Potential Use of Superheated-Steam Treatment in Underutilized Fruit of Engkala(Litsea Garciae) and Evaluation of Its Antioxidant Capacity

POTENTIAL USE OF SUPERHEATED-STEAM TREATMENT IN UNDERUTILIZED FRUIT OF ENGKALA (*Litsea garciae*) AND EVALUATION OF ITS ANTIOXIDANT CAPACITY

未活用果実のための過熱水蒸気処理の潜在的な利用とその酸化防止能力の評価

By

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بسم الله الرحمن الرحيم

In the name of Allah, the Beneficent One, the Merciful One

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Paper 1

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ABSTRACT

In the first chapter of the thesis entitled ‘Potential Use of Superheated Steam Treatment In An Underutilized Fruit of Engkala (*Litsea garciae*) and Evaluation of Its Antioxidant Capacity’, an introduction on the study was given where the link between antioxidants, free radicals and degenerative diseases, which is the increasingly deterioration of the function or structure of the affected body tissues or organ over time, such as cancer, atherosclerosis, arthritis, gastritis, diabetes mellitus and neurodegenerative diseases were discussed. The onset of these diseases was confirmed by accumulation of free radicals that are produced from oxidation process as well as from toxic present in the environment. The damaging effects of the free radicals however can be stopped by the action of antioxidants supplied by plants when incorporated in a human diet. In the introduction, the role of polyphenols as antioxidants and their source especially from fruits were also discussed and engkala and avocado fruits, which belong in the same family were introduced as source of antioxidant in this study. Drying process of food was also discussed, emphasizing the use of freeze drying and superheated steam.

In chapter 2, the characteristics of engkala fruit and its antioxidant capacity was evaluated. The physical properties, nutritional composition and minerals composition were discussed. This study showed that engkala fruit, just like avocado is a fleshy fruit where the pulp accounted for more than 50% of the fruit weight. Nutritional compositions of engkala showed that this fruit is high in moisture, protein, carbohydrate, K, Na, Fe, Zn and Ca. Polyphenol content of engkala was determined by measuring the total phenolic and total flavonoid content. Antioxidant activities of

engkala extracts were determined by using DPPH assay and ORAC assay. In DPPH assay, the concentration of the extract inhibiting 50% of the DPPH radical activity was measured as an indicator of the antioxidant activity. In this study, the edible part of the fruit which is the pulp showed highest activity when dried with superheated-steam at the temperature of 170°C. In ORAC assay, the oxygen radical absorbing capacity of the fruit extract was measured. The pulp of engkala also showed the same pattern, where superheated-steam dried pulp at the temperature of 170°C gave the highest ORAC value. This study showed that superheated-steam drying gave higher polyphenol contents and antioxidant activities in engkala pulp, while freeze drying gave higher results in the seed part of the fruit. The polyphenol compound present in engkala extract was also studies by using HPLC, TG/DTA, GC-MS and LC-MS system.

In the third chapter, polyphenol content and antioxidant activities in avocado fruit was evaluated. The avocado pulp followed the same pattern as shown by engkala pulp, where the total phenolic, total flavonoid content as well as antioxidant activities in the pulp was significantly higher when dried with superheated-steam. The peel and seed on the other hand showed significantly higher results when freeze dried.

In the fourth chapter, the antioxidant capacity of both engkala and avocado fruits were compared. The data showed that engkala pulp and seed showed higher antioxidant capacity compared to avocado. The peel on the other hand showed higher antioxidant capacity of avocado compared to engkala peel. Considering the

edible part of the fruit, engkala was ranked higher than avocado where antioxidant capacity was in concern.

The final chapters remark the conclusion of the whole study. It was concluded that both superheated-steam drying and freeze drying can offer their advantages in application of dried fruit production, where superheated-steam drying was preferred in drying the pulp of engkala and avocado, while freeze drying was the preferred method of drying for the seed and peel of engkala and avocado. The high potential of the by-products as given by the high antioxidant capacity especially when freeze dried suggested that they could be utilized and developed as natural antioxidants or food additives.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Aging process has been linked to degeneration of human biological systems. Apart from aging, many chronic and degenerative diseases including cancer, cardiovascular disease and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases have been associated with oxidative stress (Ames, Gold & Willet, 1995; Diaz et al., 1997; Christen, 2000; Lang & Lozano, 1998; Ames, Shinegana & Hagen, 1993). The presence of reactive oxygen species in the body, generated as by-products by normal cells during aerobic respiration can cause damage to biological molecules including DNA, proteins and lipids. The human body is created with a very delicate defence mechanism to protect itself from the harmful effects of the free radical. Even though it eliminates the free radicals from the body, the efficiency is not 100% (Young & Woodside, 2001; Davies, 2000). Exposure to toxins from the environment such as toxic chemicals, cigarette smoke, and air and water pollutants may also expose the body cells to harmful free radicals.

Diets rich in fruits and vegetables have been considered as excellent sources of antioxidants (Block, Patterson, & Subar, 1992; World Cancer Research Fund, 1997; Ness & Powles, 1997). Vitamin C, vitamin E, polyphenols and carotenoids have been thought to be responsible for most of the antioxidant activity in foods (Esterbauer et al., 1991; Jialal et al., 1990). While the available synthetic antioxidants may cause negative effects on human health, clinical trials on vitamin C, E and carotenoids supplements recently have provided inconsistent results (Cooper

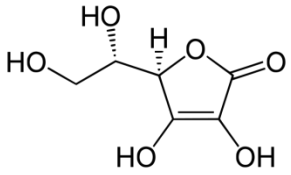
et al., 1999; Jha et al., 1995; Hercberg et al., 1999; Vivekananthan et al., 2003). Clinical trials with whole fruits and vegetables are more likely to give positive results in terms of disease prevention (Joshipura, Ascherio et al., 1999; Eastwood, 1999; Joshipura, Hu et al., 2001).

Polyphenols, being the most abundant antioxidants in a human diet, have been researched by many scientists. Fruits and vegetables are among the major sources of polyphenols. In this study, nutritional compositions, antioxidant capacity and phenolic compounds were measured and analysed for the first time in the different parts of an underutilized fruit of engkala (*Litsea garciae*). At the same time, antioxidant capacity of avocado fruit, which belongs in the same family as engkala, was also measured, and comparison was made among the two fruits.

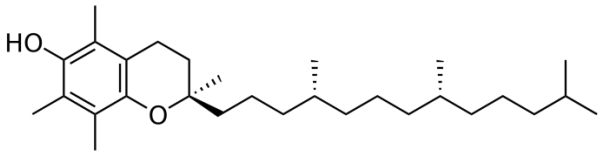
1.2. Antioxidants and free radicals

Antioxidant is something that can be defined as organic substance, such as vitamin C (ascorbic acid), vitamin E (tocopherols, tocotrienols), or beta carotene, that is capable of counteracting the damaging effects of oxidation or free radicals in living organisms. Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environment (Frie et al., 1988), produced during normal metabolism in the body (Shi et al., 1999), while other lighter antioxidants are found in the diet. Beta carotene is the most studied antioxidants, where more than 600 different carotenoids that have been discovered. Other than that, food sources of

antioxidants also come from polyphenol, selenium, glutathione, peroxidase and cystein (hprc-online.org, 2014).



Vitamin C



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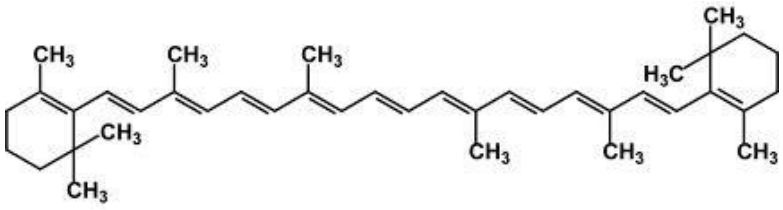


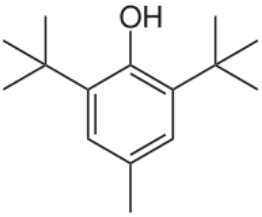
Beta carotene

Free radicals in the body on the other hand can be defined as reactive atom or group of atoms that has one or more unpaired electrons, produced in the body by natural biological processes or introduced from outside (tobacco smoke, x-ray, toxins, or pollutants) and can cause depletion of immune system antioxidants, damage cells, proteins, and DNA by altering their chemical structure (Dictionary.com, 2010). They can either donate or accept an electron from other molecules, therefore behaving as oxidants or reductants (Cheeseman & Slater, 1993). The most important oxygen-containing free radicals related to many diseases are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical (Young & Woodside, 2001). Free radicals can affect various biological functions. For example, they neutralize defence mechanisms such as enzymes (glutathione peroxidase, catalase, superoxide dismutase), glutathione, and

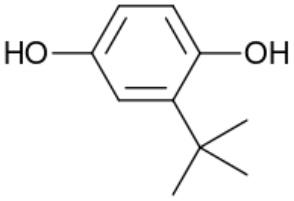
ferritin to maintain a balance (Pietta, 2000). Over production of some free radicals and their activity, particularly reactive oxygen species (ROS) can lead to oxidative stress, a condition which arises as a result of an imbalance between free radical production and antioxidant defences, making the endogenous antioxidant mechanisms insufficient for scavenging ROS (Kukic et al., 2006). Free radicals are considered as important factors in the initiation and development of aging-related diseases such as neurodegenerative diseases, cancer and inflammatory diseases (Aruoma, 1999). Many disorders in humans can be related to free radicals including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996).

Natural antioxidants in human body such as catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to nonradical forms. But due to depletion of these immune system natural antioxidants, it has become necessary to consume antioxidants as free radical scavengers (Halliwell, 1994; Kuhnan, 1976; Kumpulainen and Salonen, 1999; Younes, 1981).

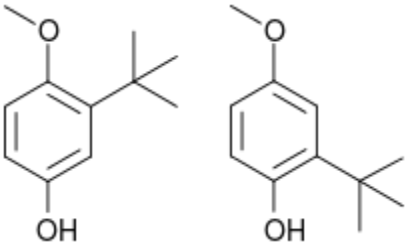
Currently available synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters show low solubility and moderate antioxidant activity. Moreover, they have been suspected to cause negative effects on human health. Thus, strong restrictions have been placed on their application and eventually, they have to be substituted with naturally occurring antioxidants (Barlow, 1990; Branen, 1975).



BHT



Tert-butylhydroquinon



BHA

1.3. Antioxidants in degenerative diseases prevention

Degenerative diseases are associated with aging where over time, oxidative damage to cell components, DNA, proteins and lipids accumulates and contributes to the degeneration of the somatic cells and the onset of these diseases (Scalbert, Johnson, & Saltmarsh, 2005; Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005). Natural defence mechanisms within the organism limit the levels of reactive oxidants and their damaging effects. Among the defences include enzymes such as superoxide dismutase, catalase and glutathione peroxidase. In addition to the protective effects of these endogenous enzymatic antioxidants, consumption of dietary antioxidants appears to be of great importance. Fruits and vegetables, which are the main source of antioxidants in the diet, are associated with a lowered risk of degenerative diseases. 172 studies in the epidemiological literature have been reviewed by Block, Patterson, & Subar (1992), which relates consistently, cancer

incidence to the lacking of adequate consumption of fruits and vegetables. Protective effect of fruit and vegetable consumption on cardiovascular disease and stroke has also been described (Gaziano et al., 1992). European countries with low fruit and vegetable intake (e.g. Scotland) have higher rates of cardiovascular disease and cancer and generally are poorer in health than countries with high intake (e.g. Greece) (James, Ferro-Luzzi, Isaksson, & Szostak, 1988).

1.4. Polyphenols as antioxidants

Polyphenols are the most abundant antioxidants in a human diet. Their sources mainly are fruits, vegetables, legumes, cereals as well as plant-derived beverages such as juices, tea, coffee and red wine. Research on the effects of dietary polyphenols on human health has strongly supports its contribution in prevention of degenerative diseases, particularly cardiovascular diseases and cancers and osteoporosis, as well as prevention of neurodegenerative diseases and diabetes mellitus. Degenerative diseases are associated with aging where over time, oxidative damage to cell components, DNA, proteins and lipids accumulates and contributes to the degeneration of the somatic cells and the onset of these diseases (Scalbert, Johnson, et al., 2005; Scalbert, Manach, et al., 2005). Antioxidants found in food can help limit this damaging effect by acting directly on reactive oxygen species or by stimulating endogenous defence systems. The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components (Kehrer & Smith, 1994). The antioxidant potency of polyphenols has been evaluated *in vitro* by measuring their ability to trap free radicals and reduce other chemicals. Their potency is compared to

that of a reference substance, usually Trolox (a water-soluble derivative of vitamin E), gallic acid, or catechin. In all cases, the reaction studied is the reduction of an oxidant by polyphenols (Scalbert, Manach, et al., 2005)

A study on *Litsea monopelata* has described antioxidant activity of extracted phenolic compounds (Arfan et al., 2008). Polyphenols retard or inhibit lipid autoxidation by acting as radical scavengers and, consequently, are essential antioxidants that protect against the propagation of the oxidative chain (Navarro et al., 2006). Experimental studies on animal and human cell lines have demonstrated that polyphenols can play a role in preventing cancer and cardiovascular diseases, when taken daily in adequate amounts (Wijngaard et al., 2009). Polyphenols have been identified to improve the status of different oxidative stress biomarkers (Williamson & Manach, 2005). In the field of cardiovascular diseases, significant progress has been achieved, where it is well established today that some polyphenols when incorporated into diet improve health status, as indicated by several biomarkers closely associated with cardiovascular risk (Vita, 2005; Keen et al., 2005; Sies et al., 2005). Furthermore, this protective effect of polyphenol consumption against cardiovascular diseases has been confirmed by epidemiologic studies (Arts & Hollman, 2005).

1.5. Fruits as source of polyphenols

Fruits contain a group of natural antioxidants that could have not only a high antioxidant activity but also a good combination or mixture of antioxidants (Hong Wang et al., 1996). Vitamins and polyphenols in fruits (and vegetables) are

considered to be responsible for their antioxidant activity, with polyphenols being the most active (Leja et al., 2003).

Wolfe et al. (2008) studied cellular antioxidant capacity of 25 fruits commonly consumed in the United States and found that pomegranate and berries (wild blueberry, blackberry, raspberry, and blueberry) had the highest cellular antioxidant activity. Li Fu et al. (2010) systematically evaluated 56 wild fruits from South China and indicated that generally, these fruits have high antioxidant capacities and total phenolic contents, where the antioxidant components in the wild fruits are capable of reducing oxidants and scavenging free radicals.

Recent epidemiological studies have associated benefits from consumption of apple and/or related products for many chronic diseases of humans, most noticeably in lowering risk of cardiovascular disease, lung dysfunctions, and various cancers, particularly prostate, liver, colon, and lung cancers (Knekt et al., 1996; Eberhardt et al., 2000; Le-Marchand et al., 2000; Xing et al., 2001). This biological impact of apple, similar to that of many other fruits, may be due largely to the presence of antioxidants (*5*), which are considered to be from phytochemicals such as polyphenolics, rather than from vitamin C, vitamin E, or beta-carotene (Hyson et al., 2000; Bors et al., 1990; Hanasaki et al., 1994; Wang et al., 1996).

1.6. Engkala (*Litsea garciae*) fruit

Litsea is a large genus of trees which belongs to the family Lauracea. It can be found in tropical Asia and eastwards to Australia and the Pacific. All *Litsea* species are aromatic and many of them have unpleasant odors. In Malaysia, some of *Litsea* spp. are applied in traditional medicine to treat boils and fever (Burkill, 1966). *Litsea cubeba* is believed to be the most popular species, which is the source of an internationally traded essential oil, flavor and fragrance material due to its pleasant citrus-like, fresh, sweet odor and taste (Coppen, 1995).

Litsea garciae Vidal or also known as Engkala is a native of Borneo, Indonesia and some say Philippines (www.tradewindsfruit.com/litsea.htm, 2010). Belonging to the Lauraceae family, which is the same family as avocado fruit, the fruit of *L. garciae* is pink to purple in colour, edible, and having a delicate avocado-like flavour. There are two recognized varieties in Borneo, known as *padi* and *bulan*. *Padi* is slightly smaller, more intense pink-fuschia colour when ripe, and reputed to be tastier, while bulan is the larger form, lighter pinkish-green when ripe, and creamier (Spanner, 2010). Traditionally, the bark of *L. garciae* is used to treat skin burns (Forest Department Sarawak, 2010), lightly burned bark is used to cure caterpillar stings, and the oil is extracted from the seeds to make soaps and candles (Hovenkamp, 2009).

The nutritional value of the fruit of *L. garciae* so far has only been studied by Voon and Kueh (1999). They reported that there are approximately 76 species of indigenous fruits found in Sarawak (Borneo), of which 16 of them were analyzed for their nutritional value. Out of all the fruits analyzed, five have been identified to have

good economic potential, and *L. garciae* is listed as one of them. Other than becoming popular and commercially important due to taste and flavour, it is also found to be highly nutritious (Voon and Kueh 1999). Another study on *L. garciae* was conducted by Lee et al. (1995) to investigate the alkaloidal contents. The study led to the isolation of bases namely laurolitsine, actinodaphnine, (+)-reticuline, isodomesticine and boldine.

Tree

Leaves

Flowers

Developing fruits

Fruits

Figure 1. *Litsea garciae* (engkala).

1.7. Avocado (*Persea Americana* Mill) fruit

Avocado fruit is classified in the Lauraceae family and is native to Mexico and Central America. The tree can grow up to 65 feet in height and the fruit vary in weight from 8 ounces to 3 pounds depending upon the variety (Chen et al. 2008; http://www.whfoods.com/genpage.php?tname=foodspice&dbid=5, 2012). Avocados are cultivated in tropical and Mediterranean climates throughout the world for their commercial importance. They ripen after being harvested where the fruit depicts a green-skinned, fleshy body that resembles the shape of either pear, egg or spherical. The trees are propagated through grafting to maintain a predictable quality and quantity of the fruit (http://en.wikipedia.org/wiki/Avocado, 2012).

Many studies have reported on the bioactive phytochemicals of this fruit. They include carotenoids (Lu et al. 2005), many phenolic acids and flavonoids (Rodriguez-Carpena et al., 2011; Kosińska et al., 2012). In medicinal usage, avocado has been applied in stimulating hair growth, wound healing, treating dysentery and diarrhoea as well as an emmenagogue and aphrodisiac (DerMarderosian & Beutler, 2002). Lutein, together with other carotenoids and vitamins found in avocado fruit extracts contributes to inhibition of prostate cancer cells (Lu et al. 2005). Many *in vitro* and *in vivo* studies have indicated that avocado contains many cancer preventing phytochemicals and it should be listed among the fruits with cancer prevention properties (Ding, Chin, Kinghorn, & D'Ambrosio, 2007). Phytochemical isolated from avocado idioblast cells have shown antifungal activities by inhibiting spores germination of a pathogenic fungus (Domergue et al. 2000). Antioxidant activities

have also been reported in avocado correlating to the high phenolic contents in the fruit (Wang et al. 2010).

1.8. Drying process of food

In food industry, drying is an important process. Drying is involved in food preparation as well as to extent its shelf life. The nutrients and quality of dried food products, however, can significantly be affected by the drying process. One of the most common techniques applied in drying process of food materials is freeze drying. This drying method enables food to maintain its colour and nutrient composition, thus producing good quality product. This attractive attribute however is not without disadvantages. The initial investment cost for freeze drying is expensive. The process also involves high energy consumption and maintenance cost (Jiang, Zhang, Liu, Mujumdar, & Liu, 2013; Zotarelli, Porciuncula, & Laurindo, 2012).

Steam drying is another drying technique which, in recent years, has been applied in many industries such as food, paper, furniture and timber as a method of drying (Mujumdar, 1995). Superheated-steam drying, is a method where steam with a temperature above the saturation or boiling point is applied as the drying medium. It offers numerous advantages by saving energy in one way and reducing energy wastage in another. It is also a safe drying method requiring low energy consumption. It is non-polluting, can improve production efficiency as well as product qualities (Tang & Cenkowski, 2000). The product quality from superheated-steam drying tends to be better than conventional hot air dried. The drying process involves no product oxidation, allows high vitamin C retention, pasteurization, sterilization and

deodorization of food products (Caixeta, Moreira, & Castell-Perez, 2002; Mujumdar & Law, 2010).

Drying process of plant materials is usually involved in many research conducted to access the polyphenols contents in fruits and vegetables, as well as their antioxidant activity. Studies have shown that the contents of polyphenols as well as antioxidant activity in dried plant materials can be higher compared to the fresh plant materials (C. H. Chang, Lin, Chang, & Liu, 2006; Choi, Lee, Chun, Lee, & Lee, 2006).

1.9. Problem statement and research objectives

The underutilized fruit of engkala is little known outside its native regions, consumed mostly by only the locals. Those outside the native regions has no or minimal information about the fruit and its potential. Although claimed to be highly nutritious, studies reported are still limited. The first objective of this study was to characterize the physical properties and nutritional composition of *Litsea garciae* fruit in order to evaluate its potential as a good source of functional food.

The use of superheated-steam in the process of food drying has captured more interest in the recent years. Reports on the application of superheated-steam drying on fruits however are very limited. The second objective of this study was to investigate on the effect of superheated-steam drying on antioxidant capacity of engkala and avocado fruits compared to the freeze dried, and also to study the phenolic compounds present in the fruit extracts.

Being an underutilized fruit, the ‘ranking’ of where engkala belongs as a source of antioxidant is unknown. Avocado, being in the same family and having similar physical properties as engkala, avocado fruit makes a good comparison for this under utilized fruit. The third objective of this study was to compare the antioxidant capacity between engkala and avocado fruits.

CHAPTER 2. ENGKALA FRUIT: EVALUATION OF ITS CHARACTERISTICS AND ANTIOXIDANT CAPACITY

2.1. Introduction

Increased attention to underutilized species can lead to great genetic diversity and heritage of indigenous knowledge. It also creates opportunity for the enhancement of the species mostly maintained by local communities. Many underutilized species are nutritionally rich. They complement the diet based on staple crops significantly by providing important vitamins and minerals (Food and Agriculture Organization of the United Nations, 1999).

The underutilized fruit of engkala is claimed to be highly nutritious (Voon & Kueh, 1999). It is usually eaten after being softened in hot water or steamed with rice. It is variably liked by first time consumers, but is a much-enjoyed delicacy to most of the locals. More studies are needed in order to help encourage the consumption of this fruit and promote its potential application as food for health as well as a potential antioxidant source. Hence, this chapter aims

- To do some characterization on engkala fruit
- To study the phenolic and flavonoid content and antioxidant activities in engkala fruit as affected by different drying processes
- To determine the phenolic compounds present in engkala fruit extracts.

2.2. Methodology

2.2.1. Chemicals

Folin-Ciocalteu's phenol reagent, gallic acid ($C_7H_6O_5$), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), rutin ($C_{27}H_{30}O_{16}$) were purchased from Sigma-Aldrich Chemie GmbH, Germany. Sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), hydrochloric acid (HCl) 37% were purchased from Merck, Germany. DPPH (2,2-diphenyl-1-picrylhydrazil) ($C_{18}H_{12}N_5O_6$), Fluorescein sodium salt ($C_{20}H_{10}Na_2O_5$), AAPH: 2,2'-Azobis(2-methyl propionamidine)dihydrochloride ($C_8H_{18}N_6$.2HCl), Trolox (6-Hydroxy -2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were purchased from Sigma-Aldrich Inc., USA. Phosphate buffer saline (PBS) was purchased from Invitrogen Corporation, CA, USA. All chemicals were of analytical grade. Phenolic standards were purchased from Sigma-Aldrich Inc. USA and Sigma-Aldrich Chemie GmbH, Germany. Methanol and acetic acid were purchased from Fisher Scientific, Leicestershire, UK, and water for chromatography was purchased from Merch, Germany, all were of HPLC grade.

2.2.2. *Litsea garciae* (Engkala) fruit

2.2.2.1. Physical Properties Study

10 of the smallest and largest individual fruits were selected from at least 7 kg of engkala fruits for determination of the physical properties. Length, width, flesh thickness, fruit mass (inclusive of pulp, peel and seed) and mass of individual parts

(pulp, peel, seed and cupule) were measured using a vernier caliper, Kern Germany, reading to 0.001mm. All mass were measured using electronic balance with a sensitivity of 0.001 g.

2.2.2.2. Sample Preparation

Fresh engkala fruits (*padi* variant) at the maturity stage were purchased from the local market in Sarawak, Malaysia. All the fruits were cleaned and manually separated into pulp, peel, seed and cupule and then were dried. The moisture loss from each part was measured using Moisture Balance MOC-120H, Shimadzu Corporation Japan.

2.2.3. Drying methods

Half of the fruits were subjected to each drying process, i.e. superheated-steam drying or freeze drying to produce superheated-steam dried (SHSD) and freeze dried (FD) samples respectively. The former process was conducted in a superheated steam oven (DC Quto QF-5200C, Naomoto, Japan) at different steam temperature of 130°C, 150°C and 170°C, while the latter treatment was operated at -50°C in a freeze dryer (EYELA FDU-1200, Tokyo Rikakikai Co. Ltd., Japan). The fruit parts were dried until they reached the final moisture content of ~10%, measured using Moisture Balance MOC-120H (Shimadzu Corporation, Japan). Dried fruit parts were ground to fine powder using Waring commercial blender 8011S (Connecticut, USA) and kept at -40°C until further use.

2.2.4. Nutritional compositions

Peroximate composition (inclusive of energy, moisture, protein, fat, carbohydrate, crude fibre and ash), minerals composition inclusive of potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zink (Zn) and sodium (Na), as well as Vitamin C were determined using laboratory procedures which were in accordance with the official method of analysis of Association of Official Analytical Chemists (AOAC) International (Horwitz, 2002).

2.2.5. Sample extraction

Ultrasonic-assisted extraction as described in chapter 3 (section 3.2.4.) was applied. One gram of dried sample was extracted in 80% ethanol with ratio of solid:liquid was at 1:30 (wt/vol). The mixture was ultrasonicated for 15 min using a 37kHz ultrasonic generator (UT-106, SHARP, Japan) and then centrifuged at 400 rpm at the temperature of 40°C for 30 min using Heidolph Instrument Unimax 1010DT orbital shaker, Germany. The extracts were then filtered using a Whatman No. 4 filter paper and kept at -40°C until further use.

2.2.6. Phytochemical Study

2.2.6.1. Total Phenolic Content (TPC)

Total phenolics were determined using the modified Folin-Ciocalteau colorimetric method (Waterhouse 2002; Wolfe et al. 2008). 0.25ml of ethanolic extract was

diluted with 1ml distilled water in a test tube. 0.25ml Folin-Ciocalteau reagent was added to the solution and allowed to stand for 6 min. Then, 2.5ml of 7% sodium carbonate solution was added into the test tubes, and the mixture was diluted to 6ml with deionized water. Each sample was allowed to stand for 90 minutes, and measured at 760 nm using a UV-Vis Spectrophotometer, Shimadzu Corporation. The measurement was compared to a standard curve of gallic acid concentrations and expressed as milligrams of gallic acid equivalents (GAE) per 100g dried sample.

2.2.6.2. Total Flavonoid Content (TFC)

The measurement of total flavonoid content was determined using a modified colorimetric method by Wolfe et al. (2003). 0.5 ml of ethanolic extract was mixed with 2.5ml of distilled water in a test tube. And this solution was mixed with 0.15ml of 5% sodium nitrite solution. After 5 minutes, 0.3ml of 10% aluminum chloride solution was added. After 6 minutes, 1ml of 1M sodium hydroxide was added and mixed. The total volume of mixture was made up to 5ml with distilled water. The sample absorbance was read immediately at 510nm using UV-Vis Spectrophotometer, Shimadzu Corporation. All measurements were compared to a standard curve of rutin solutions. The flavonoids content was expressed as milligrams of rutin equivalents (RE) per 100g dried plant sample.

2.2.7. Antioxidant Activities

2.2.7.1. DPPH radical scavenging activity

The capacity of the fruit extract to scavenge the free radical DPPH (2,2-diphenyl-1-picrylhydrazil) was carried out using colorimetric method (Othman, Ismail, Abdul Ghani, & Adenan, 2007) with modification. Briefly, 1 ml of ethanolic extract was mixed with 2 ml 0.15mM DPPH in ethanol. The mixture was left in the dark for 30 min before measuring the absorbance at 517nm using a UV-Vis spectrophotometer UV1601 (Shimadzu Corporation, Australia). The ethanol solution of DPPH served as a control. The percentage inhibition value was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = [(A_0 - A_s) / A_0] \times 100\%,$$

Where A_s is the absorbance of the sample and A_0 is the absorbance of the blank control. The inhibition percentage was plotted against the appropriate known concentrations and the sample concentration providing 50% inhibition (IC_{50}) of the DPPH radical was determined from this graph. All tests were carried out in triplicates.

2.2.7.2. Oxygen radical absorbing capacity (ORAC)

The peroxyl radical scavenging efficacy of the avocado pulp extracts were measured using the ORAC assay (Ahmad Aufa, Hassan, Ismail, Mohd Yusof, & Hamid, 2014). Briefly, a volume of 150 μL of 10nM fluorescein in 10mM sodium phosphate buffer, pH 7.4 (working buffer) was added to each well in a black, clear-bottom, 96-well

microplate. Then 25 μL of blank (working buffer), Trolox standard or sample was added to triplicate wells. No outside wells were used in order to avoid results with greater variation. The mixture was then incubated at 37°C for at least 10 min. 25 μL of freshly prepared 240 mM 2,2'-Azobis(2-methylpropionamidine)-dihydrochloride (AAPH) in working buffer were added using a 12-channel pipetter. The microplate was immediately inserted into a Fluostar Omega microplate reader (BMG LabTech GmbH, Ortenburg, Germany) at 37°C. The decay of fluorescence at emission wavelength 520nm was measured with excitation at 485 nm every 1.5 min for 3 hrs. The areas under the fluorescence versus time curve for the samples minus the area under the curve for the blank were calculated and compared to a standard curve of the areas under the curve for 25, 50, 100, 200 and 400 μM Trolox standards minus the area under the curve for blank.

ORAC value = $(AUC_{sample} - AUC_{blank}) / (AUC_{Trolox} - AUC_{blank})$, where AUC is area under the curve.

ORAC values were expressed as mean micromoles of Trolox equivalents (TE) per 1g of dried fruit sample.

2.2.8. Phenolic Compounds Analysis

2.2.8.1. Extraction of Phenolic Compounds

Extraction was performed using method as described by Chew et al. (2012) with slight modifications. 1 g of dried sample was subjected to ultrasonic-assisted extraction in 30 ml 80% ethanol (v/v) in the presence of HCl (final concentration of HCl was 1.2 N). The mixture was sonicated for 15 min using a 37kHz ultrasonic generator (UT-106, SHARP, Japan) and then shaken at 40°C using orbital shaker (Heidolph Instrument Unimax 1010DT, Germany) at 400 rpm for 30 min. The sample extract was filtered using a Whatman No. 4 filter paper. The filtrate was washed once with 60 ml hexane in a separating funnel to remove lipoidal materials. The recovered aqueous phase was further refluxed for 2 hours at 80°C and finally filtered through a 0.45-µm membrane filter prior to analysis.

2.2.8.2. Reversed Phase-High Performance Liquid Chromatography (HPLC) Analysis

Analysis of phenolic compounds was performed using Agilent 1100 Series liquid chromatographic system equipped with an Agilent 1100 Series diode array detector (DAD) HPLC system (Agilent Technologies, Germany). A reversed phase Lichrospher C-18 column (250×4 mm, i.d. and particle size 5 µm; Merck KGaA, Darmstadt, Germany) was used as a solid phase for separation. Analysis was conducted according to a method as described in a reported study (Chew et al., 2012) with slight modification. The mobile phases used were (A) 0.5% acetic acid

(v/v) and (B) methanol. The gradient elution profile was as follows: 0–20 min, linear gradient from 0% to 90% B; 20–25 min, 90% B isocratic; 25–30 min, linear gradient from 90% to 0% B and finally, washing and reconditioning of the column. The flow rate and column temperature were set at 2.0 ml/min and 30°C respectively. Wavelengths used for detection of phenolic acids and flavonoids were 280, 254 and 329 nm. UV absorption spectra were recorded from 210 to 400 nm during HPLC analysis.

The phenolic compounds in the sample extracts detected in the chromatograms were identified by comparing their retention times (t_R) and UV–vis absorption spectra with those of authentic standards, and further confirmed by spiking the sample with authentic standards. The purity of each phenolic compound identified was checked by HPLC-DAD.

2.2.8.3. Thermo Gravimetry (TG) / Differential Thermal Analysis (DTA)

Degradation temperature of substances present in the samples was analysed by using Exstar S2 TG/DTA7200 thermo gravimetry / differential thermal analyser (Hitachi High-Tech Science Corporation, Japan), where changes in mass of the samples over temperature increase were measured at the temperature range of 30 - 550°C with rate of temperature increase at 10°C/min. Nitrogen gas was used as the carrier and the sample used ranged at 5-9 mg.

2.2.8.4. Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

GC-MS analysis on the samples was done using GCMS-QP5050A, coupled with Double Pyrolizer PY-2020D, Shimadzu, Japan. Temperature was set at 60 - 550°C at the rate of 10°C/min. Sample in minute quantity was used and put into deactivated stainless-steel cup.

2.2.8.5. Liquid Chromatography – Mass Spectrometry (LC-MS) Analysis

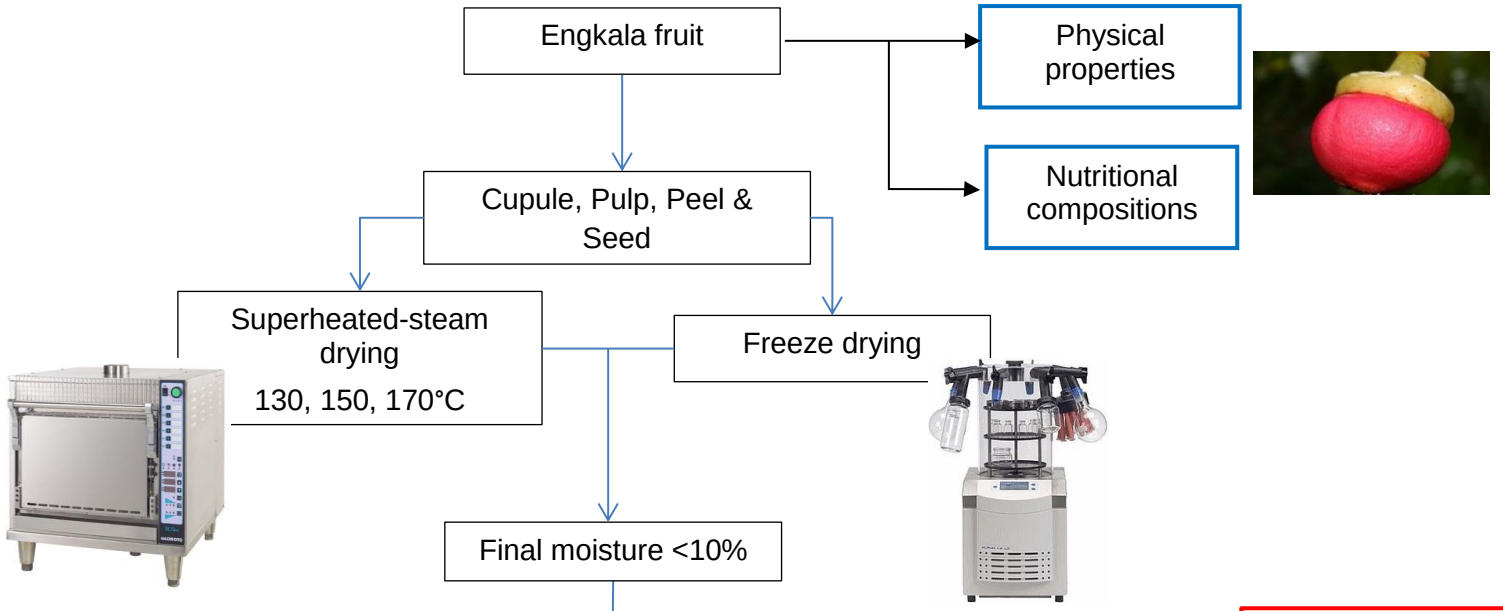
1uL sample at room temperature was injected and analysed using Acquity UPLC H-Class PDA/QDa System, Waters Corporation. Acquity UPLC HSS T3 1.8um, 2.1x100mm column was used for separation. The mobile phases used were (A) acetonitrile, (B) water and (C) 100mM ammonium formate. The gradient elution profile was as follows: 1-10 min, linear gradient from 5% to 90% A, from 85% to 0% B and 10% C isocratic; 10-15 min, linear gradient 90% to 5% A, 0% to 85% B and 10% C isocratic, and finally washing and reconditioning of the column using acetonitrile. The flow rate and column temperature were set at 0.6 mL/min and 40°C respectively. Wavelength of 280 nm was used for detection of polyphenolic compounds. UV absorption spectra were recorded from 215 to 450 nm during analysis.

The mass spectrometer was operated in both negative and positive electrospray ionization (ESI) modes, in a range of 100 - 1000 Da. The capillary temperature was set at 600°C. The run time was 20 min. In the MS analysis (negative and positive full scan modes), data was collected over a mass of 100-800 m/z. The phenolic

compounds present in the sample extracts and detected in the chromatograms were analysed by observing their retention times (t_R), UV-vis absorption spectra, and MS.

2.2.9. Statistical analysis

All experiments were performed in triplicates. The results were expressed as mean ± standard deviation (S.D). The experimental data were analyzed using analysis of variance (ANOVA) (Microsoft Excel 2010). The mean values were considered at the 95% confidence level ($p = 0.05$). Correlation between the phenolic contents and the antioxidant activity was determined using IBM SPSS Statistics (version 19).



Total Phenolic Content	Total Flavonoid Content
Measures phenolic acids content; Folin-Ciocalteau colorimetric method (Waterhouse, 2002; Wolfe et al., 2008).	**Measures flavonoids content**; colorimetric method by (Wolfe et al., 2003) with slight modification.
Absorbance at 760 nm; UV-Vis spectrophotometer	Absorbance at 510nm; UV-Vis spectrophotometer
Data expressed as mg gallic acid equivalents (GAE) /100 g dw	Data expressed as mg rutin equivalents (RE) /100g dw

DPPH Assay	ORAC Assay
Measures the capacity of fruit extract to scavenge DPPH free radical; Colorimetric method (Othman et al., 2007)	**Measures oxygen radical scavenging efficacy of fruit extract**; Ahmad Aufa et al. (2014)
Absorbance at 517nm; UV-Vis spectrophotometer	Decay of fluorescence at emission wavelength 520nm; multi-mode spectrophotometer
Data expressed as sample concentration providing 50% inhibition (IC_{50}) of the DPPH radical	Data expressed as µmol Trolox equivalents (TE) /1g dw

2.3. Results and discussion

2.3.1. Physical properties, yield and nutritional compositions

Engkala fruits are of two well-known varieties, namely *padi* and *bulan* which differ in appearance, mainly size and colour. Padi is the smaller variant with more intense pinkish skin colour, while bulan is the larger sized with lighter colour and creamier taste.

Just like avocado, it has a large and hard central seed. The edible portion of engkala is its cream-white flesh, which has a creamy but light consistency, as well as the skin, which is thin and smooth in texture. Once ripen, the fruit unfortunately does not keep long and will become rotten after about 2-3 days.

In this study, 10 of the smallest and 10 of the largest sized fruits were measured to give the characteristics of the small and large groups of both *padi* and *bulan* variants. The differences between the variants were summarized in Table 1. Generally, the round fruit of engkala was 1.9 - 3.15 mm long, 2.42 - 4.54 mm in width, weighed between 5.9 and 32.9 grams without the cupule attached, and having flesh with thickness of 0.5 – 0.84 mm.

This result upholds the character of engkala being a fleshy fruit, where the percentage of the pulp was more than 50% of the total fruit weight (excluding the cupule). The seed account for 24 – 40% of the fruit weight, while the skin was the part of the fruit which took the least of the weight, with only <20%.

Table 1. Physical properties of engkala fruit.

Properties	*Padi*		*Bulan*	
	Small	Large	Small	Large
Length, mm	1.900 - 2.317	2.500 - 2.767	2.138 - 2.427	2.862 - 3.152
Width, mm	2.417 - 2.610	3.067 - 3.533	2.570 - 3.267	4.248 - 4.538
Flesh Thickness, mm	0.460 - 0.663	0.333 - 0.467	0.453 - 0.755	0.570 - 0.840
Fruit mass, g	5.902 - 7.186	14.923 - 20.490	7.131 - 12.426	27.785 - 32.902
Pulp, g	3.083 - 4.634	7.514 - 11.445	4.718 - 7.803	16.473 - 19.592
Seed, g	1.218 - 1.941	5.964 - 8.043	1.035 - 4.669	8.385 - 11.463
Skin, g	0.995 - 1.535	1.407 - 2.078	0.769 - 1.267	1.487 - 2.077
Cupule, g	3.114 - 5.372	4.629 - 7.110	2.017 - 5.649	8.423 - 11.266

n=10 for each group

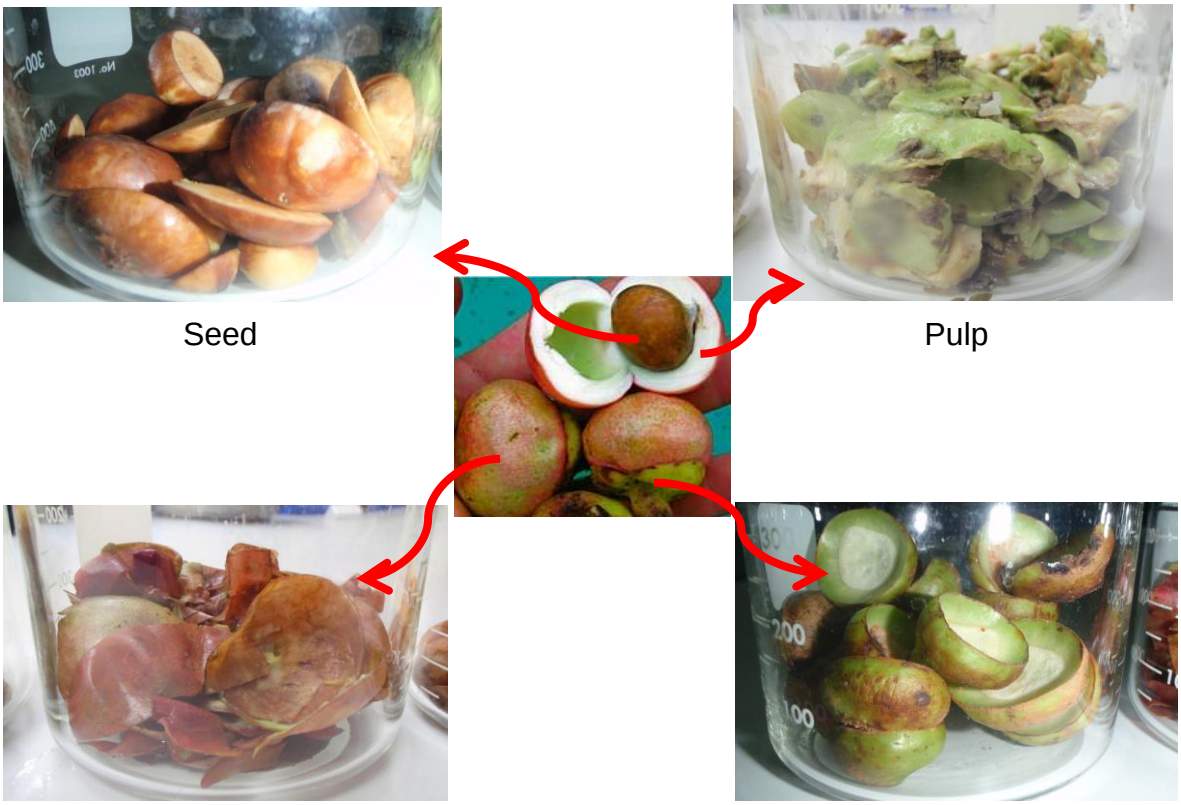


Figure 2. Engkala fruit parts.

Table 2 shows percentage yield of engkala fruit parts dried with two different methods, namely superheated-steam drying and freeze drying. In SHSD samples, seed gave the highest yield (average of 39.95%), while in FD samples, pulp (30.34%) yield the highest when dried.

It has been reported that the moisture content of engkala fruit is 78.3% (Voon & Kueh 1999).The obvious difference between superheated-steam drying and freeze drying was the duration of the fruit parts to reach the final moisture of ~10%. The pulp took 3 hrs, the cupule and seed took 2 hrs while the peel only took 1 hr to reduce their moisture content to the desired percentage. The freeze dried samples for all the fruit parts on the other hand took many days to reach the similar result (data not shown).

Table 2. Percentage yield and final moisture of SHSD and FD engkala.

		Yield (%)	Final moisture (%)	± S.D.
Cupule	FD	14.95	6.29	0.46
[a]2 hrs	SHSD, 130°C	15.47	10.32	0.11
	SHSD, 150°C	15.04	8.89	0.59
	SHSD, 170°C	14.32	5.23	0.75
Pulp	FD	30.34	4.16	0.27
[a]3 hrs	[b]SHSD, 130°C	24.27	6.94	0.52
	SHSD, 150°C	26.66	5.10	0.52
	SHSD, 170°C	25.12	3.35	0.87
Seed	FD	26.28	7.69	0.44
[a]2 hrs	SHSD, 130°C	44.35	10.89	0.08
	SHSD, 150°C	38.33	9.08	0.25
	SHSD, 170°C	37.17	7.55	0.36
Peel	FD	24.44	5.90	0.31
[a]1 hrs	SHSD, 130°C	28.33	5.32	0.46
	SHSD, 150°C	22.80	5.28	0.60
	SHSD, 170°C	20.10	4.51	0.49

Results expressed as mean ±S.D. (n=3). [a] duration of SHS treatment for the group. [b] additional 30 mins.

Table 3, 4 and 5 gave the nutritional compositions of engkala fruit. These data indicated that engkala fruit was high in moisture, protein, and carbohydrate. As for the minerals, K was the dominating one. Other minerals namely Na, Fe, Zn (highest in the pulp) and Ca (highest in the peel) were also high.

Fruits are very good source of vitamin C and polyphenols. Most fruits are usually consumed fresh. Engkala however, is usually either soaked in hot water or steamed with rice before consumption to soften the fruit and make it more palatable. For the analysis of vitamin C, the content was found to be higher in the pulp than in the seed for both FD and SHSD samples. The SHSD samples for both pulp and seed however showed significantly higher amount of vitamin C, which nearly tripled the amount compared to the FD samples.

Table 3. Peroximate composition of engkala fruit.

	per 100g fresh sample						
	Energy (kcal)	Moisture (g)	Protein (g)	Fat (g)	CHO (g)	Crude fibre (g)	Ash (g)
Pulp	93.3	65.1	2.4	0.0	20.9	3.8	2.4
Seed	83.4	68.2	3.1	0.0	17.7	4.8	1.3

Results expressed as mean (n=3).

Table 4. Minerals composition of engkala fruit.

	mg per 100g fresh sample						
	K	Ca	Mg	Fe	Cu	Zn	Na
Cupule	607.3	11.3	0.8	0.7	0.1	0.5	12.4
Pulp	652.9	4.2	3.7	4.9	1.0	1.6	91.5
Seed	331.5	2.4	1.8	1.1	0.6	1.1	6.3
Peel	531.9	12.9	2.2	1.4	0.6	1.1	22.2

Results expressed as mean (n=3).

Table 5. Vitamin C content in engkala fruit.

	Vitamin C (mg/100g fresh sample)	
	FD	SHSD
Pulp	11.8 ± 0.2	34.7 ± 2.8
Seed	4.8 ± 0.2	13.0 ± 0.3

Results expressed as mean ±S.D. (n=3).

Table 6 compares the nutritional compositions of engkala with avocado as well as other common local fruits, namely banana, papaya and durian (USDA, 2012). This study examined engkala in its different fruit parts as opposed to the study done by Voon & Kueh (1999) which study the fruit as a whole.

In this study, energy content in the pulp (93.3 kcal) of engkala was found to be higher than the seed (83.4 kcal), slightly lower than reported by Voon & Kueh (104 kcal). The energy content in engkala was lower compared to avocado (160 kcal) and durian (147 kcal) but higher than banana (89 kcal) and papaya (43 kcal).

Protein in the seed (3.1g) was higher than in the pulp (2.4g), while Voon & Kueh reported that the whole fruit has 1.4% protein content. Protein in the pulp and seed in this study was found to be higher than all the other fruits in comparison.

Engkala parts in this study however showed no content of fat, contrasting the fat content reported by Voon & Kueh (6.8%).

Carbohydrate content of the pulp (20.9g) was found to be higher than the seed (17.7g). Voon & Kueh reported that the fruit has 10% carbohydrate content. Carbohydrate of engkala in this study was found to be higher than avocado and papaya but lower than durian and banana.

Voon & Kueh reported 1% of crude fibre in engkala fruit. This study gave 3.8g crude fibre in the pulp and 4.8g in the seed.

As for mineral content, the highest was found to be potassium (K), where the highest content was in the pulp (652.9mg), followed by cupule (607.3mg), skin (531.9mg) and seed (331.5mg). Voon & Kueh reported 355mg K in the fruit. K content in the edible portion (pulp and skin) was significantly higher compared to avocado and all the local fruits.

Voon and Kueh did not report on sodium (Na) but this study found that Na content in engkala was higher than the other fruits. The highest was found in the pulp (91.5 mg), followed by the skin (22.2 mg), cupule (12.4 mg) and seed (6.3 mg).

Calcium (Ca) was also reasonable high in engkala especially the skin (12.9 mg). Earlier report only shows 1.0 mg Ca content in engkala (Voon & Kueh, 1999). This amount was higher than reported on avocado (12 mg), banana (5 mg) and durian (6 mg), but lower than papaya (20 mg).

Iron (Fe) was another mineral with high content in engkala fruit. Fe was highest in the pulp (4.9mg) followed by the skin (1.4mg). Fe content in this study was higher compared to as reported by Voon and Kueh (0.5mg) as well as the other fruits.

Zink (Zn) was another mineral found to be present higher in engkala than the other fruits. The highest amount was found in the pulp (1.6 mg). Higher amount was also found in the peel and seed (1.1 mg). This result was found to be similar to the Zn content reported earlier (1.02 mg) (Voon & Kueh, 1999).

Some differences in the amount of nutritional contents between engkala fruit reported in this study and the one reported by Voon & Kueh (1999) might be due to difference in fruit variants and sampling location. Fruits of the same species but grown at different locality might contain different nutritional contents due different environmental factors. There are many factors that can influence the nutritional composition of produce, including environmental and cultural factors. The environmental factors which are likely to affect food quality include geographical area, soil type, soil moisture, soil health, pollution, weather and climate conditions such as temperature, rainfall, flooding and drought. Cultural practices including humus management techniques, variety, seed source, fertilization, cultivation and postharvest handling are also likely to affect food quality (Diver, 2002). A study on cherry tomato grown in different environmental factors, namely temperature, solar radiation and vapour-pressure deficit shows that difference in these factors influenced nutritional quality as well as flavour of the fruit (Rosales et al., 2011).

As for the vitamin C content, Voon & Kueh (1999) has reported low vitamin C content in engkala fruit (3.4mg). This study however showed much higher vitamin C content in the fruit compared to their study. It was high in the FD pulp (11.8mg), but lower in the FD seed (4.8mg). The vitamin C content however was found to be significantly higher in both pulp (34.7mg) and seed (13.0mg) when SHSD, and giving higher vitamin C content compared to avocado (10mg) and banana (8.7mg), but lower than papaya (60.9mg).

The significant difference in vitamin C content of engkala fruit in these two different studies might be due to the different samples preparation. The preparation of fruit sample in the procedure of vitamin C determination allowed exposure of the sample to oxygen. In their study, fresh fruit samples are used to determine the nutritional compositions. By using fresh samples, ascorbic acid oxidase enzyme, found in fruits and vegetables is readily available. With the presence of oxygen, it catalyses the oxidation of vitamin C to dehydroaxcorbic acid and water (Dawson & Tokuyama, 1961). Determination of vitamin C content in this case measures the content after oxidation process takes place, hence does not reflect the actual content of the vitamin. Considering the nature of engkala fruit where oxidation can happen in a short amount of time, especially indicated by the rapid browning of the fruit, measuring the vitamin C content in its fresh form is not preferable. In this study, the low temperature of freeze drying preserved this enzyme, thus allowing oxidation process to be continued once the sample is exposed to air. The high temperature applied in the use of superheated-steam on the contrary allowed the denaturation of the ascorbic acid oxidase enzyme. A study on thermal stability of vitamin C and ascorbic acid oxidase enzyme in crushed broccoli suggested that heat treatment

above 70°C is recommended for crushed vegetable in order to prevent oxidation of ascorbic acid to dehydroxyascorbic acid, and treatment at 80°C almost completely inactivated the ascorbic acid oxidase enzyme (Munyaka, Makule, Oey, Van Loey, & Hendrickx, 2010). This indicated that the vitamin C was not really affected by the high drying temperature but instead, was destroyed by oxidation process with the presence of oxygen in the air. Since ascorbic acid oxidase enzyme catalyses the oxidation process, and could be destroyed by heat, superheated-steam thus served to protect the vitamin C.

Table 6. Nutritional compositions of engkala fruit in comparison with avocado and other common local fruits.

	per 100g fresh sample											
	Engkala							Avocado	Banana	Papaya	Durian	
	Cupule	Pulp	Seed	Skin		Voon & Kueh (1999)		USDA (2012)				
Energy	-	93.3	83.4	-	Kcal	104.0	kcal	160.0	89.0	43.0	147.0	Kcal
Moisture	86.68	65.1	68.2	75.97	%	78.3	%	73.2	74.9	88.1	65.0	%
Protein	-	2.4	3.1	-	g	1.4	%	2.0	1.1	0.5	1.5	g
Fat	-	0.0	0.0	-	g	6.8	%	14.7	0.3	0.3	5.3	g
CHO	-	20.9	17.7	-	g	10.0	%	8.5	22.8	10.8	27.1	g
Crude fibre	-	3.8	4.8	-	g	1.0	%	6.7	2.6	1.7	3.8	g (dietary fibre)
Ash	-	2.4	1.3	-	g	2.5	%	-	-	-	-	
Minerals												
K	607.3	652.9	331.5	531.9	mg	355	mg	485.0	358.0	182.0	436.0	mg
Ca	11.3	4.2	2.4	12.9	mg	1.0	mg	12.0	5.0	20.0	6.0	mg
Mg	0.8	3.7	1.8	2.2	mg	17.0	mg	29.0	27.0	21.0	30.0	mg
Fe	0.7	4.9	1.1	1.4	mg	0.5	mg	0.6	0.3	0.3	0.4	mg
Cu	0.1	1.0	0.6	0.6	mg	0.3	mg	-	-	-	-	
Zn	0.5	1.6	1.1	1.1	mg	1.0	mg	0.6	0.2	0.1	0.3	mg
Na	12.4	91.5	6.3	22.2	mg	-		7.0	1.0	8.0	2.0	mg
		SHSD										
Vit C		34.7	13.0	mg		3.4	mg	10.0	8.7	60.9	19.7	mg
		FD										
		11.8	4.8	mg								

2.3.2. Phytochemical Study

2.3.2.1. Total Phenolic Content (TPC)

Spectrophotometry is one of the relatively simple techniques for analysis of plant polyphenols. The Folin-Ciocalteu method is a widely used spectrophotometric assay to measure total phenolics in plant materials for many years. The assay is based on a chemical reduction involving reagents containing tungsten and molybdenum, where in the presence of phenolic compounds, the reduction produced a blue colored mixture with a broad light absorption spectrum around 760nm (Box, 1983; Khoddami, Wilkes, & Roberts, 2013).

Table 7 shows TPC of engkala fruit parts dried using freeze drier and superheated-steam dryer (steam temperature of 130°C, 150°C and 170°C). FD engkala yield the highest TPC in the seed at 3405.09 mg GAE/100g dried sample, whereas the TPC value for FD cupule, pulp and peel were quite similar at the value of 898.52, 986.15 and 915.30 mg GAE/100g dried sample respectively. For the seed, TPC value was drastically lower (1472.58 – 1783.15 mg GAE/100g) when samples were SHSD at all three temperatures used. Seed is the part of fruit which contains an embryonic plant in a resting condition needing appropriate temperature and water in order to undergo germination. This makes the seed sensitive towards heat, and high temperature applied in the drying process in SHSD seemed to have an injurious effect on the chemical components in the seed. Heating will reduce germination (Hill & Johnstone, 1975) and heating most probably will also destroy polyphenols in the seed. This could explain why the TPC in the seed was significantly lower when SHSD and the

TPC value decreased even lower with the increase in temperature as shown in this study.

The most targeted part of the fruit, which was the pulp on the contrary, showed significantly higher TPC when SHSD (1624.00 - 1809.33 mg GAE/100g) compared to FD (986.15 mg GAE/100g). When SHSD, the highest TPC for engkala was observed in the pulp when dried at the temperature of 170°C, giving the value of 1809.33 mg GAE/100g dried sample (83.47% increase compared to freeze dried sample). Reconstruction of molecules in nutrition can happen when heat is absorbed from the heating process involved. The energy is used to change the chemical structure, thus contributing to the increase in the concentration of the desired compounds. A study shows heating gave no effect on caffeic acid because hydrolysis of chlorogenic acid gave rise to its concentration (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008).

The peel also showed slight but not significantly higher TPC when SHSD. The cupule gave the lowest TPC value of all the fruit parts, both FD (898.52 mg GAE/100g) and SHSD (649.88 mg GAE/100g).

Table 7. Total phenolic content of engkala fruit parts dried under different conditions.

		mg GAE/100g dw	± S.D.	
Cupule	FD	898.52	29.77	a
	SHSD, 130°C	772.09	18.03	b
	SHSD, 150°C	649.88	9.40	c
	SHSD, 170°C	883.30	47.16	ab
Pulp	FD	986.15	28.75	a
	SHSD, 130°C	1624.00	25.22	b
	SHSD, 150°C	1677.27	62.71	bc
	SHSD, 170°C	1809.33	31.93	c
Peel	FD	915.30	65.75	a
	SHSD, 130°C	1047.85	31.34	a
	SHSD, 150°C	1078.06	27.07	a
	SHSD, 170°C	1050.42	10.96	a
Seed	FD	3405.09	123.94	a
	SHSD, 130°C	1783.15	19.14	b
	SHSD, 150°C	1754.24	18.93	b
	SHSD, 170°C	1472.58	5.77	c

Results expressed as mean ±S.D. (n=3). Different letters in the same column indicate significant difference ($p<0.05$).

2.3.2.2. Total Flavonoid Content (TFC)

Table 8 shows TFC of engkala fruit parts dried using freeze dryer and superheated-steam dryer (steam temperature of 130°C, 150°C and 170°C). For the FD engkala samples, highest TFC was observed in the seed part (1534.94 mg RE/100g dried sample) followed by the pulp, skin and cupule (930.08, 659.71 and 528.29 mg RE/100g dried sample respectively). For the seed, TFC value was drastically lowered to around one third (416.29 - 550.00 mg RE/100g) of the FD value (1534.94 mg RE/100g) when SHSD at all the three temperatures. Pulp of engkala, on the other hand gave significantly higher TFC when SHSD (1040.12 - 1335.92 mg RE/100g) compared to FD (930.08 mg RE/100g). The skin and cupule also showed significantly higher TFC values when SHSD. This result for TFC of engkala was similar to the result for TPC, except for the cupule.

Table 8. Total flavonoid content of engkala fruit parts dried under different conditions.

		mg RE/100g dw	± S.D.	
Cupule	FD	528.29	17.89	a
	SHSD, 130°C	556.49	44.52	a
	SHSD, 150°C	716.90	79.58	b
	SHSD, 170°C	668.16	73.34	ab
Pulp	FD	930.08	101.26	a
	SHSD, 130°C	1335.92	50.54	b
	SHSD, 150°C	1219.27	114.24	b
	SHSD, 170°C	1040.12	107.92	b
Peel	FD	659.71	17.36	a
	SHSD, 130°C	770.61	68.27	a
	SHSD, 150°C	895.63	98.48	a
	SHSD, 170°C	814.86	85.51	a
Seed	FD	1534.94	118.11	a
	SHSD, 130°C	507.02	35.04	bc
	SHSD, 150°C	550.00	59.94	b
	SHSD, 170°C	416.29	78.09	c

Results expressed as mean ±S.D. (n=3). Different letters in the same column indicate significant difference ($p<0.05$).

The superheated-steam applied in the drying process in this study caused the TPC and TFC to be significantly higher in some part of the fruit but significantly lower in some others compared to their FD counterparts. The significantly higher TPC and TFC particularly in the pulp when heat was applied in the drying process could be due to the changes in the chemical structure which transformed the molecules to become polyphenols. This could indicate that energy applied in the process is absorbed to induce the changes in the chemical structure of the molecules. On the other hand, the significantly lower TPC and TFC as evident in the seed part when SHSD compared to FD simply indicate that the polyphenols in the seed are most probably sensitive toward heat, thus destroyed by the high energy in the superheated-steam.

2.3.3. Antioxidant Study

2.3.3.1. DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a well-known stable free radical where its application in antioxidant assay is very common. The DPPH radical has a deep violet color in solution, and it becomes pale yellow when neutralized by the presence of antioxidants. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm, the wavelength where a strong absorption band is centred (Alger, 1997).

In this study, inhibition concentration (IC_{50}), which is the amount of substance required to inhibit the initial concentration of DPPH radical by half was calculated from a series of dose-response data. The lower the concentration to deplete the DPPH, the better is the antioxidant activity displayed by the substance.

Table 9 shows DPPH radical scavenging activity of engkala fruit parts dried using freeze drier and superheated-steam dryer (steam temperature of 130°C, 150°C and 170°C). The scavenging activity of engkala fruit parts showed a similar trend as portrayed by the TPC and TFC as discussed above. As presented in Table 9, seed was the part which showed the highest antioxidant activity in this study, where FD (0.22 mg/ml) and SHSD (0.56 – 0.91 mg/ml) seed gave very low IC_{50} values. This was followed by the SHSD pulp dried at the temperature of 170°C (1.82 mg/ml).

Other than the seed, freeze drying also offered advantages in the drying of the peel and cupule as indicated by the lower IC_{50} value of the FD parts compared to their respective SHSD counterparts. The edible part, which is the pulp on the contrary, favoured superheated-steam as its drying method and the antioxidant activity went higher with the increase in the steam temperature. A study conducted on tomatoes also shows that the sample dried under heat gave a higher DPPH radical scavenging activity compared to its FD and fresh sample (C.-H. Chang, Lin, Chang, & Liu, 2006).

Table 9. DPPH radical scavenging activity of engkala fruit parts.

		IC_{50} (mg/ml)	
		Mean	± S.D.
Cupule	FD	a 1.79	0.16
	SHSD, 130°C	b 3.49	0.06
	SHSD, 150°C	bc 3.79	0.05
	SHSD, 170°C	d 2.94	0.14
Pulp	FD	a 11.85	0.85
	SHSD, 130°C	b 2.52	0.15
	SHSD, 150°C	c 2.30	0.12
	SHSD, 170°C	d 1.82	0.11
Peel	FD	a 2.26	0.34
	SHSD, 130°C	a 3.05	0.08
	SHSD, 150°C	a 3.16	0.09
	SHSD, 170°C	a 3.18	0.12
Seed	FD	a 0.22	0.01
	SHSD, 130°C	b 0.56	0.03
	SHSD, 150°C	c 0.67	0.01
	SHSD, 170°C	d 0.91	0.09

Results expressed as mean ±S.D. (n=3). DPPH, 2,2-diphenyl-1-picrylhydrazil;
*p<0.05 compared to FD sample.

2.3.3.2. Oxygen radical absorbing capacity (ORAC)

The ORAC assay measures the degree of inhibition of peroxy-radical-induced oxidation by the compounds of interest via hydrogen atom transfer reaction mechanism. AAPH radical is added to initiate the reaction. Fluorescein (FL), a synthetic nonprotein probe is consumed by the radical as the reaction progresses where the FL intensity decreases. In the presence of antioxidant, the FL decay is inhibited. ORAC assay measures the performance of the compounds against a standard, Trolox (a water soluble derivative of vitamin E), and the results are reported in Trolox Equivalents (TE) (Huang, Ou, & Prior, 2005).

Table 10 shows ORAC value of engkala fruit parts dried using freeze drier and superheated-steam dryer (steam temperature of 130°C, 150°C and 170°C). The oxygen radical absorbing capacity of the fruit parts was similar to the DPPH radical scavenging activity as described above, where the seed gave the highest antioxidant activity in this study, both FD (120675 µmol TE/100g) and SHSD (42885 – 54588 µmol TE/100g). This was followed by the SHSD pulp dried at the temperature of 170°C (22446 µmol TE/100g).

The peel and cupule also favoured freeze drying as a drying method, as indicated by the higher ORAC values of the FD parts compared to their SHSD counterparts. But when SHSD, the ORAC values were found to be lower. The pulp, which is the edible part on the other hand could offer more benefit when consumed after being SHSD. The ORAC values were significantly higher (17575 - 22446 µmol TE/100g)

compared to their FD counterpart (3848 µmol TE/100g), where the values were higher with the increase in the steam temperature.

Table 10. Oxygen radical absorbing capacity (ORAC) of engkala fruit parts.

		ORAC (µmol TE/100g		
		Mean	± S.D.	
Cupule	FD	15079	335	a
	SHSD, 130°C	14793	1469	a
	SHSD, 150°C	13263	619	a
	SHSD, 170°C	11938	172	b
Pulp	FD	3848	152	a
	SHSD, 130°C	17575	1287	b
	SHSD, 150°C	19454	1001	b
	SHSD, 170°C	22446	1528	c
Peel	FD	19722	849	a
	SHSD, 130°C	16241	619	b
	SHSD, 150°C	15409	733	b
	SHSD, 170°C	17489	1045	b
Seed	FD	120675	6226	a
	SHSD, 130°C	54588	278	b
	SHSD, 150°C	50295	1556	c
	SHSD, 170°C	42885	2141	c

Results expressed as mean ±S.D. (n=3). ORAC, Oxygen radical absorbing capacity. Different letters in the same group indicate significant differences ($p<0.05$).

Comparing with other well-known fruits reported in another study (Wolfe et al., 2008), the ORAC value for FD pulp of engkala fruit in this study was found to be lower than the ORAC values for fresh samples of wild blueberry, cranberry, strawberry, blackberry, cherry, plum, raspberry, blueberry, apple and pomegranade, (with above 4000 µmol TE/100g fruit), but higher than orange, red grape, peach, lemon, pear, grapefruit, nectarine, watermelon, avocado, kiwifruit, mango, pineapple, banana, honeydew and cantelope. The SHSD pulp in this study however, gave higher ORAC value than all the fruits mentioned above (with ORAC values less than 10000 µmol TE/100g fruit).

The pulp and peel are the parts of the engkala fruit that are being consumed. This result is in agreement with the method of preparation for eating engkala fruit, where the fruits are subjected to high temperature of hot water or steam. Cooking, or application of heat on food particularly on fruits and vegetables have been shown to increase the nutritional values beside making them more palatable. Carotenoids are a type of compound which has been reported to increase in concentration when green fresh vegetables are boiled or steamed. This is due to the release of carotenoids from the matrix of carotenoid-protein complexes, leading to better extractability (Bernhardt & Schlich, 2006; Miglio et al., 2008). High temperature in sweet potatoes cooked by superheated steam and roasting also had higher total phenols and flavonoids than raw sweet potatoes, probably due to increase in reagent binding sites from the breakdown of the phenolic compounds bindings (Wang et al. 2012).

Natural compounds could become lost during processing involving heat due to the sensitivity of some compounds toward high temperature. However, recent studies have shown that certain fods especially fruits and vegetables, when thermally processed gave higher biological activities due to the various chemical changes during heat treatment (Dewanto, Wu, Adom, & Liu, 2002; Kim et al., 2000). Stahl & Sies (1992) reported that cooked tomatoes and carrots showed higher bioavailability of lycopene and b-carotene compared to the raw ones. Drying of soybean using superheated-steam has been reported to increase significantly the levels of β-glucosides (a type of isoflavone), which leads to the increase in antioxidant activity of the dried soybeans, and the increase of β-glucosides levels increases with the

increase in the drying temperature probably due to the inter-conversion of isoflavones at higher temperatures (Niamnuy et al. 2011). Other than that, steaming process has been found to inactivate enzymes responsible in oxidation of polyphenols in green tea, making the polyphenol content in green tea much higher than the others (Hoffman, 2014).

Peroxidase (POD) and polyphenol oxidase (PPO), present in many fruits and vegetables are closely associated with enzymatic colour changes with consequent loss of nutritional quality and sensorial properties (Duarte, Coelho, & Leite, 2002; Robinson, 1991). PPO enzymes generally consume many different phenolic compounds as substrates. POD on the other hand catalyses reduction of hydrogen peroxide to water while oxidizing a variety of substrates (Robinson, 1991). High temperature is capable of destroying these enzymes. Inactivation of POD and PPO has been shown in pineapple treated with high temperature in a circulated water bath up to a temperature of 95°C (Lee et al., 2009). At temperatures above 90°C, treatment with microwave in green coconut water simulated solutions reduced PPO and POD enzymatic activity to undetectable levels (Matsui, Granado, de Oliveira, & Tadini, 2007). The high temperature applied in the superheated-steam might denature these enzymes present in the fruit of engkala, hence inhibiting oxidation of polyphenols in the dried fruit. This might also contribute to the higher antioxidant activities of the SHSD pulp compared to the FD counterpart, where the enzymes were preserved at low temperature.

Superheated-steam drying may be offered in food industry as an alternative drying method. When saturated steam is heated to over 100°C, it becomes ‘superheated’,

which has the same properties as hot air in terms that it can release large amounts of heat energy when in contact with objects at low temperature. This characteristic can then be used in both heating and drying of food (Iyota et al., 2001). Yoshida & Hyodo (1966) demonstrated that drying food using superheated steam yielded better colour, lower percentage of oxidisation and nutrient loss. Fraile & Burg (1997) also reported that superheated steam had great potential for high-starch foods. The lack of oxidative reaction during dehydration with superheated steam could improve the quality of some food products (Wang et al., 2012).

2.3.4. Correlation among phenolic content, flavonoid content and antioxidant activities

The TPC of engkala when correlated with ORAC and DPPH data, Pearson correlation gave very strong correlation (r^2 = 0.899) for ORAC while the correlation was moderate (r^2 = -0.448) for DPPH. Wolfe et al. (2008) in their study on antioxidant activity of common fruits also observed that TPC are significantly correlated to ORAC values. Meanwhile, the TFC and ORAC values gave moderate correlation (r^2 = 0.328), while the TFC and DPPH IC_{50} values gave weak correlation (r^2 = 0.059). This data suggested that the radical absorbing activities in engkala might be contributed more by the phenolic than the flavonoid compounds in the fruit.

2.3.5. Phenolic Compounds Analysis

2.3.5.1. Thermal Analysis

Thermal analysis, which can be defined as experimental methods for characterizing a system (element, compound or mixture) by measuring changes in physico-chemical properties at high temperatures as a function of increasing temperature, have found wide application in analytical chemistry. The methods mainly involve thermo gravimetric (TG) analysis, in which changes in weight are measured as a function of increasing temperature, and differential thermal analysis (DTA), in which changes in heat content are measured as a function of increasing temperature. These analyses provide information relating to certain physical phenomena such as crystalline transition, second-order transition, fusion, vaporization, sublimation, absorption, adsorption and desorption. Likewise, they also provide information on certain chemical phenomena such as chemisorption, desolvation, decomposition and oxidative degradation (Coats & Redfern, 1963).

Figure 3 shows TG and DTA of engkala pulp, while figure 4 shows TG and DTA of engkala seed. In the pulp, even though the final residue of the TG curves showed similar percentage, some differences were observed in the curve of FD and SHSD pulp at the initial stage until at the temperature of around 350°C. Meanwhile, the DTA curves showed some increase and decrease in the first and second peak respectively. This data might suggest some degradation and recombination of molecular components occurred in the sample of the pulp after being SHSD. A study on the effect of steaming at 100°C on raw ginseng for 2-3 hr to produce red ginseng

has reported increase in pharmacological activities compared to air-dried ginseng, which is due to changes in the chemical components that occur during steam treatment. Ginsenosides which were absent in raw ginseng were detected in the steamed ginseng (Kim et al., 2000).

In figure 4 of the seed part, the last peak in the DTA curve showed some increase after SHSD relating to the slight decrease in the TG residue. In the seed, the first peak present in the pulp was not noticed, indicating different compounds, which were not present in the seed were present in the pulp.

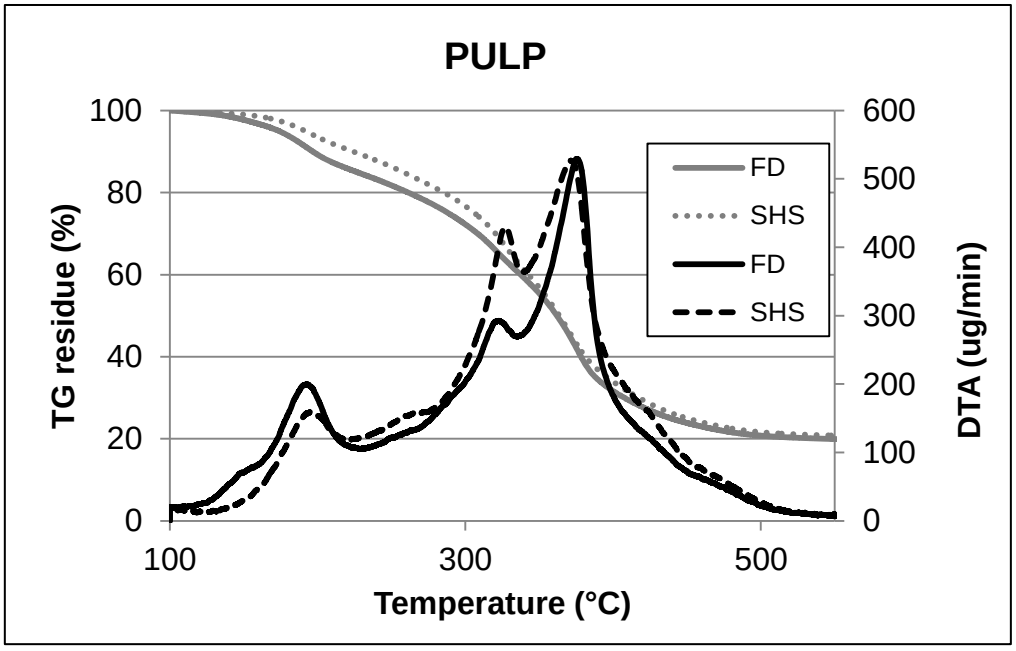


Figure 3. Thermal analysis of FD and SHSD engkala pulp.

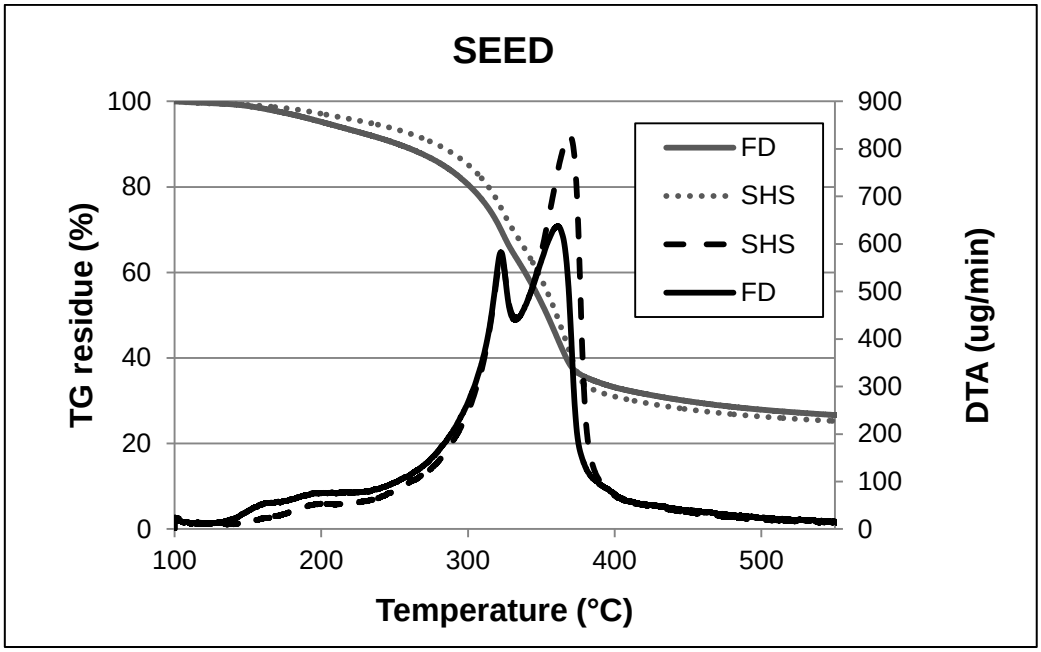
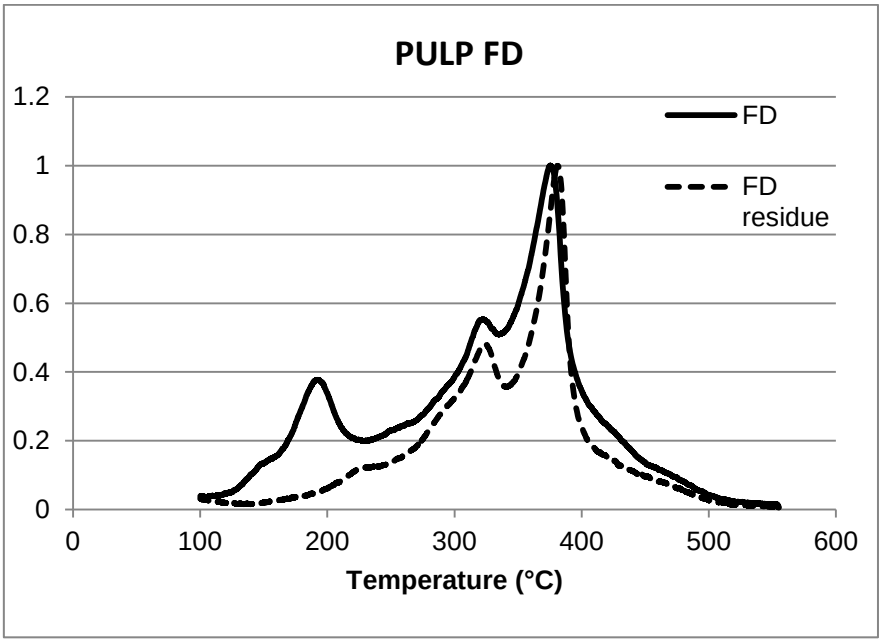
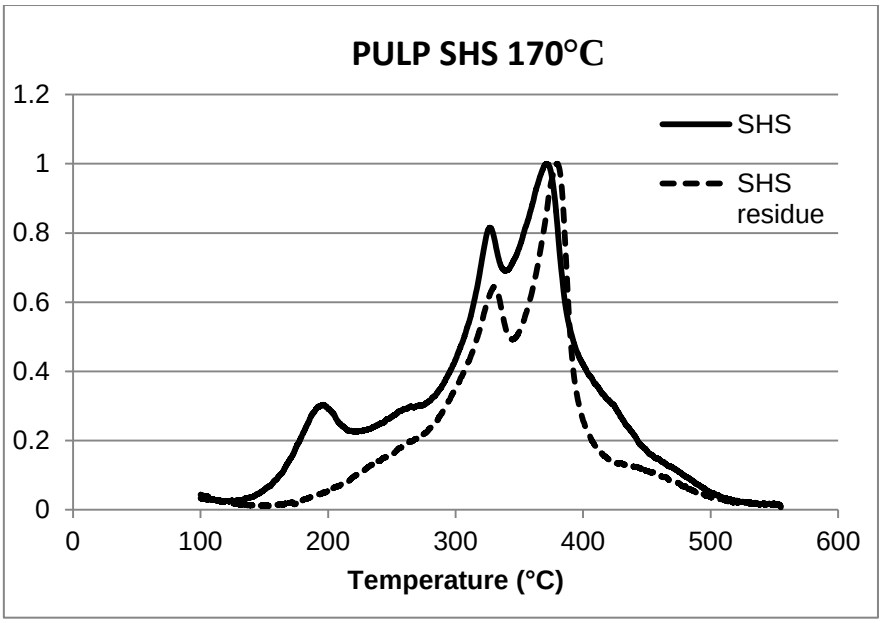


Figure 4. Thermal analysis of FD and SHSD engkala seed.

Figure 5 and 6 show DTA curves of FD and SHSD engkala pulp and seed before and after solvent extraction. From the graphs, it was noticeable that the chemical compounds present in the samples were extracted into the ethanolic solvent used in this study. In research involving extraction of polyphenols from plant materials, many solvents are used including methanol, ethanol, ethyl acetate and acetone. The highest yields are usually achieved with ethanol and methanol and their mixtures with water. Water and ethanol are most widely used with the advantage of modulating the polarity of the solvent by changing their ratios, as well the low toxicity and high extraction yield properties (Franco et al., 2008; Nur Syukriah, Liza, Harisun, & Fadzillah, 2014).

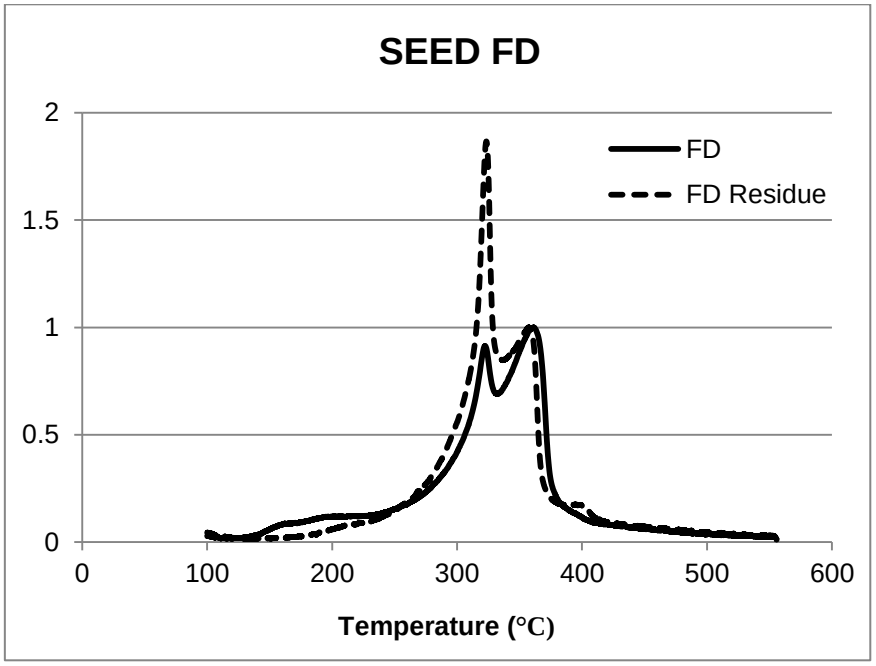


(a)

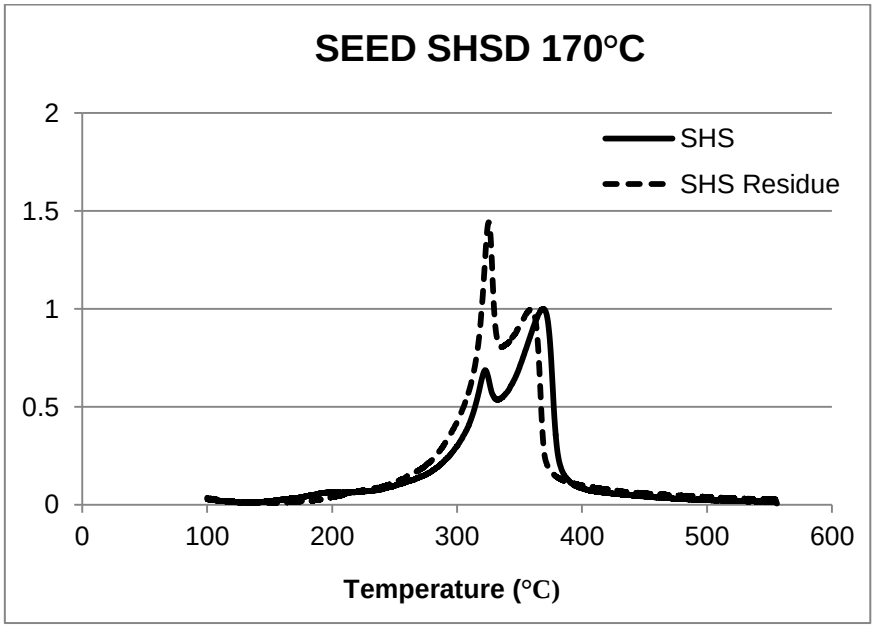


(b)

Figure 5. DTA of (a) FD and (b) SHSD engkala pulp before and after solvent extraction.



(a)



(b)

Figure 6. DTA of (a) FD and (b) SHSD engkala seed before and after solvent extraction.

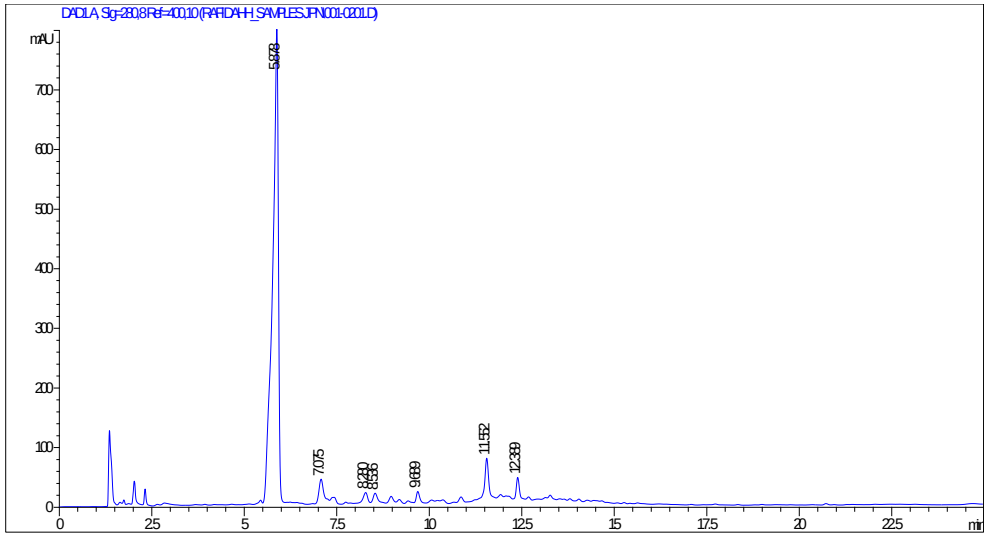
2.3.5.2. High Performance Liquid Chromatography (HPLC) Analysis

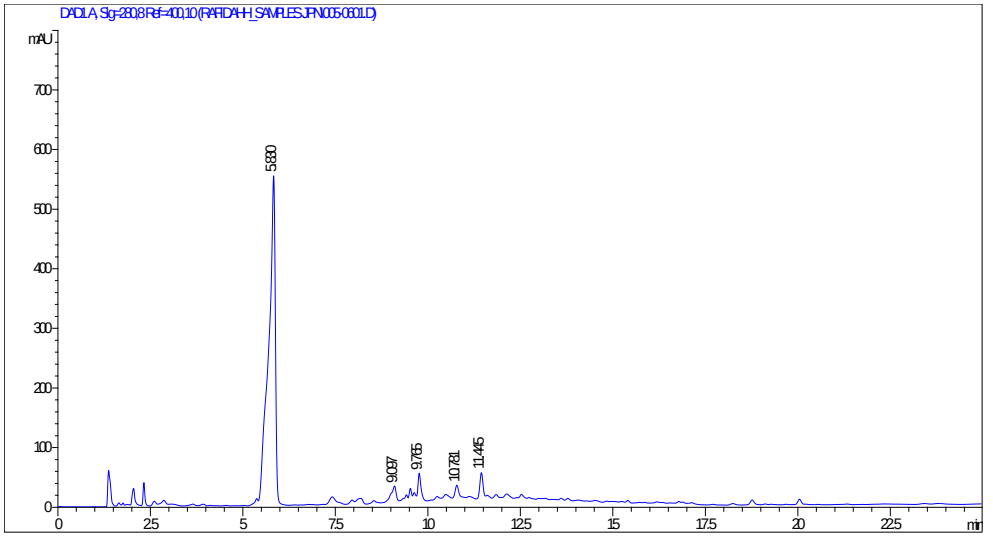
The content of phenolic compounds in biological samples can be determined by the usage of various analytical instruments. HPLC has proved to be the most appropriate, owing to the structural similarity and diversity of phenolic compounds, allowing the analysis with sufficient precision, selectivity and done within a reasonable time. Detectors coupled with HPLC such as ultraviolet-visible (UV) and DAD (photodiod-array-detector) are among the most useful and common ones in ordinary laboratories (Zhang et al., 2013).

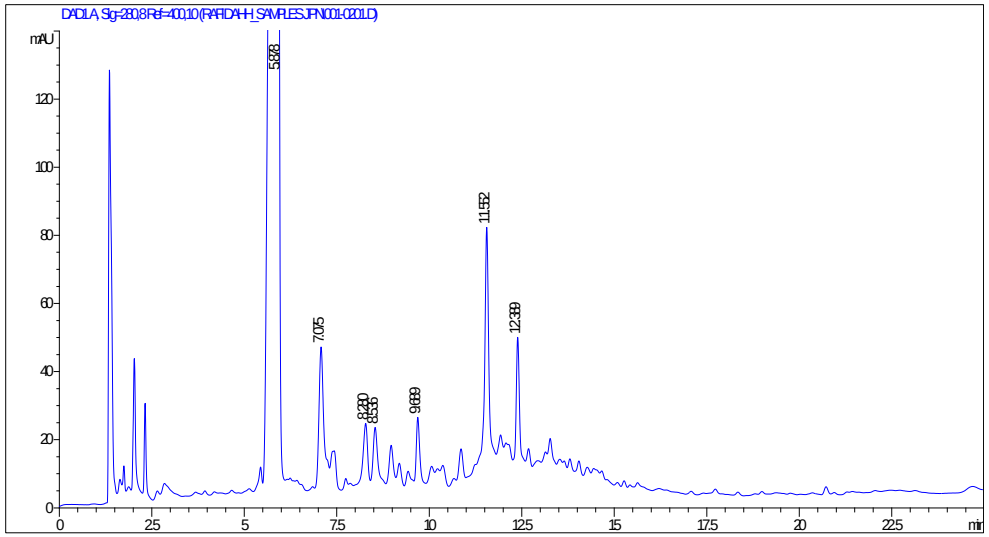
Looking at the HPLC chromatograms in figure 7, it was observed that the significantly higher TPC values in SHSD pulp (Figure 7b) were probably contributed partially by the high concentration of compound given by the peak at 5.9 min of retention time. Appearance of peak at 12.5 min and increased in the peaks at 7.04 min and 11.6 min as well as other minor peaks were observed when the pulp was SHSD could explain the significantly higher TPC and antioxidant activities. The increased TPC in SHSD peel (Figure 7c) saw the uprise of peaks at 5.9, 7.4, 11.4, 12.3, and 13.6 min of retention time. On the other hand, in the seed part of engkala (Figure 7d), most peaks within the retention time of 8-13.5 min range were higher in the FD sample. When SHSD however, appearance of peak at 5.9 min but decreased in most of the peaks existed in the FD seed were observed. This decrease could explain the significantly lower TPC values when SHSD. The high TPC in the FD seed could be due to the present of peaks in the 8-13 min range. Table 11 summarizes the peaks of HPLC chromatograms for the different parts of FD and SHSD engkala fruit.

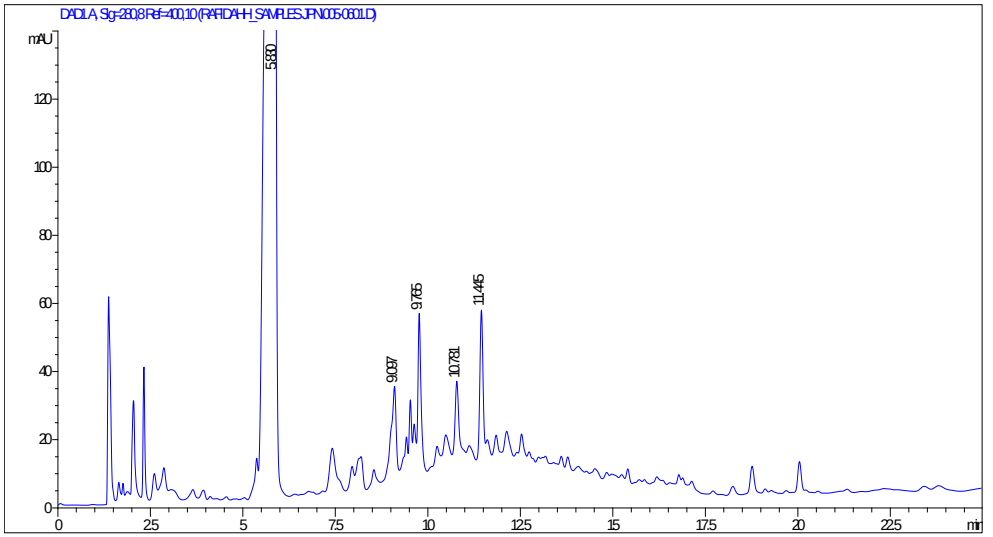
Studies on avocado, which is a fruit belonging to the same family as engkala has reveal by using HPLC system the presence of flavanol monomers, proanthocynaidins, hydroxycinnamic acids and flavonol glycosides in the fruit (Kosińska et al., 2012). Another study detected the presence of catechins, procyanidins and hydroxycinnamic acids in the peel and seed, whereas the pulp is rich in hydroxybenzoic and hydroxycinnamic acids and procyanidins detected by using UPLC system (Rodríguez-Carpena, Morcuende, Andrade, Kylli, & Estévez, 2011).

Spiking authentic standards with engkala fruit samples was done in order to identify the compounds in the samples. Due to limitation of authentic standards, unfortunately no peaks were identified from the HPLC analysis in this study. Studies were further continued with mass spectrometry analysis.



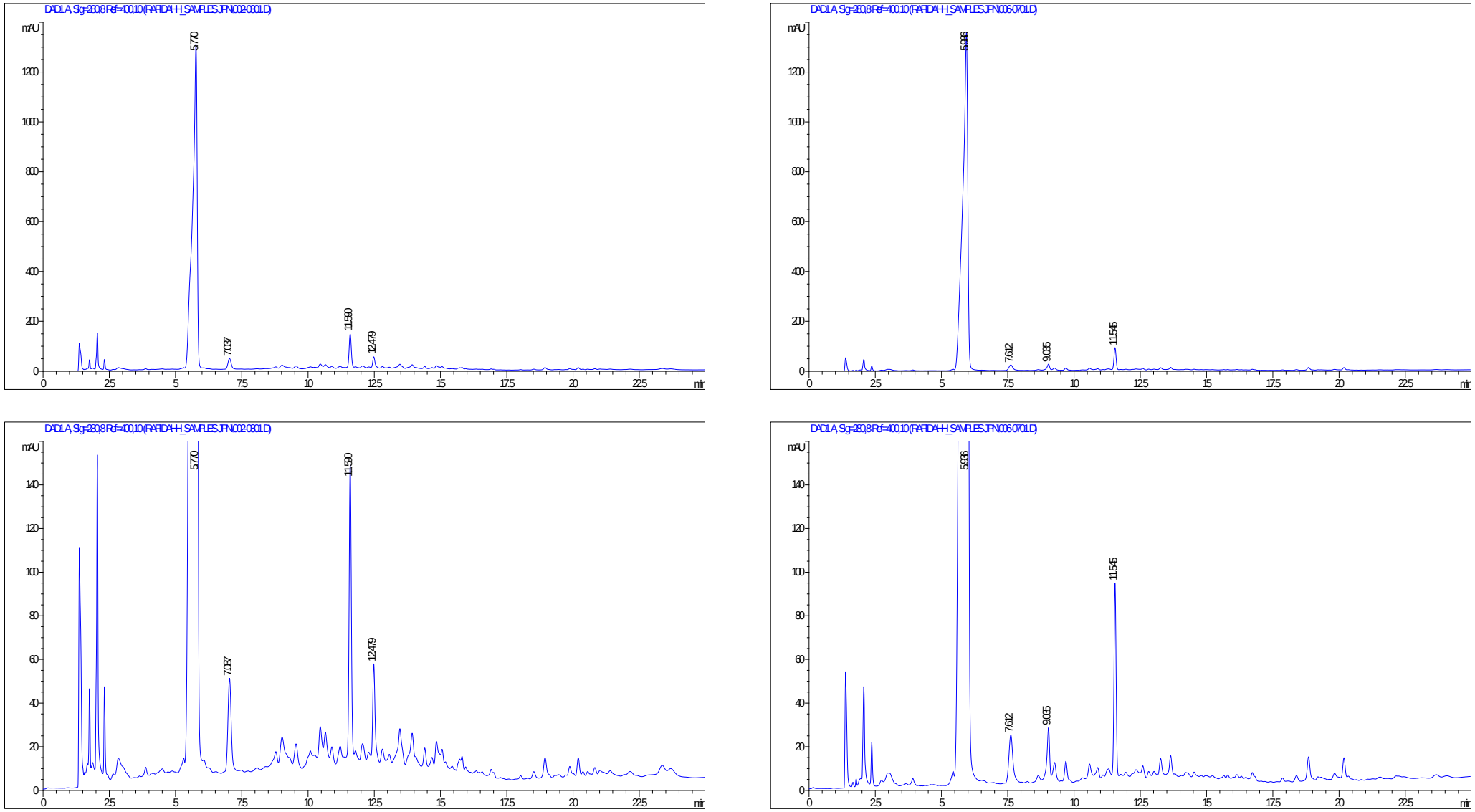






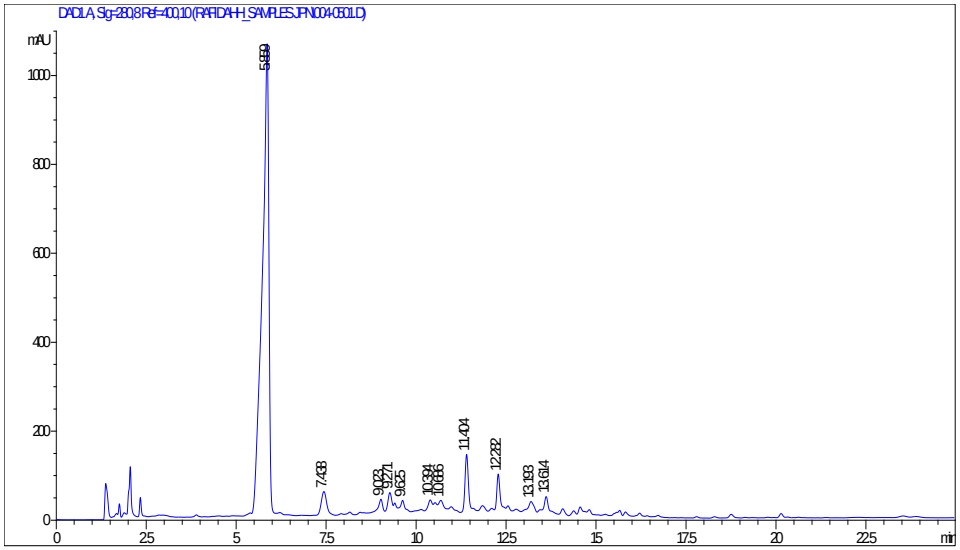
SHSD FD

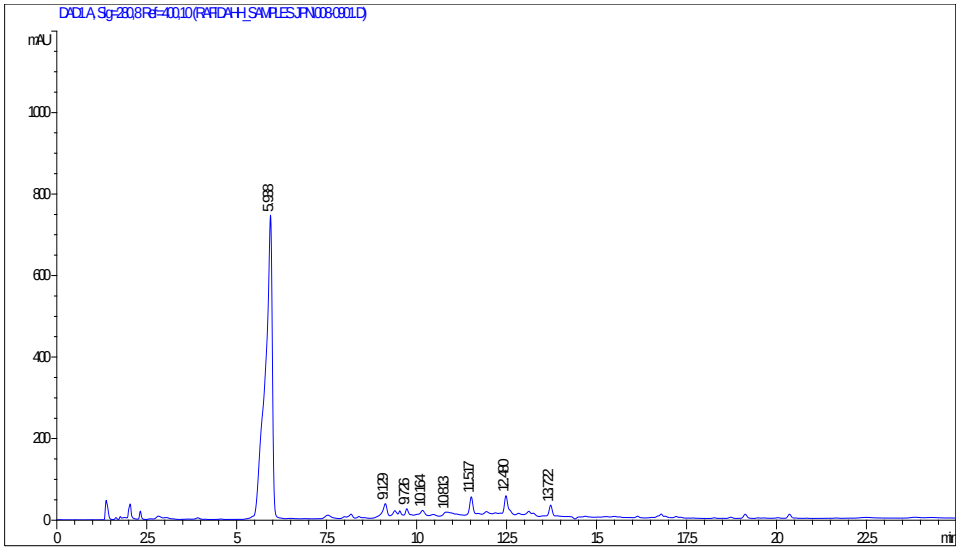
Figure 7(a). HPLC chromatograms of SHSD and FD cupule of engkala fruit.

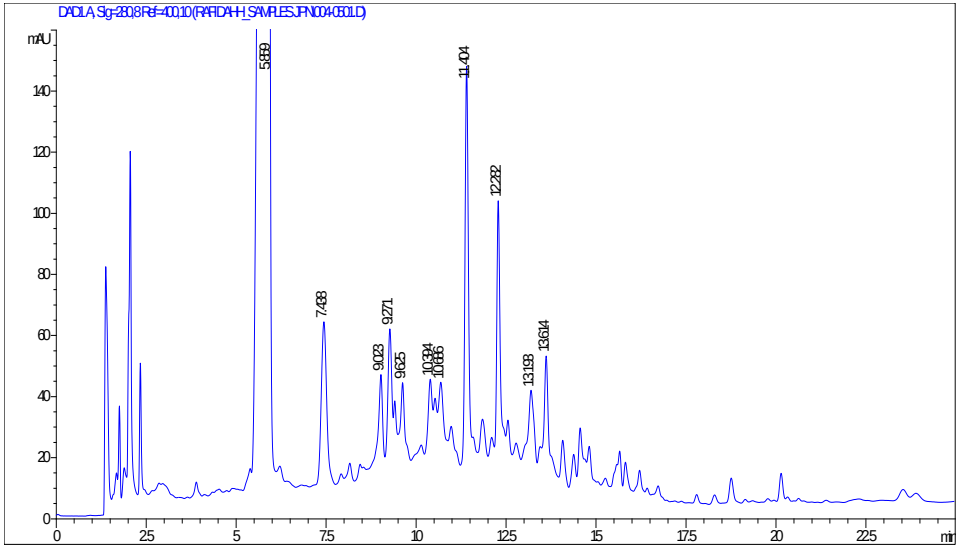


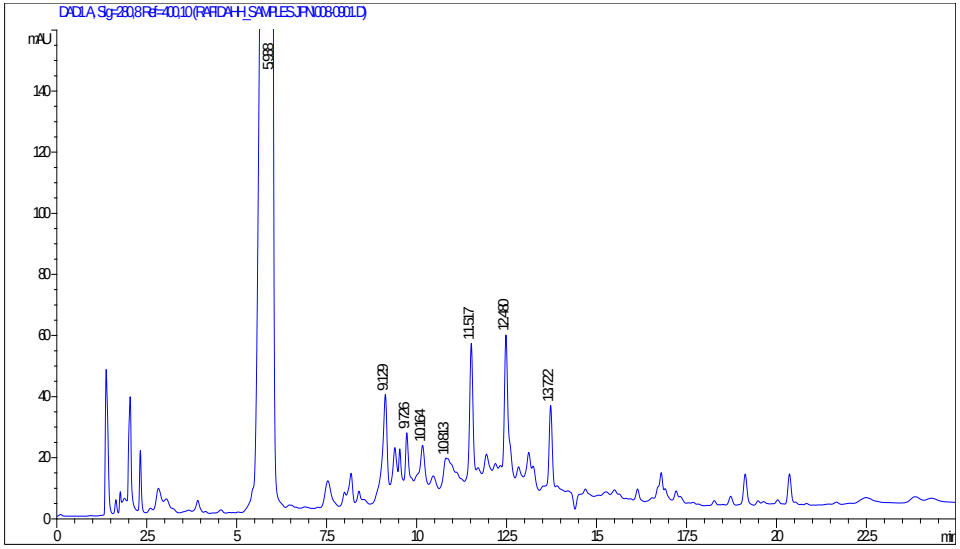
SHSD FD

Figure 7(b). HPLC chromatograms of SHSD and FD pulp of engkala fruit.



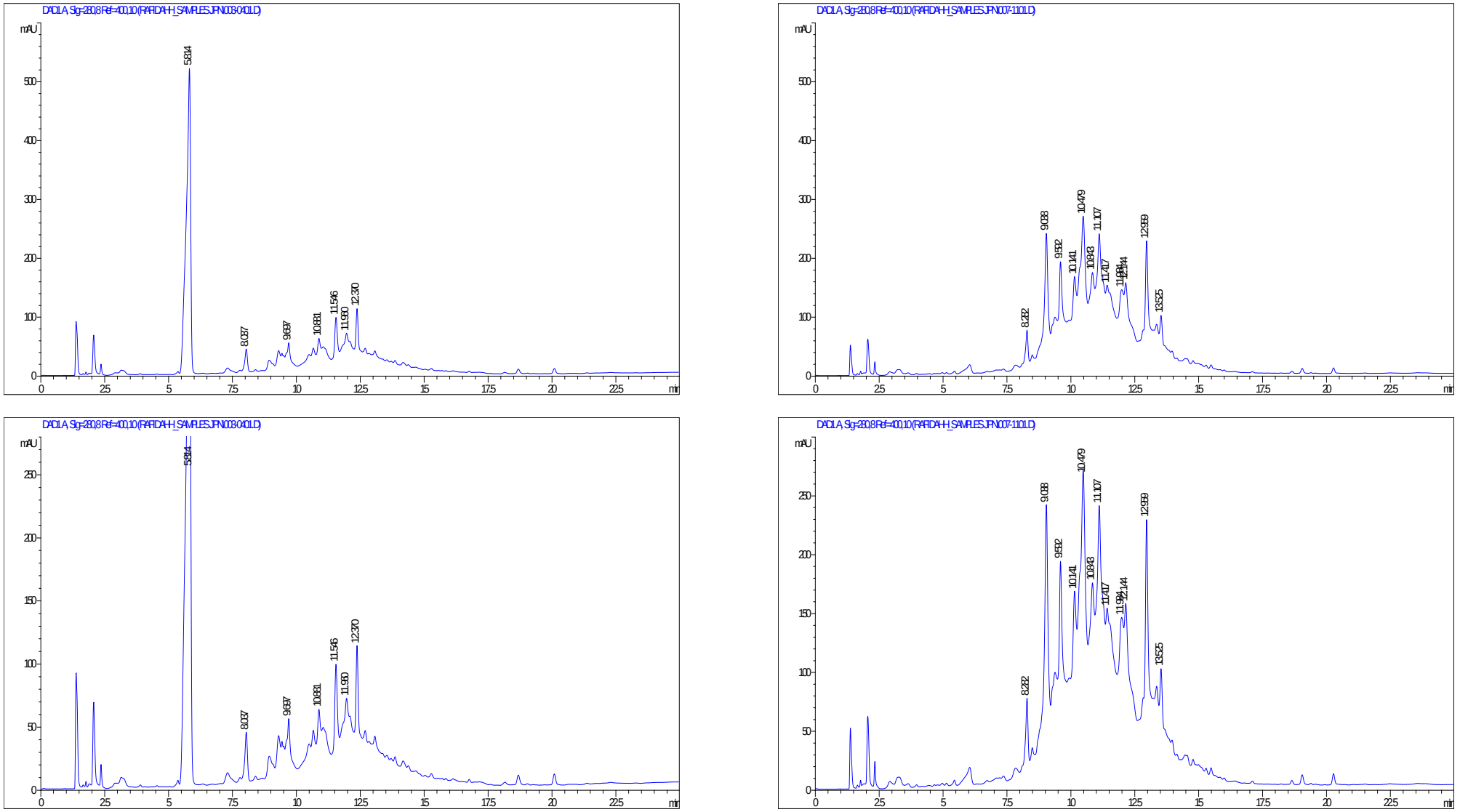






SHSD FD

Figure 7(c). HPLC chromatograms of SHSD and FD peel of engkala fruit.



SHSD FD

Figure 7(d). HPLC chromatograms of SHSD and FD seed of engkala fruit.

Table 11. Peaks of HPLC chromatograms for FD and SHSD engkala fruit parts.

		FD		SHSD	
Sample	Compound	t_R (min)	Area (mAU*s)	t_R (min)	Area (mAU*s)
Cupule	1	5.83	7151	5.88	8795
	2	9.10	495	7.08	490
	3	9.77	448	8.28	287
	4	10.78	486	8.54	271
	5	11.45	475	9.69	222
	6	12.53	346	11.55	1010
	7			12.39	433
Pulp	1	5.94	18360	5.77	16236
	2	7.61	372	7.04	633
	3	9.04	258	8.78	410
	4	11.55	588	9.02	469
	5			11.59	1012
	6			12.48	480
	7			13.47	445
	8			13.93	461
Peel	1	5.94	10422	5.86	14023
	2	9.13	444	7.44	1002
	3	9.73	250	9.02	740
	4	10.16	342	9.27	569
	5	10.81	535	9.63	607
	6	11.52	485	10.39	483
	7	12.48	586	10.69	583
	8	13.72	350	11.40	1406
	9			12.28	922
	10			13.19	735
	11			13.61	713
Seed	1	8.27	791	5.81	6319
	2	9.04	3375	8.04	390
	3	9.59	2638	8.94	421
	4	10.14	2014	9.30	423
	5	10.48	4799	9.43	308
	6	10.84	2433	9.70	838
	7	11.11	4316	10.88	623
	8	11.42	3438	11.05	869
	9	11.984	1960	11.55	1039
	10	12.144	3117	11.96	1775
	11	12.959	3663	12.37	1188
	12	13.53	1598		

2.3.5.3. Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

Pyrolysis Gas Chromatography (Py-GC) used in this study allows analysis of almost all sorts of materials including insoluble materials and complex materials at trace levels without any pre-treatment of samples, and information otherwise unobtainable by other techniques can be obtained. Py-GC technique is therefore becoming an important technique in the area of characterization (Frontier Lab, 2008).

Figures 7 and 8 show Py-GC chromatograms of engkala pulp and seed respectively. In figure 7 of the pulp part, peak A appeared at around 8 min of retention time but not observed in the chromatogram of the seed (figure 8). Other than that, for both the pulp and seed parts, the chromatograms for FD and SHSD samples for each part showed similar patterns of peaks (B, C and D) appearing between 20 and 25 min of retention time, with difference in intensity. From the database (NIST107 and NIST21) peak B, C and D were identified as fatty acid derivatives. Peak A however was not identified and marked as unknown. Figures 9 and 10 show the mass spectra corresponding to the peaks obtained from the pulp and seed respectively.

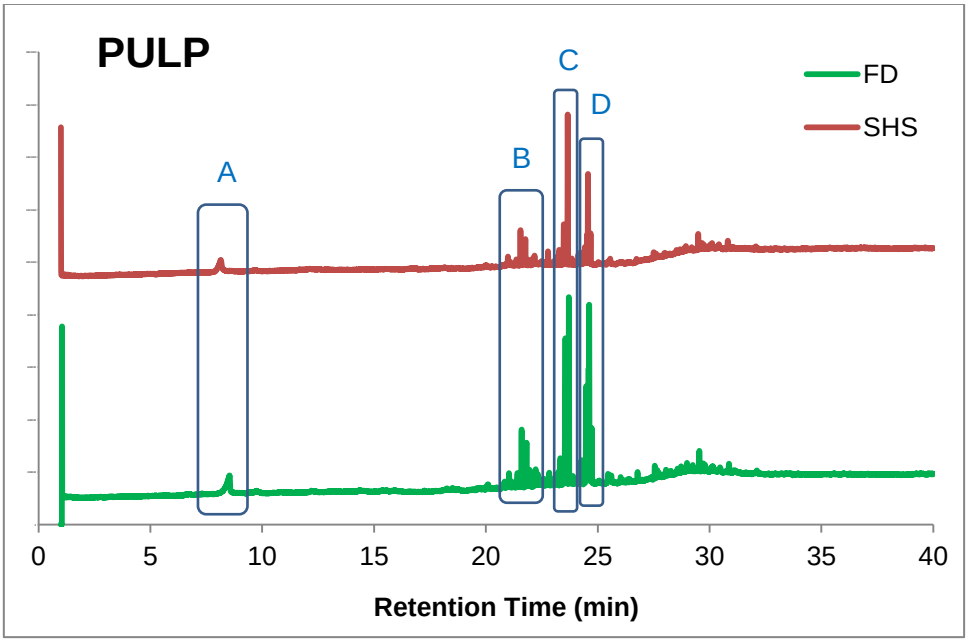


Figure 8. Py-GC chromatograms of FD and SHSD engkala pulp.

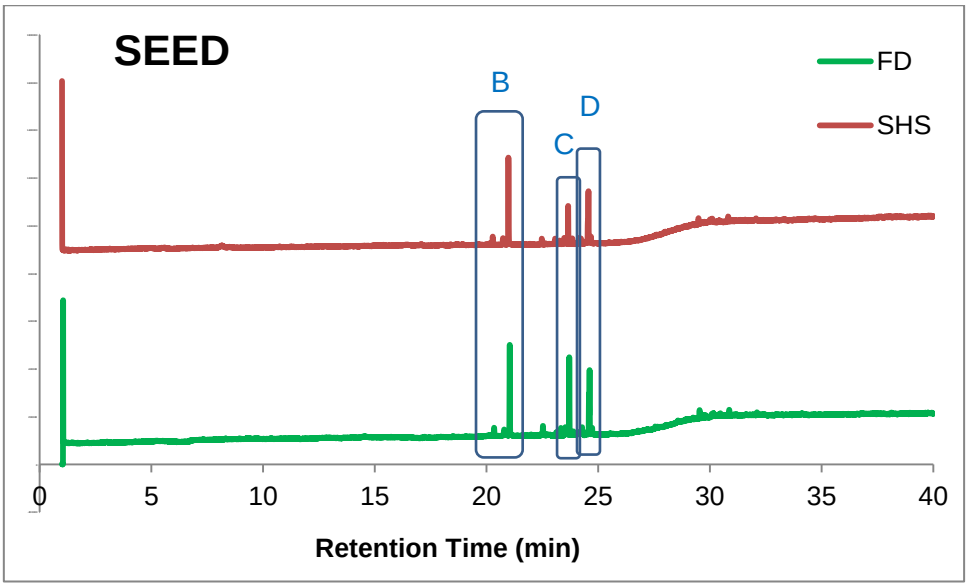
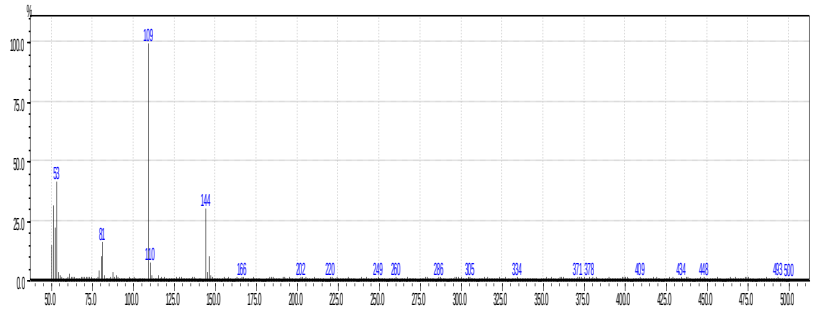
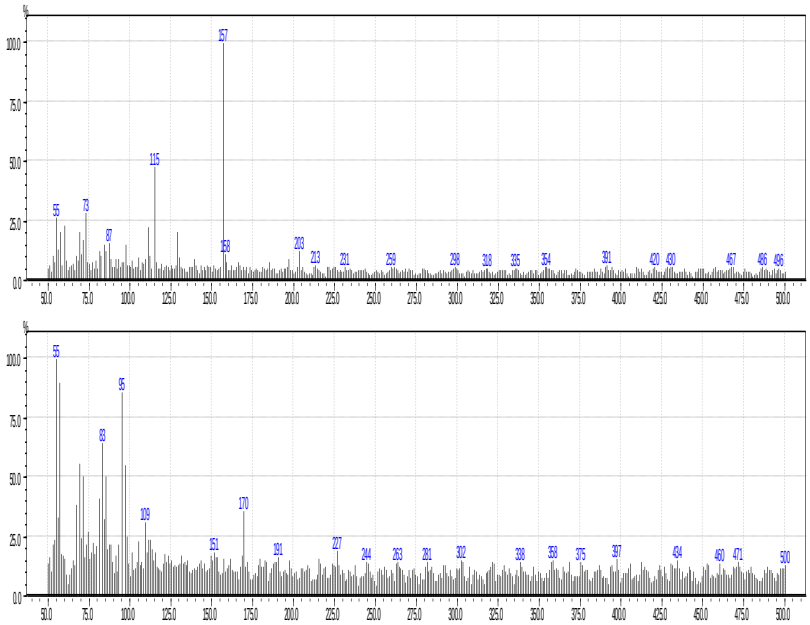


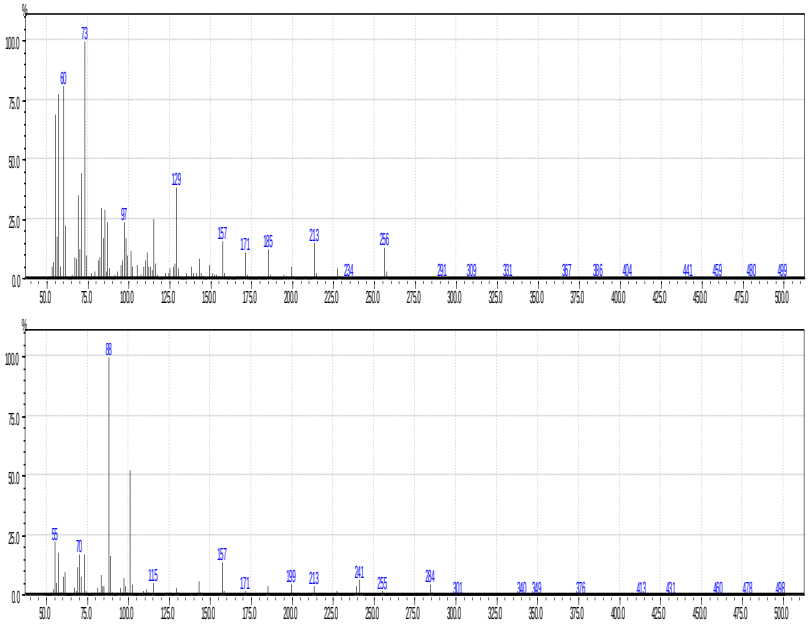
Figure 9. Py-GC chromatograms of FD and SHSD engkala seed.



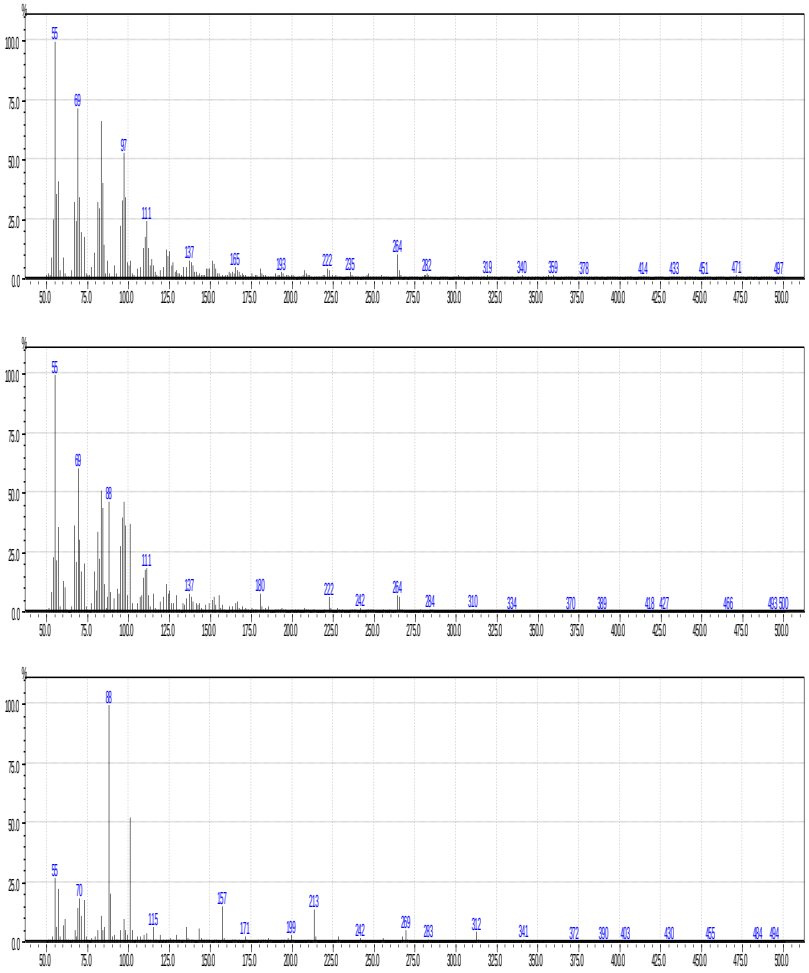
(A)



(B)

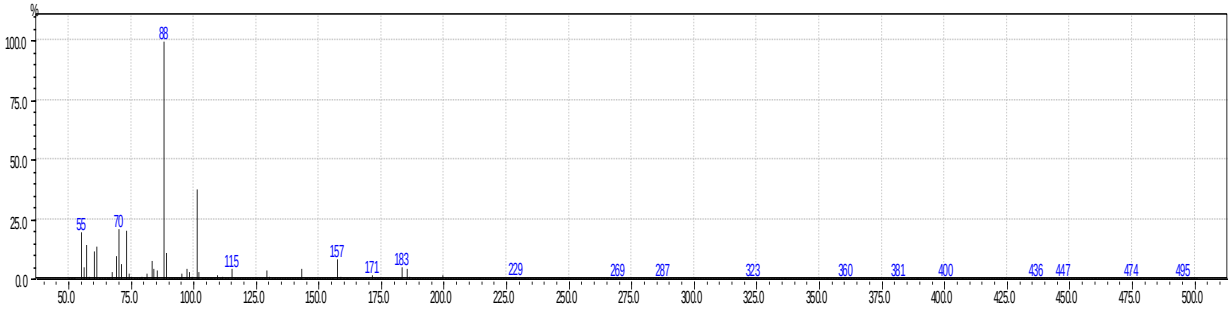


(C)

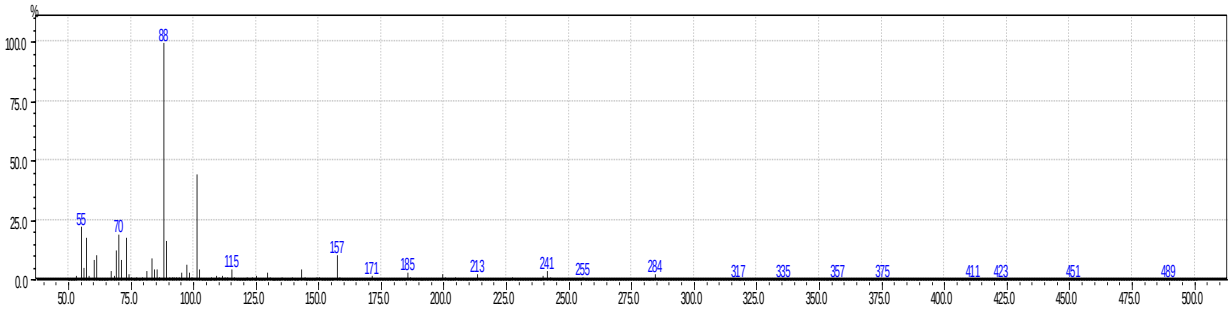


(D)

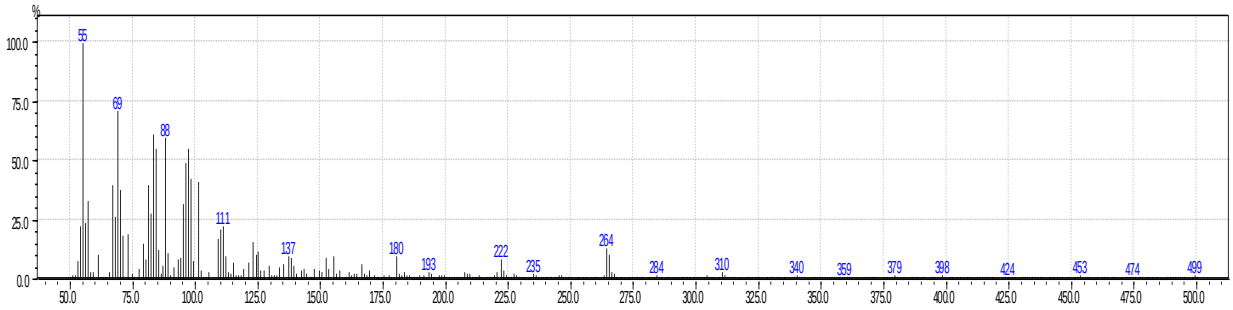
Figure 10. Mass spectra corresponding to the Py-GC chromatograms of engkala pulp.



(A)



(B)



(C)

Figure 11. Mass spectra corresponding to the Py-GC chromatograms of engkala seed.

2.3.5.4. Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

Liquid Chromatography-Mass Spectrometry (LC-MS) is a powerful analytical technique that has very high sensitivity and selectivity, therefore useful in many applications. It combines the physical separation capabilities of liquid chromatography with mass analysis capabilities of mass spectrometry, where its application aims toward separation, general detection and potential identification of chemicals of particular masses. It applies usually in analysis of complex mixtures such as natural products extracts.

In this study, LC-MS analysis was performed to detect the presence of polyphenolic compounds in the sample extracts of engkala fruit. Figure 12, 14, 16 and 18 show UV chromatograms of SHSD and FD engkala pulp, seed, cupule and peel respectively. The UV chromatograms showed similar patterns as the results obtained in the HPLC analysis described earlier. Table 12 summarizes the phenolic compounds detected in FD and SHSD engkala pulp as indicated by the peaks in the UV chromatograms (Figure 12). In engkala pulp, peak 2, 4, 5 and 8 showed increase in intensity in the SHSD sample compared to the FD, while four new peaks, peak 3, 6, 9 and 10, which were not detected in the FD sample appeared in the SHSD pulp.

Table 12. Phenolic compounds in FD and SHSD engkala pulp.

UPLC	PULP		MW	
Peak	FD (t_R)	SHSD (t_R)	(m/z)	Suspected compound
1	1.209	1.208		
2	1.603	1.603		
3		2.176	454.3	Viniferin (stilbene)
4	2.435	2.434	194.1	Ferulic acid (phenolic acid)
5	3.337	3.337	231.1	
6		3.473	137.0	
7	3.605	3.604	539.3	
8	3.673	3.669		
9		5.436	250.2	
10		5.734	264.2	
11	5.803	5.798	287.2	Cyanidin (flavonoid)

t_R, retention time (min); MW, molecular weight

The increased intensity in some of the peaks as well as detection of new peaks in the SHSD pulp compared to its FD counterpart might suggest more phenolic compounds of the same type as well as of different types were present in the SHSD pulp. It was suggested that the higher TPC and antioxidant activities in the SHSD pulp was probably due to this reason. Figure 13 shows mass spectrometry (MS) chromatograms of SHSD and FD engkala pulp. Some suspected phenolic compounds present in the pulp could be viniferin, ferulic acid and cyanidin.

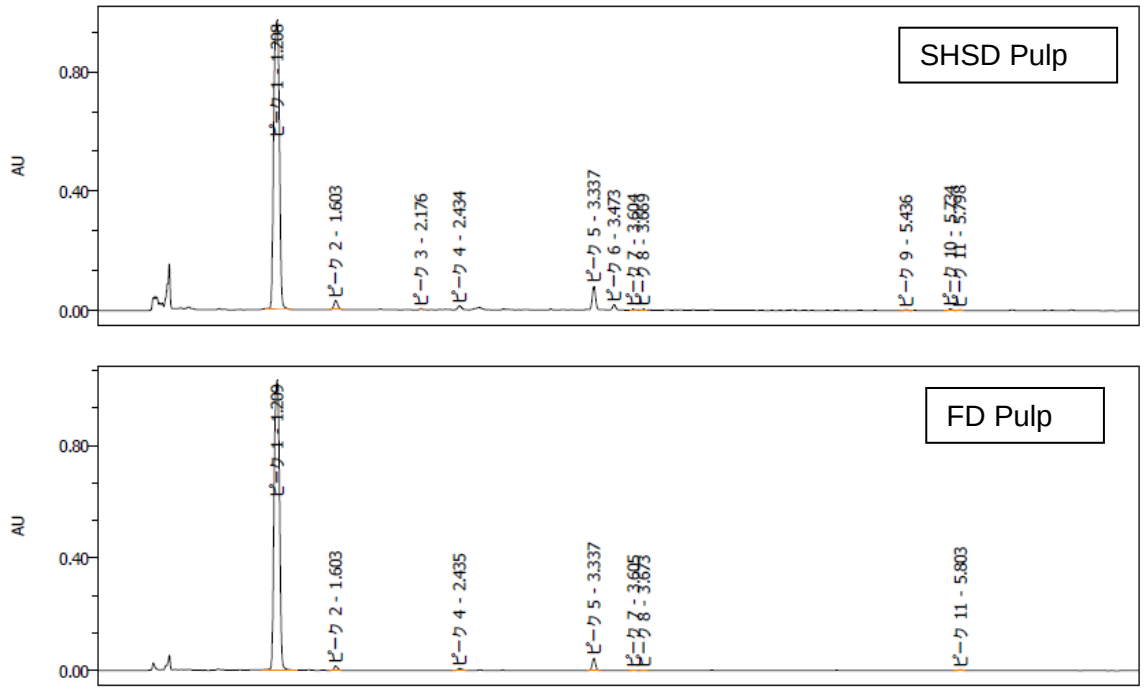


Figure 12(a). UV chromatograms of SHSD and FD engkala pulp.

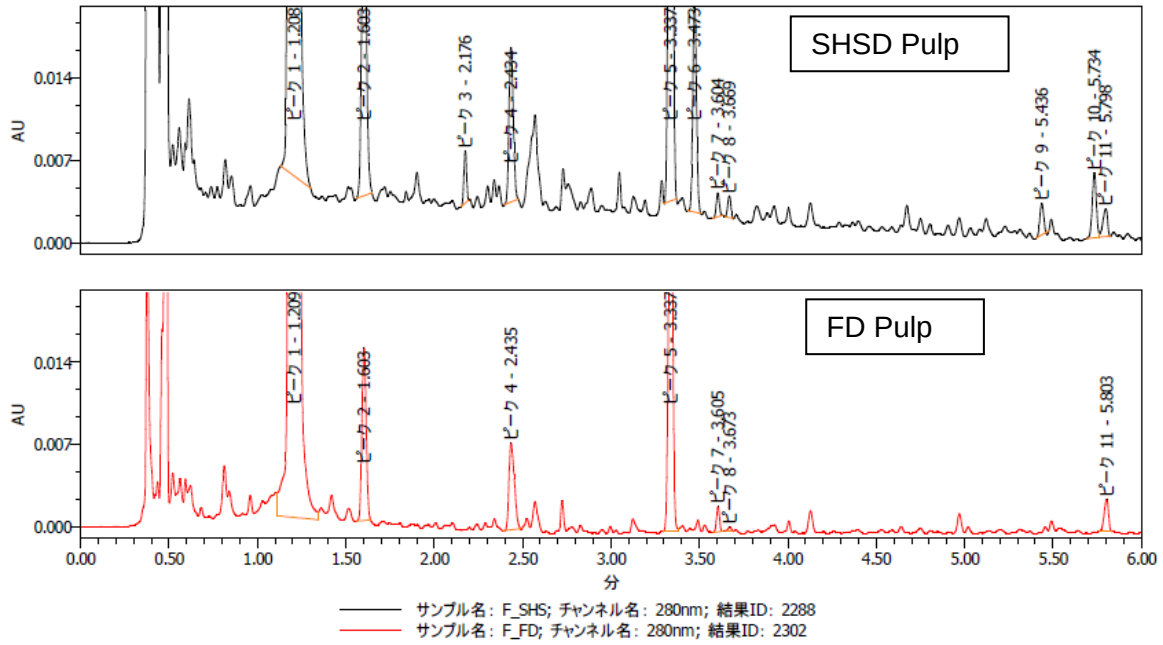
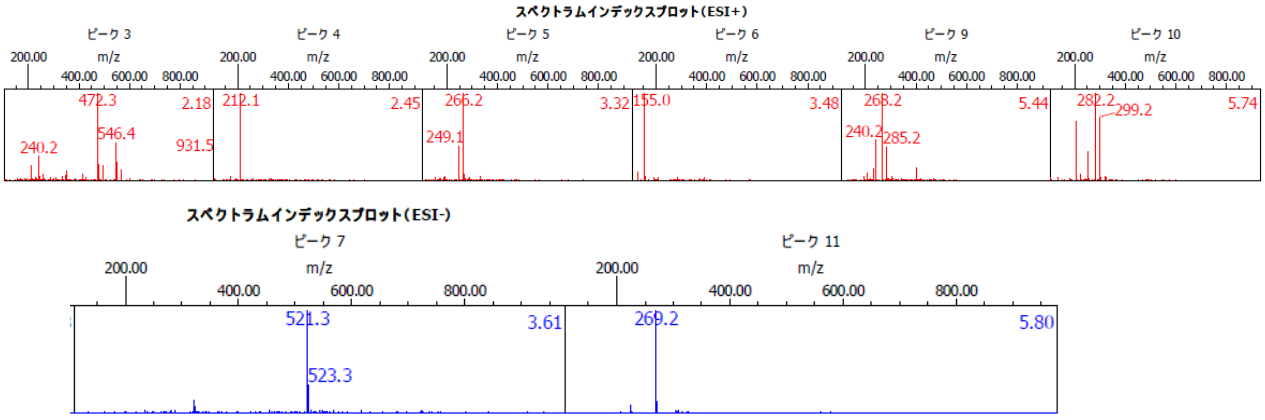
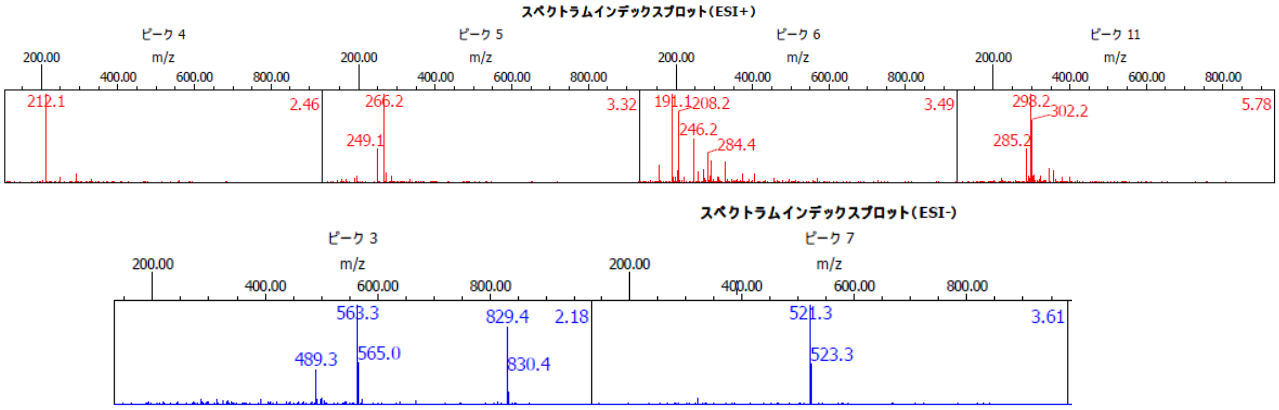


Figure 12(b). UV chromatograms of SHSD and FD engkala pulp (close-up).



(a) SHSD Pulp



(b) FD Pulp

Figure 13. MS chromatograms of (a) SHSD and (b) FD engkala pulp.

Table 13 summarizes the phenolic compounds detected in the FD and SHSD engkala seed as indicated by the peaks in the UV chromatograms (Figure 14). While some increase in intensity was detected in peak 1, 10 and 11, engkala seed saw more decrease in intensity in the peaks of the SHSD sample compared to the FD, as observed in peak 2, 4, 7, 9 and 13. Furthermore, peak 3, 5, 6 and 14 which were detected in the FD seed were not detected in the SHSD sample.

Table 13. Phenolic compounds in FD and SHSD engkala seed.

UPLC	SEED			
Peak	FD (t_R)	SHSD (t_R)	MW	Suspected compound
1	1.230	1.210	234.1	
2	1.357	1.367		
3	1.419		176.1	
4	1.523	1.531		
5	1.984		172.1	
6	2.041		304.2	Dihydroquercetin (Flavonoid)
7	2.552	2.545	296.2	p-Coumaroyl tartaric acid (Phenolic acid)
8	2.582	2.587		
9	2.917	2.957	312.2	Caffeoyl tartaric acid (Phenolic acid)
10	3.343	3.339	214.1	
11	3.489	3.476	266.3	
12	3.519	3.521	310.2	Cinnamoyl glucose (Phenolic acid)
13	3.791	3.795	348.1	
14	4.320		308.2	

t_R, retention time (min); MW, molecular weight

This result showed that more phenolic compounds of the same type as well as of different types were present in the FD seed compared to the SHSD seed. This might suggest that the phenolic compounds present in the seed of engkala were probably sensitive towards heat, thus either reduced in amount or destroyed by the high temperature of the superheated-steam. This might explain the lower TPC and antioxidant activities in the SHSD seed compared to its FD counterpart described earlier. Figure 15 shows MS chromatograms of SHSD and FD engkala seed. Some

suspected phenolic compounds present in the seed could be dihydroquercetin, p-coumaroyl tartaric acid, caffeoyl tartaric acid and cinnamoyl glucose.

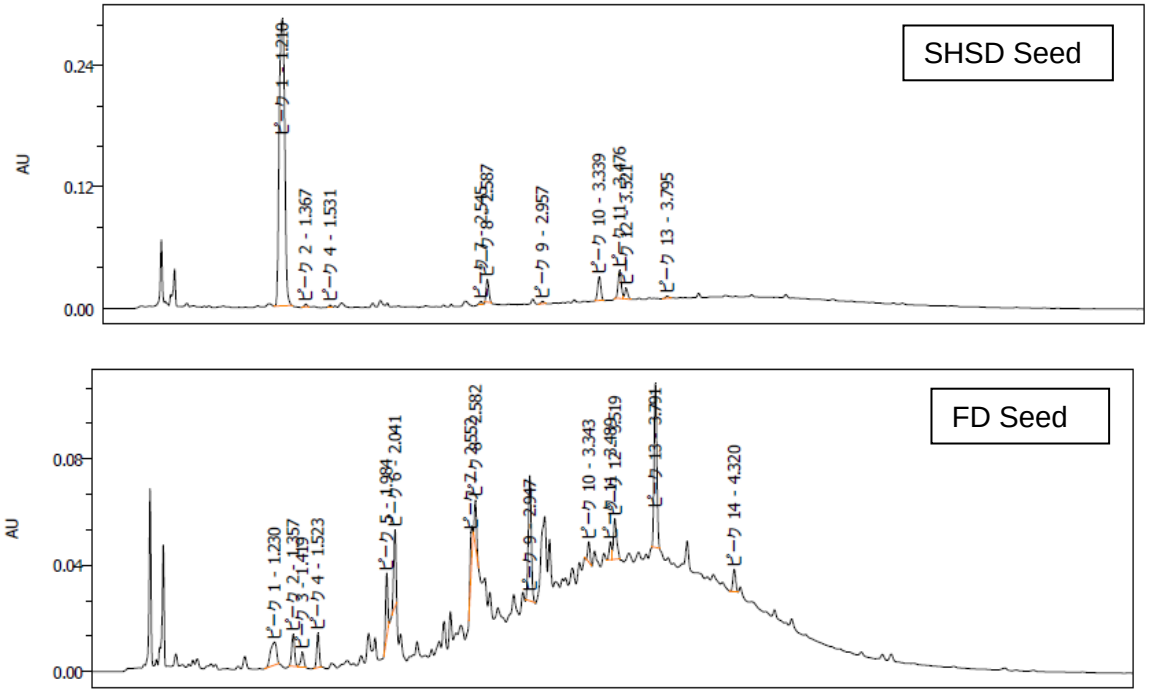


Figure 14(a). UV chromatograms of SHSD and FD engkala seed.

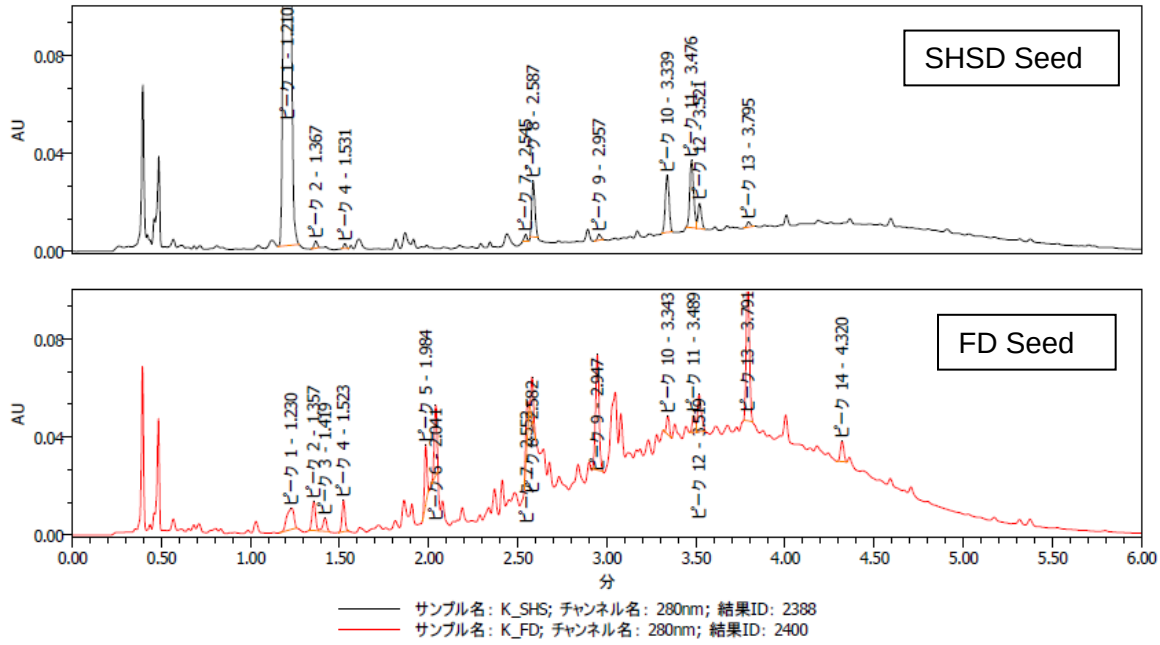
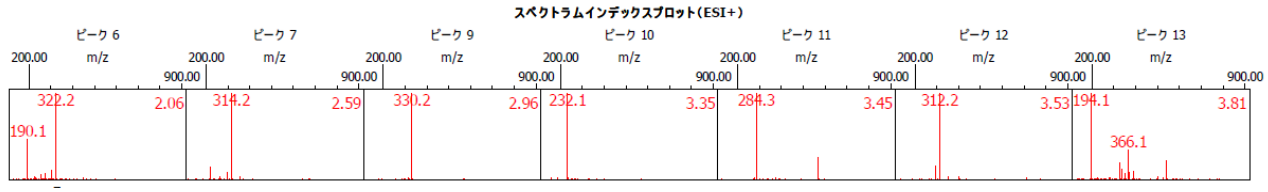
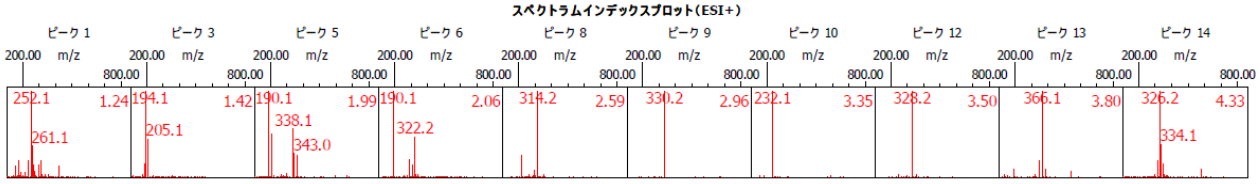


Figure 14(b). UV chromatograms of SHSD and FD engkala seed (close-up).



(a) SHSD Seed



(b) FD Seed

Figure 15. MS chromatograms of (a) SHSD and (b) FD engkala seed.

Table 14 summarizes the phenolic compounds detected in the FD and SHSD engkala cupule as indicated by the peaks in the UV chromatograms (Figure 16). The chromatograms detected two increase in the SHSD cupule, namely peak 2 and 5, but in the FD cupule, the chromatogram detected peak 9, which was not present in the SHSD cupule. Not much difference was observed in the intensity of the other peaks in both samples. The TPC and the antioxidant activities of the cupule described earlier showed not much difference in the trends between the FD and SHSD samples, which might be owed to the suggestion that mostly similar type of phenolic compounds with similar intensity were present in the FD and SHSD cupule. Figure 17 shows MS chromatograms of SHSD and FD engkala cupule. Some suspected phenolic compounds present in the cupule could be ferulic acid, cinnamoyl glucose and epigallocatechin.

Table 14. Phenolic compounds in FD and SHSD engkala cupule.

UPLC	CUPULE			
Peak	FD (t_R)	SHSD (t_R)	MW	Suspected compound
1	1.210	1.210		
2	1.608	1.604		
3	2.447	2.434	194.1	Ferulic acid (phenolic acid)
4	2.585	2.587	172.1	
5	3.335	3.337	310.2	Cinnamoyl glucose (phenolic acid)
6	3.474	3.484	137.0	
7	3.515	3.515	294.2	
8	3.606	3.604	306.2	Epigallocatechin (flavanols)
9	5.545		269.2	

t_R, retention time (min); MW, molecular weight

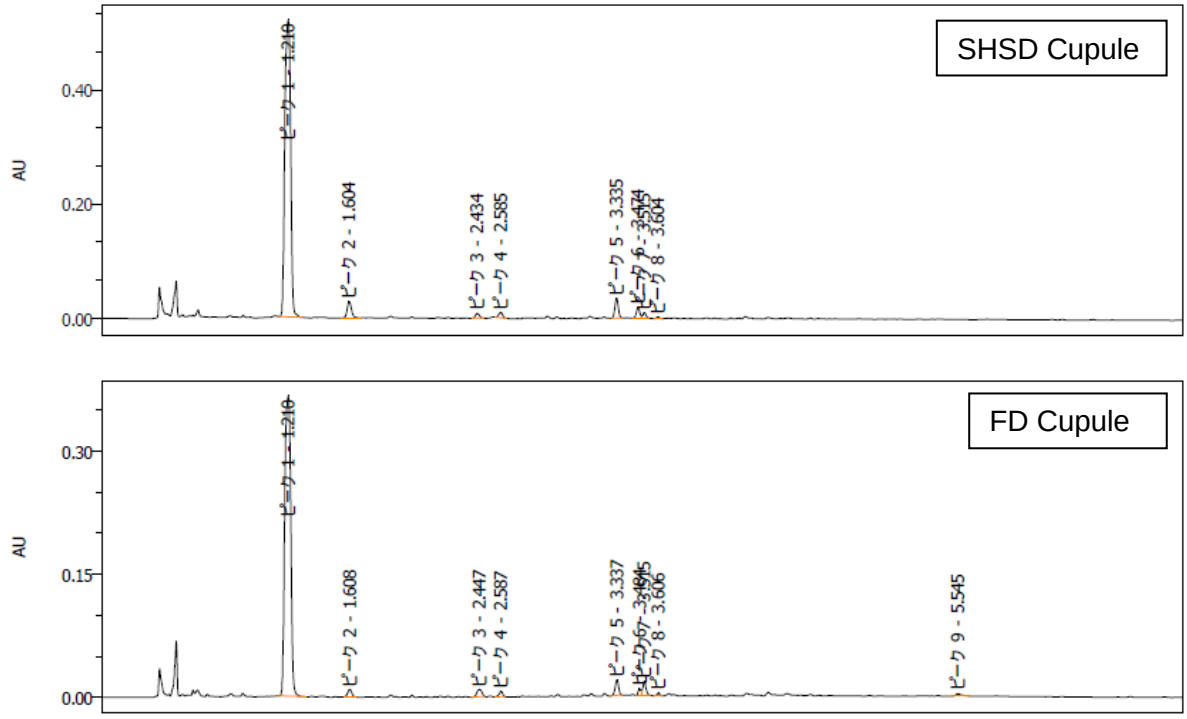


Figure 16(a). UV chromatograms of SHSD and FD engkala cupule.

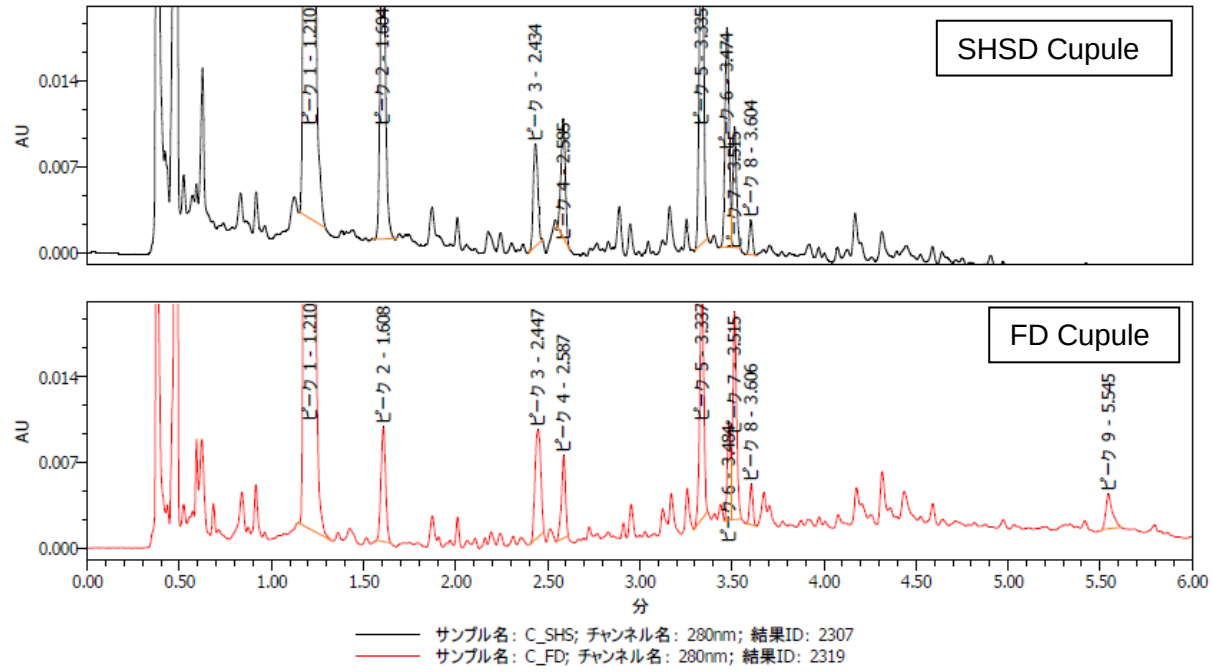
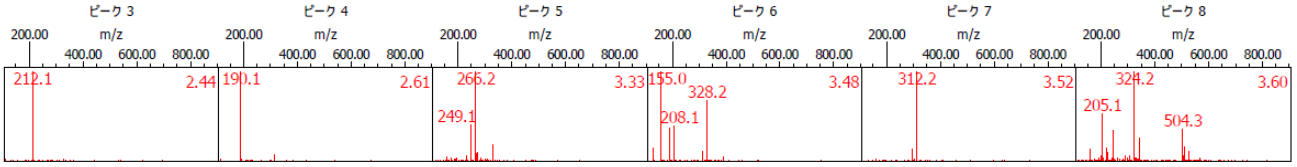
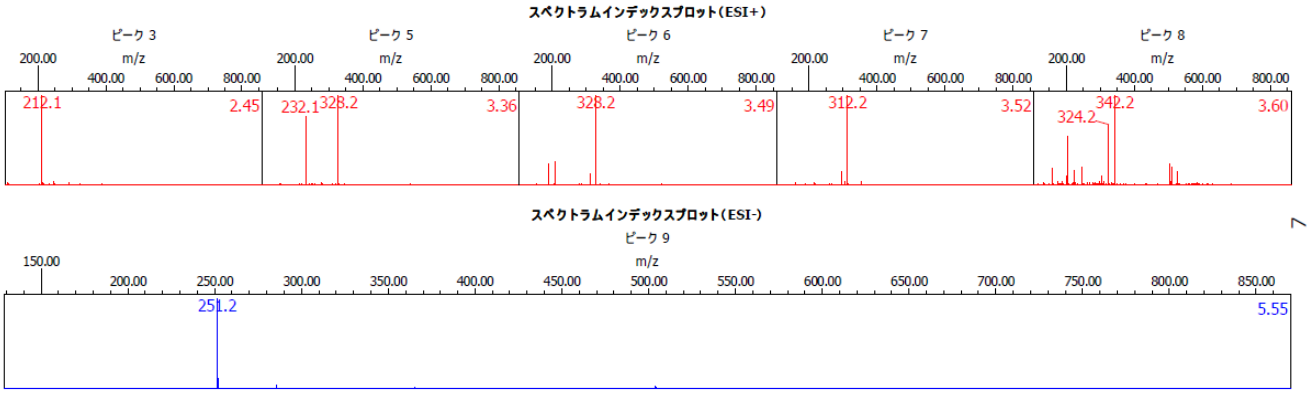


Figure 16(b). UV chromatograms of SHSD and FD engkala cupule (close-up).



(a) SHSD Cupule



(b) FD Cupule

Figure 17. MS chromatograms of (a) SHSD and (b) FD engkala cupule.

Table 15 summarizes the phenolic compounds detected in the FD and SHSD engkala peel as indicated by the peaks in the UV chromatograms (Figure 18). Some increase in intensity was detected in peak 2, 7 and 9 in the SHSD engkala peel. However, five peaks were observed in the FD peel, namely peak 3, 6, 8, 10 and 11, which were not present in its SHSD counterpart, while four peak were also observed to be present in the SHSD peel, namely peak 4, 13, 14 and 15, which were not present in its FD counterpart.

Table 15. Phenolic compounds in FD and SHSD engkala peel.

UPLC	PEEL			
Peak	FD (t_R)	SHSD (t_R)	MW	Suspected compound
1	1.210	1.209		
2	1.608	1.604		
3	2.079		172.1	
4		2.176	454.3	Viniferin (stilbene)
5	2.721	2.726	200.1	
6	2.912		627.4	Delphinidin 3,5-O-diglucoside (flavonoid)
7	3.339	3.339	248.2	
8	3.387		294.2	
9	3.493	3.475	310.2	Cinnamoyl glucose (phenolic acid)
10	3.527		294.2	
11	4.505		409.3	
12	4.821	4.817	276.2	
13		5.126		
14		5.441	250.2	
15		5.738	264.2	

t_R, retention time (min); MW, molecular weight

Even though significant difference was observed in the ORAC antioxidant activity, however no significant difference was observed in the TPC, TFC and DPPH antioxidant activity as described earlier, suggesting that similar amount of phenolic compounds were present in the FD and SHSD peel. This result also suggested that more difference in the type of phenolic compounds between the FD and SHSD

samples present in the peel compared to the other parts. Figure 19 shows MS chromatograms of SHSD and FD engkala peel. Some suspected phenolic compounds present in the peel could be viniferin, delphinidin 3,5-O-diglucoside and cinnamoyl glucose.

Superheated-steam treatment in engkala fruit in this study have brought some changes in the phenolic compounds profile of the fruit, where the high energy from the superheated-steam could cause some compounds either to be destroyed or induce some inter-conversion of phenolic compounds to produce new structure. The higher antioxidant capacity particularly in the pulp of engkala could indicate that phenolic compounds with better antioxidant capacity could be produced in the process. A study done on red ginseng shows strongest antioxidant activity when ginseng is steamed at 120°C, which is the highest temperature used in the study. HPLC results saw changes in elution profile where levels of certain ginsenosides decrease, while levels of others increase after steam treatment (Kim et al., 2000).

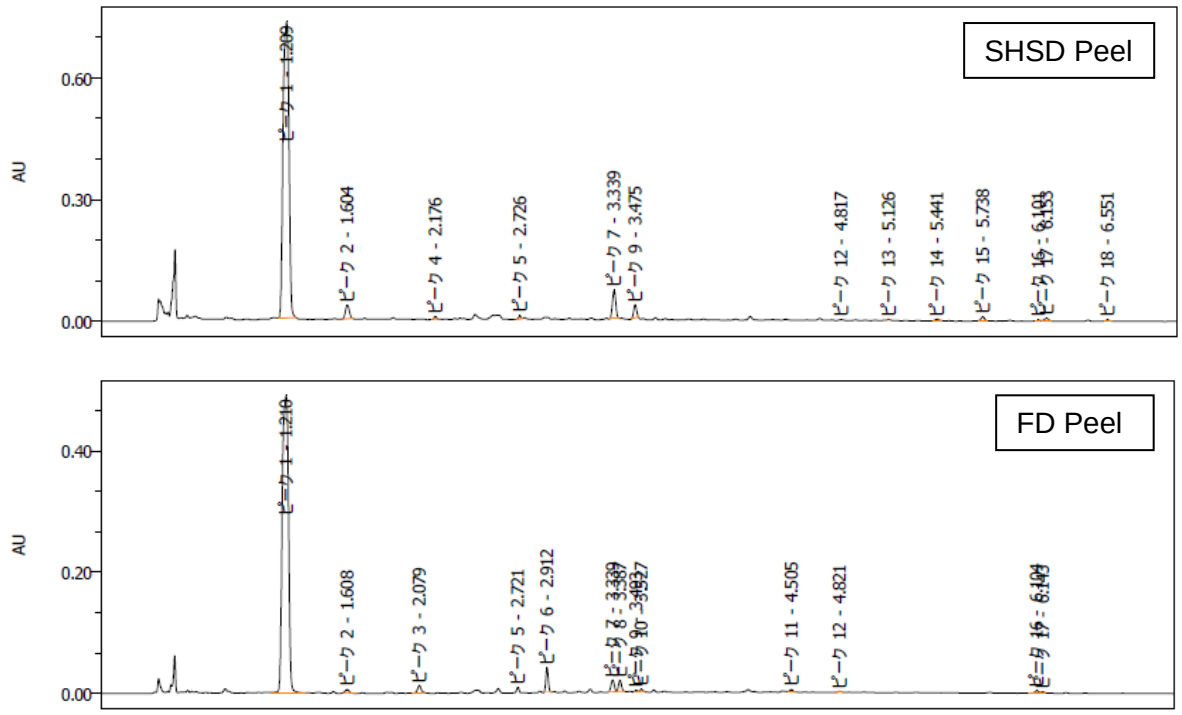


Figure 18(a). UV chromatograms of SHSD and FD engkala peel.

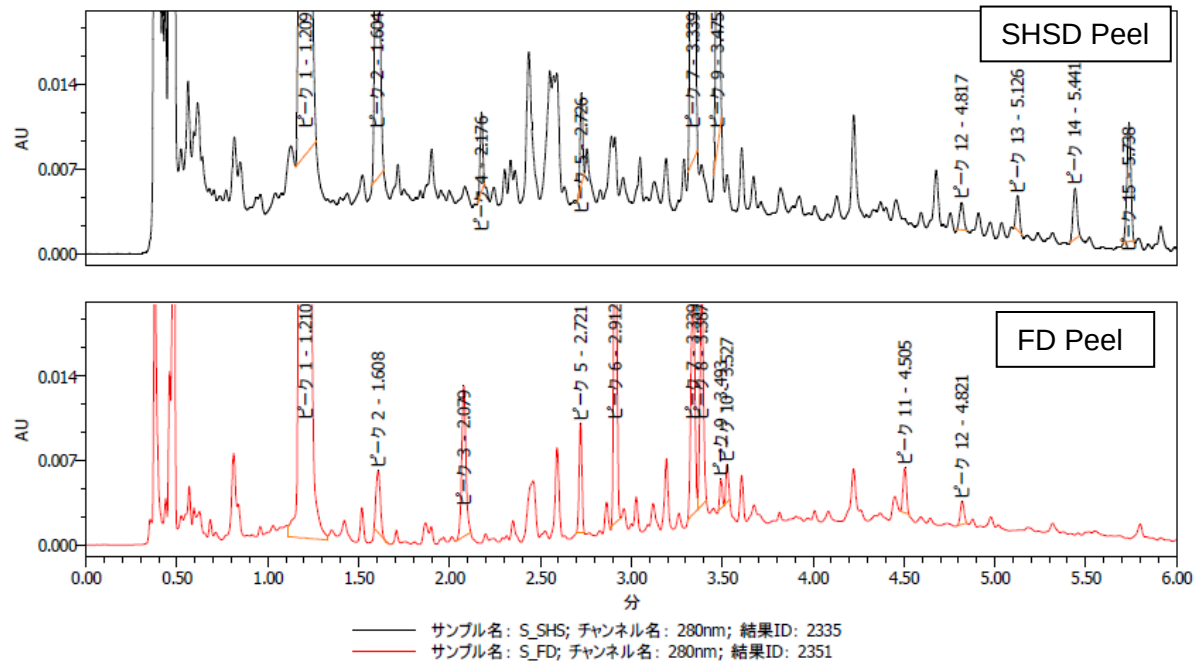
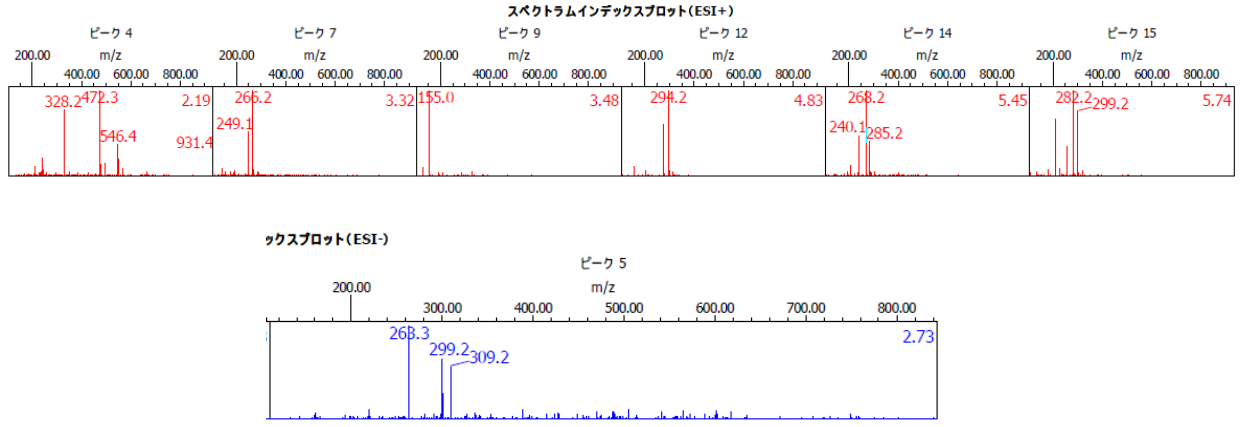
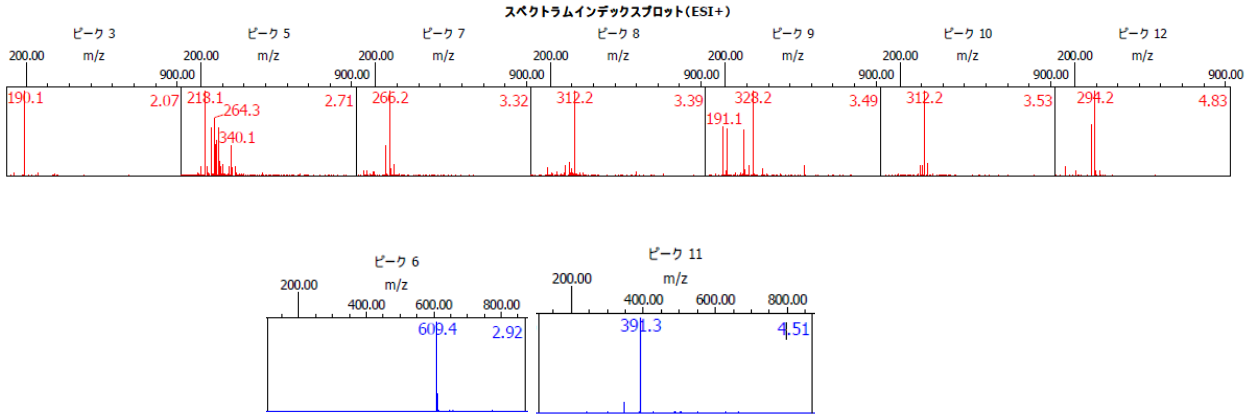


Figure 18(b). UV chromatograms of SHSD and FD engkala peel (close-up).



(a) SHSD Peel



(b) FD Peel

Figure 19. MS chromatograms of (a) SHSD and (b) FD engkala peel.

2.4. Conclusions

The result obtained in this study was in agreement with Voon & Kueh (1999), where engkala fruit was found to be highly nutritious. It contained high protein, carbohydrate, K, Na, Fe, Zn, and vitamin C. The vitamin C content in the SHSD engkala was found to be significantly higher compared to the FD samples was probably due to denaturation of ascorbic acid oxidase enzyme by the high temperature of the superheated-steam, thus protecting the vitamin from oxidation process when exposed to oxygen in the air.

The current method of preparation for eating engkala where the fruits are subjected to high temperature of hot water or steam was found to be appropriate as treatment with superheated-steam lead to significantly higher TPC, TFC and antioxidant activities in the pulp. Hence superheated-steam drying was considered as the preferred method of drying for production of engkala pulp products. The significantly high antioxidant capacity in the SHSD engkala pulp compared to the FD might be due the increase in certain polyphenols as well as the presence of new polyphenols which were not found in the FD pulp, acquired through inter-conversion of phenolic compounds in the pulp.

This study also showed that among all the parts, the FD seed of engkala fruit contained the highest antioxidant capacity. When SHSD, the seed showed significantly lower TPC, TFC and antioxidant activities compared to the FD samples. The elusion profile of the phenolic compounds showed by the seed might suggest that the phenolic compounds present were probably heat sensitive.

CHAPTER 3. EVALUATION OF POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY IN AVOCADO FRUIT DRIED WITH SUPERHEATED-STEAM

3.1. Introduction

Avocado extract is usually obtained by solid-liquid extraction with different solvents. In research involving plants, various extraction methods have been developed for the extraction of phytochemicals in order to increase the extraction yield, shorten the extraction time as well as enhance the quality of extracts. Ultrasound- assisted extraction is among the methods used to enhance the process of extraction. It is inexpensive, simple and efficient alternative to conventional extraction techniques. Like soxhlet extraction, it can be used with any solvent for extracting a wide variety of natural compounds. In solid-liquid extraction, it offers advantages which include increased yield and faster kinetics. The apparatus is cheaper and easier to operate compared to other novel extraction techniques such as microwave-assisted extraction and supercritical fluid extraction (L. Wang & Weller, 2006). Ultrasound-assisted extraction is widely used in the extraction of plant compounds. However, no information is available on the effect of ultrasound-assisted extraction on polyphenol content in avocado.

Drying process of plant materials is involved in many research conducted to access the polyphenol contents in fruits and vegetables, as well as their antioxidant activities. Studies have shown that the contents of polyphenols and antioxidant activity in dried plant materials can be higher compared to the fresh plant materials (C. H. Chang et al., 2006; Choi et al., 2006). Freeze drying is based on the

dehydration by sublimation of a frozen product. Most deterioration and microbial reactions are prevented due to the absence of liquid water and the low temperatures required for the process. These in return produce a final product of excellent quality. Unfortunately, freeze drying has always been recognized as the most expensive drying process (Ratti, 2001). Studies on avocado fruit usually involve either freeze dried or fresh fruit samples. No information is available on the effects of drying with higher temperature on avocado.

Hence, this chapter aims

- To evaluate the usage of ultrasound in overcoming the limitation of conventional extraction of avocado fruit
- To evaluate antioxidant capacity of avocado fruit undergoing heat treatment, superheated-steam in particular, and freeze drying was used as reference.

3.2. Methodology

3.2.1. Chemicals

Folin-Ciocalteu's phenol reagent, gallic acid ($C_7H_6O_5$), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), rutin ($C_{27}H_{30}O_{16}$) were purchased from Sigma-Aldrich Chemie GmbH, Germany. Sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH) were purchased from Merck, Germany. DPPH (2,2-diphenyl-1-picrylhydrazil) ($C_{18}H_{12}N_5O_6$), Fluorescein sodium salt ($C_{20}H_{10}Na_2O_5$), AAPH: 2,2'-Azobis(2-methyl propionamidine)dihydrochloride ($C_8H_{18}N_6.2HCl$), Trolox (6-Hydroxy -2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were purchased from Sigma-Aldrich Inc.,

USA. Phosphate buffer saline (PBS) was purchased from Invitrogen Corporation, CA, USA. All chemicals were of analytical grade.

3.2.2. Preparation of Avocado fruit sample

Three kilogram of fresh avocado fruits (Hass avocado of Mexican strain) were purchased from the local grocery store in Kitakyushu, Japan and left at room temperature to ripen naturally. Once ripened, as indicated by the softened fruit and darkened skin, all the fruits were cleaned and manually separated into pulp, peel and seed. The fruit parts were then dried and the moisture loss was measured using Moisture Balance MOC-120H (Shimadzu Corporation, Japan).

3.2.3. Drying methods

Refer to section 2.2.3. (page 18).

3.2.4. Sample extraction

One gram of dried sample was extracted in 80% ethanol with ratio of solid:liquid was at 1:30 (wt/vol). The mixture was centrifuged at 400 rpm under 30°C and 40°C for 2 hours using Heidolph Instrument Unimax 1010DT orbital shaker, Germany.

As another extraction approach, ultrasound assisted extraction (UAE) method was applied, where the ethanolic solution was ultrasonicated for 5, 10, 15 and 20 minutes using a 37kHz ultrasonic generator (UT-106, SHARP, Japan). Extraction process

was continued using orbital shaker following the procedure as mentioned above but the duration was reduced to only 30 minutes. The extracts were then filtered using a Whatman No. 4 filter paper and the resulting ethanolic extract was analyzed for total phenolic content (preliminary study). The extraction temperature and ultrasonication duration which give the highest total phenolic content was selected as a standard extraction method in this study.

3.2.5. Phytochemical Study

3.2.5.1. Total Phenolic Content (TPC)

Refer to section 2.2.6.1. (page 19).

3.2.5.2. Total Flavonoid Content (TFC)

Refer to section 2.2.6.2. (page 20).

3.2.6. Antioxidant Activities

3.2.6.1. DPPH Radical Scavenging Activity

Refer to section 2.2.7.1. (page 21).

3.2.6.2. Oxygen Radical Absorbing Capasity (ORAC)

Refer to section 2.2.7.2. (page 21).

3.2.7. Statistical analysis

Refer to section 2.2.9. (page 26).

Avocado (Hass)

Pulp, Peel & Seed

Superheated-steam drying

130, 150, 170°C

Freeze drying

Final moisture <10%

Sovent extraction, 80% EtOH

Total Phenolic Content	Total Flavonoid Content
Measures phenolic acids content; Folin-Ciocalteau colorimetric method (Waterhouse, 2002; Wolfe et al., 2008).	**Measures flavonoids content**; colorimetric method by (Wolfe et al., 2003) with slight modification.

DPPH Assay	ORAC Assay
Measures the capacity of fruit extract to scavenge DPPH free radical; Colorimetric method (Othman et al., 2007)	**Measures oxygen radical scavenging efficacy of fruit extract**; Ahmad Aufa et al. (2014)

3.3. Results and discussion

3.3.1. Moisture Content and Yield

Moisture is a major component of avocado fruit, as indicated by the high moisture content (David Klein 1998). The moisture content of the different parts of fresh avocado fruit is shown is Table 12. The highest moisture content of avocado was shown in the pulp, followed by the peel and seed. The given data was similar to a reported moisture content of avocado (Rodríguez-Carpena et al., 2011).

Table 16. Moisture content of fresh avocado fruit parts.

	Avocado	
	Moisture content (%)	± S.D.
Pulp	74.43	2.18
Peel	72.70	2.36
Seed	52.43	4.04

Results expressed as mean ±S.D. (n=3).

Table 13 shows percentage yield of FD and SHSD (steam temperature of 130°C, 150°C and 170°C) avocado fruit parts. Yield for the seed was higher than the pulp and peel, all relating to their respective moisture content. There were no significant difference between the yield obtained from freeze drying and superheated-steam drying. In the SHSD samples, duration of the drying process taken for the samples to reach their final moisture of ~10% was 3 hours for the pulp, 2 hours for the seed and 1 hour for the peel. For the FD samples however, it was observed that the drying process took many days (data not shown) for the samples to reach similar results. The process was a highly time consuming in freeze drying compared to

superheated-steam drying. Energy saving is an advantage that superheated-steam drying is very well known for, apart from many other advantages (Mujumdar, 2007).

Table 17. Percentage yield and final moisture of SHSD and FD avocado fruit parts.

		Yield (%)	Final moisture (%)	± S.D.
Pulp	FD	26.97	4.03	0.83
[a]3 hrs	[b]SHSD, 130°C	31.67	2.29	0.25
	SHSD, 150°C	27.18	2.89	0.53
	SHSD, 170°C	26.92	1.49	0.15
Seed	FD	47.92	10.24	0.30
[a]2 hrs	SHSD, 130°C	52.15	3.74	0.70
	SHSD, 150°C	49.87	6.68	1.17
	SHSD, 170°C	48.63	9.31	0.38
Peel	FD	28.92	7.12	0.36
[a]1 hrs	SHSD, 130°C	26.76	6.67	0.49
	SHSD, 150°C	28.60	5.86	0.55
	SHSD, 170°C	26.65	4.90	0.31

Results expressed as mean ±S.D. (n=3). [a] duration of SHS treatment for the group.
[b] additional 30 mins.

3.3.2. Total Phenolic Content (Preliminary Study)

As presented in Table 14, solid:liquid (wt/vol) ratio that gave a higher TPC reading was 1:30 at both temperatures used. TPC was higher at the temperature of 40°C for both solid:liquid ratio. Thus, TPC was highest at solid:liquid ratio of 1:30 and at the temperature of 40°C. This condition gave the best result and it was taken as the best condition to perform avocado fruit extraction. This condition was therefore used for the extraction of all fruit samples in this study.

Table 18. Total phenolic content for non-UAE of avocado fruit.

	30°C		40°C	
Wt/vol	GAE	±S.D.	GAE	±S.D.
1:30	163.27	1.43	166.32	0.98
1:40	130.21	2.26	155.95	2.26

Results expressed as mean ±S.D. (n=3). GAE, gallic acid equivalent (mg GAE/100g dried sample).

When extraction was repeated using ultrasonic assisted extraction method, the TPC readings were higher as shown in Table 15, compared to the non-ultrasonic (166.32 mg GAE/100g dried sample extracted using 1:30 ratio at 40°C), where the increase was at ~31% – 41% when 5 to 20 mins of sonication was applied. Among the four different durations used for sonication, the duration of 15 mins gave the highest TPC reading at 235.77 mg of GAE/100g dried sample, and this value was significantly higher when compared to the other duration. This duration was used to perform sonication in the preparation of all fruit extracts.

Table 19. Total phenolic content for UAE of avocado fruit with different ultrasonication duration.

Ultrasonication duration	GAE	±S.D.	% Increase
5 mins	226.69	0.56	36.30
10 mins	219.19	0.28	31.79
15 mins	235.77	0.80	41.75
20 mins	222.44	0.85	33.74

Results expressed as mean ±S.D. (n=3). GAE, gallic acid equivalent (mg GAE/100g dried sample).

Ultrasound assisted extraction is an attractive alternative to conventional extraction techniques because it is easy, inexpensive and efficient. The main benefit of including ultrasound in extraction is it increases yield and extraction process can be done at a faster rate (L. Wang & Weller, 2006). In this study, the usage of ultrasound has successfully enhanced the extraction process as indicated by the significantly

higher TPC values and furthermore, has reduced the centrifuge time from 2 hours to 30 minutes (75% reduction).

This is made possible due to the propagation of ultrasound pressure waves through the solvent resulting in cavitation phenomena. The controlling mechanism of ultrasound-assisted extraction is generally attributed to mechanical, cavitation, and thermal effects which can result in disruption of cell walls, particle size reduction, and enhanced mass transfer across cell membranes, which leads to target compounds dissolving in the solvent, hence increasing yield with shorter time (Shirsath, Sonawane, & Gogate, 2012). Research have found that ultrasonication is a critical pretreatment to obtain high yields of oils from almond, apricot and rice bran (Sharma & Gupta, 2004). For extraction of saponin from ginseng assisted by ultrasound, the total yield and saponin yield increased by 15 and 30%, respectively (Hui Li, Ohdaira, & Ide, 1994). The yield of oil extracted from soybeans also increased significantly when ultrasound was applied (Haizhou Li, Pordesimo, & Weiss, 2004).

3.3.3. Phytochemical Study

3.3.3.1. Total Phenolic Content (TPC)

Table 16 shows TPC of avocado fruit parts dried using freeze drier and superheated-steam dryer at the temperature of 130°C, 150°C and 170°C. The pulp and seed part of avocado showed similar pattern for TPC values as engkala fruit as described in the earlier chapter. In the edible part of avocado (pulp), the TPC was significantly higher in SHSD pulp (735.06 - 934.61 mg GAE/100g dried sample) compared to the

FD pulp (520.55 mg GAE/100g dried sample), whereas the TPC of FD seed (3251.15 mg GAE/100g dried sample) was significantly higher compared to the SHSD (1315.45 - 1481.91 mg GAE/100g dried sample). The peel when FD gave the highest TPC in avocado at 4065.70 mg GAE/100g dried sample, but the TPC value was significantly lower when the peel was SHSD (2405.03 - 2761.64 mg GAE/100g dried sample).

Table 20. Total phenolic content of avocado fruit parts.

		GAE/100g	± S.D.	
Pulp	FD	520.55	48.59	a
	SHSD, 130°C	735.06	41.70	b
	SHSD, 150°C	739.33	22.81	b
	SHSD, 170°C	934.61	14.56	c
Peel	FD	4065.70	83.64	a
	SHSD, 130°C	2405.03	19.00	b
	SHSD, 150°C	2748.61	2.29	c
	SHSD, 170°C	2761.64	1.82	c
Seed	FD	3251.15	82.95	a
	SHSD, 130°C	1481.91	21.95	b
	SHSD, 150°C	1334.24	12.59	c
	SHSD, 170°C	1315.45	49.64	c

Results expressed as mean ±S.D. (n=3). GAE, Gallic acid equivalent.

The result on FD avocado in this study was in agreement with studies on avocado done by Kosińska et al. (2012) reporting that TPC for FD peel is higher than the seed, while its pulp contains much less phenolic compounds (W. Wang, Bostic, & Gu, 2010). Although the pulp part contained the lowest amount of TPC in avocado fruit, the value from this finding was higher in both SHSD and FD pulp compared to a study conducted on fresh pulp of avocado (Wolfe et al. 2008), suggesting that drying might give positive effect on total phenolic of avocado pulp.

3.3.3.2. Total Flavonoid Content (TFC)

Table 17 shows TFC of avocado fruit parts dried using freeze drier, and superheated-steam dryer at the temperature of 130°C, 150°C and 170°C. The result for TFC followed the same pattern as the above described TPC. The highest TFC was observed in the FD peel (2505.18 mg RE/100g dried sample) followed by the FD seed (1788.57 mg RE/100g dried sample). These values however, showed dramatic downturn when the peel and seed were SHSD, where the TFC were significantly lower (1149.92 - 1687.67 mg and 453.39 - 562.33 mg RE/100g dried sample in the peel and seed respectively). However in the edible part, just like in the TPC, the same pattern was also observed in the pulp where the FD pulp gave the lowest TFC value for the FD samples (492.08 mg RE/100g dried sample), but this value was significantly higher when the pulp was SHSD (643.92 - 731.31 mg RE/100g dried sample).

Table 21. Total flavonoid content of avocado fruit parts.

		mg RE/100g dw	± S.D.	
Pulp	FD	492.08	41.76	a
	SHSD, 130°C	688.86	28.20	b
	SHSD, 150°C	643.92	54.00	b
	SHSD, 170°C	731.31	73.06	b
Seed	FD	1788.57	53.24	a
	SHSD, 130°C	562.33	42.62	b
	SHSD, 150°C	453.39	34.33	c
	SHSD, 170°C	472.82	12.34	c
Peel	FD	2505.18	39.41	a
	SHSD, 130°C	1149.92	60.12	b
	SHSD, 150°C	1642.49	25.63	c
	SHSD, 170°C	1687.67	99.95	c

Results expressed as mean ±S.D. (n=3). RE, Rutin equivalent.

In this study, superheated-steam drying gave significantly higher TPC and TFC in the edible part of avocado, which is the pulp, and the values increased with the increase in the steam temperature. Freeze drying on the other hand gave significantly higher TPC and TFC in the peel and seed. This result suggested that chemical components in the peel and seed seemed to be sensitive toward heat, thus destroyed by the high energy in the superheated-steam. This is evident especially in the seed where TPC value was found to be lower with the increase in steam temperature. The process of freeze drying on the other hand did not apply any heat on the samples, thus allowing more of the compounds to be preserved.

3.3.4. Antioxidant Study

3.3.4.1. DPPH radical scavenging activity

Table 18 shows DPPH radical scavenging activity of avocado fruit parts dried using freeze drier and superheated-steam dryer at the temperature of 130°C, 150°C and 170°C. The scavenging activity of avocado fruit parts showed a similar trend as portrayed by the TPC and TFC as discussed above. As presented in the table, freeze drying seemed to offer advantages in the drying of the seed and skin as indicated by the lower IC_{50} value of the FD parts compared to their respective SHSD counterparts. When these parts were SHSD, the IC_{50} values were found to be higher (lower antioxidant activity). The pulp on the contrary favoured superheated-steam as its drying method as the IC_{50} value was significantly lower compared to its FD counterpart, and this value went even lower with the increase in the steam temperature.

Table 22. DPPH radical scavenging activity of avocado fruit parts.

		IC_{50} (mg/ml)		
		Mean	± S.D.	
Pulp	FD	35.02	3.409	a
	SHSD, 130°C	11.79	0.419	b
	SHSD, 150°C	12.16	0.471	bc
	SHSD, 170°C	6.69	0.216	d
Peel	FD	0.09	0.001	a
	SHSD, 130°C	0.50	0.011	b
	SHSD, 150°C	0.27	0.014	c
	SHSD, 170°C	0.26	0.005	cd
Seed	FD	0.22	0.019	a
	SHSD, 130°C	1.47	0.020	b
	SHSD, 150°C	1.47	0.070	b
	SHSD, 170°C	1.56	0.053	b

Results expressed as mean ±S.D. (n=3). DPPH, 2,2-diphenyl-1-picrylhydrazil; *p<0.05 compared to FD sample.

3.3.4.2. Oxygen radical absorbing capacity (ORAC)

Table 19 shows ORAC value of avocado fruit parts dried using freeze drier and superheated-steam dryer at the temperature of 130°C, 150°C and 170°C. The oxygen radical absorbing capacity of the fruit parts was similar to the DPPH radical scavenging activity as described above, where the seed and peel favoured freeze drying as a drying method, as indicated by the higher ORAC value of the FD parts. But when SHSD, the ORAC values were found to be lower. The pulp, which is the edible part the other hand could offer more benefit when SHSD. The pulp showed comparable radical absorbing capacity between the FD and the SHSD at 150°C. However, when the steam temperature was increased to 170°C, the ORAC value was significantly increased by 43% (from 1513 to 2658 µmol TE/100g) in the SHSD pulp.

Considering the edible part of avocado fruit, ORAC values of the FD and SHSD pulp in this study both were higher than the ORAC value for fresh avocado sample reported in another study (Wolfe et al., 2008).

Table 23. Oxygen radical absorbing capacity (ORAC) of avocado fruit parts.

		ORAC (μmol TE/100g)		
		Mean	± S.D.	
Pulp	FD	1518	185	a
	SHSD, 130°C	537	76	b
	SHSD, 150°C	1513	124	ac
	SHSD, 170°C	2658	107	d
Peel	FD	212362	9604	a
	SHSD, 130°C	32720	1087	b
	SHSD, 150°C	50239	3360	c
	SHSD, 170°C	53143	1840	cd
Seed	FD	92782	8210	a
	SHSD, 130°C	15083	981	b
	SHSD, 150°C	16393	334	bc
	SHSD, 170°C	18607	171	d

Results expressed as mean ±S.D. (n=3). Different letters in the same group indicate significant differences ($p<0.05$).

The DPPH and ORAC assays showed that the highest antioxidant activities in avocado was found in the peel, followed by the seed and finally the pulp part, which contained the lowest. This result was in agreement with the DPPH and ORAC data given by another study on FD avocado (W. Wang et al., 2010).

The antioxidant activities in both DPPH and ORAC assays were in parallel with the results from the TPC and TFC studies described above, suggesting correlation between them. The targeted part of the fruit, which was the pulp, showed that superheated-steam drying was a better choice of drying method. The by-products of avocado fruit, the peel and seed, however both gave results which contrast with the result for the edible part, where freeze drying appeared to be a better choice of

drying method. A recent study also showed freeze dried mango peel and kernel gave higher total phenolics and antioxidant activities compared to hot air, vacuum and infra-red drying (Sogi, Siddiq, Greiby, & Dolan, 2013).

In avocado, pulp is the part of the fruit which is normally consumed for its nutritional benefit. Its peel and seed are considered as by-products and usually discarded as wastes. This study have shown that the phenolic and flavonoid content as well as antioxidant activities of the peel and seed of avocado were much higher compared to the data on pulp, especially when freeze dried. This is in agreement with another study on avocado where TPC and antioxidant activities of the peel and seed are much higher than the pulp part (Rodríguez-Carpena et al., 2011). A study on bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus)* fruits also showed that total phenolic, total flavonoid and antioxidant activities are higher in the by-products compared to the flesh (Abu Bakar, Mohamed, Rahmat, & Fry, 2009).

This study indicated that avocado fruit has good economic potential where not just the pulp consumed, but the by-products can be utilized as well. This can help minimize wastes produced from eating avocado fruit. The potential of these by-products may be explored and utilized especially in food industry as natural antioxidant or food additives. Previous studies on avocado by-products application on burgers have shown their effectiveness in preventing oxidation and microbial growth (Rodríguez-Carpena et al., 2011). Other than that, avocado seeds have also been found to possess insecticidal, fungicidal, and anti-microbial activities (Dabas, Shegog, Ziegler, & Lambert, 2013). On another note, this study also indicated that

the peel and seed of avocado could benefit from freeze drying process for preservation of their antioxidant capacity.

3.3.5. Correlation among phenolic content, flavonoid content and antioxidant capacities

The TPC and TFC when correlated with ORAC, Pearson correlation gave very strong correlation (r^2 = 0.881 and 0.893 respectively). This suggests that the radical absorbing activity might be contributed by the phenolic and flavonoid contents in avocado. Meanwhile, the TPC and DPPH IC_{50} values gave strong correlation (r^2 = -0.623), while the TFC and DPPH IC_{50} were moderately correlated with each other (r^2 = -0.446).

3.4 Conclusions

The TPC of avocado pulp extracted with and without the application of ultrasonic were compared in this study. The results clearly indicated that the values of TPC were significantly higher when the extraction procedure included ultrasonic as assistance in the method. This study showed that avocado extraction can benefit from UAE especially by reducing the extraction time.

The antioxidant capacity of the different avocado fruit parts was assessed by evaluating the TPC, TFC and antioxidant activities of the FD and SHSD avocado pulp, peel and seed. In the edible portion of avocado, namely the pulp, the results showed that antioxidant capacity was significantly higher when the pulp was SHSD in comparison with FD, suggesting that superheated-steam drying is a preferred drying method for the pulp. On the other hand, in the by-products of avocado, namely the peel and seed, the antioxidant capacity was significantly higher when the peel and seed were FD compared to SHSD, suggesting that freeze drying is a preferred drying method for the peel and seed. The high antioxidant capacity in the FD peel and seed suggests their high potential as natural antioxidant. Comparing among the two parts, the peel gave higher antioxidant capacity compared to the seed.

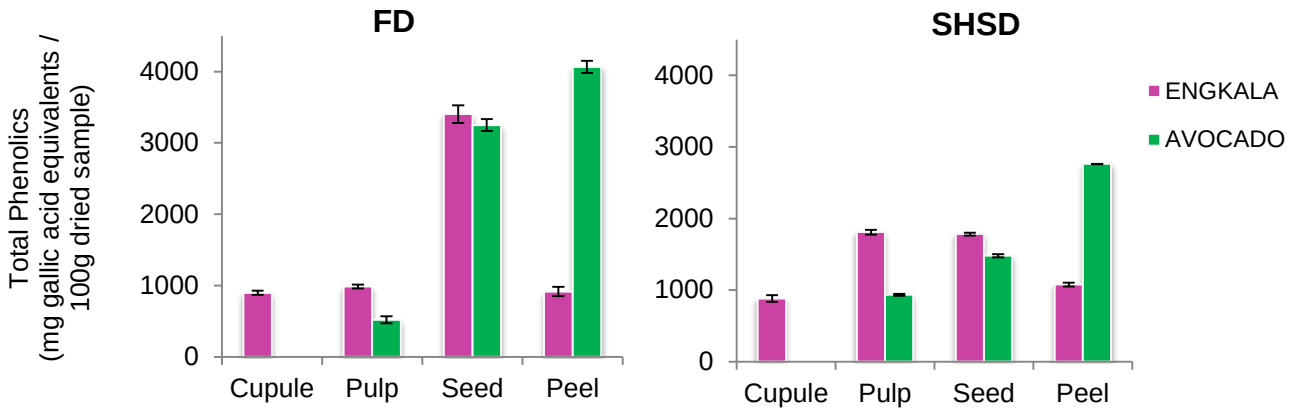
4.0. FRUITS COMPARISON AND GENERAL DISCUSSION

4.1. Comparison between the antioxidant capacity of engkala and avocado fruits

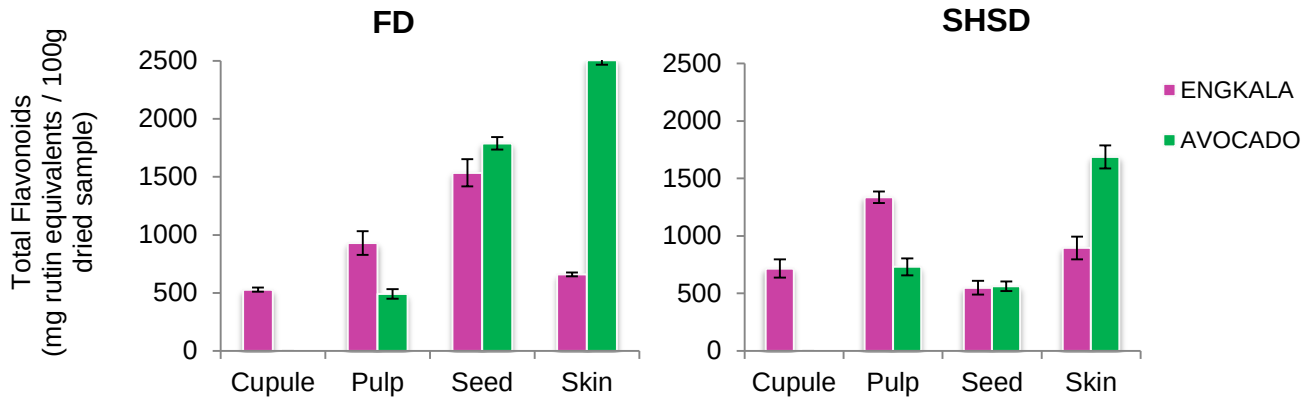
Engkala, being an underutilized fruit, the scientific data has not been much available, thus its value and ranking in terms of antioxidant capacity among the fruits commonly available is still unknown. Being in the same family as avocado fruit and having some similarities in physical properties, avocado was selected as a comparison and the results obtained on both engkala and avocado fruits in this study were compared.

4.1.1. Phytochemical study

Figure 4 shows (a) TPC and (b) TFC of FD and SHSD engkala in comparison with avocado fruit. The pulp of engkala, both FD and SHSD showed higher values in both TPC and TFC compared to avocado. FD and SHSD engkala seed gave higher TPC but lower TFC values compared to avocado. The skin of avocado, which was the part containing the highest total phenolic and flavonoid in the fruit on the other hand, showed higher TPC and TFC values compared to engkala. The cupule, present only in engala fruit was not compared.



(a) TPC



(b) TFC

Figure 20. (a) Total phenolic content (TPC) and (b) Total flavonoid content (TFC) of FD and SHSD engkala and avocado fruit parts.

4.1.2. Antioxidant activities

In the antioxidant studies, both FD and SHSD engkala pulp again showed significantly higher radical scavenging activities compared to the avocado pulp in both DPPH and ORAC assays as shown in tables 20 and 21. The seed of FD

engkala and avocado showed similar DPPH radical scavenging activity, but in the ORAC assay, FD seed of engkala gave higher ORAC value than FD avocado seed. The SHSD seed of engkala also showed significantly higher antioxidant activities in both DPPH and ORAC assays compared to SHSD seed of avocado. The peel on the other hand showed significantly higher radical scavenging activities in avocado for both FD and SHSD compared to the engkala peel in both DPPH and ORAC assays, as indicated by the lower IC_{50} values and higher ORAC values.

Table 24. DPPH radical scavenging activity of engkala and avocado fruit parts.

		IC_{50} (mg/ml)			
		Engkala		Avocado	
		Mean	± S.D.	Mean	± S.D.
Cupule	FD	[a] 1.79	0.162	-	-
	SHSD, 130°C	[b] 3.49	0.058	-	-
	SHSD, 150°C	[bc] 3.79	0.053	-	-
	SHSD, 170°C	[d] 2.94	0.144	-	-
Pulp	FD	[a] 11.85	0.851	[a] 35.02	3.409
	SHSD, 130°C	[b] 2.52	0.149	[b] 11.79	0.419
	SHSD, 150°C	[c] 2.30	0.118	[bc] 12.16	0.471
	SHSD, 170°C	[d] 1.82	0.113	[d] 6.69	0.216
Seed	FD	[a] 0.22	0.006	[a] 0.22	0.019
	SHSD, 130°C	[b] 0.56	0.028	[b] 1.47	0.020
	SHSD, 150°C	[c] 0.67	0.013	[b] 1.47	0.070
	SHSD, 170°C	[d] 0.91	0.092	[b] 1.56	0.053
Peel	FD	[a] 2.26	0.340	[a] 0.09	0.001
	SHSD, 130°C	[a] 3.05	0.084	[b] 0.50	0.011
	SHSD, 150°C	[a] 3.16	0.086	[c] 0.27	0.014
	SHSD, 170°C	[a] 3.18	0.122	[cd] 0.26	0.005

Results expressed as mean ±S.D. (n=3). Different letters in the same group indicate significant differences ($p<0.05$).

Table 25. Oxygen radical absorbing capacity (ORAC) of engkala and avocado fruit parts.

		ORAC (µmol TE/100g)					
		Engkala			Avocado		
		Mean	± S.D.		Mean	± S.D.	
Cupule	FD	15079	335	a	-	-	
	SHSD, 130°C	14793	1469	a	-	-	
	SHSD, 150°C	13263	619	a	-	-	
	SHSD, 170°C	11938	172	b	-	-	
Pulp	FD	3848	152	a	1518	185	a
	SHSD, 130°C	17575	1287	b	537	76	b
	SHSD, 150°C	19454	1001	b	1513	124	ac
	SHSD, 170°C	22446	1528	c	2658	107	d
Seed	FD	120675	6226	a	92782	8210	a
	SHSD, 130°C	54588	278	b	15083	981	b
	SHSD, 150°C	50295	1556	b	16393	334	c
	SHSD, 170°C	42885	2141	b	18607	171	cd
Peel	FD	19722	849	a	212362	9604	a
	SHSD, 130°C	16241	619	b	32720	1087	b
	SHSD, 150°C	15409	733	c	50239	3360	bc
	SHSD, 170°C	17489	1045	d	53143	1840	c

Results expressed as mean ±S.D. (n=3). Different letters in the same group indicate significant differences ($p<0.05$).

In this study, engkala pulp and seed showed higher antioxidant capacity compared to their counterparts in avocado. However, antioxidant capacity of avocado peel was higher compared to engkala peel.

4.2. General discussion

Attempts have been made to rank fruits and other food categories in order to help consumers in adding more antioxidants to their daily diet. USDA scientists measured antioxidant concentration as well as antioxidant capacity per serving size of more than 100 different foods including fruits (Wu et al., 2004). In the fruit category in the study, avocado dried using freeze dryer is mid-positioned. ORAC values are 1381 and 552 µmol TE/100g for lipophilic and hydrophilic ORAC respectively. Among the

fruits studied, blueberries, cranberries and blackberries are ranked highest in antioxidant capacity. Avocado, following behind raspberry, strawberry, apples, sweet cherries and plums, but having higher total antioxidant capacity than pears, orange, grapes, grapefruit, peaches, mango, apricot, tangerines, pineapples, bananas, nectarines, cantaloupe, honeydew and watermelon. The melons are reported to be the group where antioxidant capacity is relatively low.

In this study, avocado pulp gave ORAC value of 1518 µmol TE/100g when FD, and 537 - 2658 µmol TE/100g when SHSD. Engkala pulp on the other hand gave 3848 µmol TE/100g when FD and 17575 – 22446 µmol TE/100g ORAC values when SHSD. This result can help in putting engkala fruit in a ranking above avocado. Being an underutilized fruit unknown to most part of the world, there is no idea of where engkala fruit currently stands in its category. By knowing the ranking of avocado among the common fruits, comparing engkala with avocado pulp in this study gave some insight on where engkala fruit possibly ranks among the other fruits. Note that this ranking of engkala might only gave hints but the actual ranking must to be done through proper studies with the other fruits.

4.3. Consclusion

While the pulp of engkala and avocado fruits are readily eaten by many for their nutritional benefit, the seed of engkala and the peel of avocado could be utilized and developed as good source of natural antioxidants. In this study, it could be seen that engkala fruit sat higher in ranking than avocado when the edible part was in concerned.

5.0. CONCLUDING REMARKS AND SUGGESTIONS FOR FUTURE RESEARCH

The potential of some underutilized species to become commodity crops should not be underestimated. Given adequate research and development (including marketing and commercialisation), economic returns is very likely for these species at national, regional or international level. The world has seen many once underutilized species now have become valuable. Hulled wheat (*Triticum monococcum, T. dicoccum*) being used in making biscuits and pasta, its cultivation in Italy has increased dramatically and at the same time raised interest in other countries, thanks to processing technologies and marketing strategies. The spicy vegetable rocket (*Eruca sativa* and *Diplotaxis* species) once only popular at local level across the whole Mediterranean has improve its level of use in Italy, thanks to research efforts on the improvement of agricultural practices and its commercialisation. Roselle (*Hibiscus sabdariffa*) known for centuries in Sub Sahara Africa has become a well-established beverage in Europe thanks to simple marketing strategies. Okra (*Abelmoscus esculentus*), a traditional African vegetable, is now accepted in most markets around the world. The seeds of carob tree (*Ceratonia siliqua*, a multipurpose species from the Mediterranean region) which contain high quality natural resins and gums are generating significant market demands for the pods of this tree and are contributing sensibly to the rediscovering of this valuable species (Padulosi and Frison, 1999).

The underutilized fruit of engkala (*Litsea garciae*) in this study has shown good potential as a functional food. Being a fruit with high protein, carbohydrate, potassium, vitamin C and other minerals, its consumption could provide good

nutrition. The high total phenolic and flavonoid contents as well as antioxidant activities also could make it a good source of antioxidants. The current method of eating the fruit, which is by subjecting the fruit to steam or hot water was also found to be appropriate. The need of complex cooker or new preparation method seemed unnecessary. Comparison of the antioxidant capacity between engkala and avocado fruits had put engkala in a ranking above avocado, thus most probably putting it higher above other fruits which were ranked below avocado.

This study also showed that heat is not necessarily the damaging factor of nutrients in food. This is especially evident in the edible part of the fruit, where the TPC, TFC and antioxidant activities of both engkala and avocado fruits were significantly higher in the SHSD samples compared to the FD ones. In engkala fruit, vitamin C content in the SHSD pulp and seed almost three-folds the content in the FD ones. The World's Healthiest Foods (whfoods.org, 2014) suggests that avocado fruit to be eaten raw to preserve the health benefits. Other researchers have shown that freeze dried fruit can provide higher antioxidant activities compared to the fresh one, and heat treated fruit can have higher antioxidant activities compared the FD. This study might change the perception on the way of eating avocado, where eaten in its raw form might not be the best way, as superheated-steam drying seemed favorable.

The by-products of engkala and avocado fruits have shown good potential to be utilized as a source of natural antioxidant. In the future, by-products from fruit processing need to be reduced in order to move towards sustainable environment. The by-products of engkala and avocado, particularly the FD engkala seed and

avocado peel gave significantly high antioxidant capacity as shown this study. In the future, they could be developed as natural antioxidants or food additives.

Drying is widely used to extend the shelf life of fruits. In this study, superheated-steam drying was preferred in drying the edible part of the fruits of both engkala and avocado, while freeze drying was more favorable in drying the by-products. The difference in the preferences of drying method could be due to the different compounds present in the different parts of the fruits. The treatment with high temperature of superheated-steam provided the fruit parts with high energy which could destroy the heat sensitive compounds, as observed in the seed part, or could be able of degrading or altering the bondings between or among the chemical compounds, as observed in the pulp part, thus making inter-conversion or recombination of polyphenols as well as increasing reagent-binding sites in the phenolic compounds possible.

As an overall conclusion, both superheated-steam drying and freeze drying have their advantages in application of dried fruit production. Superheated-steam drying was preferred for production of dried pulp products, while freeze drying was preferred for production of dried fruit by-products. Drying of exotic fruits has not been researched as extensively as the common fruits. However, it is expected that as a result of globalization, exotic fruits native to certain developing regions will become known to the other parts of the world. This study was the first to give scientific data on the effect of steam treatment in engkala and also avocado fruit. It is hoped that the consumption of the underutilized fruit of engkala can be encouraged and its potential as antioxidant source and food for health be promoted.

Epilogue

The knowledge obtained in this study apart from to contribute to the pool of knowledge in the scientific community is also aimed to serve the local community at which this underutilized fruit is grown. Creating a market for this engkala fruit in the local area where it is grown is not a problem since this fruit is always in demand by the locals when in season. It is in the neighbouring region that it is hoped awareness can be created on this underutilized fruit and the benefit it can offer, therefore creating a new market outside its locality. Once this is achieved, demand on the fruit will increase, and increase in demand will encourage local growers to increase production, furthermore can encourage the local government to start organizing proper cultivation of the crop. On top of that, this will attract more research and monetary funding. Local chefs can also play their part in further promoting the fruit in culinary applications. When chefs bring the knowledge back and apply it to their kitchens, restaurants or hotel operations, the visitors will also benefit from their experience with this underutilized fruit. In the end, the consumers will benefit from the knowledge on the healthy locally grown fruit, the growers will benefit from the increased revenue from sales, and the state will benefit from the growers increased revenue. Perhaps in the near future, engkala fruit can be a well-known fruit standing at par with avocado.

The author is a local people of the region where this engkala fruit is grown and a consumer to this underutilized fruit.

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