EXTRACTION OF GREEN PIGMENT FROM PANDANUS ODORUS

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Chemical Engineering

Faculty of Chemical and Natural Resources Engineering

UNIVERSITI MALAYSIA PAHANG

APRIL 2010

ABSTRACT

The objective of this research is to find the best condition to extract green pigment so that it optimize the yield of pigment extract. Pandanus odorus is a typical plant used in everyday cooking in Malaysia. This research project is designated in extracting organic green pigment from Pandanus Odorus or Pandanus Amaryllifolius by using Soxhlet extraction techniques. Chlorophyll were divided into two, chlorophyll a and chlorophyll b, both gives the green color to leaves. The parameters that being studied in this research are the time of extraction, the type of solvents, the size of raw materials and the solvent/raw material ratio. The extract then was separated with the solvents using a vacuum rotary evaporator. The weight of the extract was taken and the extract was diluted with acetone 80% to get the reading of absorbance using UV-Vis to calculate the amount of chlorophyll in the extract. The result shows that the longer time taken for the extract, the smallest size of raw material particle, using high amount of solvent and extract using ethanol 95% will result in the highest yield of green pigment extract.

ABSTRAK

Tujuan dari penelitian ini adalah untuk mendapatkan keadaan terbaik untuk mengekstrak pigmen hijau sehingga mengoptimumkan hasil ekstrak pigmen. Pandanus odorus adalah tanaman khas yang digunakan dalam memasak harian di Malaysia. Projek Sarjana Muda ini direka untuk menghasilkan pigmen hijau organik dari Pandanus Odorus atau Pandanus Amaryllifolius dengan menggunakan teknik ekstraksi Soxhlet. Klorofil dibahagi menjadi dua, klorofil a dan klorofil b,yang memberi warna hijau untuk daun. Pemboleh ubah yang diteliti dalam kajian ini adalah waktu ekstraksi, jenis pelarut, saiz bahan mentah dan nisbah pelarut / bahan mentah. Ekstrak kemudian dipisahkan dengan pelarut menggunakan vakum rotari. Berat ekstrak diambil, kemudian ekstrak dicairkan dengan acetone 80% untuk mendapatkan pembacaan absorbansi dengan menggunakan UV-Vis untuk mengira jumlah klorofil dalam ekstrak. Keputusan kajian menunjukkan bahawa semakin lama waktu yang diambil untuk ekstrak, saiz terkecil dari partikel bahan-bahan mentah, dengan menggunakan jumlah yang tinggi dan ekstrak menggunakan pelarut etanol 95% akan menghasilkan keputusan yang tertinggi pigmen hijau ekstrak.

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^{o}C	= Degree Celsius
%	= Percentage
EtOH	= Ethanol
EtAc	= Ethyl Acetate
g	= Gram
L	= Liter
min	= Minutes
hr	= Hours
m	= Meter
µm	= Micron- meter
mm	= Mili-Meter
cm/s	= Centi-Meter Per Second
^{o}C/min	= Degree Celcius Per Minutes
µL	= Micro-Liter
V	= Volume

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CHAPTER I

INTRODUCTION

1.1 Background of Study

Extraction of green pigment from pandanus odorus (pandan) is a project designated to extract good quality green colored plant pigment from the leaves of pandanus adorus. The type of green pigment that will be produced is organic pigment. Organic pigments, also known simply as pigments or biochromes are substances produced by living organisms that have a color resulting from selective color absorption. Biological pigments include plant and flower pigments. Many biological structures, such as skin, eyes, fur and hair contain pigments such as melanin in specialized cells called chromatophores.

Industrial pigments are dispersed in paints to provide characteristics such as color, hiding power, bulk, durability, and corrosion resistance. Common colors for industrial pigments include black, blue, bronze, brown, copper, gray, green, orange, purple, red, silver, white, and yellow. Important specifications for industrial pigments include pH and oil absorption, the amount of oil required to make paint with a pigment. Typically, oil absorption depends upon the size of the pigment particle, the type of binder, and the pigment's physical and chemical properties. Smaller pigment particles require greater amounts of binder in order to cover larger surface areas and wet each of pigment particles (Eun-Jin et al, 2006).

Industrial pigments are available in several forms. Examples include dry powders, granules, liquids, pastes, pellets, and chips. Dry powders and granules are traditional forms that are available in the widest variety of grades. Free-flowing pourable liquids and high-viscosity pastes are master batches or concentrated dispersions of organic or waterborne pigments that contain solvents or diluents. They may also contain resins, additives or surfactants. Pellets and chips do not contain solvents, but may contain additives or plasticizers. These solid industrial pigments are easy to handle and feature a high pigment-to-binder ratio. Depending on the pigment and resin type, pigment levels of between 50% and 75% are achievable (Isabel, 2004).

Chlorophyll is the primary pigment in plants; it is a porphyrin that absorbs yellow and blue wavelengths of light while reflecting green (Anonim., 1999). It is the presence and relative abundance of chlorophyll that gives plants their green color. All land plants and green algae possess two forms of this pigment: cholorphyll *a* and chlorophyll *b*. Chlorophyll molecules are specifically arranged in and around photosystems which are embedded in the thylakoid membranes of chloroplasts. In these complexes, chlorophyll serves two primary functions. The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystems. Because of chlorophyll's selectivity regarding the wavelength of light it absorbs, areas of a leaf containing the molecule will appear green (Eun-jin et al, 2004).

Pandanus odorus or commonly known to Malaysians as pandan, is one of those long-domesticated plants and is the only Pandanus species with fragrant leaves. Pandanus leaves are a popular flavouring in tropical Asia, from South India to New Guinea. They are used for rather different purposes, but mostly in connection with rice, since rice can profit most from the hay-like odour of pandanus. Pandanus leaves have their center of usage in South East Asia: In Thailand, Malaysia and Indonesia, pandanus leaves are valued because their fragrance enhances the flavour of rice (Mohsin et al, 2008).

1.2 Problem statements

Pandanus Amaryllifolius or pandanus odorus(Pandan) widely used as cooking material in Malaysia. The climate of Malaysia is suitable for them to grow. They can be easily grown and need not much care for their growth (Rahman et al, 2001). This project is conducted due to these facts:

1.2.1 The availability of Pandanus Odorus in Malaysia

It’s a fact that many of this plant exist in Malaysia and actually only a small amount of this plant is need in cooking. So, there are enormous amount of pandan to be used as the raw material. The plant can be easily grown without much care, and very suitable in Malaysian weather and temperature.

1.1.2 Toxicity and hazards of pigments

The fact that mainly the pigments used in art tools nowadays are transition metal based. Most of them are toxic and hazardous to a person's health. By introducing an environmentally safe pigment, it will reduce the hazardousness and toxicity of pigment related works. As we know, plants are environmental friendly and are biodegradable.

1.1.3 Low cost extraction techniques

Using Soxhlet extraction techniques and vacuum rotary evaporator, this research can be classified as cost saving, because by using vacuum rotary evaporator, the solvents can be recycled about 95%, the other 5% should be disperse in the air. This means that the solvents can be use again and again. This saves the cost of expenses for the solvents.

Due to these facts, the initiative to invent a solution of nonhazardous pigments from natural material, in this case Pandanus Odorus is conducted. We hope for environmental friendly product which will not harm person, especially children from toxicity.

1.3 Objectives

The goals of this research are to:

1) To extract green pigment from pandanus odorus using Soxhlet extraction method with ethanol 95% and ethyl acetate 90% as the solvents.
2) To investigate the effect of size of raw material, time of extraction, and ratio of the solvents with raw material to the yields of green pigment.
3) To determine the amount of chlorophyll in the extract of green pigment.

1.3 Research Advantages

The green pigment produces through this research will be used as industrial pigments that are widely used in coloring material and arts:

1) This research gives an advantage as it can provide an environmental friendly condition for more inexpensive and ecologically safe solutions.
2) Pandanus odorus plant is a type of plant that can be easily grown in Malaysia’s weather and condition. It also does not require any special and burdensome treatment for their growth (Rahman et al, 2001).
3) Green pigment also being used in art tool such as colour pencils, pastels, and etc. The amount of hazardous material in the present pigments may cause sickness to children if inhaled or digested these pigments. The usage of pandanus odorus will not affect the health of children.
4) The solvent used are ethanol 95% and ethyl acetate 90%, which is easy to obtain, and can be recycled back to be used after separation with rotary evaporator.
5) The operating system, Soxhlet extraction method is simple.

1.4 Research Scopes

The scopes of this research will be as follows:

1.4.1 The effect of time of extraction of pigment to the yield of pigment extract.

1.4.2 The effect of size of raw material/Pandanus odorus to the yield of extract.

1.4.3 The effect of ratio of raw material/solvents to the yield of pigment extract.

1.4.4 The quantity of chlorophyll a and chlorophyll b extracted from Pandanus odorus.

1.4.5 The optimum condition to obtain the maximum amount of extract.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of pigments

A pigment is the material that changes the color of light it reflects as the result of selective color absorption. This physical process differs from fluorescence, phosphorescence, and other forms of luminescence, in which the material itself emits light. Many materials selectively absorb certain wavelengths of light. Materials that humans have chosen and developed for use as pigments usually have special properties that make them ideal for coloring other materials. A pigment must have a high tinting strength relative to the materials it colors. It must be stable in solid form at ambient temperatures (Anonim., 2006).

Pigments appear the colors they are because they selectively reflect and absorb certain wavelengths of light. White light is a roughly equal mixture of the entire visible spectrum of light. When this light encounters a pigment, some wavelengths are absorbed by the chemical bonds and substituents of the pigment, and others are reflected. This new reflected light spectrum creates the appearance of a color. . Pigments, unlike fluorescent or phosphorescent substances, can only subtract wavelengths from the source light, never add new ones.

The appearance of pigments is intimately connected to the color of the source light. Sunlight has a high color temperature, and a fairly uniform spectrum, and is considered a standard for white light. Artificial light sources tend to have great peaks in some parts of their spectrum, and deep valleys in others. Viewed under these conditions, pigments will appear different colors.

Pigments are used for coloring paint, ink, plastic, fabric, cosmetics, food and other materials. Most pigments used in manufacturing and the visual arts are dry colourants, usually ground into a fine powder. This powder is added to a vehicle (or matrix), a relatively neutral or colorless material that acts as a binder. The worldwide market for inorganic, organic and special pigments had a total volume of around 7.4 million tons in 2006. Asia has the highest rate on a quantity basis followed by Europe and North America. In 2006, a turnover of 17.6 billion US$ (13 billion Euro) was reached mostly in Europe, followed by North America and Asia (Anonim., 2006).

Naturally occurring pigments such as ochres and iron oxides have been used as colorants since prehistoric times. Archaeologists have uncovered evidence that early humans used paint for aesthetic purposes such as body decoration. Pigments and paint grinding equipment believed to be between 350,000 and 400,000 years old have been reported in a cave at Twin Rivers, near Lusaka, Zambia (Kassinger, 2003).

2.2 Organic pigments

Among the most important molecules for plant function are the pigment Plant pigments include a variety of different kinds of molecules, including porphyrins, carotenoids, anthocyanins and [betalain]s. All biological pigments selectively absorb certain wavelengths of light while reflecting others. The light that is absorbed may be used by the plant to power chemical reactions, while the reflected wavelengths of light determine the color the pigment will appear to the eye. Pigments also serve to attract pollinators (Anonim., 2006).

Chlorophyll is the primary pigment in plants; it is a porphyrin that absorbs yellow and blue wavelengths of light while reflecting green. It is the presence and relative abundance of chlorophyll that gives plants their green color. All land plants and green algae possess two forms of this pigment: cholorphyll *a* and cholorphyll *b*. Kelps,diatoms,and other photosynthetic heterokonts contain chlorophyll *c* instead of *b*, while red algae possess only chlorophyll *a*. All chlorophylls serve as the primary means plants use to intercept light in order to fuel photosynthesis. Chlorophyll is the reason most plants are of the color green (Anonim., 2006).

Carotenoids are red, orange, or yellow tetraterpenoids. They function as accessory pigments in plants, helping to fuel photosynthesis by gathering wavelengths of light not readily absorbed by chlorophyll. The most familiar carotenoids are carotene (an orange pigment found in carrots), lutein (a yellow pigment found in fruits and vegetables), and lycopene (the red pigment responsible for the color of tomatoes). Carotenoids have been shown to act as antioxidants and to promote healthy eyesight in humans (Anonim., 2006).

2.3 (Leaching) Extraction process

Removal of materials by dissolving them away from solids is called leaching. The chemical process industries use leaching but the process is usually called extraction, and organic solvents are often used. The theory and practice of leaching are well-developed because for many years leaching has been used to separate metals from their ores and to extract sugar from sugar beets. Environmental engineers have become concerned with leaching more recently because of the multitude of dumps and landfills that contain hazardous and toxic wastes. Sometimes the natural breakdown of a toxic chemical results in another chemical that is even more toxic. Rain that passes through these materials enters ground water, lakes, streams, wells, ponds, and the like (Anonim., 1997).

For single stage leaching, two steps are involved:

1) contact of solid and solvent for transfer of solute to solvent

2) separation of resulting solution from the residual solid

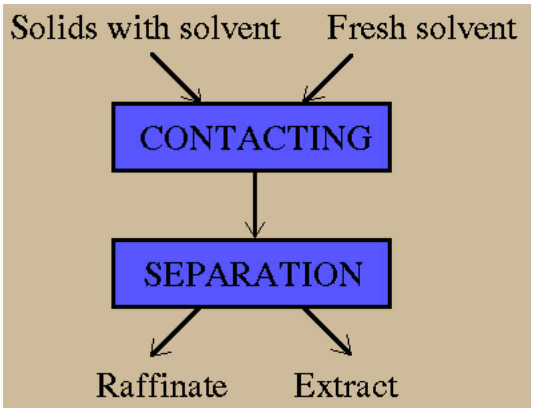

Figure 2.1: Leaching steps

The Extract is the solvent phase. The Raffinate is the solid material and its adhering solution (Anonim., 1997).

2.4 Soxhlet Extractor

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance (Jensen, 2007).

Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded (Jensen, 2007).

2.5 Pandanus Odorus (Pandanus Amaryllifolius)

Pandanus amaryllifolius is a tropical plant in the screwpine genus which is known commonly as pandan and used widely in Southeast Asian cooking. It is an erect green plant with fan-shaped sprays of long, narrow, bladelike leaves and woody aerial roots. The plant is sterile, flowers only very rarely, and is propagated by cuttings (Anonim., 1999).

The plant is rare in the wild but cultivated widely for use as a flavoring in cooking. The leaves are used fresh or wilted, and are commercially available in frozen form in Asian grocery stores in nations where the plant does not grow. They have a nutty, botanical fragrance which enhances the flavor of Indonesian, Filipino, Malaysian, Thai, Vietnamese and Burmese foods, especially rice dishes and cakes. The leaves are sometimes steeped in coconut milk, which is then added to the dish. They may be tied in a bunch and cooked with the food. They also may be woven into a basket which is used as a pot for cooking rice. Pandan chicken, or gai ob bai toey, is a Thai dish with chicken wrapped in pandan leaves and fried. The leaves are also used as a flavoring for desserts such as pandan cake and sweet beverages. In Indonesian it is called pandan wangi, soon-mhway in Burmese, and in Vietnamese it is called lá dứa. The leaves of the plant have a repellent effect on cockroaches.

The characteristic aroma of pandan is caused by the aroma compound 2-acetyl-1-pyrroline which also gives white bread, jasmine rice and basmati rice, and bread flowers (Vallaris glabra) their typical smell. Bottled pandan extract is also available in shops, but it often contains artificial green food coloring. Magnolia Ice Cream makes a pandan flavored ice cream (Anonim., 1999).

2.6 Chlorophylls

Chlorophyll is vital for photosynthesis, which allows plants to obtain energy from light. Chlorophyll molecules are specifically arranged in and around photosystems which are embedded in the thylakoid membranes of chloroplasts. In these complexes, chlorophyll serves two primary functions. The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystems. Because of chlorophyll's selectivity regarding the wavelength of light it absorbs, areas of a leaf containing the molecule will appear green (Hojnik et al, 2007).

The two currently accepted photosystem units are Photosystem II and Photosystem I, which have their own distinct reaction center chlorophylls, named P680 and P700, respectively. These pigments are named after the wavelength (in nanometers) of their red-peak absorption maximum. The identity, function and spectral properties of the types of chlorophyll in each photosystem are distinct and determined by each other and the protein structure surrounding them. Once extracted from the protein into a solvent (such as acetone or methanol), these chlorophyll pigments can be separated in a simple paper chromatography experiment, and, based on the number of polar groups between chlorophyll a and chlorophyll b, will chemically separate out on the paper (Speer, 1997).

The function of the reaction center chlorophyll is to use the energy absorbed by and transferred to it from the other chlorophyll pigments in the photosystems to undergo a charge separation, a specific redox reaction in which the chlorophyll donates an electron into a series of molecular intermediates called an electron transport chain. The charged reaction center chlorophyll is then reduced back to its ground state by accepting an electron (Marker, 1972).

The electron flow produced by the reaction center chlorophyll pigments is used to shuttle H^+ ions across the thylakoid membrane, setting up a chemiosmotic potential mainly used to produce ATP chemical energy, and those electrons ultimately reduce CO_2 into sugars as well as for other biosynthetic reductions (Speer, 1997).

Reaction center chlorophyll-protein complexes are capable of directly absorbing light and performing charge separation events without other chlorophyll pigments, but the absorption cross section (the likelihood of absorbing a photon under a given light intensity) is small. Thus, the remaining chlorophylls in the photosystem and antenna pigment protein complexes associated with the photosystems all cooperatively absorb and funnel light energy to the reaction center. Besides chlorophyll *a*, there are other pigments, called accessory pigments, which occur in these pigment-protein antenna complexes (Fleming, 1967).

2.7 Carotenoids

Carotenoids are organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria. There are over 600 known carotenoids; they are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen). Carotenoids in general absorb blue light. They serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage.In humans, four carotenoids (beta-carotene, alpha-carotene, gamma-carotene, and beta-cryptoxanthin) have vitamin A activity (meaning they can be converted to retinal), and these and other carotenoids can also act as antioxidants (Fleming, 1967).

People consuming diets rich in carotenoids from natural foods, such as fruits and vegetables, are healthier and have lower mortality from a number of chronic illnesses. However, a recent meta-analysis of 68 reliable antioxidant supplementation experiments involving a total of 232,606 individuals concluded that consuming additional β-carotene from supplements is unlikely to be beneficial and may actually be harmful, although this conclusion may be due to the inclusion of studies involving smokers. With the notable exception of Vietnam Gac and crude palm oil, most carotenoid-rich fruits and vegetables are low in lipids. Since dietary lipids have been hypothesized to be an important factor for carotenoid bioavailability, a 2005 study investigated whether addition of avocado fruit or oil, as lipid sources, would enhance carotenoid absorption in humans. The study found that the addition of both avocado fruit and oil significantly enhanced the subjects' absorption of all carotenoids tested (α-carotene, β-carotene, lycopene, and lutein).

Carotenoids belong to the category of tetraterpenoids (i.e. they contain 40 carbon atoms). Structurally they are in the form of a polyene chain which is sometimes terminated by rings. Carotenoids with molecules containing oxygen, such as lutein and zeaxanthin, are known as xanthophylls. The unoxygenated (oxygen free) carotenoids such as α-carotene, β-carotene and lycopene are known as carotenes. Carotenes typically contain only carbon and hydrogen (Fleming, 1967).

Probably the most well-known carotenoid is the one that gives this second group its name, carotene, found in carrots (also apricots) and are responsible for their bright orange colour. Crude palm oil, however, is the richest source of carotenoids in nature in terms of retinol (provitamin A) equivalent. Vietnamese Gac fruit contains the highest known concentration of the carotenoid lycopene. Their colour, ranging from pale yellow through bright orange to deep red, is directly linked to their structure. Xanthophylls are often yellow, hence their class name. The double carbon-carbon bonds interact with each other in a process called conjugation, which allows electrons in the molecule to move freely across these areas of the molecule. As the number of double bonds increases, electrons associated with conjugated systems have more room to move, and require less energy to change states. This causes the range of energies of light absorbed by the molecule to decrease. As more frequencies of light are absorbed from the short end of the visible spectrum, the compounds acquire an increasingly red appearance (Speer, 1997).

2.8 Ultra Violet Visible Spectophometer

UV/Vis spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds. Solutions of transition metal ions can be coloured (i.e., absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another. The colour of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the colour of a dilute solution of copper sulfate is a very light blue; adding ammonia intensifies the colour and changes the wavelength of maximum absorption (λ_{max}) (Skoog et al, 2007).

Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water soluble compounds, or ethanol for organic-soluble compounds. (Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths.) Solvent polarity and pH can affect the absorption spectrum of an organic compound. Tyrosine, for example, increases in absorption maxima and molar extinction coefficient when pH increases from 6 to 13 or when solvent polarity decreases (Skoog et al, 2007).

While charge transfer complexes also give rise to colours, the colours are often too intense to be used for quantitative measurement. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/VIS spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how quickly the absorbance changes with concentration. This can be taken from references (tables of molar extinction coefficients), or more accurately, determined from acalibration curve (Wittung et al,, 2009).