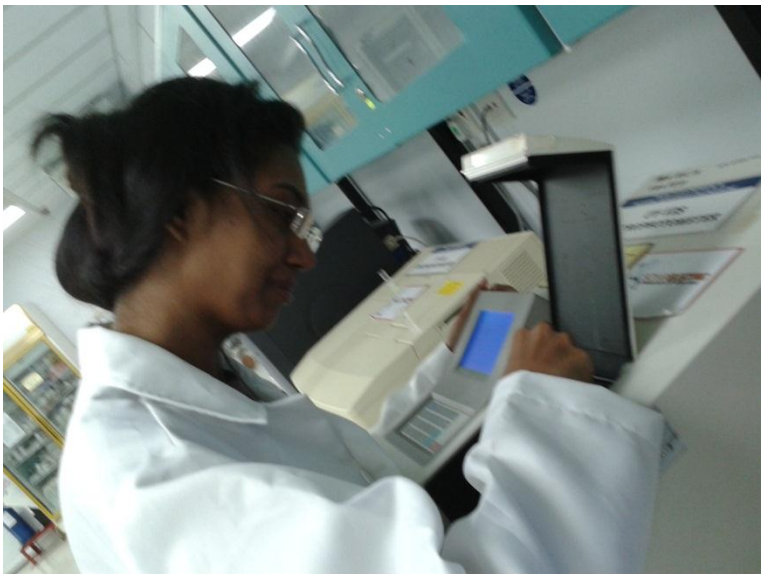

Figure 3.15 Adjusting the vial position in the UV-Vis Spectrometer

3.7.2 Analysis to determine viscosity of Natural Dye

Viscometer was used to determine the viscosity of the natural before and the enzymatic reaction. As for the determination of the viscosity of the natural dye, no calculation needed because the equipment itself will show the value of viscosity of the natural dye in centripoise (cp) unit. The same sample that has been used for UV-Vis analysis has been implemented for this analysis (Kareem and Adebowale, 2007). Figure 3.16 shows how the viscosity of the natural dye has been measured.

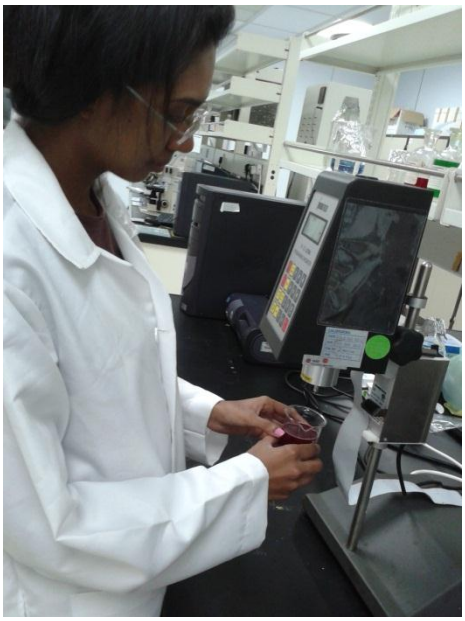

Figure 3.16 Measuring Viscosity of the natural Dye

3.8 Enzymatic Reaction for Natural Dye

3.8.1 Preparation of Buffer solution for enzymatic Reaction

Citrate buffer with pH 5.835 will be used for this enzymatic reaction..6 g Disodium hydrogen phosphate ($Na_2HP0_4.2H_20$) are dissolved in 1000 ml of water (A). 21 g of Citric acid monohydrate are dissolved in 1000 ml of water (B). 57 ml of solution A and 43 ml of solution B are mix to obtain working buffer solution. pH can be adjusted to 5.8 by adding one of the aforesaid solutions (Jamilah et al., 2011).

3.8.2 Effect of Enzymatic Concentration

35g of pectinase will be needed because there are seven different concentrations including the control experiment. 5ml of 5.00% pectinase and 15ml of buffer solution. For each test tube a 2.5ml solution of pectinase and distilled water will be made up in order to make varying concentrations of pectinase (Viajayanand et al.,2010). This is a summary of the 6 test tubes:

Test Tube 1 - 5.00% - 2.5ml of pectinase

Test Tube 2 - 2.50% - 1.25ml of pectinase and 1.25ml of buffer solution.

Test Tube 3 - 1.00% - 0.5ml of pectinase and 2.0ml of buffer solution.

Test Tube 4 - 0.50% - 0.25ml of pectinase and 2.25ml of buffer solution.

Test Tube 5 - 0.25% - 0.125ml of pectinase and 2.375ml of buffer solution.

Test Tube 6 - 0.10% - 0.05ml of pectinase and 2.45ml of buffer solution.

A volume of 2.5ml of solution is chosen because it is a sufficient volume to have an effect on the 5ml of natural dye. Percentages are often used to give concentrations in solution. The volume of pectinase is taken from the original 5.00% pectinase and the distilled water is used to make up the solution to 2.5ml. This changes the concentration of the pectinase but not the volume of the solution. The range from 0.10% to 5.00% was chosen because this is a wide range and therefore the difference in results obtained should be large enough to find valid conclusions.

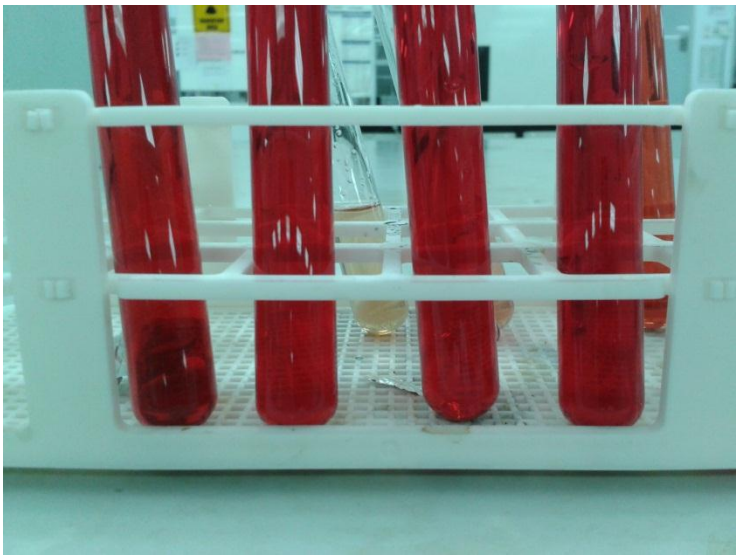

Figure 3.17 Mixture to test the effect of enzyme Concentration

3.8.3 Effect of Temperature

8 test tubes were prepared. For each test tube, 5 ml of pectinase were placed and 15 ml of distilled water were added in the same test tube. The test tubes were labeled 1 -10. Test tube 1 is placed in an environment of 30^0C and test tube 2 were placed in 40^0C and accordingly until the 8^{th} test tube is placed in 100^0C. The turbidity of the solution is check using UV-Vis spectrophotometer at 560 nm (Koshi et al., 2010).

3.9 Feasibility Study

For this qualitative analysis, it is ensure that the viscosity is the main reason that the natural dye is not fasting to the fabric. So, here, two types of natural dye is prepared. First is the initial natural dye without any further reaction or addition. Secondly, is the natural dye which is treated with enzyme with an optimum concentration of 2.5% (w/v). temperature of 55^{o}C and pH of 5.5. Immediately after the enzymatic reaction, vacuum filtration has been carried out to remove the enzyme which is in solid state. Vacuum filtration is carried out rather than denatured the enzyme at high temperature at 100^{0}C because the betacyanin is sensitive towards high temperature. The betacyanin will degrade at high temperature and the natural dye will yield a faded colour. Figure 3.18 shows the natural dye which is treated with pectinase on the left side and the natural dye without ant treatment on the right side.

Figure 3.18 Two types of natural dye. (on left side treated with enzyme and on right side, initial natural dye)

Next, two cloths or fabric with similar measurement and composition are prepared. Figure 3.19 shows the cloth that used for this analysis.

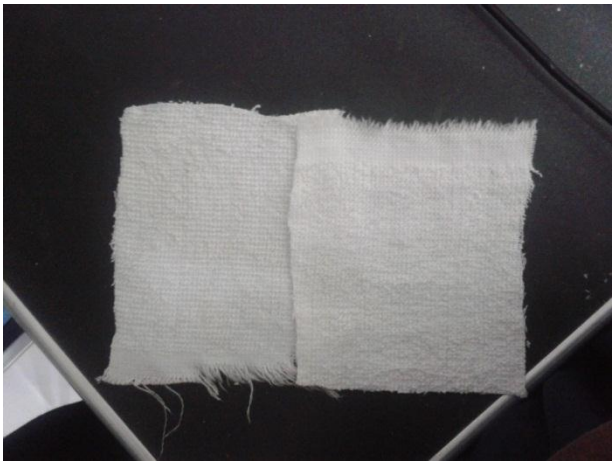

Figure 3.19 Fabrics that has been used for this analysis

One of the fabrics is inserted in the initial natural dye beaker and the other fabric is inserted in the natural dye that has been treated with enzyme, simultaneously. The two beakers of 50 ml which each of it is immersed with a cloth is left for 24 hours as shown in Figure 3.20.

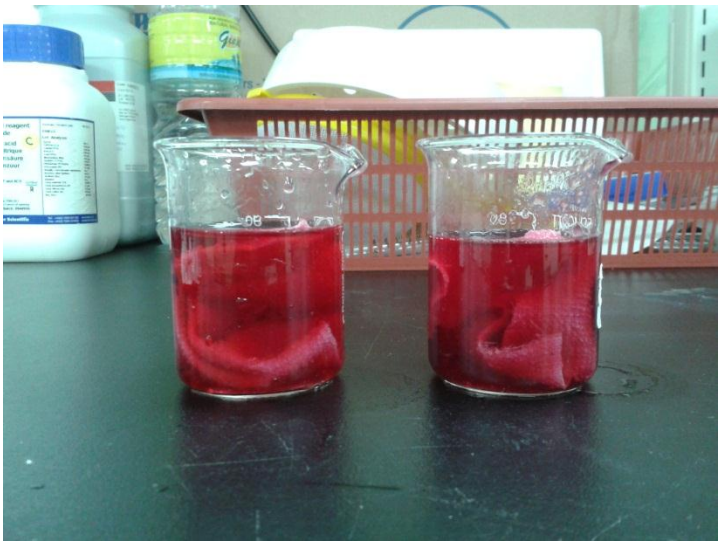

Figure 3.20 Cloths that has been immersed in natural dye

After 24 hours, the fabric is let another 24 hour to dry as shown as Figure 3.21.

Figure 3.21 The cloths that let to be dry

After the cloth has been let for 24 hours to dry, the two cloths must be washed in tap water to compare the fastness of the two type of the dye on the similar type and size of cloth as shown in Figure 3.22.

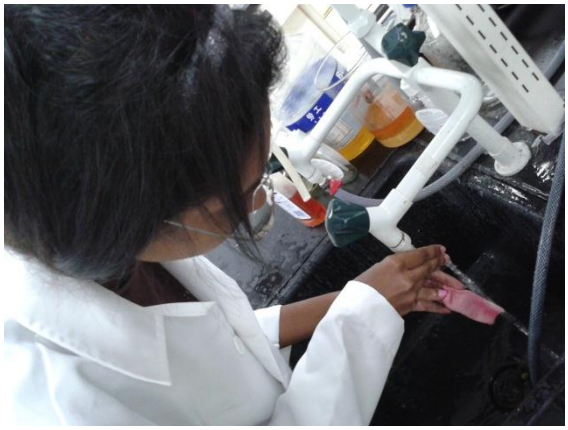

Figure 3.22 Washing the cloth that has been let to dry for 24 hours

3.10 Supporting Analysis (FTIR)

In order to support this result and hypothesis made during the discussion and conclusion, supporting analysis has to be done to prove and support the result obtained from this study. So, FTIR analysis has to be done for each and every sample after the enzymatic reaction. Figure 3.33 shows the hoe the FTIR analysis can be carry out.

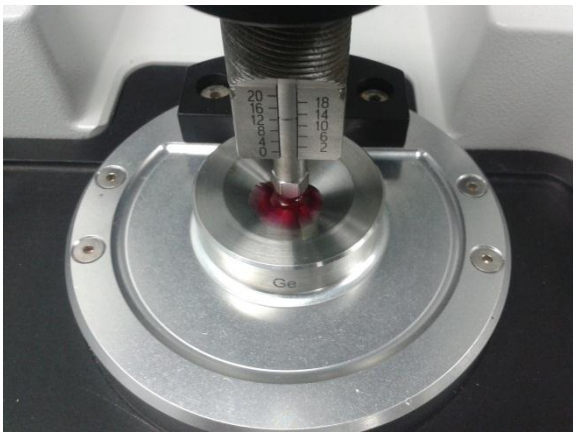

Figure 3.23 FTIR analysis for one of the sample.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Result

The application of UV-Vis Spectrometer is a fast and easy method to determine the betacyanin content in natural dye sample. Betacyanin pigments which is responsible for reddish to violet colours. The betacyanin pigment can be measured in the wavelength of 567 nm. Whereas, the viscosity of the natural dye, can be measured easily and effectively by using Viscometer. Viscosity is a measure of the resistant of a fluid which is being deformed by either shear stress or tensile stress. In everyday term, viscosity is termed as “thickness” or “internal friction”.

4.2 Results for Initial Viscosity of Natural Dye

Initial viscosity of the natural dye has been determined by using viscometer. The initial viscosity mentioned here is defined as the viscosity of the natural dye extracted from the pitaya waste before any enzymatic reaction take place. The initial viscosity of the natural dye obtained from the viscometer is 21.6 cP. Before the

viscosity of the natural dye has been determined, viscosity of water has been measured by the same viscometer, to make sure the result obtained was accurate. Each time before the viscosity of natural dye is obtained, it is necessary to check the viscosity of the water (μ = 1 cP) is same with the mention viscosity is acquired in order to get accurate reading.

4.2 Betacyanin content of Natural Dye

Whereas, the betacyanin content was determined by using UV-Vis spectrophotometer. The reading from the UV-Vis is then inserted in the equation shown below to get betacyanin content in mg/L. This UV-Vis analysis has to be done in triplet for each sample to get more accurate result for betacyanin content. The UV-Vis reading for the natural dye is 2.880, 2.886 and 2.884. The average of these three readings is 2.883. This latest absorbance value has to be inserted in the equation shown below (Jamilah et al., :

$$BC\ (mg\ L^{-1}) = \frac{\mathrm{A} \times \mathrm{MW} \times 1000 \times \mathrm{DF}}{\epsilon \times \mathrm{l}}$$

Where:

A = Absorbance

DF = Dilution Factor

MW = Molecular Weight of beta cyanin

= 550 $gmol^{-1}$

ϵ = Molar Extinction Coefficient

= 60, 000 L/mol cm in H_2O

l = Path length of cuvette

= 1cm

After inserted all the value in the equation, the beta cyanin content was determined. The initial beta cyanin content of the natural dye is 26.428 mg/L.

4.3 Results for Natural Dye After Enzymatic Reaction

4.3.1 Effect of Enzyme Concentration on Pitaya Waste Extract viscosity

This part of research studied and produced the results for the reduction of the viscosity and changes in betacyanin content for ascending order of enzyme concentration from 0.1 % to 0.5% (w/v). The results obtained for the explained conditions, in particularly, reduction of viscosity and optical density reading are tabulated in the Table 4.1 below.

Table 4.1 Results for Enzyme Concentration

Enzyme Concentration (w/v)	Viscosity, cP (after)	Optical Density readings		
0.1	14.3	2.653	2.656	2.650
0.25	12.4	2.590	2.591	2.592
0.5	9.9	2.500	2.502	2.504
1.0	5.8	2.220	2.221	2.222
2.5	4.28	2.117	2.119	2.121
5.0	4.25	1.827	1.829	1.831

Hence, table 4.2 tabulated the reduction in the viscosity of the natural dye according to the enzyme concentration tested and betacyanin content which are calculated from optical density reading from Table 4.1.

Table 4.2 Overall results for Enzyme Concentration

Enzyme Concentration (w/v)	Viscosity, cP (Before)	Viscosity, cP (after)	Betacyanin content, mg/L (Before)	Betacyanin content, mg/L (After)
0.1	21.6	14.3	26.428	25.324
0.25	21.6	12.4	26.428	24.754
0.5	21.6	9.9	26.428	23.932
1.0	21.6	5.8	26.428	22.359
2.5	21.6	4.28	26.428	21.424
5.0	21.6	4.25	26.428	15.765

The results that tabulated are then presented in graph for better understanding and clearer view of the trend of viscosity reduction and difference in betacyanin content according to the enzyme concentration. The results shown in Table 4.2 are summarized in Figure 4.1 below.

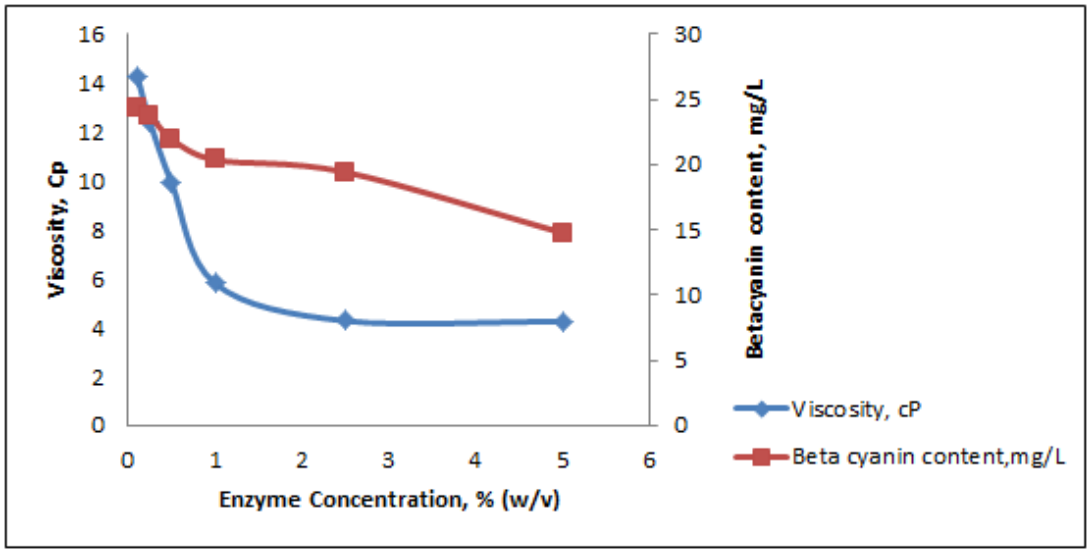

Figure 4.1 Graph of Viscosity and Betacyanin Content Against Enzyme Concentration

Figure 4.1 illustrate the results obtained for the enzymatic reaction for various enzyme concentrations on the reduction of the viscosity and changes in betacyanin content of the natural dye. In this part of experiment, the temperature and pH of the reaction is set at 50 ^{0}C and 5.5 respectively because it is the optimum temperature and optimum pH of pectinase. In addition, the reaction time for all the reaction is set to 60 minutes. Based on the Figure 4.1, the reduction of viscosity of the natural dye based on the enzyme concentrations can be separated by three phase. The first phase shows a sharp decrease in viscosity of the natural dye. This first phase trend can be observed in enzyme concentration from 0.1 to 1.0% (w/v). The amount of reduction of the viscosity in first phase shows 34% for 0.1% enzyme concentration, 43% for 0.25% enzyme concentration, 54% for 0.5% enzyme concentration and 73% for 1.0% enzyme concentration.

Next, based on the same Figure 4.1, the second phase fall on or covers the reduction of the viscosity when the enzyme concentration is from 1% to 2.5%. This second phase shows a small reduction in the viscosity of the natural dye compared to the previous reduction in the first phase. The amount of reduction of the viscosity in first phase shows 80% for 2.5% enzyme concentration.

Finally, the third phase in the Figure 4.1 exhibits a small reduction in viscosity which is negligible. This third phase of the viscosity reduction can be exhibit from 2.5% to 5% of enzyme concentration. The amount of reduction of the viscosity in first phase shows 80.3% for 5% enzyme concentration. When viewed the Figure 4.1 overall, it can be deduced that, when the enzyme concentration increases, from 0.1 % (weight per volume) to 5%, the reduction in the viscosity of the natural dye is observed until certain level then the reduction decreases and finally stop when the enzyme concentration reach 2.5%. This is the trend of the viscosity reduction in the natural dye according to the enzyme concentration.

Pectinase are introduced in this research to breakdown the pectin which shows up the viscosity in natural dye. This viscosity is believed to prevent the natural dye from fasting towards the cloths/fabrics. Pectin are complex polysaccharides consists of partially methyl esterify α (1,4) linked homogalacturonic acid backbone and branched neutral sugar side chains(Norazila t al., 2012). They are important components of cell wall and middle lamella, and can be found in fruits and vegetables. Enzymes cleaving pectic substances are called as pectinolytic enzymes or pectinases (Saad et al.,2007). Commercialize pectinase normally called polygalacturonase catalyse hydrolysis α-1,4-glycosidic linkages in polygalacturonic

acid (pectin) producing D- galacturonate (Cautinho et al.,2008). The mechanism of pectinase are shown in Figure 4.2 below.

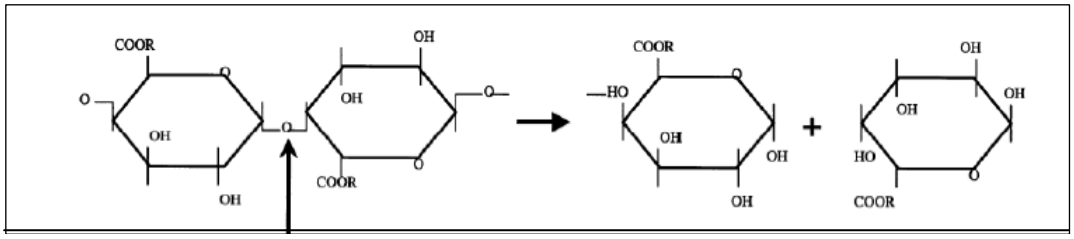

Figure 4.2 Hydrolase of pectin by pectinase

According to the results obtained, it tallies with theory. As the enzyme concentration increases, the viscosity reduction will increase (Danielle et al., 2009). This agrees with theory which state as the enzyme concentration increase the rate will also increase. When enzyme concentration is low, low concentration of pectin will be degraded, which lead to small reduction of viscosity of the natural dye. As the enzyme concentration increase, a higher concentration of pectin will be degraded, thus a higher reduction of viscosity will be experienced. But this trend can only be observed until one specific level of enzyme concentration that is 2.5%. After that level of enzyme concentration, although the enzyme concentration increased up to 5%, an insignificant amount of viscosity reduction is observed. From here, it can be deduced that, 2.5 % of enzyme concentration is the optimum amount to break down the pectin found in 15 ml of the natural dye sample. So, when 5% of enzyme concentration is used, it is assumed that only 50% of the overall enzyme present will be saturated in order to break down the pectin.

Whereas, in Figure 4.1, also illustrates the trend of betacyanin content of the natural dye, when the enzyme concentration varies, in increasing order from 0.1% to

5% concentration. In this betacyanin part, the Figure 4.1 can also divided into two phase. The first phase describes a slow and small decrease in the betacyanin content for the enzyme concentration from 0.1% to 2.5%. The reduction in betacyanin content are 6% for 0.1% of enzyme concentration, 9% for 0.25 % of enzyme concentration, 11% for 0.5% of enzyme concentration, 13% for 1.0% of enzyme concentration and 16% for 2.5% of enzyme concentration.

Whereas the second phase of the betacyanin reduction can be witnessed from the enzyme concentration from 2.5% till 5.0%. This phase of betacyanin reduction which can be observed from the enzyme concentration of 2.5% to 5% shows a massive reduction compared to previous reduction rate. The amount of reduction when the enzyme concentration increase to 5% is 40% of reduction compared to the initial betacyanin content in the natural dye.

The massive reduction in betacyanin content can be viewed when the enzyme concentration increased to 5%. It is hypothesized that, when excessive enzyme present in the natural dye, it will start to breakdown the betacyanin bond which will cause the reduction in the betacyanin reduction when enzyme concentration start increase. The betacyanin molecule will be surrounded by pectin molecule which will cause the viscosity of the natural dye to increase. When sufficient pectinase present in the natural dye, it will breakdown the pectin which surround the betacyanin. And when excessive pectinase present, the pectin surround by the betacyanin will be degraded and the excessive pectinase will start degrade the betacyanin structure.

This hypothesis will be supported by FTIR analysis. The outcome for FTIR analysis for natural dye before any enzymatic reaction is shown in the Figure 4.3 below.

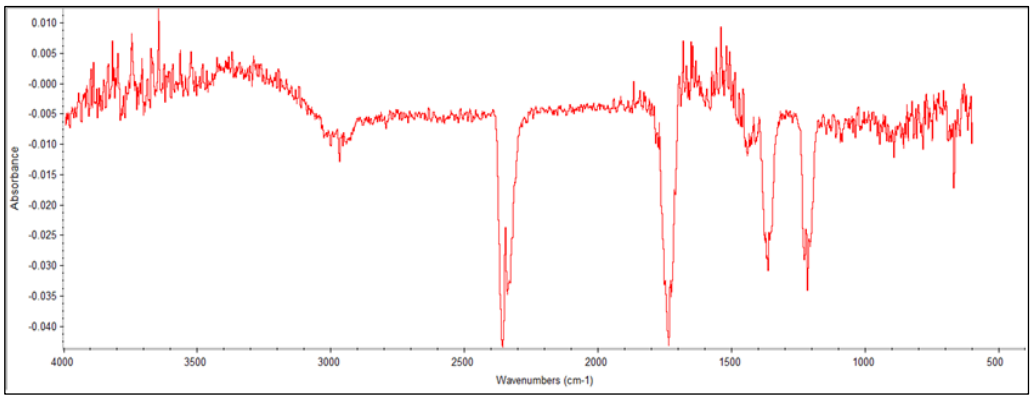

Figure 4.3 FTIR diagram for natural dye before enzymatic reaction

The peaks that shown in the Figure 4.3, will be represented in the respected functional group in the Table 4.3 below.

Table 4.3 Functional Group of the peaks present in the natural dye

Peaks (Wavelength cm^{-1})	Compounds
1210	C-O
1350	C-C
1730	C=O
2400	NH

The compounds presents as the peaks in the FTIR analysis shows the functional group of betacyanin that is C-O, C=O, NH and C-C. Hence, these compounds also present in the pectin component as its functional group which is similar to betacyanin except NH group. Figure 4.4 below shows FTIR diagram which will show peaks for pectin component from citrus peel. This prove that pectin contains C-O bond, C=O bond which is also present in the natural dye with same peak length.

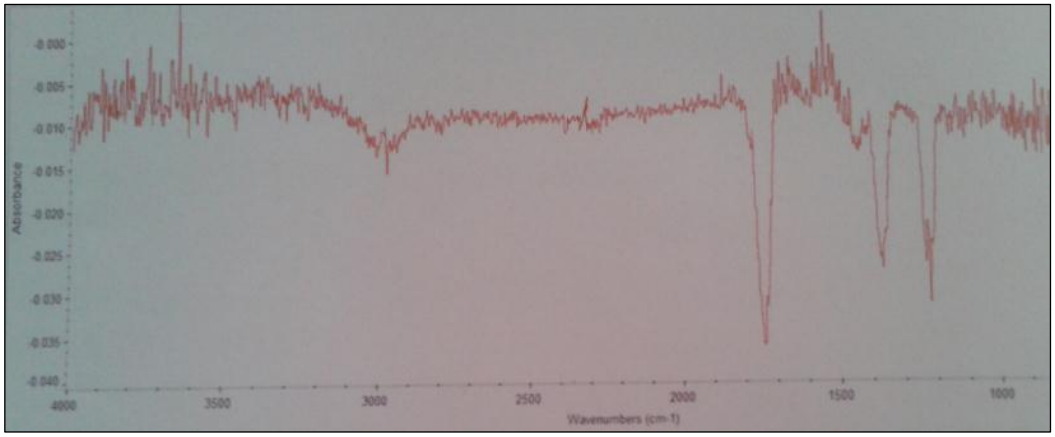

Figure 4.4 FTIR diagram for pectin from citrus peel.

The FTIR analysis after the enzymatic reaction has shown in Figure 4.5. In the first phase, only the reduction of viscosity can be seen abruptly whereas the betacyanin reduction is in significant. The reduction in viscosity can be seen in the difference in the peak height produced in FTIR analysis for group C=O and C-O of the natural dye before and after the enzymatic reaction. Whereas the difference in the peak height for NH group which stand for betacyanin group is almost not any.

In the second phase not much different can be seen from the first phase as viscosity reduction in the second phase has been reduced as the enzyme concentration increases. Hence, the reduction in the betacyanin content also remains insignificant as in the phase one as shown in Figure 4.6. Whereas, in the third phase, the pectin breakdown is very little compared to previous concentration, but the reduction of betacyanin content is quite clear. This can be proved in the peak height reduction of NH group as shown in Figure 4.7 below.

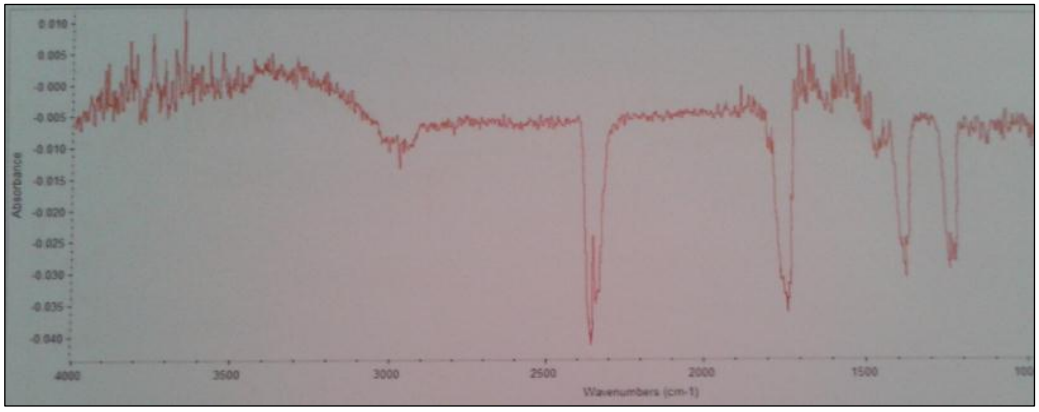

Figure 4.5 FTIR diagram for phase one for viscosity and betacyanin reduction for enzyme concentration for first phase.

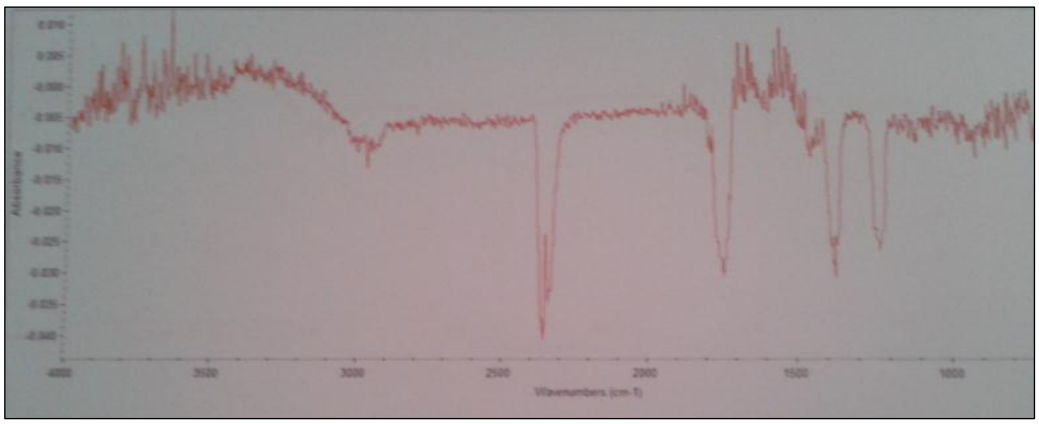

Figure 4.6 FTIR diagram for phase two for viscosity and betacyanin reduction for enzyme concentration.

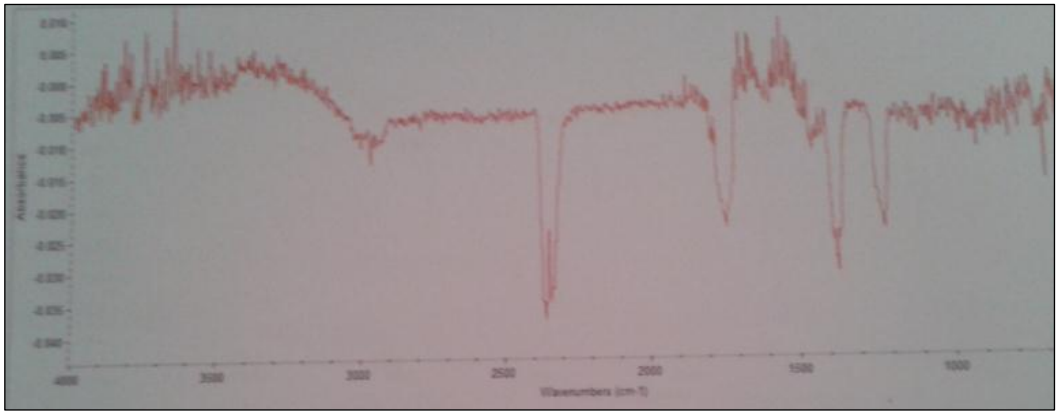

Figure 4.7 FTIR diagram for phase three for viscosity and betacyanin reduction for enzyme concentration.

4.3.2 Effect of Temperature on the Pitaya Peel Viscosity

This part of research studied and produced the results for the reduction of the viscosity and changes in betacyanin content for ascending order of reaction temperature from 30^{o}C to 100^{o}C. The results obtained for the explained conditions, in particularly, reduction of viscosity and optical density reading are tabulated in the Table 4.3 below.

Table 4.4 Results for Temperature range

Temperature,^{0}C	Viscosity, cP (after)	Optical Density readings		
30	14.30	2.548	2.548	2.549
40	10.40	2.324	2.325	2.325
50	4.32	2.091	2.092	2.092
60	4.30	1.960	1.960	1.960
70	4.28	1.149	1.150	1.145
80	4.25	0.621	0.623	0.625
90	3.91	0.351	0.352	0.352
100	3.89	0.340	0.340	0.340

Hence, Table 4.4 tabulated the reduction in the viscosity of the natural dye according to the temperature range tested and betacyanin content which are calculated from optical density reading from Table 4.1.

Table 4.5 Overall Results for Temperature range

Temperature, ^{O}C	Viscosity, cP (Before)	Viscosity, cP (after)	Beta cyanin content, mg/L (Before)	Beta cyanin content, mg/L (After)
30	21.6	14.30	26.428	24.36
40	21.6	10.40	26.428	23.31
50	21.6	4.32	26.428	21.173
60	21.6	4.30	26.428	19.967
70	21.6	4.28	26.428	10.54
80	21.6	4.25	26.428	5.7108
90	21.6	3.91	26.428	3.227
100	21.6	3.89	26.428	3.117

The results that tabulated are then presented in graph for better understanding and clearer view of the trend of viscosity reduction and difference in betacyanin content according to the temperature from 30^{o}C to 100^{o}C. The results shown in Table 4.4 are summarized in Figure 4.2 below.

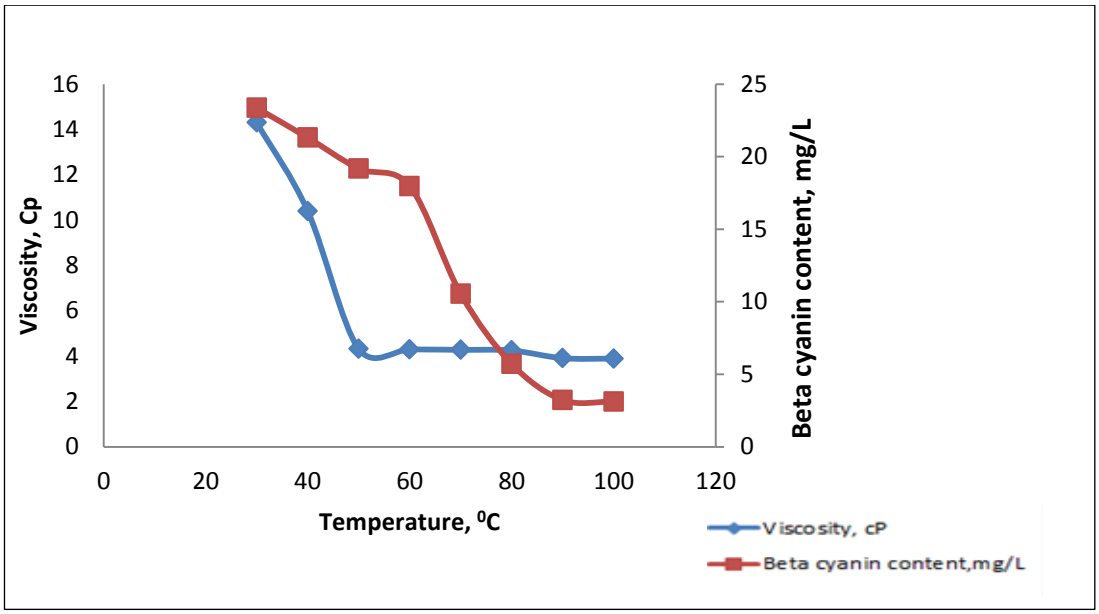

Figure 4.8 Graph of Viscosity and Betacyanin Content Against Temperature.

In this part of experiment, the enzyme concentration used is 2.5% (w/v). The particular enzyme concentration was chosen because the earlier part of the experiment, which was conducted to determine the optimum enzyme concentration for 15 ml sample and was proved to be 2.5%. The pH of the reaction is set to 5.5 as it is the optimum pH for pectinase. In addition, the reaction time was set 60 minutes for all the reactions. The temperature range from 30 ^{0}C to 100 ^{0}C was chosen because these range can clearly show the changes in reaction that took place. 0^{0}C to 20^{0}C was ignored in the range chosen because it is difficult to run the experiment or in another word, it is quite impossible to set up the environment to carry out the experiment in the mentioned temperatures.

Figure 4.8 illustrate the results obtained for the enzymatic reaction for various temperatures on the reduction of the viscosity and changes in betacyanin content of

the natural dye. Based on the Figure 4.8, the reduction of viscosity of the natural dye based on the temperatures can be separated by two phases. The first phase shows a sharp decrease in viscosity of the natural dye. This first phase trend can be observed in temperature from 30^{o}C to 50^{o}C. The amount of reduction of the viscosity in first phase shows 34% for the temperature of 30^{o}C, 52% for the temperature of 40^{o}C, and 54% for the temperature of 80^{o}C.

Whereas the second phase, the viscosity reduction is very small and almost insignificant. This phase's amount of reduction can be witnessed from the temperature of 60^{o}C to 100^{o}C. It can be observed a reduction of 80.1% for the temperature of 60^{o}C, 80.2% for the temperature of 70^{o}C, 80.3% for the temperature of 80^{o}C, 81% for the temperature of 90^{o}C and 82% for the temperature of 100^{o}C. This phase of betacyanin reduction which can be observed from the enzyme concentration of 2.5% to 5% shows a massive reduction compared to previous reduction rate. The amount of reduction when the enzyme concentration increase to 5% is 40% of reduction compared to the initial betacyanin content in the natural dye.

As detailed earlier, pectinase are used to breakdown pectin. As we all well known, an enzyme is a protein-based substance which serves as a catalyst to speed up a reaction by lowering the activation energy. Enzymes accelerate biochemical reactions by physically interacting with the reactants and products to provide a more favorable pathway for the transformation of one to the other. They increase the rates of reactions by increasing the probability that the reactant can interact properly. Enzymes ability to speed up the reaction often yields the rates which are 10^{6} to 10^{12} times faster than the corresponding to uncatalyzed reactions. The reaction rate with

enzymes, are still several times greater than those using standard chemical agents as catalysts. Enzymatic reaction pathway is shown in the figure 4.9 below which explain how enzymes facilitate a faster reaction rate. Figure 4.9 shows the difference in activation energy in both enzyme facilitated and a reaction without enzyme. In enzyme facilitated reaction, the activation energy is found much more lower which is more favorable for a reaction to take place.

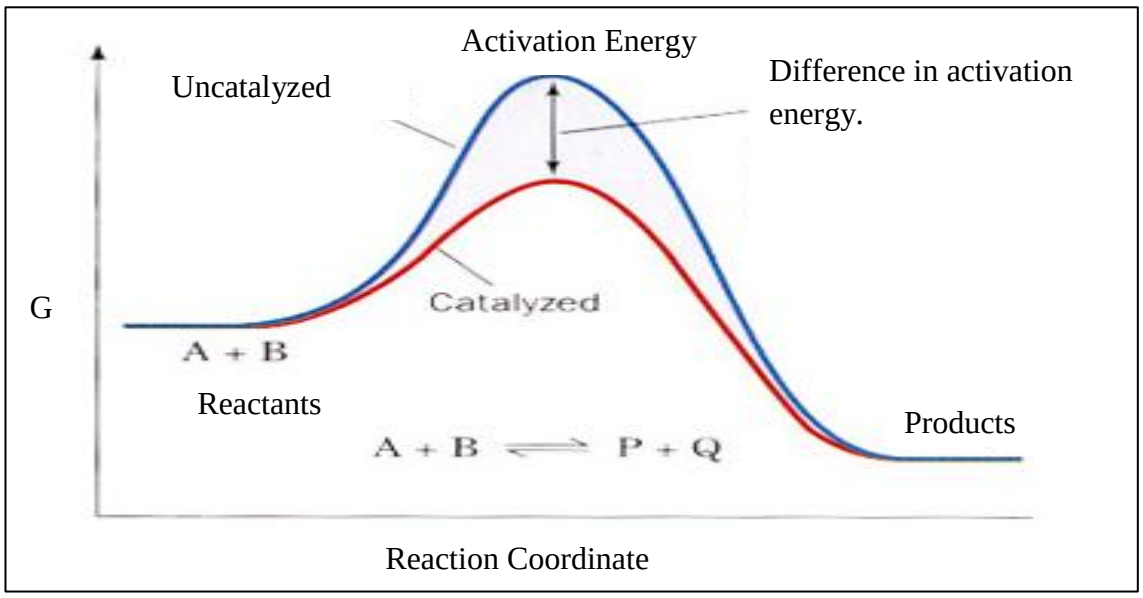

Figure 4.9 Reaction Pathway of enzyme

However, all these properties of enzymes are dependent on the external environment of the reaction. Temperature and pH both effects the rates at which a reaction takes place, and there exists an optimum value for each.

The temperature range, over which enzymes show its activity is limited between the melting point (0^oC) and boiling point (100^oC) of water. If a temperature

is too low, there can be no noticable reaction rate since the enzyme is operating at a temperature far below its optimum. If the temperature at which the enzyme is operating at is well above 100^{o}C, then thermal deactivation or denaturation can occur.

This theory tallies with the result obtained in this part of study. In the first phase of the viscosity reduction, it exhibits a sharp decrease in the viscosity of the natural dye. It is because as the temperature increases to the optimum temperature of pectinase, that is 50^{o}C, the reaction speed up which means as the temperature increases from 30^{o}C, the enzyme will get more energy to break down pectin. Note has to be taken that only in freezing point the enzyme will be inactive and there will be no reaction will take place. In low temperature such as 30^{o}C reaction will take place but less as the result appeared.

Whereas, in the second phase, the reduction in viscosity of the natural dye, become less and almost constant when the temperature start to increase from 60^{o}C to 100^{o}C. It is because when the temperature increases from the optimum temperature, although the pectinase is thermostable, it will still undergo some structure changes but will not denatured.

In the Figure 4.8 also can be observed the trend of changes in betacyanin content according to the temperature range that have been studied. The betacyanin content in the natural dye can be separated into three phases according to the Figure

4.2. The first phase shows a slow and insignificant decrease in the betacyanin content from the temperature of 30^{o}C to 50^{o}C. A reduction of 7% of betacyanin content for the temperature of 30^{o}C, 9% for the temperature of 40^{o}C, 11% for the temperature of 50^{o}C can observed.

In the second phase of the betacyanin content profile, it shows a sharp decrease in the betacyanin content when the temperature increases from 60^{o}C to 90^{o}C. A 28% of reduction in the betacyanin content can be observed at the temperature of 60^{o}C, 60% at the temperature of 70^{o}C, 78% at the temperature of 80^{o}C and 87% at the temperature of 90^{o}C.

Whereas, in the third phase of the betacyanin profile according to the temperature, a very minute reduction in the betacyanin content compared to the previous reduction can be observed. In another word, the betacyanin content can be said almost constant with the temperature before it. At 100^{o}C, the reduction of betacyanin obtained is 90% which is almost same with the betacyanin content at temperature of 90^{o}C.

Betacyanin pigment is sensitive towards temperature. At high temperature, the betacyanin pigment will degrade and the colour of the natural dye will fade. The first phase of the betacyanin profile shows a slow reduction and almost in significant reduction from the temperature from 30^{o}C to 50^{o}C, because at low temperature the

betacyanin pigment will not undergo any degradation nor alteration in its structure. Thus, the reduction in betacyanin content will be very less.

In the second phase of the betacyanin profile, the reduction of the betacyanin content in the natural dye is higher from the temperature 60^{o}C to 90^{o}C because as the temperature increase from 50^{o}C, the betacyanin starts to degrade slowly as it is sensitive to higher temperature. As the temperature increase even further to 80^{o}C and 90^{o}C, the reduction in the betacyanin content is very large as numerous and massive amount of the betacyanin pigment have degraded.

Whereas, in the third phase of the betacyanin profile, the reduction of the content is almost as same as the reduction observed at the temperature of 90^{o}C. It is because at temperature of 90^{o}C, the maximum content of the betacyanin content has been degraded. At the temperature of 100^{o}C, although a massive reduction in the betacyanin content can be observed but the reduction compared to reduction of betacyanin content at 90^{o}C is in significant.

The colour changes of the natural dye can be observed in the Figure 4.9 which shows the colour changes of the natural dye from 30^{o}C to 60^{o}C and Figure 4.10 which shows the colour changes from the temperature of 70^{o}C to 100^{o}C below. The rapid colour changes can be observed from the temperature of 70^{o}C to 100^{o}C which shows the beginning of the massive degradation of betacyanin content from the temperature 70^{o}C to 100^{o}C.

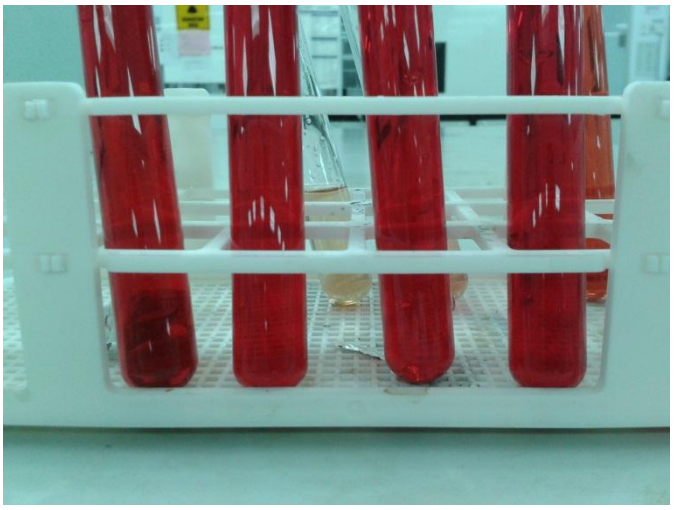

Figure 4.10 Natural dye from the enzymatic reaction from temperature of 30^{o}C (left) to 60^{o}C (right).

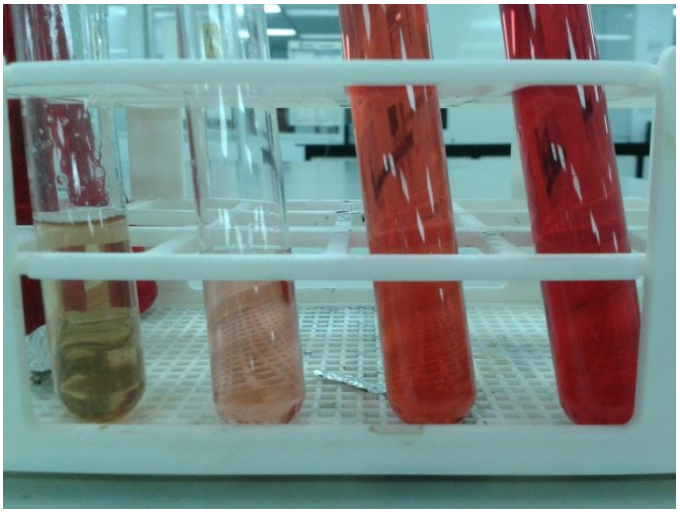

Figure 4.11 Natural dye from the enzymatic reaction from temperature of 70^{o}C (right) to 100^{o}C (left).

Supporting analysis from FTIR also has been done for this temperature study. Figure 4.11 and Figure 4.12 shows the FTIR diagram for the first and second phase of the viscosity and betacyanin reduction of the natural dye. Figure 4.9 below, exhibit the first phase where it shows a sharp reduction of the viscosity of the natural dye as can

be seen from the reduction of height of the peak of C-O bond and C=O bond compared to the peaks produced in FTIR for natural dye before enzymatic reaction. Whereas, Figure 4.10 below, exhibit the second phase where it shows a sharp reduction in betacyanin content when the temperature increase to 70^{o}C. The reduction can be proved when there is sharp reduction in the peak which represent NH bond, C-O bond and C=O bond compared to the peaks produced in FTIR for natural dye before enzymatic reaction.

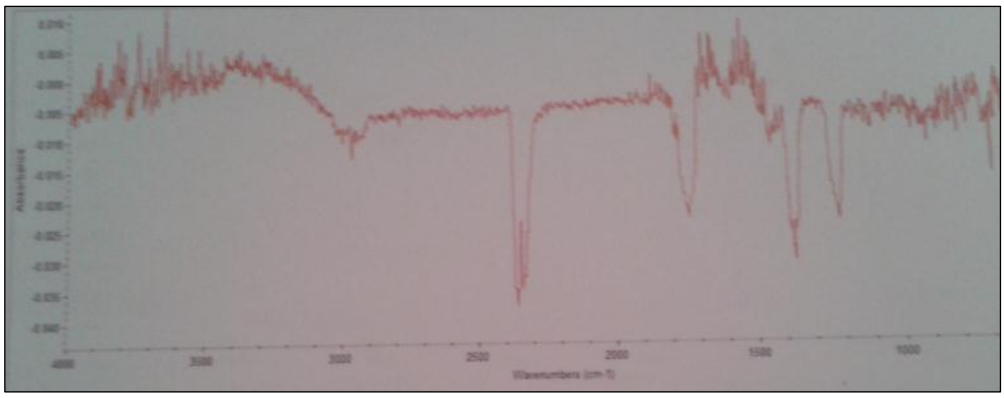

Figure 4.12 FTIR diagram for first phase for viscosity and betacyanin reduction for temperature reaction.

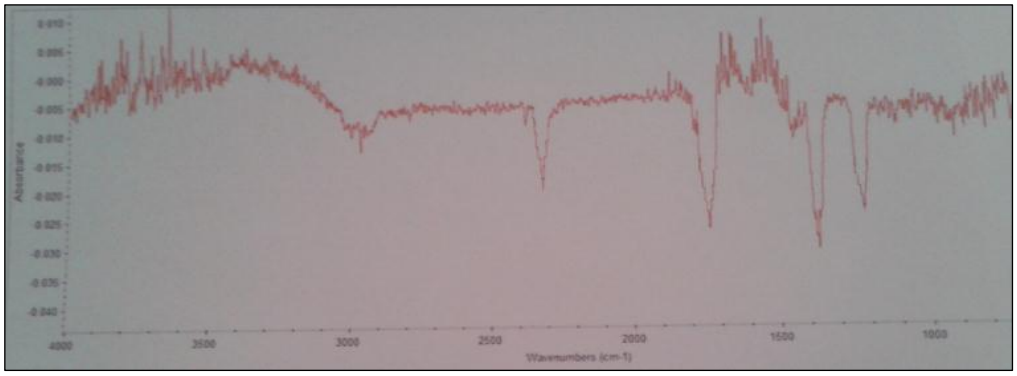

Figure 4.13 FTIR diagram for second phase for viscosity and betacyanin reduction for temperature reaction.

4.4 Feasibility Study

For a feasibility study analysis, a test has been conducted whether this research has been achieved the objective of this research. The objective of this research is to reduce the viscosity of the natural dye obtain from pitaya waste so that the natural dye will fast on the fabric. The test has been conducted by the method described in the methodology. The two type of natural dye has been prepared, that is the one without any reaction and the other natural dye is the one treated with pectinase in order to reduce viscosity. The comparison and contrast between these two types of natural dye has been summarized in the table below:

Table 4.6 Similarities and Differences between treated and untreated natural dye.

	Untreated Natural Dye	Treated Natural Dye
Viscosity	21.6	4.28
Betacyanin content	26.43	20.43
Source	Pitaya Peel	Pitaya Peel

Two white cloth with a measurement of 4cm x 4.5 cm is being immersed the two type of natural dye where each cloth is being immersed in each type of natural dye. The cloths is let to be immersed for 24 hours and let dry for another 24 hours. Then the dried coloured cloths will be washed with tap water to see the fastness of the dyes on the cloths. The cloth which is immersed in the treated natural dye has been proved to show the stronger fastness compared to the untreated natural dye. This statement can be pictured in the figure shown below:

Figure 4.14 Cloths that has been tested

The cloth that is on left side in the figure is the one which is immersed in the untreated natural dye which has the higher viscosity. Whereas, the cloth that is on the left side is the one which is immersed in the treated natural dye with pectinase to reduce the viscosity of the natural dye.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The purpose of this research is to reduce the viscosity of the natural dye so that its texture to fasten to cloth can be improved. From the result as the enzyme concentration increases, the viscosity of the natural dye is reduces. This reduction trend can only be monitored until the enzyme concentration of 2.5%. After that particular enzyme concentration level, the reduction of the viscosity of the natural dye is insignificant. Hence, 2.5% is said as the critical enzyme concentration for 5 ml of the natural dye. Concisely, the highest viscosity reduction and can be observed in the enzyme concentration of 2.5%.

Meanwhile the highest viscosity reduction can be seen in the temperature of 50 ^{o}C that was 4.25 cp which is 80% decrement compared to initial viscosity. Moreover, feasibility study has been conducted to prove that this research has achieved its objective. In this feasibility study, it is proven that the treated natural dye with pectinase gave the higher affinity towards cloth. The fasting of the treated natural has higher fastness compared to untreated natural dye.

5.3 Recommendations

In this research, water extraction has been used to extract the natural dye from pitaya peel. And then, in order to extract the natural dye, water is introduced to blended pitaya peel in the ratio of 2:1. Hence, further studies can be done to study the relationship of the water ratio which is introduced in the extraction step with the viscosity of the natural dye. The ratio of water can be varied in order to study the relationship between the mention characteristics.

In addition, for the enzymatic reaction part, more parameter can be added to broaden the horizon of the research such as pH and concentration of natural dye. This addition of parameter can help to understand better about the enzymatic reaction in natural dye in every aspect.

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APPENDIX A

Example of Calculation

For betacyanin content calculation,

$$BC\ (mg\ L^{-1}) = \frac{\mathrm{A} \times \mathrm{MW} \times 1000 \times \mathrm{DF}}{\epsilon \times \mathrm{l}}$$

Where:

A = Absorbance

DF = Dilution Factor

MW = Molecular Weight of beta cyanin

= 550 $gmol^{-1}$

ϵ = Molar Extinction Coefficient

= 60, 000 L/mol cm in H_2O

l = Path length of cuvette

= 1cm

This calculation is based on the absorbance value of the sample that has been used for the research.

Table A1 Data for enzyme Concentration(0.1% to 5.0%)

Enzyme Concentration (w/v)	Optical Density readings			Average OD readings
0.1	2.653	2.656	2.650	2.653
0.25	2.590	2.591	2.592	2.591
0.5	2.500	2.502	2.504	2.502
1.0	2.220	2.221	2.222	2.221
2.5	2.117	2.119	2.121	2.119
5.0	1.827	1.829	1.831	1.829

For enzyme concentration 0.1%, the betacyanin content in the natural dye after the enzymatic reaction is:

$$BC\ (mg\ L^{-1}) = \frac{2.653 \times 550\ \text{gmol}^{-1} \times 1000 \times 1}{60{,}000\text{L/mol} \times \text{l}}$$

$$= 24.32\ \text{g/L}$$

This is how the calculation will follow to determine betacyanin content in g/L for the rest of enzyme concentration and effect of Temperature.