

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.

The Oxidation Stability of Extra Virgin Avocado Oil

A thesis presented in the partial fulfilment of the
requirements for the degree of
Master of Technology in Food Science

At Massey University, Albany, New Zealand

Nimma Lawrence Sherpa

2002

Abstract

Extra virgin avocado oil (EVAO) is extracted from avocado fruit with minimal processing. It contains a wide range of non-lipid compounds that have a profound affect on oil stability. The deterioration of oil quality is due to autoxidation and photooxidation reactions that occur during oil storage. The objectives of this research were to determine the effect of prooxidant factors (light, temperature, oxygen level) on oil oxidation and quality; make recommendations for oil processing and packaging procedures to minimise oxidation; predict the shelf life of the oil and to determine the effect of commercial antioxidants on oil oxidation.

An accelerated oxidation reactor was developed to test the effects of fluorescent light, elevated temperature and varying oxygen levels on the peroxide value (PV) (initially 0.96 ± 0.03 meq/kg oil) and chlorophyll content (initially 16.2 ± 0.1 ppm) of EVAO. The production and packaging processes of Olivado NZ were analysed for exposure to oxidation promoting factors. EVAO was exposed to dark storage at 50°C and 60°C in order to determine Q_{10} values for oil oxidation. Several commercial antioxidants were evaluated by examining their affect on EVAO using the Rancimat oil stability index analysis and hot air oven testing.

It was found that fluorescent light at 4500 lux and aeration with dry air strongly accelerated the oxidation (determined by PV) and reduced the chlorophyll content of EVAO. The average effect of 4500 lux fluorescent light compared to 0 lux over seven hours was a PV increase of 4.5 ± 1.4 meq/kg oil and decrease in chlorophyll content by 0.9 ± 0.3 ppm. The average effect of aerated EVAO compared to EVAO stored at ambient oxygen levels over seven hours was a PV increase of 3.5 ± 1.7 meq/kg oil and a chlorophyll content decrease of 0.3 ± 0.2 ppm. Exposure to an elevated temperature of 60°C for seven hours did not cause a significant increase in PV. Recommendations were made to minimise the exposure of the oil to light, aeration, water and fruit sediment during production and packaging in order to minimise oxidation of the oil. Due to the breakdown of natural antioxidants and alternative side reactions that occurred at elevated test temperatures but not at ambient temperatures, the shelf life of the oil could not be defined. EVAO containing ascorbyl palmitate at a level of 100 ppm had a peroxide value 80 % less than control EVAO with no antioxidants after 500 hours storage at 60°C . Ascorbyl palmitate has GRAS status and was concluded to be the most effective antioxidant of those tested in EVAO.

Acknowledgements

I would like to thank both my supervisors, Dr Laurence Eyres and Dr. Marie Wong for their support throughout this project. Especially Dr Laurence Eyres for his enthusiasm and love of the subject matter and Dr Marie Wong for her guidance and technical expertise.

I would also like to thank Olivado New Zealand for all their support, especially Kurt Kupper for his quick responses and hospitality.

Thank you also to Technology New Zealand for the financial support they provided for this project through the Technology in Industry Fellowship scheme.

I would also like to extend my appreciation to other members of the Institute of Food Nutrition and Human Health at Massey University in Albany, especially Glenn Hendriks for his company and support. Also Dr. Jay Wimalasena from IFS for his help and patience during laboratory based experiments.

Finally, thanks to my family and friends for their encouragement and support.

Table of Contents

1	INTRODUCTION.....	1
2	LITERATURE REVIEW.....	4
2.1	AVOCADO OIL	4
2.2	OLIVE OIL.....	6
2.3	QUALITY LOSS AND OXIDATION IN OILS	7
2.3.1	<i>Autoxidation</i>	8
2.3.2	<i>Photooxidation</i>	9
2.3.2.1	Photosensitisation type and identification	10
2.3.2.2	Singlet oxygen chemistry.....	11
2.3.2.3	Biological consequences	14
2.3.3	<i>Chlorophyll breakdown and stability in oils</i>	14
2.3.4	<i>Effect of enzymes on oil quality</i>	16
2.3.4.1	Lipoxygenase	16
2.3.4.2	Lipase.....	18
2.3.4.3	Fruit Quality.....	18
2.3.4.4	Exogenous processing enzymes	19
2.3.5	<i>Oxygen removal</i>	20
2.3.6	<i>Nitrogen Sparging</i>	21
2.4	ANTIOXIDANTS	22
2.4.1	<i>Antiautoxidants</i>	22
2.4.2	<i>Primary antiautoxidants</i>	23
2.4.2.1	Tocopherols (vitamin E).....	23
2.4.2.2	Propyl Gallate	26
2.4.2.3	Butylated hydroxyanisole.....	26
2.4.2.4	Butylated hydroxytoluene	27
2.4.2.5	Tert-butyl hydroquinone	27
2.4.2.6	Polyphenols.....	28
2.4.3	<i>Secondary antiautoxidants</i>	28
2.4.3.1	Ascorbic acid and ascorbyl palmitate.....	28
2.4.3.2	Citric acid.....	29
2.4.3.3	Chlorophyll	30
2.4.4	<i>Antiphotooxidants</i>	31
2.4.4.1	Carotenoids	31
2.4.4.2	Rosemary	35
2.5	OXIDATION RATE MEASURING TECHNIQUES	38
2.5.1	<i>Static oxidation tests</i>	38
2.5.1.1	Peroxide value (PV).....	38

2.5.1.2	Conjugated diene products.....	39
2.5.1.3	Headspace oxygen depletion.....	40
2.5.1.4	Thiobarbituric acid test.....	40
2.5.1.5	Sensory testing.....	40
2.5.1.6	Gas chromatographic (GC) analysis.....	41
2.5.2	<i>Dynamic oxidation tests</i>	41
2.5.2.1	Induction period.....	42
2.5.2.2	Heating.....	43
2.5.2.3	Light reactors.....	45
2.6	CHLOROPHYLL MEASUREMENT.....	45
2.6.1	<i>Spectrophotometric measurement</i>	45
2.6.2	<i>Colorimetric measurement</i>	46
2.6.3	<i>High pressure liquid chromatography</i>	47
2.7	EFFECT OF PACKAGING MATERIALS ON OIL QUALITY.....	47
3	MATERIALS AND METHODS.....	48
3.1	OIL SAMPLES.....	48
3.1.1	<i>Extra virgin avocado oil</i>	48
3.1.2	<i>Refined, bleached and deodorised avocado oil</i>	48
3.2	ACCELERATED OXIDATION REACTOR.....	48
3.2.1	<i>Compressed air and nitrogen</i>	48
3.2.2	<i>Gas fittings, regulator and volumetric flow meter</i>	49
3.2.3	<i>Light source and light intensity meter</i>	49
3.2.4	<i>Water bath and stainless steel jacketed vessels</i>	49
3.2.5	<i>Reactor box and collar</i>	49
3.2.6	<i>Extraction fan</i>	50
3.3	HOT AIR OVEN APPARATUS.....	50
3.3.1	<i>Hot air oven</i>	50
3.3.2	<i>Moisture dishes</i>	50
3.4	SPECTROPHOTOMETRY EQUIPMENT.....	50
3.4.1	<i>Spectrophotometer and PC software</i>	50
3.4.2	<i>Cuvettes</i>	50
3.5	PEROXIDE VALUE (PV) DETERMINATIONS.....	51
3.5.1	<i>Solvent (isooctane)</i>	51
3.5.2	<i>Reagents</i>	51
3.6	ANTIOXIDANT EVALUATION.....	51
3.6.1	<i>Antioxidants</i>	51
3.6.2	<i>Solvent</i>	51
3.7	COLORIMETRY.....	52
3.7.1	<i>Colorimeter</i>	52
3.7.2	<i>Sample containment</i>	52

3.8	OIL STABILITY INDEX (OSI) ANALYSIS.....	52
3.8.1	<i>OSI apparatus</i>	52
3.9	METHODS.....	52
3.9.1	<i>Development of the accelerated oxidation reactor</i>	52
3.9.1.1	Light supply.....	53
3.9.1.2	Heating of the oil sample.....	54
3.9.1.3	Gas supply.....	55
3.10	OPERATING PROCEDURE FOR ACCELERATED OXIDATION REACTOR.....	57
3.10.1	<i>Start Up</i>	57
3.10.2	<i>Sampling</i>	59
3.10.3	<i>Testing</i>	59
3.11	METHOD FOR DETERMINING PEROXIDE VALUE (PV) IN AVOCADO OIL.....	60
3.11.1	<i>Modifications made to standard AOCS Cd 8b-90 method</i>	60
3.11.2	<i>Reagents</i>	60
3.11.3	<i>PV determination</i>	61
3.12	CHLOROPHYLL DETERMINATION.....	63
3.12.1	<i>Modifications made to standard AOCS Ch 4-91 method</i>	63
3.12.2	<i>Method</i>	63
3.13	OBTAINING HUNTER L A B VALUES FROM EXTRA VIRGIN AVOCADO OIL.....	65
3.14	PROCEDURE FOR ADDING ANTIOXIDANTS TO AVOCADO OIL.....	65
3.15	HOT AIR OVEN ACCELERATED OXIDATION METHOD.....	66
3.16	Q ₁₀ ANALYSIS AND ANTIOXIDANT TRIALS.....	67
3.17	OIL STABILITY INDEX (RANCIMAT) ANALYSIS.....	68
3.18	AVOCADO OIL PRODUCTION LINE ANALYSIS AND PROCESS IMPROVEMENT.....	68
4	ACCELERATED OXIDATION REACTOR TRIALS.....	70
4.1	EFFECT OF LIGHT, TEMPERATURE AND OXYGEN LEVEL ON PEROXIDE VALUE.....	70
4.2	EFFECT OF LIGHT, TEMPERATURE AND OXYGEN LEVEL ON CHLOROPHYLL LEVELS.....	75
4.3	DISCUSSION.....	81
5	MAXIMISING OXIDATIVE STABILITY OF EVAO BY REDUCING EXPOSURE TO PROOXIDANTS.....	83
5.1	EVAO PROOXIDANT FACTORS.....	83
5.2	RECOMMENDATIONS TO REDUCE EXPOSURE OF EVAO TO PROOXIDANTS DURING PRODUCTION AND PACKAGING.....	87
6	HOT AIR OVEN ACCELERATED SHELF LIFE TESTING.....	93
6.1	BACKGROUND TO ACCELERATED SHELF LIFE CONDITIONS USED.....	93
6.2	PEROXIDE VALUE RESULTS AND DISCUSSION.....	93
6.3	CHLOROPHYLL CONTENT RESULTS AND DISCUSSION.....	96
6.4	HUNTER LAB COLORIMETRY RESULTS AND DISCUSSION.....	97

7	EVAO OXIDATION KINETICS AND SHELF LIFE PREDICTION	99
7.1	Q ₁₀ REACTION KINETICS.....	99
7.2	PEROXIDE VALUE DISCUSSION.....	100
7.3	Q ₁₀ CALCULATION AND DISCUSSION.....	102
7.4	CHLOROPHYLL CONTENT RESULTS AND DISCUSSION.....	104
8	ANTIOXIDANT EVALUATION IN EVAO	106
9	RANCIMAT SCREENING TESTS.....	106
10	RESULTS AND DISCUSSION FROM ANTIOXIDANT TESTS AT 60°C.....	107
11	CONCLUSIONS AND RECOMMENDATIONS	112
12	REFERENCES.....	114
13	APPENDIX	114
13.1	PROCESS FLOW DIAGRAM FOR THE PRODUCTION AND PACKAGING OF EVAO AT OLIVADO NZ. I	
13.2	Q ₁₀ AND PREDICTED SHELF LIFE CALCULATION FOR 1 MONTH OLD EVAO.....	VII
13.3	Q ₁₀ AND PREDICTED SHELF LIFE CALCULATION FOR 10 MONTH OLD EVAO.....	VII
13.4	Q ₁₀ AND PREDICTED SHELF LIFE CALCULATION FOR 4 MONTH OLD RBD AVOCADO OIL.....	VIII

List of Figures

FIGURE 1. PROJECTED TREND OF AVOCADO PRODUCTION IN NEW ZEALAND (REQUEJO-TAPIA, 1999)	1
FIGURE 2. REACTION STEPS IN AUTOXIDATION (HAMILTON, 1994).....	8
FIGURE 3. TYPE I PHOTOSENSITISATION (CHAN 1977). SUPERSCRIPTS ¹ AND ³ REFER TO SINGLET OR TRIPLET STATE OF THAT COMPOUND.	10
FIGURE 4. TYPE II PHOTOSENSITISATION (CHAN, 1977). SUPERSCRIPTS ¹ AND ³ REFER TO SINGLET OR TRIPLET STATE OF THAT COMPOUND.	11
FIGURE 5. CHEMICAL STRUCTURE OF CHLOROPHYLL <i>A</i> WHERE R = CH ₃ AND CHLOROPHYLL <i>B</i> WHERE R=CHO. STRUCTURE ALSO CORRESPONDS TO PHEOPHYTIN <i>A</i> AND <i>B</i> WHEN MAGNESIUM IS ABSENT (HENDRY, 1993).	15
FIGURE 6. ANTIOXIDANT MECHANISM OF CHLOROPHYLL (ENDO ET AL., 1985B)	31
FIGURE 7. LIGHT FILTERING PROTECTIVE EFFECT OF β-CAROTENE AS PROPOSED BY CLEMENTS ET	33
FIGURE 8. CAROTENOIDS COMMONLY FOUND IN THE HUMAN DIET (DESHPANDE ET AL., 1995).....	34
FIGURE 9. EXAMPLE OF PV VS. TIME CURVE SHOWING EFFECT OF ANTIOXIDANT 'A' AND 'B' ON INDUCTION TIME. DOUBLE HEADED ARROWS SHOW INDUCTION PERIOD FOR EACH SAMPLE TYPE (HAMILTON, 1994).	43
FIGURE 10. OXIDATION REACTOR LID AND LIGHT SUPPLY	54
FIGURE 11. CLOSED OXIDATION BOX	54
FIGURE 12. TEMPERATURE MAINTENANCE SYSTEM FOR OXIDATION REACTOR	55
FIGURE 13. GAS SUPPLY INTO OXIDATION REACTOR BOX AND OIL SAMPLES	56
FIGURE 14. GAS SUPPLY FOR OXIDATION REACTOR.....	56
FIGURE 15. SCHEMATIC DIAGRAM OF ACCELERATED OXIDATION REACTOR	57
FIGURE 16. EFFECT OF LIGHT, TEMPERATURE AND OXYGEN LEVELS ON THE PV OF EVAO: A) EVAO OXIDISED AT 60°C, 4500 LUX, VARYING OXYGEN LEVEL; B) EVAO OXIDISED AT 60°C, 0 LUX, VARYING OXYGEN LEVEL; C) EVAO OXIDISED AT 25°C, 4500 LUX, VARYING OXYGEN LEVEL D) EVAO OXIDISED AT 25°C, 0 LUX, VARYING OXYGEN LEVEL	71
FIGURE 17. MAIN EFFECTS PLOT FOR LIGHT, TEMPERATURE AND OXYGEN LEVEL ON PV CHANGE: A) EFFECT OF 0 LUX VS. 4500 LUX FLUORESCENT LIGHT ON PV; B) EFFECT OF 25°C VS. 60°C ON PV CHANGE; C) EFFECT OF 0% VS. 21% VS. 100% OXYGEN LEVEL ON PV CHANGE.....	72
FIGURE 18. INTERACTION PLOT FOR LIGHT, TEMPERATURE AND OXYGEN LEVEL ON PV CHANGE: A) INTERACTION BETWEEN LIGHT LEVEL AND TEMPERATURE; B) INTERACTION BETWEEN LIGHT AND OXYGEN LEVEL; C) INTERACTION BETWEEN TEMPERATURE AND OXYGEN LEVEL.....	74
FIGURE 19. EFFECT OF LIGHT, TEMPERATURE AND OXYGEN LEVELS ON CHLOROPHYLL LEVELS IN EVAO: A) EVAO OXIDISED AT 60°C, 4500 LUX, VARYING OXYGEN LEVEL; B) EVAO OXIDISED AT 60°C, 0 LUX, VARYING OXYGEN LEVEL; C) EVAO OXIDISED AT 25°C, 4500 LUX, VARYING OXYGEN LEVEL; D) EVAO OXIDISED AT 25°C, 0 LUX, VARYING OXYGEN LEVEL.....	76
FIGURE 20. MAIN EFFECTS PLOT FOR LIGHT, TEMPERATURE AND OXYGEN ON CHLOROPHYLL CONTENT	

CHANGE: A) EFFECT OF 0 LUX VS. 4500 LUX FLUORESCENT LIGHT ON PV; B) EFFECT OF 25°C VS. 60°C ON PV CHANGE; C) EFFECT OF 0% VS. 21% VS. 100% OXYGEN LEVEL ON PV CHANGE	78
FIGURE 21. INTERACTION PLOT FOR LIGHT, TEMPERATURE AND OXYGEN LEVEL ON CHLOROPHYLL CONTENT CHANGE: A) INTERACTION BETWEEN LIGHT LEVEL AND TEMPERATURE; B) INTERACTION BETWEEN LIGHT AND OXYGEN LEVEL; C) INTERACTION BETWEEN TEMPERATURE AND OXYGEN LEVEL.....	80
FIGURE 22. PICTORIAL PROCESS FLOW DIAGRAM FOR EVAO PRODUCTION AT OLIVADO NZ	85
FIGURE 23. EFFECT OF DARK, AMBIENT OXYGEN LEVEL, 60°C STORAGE ON THE PV OF AVOCADO OIL SAMPLES	94
FIGURE 24. EFFECT OF DARK, AMBIENT OXYGEN LEVEL, 60°C STORAGE ON THE TOTAL CHLOROPHYLL PIGMENT CONTENT OF EVAO SAMPLES.....	96
FIGURE 25. EFFECT OF DARK STORAGE AT 60°C ON THE HUNTER A VALUE OF EVAO SAMPLES	98
FIGURE 26. EFFECT OF DARK STORAGE AT 60°C ON THE PV OF 1 AND 10 MONTH OLD EVAO AND 4 MONTH OLD RBD TO DETERMINE THE END OF SHELF LIFE BASED ON PEROXIDE VALUE.....	100
FIGURE 27. EFFECT OF DARK STORAGE AT 50°C ON THE PV OF 1 AND 10 MONTH OLD EVAO AND 4 MONTH OLD RBD TO DETERMINE THE END OF SHELF LIFE BASED ON PEROXIDE VALUE.....	101
FIGURE 28. EFFECT OF DARK STORAGE AT 50°C AND 60°C ON THE PV OF EVAO SAMPLES	105
FIGURE 29. EFFECT OF ANTIOXIDANTS ON THE PV OF EVAO STORED AT 60°C IN DARK CONDITIONS	108
FIGURE 30. EFFECT OF ANTIOXIDANTS ON THE CHLOROPHYLL CONTENT OF EVAO STORED AT 60°C IN DARK CONDITIONS	110

List of Tables

TABLE 1. FATTY ACID COMPOSITION (PERCENTAGE) OF COLD-PRESSED AVOCADO OIL AND OLIVE OIL PRODUCED IN NEW ZEALAND (EYRES ET AL., 2001).....	5
TABLE 2. CONTENTS OF TOCOPHEROLS/TRIENOLS IN SOME COMMON VEGETABLE OILS (MADHAVI ET AL., 1996).....	23
TABLE 3. RELATIVE ABILITIES OF THE 4 TOCOPHEROL GROUPS IN DIFFERENT CONDITIONS.....	25
TABLE 4. DESIGN MATRIX FOR EXPERIMENTAL TREATMENTS IN ACCELERATED OXIDATION REACTOR.....	59
TABLE 5. CHLOROPHYLL CONTENT CHANGE OVER SEVEN HOUR STORAGE FOR EVAO.....	77
TABLE 6. EXPOSURE POINTS ANALYSED FROM EVAO PRODUCTION AND PACKAGING AT OLIVADO (NZ) LTD.....	86
TABLE 7. ACCELERATED OXIDATION RESULTS FOR AVOCADO OIL STORED IN DARK AT 60°C.....	94
TABLE 8. Q ₁₀ VALUES AND PREDICTED SHELF LIFE FOR AVOCADO OIL SAMPLES.....	103
TABLE 9. OSI RANCIMAT RESULTS FOR EVAO CONTAINING ANTIOXIDANTS.....	106