ULTRASONIC EXTRACTION OF ANTIOXIDANT COMPOUND IN GUAVA

KHAIRUL ANWAR B. MOHAMAD SAID

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Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

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I declare that this thesis entitled "Ultrasonic Extraction of Antioxidant Compound in Guava" 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."

Signature : ……………………………
Name : Khairul Anwar b. Mohamad Said
Date : 2 May 2009

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ABSTRACT

The antioxidant such as ascorbic acid (AA) in guava juice has attracted a lot of interest from the public and herbal industries because of the antioxidant can inhibit the oxidation of body cells that can lead to health problem. The work done in this research investigates the most suitable operating parameter to extract the ascorbic acid from guava using ultrasonic extraction method. Different parameter volume of solvent which is ethanol at 30ml, 50ml, 200ml and 250ml. After determine the best solvent volume were investigated the experiments were conducted using different ultrasonic power at 150W, 200W, 250W and 300W after the determination of the best solvent volume. The sonication time was set at 20min, 30min, 50min and 60min. The extraction yields were analyzed using High Performance Liquid Chromatography system (HPLC). Result obtained revealed that 250 ml of ethanol, ultrasonic power of 300W and 20 minutes sonication time produce the highest yield of ascorbic acid. The extracted amount of ascorbic acid was 4.3150 mg/L. Therefore, these parameters can be concluded as the most optimum operating parameters in extracting ascorbic acid from guava using ultrasonic extraction method.

ABSTRAK

Antioksida seperti asid askorbik (AA) di dalam jus jambu batu telah menarik minat orang ramai dan industri herba disebabkan antioksida boleh menghalang pengoksidaan sel-sel badan yang boleh membawa kepada kemudaratan terhadap kesihatan. Eksperimen ini dijalankan bagi menyiasat parameter yang terbaik bagi mengekstrak antioksida yang terdapat di dalam buah jambu batu. Parameter yang pertama di dalam eksperimen ini ialah perbezaan isipadu pelarut iaitu etanol. Isipadu yang di kaji ialah 30ml, 50ml, 200ml dan 250ml. Selepas memperoleh parameter yang terbaik dari eksperimen pertama, sambung eksperimen kedua iaitu menggunakan perbezaan tenaga ultrasonik. Tenaga yang digunakan ialah 150W, 200W, 250W dan 300W. Apabila parameter optimum telah dijumpai, sambung eksperimen ketiga menggunakan kedua-dua parameter yang telah diperoleh itu. Eksperimen yang terakhir ialah menentukan masa ultrasonik yang terbaik. Masa ultrasonik yang dikaji ialah 20min, 30min, 50min dan 60min. Kesemua analisis telah dibuat menggunakan Kromatografi Cecair Berprestasi Tinggi (HPLC). Keputusan eksperimen menunjukkan 250 ml cecair pelarut etanol, 300W tenaga ultrasonik dan 20 minit masa ultrasonik menghasilkan askorbik asid yang terbanyak. Askorbik asid yang berjaya diekstrak keluar ialah 4.3150 mg/L. Oleh itu, parameter ini boleh disimpulkan sebagai parameter optimum bagi mengekstrak askorbik asid daripada jambu batu menggunakan teknik pengekstrakan ultrasonic.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

AA	-	Ascorbic acid
DCM	-	Dicloromethane
DUE	-	Direct ultrasonic extraction
ES	-	Extractive substances
HPLC	-	High performance liquid chromatography
IUE	-	Indirect ultrasonic extraction
SOX	-	Soxhlet

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

During this recent year, the use of fruit and vegetable juice has been increasing due to their health benefit to human beings. Fruits are rich with antioxidant that helps in lowering incidence of degenerative disease such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process (Feskanich *et al*., 2000; Gordon, 1996; Halliwell, 1996).

Guava (Psidium guajava L.), also known locally as jambu batu, is grown commercially and in many home gardens in Malaysia. The tree is very hardy and can grow to about 7-8 meters high with characteristic smooth, pale mottled bark that peels off in thin flakes. The fruits vary in size, shape and flavor depending on the variety. The better varieties are sweet while others may be astringent (Lim *et al*., 2006). Guava is generally used as health food usually in form of juice and dried product. The importances on healthy beverages such as guava drink are because of its pharmacological activity. The pharmacological activity is attributed to its phytochemical property such as asiaticoside and antioxidant.

The health benefit of guava is due to natural antioxidant and other type of vitamin that can be found in it. Guava is rich in ascorbic acid (vitamin c) at level far higher than most imported and local fruits (Lim *et al*., 2006). Setiawan et al., (2001) stated that Indonesian guava is an excellent source of provitamin A carotenoids. In

Japan, guava leaf tea is widely used to control blood sugar of diabetics. It has been shown to be effective in vitro, in mice and in human volunteer (Deguchi *et al*., 1998). Lycopene, the hydrocarbon of carotenoids that gives tomatoes their red color, is particularly effective at quenching the destructive potential of singlet oxygen (Di Mascio *et al*., 1998).

It is proof that most of phytochemical from plant contain antioxidant activity. Antioxidants are substances that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species, which include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non- radicals such as hydrogen peroxide, hypochlorous, etc (Lim *et al*., 2007). In food industry, usually antioxidant is used as food preservative to prevent the food from deterioration. The main cause for the oxidation of food is exposure to oxygen and sunlight. To avoid that, the food must be keep in dark place and seals it in the container but it will caused unpleasant taste to eat and have unappealing colors.

During these current years, there has been a substantial interest in discovering natural antioxidants from plant materials. The antioxidant phytochemicals from plants, particularly flavonoids and other polyphenols have been reported to inhibit the propagation of free radical reactions, to protect the human body from disease (Kinsella *et al.*, 1993; Terao and Piskula, 1997). In addition, the use of synthetic antioxidants has been questioned because of their toxicity (Valentao et al., 2002). Therefore, there have been numerous researches on this plant to seek for potential natural and possibly economic and effective antioxidants to replace the synthetic ones (Suganya *et al*., 2007).

Ultrasonic extraction can be defined as the method of extraction using ultrasonic wave. Extraction using ultrasonic method will not altered the sample properties and it is not a time consuming process rather than using Soxhlet that rely on heat and could altered the sample properties. The sample can destroyed if too much heat is added. Antioxidant is defined as the molecule that can slow or prevent the oxidation process of other molecules. Guava is the material for the purpose of extraction.

Ultrasonic extraction has proven to be equally or 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). In this research, the main objective of the study was to determine the best operating parameter for extraction of antioxidant compound from guava via ultrasonic extraction method.

1.2 Problem Statement

Antioxidants help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, inflammation and brain dysfunction. In addition, antioxidants were reported to retard ageing (Feskanich *et al*., 2000; Gordon, 1996; Halliwell, 1996) besides preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species. 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). Nowadays, the demand of antioxidant is increasing dramatically. This phenomenon happens because of the goodness of antioxidant to our body to maintain a healthy life. In order to meet the demand that keep increasing, a research need to be done in order to find the best parameter for extraction of antioxidant compound from guava. This research choose guava because it contains the highest amount of antioxidant compare to most of other import and local fruit. Extraction of antioxidant compound is very important because we can produce an antioxidant capsule for the purpose of commercial. We also can use the extract for developing a new supplement pill that based on antioxidant from guava.

1.3 Objective

The main objective of the study was to determine the best operating parameter for extraction of antioxidant compound from guava via ultrasonic extraction method.

1.4 Scope of work

For the purpose of achieving the objective, the scopes of studies are stated as below:

1. Study the effect of different solvent ratio with constant amount of sample that is 10g. The solvent amount of will be 30ml, 50 ml, 200ml, and 250ml.
2. Study the effect of different ultrasonic power (150W, 200W, 250W and 300W). The best solvent ratio obtained from no 2 will be used.
3. Study the effect of different sonication time on the extraction of antioxidant. The experiment will be done at 20, 30, 50 and 60 minute.
4. To analyze the extraction yields using High Performance Liquid Chromatography (HPLC).

CHAPTER 2

LITERATURE REVIEW

2.1 Guava (*Psidium Guajava L.)*

2.1.1 Plant Description

Psidium Guajava Linn. (family Myrtaceae), is commonly called guave, goyave or goyavier in French; guave, Guavenbaum, Guayave in German; banjiro in Japanese; goiaba, goiabeiro in Portugal; arac¸ ´a-goiaba, arac¸ ´a-guac¸ ´u, guaiaba in Brazil; guayaba, guayabo in Espa˜nol and guava in English (Killion, 2000). It grows in all the tropical and subtropical areas of the world, adapts to different climatic conditions but prefers dry climates (Stone, 1970). Psidium guajava is a small tree which is 10m high with thin, smooth, patchy, peeling bark. Leaves are opposite, short-petiolate, the blade oval with prominent pinnate veins, 5–15 cm long. Flowers are somewhat showy; petals whitish up to 2 cm long, stamens numerous (Stone, 1970).

2.1.2 Medicinal Application

More recent ethnopharmacological studies show that Psidium guajava is used in many parts of the world for the treatment of a number of diseases, e.g. as an anti-inflammatory, for diabetes, hypertension, caries, wounds, pain relief and reducing fever

(Table 1). Some of the countries with a long history of traditional medicinal use of guava include Mexico and other Central American countries including the Caribbean, Africa and Asia. Some of these uses will be outlined here in table 2.1. Table 2.1 shows the Ethnomedical uses of Psidium Guajava (Rosa *et al.,* 2008)

Table 2.1 : Ethnomedical uses of Psidium Guajava(Rosa *et al.,* 2008)

Place, country	**Part (s) used**	**Ethnomedical uses**	**Preparation(s)**	**Reference(s)**
Colombia, Mexico	Leaves	Gastroenteritis, diarrhoea, dysentery, rheumatic pain, wounds, ulcers, and toothache	Decoction and poultice	Heinrich et al. (1998),Aguilar et al. (1994)
Indigenous Maya, Nahuatl,	Leaves	Cough, diarrhoea	Decoction or infusion	Heinrich et al. (1998), Leontiet al. (2001)
Latin America, Mozambique	Leaves	Diarrhoea, stomach ache	Infusion or decoction	Pontikis (1996)
Mexico	Shoots, leaves, and leaves mixed, ripbark fruits	Febrifuge, expel the placenta after childbirth, cold, cough hypoglycaemic, affections of the skin, caries, vaginal haemorrhage, wounds, fever, dehydration,	Decoction, poultice	Mart´ınez and Barajas (1991), Argueta et al. (1994), Linares and Bye (1990), Leonti et al.(2001), Heinrich et al. (1998)

Place, country	Part (s) used	Ethnomedical uses	Preparation(s)	Reference(s)
South Africa	Leaves	Diabetes mellitus, hypertension	Infusion or decoction	Oh et al. (2005), Ojewole(2005)

2.2 Nutrient Composition in Guava

On average, the fruit contains 74–87% moisture, 13–26% dry matter, 0.5–1% ash, 0.4 0.7% fat and 0.8–1.5% protein (Chin and Yong, 1980). It is rich in ascorbic acid (vitamin C), at levels far higher than most imported and local fruits (Lim *et al.,* 2006). Table 2.2 shows the AEAC of 27 selected fruits using ABTS decolorization assay and their ascorbic acid content (Leong and Shui., 2001).

Table 2.2 : AEAC of 27 selected fruits using ABTS decolorization assay and their ascorbic acid content (Leong and Shui., 2001)

Variety	AEAC (mg/100g)	AA content byHPLC (mg/100g)	Percentage contribution of AEAC by AA (%)	Source	Classification by AEAC
Ciku	3396±387.9	2.0±0.7	0.06	Malaysia	Extremely high
Strawberry	472±92.9	53.9±11.2	11.4	Australia	
Plum	312±23.2	8.2±2.3	2.6	USA	
Star fruit	278±22.3	5.9±1.8	2.1	Malaysia	High
Guava	270±18.8	131±18.2	48.3	Thailand	
Grape seedless	264±83.6	0.5±0.2	0.2	USA	
Salak	260±32.5	2.4±1.5	0.9	Malaysia	
Mangosteen	150±23.3	4.1±1.2	2.7	Malaysia	
Avocado	143±16.5	9.0±2.1	6.3	Thailand	
Orange	142±22.6	36.1±15.9	25.5	Australia	
Solo papaya	141±26.7	67.8±12.6	48	Malaysia	

Variety	AEAC (mg/100g)	AA content byHPLC (mg/100g)	Percentage contribution of AEAC by AA (%)	Source	Classification by AEAC
Mango	139±21.5	19.7±9.1	14.2	Philippines	
Kiwi fruit	136±18.2	52.8±22.5	38.7	New Zealand	
Cempedak	126±19.1	6.2±0.9	4.83	Malaysia	Medium
Pomelo	104±34.7	36±7.5	34.7	Malaysia	

The fruit, in particular the pink flesh cultivar without doubt has a fair amount of vitamin A (beta-carotene). There also some vitamin B such as thiamin (B1), riboflavin (B2), niacin and pantothenic acid are found in the fruit. In addition, it also contains a fair amount of phosphorous, calcium, iron, potassium and sodium (Lim and Khoo, 1990).

These are characterized by a low content of carbohydrates (13.2%), fats (0.53%), and proteins (0.88%) and by high water content (84.9%), (Medina and Pagano, 2003). Food value per 100 g is: Calories 36–50 kcal, moisture 77–86 g, crude fibre 2.8–5.5 g, ash 0.43–0.7 g, calcium 9.1–17 mg, phosphorus (Conway, 2002), 17.8–30 mg, iron 0.30–0.70 mg (Iwu, 1993), vitamin A 200–400 I.U., thiamine 0.046 mg, riboflavin 0.03–0.04 mg, niacin 0.6–1.068 mg, ascorbic acid 100 mg, vitamin B3 40 I.U. (Fujita *et al.*, 1985; Hernandez, 1971; Conway, 2002). Manganese is also present in the plant in combination with phosphoric, oxalic and malic acids (Nadkarni and Nadkarni, 1999).

2.3 Antioxidant Activity in Guava

Guava, as in many other fruits and vegetables, is also rich in antioxidants that help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, inflammation and brain dysfunction. In addition, antioxidants were reported to retard ageing (Feskanich *et al*., 2000; Gordon, 1996; Halliwell, 1996) besides preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species. These include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl, and non radicals such as hydrogen peroxide and hypochlorous acid.

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). Among the most abundant antioxidants in fruits are polyphenols and ascorbic acid. The polyphenols, most of which are flavonoids, are present mainly in ester and glycoside forms (Fleuriet and Macheix, 2003). In the case of guava, free elagic acid and glycosides of myricetin and apigenin are found to be present (Koo and Mohamed, 2001).

2.4 What is Ultrasonic

Ultrasonic is defined as that band above 20 kHz. It continues up into the MHz range and finally, at around 1 GHz, goes over into what is conventionally called the hypersonic regime. Ultrasonic happens in a very broad range of disciplines, covering chemistry, physics, engineering, biology, et cetera. Nearly all these applications are based on two unique features of ultrasonic waves:

1. Ultrasonic waves travel slowly about 100000 times slower than electromagnetic waves. This provides a way to display information in time, create variable delay, etc.

2. Ultrasonic waves can easily penetrate opaque materials, where as many other types of radiation such as visible light cannot. Since ultrasonic wave sources are inexpensive, sensitive and reliable, this provides a highly desirable way to probe and image the interior of opaque objects. (David and Cheeke, 2002)

2.5 Type of Extraction Method

There are several methods that feasible for extraction purposes such as indirect ultrasonic extraction (IUE), Soxhlet (SOX) extraction and direct ultrasonic extraction (DUE).

2.5.1 Direct Ultrasonic Method

The direct ultrasonic extraction (DUE) was conducted using two different polar extractants under optimized conditions: 50 W ultrasonic power and total 15 min extraction duration. It has been proved that the extraction of polar and non-polar components by the DUE technique with dichloromethane (DCM) and dichloromethane/methane (DCM) results in comparable or slightly higher recoveries than by SOX extraction. The significant advantages of DUE over SOX include the saving of extraction time and solvent because DUE only use 100ml solvent compare to SOX that use 250-500ml solvent (Lee *et al*., 2001). Both IUE and DUE are more efficient for the extraction of various polar organics. Compared to conventional IUE, DUE can be performed within 15 min, which is an advantage over the IUE method (Zheng *et al*., 1997; Cheng *et al*., 1996; Abas *et al*., 1995; Marvin *et al*., 1992). Moreover, only 50 W power is needed for the DUE.

2.5.2 Soxhlet Extraction

SOX extraction is one of the oldest and most widely used approaches for conventional extraction of solid samples. The advantages of this method are: (a) the sample phase is always in contact with fresh solvent, thereby enhancing the displacement of the target compounds from the matrix and (b) the compounds are not decomposed due to moderate extraction conditions. The drawbacks of this technique are that the water content in the sample can affect the extraction efficiency and a long extraction time is needed (usually 8 h or more) (Lee *et al*., 2001).

2.5.3 Indirect Ultrasonic extraction

Ultrasonic extraction (IUE) can be used for both liquid and solid samples, and for the extraction of either inorganic or organic compounds (Harper *et al*., 1983). IUE is fast, inexpensive, and efficient alternative to conventional Soxhlet processes (Munoz *et al*., 2006). It is expected that the use of IUE for sample preparation purposes in environmental analytical chemistry will become more widespread, owing to its simplicity, ease of use, speed, and enhanced safety when compared with other, more traditional sample preparation procedures (Ashley, 1998). Ultrasonic extraction has proven to be equally or more efficient than 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. 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) (Lee *et al*., 2000). Table 2.3 shows the comparison of the relative advantages of three methods (Lee *et al*., 2001).

Table 2.3 : Comparison of the relative advantages of three methods (Lee *et al*., 2001).

Method	Total extract. Time	Solvent type	Solvent volume (mL)	Method comments
SOX	16-24 hr	various solvent	250-500	reflux heater needed; automation available; room temperature extraction
DUE	15 min	DCM (dichloromethane)	100	no heater required; easy-assembling; automation available;high extraction temperature; low noise (50 W)
	15 min	DCM/methanol	100	
IUE	45 min	DCM	150	no heater required; commercial available; low extraction temperature; high noise (200–300 W)
	80 min	DCM/methanol	200	
	60 min	DCM	400	
	60 min	DCM	600	

2.6 Factor Affecting Ultrasonic Extraction Efficiencies

Several factors that can affect the efficiencies of ultrasonic extraction are ultrasonic power, solvent, amount of sample, ratio of solvent to sample and sonication time.

2.6.1 Ultrasonic Power

Xiuli *et al*., 2008 stated that ultrasonic power of 150 W, 40 kHz was the optimum power for extraction of flavonoids. Flavonoid is one type from many type of antioxidant. Figure 2.1 shows the extraction yields obtained at different power (Xiuli *et al*., 2008).