TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID OF *H. POLYRHIZUS* WASTE EXTRACT BY USING ULTRASONIC SOLVENTS EXTRACTION

LEE QIAO HUI

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Faculty of Chemical Engineering and Natural Resourses
Universiti Malaysia Pahang

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I declare that this thesis entitled “Total Phenolic Content and Total Flavonoid of *H. polyrhizus* Waste Extract by using Ultrasonic Solvents Extraction’’ is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :
Name : Lee Qiao Hui
Date : 18/4/09

To my beloved family.

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Praise to God for His help and guidance that finally I will able to complete my final year project which is one of the requirement to complete my study.

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ABSRACT

Research in extracting colour from natural fruits has been actively conducted nowadays. In this research, red pitaya peels was selected as a raw material. Red pitaya peels was selected because of red pitaya easily find in local market and its can reduce waste production in the world, which is environmental friendly. This research is to analyze the total phenolic contents and total flavonoids from red pitaya peels. Its also analyze the effect of the different solvents to the production of natural color from red pitaya waste. Four different solvents: n-hexane, ethanol, propanol and acetone has been used in extracting total phenolic contents and total flavonoid from red pitaya waste. Three different parameter of ultrasonic extractor (25, 68, 132 kHz) were used in extracting red pitaya peels with four solvents respectively. The analysis was conducted in University Malaysia Pahang laboratory using the ultraviolet-visible spectrophotometry. Most important step in this research is the analysis of total phenolic and total flovonoid contents for twelve samples. Based on the result of this research, the best solvent for total phenolics and flavonoids from red pitaya waste is ethanol, followed by propanol and acetone. This is due to the highest polarity of ethanol. On the other hand, n-hexane extracts show the lowest total phenolic and flavonoids contents. 132kHz ultrasonic extractor extracts more total phenolic and flavonoids contents, follow by 68kHz and 25kHz ultrasonic extractor. As a conclusion, the highest polarity of the solvents and frequency extract more color pigments, total phenolic and flavonoids from red pitaya waste.

ABSTRAK

Pengekstrakan warna daripada buah-buahan semakin banyak dilakukan pada masa kini. Dalam penyelidikan ini, kulit buah naga merah dipilih untuk mengendalikan eksperimen ini. Kulit buah naga merah dipilih berdasarkan keadaannya yang mudah di jumpai di pasaran tempatan. Di samping itu, kulit yang digunakan untuk ekstrak warna boleh mengurangkan masalah pencemaran alam sekitar yang semakin berleluasa. Kajian ini adalah untuk mengkaji and menganalisis kandungan finolik dan flavonok di dalam kulit buah naga. Ia juga menganalisis empat jenis pelarut : n-heksana, propanol, etanol dan acetone digunakan untuk mengekstrak kandungan finolik dan flavonok daripada kulit buah naga. Tiga jenis ultrasonik yang berlainan frekuensi: 25, 68, 132 kHz digunakan untuk estrak kulit buah naga merah. Analisa ini dilakukan di makmal Universiti Malaysia Pahang dengan menggunakan ultraviolet spectrophotometer. Tujuan kajian ini adalah untuk mengenalpasti ciri-ciri dan menganalisa kandungan finolik dan flavonok di dalam kulit buah bagi dua belas sampel. Berdasarkan keputusan kajian ini, pelarut yang paling sesuai untuk mengekstrak kandungan finolik dan flavonok in dalam kulit buah naga adalah etanol, seterusnya ialah propanol dan acetone. Ini adalah disebabkan etanol mempunyai polariti berbanding dengan pelarut yang lain. n-heksana adalah pelarut yang ekstrak pigmen buah naga yang paling sedikit. Ultrasonik yang berfrekuensi 132kHz ektrak paling banyak kandungan finolik dan flavonok berbanding dengan 68kHz dan 25kHz ultrasonik. Sebagai kesimpulannya, polariti pelarut dan frekuensi ultrasonik adalah berkadar terus dengan kandungan finolik dan flavonok.

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CHAPTER 1

INTRODUCTION

1.1 Background of Research

Research in pitaya waste are very important nowadays because color properties in pitaya waste are very useful in many field of industries such as cosmetic, medicine, natural dye in food industries and also health. Color properties of pitaya waste can be an alternative way to replace synthetic chemical in many field of industries. Production of natural dye from pitaya waste can be optimize since there is a growing interest in the use of natural pigments for food coloring 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

Nowadays, synthetic chemical and drugs are largely being use as medicine and cosmetic products which give the bad impact to the human health if it takes over dosage. In order to reduce the usage of synthetic chemical as medicine and cosmetic usage, there are alternative ways by using natural resources such as pitaya waste.

Pitaya waste has been chosen to study whether it has potential to become alternative medicine and cosmetic by study of their color properties, total phenolic content, and total flavonoids content in pitaya waste. The red flesh pitaya have been chosen for the study. Nearly all dyestuff is currently produced from synthetic compounds which can cause environmental issues such as green house effect.

1.3 Objective

(i) To determine the effect of solvents to the total phenolic content and total flavonoid.

(ii) To determine the effect of ultrasonic frequency to the total phenolic content and total flavonoid.

1.4 Scope of Study

The scopes of study are:

(i) Red pitaya waste is used.
(ii) Extract total phenolic content and flavonoids from red pitaya waste by using ultrasonic extractor.
(iii) Ratio of the solvent mixed with pitaya waste was 1:1.
(iv) Rotary evaporator use to remove solvents.
(v) Different solvents (n-hexane, ethanol, isopropanol, acetone) was use to extract phenolic and flavonoids contents from pitaya waste.
(vi) Spray dryer use to dry the extracted solution in order to analyze total phenolic and total flavonoids contents.
(vii) Determine and analyze the total phenolic and total flavonoids contents by using ultraviolet-visible spectrophotometer.

1.5 Significant of the Study

Pitaya waste currently drew much attention of worldwide researchers, not only because of their red-purple color as natural dye, but also for phenolic and flavonoids contents in pitaya waste. New products with properties that may contribute to good health are much needed in the application of food industry. Fruits are rich with antioxidants that can prevent or delay oxidative damage of lipid, protein and nucleic acids. Polyphenolic compounds have been found to have potential health benefits that are believed to arise mainly from their antioxidants activity (Liu, 2003). Today, synthetic dyeing is a complex, specialized science.

Nowadays, customers have more awareness of environmental issues are now demanding natural products and natural source which are more environmental friendly. Natural dyes in pitaya waste can offer not only a rich and varied source of

dyestuff, but also the possibility of an income through sustainable harvest of the dye plants. Also, pitaya waste has a far superior aesthetic quality, which is much more pleasing to the eye. Nature provides a wealth of plants which will yield colors for the purpose of dyeing.

Pitaya waste that collected from industrial area commercially the others usage can reduce the use of toxics since starting materials are environmentally begin with associated benefits in terms of waste disposal and occupational safety. Furthermore production can be “decentralized” resulting in savings in transportation costs. After extraction of the dye, the biomass can be used for energy generation (through anaerobic treatment to generate methane, which in turn, can be sued as a fuel) and the growth media can be recycled; thus, there are virtually no wastes generated. Possible beneficial aspects such as higher UV absorption by the fabric (which contains natural dye) can result in reduced incidence of melanoma. It is clear that if natural dyes are to be considered as an alternative to the synthetic dyes used today.

Phenolic compounds can act as antioxidants by many potential pathways such as free radical-scavenging, oxygen radical absorbance, and chelating of metal ions (Halliwell *et al.,* 1995). Phenolic compounds are secondary metabolites of plants. They are naturally present in fruits and vegetables. These compounds are a part of the everyday diet and also used as medicines or supplements. Research has shown that fruits and vegetables contain other antioxidant nutrients, in addition to vitamins C and E, and carotenoids, which significantly contribute to their total antioxidant capacity (Cao *et al.,* 1996; Wang *et al.,* 1996). The major part of those antioxidant nutrients is polyphenolic compounds, which are components of fruits and vegetables having strong antioxidant capacity (Cao *et al.,* 1997; Wang *et al.,* 1997).

Pitaya plants are rich in naturally-occurring flavonoids, which are primarily found in peel. Flavonoids have a wide range of biological activities, such as cell-proliferation-inhibiting, apoptosis-inducing, enzyme-inhibiting, antibacterial, and antioxidant effects (Cook and Samman, 1996; Havsteen, 2002; Middleton and Kandaswami, 1993). Moreover, some findings indicate that flavonoids possess

various clinical properties, such as antiatherosclerotic, antiinflammatory, antitumour, antithrombogenic, antiosteoporotic, and antiviral effects (Cook and Samman, 1996; Havsteen, 2002). Numerous epidemiological studies confirm significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular risk (Cook and Samman, 1996). The formulation of preventive and healthy nutrition requires information about phenolic and flavonoid composition in the pitaya waste.

CHAPTER 2

LITERATURE REVIEW

2.1 Pitaya Fruit

Dragon fruit (*Hylocereus undatus*) or pitaya is a tropical cactus from the rainforests of Central and Northern South America. Pitaya is commercially grown in Central America, from Mexico and Texas to Peru and Argentina. Vietnam is also a big commercial producer of Pitaya (Drew *et al*., 2001) since it was introduced by French hundred years ago (Mizrahi *et al*., 1997). It is also grown in Israel and Australia. Pitaya is a segmented, vine like crawling cactus with aerial roots. Being an epiphyte, it clings to its support and can obtain nutrients from cracks where organic material concentrates. The fleshy succulent stems are three sided (occasionally four or five) and lobed along the ridges, which have small swellings equipped with short spines. Flowers appear from these swellings and are large, perfumed, ivory white with yellow centre containing a large number of stamens. The flowers open in the evening and are finished in the early morning, lasting only one night. Pitaya are very spectacular and have earned the name moonflower or Queen of the night. Fruit are large, between one hundred to hundred sixty gram and brightly colored pink, crimson or red with fleshly green scales. The flesh is white or red with many tiny black seeds (Hoa.*et.al*, 2006). There are many clones which can differ in the stem type, color,

fruit shape, skin thickness and scale expression. There are however two different species, *H. undatus* which has white flesh and *H. polyrhizus* which has red flesh. There are also several other fruiting cactus genera that are called ‘pitaya’ one of these is *Selenicereus megalanthus* which has smaller fruit with yellow skin, white flesh and clusters of spines on the fruit that brush off when ripe. Pitaya prefers a dry tropical climate with an average temperature of 21 to 29ºC, but can withstand temperature of 38 to 40ºC, and as low as 0ºC for short periods. Rainfall requirements are 600 to 1300mm with alternating wet and dry seasons. Pitaya plant like lots of sunshine, but can be damaged by high levels of light intensity so require some shading. There is a positive response in growth to organic matter and the sand content of the soil.

Pitaya is best grown from healthy green cuttings, as seedlings are very slow growing and are unreliable producers. Cuttings of 30 to 50 cm are cured in a dry place for a couple of weeks, and then potted into a free draining mix. Pitaya require shade and minimal water until roots have developed. Once this has occurred pitaya can be sun hardened and a week foliar fertilizer spray can be applied. When developed a shoot pitaya can be planted out into a well-drained mound of sand and organic material. A wooden or concrete post is used for support, with a wooden frame at the top to train the branches over. A single stem is grown up the post then allowed to branch and hang down over the frame. When the branches hang they will flower, which is about twelve to fifthteen months after planting the cutting. Many pests are known to attack cacti, and around Darwin, meat ants, ginger ants, caterpillars and mites have been recorded as causing damage. A watery rot has also been recorded if conditions are too wet or the plant has suffered injury. Regular monitoring of plants and appropriate control measures applied will reduce the problems. Birds have also been known to eat the ripe fruit.

Current research into fruit maturity indicates that the optimal harvest time for local markets is about twenty eight to thirty days after flowering. Pitaya should be stored at 7 to 10ºC and ninety to ninety eight percent relative humidity, and can be stored for two to three months. The fruit is generally eaten fresh, it can be chilled and

cut in half to reveal the attractive flesh and either sliced or scooped out with a spoon. The delicately sweet flesh is crisp and refreshing, and can also be used in fruit salads, marmalades, jellies, ices and soft drinks. Figure 2.1 and 2.2 show red and white flesh of pitaya species.

Figure 2.1 *Hylocereus. Polyrhizus*

Figure 2.2 *Hylocereus undatus*

2.2 Natural Dye

There is a growing interest in the use of natural pigments for food 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 food manufacturers and consumers in the continuing replacement of synthetic dyes (Duhard *et al.*, 1997; Stintzing and Carle, 2004). These are due to manufacturing of synthetic dyes suffers from the following limitations.

2.2.1 Environmentally Unfriendly

The production of synthetic dyes requires strong acids, alkalis, solvents, high temperatures, and heavy metal catalysts. For example, production of a dye designated as Color Index Mordant Blue twenty three states, treat 4, 8-diamino-1, 3, 5, 7-tetrahydroxy-2, 6-anthraquinonedisulfonic acid with boiling alkali or dilute acid and convert to the sodium salt or treat 1, 5-dinitro-anthraquinone with fuming sulfuric acid in the presence of sulfur sufficient to produce S2O3 at 130ºC, hydrolyze with water, and convert to the sodium salt.

2.2.2 Increase in Cost of Feedstock or Energy

Petroleum is the starting material for all synthetic dyes and thus the price of dyes is sensitive to the price of petroleum. Also, since synthesis is energy intensive

(uses super-heated steam and boiling acids), the process is sensitive to energy prices and also generates greenhouse gases.

2.2.3 Hazardous Waste Generation

Since synthetic production of dyes needs very toxic and hazardous chemicals, it also generates a hazardous waste, the disposal of which is a major environmental and economic challenge. Moreover, some facilities that produced dyes in the past are now “Superfund” sites due to intentional dumping or accidental spills of toxic and hazardous wastes.

2.2.4 Increasing Transportation Costs

Since dyes are hazardous materials and are produced at central facilities, transportation of dyes from manufacturing plants to textile dyeing and printing facilities is a major cost item and a logistic challenge.

2.2.5 Toxic and Allergic Reactions

There are occupational safety issues involved since production processes use the toxic and hazardous materials and conditions described above. Thus, if

bioengineered natural, ‘green’ dyes can be produced at a comparable price, the following benefits will be realized.

(i) Reduce the use of toxics since starting materials are environmentally benign with associated benefits in terms of waste disposal and occupational safety.

(ii) Production can be “decentralized” resulting in savings in transportation costs.

(iii) After extraction of the dye, the biomass can be used for energy generation (for example: through anaerobic treatment to generate methane, which in turn, can be sued as a fuel) and the growth media can be recycled; thus, there are virtually no wastes generated.

(iv) Possible beneficial aspects such as higher ultra violet absorption by the fabric (which contains natural dye) can result in reduced incidence of melanoma.

In particular, the so-called coloring foodstuffs representing aqueous or oily plant extracts extend their market share with red-colored preparations being particularly requested. In this regard, Fruits from the Cactaceae have been proposed as a promising betalain source (Schieber and Carle, 2003; Stintzing, 2001), offering preparations with a broader colour spectrum and being devoid of the mentioned drawbacks (Stintzing *et al.,* 2001). In addition, cactus pears, purple-fleshed pitayas (Hylocereus polyrhizus have very recently been suggested as viable betalain sources (Stintzing *et al.,* 2002; Wybraniec *et al.*, 2001). However, variability in the pigment composition within members of the genus Hylocereus has been scarcely addressed. So far, Wybraniec and Mizrahi (2002) reported the color characteristics of several Hylocereus clones range from purple to red.

Pitaya fruits from the genotype Hylocereus have been proposed as promising color sources, recently. To secure the authenticity of the plant material, a reliable differentiation of pitayas from different origins is required. In addition, Hylocereus

fruits may differ in their color quality and pigment content, the knowledge of which is crucial for the selection of appropriate plants for an emerging pitaya market.

However, substitution of synthetics with natural alternatives presents a challenge due to higher stability of the former with respect to light, oxygen, temperature, and pH (Cevallos and Cisneros, 2004). An additional important aspect which also has to be considered by an imaginary supplier of natural dyes has been identified during an extensive study of possible future use of natural dyes (Ganglberger, 2003; Rappl *et al.*, 2005), namely that the plant material which contains the natural dye needs the same level of standardization as modern synthetic dyes already have achieved at present.

Important parameters which have to be adjusted to a fixed level and confirmed within defined limits by analysis and standardization procedures are as follows: tinctorial strength of the plant material, shade of the dyeing on various textile substrates, fastness properties of the dyeing. Although numerous papers have described the selection of plant raw materials, dyeing procedures, shade of dyeing and fastness properties (Gulrajani *et al.*, 2001; Ra¨isa¨nen *et al.*, 2001), only little information is available in the literature concerning: variations among different crops of the same plant, reproducibility of dyeing, simple techniques to analyze and standardize a given plant material.

Standardization of the plant material requires the elaboration of methods to evaluate the properties of a certain batch of plant material with regard to particular dyeing properties (color strength and shade). Such procedures will be based on analytical methods which depend on the type of plant material. In an ideal case the characterization of the extract permits the manufacturer of the natural dyestuff production to adjust a certain batch of material to a desired standardized color strength and shade, analogous to the standardization of a synthetic dyestuff during the finishing of dyestuff. A set of simple analytical procedures and variations in application of the dyes were compared with regard to a possible correlation of the

dyeing results. Different photometric methods, including an analysis of the total phenolic contents (TPC) extracted from the material, were studied to predict the shade and color strength (Slinkard *et al.,* 1977; Waterhouse *et al.,* 2001). In addition, coloring fruit or vegetable extracts may contain additional ingredients of nutritional value as recently demonstrated for cactus fruits (Butera *et al*., 2002; Galati *et al*., 2003; Schieber and Carle, 2001; Stintzing, 2001).

2.3 Solvent Extraction

Solvent extraction is usually used to recover a component from either a solid or liquid. The sample is contacted with a solvent that will dissolve the solutes of interest. Solvent extraction is of major commercial importance to the chemical and biochemical industries, as it is often the most efficient method of separation of valuable products from complex feedstock or reaction products. Some extraction techniques in involve partition between two immiscible liquid, others involve either continuous extractions or batch extractions. Because of environmental concerns, many common liquid/liquid processes have been modified to either utilize benign solvents, or move to more frugal processes such as solid phase extraction. The solvent can be a vapor, supercritical fluid, or liquid, and the sample can be a gas, liquid or solid. There are a wide range of techniques used, and details can be found in Organic Vogel, Perry, and most textbooks on unit operations.

There are various type of separation process such as distillation, drying, adsorption, filtration and extraction (Geankoplis, 2003). Extraction is one of the process that being use to extract the color from pitaya peels. This is because there are found chemical compound inside the mixture in solid phase. Then, to get the compound inside the mixture in solid phase material, it needs solvent as a medium to get the natural dye in the pitaya peels.

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n-Hexane is one of the solvent that can be use as a solvent extraction. This solvent has low boiling point which is 69ºC. It usually use as solvent because it can prevent the chemical compound from damage or overheated. Extraction produces high quality of natural dye which solvents that being use such as hexane, acetone, isopropanol, and ethanol was able to extract color from pitaya peel effectively. Table 2.1 shows the example of solvents extraction in food and fruit.

Table 2.1: Examples of solvents extraction

No	Description	Reference
1	Deactivation of sunflower oil by solvent extraction.	Mohsen *et al*., 2008
2	Synthesis of Perrhenic Acid using solvent extraction.	Katarzyna *et al*., 2008
3	Residue of grape (*Vitis vinifera L.)* seed oil production as a valuable source of phenolic antioxidants.	Thorsten *et al*., 2009
4	Total phenolic contents and antioxidant activity of corn tassel extracts.	Sobhy *et al*., 2009
5	Extractability of African yam bean (*Sphenostylis stenocarpa*) protein in acid, salt and alkaline aqueous media.	Eromosele *et al*., 2008
6	Extraction optimization of watermelon seed protein using response surface methodology.	Ali *et al*., 2008
7	Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used.	Gordana *et al.,* 2008
8	Extraction of natural antioxidants from hazelnut (*Corylus avellana L.*) shell and skin wastes by long maceration at room temperature.	Marina *et al*., 2008

2.4 Ultrasonic Cleaning

An ultrasonic cleaner, often colloquially referred to as a sonicator, is a cleaning device that uses ultrasound (usually from 15 to 400 kHz) and an appropriate cleaning solution to clean delicate items. The ultrasound is not effective without the cleaning solution; it enhances the effect of a solution appropriate for the item to be cleaned and the soiling.

In an ultrasonic cleaner, the object to be cleaned is placed in a chamber containing a suitable ultrasound conducting fluid (an aqueous or organic solvent, depending on the application). In aqueous cleaners, the chemical added is a surfactant which breaks down the surface tension of the water base. An ultrasound generating transducer built into the chamber, or lowered into the fluid, produces ultrasonic waves in the fluid by changing size in concert with an electrical signal oscillating at ultrasonic frequency. This creates compression waves in the liquid of the tank which ‘tear’ the liquid apart, leaving behind many millions of microscopic ‘voids’ or ‘partial vacuum bubbles’. These bubbles collapse with enormous energy; temperatures of 10,000 K and pressures of 50,000 lbs per square inch have been reported; however, they are so small that they do no more than clean and remove surface dirt and contaminants. The higher the frequency, the smaller the nodes between the cavitations points, which allows for cleaning of more intricate detail.

Ultrasonic transducers showing 20 kHz and 40 kHz stacks. The active elements (near the top) are two rings of lead zirconate titanate, which are bolted to an aluminium coupling horn.

Transducers are usually piezoelectric material (for example, lead zirconate titanate or barium titanate), and sometimes magnetostrictive (made of a material such as nickel or ferrite). The often harsh chemicals used as cleaners in many industries are not needed, or used in much lower concentrations, with ultrasonic agitation.

Ultrasonic are used for industrial cleaning, and also used in many medical and dental techniques and industrial processes.

Industrial ultrasonic cleaners are used in the automotive, sporting, printing, marine, medical, pharmaceutical, electroplating, disk drive components, engineering and weapons industries. Cleaners are also used to experimentally determine the elastic constants of many anisotropic materials. Ultrasonic waves can usually only be sent through a material at right angles to the material's surface (normal incidence). In water the angle of incidence for a longitunal wave can be set, inducing both longitudinal and transverse waves in the material. Then by measuring the time of flight for both waves, the elastic constants can be determined. Suitable materials for ultrasonic cleaning are stainless and mild steel, aluminium, copper, brass and other alloys, wood, plastics, rubber, and cloth. Figure 2.3 below show the ultrasonic cleaning in FKASA lab.

Figure 2.3 Ultrasonic cleaning

2.5 Rotary Evaporator

A rotary evaporator (or rotavap) is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. When referenced in the chemistry research literature, description of the use of this technique and equipment may include the phrase "rotary evaporator", though use is often rather signaled by other language (for example, the sample was evaporated under reduced pressure).

A simple rotary evaporator system was invented by Lyman (1950). It was first commercialized by the Swiss company Büchi in 1957, and patented in 1964. The Büchi Rotavapor continues to be the most widely used rotary evaporator, so much so that "Rotavap" has become a synonym for such instruments. Other rotary evaporator manufacturers include Heidolph, Yamato, IKA, Stuart and EYELA. The most common form is the bench-top unit, though large scale (20 to 50L) versions are available and are used in pilot plants in commercial chemical operations.

2.5.1 Main Component in Rotary Evaporator

The main components of a modern rotary evaporator are: a motor unit which rotates the evaporation flask or vial containing ones sample, a vapor duct which acts both as the axis for sample rotation, and as vacuum-tight conduit for the vapor being drawn off of the sample, a vacuum system, to substantially reduce the pressure within the evaporator system, a heated fluid bath, generally water, to heat the sample being evaporated, a condenser with either a coil through which coolant passes, or a "cold finger" into which coolant mixtures like dry ice and acetone are placed, a condensate collecting flask at the bottom of the condenser, to catch the distilling

solvent after it re-condenses; and a mechanical or motorized mechanism to quickly lift the evaporation flask from the heating bath.

2.5.2 Function of Component in Rotary Evaporator

The vacuum system used with rotary evaporators can be as simple as a water aspirator with a trap immersed in a cold bath (for non-toxic solvents), or as complex as a regulated mechanical vacuum pump with refrigerated trap. Glassware used in the vapor stream and condenser can be simple or complex, depending upon the goals of the evaporation, and any propensities the dissolved compounds might give to the mixture. Various commercial instruments are available that include the basic features, and various designs of traps are manufactured to insert between the evaporation flask and the vapor duct. In addition, modern equipment often adds features such as digital control of vacuum, digital display of temperature and rotational speed, and even vapors temperature sensing.

Vacuum evaporators as a class function because lowering the pressure above a bulk liquid lowers the boiling points of the component liquids in it. Generally, the component liquids of interest in applications of rotary evaporation are research solvents that one desires to remove from a sample after an extraction, for instance, following product isolation or a step in an organic synthesis. Therefore allows liquid solvents to be removed without excessive heating of what are often complex and sensitive solvent-solute combinations.

Rotary evaporation is most often and conveniently applied to separate "low boiling" solvents such as n-hexane or ethyl acetate from compounds which are liquid at room temperature and pressure. However, careful application also allows removal of a solvent from a sample containing a liquid compound if there is minimal co-

evaporation, and a sufficient difference in boiling points at the chosen temperature and reduced pressure.

Solvents with higher boiling points such as water (100°C at standard atmospheric pressure, 760 torr), dimethylformamide (DMF, 153°C at standard atmospheric pressure, 760 torr), or dimethyl sulfoxide (DMSO, 189°C at 760 torr), can also be evaporated if the unit's vacuum system is capable of sufficiently low pressure. However, more recent developments are often applied in these cases evaporation while centrifuging or vortexing at high speeds. Rotary evaporation for high boiling hydrogen bond-forming solvents such as water is often a last recourse, as other evaporation methods or freeze-dry are available. This is partly due to the fact that in such solvents, the tendency to "bump" is accentuated. The modern centrifugal evaporation technologies are particularly useful when one has many samples to do in parallel, as in medium to high throughput synthesis now expanding in industry and academia.

2.5.3 Advantages of using Rotary Evaporator

Evaporation under vacuum can also, in principle, be performed using standard organic distillation glassware without rotation of the sample. The key advantages in use of a rotary evaporator are that the centrifugal force and the frictional force between the wall of the rotating flask and the liquid sample result in the formation of a thin film of warm solvent being spread over a large surface, and the forces created by the rotation suppress violent, unpredicted boiling ("bumping"). The combination of these characteristics and the conveniences built into modern rotary evaporators allow for quick, gentle evaporation of solvents from most samples, even in the hands of even relatively inexperienced users. Solvent remaining after rotary evaporation can be removed by exposing the sample to even deeper vacuum, on a more tightly sealed vacuum system, at ambient or higher temperature.

2.5.4 Disadvantages of using Rotary Evaporator

A key disadvantage in rotary evaporations, besides its single sample nature, is the potential of some sample types to bump, for example ethanol and water, which can result in loss of a portion of the material intended to be retained. Even professionals experience periodic mishaps during evaporation, especially bumping, though experienced users become aware of the propensity of some mixtures to bump or foam, and apply precautions that help to avoid most such events. In particular, bumping can often be prevented by taking homogeneous phases into the evaporation, by carefully regulating the strength of the vacuum (or the bath temperature) to provide for an even rate of evaporation, or, in rare cases, through use of added agents such as boiling chips (to make the nucleation step of evaporation more uniform). Rotary evaporators can also be equipped with further special traps and condenser arrays that are best suited to particular difficult sample types, including those with the tendency to foam or bump.

There are dangers associated even with simple operations such as evaporation. These include implosions resulting from use of glassware that contains flaws, such as star-cracks. Explosions may occur from concentrating unstable impurities during evaporation. This can also occur when taking certain unstable compounds, such as organic azides and acetylides, nitro-containing compounds, molecules with strain energy to dryness. Figure 2.4 below show the rotary evaporator in FKKSA lab.

Figure 2.4 Rotary Evaporator

2.6 Centrifugation

A centrifuge is a piece of equipment, generally driven by motor, that puts an object in rotation around fixed axis, applying a force perpendicular to the axis. The centrifuge works using the sedimentation principle, where the centripetal acceleration is used to evenly distribute substances (usually present in a solution for small scale applications) of greater and lesser density. There are many different kinds of centrifuges, including those for very specific purposes. It can be used for viable counts, when shaking the culture.

Protocols for centrifugation typically specify the amount of acceleration to be applied to the sample, rather than specifying a rotational speed such as revolutions

per minute. The acceleration is often quoted in multiples of *g*, the standard acceleration due to gravity at the Earth's surface. This distinction is important because two rotors with different diameters running at the same rotational speed will subject samples to different accelerations. The acceleration can be calculated as the product of the radius and the square of the angular velocity. Figure below show refrigerated centrifuge in the FKKSA lab.

Figure 2.5 Refrigerated Centrifuge

2.6.1 Types of Centrifuge

There are at least five types of centrifuge:

(i) Tabletop/clinical/desktop centrifuge or microcentrifuge

(ii) High-speed centrifuge

(iii) Cooling centrifuge

(iv) Ultracentrifuge

(v) Geotechnical centrifuge

2.6.2 Types of Separation Centrifuge

Industrial centrifuges may otherwise be classified according to the type of separation of the high density fraction from the low density.

2.6.2.1 Screen Centrifuge

Centrifugal acceleration allows the liquid to pass through a screen of some sort, through which the solids cannot pass by due to granulometry larger than the screen gap or due to agglomeration. Common types are pusher centrifuges and Peeler centrifuges.

2.6.2.2 Decanter Centrifuge

There is no physical separation between the solid and liquid phase, rather an accelerated settling due to centrifugal acceleration. Common types are solid bowl centrifuges and conical plate centrifuges.