PIGMENT COMPOSITION OF 'HASS' AVOCADO AND THE EXTRACTED OIL

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Dedication
To my beloved deceased parents

Abstract

The changes in the concentration of pigments in the skin and the three pulp sections of the *Persea americana* (Hass var.) and the extracted oil up to 13 days after harvest at 20°C were identified and quantified by High Pressure Liquid Chromatography (HPLC). In the fresh tissue, seven pigments identified belonging to the carotenoid family were lutein, β-carotene, neoxanthin, violaxanthin, zeaxanthin, antheraxanthin and α-carotene. Chlorophyll *a* and chlorophyll *b*, pheophytin *a* and pheophytin *b*, chlorophyllide *a* and chlorophyllide *b* were identified and measured. In the oil extracted using Accelerated Solvent Extraction (ASE), lutein and antheraxanthin were identified. Neoxanthin, violaxanthin and zeaxanthin were not present. The β-carotene and α-carotene were not tested due to the limitation of the method used in the oil determination. Chlorophyllide *a* and chlorophyllide *b* were absent. In the pulp starting from the dark pulp adjacent to the skin towards the stone, the carotenoid and chlorophyll concentrations in the fresh tissue and the extracted oil both showed a decreasing pattern. The highest oil yield was found to be between days 6 and day 8 after harvest. The pigment composition patterns of the avocado fresh tissue correspond to that of the extracted oil. There was an on-going increase in the concentration of the total anthocyanins in the avocado skin. The major anthocyanin identified was cyanidin 3-*O*-glucoside.

Cold pressed avocado oil in the laboratory and in a commercial factory with different levels of skin included during the malaxing were produced. The peroxide value, free fatty acid value, fatty acid composition and antifungal diene were measured to determine the quality of avocado oil with different levels of skin. These showed that the addition of skin had no effect on the quality of oil produced. The addition of skin during cold pressed extraction increased the pigment levels. The stability of factory cold pressed avocado oil with added skin was investigated using accelerated shelf life testing.

The addition of skin during avocado oil production in the factory shortened the shelf life. Storage of avocado oil under dark and at lower temperatures ($\leq$20°C) provided greater stability for avocado oil pigments. The levels of antifungal diene in the avocado oil were undetectable. The comprehensive data regarding pigment composition obtained

in this research may be of used in food technology, nutrition, postharvest management and gene technology.

Keywords: avocado fruit, avocado oil, pigments, colour, carotenoids, chlorophylls, anthocyanin, lutein, chlorophyll *a* chlorophyll *b*, chlorophyllides, pheophytins, accelerated solvent extraction, cold pressed extraction, diene, peroxide value, shelf life.

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Chapter 1. General Introduction

Pigment degradation during ripening of fresh fruit and during processing results in colour deterioration, quality and reduces shelf life (Heaton and Marangoni, 1996). Strategy and efforts to maximise colour retention in fresh fruit and processed food is essential as colour is most of the time the first among many criteria in determining the acceptability and hence marketability of the product (von Elbe and LaBorde, 1989; Heaton and Marangoni, 1996 and Artes et al., 2002). Colour often indicates the ripeness or freshness of fruit (Artes et al., 2002). The chemical bases of colours are the pigments anthocyanin, carotenoids and chlorophylls (Jen, 1989).

Knowledge of the pigments present and composition of fruit at different stages of ripening would be very useful in the postharvest management in terms of colour retention and consequently prolonging the shelf life of fruits and the products derived from it (Artes et al., 2002). Understanding changes of the pigment composition of the fresh fruit and their derivatives during ripening would be of use in optimizing treatment after harvest, handling and storage. This knowledge is also of importance to optimise handling and storage of the processed product (Jen, 1989). It is critical to understand the pigment composition and stability during ripening. In the oil extracted from avocado, it is imperative to understand the pigment stability during processing and what critical factors need to be controlled.

Normally, during ripening of fruit, the chloroplast is being converted to chromoplast. In ripe avocado fruit, chloroplast still dominates (Artes et al., 2002). Observation of the fruit flesh reveals a graduated change in colour from that immediately under the skin through to the stone. The flesh falls into three colour sections indicating differing pigment levels (Cran and Possingham, 1973). Data has been published about the pigments and their concentrations in the total fruit flesh with scant information about their relative levels in the different flesh sections and there is no data about the oil content in the different flesh sections. None has been published about pigment compositions in the skin and pulp sections and the extracted oil of 'Hass' avocado in New Zealand.

After harvest, colour and firmness change during ripening. Little has been published about the optimum period after harvest for extracting the greatest amount of oil from the avocado. The degree of ripeness is therefore of interest for extracting oil from the fruit. Information about pigment composition of avocado oil with flesh/skin mixtures is not available. Little is known about the effect of pigment concentrations on the oil by the addition of skin to the flesh section mixture prior to oil extraction and its effect on oil quality, shelf life and toxic compounds such as antifungal diene present in great quantities in avocado skin.

The objectives of this research are to determine the pigment composition of the 'Hass' avocado skin and the fresh flesh tissue and the extracted oil during ripening.

The pigment composition of fresh avocado tissue and the extracted oil during ripening will be compared. Likewise, the fate of the pigments of avocado oil with three levels of skin during storage at different temperatures will also be compared. The effects of skin levels during extraction on the concentration of pigments, stability and shelf life of the avocado oil will be investigated.

Chapter 2. Literature Review

Fruit undergoes changes in chemical and physical attributes in aroma, firmness, colour and taste during ripening. The most noticeable factor during ripening is the change in colour. A change in colour is due to pigments being synthesized (Belitz and Grosch, 1999a). Chlorophylls, carotenoids and anthocyanins, are the pigments responsible for green, red to yellow and red to purple colours, respectively, in fruits and vegetables (Gross, 1987).

2.1 Chlorophyll

Chlorophyll is the pigment involved in photosynthesis and is an indication of the health of plants (Scheer, 1991). During photosynthesis, chlorophyll is integral to the process whereby light energy from the sun is converted to chemical energy necessary for life processes. The first two chlorophylls that had been isolated are known today as chlorophyll *a* and *b*, which are present in the approximate ratio of 2:1 in plant green leaves (Jackson, 1976).

2.1.1 Chemistry of Chlorophyll

The tetrapyrole ring is the basic structure of porphyrin chlorophyll. The ring is connected by groups of methines (-C=). A Mg^{2+} ion links together the four atoms of nitrogen (Gross, 1987; Artes et al., 2002). At C_7, there is a lipophilic side chain derived from $C_{20}H_{39}OH$ (phytol). Chlorophyll is slightly soluble in water (Gross, 1987; Artes et al., 2002). Chemically, chlorophyll is identified by the presence of a magnesium atom in the centre of porphyrin molecule. Chlorophyll *a* and *b* have similar structures. They only differ at position 3, where chlorophyll *a* has a methyl group (-CHO) whereas chlorophyll *b* has an aldehyde group (Figure 2.1) (Gross, 1987; Artes et al., 2002).

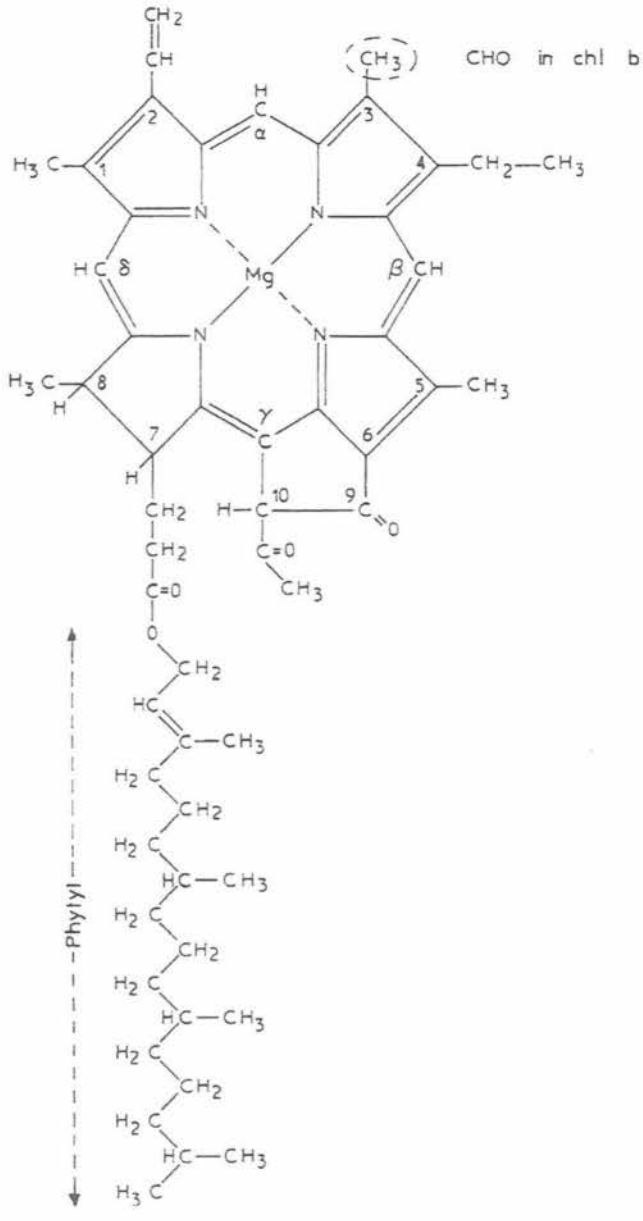
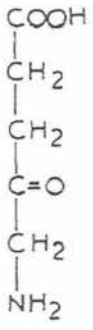


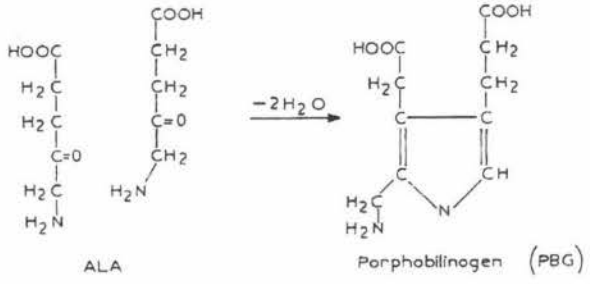
Figure 2.1.Chemical structure of chlorophyll (Gross, 1987)

2.1.2 Chlorophyll Biosynthetic Pathway

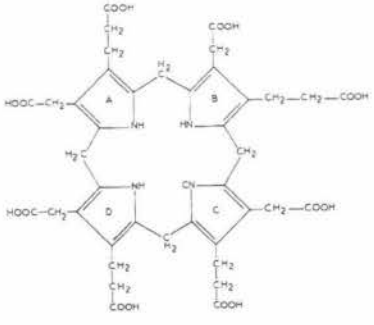
Each step in the biosynthesis of chlorophyll involves specific enzymes. The steps in the reaction are illustrated in Figure 2.2a to 2.2e.



(a) Synthesis of δ-aminolevulenic acid (ALA) precursor (Gross, 1987)



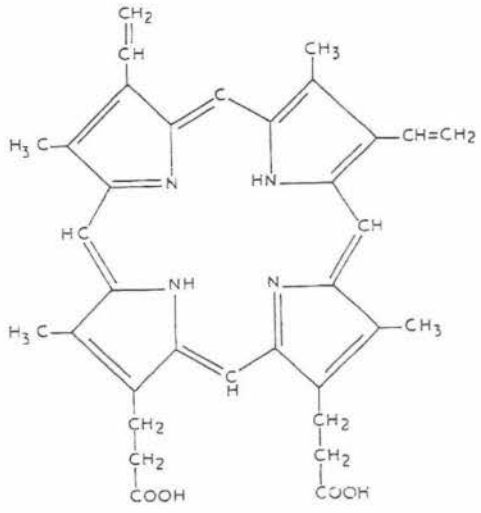
(b) Condensation of two molecules of ALA produced porphobilinogen (PBG) pyrrole precursor (Gross, 1987)



(c) Uroporphyrinogen

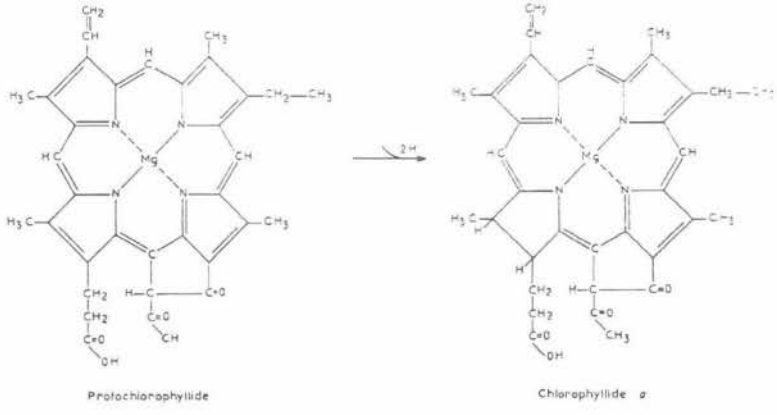
Condensation of four molecules of PBG and loss of NH_3 produce uroporphyrinogen III (Urogen), attached on each pyrrole ring is one acetic and propionic acid residue (Gross, 1987)

Figure 2.2a to c. Chlorophyll biosynthetic pathway (Gross, 1987)



(d) Protophorphyrin IX

Carbon atoms are removed from acetic side-chains to form methyl groups, propionic acid residue removal of carbon by oxidation forms vinyl groups in ring A and B. Further oxidation produces a macrocycle protoporphyrin (Gross, 1987).



(e) Protophorphyrin chelates with Mg^{2+} forming a protochlorophyllide with cyclic pentanone ring characteristic of chlorophyll. The photoreduction of ring D in Figure 2.2.c resulted in the formation of chlorophyllide *a* (Gross, 1987). Further, chlorophyllase with phytol esterifies the chlorophyllide 7-propionic acid residue followed by chlorophyllide phytylation and phytyl group formation on the side chain by reduction and hence the formation of chlorophyll (Gross, 1987).

Figure 2.2d to e. Chlorophyll biosynthetic pathway (Gross, 1987)

2.1.3 Chlorophyll Degradation

Chlorophyll degradation is not yet completely understood (Gross, 1987). The initial step in chlorophyll degradation is thought to be the release of chlorophyll from the protein-chlorophyll binding complex (Gross, 1987). The basic steps in chlorophyll degradation are the release of phytol by chlorophyllase enzyme resulting in chlorophyllide formation and the release of magnesium under acidic conditions to form pheophorbide. Chlorophyll oxidation, isomerisation and decomposition produce low molecular weight colourless compounds due to the destruction/cleavage of the chlorophyll porphyrin ring. This is caused by an oxygenase reaction and its derivative (Simpson et al., 1976; Heaton and Marangoni, 1996). In several studies, chlorophyll degradation has been attributed to the presence of oxygen, light and gamma irradiation (Simpson et al., 1976).

The degradation of chlorophyll in senescent tissues is a natural phenomenon and may be enhanced, but not regulated, by environmental factors. The changes in chlorophyll are regulated by the cell and external factors and the relevance of chlorophyll is not related to their role in photosynthesis but to their role in fruit and vegetable colours (Heaton and Marangoni, 1996). Water activity and temperature during processing influences the degradation of chlorophyll. The process of chlorophyll degradation (discoloured products) during processing involves pheophytinization. Chlorophyll is converted to pheophytin wherein the hydrogen ion replaces the magnesium ion in the porphyrin ring producing the derivative pheophytin. Pheophytinization is not the only cause of chlorophyll degradation in food. Pheophorbide is formed by the action of acid and chlorophyllase enzyme (Simpson et al., 1976; Ferruzi and Schwartz, 2001).

The effects of ethylene, free fatty acids and some enzymes on chlorophyll degradation of two kinds of spinach were investigated by Baartdseth and von Elbe (1989). Chlorophyll degradation in both varieties of spinach occurred over time. It was suggested that oxygen was involved in the discolouration as evidenced by the lower degradation rate when superoxide dismutase, antioxidants and catalase were added (Baartdseth and von Elbe, 1989).

Stability of spinach puree chlorophylls and chlorophyllides in heat 100°C to 145°C and 80°C to 115°C, respectively, were studied by Canjura et al. (1991). Pheophytin and pyropheophytin were formed from the degradation of chlorophylls and its derivatives. Pheophorbide and pyropheophorbide are the degradation products of chlorophyllides. Chlorophyll *b* and chlorophyllides degraded slower compared to chlorophyll *a* (Canjura et al., 1991).

At temperatures of 70°C, 80°C and 90°C, chlorophyll and green colour (Hunter-a) loss in peas were attributed to the conversion of chlorophyll to pheophytin (Steet and Tong, 1996). The temperature effect on chlorophyll degradation followed first order reaction kinetics and an Arrhenius relationship (Steet and Tong, 1996; Ryan-Stoneham and Tong, 2000). Another study on peas was completed by Ryan-Stoneham and Tong, (2000), who found the rate of degradation in peas was dependent on the pH. The loss of chlorophyll was inversely proportional to pH. In this study, the loss of chlorophyll *a* was always faster (2.5 times) than the loss of chlorophyll *b* regardless of pH and temperature.

2.1.4 Chlorophyll in Avocado

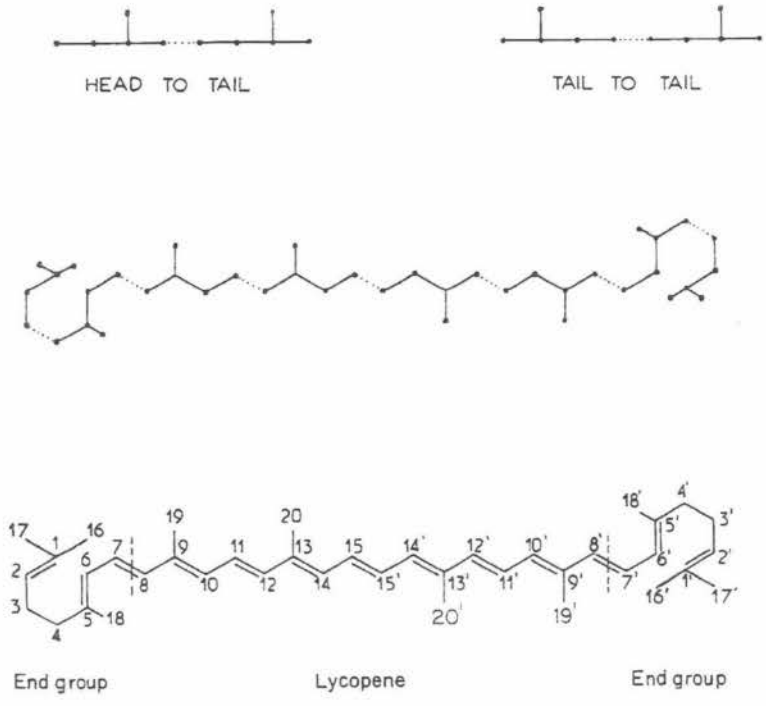
The variation of the colour of avocado fruit sections indicates the different levels of chlorophyll. The skin has the highest concentration of chlorophyll and lower concentrations of chlorophyll occur in the yellow portion of the flesh (Cran and Possingham, 1973). The chlorophyll content of the sections of mature avocado fruit (cv. Fuerte) by fresh weight were found to be 316μg g^{-1} (skin), 101μg g^{-1} (dark pulp/green), 51μg g^{-1} (pale pulp/green) and 38μg g^{-1} fresh weight (yellow pulp) (Cran and Possingham, 1973).

2.2 Carotenoids

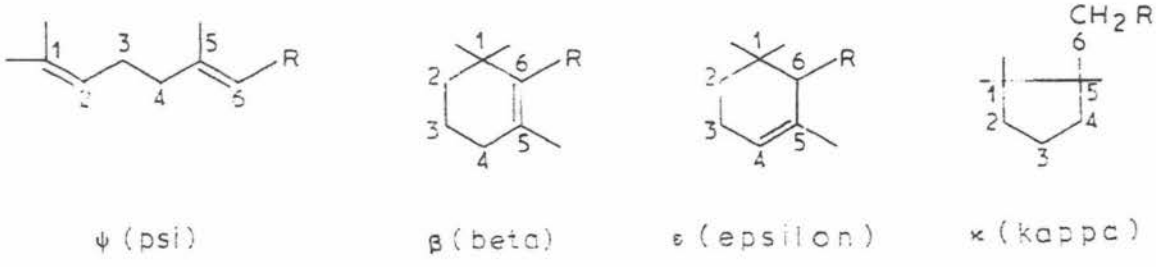
The red, yellow and orange colours of most fruits are due to the presence of carotenoids. The name carotenoid was derived from β-carotene, the principal representative of this group of pigments which was first identified in carrots (*Daucus carota*) (Gross, 1987). Carotenoids are very soluble in oil. Hence, they are denoted "lipochromes". The major carotenoids are the carotenes and the yellow xanthophylls known as oxygenated carotenoids. They are β-carotene ($C_{40}H_{56}$) and lutein ($C_{40}H_{56}O_2$) (Simpson et al., 1976; Gross, 1987).

2.2.1 Chemistry of Carotenoids

There are eight C_5 isoprene units which are joined together to form polyene isoprenoid carotenoids (Gross, 1987).



(a) Linking of isoprenoid units (Gross, 1987)



(b) End group designation of some carotenoids (Gross, 1987)

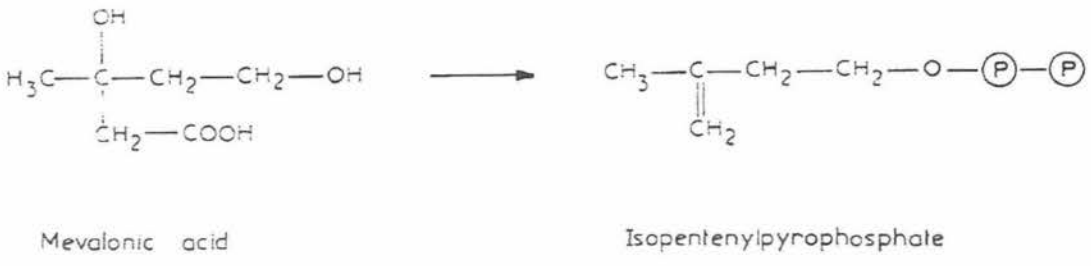
Figure 2.3.Chemistry of carotenoids

The isoprene units are linked in a regular head to tail manner (Figure 2.3a) except in the centre of the molecule where the order is inverted tail to tail, so that the molecule is symmetrical. In lycopene ($C_{40}H_{56}$; Figure 2.3a), six carbon atoms in the middle of polyene

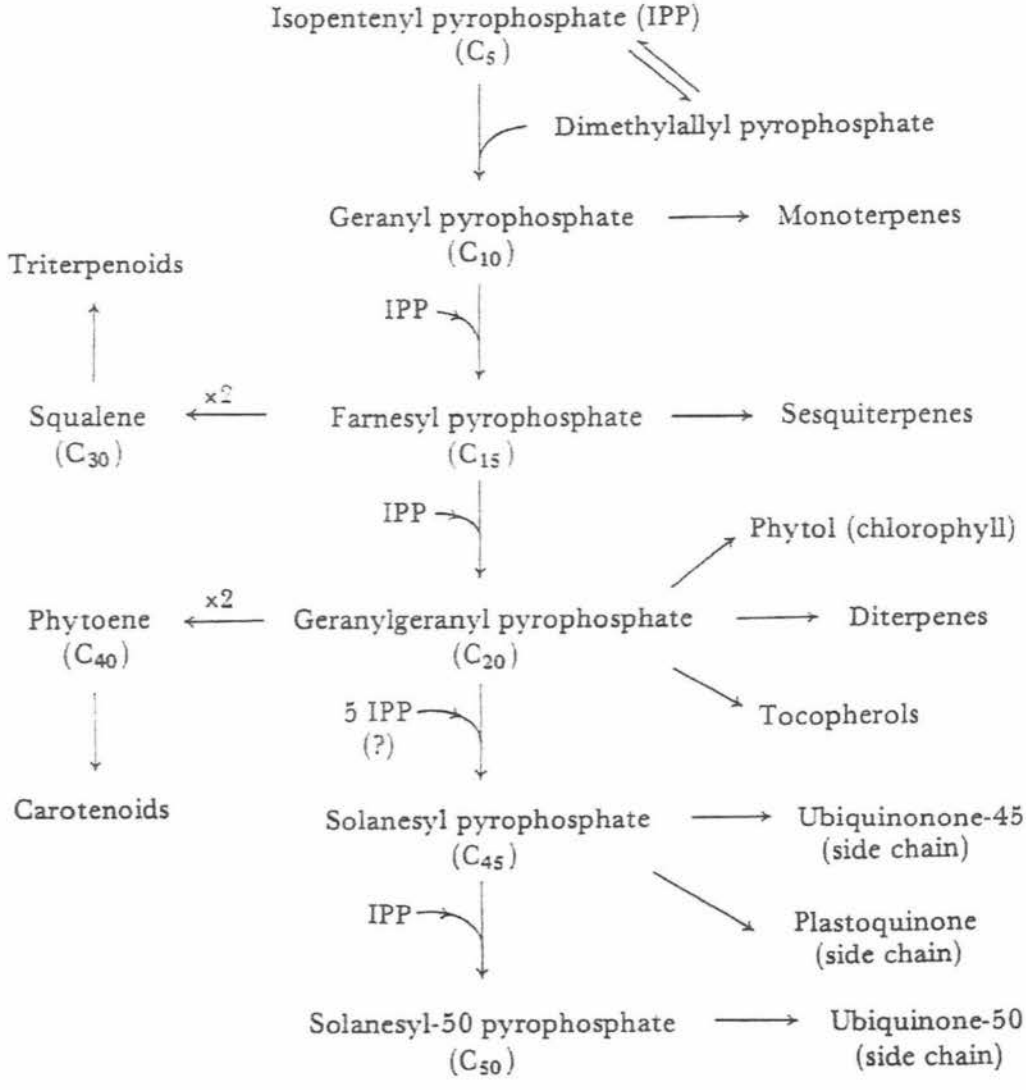
separate the two methyl groups. The numbering of carbon molecules is from the centre to the side (15 descending to 1), symmetrical part (1' to 20') and for the methyl group (16 to 20). Carotenoids are derived from this basic structure by the process of oxidation, hydrogenation and cyclisation (Gross, 1987). The name of the end groups have Greek letter prefixes acyclic ψ (psi), cyclohexane β (beta), Є (epsilon), cyclopentane κ (kappa) of two C_9 groups (Figure2.3b).

2.2.2 Carotenoid Biosynthetic Pathway

The precursor of terpenoids is isopentyl pyrophosphate (IPP), which originates from mevalonic acid (MVA) (Gross, 1987). The biological synthesis pathway of the majority of tetraterpene (C_{40}) carotenoids originates from the 'isoprene unit'(C_5).Carotenoid biosynthesis starts from four successive additions of C_5 carbons to C_{20} then condensation of two C_{20} produces the C_{40} frame, characteristic of carotenoids (Figure 2.4a and 2.4c). The initial product formed is phytoene, a C_{40} carotenoid frame. This is catalyzed by phytoene synthase (Artes et al., 2002). The prototype lycopene is the end product produced via phytofluene, ζ-carotene and neurospene by the catalytic action of the intermediate desaturase enzyme. Cyclization of lycopene, produces different xanthophylls formed with six carbon rings i.e. β, Є, α, ψ on both or one end (Figure 2.4c; Artes et al., 2002).

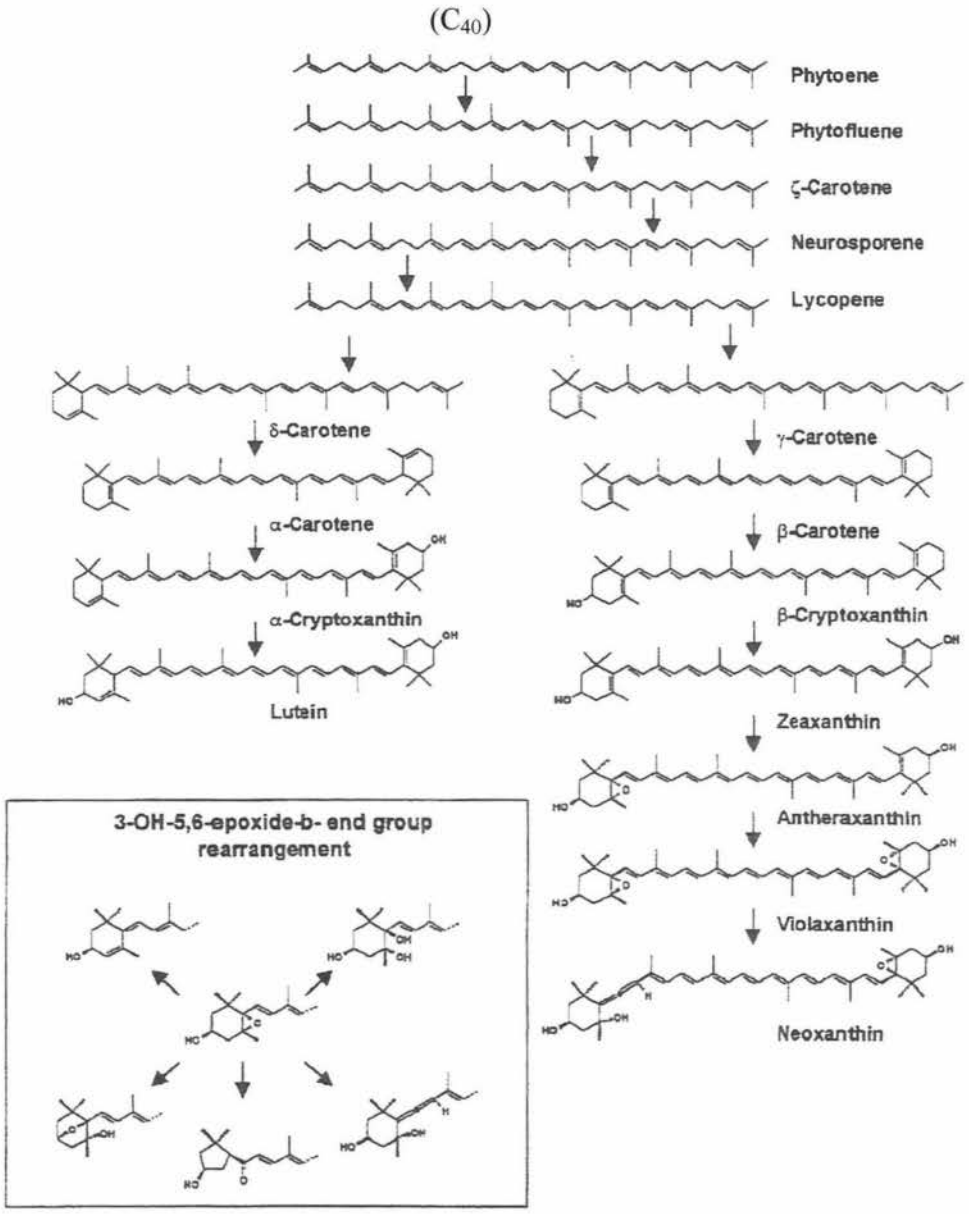


(a) Mevalonic conversion to isopentyl pyrophosphate (Gross, 1987)



(b) General scheme for terpenoids biosynthesis (Britton, 1976)

Figure 2.4 a to b. Carotenoids biosynthetic pathways



(c).Biosynthesis of main carotenoids found in fruits and vegetables (Artes et al., 2002).

Figure 2.4c. Carotenoids biosynthetic pathways

2.2.3 Carotenoid Degradation

During maturation and ripening in fruit, chlorophyll levels drop, followed by an increase in esterified xanthophylls and carotene. Most stable carotenoids are in the all-trans form, with few existing as poly-cis carotenes. The degradation of all-trans to all-cis isomers is enhanced by light, temperature, acid or iodine catalyst and is evident due to a decrease of hue in fruit colour. A suggestion that enzymes are involved in carotene degradation was observed in the protective effect of blanching the material containing carotenoids. It was also demonstrated in some studies that ionizing radiation does not enhance carotenoid degradation (Simpson et al., 1976).

At high temperatures carotenoids are stable when light and oxygen are excluded. In a lipid food system the rate of degradation (discolouration) is faster due to free radicals present from the oxidation reactions (Belitz and Grosch, 1999). Acid can degrade pure carotenoids as well as alkali, light and oxygen to some extent. Carotenoid degradation in intact tissue is slower. Carotenoids in disrupted tissues are eventually degraded. During storage and senescence carotenoid degradation was due to the presence of light. A decrease of carotenoids during fruit development would be expected to follow an increase in maturity (Simpson et al., 1976).

The major steps in carotenoid breakdown are the formation of epoxides, formation and esterification of xanthophylls, cis-trans isomerisation and enzymic changes (Simpson et al., 1976). Antheraxanthin 5, 6,-epoxide and violaxanthin 5, 6, 5', 6'-diepoxide are the common epoxides derived from zeaxanthin or the allenic monoepoxide neoxanthin. The concentration change in violaxanthin was caused by light (Simpson et al., 1976).

2.3 Anthocyanins

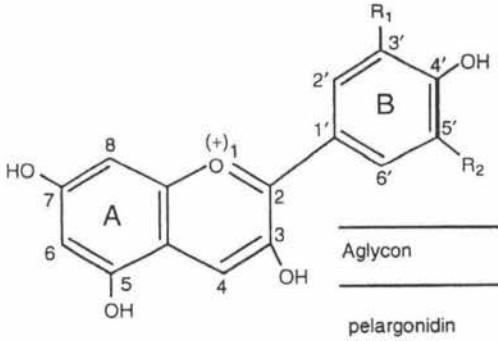
Anthocyanins belong to the group of compounds known as flavonoids. The colour derived by the presence of anthocyanin range from oranges and reds through to blues, purples and violets (von Elbe and Schwartz, 1996). The purple colour of ripe avocado skin is attributed to anthocyanins (Prabha et al., 1980). Anthocyanins are water soluble, and complex with cytosolic fraction in vacuoles outside chloroplasts and other lipid systems (Simpson et al., 1976). The majority of anthocyanin is present in the vacuole. Anthocyanin synthesis

increases with maturity. The highest content is detected when fruit is ripe. The level of anthocyanin present in fruit is an indicator of maturity and ripeness (Gross, 1987).

2.3.1 Chemistry of Anthocyanin

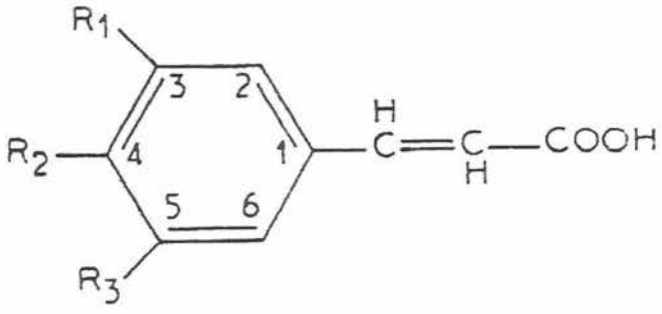
Anthocyanins have a $C_6C_3C_6$ basic structure (von Elbe and Schwartz, 1996). Two aromatic rings A and B connected together by 3 units of carbon form the characteristic flavan nucleus. Anthocyanidins are hydroxylated anthocyanins. Their identity depends on the hydroxylation at ring B. The hydroxide group on the A and B rings are positioned at 5, 7 and 3', 4', 5' carbons (Figure 2.5; Rodriguez-Saona and Wrolstad, 2001).

Glycosylation is normally at position 3 but position 5 and 7 may also be glycosylated. The sugar moiety can be acylated either by caffeic, cinnamic, ferulic, sinapic and *p*-coumaric acids or the acyl group derivatives of cinnamic acid (Figure 2.6; Gross, 1987).



Aglycon	Substitution pattern R1	R2	λ_{max} (nm) visible spectra
pelargonidin	H	H	494 (orange)
cyanidin	OH	H	506 (orange-red)
delphinidin	OH	OH	508 (blue-red)
peonidin	OCH_3	H	506 (orange-red)
petunidin	OCH_3	OH	508 (blue-red)
malvidin	OCH_3	OCH_3	510 (blue-red)

Figure 2.5.Generalized structure for anthocyanin pigments (Rodriguez-Saona and Wrolstad, 2001)



Cinnamic	$(R_1 = R_2 = R_3 = H)$
p-Coumaric	$(R_1 = R_3 = H;\ R_2 = OH)$
Caffeic	$(R_1 = R_2 = OH;\ R_3 = H)$
Ferulic	$(R_1 = OCH_3;\ R_2 = OH;\ R_3 = H)$
Sinapic	$(R_1 = R_3 = OCH_3;\ R_2 = OH)$

Figure 2.6.General structure of acyl group derivatives (Gross, 1987)

2.3.2 Anthocyanin Biosynthesis

Precursors of rings A and B are acetate units (malonyl-CoA) and phenylpropanoid (p coumaryl-CoA), respectively. By the condensations of these two precursors the two rings are formed. Condensation of acyl residues from the three molecules of malonyl-CoA and one molecule of 4-coumaryl-CoA is the main reactions in the biosynthesis of flavonoid (Figure 2.7).

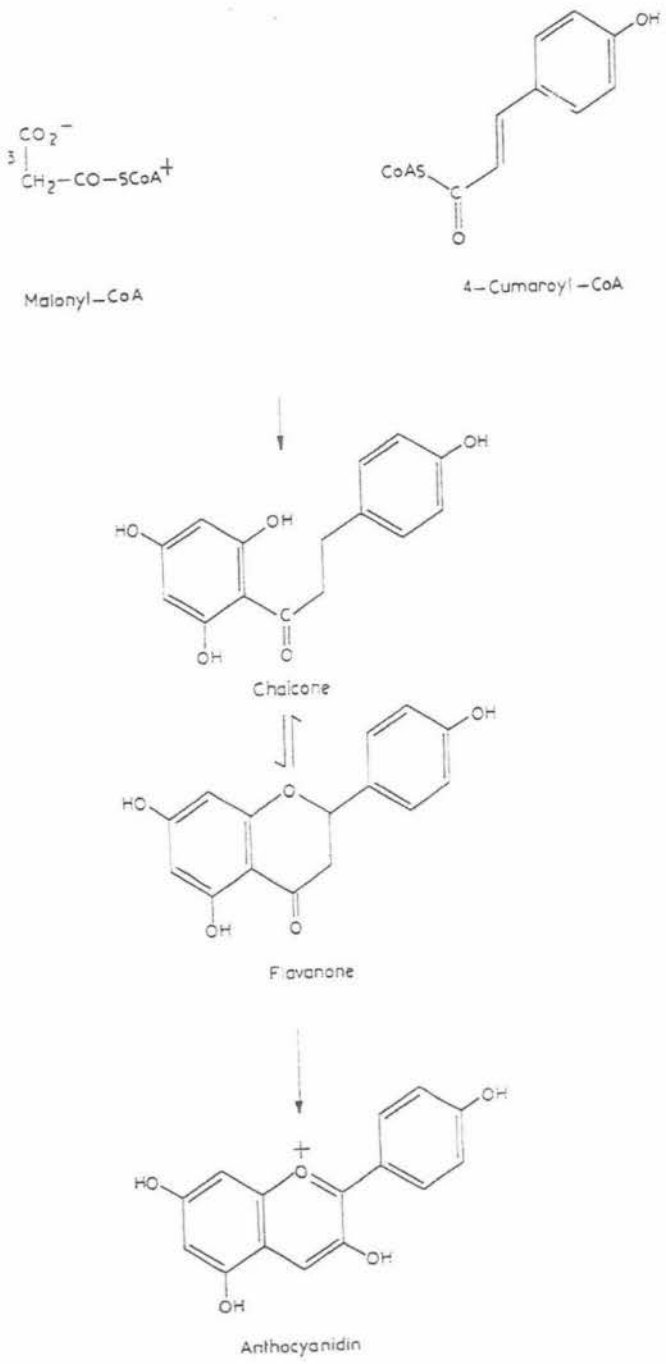


Figure 2.7.Anthocyanin biosynthetic pathways (Gross, 1987)

2.3.3 Anthocyanin Degradation

Anthocyanin stability depends on the presence of enzyme, ascorbic acid, metals, sulphite and on pH and temperature (Simpson et al., 1976). Anthocyanin is susceptible to degradation due to the OH group at position 3'. The methoxyl group was found to have both protective and deleterious effects depending on the kind of anthocyanidin. Degree of stability is also dependent on the type of sugar and the glycoside group. Diglucosides are more stable in heat and light compared to mono-glucosides. Between diglucosides, acylated forms are more resistant to the deleterious effects of light than the non-acylated (Simpson et al., 1976).

Two enzymes, glucosidases/anthocyanase and polyphenol oxidases/phenolases were found to be responsible for the discolouration of anthocyanins. Glycosidase through a hydrolysis catalyse conversion of anthocyanin to anthocyanidin. The glycoside group is removed resulting in anthocyanidin aglycone destabilisation and discolouration. The decolourising power of polyphenol oxidases was aided by catechol or other o-dihydroxyphenols and was strong between pH 6 to 7 (Simpson et al., 1976).

Anthocyanin is vulnerable to attack at its nucleus by compounds present in plant or food systems such as ascorbic acid. Ascorbic acid has been found to be responsible for anthocyanin degradation in fruits and degradation is accelerated in the presence of high oxygen concentration and copper ions (Simpson et al., 1976). Condensation of anthocyanin and ascorbic acid, and the formation of hydrogen peroxide due to the autooxidation effect of ascorbic acid on anthocyanins are the two main degradation mechanisms. In contrast, ascorbic acid has also been found to be degraded by anthocyanin (Simpson et al., 1976).

2.3.4 Anthocyanin in Avocado

Prabha et al. (1980) had separated and identified two anthocyanins in avocado peel of an unnamed purple variety. Both had maximum absorption at 538nm, an indication that they were the same aglycone. The aglycone was identified as cyanidin for the two anthocyanins. The sugars moieties were identified as galactose and glucose. A distinct absorption at 264nm was observed and was identified as p-coumaric acid. The two anthocyanins were

identified as cyanidin-3-galactoside and cyanidin-3, 5-diglucoside-p-coumarate. At the ripe stage the distribution of these two anthocyanins in avocado skin was 90% and 10% respectively.

The anthocyanin levels in skin from an unspecified variety of avocado were undetectable in green avocado, red skin (296μg g^{-1} and 358μg g^{-1}) and purple skin (706μg g^{-1}) (Lancaster et al., 1997).

2.4 Chlorophyll Analysis

Advanced analytical methods such as thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC) are considered modern instruments for the analysis of chlorophyll. Studies of chlorophyll structure utilize spectroscopic properties in visible spectra as well as nuclear magnetic resonance, infrared, x-ray crystallography and mass spectrometry (Jackson, 1976).

Solvents used in the extraction of chlorophyll were petroleum ether and methanol (Jackson, 1976). Extraction of chlorophyll pigments using acetone was used for spinach by Canjura et al. (1991). A spinach thermal degradation study, twelve chlorophyll derivatives were characterized and four components chlorophylls *a* and *b* and chlorophyllides *a* and *b* were quantified with the use of HPLC (Canjura et al., 1991). HPLC was used in the analysis of the rate of degradation of chlorophyll *a* and *b* in peas (Steet and Tong, 1996).

2.4.1 Extraction and Isolation

Extraction of photosynthetic tissue such as chlorophylls required polar solvents such as methanol, ethanol and acetone (Lichtenthaler and Buschmann, 2001). These solvents used for extraction of chlorophyll are also applicable for the extraction of carotenoids. Detailed steps to perform extraction, spectrophotometric analysis and sample storage were described by Lichtenthaler and Buschmann (2001).

2.4.2 Spectrophotometry

Spectrophotometric determination was described for chlorophyll *a* and *b* determination as well as for carotenoids (Lichtenthaler and Buschmann, 2001). There are two methods for spectrophotometric determination of chlorophyll in oil available in the American Oil

Chemist Society Methods AOCS (1997). The first method applicable to refined, bleached and expelled oils was described in AOCS Official Method Cc 13d-55 (AOCS, 1997). In this method, absorption is measured at 630, 670 and 710nm. The second method AOCS Recommended Practice Cc 13i-96 (AOCS, 1997) is applicable to crude vegetable oil with chlorophyll contents greater than 1mg/kg oil. In this method absorption is measured at 670nm. All measurements are expressed in mg pheophytin/kg of oil.

2.4.3 High Pressure Liquid Chromatography (HPLC)

The major groups in chlorophyll that can be taken into consideration were the chlorophyll *a* and *b*, pheophytins and pyropheophytins (non-polar chlorophyll derivatives), chlorophyllides and pheophorbides (polar chlorophyll derivatives) and Cu^{2+} and Zn^{2+} pheophytins (metalloporphyrin derivatives) (Ferruzzi and Schwartz, 2001).

One reported method of determining chlorophyll compounds by HPLC uses the stationary reverse phase of octadecyl-bonded column with water and methanol. The mobile phase was ethyl acetate. The detector was UV/visible and photosynthetic pigment detection was at 654 nm (Ferruzzi and Schwartz, 2001). Synthesis of Cu^{2+} and Zn^{2+} pheophytins standards which are not available commercially and the most frequently used standard is from the parent Mg-chlorophyll standard. Intensive cleaning of the C_{18} column for optimum performance was also described (Ferruzzi and Schwartz, 2001).

2.5 Carotenoids Analysis

Chlorophyll and carotenoids were extracted by acetone/tetrahydrofuran (1:1), separated and quantified by HPLC and characterized by mass spectrophotometer in a comprehensive analysis of these pigments in four varieties of Kiwifruit (*Actinidia*) (McGhie and Ainge, 2002). It was suggested that the yellow colour of gold *Actinidia* (Hort 16A) is not due to the increase in carotenoids but due to the degradation of chlorophyll. The chlorophyll pigment is retained in 'Hayward' Kiwifruit.

2.5.1 Extraction and Isolation

Benzene, petroleum ether, ether, ethanol and acetone are suitable solvents for extraction of carotenoids from plant samples (Belitz and Grosch, 1999b).

Extraction and isolation of samples without water followed by saponification to isolate the carotenoids has been described (Rodriguez, 2001). In the case of hydrated samples, removal of water with the use of a vacuum oven for samples that could be powdered and use of alcohol for samples containing lipids, sugars or waxes were employed (Rodriguez, 2001). Procedures for extraction and saponification could be followed after sample dehydration (Rodriguez, 2001).

2.5.2 Spectrophotometry

Individual carotenoid and total carotenoids could be measured using the methods described by Scott (2001). Preparation and dilution of samples and standards for both methods were similar.

2.5.3 High Pressure Liquid Chromatography (HPLC)

A C_{18} polymeric column for carotenoids separation was used. Tocopherols were analyzed simultaneously with a diode-array detector. The accuracy of analysis depended on the preparation of standards and calibration as well as sample preparation. Spherisorb ODS2 was used for isocratic separation of carotenoids as well as tocopherol and retinol separation (Craft, 2001). Higher resolution C_{30} column was employed in gradient separation of carotenoids (Craft, 2001). Normal-phase separation was recommended if the xanthophyll fraction was of interest. Separation in normal-phase allowed quick elution of carotene, the non polar constituents and polar constituents were left allowing efficient separation of polar carotenoids (Craft, 2001).

2.6 Anthocyanin Analysis

Original methods were used to analyse anthocyanin in 'Royalty' grapes and apple (Chen and Luh, 1967; Sun and Francis, 1967).

In the analysis of anthocyanin in 'Royalty' grapes, methanol in 1% HCl was the solvent used for extraction. Purification by adsorption of anthocyanin and separation of pigment was achieved with the use of cation-exchange resin and paper chromatography respectively. Acyl and sugar moieties were identified by acid hydrolysis and again by paper chromatography. Absorption in the visible and ultra violet region was measured by a spectrophotometer (Chen and Luh, 1967). Malvidin and peonidin were identified and the

presence of cyanidin, delphinidin and petunidin were indicated. 'Royalty' grapes had monoside and biosides glycoside units and an acylation by *p*-coumaric acid (Chen and Luh, 1967). In apple anthocyanins were identified by Sun and Francis (1967) as cyanidin-7-arabinoside. Anthocyanins were extracted using the same solvent used in 'Royalty' grapes anthocyanin extraction and paper chromatography using Whatman No.3 as in the work of Chen and Luh (1967).

2.6.1 Extraction and Isolation

Extraction, isolation and purification of anthocyanin from plant materials were key steps before a pigment can be quantified and characterized (Rodriguez-Saona and Wrolstad, 2001). Extractions of anthocyanin can be carried out using acetone and subsequent partitioning using chloroform. This method also allowed removal of other oil soluble pigments and concentration of anthocyanin. Another method of extraction was by the use of methanol (Rodriguez-Saona and Wrolstad, 2001). This method was rapid due to the low boiling point of methanol but it required purification because it could only extract crude anthocyanin and since it used HCl, hydrolysis of sensitive acyl linkages may occur. Crude anthocyanin extract can be purified by the use of solid-phase extraction (Rodriguez-Saona and Wrolstad, 2001).

2.6.2 Spectrophotometry

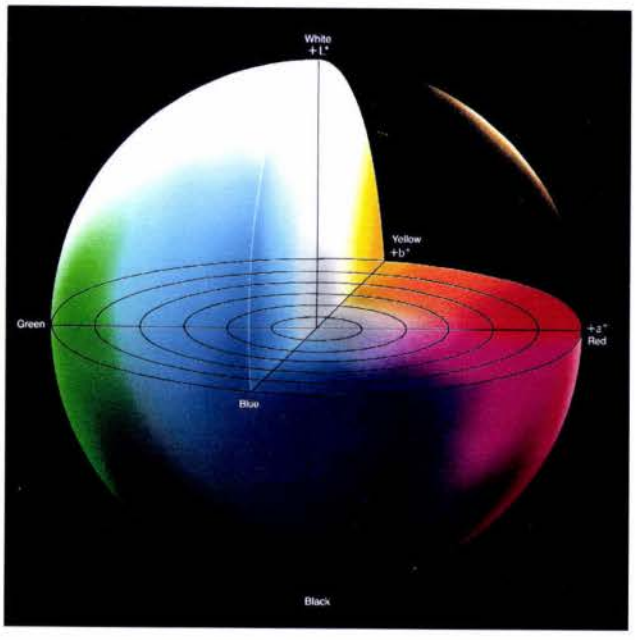
The pH differential method is used to determine total monomeric anthocyanin (Giusti and Wrolstad, 2001). This method is rapid and even when other compounds and degraded pigments are present it provided accurate measurements. Giusti and Wrolstad (2001) described measurement of anthocyanin degradation (browning and polymerization) of a sodium bisulphite treated sample by spectrophotometry.

2.6.3 High Pressure Liquid Chromatography (HPLC)

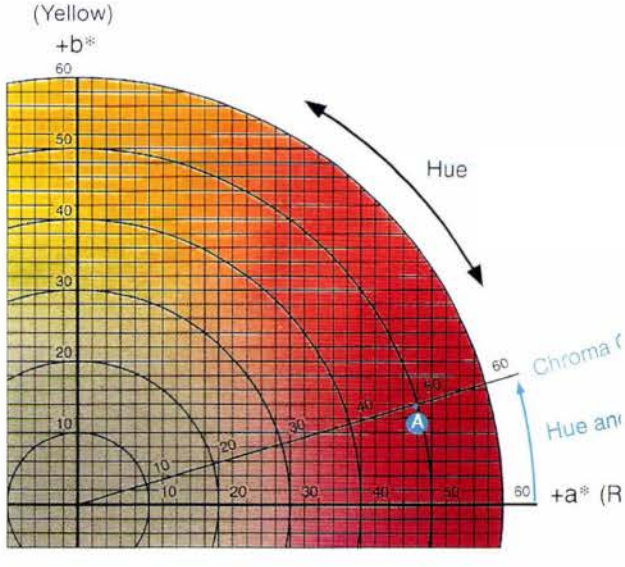
A C_{18} column is applicable for anthocyanin with simple glycoside while a polymeric C_{18} column is applicable for acylated anthocyanin. Steps included dilution, solid phase extraction, acid hydrolysis, filtration and reversed-phase HPLC analysis. Acylated anthocyanins can be removed by base saponification (Durst and Wrolstad, 2001).

2.7 Colour Measurement

The L, a and b colour scale and the L*c*h colour space are shown in Figure 2.8a and 2.8b.



(a) Hunter L*, a*, and b* Colour Space (Minolta Co. Ltd, 1998)



(b) L*c*h colour space (Minolta Co. Ltd, 1998)

Figure 2.8.The Hunter L*a*b* and L*c*h Colour Spaces

A colour space created by a continuum of colour allows the attributes of saturation and lightness to be plotted on numerical scales. Colour space represents all colours and has a three dimensional configuration. Any particular colour can therefore be plotted as a point in the colour space (Gullet, 1991).

The value of the *L* (lightness) ranges from white with a value of 100, to black with a value of 0. This scale forms a vertical axis with "0" at the bottom. The axes *a* and *b* are at right angles forming a rectangular grid with the L axis passing vertically through the centre. The positive *a* axis is in the red direction with the negative *a* towards green. The positive *b* axis is in the yellow direction with the negative *b* towards blue. The grid origin where the three axes meet is chromatic (grey), and as *a* and *b* values increase the intensity rises (Gullet, 1991).

The L*c*h colour space (Figure 2.8b) is similar to L*, a*, and b* colour space. Both of them used rectangular and cylindrical coordinates respectively. L*indicated lightness, Chroma, C*=square root $[(a^*)^2 + (b^*)^2]$, and Hue angle, $h_{ab} = \tan^{-1}(b^*/a^*)$ (Minolta Co. Ltd, 1998). Chroma decreases towards the centre. The chroma value at the centre is 0. The angle of hue expressed is in 0^o, 90^o, 180^o and 270^o correspond to red (+a*), yellow (+b*), green (-a*) and blue (b*) respectively (Minolta Co. Ltd, 1998).

The two common methods for colour measurement are colorimetric and spectrophotometric. The colorimeter method is simple and rapid on the other hand, the spectrophotometer provides greater accuracy (Giese, 2000). However, a colorimeter is cheaper (Giese, 2000). Minolta Chroma Meter CR300 (Minolta, Osaka-light source D_{65}) has been used to measure avocado skin colour (Cox et al., 2004). Steet and Tong, (1996) used a Tristimulus Colorimeter (Model CR210, Minolta Corporation, Ramsey, New Jersey) for the colour analysis of peas to determine rates of chlorophyll degradation.

An inexpensive method for the measurement of food colour uses a lighting system, a high resolution digital camera and Adobe Photoshop software to obtain L*a*b* colour space values has been developed (Spyridon et al., 2000).

There is no legal requirement for colour measurement of oil or levels of pigment measurement. However, measurements of these parameters may be of use as this could serve as an index of quality during processing and storage (Swisher, 1988). Colour measurement of oil is monitored during processing to achieve uniform quality. The colour of fats and oil is an important characteristic. Consumers indicated a preference for shades according to their expectancy for that kind of oil. In the case of avocado oil, the green chlorophyll layer under the skin imparted considerable green colour to the crude oil. After refining, the oil was more of a yellow green (Swisher, 1988).

Each type of oil has its distinctive colour. The colour of light transmitted at a certain level in oil is matched to the colour from the same origin transmitted to a range of coloured glasses. First grade peanut oil used for cooking is 2 Lovibond red or less and set to Lovibond yellow 20 based on Chinese national standard GB5525-85. First grade peanut oil used for salads was 1.5 Lovibond red or less and set to Lovibond yellow 15 (Fengxia et al., 2001). Colour measurement of vegetable fats and oils using Lovibond was described in AOCS Method Cc 13e-92 (AOCS, 1997).

2.8 Avocado Fruit

Avocado (*Persea americana.*) belongs to the family of Lauraceae. There are three major races (Popenoe, 1974). Each individual race has unique attributes. The Mexican race is suitable to cool temperatures with thick, smooth and shiny skin. The size of the fruit is small, about 85-340g. Guatemalan race is suitable for temperate climates. The weight of the fruit is about 340-560g and the skin grain is coarse with a thickness of about 1.6mm. West Indian race is suitable for high temperature tropical conditions. The size of the fruit was medium and the texture of skin is leather like (Kadam and Salunkhe, 1995). Many of the cultivated varieties are hybrids of the three races which are of economic importance (Popenoe, 1974; Kadam and Salunkhe, 1995). Fuerte, a mixture of Guatemalan and Mexican race is one of the most known avocado varieties cultivated worldwide (Popenoe, 1974; Kadam and Salunkhe, 1995).

'Hass' is a hybrid of Mexican and Guatemalan with dominating genes of the latter. The fruit have an oblong shape and have no neck (Kadam and Salunkhe 1995; Newett et al.,

2002). The skin is thick with a rough texture and the size of the fruit is medium (140-400g) (Newett et al., 2002). During ripening the skin changes in colour from green to purple (Cox et al., 2004). 'Hass' was named after Rudolph Hass who selected the seedling because of its high percentage of flesh, at La Glabra Heights, California. The yield is higher and it matures later than 'Fuerte'. It was patented in 1935 (Newett et al., 2002). 'Fuerte' and 'Hass' are both suited to cool climate temperatures (Kadam and Salunkhe, 1995).

2.8.1 Maturity at Harvest

The maturity at harvest indicates the ability of the avocado to ripen (physiological maturity) (Woolf et al., 2003).

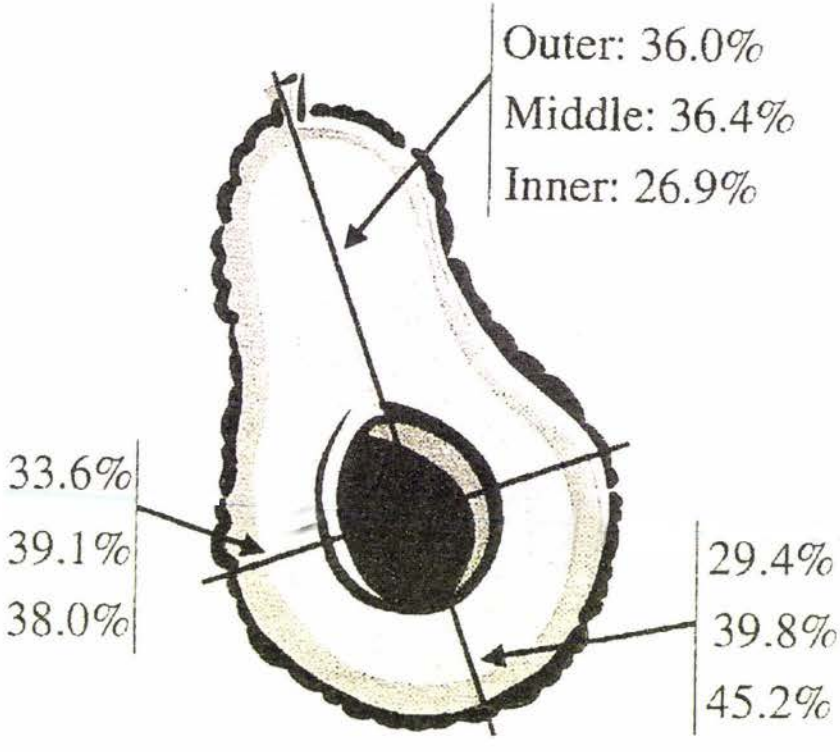
In 1925, an avocado with an oil content of 8% w/w was the minimum harvest criteria for avocado fruit (Lee, 1981). This criterion was based on comparisons of results obtained by several methods of oil extraction namely Halowax, Nuclear Magnetic Resonance (NMR), Soxhlet and Oil-Plus-Water Constant methods (Lee, 1981). It was found that the most accurate method for oil determination was by the Soxhlet solvent extraction. However, it was not economical as it took a long time to obtain results. The Halowax and NMR methods were not feasible for use by ordinary growers. The simple method of dry matter determination used today was derived from the Oil-Plus-Water Constant Method (% Oil plus % Water = Constant therefore, % Oil = Constant -% Water) results (Lee, 1981).

In 1983, it was found from sensory evaluation of avocado fruit that the 8% w/w oil criterion for acceptable maturity was too low. The taste of several avocado varieties such as 'Hass', 'Fuerte' and 'Zutano' was acceptable when the oil content was 11.8%, 11.1% and 10.4% respectively and with a percent dry weight of 22.8%, 21% and 20.2% respectively (Lee and Young, 1983). In the same year, the California avocado industry adopted percent dry matter as the official method for the determining minimum avocado maturity which was based on the close relationship between percent oil content and percent dry matter during fruit growth and maturation (Lee and Young, 1983).

A three-year study of major California avocado cultivars was undertaken by Ranney et al. (1992). It evaluated the correlation between physiological maturity and the percent dry matter of the fruit flesh. Although there were discrepancies in different years and different locations, assignment of dry matter as a maturity index was developed for California: 'Hass', 21.6%; 'Fuerte', 19.9%; 'Zutano', 18.8%; 'Reed', 19.8%; 'Gwen', 25.9%; 'Bacon', 18.5% and 'Pinkerton', 23.0% .

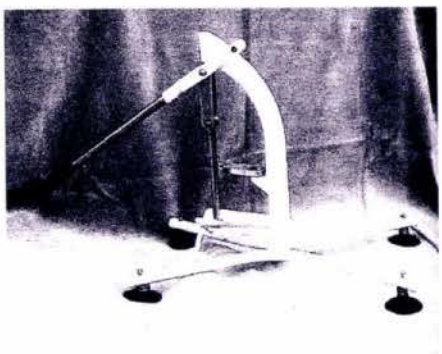
2.8.1.1 Dry Matter Determination

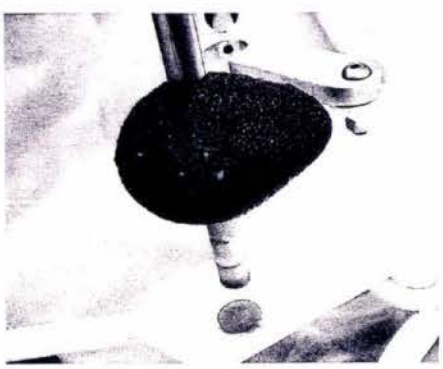
Dry matter content varies within an avocado fruit (Figure 2.9a to 2.9c) (Woolf et al., 2003).



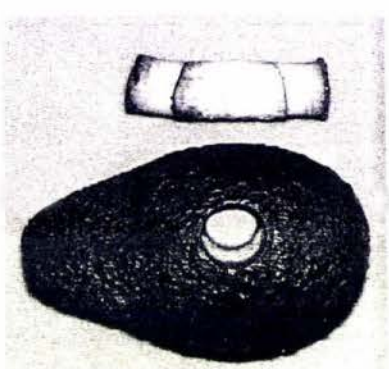
(a) Mean dry matter content in 'New Zealand 'Hass' avocado showing variation of concentration within the fruit.

Figure 2.9a. A recent development in the sample preparation for dry matter determination with the use of a Hofshi Coring Machine (Woolf et al., 2003)





(b) Sampling the core of the avocado flesh (equatorial) with a sharp metal 18.88 mm in diameter.



(c)Tissue (two plugs of pulp) about 5g were obtained using Hofshi machine. Flesh was cut and dried for dry matter determination.

Figure 2.9b to c. A recent development in the sample preparation for dry matter determination with the use of a Hofshi Coring Machine (Woolf et al., 2003)

2.8.2 Avocado Fruit Ripening

During fruit development cells divide and expand/mature before ripening (Bryant and Cumming, 1999). Ripening of fleshy fruits included physical and chemical changes such as colour, texture, aroma and flavour (Bryant and Cumming, 1999). Fruit ripening includes complicated physiological and biochemical changes which are manifested in the modification of structures at a cellular level (Platt-Aloia and Thomson, 1992).

Avocado fruit does not ripen while on the tree. It is only when they were picked that the ripening process starts (Platt-Aloia and Thomson, 1992; Cutting and Dixon, 1997). Several factors affect ripening of avocado such as storage temperature, carbon dioxide and ethylene (anti and pro ripening gases, respectively), and physiological condition of the fruit. One to 3 days after harvest ripening starts and the avocado becomes susceptible to ethylene and is edible six days after harvest (Kadam and Salunkhe, 1995; Cutting and Dixon, 1997).

Avocado is a climacteric fruit (Cutting and Dixon, 1997; Belitz and Grosch, 1999a).The respiration of avocado during expansion/maturation declines and increases again during ripening. After ripening, the respiration decreases again (Bryant and Cumming, 1999). During ripening, internal changes and softening of fruit mesocarp occur (Cutting and Dixon, 1997). Biochemical analysis of avocado during ripening revealed increasing activities of wall hydrolytic enzymes. There was a direct correlation between high activity of enzyme with the cell wall dissolution and subsequent softening (Platt-Aloia and Thomson, 1992). Nothangel (1988) showed that the principal enzyme that was responsible during avocado ripening was cellulase and demonstrated that the appearance of cellulase during ripening started at the bottom part of the fruit and then moved upwards to the peduncle. Hence, the bottom part of the fruit softens first.

Avocado ripening is accompanied by increasing fatty acid and free fatty acids contents (Kadam and Salunkhe, 1995). The vessel containing oil has a cell wall that becomes easier to rupture as the fruit ripens (Human, 1987). The wall swells and appears to be more threadlike but does not degrade totally (Platt-Aloia and Thomson, 1992). From 1 to 4 days of ripening there is an increase in water-soluble pectin and a fast decline in protopectin. The

cell softens and the cell wall is then more easily ruptured therefore the release of oil becomes easier (Human, 1987). The oil cells are generally easy to extract from the tissue by the mechanical effect of grinding which can then be separated by various methods e.g. filtration and centrifugation (Platt-Aloia and Thomson, 1992).

2.8.2.1 Ethylene in Fruit Ripening

Ethylene gas can either be natural or applied. Fruits such as avocado produce ethylene gas during ripening and respond to ethylene even at low concentration (0.1ppm). Initial ethylene production triggers and accelerates ethylene production thereby speeding up ripening. The avocado fruit is capable of producing ethylene at a rate of 100 ppm per hour. Ripe avocados stored at low temperature have been found to produce 0.1ppm to 1.8ppm ethylene. The production of ethylene and the response of avocados to ethylene production is directly proportional to temperature (Cutting and Dixon, 1997).

The effects of ethylene on avocado fruits is affected by several factors such as fruit maturity, length of time taken between harvest to ethylene treatment, concentration of ethylene, length of ethylene treatment, treatment temperature, carbon dioxide level, storage duration at ambient temperature after ethylene treatment (White et al., 1999).

2.8.2.2 Firmness

Different methods of firmness measurements have been used by researchers internationally. In several countries a Firmometer with various probes has been used. Probes includes the WFC rounded probe (Australia), Effegi probe (U.S.A), Chatillon pointed probe (Israel). Whole fruit compression method has been used in South Africa. The relationship between Firmometer and hand measurement for measuring firmness of avocado was considered to be very close. In New Zealand, use of Firmometer was increasing (White et al., 1999).

2.9 Avocado Oil

2.9.1 Extraction

The Soxhlet method uses organic solvents to extract the oil from the material due to the solubility of lipids in the solvent. Generally the basic steps involved drying of material to remove the moisture. Each extraction cycle takes about 5 to 20min and is repeated for 7 to

12 hours for maximum extraction (James, 1995; Shahidi, 2001). The most common organic solvents for oil extraction are hexane and petroleum ether, but since these solvents are not allowed in some parts of the U.S.A., ethanol, ethyl acetate and isopropanol are used as alternatives (Werman and Neeman, 1987).

The Accelerated Solvent Extraction (ASE) method is used in the extraction of organic materials from solid to semisolid sources. Application of high temperatures during extraction accelerates extraction. High pressure sustains the solvent in the fluid condition during sample extraction (Dionex, 2000). The ASE is shown in Figure 2.10.

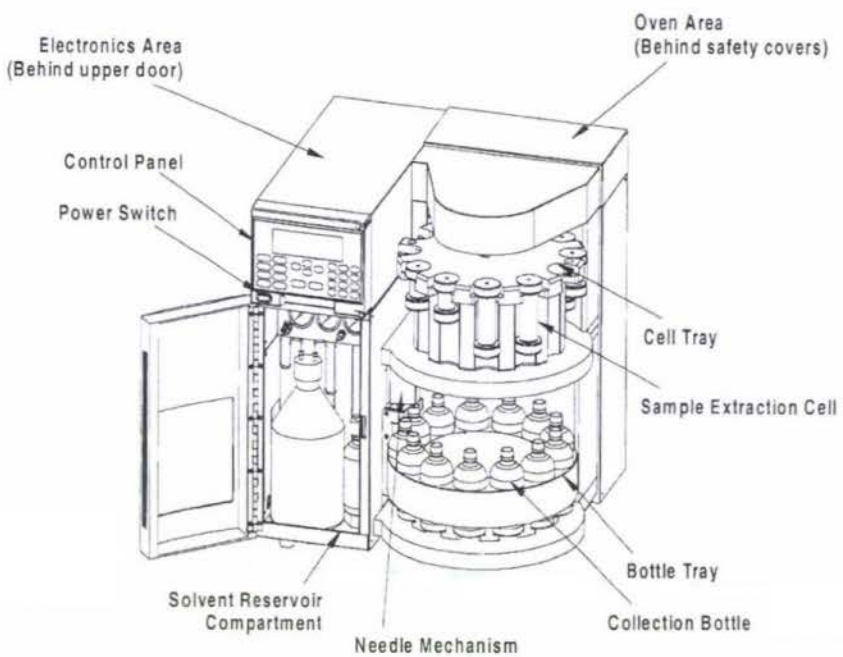


Figure 2.10.ASE 300 (Dionex, 2000)

The supercritical fluid extraction method of avocado oil was performed at supercritical conditions of 37°C/350atm, and 81°C/350atm, and, at 37°C/540atm and 81°C/540atm by Botha and McCrindle (2003). So as to eliminate any moisture the oil extracted was exposed to evaporation by vacuum for half an hour as well as carbon dioxide in solution. A steady flow rate of 4.5ml/minute was maintained. Mesocarp samples of 4g oven dried ripe avocados were used in every extraction performed.

Successful enzymatic extraction of avocado oil had been reported by Buenrostro and Lopez-Munguia (1986). The extraction process was carried out by partially hydrolysing of the oil-macromolecules complex by the catalyzing action of polygalacturonases, α-amylase and protease enzyme (Buenrostro and Lopez-Munguia, 1986).

Avocado oil production using a small expeller (Figure 2.11) requires sample preparation such as drying and slicing. The wormshaft transports the avocado pulp. There is a horizontal metal cage with a small space for the oil to pass through. The machine outlet has a choke that can be adjusted wherein the back pressure can be increased. The oil separated from the cake by expulsion. The machine resulted in oil extraction yields of 79.4-90.3% w/w of flesh (Southwell et al., 1990).

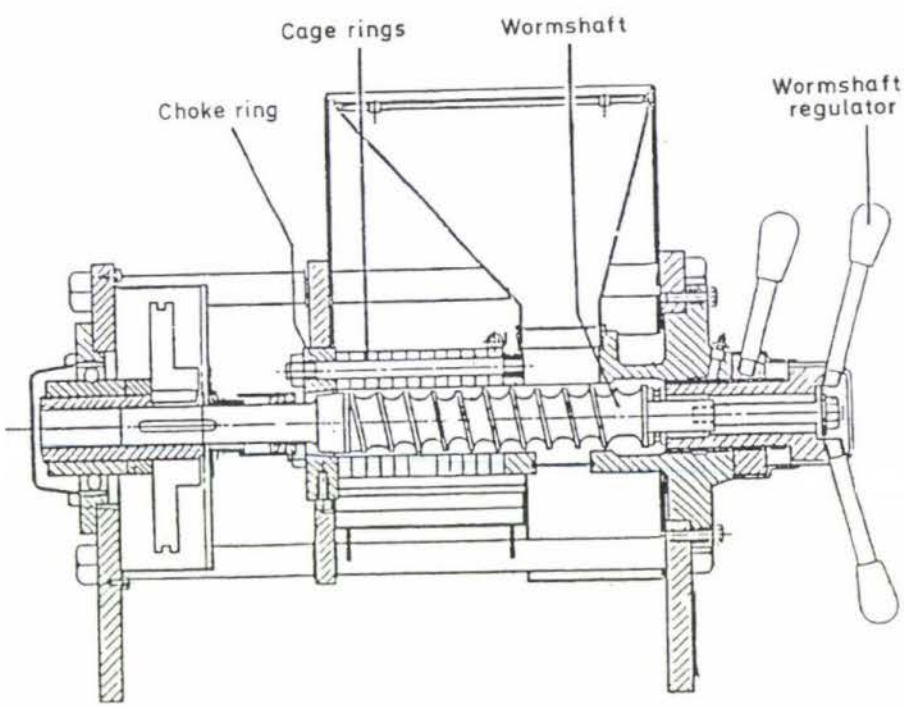


Figure 2.11.Mini-40 expeller (Southwell et al., 1990)

Aqueous extraction can be used for avocado oil extraction of small quantities e.g. 3kg up to 40kg. The oil output was about 1.8 to 11.6% w/w of flesh collected (Kameni and Tchamo, 2003). The process is simple and was applicable for home based avocado oil processing. Avocado pulp and 10% w/w Eucalyptus saw dust was mixed and pounded by mortar. The mixture was then transferred to a muslim cloth and dipped for 15 to 20 minutes in boiling

water. The mixture was placed in a hollow wooden vessel and pressure was applied with the use of a board made from wood. The oil and water extracted passed through holes in the vessel. The cake was extracted again by putting it back to a hot water and repeating the process until almost all the oil/water mixture had been extracted. The liquid phase was then allowed to separate for 12 hours. The upper layer was then transferred to a pot and boiled to allow the dried particles to settle. The upper layer (oil) was then collected (Kameni and Tchamo, 2003).

Centrifugal separation basically uses centrifugal force to separate oil from the solid mass. Avocado pulp and water is mixed, blended and pressed then stirred for about half an hour at a specific temperature, pH and salt level (Werman and Neeman, 1987). Thereafter, the oil is separated by the use of a centrifuge (6,000 x g). The optimum malaxing temperature and pH were found to be 75°C and pH 5.5 respectively. It was suggested that this temperature could deactivate enzymes and at this pH an emulsion was not formed (Werman and Neeman, 1987).

Eyres et al. (2001) described oil extraction where water was added to the fruit then the mixture was crushed by a grinder at a fast rate then transferred into the malaxers where it was slowly stirred. Thereafter, solids were separated from the oil/water phase by a decanting centrifuge. The oil/water phase was then re-centrifuged to produce separated oil and water.

2.9.2 Fatty Acids of Avocado Fruit and Oil

Thin layer chromatography of the lipid extract of isolated avocado oil cells indicated a complex lipid composition (Platt-Aloia and Thomson, 1992). The oil and the fatty acid composition of the lipids of avocado fruit differed with cultivar, stage of ripening, anatomical region of the fruit, geographic location, harvesting time and post harvest ripening period (Kadam and Salunke, 1995; Knight, 2002; Ozdemir and Topuz, 2004).

The oil content of avocado fruit grown in cool subtropical climates is high and increases with delayed harvest, reaching 25-30% w/w of fruit for 'Hass' (Knight, 2002). The major fatty acids were oleic, palmitic and linoleic (Kadam and Salunkhe, 1995; Knight, 2002).

The fatty acid identified in trace quantities were arachidic, arachidonic, myristic and stearic (Kadam and Salunkhe, 1995; Ozdemir and Topuz, 2004). Avocado seeds contained more linoleic and linolenic acids and less oleic acid compared to avocado pericarp (Kadam and Salunkhe, 1995). During the ripening process the level of palmitic acid decreased while the oleic acid content increased (Vekiari et al., 2004). The fatty acid composition is shown in Table 2.1 (Eyres et al., 2001).

Table 2.1.Fatty Acid Composition of Cold Pressed Avocado Oil Produced in New Zealand. Oleic Value for Avocado Included 5% 18:1 Isomer (Eyres et al., 2001)

Fatty acid	Formula	% of Total
Palmitic	16:0	12.5-14.0
Palmitoleic	16:1	4.0-5.0
Stearic	18:0	0.2- 0.4
Oleic	18:1	70.0-74.0
Linoleic	18:2	9.0-10.0
α-Linolenic	18:3	0.3-0.6
Arachidic	20:0	0.1
Gadoleic	20:1	0.1

2.9.2.1 Fatty Acid Analysis

Determination of fatty by gas Chromatography (GC) required conversion of fats to methyl esters by the process of methylation or direct transesterification (Li and Watkins, 2001). Three preparations of methyl esters were described which used boron trifluoride, sodium methoxide and tetramethylguanidine as catalysts (Li and Watkins, 2001).

The review of Christie (1989) on analysis of lipids and gas and chromatography was intensive. It described the saponification of lipids (hydrolysis), preparation of methyl esters of fatty acids by acid-catalyzed esterification and transesterification, base-catalyzed transesterification and diazomethane as well as gas chromatographic analysis of the fatty acid derivative which included column and other instrumental considerations, provisional identification using standards and retention time relationships.

Gas chromatographic study of the fatty acid composition was also described in James (1995) with procedures for methyl ester transesterification.

The fatty acid composition of avocado fruit before harvest and during post-harvest ripening period were determined after a solution of methyl alcohol, benzene, 2, 2-dimethoxy-propane, sulphuric acid and n-heptane were used to prepare methyl esters (Ozdemir and Topuz, 2004). The gas chromatography unit used for analysis was equipped with fused silica capillary detector (FID). The temperature of the injector port was kept at 260°C. Column temperature started from 150°C and increased incrementally by 5°C until it reached 200°C. The flow rate of helium, hydrogen and air were 1ml/min, 30ml/min, and 300ml/min, respectively.

An automated method which used the sodium methylate procedure in the determination of fatty acid methyl ester and cis/trans methyl ester composition of fats and oil had been reported (de Koning et al., 2001). After oil was weighed and added manually, the addition of reagent, shaking, sample set up and injection into the gas chromatograph were all done with the XYZ robotic auto sampler. This automated procedure was based on the Schulte and Weber method. The analysis time was shorter and the results were in agreement with the manual method (de Koning et al., 2001).

2.9.3 Free Fatty Acid (FFA)

Free fatty acid is a measure of the extent of lipolysis (Belitz and Grosch, 1999b). FFA content is a measure of oil quality and oil processing efficiency (O'Brien, 2000). Oils with FFA contents that exceed 1% are designated as crude oils and free fatty acid content lower than 1% was an indication that the quality of the oil extracted from avocado is good (Belitz and Grosch, 1999b). The presence of FFA is due to lipase activity or other hydrolytic action (Shahidi and Wanasundara, 2002).

Avocado oil extracted from fruits with physiological disorders could have a rancid odour and high free fatty acid (Human, 1987). FFA may be due to the fungal lipase which promoted lipolysis. Free fatty acid increase during oil processing is due to the hydration of oil (moisture) and the formation of free fatty acid is made faster with heat and pressure. The

conditions of fruits during harvest, storage and transportation result in an increase in free fatty acid content in crude oils. In avocado oil processing, free fatty acid content may serve as an index of the fruit quality used in the extraction and the effectiveness of the parameters used in oil extraction (% of water added, time in the malaxers and malaxing temperature). Moisture, enzymes and heat promote cleavage of the glycerol backbone of the triglycerides of fatty acid (hydrolysis) forming free fatty acids (Nawar, 1996). The trace amounts of free fatty acid had negligible effect on oil stability (Nawar, 1996).

2.9.3.1 FFA Analysis

FFA is expressed as the mg of potassium hydroxide used to neutralize acid in 1g of sample (Shahidi and Wanasundara, 2002). FFA can be measured by colorimetric, titrimetric and spectroscopy methods.

The colorimetric method involves dissolving oil in chloroform or benzene. Cupric acetate solution then reacts with FFA. The FFA-cupric ion complex which absorbs at 640 and 690 nm results in a blue colour change of the organic solvent. FTIR (Fourier Transform IR spectroscopy) is another method for FFA determination as there is a COOH (carboxyl) group positioned in the middle of the mid-IR spectrum (Shahidi and Wanasundara, 2002).

Free fatty acid in oils using the titration method is described by the AOCS Ca 5a-40 Method (AOCS, 1997). Procedure for sodium hydroxide standardization can be found in AOCS Specification H12-52 (AOCS, 1993).

A procedure is available to measure both fatty acids which are not attached to the glycerol backbone (free) and the acid value which is a reflection of total acidity (Li and Watkins, 2001).

2.9.4 Lipid Oxidation

The major steps in lipid oxidation are initiation, propagation and termination (Erickson, 2002). R and H represented a triglyceride and hydrogen molecules, respectively (Figure 2.12).

Initiation:	$In^{\cdot} + RH \rightarrow InH + R^{\cdot}$
Propagation:	$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$
	$ROO^{\cdot} + RH \rightarrow R^{\cdot} + ROOH$
Termination:	$2ROO^{\cdot} \rightarrow O_2 + ROOR$
	$ROO^{\cdot} + R^{\cdot} \rightarrow ROOR$

Figure 2.12.Mechanisms of fat oxidation (Erickson, 2002)

It has been long known that triplet oxygen is involved in oil oxidation. Rawls and van Santen (1970) reported that singlet oxygen also initiated hydroperoxide formation which is essential for free radical formation so oxidation can proceed. The rate of triplet oxidation is much slower compared to singlet oxidation.

2.9.5 Pigments in Oil

The major carotenoid pigments found in virgin olive oil are β-carotene and lutein. Their ratio in different cultivars was suggested to be related to biosynthesis and catabolism (Psomiadou and Tsimidou, 2001).

The concentration of carotenoids and chlorophylls in olives and the oil produced decreased during ripening. The degradation of chlorophyll was faster than carotenoids. Chlorophyll *b* degraded faster than chlorophyll *a* in the oil. The degradation of lutein was faster compared to that of β-carotene (Gallardo-Guerrero et al., 2002).

In Greek virgin olive oil, the main chlorophyll derivative was pheophytin *a* and trace amounts of pheophytin *b*. Chlorophyll *a* was not present but chlorophyll *b* was present in trace quantities. The wide concentration ranges in pheophytin *a* were due to cultivar and primarily the degree of ripeness (Psomiadou and Tsimidou, 2001).

The effect of chlorophyll in olive oil on the peroxide formation had been investigated by Fakaourelis et al. (1987). Different concentrations of chlorophyll 0, 2, 4mg/kg oil gave peroxide values of 4.50, 14.75 and 17.20meq/kg, respectively and the disappearance of oxygen in the headspace of samples bottles with higher concentrations of chlorophyll was higher after 70 hours of storage under light at 25°C. This indicated that chlorophyll enhanced oxidation under light. On the contrary, different concentrations of β-carotene, 0,

5, 10, 20ppm in the purified olive oil (chlorophyll free) gave corresponding peroxide values of 7.80, 5.20, 4.95 and 4.02meq/kg respectively and the retention of sample bottle headspace oxygen was higher in the samples with highest β-carotene concentrations after 84 hours of storage under light at 25°C. This suggested that lipid oxidation was minimised by β-carotene under light (Fakaourelis et al., 1987).

Avocado oil extracted from New Zealand grown 'Hass' avocado have low free fatty acid content (0.08-0.17% as oleic) and low peroxide value (0.1-0.2mEq/kg fat) (Eyres et al., 2001). Fatty acid composition present was found to be palmitic, palmitoleic, stearic, oleic, linoleic and α-linolenic. Analyses of extra virgin avocado oil showed 40-60mg/kg oil chlorophyll content (Eyres et al., 2001).

The chlorophyll content in avocado oil extracted with hexane and petroleum ether was found to be 69.2 mg/kg oil and 68.8mg/kg oil, respectively. Ethanol was the solvent that yielded the greatest chlorophyll content of 192.9mg/kg oil in a laboratory extraction (Werman and Neeman, 1987).

The stability of crude avocado oil produced by an unspecified method was determined at ambient temperature, with peroxide values serving as an indicator of oil quality (Werman and Neeman, 1986). After 31 days of storage under different conditions, an inverse relationship was observed between chlorophyll concentration and peroxide value. Chlorophyll concentration of the crude avocado oil "on the shelf" decreased from 41.36 mg/kg oil to 29.97mg/kg oil while the peroxide value increased from 5.85meq/kg to 62.75meq/kg. Chlorophyll decreased very rapidly under fluorescent light from 41.3mg/kg oil to 1.95mg/kg oil while the peroxide value increased from 5.85meq/kg to 208.34meq/kg. The crude avocado oil chlorophyll under absolute darkness decreased very slowly from 41.36ppm to 34.27ppm while the peroxide value increased from 5.85meq/kg to 7.60meq/kg. This may be due to the chlorophyll acting as an antioxidant in the dark. Chlorophyll became excited when exposed to light in the UV range. Excited chlorophyll triggered excitation of oxygen, resulting in singlet oxygen which then reacted with lipids to produce hydroperoxides. The consequent peroxy-radicals caused chlorophyll degradation (discolouration) (Werman and Neeman, 1986).

Carotenoids trap free radicals and quench singlet oxygen and these mechanisms are considered to be primary and secondary antioxidant actions respectively. At <150mmHg (low oxygen pressure) carotenoids act as free radicals scavenger (Reische et al., 2002). A resonance-stabilised carotene is formed as the result of attack by peroxy radical on the conjugated carotenoids double bond. This product stops the reaction and the peroxy radicals are diverted to lesser destructive side reactions (Reische et al., 2002). In food systems carotenoids are well known for quenching singlet oxygen. One thousand molecules of singlet oxygen can be quenched by β-carotene. Free radicals are formed by the reaction of singlet oxygen and lipids. Triplet state β-carotene is formed when singlet oxygen transferred energy (transfer of electron) to β-carotene. The triplet state β-carotene then returned to the unexcited state through the release of heat energy (Figure 2.13) (Reische et al., 2002).

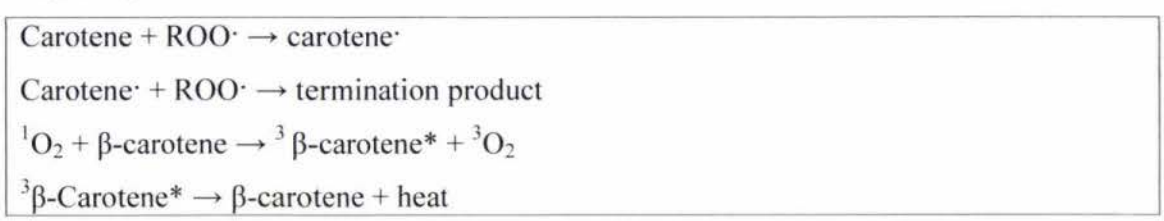


Figure 2.13.Carotenoids mechanism of trapping free radicals and quenching singlet oxygen (Reische et al., 2002).

The mechanism of photooxidation of soybean oil was studied by Lee and Min (1988). The results showed that β-carotene quenched singlet oxygen reducing soybean oil oxidation. However, β-carotene had no quenching effect in oil containing chlorophyll.

The inhibitory effects of chlorophyll and pheophytin on oxidation in the dark (oven test) were investigated using methyl linoleate (ML) and/or triglycerides as substrate were compared (Endo et al., 1985). Both chlorophyll and pheophytin inhibited oxidation in ML. In triglycerides substrate, chlorophyll slowed down the oxidation of rapeseed and soybean oils at 30°C. Chlorophyll *a* offered more protection against oxidation. At the concentration of 2.2×10^{-7}mol/g ML, all chlorophylls acted as antioxidants (Endo et al., 1985).

Photosensitizer-free rapeseed and soybean oil stored in the dark showed that chlorophyll *a* and *b* prevented the oxidation for both oils similar to that of t-butyl hydroxy toluene (BHT), a popular synthetic antioxidant. Pheophytin *a* and *b* did not reduce autooxidation of the same oil at a concentration of 2.2 x 10^{-8}mol/g oil. The rate of oxidation of rapeseed and soybean oils was inhibited by chlorophyll *a* and *b* by one quarter and one half, respectively (Endo et al., 1985).

It was found that oxidation was fast under light storage due to the avocado oil high chlorophyll pigment content. It was considered that the oxidation was catalyzed by light and followed the path of singlet oxygen (Eyres et al., 2001). Conversely, refined avocado oil (light yellow) was found to be more stable (Eyres et al., 2001).

2.9.5.1 Storage Trial (Oven Test)

A procedure to determine oil stability is described in AOCS Recommended Practice Cg 5-97 (AOCS, 1997). It is applicable also to edible fats. Oil is stored at high temperature 60°C over time and tested to measure the degree of oxidation by determining the peroxide value, measurement of volatiles by Gas Chromatography (GC).

2.9.5.2 Peroxide Value (PV)/Quantity of Peroxide Oxygen

The PV is determined by titrimetric and spectrophotometric methods. The PV is a measure of the hydroperoxide content. In the iodometric method, the concentration of the liberated iodine (I_2) on the reduction of hydroperoxide group (ROOH) with iodide (I^-) ion was proportional to the amount of peroxide. The standardized solution of sodium thiosulfate ($Na_2S_2O_3$) was used to titrate the sample to assess released I_2 by the use of a starch indicator (Figure 2.14) (Pegg, 2001; Shahidi and Wanasundara, 2002).

$$ROOH + 2H + 2KI \rightarrow I_2 + ROH + H_2O + 2K^+$$

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\,NaI$$

Figure 2.14.Peroxide formation (Shahidi and Wanasundara, 2002)

The ability of peroxides with iron (II) to be oxidized to iron (III) was the basis of the spectrophotometric method. Ferric ion and xylenol orange formed a complex detectable by spectrophotometer. This method was sensitive and could be used in low peroxide

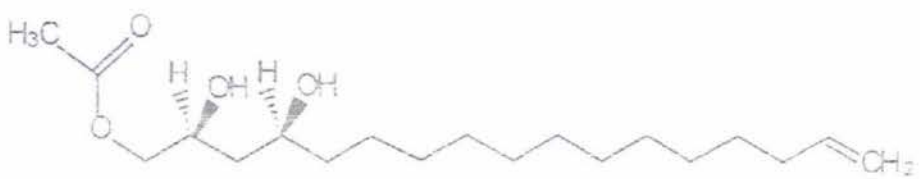
concentrations (0.1meq active oxygen/kg oil; Shahidi and Wanasundara, 2002). AOCS Official Method Cd 8-53 (AOCS, 1997) and AOAC Official Method 965.33 (AOAC, 2000) described PV determination by titration method using acetic-acid chloroform method and AOCS Official Method Cd 8b-90 (AOCS, 1997) using acetic acid-isooctane.

2.10 Antifungal Compounds (Yne, Trienes, Dienes and Monoenes)

There were four antifungal compounds isolated from avocado fruit. The first two compounds are Z, Z-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12, 15 diene ("persin") with a molecular formula of $C_{23}H_{38}O_4$ which are present in unripe avocado fruit pericarp (Prusky et al., 1982) and 1-acetoxy-2, 4-dihydroxy-n-heptadeca-16-ene with a molecular formula of $C_{19}H_{36}O_4$ was isolated by Kobiler et al. (1993). The other two compounds are 1 acetoxy-2, -dihydroxy-n-heptadeca-16-yne and Z, Z, E-1-acetoxy-2-hydroxy-4-oxo-heneicosa-5, 12, 15-triene with a molecular formula $C_{19}H_{34}O_4$ and $C_{23}H_{38}O_4$ respectively were isolated from the idioblast cell of avocado fruit. These four compounds were sequestered in high concentrations in the oil cells of avocado flesh (Figure 2.15; Domergue et al., 2000).

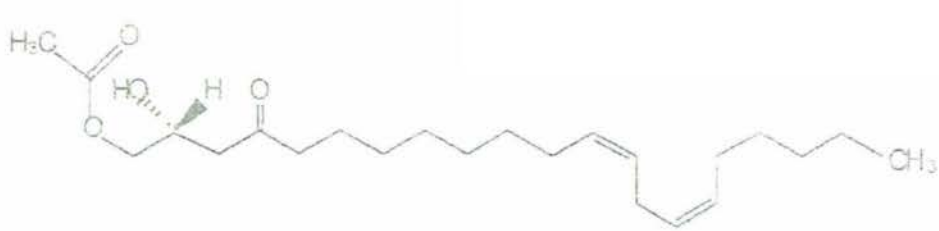
Antifungal compounds are known to inhibit *Colletotrichum gloeosporioides* spore formations, the most effective is "persin" (Leiken-Frenkel and Prusky, 1998; Domergue et al., 2000) followed by the triene compound. They have similar activity at higher concentration (Domergue et al., 2000). Antifungal compounds present in avocado pericarp (peel) are free to resist fungal pathogens (Kobiler et al., 1993). During ripening antifungal compound concentration in avocado pericarp decreases (Prusky et al., 1991). *Colletotrichum gloeosporioides* started to germinate and penetrated avocado fruit through the skin during development until harvest. During fruit ripening, the fungus was activated as evidenced by physiological disorders (rotten fruits) (Leiken-Frenkel and Prusky, 1998). Lipoxygenase enzyme catalyzed degradation of antifungal diene resulted in a decrease in pathogen resistance. Epicatechin (a non-specific inhibitor) in the peel which was believed to regulate lipoxygenase enzyme also decreased during ripening (Leiken-Frenkel and Prusky, 1998).

1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-yne, 1



1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-ene, 2

(*Z, Z, E*) -1-acetoxy-2-hydroxy-4-oxo-henelcosa-5,12,15-triene, 3



(*Z, Z*) -1-acetoxy-2-hydroxy-4-oxo-henelcosa-12,15-diene, 4

Figure 2.15.Chemical structure of antifungal compounds isolated in avocado oil idioblast cells (Domergue et al., 2000)

The levels and pattern of antifungal compounds are affected by ethylene gas and carbon dioxide (Prusky et al, 1991; Leiken-Frenkel and Prusky, 1998; Ardi et al., 1998; Domergue et al., 2000). The effect is dependent on the ethylene concentration and temperature. 'Fuerte' avocado that have been exposed for 3 hours to 40µl/l ethylene were found to enhance 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12, 15 diene only in the fruit peel of the whole avocado exposed. When 1-2mm slices of peel and flesh were exposed to ethylene, only the antifungal diene in the mesocarp was enhanced (Leiken-Frenkel and Prusky, 1998). It was also found that antifungal diene in the idioblast was enhanced by ethylene. Fifty percent increase of antifungal diene after three hours of the idioblast exposure to ethylene was observed. The increase was directly proportional to temperature. There was a suggestion that antifungal diene that had been synthesized in idioblasts was exported to the peel of the fruits (Leiken-Frenkel and Prusky, 1998).

CO_2 treatment was found to enhance antifungal compounds of fruits exposed for 24 hours to 30% CO_2 (Prusky et al., 1991). Exposure of avocado fruits to ethylene or CO_2 both enhanced concentrations of antifungal diene in the peel but the rate of increase is different (Ardi et al., 1998).

Recently, Woolf et al. (2000) found that exposure of avocado fruits to sun affected the antifungal diene pattern during storage. The antifungal diene level in the pulp of sun fruit is always higher compared to shade fruit. In the skin, the diene level after one week is decreasing at the same rate. Thereafter, sun fruit diene level rose and the concentration was 50% more than the shade fruit.

Isolations of idioblast cells and antifungal compounds in the idioblast cells was described in Platt-Aloia and Thomson (1992) and Domergue et al. (2000).

There are reports of toxicity of 'Hass' leaves in animals (Carman and Handley, 1999) and avocado oil causing liver metabolism alteration in rat (Werman et al., 1989; Werman et al., 1991). Whether the level of antifungal diene present in the avocado fruit and oil is harmful or affects the metabolism of humans is unknown.

2.11 Conclusion

The importance of pigments in the fresh fruit and the product derived from it have highlighted that a thorough investigation of the pigment composition of avocado fruit and the extracted oil is warranted.

Chapter 3. Materials and Methods

3.1 Experimental Overviews

In carrying out the experiment for the pigment compositional analysis of the skin and flesh tissues of 'Hass' avocado and the extracted oil, post harvest fruit quality such as dry matter, colour and firmness were assessed. The pigment compositions of fresh 'Hass' avocado sections were analysed by HPLC. Carotenoids and chlorophylls in the pulp and anthocyanin in the skin were analysed by HPLC. The oil from the different sections of Hass' avocado was extracted using Accelerated Solvent Extraction (ASE). Carotenoid and chlorophyll pigments composition of the extracted oil were also analysed using HPLC. Likewise, in carrying out the experiment for avocado oil with skin addition, colour and firmness of avocado fruit to be extracted were tested. Oil with added skin was extracted by cold pressed method in the laboratory and in the factory. The extracted oil with different levels of skin were analysed for carotenoid and chlorophyll pigment composition using HPLC and spectrophotometer.

The concentrations of standards for HPLC analysis were measured by spectrophotometer and calculated using published extinction coefficients (Jeffrey, 1997; Giusti and Wrolstad, 2001).

3.2 Materials

The materials, chemicals (all analytical grade), brand and manufacturers were as follows:

- Ammonium acetate and butylated hydroxyl toluene (BHT), sodium carbonate, sodium hydroxide, sodium sulfate anhydride (BDH Laboratory Supplies Poole, England)
- Acetone, acetonitrile, diethyl ether, ethanol, glacial acetic acid, hexane, methanol, phosphoric acid, potassium hydroxide and sulphuric acid (Merck, Darmstadt, Germany)
- Dichloromethane, potassium iodide, sodium thiosulphate, wheat unmodified starch (Sigma-Aldrich Chemie, Steinheim, Germany)
- Ammonium chloride, ethyl acetate, phenolphthalein indicator, potassium hydrogen phthalate, sodium hydroxide (Scharlau Chemie S.A., Barcelona, Spain)

- Magnesium sulphate dried (May & Baker Ltd, Dagenham, England), Isooctane, (Ajax Fine Chemicals, Sydney, Australia)
- Solid Phase Extraction (SPE) cartridge, Alltech Extract clean C18 500mg/8.0ml PN 205350, Illinois U.S.A.
- Lutein, β-carotene, chlorophyll *a* and chlorophyll *b* standard (Sigma, Sydney, Australia)
- Cyanidin 3-*O*-glucoside anthocyanin standard (Extrasynthese, Genay, France)

3.2.1 Avocado Fruit

The 'Hass' avocados (*Persea americana*) were harvested from a Whangarei, New Zealand orchard on September (spring) and transported to HortResearch, Mt Albert Research Centre Auckland, New Zealand for processing. On arrival two days postharvest, fruit were randomised and graded for uniformity/quality and were tested for dry matter, skin colour and firmness then sampled.

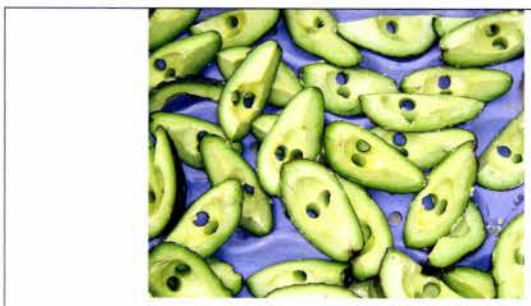
The remaining avocados were placed in trays for storage and treated with ethylene to accelerate ripening and reduce variability. Ethylene treatment of 100µl l^{-1} was carried out at 20°C ± 2°C for 2 days in 360 litre tubs and lime in bags were included to minimise CO_2 accumulation. After removal from ethylene the fruit was held for 13 days at 20°C ± 2°C.

Four trays were withdrawn 3, 4, 6, 8, 9 and 13 days postharvest. Three replicates (15 fruit per replicate) were tested as whole fruit for firmness and skin colour before skin and pulp colour measurement and sampling.

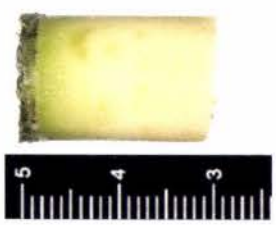
The dry matter was determined using the method described by Woolf et al. (2003). The mean dry matter content of the avocado fruit was found to be 35.2% ± 0.3%.

3.2.2 Tissue Sampling

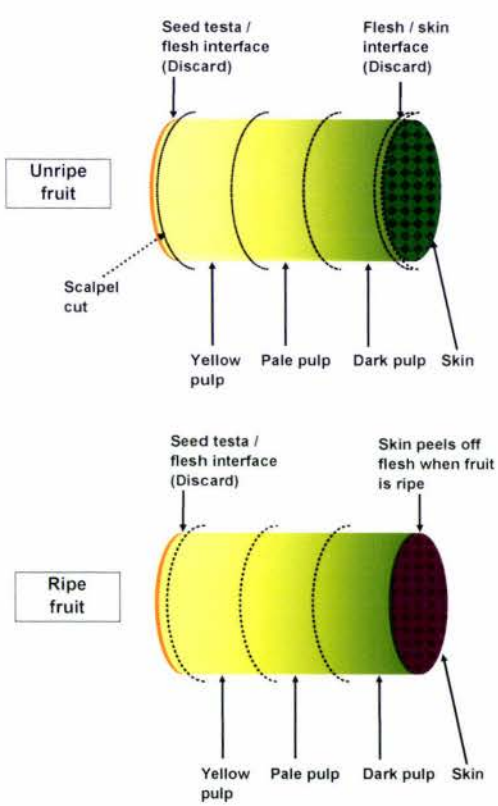
There were two methods employed for obtaining fruit plugs from which skin and pulp samples were taken. The method used for unripe fruit sampling varied from that for the ripe fruit as this method would have crushed the pulp of ripe fruit and hence sampling of the different sections of pulp would have become unreliable. Avocado tissue sampling is shown in Figure 3.1a to c.



(a) Ripe avocado quarter



(b) Ripe avocado plug



(c) Cutting of different sections

Figure 3.1a to c. Avocado tissue sampling

Whole unripe avocados (2-4 days) were plugged through the equator using a cork borer (7mm diameter). Six plugs were taken per fruit. In avocados which were considered to be ripe (6 to 13 days) fruit were cut twice at right angle longitudinally producing four segments/quarters, and the stone was discarded. Each segment was sampled twice (equatorial) using a 7mm diameter cork borer working from the inside through to the skin (Figure 3.1a). This gave 8 plugs per fruit (Figure 3.1b).

Each plug of fruit was divided into four sections (Figure 3.1c). The skin, and the three sections of flesh namely dark pulp, pale pulp and yellow respectively. The testa and stone were discarded. Each section from the 15 fruit was pooled together. The pooled samples of avocado skin and the three pulp sections were placed separately in glass scintillation vials. The samples were flash frozen immediately with liquid nitrogen and stored at -80°C until analysed.

3.3 Post Harvest Assessments

3.3.1. Dry Matter Determination

Fifteen fruit (3 replicates of 5 fruits each) were plugged twice at right angles through the equator of the fruit by a sharp 18.88mm diameter metal tube of the Hofshi Coring Machine producing two cores of tissue (4 plugs of pulp). The skin and stone were removed and the flesh then sliced and dried at 65°C in an oven (Contherm Digital Series Oven). The weight before and after 48 hours of drying or until constant weight, was recorded to four decimal places. The percent dry matter was then calculated (Woolf et al., 2003).

3.3.2 Colour Measurement

A chroma meter (Minolta Co., Ltd., 3-13, 2-Chome, Azuchi-Machi, Chuo-Ku, Osaka, Japan) was used for testing the colour of the whole fruit at three points on the equator.

Both sides of twenty cylindrical discs of each section were measured for colour. The average reading of both sides was taken as the colour for each section.

3.3.3 Firmness Measurement

The firmness of fruit was measured using an Anderson digital Firmometer (Anderson Manufacturing, Tauranga, New Zealand). The firmness measurement of the fruit was carried out using a 200g weight as outlined by White et al. (2005).

The Firmometer reading (mm displacement) multiplied by 10 is the Firmometer value (FV). The FV is directly proportional to the softening of fruits (White et al., 2005). Note that as the fruit softens FV increases.

3.4 Pigment Compositional Analysis of 'Hass' Avocado Fresh Tissue

The skin and the three pulp sections were analysed for chlorophylls and carotenoids over a period of 13 days following harvesting. Anthocyanins were analysed in the skin over the same period.

3.4.1 Carotenoids and Chlorophyll Analysis (HPLC)

The method for chlorophylls and carotenoids in kiwifruit samples by Cano (1991) with some modifications was used in the extraction of carotenoids and chlorophylls in avocado tissue and in the HPLC determination.

3.4.1.1 Sample Preparation/Extraction

The equipment used was a Labofuge GL, Heraeus Christ, Germany centrifuge, HPLC (Waters 2690 Separation Module, Milford, MA, USA) and an Ultra Turrax blender (Jancke and Kundel, Munich, Germany). Extraction and sample preparation were all done under safe (yellow) lights.

Defrosted tissue was cut into small pieces. About 1g was weighed accurately on a four decimal place balance in a 10ml screw-capped test tube. Five ml of 0.1% BHT in acetone and 100mg $NaCO_3$ were added to the samples. The mixture was blended using an Ultra Turrax homogenizer until the tissue was broken down, then 500mg Na_2SO_4 was added and the test tube was flushed with N_2 and stored in the refrigerator at 4°C±0.2°C to extract overnight. Samples were warmed to room temperature the following morning.

Two ml of each extract were transferred to a labelled 10ml screw-capped test tube. Two ml of diethyl ether and 8ml of 10% NaCl were added to the extract and mixed by shaking. The solution was centrifuged for 10 minutes at 3000rpm (2900g). The upper layer was transferred to another labelled screw-capped test tube. Extraction was repeated by adding 2ml of diethyl ether to the remaining aqueous layer. The solution was centrifuged for 10 minutes at 3000rpm and the upper layer was transferred to the labelled tube containing the first diethyl ether extract. The diethyl ether extract was evaporated to dryness using a gentle flow of N_2 at 30°C. The residue was re-dissolved in 0.8ml of 0.1% BHT/acetone, and was vigorously mixed using a vortex and centrifuged for 20 minutes at 3000rpm. The supernatant was transferred to an auto sampler vial and then analysed by HPLC.

3.4.1.2 HPLC Determination

The HPLC (Waters, Model 2690) with Photodiode Array (PDA) (Model 996) and fluorescence detection (Model 474) and a Waters Spherisorb S5 ODS2 4.0 x 250mm column were used. To measure carotenoids, chromatograms were detected at 455nm and the identified carotenoid components quantified as lutein equivalents. Chlorophylls were measured by fluorescence (excitation 440nm, emission 460nm) and quantified as chlorophyll *b* equivalents.

All chromatographic data was processed on a Waters Millennium Chromatography Manager (V4.0) data system.

Table 3.1.Solvent Programme for HPLC Determination of Carotenoids and Chlorophyll

Time	%A	%B	%C
Start	100	0	0
4min	0	100	0
18min	0	20	80
22min	0	20	80
25min	0	100	0
29min	100	0	0
40min	100	0	0

A ternary solvent system A (20% 0.5M ammonium acetate, 80% methanol), B (10% H_2O, 90% acetonitrile), and C (ethyl acetate) were used. Percentages of each solvent supply were programmed with time intervals as shown in Table 3.1. A flow rate of 1ml/min and pressure of 2650psi were used. The injection volume was 10µl. The sample chamber was set at 6°C and the column was in a heated chamber set at 35°C.

3.4.2 Anthocyanins Determination (HPLC)

3.4.2.1 Sample Preparation/Extraction

The method of sample preparation described by Cox et al. (2004) with some modifications was used. About 1g of avocado skin tissue was weighed into a 10ml screw capped test tube. Eight ml of ethanol/H_20/acetic acid (80/20/1) was added then blended with an Ultra Turrax blender (18mm shaft) until the tissues were broken down. The bottle was capped tightly and stored in a refrigerator at 4°C±0.2C for 48 hours. The supernatant was transferred to another 10 ml screw capped test tube and centrifuged for 20 minutes at 3000rpm (2900g). The clear supernatant was transferred to an auto sampler vial and analysed by HPLC.

3.4.2.2. HPLC Determination

Extracts were separated and analysed similar to that described by Cox et al. (2004) but using different HPLC model (Waters, Model 2690), equipped with PDA (Model 996) and a Merck LiChrospher 100, RP18e 5µm, 250 mm column.

The mobile phase was a binary solvent system consisting of A (1.5% phosphoric acid) and B (acetic acid/acetonitrile/phosphoric acid/H_2O (20:24:1.5:54.5). Percentages of each solvent supply are shown in Table 3.2. A flow rate of 1ml/min and pressure of 2650psi was used. The injection volume was 10µl. The sample chamber was set at 6°C and the column was in a heated chamber set at 35°C.

To measure anthocyanin concentrations, chromatograms were measured at 530nm and quantified with using cyanidin 3-*O*-galactoside as a reference. All chromatographic data was processed on a Waters Millennium Chromatography Manager (V4.0) data system.

Table 3.2.Solvent Programme for HPLC Determination of Anthocyanin

Time	%A	%B
Start	80	20
25min	30	70
30min	10	90
35min	10	90
40min	80	20

3.5 Pigment Compositional Analysis of Oil Extracted from 'Hass' Avocado Sections

3.5.1 Solvent Extraction of Avocado Oil (ASE)

3.5.1.1 Freeze Drying

Pooled sections were flash frozen with liquid nitrogen and sample placed at -25°C until they were freeze dried (typically 48 hours at -80°C). The freeze dried samples were flushed with N_2 and sealed in a laminated foil metallized bags (81 micron, Caspak, Auckland, New Zealand) with an oxygen absorber sachet (Ageless®, FX-20E, Mitsubishi Gas Chemical Company Inc. Tokyo, Japan). The laminated foil metallized bag was again sealed manually using a heat sealer. Prior to solvent extraction, samples were ground to a powder using a Breville coffee 'n spice grinder.

3.5.1.2 Accelerated Solvent Extractor (ASE)

Four temperatures were tested (40, 45, 50 and 60°C) for the extraction of oil and pigments. The temperature which gave the highest total chlorophyll concentration determined by spectrophotometer AOCS Ch 4-91 method (AOCS, 1997) was 60°C (31.2mg/kg oil).

After grinding, the samples were weighed, mixed with diatomaceous earth. The sample was loaded in Accelerated Solvent Extractor (ASE 300, Dionex Corporation, U.S.A) as per the manufacturer instructions:

- A method for each sample extraction which consisted of a set of operating parameters was created (Table 3.3).
- The solvent reservoir was filled with the solvent (hexane).
- The disposable filter was inserted into the cell with the aid of the insertion tool after the bottom cell cap was hand-tighten.

- The aluminum funnel was used to place the sample inside the cell and the top cap was hand screwed and tightened. The extraction cells with samples were hung in a vertical position in the tray slots, the rinse bottle was loaded in the slot labelled R1. It was ensured that the cap with the cellulose filter was at the bottom.

Table 3.3.ASE Operating Conditions Used for Solvent Extraction of Avocado Oil

PARAMETERS	VALUE RANGE
Heat	5 minutes
Temperature	60°C
Static solvent extraction time	20 minutes
Flush volume (%)	70
Purge (Flushing)	90 seconds
Cycles	5
Solvent	Hexane (100%)
Pressure	1500psi

The cell and bottle started to rotate when the oven reached the desired temperature.

3.5.1.3. Oil Drying/Storage

After oil extraction, the solvent was removed from the oil for 2 hours at 30°C at a speed of 65rpm using a rotary evaporator (RapidVap Vacuum, N_2 and N_2/48 Evaporation Systems, Labconco Corp. Kansas City, MO) under N_2. The % oil was expressed as % oil of dry weight avocado tissue. Oil samples extracted were stored in 21ml dark amber glass bottles, flushed with N_2, sealed and stored at -80°C until analysis.

3.5.2 Carotenoid and Chlorophyll Analysis of Avocado Oil (HPLC)

3.5.2.1 Sample Preparation/Extraction

Solid phase extraction (SPE) was used to isolate lutein and chlorophylls from oil followed by HPLC to quantify the pigment concentrations. Avocado oil (approx 0.8g) was diluted with 4 ml hexane (HPLC grade) containing 1% BHT and applied to a SPE cartridge that had previously been conditioned with 8ml acetone followed with 8ml hexane. The SPE cartridge was then washed with 4ml hexane and the lutein and chlorophyll eluted with 4ml acetone. The total volume of the extract was adjusted to 5.0ml with acetone containing 0.8% BHT.

3.5.2.2 HPLC Determination:

The analytical column used was Waters Spherisorb ODS2 5μm 4.6mm i.d. x 250mm and a ternary solvent gradient system described by Wright et al. (1991) was used. Refer to section 3.4.1.2 for detailed methodology.

3.6 Cold Pressed Laboratory Scale Oil Extraction Process

3.6.1 Calculations

Skin (0%, 5%, 10%, 20%, 40%, 70% and 100%) was added to the flesh during the malaxing stage of cold pressed avocado oil extraction. For each 100g of avocado, the average distribution of stone, skin and flesh determined from the selection of 45 avocado fruit have been established as 18%, 14% and 68% respectively. The level of skin in the malaxer varied between 0-100%, where 100% skin was equal to 18g/100g (skin + flesh).

Table 3.4.Skin and Flesh Proportion for Cold Pressed Extraction Laboratory Scale of Avocado Oil

Skin percentage of avocado (%)	Skin (g/1000g)	Flesh (g/1000g)
0	0	1000
5	9	991
10	18	982
20	36	964
40	72	928
70	126	874
100	181	819

3.6.2 Avocado Fruit

On the day after harvest, 21 January 2005, 11 trays each containing 20 avocados from Whangarei were delivered to MARC. The fruit were immediately randomised and ethylene treatment of 100µl l^{-1} was carried out at 8.8°C for 2 days in 360 litre tubs and lime in bags to minimise CO_2 accumulation. The trays were stored at 20°C ± 2°C for 72 hours. After removal from ethylene the fruit was held for 3 days at 20°C ± 2°C and the fruit were placed in a refrigerated cabinet at 5.5°C ± 0.2 °C until cold press extraction.

A total of 70 fruit each day were cold pressed over 3 successive days. Seventy avocado fruit each day were cold pressed on three successive days (10 fruit per trial). Each group was assigned a percentage by weight of skin to flesh, namely 0%, 5%, 10%, 20%, 40%, 70% and 100%.

3.6.3 Oil Extraction

The avocado fruit were cut into quarters. Skin and pulp were separated by hand peeling (Figure 3.2a and Figure 3.2b). The skins were cut into small pieces before grinding (Figure 3.2c). A spatula was used to push the pulp and the skin into the hammer mill grinder (Tech Services, HortResearch, Auckland, New Zealand) (Figure 3.2d). The ground pulp and skin was collected in a plastic container (Figure 3.2e). The mixture of pulp and skin (1000g) was weighed in a plastic beaker (Figure 3.2f) and 300g of distilled water was added achieving a pulp/water ratio of 10:3 (Sherpa, 2000). The mixture was mixed and poured into the malaxer and was stirred with the overhead mixers at a speed of 90rpm for 60 minutes (Figure 3.2g). The malaxers were maintained at 45-48°C by circulating water through a jacketed stainless steel vessel. After malaxing, the mixture was weighed again and distilled water was added to the pulp in a 1:1 ratio. The mixture was then poured equally into six (500ml) plastic centrifuge bottles (Figure 3.2h) and centrifuged for 20 minutes at 8600rpm (12500g) (Sorval Dupont centrifuge, Model RC 5C, Rotor GS-3, Kendro Quality Products –Lifetime Care, Wilmington, Delaware, 19898 U.S.A) (Figure 3.2i) at 40°C.

The oil layer (Figure 3.2j) was transferred to a 50ml clear centrifuge tube using a 5ml pipetter and re-centrifuged for 10 minutes 5000rpm (2720g) at 20°C, (Model MR 1822, Jouan, Z.I. de Brais, BP 403, 44 611 Saint-Nazaire Cedex, France) (Figure 3.2k). The oil layer (Figure 3.2l) was removed and was stored in 21ml brown bottles, flushed with N_2 and stored in a freezer at -80°C until analysis.

(a)Avocado cutting and peeling

(b)Avocado skin quarters

(c) Avocado skin pieces

(d)Grinding

(e) Ground skin

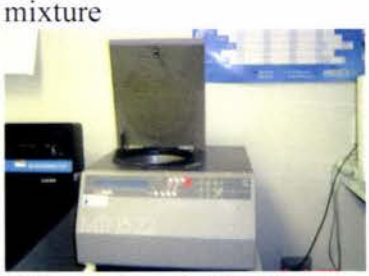
(f)Ground pulp and skin mixture

(g) Malaxing

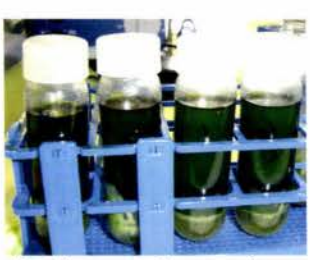
(h)Equally subdividing the mixture

(i)First centrifugation

(j)Separation-oil was separated from pulp after centrifugation



(k)Second centrifugation



(l)Oil green layer after second centrifugation

Figure 3.2 a to l. Avocado oil cold press laboratory scale process

3.7 Commercial/Factory Extraction

3.7.1. Experimental Overview

Three formulations of avocado oil were produced in the factory. Percent free fatty acid (% FFA as oleic acid), fatty acid composition, peroxide value (PV), total chlorophyll, colour and diene content were determined on the oil collected at day 0.

The three oil formulations were stored at different temperatures (-20°C to 60°C) for 97 days and were analysed at regular intervals for PV and colour. Carotenoids and chlorophyll composition were analysed using HPLC. The total chlorophyll concentration was analysed using a spectrophotometer.

3.7.2 Oil Formulations/Identifications (100%, 40% and 5% skin)

Nine bins of fruit were used to produce three formulations of commercially extracted oils. All of the skin from the destoner was collected and the weight was recorded. All of the skin collected was added during the malaxing stage to produce the oil with 100% skin. The total weight of the skin collected during the production of the oil with 100% skin was the basis for calculating the amount of skin that was to be added to produce the oil with 40% of skin (actual, 43%). During the removal of the stones a quantity of skin found its way into the pulp before malaxing. Previously, it was estimated that this was 5% of the skin. However, the sample taken during factory trial was found to contain 11% of skin. Skin was not added manually for the third formulation. The amount of skin included in the malaxer for each formulation is shown in Table 3.5.

Table 3.5.Calculation for Different Levels of Skin for Cold Pressed Extraction Factory Scale

Oil Formulations (Identifications)	Weight of fruit	Skin added (kg)	Actual percentage of skin
100%	789kg	54kg	100%
40%	764kg	22kg	43%
5%	775kg	None	11%

The three commercial production runs were completed and the resulting oil was collected and sparged with nitrogen. The process flow diagram for avocado oil production is shown in Figure 3.3.

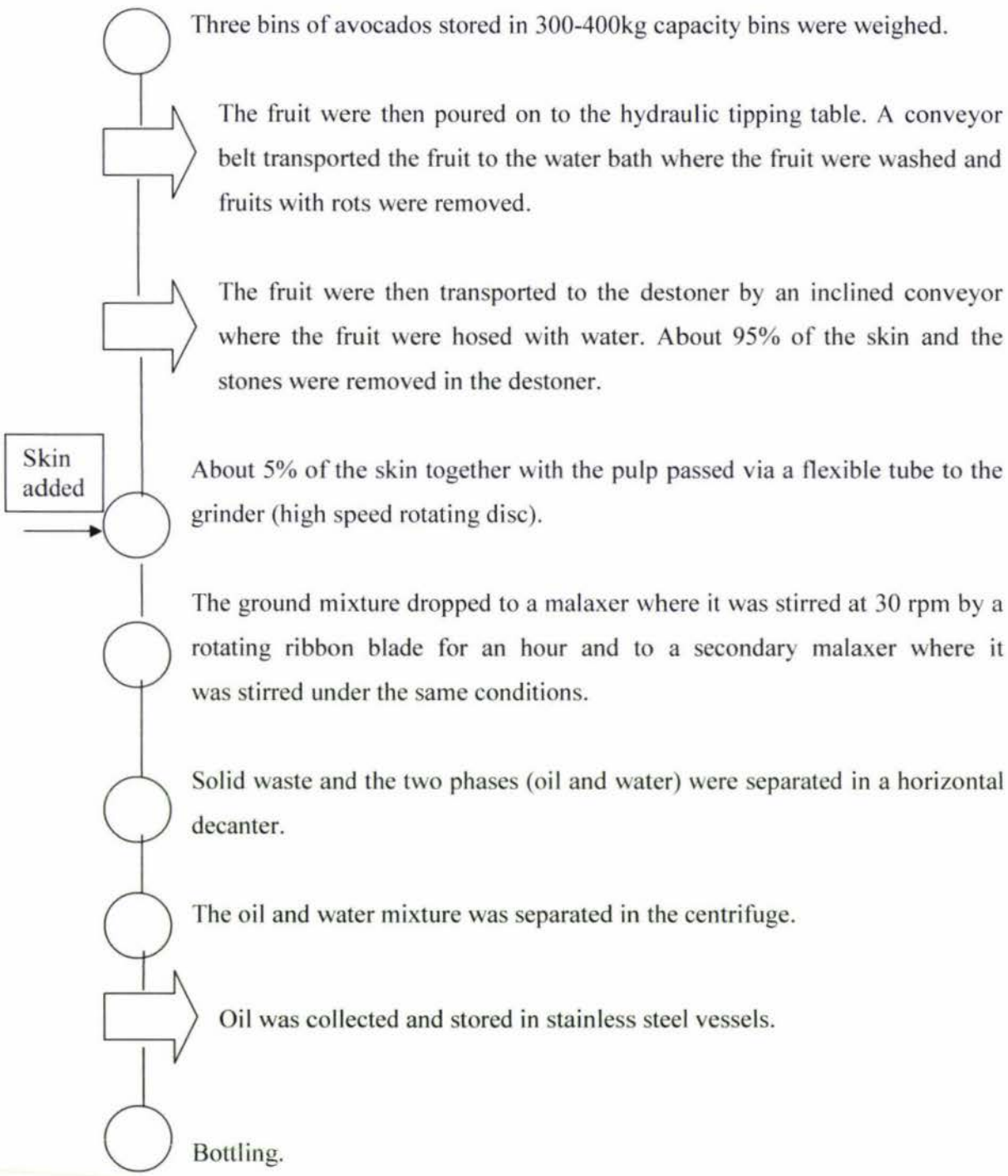


Figure 3.3.Process flow diagram of commercial avocado oil processing (Sherpa, 2002)

3.7.3 Free Fatty Acid (FFA)

FFA value is often express as the % of dominant fatty acid (usually as oleic acid with a molecular weight of 282) present in the source of oil (Karl, 2001).

3.7.3.1 Reagents

0.5M NaOH: About 2g of NaOH was weighed and dissolved in a litre volumetric flask.

1% phenolphthalein indicator: 1g phenolpthalein was dissolved in 100ml 95% ethanol.

Standardisation of NaOH was carried out using AOCS Specification H12-52 (AOCS, 1997). About 0.2g potassium hydrogen phthalate (KHP) that had been previously dried in the oven for 2 hours and cooled was weighed and dissolved using 25-30ml of distilled water in a 100ml conical flask on a heating plate. Several drops of 1% phenolphthalein indicator were added then titrated with 0.5M NaOH. The exact molarity of sodium hydroxide was calculated by the following formulae:

$$\text{Molarity of NaOH} = \frac{\text{Weight of KHP in g} \times 1000}{\text{Molecular weight of KHP} \times \text{V NaOH (ml)}}$$

$$= \frac{\text{Weight of KHP in g} \times 1000}{204.22 \times \text{V NaOH (ml)}}$$

3.7.3.2 FFA Determination

AOCS Official Method Ca 5a-40 (AOCS, 1997) was used to determine FFA. A sample of oil of about 10 to 15g was weighed into an Erlenmeyer flask. Previously neutralised alcohol was added followed by several drops of phenolphthalein indicator. The mixture was heated to boiling and titrated immediately with 0.5M NaOH to a faint pink colour. The volume of NaOH was read when the faint pink colour persisted for 30 seconds.

The percent FFA was calculated using the formula below:

$$\%\,\text{FFA as Oleic acid} = \frac{\text{Volume of 0.5 M NaOH (ml)} \times \text{Molarity of NaOH} \times 282}{\text{Sample weight (g)} \times 10}$$

3.7.4 Storage Trial of Avocado Oil

Using dark bottles, avocado oil for the three levels of skin content extracted by cold pressed extraction in the factory were stored for up to three months at -20°C, 4°C, 40°C, 50°C, and 60°C in darkness. Oil was also stored at 20°C under 400 lux light conditions. Bottles were withdrawn at regular intervals and analysed for colour, pigment level, peroxide value (PV) as shown in Table 3.6.

Table 3.6.Avocado Oil Testing Intervals of Three Oil Formulations

Testing	Time (Day)	Temperatures tested
2	6	20°C light and dark, 40°C, 50°C and 60°C
3	13	20°C light and dark, 40°C, 50°C and 60°C
4	20	20°C light and dark, 40°C, 50°C and 60°C
5	27	-20°C, 4°C, 20°C light and dark, 40°C, 50°C and 60°C
6	41	20°C light and dark, 40°C, 50°C and 60°C
7	55	-20°C, 4°C, 20°C light and dark, 40°C, 50°C and 60°C
8	69	20°C, light and dark, 40°C, 50°C and 60°C
9	83	-20°C, 4°C, 20°C light and dark, 40°C, 50°C and 60°C
10	97	-20°C, 4°C, 20°C light and dark, 40°C, 50°C and 60°C

3.8 Tests for both Cold Pressed and Commercial/Factory Oil Extracted Avocado

Oil were analysed for colour, total chlorophyll determination, carotenoid and chlorophyll, peroxide value (PV), free fatty acid and free fatty acid composition.

3.8.1 Total Chlorophyll Determination (Spectrophotometer)

Pheophytin is the main chlorophyll pigment in crude vegetables oils. The total chlorophyll pigment content in vegetable oil was expressed as mg of pheophytin *a* in 1kg of oil AOCS Ch 4-91 (AOCS, 1997).

The division constant was 0.101 (a function of the spectrophotometer) as used in virgin olive oil analysis (Sherpa, 2002). The total chlorophyll concentration was analysed using AOCS Ch 4-91 with some modifications (AOCS, 1997; Sherpa, 2002). The avocado oil was diluted (two ml of avocado oil and 3ml of isooctane were mixed). The absorbance was

measured using a Spectrophotometer (UV-1601 PC, Zhimadzu UV-Visible).The absorbance value at wavelengths 630, 670 and 710nm was recorded. The total chlorophyll concentration was calculated using the equation below:

$$C_{670} = \frac{A_{670} - 0.5\,(A_{630} - A_{710})}{0.101 \times L \times D}$$

Where:

C_{670} = Total chlorophyll pigment content (ppm)

A_n = Absorbance of oil at wavelength n

L = Path Length (1cm)

D = Dilution = 0.4

3.8.2 Colour Measurement of Oil

The Minolta CR300 colorimeter (Minolta Japan), was used to measure the colour of the oil. It was first calibrated using a standard white tile prior to taking oil measurements. The 4ml polystyrene cuvettes (10x10mm optical pathway) were filled with oil and were each in turn positioned in the liquid sample holder and colour measurements were carried out. The average reading of three measurements for each replicate was recorded.

3.8.3 Peroxide Value

Peroxide value (PV) is an index of oil quality after production and consequently during storage (Karl, 2001). AOCS Official Method Cd 8-53 (AOCS, 1997) was used to test PV. Dichloromethane was used as a solvent as this is less toxic compared to chloroform.

3.8.3.1 Reagents

- 0.002M sodium thiosulphate: 2ml of 0.1M sodium thiosulphate was measured into a 100ml volumetric flask and filled to the mark with distilled water.
- Saturated potassium iodide (KI) solution: 10g of KI was weighed, 5ml of water was added and the solution was stirred. The solution was saturated as evidenced by the crystals present.

- Starch indicator: 1g of unmodified starch was weighed and mixed with 2g cold water until it formed slurry, 100ml of boiled water was added and boiled for a further 2 minutes.
- Glacial acetic acid/dichloromethane solvent: 6ml glacial acetic acid was measured and mixed with 4ml dichloromethane.

3.8.3.2 PV Determination

Two grams avocado of avocado oil was weighed into a 250ml Erlenmeyer flask. Ten ml acetic/dichloromethane solvent was added to dissolve the oil, two drops of potassium iodide solution were added and the flask was swirled and left in a dark cupboard for exactly two minutes. One ml of 1% starch solution (brown colour developed) and 30ml distilled water was added (dark blue/black colour developed). The solution was titrated with sodium thiosulphate until the blue/black colour of iodised starch turned colourless. PV was calculated using the equation below:

$$\text{PV (mEq/kg oil)} = \frac{\text{Sodium thiosulphate (ml)} \times 0.002 \times 1000}{\text{Weight of oil}}$$

3.8.4 Fatty Acid Composition Determination

The methods for fatty acid analysis described in Christie (1989) and AOAC Official Method 963.22 (AOAC, 2000) were used.

About 2g of ammonium chloride was dissolved in 60ml methanol and 3ml of concentrated sulphuric acid was added and refluxed for 15 minutes to prepare Hartman reagent. Methanolic-potassium hydroxide was prepared by dissolving 28.05g of potassium hydroxide into 1 litre of methanol.

About 0.1g of oil was added into a Kimax tube, 0.5ml of 0.5M methanolic-potassium hydroxide was added, mixed and was placed in a water bath for 10 minutes at 70°C. Five ml of Hartman reagent was added and again heated for 10 minutes in the same water bath. Two ml of hexane was added and then shaken followed by 5ml of distilled water.

Gas chromatography (GC): Fatty acid composition was analysed using GC equipped with DB wax column heated at 250°C. Analysis time per sample was 40 minutes. The sample was analysed as per the manufacturer's instructions. The three gases used were hydrogen (H_2), the mobile gas at 50 to 60psi, Nitrogen (N_2) at 100psi was the carrier gas and air at 50 to 60psi. A 0.5µl aliquot from the upper layer was injected in to the GC. Fatty acid compositions of the oil sample were characterised by comparing the retention to standard avocado oil compositions. The fatty acid percent composition was calculated using the % peak area.

3.9 Diene Analysis of Avocado Oil

The diene extraction and determination was carried out using the method of Prusky (2005).

3.9.1 Diene Extraction and Determination

10ml of avocado oil was mixed and homogenized in 100ml of technical ethanol (96%) using a blender. The ethanol extract was filtered through a Buchner funnel with Whatman No 1 filter paper. The filtrate was collected in a separation funnel and allowed to settle until two phases were formed. The lower phase (ethanol part) was decanted.

All the ethanol part of the homogenate was dried at 40°C using a rotary evaporator. The dried material was dissolved in 50ml of dichloromethane, and 10ml of distilled water was added and transferred to a separation funnel. When the two phases had separated the organic phase (the lower one) was collected and the water phase (the upper one) was washed again with dichloromethane, separated and the organic phase added to the first one. Dried magnesium sulphate was added to the dichloromethane and the solution was filtered through Whatman No 1 filter paper and the organic/dried solution dried in a rotary evaporator. The material was dissolved in about 5ml of ethyl acetate and put into a vial and dried under nitrogen and then injected to the HPLC.

3.10 Statistical Analysis

The Independent Sample t test (Urdan, 2001) for a 95% confidence level was used to statistically analyse the data of the fresh tissue sections and the extracted oil to determine whether there were significant changes (increased and deceased) in the firmness, colour, and the concentrations of carotenoids, chlorophylls and anthocyanins from day 0 to day 13. Likewise, the Independent Sample t test (Urdan, 2001) for a 95% confidence level was used to statistically analyse the data for the laboratory and commercially produced oil to determine whether there were significant changes (increased and decreased) in the colour, peroxide value and the concentrations of carotenoids, chlorophylls from day 0 to day 97. The results for oil samples during the 97 days period stored at different temperatures were analysed by regression using Excel to determine the rate of reaction of PV and determine the shelf life and to calculate the total chlorophyll at the end of the shelf life.

Chapter 4. Pigment Composition of Skin and Flesh Tissues of 'Hass' Avocado and the Extracted Oil

4.1 Introduction

Avocado (*Persea americana*) have been found to contain considerable quantities of chlorophyll, carotenoids and anthocyanin (Gross et al., 1972, Cran and Possingham, 1973; Gross et al., 1973; Prabha et al., 1980; Cox et al., 2004). The oil content of avocado fruit is high (15-30%; Werman and Neeman, 1986). New Zealand companies have recently started extraction of high value culinary cold pressed avocado oil. The level and nature of pigments in the oil of avocado are an important factor in its quality and marketability (Eyres et al., 2001).

Components present in avocado fruit and avocado oil may have considerable health benefits such as prevention of coronary heart disease, cataracts, diabetes, chemoprevention, prostate cancer and age related macular disease (Bendich, 1993; Birbeck, 2002; Semba and Dagnelie, 2003; Brown et al., 2004; Qing-Yi Lu et al., 2005; Monro, 2005). The pigment compositions of New Zealand 'Hass' avocado fruit and the pigments are therefore of interest. There is little published information available on the pigment composition of skin and flesh tissues of 'Hass' avocado and the extracted oil.

Qing-Yi Lu et al. (2005) found that Californian 'Hass' avocado fruit contained lutein (2.93μg g^{-1}), zeaxanthin (0.11μg g^{-1}), β-cryptoxanthin (0.25μg g^{-1}), α-carotene (0.25μg g^{-1}) and β-carotene (0.60μg g^{-1}). Earlier investigations showed that the crude carotene concentration in avocado oil was about 2.36μg g^{-1} of oil of which 0.54μg g^{-1} was β-carotene (Gross et al., 1972). In investigations using the 'Nabal' avocado, it was found that in the pulp, lutein was the most discernable carotenoid (Gross et al., 1972). The total carotenoids in the pulp ranged from 10-14μg g^{-1} of fresh weight (Gross et al., 1972). Investigations revealed that the total concentration of carotenoids in the skin was 40ug g^{-1} fresh weight. The concentration of lutein in the pulp was about 50% less than in the peel (Gross et al., 1973). Woolf and Laing (1996) found that the chlorophyll contents of unripe and ripe 'Hass' avocado skin were almost the same 354mg chlorophyll/m^2 and 357mg chlorophyll/m^2 respectively. Cox et al. (2004) characterized anthocyanin and chlorophyll pigments in the skin of 'Hass' avocado. Chlorophyll concentrations of avocado skin taken straight from the tree were a little higher

(0.63μg g^{-1}) compared to the fruit showing colour change (0.50μg g^{-1}-0.57μg g^{-1}). The chlorophyll content of the sections of mature avocado fruit (cv. Fuerte) by fresh weight were found to be 316μg g^{-1} (skin), 101μg g^{-1} (dark pulp), 51μg g^{-1} (pale pulp) and 38μg g^{-1} fresh weight (yellow pulp) respectively (Cran and Possingham 1973). The carotenoid contents of each section were not studied.

Oil extracted from the flesh of 'Hass' avocado contains high levels of fat soluble pigments including chlorophyll (40μg g^{-1} to 60μg g^{-1}) (Werman and Neeman, 1987; Eyres et al., 2001). Oil extracted from the flesh of 'Hass' avocado also contains lutein (Requejo et al., 2003).

Here we sought to determine the pigment composition of 'Hass' avocado fruits at different stages of ripening (anthocyanin, chlorophyll and carotenoid pigment distributions in the skin). Chlorophyll and carotenoid pigment compositions in the three sections of the pulp (dark pulp adjacent to the peel, pale pulp and yellow pulp adjacent to the stone) and to determine the pigment composition of the oil extracted from these sections.

4.2. Materials and Methods

4.2.1 Experimental Overview

Post harvest fruit quality such as dry matter, colour and firmness were assessed. The pigment compositions in the skin and flesh tissue sections of 'Hass' avocado and the extracted oil were analysed by HPLC. Refer to Chapter 3 for detailed methodology.

4.2.2 Tissue Sampling

Tissue from unripe avocados (2-4 days) was sampled by removing a plug of tissue through the equator using a cork borer (7mm diameter). Six plugs were taken from each fruit. For ripe fruit (6 to 13 days) the fruit were cut into quarters. The stone was discarded and 2 plugs taken from each segment using a cork borer working from the inside through to the skin. This produced 8 plugs per fruit. Each plug of tissue was divided into four sections - the skin, and the three sections of pulp namely dark pulp, pale pulp and yellow pulp, respectively.

4.3 Results

4.3.1 Firmness of 'Hass' Avocado during Ripening

The Firmometer values of the avocados measured from day 2 to day 13 increased from 9 to 100 as the fruit ripened (Figure 4.1).

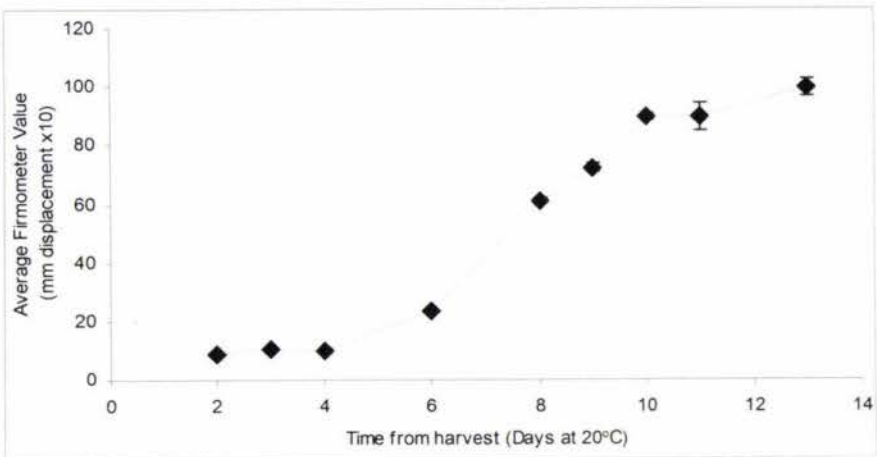


Figure 4.1.Softening of 'Hass' avocado during different stages of ripening as measured by an Anderson digital Firmometer (n=45)

4.3.2 Colour of 'Hass' Avocado during Ripening

The changes in lightness, chroma and hue angle over 13 days were measured by chroma meter and the results shown in Figure 4.2. The skin of the whole fruit decreased in lightness (40 to 27) and chroma (15 to 3) but the hue angle increased (123 to 249) over 13 days. Measurement of the cut skin (average of inner and outer skin surface) showed a decrease in lightness (44 to 42) and chroma (24 to 12), and increased hue angle (106 to 216) over 13 days. The dark pulp decreased in lightness and chroma (72 to 63) and (42 to 34) over 13 days .The hue angle did not change, was 113 at day 2 and 114 at day 13. The pale pulp decreased in lightness and chroma (82 to 75) and (41 to 35) respectively over 13 days. The hue angle did not change, was 106 at day 2 and 105 at day 13. The yellow pulp decreased in lightness (83 to 74) and chroma (42 to 36) over 13 days. The hue angle did not change, was 101 at day 2 and 99 at day 13. The changes in lightness and chroma over 13 days period of ripening for the whole fruit and all sections were all significant ($p<0.05$). During avocado ripening the lightness of the skin surface of the whole avocado fruit decreased as it became darker changing from green to olive to a purple/black colour. Similarly, the inside surface of the skin and the three pulp sections became darker and duller or less vivid during ripening as the lightness values and chroma decreased and as the hue angle increased.

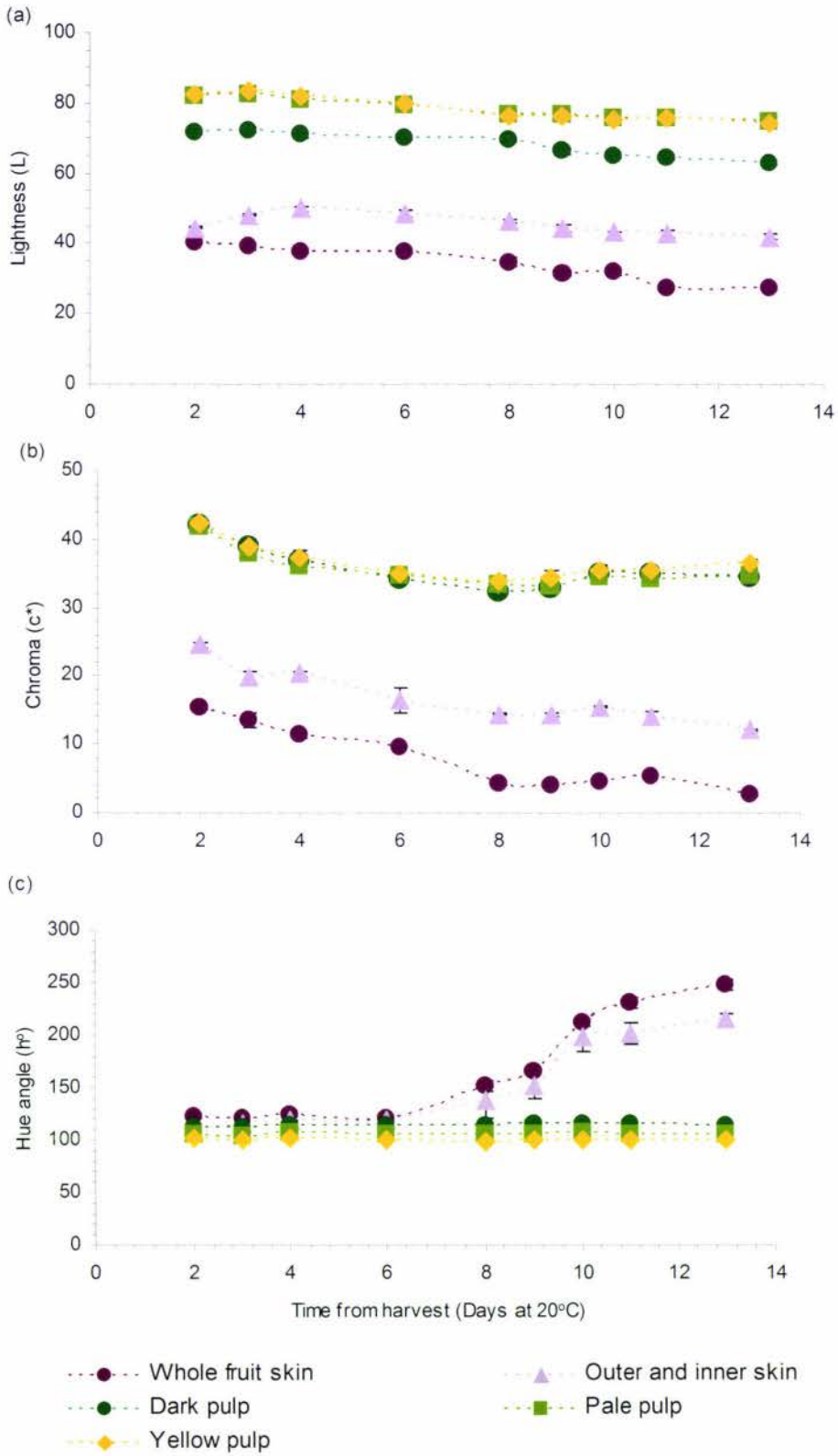


Figure 4.2.Colour changes. (a) Lightness L, (b) Chroma, c, (c) Hue angle of 'Hass' avocado (n=60).

4.3.3 Carotenoids in Fresh Tissue

Carotenoids were analysed using HPLC and the results are shown in Figure 4.3. (Raw Data, Appendix 1 and Chromatograms, Appendix 7)

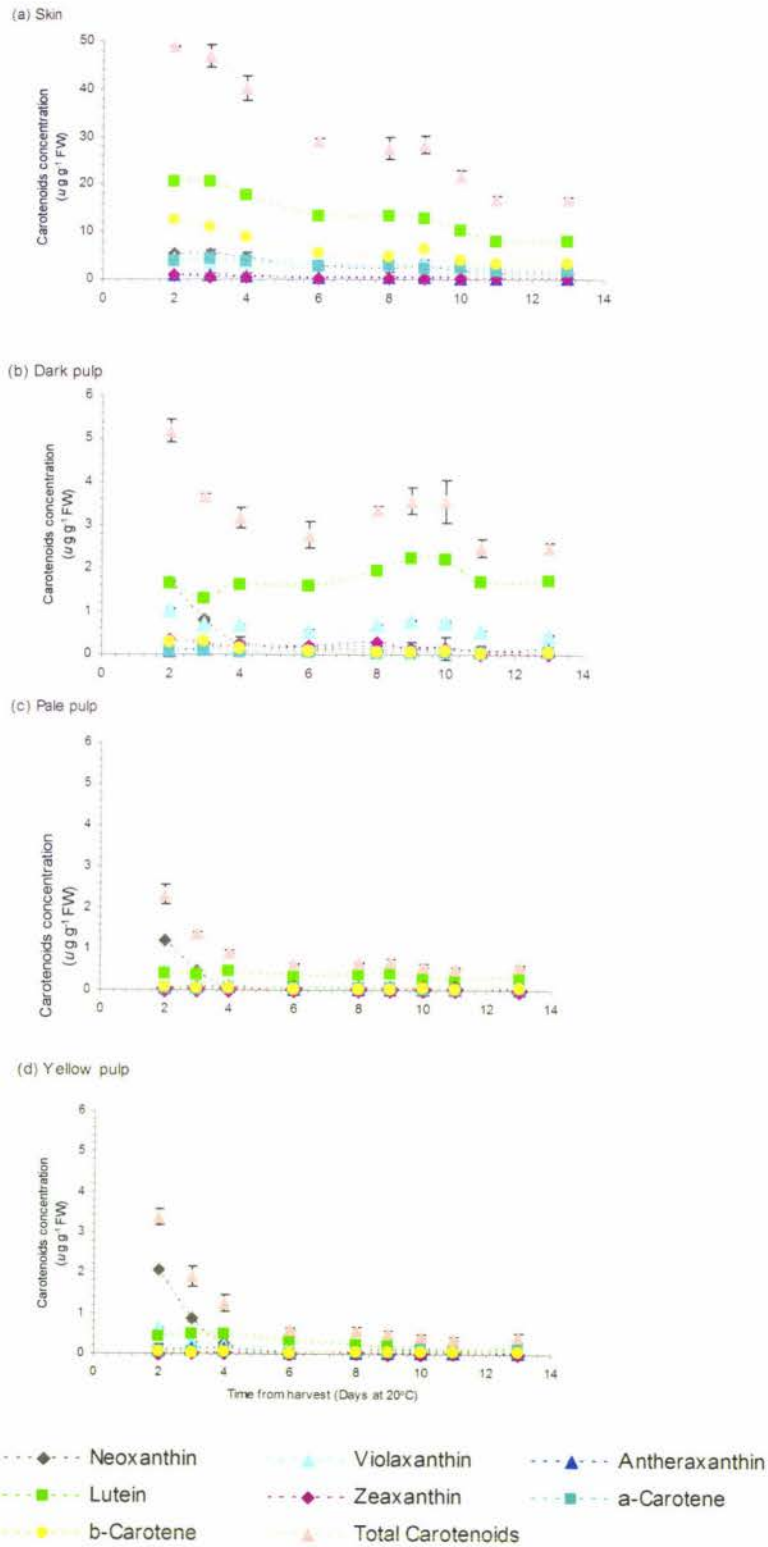


Figure 4.3.Carotenoids concentrations in 'Hass' avocado (a) skin, (b) dark pulp, (c) pale pulp and (d) yellow pulp (n=3)

Lutein was the most abundant of the carotenoids found in 'Hass' avocado fruit. The highest concentration was found to be in the skin (Figure 4.3a). The concentration of lutein in the skin declined (20.54μg g^{-1} to 8.02μg g^{-1}) after 13 days. The second most abundant carotenoid pigment in the skin was found to be β-carotene, the concentration decreased steadily from 12.53μg g^{-1} to 3.42μg g^{-1} after 13 days. The β-carotene level was approximately half that of lutein. Initially at day 2 the concentration of neoxanthin in the skin was the third highest carotenoid. The initial concentration of neoxanthin was 5.52μg g^{-1} and decreased to about 25% of its original level (1.26μg g^{-1}) after 13 days. The violaxanthin in the skin decreased from 4.49μg g^{-1} to 1.69μg g^{-1} after 13 days. Zeaxanthin had the lowest concentration of all the carotenoids in the skin identified at day 2 (0.92μg g^{-1}). The concentration declined throughout the period of the tests to about 15% of its initial level (0.13μg g^{-1}) by day 13. Antheraxanthin was the second least abundant carotene in the skin identified (1.04μg g^{-1}) at day 2 and decreased to 0.24μg g^{-1} after 13 days. The concentration of α-carotene in the skin was 3.87μg g^{-1} at day 2. By day 13 the concentration of α-carotene had declined to 1.93μg g^{-1}. From day 2 to day 13 the changes in all carotenoids were significant ($p<0.05$).

In the dark pulp (Figure 4.3b), the lutein concentration was lower than the lutein in the skin (1.65μg g^{-1}) at harvest, less than 10% of that in the skin at day 2 and did not change significantly throughout the test period to day 13. The concentrations of other carotenoids in the dark pulp decreased significantly over the 13 days period ($p<0.05$). Neoxanthin decreased from 1.68μg g^{-1} to 0.07μg g^{-1}, violaxanthin decreased from 1.06μg g^{-1} to 0.45 μg g^{-1}, zeaxanthin fell from 0.35μg g^{-1} to zero, α-carotene decreased from 0.08μg g^{-1} to 0.05μg g^{-1} and β-carotene decreased from 0.28μg g^{-1} to 0.06 μg g^{-1} ($p<0.05$). However, the concentration of antheraxanthin in the dark pulp did not change throughout the test period (0.09μg g^{-1} to 0.11μg g^{-1}).

Lutein, α-carotene, zeaxanthin and antheraxanthin in the pale pulp did not change significantly over the storage period (Figure 4.3c). The concentration of lutein in the pale pulp was 0.41μg g^{-1} at day 2 and 0.34μg g^{-1} at day 13. The α-carotene concentration in the pale pulp was 0.05μg g^{-1} at day 2 and at day 13, 0.06μg g^{-1}. Zeaxanthin concentration in the pale pulp was 0.02μg g^{-1} and zero at day 13. Antheraxanthin concentration in the pale pulp was 0.05μg g^{-1} at day 2 and 0.02μg g^{-1} at day 13. In the pale pulp neoxanthin concentration decreased from 1.21μg g^{-1} to

0.03μg g^{-1} and violaxanthin concentration decreased from 0.49μg g^{-1} to 0.09μg g^{-1} ($p<0.05$).

In the yellow pulp (Figure 4.3d) the concentration of lutein at day 2 was 0.45μg g^{-1}. By day 13, the concentration of lutein decreased to 0.14μg g^{-1} ($p<0.05$). The concentration of β-carotene in the yellow pulp remained unchanged (0.06μg g^{-1}). The concentrations of neoxanthin decreased from 2.08μg g^{-1} to 0.03μg g^{-1}, violaxanthin fell from 0.63μg g^{-1} to 0.05μg g^{-1}, antheraxanthin decreased from 0.10μg g^{-1} reaching a level of 0.02μg g^{-1} and the concentration of α-carotene increased from 0.06μg g^{-1} to 0.14μg g^{-1} ($p<0.05$). Zeaxanthins in the pale pulp was 0.02μg g^{-1} and became undetectable by day 8.

The sum of all carotenoids analysed in the skin, dark pulp, pale pulp and yellow pulp is shown in Figure 4.3 a to d. The total carotenoid concentration in the skin at day 2 was 48.91μg g^{-1} and 16.69μg g^{-1} at day 13. In the dark pulp the total carotenoid concentration was 5.19μg g^{-1} at day 2 and 2.47μg g^{-1} at day 13. The pale pulp total carotenoid concentration was 2.33μg g^{-1} at day 2 and 0.59μg g^{-1} at day 13. In yellow pulp the total carotenoid concentration was 3.38μg g^{-1} at day 2 and 0.44μg g^{-1} at day 13.

4.3.4 Chlorophylls in Fresh Tissue

Chlorophylls were analysed using HPLC and the results are shown in Figure 4.4. (Raw Data, Appendix 2 and Chromatograms, Appendix 7).

The chlorophyll *a* concentration in the skin did not changed significantly being at day 2, 23.00μg g^{-1} and 17.68μg g^{-1} at day 13. The chlorophyll *b* in the skin at day 2 was 8.55μg g^{-1} and 6.99μg g^{-1} at day 13 and did not significantly change ($p>0.05$). The chlorophyll *a* concentration of the dark pulp did not change significantly ($p>0.05$) with a concentration of 23.03μg g^{-1} at day 2 and 21.02μg g^{-1} at day 13. Chlorophyll *b* in the dark pulp increased from 5.30μg g^{-1} at day 2 to 9.45μg g^{-1} at day 13 ($p<0.05$). The chlorophyll *a* concentrations in the pale pulp at day 2 and 13 did not change significantly (3.58μg g^{-1} and 4.79μg g^{-1}) respectively. The chlorophyll *b* concentration in the pale pulp increased from 0.45μg g^{-1} at day 2 to 1.82μg g^{-1} at day 13. In the yellow pulp the chlorophyll *a* concentration did not change significantly being 0.73μg g^{-1} at day 2 and 0.90μg g^{-1} at day 13. The chlorophyll *b* concentration at day 2 and 13 were 0.06μg g^{-1} to 0.21μg g^{-1} and did not changed significantly ($p>0.05$) respectively.

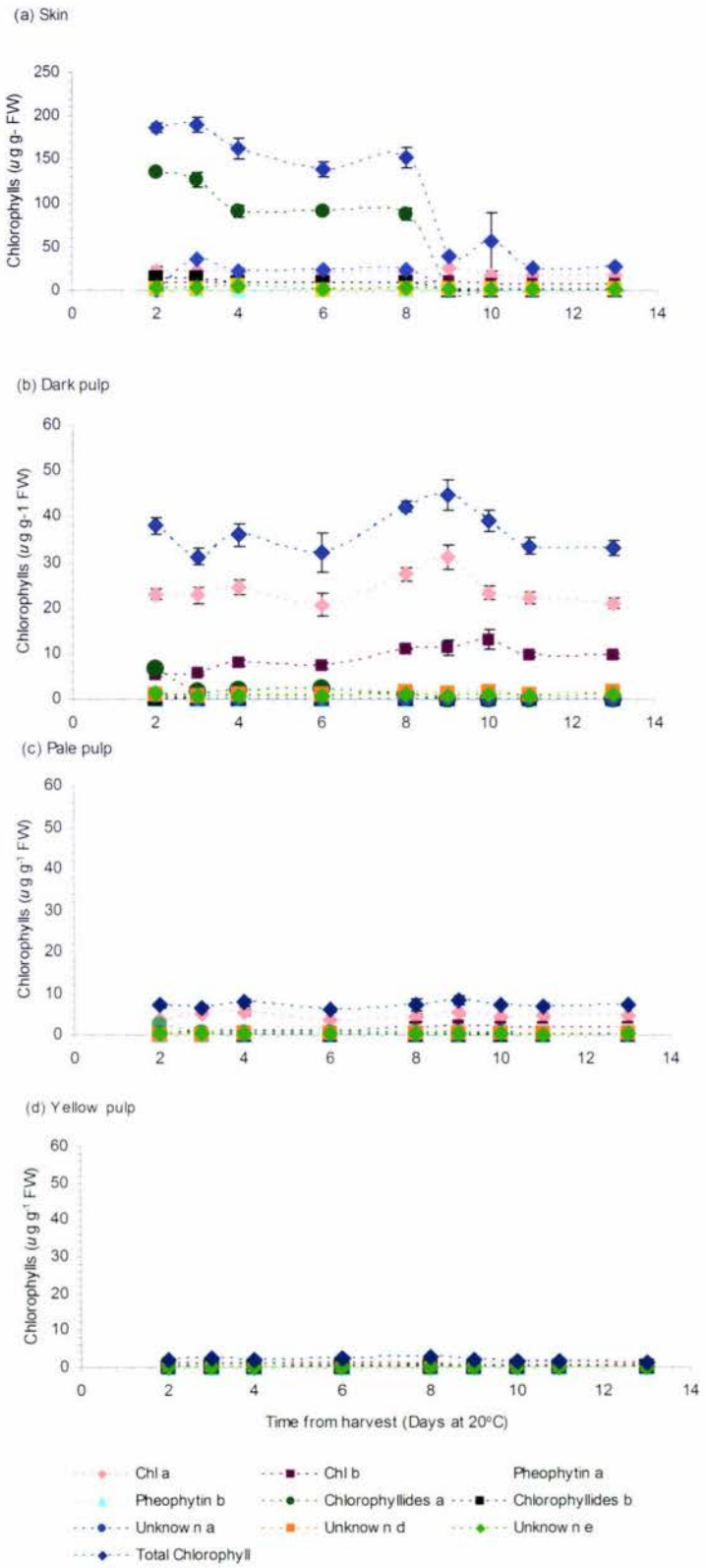


Figure 4.4.Chlorophyll concentrations in the (a) skin, (b) dark pulp, (c) pale pulp (d) yellow pulp of 'Hass' avocado (n=3)

Pheophytins and chlorophyllides concentrations are also shown Figure 4.4 a to d. Pheophytin *a* in the skin was detected in small quantities in the skin on days 2, 3 and 4 (0.10μg g^{-1} or less), then was not detected thereafter. However, pheophytin *b* was detected in small concentrations throughout the test period ranging from 0.05μg g^{-1}-0.12μg g^{-1}. Chlorophyllide *a* in the skin at day 2 was 135.83μg g^{-1} and fell to zero at day 13. The concentration of chlorophyllide *b* in the skin was about 10% of the chlorophyllide *a* concentration until day 8. By day 9 the concentration of chlorophyllide *b* in the skin had decreased to zero. No pheophytin *a* or *b* was detected in the dark pulp. The concentration of chlorophyllide *a* in the dark pulp was about 2% of that in the skin until day 8. By day 9 the concentration had fallen to or to zero where it remained. The concentration of chlorophyllide *b* in the dark pulp section was 0.15μg g^{-1} at day 2 and 0.02μg g^{-1} at day 13. No pheophytin *a* or *b* was detected throughout the whole period of testing of the pale pulp. The concentration of chlorophyllide *a* on day 2 was 2.51μg g^{-1} in the pale pulp had fallen to zero by day 11. Chlorophyllide *b* was not found in the pale pulp section except a trace on day 6. No pheophytin *a* or *b* was detected in the yellow pulp sections throughout the whole period of testing. Chlorophyllide *a* in the yellow pulp section was found to be about 1.11μg g^{-1} at day 2 and zero at day 11. Chlorophyllide *b* was not found in the yellow pulp section except for a trace on day 6, the same on which a trace was found in the pale pulp section.

There were three unknown chlorophylls detected on the chromatograms (Appendix 8). They were assigned as unknown 'a','d' and 'e' as follows and have yet to be identified (Appendix 8). These three unknowns could either be the pheophytin and chlorophyllides derivatives pyropheophytin, pheophorbide, pyropheophorbide or other chlorophyll epimer (Ferruzi and Schwartz, 2001).

The unknown chlorophyll 'a' had a retention time of 5.517 minutes (Appendix 8). Unknown chlorophyll 'a' appeared consistently in the skin on the chromatographs and the three pulp areas on days 2, 3 and 4 but did not appear from day 8 in the dark and pale pulp. It was not detected from day 6 in the yellow pulp area (Appendix 2). There were two isolated unknown chlorophyll 'a' readings on days 10 and 12, both in the skin. Unknown chlorophyll 'a' appeared most abundant in the skin (23.10μg g^{-1}-36.31μg g^{-1}) with considerably lower concentrations in the dark pulp (0.07μg g^{-1}-0.64μg g^{-1}) at day 2 (Appendix 2). The concentration was less, in the pale and yellow areas, than the dark

pulp area, with maximums of 0.59μg g^{-1} and 0.26μg g^{-1} respectively on day 2 (Appendix 2).

The unknown chlorophyll 'd' had a retention time of 13.920 minutes (Appendix 8). The concentration of unknown chlorophyll 'd' over 13 days in the skin was between 0.36μg g^{-1}-5.74μg g^{-1} (Appendix 2). The dark pulp section concentration of unknown chlorophyll 'd' was similar to that in the skin (0.73μg g^{-1}-1.74μg g^{-1}) during the test period. The dark pulp concentration was similar to that for the skin (Appendix 2). In the pale pulp unknown chlorophyll 'd' on day 2 was lower (0.07μg g^{-1}) than that in the dark pulp and rose to about 15-30% of that in the skin (0.41μg g^{-1}) by day 13 (Appendix 8). The yellow pulp unknown chlorophyll 'd' concentration was not detectable initially but appeared on day 6 to day 13 with concentrations between 0.04μg g^{-1} to 0.12μg g^{-1} which was less than 10% of that in the skin (Appendix 2).

The unknown chlorophyll 'e' had a retention time of 14.699 minutes (Appendix 8). The concentration of unknown chlorophyll 'e' in the skin was 3.15μg g^{-1} at day 2 and 1.25μg g^{-1} at day 13 (Appendix 2). The concentration of unknown chlorophyll 'e' in the dark pulp at day 2 was 1.25μg g^{-1}, which was about 40% of that in the skin at day 2. The concentration of unknown chlorophyll 'e' in the dark pulp varied between 0.56μg g^{-1} and 1.25μg g^{-1} over 13 days (Appendix 2). Unknown chlorophyll concentrations 'e' in the pale pulp varied between (0.17μg g^{-1}-0.30μg g^{-1}). The initial concentration at day 2 (0.28μg g^{-1}) was about 10% of that in the skin. The concentration fluctuated over the test period (Appendix 2). The concentration of unknown chlorophyll 'e' in the yellow pulp remained steady (0.03μg g^{-1}-0.09μg g^{-1}) concentration of unknown chlorophyll 'e' in the skin (Appendix 2).

4.3.5 Ratio of Chlorophyll *a* and *b*

A comparison of the ratio of chlorophyll *a* to chlorophyll *b* in the skin, dark pulp, pale pulp and yellow pulp are shown in Table 4.1.

Table 4.1.Ratio of Chlorophyll *a* and *b* in the 'Hass' Avocado Skin, Dark, Pale and Yellow Pulp

Time from harvest (Days at 20°C)	Skin	Dark Pulp	Pale Pulp	Yellow Pulp
2	2.7	4.3	8.0	12.2
3	2.4	4.1	7.3	13.8
4	2.5	3.1	4.8	8.5
6	2.6	2.9	3.5	2.6
8	2.5	2.5	2.5	2.6
9	2.5	2.8	2.5	2.6
10	2.4	1.8	2.5	2.3
11	2.6	2.3	2.5	2.5
13	2.5	2.2	2.6	4.3

The ratio of chlorophyll *a* to chlorophyll *b* in the skin, on each day revealed that this was within a narrow range of 2.4:1-2.7:1 with a mean of 2.5:1 (Table 4.1). A ratio for chlorophyll *a* to chlorophyll *b* was reported to be 2:1 in skin tissue (Cox et al., 2004). A ratio of 2.5:1 to 3.5:1 has been established as the norm for the chlorophyll *a* to *b* ratio in most plants (Simpson et al., 1976).

It is noted that at the beginning of the test period (day 2) the ratio of chlorophyll *a* to chlorophyll *b* was the least in the skin and increased through the dark to pale to yellow pulp which had the greatest initial ratio. The ratio in the skin remained relatively constant throughout the test period whereas, the ratio fell in each pulp section.

4.3.6 Anthocyanins

Anthocyanins in avocado skin were analysed by HPLC. The results are shown in Figure 4.5 (Raw Data, Appendix 3 and Chromatograms, Appendix 9).

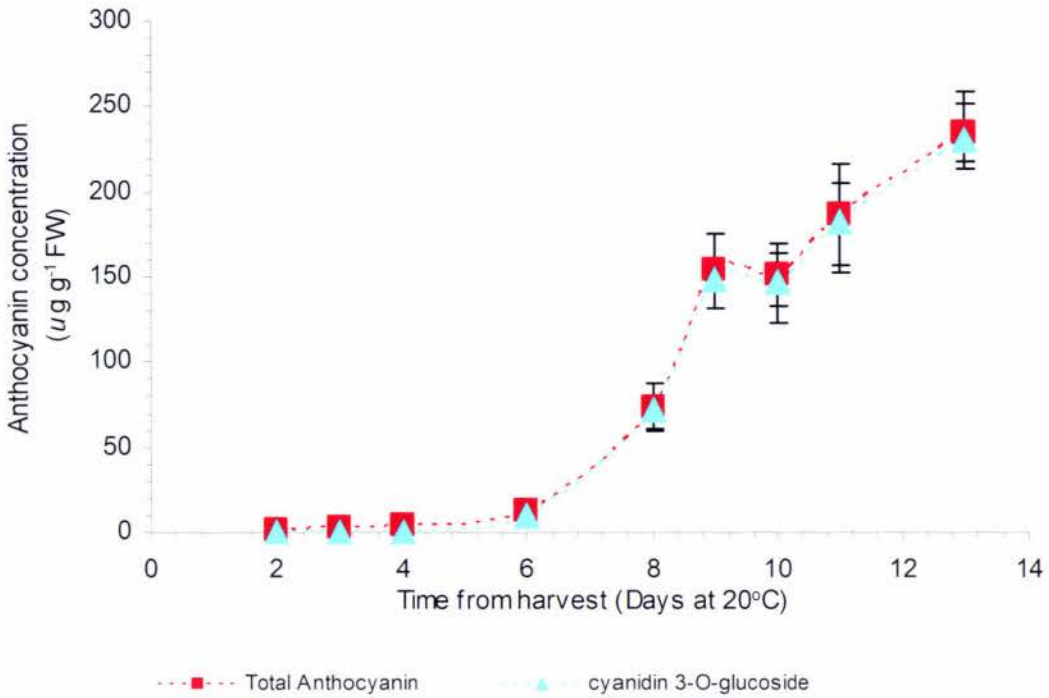


Figure 4.5.Total anthocyanins and cyanidin 3-*O*-glucoside of 'Hass' avocado skin (n=3)

Cyanidin 3-*O*-glucoside accounted for the major component of the total anthocyanin in ripe fruit. As the fruit ripened the cyanidin 3-*O*-glucoside concentration followed a similar curve to that of the total anthocyanins. The cyanidin 3-*O*-glucoside concentration was 230.04μg g^{-1} at day 13.

4.3.7 Oil (%) of 'Hass' Avocado Sections

Oil was extracted from each section of 'Hass' avocado using ASE. Results are shown Figure 4.6.

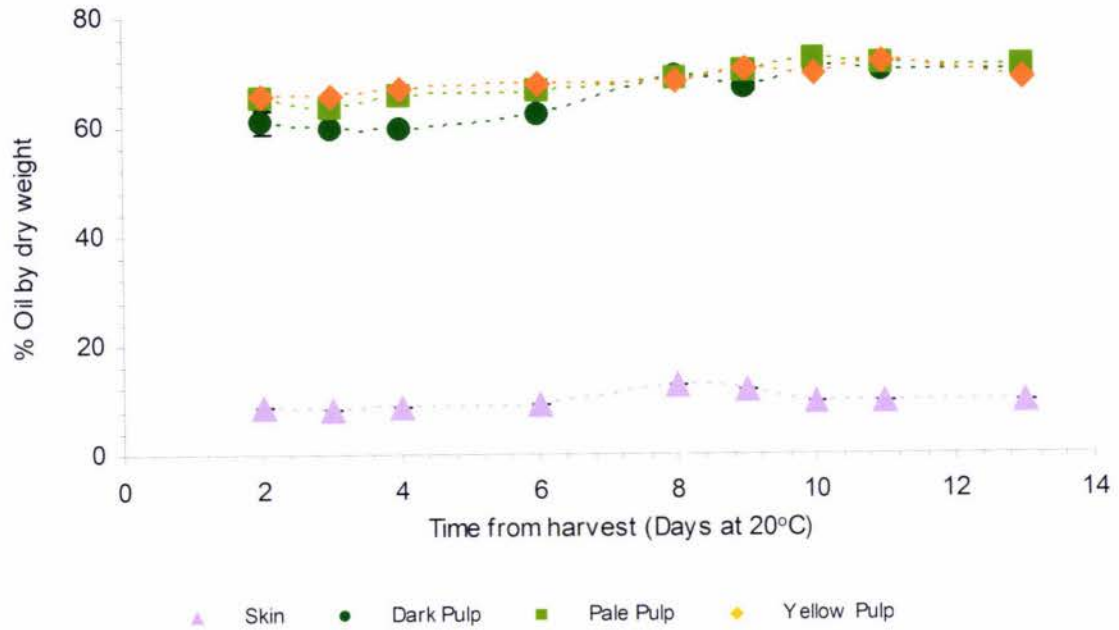


Figure 4.6.Percent oil in the skin, dark pulp, pale pulp and yellow pulp of 'Hass' avocado extracted using ASE (n=3) except skin where n=1

The oil extracted from the skin at day 2 to day 13 increased from 8.7% to 9.8%, dark pulp increased from 60.8% to 70.1%, pale pulp increased from 65.0% to 70.8% and yellow pulp increased from 65.5% to 68.9% ($p<0.05$) (Figure 4.6).

4.3.8 Carotenoids in the Extracted Oil

The carotenoids determined in the oil extracted by ASE for various sections during 13 days of ripening are shown in Figure 4.7 (Raw Data, Appendix 4).

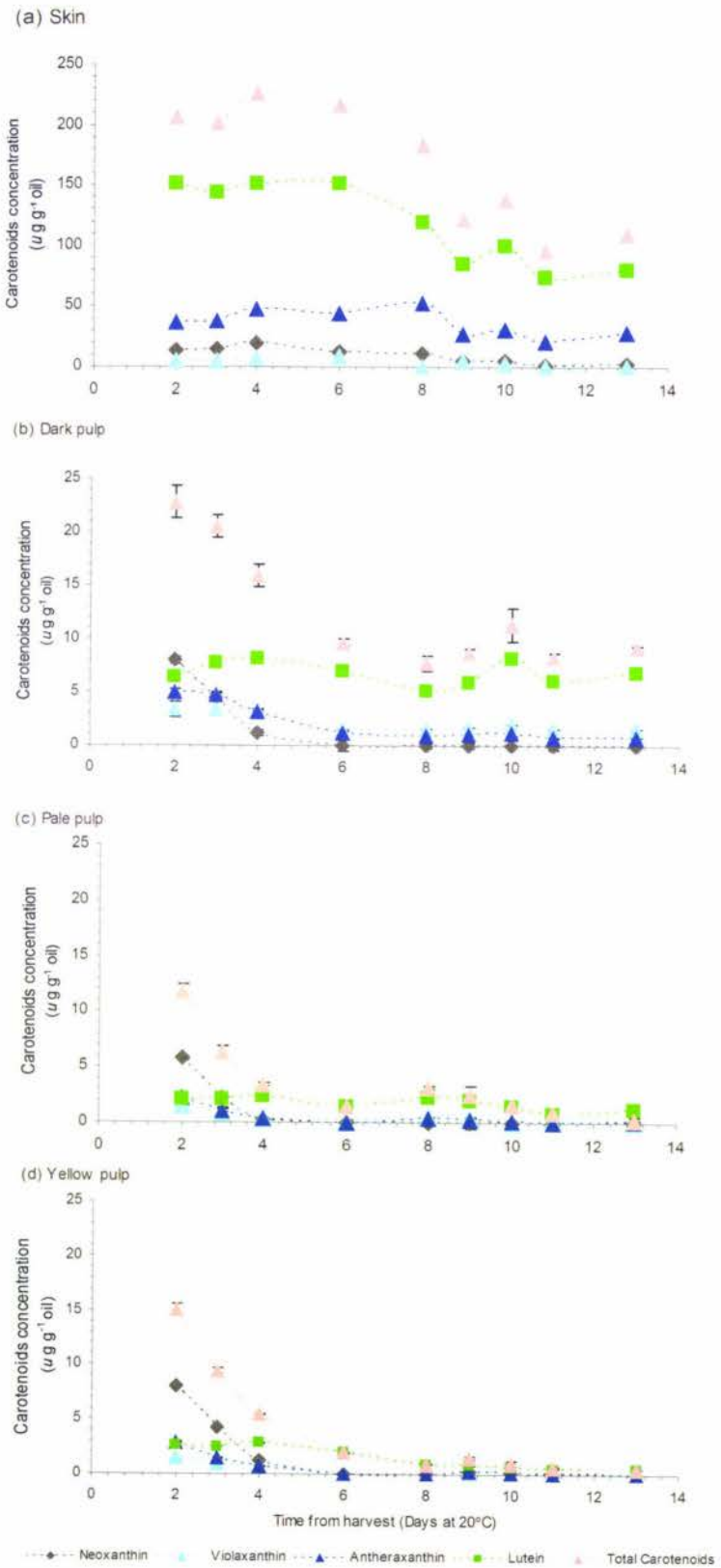


Figure 4.7.Carotenoids concentrations in the oil extracted from 'Hass' avocado (a) skin, (b) dark pulp, (c) pale pulp and (d) yellow pulp (n=3) except skin where n=1

Lutein was the most abundant carotenoid in avocado oil extracted from the skin with concentrations of 151.0μg g^{-1} and 79.7μg g^{-1} at days 2, and 13 respectively (Figure 4.7a). Antheraxanthin was the next most abundant carotenoid in oil from the skin with concentrations of 37.3μg g^{-1} (day 2) and 28.1μg g^{-1} (day13). The third most abundant carotenoid in the oil extracted from the skin was neoxanthin which had a concentration on day 2 of 13.9μg g^{-1} and 2.1μg g^{-1} on day 13. The second least abundant carotenoid in oil from the skin was violaxanthin which had a concentration at day 2 of 4.6μg g^{-1} which decreased until day 11 when the concentration became zero. All of the decreases in concentration of carotenoids in the skin were significant ($p<0.05$). The concentration of lutein in the oil extracted from the dark pulp did not change significantly being 6.43μg g^{-1} at day 2 and 6.80μg g^{-1} at day 13 (Figure 4.7b). Initially, at day 2 neoxanthin was the most abundant carotenoid from the dark pulp with a concentration of 7.97μg g^{-1}. By day 6 the concentration had declined to zero. Antheraxanthin was initially the third most abundant carotenoid in oil from the dark pulp with a concentration of 5.03μg g^{-1} at day 2. This declined to a concentration of 0.83μg g^{-1} by day 13. The fourth most concentrated carotenoid in oil from the dark pulp was violaxanthin at 3.37μg g^{-1} at day 2 and 1.53μg g^{-1} at day 13. The decreases in concentration of neoxanthin, antheraxanthin and violaxanthin in the dark pulp were significant ($p<0.05$). Neoxanthin at day 2 was 5.77μg g^{-1} in the pale pulp (Figure 4.7c). By day 6 this had decreased to zero. In the pale pulp, on day 2 antheraxanthin in oil had the second highest concentration (2.27μg g^{-1}) which decreased to 0.13μg g^{-1} at day 13. Lutein in the oil from the pale pulp was the third most abundant carotenoid at day 2, 2.10 μg g^{-1} and 1.23μg g^{-1} by day 13. Violaxanthin in the pale pulp decreased from 1.60μg g^{-1} at day 2 to 0.07μg g^{-1} at day 13. All of the decreases in concentration of carotenoids in the pale pulp were significant ($p<0.05$). Neoxanthin in oil from yellow pulp was the carotenoid with the highest concentration at 8.07μg g^{-1} at day 2 and decreased rapidly to day 4 (1.20μg g^{-1}) and then to zero by day 6 (Figure 4.7d). Antheraxanthin had the second highest abundance in oil from the yellow pulp at day 2 at 2.93μg g^{-1} and decreased to zero by day 6. At 2.63μg g^{-1} on day 2, lutein was the third most concentrated carotenoid in oil from the yellow pulp and decreased to 0.53μg g^{-1} by day 13. Violaxanthin in the pale pulp decreased from 1.50μg g^{-1} at day 2 to zero at day 11. All of the decreased in concentration of carotenoids in the yellow pulp are significant ($p<0.05$).

Zeaxanthin was not detected in the oil extracted from the skin, dark pulp, pale pulp and yellow pulp.

4.3.9 Chlorophyll in the Extracted Oil

The chlorophyll content of avocado oil extracted from different sections was analysed by HPLC and results are shown in Figure 4.8 (Raw Data, Appendix 5).

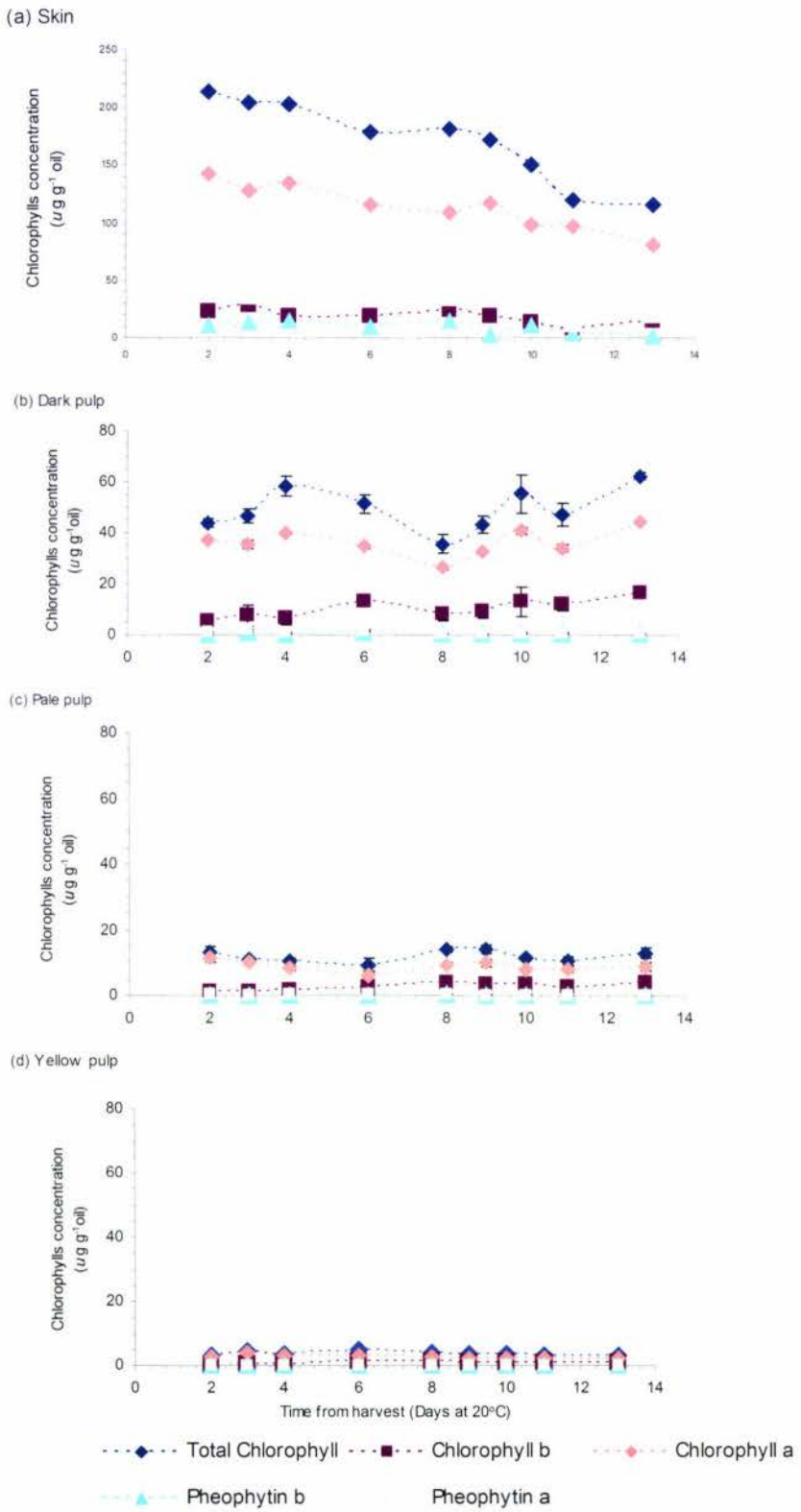


Figure 4.8.Total chlorophyll, chlorophyll *a*, chlorophyll *b*, pheophytin *a* and pheophytin *b* in oil extracted from 'Hass' avocado (a) skin, (b) dark pulp, (c) pale pulp and (d) yellow pulp (n=3) except skin where n=1

Chlorophyll *a* in the oil from skin concentration at day 2 was 143.0μg g^{-1} and 81.1μg g^{-1} at day 13. The concentration in oil of chlorophyll *b* from the skin was 22.7μg g^{-1} at day 2 and at day 13, 14.3μg g^{-1} (Figure 4.8a). At day 2, the pheophytin *b* concentration in oil from the skin was 11.1μg g^{-1} and at day 13, 1.6μg g^{-1}. Pheophytin *a* concentration was 37.0μg g^{-1} at day 2 and 18.6μg g^{-1} at day 13. All of the decreases in concentration of chlorophylls in oil from the skin were significant ($p<0.05$).

In the oil extracted from the dark pulp (Figure 4.8b) at day 2 the chlorophyll *a* content was 37.03μg g^{-1} and at day 13, 44.60μg g^{-1}. The concentration in oil of chlorophyll *b* at day 2 was 5.70μg g^{-1} and at day 13, 16.73μg g^{-1}. Both chlorophyll *a* and *b* in the oil from dark pulp decreased significantly ($p<0.05$) (Figure 4.8b). For pheophytin *b*, the concentration of oil from dark pulp at day 2 was 0.17μg g^{-1} and 0.50μg g^{-1} at day 13. Pheophytin *a* concentration was 0.90μg g^{-1} at day 2 to 0.63μg g^{-1} at day 13.

At day 2 the chlorophyll *a* content of oil extracted from the pale pulp did not change significantly being 11.37μg g^{-1} at day 2 and 8.73μg g^{-1} at day 13 ($p>0.05$). Chlorophyll *b* from the pale pulp had increased significantly from 1.43μg g^{-1} at day 2 to 3.97μg g^{-1} at day 13 ($p<0.05$) (Figure 4.8c). Pheophytin *b* in oil at day 2 was zero and 0.10μg g^{-1} at day 13. Pheophytin *a* concentration in oil was 0.27μg g^{-1} at day 2 and 0.13μg g^{-1} at day 13) (Figure 4.8c).

Chlorophyll *a* and *b* in the oil extracted from yellow pulp sections (Figure 4.8d) did not change significantly throughout the test period ($p>0.05$). Chlorophyll *a* from the yellow pulp concentrations was 2.70μg g^{-1} at day 2 and 2.23μg g^{-1} at day 13. Chlorophyll *b* from the yellow pulp concentrations at day 2 was 0.17μg g^{-1} and 1.00μg g^{-1} at day 13 (Figure 4.8d). Pheophytin *b* had zero content until day 4 was zero. By day 13 this had decreased to 0.03μg g^{-1}. Pheophytin *a* concentration remained the same being 0.03μg g^{-1} at day 2 and day 13.

In the oil extracted from the skin (Figure 4.8a) the total chlorophyll concentration decreased throughout the test period with a concentration of 213.8μg g^{-1} at day 2 and 115.6μg g^{-1} at day 13 ($p<0.05$). The oil extracted from the dark pulp section (Figure 4.8b) had a total chlorophyll content of 43.80μg g^{-1} at day 2 which increased considerably to 62.50μg g^{-1} at day 13 ($p<0.05$). The total chlorophyll in the oil extracted

from the pale pulp section (Figure 4.8c) did not change significantly with concentrations of 13.07μg g^{-1} at day 2 and 12.93μg g^{-1} at day13 ($p>0.05$). The oil extracted from yellow pulp section (Figure 4.8d) had the lowest total chlorophyll which did not change, was 2.97μg g^{-1} at day 2 and day 13. The total chlorophyll in the oil extracted from each section was composed mainly of chlorophyll *a* providing 70-75% of the total.

Chlorophyllides *a* and *b* were not detected in the extracted oil.

4.4 Discussion

4.4.1 Fruit Quality Assessment

The increase in firmness value followed a similar curve to Cox et al. (2004) but with a delay in the commencement of the softening process of about two days which may be due to the different seasons in which the fruit was harvested. It was observed that some fruits were ripe but the colour was still green and some fruit which were changing to a purple colour were not soft but generally the skin became darker over the 13 days of ripening which is in agreement with the value of lightness, chroma and hue angle.

4.4.2 Pigments in Fresh Tissue Sections

4.4.2.1 Carotenoids

The skin was found to have the highest concentration of all carotenoid pigments with a lesser concentration in the dark pulp area under the skin. In the pale and yellow areas of the pulp, the carotenoid levels were much lower. The amount of chloroplast per cell decreases from the skin to the centre of the fruit therefore the carotenoids declined.

Lutein was the most abundant carotenoid followed by β-carotene in avocado as the major components of this fruit are xanthophylls (Simpson et al., 1976). The β-carotene concentration was approximately half that of lutein. Throughout the 13 days ripening period the concentration of these carotenoids decreased steadily. The concentrations of all carotenoids in all three pulp sections followed the same pattern of steadily declining although in the two innermost areas the decline was small due to the initial low concentration. The concentration of neoxanthin was the third highest carotenoid in the skin. Neoxanthin in the three pulp sections followed similar patterns to each other but were different to that in the skin. Neoxanthin was the only carotenoid which had a

concentration significantly greater than lutein at any time in the whole ripening period and then only between day 2 and day 4 in the pale and yellow pulp areas. Lutein was the most abundant carotenoid.

4.4.2.2 Chlorophyll

The avocado skin changed from green to purple/black as shown by the lightness, chroma and hue values. The flesh retained chlorophyll at the ripe stage. The chlorophyll pigment levels varied with depth in the avocado fruits. The layer adjacent to the skin had the darkest green colour, the flesh adjacent to the stone was yellow and the flesh between these layers was pale green as shown by the lightness, chroma and hue. The presence of the various plastid types may be related to different levels of sunlight penetration within the fruit (Cran and Possingham, 1973). Plastids are organs in the plant that self–reproduce and contain pigments, oil and protein. Plastids could be identified based on their colour (Newcomb, 1990). Chlorophyll pigment was present in the green plastid (chloroplasts). Carotenoid pigments are present in the yellow plastid (chromoplast). The pale yellow plastid (etioplast) is produced under dark (Newcomb, 1990). Under natural light conditions the avocado green flesh received sufficient light. It is likely that the pale pulp intermediate zone receives very low intensities of light. It was probable that the degree of light penetration to the pale pulp section was sufficient to effect a slow conversion of protochlorophyll to chlorophyll (Cran and Possingham, 1973). The central yellow zone did not receive any light and hence the various photochemical processes such as the conversion of protochlorophyll to chlorophyll could not proceed (Cran and Possingham, 1973). The degradation of chlorophyll in senescent tissues is a natural phenomenon and may be enhanced, but not regulated, by environmental factors (Heaton and Marangoni, 1996). The formation of chlorophyll derivatives chlorophyllide *a* and *b* in the skin and flesh tissue may be due to the chlorophyllase enzyme reaction whereas the pheophytin *a* and *b* derivatives may be due to the heat and considerable time during blending to extract the chlorophyll pigments (Ferruzi and Schwartz, 2001; Artes et al., 2002).

From day 6 to day 8 the ratio of chlorophyll *a* and *b* in the three pulp sections all fell within the same range which was that for the skin throughout the 13 days ripening period. It seemed that as the ripening progressed, the ratio of chlorophyll *a* and *b* in the pulp when it reached that of the ratio of skin was an indication of ripeness.

4.4.2.3 Anthocyanin

The anthocyanin concentration of New Zealand 'Hass' avocado skin tissue increased steadily from day 2 to day 13. This increase was due almost entirely to a single anthocyanin; cyanidin 3-*O*-glucoside. The colour changes from green to purple, then black, resulted from an initial decrease in chlorophyll content, followed by an increase in the levels of the anthocyanin. The cyanidin 3-*O*-glucoside represented about 98% of the total anthocyanins present in ripe 'Hass' avocado skin. However, at harvest and for about 4-6 days afterwards the concentration of cyanidin 3-*O*-glucoside was at a considerably lower proportion, indicating that cyanidin 3-*O*-galactoside could have been synthesised (Prabha et al., 1980).

The total anthocyanin concentrations obtained were consistently lower than the results of Cox et al. (2004) taking about 2 days longer to reach the equivalent total anthocyanin concentrations. The fruit harvested in October were still green without signs of colour change commencing, indicating little or no anthocyanin development at that stage. Prior to harvesting skin darkening was apparent in the late season harvest showing an increase in the total anthocyanin concentrations whilst the avocados were still on the tree (Cox et al., 2004). It was noted that similar patterns were followed in the development of anthocyanins in the fruit during ripening with the difference which may be due to the season of harvesting.

The anthocyanin level during ripening had a direct relationship with the firmness and an inverse relationship with carotenoid and chlorophyll levels in the skin over 13 days of ripening.

4.4.3 Pigments in the Extracted Oil (ASE)

4.4.3.1 Carotenoids

The highest concentration of lutein was in the oil extracted from the skin and decreased through the three pulp sections towards the stone (Figure 3.1c). The concentration of carotenoids in the oil extracted from the skin, dark, pale and yellow pulp sections at day 13 had all decreased. In the oil extracted from all sections the concentration of lutein was the highest of all the carotenoids at day 13 even though the day 2 concentrations of neoxanthin in the pale and yellow sections were greater than lutein. Also in the oil from pale and yellow pulp sections the concentrations of antheraxanthin were greater than lutein at day 2. The pattern of concentration levels of carotenoids in the oil extracted from each section corresponds to the pattern of level of carotenoids found in each section of fresh tissue.

4.4.3.2 Chlorophyll

The chlorophyll derivative pheophytin present in the oil may be due to the heating during accelerated solvent extraction (Ferruzi and Schwartz, 2001; Artes et al., 2002). The chlorophyll degradation during processing is indicated by the conversion of chlorophyll to pheophytin whereby the hydrogen ion replaced the magnesium ion in the porphyrin ring. The degradation of chlorophyll is related to temperature (Simpson et al., 1976).

4.4.4 Comparison of Pigments in the Fresh Tissue Sections and the Extracted Oil

The carotenoids and chlorophylls concentration values of the fresh tissue sections which are expressed in $\mu g\ g^{-1}$ FW were converted to $\mu g\ g^{-1}$ oil to enable comparison with the concentration of pigments in the extracted oil (Appendix 6).

The total carotenoids concentration in the dark pulp, pale pulp and yellow pulp showed that extracted oil resulted in a higher concentration of carotenoids at unripe stage considering that the values did not include α-carotene, β-carotene and zeaxanthin (Appendix 6). This may be that the temperature and pressure of ASE were efficient in the extraction of carotenoids from hard flesh tissue. At ripe stage, the carotenoid concentrations in the fresh tissue were greater than in the extracted oil. The Ultra Turrax

used in the extraction of pigment in the fresh tissue is efficient when the fruit was ripe as evidenced by the quick disintegration of tissue during blending.

The total chlorophylls concentration in the dark pulp, pale pulp and yellow pulp of the fresh tissue was higher than the total chlorophylls in the extracted oil during the 13 days period (Appendix 6). This may be because, numerically, aside from chlorophyll *a* and *b*, total chlorophyll in the fresh tissue consisted of other derivatives such as chlorophyllides a and *b* and three unknowns which could be the breakdown product of pheophytin and chlorophyllides which were not present in the oil such as pyropheophytin, pheophorbide and pyropheophorbide or other chlorophyll epimers. Also, it would seem that chlorophylls have been degraded during accelerated extraction due to the temperature.

The total chlorophyll and total carotenoid concentrations in the oil extracted from the skin were considerably lower than the concentrations extracted from the fresh tissue (Appendix 6). This may be because the chlorophylls and carotenoids in the skin were bound. The pressure and temperature in ASE may not be as efficient in the extraction of fresh tissue which had been left soaking in solvent over a long period of time (overnight) and thereby through osmosis was able to penetrate the tight tissue of the skin resulting in a higher carotenoid and chlorophyll concentrations in the fresh tissue.

Chlorophyllides *a* and *b* were detected in the fresh tissue but mostly in skin. The inner surface of the skin turned brown rapidly. Browning is usually thought to be due to the oxidation of phenolics by the enzyme polyphenol oxidase (Simpson et al., 1976). In avocado fresh tissue browning might be due to the degradation of chlorophyll (chlorophyllides formation). Chlorophyllides are formed due to chlorophyllase enzymes which is specific in the mechanism of chlorophyll degradation. In the pulp of fresh tissue, chlorophyllides sometimes appeared and were present in trace quantities or were absent. This may be because chlorophyllase enzymes in the pulp were present in trace amounts and were not as active. Alternatively, the skin tissue may be more susceptible to chlorophyllase enzymes than the pulp tissue.

In the pigment trials in fresh tissue there may be a change in chlorophyllase activity because once the fruit had ripened the skin samples were being peeled from rather than

being cut from the pulp. This would mean less tissue damage and thus lower levels or absence of chlorophyllide.

In the oil chlorophyllides *a* and *b* were not detected. It may be chlorophyllase enzymes were deactivated by the temperature during accelerated solvent extraction. Chlorophyllides are water soluble so it could be that during accelerated solvent extraction, the chlorophyllides were absorbed by the diatomaceous earth mixed in the powdered freeze dried avocado samples together with moisture.

4.5 Conclusions

The pigments identified during the 13 days of ripening at 20°C in the fresh avocado tissue were chlorophyll *a* and *b*, pheophytin *a* and *b*, chlorophyllides *a* and *b*, lutein, β-carotene, neoxanthin, violaxanthin, zeaxanthin, antheraxanthin and α-carotene.

The major anthocyanin identified in the skin was cyanidin 3-*O*-glucoside and the changes in this pigment were highly correlated with ripening. There was an on-going increase in the concentration of the total anthocyanins.

The pigments chlorophyllides *a* and *b* were absent in the extracted oil as were violaxanthin, zeaxanthin and neoxanthin. There was a general decrease in the carotenoids and chlorophylls concentrations in the fresh tissue and the extracted oil. The pigment composition of the avocado fresh tissue corresponded to that of the extracted oil. Also, the colour patterns of the fresh tissue and extracted oil corresponded to the pigment composition.

New Zealand 'Hass' avocado contained high amounts of chlorophyll and the carotenoid, lutein. The highest concentration of chlorophylls and carotenoids were found to be in the skin and decreased towards the fruit centre. The optimum time to extract the oil was between days 6 and 8 after harvest corresponding to the firmness value between 24 and 60 in order to maximise the chlorophyll and carotenoids levels and the oil yield.

Chapter 5 Avocado Oil with Skin Addition

5.1 Introduction

The principal factors that cause lipid oxidation are oxygen, enzymes, temperature and light (Erickson, 2002; Min and Boff, 2002).

Volatile compounds are formed rapidly in soybean oil in the presence of light, chlorophyll, and oxygen due to the oxidation of singlet oxygen (Min and Boff, 2002). Chlorophyll *a* and *b* and pheophytin *a* and *b* derivatives in commercial edible oils both showed antioxidant capabilities under dark conditions (Endo et al., 1985). Chlorophyll *a* was found to offer the strongest protection against oxidation. They suggested that chlorophyll prevented deterioration due to oxidation in vegetable oils provided they were stored at low temperature and in the dark. It was suspected that chlorophyll inhibited autooxidation of oil by acting as a free radical scavenger (Endo et al., 1985).The antioxidant effect of chlorophyll and pheophytin was lower at higher temperature (50°C). Conversely, the decomposition rates of chlorophyll and pheophytin during autooxidation at 50°C were greater than those at 30°C. The decreased inhibitory effect of chlorophyll was not only due to the formation of hydroperoxides, but also to their instability at high temperature (Endo et al., 1985). It was suggested that chlorophyll and pheophytin are considered as antioxidants in oil with a high level of unsaturated fatty acids (Endo et al., 1985).

The estimation of shelf life involves consideration of the storage conditions and specific kinetic equations for the deteriorative reaction studied in the specific food product. A common method of determining data for shelf life calculations is by accelerated storage tests whereby products are stored at elevated temperatures (60°C-70°C) which encourages degradation (Kristott, 2000; Man, 2002; Tauokis and Giannakourou, 2004). Samples are removed from storage at pre-determined intervals for testing to determine a point where the fat or oil becomes unacceptable (Kristott, 2000).

Hydroperoxides, the initial degradation product is measured by peroxide value (PV) tests (Kristott, 2000). Other tests often employed for determining oil quality are free fatty acid concentration and anisidine value (AnV), thiobarbituric acid test, extinction at 230-270nm and total oxidation value (Kristott, 2000). In some cases where there is

more than one important factor and therefore different activation energy and rates for each deteriorative reaction, there is the potential for one to dominate at a particular temperature (Taoukis and Labuza, 1996).

The aim of this research was to determine the influence of levels of skin tissue present during cold pressed extraction on oil quality, pigment levels, colour, antifungal compound (diene levels) and stability of oil by accelerated storage testing.

5.2 Materials and Methods

5.2.1 Experimental Overview

Fruit quality was assessed immediately prior to extraction. Cold pressed extraction was carried out in the laboratory with different levels of skin. In addition, cold pressed extraction in the factory with three levels of skin was carried out. Oil was analysed for free fatty acid (FFA), fatty acid composition (FAMES), peroxide value (PV), chlorophylls, carotenoids, colour, and diene levels. Oil was stored at different temperatures for 97 days and tested for change in quality.

Refer to Chapter 3 for detailed methodology.

5.2.2 Laboratory Cold Pressed Extraction of Avocado Oil

5.2.2.1 Calculations

For each 100g the average distribution of stone, skin and flesh determined from the selection of 45 avocado fruit was determined to be 18%, 14% and 68%, respectively.

The level of skin in the malaxer was varied between 0-100%, where 100% skin was equal to 18g/100g (skin/flesh). The amounts of skin added per 1000g mixture of skin and flesh for 0%, 5%, 10%, 20%, 40%, 70% and 100% were 0g, 9g, 18g, 36g, 72g, 126g and 181g. The fruit was held at 5.5°C over 3 days of extraction. One replicate for each level of skin was extracted each day.

5.2.3 Commercial/Factory Extraction

Nine bins of fruit were used to produce three formulations (≈750kg/3 bins) of commercially extracted oils. The weight of fruit used for 100%, 40% and 5% skin were 789kg, 764kg and 775kg respectively.

5.3 Results

5.3.1 Cold Pressed Laboratory Scale

5.3.1.1 Avocado Fruit Firmness and Colour

The lightness and chroma decreased ($p<0.05$) due to the colour of the skin becoming darker and less vivid as the fruit ripened, The hue angle of the fruit used over the 3 days of cold pressed laboratory scale oil extraction was the same ($p>0.05$). The firmness values of the fruit used for avocado oil extraction increased over the three days of oil extraction. This demonstrated increased ripeness of the fruit (softening) from day 1 to 3. The firmness and the colour correlated with the ripening of fruit (Table 5.1).

Table 5.1.Colour and Firmness of Fruit Used in Cold Pressed Extraction of Avocado Oil (n=10)

Day of Extraction	Colour			
	Lightness	Chroma	Hue Angle	Firmness
Day 1	103 ±2	10 ±2	281 ±19	71 ±19
Day 2	92 ±7	12 ±2	173 ± 27	75 ±27
Day 3	87 ±7	6 ±6	199 ±33	80 ±33

5.3.1.2 Free Fatty Acid (FFA), Peroxide Value (PV) and Colour

The FFA for cold pressed oil extracted from avocados in the laboratory for 5% to 100% skin additions ranged from 0.18 to 0.23 % FFA as oleic acid (Figure 5.1).

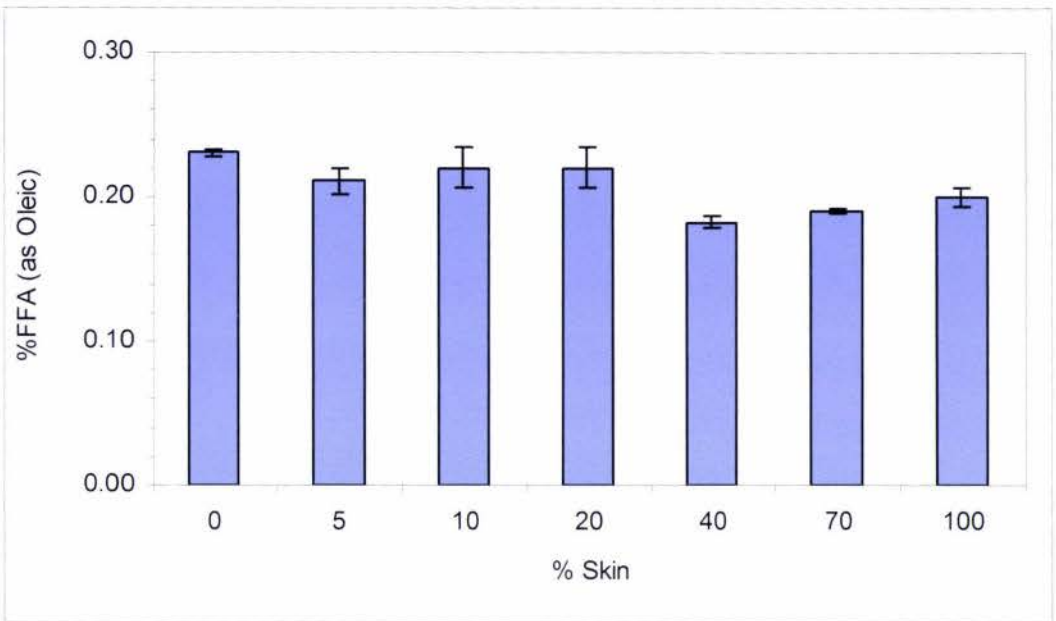


Figure 5.1.Mean free fatty acid of avocado oil from cold pressed extraction in the laboratory (n=2)

The peroxide value for cold pressed oil extracted from avocados in the laboratory for 5% to 100% skin addition were also within a narrow range of 1.82 to 1.99 meq/kg oil and not significantly different indicating that the quality of oil for the seven levels of skin addition were similar (Figure 5.2).

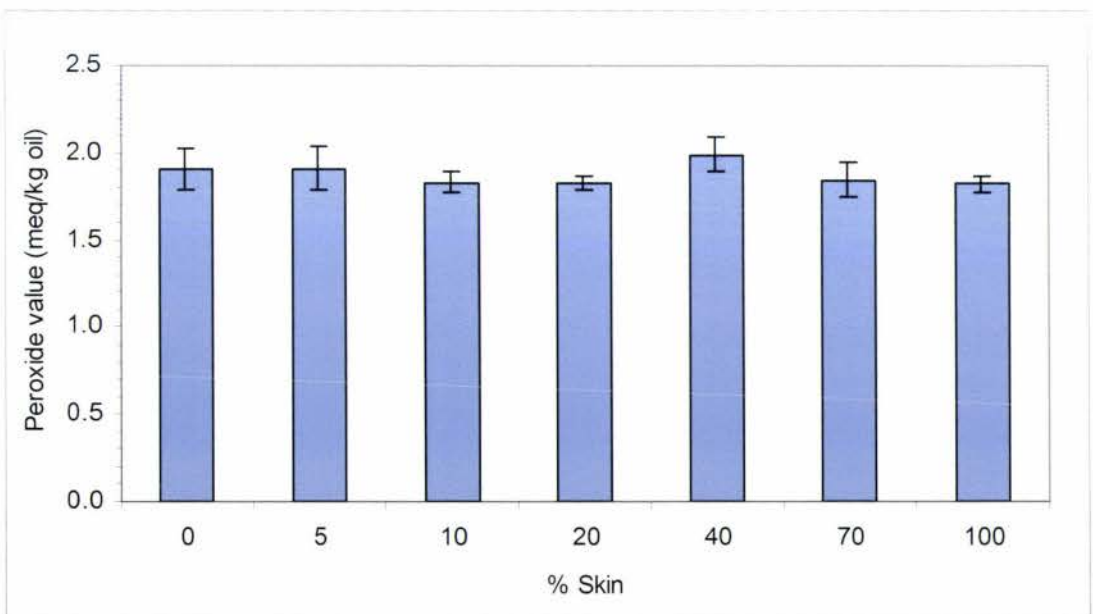


Figure 5.2.Mean peroxide values of avocado oil from cold pressed extraction in the laboratory (n=2)

The colour of the oil (Figure 5.3) with increasing levels of skin added from 0 to 100% obtained from cold pressed extraction in the laboratory was significantly different ($p<0.05$). For 0% to 100% skin additions, the colour space *a* value decreased from -0.12 to -5.47 and the colour space *b* values decreased from 9.31 to -1.87. The lightness (L) decreased from 106.02 to 99.63, chroma (c) decreased from 14.73 to 11.90 and hue angle increased from 171.33 to 222.03 for 0% to 100% skin additions. Overall, the changes in these colour spaces indicated that the colour of the oil became a darker green/with increasing skin additions due to the increased chlorophyll extracted from the avocado skin.

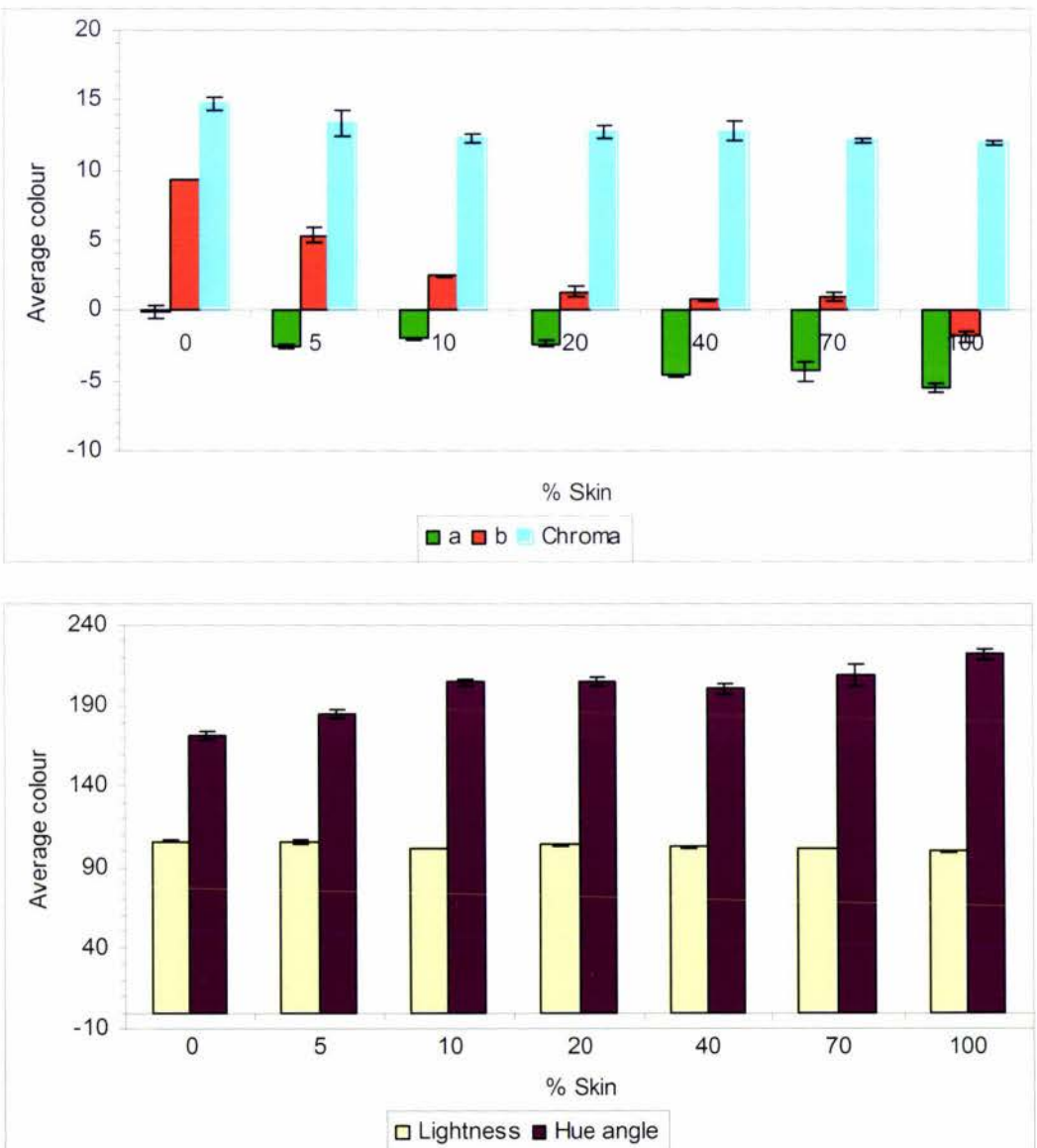


Figure 5.3 Colour of avocado oil from cold pressed extraction in the laboratory (n=3)

5.3.1.3 Carotenoids (HPLC)

Carotenoids were analysed using HPLC and the results are shown in Figure 5.4 (Raw Data, Appendix 10).

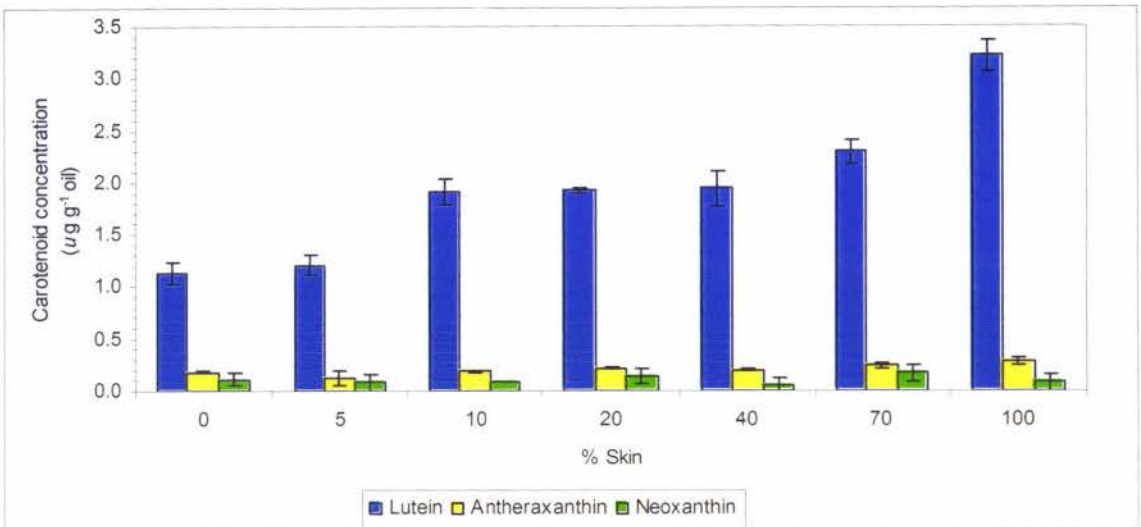


Figure 5.4.Carotenoids concentration. Lutein, antheraxanthin and neoxanthin concentration of avocado oil produced by cold pressed extraction with different levels of skin addition (n=3)

As the percentage of skin addition increased from 0% to 100% during cold pressed oil extraction, the lutein concentration in the oil increased from 1.13µg g^{-1} to 3.21µg g^{-1} (Figure 5.4).This would be expected as the concentration of lutein in avocados is highest in the skin. The concentration of antheraxanthin in the extracted oil increased as the percentage of skin addition increased and was about 10% of that for lutein at various skin content percentages. Of the three carotenoids detected in the oil, neoxanthin had the lowest concentration being 5% of that for lutein (Figure 5.4). Violaxanthin and zeaxanthin were not detected in the cold pressed avocado oil produced in the laboratory.

5.3.1.4 Chlorophyll (HPLC)

Chlorophylls were analysed using HPLC and the results are shown in Figure 5.5 (Raw Data, Appendix 10).

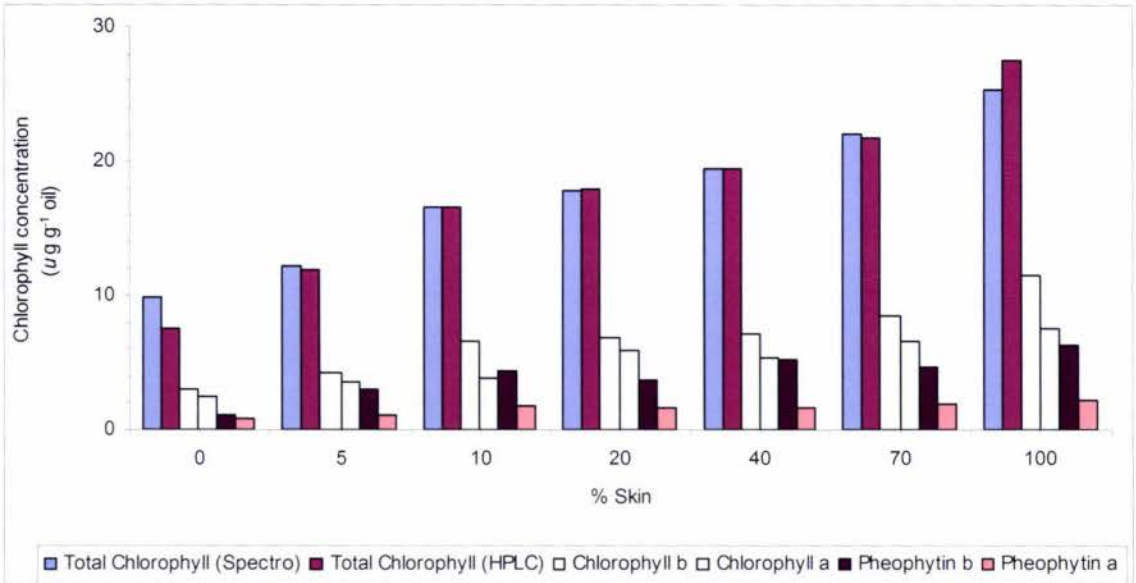


Figure 5.5.Total chlorophyll, chlorophyll *b*, chlorophyll *a*, pheophytin *b* and pheophytin *a* in avocado oil with different levels of skin addition (n=3)

The total chlorophyll in avocado oil with various percentages of skin addition was also analysed by UV spectrophotometer and results were in agreement with HPLC ($p>0.05$) (Figure 5.5).

The total chlorophyll in the cold pressed extracted oil increased as the percentage of skin addition increased. The chlorophyll *a* concentration increased from 2.52μg g^{-1} at 0% skin addition to 7.51μg g^{-1} at 100% skin addition, an increase of 2.98 times. Chlorophyll *b* increased in concentration from 3.00μg g^{-1} for 0% skin addition to 11.48μg g^{-1} for skin addition of 100%. The concentration of pheophytin *a* increased from 0.86μg g^{-1} at 0% skin addition to 2.25μg g^{-1} at 100% skin addition an increase of 2.62 times. Likewise the concentration of pheophytin *b* at 0% skin addition was 1.13μg g^{-1} and 6.24μg g^{-1} at 100% skin addition. In all cases the contents of chlorophyll *a* and *b* and pheophytin *a* and *b* increased resulting in an overall increase of total chlorophylls of 3.66 times from 0% addition to 100% skin addition ($p<0.05$).

5.3.2 Factory Trial

5.3.2.1 Avocado Fruit and Oil Quality (Colour, Firmness)

The lightness of the fruit used for cold pressed extraction of three levels of skin starting from 5%, 40% and 100% decreased from 93.11 to 86.49 ($p<0.05$) whereas the chroma increased from 16.00 to 18.19 ($p<0.05$) and the hue angle was similar 314.44, 315.41 and 317.03 respectively ($p>0.05$). In all cases n=45.

Fruit firmness was similar ($p>0.05$) indicating the same degree of ripeness. The firmness for each formulation at 5%, 40% and 100% skin addition were 80 ± 0.2, 75 ± 0.2 and 65 ± 0.2 FV respectively. In all cases n=45.

5.3.2.2 Fatty Acid Compositions (FA), Free Fatty Acid (FFA) and Peroxide Value (PV)

The FA compositions were also relatively the same as shown in Table 5.2 ($p>0.05$).

Table 5.2.Fatty Acid Composition (%) of Avocado Oil Day 0

Formulations	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic
5% Skin	12.20	4.58	0.29	73.70	9.36	0.37
40% Skin	10.77	3.81	0.27	75.63	9.08	0.44
100% Skin	10.85	4.07	0.25	75.39	9.09	0.44

The FFA and PV for cold pressed oil extracted from avocados in the factory for all formulations were the same with PV of 0.37meq/kg oil and FFA of 0.1%.

5.3.2.3 Peroxide Value during Storage

The peroxide values during storage are shown in Figure 5.6 (Raw Data, Appendix 11).

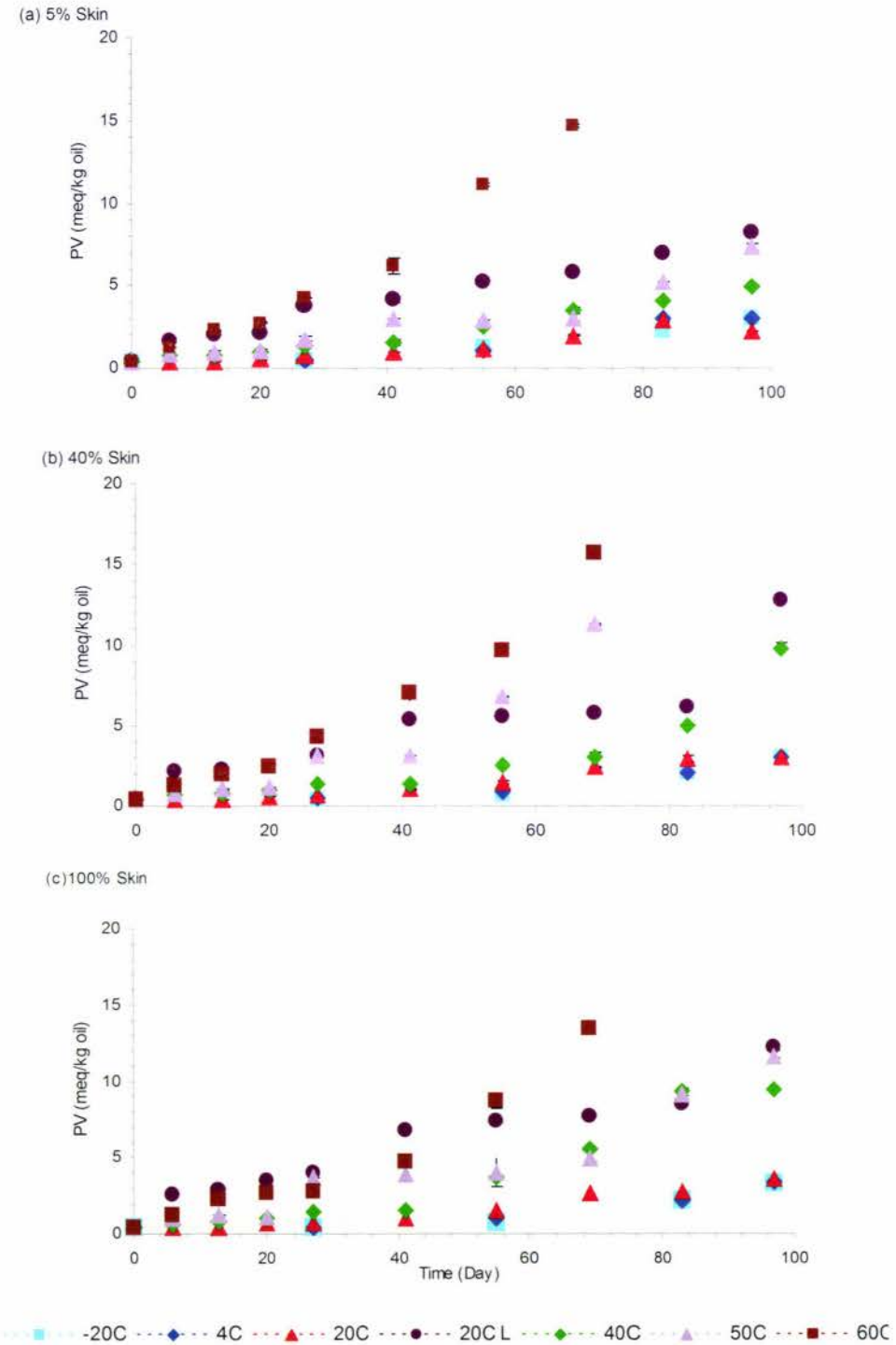


Figure 5.6.PV of avocado oil with (a) 5% skin, (b) 40% skin and (c) 100% skin addition stored at -20°C, 4°C, 20°C, 20°C light, 40°C, 50°C and 60°C (n=2)

For oil with 5% skin (Figure 5.6a) after 97 days of storage in the dark, as the storage temperature increased so did the PV of the oil. At day 97 the PV was at -20°C (3.01meq/kg), 4°C (3.02meq/kg), 20°C (2.26meq/kg), 40°C (4.88meq/kg) and 50°C (7.36meq/kg). The PV for the samples stored at 60°C reached a value of 11.09meq/kg by day 55. The sample stored at 20°C in the light behaved in the same manner with the PV increasing with time, reaching 8.25meq/kg by day 97, but at a higher PV than the sample stored at 20°C in the dark and also higher than the samples stored at 40°C and 50°C in the dark.

For oil with 40% skin (Figure 5.6b), after 97 days of storage it was noted that for all the samples stored in the dark that as the storage temperature increased so did the PV. At day 97 the PV were at -20°C (2.98meq/kg), 4°C (3.00meq/kg), 20°C (2.99meq/kg), 40°C (9.79meq/kg). At 50°C the PV was 11.27meq/kg after 69 days. Also at day 69 the PV for the samples stored at 60°C reached a value of 15.69meq/kg. The sample stored at 20°C in the light behaved in the same manner with the PV increasing with time, reaching 12.74meq/kg by day 97, but at a higher PV than the sample stored at 20°C in the dark and also higher than the samples stored at 40°C in the dark.

For oil with 100% skin (Figure 5.6c), after 97 days of storage it was noted that for all the samples stored in the dark that as the storage temperature increased so did the PV. At day 97 the PV at -20°C was 3.25meq/kg; at 4°C, 3.41meq/kg; at 20°C, 3.54meq/kg; at 40°C, 9.48meq/kg; at 50°C, 11.63meq/kg. The PV for the samples stored at 60°C reached a value of 13.44meq/kg by day 69. The sample stored at 20°C in the light behaved in the same manner with the PV increasing with time, reaching 12.20meq/kg by day 97, but at a higher PV than the sample stored at 20°C in the dark and also higher than the samples stored at 40°C and 50°C in the dark.

The avocado oil extracted from flesh tissue with lower level of skin addition was more stable against oxidation than oil with higher percentages of skin addition.

5.3.2.4 Total Chlorophyll (Spectrophotometer)

The total chlorophyll concentration of factory produced avocado oil were analysed by spectrophotometer. Results are shown in Figure 5.7 (Raw Data, Appendix 16, and Chromatogram, Appendix 17).

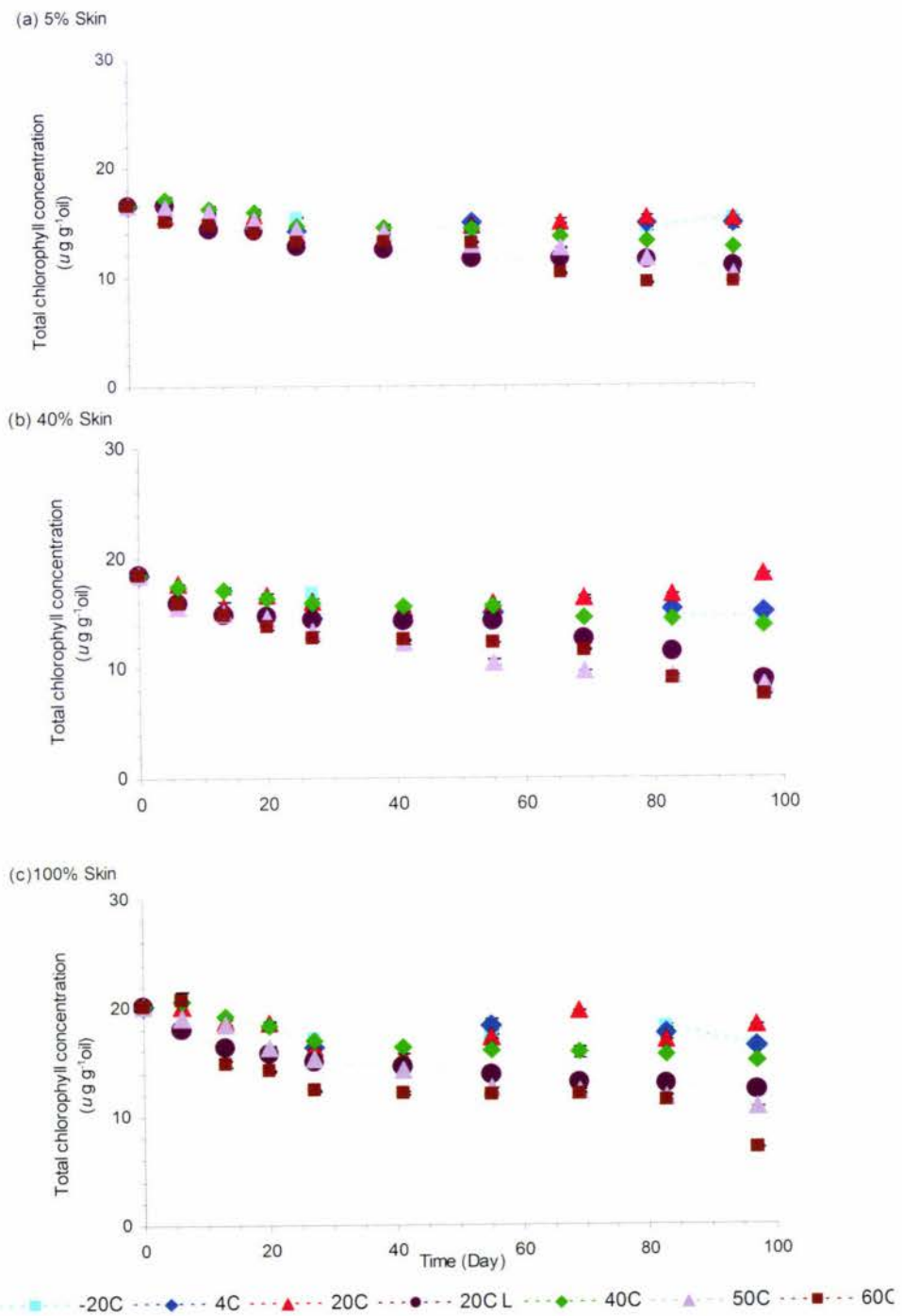


Figure 5.7.Total chlorophyll of avocado oil determined by spectrophotometer with (a) 5% skin, (b) 40% skin and (c) 100% skin stored at -20°C, 4°C, 20°C, 20°C light, 40°C, 50°C and 60°C (n=2)

The total chlorophyll concentration of factory produced avocado oil at day 0 as determined by spectrophotometer for the three formulations 5%, 40% and 100% skin were 16.54μg g^{-1}, 18.69μg g^{-1} and 20.08μg g^{-1} respectively.

By day 97, the concentrations for the 5% skin addition avocado oil, had decreased to 15.16μg g^{-1}, 14.76μg g^{-1}, 15.16μg g^{-1}, 12.64μg g^{-1}, 10.15μg g^{-1} and 9.32μg g^{-1} for the samples stored in the dark at -20°C, 4°C, 20°C, 40°C, 50°C and 60°C respectively. The total chlorophyll concentration for the sample stored at 20°C in light was 10.79μg g^{-1}. It was noted that for the lower storage temperatures (-20°C, 4°C and 20°C) the retention of chlorophylls were similar and were greater than at higher temperatures. At temperatures 40°C, 50°C and 60°C the retention decreased as the temperature increased. The retention at 20°C for the sample stored in light was less than the sample stored at 40°C.

By day 97, the concentrations for the 40% skin addition avocado oil, had decreased to 14.54μg g^{-1}, 15.00μg g^{-1}, 18.47μg g^{-1}, 13.79μg g^{-1}, 8.28μg g^{-1} and 7.44μg g^{-1} for the samples stored in the dark at -20°C, 4°C, 20°C, 40°C, 50°C and 60°C respectively. The total chlorophyll concentration for the sample stored at 20°C in light was 8.28μg g^{-1}. It was noted that for the lower storage temperatures (-20°C, 4°C and 20°C) the retention of chlorophylls were similar and were greater than at the three highest temperatures. The retention of total chlorophylls was greatest at 20°C at 18.47μg g^{-1}. At the temperatures 20°C, 40°C, 50°C and 60°C the retention decreased as the temperature increased. The retention at 20°C for the sample stored in light was less than the sample stored at 40°C but slightly more than that stored at 50°C.

By day 97, the concentration for the 100% skin addition avocado oil, had decreased to 16.32μg g^{-1}, 16.43μg g^{-1}, 18.40μg g^{-1}, 14.93μg g^{-1}, 10.73μg g^{-1} and 7.03μg g^{-1} for the samples stored in the dark at -20°C, 4°C, 20°C, 40°C, 50°C and 60°C respectively. The total chlorophyll concentration for the sample stored at 20°C in light was 12.25μg g^{-1}. It was noted that for the lower storage temperatures (-20°C, 4°C and 20°C) the retention of chlorophylls in the oil were similar and were greater than at the three highest temperatures. At temperatures 20°C, 40°C, 50°C and 60°C the retention decreased as

the temperature increased. The retention at 20°C for the sample stored in light was less than the sample stored at 40°C.

5.3.2.5 Shelf Life Based on PV, Total Chlorophyll Concentration at the End of Shelf Life and Activation Energy

Four rates of reaction equations can be written depending on the order of reaction (Levenspiel, 1999).

1. Zero order reaction formula: $C_A=C_{A0}-kt$
2. Half order reaction formula: $\sqrt{C_A} = -0.5 \times kt + \sqrt{C_{Ao}}$
3. First order: $\ln C_A=\ln C_{A0} -kt$
4. Second order: $\frac{1}{C_A} = \frac{1}{C_{Ao}} + kt$

Where:

C_A = Concentration of component A

C_{AO} = Concentration at time 0

t = Time

k = Rate of change of concentrations

Based on linear regression (Raw data, Appendix 11; Summary, Appendix 13) for the peroxide value (PV) for avocado oil with 5%, 40% and 100% skin added and stored in the dark at -20°C, 4°C, 20°C, 40°C, 50°C and 60°C temperatures and also at 20°C stored in the light, zero order reaction (concentration versus time) was used/tested to predict shelf life of avocado oil. The linear regression of the same formulations and temperatures for 0.5 order (square root of concentration versus time), first order (natural logarithm of concentration versus time) and second order (reciprocal of concentration versus storage time) had been provided in Appendix 13 to calculate shelf life if any of these order is to be used/tested.

Given C_A=20meq/kg for PV which is the maximum concentration used to predict the shelf life of olive oil based on IOOC standards (IOOC, 2003) and which has been adopted for avocado oil. The initial PV concentration of component C_{A0} was 0.37meq/kg oil for 5%, 40% and 100% skin addition. The rates of reaction constants (k) at 20°C obtained from the zero order plots were 0.025meq kg^{-1} day^{-1}, 0.031meq kg^{-1} day^{-1} and 0.034meq kg^{-1} day^{-1} for 5%, 40% and 100% skin respectively.

The shelf life of avocado oil stored at 20°C was determined using the zero order reaction equation (Levenspiel, 1999) and were found to be 780 days (26 months), 630 days (21 months) and 570 days (19 months) for avocado oil with 5%, 40% and 100% skin respectively (Calculation, Appendix 14a).

Based on the shelf life of avocado oil determined using the PV for 5%, 40% and 100% skin additions, a check on the quantities of the total chlorophylls remaining were calculated on reaching the respective she1f lives. The total chlorophyll concentrations using the values obtained from HPLC (Appendix 18) were initially 13.3μg g^{-1} (5% skin), 15.7μg g^{-1} (40% skin) and 16.3μg g^{-1} (100% skin). At the end of shelf lives the total chlorophylls were calculated using second order reactions gave 4.32μg g^{-1}, 3.96μg g^{-1} and 3.46μg g^{-1} of avocado oil respectively (Calculation, Appendix 14b).

The linear regression ln k versus 1/T for avocado oil with 5%, 40% and 100% skin added was shown in Figure 5.8.

The hydroperoxide formation determined by peroxide value found to follow Arrhenius relationship. From the formula slope $= \frac{-Ea}{R}$, using the values of slope from Figure 5.8 plotted using the values from zero order, the activation energy was found to be 1386.8 joule mol^{-1}, 2060.2 joule mol^{-1} and 1605.1 joule mol^{-1} for avocado oil with 5%, 40% and 100% skin respectively (Calculation, Appendix 14c).

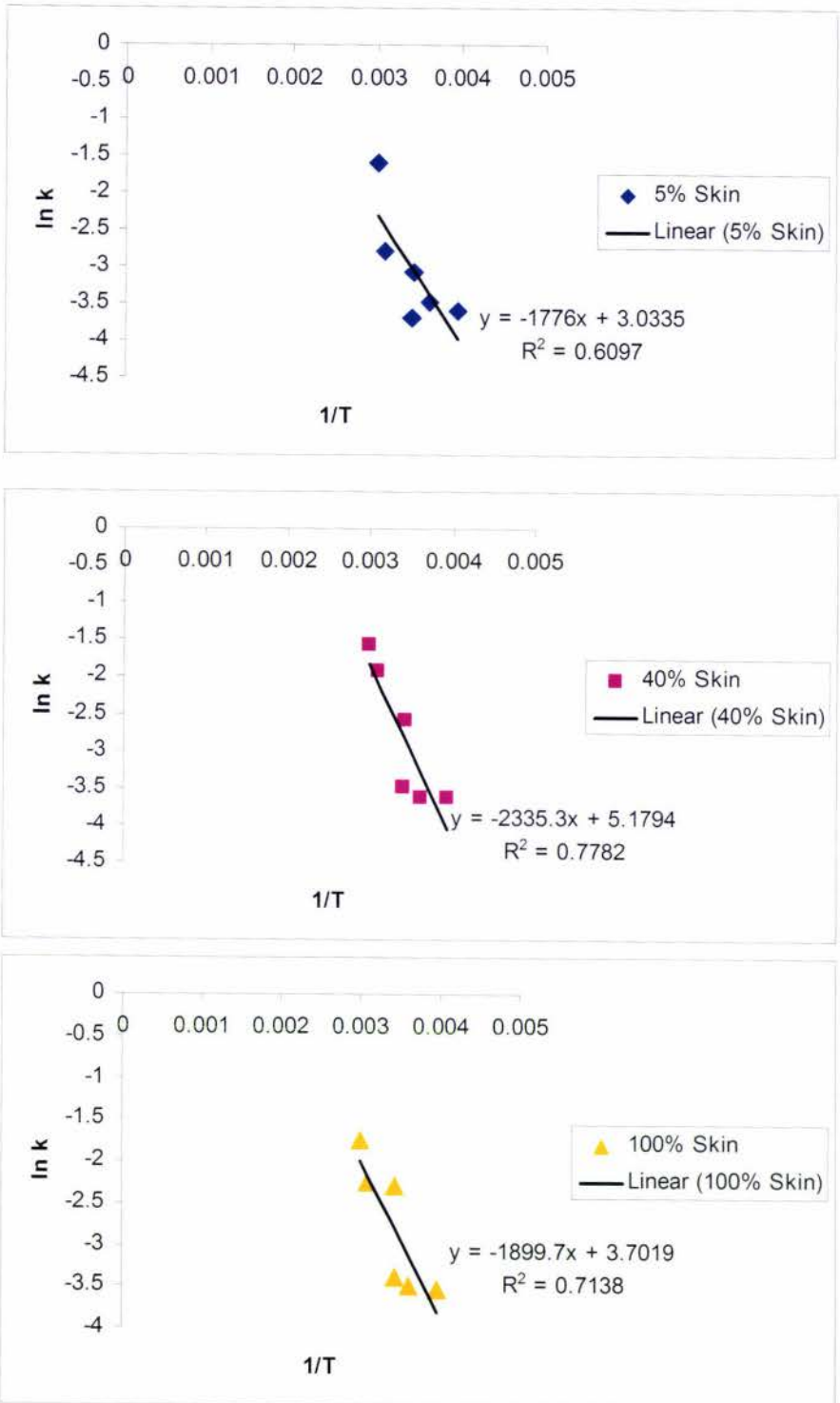


Figure 5.8.The Arrhenius plots for the peroxide formation of avocado oil with 5% skin, 40% skin and 100% skin

5.3.2.6 Chlorophylls and Carotenoids (HPLC)

The total chlorophyll concentrations of factory produced avocado oil were also analysed by HPLC. Results are shown in Figure 5.9 (Raw Data, Appendix 18).

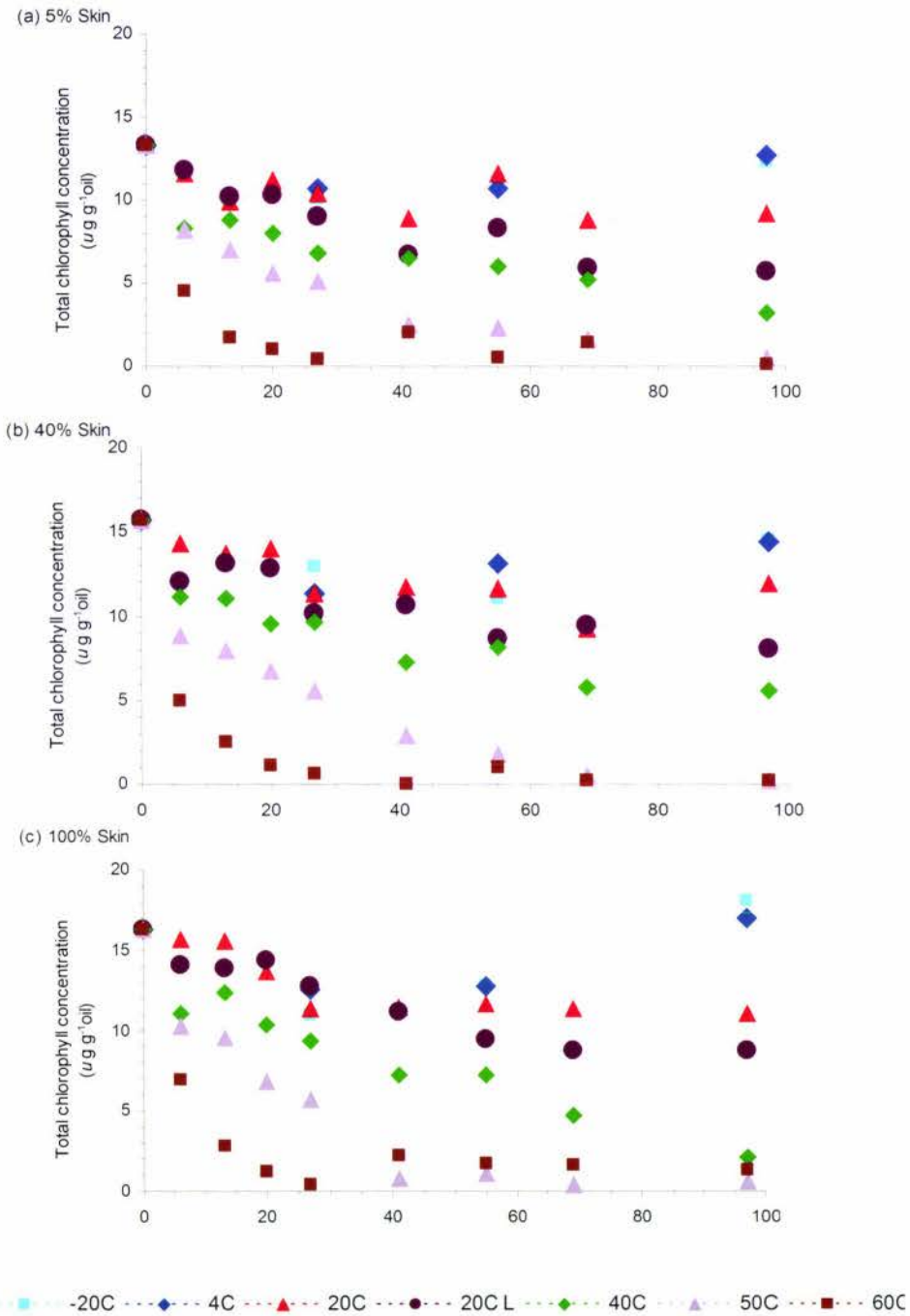


Figure 5.9.Total chlorophyll of avocado oil determined by HPLC with (a) 5% skin, (b) 40% skin and (c)100% skin addition stored at -20°C, 4°C, 20°C, 20°C light, 40°C, 50°C and 60°C (n=1)

The total chlorophylls measured by HPLC (Figure 5.9a to c) at day 0 for the three formulations (5%, 40% and 100%) skin additions were 13.3μg g^{-1}, 15.7μg g^{-1} and 16.3μg g^{-1} respectively. The concentrations of chlorophyll *a* were 4.9μg g^{-1}, 4.8μg g^{-1} and 5.4μg g^{-1} for the 5%, 40% and 100% skin addition respectively at day 0. For the chlorophyll *b*, the concentrations at day 0 were 5.1μg g^{-1}, 6.0μg g^{-1} and 6.2μg g^{-1} for the 5%, 40% and 100% formulations respectively. Pheophytin *a* concentrations at day 0 for the 5%, 40% and 100% formulations the concentrations were 1.1μg g^{-1}, 1.4μg g^{-1} and 1.6μg g^{-1} respectively. For the 5%, 40% and 100% formulations the concentrations of pheophytin *b* were 2.2μg g^{-1}, 3.4μg g^{-1} and 3.0μg g^{-1} at day 0 respectively. As the percentage of skin addition increased so did the concentration of the chlorophylls generally increased. For each of the samples stored in the dark at day 97 at low temperatures (-20°C and 4°C), the chlorophyll concentrations remained the same. At 20°C there was a decline in chlorophyll concentrations to about 70% of the day 0 values. At 40°C, the chlorophylls had declined to levels which were about 25%-35% of the day 0 values. At 50°C and 60°C the chlorophylls at day 97 had trace concentration levels. The chlorophylls concentration in the oil stored at 20°C in the light were all lower than those stored at the same temperature in the dark but higher than those stored at 40°C at day 97.

Chlorophylls identified in the avocado oil produced from the factory trial were chlorophylls *a* and *b* and pheophytins *a* and *b*. Chlorophyllides *a* and *b* were not detected in the avocado oil produced from the factory. This showed that water was removed efficiently after centrifugation and chlorophyllides could have been washed out being water soluble chlorophyll derivatives (Table 5.3).

The five carotenoids neoxanthin, violaxanthin, antheraxanthin, lutein and zeaxanthin found in the skin and pulp of 'Hass' avocados were analysed in the cold pressed avocado oil by HPLC for all formulations produced in the factory (Table 5.4).

Neoxanthin, violaxanthin and zeaxanthin were not present at day 0. Antheraxanthin remained unchanged from day 0 to day 97 storage in all storage conditions indicating that neither light nor temperature were contributory factors to the degradation of antheraxanthin. The concentration of lutein stored under dark conditions from -20°C up to 40°C and oil stored at 20°C in the light were the same from day 0 to day 97, however,

the retention of lutein in the oil stored at 50°C and 60°C was lower, but only slightly. Overall, this lutein was stable under all storage conditions (Table 5.4).

Table 5.3.Concentrations of Chlorophylls Compounds in Avocado Oil with 5%, 40% and 100% Skin Addition at Day 0 and Day 97 at Different Temperatures

Chlorophylls	% Skin	Day 0	Day 97						
		Day 0	-20C	4C	20C	20C L	40C	50C	60C
		μg g^{-1}							
Chl a	5%	4.9	4.5	3.7	2.7	2.1	1.1	0.0	0.0
	40%	4.8	4.5	4.4	3.9	2.7	1.2	0.0	0.1
	100%	5.4	7.3	6.7	1.3	2.8	0.6	0.1	0.0
Chl b	5%	5.1	4.5	4.5	2.7	2.5	1.3	0.1	0.0
	40%	6.0	4.9	5.1	3.8	3.0	2.2	0.1	0.0
	100%	6.2	6.9	6.3	3.6	3.0	1.0	0.3	0.1
Pheo a	5%	1.1	1.2	1.2	0.8	0.7	0.3	0.1	0.0
	40%	1.4	1.6	1.3	0.9	0.7	0.6	0.1	0.0
	100%	1.6	1.6	1.5	1.4	0.9	0.4	0.1	1.2
Pheo b	5%	2.2	2.1	3.2	1.8	0.4	0.4	0.2	0.0
	40%	3.4	3.3	3.6	3.2	1.7	1.6	0.0	0.0
	100%	3.0	2.3	2.6	4.9	2.1	0.1	0.2	0.0
Total Chl	5%	13.3	12.3	12.7	9.2	5.7	3.2	0.5	0.1
	40%	15.7	14.5	14.4	11.9	8.1	5.6	0.2	0.2
	100%	16.3	18.1	17.0	11.1	8.7	2.1	0.6	1.3

Table 5.4.Concentration of Carotenoids Compounds in Avocado Oil with 5%, 40% and 100% Skin Addition at Day 0 and Day 97 at Different Temperatures

Carotenoids	% Skin	Day 0	Day 97						
			-20C	4C	20C	20C L	40C	50C	60C
		μg g^{-1}							
Neoxanthin	5%	0.0	0.1	0.1	0.3	0.1	0.0	0.0	0.0
	40%	0.0	0.2	0.0	0.1	0.2	0.0	0.1	0.1
	100%	0.0	0.1	0.0	0.0	0.3	0.1	0.1	0.1
Violaxanthin	5%	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
	40%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	100%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Antheraxanthin	5%	0.2	0.2	0.2	0.3	0.2	0.0	0.2	0.2
	40%	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	100%	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.4
Lutein	5%	1.6	1.9	1.8	1.9	1.7	1.8	1.6	1.3
	40%	1.5	1.5	1.7	2.0	1.7	1.8	1.2	1.3
	100%	1.6	1.7	1.8	1.8	1.7	1.5	1.5	1.0
Zeaxanthin	5%	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
	40%	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
	100%	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0

HPLC results of the most discernible carotenoids lutein are shown in Figure 5.10 (Raw Data, Appendix 18).

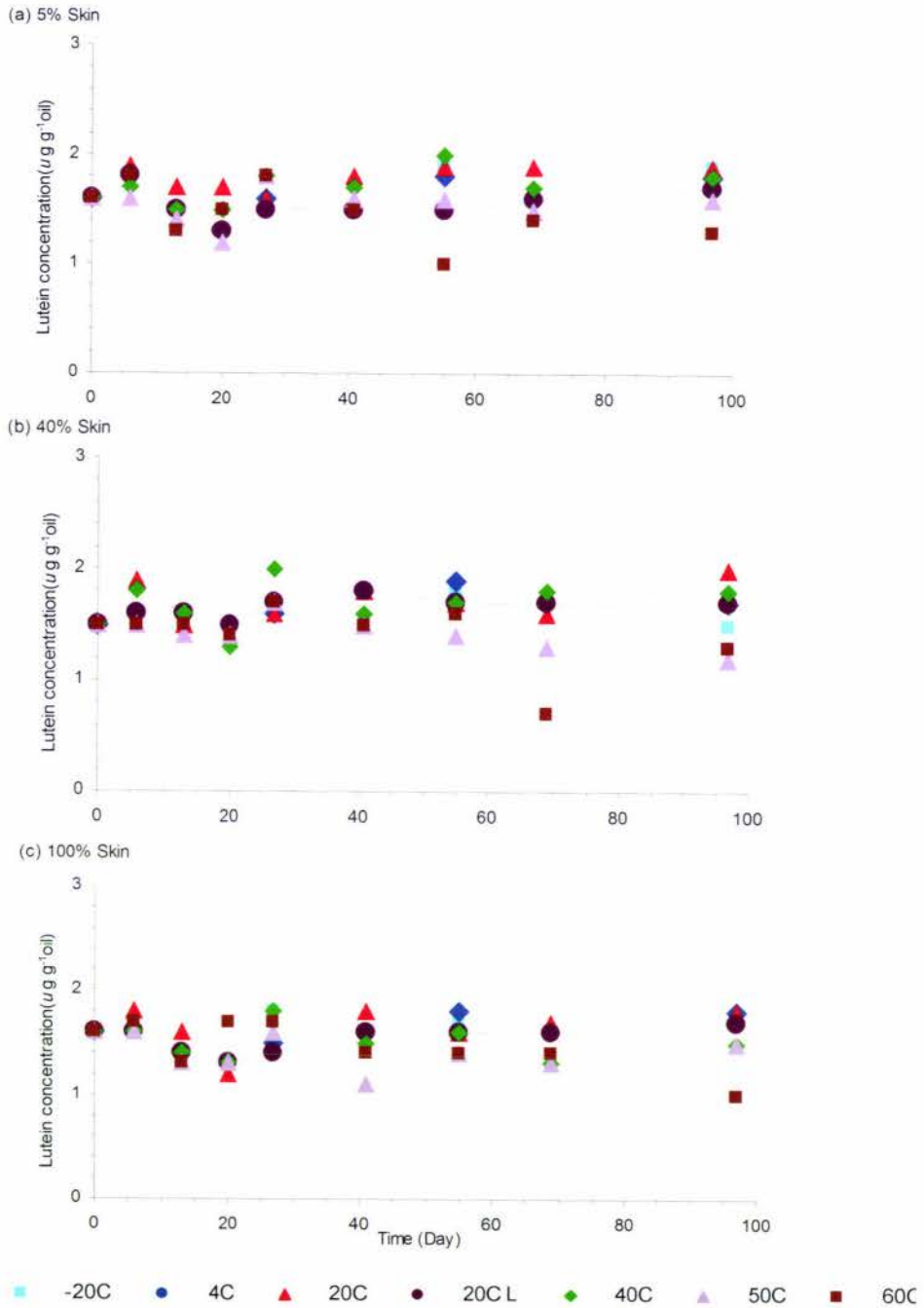


Figure 5.10.Lutein in avocado oil with (a) 5% skin, (b) 40% skin and (c) 100% skin addition stored at -20°C, 4°C, 20°C, 20°C light, 40°C, 50°C and 60°C (n=1)

5.3.2.7 Colour

The colour of the oils during the 97 day storage period at -20°C and 4°C did not change. The oil stored at 20°C in the dark experienced the smallest change in colour from green to less green whilst the oils stored at 40°C, 50°C and 60°C all changed from the green area of the colour space towards the less green area. Oil stored at 20°C in light showed a change between those for 20°C and 40°C stored in the dark.

The colour space *a* value was measured during 97 days of storage and is shown in Figure 5.11a, b and c. The avocado oil with 5%, 40% and 100% skin had initial colour space *a* values of -1.29, -2.06 and -2.68 respectively. After 97 days of storage in the dark at -20°C, 4°C, and 20°C the colour space *a* values changed to -0.82, 1.65 and 0.40 for the 5% skin, -2.30, 0.24 and 0.65 for the 40% skin and -1.82, 0.95 and 0.24 for the 100% skin respectively. All changes were from the green to the less green area of the colour space. The changes in the colour space *a* values for the oil stored for 97 days at 40°C, 50°C and 60°C in the dark were to 4.38, 5.48 and 6.32; 5.04, 5.83 and 6.63; and, 3.77, 5.85 and 5.82 for the 5%, 40% and 100% skin respectively. All the changes were greater than for the oils stored at lower temperatures and were all from the green towards the less green area of the colour space. For the oil stored in light at 20°C the changes in *a* value were to 2.21, 5.94 and 3.26 over the 97 days storage for the 5%, 40% and 100% skin additions respectively. In all cases the changes were greater than the oil stored at 20°C and less than that at 40°C in the dark. The change was from green towards the less green area of the colour space. The change in the colour space *a* values at each storage condition showed an increase towards the less green area from the green area of the colour space as the percentage of skin addition increased and as the temperature increased (Figure 5.11a, b and c).

The colour space *b* value was measured during the 97 days and is shown in Figure 5.12a, b and c. The initial colour space *b* values were 1.79, -0.53 and -2.42 for the oil stored with 5%, 40% and 100% skin. After 97 days the colour space *b* values for oils stored in the dark at -20°C, 4°C and 20°C changed to -0.94, -4.10 and 1.98; -1.44, -4.46 and -1.56; and, -1.41, -4.29 and -3.69 for 5%, 40% and 100% skin respectively. For oil stored at 40°C, 50°C and 60°C in the dark the colour space *b* changes were to 2.76, 5.09 and 6.51; 3.19, 7.99 and 7.91; and, 2.57, 4.33 and 6.79 for the 5%, 40% and 100% skin respectively. These changes were all larger than those for the lower temperatures. The colour space *b* changes were to 2.84, 3.72 and 2.01 for the oil stored at 20°C in light.

These changes were larger than those for the 20°C but less than 40°C storage temperatures in the dark. In all cases the changes in the colour space *b* were from the green towards the less green area with the exception of the two lower temperatures (Figure 5.12a, b and c).

The vividness value was measured during the 97 days and is shown in Figure 5.13a, b and c. The initial vividness (chroma) values were 1.89, 2.03 and 3.08 for the extracted oil with 5%, 40% and 100% skin respectively. After 97 days in the dark at -20°C, 4°C and 20°C the values were 2.12, 2.22 and 2.03; 2.44, 2.10 and 2.66; and, 2.78, 3.22 and 3.21 for the 5%, 40% and 100% skin respectively. The vividness (chroma) values after 97 days stored in the dark at 40°C, 50°C and 60°C were for the 5%, 40% and 100% skin, 4.49, 7.49 and 9.28; 4.48, 9.87 and 8.48; and 4.87, 6.33 and 9.31 respectively. Oil stored at 20°C in light had vividness (chroma) values at the end of the storage period of 3.33, 4.27 and 3.49 for the 5%, 40% and 100% skin respectively. These values were all greater than those for oil stored at 20°C and less than those for 40°C in the dark (Figure 5.13a, b and c).

The hue angle was measured during the 97 days and is shown in (Figure 5.14a, b and c). The initial hue angles for the 5%, 40% and 100% skin were 110.45, 117.35 and 117.05 degrees, respectively. For the oil with 5%, 40% and 100% skin at -20°C, 4°C the hue angle at day 97 were 186.95 and 192.05; 160.10 and 180.20; 156.95 and 154.55 degrees, respectively. The hue angles at day 97 for the oil stored at 20°C, 40°C, 50°C and 60°C were 80.05, 50.25, 36.55 and 45.45; 91.15, 48.90, 55.50 and 42.95; 129.75, 34.70, 25.80 and 48.30 for the 5%, 40% and 100% skin, respectively. The oil stored at 20°C in light hue angle at day 97, were 65.10, 105.80 and 57.55 for 5%, 40% and 100% skin respectively. The hue angle of oil stored at higher temperatures showed a change from green to less green area.

The lightness value was measured during the 97 days and is shown in Figure 5.15a, b and c. The initial lightness values of the extracted oil with 5%, 40% and 100% skin were 99.90, 101.53 and 98.50 respectively. The oils stored at the various temperatures for 97 days all showed a decrease in the lightness value for all skin formulations. The maximum decreases were 2.0%, 5.1% and 3.3% for 5%, 40% and 100% skin respectively. The oil stored at 20°C in light showed decreases of 2.6%, 4.7% and 2.5% for the 5%, 40% and 100% skin respectively (Figure 5.15a, b and c).

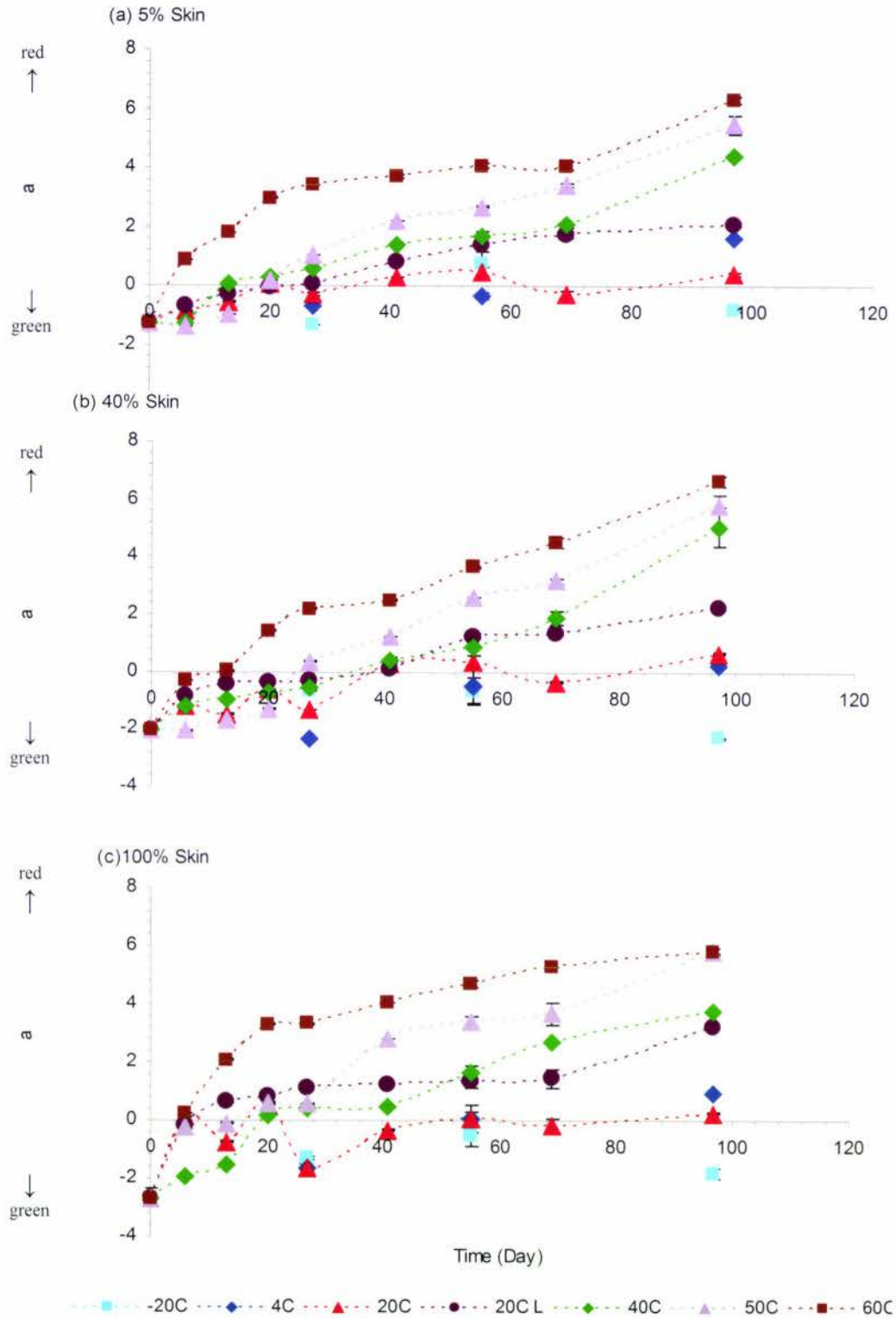


Figure 5.11.Greenness (-a) and redness (+) of avocado oil with (a) 5% skin, (b) 40% skin and (c) 100% skin addition stored at -20°C, 4°C, 20°C, 20°C light, 40°C, 50°C and 60°C (n=2)

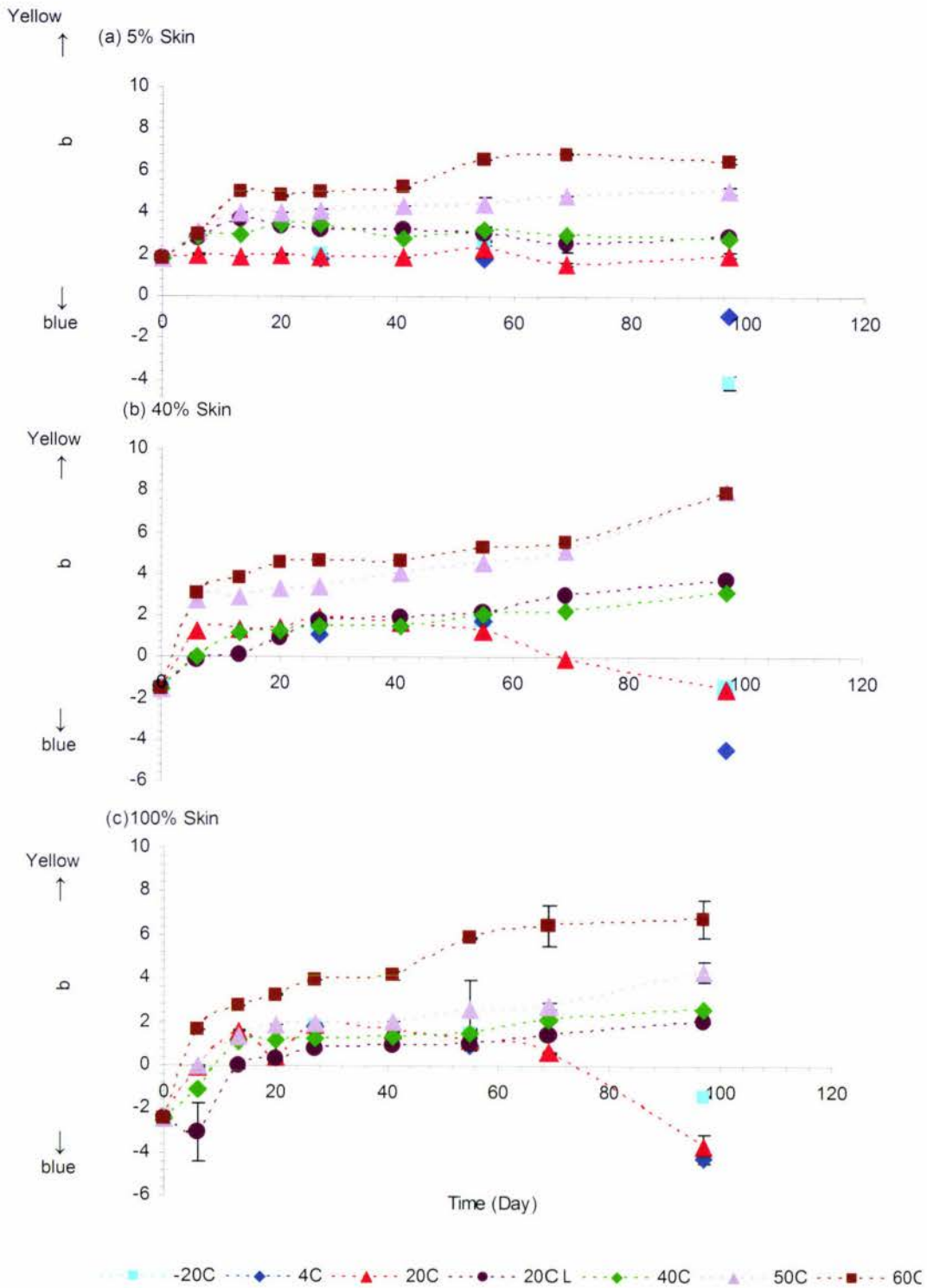


Figure 5.12.Yellowness (+b) and blueness (-b) of avocado oil with (a) 5% skin, (b) 40% skin and (c) 100% skin addition stored at -20°C, 4°C, 20°C, 20°C light, 40°C, 50°C and 60°C (n=2)

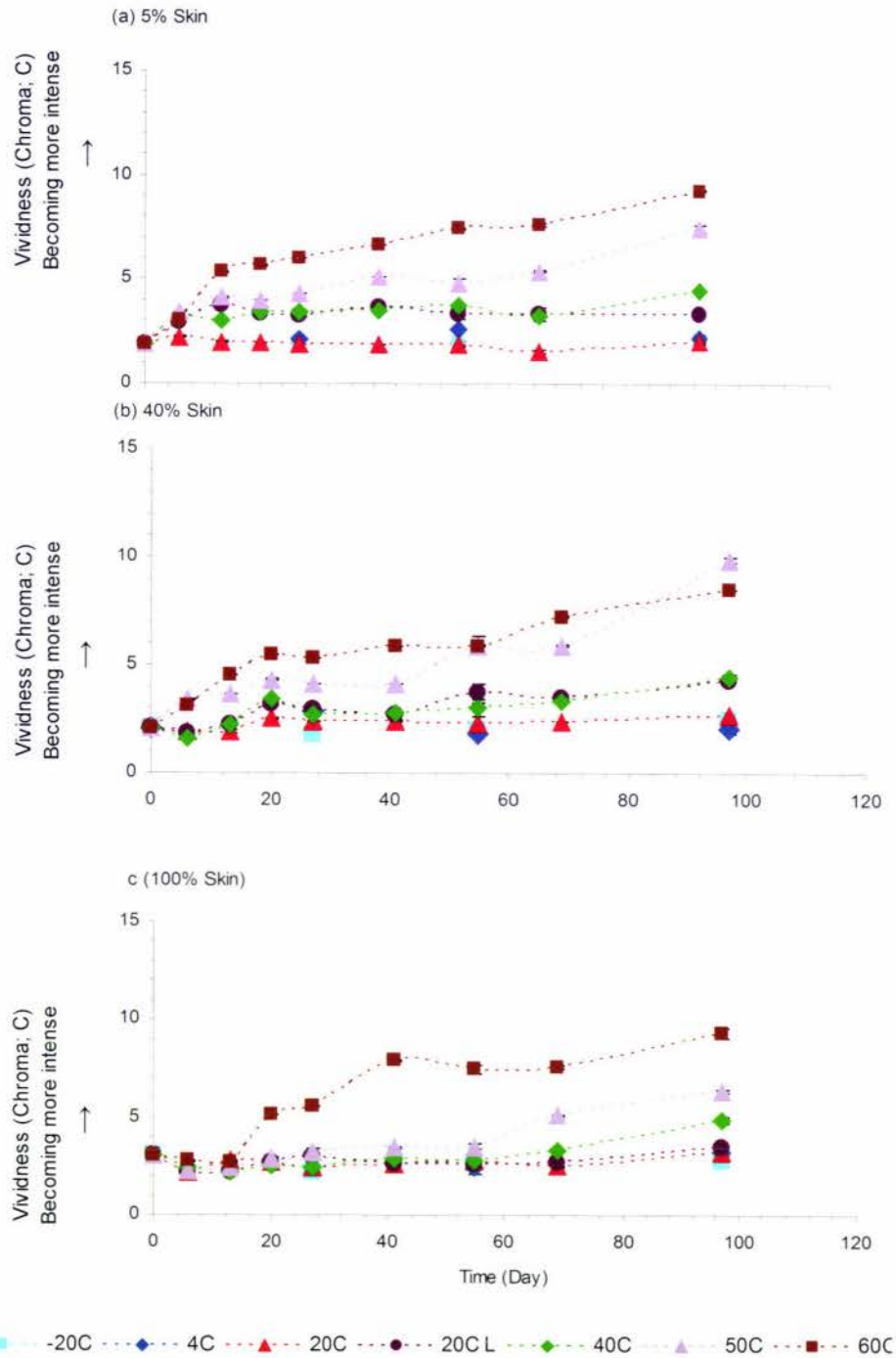


Figure 5.13.Vividness (Chroma; C) of avocado oil with (a) 5% skin, (b) 40% skin and (c) 100% skin addition stored at -20°C, 4°C, 20°C, 20°C light, 40°C, 50°C and 60°C (n=2)

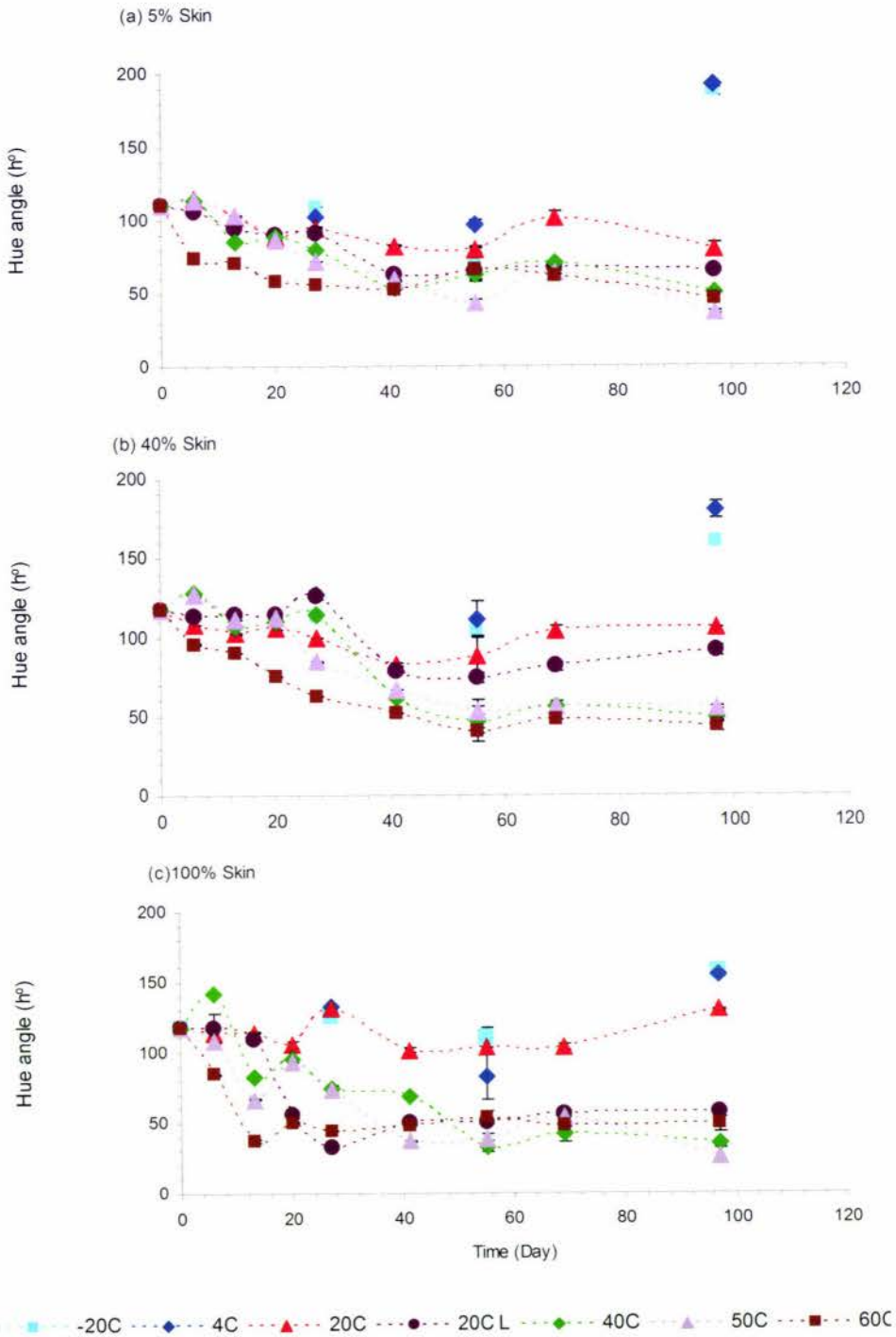


Figure 5.14.Hue angle (h°) of avocado oil with (a) 5% skin, (b) 40% skin and (c) 100% skin addition stored at -20°C, 4°C, 20°C, 20°C light, 40°C, 50°C and 60°C (n=2)

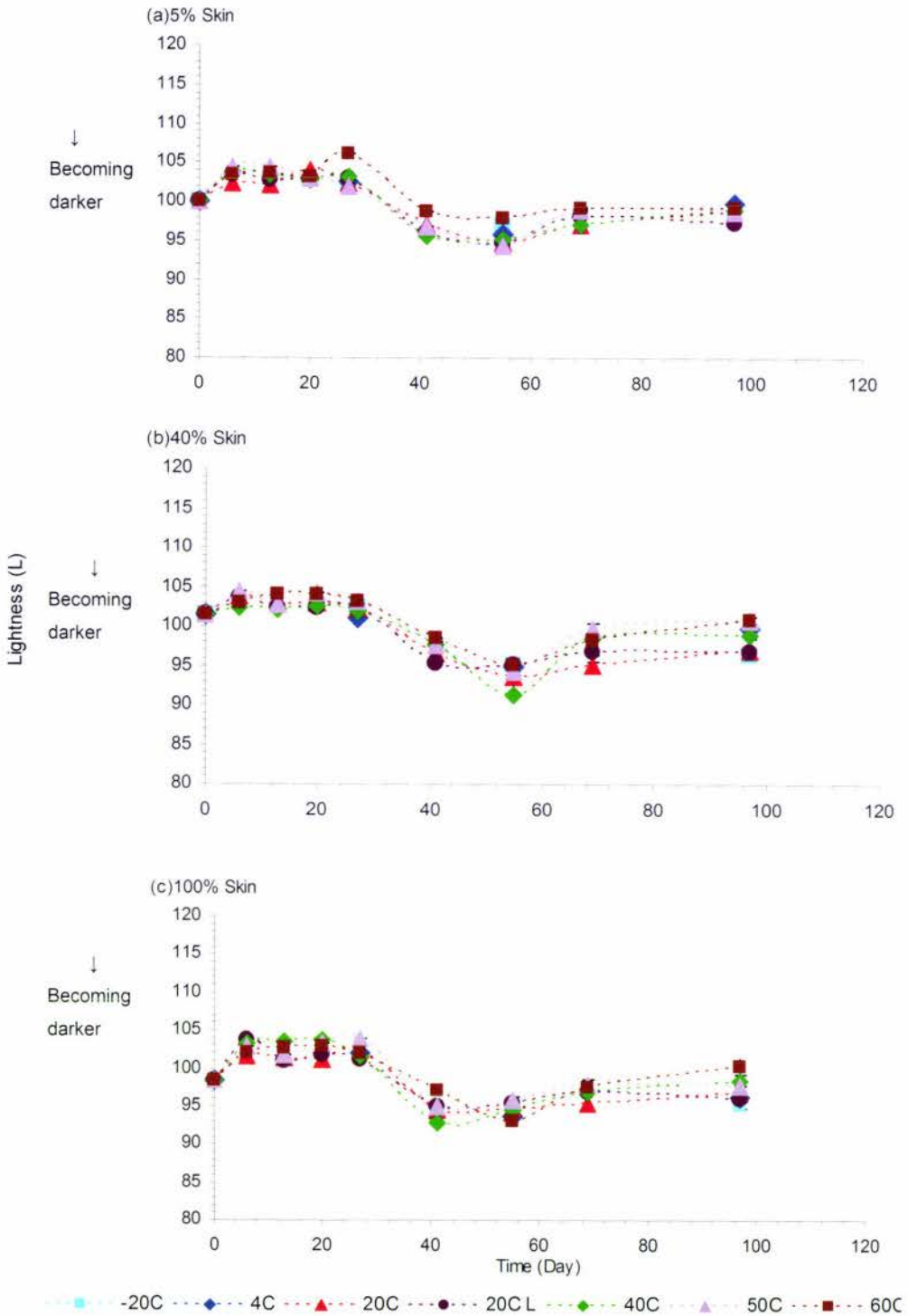


Figure 5.15.Lightness (L) of avocado oil with (a) 5% skin, (b) 40% skin and (c) 100% skin addition stored at -20°C, 4°C, 20°C, 20°C light, 40°C, 50°C and 60°C (n=2)

5.3.2.8 Diene

The antifungal compound diene was not detected in the avocado oil with 5% skin, 40% skin and 100% skin.

5.4 Discussion

5.4.1 Laboratory and Factory Scale Comparison

The quality of oil produced from 5%, 40% and 100% skin additions by the cold pressed extraction method in the laboratory in terms of PV and FFA were similar. Likewise, for the factory scale extraction of oil, the quality of the oil in terms of PV and FFA with 5%, 40% and 100% addition of skin were similar indicating that the addition of skin did not have a negative effect on the quality of oil. However, in the cold pressed extraction of oil from avocados in the laboratory the PV and FFA values were considerably higher compared to those obtained for factory cold pressed oil extraction. This may be due to an uninterrupted unit operation in the factory therefore less handling delay as opposed to segregated operations in the laboratory. As the percentage of skin addition increased from 5% to 100% during cold pressed oil extraction in the laboratory, the carotenoids and chlorophylls concentrations in the oil increased. Carotenoid and chlorophyll concentration in the oil produced in the laboratory were consistently higher compared to the avocado oil produced in the factory for 40% and 100% skin addition. Both the avocado oil produced in the laboratory and the factory had the same chlorophyll and carotenoid compounds present. The carotenoids and chlorophyll levels of avocado oil with 5% skin produced in the laboratory were lower compared to the avocado oil produced in the factory. The skin percentage of 5% in avocado oil produced in the factory was an estimate. It would seem that the skin which passed through with the pulp to the malaxer was more than 5% (i.e. about 11% as quantified from the sample taken from the malaxer). In this case, even the avocado oil produced in the laboratory with 5% skin addition had higher carotenoids and chlorophylls compared to the avocado oil produced in the factory. Overall, pigment composition of avocado oil produced in the laboratory was higher compared to the oil produced in the factory. This may be due to the accurate weighing of skin addition and good quality of the skin added in the laboratory. In the factory the skin tissue could have been damaged through mechanical pressure in the destoner and caused pigment deterioration.

5.4.2 Peroxide Value during Storage

For all formulations 5%, 40% and 100% skin, after 97 days of storage it was noted that for all the samples stored in the dark that as the storage temperature increased so did the PV which indicated that temperature was one major cause of oil deterioration. In all cases the sample stored at 20°C in the light behaved in the same manner with the PV increasing with time by day 97, but at a higher PV than the sample stored at 20°C in the dark and also higher than the samples stored at 40°C and 50°C in the dark. This showed that light accelerates degradation.

For the oils produced with 5%, 40% and 100% skin additions as the storage temperature increased, the slope for the peroxide values increased with time. The slopes of the graphs were between 6 to 8 times higher for the oils stored at 60°C compared with those at -20°C. The rate of peroxide formation of oils stored at 20°C in the light was faster than the oils stored at 40°C but slower than the oils stored at 60°C. The indication was that the oxidation was faster at high temperature and under light. The slope of the graph increased as the percentage of skin addition increased indicating that the oxidation of higher levels of skin was faster.

The activation energy of avocado oil with 100% skin was higher compared to that with 5% skin. The avocado oil with 40% skin had the highest activation rate and the initial colour space *a* value showed that this formulation appeared to be greener compared to the avocado oil with 100% skin. Other factors such as temperature during the production may have influenced the increase in green colour but not necessarily the pigment concentration. It had been confirmed that the concentration of pigments of avocado oil with 100% skin was higher compared to that with 40%. In all cases the avocado oil with 5% skin had the lighter green colour and lowest activation energy.

5.4.3 Total Chlorophyll (Spectrophotometer and HPLC)

The spectrophotometer method used measures the total chlorophyll content. The chlorophyll *a* and *b* was being converted to pheophytins and other chlorophyll derivatives such as pyropheophytin but still being measured as chlorophylls. The total chlorophyll obtained by HPLC was only the sum of chlorophyll *a* and *b* and pheophytin *a* and *b*. Hence, it appeared that in terms of readings, the total chlorophyll obtained by spectrometer method was always higher compared to the total chlorophylls obtained by HPLC for the oil used in storage trial.

Generally, the concentration of avocado oil analysed by both methods which was stored in the dark at low temperature (-20°C and 4°C and 20°C) showed that the chlorophyll concentrations remained relatively stable over 97 days. As the storage temperature increased, the slope (rate of degradation) of the chlorophyll concentration increased with time. The rate of degradation was higher for the oils stored at 60°C, 50°C 20°C in the light and 40°C compared with those at -20°C, 4°C and 20°C. The slope of the graph increased as the temperature increased indicating that the degradation of pigment was faster at high temperature and under light. At high chlorophyll level (high % skin) the rate of oxidation was faster. This may be due to the chlorophyll acting as an antioxidant in the dark. This is in agreement with the findings of Endo et al. (1985) and Werman and Neeman (1986). Chlorophylls in commercial edible oils acted as antioxidants under dark (Endo et al., 1985). There was an inverse relationship between PV and pigments concentrations during storage similar to that which was found by Werman and Neeman (1986). It was suggested that in the crude avocado oil, chlorophyll acted as an antioxidant in absolute darkness as evidenced by a slow decrease of chlorophyll and slow increase in the peroxide value (Endo et al., 1985; Werman and Neeman 1986). In the dark and at low temperature, chlorophyll and pheophytin prevented oxidative deterioration of olive oil but acted as a photosynthesizer for singlet oxygen formation under light (Psomiadou and Tsimodou, 2001). In olive oil, chlorophyll enhanced peroxide formation/oxidation under light (Fakaourelis et al., 1987). This may explain why the degradation of chlorophyll in avocado oil with added skin in the dark was slower while the degradation of avocado oil in the light and at high temperature was faster. Chlorophyll becomes excited when exposed to light in the UV range. Excited chlorophyll triggers further excitation of oxygen, resulting in singlet oxygen

oxygen which then reacts with lipids to produce hydroperoxide. The consequent formation of peroxy-radicals causes chlorophyll degradation (discolouration) (Werman and Neeman, 1986).

5.4.4 Carotenoids (HPLC)

Lutein which is the major carotenoid present in the avocado oil was found to be more stable even at high temperatures i.e. 40°C after 97days compared to chlorophylls. Lutein may be trapping free radicals and quenched singlet oxygen and thereby also acting as an antioxidant (Reische et al., 2002). Carotenoids are particularly effective at quenching singlet oxygen (Viljanen et al., 2002; Reische et al., 2002). One thousand molecules of singlet oxygen can be quenched by β-carotene. Carotenoids trapped free radicals (Reische et al., 2002). Free radicals are formed by the reaction of singlet oxygen and lipids. Triplet oxygen is formed when singlet oxygen transfers energy (transfer of electron) to β-carotene. The β-carotene then returns to the unexcited state through the release of heat energy (Reische et al., 2002). Lutein behaved as antioxidants (Viljanen et al., 2002). The amount of antioxidation is related to the concentration of the carotenoids (Viljanen et al., 2002). There is little or no difference in the antioxidation effects at 10ppm or 20ppm for lutein and lycopene, but there is considerable improvement at 40ppm. However, β-carotene at various concentrations showed noticeably different antioxidation activity and the most efficient at 40ppm of these three carotenoids (Viljanen et al., 2002).

5.4.5 Colour

Based on values of colour space *a*, *b*, chroma, hue angle and lightness, it was noted that the avocado oils stored in the dark at -20°C, 4°C showed retention and unexpected enhancement of green colour. As the percentage of skin increased the rate of colour change from green to less green area was slower for avocado oil with higher percentage of skin at lower temperatures. In all cases, the avocado oils stored in the dark at higher temperatures (40°C, 50°C and 60°C) and at 20°C under light showed a significant increase of colour change from green to less green area. The rate colour deterioration was faster at higher percentages of skin and at high temperatures.

The change in colour was proportional to the degradation of pigments i.e. chlorophylls and formation of hydroperoxides. At high temperature storage and under light, the chlorophylls *a* and *b* may have been converted to chlorophyll derivatives such as

pheophytin, pyropheophytin and possibly other chlorophylls epimers which resulted in a shift green to less green area of the avocado oil. Likewise, the hydroperoxide formation may also have caused the shift to this colour. The avocado oil stored at low temperatures did not change over 97 days of storage which suggested that the formation of breakdown products of pigments and hydroperoxides had not occurred during the 97 days test period.

5. 5 Conclusions and Recommendations

The pigments identified in the cold pressed avocado oil were chlorophyll *a* and *b*, pheophytin *a* and *b*, lutein and antheraxanthin. The chlorophyllides *a* an *b*, violaxanthin, zeaxanthin and neoxanthin were not detected.

The level of pigments was higher in the cold pressed avocado oil extracted in the laboratory compared to the oil extracted in the factory. The additions of skin during the malaxing stage of cold pressing had no effect on the quality of the oil produced immediately after extraction, but had increased pigment levels and decreased shelf life. Storage at low temperature and in darkness of avocado oil is recommended.

The pattern of colour change of avocado oil corresponded to the pigment degradation during storage.

Due to time constraints, only basic statistical analysis was used and the data collated could be subjected to further statistical analysis.

Chapter 6. Output/Future Research

The comprehensive data collated in this research may be of use in food, nutrition, postharvest and gene technologies.

If the technology in segregating the avocado sections would be developed, commercial cold pressed extraction could be viable and could cater to a wider market in terms of colour preferences and nutritional needs.

The pigment composition of the different sections of avocado fruit may be linked to expression of certain genes and would aid in finding a correlation between colours and patterns of genes expression for the biosynthetic enzymes. Consequently, the markers could be obtained for new varieties for the increase or decrease of colour.

In the avocado oil industry, comprehensive information of the oil pigment composition, colour, and absence of toxic substances, oil stability and in general, oil quality of the cold pressed avocado oil may be of use as a basis for quality assurance, product development and marketing. It could be the basis in optimising storage conditions to increase oil shelf life.

Using the comprehensive data collated from this research, suggested future research would be to study the response of human genotype to avocado fruit and the extracted oil by carrying out investigations as to whether a diet which includes this food can modify genetic susceptibility to prostate cancer using Prostate Specific Antigen (PSA) in blood for different age groups of males in New Zealand as an index.

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Chapter 8. Appendices

Appendix 1.Carotenoids concentration in 'Hass' avocado fresh tissue

(a) Skin								
Days	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	α-Carotene	β-Carotene	Total Carotenoids
	μg g^{-1} FW							
2	5.52	4.49	1.04	20.54	0.92	3.87	12.53	48.91
3	5.35	3.71	1.02	20.53	0.64	4.47	11.20	46.92
4	4.61	3.54	0.82	17.70	0.56	3.85	9.10	40.18
6	2.94	3.31	0.55	13.37	0.27	2.78	5.79	29.00
8	2.44	3.03	0.50	13.34	0.27	3.07	4.89	27.53
9	2.48	3.53	0.50	12.77	0.31	2.39	6.40	28.38
10	1.83	2.41	0.32	10.20	0.15	2.54	4.12	21.56
11	1.27	1.80	0.28	8.05	0.17	1.74	3.45	16.76
13	1.26	1.69	0.24	8.02	0.13	1.93	3.42	16.69
Days	SE							
2	0.04	0.16	0.02	0.09	0.08	0.18	0.10	0.18
3	0.36	0.25	0.02	0.74	0.12	0.13	0.78	2.30
4	0.34	0.24	0.02	1.05	0.10	0.31	0.63	2.58
6	0.08	0.05	0.04	0.21	0.02	0.20	0.76	0.59
8	0.21	0.23	0.05	0.94	0.02	0.22	0.74	2.35
9	0.20	0.20	0.01	1.09	0.03	0.35	0.48	1.87
10	0.26	0.08	0.03	1.07	0.02	0.39	0.51	1.32
11	0.04	0.06	0.01	0.34	0.02	0.15	0.38	0.81
13	0.11	0.08	0.00	0.02	0.00	0.04	0.40	0.56
(b) Dark pulp								
Days	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	α-Carotene	β-Carotene	Total Carotenoids
	μg g^{-1} FW							
2	1.68	1.06	0.09	1.65	0.35	0.08	0.28	5.19
3	0.83	0.70	0.15	1.32	0.26	0.09	0.31	3.66
4	0.27	0.69	0.21	1.62	0.20	0.06	0.14	3.18
6	0.11	0.55	0.17	1.60	0.21	0.05	0.09	2.78
8	0.13	0.69	0.19	1.96	0.28	0.04	0.06	3.34
9	0.14	0.80	0.18	2.23	0.12	0.04	0.06	3.56
10	0.14	0.76	0.17	2.21	0.15	0.05	0.08	3.56
11	0.07	0.54	0.10	1.70	0.00	0.03	0.04	2.48
13	0.07	0.45	0.11	1.73	0.00	0.05	0.06	2.47
Days	SE							
2	0.12	0.08	0.01	0.06	0.03	0.01	0.02	0.27
3	0.05	0.01	0.00	0.04	0.01	0.01	0.03	0.06
4	0.04	0.04	0.02	0.14	0.02	0.00	0.00	0.24
6	0.03	0.09	0.02	0.15	0.04	0.02	0.03	0.30
8	0.02	0.01	0.02	0.06	0.02	0.01	0.01	0.10
9	0.02	0.07	0.03	0.16	0.02	0.01	0.01	0.31
10	0.04	0.08	0.03	0.26	0.03	0.02	0.04	0.49
11	0.01	0.04	0.01	0.15	0.00	0.01	0.01	0.20
13	0.00	0.02	0.00	0.10	0.00	0.01	0.01	0.13

Appendix 1.Carotenoids concentration in 'Hass' avocado fresh tissue continuation

(c.) Pale pulp)								
Days	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	α-Carotene	β-Carotene	Total Carotenoids
	μg g^{-1} FW							
2	1.21	0.49	0.05	0.41	0.02	0.05	0.10	2.33
3	0.46	0.27	0.10	0.39	0.04	0.04	0.07	1.37
4	0.07	0.16	0.08	0.47	0.03	0.05	0.05	0.92
6	0.04	0.08	0.03	0.36	0.04	0.05	0.04	0.64
8	0.04	0.10	0.04	0.38	0.04	0.05	0.04	0.67
9	0.04	0.11	0.03	0.40	0.03	0.06	0.04	0.70
10	0.04	0.09	0.03	0.33	0.02	0.04	0.05	0.60
11	0.03	0.10	0.02	0.30	0.02	0.03	0.03	0.53
13	0.03	0.09	0.02	0.34	0.00	0.06	0.05	0.59
Days	SE							
2	0.15	0.05	0.01	0.03	0.00	0.00	0.01	0.23
3	0.04	0.03	0.01	0.01	0.01	0.01	0.01	0.05
4	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.05
6	0.02	0.01	0.00	0.02	0.01	0.01	0.01	0.02
8	0.00	0.01	0.00	0.07	0.00	0.01	0.01	0.01
9	0.01	0.01	0.01	0.05	0.01	0.00	0.00	0.06
10	0.02	0.01	0.01	0.03	0.01	0.01	0.01	0.04
11	0.01	0.00	0.01	0.02	0.00	0.00	0.00	0.02
13	0.01	0.01	0.00	0.01	0.00	0.02	0.01	0.02
d) Yellow pulp								
Days	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	α-Carotene	β-Carotene	Total Carotenoids
	μg g^{-1} FW							
2	2.08	0.63	0.10	0.45	0.00	0.06	0.06	3.38
3	0.86	0.28	0.15	0.49	0.03	0.06	0.04	1.91
4	0.25	0.20	0.15	0.50	0.02	0.06	0.07	1.25
6	0.07	0.07	0.03	0.35	0.01	0.04	0.03	0.60
8	0.09	0.08	0.03	0.24	0.02	0.06	0.07	0.59
9	0.11	0.07	0.03	0.19	0.00	0.07	0.05	0.52
10	0.08	0.05	0.02	0.14	0.00	0.11	0.05	0.45
11	0.06	0.05	0.02	0.11	0.02	0.08	0.05	0.39
13	0.03	0.05	0.02	0.14	0.00	0.14	0.06	0.44
Days	SE							
2	0.13	0.04	0.02	0.04	0.01	0.00	0.01	0.21
3	0.13	0.04	0.01	0.06	0.00	0.01	0.01	0.24
4	0.08	0.05	0.06	0.03	0.00	0.01	0.01	0.21
6	0.01	0.01	0.00	0.04	0.00	0.02	0.01	0.03
8	0.01	0.02	0.01	0.02	0.00	0.01	0.01	0.07
9	0.02	0.02	0.01	0.02	0.00	0.02	0.01	0.07
10	0.02	0.00	0.00	0.02	0.00	0.03	0.01	0.05
11	0.01	0.00	0.00	0.00	0.00	0.02	0.02	0.04
13	0.00	0.01	0.00	0.06	0.00	0.02	0.02	0.09

Appendix 2.Chlorophylls concentration in 'Hass' avocado fresh tissue

(a) Skin										
Days	Chl *a*	Chl b	Pheophytin a	Pheophytin b	Chlorophyllides a	Chlorophyllides b	Unknown a	Unknown d	Unknown e	Total Chlorophyll
	μg g^{-1} FW									
2	23.00	8.55	0.10	0.05	135.83	13.92	0.07	1.91	3.15	186.52
3	22.14	9.18	0.03	0.06	126.58	13.72	36.31	1.94	3.98	189.68
4	22.14	9.00	0.10	0.05	90.65	8.23	23.10	5.74	5.93	163.03
6	20.29	7.82	0.00	0.00	91.17	8.75	24.72	0.36	2.02	138.64
8	20.28	8.04	0.00	0.04	87.37	7.81	24.76	1.60	2.57	152.44
9	24.94	9.85	0.04	0.09	0.00	0.00	0.00	1.84	1.96	38.66
10	16.81	7.09	0.00	0.04	0.00	0.00	0.00	1.30	1.34	55.67
11	16.80	6.43	0.00	0.08	0.02	0.02	0.05	1.17	1.25	25.70
13	17.68	6.99	0.00	0.12	0.00	0.00	0.01	1.30	1.25	27.33
	SE									
2	4.30	1.56	0.01	0.00	1.74	0.67	0.02	0.32	0.59	5.65
3	1.82	0.71	0.00	0.00	7.89	0.80	0.00	0.17	0.54	8.89
4	0.00	0.00	0.01	0.02	6.25	0.92	3.11	1.47	1.01	11.76
6	2.33	0.71	0.00	0.00	1.79	0.09	0.00	0.03	0.07	9.26
8	2.21	0.81	0.00	0.00	6.38	0.62	1.71	0.18	0.20	12.06
9	1.06	0.12	0.00	0.04	0.00	0.00	0.00	0.07	0.17	1.46
10	2.78	1.58	0.00	0.00	0.00	0.00	0.00	0.24	0.11	33.71
11	0.60	0.17	0.00	0.00	0.00	0.00	0.00	0.02	0.07	0.84
13	0.75	0.33	0.00	0.04	0.00	0.00	0.00	0.07	0.09	1.23
Dark pulp										
Days	μg g^{-1} FW									
2	23.02	5.30	0.00	0.00	6.53	0.15	0.64	1.14	1.25	38.03
3	22.71	5.56	0.00	0.00	1.54	0.00	0.12	0.73	0.61	31.26
4	24.50	7.94	0.00	0.00	2.10	0.09	0.07	0.85	0.56	36.04
6	20.64	7.24	0.00	0.00	2.46	0.08	0.12	1.00	0.66	32.16
8	27.35	11.10	0.00	0.00	1.01	0.06	0.15	1.63	0.93	42.21
9	31.19	11.30	0.00	0.00	0.00	0.10	0.00	1.45	0.78	44.77
10	23.36	13.06	0.00	0.00	0.00	0.04	0.00	1.74	0.95	39.12
11	22.15	9.76	0.00	0.00	0.00	0.00	0.00	1.12	0.59	33.62
13	21.02	9.45	0.00	0.00	0.00	0.02	0.00	1.64	1.05	33.17
	SE									
2	1.27	0.32	0.00	0.00	0.62	0.01	0.04	0.07	0.07	1.90
3	1.69	0.32	0.00	0.00	0.18	0.00	0.03	0.03	0.04	1.86
4	1.56	0.65	0.00	0.00	0.18	0.05	0.00	0.12	0.07	2.57
6	2.56	0.57	0.00	0.00	0.73	0.04	0.06	0.18	0.18	4.22
8	1.38	0.18	0.00	0.00	0.50	0.03	0.04	0.08	0.06	1.12
9	2.67	1.63	0.00	0.00	0.00	0.05	0.00	0.14	0.08	3.38
10	1.38	2.22	0.00	0.00	0.00	0.00	0.00	0.52	0.36	2.30
11	1.23	0.53	0.00	0.00	0.00	0.00	0.00	0.16	0.11	1.95
13	1.20	0.50	0.00	0.00	0.00	0.00	0.00	0.08	0.13	1.66

Appendix 2.Chlorophylls concentration in 'Hass' avocado fresh tissue continuation

(c)Pale pulp										
Days	Chl a	Chl b	Pheophytin a	Pheophytin b	Chlrophyllides a	Chlorophyllides b	Unknown a	Unknown d	Unknown e	Total
	μg g^{-1} FW									
2	3.58	0.45	0.00	0.00	2.51	0.00	0.31	0.07	0.28	7.20
3	5.09	0.70	0.00	0.00	0.55	0.00	0.04	0.11	0.20	6.63
4	5.51	1.15	0.00	0.00	0.89	0.00	0.00	0.19	0.23	7.96
6	3.76	1.08	0.00	0.00	0.78	0.05	0.03	0.20	0.19	6.05
8	4.20	1.65	0.00	0.00	0.81	0.00	0.05	0.26	0.19	7.16
9	5.39	2.18	0.00	0.00	0.81	0.00	0.00	0.30	0.21	8.36
10	4.40	1.75	0.00	0.00	0.59	0.00	0.59	0.30	0.29	7.14
11	4.60	1.81	0.00	0.00	0.00	0.00	0.00	0.26	0.17	6.83
13	4.79	1.82	0.00	0.00	0.00	0.00	0.00	0.41	0.30	7.32
Days	SE									
2	0.34	0.06	0.00	0.00	0.18	0.00	0.09	0.01	0.05	0.60
3	0.45	0.10	0.00	0.00	0.05	0.00	0.00	0.05	0.02	0.57
4	0.68	0.16	0.00	0.00	0.10	0.00	0.00	0.01	0.04	0.91
6	0.35	0.12	0.00	0.00	0.06	0.00	0.01	0.01	0.02	0.46
8	0.96	0.42	0.00	0.00	0.08	0.00	0.02	0.03	0.02	1.51
9	0.59	0.25	0.00	0.00	0.00	0.00	0.00	0.04	0.03	1.02
10	0.26	0.13	0.00	0.00	0.00	0.00	0.00	0.03	0.09	0.03
11	0.51	0.20	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.77
13	0.22	0.05	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.24
(d) Yellow										
Days	μg g^{-1} FW									
2	0.73	0.06	0.00	0.00	1.11	0.00	0.26	0.00	0.06	2.22
3	1.24	0.09	0.00	0.00	0.82	0.00	0.08	0.00	0.06	2.30
4	1.10	0.13	0.00	0.00	0.86	0.00	0.04	0.00	0.07	2.19
6	0.99	0.38	0.00	0.00	1.06	0.04	0.10	0.04	0.03	2.56
8	1.39	0.53	0.00	0.00	0.67	0.00	0.00	0.10	0.09	2.78
9	1.32	0.51	0.00	0.00	0.00	0.00	0.00	0.08	0.09	2.00
10	1.13	0.49	0.00	0.00	0.02	0.00	0.00	0.12	0.09	1.79
11	1.03	0.41	0.00	0.00	0.00	0.00	0.00	0.07	0.04	1.55
13	0.90	0.21	0.00	0.00	0.00	0.00	0.00	0.09	0.09	1.25
Days	SE									
2	0.13	0.00	0.00	0.00	0.08	0.00	0.04	0.00	0.01	0.21
3	0.06	0.01	0.00	0.00	0.08	0.00	0.04	0.00	0.01	0.15
4	0.31	0.08	0.00	0.00	0.08	0.00	0.00	0.00	0.03	0.45
6	0.16	0.04	0.00	0.00	0.32	0.00	0.10	0.02	0.00	0.22
8	0.26	0.12	0.00	0.00	0.09	0.00	0.00	0.03	0.00	0.43
9	0.40	0.12	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.58
10	0.35	0.18	0.00	0.00	0.00	0.00	0.00	0.05	0.03	0.59
11	0.09	0.04	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.12
13	0.14	0.06	0.00	0.00	0.00	0.00	0.00	0.04	0.02	0.22

Appendix 3.Total anthocyanins and cyanidin 3-*O*-glucoside of 'Hass' avocado skin

Days	Total Anthocyanin	Cyanidin 3-*O*-glucoside
	ug g^{-1} FW	
2	2.12	0.58
3	3.36	0.58
4	4.43	0.64
6	12.96	10.39
8	74.21	71.71
9	153.87	148.65
10	151.42	146.71
11	186.36	181.89
13	234.66	230.04
	SE	
2	0.67	0.00
3	0.14	0.00
4	1.63	0.00
6	2.09	2.09
8	13.94	12.75
9	21.91	17.19
10	18.00	23.95
11	29.88	28.67
13	17.06	16.24

Appendix 4.Carotenoids concentration in oil extracted from 'Hass' avocado sections

(a) Skin	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Total Carotenoids
Days			$\mu g\ g^{-1}$ oil		
2	13.90	4.60	37.30	151.00	206.80
3	14.40	4.80	38.00	144.50	201.70
4	19.90	7.30	48.40	150.90	226.50
6	12.00	8.50	44.40	151.50	216.40
8	11.10	0.00	53.00	119.00	183.10
9	5.40	4.70	26.80	85.40	122.30
10	4.80	2.60	30.80	99.60	137.80
11	1.00	0.00	21.50	73.50	96.00
13	2.10	0.00	28.10	79.70	109.90
Oil extracted from the skin is not enough to do replication hence there's no SE					
(b) Dark pulp					
Days	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Total Carotenoids
			$\mu g\ g^{-1}$ oil		
2	7.97	3.37	5.03	6.43	22.80
3	4.50	3.43	4.77	7.80	20.50
4	1.17	3.30	3.20	8.20	15.87
6	0.00	1.47	1.17	7.03	9.67
8	0.00	1.63	0.93	5.13	7.70
9	0.00	1.73	1.10	5.87	8.70
10	0.00	1.97	1.20	8.13	11.30
11	0.00	1.53	0.73	6.07	8.33
13	0.00	1.53	0.83	6.80	9.17
			SE		
2	0.68	0.27	0.33	0.35	1.53
3	0.61	0.23	0.29	0.38	1.08
4	0.00	0.20	0.03	0.22	1.05
6	0.00	0.17	0.07	0.47	0.39
8	0.00	0.07	0.06	0.23	0.70
9	0.00	0.07	0.06	0.23	0.35
10	0.00	0.22	0.15	0.19	1.55
11	0.00	0.09	0.03	0.33	0.38
13	0.00	0.03	0.03	0.12	0.18

Appendix 4.Carotenoids concentration in oil extracted from 'Hass' avocado sections continuation

(c)Pale pulp	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Total Carotenoids
Days			μg g^{-1} oil		
2	5.77	1.60	2.27	2.10	11.73
3	2.20	0.97	1.07	2.13	6.37
4	0.30	0.40	0.40	2.37	3.47
6	0.00	0.00	0.00	1.47	1.47
8	0.00	0.57	0.37	2.27	3.20
9	0.00	0.37	0.27	1.93	2.57
10	0.00	0.00	0.13	1.40	1.53
11	0.00	0.13	0.00	0.73	0.87
13	0.00	0.07	0.13	1.23	0.24
Days			SE		
2	0.15	0.06	0.29	0.15	0.64
3	0.35	0.09	0.03	0.09	0.46
4	0.06	0.06	0.00	0.12	0.13
6	0.00	0.00	0.00	0.33	0.33
8	0.00	0.03	0.03	0.12	0.17
9	0.00	0.17	0.07	0.48	0.72
10	0.00	0.00	0.07	0.06	0.12
11	0.00	0.13	0.00	0.37	0.24
13	0.00	0.07	0.03	0.15	0.24
(d) Yellow pulp					
Days			μg g^{-1} oil		
2	8.07	1.50	2.93	2.63	15.13
3	4.33	1.07	1.47	2.53	9.40
4	1.20	0.73	0.73	2.83	5.50
6	0.00	0.00	0.00	2.00	2.00
8	0.00	0.00	0.00	0.93	0.93
9	0.30	0.13	0.27	0.83	1.53
10	0.17	0.07	0.10	0.67	1.00
11	0.13	0.00	0.00	0.53	0.67
13	0.00	0.00	0.00	0.53	0.53
			SE		
2	0.23	0.10	0.18	0.03	0.48
3	0.22	0.03	0.03	0.03	0.25
4	0.10	0.03	0.03	0.09	0.06
6	0.00	0.00	0.00	0.06	0.06
8	0.00	0.00	0.00	0.03	0.03
9	0.06	0.07	0.03	0.03	0.15
10	0.09	0.07	0.10	0.07	0.31
11	0.07	0.00	0.00	0.03	0.03
13	0.00	0.00	0.00	0.03	0.03

Appendix 5.Chlorophylls concentration in oil extracted from 'Hass' avocado sections

(a) Skin	Total Chlorophyll	Chlorophyll *b*	Chlorophyll *a*	Pheophytin *b*	Pheophytin *a*
Days	μg g^{-1} oil				
2	213.80	22.70	143.00	11.10	37.00
3	204.40	27.90	128.30	13.80	34.40
4	203.40	19.40	134.50	14.80	34.70
6	179.30	18.20	115.90	9.80	35.40
8	181.60	24.40	109.00	14.40	33.80
9	171.60	18.20	116.60	3.30	33.50
10	150.70	13.00	98.10	10.50	29.10
11	119.80	8.50	96.50	4.60	10.20
13	115.60	14.30	81.10	1.60	18.60
Oil extracted from the skin is not enough to do replication hence there's no SE					
(b) Dark pulp					
Days	μg g^{-1} oil				
2	43.80	5.70	37.03	0.17	0.90
3	46.47	7.70	35.53	1.23	2.07
4	58.37	6.63	40.23	0.20	1.03
6	51.47	13.50	34.80	1.33	1.80
8	35.77	8.27	26.50	0.40	0.63
9	43.40	9.43	33.03	0.30	0.60
10	55.37	13.07	41.07	0.57	0.67
11	47.30	12.07	34.03	0.37	0.77
13	62.50	16.73	44.60	0.50	0.63
	SE				
2	1.66	0.32	1.38	0.03	0.06
3	2.73	1.54	4.12	1.08	1.07
4	3.88	0.55	2.72	0.00	0.15
6	3.57	0.90	2.30	0.35	0.06
8	3.56	0.83	2.61	0.10	0.12
9	3.20	0.47	2.74	0.06	0.06
10	7.48	1.37	5.88	0.18	0.07
11	4.40	1.47	2.72	0.18	0.15
13	1.19	0.54	0.61	0.06	0.07

Appendix 5.Chlorophylls concentration in oil extracted from 'Hass' avocado sections continuation

(c)Pale pulp					
Days	Total Chlorophyll	Chlorophyll *b*	Chlorophyll *a*	Pheophytin *b*	Pheophytin *a*
	μg g^{-1} oil				
2	13.07	1.43	11.37	0.00	0.27
3	10.93	1.17	10.23	0.03	0.20
4	10.37	1.90	8.23	0.00	0.27
6	9.27	2.67	6.07	0.20	0.30
8	14.10	4.07	9.40	0.27	0.30
9	13.90	3.70	9.90	0.07	0.20
10	11.50	3.40	7.90	0.05	0.15
11	10.63	2.47	8.00	0.03	0.07
13	12.93	3.97	8.73	0.10	0.13
	SE				
2	1.74	0.26	1.41	0.00	0.09
3	0.62	0.12	0.09	0.03	0.06
4	0.95	0.17	0.75	0.00	0.03
6	2.29	0.64	1.49	0.12	0.10
8	0.78	0.28	0.59	0.03	0.00
9	1.30	0.29	1.01	0.07	0.06
10	0.40	0.00	0.40	0.05	0.15
11	1.28	0.67	0.84	0.03	0.07
13	1.55	0.38	1.19	0.00	0.07
(d) Yellow pulp					
Days	μg g^{-1} oil				
2	2.97	0.17	2.70	0.00	0.03
3	4.33	0.33	3.87	0.00	0.17
4	3.63	0.60	2.93	0.00	0.10
6	4.87	1.53	3.07	0.10	0.17
8	4.20	1.33	2.63	0.10	0.10
9	3.43	0.97	2.40	0.03	0.00
10	3.47	1.10	2.30	0.03	0.03
11	3.07	0.90	2.20	0.00	0.03
13	2.97	1.00	2.23	0.03	0.03
	SE				
2	0.20	0.03	0.21	0.00	0.03
3	0.26	0.07	0.23	0.00	0.03
4	0.15	0.06	0.20	0.00	0.00
6	0.41	0.18	0.27	0.06	0.03
8	0.26	0.12	0.15	0.06	0.00
9	0.18	0.09	0.12	0.03	0.00
10	0.27	0.06	0.15	0.03	0.03
11	0.33	0.06	0.25	0.00	0.03
13	0.39	0.00	0.09	0.03	0.03

Appendix 6.Comparison of the pigment in the fresh tissue and the extracted oil

The mean dry matter content (% w/w) of avocado pulp sections (p5 and p3) for late season fruit obtained by Phetsomphou (2000) was used for this calculation. The dry matter content of the skin was determined separately.

Basis:

Days	Skin		Dark pulp		Pale pulp		Yellow pulp	
	% Dry matter	% Oil	% Dry matter	% Oil	% Dry matter	% Oil	% Dry matter	% Oil
2	35	8.66	39.6	60.84	40.30	65.02	34.60	65.53
3	35	8.29	39.6	59.54	40.30	63.31	34.60	65.50
4	35	8.61	39.6	59.49	40.30	65.50	34.60	66.84
6	35	9.15	39.6	62.31	40.30	66.65	34.60	67.94
8	35	12.40	39.6	68.94	40.30	68.57	34.60	68.47
9	35	11.66	39.6	67.08	40.30	70.07	34.60	70.54
10	35	9.71	39.6	70.68	40.30	72.09	34.60	69.53
11	35	9.78	39.6	69.91	40.30	71.40	34.60	71.95
13	35	9.75	39.6	70.11	40.30	70.83	34.60	68.86

For example, dark pulp

Given:

Weight fresh tissue = 100 grams

Dry matter of dark pulp = 39.6%

Oil yield at day 2 = 60.84%

Dry tissue = 100 x .396
= 39.6g

Weight of oil = 39.6g x 0.6084
= 24.09g/100g
= 0.2409g/g

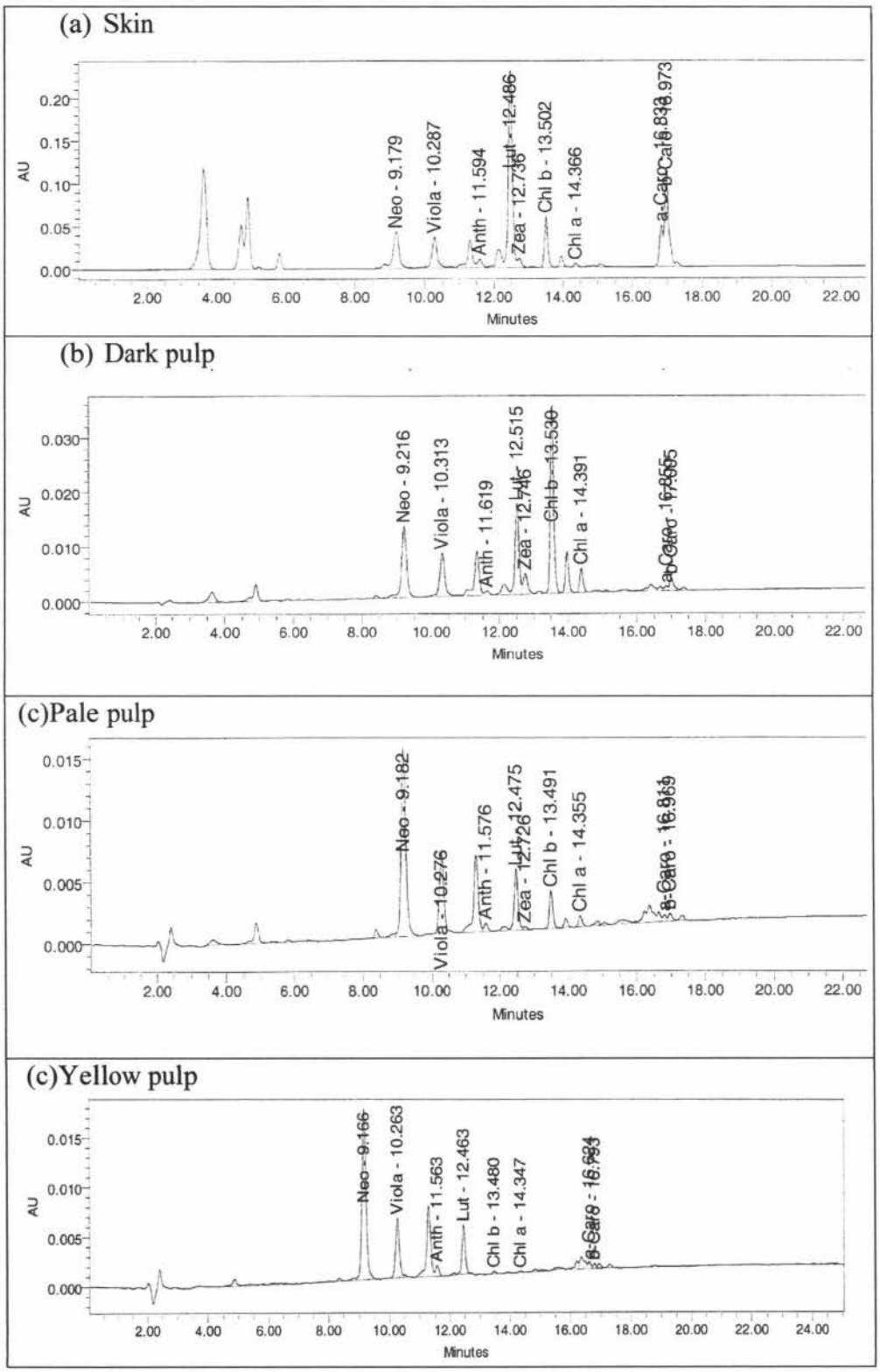
Conversion Factor ($\mu g\ g^{-1}$ FW to $\mu g\ g^{-1}$ oil)

Days	Skin	Dark pulp	Pale pulp	Yellow pulp
2	0.0303	0.2409	0.2620	0.2267
3	0.0290	0.2358	0.2552	0.2266
4	0.0301	0.2356	0.2640	0.2313
6	0.0320	0.2467	0.2686	0.2351
8	0.0434	0.2730	0.2763	0.2369
9	0.0408	0.2656	0.2824	0.2441
10	0.0340	0.2799	0.2905	0.2406
11	0.0342	0.2768	0.2877	0.2489
13	0.0341	0.2776	0.2854	0.2383

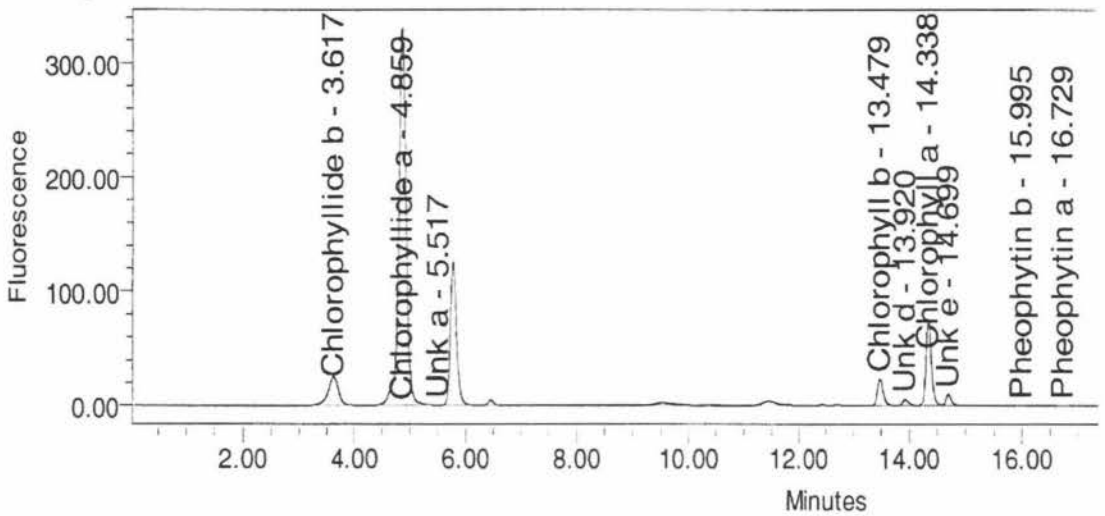
Appendix 6.Comparison of the pigment in the fresh tissue and the extracted oil continuation

Total Carotenoids				Total Chlorophylls		
(a) Skin						
Days	Fresh Tissue		Oil	Fresh Tissue		Oil
	μg g^{-1} FW (measured)	μg g^{-1} oil (converted)	μg g^{-1} oil (measured)	μg g^{-1} FW (measured)	μg g^{-1} oil (converted)	μg g^{-1} oil (measured)
2	48.91	1614.19	206.80	186.52	6155.78	213.80
3	46.92	1548.51	201.70	189.68	6540.69	204.40
4	40.18	1326.07	226.50	163.03	5416.28	203.40
6	29.00	957.10	216.40	138.64	4332.50	179.30
8	27.53	908.80	183.10	152.44	3512.44	181.60
9	28.38	936.63	122.30	38.66	947.55	171.60
10	21.56	711.55	137.80	55.67	1637.35	150.70
11	16.76	553.14	96.00	25.70	751.46	119.80
13	16.69	550.83	109.90	27.33	801.47	115.60
(b) Dark pulp						
Days	Fresh Tissue		Oil	Fresh Tissue		Oil
	μg g^{-1} FW	μg g^{-1} oil	μg g^{-1} oil	μg g^{-1} FW	μg g^{-1} oil	μg g^{-1} oil
2	5.19	21.54	22.80	38.03	157.87	43.80
3	3.66	15.52	20.50	31.26	132.57	46.47
4	3.18	13.50	15.87	36.04	152.97	58.37
6	2.78	11.27	9.67	32.16	130.36	51.47
8	3.34	12.23	7.70	42.21	154.62	35.77
9	3.56	13.40	8.70	44.77	168.56	43.40
10	3.56	12.72	11.30	39.12	139.76	55.37
11	2.48	8.96	8.33	33.62	121.46	47.30
13	2.47	8.90	9.17	33.17	119.49	62.50
(c)Pale pulp						
Days	Fresh Tissue		Oil	Fresh Tissue		Oil
	μg g^{-1} FW	μg g^{-1} oil	μg g^{-1} oil	μg g^{-1} FW	μg g^{-1} oil	μg g^{-1} oil
2	2.33	8.89	11.73	7.20	27.48	13.07
3	1.37	5.37	6.37	6.63	25.98	10.93
4	0.92	3.48	3.47	7.96	30.15	10.37
6	0.64	2.38	2.44	6.05	22.52	9.27
8	0.67	2.42	3.20	7.16	25.91	14.10
9	0.70	2.48	2.57	8.36	29.60	13.90
10	0.60	2.07	1.53	7.14	24.58	11.50
11	0.53	1.84	0.87	6.83	23.74	10.63
13	0.59	2.07	0.24	7.32	25.65	12.93
(d) Yellow pulp						
Days	Fresh Tissue		Oil	Fresh Tissue		Oil
	μg g^{-1} FW	μg g^{-1} oil	μg g^{-1} oil	μg g^{-1} FW	μg g^{-1} oil	μg g^{-1} oil
2	3.38	14.91	15.13	2.22	9.79	2.97
3	1.91	8.43	9.40	2.30	10.15	4.33
4	1.25	5.40	5.50	2.19	9.47	3.63
6	0.60	2.55	2.00	2.56	10.89	4.87
8	0.59	2.49	0.93	2.78	11.73	4.20
9	0.52	2.13	1.53	2.00	8.19	3.43
10	0.45	1.87	1.00	1.79	7.44	3.47
11	0.39	1.57	0.67	1.55	6.23	3.07
13	0.44	1.85	0.53	1.25	5.25	2.97

Appendix 7.Carotenoids and chlorophylls HPLC chromatogram of 'Hass' avocado skin, dark pulp, pale pulp, yellow pulp determined simultaneously

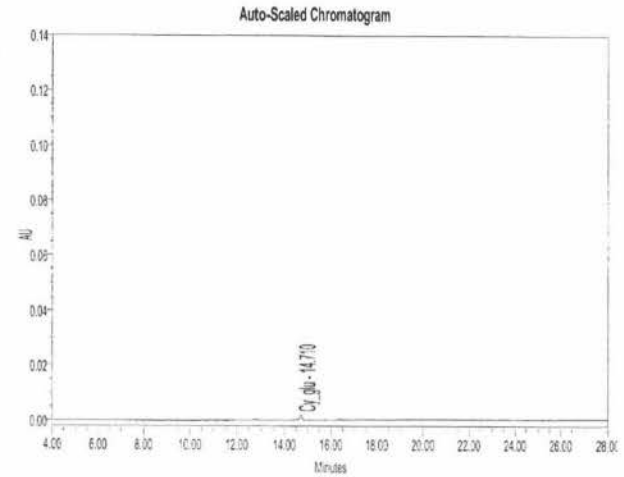


Appendix 8 HPLC chromatogram of chlorophylls and unknown (unknown 'a','d' and 'e')

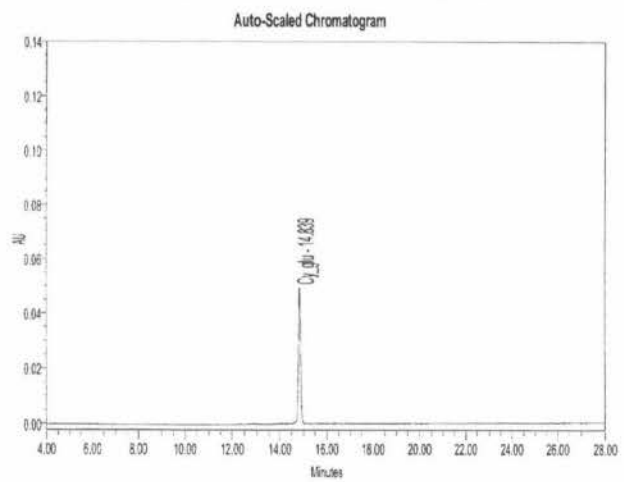


Appendix 9.Anthocyanins HPLC chromatogram of 'Hass' avocado skin

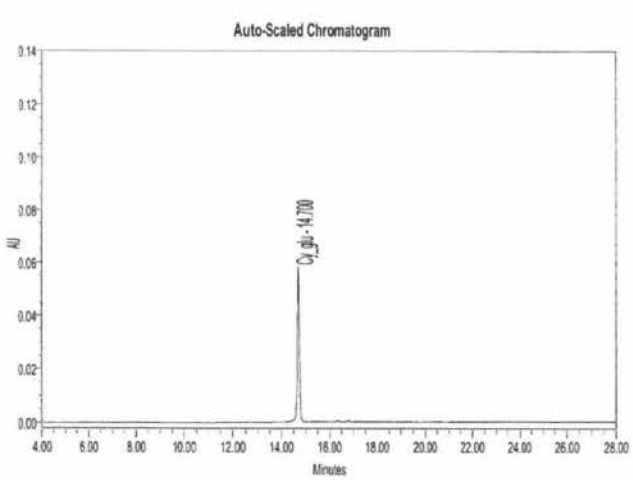
(a) Unripe/green avocado (day 2)



(b) Partially ripe/ripe avocado (day 6)



(c)Ripe/senescent avocado (day 13)



Appendix 10.Carotenoids and chlorophylls concentration of avocado oil produced by cold pressed extraction with different levels of skin addition and comparison of avocado oil produced by cold pressed laboratory vs factory scale

Carotenoids			
% Skin	Neoxanthin	Antheraxanthin	Lutein
	ug g^{-1} oil		
0	0.11	0.18	1.13
5	0.08	0.13	1.21
10	0.08	0.19	1.91
20	0.14	0.22	1.92
40	0.06	0.20	1.94
70	0.17	0.24	2.29
100	0.08	0.28	3.21
	SE		
0	0.06	0.01	0.11
5	0.08	0.07	0.09
10	0.00	0.01	0.12
20	0.07	0.01	0.03
40	0.06	0.01	0.17
70	0.08	0.02	0.11
100	0.08	0.03	0.15

Chlorophylls						
% Skin	Total Chlorophylls		Chlorophyll b	Chlorophyll a	Pheophytin b	Pheophytin a
	Spectrophotometer	HPLC	HPLC			
	µg g^{-1}oil					
0	9.81	7.52	3.00	2.52	1.13	0.86
5	12.22	11.94	4.22	3.57	3.08	1.07
10	16.51	16.58	6.59	3.81	4.43	1.75
20	17.77	17.94	6.83	5.83	3.70	1.58
40	19.5	19.50	7.19	5.35	5.26	1.70
70	22.01	21.73	8.47	6.59	4.72	1.95
100	25.34	27.48	11.48	7.51	6.24	2.25
	SE					
0	0.18	0.40	0.19	0.47	0.49	0.09
5	0.22	0.97	0.29	0.87	0.67	0.02
10	0.24	0.39	0.51	1.03	0.24	0.11
20	0.26	0.20	0.07	0.64	0.57	0.09
40	0.31	0.16	0.54	1.35	1.48	0.07
70	0.27	0.68	0.45	1.04	1.03	0.19
100	0.49	2.00	1.23	0.99	0.58	0.23

20°C	Days	**Total Chlorophylls**	**Chl *b***	**Chl *a***	**Pheo-*b***	**Pheo-*a***	**Lutein**	**Antheraxanthin**
		µg g^{-1} oil						
5%	Laboratory scale	11.94±0.97	4.22±0.29	3.57±0.87	3.08±0.67	1.07±0.02	1.13±0.09	0.13±0.07
	Factory trial	13.3	5.1	4.9	2.2	1.1	1.6	0.2
40%	Laboratory scale	19.50±0.16	7.19±0.54	5.35±1.35	5.26±1.48	1.70±0.07	1.94±0.17	0.20±0.01
	Factory trial	15.7	6.0	4.8	3.4	1.4	1.5	0.3
100%	Laboratory scale	27.48±2	11.48±1.23	7.51±0.99	6.24±0.58	2.25±0.23	3.21±0.15	0.28±0.03
	Factory trial	16.3	6.2	5.4	3.0	1.6	1.6	0.2

Appendix 11.Peroxide value used for linear regression analysis of avocado oil

5% Skin

Time (Day)	Zero order C_A meq/kg oil	Half order Square root (C_A)	First order ln C_A	Second order $1/C_A$
	-20°C			
0	0.38	0.62	-0.97	2.63
0	0.37	0.61	-0.99	2.70
27	0.48	0.69	-0.73	2.08
27	0.45	0.67	-0.80	2.22
55	1.27	1.13	0.24	0.79
55	1.30	1.14	0.26	0.77
83	2.47	1.57	0.90	0.40
83	1.96	1.40	0.67	0.51
97	3.02	1.74	1.11	0.33
97	3.00	1.73	1.10	0.33
	4°C			
0	0.38	0.62	-0.97	2.63
0	0.37	0.61	-0.99	2.70
27	0.56	0.75	-0.58	1.79
27	0.49	0.70	-0.71	2.04
55	1.04	1.02	0.04	0.96
55	1.03	1.01	0.03	0.97
83	3.10	1.76	1.13	0.32
83	2.86	1.69	1.05	0.35
97	4.08	2.02	1.41	0.25
97	5.96	2.44	1.79	0.17
	20°C			
0	0.38	0.62	-0.97	2.63
0	0.37	0.61	-0.99	2.70
6	0.37	0.61	-0.99	2.70
6	0.39	0.62	-0.94	2.56
13	0.43	0.66	-0.84	2.33
13	0.37	0.61	-0.99	2.70
20	0.69	0.83	-0.37	1.45
20	0.53	0.73	-0.63	1.89
27	0.75	0.87	-0.29	1.33
27	0.76	0.87	-0.27	1.32
41	1.00	1.00	0.00	1.00
41	1.00	1.00	0.00	1.00
55	1.14	1.07	0.13	0.88
55	1.26	1.12	0.23	0.79
69	1.99	1.41	0.69	0.50
69	1.94	1.39	0.66	0.52
83	2.81	1.68	1.03	0.36
83	2.92	1.71	1.07	0.34
97	2.25	1.50	0.81	0.44
97	2.26	1.50	0.82	0.44

Appendix 11.Peroxide value used for linear regression analysis of avocado oil continuation (a) 5% Skin

	Zero order C_A meq/kg oil	Half order Square root (C_A)	First order ln C_A	Second order $1/C_A$
Time (Day)	20°C L			
0	0.38	0.62	-0.97	2.63
0	0.37	0.61	-0.99	2.70
6	1.55	1.24	0.44	0.65
6	1.79	1.34	0.58	0.56
13	1.98	1.41	0.68	0.51
13	2.01	1.42	0.70	0.50
20	2.15	1.47	0.77	0.47
20	2.11	1.45	0.75	0.47
27	3.75	1.94	1.32	0.27
27	3.74	1.93	1.32	0.27
41	3.94	1.98	1.37	0.25
41	4.33	2.08	1.47	0.23
55	5.16	2.27	1.64	0.19
55	5.19	2.28	1.65	0.19
69	5.76	2.40	1.75	0.17
69	5.81	2.41	1.76	0.17
83	6.85	2.62	1.92	0.15
83	6.99	2.64	1.94	0.14
97	8.35	2.89	2.12	0.12
97	8.15	2.85	2.10	0.12
Time (Day)	40°C			
0	0.38	0.62	-0.97	2.63
0	0.37	0.61	-0.99	2.70
6	0.72	0.85	-0.33	1.39
6	0.89	0.94	-0.12	1.12
13	0.78	0.88	-0.25	1.28
13	0.75	0.87	-0.29	1.33
20	0.94	0.97	-0.06	1.06
20	0.97	0.98	-0.03	1.03
27	1.37	1.17	0.31	0.73
27	1.39	1.18	0.33	0.72
41	1.71	1.31	0.54	0.58
41	1.34	1.16	0.29	0.75
55	2.54	1.59	0.93	0.39
55	2.56	1.60	0.94	0.39
69	3.30	1.82	1.19	0.30
69	3.68	1.92	1.30	0.27
83	4.07	2.02	1.40	0.25
83	4.08	2.02	1.41	0.25
97	4.82	2.20	1.57	0.21
97	4.94	2.22	1.60	0.20

Appendix 11.Peroxide value used for linear regression analysis of avocado oil continuation (a) 5% Skin

	Zero order C_A meq/kg oil	Half order Square root (C_A)	First order ln C_A	Second order $1/C_A$
Time (Day)	50°C			
0	0.38	0.62	-0.97	2.63
0	0.37	0.61	-0.99	2.70
6	0.87	0.93	-0.14	1.15
6	0.91	0.95	-0.09	1.10
13	1.06	1.03	0.06	0.94
13	0.79	0.89	-0.24	1.27
20	1.17	1.08	0.16	0.85
20	0.95	0.97	-0.05	1.05
27	1.64	1.28	0.49	0.61
27	1.89	1.37	0.64	0.53
41	2.98	1.73	1.09	0.34
41	2.98	1.73	1.09	0.34
55	2.94	1.71	1.08	0.34
55	2.86	1.69	1.05	0.35
69	2.78	1.67	1.02	0.36
69	3.28	1.81	1.19	0.30
83	5.85	2.42	1.77	0.17
83	4.51	2.12	1.51	0.22
97	7.18	2.68	1.97	0.14
97	7.54	2.75	2.02	0.13
	60°C			
0	0.38	0.62	-0.97	2.63
0	0.37	0.61	-0.99	2.70
6	1.26	1.12	0.23	0.79
6	1.23	1.11	0.21	0.81
13	2.31	1.52	0.84	0.43
13	2.26	1.50	0.82	0.44
20	2.68	1.64	0.99	0.37
20	2.75	1.66	1.01	0.36
27	4.25	2.06	1.45	0.24
27	4.24	2.06	1.44	0.24
41	5.72	2.39	1.74	0.17
41	6.62	2.57	1.89	0.15
55	11.20	3.35	2.42	0.09
55	10.98	3.31	2.40	0.09

Appendix 11.Peroxide value used for linear regression analysis of avocado oil continuation (b) 40% Skin

Time (Day)	Zero order C_A meq/kg oil	Half order Square root (C_A)	First order ln C_A	Second order $1/C_A$
	-20°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
27	0.38	0.62	-0.97	2.63
27	0.38	0.62	-0.97	2.63
55	0.67	0.82	-0.40	1.49
55	0.64	0.80	-0.45	1.56
83	1.95	1.40	0.67	0.51
83	2.09	1.45	0.74	0.48
97	2.94	1.71	1.08	0.34
97	3.02	1.74	1.11	0.33
	4°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
27	0.47	0.69	-0.76	2.13
27	0.49	0.70	-0.71	2.04
55	0.93	0.96	-0.07	1.08
55	0.90	0.95	-0.11	1.11
83	2.18	1.48	0.78	0.46
83	1.97	1.40	0.68	0.51
97	2.89	1.70	1.06	0.35
97	3.11	1.76	1.13	0.32
	20°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	0.37	0.61	-0.99	2.70
6	0.38	0.62	-0.97	2.63
13	0.43	0.66	-0.84	2.33
13	0.35	0.59	-1.05	2.86
20	0.58	0.76	-0.54	1.72
20	0.56	0.75	-0.58	1.79
27	0.71	0.84	-0.34	1.41
27	0.68	0.82	-0.39	1.47
41	1.09	1.04	0.09	0.92
41	0.96	0.98	-0.04	1.04
55	1.42	1.19	0.35	0.70
55	1.59	1.26	0.46	0.63
69	2.47	1.57	0.90	0.40
69	2.38	1.54	0.87	0.42
83	3.08	1.75	1.12	0.32
83	2.75	1.66	1.01	0.36
97	3.05	1.75	1.12	0.33
97	2.92	1.71	1.07	0.34

Appendix 11.Peroxide value used for linear regression analysis of avocado oil continuation (b) 40% Skin

	Zero order C_A meq/kg oil	Half order Square root (C_A)	First order ln C_A	Second order $1/C_A$
Time (Day)	20°C L			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	2.04	1.43	0.71	0.49
6	2.16	1.47	0.77	0.46
13	2.54	1.59	0.93	0.39
13	2.01	1.42	0.70	0.50
20	2.48	1.57	0.91	0.40
20	2.39	1.55	0.87	0.42
27	3.05	1.75	1.12	0.33
27	3.23	1.80	1.17	0.31
41	5.52	2.35	1.71	0.18
41	5.24	2.29	1.66	0.19
55	5.48	2.34	1.70	0.18
55	5.56	2.36	1.72	0.18
69	5.81	2.41	1.76	0.17
69	5.68	2.38	1.74	0.18
83	6.10	2.47	1.81	0.16
83	6.18	2.49	1.82	0.16
97	12.99	3.60	2.56	0.08
97	12.49	3.53	2.52	0.08
Time (Day)	40°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	0.73	0.85	-0.31	1.37
6	0.54	0.73	-0.62	1.85
13	0.74	0.86	-0.30	1.35
13	0.77	0.88	-0.26	1.30
20	0.98	0.99	-0.02	1.02
20	0.96	0.98	-0.04	1.04
27	1.39	1.18	0.33	0.72
27	1.33	1.15	0.29	0.75
41	1.37	1.17	0.31	0.73
41	1.40	1.18	0.34	0.71
55	2.55	1.60	0.94	0.39
55	2.60	1.61	0.96	0.38
69	3.07	1.75	1.12	0.33
69	3.00	1.73	1.10	0.33
83	4.99	2.23	1.61	0.20
83	5.02	2.24	1.61	0.20
97	9.45	3.07	2.25	0.11
97	10.11	3.18	2.31	0.10

Appendix 11.Peroxide value used for linear regression analysis of avocado oil continuation (b) 40% Skin

	Zero order C_A meq/kg oil	Half order Square root(C_A)	First order ln C_A	Second order $1/C_A$
Time (Day)	50°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	0.76	0.87	-0.27	1.32
6	0.77	0.88	-0.26	1.30
13	1.00	1.00	0.00	1.00
13	1.10	1.05	0.10	0.91
20	1.10	1.05	0.10	0.91
20	1.21	1.10	0.19	0.83
27	3.14	1.77	1.14	0.32
27	3.16	1.78	1.15	0.32
41	3.17	1.78	1.15	0.32
41	3.15	1.77	1.15	0.32
55	6.87	2.62	1.93	0.15
55	6.73	2.59	1.91	0.15
69	11.28	3.36	2.42	0.09
69	11.26	3.36	2.42	0.09
	60°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	1.24	1.11	0.22	0.81
6	1.21	1.10	0.19	0.83
13	1.92	1.39	0.65	0.52
13	1.98	1.41	0.68	0.51
20	2.38	1.54	0.87	0.42
20	2.49	1.58	0.91	0.40
27	4.23	2.06	1.44	0.24
27	4.28	2.07	1.45	0.23
41	6.64	2.58	1.89	0.15
41	7.34	2.71	1.99	0.14
55	9.88	3.14	2.29	0.10
55	9.46	3.08	2.25	0.11
69	15.60	3.95	2.75	0.06
69	15.78	3.97	2.76	0.06

Appendix 11.Peroxide value used for linear regression analysis of avocado oil continuation (c) 100% Skin

Time (Day)	Zero order C_A meq/kg oil	Half order Square root (C_A)	First order ln C_A	Second order $1/C_A$
	-20°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
27	0.46	0.68	-0.78	2.17
27	0.46	0.68	-0.78	2.17
55	0.65	0.81	-0.43	1.54
55	0.77	0.88	-0.26	1.30
83	2.26	1.50	0.82	0.44
83	2.11	1.45	0.75	0.47
97	2.86	1.69	1.05	0.35
97	3.63	1.91	1.29	0.28
	4°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
27	0.45	0.67	-0.80	2.22
27	0.38	0.62	-0.97	2.63
55	1.06	1.03	0.06	0.94
55	1.06	1.03	0.06	0.94
83	2.03	1.42	0.71	0.49
83	2.37	1.54	0.86	0.42
97	3.51	1.87	1.26	0.28
97	3.32	1.82	1.20	0.30
	20°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	0.37	0.61	-0.99	2.70
6	0.38	0.62	-0.97	2.63
13	0.42	0.65	-0.87	2.38
13	0.46	0.68	-0.78	2.17
20	0.68	0.82	-0.39	1.47
20	0.66	0.81	-0.42	1.52
27	0.72	0.85	-0.33	1.39
27	0.79	0.89	-0.24	1.27
41	0.99	0.99	-0.01	1.01
41	1.01	1.00	0.01	0.99
55	1.70	1.30	0.53	0.59
55	1.40	1.18	0.34	0.71
69	2.69	1.64	0.99	0.37
69	2.69	1.64	0.99	0.37
83	2.80	1.67	1.03	0.36
83	2.84	1.69	1.04	0.35
97	3.44	1.85	1.24	0.29
97	3.63	1.91	1.29	0.28

Appendix 11.Peroxide value used for linear regression analysis of avocado oil continuation (c) 100% Skin

	Zero order C_A meq/kg oil	Half order Square root (C_A)	First order ln C_A	Second order $1/C_A$
Time (Day)	20°C L			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	2.62	1.62	0.96	0.38
6	2.60	1.61	0.96	0.38
13	2.85	1.69	1.05	0.35
13	2.93	1.71	1.08	0.34
20	3.45	1.86	1.24	0.29
20	3.52	1.88	1.26	0.28
27	4.06	2.01	1.40	0.25
27	3.87	1.97	1.35	0.26
41	6.76	2.60	1.91	0.15
41	6.76	2.60	1.91	0.15
55	7.27	2.70	1.98	0.14
55	7.42	2.72	2.00	0.13
69	7.68	2.77	2.04	0.13
69	7.80	2.79	2.05	0.13
83	8.38	2.89	2.13	0.12
83	8.67	2.94	2.16	0.12
97	12.00	3.46	2.48	0.08
97	12.40	3.52	2.52	0.08
Time (Day)	40°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	0.56	0.75	-0.58	1.79
6	0.57	0.75	-0.56	1.75
13	0.76	0.87	-0.27	1.32
13	0.78	0.88	-0.25	1.28
20	0.96	0.98	-0.04	1.04
20	0.99	0.99	-0.01	1.01
27	1.36	1.17	0.31	0.74
27	1.42	1.19	0.35	0.70
41	1.57	1.25	0.45	0.64
41	1.56	1.25	0.44	0.64
55	3.79	1.95	1.33	0.26
55	3.54	1.88	1.26	0.28
69	5.47	2.34	1.70	0.18
69	5.62	2.37	1.73	0.18
83	9.22	3.04	2.22	0.11
83	9.52	3.09	2.25	0.11
97	9.49	3.08	2.25	0.11
97	9.47	3.08	2.25	0.11

Appendix 11.Peroxide value used for linear regression analysis of avocado oil continuation (c) 100% Skin

Time (Day)	Zero order C_A meq/kg oil	Half order Square root (C_A)	First order ln C_A	Second order $1/C_A$
	50°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	1.09	1.04	0.09	0.92
6	0.86	0.93	-0.15	1.16
13	1.13	1.06	0.12	0.88
13	1.09	1.04	0.09	0.92
20	1.22	1.10	0.20	0.82
20	1.29	1.14	0.25	0.78
27	3.65	1.91	1.29	0.27
27	3.67	1.92	1.30	0.27
41	3.59	1.89	1.28	0.28
41	4.14	2.03	1.42	0.24
55	3.04	1.74	1.11	0.33
55	4.91	2.22	1.59	0.20
69	4.72	2.17	1.55	0.21
69	5.03	2.24	1.62	0.20
83	9.00	3.00	2.20	0.11
83	9.23	3.04	2.22	0.11
97	11.78	3.43	2.47	0.08
97	11.48	3.39	2.44	0.09
	60°C			
0	0.37	0.61	-0.99	2.70
0	0.37	0.61	-0.99	2.70
6	1.29	1.14	0.25	0.78
6	1.21	1.10	0.19	0.83
13	2.24	1.50	0.81	0.45
13	2.33	1.53	0.85	0.43
20	2.48	1.57	0.91	0.40
20	2.92	1.71	1.07	0.34
27	2.80	1.67	1.03	0.36
27	2.83	1.68	1.04	0.35
41	4.72	2.17	1.55	0.21
41	4.78	2.19	1.56	0.21
55	8.22	2.87	2.11	0.12
55	9.22	3.04	2.22	0.11
69	13.39	3.66	2.59	0.07
69	13.49	3.67	2.60	0.07

Appendix 12.Example of linear regression using peroxide value of 20°C of avocado oil with 5% skin over 97 days of storage.

5% Skin

Regression Statistics		:20 versus day
Multiple R	0.94614608	
R Square	0.8951924	
Adjusted R Square	0.88936975	
Standard Error	10.9023988	
Observations	20	

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	18274.2786	18274.28	153.7433	2.97401E-10
Residual	18	2139.521403	118.8623		
Total	19	20413.8			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.36703351	4.203971659	-0.325177	0.748797	-10.19925706	7.46519	-10.19925706	7.465190045
X Variable 1	35.9737683	2.901268199	12.39933	2.97E-10	29.8784253	42.06911	29.8784253	42.06911135

Regression Statistics		:SQRT20 versus day
Multiple R	0.96870663	
R Square	0.93839253	
Adjusted R Square	0.93496989	
Standard Error	8.3587699	
Observations	20	

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	19156.15738	19156.16	274.1724	2.43988E-12
Residual	18	1257.642617	69.86903		
Total	19	20413.8			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-43.2424944	5.425806921	-7.969781	2.59E-07	-54.64170057	-31.84329	-54.64170057	-31.84328823
X Variable 1	82.6881295	4.993802236	16.55815	2.44E-12	72.19653215	93.17973	72.19653215	93.17972676

5% Skin

Regression Statistics		:ln20 versus day
Multiple R	0.97236075	
R Square	0.94548544	
Adjusted R Square	0.94245685	
Standard Error	7.86288533	
Observations	20	

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	19300.95062	19300.95	312.187	8.08754E-13
Residual	18	1112.849384	61.82497		
Total	19	20413.8			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	45.0780969	1.772551798	25.43119	1.47E-15	41.35410086	48.80209	41.35410086	48.80209291
X Variable 1	42.7762793	2.421004517	17.66881	8.09E-13	37.68993365	47.86263	37.68993365	47.86262502

Regression Statistics		:$1/C_A$ versus day
Multiple R	0.92486248	
R Square	0.85537061	
Adjusted R Square	0.84733564	
Standard Error	12.807193	
Observations	20	

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	17461.36452	17461.36	106.456	5.50684E-09
Residual	18	2952.435484	164.0242		
Total	19	20413.8			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	87.6667845	5.345165776	16.40113	2.87E-12	76.43699927	98.89657	76.43699927	98.89656983
X Variable 1	-33.3948027	3.23663516	-10.31775	5.51E-09	-40.19472606	-26.59488	-40.19472606	-26.59487925

Appendix 13.Summary of linear regression analysis (rate of reaction) for peroxide value of avocado oil (R square values and slopes)

(a) Zero order

Temperature	°K	1/T	R square values	Slope (k)	ln k	R square values	Slope (k)	ln k	R square values	Slope (k)	ln k
			5% Skin			40% Skin			100% Skin		
-20°C	253	0.003953	0.9256	0.0275	-3.593569	0.8095	0.0265	-3.630611	0.8166	0.0289	-3.543914
4°C	277	0.003610	0.8776	0.0310	-3.473768	0.8685	0.0266	-3.626844	0.8615	0.0304	-3.493313
20°C	293	0.003413	0.8974	0.0250	-3.688879	0.9548	0.0310	-3.473768	0.9496	0.034	-3.381395
20°CL	293	0.003413	0.9971	0.0741	-2.602340	0.8331	0.0938	-2.366590	0.9455	0.1018	-2.284745
40°C	313	0.003195	0.9741	0.0462	-3.074775	0.7863	0.0762	-2.574394	0.9082	0.101	-2.292635
50°C	323	0.003096	0.9039	0.0621	-2.779009	0.8900	0.1470	-1.917323	0.8973	0.1041	-2.262403
60°C	333	0.003003	0.9635	0.2046	-1.586698	0.9526	0.2101	-1.560172	0.92	0.1743	-1.746977

(b) Half order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.9596	0.0124	0.8654	0.0116	0.9695	0.0117
4°C	0.9211	0.0150	0.9326	0.0117	0.9136	0.0127
20°C	0.9379	0.0111	0.9736	0.0136	0.9762	0.0140
20°CL	0.9165	0.0199	0.8581	0.0222	0.6890	0.0227
40°C	0.9935	0.0160	0.9412	0.0215	0.9687	0.0273
50°C	0.9388	0.0190	0.9592	0.0384	0.9120	0.0253
60°C	0.9740	0.0451	0.9925	0.0458	0.9704	0.0396

(c)First order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.9733	0.0238	0.9072	0.0228	0.9105	0.0257
4°C	0.9715	0.0258	0.9699	0.0255	0.9642	0.0238
20°C	0.9462	0.0215	0.9749	0.0250	0.9709	0.0255
20°CL	0.7527	0.0236	0.7204	0.0241	0.6646	0.0239
40°C	0.9502	0.0243	0.9660	0.0267	0.9769	0.0343
50°C	0.9013	0.0255	0.9499	0.0468	0.8647	0.0294
60°C	0.8758	0.0527	0.9052	0.0474	0.8880	0.0431

(d) Second order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.9446	-0.0256	0.9466	-0.0275	0.9792	-0.0259
4°C	0.9790	-0.0253	0.9792	-0.0258	0.9636	-0.0266
20°C	0.8512	-0.0247	0.8849	-0.0269	0.8650	-0.0268
20°CL	0.3669	-0.0136	0.3192	-0.0131	0.2860	-0.0126
40°C	0.6972	-0.0186	0.7177	-0.0196	0.7563	-0.0219
50°C	0.5829	-0.0168	0.6722	-0.0294	0.5078	-0.0166
60°C	0.4886	-0.0319	0.4705	-0.0247	0.4520	-0.0239

Appendix 14.Calculation of (a) shelf life and (b) energy of activation in terms of peroxide value and (c) total chlorophyll at the end of shelf life

(a) Shelf life

The order of degradation for avocado oil was determined to be, zero order.

The equations for calculating shelf life are:

Zero Order: $C_A = C_{AO}-kt$

0.5 Order: $\sqrt{C_A} = -0.5kt + \sqrt{C_{AO}}$

First Order: $\ln C_A = \ln C_{AO} - kt$

Second Order: $\frac{1}{C_A} = \frac{1}{C_{AO}} + kt$

Where:

C_A = Concentration of component A (IOOC 2002) = 20meq/kg

C_{AO} = Concentration at time 0

t = Time (Shelf Life)

k = Rate of change of concentrations

1.) 5% skin addition to avocado pulp in cold pressed oil extraction, factory trial.

Extracted oil stored at 20°C in the dark.

Zero Order $C_A = C_{AO}-kt$

Where:

C_A = Concentration of component A (IOOC 2002) = 20meq/kg

C_{AO} = Concentration at time 0= 0.37meq/kg

t = Time (Shelf Life) (days)-unknown

k = Rate of change of concentrations 0.025meq/kg /day

Substituting into: $C_A = C_{AO} - kt$, $20 = 0.37 - 0.025t$

$\therefore t = 785.2$ days, say 780 days or 26 months.

2.) 40% skin addition to avocado pulp in cold pressed oil extraction, factory trial.

Extracted oil stored at 20°C in the dark.

Zero Order $C_A = C_{AO}$-kt

Where:

C_A = Concentration of Component A (IOOC 2002) = 20meq/kg

C_{AO} = Concentration at time 0 = 0.37meq/kg

t = Time (Shelf Life) (days) - unknown

k = Rate of change of concentrations 0.031meq/kg /day

Substituting into: $C_A = C_{AO}$ -kt, 20=0.37-0.031t

$\therefore$ t=633.2 days, say 630 days or 21 months.

3.) 100% skin addition to avocado pulp in cold pressed oil extraction, factory trial.

Extracted oil stored at 20°C in the dark.

Zero Order $C_A = C_{AO}$-kt

Where:

C_A = Concentration of Component A (IOOC 2002) = 20meq/kg

C_{AO} = Concentration at time 0 = 0.37meq/kg

t = Time (Shelf Life) (days) -unknown

k = Rate of change of concentrations 0.034meq/kg /day

Substituting into: $C_A = C_{AO}$ - kt, 20=0.37-0.034t

$\therefore$ t=577.4 days, say 570 days or 19 months.

(b) Total chlorophyll at the end of shelf life (HPLC)

R square values and slope from Appendix 15 (d) were used and substituted using

formula $\frac{1}{C_A} = \frac{1}{C_{Ao}} + kt$

1.) 5% skin

$$\frac{1}{C_A} = \frac{1}{13.3} + 0.0002 \times 780 \quad = \frac{1}{0.2312} = 4.32 \mu g\ g^{-1}$$

2.) 40% Skin

$$\frac{1}{C_A} = \frac{1}{15.7} + 0.0003 \times 630 \quad = \frac{1}{0.2527} = 3.96\mu g\ g^{-1}$$

2.) 100% Skin

$$\frac{1}{C_A} = \frac{1}{16.3} + 0.0004 \times 570 \quad = \frac{1}{0.2894} = 3.46\mu g\ g^{-1}$$

(c)Energy of activation

Using the formula Slope = $\frac{-Ea}{R}$

1.) 5% Skin

$-Ea = -slope \times R$

$= -1776 \times \sqrt{0.6097}$

$Ea = 1776 \times 0.7808$

$= 1386.75$ joules mol^{-1}

2.) 40% Skin

$-Ea = -slope \times R$

$= -2335.3 \times \sqrt{0.7782}$

$Ea = 2335.3 \times 0.8822$

$Ea = 2060.2$ joules mol^{-1}

3.) 100% Skin

$-Ea = -slope \times R$

$= -1899.7 \times \sqrt{0.7138}$

$Ea = 1899.7 \times 0.8449$

$= 1605.1$ joules mol^{-1}

Appendix 15.Summary of R square values and slope of total chlorophylls (the sum of chlorophyll *a* and *b*, and pheophytin *a* and *b*) by HPLC.

(a) Zero order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.0108	-0.0035	0.0583	-0.0119	0.1436	0.0295
4°C	0.0050	-0.0023	0.0050	-0.0032	0.0539	0.0129
20°C	0.4249	-0.0306	0.5538	-0.0453	0.6705	-0.0550
20°CL	0.8238	-0.0746	0.7483	-0.0655	0.8828	-0.0813
40°C	0.7700	-0.0770	0.7652	-0.0853	0.9107	-0.1254
50°C	0.7661	-0.1093	0.7718	-0.1357	0.7287	-0.1481
60°C	0.3272	-0.0740	0.3524	-0.0932	0.3097	-0.0873

(b) Half order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.0078	-0.0004	0.0512	-0.0015	0.1305	0.0037
4°C	0.0057	-0.0004	0.0029	-0.0003	0.0481	0.0016
20°C	0.4310	-0.0047	0.5421	-0.0065	0.6781	-0.0076
20°CL	0.8436	-0.0125	0.7750	-0.0098	0.8959	-0.0119
40°C	0.8507	-0.0148	0.8198	-0.0141	0.9498	-0.0233
50°C	0.9101	-0.0270	0.9293	-0.0344	0.8073	-0.0354
60°C	0.4519	-0.0210	0.4765	-0.0259	0.3165	-0.0181

(c) First order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.0049	-0.0002	0.0544	-0.0009	0.1312	0.0020
4°C	0.0046	-0.0002	9E-05	-3E-05	0.0473	0.0008
20°C	0.4350	-0.0029	0.5245	-0.0035	0.6731	-0.0041
20°CL	0.8566	-0.0087	0.7979	-0.0059	0.9063	-0.0071
40°C	0.9040	-0.0117	0.8915	-0.0095	0.9457	-0.0185
50°C	0.9787	-0.0308	0.9787	-0.0446	0.8324	-0.0404
60°C	0.5966	-0.0341	0.7555	-0.0385	0.2402	-0.0163

(d) Second Order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.0450	-5E-05	0.0876	9E-05	0.0308	-6E-05
4°C	0.0109	-1E-05	0.0148	4E-05	0.0109	-3E-05
20°C	0.5014	0.0002	0.4218	0.0003	0.6371	0.0004
20°CL	0.8780	0.0011	0.8533	0.0006	0.8761	0.0006
40°C	0.8963	0.0019	0.9248	0.0012	0.8008	0.0036
50°C	0.7772	0.0166	0.7920	0.0450	0.7317	0.0234
60°C	0.5676	0.7320	0.8118	0.0539	0.0227	0.0034

Appendix 16.Summary of R square values and slope of total chlorophyll (Spectrophotometer)

(a)Zero order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.8052	-0.0230	0.7730	-0.0418	0.4625	-0.0248
4°C	0.2719	-0.0117	0.4161	-0.0269	0.3496	-0.0229
20°C	0.3236	-0.0205	0.0001	-0.0003	0.0833	-0.0129
20°CL	0.8585	-0.0621	0.8786	-0.0789	0.7893	-0.0636
40°C	0.9432	-0.0419	0.8612	-0.0427	0.8634	-0.0506
50°C	0.9647	-0.0618	0.9105	-0.0931	0.9253	-0.0937
60°C	0.9437	-0.0720	0.8941	-0.0896	0.7388	-0.1044

(b)Half order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.8146	-0.0030	0.7732	-0.0052	0.4617	-0.0029
4°C	0.2855	-0.0015	0.6821	-0.0124	0.3349	-0.0027
20°C	0.3244	-0.0027	5E-06	9E-06	0.0806	-0.0015
20°CL	0.8754	-0.0085	0.8803	-0.0108	0.8150	-0.0081
40°C	0.9486	-0.0055	0.8870	-0.0054	0.8733	-0.0061
50°C	0.9656	-0.0084	0.9344	-0.0132	0.9423	-0.0122
60°C	0.9439	-0.0103	0.9105	-0.0129	0.7674	-0.0143

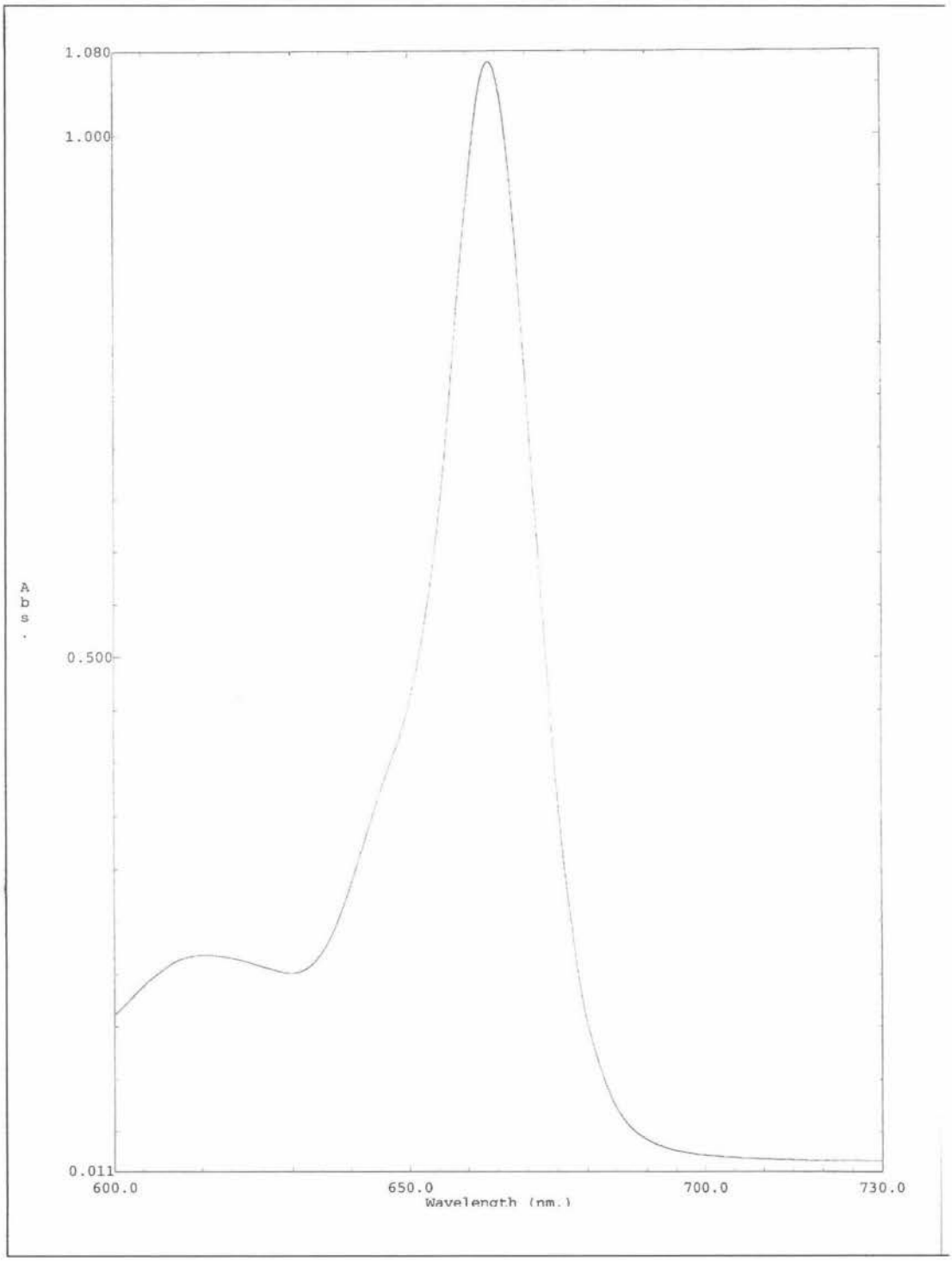
(c) First order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.8052	-0.0016	0.7453	-0.0025	0.4866	-0.0014
4°C	0.2693	-0.0008	0.4043	-0.0016	0.3191	-0.0012
20°C	0.3065	-0.0014	2E-05	9E-06	0.0842	-0.0007
20°CL	0.8938	-0.0047	0.8730	-0.0060	0.8505	-0.0042
40°C	0.9521	-0.0029	0.8829	-0.0027	0.8793	-0.0029
50°C	0.9598	-0.0046	0.9490	-0.0076	0.9559	-0.0063
60°C	0.9448	-0.0059	0.9125	-0.0075	0.7821	-0.0081

(d)Second order

Temperature	R square values	Slope (k)	R square values	Slope (k)	R square values	Slope (k)
	5% Skin		40% Skin		100% Skin	
-20°C	0.7977	0.0001	0.8378	0.0002	0.0626	3E-05
4°C	0.5428	8E-05	0.5428	0.0002	0.5428	8E-05
20°C	0.6688	0.0002	0.0075	-1E-05	0.0706	4E-05
20°CL	0.9142	0.0004	0.8225	0.0004	-.8090	0.0003
40°C	0.8716	0.0002	0.7803	0.0002	0.6984	0.0002
50°C	0.9444	0.0004	0.9140	0.0008	0.8781	0.0004
60°C	0.9272	0.0005	0.8724	0.0006	0.7389	0.0006

Appendix 17.Chromatogram of total chlorophyll (Spectrophotometer)



Appendix 18.Summary of carotenoids and chlorophylls of avocado oil (5%, 40% and 100% skin) during storage at different temperatures (HPLC)

	Days	Total Chlorophylls	Chl b	Chl a	Pheop-b	Pheop-a	Lutein	Antheraxanthin
		$\mu g\ g^{-1}$ oil						
20°C								
5%	0	13.3	5.1	4.9	2.2	1.1	1.6	0.2
	6	11.6	4.9	2.8	2.9	1.4	1.9	0.2
	13	10.9	4.2	4.1	1.3	1.1	1.7	0.2
	20	11.2	4.1	4.4	1.4	1.1	1.7	0.2
	27	10.4	3.4	3.6	4.3	1.1	1.6	0.2
	41	8.9	2.9	3.2	1.9	0.9	1.8	0.2
	55	9.6	3.3	3.4	1.9	1.3	1.9	0.2
	69	8.8	3.2	2.4	2.3	0.9	1.9	0.2
	97	9.2	2.7	2.7	1.8	0.8	1.9	0.3
40%	0	15.7	6.0	4.8	3.4	1.4	1.5	0.3
	6	14.3	6.2	4.3	2.6	1.8	1.9	0.4
	13	13.7	5.3	5.1	1.8	1.2	1.5	0.2
	20	14.0	5.3	5.8	1.3	1.1	1.4	0.1
	27	11.9	4.4	4.9	1.3	1.3	1.6	0.2
	41	11.7	3.9	4.7	2.0	1.1	1.8	0.2
	55	11.6	3.7	3.4	3.3	1.3	1.7	0.2
	69	11.3	3.8	3.0	3.0	1.4	1.6	0.2
	97	11.9	3.8	3.9	3.2	0.9	2.0	0.2
100%	0	16.3	6.2	5.4	3.0	1.6	1.6	0.2
	6	15.7	6.6	4.2	3.0	1.8	1.8	0.3
	13	15.6	6.0	6.0	2.1	1.5	1.6	0.2
	20	13.7	5.0	5.2	2.2	1.3	1.2	0.0
	27	11.4	3.7	3.3	2.9	1.5	1.6	0.1
	41	11.5	4.1	3.7	2.4	1.3	1.8	0.2
	55	11.4	4.0	3.5	2.8	1.1	1.6	0.2
	69	11.4	3.8	3.3	3.1	1.1	1.7	0.2
	97	11.2	3.6	3.3	3.0	1.4	1.8	0.2
20C L								
5%	0	13.3	5.1	4.9	2.2	1.1	1.5	0.2
	6	11.8	4.5	4.8	2.0	1.2	1.6	0.2
	13	10.2	3.9	3.5	1.7	1.0	1.6	0.2
	20	10.3	3.9	4.2	1.1	0.8	1.5	0.2
	27	9.0	2.5	2.3	3.2	1.5	1.7	0.1
	41	6.7	1.9	1.2	2.8	0.8	1.8	0.2
	55	6.8	2.0	1.0	2.6	0.8	1.7	0.2
	69	5.9	1.9	0.6	2.6	0.8	1.7	0.2
	97	5.7	2.5	2.1	0.4	0.7	1.7	0.2
40%	0	15.7	6.0	4.8	3.4	1.4	1.5	0.3
	6	12.0	5.2	3.4	2.7	1.3	1.6	0.2
	13	13.1	5.2	6.1	0.3	1.1	1.6	0.2
	20	12.8	4.9	5.2	1.4	1.0	1.5	0.1
	27	10.1	2.9	1.7	4.1	1.4	1.7	0.2
	41	10.6	3.1	2.0	4.3	1.1	1.8	0.2
	55	8.7	3.2	3.0	1.7	0.9	1.7	0.2
	69	9.5	3.3	1.2	4.1	1.0	1.7	0.2
	97	8.1	3.0	2.7	1.7	0.7	1.7	0.2
100%	0	16.3	6.2	5.4	3.0	1.6	1.6	0.2
	6	14.4	5.8	5.0	2.0	1.6	1.6	0.2
	13	13.9	5.3	5.5	2.0	1.2	1.4	0.0
	20	14.4	5.3	6.0	1.8	1.4	1.3	0.1
	27	12.8	4.3	3.6	3.8	1.1	1.4	0.1
	41	11.2	3.7	2.8	3.4	1.2	1.6	0.2
	55	9.4	3.1	2.2	3.2	0.9	1.6	0.2
	69	8.7	3.0	1.3	3.4	1.1	1.6	0.2
	97	8.7	3.0	2.8	2.1	0.9	1.7	0.2

Appendix 18.Summary of carotenoids and chlorophylls of avocado oil (5%, 40% and 100% skin) during storage at different temperatures continuation (HPLC)

	Days	Total Chlorophylls	Chl b	Chl a	Pheop-b	Pheop-a	Lutein	Antheraxanthin
		μg g^{-1} oil						
40°C								
5%	0	13.3	5.1	4.9	2.2	1.1	1.6	0.2
	6	8.8	3.1	2.5	2.1	1.1	1.7	0.2
	13	8.5	3.1	2.7	1.5	1.0	1.5	0.2
	20	8.0	2.9	3.5	0.8	0.7	1.5	0.2
	27	6.8	2.4	1.8	1.7	0.8	1.8	0.2
	41	6.5	1.8	1.0	2.9	0.8	1.7	0.2
	55	6.0	1.6	1.3	2.1	1.0	2.0	0.0
	69	5.2	1.5	0.3	2.7	0.7	1.7	0.2
	97	3.2	1.3	1.1	0.4	0.3	1.8	0.0
40%	0	15.7	6.0	4.8	3.4	1.4	1.5	0.3
	6	11.1	4.2	3.0	2.8	1.1	1.8	0.2
	13	11.0	4.2	3.8	1.7	0.7	1.6	0.4
	20	9.6	3.6	4.1	0.9	0.7	1.3	0.4
	27	9.7	3.5	3.6	1.6	0.9	2.0	0.2
	41	7.3	2.4	1.2	2.8	0.9	1.6	0.2
	55	8.2	2.7	1.3	3.2	1.0	1.7	0.3
	69	5.8	1.9	0.4	2.7	0.8	1.8	0.2
	97	5.6	2.2	1.2	1.6	0.6	1.8	0.2
100%	0	16.3	6.2	5.4	3.0	1.6	1.6	0.2
	6	13.2	4.7	4.4	2.6	1.5	1.6	0.2
	13	12.4	4.4	3.9	2.6	1.4	1.4	0.4
	20	10.4	4.0	4.3	1.2	0.8	1.3	0.4
	27	9.3	3.4	2.9	1.9	1.0	1.8	0.2
	41	7.2	2.8	1.2	2.0	1.1	1.5	0.2
	55	7.2	2.6	1.1	2.5	1.0	1.6	0.2
	69	4.7	1.7	0.4	2.0	0.7	1.3	0.2
	97	2.1	1.0	0.6	0.1	0.4	1.5	0.2
50°C								
5%	0	13.3	5.1	4.9	2.2	1.1	1.6	0.2
	6	8.2	3.3	2.6	1.7	1.1	1.6	0.2
	13	7.0	2.8	2.4	1.0	0.6	1.4	0.2
	20	5.6	2.0	1.9	0.9	0.7	1.2	0.2
	27	5.1	1.7	1.3	1.4	0.6	1.8	0.3
	41	2.5	0.7	0.3	1.1	0.4	1.6	0.2
	55	2.3	0.9	0.3	0.9	0.2	1.6	0.3
	69	1.6	0.5	0.1	0.8	0.2	1.5	0.2
	97	0.5	0.1	0.0	0.2	0.1	1.6	0.2
40%	0	15.7	6.0	4.8	3.4	1.4	1.5	0.3
	6	8.9	3.7	2.3	2.0	0.7	1.5	0.4
	13	8.0	3.1	2.9	1.1	0.6	1.4	0.4
	20	6.8	2.6	2.4	0.9	0.6	1.4	0.1
	27	5.6	2.1	1.9	1.4	0.7	1.7	0.2
	41	2.9	0.9	0.6	1.2	0.1	1.5	0.2
	55	1.8	0.6	0.4	0.6	0.2	1.4	0.2
	69	0.5	0.1	0.1	0.2	0.2	1.3	0.2
	97	0.2	0.1	0.0	0.0	0.1	1.2	0.2
100%	0	16.3	6.2	5.4	3.0	1.6	1.6	0.2
	6	10.3	4.4	2.1	1.4	1.3	1.6	0.4
	13	9.5	3.6	3.7	1.4	0.8	1.3	0.4
	20	6.8	2.9	2.7	0.5	0.8	1.3	0.1
	27	5.7	2.1	1.8	1.1	0.8	1.6	0.2
	41	0.8	0.0	0.0	0.0	0.8	1.1	0.1
	55	1.1	0.4	0.2	0.4	0.2	1.4	0.2
	69	0.4	0.2	0.0	0.2	0.1	1.3	0.2
	97	0.6	0.3	0.1	0.2	0.1	1.5	0.2

Appendix 18.Summary of carotenoids and chlorophylls of avocado oil (5%, 40% and 100% skin) during storage at different temperatures continuation

	Days	Total Chlorophylls	Chl b	Chl a	Pheop-b	Pheop-a	Lutein	Antheraxanthin
		μg g^{-1} oil						
60°C								
5%	0	13.3	5.1	4.9	2.2	1.1	1.6	0.2
	6	4.5	1.6	1.4	1.2	0.5	1.8	0.2
	13	1.7	0.6	0.5	0.4	0.2	1.3	0.2
	20	1.0	0.3	0.3	0.3	0.1	1.5	0.2
	27	0.4	0.2	0.1	0.1	0.0	1.8	0.2
	41	2.0	0.0	0.0	0.0	0.0	1.5	0.2
	55	0.5	0.0	0.0	0.1	0.5	1.0	0.2
	69	1.4	0.0	0.0	0.0	1.3	1.4	0.2
	97	0.1	0.0	0.0	0.0	0.0	1.3	0.2
40%	0	15.7	6.0	4.8	1.4	3.4	1.5	0.3
	6	5.0	1.5	0.4	0.3	2.5	1.5	0.1
	13	2.5	0.8	1.0	0.1	0.4	1.5	0.4
	20	1.1	0.2	0.7	0.1	0.0	1.4	0.3
	27	0.6	0.2	0.1	0.0	0.3	1.7	0.2
	41	0.0	0.0	0.0	0.0	0.0	1.5	0.2
	55	1.0	0.0	0.3	0.6	0.1	1.6	0.2
	69	0.2	0.1	0.1	0.5	0.0	0.7	0.4
	97	0.2	0.0	0.1	0.0	0.0	1.3	0.2
100%	0	16.3	6.2	5.4	3.0	1.6	1.6	0.2
	6	6.9	2.5	1.9	0.5	0.8	1.7	0.3
	13	2.8	1.1	0.9	0.5	0.3	1.3	0.1
	20	1.2	0.2	0.2	0.1	0.7	1.7	0.3
	27	0.9	0.1	0.1	0.1	0.5	1.7	0.2
	41	2.2	0.1	0.6	1.0	0.5	1.4	0.1
	55	1.7	0.1	0.0	0.0	1.6	1.4	0.1
	69	1.6	0.1	0.1	0.0	1.5	1.4	0.1
	97	1.3	0.1	0.0	0.0	1.2	1.0	0.4

Appendix 18.Summary of carotenoids and chlorophylls of avocado oil (5%, 40% and 100% skin) during storage at different temperatures continuation

	Days	Total Chlorophylls	Chl b	Chl a	Pheop-b	Pheop-a	Lutein	Antheraxanthin
		μg g^{-1} oil						
-20C								
5%	0	13.3	5.1	4.9	2.2	1.1	1.6	0.2
	27	10.1	3.2	2.1	3.6	1.2	1.8	0.2
	55	11.1	2.9	2.6	4.3	1.0	1.9	0.2
	97	12.3	4.5	4.5	2.1	1.2	1.9	0.2
40%	0	15.7	6.0	4.8	3.4	1.4	1.5	0.3
	27	12.9	4.4	3.6	3.5	1.5	1.7	0.2
	55	11.0	3.6	3.8	2.7	0.9	1.8	0.2
	97	14.5	4.9	4.5	3.3	1.6	1.5	0.2
100%	0	16.3	6.2	5.4	3.0	1.6	1.6	0.2
	27	11.0	4.7	2.0	2.5	1.8	1.8	0.2
	55	12.8	4.5	4.2	2.7	1.4	1.7	0.2
	97	18.1	6.9	7.3	2.3	1.6	1.7	0.2
4C								
5%	0	13.3	5.1	4.9	2.2	1.1	1.6	0.2
	27	10.4	3.0	2.1	2.9	1.3	1.7	0.2
	55	10.7	2.9	2.6	2.8	1.1	1.8	0.2
	97	12.7	4.5	4.5	3.2	1.2	1.8	0.2
40%	0	15.7	6.0	4.8	3.4	1.4	1.5	0.3
	27	11.3	4.4	3.6	4.2	1.2	1.6	0.1
	55	13.1	3.6	3.8	3.6	1.4	1.9	0.2
	97	14.4	4.9	4.4	3.6	1.3	1.7	0.2
100%	0	16.3	6.2	5.4	3.0	1.6	1.6	0.2
	27	12.6	4.7	2.0	4.0	1.5	1.5	0.1
	55	12.8	4.5	4.2	3.0	1.1	1.8	0.3
	97	17.0	6.9	7.3	2.6	1.5	1.8	0.2