1. Introduction and Literature Review

1.1 Statement of the problem

The avocado (*Persea americana Mill.*) belongs to the Lauraceae family and is placed in one of three groups or races for horticultural purposes: *americana* (West Indian), *guatemalensis* (Guatemalan) and *drymifolia* (Mexican) (Sippel, 2001). South Africa produces 34 000 tons of avocado fruit per year (Mr. Derek Donkin, 2004, South African Avocado Growers' Association, personal communication) of which the main cultivars are 'Fuerte' (Guatemalan/Mexican hybrid) and 'Hass' (Guatemalan), which make up 38% and 36% of the area planted to avocados respectively. Of this fruit 13 800 tons are used for the production of oil in local oil factories. When seasonal variation is taken into account, these avocado oil-producing factories have an average yield of 10% oil per ton fruit utilised (Mr. Dennis Gilbert, 2003, Specialised Oil, Personal Communication; Mr. Daan Jacobs, 2003, Hans Merensky Oils, Personal Communication).

The two main processes used for the production of avocado oil include a centrifugal extraction process and a cold press extraction of heat-dried avocado fruit. Ripe fruit is used for the centrifugal process, whilst unripe fruit is used for the cold press extraction method. It is a recognized fact that the unsaponifiable fraction of avocado oil is rich in phytochemicals, including polyphenols, sterols and tocopherols (Farines, Soulier, Rancurel, Montaudoin & Leborgne, 1995; Eyres, Sherpa & Hendriks 2001). However, neither of these two processes produces a product containing significant amounts of antioxidants (Prof. Ben Botha, 2003, Tshwane University of Technology, personal communication). Factors influencing the breakdown of these compounds might include heat drying, which is used as a pre-treatment before avocado oil extraction with the cold press method. The ripening stage of the fruit may also play a significant role.

A few studies have been undertaken to distinguish between compositional differences during the maturation stages of the avocado, mainly the fatty acid profile (Lozano, Dhuique Mayer, Bannon & Gaydou, 1993; Poiana, Giuffre & Mincione;

1999). Like all climacteric fruit, avocado only starts to ripen after harvesting, which takes place at horticultural maturity (Awad & Lewis, 1980; Sippel, 2001; Ozdemir & Topuz, 2004). The carotenoid and chlorophyll content seems to decrease during ripening (Ashton, Wong, McGhie, Vather, Wang, Requejo-Jackman, Ramankutty, & Woolf, 2006). Heat drying of the unripe fruit, on the other hand, might lead to the degradation of tocopherols, carotenoids and polyphenols. Preservation of these antioxidants in avocado oil as an edible oil is important, not only because they influence the oxidative stability of the oil, but also for their function in the prevention of ailments like cardiovascular disease caused by atherosclerosis (Ohr, 2002).

Other extraction methods for avocado oil include solvent extraction using organic solvents like hexane and supercritical fluid extraction with carbon dioxide. Although hexane extraction is a mild, well-known extraction method, large amounts of solvent is needed which is expensive and environmentally hazardous. Carbon dioxide is a non-toxic and environmentally compatible fluid for the extraction of edible oils (Garcia, Lucas, Rincon, Alvarez, Gracia & Garcia 1996; King, 1997). Supercritical carbon dioxide (SC-CO_2) extraction has been proven to be a viable alternative for hexane as avocado oil extracted with these two methods have been shown to have similar fatty acid profiles (Botha & McCrindle, 2003). The micro-component content and composition as well as oxidative stability of avocado oil extracted with SC-CO_2 have, however, not been determined. Furthermore, the effects of progressive extraction on the micro-component distribution and oxidative stability is not known.

South Africa has a large agricultural sector and there is increasing interest in the growth of small and developing farmers. If a new market for avocado fruit can be created by production of high quality avocado oil at a premium price, more small and developing farmers would be interested in cultivating avocado fruit. This will in turn benefit the agricultural and economic sectors in the long term. Oil production can also create an alternative market for the commercial farmer, which has the benefit of less risk, compared to the fresh fruit market where visual appearance of the fruit is very important.

1.2 Literature review

1.2.1 Avocado fruit and avocado oil

Although known to the natives of tropical America, the avocado was described for the first time in 1499 when it was observed growing in a small harbour at the foot of the Sierra Nevada de Santa Marta, USA (Sippel, 2001). Major avocado growing areas in the world include Mexico, the USA, Brazil, Israel, Chile, South Africa, Spain and Australia (Knight, 2002). Little has been recorded about early introductions of avocado into South Africa, but it is accepted that the first trees were West Indian race seedlings planted on the coastal strip of KwaZulu Natal, especially around Durban, in the late 19th century. Avocados are now widely grown in South Africa, principally in the Limpopo Province and Mpumalanga and to a lesser extent in KwaZulu Natal. Trees of Mexican and Guatemalan origin proved to be better adapted to South African climatic conditions. Production in South Africa is dominated by two cultivars of the genus and species *Persea americana* Mill, namely *Fuerte* and *Hass*.

The avocado fruit has been accredited with several health claims (Eyres *et al*., 2001). One of these claims include the lowering of total cholesterol and low-density lipoprotein levels, without changing high-density lipoprotein levels, when included in the diet (Colquhoun, Moores, Somerset, & Humphries, 1992).

Avocado is one of the few cultivated fruits in which oil is a main component on dry basis (Werman & Neeman, 1987). The oil content is in the range of 15-30% depending on the variety, and is mainly mono-unsaturated with the predominant fatty acid being oleic acid (Werman & Neeman, 1987). According to Werman and Neeman (1987), of all fruits only olive and palm can rival the avocado in oil content.

Avocado oil is valued as an edible oil due to its health-enhancing qualities and is especially used in the treatment of connective tissue diseases (Maheu, Le Loet & Loyau, 1995). This oil is of good quality because the processed fruit from which the oil is obtained is still intrinsically sound and is only termed second grade because of

its appearance (black or brown spots, rough skin, shape and size), which is not appealing to the consumer (Eyres *et al*., 2001).

Avocado cultivars produced in South Africa and their oil contents are listed in Table 1.1.

Table 1.1: Oil content and harvesting time of Avocado cultivars produced in South Africa (Kaiser, Keevil, Levin & Wolstenholme, 1996)

Cultivar	Oil content (% wet basis)	Harvesting time (warm climates)
Fuerte	26 – 40	June – September
Hass	± 25	June – October
Edranol	Max 20	June – September
Pinkerton	± 20	June
Nabal	10 – 15	October – November
Ettinger	± 24	March – June
Bacon	Max. 22	April – June
Alboyce	Max. 22	March – August
Ferdyn	± 22	March – July
Sharwill	± 22	April – June
Teague	± 10	February – May
Santana	± 12	April – May
Wurtz	Max. 21	July – September

1.2.2 Morphology of avocado fruit

The avocado fruit consists of a skin, mesocarp (which is divided into a green part near the skin and a yellow part near the stone) and a large stone (Figure 1.1) (Somogyi, Barrett & Hui, 1996). The mesocarp of the avocado fruit has a fairly uniform cellular composition, consisting primarily of large parenchyma cells and idioblast cells (which comprise 2% of the mature fruit volume). Parenchyma cells

differ in two major ways from idioblast cells, namely their structure and their contents.

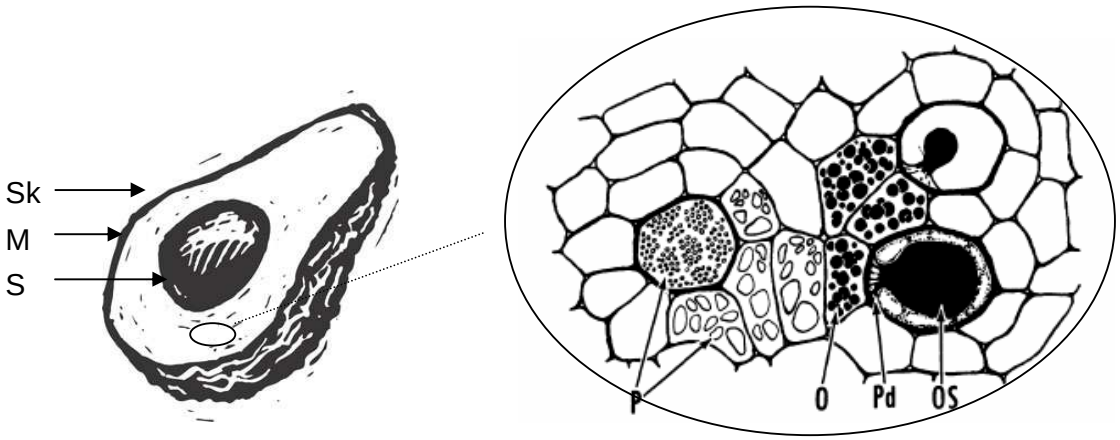


Figure 1.1: Diagram of avocado fruit morphology and avocado mesocarp cells where Sk, Skin; M, Mesocarp; S, Stone; P, pits; O, oil; OS, oil sac in idioblast cell; Pd, plasmodesmata (Scott, Bystom & Bowler, 1963)

Parenchyma cells only have thin, primary walls (Mauseth, 1995). The idioblast cells on the other hand, are surrounded by a specialised cell wall composed of the primary cellulosic wall, a secondary suberin layer and a tertiary wall (Platt-Aloia, Oross & Thomson, 1983). During ripening, which only occurs after the fruit is picked, the primary walls of the parenchyma cells are degraded due to the activities of the cell wall hydrolytic enzymes, namely cellulase and polygalacturonase and fruit softening occurs. The suberised wall of the idioblast oil cells is, however, immune to the activity of these enzymes and remains intact during ripening (Platt & Thomson, 1992).

Parenchyma cells mainly contain numerous droplets of lipid substances, mostly triacylglycerol (Platt & Thomson, 1992). The oil in the idioblast cells occur as a single large drop filling the cell, has a different appearance in freeze fracture replicas compared with the triacylglycerols and is therefore thought to have a different

composition (Platt-Aloia *et al*., 1983). Scott *et al*. (1963) noted a difference in staining between the content of the parenchyma and idioblast cells and attributed this to possible traces of terpene compounds present in the oil sacs of the idioblast cells. Werman and Neeman (1987), however, claim that most of the oil in avocado fruit is located in the idioblast cells and that only small droplets of oil can be detected in the parenchyma cells. Compounds isolated from the idioblast oil cells include those with anti-fungal, antifeedant and insecticidal activity (Prusky, Plumbley & Kobiler, 1991; Rodriguez-Saona, Millar & Trumble, 1997; Rodriguez-Saona, Millar & Trumble, 1998[a]; Rodriguez-Saona, Millar, Maynard, & Trumble 1998[b]). These effects have been attributed to the presence of unique compounds present in the idioblast cells including persin ((12Z,15Z)-1-acetoxy-2-hydroxy-4-oxo-heneicoxa-12,15-diene) and several 2-alkylfurans (Rodriguez-Saona *et al*., 1998[a]).

1.2.3 Avocado oil production

The first step in production of avocado oil involves extraction of the oil from the fruit using methods including solvent extraction, mechanical pressing, centrifugation of pulp slurries and enzymatic extraction using a mixture of polygalacturonases, α-amylase and a protease. (Bizimana *et al*., 1993; Buenrostro & López-Munguia, 1986). Hard, mature, fruit are used for solvent extraction and mechanical extraction, while soft, mature seeded fruit are used for oil separation by centrifugation (Werman & Neeman, 1987).

Current methods used for extraction of avocado oil in South Africa include cold pressing of heat dried unripe fruit and the centrifugal extraction of ripe fruit (Mr. Dennis Gilbert, 2003, Specialised Oil, Personal Communication; Mr. Daan Jacobs, 2003, Hans Merensky, Personal Communication). However the application of heat in these methods could affect the functional properties of the micro-components in avocado oil negatively.

After extraction, the crude avocado oil undergoes refinement, bleaching and deodorisation to yield an edible oil. Similar to other well-known edible oils, avocado

oil is sensitive to oxidative processes resulting in rancidity, production of undesirable flavours and quality losses during storage (Werman & Neeman, 1986).

1.2.4 Methods of extraction of avocado oil

1.2.4.1 Mechanical pressing

In general, mechanical extraction of vegetable oils includes two methods namely hydraulic expelling and screw pressing. Mechanical pressing is usually used for materials exceeding an oil content of 20%, while solvent extraction is recommended for products like soybeans or press cakes having an oil content of less than 20% (Carr, 1997).

Screw presses are used in higher technology areas throughout the world for expulsion of oil from copra, palm kernel, peanut, cottonseed and flax seed amongst others (Carr, 1997). Avocado oil has also been successfully expelled from sun-dried, destoned avocado fruit using a screw press (Southwell, Harris & Swetman, 1990). Due to the high water content of avocados, pressing of the raw flesh is problematic, and fruit is normally air dried prior to screw pressing (Southwell, Harris & Swetman, 1990). The effect of the air drying on the oxidative stability of the oil is not clear. In principle, a screw press is a continuous screw auger designed to accept feed and subject it to gradually increasing pressure as it is conveyed through a barred cage. Disrupted or distorted oil cells act as capillaries which are reduced in volume as pressure is applied and the oil is expelled (Ward, 1976).

Hydraulic pressing uses the principle of gradually increasing pressure on the incoming material as it progresses through the interior of a closed barrel. Oil extracted in this manner is traditionally called “cold pressed” oil (Carr, 1997). Hydraulic pressing of avocado fruit has been well documented and implemented for the extraction of avocado oil for several years (Love, 1944). Avocado oil recovery from mechanical pressing varies between 79.4 – 90.3 % (Southwell, Harris & Swetman, 1990).

1.2.4.2 Solvent extraction

Together with mechanical extraction using centrifugal force, solvent extraction was probably, until recently the most common method of extracting oil from avocado fruit (Southwell, Harris & Swetman, 1990). Hexane has become the solvent of choice for solvent extraction because of high stability of the solvent, low evaporation loss, low corrosiveness, little greasy residue and better odour and flavour of the extracted products (Johnson, 1997).

Solvent extraction has several drawbacks including high capital equipment cost and operational expenditures, the perpetual hazard of fire and/or explosion as well as the residual solvents associated with both the oil and the meal including endocrine-disrupting compounds like phthalates that can lead to the production of androgens in the body (Owusu-Ansah, 1997; Petrovic, Eljarrat, Lopez de Alda & Barceló, 2004).

The primary prerequisite for solvent extraction for oils is the rupturing of the seed or feed material to render the cell wall more porous. According to Diosady, Rubin, Ting and Trass (1983), complete rupturing of the cell wall is necessary for rapid solvent extraction. In a study conducted by Ortiz, Dorantes, Gallndez and Cárdenas (2004), the shape of the idioblast cells of avocado fruit became irregular and rough-shaped after hexane extraction. According to Ortiz *et al*. (2004), hexane extraction of an unknown avocado cultivar yielded approximately 59% oil from the avocado pulp. An avocado oil yield of 74-75% (dry basis) from the *Fuerte* variety has been obtained using petroleum ether for an extraction time of four hours (Lewis, Morris & O'Brien, 1978).

1.2.4.3 Centrifugation of pulp slurries

Centrifugation of pulp slurries is mostly used in the olive oil industry and is used in South Africa for the extraction of avocado oil from ripe fruit (Mr. Dennis Gilbert, 2003, Specialised Oil, Personal Communication; Mr. Daan Jacobs, 2003, Hans Merensky Oils, Personal Communication). This process is also lately referred to as "cold pressing" (Eyres *et al*., 2001). The olive or avocado fruit is first put through a hammer mill and the paste is pumped to a malaxeur where it is warmed and beaten

or mixed until the oil begins to separate (Benedito, Mulet, Clemente & García-Perez, 2004). The paste is then pumped to a centrifuge where the solids are separated from the liquids. In some instances water is added and this process is also referred to as the "aqueous extraction process" (Cater, Rhee, Hagenmaier & Mattil, 1974). The vegetable water and oil are further separated in a final centrifugal process. The yield obtained from this method is generally high, it requires limited labour and is continuous and automated (Ranalli & Martinelli, 1995). It is however expensive, has a high energy consumption, yields a varying amount of vegetable water to be disposed of and has reduced antioxidant levels due to added water.

Modern centrifugal olive oil processing units have been modified to suit the parameters of the avocado fruit (Eyres *et al*., 2001). The adaption of certain parameters and the addition of chemicals have been introduced to optimize oil extraction from the avocado fruit. The effects of centrifugation rate, pH and sodium chloride on extraction yield were extensively studied by Werman and Neeman, (1987). The addition of inorganic salts like $CaCO_3$ and $CaSO_4$ has also been proven to increase oil yield (Bizimana *et al*., 1993). There was no indication in any studies of how these parameters affected oxidative stability.

1.2.4.4 Enzymatic extraction

Enzymes are generally considered environmentally friendly and the utilization of enzymes for oil extraction with regards to increasing yields and reducing side products have long been recognised. They are probably the most efficient way to rupture cell walls, even at molecular level (Fullbrook, 1983) and can be synergistically used with other solvents or physical means to extract oils and fats from plant material (Owusu-Ansah, 1997). Due to the structural complexity of plant material, the extent of enzymic degradation of the cell wall is determined by the structural details, such as the chemical constituents and the type of source of the enzymes. Some of the most widely used enzyme actions used in extraction of vegetable oils include protease, cellulase, polygalacturonase and amylase activity (Owusu-Ansah, 1997). Enzymes used in the extraction of avocado oil include α-

amylase and a mixture of protease and cellulase (Buensrostro & López-Munguía, 1986).

Enzymic extraction of fat can be divided into three categories namely: Enzyme-Enhanced Solvent Extraction, Enzyme-Assisted Expelling and Enzyme Assisted Aqueous Extraction. In all of these, the objective of using enzymes is to break the cell wall and release the oil by some mechanical means (Owusu-Ansah, 1997).

Enzyme aided aqueous extractions that have been carried out include those on coconut (McGlone, Canales & Carter, 1986), melon seed (Fullbrook, 1983) and avocado (Buenrostro & Lopez-Munguia, 1986). It seems that the enzyme assisted extraction process is more conducive for materials with higher oil-to-protein ratios. Satisfactory yields have, for instance, not been obtained for soybeans unless excessive hydrolysis of the proteins is effected (Owusu-Ansah, 1997).

The extraction of oil from fruits like avocado is enhanced by partially accelerating the natural enzymatic breakdown processes within the avocado paste, so favouring the separation of oil from other macromolecules to which oil is linked (Domínguez, Núñez & Lema, 1994). Although olives are the most studied of the oil fruits, avocado oil extraction studies on laboratory scale have indicated an increase of 4-5 times superior to olive oil after enzymatic treatment. α- Amylase proved to be the best enzyme for the optimization of oil extraction from avocados (Domínguez *et al*., 1994).

1.2.4.5 Supercritical fluid extraction

Supercritical fluid extraction is a unitary mass transfer operation based on the use of fluids at temperatures and pressures above the critical values. These conditions make the supercritical fluid present peculiar physicochemical properties between the gas and liquid states, which confer them with exceptional solvent characteristics (Gómez & De la Ossa, 2002). Supercritical fluid extraction (SFE) has been used since the early eighties when extensive work was performed in industrial research laboratories (Manigold, 1983). Supercritical fluid extraction is an alternative to

traditional separation processes, which is used when the separation of thermally labile substances and attainment of high-purity products is the target (Araujo *et al*., 2001). The technology of supercritical fluid extraction has been applied to fragrances, cosmetics, food and various chemical industries (Palmer & Ting, 1995; Garcia *et al*., 1996; Turkay, Burford & Sangum, 1996).

Although supercritical fluids like methylene chloride (CH_2Cl_2) have been used in the past for the removal of caffeine from coffee, it is no longer used because it is hazardous in the workplace and difficult to remove completely from the coffee (Kotz & Treichel, 1999). Currently, supercritical carbon dioxide (SC-CO_2) is finding more application in the food industry; the best known application probably being the decaffeination of coffee (Kotz & Treichel, 1999). SC-CO_2 has also been used for the enrichment of oil in micro-components to obtain products with improved functionality for specific applications or with better nutritional values (Ibáñez, Benavides, Señoráns, & Reglero, 2002). Co-solvents used for the SC-CO_2 extraction of lipids from plant material include isopropanol and methanol (Kuk & Hron, 1994).

The principle of supercritical fluid extraction can be explained by using a phase diagram for carbon dioxide (Figure 1.2): The boiling point of carbon dioxide at 1 bar is -78.5ºC, its critical pressure is 72.9 bar and its critical temperature is 31.1ºC (Manigold, 1983).

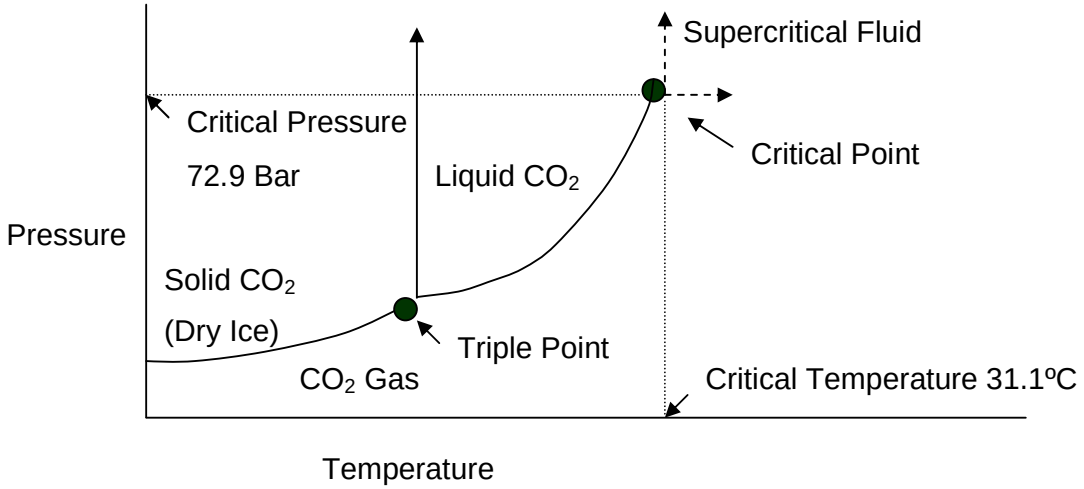


Figure 1.2: Phase diagram for carbon dioxide (King, 1997).

A phase of a substance is a form of matter that is uniform throughout in chemical composition and physical state and matter is usually referred to in its gaseous, liquid or solid state (Atkins, 1994). A spontaneous phase transition occurs at a characteristic temperature for a given pressure. The phase diagram shows the regions of pressure and temperature at which various phases are thermodynamically stable. The boundaries between phases show the values at which two phases coexist in equilibrium.

When pressure is applied to a heated liquid in a sealed vessel, the conditions differ from that of liquid in an open vessel. When liquid is heated in a sealed vessel, boiling does not occur. Instead, the temperature, vapour pressure and the density of the vapour rise continuously. Simultaneously, the density of the liquid decreases as a result of its expansion. When the density of the vapour is equal to that of the remaining liquid, the surface between the two phases disappears. The temperature and pressure at this point (the critical point) is called the critical temperature and critical pressure (shown in Figure 1.2 for CO_2). At and above this temperature a single phase exists and an interface no longer exists (Araujo *et al*., 2001).

Above the critical point, the liquid and gaseous phases become identical and indistinguishable. This phase has the high density of a liquid, but the low viscosity of a gas and is known as a supercritical fluid (Petrucci & Harwood, 1997). Molecules in supercritical fluids are in much closer proximity than in ordinary gases and exert strong attractive forces on the molecules of a liquid or solid solute. Therefore, both liquids and solids become much more soluble in a gas above its critical pressure and temperature i.e. in the supercritical region (King, 1997).

The main advantage of supercritical fluids over liquid solvents is that their high diffusivity, low viscosity and low surface tension can speed up mass transfer-limited extractions (Garcia *et al*., 1996). A single supercritical fluid may also substitute for a variety of liquid solvents because it offers the possibility of modifying product solubilities through altering the pressure and/or temperature (Garcia *et al*., 1996). In

general, the solubility of a substance in supercritical fluids increases with pressure when kept at a constant temperature (Garcia *et al*., 1996).

The procedure used in the extraction of natural products with SC-CO_2 is simple. The carbon dioxide is condensed in a diaphragm compressor to a pressure in excess of its critical pressure and a temperature in excess of its critical temperature. The fluid or SC-CO_2 flows through an extraction vessel containing the plant material. By lowering the pressure in two stages, below the critical pressure of carbon dioxide, the extracted oil is recovered from its solution (Manigold, 1983). It is also possible to isolate the oil by decreasing the pressure and increasing the temperature simultaneously. The released gas is condensed and recompressed, thus completing the cycle (Manigold, 1983).

According to Gómez and de la Ossa (2002), the yield of seed oil obtained with SC-CO_2 increases with increasing operating pressure. A higher flow rate also led to a somewhat higher yield, but with a much higher solvent (SC-CO_2) consumption. A lower flow rate, on the other hand, reduced solvent consumption but produced notably lower yields. An increase in particle size of the seed to be extracted is associated with a decrease in extraction yield due to the increase of the mass transfer resistance between the surface of the seed and the SC-CO_2 (Gómez, López & de la Ossa, 1996).

It appears the only work done on SC-CO_2 extraction of avocado oil was that reported by Botha and McCrindle (2003). The optimum extraction pressure was determined to be 540 atmosphere (atm), yielding a 95% complete extraction after one hour. A 94% extraction was complete within two hours when the pressure was reduced to 350 atm. A substantial decrease in capital investment was achieved when the pressure was reduced, even though this resulted in a longer extraction period (Botha & McCrindle, 2003). They also proposed supercritical extraction method as an alternative for hexane extraction of avocado oil as no significant difference in fatty acid profile was reported.

1.2.4.6 Yield and composition of plant oils extracted with SC-CO_2 compared with hexane extracts

The apparent yield from SC-CO_2 extraction of seed oil is slightly lower than extraction with hexane (Friederich & List, 1982; Gómez & de la Ossa, 2002; Bravi, Spinoglio, Verdone, Adami, Aliboni, D'Andrea, De Santis & Ferri, 2005). This has been attributed to the fact that as an extraction solvent, hexane is much less selective than SC-CO_2 (Gómez & de la Ossa, 2002) and is able to extract a wider variety of compounds such as phospholipids, waxes and pigments, thus contributing to higher oil yield. This selectivity is further demonstrated by the lower acidity index of oils extracted with SC-CO_2 (Bernardo-Gil, Grenha, Santos, & Cardoso, 2002). A darker green oil, possibly related to the higher chlorophyll content was reported for avocado oil extracted with hexane when compared to SC-CO_2. (Botha & McCrindle, 2003). Bhattacharjee, Singhal and Tiwari (2007) also reported increased pigmentation with the average gossypol content of hexane-extracted cotton oil being 0.242% while that of SC-CO_2-extracted cotton oil was 0.015%.

The amount and composition of triglycerides in oils extracted with SC-CO_2 and hexane is very similar (Botha & McCrindle, 2003). Significant differences have been observed for the unsaponifiable fraction of oils extracted with these two methods (Gómez *et al*., 1996; Gómez & de la Ossa, 2002). More unsaponifiables were obtained with hexane extraction which is probably also due to the lower selectivity of hexane. Oil extracted with SC-CO_2 shows significantly less phosphorus and correspondingly less chromatographic refining loss than hexane-extracted oil (Friederich & List, 1982). Due to the insoluble nature of phospholipids in SC-CO_2, oil extracted with SC-CO_2 is normally low in phospholipids, resulting in a lower oxidative stability than that of oils extracted with conventional solvent or screw press methods (List & Friedrich, 1989). The SC-CO_2 extracted oil, therefore, has the advantage of being essentially equivalent to a degummed, hexane-extracted crude oil.

1.2.4.7 Enrichment of oils with micro-components during SC-CO_2 extraction

SC-CO_2 can be used in the extraction of oils to produce oil fractions enriched in certain micro-components. Przybylski, Lee and Kim (1998) reported high colour measurements (values of tristimulus yellow, red and blue) and the presence of chlorophyll pigments in the last fractions of canola oil extracted with SC-CO_2. This suggests that during SC-CO_2 extraction of oil from plant material, pigments tend to be extracted towards the end of the process which results in the latter oil fractions becoming enriched in these components. This can be explained by observations on the partially extracted flakes, which suggest that the column of flakes acts much like the stationary phase of a chromatography column, with the SC-CO_2 eluting the triglycerides to a considerable extent before elution of the pigments and other unsaponifiables (Gómez *et al*., 1996).

Chuang and Brunner (2006) reported the concentration of minor constituents in palm oil using SC-CO_2. An enrichment of 550 to 105 000 ppm β-carotene and an enrichment of 300 to 30 000 ppm of sterols were obtained after three steps of extraction (transesterification, followed by two consecutive extractions). The higher rates of extraction of compounds containing polyunsaturated fatty acids than monounsaturated and saturated fatty acids at the beginning of extraction has also been reported in canola oil. (Przybylski *et al*., 1998). These differences in rates of extraction of different compounds during SC-CO_2 extraction may also be attributed to the different solubilities of these compounds in SC-CO_2 (Brunetti, Daghetta, Fedeli, Kikic & Zanderidghi, 1989).

A resultant effect of the different rates of extraction of different compounds during SC-CO_2 extraction of plant oils is the influence on oxidative stability of the oil. Przybylski *et al*., (1998) reported a slight increase in triglyceride content and decrease in free fatty acids from 2.03 to 0.73% between the first and last fractions of canola oil extracted with SC-CO_2. The contents of phospholipids increased as the extraction progressed and the total phospholipid content increased by a factor of 28 in the last fraction, compared to the first (Przybylski *et al*., 1998). The first fractions of the canola oil had lower oxidative stability, and this was attributed to their higher

contents of polyunsaturated fatty acids, higher levels of free fatty acids and absence of phospholipids. The most oxidatively stable canola oil fraction (the last fraction), contained the highest amount of phospholipids and sterols (Przybylski *et al*., 1998).

1.2.5 Lipid oxidation

Lipid oxidation is one of the major causes of food spoilage and is of great concern to the food industry, as it leads to the development of off-odours and decreases the nutritional value of food (Nawar, 1985). It is generally agreed that reaction with molecular oxygen and subjection to elevated temperatures are the main factors influencing the oxidative deterioration of lipids. In order to produce a healthier product for the consumer, the shelf life or oxidative stability of vegetable fats is the top priority for the oil manufacturer (Gunstone, 1996).

Lipid oxidation can be divided into three steps: Initiation, Propagation and Termination (McClements & Decker, 2000). During initiation, highly reactive free radicals are created when oxygen reacts with a substrate (fatty acids) (reaction equation 1).

Initiation $O_2 + RH \longrightarrow R^{\bullet} + HOO^{\bullet}$ …1

These free radicals are highly reactive in their short lifespan in search of another unpaired electron (Gunstone, 1996). The initiation step cannot be stopped by additives. Only the exclusion of radical formers can inhibit free radical production. During propagation, the atmospheric oxygen reacts with the free radicals to form peroxy radicals ($ROO^{\bullet}$) (reaction equation 2). These highly reactive free radicals go on to react with other unsaturated fatty acids where a hydrogen (H)-atom is removed from a fatty acid molecule to form hydroperoxides or primary oxidation products (ROOH) (reaction equation 3), which are odourless and tasteless. This H-abstraction is the slowest and, hence, the limiting step in radical ($R^{\bullet}$) formation (Belitz, Grosch & Schieberle, 2004[a]). This second step in the propagation process (reaction equation 3) will continue until oxygen is depleted or a reaction with a stable antioxidant occurs. Peroxidation of unsaturated fatty acids (reaction equation 3) is

accelerated autocatalytically by radicals generated from the degradation of hydroperoxides by a monomolecular reaction mechanism (reaction equation 4). The degradation of hydroperoxides is prompted by heavy metal ions (Berger, 1994) and is considered to be a starting point for the formation of volatile reaction products. The latter are usually powerfully odorous compounds (consisting of ketones, aldehydes, alcohols and acids created by either peroxide scission alone or simultaneous peroxide and chain scission) perceived as rancidity by the consumer. After a while, the hydroperoxide concentration reaches a level at which it begins to generate free radicals by a bimolecular degradation mechanism (reaction equation 5). However, in food products, reaction equation 5 is of no relevance, since lipid oxidation makes food unpalatable well before reaching the necessary hydroperoxide level for this to occur.

Propagation	$R^{\cdot} + O_2$	$\longrightarrow$	$ROO^{\cdot}$	…2
	$ROO^{\cdot} + RH$	$\longrightarrow$	$ROOH + R^{\cdot}$	…3
	$ROOH$	$\longrightarrow$	$RO^{\cdot} + {}^{\cdot}OH$	…4
	$ROOH$	$\longrightarrow$	$RO^{\cdot} + ROO^{\cdot} + H_2O$	…5

The increase in free radicals leads to them reacting with each other to form stable end products. This reaction step is known as termination (reaction equation 6) and usually plays a role when the oxygen level is low.

Termination	$R^{\cdot} + RO^{\cdot} + ROO^{\cdot}$	$\longrightarrow$	Stable Species	…6

Factors influencing oxidation include oxygen concentration, chemical structure of lipids including unsaturation and chain length, packaging, metal ions, moisture, light, temperature and antioxidants. Oxidation can be inhibited during the initiation step and it is therefore important to ensure correct processing and storage conditions which minimize or eliminate these factors. The addition of an antioxidant will prevent the breakdown of hydroperoxides by inhibiting the propagation process and subsequently prevent rancidity by breaking the oxidation chain. These chain breaking antioxidants include tocopherols, polyphenols, carotenoids and flavonoids

(Murcia, Jiménez, & Martínez-Tomé, 2001). These antioxidants can also occur in avocado oil and will therefore influence the oxidative stability depending on the combination and concentration of these compounds in the oil.

1.2.6 Factors affecting oxidative stability of avocado oil

The oxidative stability of avocado oil can be influenced by various factors. These include the chemical composition of the oil (unsaponifiables, saponifiables, metals), ripeness of the avocado fruit from which oil is extracted and pre-treatment of the avocado fruit prior to oil extraction.

1.2.6.1 Effect of composition of the unsaponifiable matter on oxidative stability of avocado oil

Unsaponifiable matter refers to those substances frequently found dissolved in fats and oils, which cannot be saponified by alkali treatment, but are soluble in ordinary fat and oil solvents (Farines *et al*., 1995). These compounds include higher aliphatic alcohols, sterols, tocopherols, carotenoids, pigments and hydrocarbons (Farines *et al*., 1995). The analysis of the unsaponifiable fraction of vegetable oils is widely recognized as crucial in determining their origin and possible adulteration (Frega, Bocci, Giovannoni & Lercker, 1993). It is also important in predicting the oxidative stability because complementary to the fatty acid profile, it gives an indication of the antioxidant potential inherent to the oil. This is often based on antioxidants (tocopherols, carotenoids) or prooxidants (metal ions) present in the unsaponifiable fraction.

According to Lozano *et al*. (1993) the unsaponifiable matter in avocado oil extracted from young fruit was higher than for mature fruit. In oil extracted from fresh avocado fruit, the unsaponifiables amounted to 1 – 2 % compared to the 3 – 7 % in oil extracted from dried avocado fruit (Farines *et al.*, 1995). This difference is associated with the formation of a new class of compounds, consisting of a long aliphatic chain attached to a furyl nucleus, which accounts for up to 50 % of the unsaponifiable part. The presence of these components can be linked with

pharmacological activity of the unsaponifiable part of the avocado lipids and is formed during heating before extraction (Farines *et al.*, 1995). The unsaponifiable matter of avocado oil is currently used in various pharmaceutical and cosmetic preparations (Farines *et al.*, 1995). The inclusion of the seed during extraction increases the unsaponifiable fraction as the seed contains 55% unsaponifiable material (Werman & Neeman, 1987).

Lozano *et al*. (1993) analyzed avocado oil using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) and measured decreases in unsaponifiable matter in commercial crude avocado oil after industrial treatment. Four major groups of peaks were observed for the unsaponifiable fraction of avocado oil namely, hydrocarbons, tocopherols and sterols (Δ-5 and Δ-7 sterols). HPLC was not selective enough to separate all the fractions of the unsaponifiable matter together with the individual sterol molecules contained in each of these fractions in a single run.

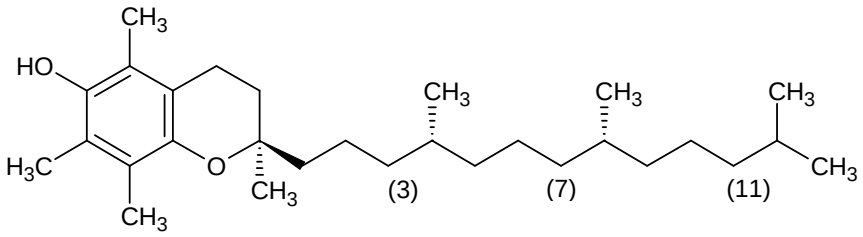
1.2.6.1.1 Tocopherols (Vitamin E)

The methyl derivatives of tocol are denoted tocopherols (Belitz *et al*., 2004[a]). Tocols are 2-methyl-2(4',8',12'-trimethyltridecyl)chroman-6-ols. Tocotrienols are identical except for the occurrence of double bonds at positions 3', 7', and 11' in the side chain (Gregory, 1996) (Figure 1.3). All the isomers of vitamin E are pale yellow, clear, viscous, oily substances, with a boiling point of 200-220 ºC. The eight naturally occurring isomers are α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol (Bramley, Elmadfa, Kafatos, Kelly, Manios, Roxborough, Schuch, Sheehy & Wagner, 2000). The isomers of tocopherols and tocotrienol differ according to the number and position of the methyl groups and thus differ significantly in vitamin E activity (Gregory, 1996).

Vitamin E is a fat-soluble vitamin and is present in nearly all food materials, but found at high concentrations in vegetable oils, nuts, plant tissues and fruits (Tawfik

& Huyghebaert, 1997). The richest sources of vitamin E in the human diet are vegetable oils and the products made from them (Bramley *et al*., 2000).

a)



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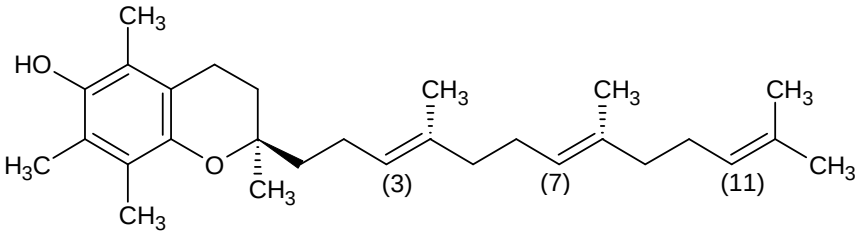


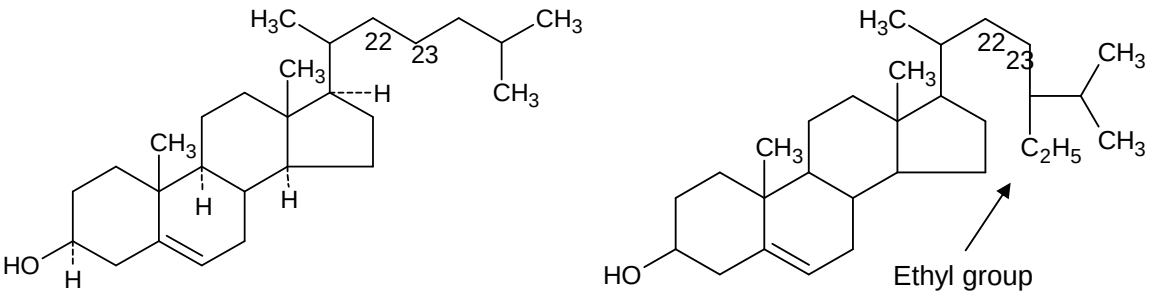
Figure 1.3: Tocopherol (a) and Tocotrienol (b)

The tocopherol content of crude oil is higher than that of refined oils due to losses during deodorization (O'Brien, 1998). Good sources of vitamin E in refined oils include wheat germ (257 mg/100 g), maize (168 mg/100 g) and walnut oil (161 mg/100 g) (Gunstone, 1996). In comparison, crude avocado oil has a tocopherol content of 5.7 – 10.3 mg/100 g oil for mature fruit and 20.1 – 45.6 mg/100 g oil for immature fruit. No significant variation has been detected between the tocopherol content of the varieties *Zutano*, *Bacon*, *Fuerte* and *Lula* (Lozano *et al.*, 1993).

Tocopherols are the best known and most widely used antioxidants (Murcia *et al*., 2001). Tocopherols function as antioxidants by donating the hydrogen of the hydroxyl group to the lipid peroxyl radical. The hydrogen-donating power of tocopherols in fats and oils is in the order δ>β~γ>α (Murcia *et al*., 2001). Tocopherols can also function as inhibitors of lipid oxidation by scavenging singlet oxygen molecules (Kamal-Eldin & Appelqvist, 1996; Fukuzawa, Inokami, Tokumura, Terao & Suzuki, 1998) and free radicals (Schuler, 1990).

1.2.6.1.2 Plant Sterols

Sterols are crystalline, neutral, unsaponifiable alcohols of high melting points with properties resembling those of cholesterol (Sonntag, 1997[a]). The chemical structures of these sterols differ from cholesterol only by an additional methyl-group (campesterol) or ethyl-group (sitosterol) at the C24-position (Figure 1.4), or by an additional double bond at the C22-position (stigmasterol) (Jansen, Lüjohann, Abildayeva, Vanmierlo, Plösch, Plat, Von Bergmann, Groen, Ramaekers, Kuipers & Mulder, 2006). This small structural difference leads to very divergent metabolic fates of plant sterols and cholesterol in mammals including the lowering of serum cholesterol which is attributed to plant sterols (Gylling, Radhakrishnan & Miettinen, 1997).



a.) b.)

Figure 1.4: Typical sterol structure represented by (a) cholesterol and (b) β-sitosterol (Przybylski, 2006)

Plant sterols comprise the bulk of the unsaponifiable matter in many fats and oils and are found in relatively large amounts in nuts and avocados (Jansen *et al*., 2006). Plant sterols, also called phytosterols have been reported to include over 250 different compounds. Although sitosterol is the main sterol in plant materials, stigmasterol, campesterol, brassica- and avenasterols are generally also present (Piironen, Toivo & Lampi, 2000).

The sterol content is less in the crude oil of mature avocado compared to immature fruit (Lozano *et al.*, 1993). This is a direct consequence of the tremendous drop of unsaponifiable content in the oil between the two stages of maturity of the fruits. According to Lozano *et al*. (1993), hexane-extracted oil from freeze dried immature *Fuerte* avocados contain 1.1% sterols, while oil extracted from mature fruit contain 0.9% sterols. The composition of the sterol fraction of avocado oil is given in Table 1.2.

Table 1.2: Composition (%) of the sterol fraction of avocado oil calculated on the basis of the high resolution gas chromatography (HRGC) peak areas (Frega *et al*., 1993).

Sterols	**Percentage (%)**
Cholesterol	0.6
X1*	1.2
Campesterol	2.8
X2*	0.5
Stigmasterol	0.2
B-sitosterol	81.4
X3*	Trace
X4*	Trace
Δ^5-avenasterol	9.0
Δ^7-stigmasterol	Trace
X5*	Trace
Δ^7-avenasterol	Trace
Other	4.3

* Fractions not identified

Due to the presence of oxidatively sensitive hydrophilic hydroxyl groups and double bonds in their chemical structure, phytosterols are susceptible to oxidation in oils and food products (Dutta, 1997). Several studies have confirmed the stabilizing effect of some sterols in oil subjected to prolonged heating (Sims, Fioriti & Kanuk,

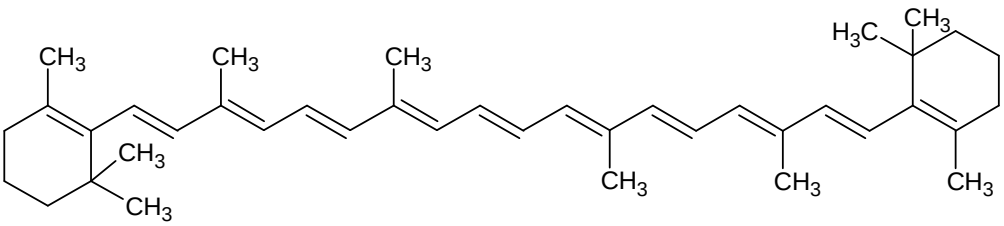
1972; Boskou & Morton, 1976; White & Armstrong, 1986). Sterols such as fucosterol, Δ-5 avenasterol, vernosterol, fucosterol and citrostadienol have been shown to have antioxidant activity in oils at 180ºC (Rajalakshmi & Narasimhan, 1995; White & Armstrong, 1986) whereas stigmasterol and cholesterol did not exhibit any antioxidant activity (Gordon & Magos, 1983). It has also been suggested that sterols function by forming a monolayer at the surface of oils to inhibit oxidation by acting as hydrogen donors. However, Cercaci, Tassalcqua, Poerio, Rodriguez-Estrada & Lercker (2007) found a minor, non-significant effect of total sterols obtained from extra virgin olive oil on the oxidative stability of vegetable model systems when determined by the oxidative stability index (OSI). They focused on considering the synergistic or antagonistic effects of the various sterols, thus hiding their single antioxidant properties, which might provide a more realistic picture of the overall antioxidant capacities of sterols.

1.2.6.1.3 Carotenoids

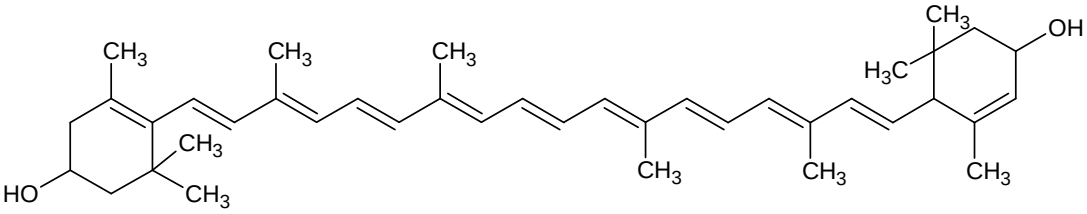
Carotenoids are structurally unique molecules consisting of a system of conjugated double bonds and are responsible for the yellow, orange or red colour in plants. They have a 40-C skeleton and consist of eight isoprene units, arranged in a head-to-tail manner to create a symmetrical molecule (Stahl & Sies, 1996). Carotenoids are divided into two main classes: carotenes and xanthophylls (Belitz *et al*., 2004[a]). Xanthophylls contain oxygen in the form of hydroxyl, epoxy or oxo groups, while carotenes are pure polyene hydrocarbons (Belitz *et al*., 2004[a]) (Figure 1.5). Carotenoids are present in plants as a complex mixture and often occur as esters of fatty acids (Belitz *et al*., 2004[a]).

The carotenoid content of the avocado is higher in the yellow part of the mesocarp than in the green part under the skin. It was this obvious yellow-green colour, normally attributed to lutein, which prompted Lu, Arteaga, Zhang, Huerta, Go & Heber (2005), to quantify the carotenoids present in the mesocarp of the avocado and specifically investigate the lutein content. Among five carotenoids measured

namely: α- and β-carotene, lutein, zeaxanthin and β-cryptoxanthin, lutein accounted for 70% of total caotenoids.



a.)



b.)

Figure 1.5: Examples representing the two main classes of carotenoids (a) ß-carotene (representing the carotenes) and (b) Lutein (representing the xanthophylls) (Belitz *et al*., 2004[a])

Although seasonal variation was observed, contents of lutein varied from 232 to 335 µg/100g fruit. The USDA-NCC Carotenoid Database for US Foods (2002) indicates a β-carotene content of 34 µg/100g and a lutein content of 320 µg/100g for avocado oil. According to Gross, Gabai and Lifshitz (1972;1973), lutein and chrysanthemaxanthin each comprise about 21-25% respectively, of the total carotenoids of avocado mesocarp, while the other types of carotenoids each comprise about 1-10%. Ashton *et al*. (2006) reported a decrease in the total carotenoid content of peel (200-100 mg/kg) and mesocarp (green flesh: 23-10 mg/kg and yellow flesh: 15-1 mg/kg) during ripening, in oil extracted from freeze dried *Hass* avocado.

Carotenoids are able to deactivate radical-mediated reactions and thus inhibit lipid peroxidation. They may also protect against the formation of singlet oxygen by

preventing exposure to light through their function as a natural light filter, which is effective for wavelengths from 400 nm to 500 nm (Zambiazi & Przybylski, 1998). Among the various defence strategies, carotenoids are most likely involved in the scavenging of two of the reactive oxygen species, singlet moleculer oxygen and peroxyl radicals. Carotenoids are sensitive to oxygen and light and are stable in food even at high temperatures, when these two factors are excluded (Belitz *et al*., 2004[a]). Suzuki and Shioi (2003), however observed a structural change of carotenoids due to heat as well as a decrease in lutein after the heat treatment involved in the processing of Japanese teas (Suzuki & Shioi, 2003). It has been established by Chen and Chen (1993) that epoxy-containing carotenoids are more susceptible to heat than other carotenoids. Warner and Frankel (1987) reported that the presence of 5 to 20 ppm of β-carotene had a significant effect in protecting soybean oil against light deterioration. In a comparative study on carotenoids and tocopherol the order of antioxidant potency was described in the following order: α-tocopherol > α-carotene > lutein > zeaxanthin = β-carotene (Farombi & Britton, 1999). All carotenoids however, showed antioxidant potential by significantly reducing the rate of peroxyl formation.

1.2.6.1.4 Chlorophyll

Chlorophylls are magnesium complexes derived from porphin which is a fully unsaturated macrocyclic structure that contains four pyrrole rings linked by single bridging carbons (Von Elbe & Schwartz, 1996) (Figure 1.6). According to Von Elbe and Schwartz (1996), chlorophylls are the major light-harvesting pigments in green plants and other photosynthetic organisms. Several chlorophylls and their derivatives are found in nature including chlorophyllides, pheophorbides, pheophytins and pyropheophytins (Gunstone, 2004). The level of pheophytin, is an important criterion in determining the quality of crude oils, especially canola oil (Daun, 1982).

Refined avocado oil has a chlorophyll content of 0.3 mg/kg (Smith & Winter, 1970), while the crude oil has chlorophyll values of 40-60 mg/kg (Eyres *et al*., 2001).

Chlorophyll seems to decrease with fruit ripening (Ashton *et al*., 2006). A decrease of 214 to 116 mg/kg oil in total chlorophyll content of oil extracted from freeze dried *Hass* avocado peel during ripening (14 days at 20℃) has been observed (Ashton *et al*., 2006).

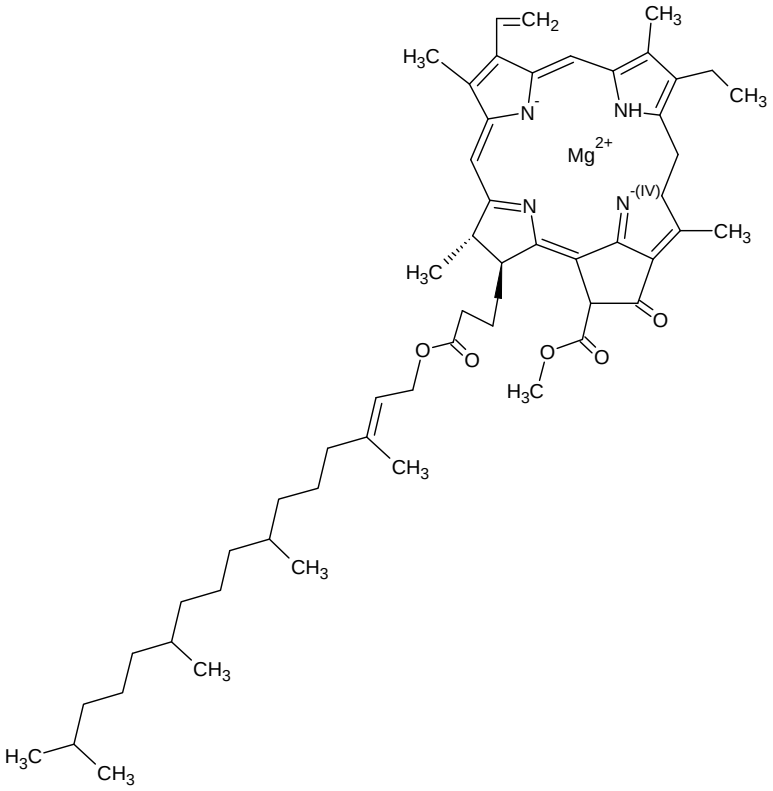


Figure 1.6: Chlorophyll, with the active porphyrin site surrounding the Mg^{2+} complex (Belitz *et al*., 2004[b])

Chlorophyll derivates are formed during processing due to heat, acid and enzymatic actions (Figure 1.7). The formation of pheophytin *a* due to heat has been well established (Suzuki & Shioi, 2003). Chen and Chen (1993), showed that when sweet potato leaves were heated briefly in a microwave, the concentrations of epimers of chlorophylls *a* and *b* and pheophytin *a* increased. Epimer formation of chlorophylls *a* and *b* in tea leaves is considered to be due to heating during processing of tea (Suzuki & Shioi, 2003). Chlorophyll *a* is more susceptible to heat

and the conversion rate of chlorophyll *a* to pheophytin *a* is higher than that of chlorophyll *b* to pheophytin *b*.

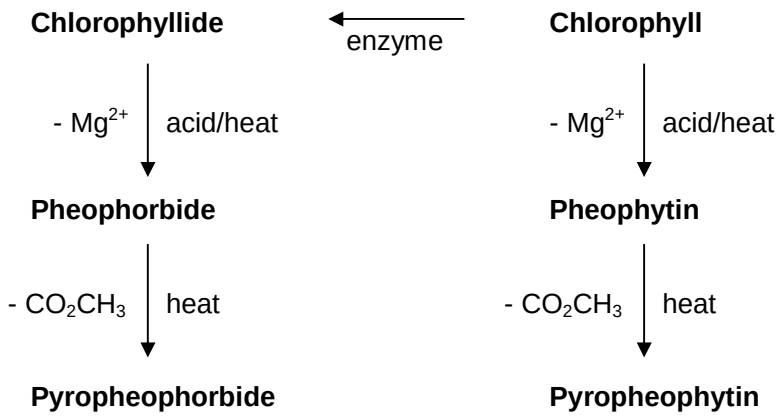


Figure 1.7: Formation of chlorophyll derivatives due to heat, acid and enzymes (Von Elbe & Schwartz, 1996)

Chlorophyll is a strong prooxidant. The oxidation reaction, in which chlorophyll acts as oxidising agent, is catalyzed by light (Smouse, 1995). The oxidative stability of vegetable oils is greatly affected by the presence of chlorophylls and their derivatives which have the ability to transfer energy from light to other molecules (Foote, 1979). The most detrimental product formed during this transfer of energy is singlet oxygen, which initiates the oxidation of oils. Oxidation then proceeds by an ene reaction forming a *trans* configuration of an unsaturated hydroperoxide (Frankel, Neff, Selke & Weisleder, 1982). These reactions are not affected by antioxidants, but can be inhibited by singlet oxygen quenchers such as carotene.

Chlorophyll however, seems to have antioxidant potential when the autoxidation reaction occurs in the dark (Endo, Usuki & Kaneda, 1984; Endo *et al*., 1985[a]; Zambiazi & Przybylski, 1998; Psomiadou & Tsimidou, 2002). Chlorophyll, as well as its derivatives, such as pheophytin, protoporphyrin methyl ester and magnesium chelated porphyrin methyl ester have been proven to retard the formation of peroxides and carbonyl compounds during autoxidation of methyl linoleate in the

dark at 30ºC (Endo *et al*., 1985[b]). It was concluded that the antioxidant effect of chlorophyll may not be ascribed to the decomposition of hydroperoxides, but rather to the chain breaking reaction by donating electrons to reduce free radicals. The essential structure for antioxidant activity of chlorophyll derivatives was found to be the porphyrin compounds, possibly strengthened by magnesium, but only in the chelated form. Antioxidant activities of chlorophyll and pheophytin were also demonstrated in oven tests with magnesium linoleate as substrate (Endo *et al*., 1985[a]).

1.2.6.2 Effect of composition of the saponifiable matter on oxidative stability of avocado oil

Saponifiable matter refers to lipid substances that can be saponified by caustic treatment. This fraction usually consists of the triglycerides and phospholipids.

1.2.6.2.1 Fatty acids

A fatty acid is a carboxylic acid often with a long unbranched aliphatic tail (chain), which is either saturated (no double bonds) or unsaturated (one or more double bonds) (Gunstone, 1996). Saturated fatty acids form straight chains, while unsaturated ones can take up different forms. Avocado oil is classified as a monounsaturated oil together with other oils such as olive, apricot kernel and macadamia (Gunstone, 1996). The predominant fatty acids in avocado oil are oleic acid (65-75%) (Figure 1.8), linoleic acid (10-18%) and palmitic acid (12-18%) (Werman & Neeman, 1987).

The fact that the oil is more mono-unsaturated will promote its oxidative stability (Werman & Neeman, 1986) in contrast to polyunsaturated oils such as sunflower and grape seed oil. The ease and rapidity with which an oil oxidizes depend primarily on the number of double bonds of the fatty acids and their arrangement (Sonntag, 1979[b]).

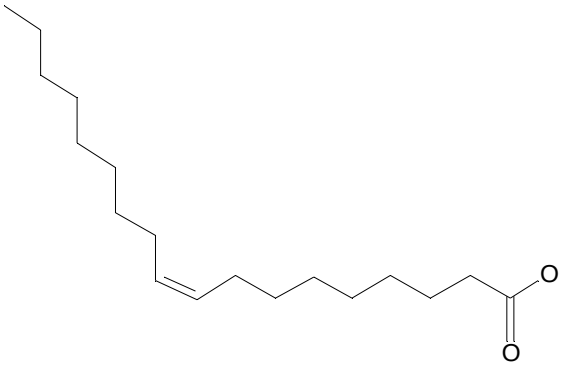


Figure 1.8: Oleic acid, a monounsaturated fatty acid with one double bond

Polyunsaturated fatty acids are oxidized more rapidly than monounsaturated or saturated fatty acids resulting in polyunsaturated fatty acids being the focal point in autoxidation of fats and oils (Sonntag, 1979[b]). The main polyunsaturated component of principal edible oils is linoleic acid and therefore the mechanism of autoxidation of this acid is of major importance in oxidative rancidity (Sonntag, 1979[b]). The rate of autoxidation of methyl interrupted polyunsaturated systems is much higher than that of monounsaturated systems because a methylene group is activated by surrounding double bonds. According to Gunstone (1996), the rates of oxidation of methyl oleate, methyl linoleate and methyl linolenate are 1:12:25. Being a monounsaturated oil, containing high amounts of oleic acid, avocado oil will oxidise slower than polyunsaturated oils, containing more linoleic or linolenic acid.

1.2.6.2.2 Phospholipids

Phospho- and glycolipids, together with proteins, are the building blocks of biological membranes (Garret & Grisham, 1995). They are surface-active compounds and contain hydrophobic moieties and hydrophilic portions, forming bilayer structures in all biological membranes. Crude oils generally contain phospholipids, which are removed during refining at the degumming stage (Gunstone, 2004). The major components are phosphatidylcholines (Figure 1.9), phosphatidylethanolamines and phosphatidylinositides accompanied by smaller proportions of other phospholipids (Gunstone, 2004).

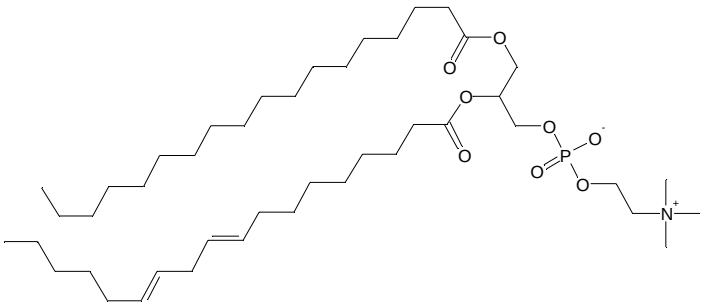


Figure 1.9: Phosphatidylcholine (lecithin), extracted from soybeans

According to Du Plessis (1980), *Fuerte* contained much higher phospholipid concentrations when compared to other avocado varieties. The phospholipid content was also proven to be higher in solvent extracted avocado oil (4.0-7.2%) than in centrifugal extracted avocado oil (0.4-2.5%) (Du Plessis, 1980).

The synergistic antioxidative effects of phospholipids are well documented (Chu, 1991; Hara, Okada, Hibino, & Totani, 1992). Various antioxidative mechanisms have been proposed for phospholipids, for example metal-chelating properties (Zambiazi & Przybylski, 1998), oxygen barrier effect, assisting in the dispersion of other antioxidants in emulsion systems as well as limiting the propagation of free radicals in the medium (Hamilton, Kalu, McNeill, Padley & Pierce, 1998). Furthermore, when heating oil at high temperatures, aldehydes can form complexes with phospholipids through carbonyl and amino group interactions and generate malanophosphatides, which themselves inactivate hydroperoxides (King, Boyd & Sheldon, 1992[a]). A synergistic effect of lecithins with phenolic antioxidants (King, Boyd & Sheldon, 1992[b]) as well as tocopherols (Judde, Villeneuve, Rossignol-Castera & Le Guillou, 2003) has also been observed. The addition of standard crude lecithin (containing approximately 60% phospholipids) was used by Judde *et. al.* (2003) to illustrate the synergistic effect of phospholipids during lipid oxidation. When tested with the Rancimat (accelerated oxidation test) rapeseed oil alone had an induction time of 8.4 hours, while this time increased to 37.6 hours, with the addition of 5% crude lecithin (Chu & Hsu, 1999).

1.2.6.3 Effect of avocado fruit ripeness on oxidative stability of avocado oil

Unripe avocado fruit is used for the pressing of avocado oil (Carr, 1997), while ripe fruit is used for the centrifugal (Benedito *et al*., 2004) and solvent extraction of the oil (Owusu-Ansah, 1997). There is a distinct difference between maturity of fruit and ripeness. The avocado is a climacteric fruit, and only starts to ripen after harvesting (Awad & Lewis, 1980; Sippel, 2001; Ozdemir & Topuz, 2004). Ripening is often completed 5 – 7 days following harvest (Seymore & Tucker, 1993) although Ozdemir and Topuz (2004) allowed 8 days at 18 – 22°C for ripening of avocado fruit when they investigated the changes in fatty acid composition. Ripening is accompanied by physical and chemical changes in the fruit and it is the chemical changes that can influence oxidative stability of oil extracted from the fruit.

Ripening might have a negative effect on the oxidative stability as the micro-components known for their antioxidant potential decrease during ripening. Ashton *et al*. (2006) reported a decrease in the total carotenoid content of peel and mesocarp during ripening, in oil extracted from freeze dried *Hass* avocado. These authors also observed a decrease in total chlorophyll content of oil extracted from freeze dried *Hass* avocado peel during ripening (14 days at 20℃). T he decrease of chlorophyll, on the other hand might increase the oxidative stability.

1.2.6.4 Effect of avocado fruit pre-treatment on oxidative stability of avocado oil

The drying method or pre-treatment and storage of avocado fruit prior to extraction plays a large role in the quality and characteristics of the extracted oil. Oven drying is a well-known and relatively cost-effective drying technique used in the oil industry prior to mechanical pressing. This can be both detrimental and advantageous where oxidative stability of oil is concerned, as heat is known to destroy tocopherols, but elevated temperatures also inactivate enzymes that lead to hydrolysis of fatty acids from triglycerides (Gunstone, 1996). Freeze drying on the other hand, is a milder drying technique where the product is not exposed to elevated temperatures and has been proven to preserve phenolic (Ferreira, Nogueira, Souza & Batista, 2004) and carotenoid (Çinar 2004) compounds better than oven drying. Although

freeze drying would seem like a superior drying technique it is important to note that the drying temperatures are too low to destroy enzymes which could lead to hydrolysis of fatty acids from glycerol (Belitz *et al*., 2004[a]). According to Çinar (2004), phytochemicals like phenols are best preserved in freeze dried samples and storage in dark, cool places is advised. Refrigeration is recommended (Ferreira *et al*., 2004), but this is often difficult to execute in industry.

Heat treatment of avocado fruit prior to oil extraction seems to lead to the formation of a compound consisting of a furyl nucleus fixed to a linear hydrocarbon-based saturated or unsaturated chain comprising one or more ethylenic or acetylenic unsaturations (Rancurel, 1993). This compound has, however, not been proven to have antioxidant activity.

Extraction method of oil is closely linked to the pre-treatment (fruit ripeness and drying method in this study) of the fruit prior to extraction and the quality of the oil is often already established before extraction commences.

1.2.7 Hypotheses

1. Oil yield obtained from avocado fruit will be influenced by ripeness of the fruit, drying method prior to oil extraction and the extraction method used. Enzymatic degradation of cellular structure during ripening will expose oil cells in ripe fruit and make them more accessible, leading to higher oil yields in ripe fruit compared to unripe. More oil will be extractable from freeze-dried fruit than from oven-dried fruit because plant material will be more porous after freeze-drying than after oven-drying, where denaturing (Belitz *et al*., 2004[c]) and cross-linking of proteins (Duodu, Taylor, Belton, & Hamaker, 2003) and starch, will form barriers around oil cells in the fruit, which will decrease the extractability. Hexane extraction will produce a higher oil yield than SC-CO_2 extraction. Hexane is less selective as an extraction solvent (Gómez & de la Ossa, 2002) compared to SC-CO_2 and will extract a higher content of unsaponifiables leading to higher oil yield for hexane extracts.
2. The micro-component (tocopherols, sterols, carotenoids, chlorophyll) content of the avocado oil extracted from ripe fruit will be lower than the micro-component content of oil extracted from unripe fruit (Ashton *et al.,* 2006) because enzymes such as lipoxygenases which are known to reduce the tocopherol, carotenoid and chlorophyll levels, increase in ripe avocado fruit compared to unripe. The tocopherol, carotenoid and sterol content of the oil extracted from oven-dried fruit will be lower than that of the oil extracted from freeze-dried fruit because these components might be oxidised by the heat treatment (80℃) during oven-drying. Progressive e xtraction will yield an oil enriched in chlorophyll and carotenoids, while the sterol and tocopherol content will remain unchanged throughout progressive extraction. This difference in separation is based on a lower initial availability of the pigments in the plant material. Tocopherols and sterols form part of the cell membrane (Kumar, Raclaru, Schüßeler, Gruber, Sadre, Lühs, Zarhloul, Friedt, Enders, Frentzen & Weier, 2005; Taiz & Zeiger, 2006) where they are readily available for extraction, while chlorophyll and carotenoids are located in

chloroplasts and chromoplasts respectively where thick cell walls will make them less available during the first phases of progressive extraction.

3. The oxidative stability of the oil extracted from unripe fruit will be higher than that of oil extracted from ripe fruit because the antioxidants which protect the oil (carotenoids, tocopherol) will be present at higher levels in unripe fruit and lower levels in ripe fruit due to oxidation by increased levels of lipoxygenase enzymes during ripening (Lajolo & Anfer-Marquez, 1982; Lopez-Ayerra, Murcia & Garcia-Carmona, 1998; Ashton *et al.*, 2006). The oxidative stability of oil extracted from freeze-dried fruit will be higher than that of oil extracted from oven-dried fruit because freeze-drying is a milder drying technique which eliminates the exposure of the fruit to high temperatures which is known to increase the oxidative and hydrolytic deterioration rate of oils (Berger, 1994). Progressive extraction will yield an oil with an increased oxidative stability due to the absence of free fatty acids and an increase in micro-components with antioxidant activity, including carotenoids, in the last fractions (Przybylski *et al*., 1998).

1.2.8 Objectives

1. To determine the oil yield obtained from SC-CO_2 or hexane extraction of avocado as influenced by fruit ripeness and method of fruit drying (freeze-drying or oven-drying).
2. To determine the micro-component (tocopherols, sterols, carotenoids, chlorophyll) content of avocado oil extracted with SC-CO_2 and how these are influenced by fruit ripeness, method of fruit drying and progressive extraction.
3. To determine the oxidative stability of avocado oil extracted with SC-CO_2 as influenced by fruit ripeness, method of fruit drying, progressive extraction and micro-component content.