COMPARATIVE STUDY ON THE EXTRACTION OF β-CAROTENE IN THE FLESH AND PEEL OF PAPAYA

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Date : 30 APRIL 2010

COMPARATIVE STUDY ON THE EXTRACTION OF β-CAROTENE IN THE FLESH AND PEEL OF PAPAYA

MOHD RAZMAN BIN FADZIL

A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
University Malaysia Pahang

APRIL 2010

I declare that this thesis entitled “Comparative study on the extraction of β-carotene in the flesh and peel of papaya” 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 objective of this study was to compare and optimize the extraction conditions for β-carotene compounds from flesh and peel of papaya (*Carica* papaya) using extraction process and HPLC analysis also to determine mineral content and microbe affected. Extraction is a process to obtaining something from mixture or compound by chemical or physical methods. Liquid-liquid extraction is used to extract and purify carotenoids from flesh and peel of Papaya and then this sample will be analysis in High Performance Liquid Chromatographer (HPLC).Several parameter was applied to determine the effects of acetone as a solvent, acetone ratio/ concentration (%), extraction temperature (°C), and extraction time (minutes) on β-carotene content from flesh and peel of papaya (*Carica* papaya). The independent variables were coded at five levels and their actual values were selected based on the results of single factor experiments. Results showed that acetone concentration was the most significant factor affecting the concentration of β-carotene. The optimum extraction conditions were found to be acetone concentration of 60%, extraction temperature of 40°C, and extraction time of 60 minutes. Under the optimized conditions, the experimental value for β-carotene content was ($3.45x10^{-5}$ mg/L) for peel and ($3.01x10^{-5}$) mg/L for flesh of papaya

ABSTRAK

Tujuan kajian ini adalah untuk membandingkan dan menentukan nilai kandungan molekul β-carotene dalam keadaan yang optimum dari isi dan kulit daripada buah betik dengan menggunakan proses pengekstrakan dan menganalisis menggunakan alat HPLC dan juga menentukan nilai kandungan mineral. Dari aspek bioteknologi, kajian ini juga mengenalpasti kesan dan tindakbalas hidupan organisma merbahaya seperti bakteria dapat hidup atau sebaliknya. Pengekstrakan ini adalah satu proses penyerapan molekul-molekul tertentu berdasarkan sifat kimia dan fizikal sesuatu molekul dalam larutan kimia.kajian ini adalah salah satu cara mengekstrak molekul β-carotene dan secara langsung dapat menentukan nilai kepekatan molekul β-carotene antara isi dan kulit buah betik. Beberapa parameter telah digunakan dalam kajian ini bagi menentukan kesan optimum terhadap jenis larutan yg digunakan, nisbah larutan dan sample, kesan terhadap masa dan kesan terhadap suhu. Pembolehubah ini direkodkan pada 5 tahap dan nilai ini dipilih berdasarkan daripada setiap keputusan kajian dan ini mendapati larutan acetone memberikan nilai paling terbaik iaitu 60% pada bersuhu 40°C dan pada masa 60 minit. Dibawah keadaan optimum ini, nilai kepekatan molekul β-carotene pada kulit dan isi 3.45×10^{-5} mg/L dan 3.01×10^{-5} mg/L masing-masing

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A	—	Absorbance
ε	—	Molar absorptivity
λ	—	Wavelength
c	—	Concentration
∫	—	Length through the sample

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cultivated papaya, *Carica papaya* L., sometimes known as paw paw (or papaw), is a fast-growing tree-like herbaceous plant in the family Caricaceae. In Australia, red and pink fleshed cultivars are often known as 'papaya' to distinguish them from the yellow-fleshed fruits, known as 'paw paw', but both of these common names refer to the same plant species. Irrespective of its flesh colour, *C. papaya* is generally known as 'papaya' in other countries. In some areas, an unrelated plant, *Asiminia triloba* (Annonaceae), native to north America, is also called paw paw.

Until recently, the Caricaceae was thought to comprise 31 species in three genera (namely Carica, Jacaratia and Jarilla) from tropical America and one genus, Cylicomorpha, from equatorial Africa (Nakasone & Paull 1998). However, a recent taxonomic revision proposed that some species formerly assigned to Carica were more appropriately classified in the genus Vasconcella (Badillo 2002). Accordingly, the family's classification has been revised to comprise Cylicomorpha and five South and Central American genera (Carica, Jacaratia, Jarilla, Horovitzia and Vasconcella) (Badillo 1971), with Carica papaya the only species within the genus Carica (Badillo 2002).

Although opinions differ on the origin of *C. papaya* in tropical America (see Garrett 1995), it is likely that *C. papaya* originates from the lowlands of eastern Central America, from Mexico to Panama (Nakasone & Paull 1998). Its seeds were

distributed to the Carribean and south-east Asia during Spanish exploration in the 16th Century, from where it spread rapidly to India, the Pacific and Africa (Villegas 1997).

Figure 1 β-carotene in Papaya

β-carotene is the compound responsible for orange colour in papaya, carrot and other fruits, and it is also used as a colour ingredient in many food formulations. A great interest has recently been focused on β-carotene due to its preventive activity against several pathologies, such as cardiovascular disease, hepatic fibrogenesis, solar light induced erythema, human papillomavirus persistence and some cancer types, such as prostate, gastrointestinal and epithelial. β-carotene has also been recently reported to play a role in lung function as well as in foetal growth. Finally, it is also important to consider the synergic action of carotenoids with other bioactive compounds present in fruits and vegetables.

Carotenoid analysis in food products may be carried out by different methods: HPLC, or colour evaluation. Although spectrophotometry or colorimetry can be used to rapidly assess the β-carotene content of products derived from papayas, a highly versatile, sensitive and selective method such as HPLC is needed for reliable analysis of food samples. HPLC analysis of carotenoids is usually done with C18 or C30 RP-columns, operated with isocratic or gradient elution with a wide variety of mixtures of different organic solvents as mobile phases, using UV–vis (450 nm) or photodiode array or MS detection. Heating the column is sometimes

used to improve pigment separation as well as to standardize the separation conditions. For the extraction of carotenoids from the samples, different systems can be used, like liquid–liquid extraction, solid phase extraction or supercritical fluid extraction.

AOAC (1993) recommends methanol/tetrahydrofuran (THF) (50:50 v/v) for extracting the carotenoids, while other sources use ethyl acetate (100%) or different mixtures of ethanol/hexane, acetone/ethanol/hexane, ethyl acetate/hexane or acetone/hexane.

The instability of β-carotene during processes of extraction, handling, and elimination of organic solvents makes the preparation of a sample for analysis an extremely delicate task, often requiring successive and complex procedures to ensure that all the carotenoids are extracted. Besides, not all the analytical methods available for carotenoid analysis in food products are suitable for β-carotene rich foods due to the low solubility of β-carotene in some of the solvents employed as in the case of methanol and due to the fact that the use of other solvents may interfere with the mobile phases applied for carotenoid separation. There are many HPLC methods that can be applied to the determination of carotenoids.

However, this kind of compounds needs a very careful and tedious manipulation due to their chemical lability. Therefore the development of new methodologies of extraction/separation is of relevance and the necessity for a reliable and rapid analysis method for β-carotene in vegetable products has been recognized. This work is aimed at solving the problems above mentioned with a quite simple preparation of the samples, the selection of extraction solvent mixtures more compatible with mobile phase, and short run times, by developing a suitable, reliable, rapid and simple HPLC method for β-carotene analysis and its compare it with a reference spectrophotometric method.

West Macedonia and Epirus regions located in Northwestern Greece have a mild and rainy climate in spring and autumn, providing nearly ideal conditions for fungal growth, with temperatures ranging between 8 and 25 1C.Driven by the growing interest in natural, tropical and ethnic foods, the market in Greece for wild edible papaya is expanding, offering larger varieties of papaya to consumers. In addition, papaya consumption has increased during recent years, due to their delicate flavor and texture as well as their high content of trace minerals. Therefore, it is necessary to investigate the metal content in wild species, given the fact that many of them are known to accumulate high levels of heavy metals, such as cadmium, mercury, lead and copper (Kalaˇ c and Svoboda, 2000). These elements are known to have severe toxicological effects on human health even at very low concentrations. Several factors may affect the accumulation and concentration of trace elements and heavy metals in papaya. Concentrations of the elements are generally assumed to the species-dependent, but substrate composition is also considered to be an important factor (Kalaˇ c and Svoboda, 2000; Cocchi et al., 2006).

Preliminary FoodNet data on the incidence of food borne illness show Salmonella at the top of the overall incidence in the United States (Vugia et al., 2002). Reflecting a worldwide trend in the United States, the proportion of those Salmonella isolates that were Salmonella Enteritidis (SE) increased from 6% in 1980 to 25% in 1995 (Altekruse, Cohen, & Swerdlow, 1997). The risk of acquiring a food borne disease has increased greatly. Pathogen survival depends on many factors, including the physical and chemical characteristics of the fruit or vegetable, the post harvest processes applied and consumer handling practices (FDA/ CFSAN, 1999).

Watermelon, melon and papaya are highly popular fruits in Brazil. These fruits are low acid with an average pH above 4.5, and often served sliced in food establishments in fresh pieces in mixes for salad bars, at deli counters and as a pulp juice. Salmonella spp. can survive and grow in these fruits as described by Escartin, Ayala, and Lozano, 1989; Golden, Rhodehamel, and Kautter, 1993; Leverentz et al., 2001; Ukuku and Sapers, 2001 and Viswanathan and Kaur, 2001.

1.2 Objectives of This Study

- To extract β-carotene in the flesh and peel of papaya

1.3 Scopes of This Study

- To study the effect of solvents type in the extraction process to the sample.
- To determine the most optimum parameters that produces high yield of β-carotene in the extraction process
- To investigate mineral content in the flesh and peel of papaya

1.4 Problem Statement

- Privies researchers more focus on flesh and seed of papaya
- Lack study on comparison of mineral content in papaya in other local fruit
- The peel has potential to be converted into added value product
- The demand on β-carotene has increased significantly, with increased of consumer awareness about cancer.

In early this century, scientist found that some cancers can be avoided by β-carotene. The major sources of β-carotene came from papaya. But β-carotene also can be finding in other orange fruits such as orange and carrot. Peel and flesh of papaya containing amount of β-carotene because it is orange in color.

Every single day, we can hear many peoples dies cause of cancers. The increasing of human awareness makes the demand on β-carotene increase significantly with it. So, many companies are trying to get more sources to get this antioxidant. Nowadays, Malaysia is among the larger country that produced the β-carotene product.

The skin of papaya is excellent for treating skin wounds and places that do not heal quickly. All the parts of the papaya fruit are useful and beneficial. Right from the seeds to the papaya leaves and the flesh of the fruit, all of it has some value. Both the inside and the outside of the fruit can be utilized .Thus no part of the fruit is useless or goes as a waste.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and Distribution

The name for papaya is *Carica papaya* L. It is a tree-like herbaceous plant, a member of the small family Caricaceae and widely cultivated for its edible fruits it was believed in 16th century the papaya seeds were brought to Melaka from Philippines. Papaya is called as “betik “in Malaysia. Papaya is rich in enzyme called as papin (help in tenderizing meat), vitamin C (more than orange that is 71 mg verses 39.6 mg) and a lot of fibres. You can easily see the Papaya tree everywhere in Malaysia. Papaya, native to Central America and Mexico but it is now a very common fruit grown in most of the tropical and subtropical countries. Some countries called it 'pawpaw' but do not be confused with another unrelated species of fruit that goes by this actual name too.

Papaya is green when young and will turn yellowish-orange when ripe while its flesh is yellow, orange or red, depending on the various cultivars. Some varieties can grow to an enormous size (above 4kg/10 lbs), especially those from the South America origins. There are numerous small black seeds clustered in the center. It is edible and tastes spicy, similar to black pepper but it is usually not well appreciated. The papaya tree is an unbranch tree and can grow till 10 m tall. The 7 lobes leaves are large and place on top of the trunk. It’s about 45cm to 65cm. The flower is small and a bit waxy. We can see them on the axils of the leaves. The lower trunk is filled with the old scars and you can see them when the tree grows higher and higher. These scars were made by the old leaves or the borne fruits. If you plant this papaya

from the seed then within 6 to 12 months, it will be mature enough to produce the fruit.

Although opinions differ on the origin of *C. papaya* in tropical America (see Garrett 1995), it is likely that *C. papaya* originates from the lowlands of eastern Central America, from Mexico to Panama (Nakasone & Paull 1998). Its seeds were distributed to the Carribean and south-east Asia during Spanish exploration in the 16th Century, from where it spread rapidly to India, the Pacific and Africa (Villegas 1997).

Papaya is now grown in all tropical countries and many sub-tropical regions of the world. It was deliberately introduced to Australia more than a century ago as a horticultural crop for fruit production (Garrett 1995)

2.1.1 Uses of Papaya

Economically, *Carica papaya* is the most important species within the Caricaceae, being cultivated widely for consumption as a fresh fruit and for use in drinks, jams candies and as dried and crystallised fruit (Villegas 1997). Green fruit and the leaves and flowers may also be used as a cooked vegetable (Watson 1997). Nutritionally, papaya is a good source of calcium and an excellent source of vitamins A and C (Nakasone & Paull 1998). The vitamin A and C content of one medium papaya approaches or exceeds USDA minimum daily requirements for adults (see OECD 2003). The fruit of some species of *Vasconcella* may be used as a food source, particularly in some regions of South and Central America, but such usage is relatively limited.

Papaya also has several industrial uses. Biochemically, its leaves and fruit are complex, producing several proteins and alkaloids with important pharmaceutical and industrial applications (El Moussaoui et al. 2001). Of these, however, papain, is a particularly important proteolytic enzyme that is produced in the milky latex of

green, unripe papaya fruits (note that ripe papaya fruit contain no latex or papain). The latex is harvested by scarifying the green skin to induce latex flow, which is allowed to dry before collection for processing (Nakasone & Paull 1998). Evolutionarily, papain may be associated with protection from frugivorous predators and herbivores (El Moussaoui et al. 2001). Commercially, however, papain has varied industrial uses in the beverage, food and pharmaceutical industries including in the production of chewing gums, in chill-proofing beer, tenderising meat, drug preparations for various digestive ailments and the treatment of gangrenous wounds. Papain has also been used in the textiles industry, for degumming silk and for softening wool (Villegas 1997) and in the cosmetics industry, in soaps and shampoo.

2.1.2 Morphology of Papaya

Carica papaya is a soft-wooded perennial plant that lives for about 5-10 years, although commercial plantations are usually replanted sooner (Chay-Prove et al. 2000). Papayas normally grow as single-stemmed trees with a crown of large palmate leaves emerging from the apex of the trunk, but trees may become multi-stemmed when damaged (Villegas 1997). The soft, hollow, cylindrical trunk ranges from 30 cm diameter at the base to about 5 cm diameter at the crown. Under optimal conditions, trees can reach 8-10 metres in height but in cultivation, they are usually destroyed when they reach heights that make harvesting of fruit difficult (Villegas 1997). Cultivated trees in Australia are usually replaced before exceeding 4 m in height.

Papaya flowers are born on inflorescences which appear in the axils of the leaves. Female flowers are held close against the stem as single flowers or in clusters of 2-3 (Chay- Prove et al. 2000). Male flowers are smaller and more numerous and are born on 60- 90 cm long pendulous inflorescences (Nakasone & Paull 1998). Bisexual flowers are intermediate between the two unisexual forms (Nakasone & Paull 1998). The functional gender of flowers can be altered or reversed, depending on environmental conditions, particularly temperature.

Fruit are ready to harvest five to six months after flowering, which occurs five to eight months after seed germination (Chay-Prove et al. 2000). The fruits range in size from 7-30 cm long and vary in mass from about 250 to 3000g (OECD 2003). Fruit from female trees are spherical whereas the shape of fruit from bisexual trees is affected by environmental factors, particularly temperature, that modify floral morphology during early development of the inflorescence (Nakasone & Paull 1998)

2.2 Plants as Sources of Antioxidants

Natural antioxidants may be found in any plant part. Fruits, vegetables, spices, nuts, seeds, leaves, roots and barks have been considered as potential sources of natural antioxidants (Pratt and others 1990). Antioxidants in flaxseed, sunflower, soybean, cottonseed and papaya typify those found in oilseeds. The majority of natural antioxidants are phenolic compounds, and the most important groups of natural antioxidants are the tocopherols, flavonoids and phenolic acids that are common to all plant sources (Naczk and Shahidi 2006).

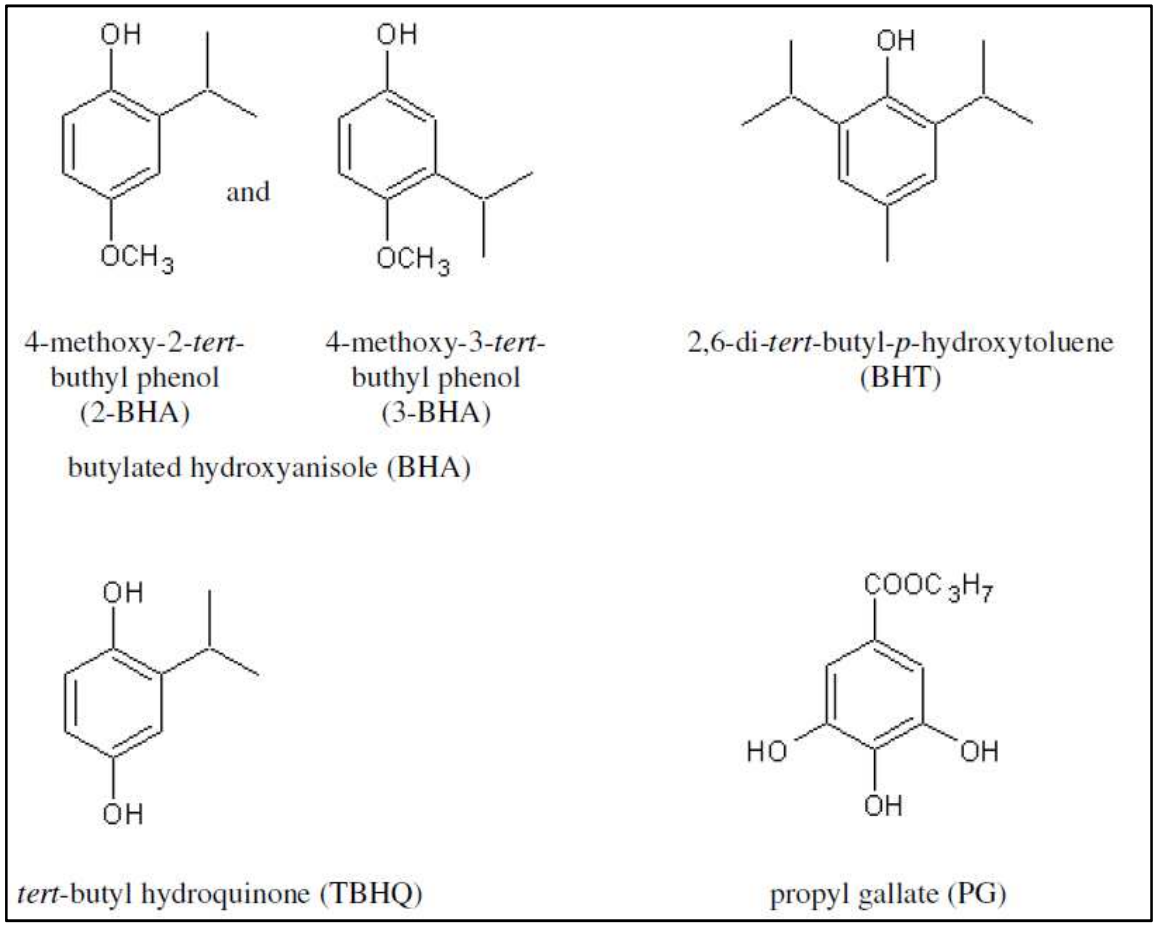


Figure 2.2 Chemical structures of food-grade synthetic phenolic antioxidants (modified from Yanishlieva 2001)

Flavonoids represent a large group of phenolics that occur naturally in plants andare found in fruits, vegetables, grains, barks, roots, stems, flowers, tea and wine (Blokhina and others 2003). They are characterized by the carbon skeleton C6–C3–C6.The basic structure of these compounds consists of two aromatic rings linked by a threecarbonaliphatic chain (Yanishlieva 2001). Several classes of flavonoids are delineatedon the basis of their molecular structure, but the four main groups that occur in planttissues are flavones, flavanones, catechins and anthocyanins (Figure 2.3) (Nijveldt andothers 2001).

Phenolic acids and their derivatives occur widely in the plant kingdom, *e.g.*,legumes, cereals, fruits and plant products such as tea, cider, oil, wine, beverages and medicinal plants (Odaci and others 2007). Phenolic acids (Figure 2.4) can found

in free and conjugated forms in cereals (Naczk and Shahidi 2006). They are present in highest concentration in the aleurone layer of grains, but are also found in the embryo and seed coat (Naczk and Shahidi 2006). The level of phenolics in plant sources also depends on such factors as cultivation techniques, cultivar, growing conditions, ripening process, processing and storage conditions, as well as stress conditions such as UV radiation, infection by pathogens and parasites, wounding, air pollution and exposure to extreme temperatures (Naczk and Shahidi 2006).

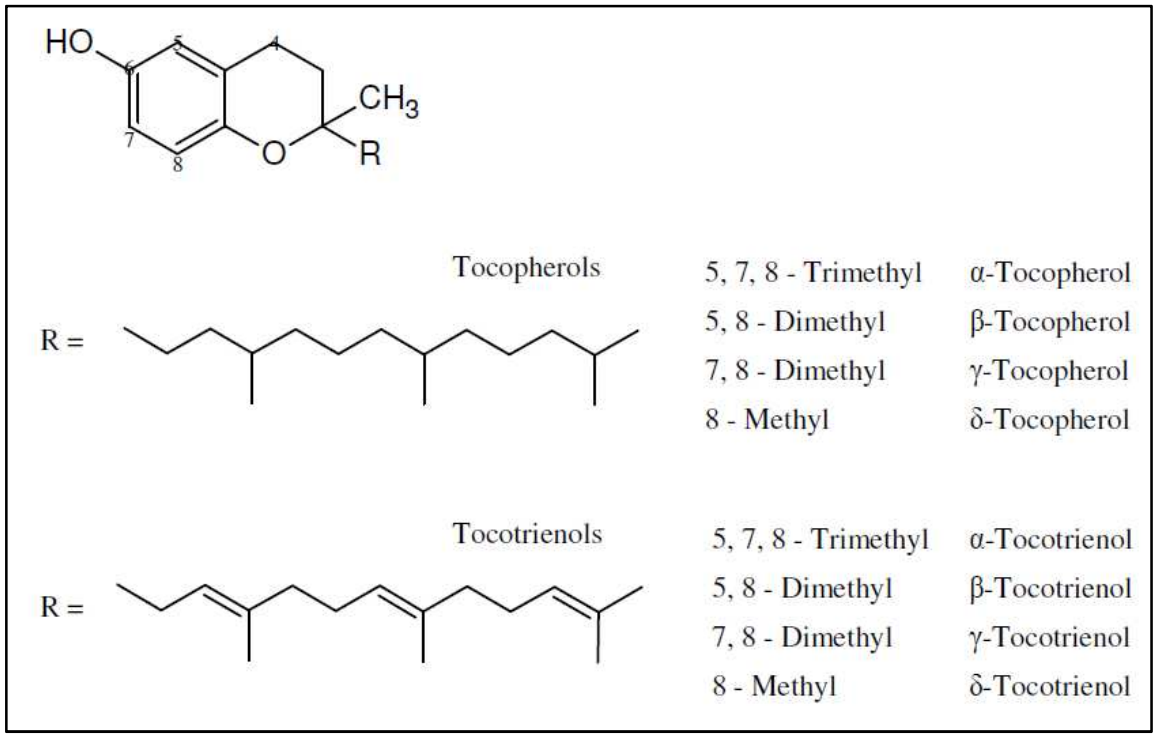


Figure 2.3 Chemical structures of tocopherols and tocotrienols and their isomers (modified from Yanishlieva 2001).

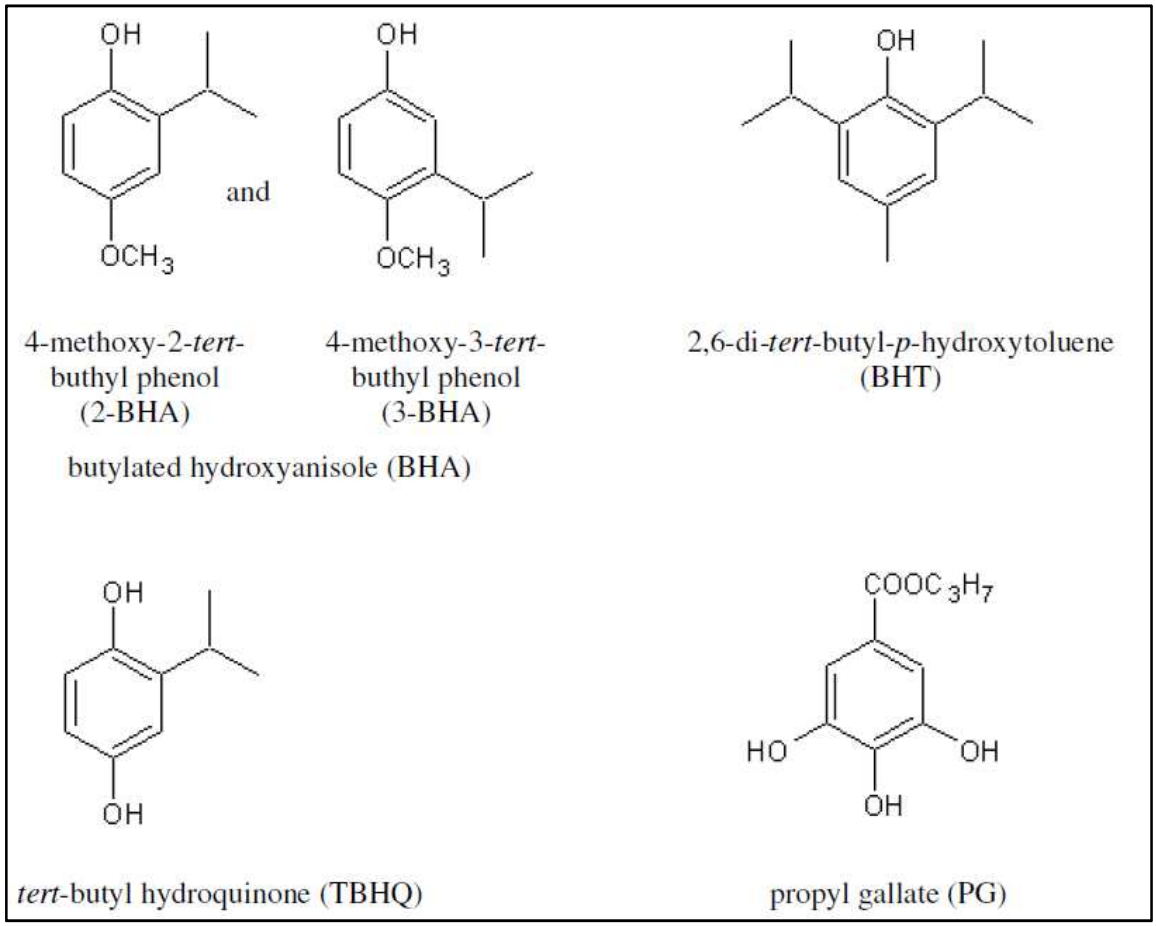


Figure 2.4 The molecular structures of the four main flavonoid groups (modified from Nijveldt and others 2001)

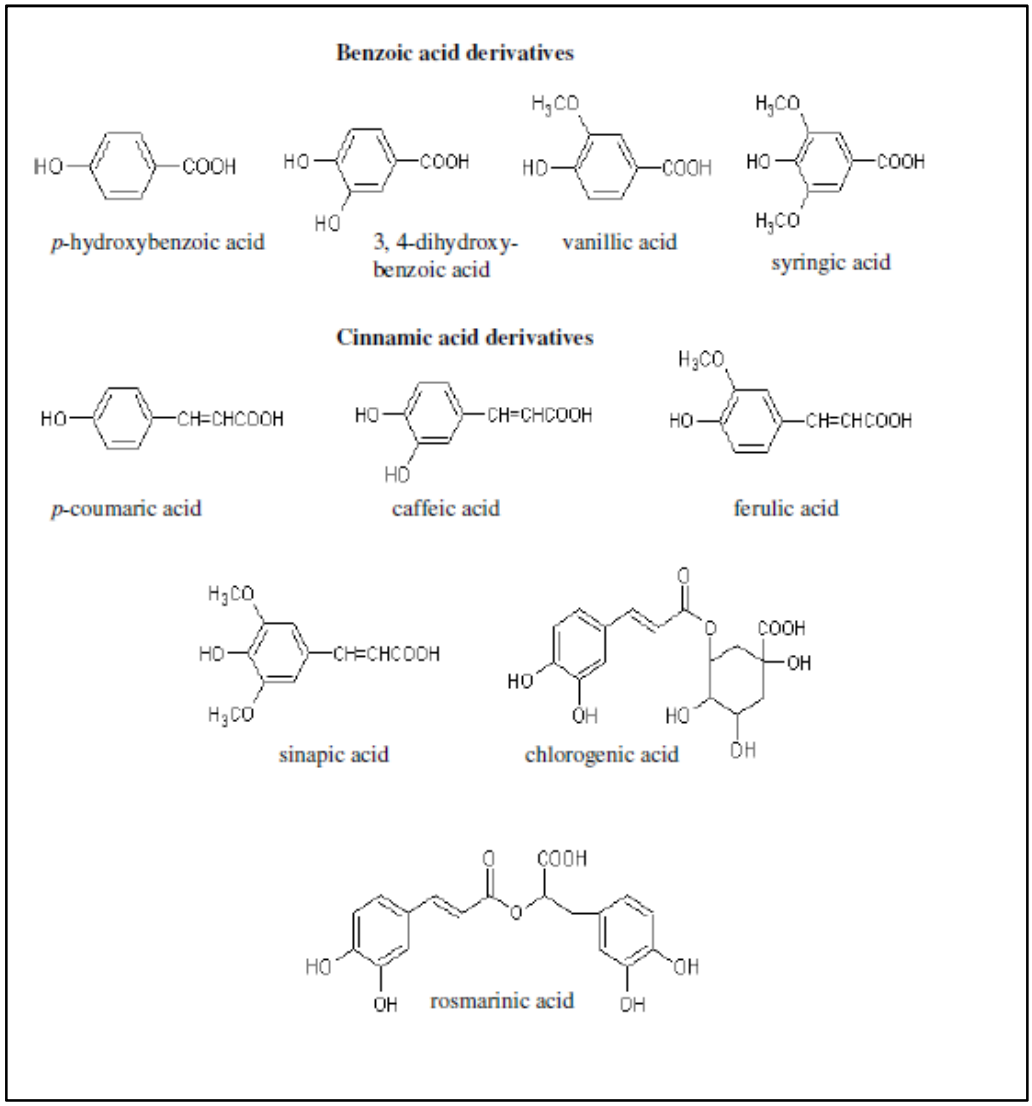


Figure 2.5 Phenolic acids as examples of common natural antioxidants (modified from Yanishlieva 2001)

2.2.1 Antioxidants in Papaya

Papaya is a derivative of rapeseed with low glucosinolate and erucic acid contents (Hall 2001). Antioxidant compounds identified in papaya include phenolic acids (both benzoic and cinnamic acid derivatives) (Kozlowska and others 1988), flavonoids (Hall 2001) and condensed tannins (Shahidi and Naczk 1989). A diversity of phenolic compounds is present in papaya and rapeseed flours (dehulled, defatted seed), meals (defatted, whole seed) or extracts, indicating that these products could

protect a food against rancidity by any of several mechanisms (Table 2.1). The high antioxidant activity of a papaya fraction containing several groups of phenolics demonstrated protection via multiple mechanisms (Amarowicz and others 2003).

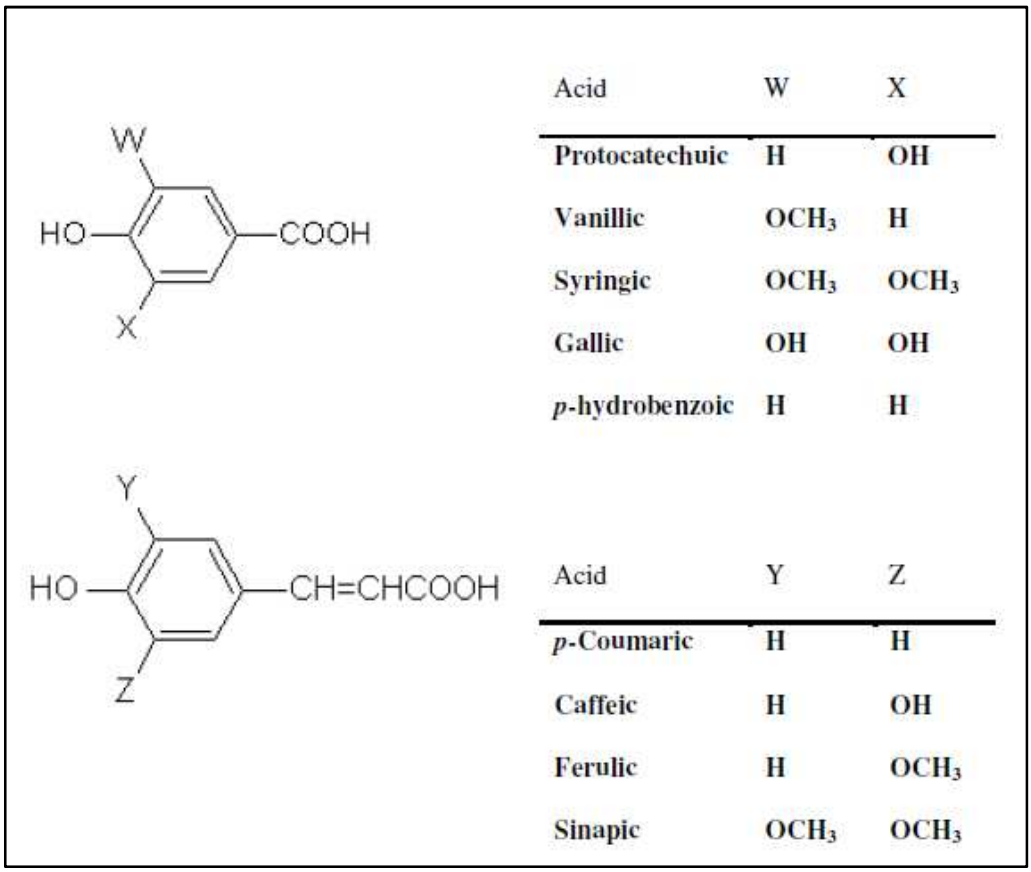
Papaya meal has been reported to contain 15.4-18.4 g/kg (dry basis, defatted meal) of phenolic acids (Shahidi and Naczk, 1992). The phenolic compounds of flesh and peel include hydroxylated derivatives of benzoic acid and *trans*-cinnamic acid, coumarins, flavonoids and lignans (Pink and others 1994). Phenolic acids in papaya meal are found in free, esterified or insoluble-bound forms (Naczk and others 1998). These authors reported that papaya meal may contain more than 2 g of free phenolic acids per kg of meal, more than 15 g of esterified phenolic acids per kg of meal and approximately 1 g of insoluble-bound phenolic acids per kg of meal (dry basis in all cases).

A high concentration of sinapic acid in papaya meal was reported by Naczk and others (1992). These authors also reported that sinapic acid, the predominant phenolic acid found in papaya, exists in free form, in esterified form, and in soluble-bound form. Wanasundara and others (1995) reported that sinapic acid and its analogues contributed significantly to antioxidant in papaya meal. Several compounds with high antioxidant were identified as phenolic compounds having one, two or three 14hydroxy groups (Figure 2.5), which were identified (by thin layer chromatography) assinapic acid, *p*-hydroxybenzoic acid, flavonoids and 1-*O*-_-D-glucopyranosyl sinapate (Wanasundara and others 1995).

Wanasundara and Shahidi (1994) reported that the antioxidant of a crude ethanolic extract of papaya meal (500 and 1000 ppm) against the oxidation of papaya flesh was equivalent to that of TBHQ (200 ppm), and stronger than that of BHA (200 ppm),BHT (200 ppm) or BHA/BHT/monoglyceride citrate (MGC) (250 ppm) on a mass basis. Wanasundara and others (1994) isolated the most active component of the extract and identified it as 1-*O*-_-D-glucopyranosyl-3, 5-dimethoxy-4-hydroxycinnamate (1-*O*-_-Dglucopyranosylsinapate; Figure 2.6). Shahidi and others (1995) observed that the addition of 0.5-5% papaya provided 73-97% inhibition of

fat oxidation in meat. The amount of phenolic acids in papaya flesh ranged from approximately 6.2 to 12.8 g per kg of meal (Naczk and others 1998).

Papaya also contains tocopherols (Hall 2001) and condensed tannins (Shahidiand Naczk 1989). Tocopherol contents in papaya meal ranged from 580 to 850 ppm and Gamma-tocopherol represented 66% of the tocopherols, and α- and β-tocopherolsaccounted for 32 and 2%, respectively (Warner and Mounts 1990). Condensed tannins in papaya meal ranged from 0.2 to 22% of hulls. However, the level of condensed tannin sex tractable by solvent systems commonly used for isolation of poly phenols was not more than 0.1% (Naczk and others 1998). Naczk and others (1998) mentioned that discrepancies in the reported data on tannin contents may be due to the different solvent systems employed for extraction or the quantification methods used for tannin analysis. The same authors indicated that papaya meals contained 0.68-0.77% of condensed tannins.



Acid	W	X
Protocatechuic	H	OH
Vanillic	OCH_3	H
Syringic	OCH_3	OCH_3
Gallic	OH	OH
p-hydrobenzoic	H	H

Acid	Y	Z
p-Coumaric	H	H
Caffeic	H	OH
Ferulic	H	OCH_3
Sinapic	OCH_3	OCH_3

Figure 2.6 Structures of phenolic acids found in papaya (modified from Naczk and others 1998).

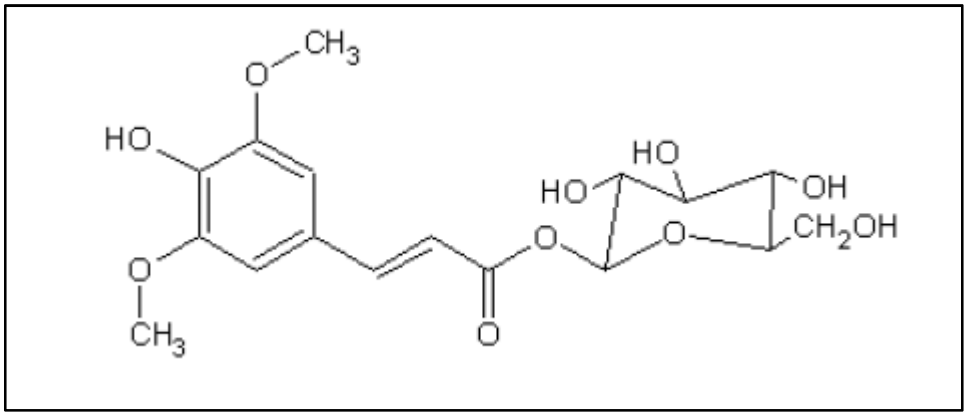


Figure 2.7 1-O--D-Glucopyranose sinapate isolated from papaya meal and characterized as an antioxidant (adapted from Wanasundara and others 1995).

2.2.2 Antioxidant Mechanisms

Antioxidants represent a class of compounds that vary widely in chemical structure and have varied mechanisms of action. The most important mechanism is their 5 reactions with lipid free radicals, forming inactive products (Pokorný and Korczak 2001). The mechanisms of antioxidant activity are shown in Table 2.1. Antioxidants can be classified into two groups based on the way they inhibit or retard oxidation. The first group is primary (chain-breaking) antioxidants, which react directly with lipid radicals and convert them into stable products. The second group is secondary (preventive) antioxidants, which can lower the rate of oxidation by different mechanisms. Direct scavenging of free radicals is not involved (Decker and others 2005). Most primary antioxidants act by donating a hydrogen atom, and are consume during the induction period (Gordon 2001).

Secondary antioxidants may act by binding metal ions able to catalyze oxidative processes, by scavenging oxygen, by absorbing UV radiation, by inhibiting enzymes or by decomposing hydroperoxides (Schwarz and others 2001). It has been reported that some natural phenolic compounds function as both primary and secondary antioxidants (Gordon 2001a). Antioxidant capacity assessment monitors either a decrease in the radical or the antioxidant, or the formation of products (Decker and others 2005).

2.2.3 Antioxidants (oxidation inhibitors)

Several degradation reactions, which may occur on heating or during long term storage, deteriorate fats and oils and the lipid constituents of foods. Oxidation reactions and the decomposition of oxidation products are the main processes which result in decreased nutritional value and sensory quality (Gordon 2001a). Research has implicated oxidative and free-radical-mediated reactions in degenerative processes related to ageing and diseases such as cancer, coronary heart disease and neurodegenerative disorders such as Alzheimer's disease (Lachance and others 2001).

The prevention or retardation of these oxidation processes is essential for the food producer and almost everyone involved in the entire food chain from “farm to fork”. Various methodologies may be employed to inhibit oxidization, including prevention of oxygen access, use of lower temperature, inactivation of enzymes catalyzing oxidation, reduction of oxygen pressure and the use of suitable packaging (Yanishlieva 2001). Another common method of protection against oxidation is to use specific additives which inhibit or retard oxidation. These oxidation inhibitors are generally known as antioxidants (Huang 2005).

2.2.4 Characteristics of Phenolics as Antioxidants

The overall effectiveness of a natural antioxidant (*e.g.*, poly phenolics and phenolic acid derivatives) depends on the involvement of the phenolic hydrogen in radical reactions, the stability of the natural antioxidant radical formed during radical reactions, and chemical substitutions present on the structure (Hall 2001). The substitutions on the structure are probably the most significant with respect to the ability of a natural antioxidant to participate in the control of radical reactions and to form resonance-stabilized, natural antioxidant radicals (Barlow 1990).

2.2.5 Extraction of Antioxidants

Antioxidant compounds are usually present in rather low amounts in natural materials. Therefore, antioxidants materials, in non-concentrated form, need to beam ployed in relatively large amounts to obtain a significant improvement in stability against oxidation. However, there could be a negative effect on the flavour or functional properties of a product by using such a large amount of an antioxidant preparation. The easiest way to prepare materials that are more concentrated is to remove water by a suitable drying procedure. The next most optimal procedure is extraction, where the choice of extraction solvent is of critical importance.

2.2.6 Extraction with Organic Solvents

Organic solvents are commonly used for the extraction of antioxidants from plant material. Two preliminary factors determine the choice of solvent for extraction, namely the nature of the antioxidants and that of the plant material (Pokorný and Korczak 2001).Table 2.2 provides some examples which demonstrate that even in such closely related materials as rosemary and sage leaves, the optimum solvent may be different (Pokorný and Korczak 2001).

Organic solvents, including, hexane, acetone, ethyl acetate and methanol, have been compared for the extraction of antioxidants (Pokorný and Korczak 2001). These authers reported that solvents of intermediate polarity seemed to be preferable to either non-polar or highly polar solvents. For the extraction of antioxidants from lentil seed, mixtures of acetone, methanol or ethanol with water (8:2, v/v) were tested, and aqueous acetone was found to be the best (Amarowicz and others 2003).

Duh and Yen (1997) used methanol for extraction of phenolic antioxidants fro peanut hull. The same solvent was used for extraction of antioxidant compounds from spices (Banias and others 1992). Pokorný and Korczak (2001) reported that less polar solvents (*e.g.*, acetone or ethyl acetate) were more suitable for spices. They also reported that using chloroform or ethyl acetate for extraction of tea leaf catechins

was more effective than using methanol. Oregano leaves were extracted with ethanol, and the obtained extract was extracted with petroleum ether, diethyl ether, ethyl acetate or butanol. The diethyl ether extract was found to be very efficient in lard in terms of antioxidant activity (Vekiari and others 1993).There exist many procedures to remove from extracts the impurities which may cause strong odour, bitter taste or undesirable colour. It was proposed that washing of crude extracts prepared by organic solvent extraction with cold or hot water removed bitter substances (López-Sebastián and others 1998). The water soluble fraction from the same material exhibited weak antioxidant activity in comparison with the starting material and the water-insoluble fraction. Therefore, antioxidant constituents extracted by organic solvent extraction were not extractable with water.

In order to remove sugars and other undesirable water-soluble, inactive substances, extracts obtained by organic solvent extraction may be concentrated by a subsequent extraction with water (López -Sebastián and others 1998). However, some efficient antioxidants may also be removed in this step (Pokorný and Korczak 2001), hence an increase in the activity of re-extracted material may not occur. The removal of interfering components occasionally compensates for this disadvantage. For example, sugars as initiators of Maillard reactions could impart foreign flavours to the product and could cause the deterioration of colour (Kitts and Hu 2005).

Aqueous alkaline solution has been used to extract antioxidants from rosemary and sage leaves (Pokorný and Korczak 2001). Alkaline solution also can be used to wash the active acidic fraction from a crude extract prepared by organic solvent extraction (Stashenko and others 1999). Antioxidant constituents from rosemary and sage can also be extracted during the process of aromatizing vinegar with these spices (Pokorný and Korczak 2001). It was reported by these authors that aromatized vinegar extended the shelf-life of mayonnaise by inhibiting lipid oxidation.

Aeschbach and Rossi (1996) extracted hydro soluble (polar) antioxidants, using propylene glycol as a polar carrier, by a purely mechanical procedure from herbs, spices, tea, coffee, fruit and vegetable peel, and cereals. They recommended these extracts for direct application in food systems.

Strong antioxidative activity of ethanolic and methanolic extracts of rape seed phenolics has been reported by Naczk and others (1998) and Nowak and others (1992). Moreover, Shahidi and others (2000) observed that the addition of 0.5-5% of papaya meat resulted in a 73-97% inhibition of lipid oxidation. Therefore, extraction of phenolic compounds from papaya meal and their possible use as natural antioxidants to delay lipid oxidation may present a new opportunity for the papaya industry.

The utility of the extraction should be estimated for every case of industrial application since the costs are rather high, and sometimes increased by a subsequent re extraction to remove impurities. In some cases, the extracts may not be considered as natural food materials, but extracts from spices would probably be acceptable as they have already been used as food ingredients for other purposes (Shan and others 2005).

2.3 General Review of β-Carotene

β-carotene is the most available and therefore important source of provitamin A in the diet of most people living in developing countries, providing about 66% of vitamin A in their diets. The carotenoids (e.g. beta-carotene and lycopene) are micronutrient antioxidants that have integral role in regulating vital metabolic reactions in the body (Abiaka et al., 2002). Nowadays, the major interest in carotenoids which are found in plants is not only due to their provitamin A activity but also their antioxidant action of scavenging oxygen radicals and reducing oxidative stress in the organism (Rao and Honglei, 2002). Epidemiological evidence also suggests that carotenoids-rich foods protect against some chronic diseases;

including certain type of cancer, cardiovascular disease and age-related macular degeneration (National Research Council, 1982). In West Africa much carotene is obtained from red palm oil, which is widely used in cooking (Latham, 1997).

However, it has been reported that the available plant sources of provitamin A are very often not eaten by children and pregnant women who are vulnerable groups at risk of vitamin A deficiency (WHO, 1976). Thus, it is common to find destruction of eyes by xerophthalmia due to Vitamin A Deficiency (VAD) in environments where carotene-rich green leaves are abundant. The prevalence of VAD in Nigeria was given as 28.1% for preschool children and 4.7% for mothers (UN/SCN, 2004). Maziya-Dixon et al. (2006) similarly reported 29.5% prevalence of VAD in children less than five years of age in Nigeria, the proportion of which was highest in the northwest dry savannah agro-ecological zone of Nigeria. It has been suggested that the high prevalence of VAD in Nigerian communities was due to low dietary intake of vitamin A, dominant dietary staple being cassava and other carbohydrate-dense foods that are virtually devoid of vitamin A and carotenoids (Tee, 1995). VAD has also been described as essentially a consequence of poor socio-economic environment (Oomen, 1976).

Since dietary diversification has been recognized as the long term solution to VAD for supporting VAD prevention and control programmes emphasized by the International Vitamin A Consultative Group (IVACG), it would therefore be necessary to identify and determine the beta carotene content of commonly consumed foods and soups in Nigeria. However, there is little published information on the carotenoid composition of foods that constitute the bulk of the diets in Nigeria and such information is important for evaluation of diets, establishment of locally relevant dietary guidelines and future nutritional research on the relationship between diet, health and disease.

Carotene is an important member of the carotenoid terpenes, numerous examples of which exist in plant and animal pigments (Bauernfiend, 1981a, b; Gordon and Bauernfiend, 1982). The carotenoids are biologically important to mammals, and an enormous amount of work has been invested in studies of their

photophysics, photochemistry, biochemistry, and antioxidant properties.-Carotene is the most efficient precursor to vitamin A known, and is also of interest as a biologically active compound in its own right. It is widely used as a colorant in processed foods, and is sold as an over-the-counter dietary supplement. It has been touted as a potential anticancer drug but recent clinical investigations failed to establish efficacy in this area (Omenn et al., 1996;Heinonen et al., 1994; Hennekens et al., 1996). It has been synthesized commercially for about 40years, presently on a scale of approximately 450metric tons per year (Paust, 1991). Surprisingly few publications have dealt with the physical chemistry of β-carotene (Stitt et al., 1951; Marty and Berset, 1986), although this information is evidently relevant to both its behavior in biological systems and its commercial use.

We became interested in this subject as it relates to the path by which β-carotene is absorbed in the body from the small intestine, and how coingestedolestra non-digestible fat-like high polyester of sucrose) might have diminished this absorption (Mattson et al., 1979; Jandacek, 1982; Mutter etal., 1958). In pursuing this work we attempted to perform elementary quantitative physical studies, and encountered numerous and surprising difficulties.

First, we note that the existing assay method for standard samples of β-carotene is based upon absorbance data in the visible spectrum at one or two wavelengths (Bauernfiend, 1981a, p. 835)).Such limited spectroscopic data would a priori seem unlikely to provide reliable assays of a compound having the molecular and stereo chemical complexity of β-carotene and its numerous oxidation products (Mordi et al., 1993; Doering et al., 1995). These fears were indeed justified by our data.

Having good standard samples is critically important to the calibration of analytical methods such as HPLC, for this method (as used with the usual diode array detectors) is blind to the oxidation products of -carotene. Second, samples of sufficiently high quality to perform the desired physical studies were unavailable in the necessary quantities. To illustrate, initial attempts to measure the solubilization of supposedly pure -carotene into aqueous micellar solutions resulted, instead, in the

selective dissolution of polar oxidation products. The dissolution of -carotene itself was not observed.

Faced with this situation, we fell back on quantitative UV–Vis spectroscopy to characterize β-carotene samples. Spectral data of this sort have been used for this purpose since the pioneering work of Zechmeister (1962), but these studies disclosed yet another problem: both commercial laboratory samples of β-carotene, and recrystallized samples, severely violate Beer's Law in the UV. If one truncates -carotene spectra at the lower limit of the VIS region (as is often done in published spectra), they are very well behaved. If, however, one analyzes the entire UV–Vis spectrum, then serious problems emerge.

The analytical issue was resolved by developing a new assay method for -carotene (Laughlin, 2002; Laughlin, et al., 2002). This was accomplished using Thin Layer Chromatography with Flame Ionization Detection (TLC-FID) analysis (to determine the fraction of hydrocarbon carotenoids present), in combination with HPLC analysis (to analyze for stereoisomers). Success in the TLC-FID analysis hinged upon successfully controlling the influence of water. This old, and still serious, problem was resolved by using 4-Phase Development or 4PD a robust new development principle that utilizes a developing solvent which exists within a thermodynamically invariant mixture of four phases. The toluene sodium chloride water system proved to be useful for β-carotene assays.

The papaya and their products in general are a complex source of β-Carotene pigments, with the largest number of them reported for any fruit (Gross, 1987). Papaya undoubtedly stand out among them all for being one of the most globally accepted fruit products and because their consumption is increasing worldwide (Moulyet al., 1999). The importance of β-Carotene on the flesh an peel colour and the renewed interest in these pigments due to their health benefits, in conjunction with the nutritional value of papaya, have furthered the development of a wide variety of analytical methods for their analysis, as the correct characterization of these compounds is necessary to obtain reliable compositional data for realistic and valuable conclusions in nutritional studies.

The analysis of β-Carotene comprises a series of stages, typically sampling, extraction, saponification, chromatographic analysis, identification and quantification. The assessment of these pigments in citrus products is more difficult as compared to other foodstuffs not only because of their complex β-Carotene profile, but also because of the inherent acidity of these products, which promotes isomerization reactions and makes identification even more complicated. Due to the importance of papaya as source of these compounds and the added difficulties in relation to their analysis, the aim of this paper is to review the various techniques used in the determination of β-Carotene pigments in papaya.

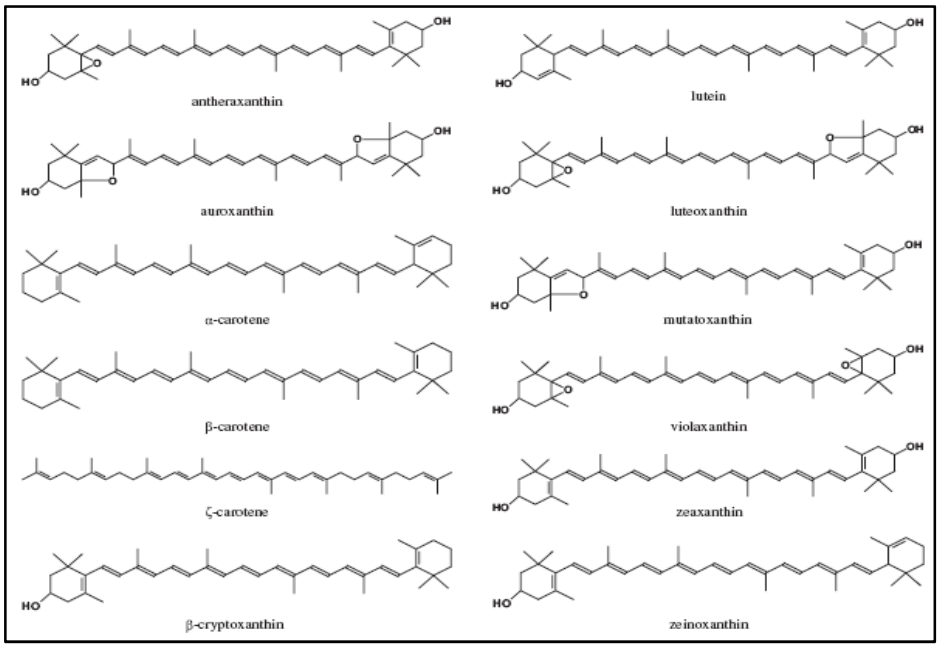


Figure 2.8 Chemical structures of some typical Papaya carotenoids

β-carotene is the molecule that gives carrots their orange color. It is part of a family of chemicals called the carotenoids, which are found in many fruit and vegetables, as well as some animal products such as egg yolks. Carotenoids were first isolated in the early 19th century, and have been synthesized for use as food colorings since the 1950s. Biologically, beta-carotene is most important as the precursor of vitamin A. It also has antioxidant properties and may help in preventing cancer and other diseases although much controversy continues in this area (see below).

β-carotene plays a crucial role as a photosynthetic pigment, important for photosynthesis. . It does not actively contribute in photosynthesis, but instead it transmits the energy it absorbs to chlorophyll and also plays a protective role for chlorophyll being a powerful antioxidant that protects organic molecules from being destroyed by oxidation. Carotene is the dimer of vitamin A and comes in two forms alpha and β-carotene. Both types can be stored in the liver, and unlike vitamin A, excess carotene is non-toxic and can also be converted to vitamin A as needed.

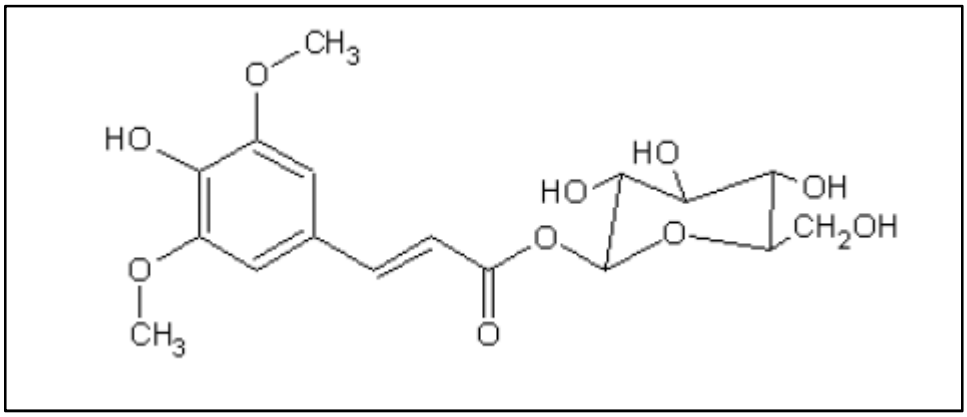


Figure 2.9 Structure of β-Carotene

β-carotene is one of over 500 of the carotenoid family. These substances form the coloring pigments for deep yellow, orange and dark green fruits and vegetables. In its natural form, β-carotene can be found in strawberries, cantaloupe, broccoli, squash, sweet potatoes, carrots, etc. β-Carotene is believed to act as an antioxidant and an immune system booster. Other members of the antioxidant carotenoid family include cryptoxanthin, alpha-carotene, zeaxanthin, lutein, and lycopene. However, unlike β-carotene, these nutrients are not converted to vitamin A in significant amounts. Although β-carotene supplements are widely available commercially, the preferred form is that found in fresh fruits and vegetables

There have been conflicting reports on the effectiveness of β-carotene supplements with some research findings that the supplements may even increase the risk for some cancers; studies have shown that synthetic β-carotene increased the incidence of lung cancer and those exposed to asbestos. There are many types of antioxidants, and they do different kinds of work. What marketers of supplements

never tell you is that not all of it is good work. Antioxidants can certainly deactivate free radicals in a test tube, but in the human body they can sometimes have the opposite effect acting as pro oxidants (see below) instead of antioxidants. That's what the two β-carotene studies showed. Published in 1996, the CARET study (full name: β-Carotene and Retinol Efficacy Trial) tested β-carotene and vitamin A supplements in people at high risk for lung cancer smokers, former smokers, and asbestos-industry workers.

The study was halted when it became clear that taking β-carotene (not even a high dose just 30 milligrams a day) actually had a higher rate of lung cancer and higher mortality rate than those taking a placebo. The investigators found that β-carotene can turn into a pro-oxidant or form oxidized by-products, particularly if adequate amounts of vitamins C and E aren't present. It's well known that C and E work together to produce their antioxidant effect. Recent research strongly suggests that these vitamins can also help limit the oxidation of beta carotene and/or recycle it after it is oxidized, so that it won't damage cells. (In fact, any antioxidant can become a pro-oxidant under certain conditions in the body especially if other antioxidants are lacking, since they protect one another from oxidation.)

It's better to get β-carotene from food (carrots, spinach, cantaloupe, etc.) than from pills. There has never been any evidence that the beta carotene in foods poses any danger, perhaps because it comes with many other plant chemicals that all work together. In fact, virtually every study shows that foods rich in beta carotene help keep people healthy and even reduce the risk of lung cancer. β-carotene is separable by sublimation even though their melting points differ by only 2 °C. The separation is possible because the compounds have very different vapor pressure. β-carotene has a very low vapor pressure over a wide range of temperatures. As a result, β-carotene does not readily undergo a change of state from solid-to-vapor. Rather, it changes from a solid to a liquid at its melting point of 178–179 °C, molecular formula $C_{40}H_{56}$ and molecular weight is 536.88.

2.3.1 β- Carotene is Antioxidant

There are several plausible reasons why there may be an association between fruit and vegetable consumption and reduced risk of chronic disease, apart from a possible influence on the relationship of other associated factors, such as tobacco use and physical inactivity among those with low fruit and vegetable intake (known as confounding) (Lampe 1999). A popular explanation among scientists, and more recently in the media, has been that food components with antioxidant properties (including vitamins C and E, selenium and ß-carotene) present in these foods may prevent some of the processes involved in the development of cancer (e.g. by protecting DNA from oxidative damage) and in the development of cardiovascular disease (e.g. by inhibiting oxidative damage to LDL-cholesterol).

Normal oxidative metabolism in the human body produces large quantities of potentially damaging toxins (‘free radicals’ or ‘pro-oxidants’) that can cause damage to cells by disrupting their normal repair mechanisms. This damage is known as oxidative stress. The delicate balance between pro and antioxidants in the cell determines the degree of oxidative stress to the cell, and the balance between pro- and antioxidants has been implicated in the development of many chronic diseases, including heart disease, diabetes, cancer and the ageing process (Jackson 2003).

Free radicals are highly reactive molecules that react with and damage cells throughout the body. They are suspected of causing cardiovascular disease, cancer, neurological disorders, cataracts, arthritis, aging and other conditions such as muscle damage and fatigue that could inhibit performance. Antioxidants are molecules, which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.

Many researchers believe that vitamin supplements can dramatically reduce free radical damage, prevent and delay the onset of chronic degenerative diseases, and possibly extend lifespan. Numerous epidemiological studies have demonstrated

an association between higher intakes or higher blood concentrations of certain antioxidants and a lower incidence of certain degenerative diseases. Clinical studies have shown that supplemental levels of antioxidant vitamins reduce an individual's risk for certain cancers and cardiovascular diseases. Moreover, studies have shown that fruit and vegetable consumption (the major source of antioxidant nutrients) has a protective effect against cancer .Evidence also suggests that vitamins C, E and β-Carotene supplementation have ergogenic or performance enhancing effects.

High-performance liquid chromatography (HPLC) combined with a UV-vis detector is the most common method for identification and quantification of antioxidant vitamins in biological fluids. Several HPLC methods have been presented for the determination of vitamin C in serum or plasma. The procedures for simultaneous measurement of vitamin E and β-Carotene by HPLC have also been described. Recently, an isocratic liquid chromatographic method was reported for the simultaneous determination of vitamins C, E and β-Carotene in pharmaceutical preparations However, no reports for the simultaneous determination of these three antioxidant vitamins inhuman biological fluids were described. This is attributed to the extraction and reconstitution difficulties from biological fluids that are linked to their chemical properties (Vitamin C is water-soluble; Vitamin E and β-Carotene are fat-soluble).

2.3.2 β-carotene in Papaya

β-Carotene as the main groups of coloring substances in nature, are responsible for many of the red, orange and yellow colors of fruits and vegetables. They have been attracted many researchers because of their commercially desirable properties, such as their natural origin, null toxicity and high versatility, providing both lipo- and hydro-soluble colorants and pro vitamin A activity. The restrictions on several certified food colors have stimulated the interest in commercial production and storage stability of these natural β-Carotene pigments. Natural food colorants do not need to be certified and can be listed in ingredients simply as ‘colorants’’.β-

Carotene is an organic compound and classified as a terpenoid. It is a strongly-coloured red-orange pigment abundant in plants and fruits.

As a carotene with beta-rings at both ends, it is the most common form of carotene. It is a precursor (inactive form) of vitamin A. The structure was deduced by Karrer et al. In nature, β-carotene is a precursor to vitamin A via the action of β-carotene 15,15'-monooxygenase. β-Carotene is also the substance in carrots that colours them orange. β-Carotene is biosynthesized from geranylgeranyl pyrophosphate. Isolation of β-Carotene from fruits abundant in β-Carotene is commonly done using column chromatography. The separation of β-carotene from the mixture of β-Carotene is based on the polarity of a compound. β-carotene is a non-polar compound, so it is separated with a non-polar solvent such as hexane. Being highly conjugated, it is deeply colored, and as a hydrocarbon lacking functional groups, it is very lipophilic. (Tang and Chen, 2000).

The importance of β-Carotene on the papaya and the renewed interest in these pigments due to their health benefits, in conjunction with the nutritional value of papaya, have furthered the development of a wide variety of analytical methods for their analysis, as the correct characterization of these compounds is necessary to obtain reliable compositional data for realistic and valuable conclusions in nutritional studies. The analysis of β-Carotene comprises a series of stages, typically sampling, extraction, chromatographic analysis, identification and quantification.

An HPLC gradient elution system with monomeric C18 column and UV detection at 450nm were used for separation and quantification of the carotenoids. Due to the importance of papaya as source of these compounds and the added difficulties in relation to their analysis, the aim of this paper is to verify methods, investigate the content of the papaya and optimization process in the various techniques used in the determination of β-Carotene pigments in papaya.

2.3.3 Subcritical Water Extraction (SWE)

Most conventional methods for extraction of natural antioxidants from plants are based on organic solvents, which may have undesirable effects on the environment and on food components. Several methods have been employed to extract antioxidants from aromatic plants. These include solid-liquid extraction, aqueous alkaline extraction, extraction with vegetable oils, extraction with aqueous alkanol solutions and supercritical fluid extraction (SFE) (Kubátová and others 2001). Water may be used a san extraction solvent, and has gained an increasing amount of attention due to its unique solvation properties, which can be altered by changing the temperature (Rovio andothers 1999). Subcritical water extraction is considered a recent alternative for the isolation of antioxidant constituents.

Subcritical water extraction, also known as hot water extraction, pressurized (hot) water extraction, pressurized low polarity water extraction, high-temperature water extraction, superheated water extraction or hot liquid water extraction, is a promising “green” technique based on the use of water as the sole extraction solvent (Smith 2002). Temperatures between 100 and 374°C (the critical point of water is at 374°C and 22 MPa) are generally applied and the pressure is sufficient to keep water in the liquid state (Ramos and others 2002).

In some reports, room temperature (25°C) has been employed for SWE (Ibáñez and others 2003). At temperatures above 100°C, the dielectric constant of water, ε, (*i.e.*, its polarity) cans be lowered easily and significantly by increasing the temperature. Pure water at ambient temperature and pressure has a ε of 79, whereas increasing the temperature to 250°C at a pressure of 5 MPa (necessary to maintain the liquid state) yields a significant reduction in ε to ~27. This value is similar to that of ethanol at 25°C and 0.1 MPa and,24 consequently, low enough to dissolve many compounds of intermediate or low polarity(Ramos and others 2002).

Subcritical water extraction has been applied in the determination of organic pollutants in soils, sludges and sediments, and also is used for the extraction of volatiles from plant material (Rovio and others 1999). Ibáñez and others (2003) extracted the most active antioxidant compounds from rosemary, such as carnosol, rosmanol, carnosicacid, methyl carnosate and flavonoids such as cirsimaritin and genkwanin, by SWE.

Kimand Mazza (2006) reported that SWE of phenolic compounds, including *p*hydroxybenzaldehyde, vanillic acid, vanillin, acetovanillone and ferulic acid, from flaxshive was maximized at the combined conditions of high temperature and high NaOH concentration. Lignans were also extracted from whole flaxseed by SWE (Cacace and Mazza 2006). Maximum amounts of lignans and other flaxseed bioactives, including proteins, were extracted at 160°C. However, these authors reported that on a dry weight basis, the most concentrated extracts in terms of lignans and other phenolic compounds were extracted at 140°C. Ho and others (2007) extracted lignans, carbohydrates and proteins from flaxseed meal.

The maximum yield of lignans and proteins was obtained at pH 9 at temperatures of 170°C and 160°C, respectively. Maximum recovery of carbohydrates was at pH 4 and 150°C. In another study, Rodriguez-Meizoso and others (2006) demonstrated that the combined use of SWE and high performance liquid chromatography-diode array detection (HPLC-DAD) was a suitable protocol to obtain and characterize nutraceuticals from natural sources, *i.e.*, oregano. They also reported that changing the water temperature could be used as a means of fine tuning the extraction selectivity of pressurized water for the extraction of antioxidant compounds from oregano using SWE. Subcritical water has been applied as an HPLC analytical solvent to extract and quantify caffeine, chlorophenols and anilines (Li and others 2000).

García-Marino and others (2006) stated that SWE would be an appropriate extraction technique for obtaining a greater quantity of poly phenolic compounds (catechins and proanthocyanidins) from winery by-products, and compared SWE with extraction with MeOH/H2O (75:25, v/v). Pongnaravane and others (2006)

compared the effectiveness of SWE of anthraquinones from *Morinda citrifolia* with that of other extraction methods, such as ethanol extraction in a stirred vessel, Soxhlet extraction and ultrasound-assisted.

Subcritical water extraction has been used to extract ginsenosides from American ginseng (Choi and others 2003); catechins and epicatechinfrom tea leaves and grape seeds (Piñeiro and others 2004); anthraquinones (antibacterial, antiviral and anticancer compounds) from roots of *Morinda citrifolia* (Shotipruk and others 2004); and, flavones, anilines and phenols from orange peels (Lamm and Yang 2003)

A major disadvantage of SWE is the high operating pressure, which requires expensive equipment (Smith 2002). Thus, the cost of the process is relatively high, making it unsuitable for the extraction of major food components, such as lipids. In the case of antioxidants, price would not play a crucial role, since they are an expensive group of food compounds and costs are compensated by other advantages, such as the high purity of extracts and the efficiency of the process (Ramos and others 2002). The 26 use of water also allows for a substantial saving in maintenance costs, although maintaining high pressure can be expensive (Ramos and others 2002). The possibility of fine tuning the selectivity of antioxidant extraction through a small change in water temperature is another advantage of SWE (Smith 2002; Ramos and others 2002).

2.3.4 Analysis of β-Carotene

The *β*-carotene in the sample was extracted according to the method described by Tee *et al*. (1996) with slight modifications. The sample (10 g) was added with 40 ml of 99.8% ethanol and 10 ml of 100% (w/v) potassium hydroxide, and homogenised for 3 min using a blender. The mixture was saponified by means of a refluxing apparatus, and heated using a heating mantle for 30 min, and then cooled to room temperature. The mixture was frequently agitated to avoid any aggregation. For the extraction step, the mixture was transferred into a separation funnel and 50 ml of n-hexane was added. The funnel was inverted, vented and then shaken

vigorously for a few seconds, and the layers were allowed to separate. The upper layer (hexane extract) was pipetted out, and the aqueous layer was re-extracted twice, each time with 50 ml of n-hexane. Then, the upper layer was pooled and washed with distilled water until free of alkali. Phenolphthalein solution (1%) was used to check for any alkali. The presence of alkali turns this indicator to pink. The extract was then filtered through anhydrous sodium sulphate to remove any water residue. The hexane residue was removed under reduced pressure at 45°C using a rotary evaporator (Laborata 4000, Heidolph Instruments GmbH & Co. KG, Germany). The resulting extract was diluted to 10 ml with n-hexane. All samples were carried out in triplicates.

2.4 Mineral Content of Papaya

The papaya is a very nutritious fruit, containing high amounts of vitamins (A, B, C and E), and minerals (potassium and magnesium). In fact, weights for weight, papayas have more vitamin C content than oranges. 100 gm of papaya has 71mg of vitamin C, while 100gm of orange has only 40mg of vitamin C. Papayas also contain lycopene (normally associated with tomatoes), which is believed to lower the risk of prostate cancer. The papaya fruit is also a good source of dietary fiber. A cup of the cut papaya fruit (approx. 150 gm) has the dietary fiber equivalent of half a cup of cooked barley, or 4 cups of cooked white rice. Papayas also contain papain and chymopapain. These are protein-digesting enzymes, commonly used as meat tenderizers. These enzymes are found in the unripe papaya fruit, and in the papaya leaves. Wrapping meat in papaya leaves before cooking helps to tenderize the meat.

Papaya are valuable health foods, low in calories and high in vegetable proteins, vitamins, iron, zinc, selenium, sodium, chitin, fibers and minerals (Racz et al.,1996; Mendil et al., 2004; Ouzouni, 2004). Elements are of great biochemical interest and having nutritional and clinical importance. The content of metals is related to species of Papaya, collecting site of the sample, age of fruiting bodies and mycelium, and distance from sources of pollution (Kalaˇ c et al., 1991). It is also

influenced by species physiology and particularly by fungi ecosystem pattern (Turkekul et al., 2004).

Metals occur in different forms: as ions dissolved in water, as vapors, or as salts or minerals in rock, sand and soil. They can also be found in organic or inorganic molecules, or attached to particles in the air. Both natural and anthropogenic processes emit metals into air and water (Demirbas, 2001). Heavy metal concentrations in peel and flesh are also considerably higher than those in agricultural crop plants and vegetables. This suggests that Papaya possess a very effective mechanism that enables them readily to concentrate certain heavy metals from the ecosystem, compared to green plants growing in similar conditions. (Svoboda et al, 2000).

According to Stije and Besson (1976), the mechanism by which some heavy metals are accumulated is somewhat obscure although it seems to be associated with a chelation reaction with sulfhydryl groups of protein and especially with methionine. Thus considerable effort has been focused to evaluate the possible risk to human health from the consumption of Papaya with regard to their heavy metal content (Gast et al., 1988). Determination of metal content in various food substrates has been performed by atomic absorption spectrophotometer (AAS) using flame atomization and graphite furnace. Alternative methods to conventional AAS technique for the determination of metal content in foodstuffs include: inductively coupled plasma (ICP), inductively coupled plasma mass spectrometry(ICP-MS), neutron activation analysis (NAA),X-ray fluorescence methods, etc. (Knapp, 1991; Soylacet al., 2005).

In addition, Papaya consumption has increased during recent years, due to their delicate flavor and texture as well as their high content of trace minerals. Therefore, it is necessary to investigate the metal content in wild species, given the fact that many of them are known to accumulate high levels of heavy metals, such as cadmium, mercury, lead and copper (Kalaˇ c and Svoboda, 2000). These elements are known to have severe toxicological effects on human health even at very low concentrations. Several factors may affect the accumulation and concentration of

trace elements and heavy metals in Papaya. Concentrations of the elements are generally assumed to the species-dependent, but substrate composition is also considered to be an important factor (Kalaˇ c and Svoboda, 2000; Cocchi et al., 2006). The present study relates to the determination of calcium, Ca and ferum, Fe content in the fruiting bodies in the peel and flesh of Papaya using the AAS method.

Table 2.4: List of metals and respective wavelength

Metal	Wavelength (nm)
Iron	248.3
Zinc	285.2
Magnesium	213.9
Cadmium	228.8
Lead	283.3
Chromium	357..9
Manganese	240.7
Cobalt	232
Nickel	324.8
Copper	193.7
Arsenic	224.6
Tin	309.3

The wavelengths used for the determination of iron, zinc, magnesium, cadmium, lead, chromium, manganese, cobalt, nickel, copper, arsenic, tin and aluminum are shown in Table 2.4. Standards used to construct appropriate calibration curves were purchased from Perkin-Elmer (MA, USA). To investigate the metal content in wild species, given the fact that many of them are known to accumulate high levels of heavy metals, such as cadmium, mercury, lead and copper (Kalaˇ c and Svoboda, 2000). These elements are known to have severe toxicological effects on human health even at very low concentrations.

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2.4.1 Chemical Properties of Calcium

The chemical element Calcium (Ca), atomic number 20, is the fifth element and the third most abundant metal in the earth’s crust. The metal is trimorphic, harder than sodium, but softer than aluminium. A well as beryllium and aluminium, and unlike the alkaline metals, it doesn’t cause skin-burns. It is less chemically reactive than alkaline metals and than the other alkaline-earth metals.

Calcium ions solved in water form deposits in pipes and boilers and when the water is hard, that is, when it contains too much calcium or magnesium. This can be avoided with the water softeners. In the industry, metallic calcium is separated from the melted calcium chloride by electrolysis. This is obtained by treatment of carbonated minerals with chlorhydric acid, or like a sub product of the carbonates Solvay process

2.4.2 Health Effects of Calcium

Calcium is the most abundand metal in the human body: is the main constituent of bones and it has keys metabolic functions. Calcium is sometimes referred to as l time. It is most commonly found in milk and milk products, but also in vegetables, nuts and beans. It is an essential component for the preservation of the human skeleton and teeth. It also assists the functions of nerves and muscles. The use of more than 2, 5 grams of calcium per day without a medical necessity can lead to the development of kidney stones and sclerosis of kidneys and blood vessel

A lack of calcium is one of the main causes of osteoporosis. Osteoporosis is a disease in which the bones become extremely porous, are subject to fracture, and heal slowly, occurring especially in women following menopause and often leading to curvature of the spine from vertebral collapse.

Unlike most of the people think, there is an intense biological activity inside our bones. They are being renewed constantly by new tissue replacing the old one. During childhood and adolescence, there’s more production of new tissue than destruction of the old one, but at some point, somewhere around the 30 or 35 years of age, the process is inverted and we start to loose more tissue than what we can replace. In women the process is accelerated after the menopause (he period marked by the natural and permanent cessation of menstruation, occurring usually between

the ages of 45 and 55); this is because their bodies stop producing the hormone known as estrogen, one of which functions is to preserve the osseous mass.

Evidence suggests that we need a daily intake of 1,000 milligrams of calcium in order to preserve the osseous mass in normal conditions. This is both for man and pre-menopausic women. The recommended daily intake rises to 1,500 for menopausic woman. The main calcium sources are the dairy products, but also nuts, some green vegetables like spinach, and cauliflower, beans, and lentils.

Calcium works together with magnesium to create new osseous mass. Calcium should be taken together with magnesium in a 2:1 rate, that is to say, if you ingest 1000 mg of calcium, you should also ingest 500 mg of magnesium. Some magnesium sources in the diet are seafood, whole-grains, nuts, beans, wheat oats, seeds and green vegetables.

2.4.3 Chemical Properties of Iron

Iron is a lustrous, ductile, malleable, silver-gray metal (group VIII of the periodic table). It is known to exist in four distinct crystalline forms. Iron rusts in dump air, but not in dry air. It dissolves readily in dilute acids. Iron is chemically active and forms two major series of chemical compounds, the bivalent iron (II), or ferrous, compounds and the trivalent iron (III), or ferric, compounds.

2.4.4 Health Effect of Iron

Iron can be found in meat, whole meal products, potatoes and vegetables. The human body absorbs iron in animal products faster than iron in plant products. Iron is an essential part of hemoglobin; the red colouring agent of the blood that transports oxygen through our bodies.

Iron may cause conjunctivitis, choroiditis, and retinitis if it contacts and remains in the tissues. Chronic inhalation of excessive concentrations of iron oxide fumes or dusts may result in development of a benign pneumoconiosis, called siderosis, which is observable as an x-ray change. No physical impairment of lung function has been associated with siderosis. Inhalation of excessive concentrations of iron oxide may enhance the risk of lung cancer development in workers exposed to pulmonary carcinogens

2.5 Antimicrobial in Papaya

According to the United States Centers for Disease Control and Prevention (CDC), the number of reported produce-associated food borne outbreaks per year has increased in the last few years in the USA and doubled between the periods 1973–1987 and 1988–1991 (Tauxe et al., 1997). Several outbreaks of *Escherichia coli* have been associated with the consumption of cut cantaloupe and watermelon. In 1991 an *Escherichia coli* Javiana outbreak among school children revealed a strong association with the consumption of watermelon (Blostein, 1993). During June and July 1991, more than 400 laboratory-confirmed infections with *Escherichia coli* Poona occurred in 23 USA states and in Canada, related to the consumption of cantaloupes (Francis et al., 1991). In 1997 an outbreak of *Escherichia coli* sero group Saphra was related to cantaloupe from Mexico. Twenty-four consumers showed the onset of illness (Mohle-Boetani et al., 1999). Deeks et al. (1998) reported an S. Oranienburg outbreak in Canada due to the consumption of imported cantaloupes. In 2000 cantaloupe from Mexico was the food.

CHAPTER 3

METHODOLOGY

3.1 Sample Preparation

3.1.1 Plant Material

Fresh papaya (3.5 kg) was purchased from a wet market in Gambang, Pahang, Malaysia. The fruits of uniform shape and colour were selected whereas blemished and diseased fruits were eliminated. The chosen fruits had an average length of 4.0-5.6 cm, width of 3.8-5.8 cm and weight of 34-90 g.

3.1.2 Chemical Reagents

All the solvents and chemicals used were of analytical grade. Deionized water used for the preparation of all the solutions was purified by Milli-Q purification system (Millipore) (Massachusetts, USA).

3.1.3 Pretreatment

Upon arrival at the laboratory, samples were thoroughly washed with tap water, manually peeled and the peels were cut into small pieces of about 1.0 cm^2. The fresh peels were dried at 25°C for 24 h in a convection oven (Memmert,

Germany). After drying, the dried peels were blended to find liquid form (0.5 mm) with a blender (Model MF 10 basic; IKA®WERKE, Germany) at 20 rpm. Solutions of the sample were filtered using tea filter. The samples were stored at room temperature until use.

3.1.4 Solvent Extraction

Approximately 20 g of dried sample was weighed and extracted with 40 mL of the extracting solvent in a conical flask. Conical flask was covered with parafilm (Pechiney plastic packaging) and aluminium foil to prevent light exposure. The mixture was shaken at constant rate using a water bath shaker (Memmert, Germany) for different times at required temperature. After the extraction, the papaya peels extract was then filtered through a Whatman No. 1 filter paper, and the clear solution was collected in an amber reagent bottle. The filtrate was subsequently used for the determination of β-carotene. All the extractions were replicated three times.

3.1.5 Experimental Design

The experimental design for this study was divided into two major parts. Firstly, single factor experiments were performed to determine the appropriate range of conditions for papaya peels β-carotene extraction, namely, solvent type, solvent ratio, extraction time, and extraction temperature by varying one independent variable at a time while keeping the others constant in separation process using rotary evaporator. Secondly, the mineral content of papaya extraction was carried out using AAS and a second antimicrobial testing was developed.

3.1.5.1 Single Factor Experiments

(a) Selection of solvent type

By fixing extraction time (30 min) and extraction temperature (25°C), samples were extracted with 60% (v/v) water, 60% (v/v) acetone, and 60% (v/v) ethanol respectively. The extraction procedures were described in solvent extraction section. The best solvent type was selected according to the value from optical density (OD) of Uv-vis Spectrophotometer at wavelength 450 nm.

(b) Effect of solvent ratio on extraction of β-carotene compound

Using the best solvent type selected in single factor experiments section (a), samples were extracted with solvent ranging from 1:1, 1:2, 1:3, 1:4 (sample: solvent) by fixing the extraction time and extraction temperature at 30 min and 25°C, respectively. The best solvent ratio was selected according to the value from optical density (OD) of Uv-vis Spectrophotometer at wavelength 450 nm.

(c) Effect of extraction time on extraction of β-carotene compound

Samples were extracted using the best solvent type and the best solvent ratio selected in single factor experiments sections (a) and (b), respectively. The extraction procedures were repeated as described in section of single factor experiments by varying the extraction time from 20 to 60 min while fixing the extraction temperature constant at 25°C. The best extraction time was selected according to the value from optical density (OD) of Uv-vis Spectrophotometer at wavelength 450 nm.

(d) Effect of extraction temperature on extraction of β-carotene compound

Using the best solvent type and the best solvent ratio selected in single factor experiments sections (a) and (b), samples were extracted at various extraction temperature ranged from 25 to 60°C at the optimum time determined in single factor experiments section (c). The extraction procedures were repeated as described in solvent extraction section. The best extraction temperature was selected according to the value from optical density (OD) of Uv-vis Spectrophotometer at wavelength 450 nm.

Based on the results of single factor experiment, the ranges of three factors (selective type of solvent, solvent ratio, extraction temperature and extraction time) were determined for concentration of β-carotene from using value OD using equation The Beer Lambert Law =.

$$A = \varepsilon cl$$

The maximum absorbance is usually expressed as the molar adsorptivity, €, with the wavelength called λmax. The Beer's Lambert Law related to absorbance A, the molar concentration c, and the path length ∫ in centimeters through the sample, as shown in equation above. β-carotene has a λmax 450 nm with a €=145 000.

3.2 Chromatography Analysis Instruments

Figure 3.2 HPLC Equipment for analysis method

The HPLC pump was an Altex 11OA (Altex Scientific, Berkeley, CA 94710), equipped with a pulse dampener. The injector was an Altex 210, with a 100-‘Use L loop. Injection was with a 50-L syringe (Hamilton Co., Reno, NV 89510). We used the Model 450 variable-wavelength detector (Waters Associates, Milford, MA 01757), a Beckman recorder (Beckman Instruments, Fullerton, CA 92634), and the Waters Associates Model 730 integrating recorder. The column was 3.9 x 250 mm, prepacked with Waters Bondapak C18 (10-pm particle size). A guard column (Waters), 2 x 22 mm, packed with Waters C18 Corasil, was attached on-line before the main column. Elution was performed isocratically with acetonitrile/methylene chloride (89/11 by vol) at a flow rate of 2 mL/min at a pressure of about i07 Pa (95 bar). The absorbance of the eluting compounds was monitored at 450 nm.

3.2.1 Sample Preparation

Figure 3.2.1 Sample β-carotene in peel and flesh of papaya

Pipet 100 uL of serum into a 10 x 75 mm test tube. Add 100 L of absolute ethanol containing a known amount of dimethyl-beta-carotene (300 to 600 g/L) and ascorbic acid (1 g/L). Vortex-mix the tube’s contents for a few seconds, add 200 L of hexane, and vortex-mix again for 30 s. Centrifuge the mixture for 1 mm at 1000 x g, then use a Pasteur pipet to transfer about 150 uL of the top layer to another 10 x 75 mm test tube. Place the tubes in a room temperature water bath, and evaporate the hexane under a stream of nitrogen. Re dissolve the residue in 100 /.LL of absolute ethanol, and inject 50 L of this solution into the chromatograph.

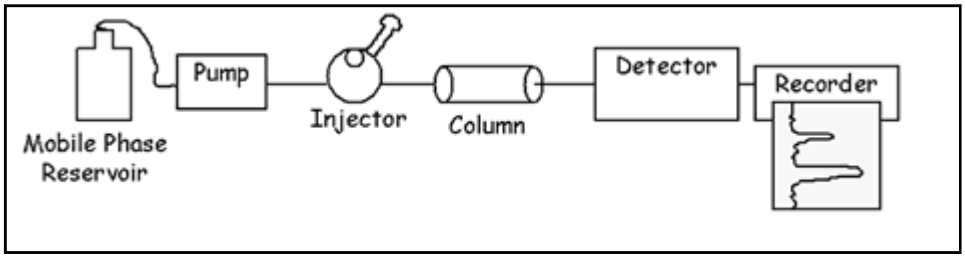

Figure 3.2 Schematic diagrams for HPLC analysis

In high performance liquid chromatography (HPLC) the liquid mobile phase is forced through the stationary phase using pressure. A simple HPLC would include a solvent reservoir to hold the liquid mobile phase, a pump to pressurize the liquid mobile phase, and injector to allow injection of a small volume of the sample mixture under high pressure, a column containing the bed of stationary phase, a detector to detect the presence of components as they exit the column, and some means to record the detector signal.

More sophistocated HPLC's may involve more than one pump to generate mobile phases mixtures, robotic arms capable of injecting perhaps 100 samples unattended (autosamplers), small ovens to control column temperature, mass spectral detectors capable of identifying components as they exit the column, and complete computer contral for automation.

3.3 Determination Mineral Contents

To determine the mineral content such as calcium and iron, procedure was same with privies method of pre-treatment, 20.0 g of flesh was extracted at 25°C (the temperature was chosen from room temperature as a control) for 30 min at 1 atm. Extractions were performed in triplicate. The concentrations of iron, zinc and magnesium in the papaya samples were determined in air-acetylene flame by the AAS method (A Perkin-Elmer Analyst 700 model atomic absorption spectrometer) using a deuterium. Manganese, cobalt, nickel, copper, arsenic, tin and aluminum

content were determined with an HGA graphite furnace, using argon as inert gas. Standards used to construct appropriate calibration curves were purchased from Perkin-Elmer (MA, USA). The same method was used for peel of papaya.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Single Factor Experiments

The value optimum using equation for concentration β-carotene was $3.54x10^{-5}$mg/L for peel and $3.01x10^{-5}$ mg/L for flesh of papaya. All the results in this study were computed from the above equation and absorbance 450nm.

4.2 Effect of Solvent Type on Extraction of β-carotene Compounds

Items	Concentration of β-carotene for Flesh			
	1	2	3	Average
Water	9.27586E-06	9.24138E-06	9.22759E-06	9.24828E-06
Acetone	1.9E-05	1.9E-05	1.9E-05	**1.9E-05**
Ethanol	1.69E-05	1.67E-05	1.72E-05	1.7E-05

Table 4.2(a) Effect concentration to type of solvent for flesh

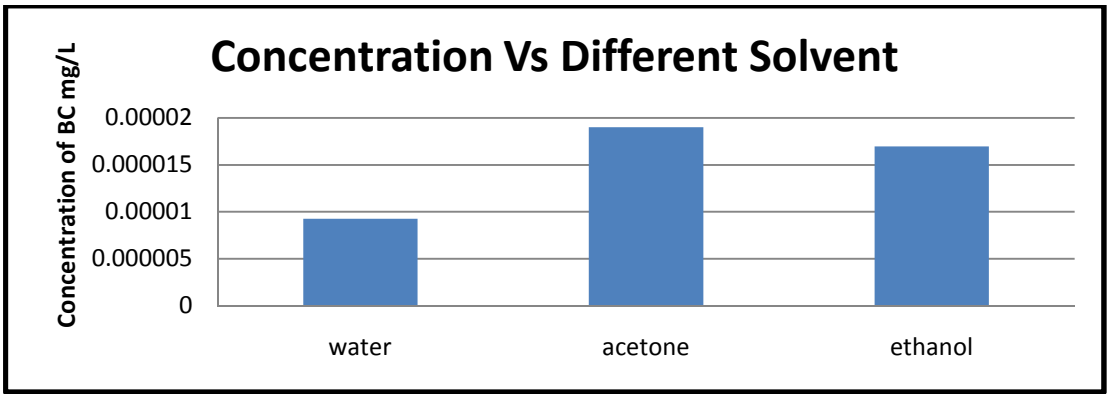

Figure 4.2(a) Effect concentrations to type of solvent for flesh

Items	Concentration of β-carotene for Peel			
	1	2	3	Average
Water	8.37E-06	8.37E-06	8.37E-06	8.37E-06
Acetone	2.45E-05	2.45E-05	2.45E-05	**2.45E-05**
Ethanol	2.06E-05	2.06E-05	2.06E-05	2.06E-05

Table 4.2(b) Effect concentration to type of solvent for peel

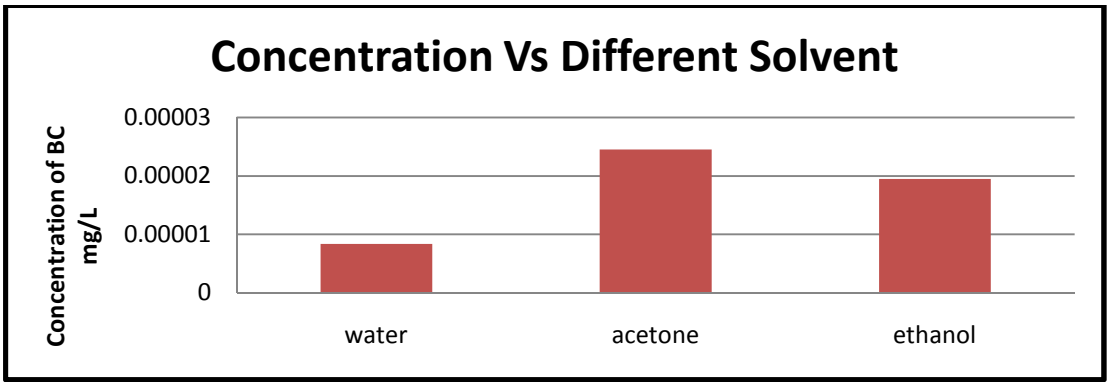

Figure 4.2(b) Effect concentrations to type of solvent for peel

The selection of extraction solvents is critical for the complex food samples as it will determine the amount and type of β-carotene compounds being extracted. Aqueous alcohols particularly acetone, ethanol and water are most commonly employed in carotene extraction from botanical materials (Naczk and Shahidi, 2004; Hayouni et al., 2007). Figure 4.2(a) and (b) showed that aqueous acetone was slightly better than aqueous water and aqueous ethanol in extracting β-carotene from flesh and peels of papaya under the same extraction conditions (1:3, 25°C, and 60 min).

However, the differences in β-carotene concentration among all the solvent extracts were not significant, indicating that β-carotene compounds from peels and flesh of papaya might present a wide coverage of polarity. The separation of β-carotene from the mixture of carotenoids is based on the polarity of a compound. β-carotene is a non-polar compound, so it is separated with a non-polar solvent such as acetone. Being highly conjugated, it is deeply colored, and as a hydrocarbon lacking functional groups, it is very lipophilic. So acetone which is categorized under GRAS (Generally Recognized as Safe) would be preferable in view of the

application in food system, (Mercadante, A.Z., Steck, A., Pfander, H, 1999). Acetone was chosen as the extraction solvent for the next experiments.

4.3 Effect of Solvent Ratio on Extraction of β-carotene Compounds

Table 4.3(a) Effect concentration of solvent ratio for flesh

Items	Concentration of β-carotene for Flesh			
	1	2	3	Average
1:1	6.9931E-06	6.9931E-06	6.9931E-06	6.9931E-06
1.2	7.64E-06	7.64E-06	7.64E-06	7.64E-06
1.3	1.53E-05	1.53E-05	1.53E-05	**1.53E-05**
1.4	1.39E-05	1.39E-05	1.39E-05	1.39E-05

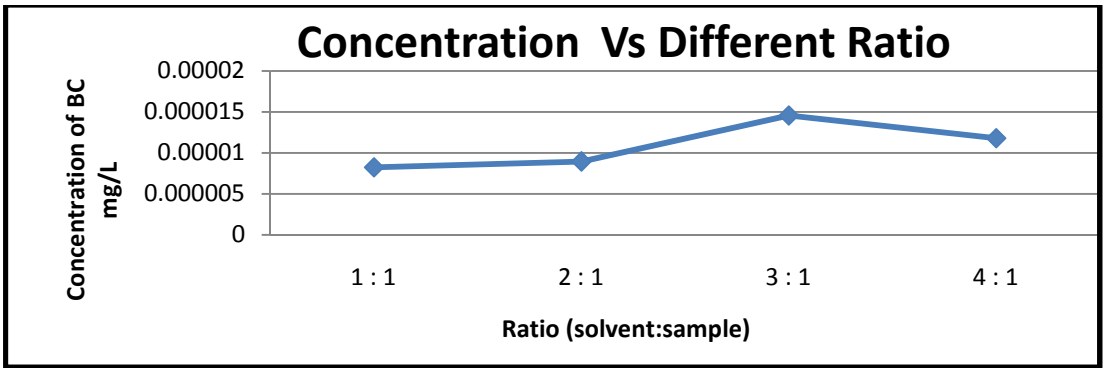

Figure 4.3(a) Effect concentrations to solvent ratio for flesh

Table 4.3(b) Effect concentration of solvent ratio for peel

Items	Concentration of β-carotene for Peel			
	1	2	3	Average
1:1	6.99E-06	6.99E-06	6.99E-06	6.99E-06
1.2	7.64E-06	7.64E-06	7.64E-06	7.64E-06
1.3	2.22E-05	2.22E-05	2.22E-05	**2.22E-05**
1.4	2.51E-05	2.51E-05	2.51E-05	1.81E-05

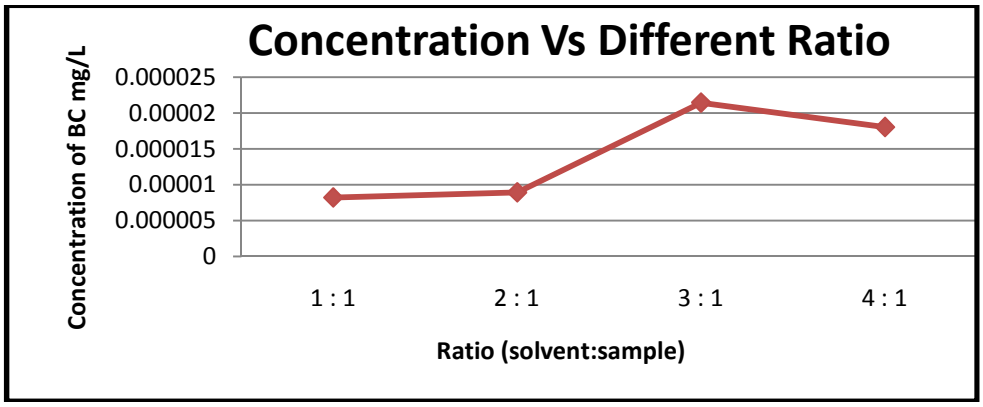

Figure 4.3(b) Effect concentrations to solvent ratio for peel

The effects of solvent ratio on extraction of β-carotene compounds from flesh and peel of papaya were shown in Figure 4.3(a) and (b). Concentration of β-carotene increased with the increment of the acetone concentration up to 75% (37.5ml solvent: 12.5ml sample) followed by a reduction until reaching a minimum of (40ml solvent: 10ml sample) at 80%. Similarly, Cacace and Mazza (2003) revealed that maximum concentration of β-carotene in carrot extracts was obtained at about 75% acetone followed by a decrease with further increase in concentration. Nepote et al. (2005) also found that increased the acetone ratio beyond 100% will dramatically reduced the amount of β-carotene extracted from carrot skins. A remarkable drop in concentration of β-carotene at 100% acetone revealed that absolute solvent do not ensure a good recovery of β-carotene compounds as compared to aqueous acetone. Thus, moderate acetone concentration of 50, 67% and 70% were selected as the lower, middle and upper levels, respectively, to be employed in solvent ratio optimization.

4.4 Effect of Extraction Time on Extraction of β-carotene Compounds

Table 4.4(a) Effect concentration of time for flesh

Items min	Concentration of β-carotene for Flesh			
	1	2	3	Average
10	7.66897E-06	8.48966E-06	8.42069E-06	8.1931E-06
20	1.46E-05	1.47034E-05	1.48345E-05	1.46989E-05
30	2.29E-05	2.07E-05	2.08E-05	2.15E-05
40	3.06E-05	3.07E-05	3.06E-05	3.06E-05
50	3.68E-05	3.68E-05	3.68E-05	3.68E-05
60	4.44E-05	4.51E-05	4.51E-05	**4.49E-05**

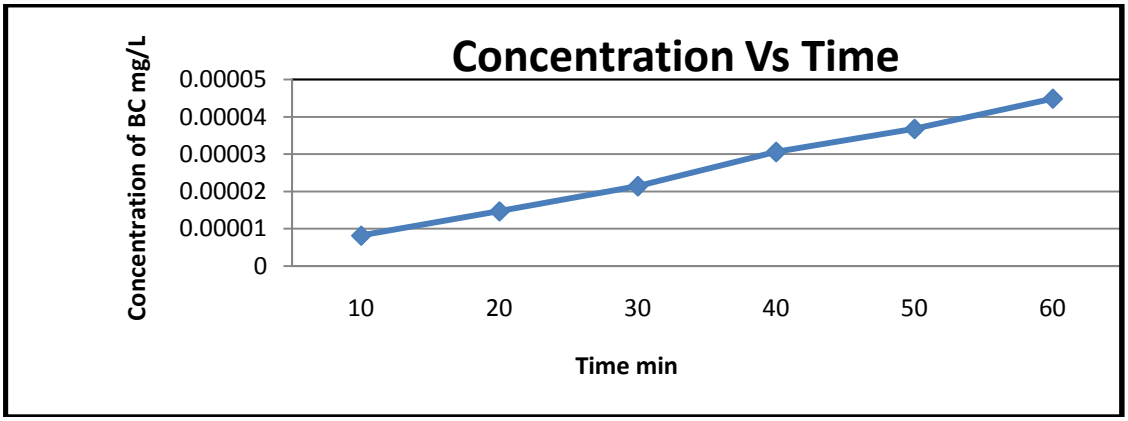

Figure 4.4(a) Effect concentrations to time for flesh

Table 4.4(b) Effect concentration of time for peel

Items min	Concentration of β-carotene for Peel			
	1	2	3	Average
10	7.66897E-06	8.48966E-06	8.42069E-06	8.1931E-06
20	1.46E-05	1.47034E-05	1.48345E-05	1.46989E-05
30	2.29E-05	2.07E-05	2.08E-05	2.15E-05
40	3.06E-05	3.07E-05	3.06E-05	3.06E-05
50	3.68E-05	3.68E-05	3.68E-05	3.68E-05
60	4.44E-05	4.51E-05	4.51E-05	**4.49E-05**

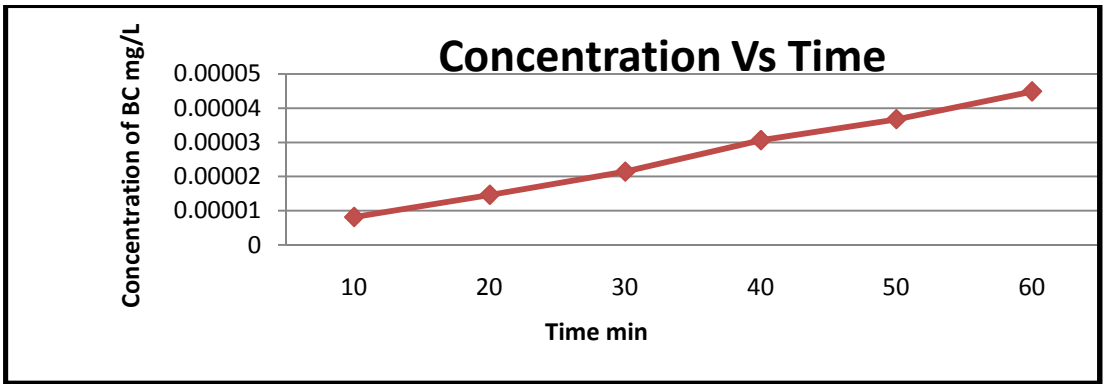

Figure 4.4(b) Effect concentrations to time for peel

Extraction time was another main parameter in the extraction procedure. The extraction time can either be as short as few minutes or very long up to 24 hours (Laponik et al., 2005; Lee et al., 2005). In this study, the range of extraction time was designed based on the practical and economical aspects. Figure 4.4(1) and (2) showed that an increase in extraction time increased from 20 to 60 min was accompanied by a small increment in concentration of β-carotene After 60 min; further increase in process duration did not significantly improve the recovery of β-carotene.

This observation was well explained by Fick's second law of diffusion, which stated that final equilibrium will be achieved between the solute concentrations in the solid matrix (plant matrix) and in the bulk solution (solvent) after a certain time, hence, an excessive extraction time was not useful to extract more β-carotene antioxidants (Silva et al., 2007). Furthermore, prolonged extraction process might lead to β-carotene oxidation due to light or oxygen exposure. Taking into account of these facts, an extraction time of 50–60 minutes was selected for extraction time optimisation. Therefore, moderate extraction time of 25, 45 and 60 minutes were chosen as the lower, middle and upper levels, respectively, to be applied in extraction time optimisation

4.5 Effect of Temperature on Extraction of β-carotene Compounds

Table 4.5(a) Effect concentration of temperature for flesh

Items °C	Concentration of β-carotene for flesh			
	1	2	3	Average
20	1.77E-05	1.76E-05	1.75E-05	1.76E-05
40	3.52E-05	3.54E-05	3.55E-05	3.54E-05
60	2.07E-05	2.08E-05	2.29E-05	2.15E-05
80	1.38E-05	1.39E-05	1.41E-05	1.39E-05
100	9.19E-06	9.19E-06	9.19E-06	9.19E-06
120	3.77E-06	3.77E-06	0.000003	3.51E-06

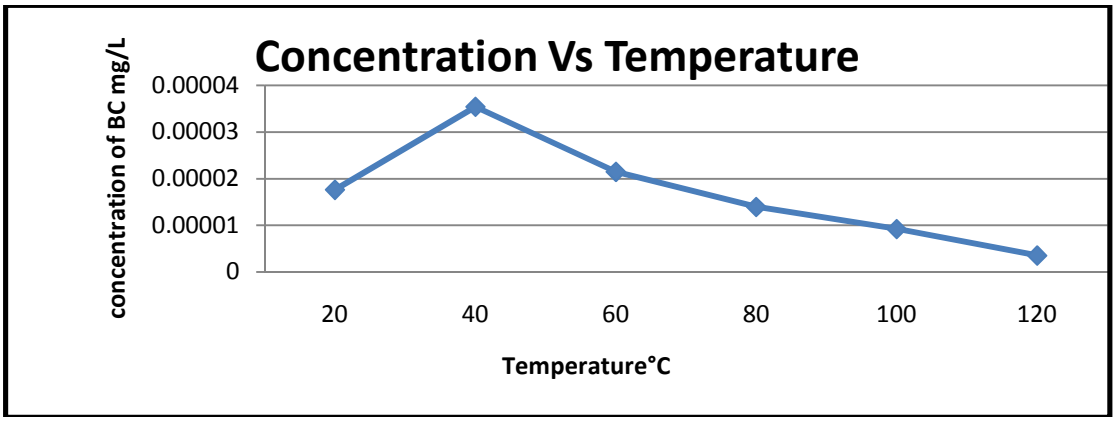

Figure 4.5(a) Effect concentrations to temperature for flesh

Items °C	Concentration of β-carotene for Peel			
	1	2	3	Average
20	1.472E-05	1.485E-05	0.0000138	1.446E-05
40	3.06E-05	3.06E-05	2.91E-05	3.01E-05
60	1.54E-05	1.39E-05	1.53E-05	1.49E-05
80	8.28E-06	8.94E-06	7.74E-06	8.32E-06
100	9.02E-06	9.02E-06	9.51E-06	9.18E-06
120	4.52E-06	4.51E-06	3.91E-06	4.31E-06

Table 4.5(b) Effect concentration of time for peel

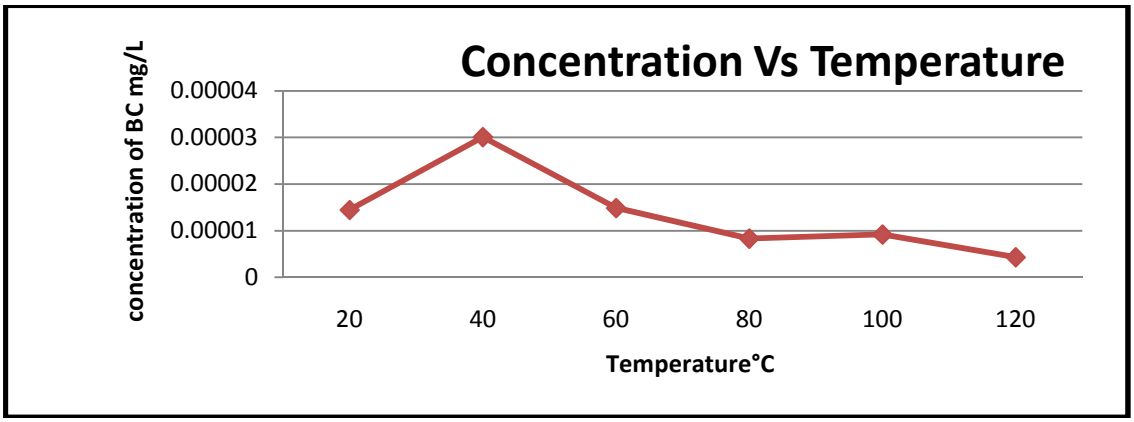

Figure: 4.5(b) Effect concentrations to temperature for peel

The selection of an appropriate extraction temperature was the final step in a series of single factor experiments. The extraction of β-carotene compounds was increased slightly when extraction temperature increased from 25 to 40 °C as reflected in Figure 4.5 (1) and (2) This result was in accordance with the study of Juntachote et al. (2006), which reported that concentration of β-carotene for flesh and peel of papaya increased at elevated temperature due to enhanced β-carotene solubility, faster diffusion rate, and increased mass transfer.

However, it should be noted that increasing the temperature beyond certain values may promoting possible concurrent decomposition of β-carotene compounds which were already mobilized at lower temperature or even the break down of β-carotene that are still remained in the plant matrix. Additionally, high temperature may encourage solvent loss through vaporization and increase the cost for extraction process from the industrialization point of view. Therefore, moderate extraction temperature of 60, 20 and 40°C were chosen as the lower, middle and upper levels, respectively, to be applied in temperature extraction optimization.

Optimum condition				
Type of solvent	Solvent ratio, ml: ml	Time extraction, minutes	Temperature extraction, °C	Concentration of β-carotene, mg/L
Acetone	**75%(1:3=sample: solvent)**	**60**	**40**	**Peel=3.54x1^{-5}** **Flesh=3.01x10^{-5}**

Table: 4.6 Optimum conditions and experimental value of response under those conditions

Verification of predictive model Table 4.6 showed that the experimental results were very close to the predicted one. This implied that there was a high fit degree between the values observed in experiment and the value predicted from the regression model. Hence, the response surface modeling could be applied effectively to predict extraction of carotene compounds from papaya peels.

4.7 Comparison Mineral Content in Local Fruits

Fruits	**Content Mineral mg/100g**						
Banana	**Calcium**	Copper	**Iron**	Magnesium	Sodium	Zinc	Potassium
Durian	20	12	0.2	0.5	2	3	134
Mangosteen	18	8	0.8	0.1	4	8	144
Rambutan	21	7	0.2	0.1	4	7	167
Cempedak	22	9	0.4	0.3	4	3	128
Papaya	**24**	10	**0.8**	0.2	3	5	176
Mango	23	14	0.1	0.5	1	4	244

Table 4.7 Comparison mineral content in Papaya and other local fruits

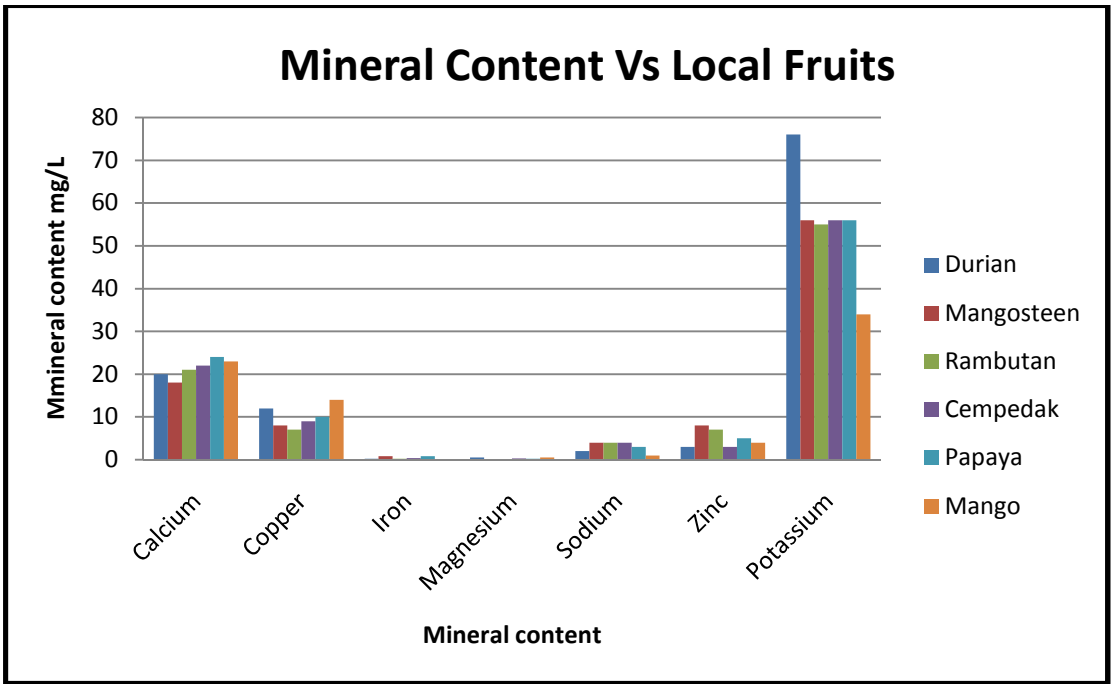

Figure 4.7 Comparison of mineral content in local fruit

Comparative the mineral content from sixth different species of type of local fruit (Durian, Mangosteen, Rambutan, Cempedak, Papaya and Mango was analyzed by AAS for their metal (Ca, Co, Mg, Fe, Zn and Na) content. The metal content of flesh and peel of papaya samples ranged from 0.2 mg for Mg, 0.8mg for Fe, 10mg.for Co, 5mg for Zn, and 24mg for Ca and 0.4 mg for Mg, 0.9mg for Fe, 12mg.for Co, 6mg for Zn, and 32mg for Ca.

4.8 Effect Microbe on β-Carotene

Weeks	Diameter, cm	
	Flesh	Peel
1	1.3	1.0
2	2.4	1.9
3	4.5	3.3

Table: 4.8 Effect diameter growth microbes in the period for flesh and peel of papaya

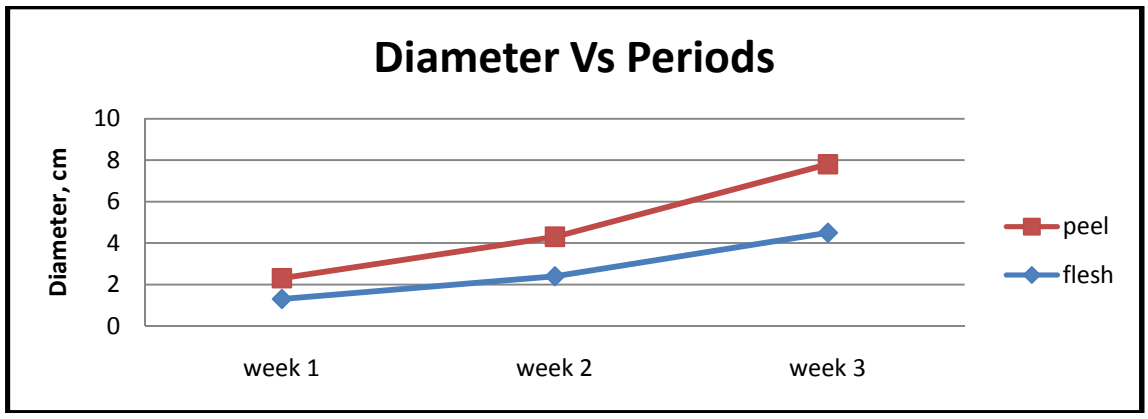

Figure: 4.8 Effect diameter growths to period

The ability *Escherichia coli* to grow on flesh and peel of papaya (Carica papaya) flesh and peel stored at significant times and significant temperatures was investigated. The flesh and peel of papaya with a normal pH were obtained aseptically, homogenized, weighed and inoculated with suspensions (approximately 102 CFU/g) of *Escherichia coli*. Viable populations of *Escherichia coli* were determined by the pour plate technique using of test portions on TSA agar. The standard temperature and other parameter was used in this procedure The results showed that *Escherichia coli* can grow on low acid fruit pulp, and that refrigeration at 30 C, although increasing the generation rate, does not inhibit its growth.

CHAPTER 5

CONCLUSIONS

The present study confirmed the advantages of experiment in optimizing the extraction conditions for β-carotene antioxidants from flesh and peel of papaya. The results from experiment showed that concentration of β-carotene papaya peels were most affected by acetone concentration followed by extraction temperature and extraction time. Using the numerical optimization method, the optimum conditions for maximum concentration of β-carotene were as follows: acetone ratio, 75%; extraction temperature, 40°C; and extraction time, 60 minutes. Under the mentioned conditions, ($3.54x10^{-5}$ mg/L) of β-carotene was extracted from the papaya peels, which well compare with the value of flesh of papaya ($3.01x10^{-5}$ mg/L) which is the optimum conditions for maximum concentration of β-carotene were as follows: acetone ratio, 75%; extraction temperature, 40°C; and extraction time, 60 minutes. The second experiment developed was satisfactory in describing and predicting the mineral content using extraction procedure from papaya peels and flesh of papaya. With the application of concentration of β-carotene from peel of papaya, the interaction effects among the extraction factors can be accessed as well the solvent usage and extraction time can be reduced as compared to single factor experiment. Further works may carry out under the optimum conditions to elucidate the identity of β-carotene compounds responsible for the antioxidant properties of peel of papaya.

CHAPTER 6

RECOMMENDATION

In any recommendation derived from this study, the following research will provide a better understanding of liquid-liquid extraction and utilization with respect to the extracted material:

- Characterization and identification of antioxidant compounds in optimum
- Parameter extracts from flesh, seed, flowers, stem, and root of papaya
- Characterization and identification of proteins in parameter extracts from peel of papaya
- Purification of extraction process extracts from papaya peel
- Exploring applications for β-carotene extracts of papaya peel
- Studying the effect of fungal and more type of microbe (mixed with water as solvent) in extraction of from peel of papaya using more efficiency process

CHAPTER 7

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APPENDICES A

Types of Papaya

APPENDICES B

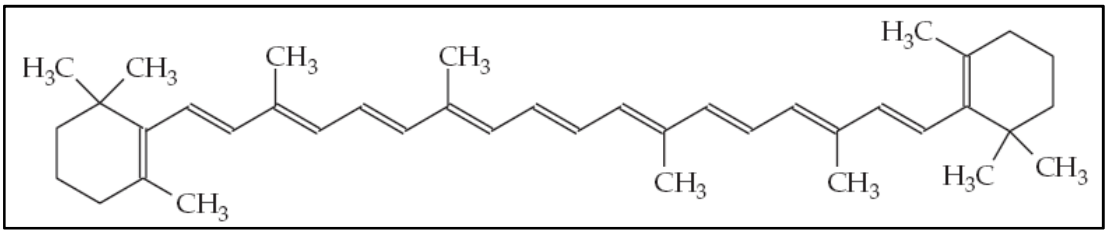

Figure molecule structure of β-carotene

Table 1
Metals and respective wavelengths

Metal	Wavelength (nm)
Iron	248.3
Zinc	285.2
Magnesium	213.9
Cadmium	228.8
Lead	283.3
Chromium	357.9
Manganese	279.5
Cobalt	240.7
Nickel	232.0
Copper	324.8
Arsenic	193.7
Tin	224.6
Aluminum	309.3

Table metal and respective wavelength

APPENDICES C

Figure the sample of β-carotene in peel of papaya

Figure the sample of β-carotene in flesh of papaya

Figure of effect microbe into β-carotene content (1 week)

Figure of effect microbe into β-carotene content (2 weeks)

Figure of effect microbe into β-carotene content (3 weeks)

APPENDICES D

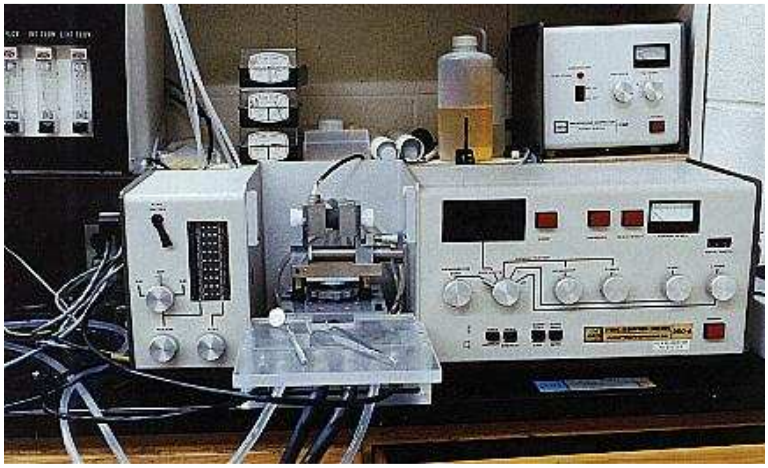

Figure of Atomic Absorption Spectrophotometer (AAS)

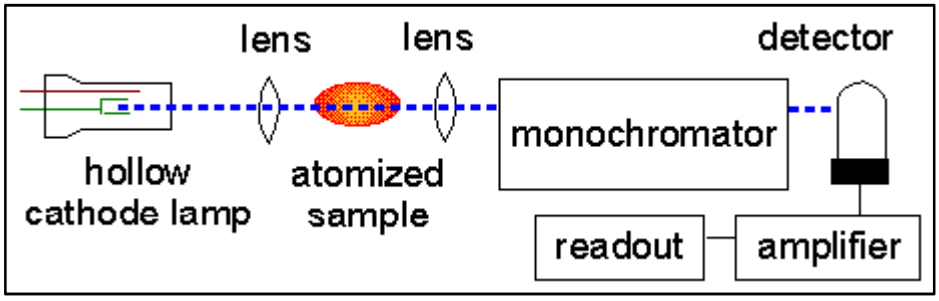

Figure of schematic diagram of AAS

Figure of high performance liquid chromatography (HPLC)

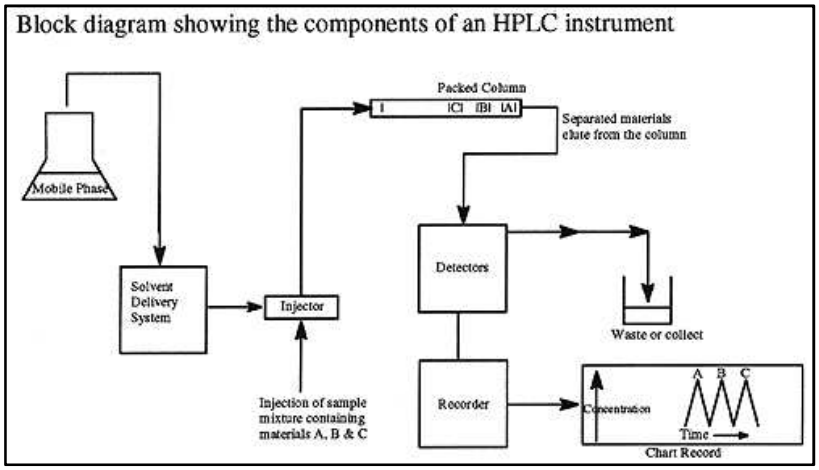

Figure of block diagram of HPLC instrument