ULTRASONIC EXTRACTION OF ANTIOXIDANT COMPOUND FROM RED PITAYA

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I declare that this thesis entitled "ultrasonic extraction of antioxidant compound from red pitaya" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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ABSTRACT

The health benefit of antioxidant's activities from the betacyanin contents in red Pitaya, attract a lot of interest from the public, food as well as pharmaceutical industries. This natural antioxidant from red Pitaya can replace the dangerous synthetic antioxidant at the market. The works done in this research explore the effects of different process parameter on antioxidant yield via ultrasonic extraction method. The extracted oil was recovered using rotary evaporator and the composition of vitamin C was determined using HPLC. The parameter studied were different solid to solvent ratio (10 gram dried red Pitaya with 30 ml ethanol, 10 gram dried red Pitaya with 50 ml ethanol, 10 gram dried red Pitaya 200 ml ethanol, 10 red Pitaya with 250 ml ethanol), different ultrasonic power (150 watt, 200 watt, 250 watt, 300 watt), and different sonification time (20 minute, 30 minute, 50 minute, 60 minute). For the control sample, sample has been put into solvent without ultrasonic extraction. The analysis show that there is no antioxidant has been detected during the experiment. Results demonstrate that the highest value of vitamin C in first parameter is 6.16380 mg/L at 10 gram of dried red Pitaya with 250 ml ethanol. Besides, the vitamin C recovery was 6.88542 mg/L obtained by using second parameter with 10 gram of dried red Pitaya and 250 ml ethanol. The best yield of vitamin C obtained was 7.13589 mg/L by using both of the best parameter which were (state what is the parameter) with different sonification time for about 50 minutes of ultrasonic extraction at 10 gram sample with 50 solvent and 300 watt.

ABSTRAK

Faedah kesihatan bagi antioksida iaitu betacynin dalam pitaya merah telah menarik minat orang awam, industri makanan termasuk industri produk kecantikan dan kesihatan. Antioksida dari sumber asli yang terjamin selamat dapat menggantikan antioksida tiruan(sintetik) yang lebih bahaya kepada pengguna. Eksperimen telah dijalankan dalam mengekplorasi parameter berbeza bagi mendapatkan hasil yang paling optimum dalam kaedah pengekstrak ultrasonic, hasil minyak ekstrak diperolehi menggunakan alat putaran pengewapan (rotary evaporator) dan kandungan vitamin C diuji menggunakan alat HPLC. Untuk sampel kawalan, sampel telah direndam di dalam etanol tanpa melalui pengekstrakan ultrasonik. Analisis menunjukkan bahawa tiada vitamin diperolehi melalui kaedah ini. Parameter yang digunakan ialah nisbah pepejal kepada pelarut yang berbeza (10 gram pitaya merah kering kepada 30 ml etanol, 10 gram pitaya merah kepada 50 ml etanol, 10 gram pitaya merah kepada 200 ml etanol, 10 gram pitaya merah 250 ml), kuasa ultrasonik yang berbeza (150 watt, 200 watt, 250 watt, 300 watt), dan perbezaan masa pengekstrakan ultrasonik (20 minit, 30 minit, 50 minit , 60 minit) telah digunakan. Nilai vitamin C dalam hasil parameter pertama ialah 6.16380 mg/L. Seterusnya, hasil ini iaitu 10 gram pitaya merah kering dengan 250 ml etanol telah digunakan untuk parameter kedua dan nilai vitamin C telah diperolehi ialah 6.88542 mg/L. Hasil terbaik dari kedua-dua parameter telah digunakan di dalam eksperiment ketiga, hasil yang telah diperolehi iaitu untuk eksperiment ini ialah 7.13589 mg/L pada 50 minit masa pengekstrakan , 250 ml etanol nisbah kepada 10 gram sampel dan 300 watt kuasa ultrasonik

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HPLC	-	High performance liquid chromatography
ORAC	-	Oxygen Radical Absorbance Capacity
^{o} C	-	Degree Celsius
min	-	Minute
ppm	-	Part per Million
kHz	-	Kilo Hertz

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Red Pitaya (*Hylocereus undatus*) is a species of Cactus. In Malaysia, it's commonly known as dragon fruit and usually consumed as juice and dessert. Red Pitaya is grown commercially in many home gardens in Malaysia. The tree is color grayish brown to black and can grow to about 2-3 meters high with characteristic branching profusely, sprawling or clambering and epidermis deep green. The fruit vary in size, shape and flavor depending on the variety. Both its skin and flesh are characterized by being a glowing, deeply red-purple color. On average, the main physicochemical characteristics of three commercial cultivars of red pitaya were assessed, including total phenolic compounds contents, total betacyanins, vitamin C and oxygen radical absorbance capacity (ORAC)(Fabrice Vaillant *et al* 2004).

Red Pitaya, as in many other fruit and vegetables, is also rich in antioxidants that help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, and inflammation and brain dysfunction. In addition, antioxidants were reported to retard ageing besides preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species. (Feskanich *et al.*, 2000; Gordon, 1996; and Halliwell, 1996)

Ultrasonic extraction has proven to be more efficient than Soxhlet (SOX) extraction. The major advantages of this method are a) the reproducibility of the technique; b) the applicability of the method to a range of sample sizes; c) the dramatic reduction in time needed to perform highly efficient extractions, and d) efficient extraction of polar organic compounds (Lee *et al*., 2001).

1.2 Objective of Research

1. To extract antioxidant compound in red pitaya (dragon fruit) by using ultrasonic extraction.

1.3 Scope of Research

In order to achieve the objective, scopes of the research have been identified as follows:

1. To extract antioxidant compound without sonication.

2. To study effect of the different solvent to sample ratio.
   i. 30 ml ethanol with 10 gram sample
   ii. 50 ml ethanol with 10 gram sample
   iii. 200 ml ethanol with 10 gram sample
   iv. 250 ml ethanol with 10 gram sample

3. To study the effect of different ultrasonic power: 150, 200, 250 and 300 watt.

4. To study the effect of different sonication time: 20 , 30, 50 and 60 minutes

5. To analyze the extraction yields using GCMS/HPLC

1.4 Problem Statement

Nowadays, natural antioxidants are widely used in the pharmaceutical and food processing. Antioxidants play a significant role to prevent many several diseases like Alzheimer disease, cancer, and even aging. Natural antioxidant from fruit is safer for human body because it's come from natural condition compared with the synthetic antioxidant. (Weerasak Samee *et al*, 2008). Therefore, this research is conducted to explore a new source of antioxidant which in red pitaya.

1.5 Rational and Significant of Research

Extraction of antioxidant in red pitaya can give big impact in agricultural and food chemistry. Red pitaya is easily got in Malaysia and plant widely. Demand for antioxidant in the pharmaceutical and food processing is very high, thus makes the antioxidant processes industry very easy to get profit and market.

Extraction of antioxidant compound from natural fruits such as red Pitaya will give source of safer antioxidant. These researches focus more to choose the best parameter in producing antioxidant to fulfill increment demand for antioxidant in future. Beside that, extraction is the best method to obtain the antioxidant compound for commercial product like antioxidant supplement pill based on natural source especially red pitaya.

CHAPTER 2

LITERATURE REVIEW

2.1 Plant Material

2.1.1 Plant Description

Red Pitaya (Hylocereus undatus) is a species of Cactus. In Malaysia, it's commonly known as dragon fruit and usually consume as juice and dessert. Red Pitaya is grown commercially and in many home gardens in Malaysia. The tree is color grayish brown to black and can grow to about 2-3 meters high with characteristic branching profusely, sprawling or clambering and epidermis deep green. The fruit vary in size, shape and flavor depending on the variety. Both its skin and flesh are characterized by being a glowing, deeply red-purple color. On average, the main physicochemical characteristics of three commercial cultivars of red pitaya were assessed, including total phenolic compounds contents, total betacyanins, vitamin C and oxygen radical absorbance capacity (ORAC)(Fabrice Vaillant *et al*, 2004).

Red Pitaya, as in many other fruit and vegetables, is also rich in antioxidants that help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, and inflammation and brain dysfunction. In addition, antioxidants were reported to retard ageing (Feskanich *et al.*, 2000; Gordon, and 1996; Halliwell,

1996) besides preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species.

2.1.2 Nutrition Composition of Red Pitaya

Red Pitaya fruit has a low vitamin C content ranging from (116 to 171) µg•g-1 of fresh pulp without seeds, but it is rich in betacyanins [(0.32 to 0.41) mg•g-1] and phenolic compounds [(5.6 to 6.8) µmol Eq gallic acid•g-1]; it has a high antioxidant ORAC value of (8.8 to 11.3) µmol Eq Trolox•g-1. Visible spectra of aqueous fruit extracts were very similar to that of pure betacyanin. Indeed, the characteristic color of juice diluted to 1% presents a high hue angle (H° = 350º ± 3) and high chroma values (C* = 79 ± 2). Thermal stability of pitahaya betacyanin decreases with pH, but it remains compatible with industrial utilization as a colorant (half-time = 22.6 min at 90 °C at pH = 5 of the fruit) and was found to be very similar to that previously reported for beetroot.(Fabrice Vaillant *et al*, 2003).

2.2 Antioxidant

2.2.1 Effect of Antioxidant to Free Radicals

Antioxidants, which can inhibit or delay the oxidation of an oxidisable substrate in a chain reaction, would therefore seem to be very important in the prevention of these diseases (Ames *et al*., 1993; Aruoma, 1998; Jacob & Burri, 1996; Steinberg *et al*., 1991; Maxwell & Lip, 1997; Pratico & Delanty, 2000; and Wang *et al*., 1996). They scavenge radicals by inhibiting initiation and breaking of chain reaction, suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Shi *et al*., 2001). It is well known that reactive oxygen species (ROS) formed in vivo, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. Tissue damage resulting from an imbalance between ROS-generating and scavenging systems has been implicated in the pathogenesis of a variety of disorders, including degenerative disorders of the CNS, such as Alzheimer's disease, cancer, atherosclerosis, diabetes mellitus, hypertension, AIDS and aging (Halliwell & Gutteridge, 1998; Mantle, Eddeb, & Pickering, 2000). High consumption of fruits and vegetables has been associated with a lowered incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction and cataracts (Ames, 1983; Ames, Shiganaga, & Hagwn, 1993; Feskanich *et al*., 2000; Gordon, 1996; Haegele *et al*., 2000; Halliwell *et al*., 1996; Michels *et al*., 2000).

2.3 Ultrasonic Extractions

The most probable mechanism for ultrasonic extraction enhancement is the intensification of mass transfer and easier access of the solvent to the plant cell (M. Vinatoru, and A. Shotipruk, *et al*, 2001). The collapse of cavitations bubbles near cell walls is expected to produce cell disruption together with a good penetration of the solvent into the cells through the ultrasonic extraction (M. Toma, *et al*, 2001). In many analytical situations, ultrasound-assisted leaching is an expeditious, inexpensive and efficient alternative to conventional extraction techniques and, in some cases, even to supercritical fluid and microwave-assisted extraction (J.L. Luque-García and M.D. Luque de Castro, 2003)

2.4 Factors Affecting Extraction Process

2.4.1 Ultrasonic Power

The application of ultrasonic waves as a priming technique for accelerating and enhancing the germination of Barley Seed shows that the efficacy of ultrasonic waves on the increase of sample weight after 36 h and 44 h of soaking was investigated using cavitation levels between 20 and 100% power setting of the device. Maximum germination was achieved with sonication at 100% for 15 min. Increasing sonication intensity and irradiation time improved germination, showing an increase from only 34% germinated seeds without sonication to 91% for seeds treated for 5 min at 100% of the power setting. Seeds that were treated for 15 min achieved near complete imbibition (increase in seed weight 96% on a dry weight basis) after 44 h of steeping. In comparison, non-sonicated seeds increased seed weight by 34% after 36 h and approached complete imbibition only after 44 h, achieving a 52% seed weight increase (Maryam Yarldagard *et al*., 2008).

This research is about global prawn production has increased to over 4.2 million tons in 2001. However, accumulated waste, which is almost 40% of the total prawn mass, has become a major concern due to its slow degradation. The extraction is time consuming and many methods have been implemented with various times, temperatures and concentrations of acid and base solvents. However, all these methods have in common that they require application of large amounts of both strong acids and strong alkali at high temperatures. Application of high-intensity ultrasound during extraction may improve yield of chitin, lower the amount of solvents, reduce processing time, improve the control over the process, and enhance uniformity of the extracted material (Gunnar Kjartansson, 2006).

Beside that, the cavitation also effect to the extraction process. Its occur because of some situations like if a sound wave is impressed upon a liquid and the intensity is increased, a point will be reached where cavitation occurs. Cavitation is the formation of a gas bubble in the liquid during the rarefaction cycle. When the compression cycle occurs, the gas bubble collapses. During the collapse, tremendous pressures are produced. The pressure may be of the order of several thousand atmospheres. Thousands of these small bubbles are formed in a small volume of the liquid. It is quite generally agreed that it is cavitation that produces most of the biological, detergent, mechanical, and chemical effects in the application of high intensity sound to various mediums (file:///C|/Web-page2000/ultrasonic_information.html).

2.4.2 Solvent to Solid Ratio

Grape byproducts were subjected to an extraction process under various different experimental conditions (namely, solvent type, temperature, solvent-to-solid ratio, time contact, and raw material) in order to study the effect of these conditions on the yield of phenolic compounds and the corresponding antiradical activity of extracts. Solvent-to-solid ratio was found to have a critical role in extraction efficiency; values 1:1 (between 1:1 and 5:1) maximized the antiradical activity of phenolic extracts (PINELO Manuel *et al.*, 2005).

According to research of extraction process of oil and antioxidant substances from Rosa rubiginosa, the extraction was carried out at three different solvent-to-solid ratios during 100 h. The best results were detected for a solvent-to-solid ratio 50:1 (36% oil and 40% total solids), more than 4-times higher than those reached at 15:1 (7% oil, 9% total solids). Higher solvent- to-solid ratios promoted an increasing concentration gradient between solvent and solid to extract. As a consequence, a major mass transfer between both phases was observed. Comparing the results taken from the two extraction procedures, higher quantities of extractable were detected in shaker. Differences could be mainly explained in basis of the stirring effects (Danial Franco *et al.*, 2007).

2.4.3 Sonication Time

Traditional ultrasonic agitation, referred to as indirect ultrasonic extraction, uses water as agitation energy transportation medium and total recovery can be reached within relatively short time (usually, 45–60 min). Nevertheless, it is still time-consuming, especially for the handling of batches of samples. For the further reduce the extraction time of organic pollutants from particulate samples, a direct ultrasonic extractor, where the transducer was bonded directly to the base of the sample vessel, is proposed here. Differing from conventional USE, the proposed direct ultrasonic probe focuses the energy on the sample zone hence providing more effective cavitations in the liquid (Lee *et al*., 2000).

Based on research of extraction the ginseng saponins from ginseng roots and cultured ginseng cells. The ginseng samples were first extracted in the two types of sonicator for different periods of time in order to determine the contact time required to achieve the maximum yield of total saponin. The extraction yields increased significantly with the sonication period extended from 15 to 45 min for both types of ginseng root and in both sonicators, but increased slightly or leveled off from 60 to 120 min. The results suggest that the ultrasonic extraction period for achieving maximum yield of saponin from the ginseng roots is about 2 h (Jianyong Wu *et al*., 2000).

Thus in this research, the extraction of red pitaya is done to get the best parameter which is solvent to solid ratio, ultrasonic extraction power and extraction time.

CHAPTER 3

METHODOLOGY

3.1 Raw Material

2 kilogram of Red Pitaya is purchases from the local supermarket at Kuantan were used in this experiment. The picture of Red Pitaya is illustrated as as in Figure 3.1. The chemical used in this experiment were, ethanol with ~99% purity, ascorbic acid, Potassium di-hydrogen phosphate and methanol with HPLC grade. Ethanol was stored in dark flasks until used (Maria *et al*., 2005). Ethanol was used as solvent for extraction and every chemical ware degassed before used.

Figure 3.1:- Red Pitaya

3.2 Equipment

Four equipments were used in this research. There were oven, ultrasonic water bath, rotary evaporator, high performance liquid chromatography (HPLC). Details descriptions of equipment are provided in section 3.2.1 to 3.2.4.

3.2.1 Oven

Oven is one of the kitchen appliances that used for cooking or heating. The concept of this appliance is using electric to heat the heating medium to produce heat for cook and heat. The temperature can be set for various heating process. In this research, oven is used for drying raw material. The picture of oven illustrated as figure 3.2.

Figure 3.2: Oven

3.2.2 Ultrasonic Bath

An ultrasonic bath is sonication devices that use ultrasound with frequency at 15 kHz and the power from 150 watt to 300 watt were used in this research to extract sample. Figure 3.3 illustrates the ultrasonic bath used in this research.

(a) **(b)**

Figure3.3: - (a) Ultrasonic bath (b) Power controller

3.2.3 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography is used for analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase). The analyzed is forced through a column by liquid at high pressure, which decreases the time to separate components remained on the stationary phase and time to spread out within the column. Thus lead of broader peaks in the HPLC's results. Less time on the column then it will be translates to narrower peaks in the resulting chromatogram and better selectivity. The peak should be compared with the standard form of peak to determine the composition in the sample. Figure 3.3 illustrate HPLC ware used in this research.

Figure 3.4:- HPLC