

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**PIGMENT COMPOSITION OF 'HASS' AVOCADO AND
THE EXTRACTED OIL**

**A THESIS
PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF TECHNOLOGY IN
FOOD TECHNOLOGY AT
MASSEY UNIVERSITY, ALBANY
NEW ZEALAND**

OFELIA BATALLA ORLINGA ASHTON

2005

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 ongoing 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 ($\leq 20^\circ\text{C}$) 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.

ACKNOWLEDGMENTS

I wish to express my heartfelt gratitude to several people for making this accomplishment possible.

My two supervisors **Dr. Marie Wong** of Massey University and **Dr. Allan Brian Woolf** of HortResearch

Dr. Marie Wong for accepting my enrolment and for finding a good environment for me to conduct my research and for guiding me during the initial stage of storage trial. Her technical skills and academic mind provided me with a positive sense of direction.

Dr. Allan Brian Woolf for arranging the funding for materials and research facilities and for drawing Figure 3.1c. I am strengthened by the moral support, understanding and kindness he had extended.

Jointly, my supervisors trusted me to carry out most of my laboratory work independently which enabled me to think in depth, work efficiently and to achieve good results. Both of their skills and guidance have contributed to the making of my thesis

Dr. Tony McGhie for guiding me in the pigment analysis and for completing the pigment analysis of oil and Professor Dov Prusky for the diene analysis of oil

Yan Wang for her company during the factory trial and Susan Bryers for her company during the fruit tissue sampling and cold pressed extraction in the laboratory

Cecilia Requejo, Mary Petley and Richard Jackman during early days of fruit tissue sampling and also Cecilia for her assistance with starting the ASE and Anne White for Firmometer training

Dr. Andrew Allan, Dr. Ken Marsh, Laura Barnet, Rosheila Vasather, Kui Lin Wang, Reginald Wibisono, Paul Pidakala, Di Barraclough and Kay Hooi for answering some of my queries

To all staff members of Massey University and HortResearch who have given assistance no matter how small that may have been

To Olivado for factory trial, HortResearch and Foundation of Research Science and Technology for funding the materials

To my brothers, sisters and friends for their support and to my lovely nieces and nephews for making me happy

I thank my husband for his love

To Almighty God for all the blessings

Table of Contents

CHAPTER 1. GENERAL INTRODUCTION.....	1
CHAPTER 2. LITERATURE REVIEW	3
2.1 CHLOROPHYLL	3
2.1.1 <i>Chemistry of Chlorophyll</i>	3
2.1.2 <i>Chlorophyll Biosynthetic Pathway</i>	5
2.1.3 <i>Chlorophyll Degradation</i>	7
2.1.4 <i>Chlorophyll in Avocado</i>	8
2.2 CAROTENOIDS	8
2.2.1 <i>Chemistry of Carotenoids</i>	9
2.2.2 <i>Carotenoid Biosynthetic Pathway</i>	10
2.2.3 <i>Carotenoid Degradation</i>	13
2.3 ANTHOCYANINS	13
2.3.1 <i>Chemistry of Anthocyanin</i>	14
2.3.2 <i>Anthocyanin Biosynthesis</i>	15
2.3.3 <i>Anthocyanin Degradation</i>	17
2.3.4 <i>Anthocyanin in Avocado</i>	17
2.4 CHLOROPHYLL ANALYSIS	18
2.4.1 <i>Extraction and Isolation</i>	18
2.4.2 <i>Spectrophotometry</i>	18
2.4.3 <i>High Pressure Liquid Chromatography (HPLC)</i>	19
2.5 CAROTENOIDS ANALYSIS	19
2.5.1 <i>Extraction and Isolation</i>	19
2.5.2 <i>Spectrophotometry</i>	20
2.5.3 <i>High Pressure Liquid Chromatography (HPLC)</i>	20
2.6 ANTHOCYANIN ANALYSIS	20
2.6.1 <i>Extraction and Isolation</i>	21
2.6.2 <i>Spectrophotometry</i>	21
2.6.3 <i>High Pressure Liquid Chromatography (HPLC)</i>	21
2.7 COLOUR MEASUREMENT	22
2.8 AVOCADO FRUIT	24
2.8.1 <i>Maturity at Harvest</i>	25
2.8.1.1 <i>Dry Matter Determination</i>	26
2.8.2 <i>Avocado Fruit Ripening</i>	28
2.8.2.1 <i>Ethylene in Fruit Ripening</i>	29
2.8.2.2 <i>Firmness</i>	29
2.9 AVOCADO OIL	29
2.9.1 <i>Extraction</i>	29
2.9.2 <i>Fatty Acids of Avocado Fruit and Oil</i>	32
2.9.2.1 <i>Fatty Acid Analysis</i>	33
2.9.3 <i>Free Fatty Acid (FFA)</i>	34
2.9.3.1 <i>FFA Analysis</i>	35
2.9.4 <i>Lipid Oxidation</i>	35
2.9.5 <i>Pigments in Oil</i>	36
2.9.5.1 <i>Storage Trial (Oven Test)</i>	39
2.9.5.2 <i>Peroxide Value (PV)/Quantity of Peroxide Oxygen</i>	39
2.10 <i>ANTIFUNGAL COMPOUNDS (YNE, TRIENES, DIENES AND MONOENES)</i>	40
2.11 <i>CONCLUSION</i>	43
CHAPTER 3. MATERIALS AND METHODS	44
3.1 <i>EXPERIMENTAL OVERVIEWS</i>	44
3.2 <i>MATERIALS</i>	44
3.2.1 <i>Avocado Fruit</i>	45

3.2.2 Tissue Sampling.....	45
3.3 POST HARVEST ASSESSMENTS	47
3.3.1 Dry Matter Determination.....	47
3.3.2 Colour Measurement.....	47
3.3.3 Firmness Measurement.....	48
3.4 PIGMENT COMPOSITIONAL ANALYSIS OF 'HASS' AVOCADO FRESH TISSUE.....	48
3.4.1 Carotenoids and Chlorophyll Analysis (HPLC).....	48
3.4.1.1 Sample Preparation/Extraction.....	48
3.4.1.2 HPLC Determination.....	49
3.4.2 Anthocyanins Determination (HPLC).....	50
3.4.2.1 Sample Preparation/Extraction.....	50
3.4.2.2 HPLC Determination.....	50
3.5 PIGMENT COMPOSITIONAL ANALYSIS OF OIL EXTRACTED FROM 'HASS' AVOCADO SECTIONS.....	51
3.5.1 Solvent Extraction of Avocado Oil (ASE).....	51
3.5.1.1 Freeze Drying.....	51
3.5.1.2 Accelerated Solvent Extractor (ASE).....	51
3.5.1.3. Oil Drying/Storage.....	52
3.5.2 Carotenoid and Chlorophyll Analysis of Avocado Oil (HPLC).....	52
3.5.2.1 Sample Preparation/Extraction.....	52
3.5.2.2 HPLC Determination:.....	52
3.6 COLD PRESSED LABORATORY SCALE OIL EXTRACTION PROCESS	53
3.6.1 Calculations.....	53
3.6.2 Avocado Fruit.....	53
3.6.3 Oil Extraction.....	54
3.7 COMMERCIAL/FACTORY EXTRACTION.....	56
3.7.1. Experimental Overview.....	56
3.7.2 Oil Formulations/Identifications (100%, 40% and 5% skin).....	56
3.7.3 Free Fatty Acid (FFA).....	58
3.7.3.1 Reagents.....	58
3.7.3.2 FFA Determination.....	58
3.7.4 Storage Trial of Avocado Oil.....	59
3.8 TESTS FOR BOTH COLD PRESSED AND COMMERCIAL/FACTORY OIL EXTRACTED AVOCADO.....	59
3.8.1 Total Chlorophyll Determination (Spectrophotometer).....	59
3.8.2 Colour Measurement of Oil.....	60
3.8.3 Peroxide Value.....	60
3.8.3.1 Reagents.....	60
3.8.3.2 PV Determination.....	61
3.8.4 Fatty Acid Composition Determination.....	61
3.9 DIENE ANALYSIS OF AVOCADO OIL.....	62
3.9.1 Diene Extraction and Determination.....	62
3.10 STATISTICAL ANALYSIS	63
CHAPTER 4. PIGMENT COMPOSITION OF SKIN AND FLESH TISSUES OF 'HASS' AVOCADO AND THE EXTRACTED OIL.....	64
4.1 INTRODUCTION.....	64
4.2. MATERIALS AND METHODS	65
4.2.1 Experimental Overview.....	65
4.2.2 Tissue Sampling.....	65
4.3 RESULTS.....	66
4.3.1 Firmness of 'Hass' Avocado during Ripening.....	66
4.3.2 Colour of 'Hass' Avocado during Ripening.....	66
4.3.3 Carotenoids in Fresh Tissue.....	68
4.3.4 Chlorophylls in Fresh Tissue.....	70
4.3.5 Ratio of Chlorophyll a and b.....	73
4.3.6 Anthocyanins.....	75
4.3.7 Oil (%) of 'Hass' Avocado Sections.....	76
4.3.8 Carotenoids in the Extracted Oil.....	77

4.3.9 Chlorophyll in the Extracted Oil	79
4.4 DISCUSSION.....	81
4.4.1 Fruit Quality Assessment.....	81
4.4.2 Pigments in Fresh Tissue Sections	81
4.4.2.1 Carotenoids	81
4.4.2.2 Chlorophyll	82
4.4.2.3 Anthocyanin.....	83
4.4.3 Pigments in the Extracted Oil (ASE)	84
4.4.3.1 Carotenoids	84
4.4.3.2 Chlorophyll	84
4.4.4 Comparison of Pigments in the Fresh Tissue Sections and the Extracted Oil.....	84
4.5 CONCLUSIONS	86
CHAPTER 5. AVOCADO OIL WITH SKIN ADDITION	87
5.1 INTRODUCTION.....	87
5.2 MATERIALS AND METHODS	88
5.2.1 Experimental Overview	88
5.2.2 Laboratory Cold Pressed Extraction of Avocado Oil.....	88
5.2.2.1 Calculations.....	88
5.2.3 Commercial/Factory Extraction.....	88
5.3 RESULTS.....	89
5.3.1 Cold Pressed Laboratory Scale.....	89
5.3.1.1 Avocado Fruit Firmness and Colour.....	89
5.3.1.2 Free Fatty Acid (FFA), Peroxide Value (PV) and Colour	89
5.3.1.3 Carotenoids (HPLC).....	92
5.3.1.4 Chlorophyll (HPLC).....	93
5.3.2 Factory Trial	94
5.3.2.1 Avocado Fruit and Oil Quality (Colour, Firmness)	94
5.3.2.2 Fatty Acid Compositions (FA), Free Fatty Acid (FFA) and Peroxide Value (PV).....	94
5.3.2.3 Peroxide Value during Storage.....	95
5.3.2.4 Total Chlorophyll (Spectrophotometer).....	97
5.3.2.5 Shelf Life Based on PV, Total Chlorophyll Concentration at the End of Shelf Life and Activation Energy	99
5.3.2.6 Chlorophylls and Carotenoids (HPLC).....	102
5.3.2.7 Colour.....	106
5.3.2.8 Diene	113
5.4 DISCUSSION.....	113
5.4.1 Laboratory and Factory Scale Comparison	113
5.4.2 Peroxide Value during Storage	114
5.4.3 Total Chlorophyll (Spectrophotometer and HPLC)	115
5.4.4 Carotenoids (HPLC).....	116
5.4.5 Colour.....	116
5.5 CONCLUSIONS AND RECOMMENDATIONS	117
CHAPTER 6. OUTPUT/FUTURE RESEARCH	118
CHAPTER 7. REFERENCES	119
CHAPTER 8. APPENDICES.....	130
Appendix 1.Carotenoids concentration in 'Hass' avocado fresh tissue.....	130
Appendix 1.Carotenoids concentration in 'Hass' avocado fresh tissue continuation	131
Appendix 2.Chlorophylls concentration in 'Hass' avocado fresh tissue	132
Appendix 2.Chlorophylls concentration in 'Hass' avocado fresh tissue continuation	133
Appendix 3.Total anthocyanins and cyanidin 3-O-glucoside of 'Hass' avocado skin	134
Appendix 4.Carotenoids concentration in oil extracted from 'Hass' avocado sections	135
Appendix 4.Carotenoids concentration in oil extracted from 'Hass' avocado sections continuation.....	136
Appendix 5.Chlorophylls concentration in oil extracted from 'Hass' avocado sections.....	137
Appendix 5.Chlorophylls concentration in oil extracted from 'Hass' avocado sections continuation.....	138
Appendix 6.Comparison of the pigment in the fresh tissue and the extracted oil.....	139
Appendix 6.Comparison of the pigment in the fresh tissue and the extracted oil continuation.....	140

Appendix 7. Carotenoids and chlorophylls HPLC chromatogram of 'Hass' avocado skin, dark pulp, pale pulp, yellow pulp determined simultaneously	141
Appendix 8 HPLC chromatogram of chlorophylls and unknown (unknown 'a', 'd' and 'e')	142
Appendix 9. Anthocyanins HPLC chromatogram of 'Hass' avocado skin	143
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	144
Appendix 11. Peroxide value used for linear regression analysis of avocado oil 5% Skin	145
Appendix 11. Peroxide value used for linear regression analysis of avocado oil continuation (a) 5% Skin ..	146
Appendix 11. Peroxide value used for linear regression analysis of avocado oil continuation (a) 5% Skin ..	147
Appendix 11. Peroxide value used for linear regression analysis of avocado oil continuation (b) 40% Skin	148
Appendix 11. Peroxide value used for linear regression analysis of avocado oil continuation (b) 40% Skin	149
Appendix 11. Peroxide value used for linear regression analysis of avocado oil continuation (b) 40% Skin	150
Appendix 11. Peroxide value used for linear regression analysis of avocado oil continuation (c) 100% Skin	151
Appendix 11. Peroxide value used for linear regression analysis of avocado oil continuation (c) 100% Skin	152
Appendix 11. Peroxide value used for linear regression analysis of avocado oil continuation (c) 100% Skin	153
Appendix 12. Example of linear regression using peroxide value of 20°C of avocado oil with 5% skin over 97 days of storage.	154
Appendix 13. Summary of linear regression analysis (rate of reaction) for peroxide value of avocado oil (R square values and slopes)	155
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	156
Appendix 15. Summary of R square values and slope of total chlorophylls (the sum of chlorophyll <i>a</i> and <i>b</i> , and pheophytin <i>a</i> and <i>b</i>) by HPLC	159
Appendix 16. Summary of R square values and slope of total chlorophyll (Spectrophotometer)	160
Appendix 17. Chromatogram of total chlorophyll (Spectrophotometer)	161
Appendix 18. Summary of carotenoids and chlorophylls of avocado oil (5%, 40% and 100% skin) during storage at different temperatures (HPLC)	162
Appendix 18. Summary of carotenoids and chlorophylls of avocado oil (5%, 40% and 100% skin) during storage at different temperatures continuation (HPLC)	163
Appendix 18. Summary of carotenoids and chlorophylls of avocado oil (5%, 40% and 100% skin) during storage at different temperatures continuation	164
Appendix 18. Summary of carotenoids and chlorophylls of avocado oil (5%, 40% and 100% skin) during storage at different temperatures continuation	165

List of Figures

Figure 2.1. Chemical structure of chlorophyll (Gross, 1987).....	4
Figure 2.2a to c. Chlorophyll biosynthetic pathway (Gross, 1987).....	5
Figure 2.2d to e. Chlorophyll biosynthetic pathway (Gross, 1987).....	6
Figure 2.3. Chemistry of carotenoids (Gross, 1987).....	9
Figure 2.4a to b. Carotenoids biosynthetic pathways.....	11
Figure 2.4c. Carotenoids biosynthetic pathways.....	12
Figure 2.5. Generalized structure for anthocyanin pigments (Rodriguez-Saona and Wrolstad, 2001).....	14
Figure 2.6. General structure of acyl group derivatives (Gross, 1987).....	15
Figure 2.7. Anthocyanin biosynthetic pathways (Gross, 1987).....	16
Figure 2.8. The Hunter L*a*b* and L*c*h colour spaces.....	22
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).....	26
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).....	27
Figure 2.10. ASE 300 (Dionex, 2000).....	30
Figure 2.11. Mini-40 expeller (Southwell et al., 1990).....	31
Figure 2.12. Mechanisms of fat oxidation (Erickson, 2002).....	36
Figure 2.13. Carotenoids mechanism of trapping free radicals and quenching singlet oxygen (Reische et al., 2002).....	38
Figure 2.14. Peroxide formation (Shahidi and Wanasundara, 2002).....	39
Figure 2.15. Chemical structure of antifungal compounds isolated in avocado oil idioblast cells (Domergue et al., 2000).....	41
Figure 3.1 a to c. Avocado tissue sampling.....	46
Figure 3.2 a to l. Avocado oil cold press laboratory scale process.....	55

Figure 3.3 Process flow diagram of commercial avocado oil processing (Sherpa, 2002).....	57
Figure 4.1. Softening of ‘Hass’ avocado during different stages of ripening as measured by an Anderson digital Firmometer (n=45).....	66
Figure 4.2. Colour changes. (a) Lightness L, (b) Chroma, c, (c) Hue angle of ‘Hass’ avocado (n=60).....	67
Figure 4.3. Carotenoids concentrations in ‘Hass’ avocado (a) skin, (b) dark pulp, (c) pale pulp and (d) yellow pulp (n=3).....	68
Figure 4.4. Chlorophyll concentrations in the (a) skin, (b) dark pulp, (c) pale pulp (d) yellow pulp of ‘Hass’ avocado (n=3).....	71
Figure 4.5. Total anthocyanins and cyanidin 3- <i>O</i> -glucoside of ‘Hass’ avocado skin n=3.....	75
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).....	76
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).....	77
Figure 4.8. Total chlorophyll, chlorophyll <i>a</i> , chlorophyll <i>b</i> , pheophytin <i>a</i> and pheophytin <i>b</i> 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).....	79
Figure 5.1. Mean free fatty acid of avocado oil from cold pressed extraction in the laboratory (n=2).....	90
Figure 5.2. Mean peroxide values of avocado oil from cold pressed extraction in the laboratory (n=2).....	90
Figure 5.3 Colour of avocado oil from cold pressed extraction in the laboratory (n=3).....	91
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).....	92

Figure 5.5.Total chlorophyll, chlorophyll <i>b</i> , chlorophyll <i>a</i> , pheophytin <i>b</i> and pheophytin <i>a</i> in avocado oil with different levels of skin addition (n=3).....	93
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).....	95
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).....	97
Figure 5.8.The Arrhenius plot for the peroxide formation of avocado oil with 5% skin, 40% skin and 100% skin.....	101
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).....	102
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).....	105
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).....	108
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).....	109
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.....	110
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).....	111
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).....	112

List of Tables

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).....	33
Table 3.1.Solvent Programme for HPLC Determination of Carotenoids and Chlorophyll.....	49
Table 3.2.Solvent Programme for HPLC Determination of Anthocyanin.....	51
Table 3.3.ASE Operating Conditions Used for Solvent Extraction of Avocado Oil.....	52
Table 3.4.Skin and Flesh Proportion for Cold Pressed Extraction Laboratory Scale of Avocado Oil.....	53
Table 3.5.Calculation for Different Levels of Skin for Cold Pressed Extraction Factory Scale.....	56
Table 3.6.Avocado Oil Testing Intervals of Three Oil Formulations.....	59
Table 4.1.Ratio of Chlorophyll <i>a</i> and <i>b</i> in the ‘Hass’ Avocado Skin, Dark, Pale and Yellow Pulp.....	74
Table 5.1.Colour and Firmness of Fruit Used in Cold Pressed Extraction of Avocado Oil (n=10).....	89
Table 5.2.Fatty Acid Composition (%) of Avocado Oil Day 0.....	94
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.....	104
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.....	104

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.