

**Studies on the Nutritional Value of the Oils And
Mesocarp of Avocado (*Persea americana*) and
Impact of
Soil Quality on Elemental Composition**



by Mageshni Reddy

*Submitted in fulfilment of the academic requirements for the degree of Master of Science in
the School of Chemistry, University of KwaZulu-Natal, Durban.*

2011

As the candidate's supervisor, I have approved this dissertation for the submission

Signed: _____ Name: _____ Date: _____

DECLARATION

I hereby declare that this dissertation is my own work, except where specifically acknowledged in the text. Neither the present dissertation nor any part thereof has been submitted by any other university for a degree.

Author: _____

Mageshni Reddy

Supervisor: _____

Prof. S.B. Jonnalagadda

Co-supervisor: _____

Roshila Moodley

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor, Prof. S.B. Jonnalagadda whose suggestions, guidance, expert knowledge and encouragement is greatly appreciated. I am also deeply indebted to Mrs R. Moodley for providing her invaluable time, feedback, comments, critiques and unlimited assistance with all facets of this research project. I am truly fortunate to have had two supportive mentors guiding me through these two years of research. Funding for this research is gratefully acknowledged from National Research Foundation (NRF)

I would like to acknowledge the following people who have assisted me in acquiring the soil and avocado samples for the study:

- Richard Saulez (Argyl Farm, Ixopo)

- Fred Kleyn and David Spence (Waterford Farm, Richmond)

- Athol Currie and Sibusiso Memela (Atherstone Farm, Thornville)

- Cecil Hackney and Lynton Freese (Everdon Estate, Howick)

- Phil Swan (Rozetta Avocadoes, Seven Oaks)

- Mr. Philip Wouter Vermaak (Emseni farm, Kranskop)

I am grateful to Sagren Moodley and Shaeen Chetty for taking the time and effort in transporting and assisting me with sample collection at the various sites.

I would also like to acknowledge with sincere thanks:

- The technical team in the School of Chemistry at UKZN (Westville) for their assistance, especially Anita Naidoo, Neil Broomhead, Raj Somaru, Gregory Moodley, Zarina Sayed, Denise Broomhead and Precious Ntombela.

- ❑ Dr. Shakira Shaik for the use of an instrument at her research lab, and Prof. N. Pammente for the advice on the plant physiological aspects of my research. (Biological Sciences Department, UKZN-Westville)
- ❑ Dr. Southway, Bhekizitha Dlamini and Zaheer Ismail for the assistance and supervision during use of instruments at UKZN (Pietermaritzburg)
- ❑ Jimmy Chetty and Prof. K.G. Moodley for the supervision during the use of instruments at DUT (Durban)
- ❑ Prof. B. Botha and Gabriel for allowing me the opportunity to learn and operate the Supercritical Fluid Extraction apparatus at TUT (Pretoria)
- ❑ ANEMS (Analytical Environmental and Material Science) research group for all their advice and encouragement.
- ❑ My colleagues and friends in the school of Chemistry for all their moral support. A special thanks goes to some of my closest friends, Lynette Komarsamy, Veresha Dukhi, Shaeen Chetty and all my postgraduate friends, for the caffeine boosts and inspirational chats provided when I really needed it.

I wish bestow my thanks with greatest sincerity to my parents who have shared with me my triumphs and encouraged me at times of frustration, my siblings , for their continuous support and to my extended family for all their help and advice I received from them. I cannot forget the devotion and patience shown by my boyfriend, Lee-Andrew Appalsami, during these years and I wish to thank him wholehearted for all sacrifices made.

ABSTRACT

This study covered both the Hass and Fuerte varieties of avocado pears. The quality of extracted avocado oil produced by different extraction techniques was assessed to determine the effect the extraction method had on the nutritional and storage value of the oil. While microwave extraction produced the highest yield of oil (70.0 %), supercritical fluid extraction produced oil with a wider range of fatty acids. Although the Hass variety produced a higher oil yield, oil extracted from the Fuerte variety was shown to have a higher monounsaturated fatty acid to saturated fatty acid ratio, which makes the latter oil more beneficial to health. Oils of the Fuerte variety also possessed a higher concentration of co-extracted metals, which makes it more susceptible to lipid oxidation. The overall choice for the most efficient extraction method was microwave extraction as it produced the highest yield and quality of oil.

The impact of soil quality on elemental uptake into locally grown avocado fruit sampled from six different locations was determined. Of the 14 selected metals investigated, avocado fruit was found not to accumulate Cd, Co, Cr, Pb and Se. Generally, the concentration of elements in both varieties of fruit was in the order of $Mg > Ca > Al > Zn > Fe > Mn > Cu > Ni > As$. Relative bioaccumulation plots were used to establish the essential and non-essential elements for normal growth of avocado fruit. It was found that the plant has an involuntary uptake mechanism for As due to similarity in ion species to P, which is an essential element. The impact of soil quality parameters pH, cation exchange capacity and soil organic matter were determined and their impact on plant-soil interactions was analysed. Statistical analysis revealed a plethora of metal interactions at the plant-soil interface. However, the plant was still seen to control uptake of specific elements such as Cu, Fe and Ca, due to its

physiological requirements. CEC was found to have a greater effect on availability of elements than pH and SOM. Geoaccumulation indices indicated moderate enrichment of Pb in soils; however this result had no bearing on the elemental uptake of the fruit at all sites. Comparisons to recommended dietary allowances (RDAs) for human diet reveal the average contribution of avocado to be 70% and 45% for Cu and Mn, respectively. Low levels of As was found in fruit which warrants continued monitoring of this element in the plant due to its similarity to P.

LIST OF PUBLICATIONS

Title: Fatty Acid Profile and Elemental Content of Avocado (*Persea Americana* Mill.) Oil -
Effect of Extraction Methods

Authors: Reddy, Mageshni; Moodley, Roshila and Jonnalagadda, Sreekanth B.

Journal: Journal of Environmental Science and Health, Part B (2012) 47, 1–9

(Appendix 4)

Title: Elemental Uptake and Distribution of Nutrients in Avocado Mesocarp and the Impact
of Soil Quality

Authors: Reddy, Mageshni; Moodley, Roshila and Jonnalagadda, Sreekanth B.

Journal: (Communicated)

Contents

<i>DECLARATION</i>	<i>ii</i>
<i>ACKNOWLEDGEMENTS</i>	<i>iii</i>
<i>ABSTRACT</i>	<i>v</i>
<i>LIST OF PUBLICATIONS</i>	<i>vii</i>
<i>LIST OF TABLES</i>	<i>1</i>
<i>LIST OF FIGURES</i>	<i>3</i>
<i>ABBREVIATIONS</i>	<i>5</i>
<i>CHAPTER 1</i>	<i>6</i>
<i>1.1 Introduction</i>	<i>6</i>
<i>1.2 Statement of the Problem</i>	<i>9</i>
<i>CHAPTER 2</i>	<i>12</i>
<i>LITERATURE REVIEW</i>	<i>12</i>
<i>2.1 Avocado</i>	<i>12</i>
<i>2.1.1 Avocado Trade in South Africa</i>	<i>12</i>
<i>2.1.2 Nutritional Value of Avocados</i>	<i>13</i>
<i>2.1.3 Fatty Acids in Avocado</i>	<i>13</i>
<i>2.1.4 Cosmetic Uses for Avocado Oil</i>	<i>14</i>
<i>2.1.5 Avocado Oil Cells</i>	<i>15</i>
<i>2.2 Extraction Methods</i>	<i>16</i>
<i>2.2.1 Traditional Soxhlet Extraction</i>	<i>17</i>
<i>2.2.2 Sonication Assisted Extraction</i>	<i>19</i>
<i>2.2.3 Microwave Assisted Extraction (MAE)</i>	<i>19</i>
<i>2.2.4 Supercritical Fluid Extraction (SFE)</i>	<i>20</i>
<i>2.2.5 Ultra-Turrax Treated Extraction</i>	<i>22</i>
<i>2.2.6 Factors Effecting Oil Quality</i>	<i>23</i>
<i>2.3 Essential and Non-Essential Elements</i>	<i>23</i>
<i>2.4 Soil and Metals</i>	<i>24</i>
<i>2.4.1 Interactions of Elements in Soil</i>	<i>28</i>
<i>2.5 Soil Quality</i>	<i>28</i>
<i>2.5.1 Soil pH</i>	<i>29</i>
<i>2.5.2 Soil Organic Matter (SOM)</i>	<i>29</i>

2.5.3 Cation Exchange Capacity (CEC).....	30
2.5.4 Total and Exchangeable Metal Concentrations in Soil.....	31
2.6 Geoaccumulation Index (I_{geo}).....	33
2.7 Essential Elements in Plants.....	34
2.8 Distribution of Nutrients in Plants.....	34
2.8.1 Bioaccumulation.....	35
2.8.2 Bioaccumulation Factors (BF).....	36
2.8.3 Accumulators and Excluders.....	36
2.9 Essential Elements in Humans.....	37
2.10 Methods.....	38
2.10.1 Walkley Black Method for Determination of SOM.....	38
2.10.2 Chapman Method for Determination of CEC.....	39
2.10.3 The Kjeldahl Method for the Determination of Protein.....	40
2.11 Instrumentation.....	40
2.11.1 Gas Chromatography (GC).....	40
2.11.2 Microwave Digestion.....	41
2.11.3 Hydride Generation Atomic Absorption Spectroscopy (HG-AAS).....	42
2.11.4 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).....	43
2.11.4. 1 Advantages of ICP–OES.....	45
2.11.4.2 Limitations and Interferences.....	45
2.11.4.3 Matrix Interferences.....	46
2.11.4.4 Physical and Chemical Interferences.....	46
2.11.4.5 Spectral Interferences.....	47
2.11.4.6 Limit of Detection (LOD).....	47
CHAPTER 3.....	49
EXPERIMENTAL.....	49
3.1 Sampling.....	49
3.1.1 Avocados used for Oil Extraction Study.....	49
3.2 Sampling Sites for Plant-Soil Study.....	49
3.3 Sample Preparation.....	51
3.4 Extractions.....	51
3.4.1 Hexane Extraction.....	52
3.4.2 Ultrasound Extraction.....	52

3.4.3 Ultra-Turrax Treatment-Hexane Combined Extraction	52
3.4.4 Microwave Assisted-Hexane Combined Extraction	52
3.4.5 Supercritical Fluid Extraction.....	53
3.5 Instrumentation for Oil Extraction Study.....	54
3.6 Metal Determination in Extracted Oils.....	54
3.7 Fatty Acid (FA) Analysis of Extracted Oils.....	55
3.8 Proximate Chemical Composition of Avocados.....	56
3.9 Statistical Data for Oil Extraction Study.....	56
3.10 Instrumentation used for Plant- Soil Study	56
3.11 Certified Reference Material (CRM) Analysis	57
3.12 Total Metal Determination in Soil, Fruit and CRM	57
3.13 Determination of As and Se.....	59
3.14 Bioavailability Determinations	60
3.15 Bioaccumulation Factors (BFs)	61
3.16 Soil Quality Assessment	61
3.16.1 Soil pH, Soil Organic Matter (SOM) and Cation Exchange Capacity (CEC).....	61
3.16.1.1 Soil pH.....	62
3.16.1.2 Walkley-Black Method for the Determination of SOM.....	62
3.16.1.3 Determination of CEC at pH 7 with Ammonium Acetate by Chapman.....	63
3.16.4 Geoaccumulation Index (I_{geo}).....	67
3.17 Statistical Data for Plant–Soil Investigation	67
CHAPTER 4.....	68
<i>FATTY ACID PROFILE AND ELEMENTAL CONTENT OF AVOCADO (PERSEA AMERICANA MILL.) OIL - EFFECT OF EXTRACTION METHODS.....</i>	68
4.1 Introduction.....	68
4.2 Extraction.....	69
4.3 Fatty Acid (FA) Profiling.....	71
4.4 Evaluation of Metals in Avocado Oil.....	77
4.5 Proximate Chemical Composition of Avocado Fruit.....	80
CHAPTER 5.....	82
<i>ELEMENTAL UPTAKE AND DISTRIBUTION OF NUTRIENTS IN AVOCADO MESOCARP AND THE IMPACT OF SOIL QUALITY.....</i>	82
5.1 Introduction.....	82
5.2 Elemental Concentration of Avocado Fruit (Hass and Fuerte) from Various Sites.	83

5.3. Bioaccumulation Factors Obtained from Different Sites	91
5.4 Soil Quality Assessment	106
5.4.1 SOM, CEC and pH.....	106
5.4.2 Geoaccumulation Index	107
5.5 Statistical Analysis.....	109
5.6 Dietary Reference Intakes.....	113
CHAPTER 6.....	115
CONCLUSION.....	115
<i>Further Work</i>	117
REFERENCES.....	118
APPENDIX 1	140
APPENDIX 2	141
APPENDIX 3	142
APPENDIX 4	143

LIST OF TABLES

Table 1. Chemical forms of metals in solid phases.....	32
Table 2. Classes with respect to soil quality.....	33
Table 3. Recommended Dietary Allowances.....	37
Table 4. Tolerable Upper Intake Levels.....	38
Table 5. Geographical coordinates, in decimal degrees, for the 6 chosen sites.....	51
Table 6. Emmision Lines selected for each element.....	58
Table 7. Parameters and oil yields obtained from different extraction techniques.....	70
Table 8. Fatty acid composition of oils obtained from different extraction methods.....	72
Table 9. Summary of the M: S ratio for each variety of avocado oil extracted by various techniques.....	75
Table 10. Comparison of measured and certified values in the CRM.....	77
Table 11. Element content of avocado mesocarp and its dissolution (%) into the oil.....	78
Table 12. Proximate chemical composition of Avocado fruit.....	80
Table 13. Elemental concentrations for chosen elements in avocado fruit and corresponding soil samples.....	83
Table 14. As concentrations in fruit and soil with bioaccumulation factors (BFs).....	92
Table 15. Cu concentrations in fruit and soil with bioaccumulation factors (BFs).....	94
Table 16. Fe concentrations in fruit and soil with bioaccumulation factors (BFs).....	96
Table 17. Mg concentrations in fruit and soil with bioaccumulation factors (BFs).....	98
Table 18. Ca concentrations in fruit and soil with bioaccumulation factors (BFs).....	100
Table 19. Ni concentrations in fruit and soil with bioaccumulation factors (BFs).....	102

Table 20. Zn concentrations in fruit and soil with bioaccumulation factors (BFs).....104

Table 21. pH, soil organic matter (SOM), and cation exchange capacity (CEC) of soil samples from each site.....106

Table 22. Total Baseline Concentrations of metals in South African soils, total concentration of soils, and geoaccumulation index (I_{geo}) for each site.....108

Table 23. Correlation matrix for concentrations of elements in soil total and exchangeable and mesocarp of Hass and Fuerte.....110

Table 24. Dietary Reference Intake, Recommended Dietary Allowance and Tolerable Upper Intake Levels of elements for most individuals and average concentration of elements in mesocarp of avocado (Hass and Fuerte varieties).....114

LIST OF FIGURES

Fig.1 Specialised idioblast oil cells of avocado	16
Fig.2 Two cultivars of avocado fruit	17
Fig.3 Conventional Soxhlet system	18
Fig.4 Supercriticalfluid extraction apparatus.....	21
Fig.5 Phase diagram of carbon dioxide.....	22
Fig.6 Ultra-turrax cell lysing device.....	23
Fig.7 A schematic look at cation exchange capacity.....	30
Fig.8 Schematic of pressurized microwave digestion system.....	41
Fig.9 Principle of the hydride generation technique.....	43
Fig.10. Atomization stages before detection.....	44
Fig.11 Map of selected sampling sites in KwaZulu-Natal.....	50
Fig.12 Schematic of the constructed supercritical fluid extractor.....	53
Fig.13 Oil yields obtained from different extraction methods.....	70
Fig.14 Comparison of the amount of the major fatty acids found in Hass and Fuerte extracted by different techniques.....	73
Fig.15 Monounsaturated Fatty Acid to Saturated Fatty Acid ratio for each variety of avocado oil extracted by various techniques.....	76
Fig.16 Percentage ash, protein, carbohydrate and oil in avocado fruit.....	81
Fig.17 Distribution of major elements in fruit at the 6 different sites.....	88
Fig.18 Distribution of minor elements in fruit at the six different sites.....	90

Fig.19 Bioaccumulation Factors vs. Total and Exchangeable Concentrations of As in soil for both varieties.....	93
Fig.20 Bioaccumulation Factors vs. Total and Exchangeable Concentrations of Cu in soil for both varieties.....	95
Fig.21 Bioaccumulation Factors vs. Total and Exchangeable Concentrations of Fe in soil for both varieties.....	97
Fig.22 Bioaccumulation Factors vs. Total and Exchangeable Concentrations of Mg in soil for Hass and Fuerte varieties.....	99
Fig. 23 Bioaccumulation Factors vs. Total and Exchangeable Concentrations of Ca in soil for Hass and Fuerte varieties.....	101
Fig.24 Bioaccumulation Factors vs. Total and Exchangeable Concentrations of Ni in soil for Hass and Fuerte varieties.....	103
Fig.25 Bioaccumulation Factors vs. Total and Exchangeable Concentrations of Zn in soil for Hass and Fuerte varieties.....	105
Fig.26: Synergistic relationships in soil.....	111
Fig.27 Antagonistic relationships between exchangeable elemental concentrations and elemental uptake into the Hass and Fuerte fruit.....	112

ABBREVIATIONS

BF – Bioaccumulation Factors

CEC – Cation exchange capacity

CHD – Coronary heart disease

CRM – Certified reference material

EDTA – Ethylenediaminetetraacetic acid

FAME – Fatty acid methyl ester

GC – Gas chromatography

HDL – High-density lipoprotein

HGAAS – Hydride generation atomic absorption spectroscopy

I_{geo} – Geoaccumulation factors

ICP-OES – Inductively coupled plasma optical emission spectroscopy

LDL – Low-density lipoprotein

MAE – Microwave assisted extraction

MUFA – Monounsaturated Fatty Acid

ppb – parts per billion

RDA – Recommended daily allowance

SFA – Saturated Fatty Acid

SFE – Supercritical Fluid Extraction

SOM – Soil organic matter

UL – Tolerable upper intake level

CHAPTER 1

1.1 Introduction

There is increasing global awareness of heart diseases especially since the release of alarming statistics that refer to heart related diseases as silent killers. Subsequently, the search for healthier low fat foods is growing in popularity as more people are recognising the need for a healthier lifestyle to prevent heart disease. Many studies have been undertaken to identify foods, especially of plant origin, that are beneficial to human health. The avocado pear has for a long time been stereotyped as a “fattening fruit” and has been avoided for fear of weight gain and the degenerative effects thereof. However, a series of scientific studies have proven that the fats and oils contained in avocado flesh/mesocarp have vast health benefits (Nuray et al. 2005, Salazar et al., 2005, Colquhoun et al., 1992).

The oils extracted from avocado fruit are similar in composition to olive oil and both are highly digestible (Sinyinda and Gramshaw, 1998). Avocados have the highest energy value of any fruit owing to their high oil content and can be recommended to diabetics as a high energy food source due to their low sugar content (Swisher, 1988). There is currently growing interest in fatty acids particularly monounsaturated fatty acids. Edible oils such as avocado and olive oils are known to contain high levels of oleic acid, a stable omega-9 monounsaturated fatty acid (Swisher, 1988), that has generated much interest due to its considerable health value. Epidemiological data obtained from a study in the Mediterranean region, where the diet includes large quantities of oils rich in monounsaturated fatty acids, show low incidences of atherosclerotic cardiovascular disease (Amunziata, 1999). Further research has shown that an avocado enriched diet could possibly reduce total and low density

lipoprotein (LDL) cholesterol levels whilst increasing high density lipoprotein (HDL) cholesterol levels hence lowering the risk of atherosclerotic cardiovascular disease (Carranza et al., 1997). The avocado fruit contains high levels of lipophilic bioactive phytochemicals which include vitamins E and C, carotenoids, and sterols, which have been shown to possess antioxidant and radical scavenging activities (Lee et al., 2004). Bergh reported from surveys done in America that people are deficient in these antioxidants that avocados alone can provide (Bergh, 1992).

Besides the nutritional aspect of avocado oil, it is much sought after in the pharmaceutical and cosmetic industries due to its therapeutic value. The oil contains phytosterols which have the same penetrating abilities as lanolin. This particular quality of avocado oil makes it suitable for skin and massage creams, massage oils and all other preparations which are used for applying to, or rubbing onto skin (Human, 1987). The oil is also known to have sun screening properties.

The above-mentioned discussion clearly confirms the cosmetic and nutritional value of avocado oil. There is therefore a growing interest in efficient extraction methods yielding high quality avocado oil by the industrial sector. The most conventional way of extracting avocado oil is by means of cold pressing (Requejo et al., 2003). Some of the negative attributes of cold pressed oil include producing oils that are fishy and rancid due to extraction from rotten fruit and oils that have oxidized (Martin-Polvillo et al., 1994). Small amounts of metals in edible oils are well known to have serious deleterious effects on the stability of oils. Reports have described the deleterious effects that trace metal contamination, particularly Fe and Cu, have on the flavor and oxidative stability of oil (Martin-Polvillo et al., 1994; Karadjova et al., 1998). According to Kanner and Rosenthal, oxidation is a free radical

reaction and is initiated by light and catalysed by transition metals (Kanner and Rosenthal, 1992). The quality of fats and oils in terms of storability, property retention and freshness can be assessed by determining the presence and quantity of metals in oils (Murillo et al., 1999). The metal ion concentration in the oil depends on the extraction method (Szentmihályi et al., 2002) hence a minor study to compare various extraction methods for the recovery of oil from avocado mesocarp was conducted in this research. Fatty acid and elemental content of extracted oils were determined to assess the quality of oil extracted from avocado fruit and to compare the efficiency of the extraction methods.

The organic constituents of the avocado fruit are of great health benefit, but consumption of the fruit for the organic constituents does not preclude intake of the inorganic constituents such as heavy metals. The latter is directly affected by quality of soil in which the fruit crop is cultivated (Reddy et al., 2011), thus an assessment of the metals present in the soil and its impact on uptake into the fruit was conducted.

Assessing soil quality involves measuring soil physical, chemical, and biological properties and using these measured values to detect changes in soil as a result of land use change or management practices (Campos et al., 2007). Soil organic matter (SOM), cation exchange capacity (CEC) and pH are important soil properties that affect the capacity of soil to supply nutrients which in turn affects uptake of nutrients into the plant (Davis et al., 1994). Soil enrichment/contamination with elements can be carried out in many ways. The most common ones are the index of geoaccumulation. The index of geoaccumulation (I_{geo}) has been used as a measure of bottom sediment contamination since the 1970s (Müller, 1969), and it has been used by various researchers to assess the contamination of soils (Aikpokpodion et al., 2011; Ahiamadjie et al., 2011). It determines contamination by

comparing current metal contents with pre-industrial levels (Loska et al., 2003). Geoaccumulation indices were used here to assess the level of enrichment of selected elements in the soil and to relate this information to elemental uptake by the plant. A major study was conducted to report the elemental composition of avocado mesocarp and the impact of soil quality on elemental uptake. The 14 elements selectively investigated were Al, As, Ca, Cd, Co, Cu, Cr, Fe, Mg, Mn, Ni, Pb, Se, and Zn.

1.2 Statement of the Problem

The commercial planting of avocado crops in South Africa has expanded steadily from the early 1970's to present, with plantings of 2 000 hectares in 1970 increasing to about 12 000 hectares in 2003 (Donkin, 2008). Numerous national and international studies have focused on the isolation and characterisation of organic constituents in avocado fruits that is important for the prevention of many diverse diseases. However, there is little information on the inorganic constituents such as nutrients and heavy metals present in the fruit. This knowledge can contribute to the understanding of the nutritional value of avocados and can also be of potential use to food consumption tables, especially in calculating the Dietary Reference Intakes (DRI's) of these nutrients. It is widely known that cultivar, geographical location, and sun exposure are factors that influence the composition of organic constituents in the avocado fruit but inorganic constituents, particularly elemental content, is mainly influenced by the plant's characteristic elemental uptake profile which is a function of geographical location and soil quality. There is therefore a need to conduct a localised study to assess the nutritional value of avocado crops produced in South Africa. A comparative study on the chemical characteristics of different cultivars of avocado fruit grown and consumed in KwaZulu-Natal has not been reported. The information gained from the study is likely to

make valuable contributions to food science and agriculture thereby attenuating this gap in information.

1.3 Aims and Objectives of the Study

The aims of the study were to assess the quality of extracted avocado oil produced by different extraction techniques to determine the suitability and feasibility of these extraction techniques for the various applications, to assess the nutritional value of locally grown avocado fruit and to determine the impact of soil quality on elemental uptake.

Objectives of this study include:

- To investigate the yield of oil produced by different extraction techniques on avocado fruit of the Hass and Fuerte variety. Techniques include traditional Soxhlet extraction, microwave treatment + Soxhlet extraction, Ultra-turrax treatment + Soxhlet extraction, ultrasound + water bath sonication and supercritical fluid extraction (using carbon dioxide gas).
- To determine the metal content in defatted fruit of both varieties of avocado and corresponding lipid fraction produced by the different extraction techniques to evaluate the suitability of extraction techniques.
- To provide a fatty acid profile for extracted oil from both varieties using the different extraction techniques to assess for oil quality.
- To determine the proximate chemical composition namely % oil, % ash % protein and % carbohydrate of the two avocado varieties.

- To determine the elemental concentration of the elements Al, As, Ca, Cd, Co, Cu, Cr, Fe, Mg, Mn, Ni, Pb, Se and Zn in avocado fruit and soil samples collected from six different sites in KwaZulu-Natal.
- Measurement of soil parameters: pH, soil organic matter and cation exchange capacity
- Determination of the distribution of nutrients in avocado fruit and impact of soil quality parameters on the chemical characteristics of the fruit.
- Assessment of the elemental content in avocado fruit to determine if they conform to recommended dietary allowances (RDAs) and to assess for potential toxicities.
- Evaluation of soil enrichment by using Geoaccumulation indices.
- Statistical analysis to evaluate the impact of soil quality parameters on the chemical composition of the avocado fruit.

The chapter that follows, Chapter 2 is a review of the literature relating to various aspects of the study. Chapter 3 details the different analytical experimental procedures, instrumentation, and methods of analyses used to meet the specific objectives of the study. Chapter 4 focuses on the results obtained for the comparative study of extraction techniques used to assess for oil quality; Chapter 5 is a comprehensive discussion of the results obtained from the soil and fruit analyses and Chapter 6 provides the conclusions of the study.

CHAPTER 2

LITERATURE REVIEW

2.1 Avocado

The avocado, *Persea americana* Miller, of the plant family Lauraceae, is a fruit with extremely high oil content which is the main component of its dry weight. The avocado tree is indigenous to tropical America, but it has been adopted and commercially cultivated by other countries such as Australia, New Zealand and South Africa. This evergreen is the only tree that bears fruit that ripens when fallen.

2.1.1 Avocado Trade in South Africa

There are three botanically distinct races of avocado that are internationally recognized namely; West Indian, Guatemalan and Mexican (Mossler et al., 2001). By the selection of these varieties and by cross-breeding, numerous avocado cultivars were developed over the years, of which mostly Fuerte, Hass and Edranol are grown commercially in South Africa (Human, 1987). Production in South Africa is an export-orientated industry where fresh avocados are exported to the European market however, processing of the fruit to produce purees and oils is a growing industry in South Africa (Donkin, 2008). Avocado trees can grow to a height of 18.3 m but it is generally maintained at 6.3 m for ease of harvest and maintenance (Swisher, 1988).

2.1.2 Nutritional Value of Avocados

The health benefits of avocado were first recognised by a study published in 1960 (Grant, 1960). This investigated the cholesterol-lowering effect of the fruit (Pieterse et al., 2003). The oil content of the fruit depends on its ecological origin and on the cultivar, for example, in Guatemalan and Mexican cultivars, the oil content varies from 10 to 13% and 15 to 25%, respectively (Biale and Young, 1971) whilst in the fruits from the Caribbean, low fat content (2.5 to 5%) has been reported (Hatton et al. 1964). The fruit is known to help to reduce low-density lipoprotein (LDL) cholesterol which is harmful, as well increase high-density lipoprotein (HDL) cholesterol. Frequent ingestion of the fruit reduces the risk of developing atherosclerosis by lowering the triglyceride content in the blood (Carranza et al., 1992). The fruit also contains large amounts of the less common heptose (C7) sugar, mannoheptulose, and its corresponding sugar alcohol, perseitol, which have been reported to have anti-cancer activity (Board et al., 1995; Ishizu et al., 2002). In addition, mannoheptulose has been associated with an insulin secretion inhibitory effect (Ferrer et al., 1993).

2.1.3 Fatty Acids in Avocado

High blood cholesterol concentrations, mainly LDL cholesterol, are widely recognized as a major risk factor for coronary heart disease (CHD). Conversely, high concentrations of HDL cholesterol protect against the development of CHD (La Rosa et al., 1990; Gordon et al., 1977). Studies have shown that when olive oil is added to a patient's diet, blood cholesterol concentrations decrease and there is conversion to HDL concentrations (Grundy et al., 1984). In Mediterranean communities, despite a moderate to high intake of fat, serum cholesterol

concentrations are low, as is the incidence of CHD. In Greece, 40% of energy on average comes from fat, particularly from olive oil. However, the saturated fatty acid intake is low (< 10% of energy) and the diet is high in olive oil (James et al., 1989; Keys, 1970). Avocado oil is similar to olive oil, which itself is an essential component of the Mediterranean diet, as it is rich in monounsaturated fatty acids and low in saturated fatty acids, and is free of cholesterol (Eyres et al., 2001). Both avocado and olive oil, thanks to their fatty acid content, help lower LDL cholesterol and increase HDL cholesterol. Oleic acid, which is the main monounsaturated fatty acid found in avocado and olive oil, increases the absorption of omega-3 polyunsaturated fatty acids in cell membranes and lowers the possibility that LDL becomes oxidised, both processes helping to reduce the risk of cardiovascular disease (Human, 1987; Alvizouri et al., 1992; Carranza, 1995; Lopez et al., 1996; Rodríguez, 1997; Lerman et al., 1994; López, 2005).

2.1.4 Cosmetic Uses for Avocado Oil

As mentioned earlier, avocado also has many uses in the cosmetic industry. The oil is of particular importance as it contains plant phytosterols which have the same penetrating abilities as lanolin. The ability to penetrate the skin is no doubt the key to the success of avocado oil as a natural and effective beauty aid. The quality of the oil makes it ideal as a carrier for other substances which are not able to permeate into the skin. The flavour of the oils is bland and can replace unpleasant smelling oils such as codliver and turtle oils. Avocado oil is also used in high-grade toilet soaps and contributes to the soap's superior lathering and cleaning qualities. Avocado oil is easy to emulsify. Its low surface tension produces smoother creams and soaps and makes a superior cosmetic oil. According to Rolfe, the impressive list of vitamins is of benefit to the cosmetic industry, because vitamin A helps

to prevent dry skin while vitamin E (Tocopherol) and vitamin D are effective against skin wrinkling. Due to the abundance of unsaturated fatty acids in the oil, its fibrous proteins act as a natural skin moisturiser (Rolfe, 1975). Moisture is necessary to make the skin look soft and young. Avocado oil also has some sun-screening properties. The ultraviolet radiation of the sun may contribute to the ageing process of the skin. It dries the skin and induces wrinkling. The oil can easily be labelled the world's finest skin nutrient and truly nature's own cosmetic (Rolfe, 1975).

2.1.5 Avocado Oil Cells

The avocado's fleshy mesocarp in the mature fruit consists mostly of uniform isodiametric idioblastic cells of about 60 μm in diameter in the mature fruit (Werman and Neeman, 1987). Scattered throughout the tissue, these are specialized oil cells which are characterised by large oil sacs, although small droplets of oil can also be detected in the parenchyma cells. The oil cells, or idioblasts, are distinguished by their large size and lignified walls (Werman and Neeman, 1987). Platt et al. found that the idioblastic oil cell wall consists of three different layers: an outer inert layer made of cellulose, an intermediate layer made of suberin, and a tertiary layer of cellulose as the inner layer (Platt et al., 1983). The specialised oil cells contains a single large oil drop which fills the cell while the parenchyma cells have smaller single droplets in their cell structure (Cummings and Schroeder, 1942). Idioblast cell oil stains with a different density and has a different appearance in freeze-fracture eplicas compared to oil present in the parenchyma cells (Platt Aloia et al., 1983; Platt and Thomson, 1992). Idioblast cell oil is therefore thought to have a different composition to oil present in parenchyma cells (Platt and Thomson, 1992). When ripening of the fruit occurs, there is a significant increase in the activities of cell wall hydrolytic enzymes which results in the

degradation of the walls of surrounding parenchyma cells; hence the fruit softens (Awad and Young, 1979; Platt-Aloia et al., 1980). However, the suberized wall of the idioblast oil cells are immune to the surrounding enzyme activity and remain intact during and after the ripening stages (Platt and Thomson, 1992).

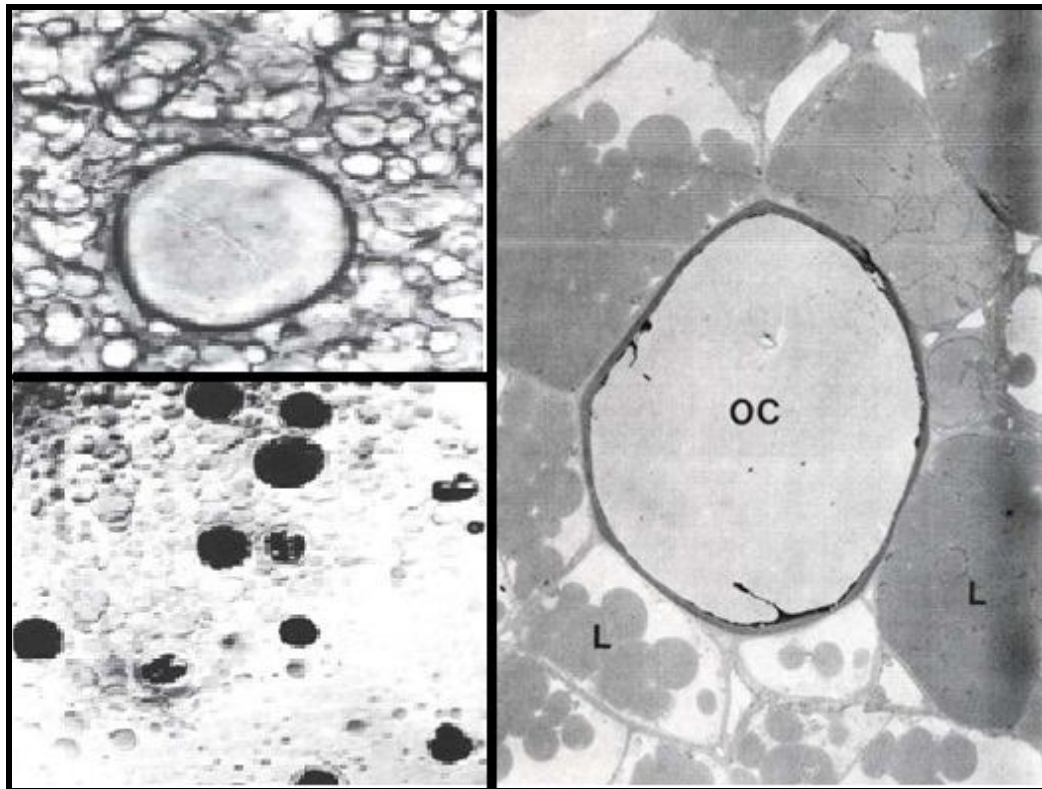


Fig.1 Specialised idioblast oil cells of avocado

Sourced from: California avocado society 1966 yearbook, A. Schroeder

2.2 Extraction Methods

There are a large number of avocado cultivars available, but only those cultivars with the highest oil content should be considered for oil extraction, hence the selection of Hass and Fuerte cultivars for the study (Fig.2). It is well known that these two varieties have the highest oil content compared to other cultivars (Human, 1987). Extraction of the oil requires disruption of both the oil cells and the finely dispersed oil emulsion in the fruit pulp (Human, 1987). Solvent extraction, mechanical pressing and centrifugation of pulp slurries have been used in processing avocados for their oil (Bizimana et al., 1993).



Fig.2 Two cultivars of avocado fruit

Sourced from: www.gofor2and5.com.au

The idea behind the study which is based on the comparison of extraction methods was to focus on unusual physical methods to lyse the specialised idioblast oil cells followed by solvent extraction. The combination approach between physical and chemical methods was investigated and compared to traditional Soxhlet extraction. The different methods of extraction used in this study will be discussed.

2.2.1 Traditional Soxhlet Extraction

Soxhlet, which is a well established technique, has for a long time been the standard reference technique to which other solid liquid extraction techniques have been compared (Luque de Castro & Garcia-Ayuso, 1998). The basic operation of the Soxhlet system is fairly simple. Plant material is placed in a thimble-holder and filled with fresh solvent from a distillation flask. When the liquid reaches the overflow level, it siphons back into the distillation flask carrying with it extracted plant contents into the solvent containing distillation flask. Extracted plant contents are separated from the solvent as the mixture is heated during distillation. Fresh solvent begins to fill back into the thimble containing plant material. The operation is repeated until complete extraction is achieved which is usually an overnight

process. The advantages of conventional Soxhlet extraction includes the maintenance of a relatively high extraction temperature, the creation of a displacement of transfer equilibrium by continuously bringing fresh solvent into contact with the plant material, no filtration after leaching step and the fact that the method is simple and cheap to conduct (Luque de Castro and Garcia-Ayuso, 1998). The main disadvantages of conventional Soxhlet extraction include the long extraction times and the requirement of an evaporation step where thermal labile plant contents may possibly decompose (Luque de Castro and Garcia-Ayuso, 1998).

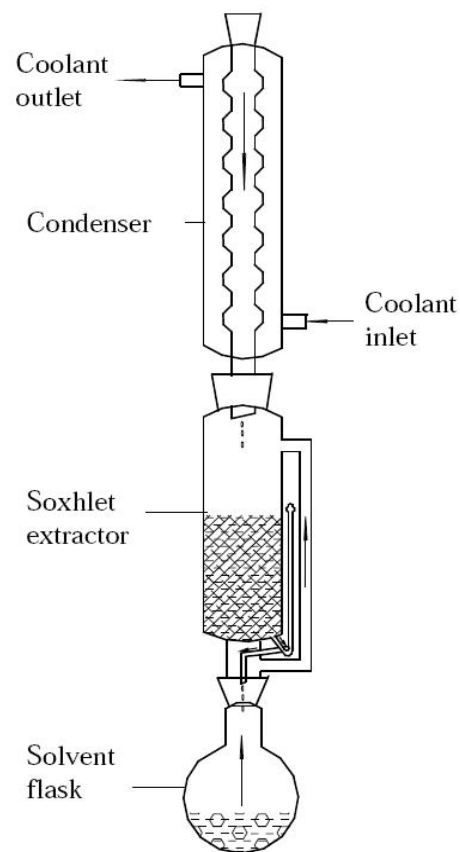


Fig.3 Conventional Soxhlet system

Sourced from Wang and Weller, 2006

2.2.2 Sonication Assisted Extraction

The use of ultrasound is well known to cause a number of physical effects such as turbulence, particle agglomeration, microstreaming and biological cell rupture. These effects result mainly from the phenomenon known as cavitation (Leighton, 1994). Sound waves which have frequencies higher than 20 kHz can cause mechanical vibrations in a solid, liquid and gas. When sound waves travel through matter, they induce expansion and compression cycles through the medium. These expansions can create microbubbles in a liquid. The violent collapse of microbubbles in a sonication liquid due to pressure fluctuations is referred to as cavitation (Leighton, 1994). When cavitation occurs in close proximity to a solid biological material, the collapse of a cavity produces tiny jets of liquid which impact strongly on the solid surface resulting in the release of cellular contents (Thompson et al., 1999; Luque-Garcia and Luque de Castro, 2003). Ultrasound-assisted extractors are available in two designs: ultrasonic baths or closed extractors fitted with an ultrasonic horn transducer. Efficient cell disruption and effective mass transfer are cited as two major factors leading to the enhancement of extraction with ultrasonic power (Mason et al., 1996). In contrast to conventional extractions, plant extracts diffuse across cell walls due to ultrasound, causing cell rupture over a shorter period (Vinatoru et al., 1999; Toma et al., 2001; Chemat et al., 2004; Li et al., 2004).

2.2.3 Microwave Assisted Extraction (MAE)

Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz. Microwaves are transmitted as waves, which can penetrate biomaterials and interact with polar molecules such as water in the biomaterials to create heat. Water within the plant matrix absorbs microwave energy, cell disruption is promoted by internal superheating, which

facilitates desorption of chemicals from the matrix, improving the recovery of cellular contents (Kaufmann et al., 2001). It was reported that microwave pretreatment of fresh orange peels led to destructive changes in the plant tissue (Kratchanova et al., 2004). These changes in the plant tissue due to microwave heating have a considerable increase in the yield of extractable pectin. Furthermore, the migration of dissolved ions increased solvent penetration into the matrix and thus facilitated the release of the chemicals. There are two types of commercially available MAE systems: closed extraction vessels under controlled pressure and temperature, and focused microwave ovens at atmospheric pressure (Kaufmann and Christen, 2002). The microwave assisted extraction used in this study does not involve simultaneous use of microwaves and solvent in a closed vessel but instead uses microwaves as a means of facilitating cellular disruption prior to solvent extraction.

2.2.4 Supercritical Fluid Extraction (SFE)

A supercritical state is achieved when the temperature and the pressure of a substance are raised over its critical value (Wang and Weller, 2006). As an extracting solvent, supercritical fluids have enhanced properties. It possesses properties similar to gas in terms of diffusion, surface tension and viscosity, but simultaneously it also has a high density similar to that of a liquid. Compared to liquid solvents, supercritical fluids have several advantages: (1) the dissolving power of a supercritical fluid solvent depends on its density, which is highly adjustable by changing the pressure or/and temperature; (2) the supercritical fluid has a higher diffusion coefficient and lower viscosity and surface tension than a liquid solvent, leading to more favourable mass transfer (Wang and Weller, 2006).

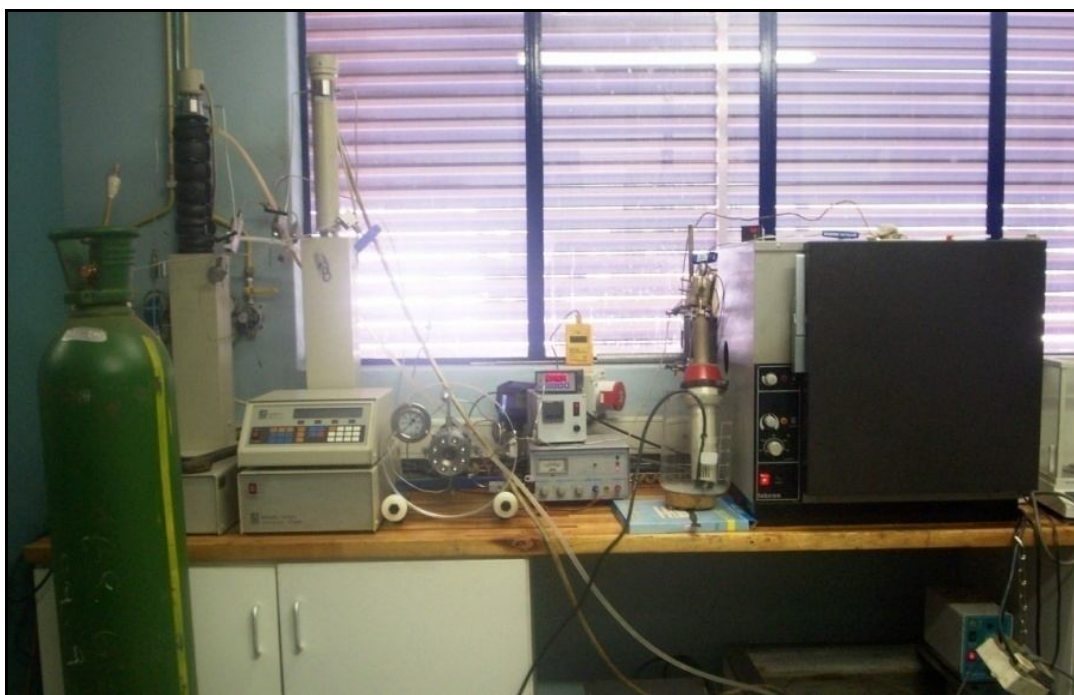


Fig.4 Supercritical fluid extraction apparatus

Fig.4 represents the SFE apparatus used in this study. Generally, raw plant material is loaded into an extraction vessel, which is equipped with temperature controllers and pressure valves at both inlet and outlet to keep the desired extraction conditions. The extraction vessel is pressurized with the fluid by a pump. The fluid and the dissolved compounds are transported to separators, where the power of the fluid is decreased by decreasing the pressure or increasing the temperature of the fluid. The product is then collected via a valve located in the lower part of the separators (Sihvonen et al., 1999). With a reduction in the price of carbon dioxide and restrictions in the use of other organic solvents, carbon dioxide has moved from some marginal applications to being the major solvent for SFE (Hurren, 1999). The critical state of carbon dioxide fluid is at a temperature of only 304 K and pressure of 7.3 MPa (Fig. 5). Also, carbon dioxide is non-flammable and non-toxic. Supercritical CO₂ is a good solvent for the extraction of non-polar compounds such as hydrocarbons (Vilegas, 1997).

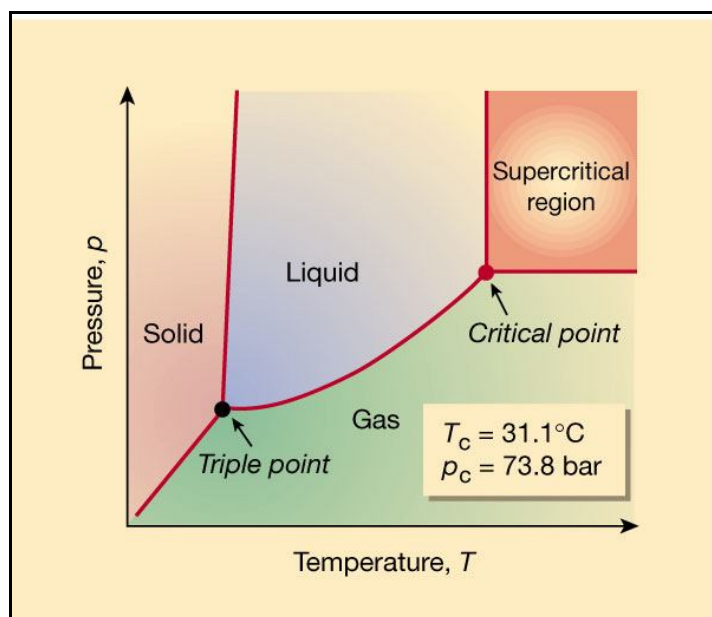


Fig.5: Phase diagram of carbon dioxide

Sourced from:<http://wikis.lawrence.edu/display/CHEM/Changes+in+Physical+State+-+phase+transitions+>
+Laura+Qiu

2.2.5 Ultra-Turrax Treated Extraction

The use of the Ultra-turrax device is usually limited to biological sample preparations where it is primarily used as a sample homogeniser (Zweck, et al., 1978). In this study, a combined extraction method, proposed by the author, involving the use of the Ultra-turrax device followed by solvent extraction will be investigated.



Fig.6 Ultra-turrax cell lysing device

Sourced from www.dotmed.com.

2.2.6 Factors Effecting Oil Quality

The transition metal content of edible oils has a significant impact on stability and shelf life of products (Nash et al., 1983). Many reports have described the deleterious effects that trace metal contamination has on the flavour and oxidative stability of oil (Wong et al., 1980; Hendrikse et al., 1991; Martin-Polvillo et al., 1994). Small amounts of metals in edible oils are well known to have serious deteriorative effects on the stability of these oils. The altered oil characteristics are expressed as changes in colour, odour, and flavour. Cu and Fe, in particular, greatly reduce the oxidative stability of oil. Therefore, accurate determination of trace metal content is very important in evaluating deteriorating effects (Ooms et al., 1983).

2.3 Essential and Non-Essential Elements

An element is essential to an organism if it cannot be synthesised in the organism and must be obtained from a food source. In the case of non-essential elements, the body has no need for

the element at all (Harrison and Mora, 1996). Some elements essential to man include Ca, Cr, Co, Cu, Mg, Mn, Ni, Se, and Zn and most of these elements are essential at low concentrations.

2.4 Soil and Metals

Soil has been defined as the upper weathered layer of the earth's crust that serves as a natural medium for the growth of land plants. Soil is the reservoir for many constituents, elemental and biological, including heavy metals (Varma, 1999). Trace metals occur naturally in soils usually at low concentrations as a result of weathering of rock from which soil develops (parent material) (Kabata-Pendias and Pendias, 1992). Metals exist in the soil solution as either free (uncomplexed) metal ions (e.g., Cd^{2+} , Zn^{2+} , Cr^{3+}), in various soluble complexes with inorganic or organic ligands (e.g., CdSO_4 , ZnCl^+ , CdCl^+), or associated with mobile inorganic and organic colloidal material (Mattigod et al, 1981). The accumulation of trace metals usually due to anthropogenic causes are of concern due to adverse health effects which include chronic accumulation of metals in the kidney and liver of humans causing disruption of numerous biochemical processes (WHO, 1992), the development of cancer (Trichopoulos, 1997) and the development of abnormalities in children (Gibbes and Chen, 1989). The metals of concern are As, Cr, Cu, Hg, Pb, Ni, Se and Zn. Essential and non-essential elements are found in soil, the concentrations of which are toxic if at elevated concentrations. These metals gain entry into human and animal food chains through crops grown on soils contaminated with them.

Arsenic is ubiquitous in the environment. Major anthropogenic sources of As distribution are metal processing, burning of coal, and the application of arsenic-based pesticides or

herbicides (Kabata-Pendias et al., 1992). An important natural origin of As contamination is volcanism (Queirolo et al., 2000). Arsenate (AsO_4^{3-}) and arsenite (AsO_3^{3-}) are the primary chemical forms occurring in soils. Soil microorganisms convert these compounds by oxidation/reduction or methylation/de-methylation reactions (Okada et al., 1994). Bioavailability, uptake and phytotoxicity of As to plants are influenced by factors such as arsenic concentration in soil, As species, plant species and soil properties, like redox potential, drainage conditions, pH and soil P content (Marin et al., 1993; Creger et al., 1994; Ernst et al., 1997; Carbonell-Barrachina et al., 1998). The concentration tolerated by plants varies from 1 to 50 mg of As per kg of soil. Arsenite is more toxic than arsenate, and both are more toxic than organic arsenical compounds (Sachs et al., 1971; Lepp et al., 1981). Arsenate is chemically similar to phosphate. It uncouples the oxidative phosphorylation by displacing phosphate in ATP synthesis (Terwelle et al., 1967). Arsenate competes with phosphate for uptake, but the affinity for phosphate is much stronger than for arsenate. An increased phosphate level leads to reduced arsenate uptake in plants and vice versa. Arsenate has been reported to reduce chlorophyll biosynthesis in maize (Meeta-Jain et al., 1997).

Lead is a heavy non-essential metal present in petrol. However, its use in petrol has been phased out in South Africa. Most of the Pb released into the environment comes from vehicle exhaust emissions and houses with paint containing Pb (Bigdeli and Seilsepour, 2008). Lead is toxic to humans since the body does not metabolize it and absorbs about 20% of ingested amounts directly into the bloodstream (Department of Environmental Affairs, 2010). At relatively low concentrations, with continuous exposure, Pb can cause neurological impairment in young children. At high levels of exposure, Pb can severely damage the brain and kidneys in adults or children and ultimately cause death (Singh, 2005).

Chromium is recognised as an essential element for humans and animals (Mertz, 1967). Cr(III) occurs naturally in many fresh vegetables, fruits, meat, and grains. Cr(VI) is most often produced by industrial processes and may be an indicator of environmental contamination as it is more leachable than Cr(III). Elevated concentrations have been found in run-off for Cr from concrete and stainless steel (Persson and Kucera, 2001). Cr(VI) is extremely toxic for all organisms and is carcinogenic (Wetterhahn and Hamilton, 1987).

Cadmium is a highly toxic element that accumulates in biologic systems and has a long half-life. Cadmium is not an essential element in plant nutrition; it is easily transferred from soil to plants, which are increasingly contaminated by cadmium from phosphate-based fertilizers. (Dalen et al., 1996). Cadmium can also be present in edible oils and fats, as a result of contamination from the environment, the refining process, the storage tank, or the packing material (Dalen et al., 1996).

Copper is a trace element essential to plants, animals and humans. Environmental sources of Cu relate to smelters and refiners with the exposure route being via inhalation of dust (Department of Environmental Affairs, 2010). Ingestion of high concentrations of Cu results in gastrointestinal disturbances and possible liver, kidney, and red blood cell damage (Department of Environmental Affairs, 2010).

Manganese is found in rock, soil, water, and food and is an essential element for humans and animals. Although Mn is essential for plant growth, high concentrations can be toxic (McLaughlin, 1999). Edible plants are direct oral sources of Mn and an important route of exposure to humans.

Anthropogenic activity has resulted in widespread distribution of Ni from the burning of oil and coal. Nickel present in refinery dust is carcinogenic. The critical effects of ingested Ni are developmental effects on the offspring of females exposed during pregnancy (Department of Environmental Affairs, 2010). Nickel released from concrete surfaces may also add to total Ni emissions (Persson and Kucera, 2001).

Selenium is both an essential nutrient for humans and animals and an environmental toxicant (Germ et al., 2007). Selenium levels in plants are influenced by plant type and soil factors such as geology, soil type and pH (Johnsson, 1991). Other influential factors include the chemical form of Se, climate, fertilizer treatment, and deposition rate of atmospheric Se (Johnsson, 1991). Selenium is important in the metabolism of cyanobacteria and some plants, being involved in their antioxidative processes (Germ et al., 2007). The essentiality of Se to higher plants, however, is still under debate (Germ et al., 2007). Although it is harmful for plants in high concentrations, it can exert beneficial effects at low concentrations. Selenium has become the primary element of concern in much environmental contamination because of its bioaccumulation in food webs (Germ et al., 2007).

Zinc is an essential element for plants and animals. In plants too much of Zn can suppress crop yields and can render the soil unproductive (Shipp and Baker, 1975). Humans have a high tolerance for Zn. Zinc is used in galvanizing processes and in alloys, including brass and bronze. Zinc exposure is primarily through ingestion. The present use of zinc oxide (ZnO) in rubber is a major source of Zn (Bergback, 2000). Zinc oxide is also widely used for concrete manufacture; addition of ZnO improves the processing time and the resistance of concrete against water (Brown, 1957).

Iron is one of the most abundant metals on earth; it is essential to most life forms and to normal human physiology. In humans, Fe is an essential component of protein involved in

oxygen transport (Institute of Medicine, Food and Nutrition Board, 2001). Excess amounts of Fe can result in toxicity and even death (Department of Environmental Affairs, 2010).

2.4.1 Interactions of Elements in Soil

Interactions between chemical elements both micro and macro have an antagonistic and/or synergistic character. Most of these interactions are associated with physiological processes in plants; some are related to soil chemistry. The latter are mainly impacts of the major elements on the distribution and forms of some trace elements in the soil (Kabata–Pendias and Mukherjee, 2007). Correlation analyses are useful tools in predicting these interactions and will be used in this study, extensively.

2.5 Soil Quality

From the advent of agriculture, there has been an innate interest in soil and land quality (Carter et al., 2004). Maintaining or improving soil quality can provide economic benefits in the form of increased productivity, more efficient use of nutrients and pesticides. The general consensus is that the soil quality concept should not be limited to soil productivity, but should encompass environmental quality (Karlen et al., 2003). In order to properly assess soil quality, it is important to differentiate between natural and dynamic soil properties (Carter et al., 2004). The dynamic nature of the soil refers to soil that has been subjected to management practices and land use while inherent nature refers to measurements that represent the ability of the soil to function in its native state. A comparison can then be made to assess the effects of land use or different management practices on similar soils (Aghasi et al., 2010). Assessing soil quality involves measuring physical, chemical, and biological soil properties

and using these measured values to detect changes in soil as a result of land use change or management practices (Adolfo et al., 2007).

2.5.1 Soil pH

Soil pH affects all chemical, physical and biological soil properties (Pietri and Brookes, 2008). The pH of the soil refers to the H^+ concentration in the solution present in soil pores (Vangheluwe et al., 2005). Positively charged hydrogen ions are constantly in equilibrium with the negatively charged surfaces of the soil particle (Vangheluwe et al., 2005). The available number of negatively charged binding sites for cations is therefore dependant on soil pH (Vangheluwe et al., 2005). Soil pH is affected by changes in the redox potential of soil, degradation of organic material in soils and weathering of parent material (Alloway, 1995). In general, heavy metal cations are most mobile under acid conditions and increasing the pH by liming reduces their bioavailability (Alloway, 1995).

2.5.2 Soil Organic Matter (SOM)

Because SOM is a major source of negative charge in many soils it acts as an important cation exchanger, which may represent a significant chemical buffer (e.g. the forest floor) (James and Riha, 1986). In addition, soil organic matter may complex trace metals thus reducing phytotoxic effects (Bloom et al., 1979). Soil organic matter is also important as a store for N and S, which may be liberated slowly upon decay. Soil organic matter can be subdivided into non-humified and humified material. Non-humified substances are not or are only slightly altered after decay of tissue from living organisms and include carbohydrates, amino acids, protein, lignin, hormones and low molecular weight organic acids (Tan, 1986).

Humified substances are decomposition products of non-humified constituents and include complex compounds such as humin, fulvic acid (FA), humatmelanic acid, humic acid (HA) and their hydroxybenzoic acid derivatives (Tan, 1986).

2.5.3 Cation Exchange Capacity (CEC)

The relative ability of soils to store the cations is referred to as cation exchange capacity or CEC. In other words, it is a measure of the number of negatively charged binding sites in the soil, which can be summed up as the nutrient holding capacity of the soil. CEC is directly proportional to the number of available negatively charged sites. Therefore, soil pH becomes a factor that determines the CEC of soil since the number of available negative charges depends on it. Other factors that effect the CEC of soil include soil organic matter, clay content, clay type, and parent material.

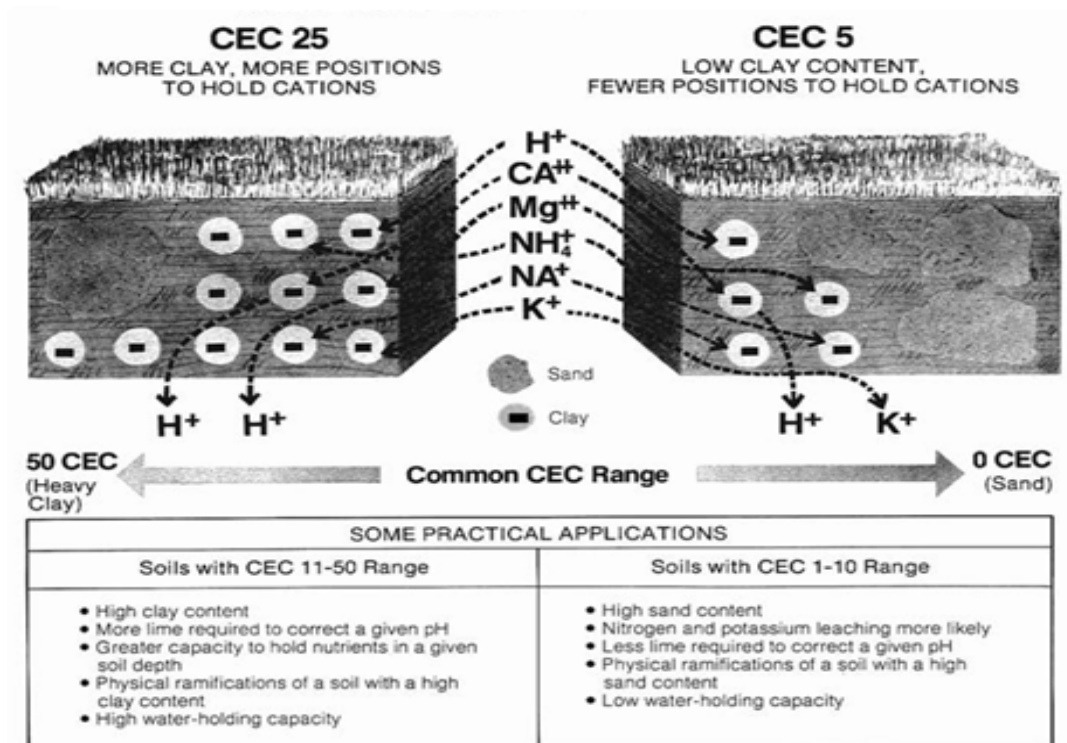


Fig.7 A schematic look at cation exchange capacity

Sourced from: http://www.spectrumanalytic.com/support/library/ff/CEC_BpH_and_percent_sat.htm

2.5.4 Total and Exchangeable Metal Concentrations in Soil

Total metal concentration refers to the metal concentration representing the total amount of metal determined in soil after digestion in a strong acid where complete destruction of the sample occurs. One of the widely used acids is nitric acid (HNO₃) because of its availability, chemical compatibility, oxidizing ability, purity and low cost. For materials that are strongly bound to the soil like silicates, then a harsher acid like hydrofluoric acid (HF) is used for digestion. On the other hand, bioavailability or the exchangeable fraction is the amount of metals that are ready for uptake by the plant. Exchangeable cations are available to plants, for example through exchange with H⁺ liberated by the roots. Exchange reactions are also responsible for the retention of freshly introduced cations into the soil solution (Mulder, 1994). In this way the CEC gives the soil a buffering capacity, which may slow down the leaching of nutrient cations and positively charged pollutants (Mulder, 1994). In order to understand bioavailability, plant materials and selective chemical leaching of soil must be analyzed and the results compared. Metal availability is determined by the way the metal complexes with the soil and depends on the abiotic factors and speciation of the metal. In soil solution, metals may form organic complexes with dissolved organic matter, inorganic complexes with dissolved anions or occur as free hydrated metal ions. The relative mobility of trace elements associated with different fractions is shown in Table 1

Table 1. Chemical forms of metals in solid phases.

		FRACTIONS	MOBILITY
T O T A L		Dissolved - in pore water	High
		Exchangeable - weakly adsorbed	High
		Associated with carbonate	High
		Associated with Fe, Mn oxides	Medium
		Complexed by organics	Medium
		Associated with sulfide	Low
		Crystalline - in the mineral lattice	Low

Adopted from Al-Yemeni et al., 2006

Soil extractants are commonly grouped according to their ability to extract cations from the various soil fractions. Cations dissolved in the soil solution can be easily isolated by centrifugation (Ure, 1996) while cations in the exchangeable fraction can be extracted by salts of strong acids and bases or neutral salts of weak acids and bases such as potassium nitrate (KNO_3) or ammonium acetate (NH_4OAc at pH 7) (Rauret, 1998, Schramel et al., 2000). In some cases, the complexing ability of an anion is used to dissolve not only the cations of the exchangeable fraction, but also the cations complexed by organics (Rauret, 1998). Chelators such as ethylenediaminetetraacetic acid (EDTA), used for mineral soils, and diethylenetriaminepentaacetic acid (DPTA) used for calcareous soils, are common extractants adopted for exchangeable metal determination (Rauret, 1998). Diluted acids partly dissolve cations associated with fractions such as exchangeable, carbonates, iron and manganese oxides as well as organic complexes (Rauret, 1998). This study uses concentrated HNO_3 for the total metal determination and a combination of ammonium acetate and EDTA as extractants for the bioavailable or exchangeable cation fractions (Beckett, 1989; Dean, 2005).

2.6 Geoaccumulation Index (I_{geo})

All soils naturally contain trace levels of heavy metals. The presence of metals in soil is, therefore, not indicative of contamination. The concentration of metals in uncontaminated soil is primarily related to the geology of the parent material from which the soil was formed (McNeal and Balistreri, 1989). The assessment of soil enrichment with elements can be carried out in many ways. The most common ones are the index of geoaccumulation and enrichment factors. The index of geoaccumulation (I_{geo}) has been used as a measure of bottom sediment contamination since the 1970s (Muller, 1969), and numerous researchers have employed it to assess for contamination of soils (Kwapuliński et al., 1996; Miko et al., 2000). I_{geo} is used to determine contamination by comparing current metal contents with pre-industrial levels (Muller, 1981). The method assesses the degree of metal pollution in seven grades (Table 2) ranging from uncontaminated to extremely contaminated. In the study, the degree of anthropogenic pollution is established by calculating the geoaccumulation index (I_{geo}).

Table 2. Classes with respect to soil quality (Muller, 1969).

I_{geo} value	Designation of soil quality
0	Uncontaminated
0-1	Uncontaminated to moderately contaminated
1-2	Moderately contaminated
2-3	Moderately to strongly contaminated
3-4	Strongly contaminated
4-5	Strongly to extremely contaminated
>5	Extremely contaminated

2.7 Essential Elements in Plants

Trace elements are essential in small concentrations for the normal healthy growth of plants, but at higher concentrations are detrimental to the plant (Saaman, 1998). Elements that have been shown to be essential to plants are: B, Br (algae), Co, Cu, F, Fe, I, Mn, Mo, Ni, Rb, Se, Si, Ti, V and Zn. However, even though these elements fulfil the criteria for essentiality, many of these, if deficient in soil, are unlikely to cause deficiency problems in agricultural crops. The essential elements which are most likely to induce deficiency problems in plants are: B, Cu, Fe, Mn, Mo and Zn (Herselman, 2007).

2.8 Distribution of Nutrients in Plants

Plant availability of trace metals differs widely among plant species and organs. Hooda et al. measured variability in the accumulation of Cd, Cu, Ni, Pb, and Zn in wheat, carrots, and spinach grown on biosolids-amended soils (Hooda et al., 1997). Cadmium, Ni, and Zn increased in plants to a greater extent than Cu and Pb compared to their background levels suggesting that Cd, Ni, and Zn might pose the greatest hazard among the trace metals studied. Similar findings were reported by Keefer et al. and Smith (Keefer et al., 1986; Smith, 1994). Sauerbeck and Hein found that Ni uptake in 13 crops was dependant on plant species and organs (Sauerbeck and Hein, 1991). Nickel concentration was higher in grain and storage organs than in vegetative plant parts. Barley accumulates low amounts of Ni, lettuce is a medium accumulator, and radish absorbs high amounts of Ni (Sauerbeck and Hein, 1991). It is therefore necessary to conduct elemental distribution of different plants, especially edible parts, in order to gain knowledge of the plants characteristic uptake patterns.

2.8.1 Bioaccumulation

Bioaccumulation is an important process since it results in chemicals affecting living organisms. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemicals concentration in the environment. Compounds accumulate in an organism if they are taken up and stored faster than they are metabolized or excreted. Bioaccumulation is a normal and essential process for the growth and nurturing of organisms. Metal bioaccumulation can apply to the entire organism, including both metal adsorbed to surfaces or absorbed by the organism. The mobility, solubility and bioaccumulation of trace elements depend on a plethora of soil, microbial and plant factors as well as the properties of the trace element.

2.8.2 Bioaccumulation Factors (BF)

The relative accumulation of metals taken up by plants can be calculated by dividing the concentration of the metal in the plant by the concentration in the soil (Timperley et al., 1973). This relative accumulation is known as the bioaccumulation factor (BF).

$$BF = \frac{[\text{Metal}]_{\text{plant}}}{[\text{Metal}]_{\text{soil}}}$$

The BF can be obtained for both total and bioavailable amounts of metals found in soil.

2.8.3 Accumulators and Excluders

Other plants have the ability to accumulate heavy metals with no known biological function, such as Cd, Cr, Pb, Co, Ag, Se and Hg (Garbisu and Itziar, 2001). By definition, a hyperaccumulator is a plant that is capable of removing metal from its surroundings and transporting it from the roots to the shoots, where it is stored at concentrations exceeding 1000 $\mu\text{g g}^{-1}$ dry matter (Brooks et al. 1977). These criteria hold for Ni, Co, Cu, Pb and Se, whereas Zn and Mn have a threshold of 10000 $\mu\text{g g}^{-1}$ and Cd 100 $\mu\text{g g}^{-1}$ respectively (McGrath et al. 2002). Regardless of why these plants have developed the ability to hyperaccumulate metals, their use provides a unique, natural opportunity for remediation of anthropogenically enriched soils. According to a study, hyperaccumulators can accumulate metals such as Ni, Zn, and Cu to a level of 1-5% of their dry weight, which is considerably higher than non-hyperaccumulator plants (Raskin et al., 1997). Metal excluder plants are usually recognised by a low incidence of a specific metal concentration in the roots than in plant shoots (Tang et al., 2001).

2.9 Essential Elements in Humans

Essential elements are divided into two categories namely, macronutrients and micronutrients (Abdulla et al., 1993). Macronutrients are known to be found in high quantities and are taken up by the major parts of the body while micronutrients are those that are present in trace amounts and play a lesser but essential role in our bodies (Abdulla et al., 1993). One must consider the entire exposure process of different metals and further assess their bioavailability and toxicity on the environment (Jonnalagadda et al., 1993). Table 2.2 and 2.3 shows the Recommended Dietary Allowances (RDAs) and Tolerable Upper Intake (UL) levels, respectively for most individuals.

Table 3. Recommended Dietary Allowances (RDAs).

Sourced from: Food and Nutrition Board, Institute of Medicine, National Academies, 2007

Life Stage	Ca	Cr	Cu	Fe	Mg	Mn	Se	Zn
Males	(mg/d)	(μ g/d)	(μ g/d)	(mg/d)	(mg/d)	(mg/d)	(μ g/d)	(mg/d)
14– 18 y	1,300	35	890	11	410	2.2	55	11
19– 50 y	1,000	35	900	8	400	2.3	55	11
>51 y	1,200	30	900	8	420	2.3	55	11
Females								
14– 18 y	1,300	24	890	15	360	1.6	55	9
19– 50 y	1,000	25	900	18	310	1.8	55	8
> 51 y	1,200	20	900	8	320	1.8	55	8

Table 4. Tolerable Upper Intake (UL^{*}) levels.

Sourced from: Food and Nutrition Board, Institute of Medicine, National Academies, 2007

Males/Females	As	Ca	Cr	Cu	Fe	Mg	Mn	Se	Zn
(Life Stage)		(g/d)		(μ g/d)	(mg/d)	(mg/d) ^{**}	(mg/d)	(μ g/d)	(mg/d)
9 - 13 y	ND	2.5	ND	5,000	40	350	6	280	23
14 - 18 y	ND	2.5	ND	8,000	45	350	9	400	34
19 - 70 y	ND	2.5	ND	10,000	45	350	11	400	40
>70 y	ND	2.5	ND	10,000	45	350	11	400	40

^{*}UL = Maximum level of daily nutrient intake that is likely to pose no risk of adverse effects.

^{**}Represent intake from a pharmacological agent only. ND = Not determinable.

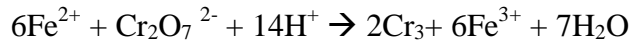
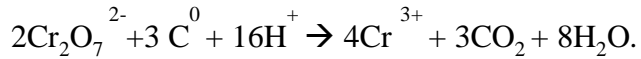
2.10 Methods

2.10.1 Walkley Black Method for Determination of SOM

This is the standard wet chemistry technique which involves the rapid dichromate oxidation of organic matter. The Walkley–Black method is perhaps the best known of the rapid dichromate oxidation methods (Diaz-Zorita, 1999). In this procedure, potassium dichromate ($K_2Cr_2O_7$) and concentrated H_2SO_4 are added to between 0.5 g and 1.0 g of soil or sediment. These amounts can vary depending on the organic content. An exothermic reaction occurs when potassium dichromate and sulfuric acids are mixed. The solution must be swirled and allowed to cool prior to adding water to halt the reaction. The addition of H_3PO_4 to the digestive mix after the sample has cooled has been used to help eliminate interferences from the ferric ion (Fe^{3+}) that may be present in the sample although in most cases, this step is not

necessary (Tiessen and Moir, 1993). Excess dichromate ions are then back titrated with ferrous iron.

The chemistry of this digestion procedure is as follows:



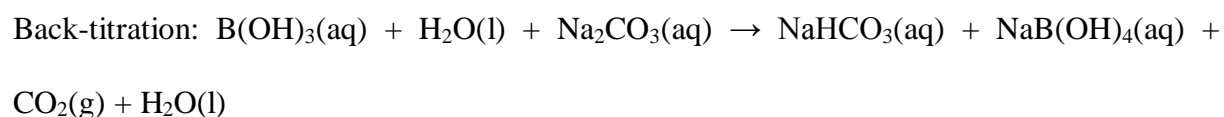
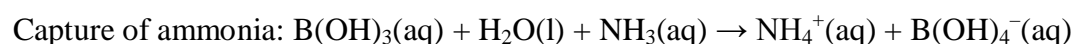
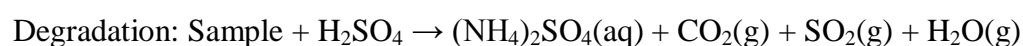
The Walkley-Black procedure is widely used because it is simple, rapid, and has minimal equipment needs (Nelson and Sommers, 1996).

2.10.2 Chapman Method for Determination of CEC

This method involves saturation of the cation exchange sites with ammonium acetate and removal of the excess ammonium ions with ethanol, replacement and leaching of exchangeable ammonium ion with protons from HCl acid (Horneck, et al., 1989). This method may be poorly suited to soils containing carbonates, vermiculite, gypsum and zeolite minerals. The pH 7.0 ammonium acetate CEC method has been widely used in the U.S. for decades (Rhoades, 1982). Consequently, a large data base exists for soil CEC by this method. Many state agencies have traditionally required CEC to be measured by this procedure (Rhoades, 1982). A significant disadvantage of this method is that it is more time-consuming than effective but can be readily adapted by most soil testing laboratories (Sumner et al., 1996). The main problem with this method is that it buffers soil at pH 7.0 causing large overestimates of CEC for highly acidic soils (Sumner et al., 1996).

2.10.3 The Kjeldahl Method for the Determination of Protein

This method is the standard method for the determination of nitrogen which dates back to its development in the late 1800's (Bremner, 1960). The Kjeldahl method is a wet oxidation method using concentrated sulfuric acid. The method consists of three basic steps: 1) digestion of the sample in H₂SO₄ with a catalyst, which results in conversion of nitrogen to ammonia; 2) distillation of the ammonia into a trapping solution; and 3) quantification of the ammonia by titration with a standard solution (Skoog et al. 1992). The general chemical reactions for the process are as follows:



Nowadays, the Kjeldahl method is largely automated and makes use of specific catalysts (mercury oxide or copper sulfate) to speed up the decomposition.

2.11 Instrumentation

2.11.1 Gas Chromatography (GC)

The two most common approaches for separation of analyte from other compounds in a sample are gas chromatography (GC) and high performance liquid chromatography (HPLC). The choice of which technique is employed is largely dependent on the analyte of interest.

Separation in GC is based on the vapour pressures of volatized compounds and their affinities for the liquid stationary phase, which coats a solid support, as they pass down the column in a carrier gas (Dean, 2003). A gas chromatograph consists of a column, typically 15-30 m long, with an internal diameter of 0.1-0.3 mm (Dean, 2003). There are many different types of columns that are available from manufacturers. GC is usually used for the routine fatty acid analysis (Metcalf and Scmitz, 1961). The formation of methyl esters is necessary prior to GC analysis in order to introduce the analyte in a more volatile form. A derivitisation step is therefore incorporated into the procedure to form the volatile fatty acid methyl esters (FAMES) which is then quantified by GC analysis (Metcalf and Scmitz, 1961).

2.11.2 Microwave Digestion

In this study, microwave-assisted digestion was utilised. This method allows for the rapid dissolution of the sample matrix, requires low volume of oxidizing reagent and with closed vessels, it allows for minimal contamination of the sample (Fig.8). The samples were subjected to microwave digestion using a CEM MARS Xpress closed vessel microwave digestion system. The maximum temperature was 260°C and maximum pressure was 75 bar.

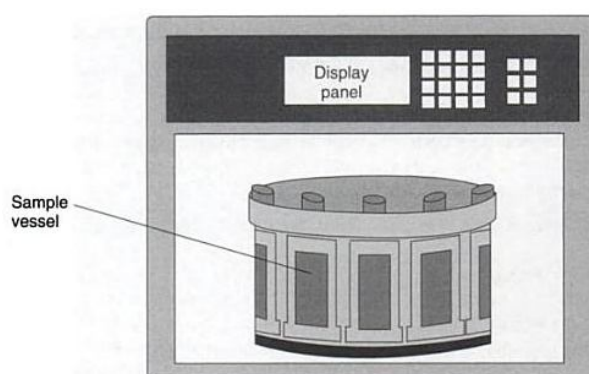


Fig.8 Schematic of pressurized microwave digestion system

Sourced from: Herbert and Hashemi, 2008

The two main systems that are used for decomposing samples are open and closed vessels. Microwave digestion (closed vessel) is preferred method for decomposing both organic and inorganic samples over directly heating on a hotplate. Microwaves come in different forms but their principal is common. In the last 30 years the use of microwaves increased significantly and most laboratories prefer this method. Microwave digestion may use sealed bombs that allow complete digestion of both organic and inorganic samples in TFM reaction vessels that operate at high temperatures and high pressures. Some of the advantages of this type of digestion are:

- Fast decomposition times - typically it may take 10-30 min to digest a sample in a microwave compared to the open vessel.
- Closed vessels prevent the loss of volatile compounds.
- Contamination is not possible since nothing can come in and out of the system.
- High temperatures allow complete digestion; therefore high precision is possible with replicate analyses.
- Less expensive chemicals are needed—HNO₃ is good enough for digestion.
- Smaller sample sizes can be analysed.

2.11.3 Hydride Generation Atomic Absorption Spectroscopy (HG-AAS)

A specialized form of atomization cell is available for a limited number of elements that are capable of forming volatile hydrides. These elements include: As, Bi, Sb, Se and Sn. In this situation, an acidified sample is reacted with a sodium tetraborohydride solution. After a short time, the gaseous hydride is liberated. The hydride is transported to the atomization cell

by means of a carrier gas. The quartz tube representing the atomization chamber can either be electrically heated or flame heated. Hydride generation atomic absorption spectrometry (HG-AAS) offers excellent promise in terms of sensitivity (0.02-2 ng), minimal matrix interferences and relatively inexpensive equipment requirements (Tingii et al., 1992; Verber et al., 1994). The most common of the HG AAS methods involves reduction of Se by sodium borohydride followed by introduction of the hydride by means of a carrier gas (nitrogen) directly into an air-entrained nitrogen-hydrogen flame (Jackson & Qiao, 1992).

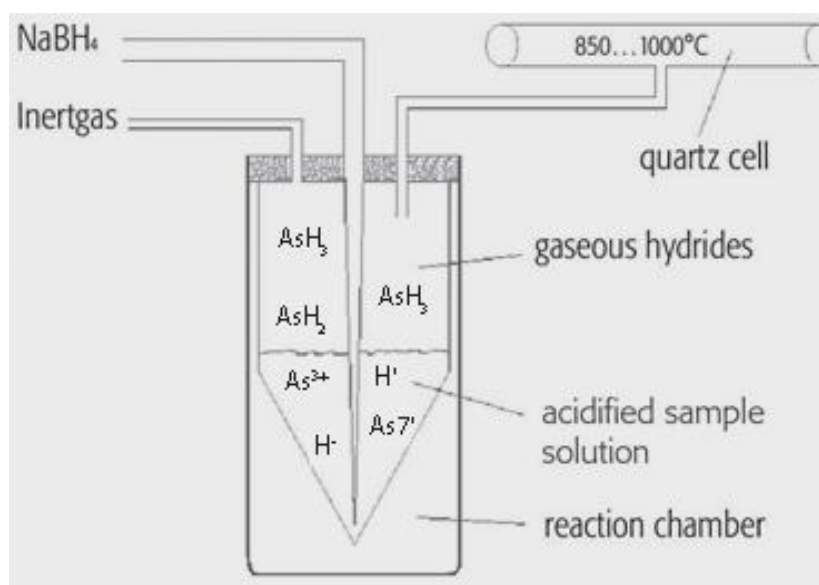


Fig.9 Principle of the hydride generation technique

Sourced from: www.Analytik Jena AG Principle.htm

2.11.4 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

The features common to the ICP-OES methodology include sample preparation, sample introduction, instrument calibration and wavelength selection which will be reviewed. ICP-OES found widespread use in laboratories from 1980s (Chunillal, 2003). This is a type of emission spectroscopy based on using plasma to excite atoms and ions which emit electromagnetic radiation at various wavelengths depending on the element. The instrument

can either be simultaneous or sequential. The principle of simultaneous multi-element instruments is based on different methods of detecting multiple signals arising from a dispersed beam while sequential instruments is based on having one detector that uses a high speed precise monochromator. This "scans" through the emission spectrum and measures the intensity of each line sequentially.

Most samples analysed using ICP-OES enter as liquids but they are nebulized into an aerosol, in order to be introduced into the ICP-OES for analysis. A conducting gaseous mixture that is highly energetic to excite elements into atoms (cation or anions) is called a plasma. The most common gas used in ICP-OES is argon; argon ions and electrons form the principal conducting species. Argon ions, once formed in the plasma are capable of absorbing sufficient power from an external source to maintain the temperature at a level which sustains the plasma. The plasma removes all the water that might still be present in the sample as solvent. The spray particles are then desolvated, vaporised and atomised, excited and ionised (Fig. 10). Once the atoms and ions are formed they emit energy in the form of electromagnetic radiation which is discriminated by a device called a monochromator. The radiation detected is converted into electronic signals with peak intensities that are then converted into concentration information for the analyst.

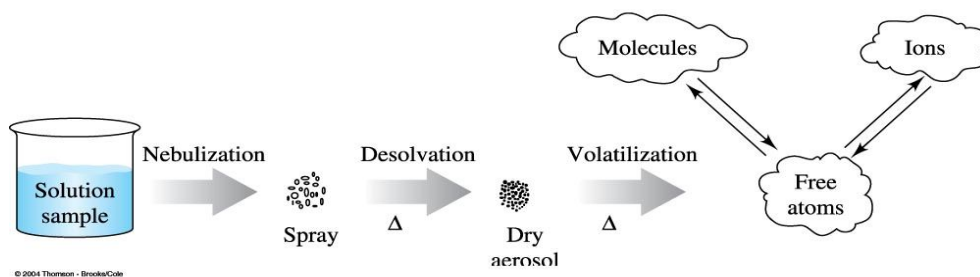


Fig.10. Atomization stages before detection

Sourced from: Boss et al., 1997

2.11.4. 1 Advantages of ICP–OES

- Atomisation occurs in a chemically inert environment therefore there are less chemical effects.
- No chemical interferences.
- Uniform temperature in the plasma, hence calibrations tend to be linear over several orders of magnitude of concentrations.
- By monitoring several wavelengths a wide range of elements can be detected including non-metals, can be detected simultaneously.
- No source lamps required unlike other emission techniques.

2.11.4.2 Limitations and Interferences

ICP-OES is a moderately sensitive technique that can analyse a wide range of elements simultaneously. Under optimum conditions it can analyse over 100 samples per day. It is important, however, to be aware of the limitations of the method. These include:

- Spectral interferences between different elements. The wavelength of one element's light emission can sometimes be close enough to that of another element to cause problems.
- Matrix effects caused by high concentrations of an element in the sample, (most commonly the easily ionisable Na, K, Mg or Ca) can change the way the sample is introduced to the plasma or its thermal characteristics and lead to over or underestimation of sample concentration.

- Optimum conditions for analysis occur for different elements under different conditions; therefore sensitivity can be compromised when running multi-element analysis.
- The high temperature used in ICP pose negative effects since the plasma is so effective in generating excited states that it produces rich emission spectra which results in interferences. Some of these interferences could be matrix, physical, chemical and spectral interferences.

2.11.4.3 Matrix Interferences

Matrix interferences are normally due to differences in viscosity, surface tension and the sample content dissolved in a solvent. However, these interferences could be corrected by using a technique called matrix matching (Boss et al., 1997). Here, solvents and concentration of acid is matched with standards and samples. In addition, the blank should also be matched with the standards used for the analysis. Standard addition or the use of an internal standard can be used if the standards do not match the samples.

2.11.4.4 Physical and Chemical Interferences

Both physical and chemical interferences are not present in ICP. Chemical interferences are eliminated by the high operating temperature of the argon plasma since it promotes breakage of chemical bonds (Boss et al., 1997).

2.11.4.5 Spectral Interferences

This is the most common form of interference that is normally encountered in ICP. This generally describes the part of that emission spectrum line that is not to be measured or observed (Koirtiyohann et al., 1981). Spectral interferences can arise from the following factors:

- Direct spectral overlap of another atomic line on the atomic line being analysed (wavelengths are too close).
- Continuous background resulting from the plasma which superimposes on the emission line of the analytical line.
- Unresolved overlap of molecular band spectra.

One way of minimizing this interference may be to carefully select wavelengths since there is the flexibility to choose from many possible emission lines or use of high resolution spectrometers and advanced background correction techniques. The correction can be done by subtracting the background i.e. finding a spectral location as close as possible to the line used. The blank signal is compared with the background measured on both sides of the analyte then the difference is subtracted. Improving the performance of the spectrometer can also decrease the intensity of the spectral background.

2.11.4.6 Limit of Detection (LOD)

The lowest concentration of analyte detectable or quantifiable with a stated degree of reliability is referred to as the limit of detection (LOD) (Arinbruster et al., 1994). The

determination of the LOD can be done using a statistical or empirical approach (Arinbruster et al., 1994). Statistically, the determination involves measuring a series of blank samples (a sample containing no analyte but has an identical matrix to the average sample being analysed) and calculating the mean and standard deviation. The LOD is then calculated as the value of the mean blank plus the value of three times the standard deviation (Arinbruster et al., 1994). The LOD should be statistically distinguishable from the blank approximately 95 to 99% of the time (Arinbruster et al., 1994). The empirical approach consists of analysing a series of samples with decreasing concentrations of the analyte. The lowest detectable concentration which is still able to satisfy predetermined acceptance criteria is taken as the LOD (Arinbruster et al., 1994).

Normally in ICP limit of detection ranges are in $\mu\text{g L}^{-1}$ (ppb). If the element has a concentration less than the LOD of the instrument it cannot be detected. A good example of this is As which is generally found in lower concentrations and cannot be detected in ICP. Another sophisticated instrument can then be used for the analysis or the sample can be pre-concentrated.

CHAPTER 3

EXPERIMENTAL

The proper execution of preliminary steps of the analytical process is vital in achieving meaningful results that can be used to develop or test a hypothesis. In analytical chemistry, four major steps is required to be undertaken. These include sample collection, sample preparation, sample analysis and the interpretation of data. The following chapter outlines procedures employed in sampling and instruments used for the analysis of samples.

3.1 Sampling

3.1.1 Avocados used for Oil Extraction Study

Fruit samples (Fuerte and Hass variety) were collected from Westfalia Organic Avocado Farm on the Everdon Estate, located five kilometres from Howick, in the KwaZulu-Natal Midlands, South Africa. The fruit was ripened, skinned, deseeded and dried in an oven at 45°C to constant mass. Dried fruit was milled in a food processor and the powder was stored in plastic bags in a refrigerator at 4°C until analysed.

3.2 Sampling Sites for Plant-Soil Study

The avocado fruit and soil samples used for the plant-soil study were collected from six different sites in KwaZulu-Natal represented in Fig.11. The chosen sites were: Site A-Kranskop, Site B-Seven Oaks, Site C-Howick, Site D-Thornville, Site E-Richmond and Site F-Ixopo. The geographical coordinates (decimal degrees) for the six sampling sites are

presented in Table 5. Phosphoric acid is injected into stems of trees which is generally done to prevent root rot. Copper based fungicides are sprayed on leaves when needed. Leaves are treated with B, Ca and Zn sprays routinely. Calcium may also be added in the form of lime application. Information on levels of added nutrition for avocado at the sites was not available. Landscape description of sites ranged from flat to varying degrees of slopes. The climate is humid and subtropical. Crops at most sites were frequently irrigated. Soils were generally sandy or loamy sand in texture. Two avocado varieties, Hass and Fuerte, and accompanying soil samples were obtained at each site from multiple (12-15) random locations within the site. The fruit were picked based on the size recommendations made by farm managers present at each site. Sampling of fruit growing in close proximity to large trees (strategically planted to serve as wind breakers) was avoided to ensure uniformity.

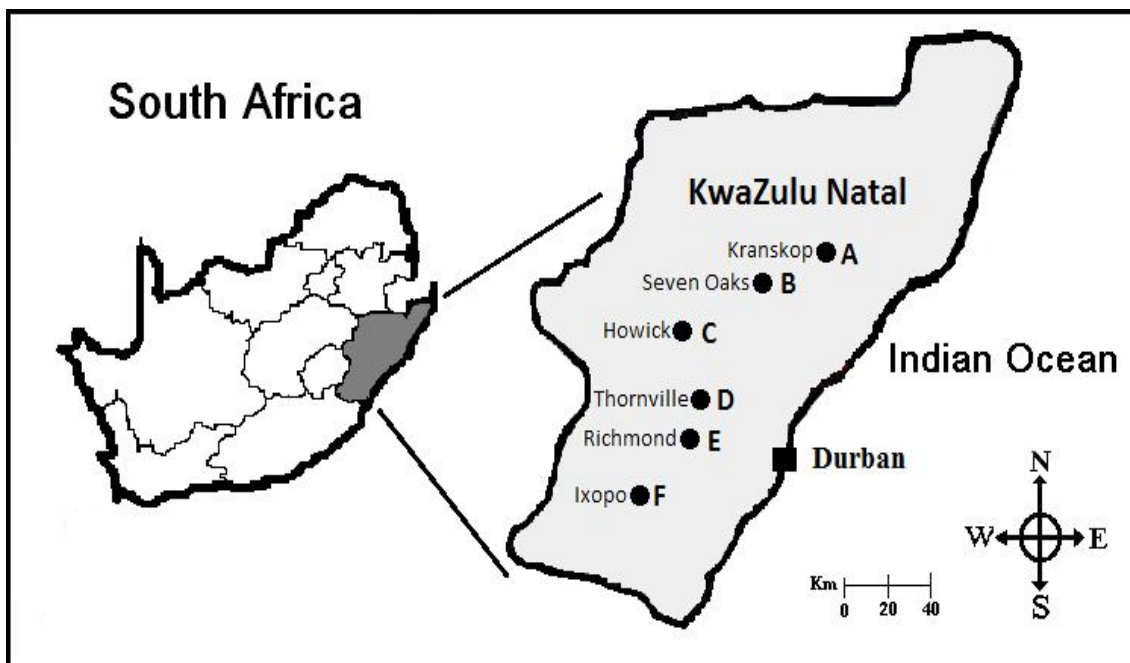


Figure 11: Map of selected sampling sites in KwaZulu-Natal.

Table 5. Geographical Coordinates, in Decimal Degrees, for the 6 chosen sites.

<i>Site Name</i>	<i>Location</i>	<i>Latitude</i>	<i>Longitude</i>
A	Kranskop	-28.96712	30.8641
B	SevenOaks	-29.209957	30.59473
C	Howick	-29.48252	30.23391
D	Thornville	-29.73653	30.38583
E	Richmond	-29.75002	30.38129
F	Ixopo	-30.15418	30.05425

3.3 Sample Preparation

The fruit was ripened for seven days before processing. Fruit were skinned, deseeded and dried at 45°C to constant mass. Dried fruit were milled into a powder by a food processor (Braun range). Processed samples were stored in the refrigerator at 4°C until analysed. Soil was collected from the drip line of the tree from a plough depth of 0.5-1.0 m in accordance with the root depth of the tree. The soil was gently crushed with a mortar and pestle, passed through a 75 µm sieve, stored in plastic zip lock bags and kept at 4°C until analysed.

3.4 Extractions

Five extraction techniques were used to obtain avocado oils that were subjected to fatty acid and metal analysis. The results were compared to evaluate the efficiency of the extraction methods.

3.4.1 Hexane Extraction

Avocado oil was extracted using the traditional exhaustive Soxhlet extraction method. A cellulose thimble containing 5.0 g dried sample was placed in the Soxhlet device and extracted with 250 mL hexane for 24 h; the extractor siphoned every 15 min. The flask was removed and the solvent was evaporated using a rotary evaporator.

3.4.2 Ultrasound Extraction

Approximately 5.0 g of dried avocado samples were sonicated in a water bath at 60°C with 10 mL of hexane as solvent for 1 h. The resultant mixture was filtered by suction and filtrate evaporated using a rotary evaporator.

3.4.3 Ultra-Turrax Treatment-Hexane Combined Extraction

Approximately 10.0 g of dried avocado samples containing 10 mL of hexane in glass bottles were treated with an Ultra-turrax tool (cell lysing apparatus) for 10 min. The slurry was dried to constant mass, of which 5.0 g was extracted with hexane by traditional Soxhlet extraction for 24 h.

3.4.4 Microwave Assisted-Hexane Combined Extraction

Freshly peeled avocados, pressed into a smooth paste was spread uniformly (5 mm thickness) on the rotary plate of a domestic microwave oven (Braun, 1000W, 2450 MHz) and heated at maximum power for 11 min. The resulting mass was ground to a fine powder, of which 5.0 g was extracted with hexane by traditional Soxhlet extraction for 24 h.

3.4.5 Supercritical Fluid Extraction

A home-built extractor, Fig.12 (Botha, 2002) was used to extract oil from avocado samples employing supercritical Ar and CO₂ as the extraction fluids. Approximately 5.0 g of dried avocado was accurately weighed and loaded into the 10.0 g capacity extraction cell. A small piece of cotton wool was lodged at both ends of the extraction cell to take up dead volume and avoid plant material blocking the entry and exit ports. All extractions were performed for 2 h with a fluid flow rate of 2.8-3.5 mL/min. Extracts were collected in glass screw cap collection vessels without solvent and dried in an oven at 150°C to constant mass. Prior to analysis the extracted oil was subjected to vacuum evaporation for 30 min to remove any water and dissolved CO₂.

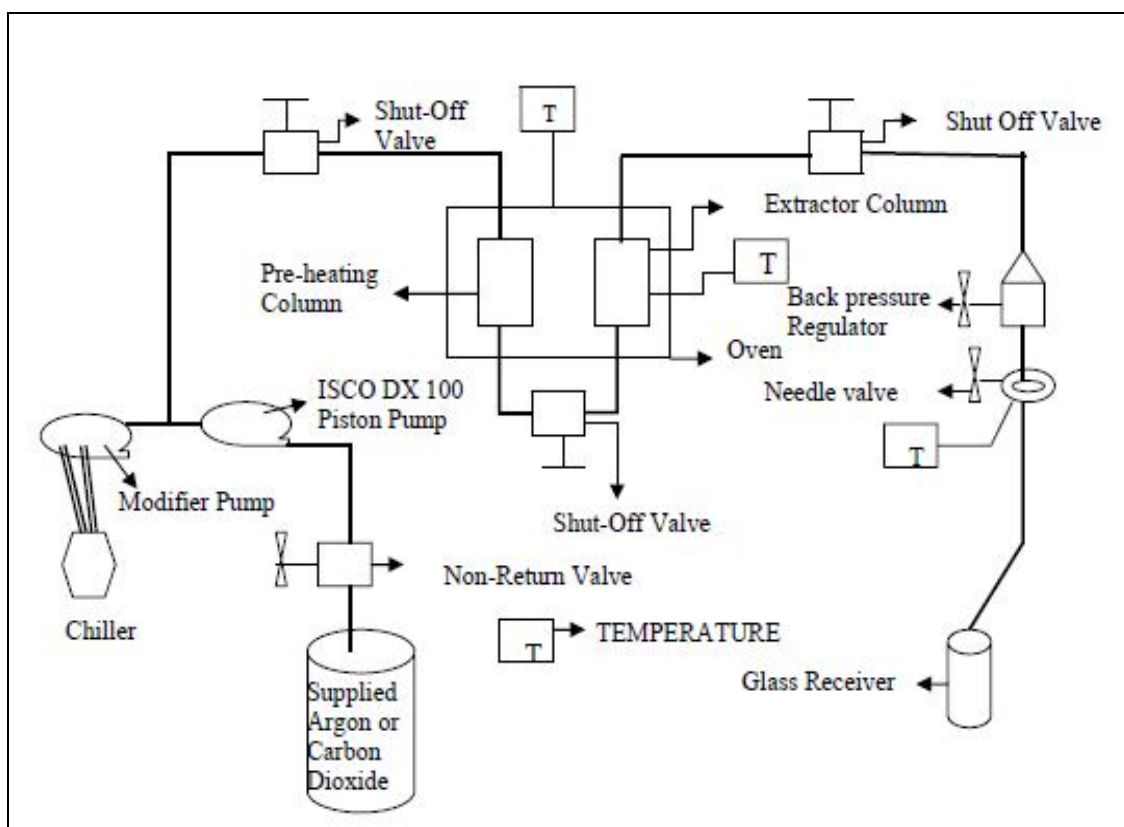


Fig.12 Schematic of the constructed supercritical fluid extractor

Sourced from: Botha, 2002

3.5 Instrumentation for Oil Extraction Study

Microwave digestion was the preferred method of digestion because it provided higher accuracy with respect to both time and recovery values. Elemental determination was by ICP-OES. Method validation was accomplished using certified reference material (CRM) *lyophilized brown bread* (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities (Appendix 1). GC, performed on a 6820 GC system (Agilent Technologies) with DB-wax fused silica capillary column (30 m x 0.25 mm i.d., 0.25 mm film thickness), was used for fatty acid analysis. The injector and flame ionization detector were set at 250°C. The column temperature program was started from 150°C where it was held for 1 min, then ramped to 200°C at 25°C/min where it was held for another 3 min. The final temperature was increased to 230°C at a rate of 15°C/min where it was held for 5 min. The pressure of N₂ carrier gas was set at 100 kPa. Supercritical extractions were done using an apparatus that was constructed in-house. The specifications of the apparatus and all accompanying parts are as described by Botha (Botha, 2002).

3.6 Metal Determination in Extracted Oils

A mass of 0.5 g of dried avocado sample, extracted oil and CRM was placed in separate Perfluoroalkoxy (PFA) vessels. To each vessel, 10.0 mL of 69% HNO₃ was added, swirled gently and left to stand for 1 min before sealing. The samples were subjected to microwave digestion using a CEM MARS Xpress closed vessel microwave digestion system. For digestion, the temperature was ramped to 200°C over 15 min where it was held for 15 min (Appendix 2). The digests obtained were filtered by gravity into 50 mL volumetric flasks and filled to the mark with double distilled water. This was transferred into plastic bottles and stored at 4°C in a refrigerator for elemental analysis. All analyses were done in triplicate.

The element dissolution in oil was calculated as follows:

$$\text{Dissolution (w/w \%)} = (O_c * O_y / M_w * M_c) * 100$$

Where O_c is the element concentration in the oil ($\mu\text{g g}^{-1}$)

O_y is the oil yield from 100 g of dried avocado mesocarp (g)

M_w is the mesocarp weight (100 g)

M_c is the element concentration in the mesocarp ($\mu\text{g g}^{-1}$)

3.7 Fatty Acid (FA) Analysis of Extracted Oils

Derivatization of the FAs into fatty acid methyl esters (FAMES) was done according to Kanchanamayoon, with some modifications (Kanchanamayoon and Kanenil, 2007). Approximately 0.5 g of avocado extract was accurately weighed into PTFE lined screw-cap bottles. 2.0 mL of 1mg/mL internal standard (prepared by dissolving 100 mg of pentadecanoic acid in toluene in a 100 mL volumetric flask) and 3.0 mL of 10% methanolic HCl (prepared by slow addition of 10.0 mL conc. HCl to 90.0 mL dry methanol with constant stirring), were added to extract, sealed and placed in a hot water bath (70°C) for 2 h. Thereafter 5.0 mL of 6% K_2CO_3 solution and 1 mL of toluene were added and vortexed for 1 min. The organic phase was separated from the aqueous phase after centrifugation at 1100 $\text{m}\cdot\text{s}^{-2}$ for 5 min. The organic phase was dried with a small amount of anhydrous Na_2SO_4 and filtered using Millipore 0.45 μm filters. An aliquot was injected (0.1 μl) into the GC. FAs were identified using individually run FA standards (Appendix 3). The internal standard was used to correct for variations in injected sample amounts as well as to validate the method (Bruckner et al., 1998). Peak areas were used to measure relative concentration of the fatty acids which can be compared to other similar studies (Szentmihályi et al., 2002).

3.8 Proximate Chemical Composition of Avocados

The lipid content was determined as per a previously described method (Kannamkumarath et al., 2002). The ash content was determined by incineration of known masses of the defatted fruit samples in a muffle furnace at 600°C for 6 h. Nitrogen in the defatted fruit samples was determined by the Kjeldahl Method (Section 2.10.3). The nitrogen value obtained was multiplied by a conversion factor and reported as mass of protein in sample. The conversion factor for vegetable protein is 6.25 (Fujihara et al., 2001). The available carbohydrate was obtained by difference. This was done by subtracting the amount of oil, ash and protein from the total dry matter (Özcan et al., 2007).

3.9 Statistical Data for Oil Extraction Study

Data generated from the FA analysis was subjected to ANOVA and Duncan's multiple range tests using the SAS program (Version 6.12, SAS Institute Inc., Cary, NC, USA). The analysis was performed to determine the significance of the extraction methods in relation to oil yield, FA content and metal extractability.

3.10 Instrumentation used for Plant-Soil Study

Microwave digestion was used in the preparation of both soil and avocado samples before analysis. The determination of the elements was done using a Perkin-Elmer ICP-OES (model Optima 5300 DV, Perkin Elmer, Shelton, Conn.), as well as a Perkin-Elmer Analyst 100 Cold Vapour Atomic Absorption Spectroscopy Hydride Generator (Perkin Elmer, AAnalyst 200, Life and Analytical Science (PTY) Ltd) for the determination of As and Se.

3.11 Certified Reference Material (CRM) Analysis

Accuracy of elemental determination was tested by analysis of a CRM, *lyophilized brown bread* (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities which was chosen due to matrix similarities (Appendix 1). Analysis of the CRM was to ensure that digestion was complete, instrument parameters were optimized and calibration errors removed (Skoog et al., 1992).

3.12 Total Metal Determination in Soil, Fruit and CRM

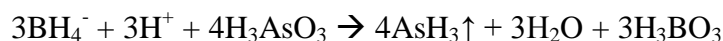
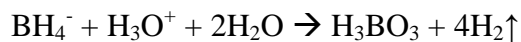
A mass of 0.5 g of dried sample (fruit or soil) was placed in separate Perfluoroalkoxy (PFA) vessels. To each vessel, a volume of 10 mL of 70% HNO₃ was added, swirled gently and left to stand for a minute before sealing each vessel. The samples were then subjected to microwave digestion using a CEM MARS Easyprep closed vessel microwave digestion system. The setting used for the heating programme was as follows: the temperature was ramped to 200°C for 15 min, and then held at 200°C for a further 15 min (Appendix 2). The digests obtained were filtered by gravity into 50 mL volumetric flasks and filled to the mark with double distilled water. This was then transferred immediately into plastic bottles and stored at 4°C until analysed by ICP–OES using axial plasma observation, less than a week later. The following elements were determined in triplicate: Al, Ca, Cd, Co, Cu, Cr, Fe, Mg, Mn, Ni, Pb and Zn, with the exception of As and Se which were analyzed using HGAAS by standard methods. ICP standards were from Merck. All working standards were made up with double-distilled water and 70% HNO₃ to match the matrix of digested samples. Emission lines were chosen based on maximum analytical performance and minimum spectral interference; lines outside the linear working range were omitted. Table 6 shows the emission lines that were selected.

Table 6. Emission lines (wavelengths) selected for each element

Element	Wavelength (nm)
Al	394.401
As	197.197
Ca	315.887
Cd	226.502
Co	228.616
Cr	283.563
Cu	324.752
Fe	259.939
Mg	280.271
Mn	259.372
Ni	231.604
Pb	217.000
Se	203.985
Zn	213.857

3.13 Determination of As and Se

HGAAS was employed for the determination of As and Se. The following equations show the reaction of NaBH₄ in acidic solution and the reduction of the hydride-forming element:



Preparation of solutions and procedure is outlined as follows:

Solutions

0.15 mol L⁻¹ (~ 1.5% V/V) Hydrochloric acid: 15 mL of concentrated HCl was carefully added to double distilled water and made up to 1 L.

0.25 mol L⁻¹ (~ 1% W/V) Sodium hydroxide solutions: 10 g NaOH pellets were dissolved in double distilled water and made up to 1 L.

0.8 mol L⁻¹ (~ 3% W/V) Sodium tetrahydroborate solution: 3 g of NaBH₄ was dissolved in 1% NaOH solution and made up to 100 mL with 1% NaOH solution.

Arsenic stock solution (1000 mg L⁻¹): 1.3203 g As₂O₃ was dissolved in a minimum volume of 20% NaOH and neutralized with HNO₃. The resulting solution was then diluted to 1 L to give 1000 mg L⁻¹ As.

Working solution (1 mg L⁻¹): 1 mg As stock solution was diluted to 1 L with 1.5% HCl.

Aliquots for calibration: 10, 25, 50 μL of working solution was diluted to 10 mL with 1.5% HCl.

Pre-reduction solution: 3 g KI and 5 g L (+)-ascorbic acid was dissolved in 100 mL of double distilled water.

Procedure

1. 1 mL of the pre-reduction solution was added to 10 mL of sample and allowed to stand for 30 min (Welz, 1993).
2. The calibration solutions were run to establish a calibration plot of either absorbance vs. mass or absorbance vs. concentration.
3. The sample solutions were then run, while making sure to properly clean the sample reagent bottle with 1.5% HCl after each determination.

Similarly determination of Se was done using the same reductant solutions but calibrating with Se stock solutions. A pre-reduction step was performed by heating with 5 mol L⁻¹ HCl for 15 min under reflux (Bye and Lund, 1988).

3.14 Bioavailability Determinations

For soil analysis both total and bioavailable concentrations of elements were determined. For determination of exchangeable metals in soil, a single extraction procedure was performed using an extractant solution containing ammonium acetate (1.0 M), EDTA, (0.05 M) and acetic acid (0.43 M) (Beckett, 1989; Dean, 2005). An extractant solution was prepared by diluting 38.542 g NH₄CO₂CH₃ (0.5 M), 25 mL CH₃COOH (96%) and 37.225 g EDTA (0.1 M) to 1 L. Approximately 1.0 g of dry soil samples were accurately weighed into plastic bottles and 10 mL of extractant solution added to each bottle. Bottles were shaken for 1 h on an orbital shaker at 30 m s⁻² and resulting mixtures centrifuged for 10 min at 600 m s⁻². The resulting mixtures were filtered through Whatman No. 41 filter paper by gravity into 50 mL volumetric flasks and filled to the mark with double distilled water. This was then transferred immediately into plastic bottles and stored at 4°C until analysed by ICP–OES.

3.15 Bioaccumulation Factors (BFs)

The magnitude of the bioavailable or exchangeable fraction relative to total soil concentration for each element is given by the ratio $([Soil]_{Ex} / [Soil]_T) \times 100$. BFs were calculated by computing the ratio of the metal content in plant and the total/exchangeable metal content in soil which indicates accumulation of the element if > 1 and exclusion if < 1 (Timperley et al., 1973). A plot of BFs versus Total/exchangeable soil concentration can give an indication of whether the element is essential or non essential to the plant. Essentiality is indicated by a rectangular hyperbola whereas non-essentiality is indicated by a linear plot parallel to the x-axis (Timperley et al., 1973).

3.16 Soil Quality Assessment

3.16.1 Soil pH, Soil Organic Matter (SOM) and Cation Exchange Capacity (CEC)

Soil pH was determined. SOM was estimated by a wet chemistry extraction technique (Walkley and Black, 1934). The CEC of each soil sample was determined at pH 7 with ammonium acetate by the Chapman method (Chapman, 1965). Determination of the concentration of NH_4-N in the KCl extract was done by distillation using the Kjeldahl method (Skoog et al., 1992). All determinations were done in triplicate. All three procedures are outlined in detail below:

3.16.1.1 Soil pH

Soil pH was determined by using a 2:1 soil water suspension then determining the pH values from a calibrated pH meter. The pH meter was calibrated using a pH 4 and pH 7 buffer system. The measurements were done as follows: the pH electrode was immersed in the suspension for a few minutes then the reading was taken. Each soil sample was analysed in triplicate (n= 3).

3.16.1.2 Walkley-Black Method for the Determination of SOM

In this reaction carbon is oxidized by the dichromate ion. Excess dichromate ion is then back titrated with ferrous ion.

Solutions

1. **Potassium Dichromate (1 M):** A mass of 49.04 g potassium dichromate ($K_2Cr_2O_7$) was weighed into a 1L volumetric flask. This was dissolved and diluted to volume with deionized water.

2. **Ferrous Ammonium Sulfate (0.5 M):** A volume of 20 mL H_2SO_4 was slowly added to a 1 L volumetric flask containing 800 L of deionized water. Thereafter, 196.1 g of ferrous ammonium sulphate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) was added, dissolved and diluted to volume with deionized water.

3. **Diphenylamine Indicator:** A mass of 0.5 g of diphenylamine ($C_6H_5NHC_6H_5$) was dissolved in 20 mL deionized water. Thereafter, 100 mL of concentrated H_2SO_4 was slowly

added. This was carefully mixed with a glass stirring rod as the solution is corrosive and can cause severe burns.

Procedure

1. A mass of 1.0 g of soil was weighed and passed through a 0.5 mm mesh sieve into a 500 mL Erlenmeyer flask.
2. Approximately 10 mL of 1 M potassium dichromate solution was added to the soil sample.
3. About 20 mL of concentrated H₂SO₄ was added and mixed by gentle rotation for 1 min, taking care not to throw soil up onto the sides of the flask. This was allowed to stand for 30 min thereafter diluted to 200 mL with deionized water.
4. A volume of 10 mL of concentrated phosphoric acid (H₃PO₄), 0.2 g sodium fluoride (NaF) and 10 drops diphenylamine indicator were added to the solution.
5. The contents in the flask were then titrated with 0.5 M ferrous ammonium sulfate solution until the colour changed from dull green to a turbid blue. The titrating solution was added dropwise until the end point was reached when the colour shifted to a brilliant green.
6. The blank was prepared in the same manner and titrated.

Calculation

$$\% \text{ Organic Matter} = 10[1(S \div B)] \times 0.67$$

Where: B = Titration of blank (mL); S = Titration of sample (mL).

3.16.1.3 Determination of CEC at pH 7 with Ammonium Acetate by Chapman

In this reaction, the normal mixture of cations on the soil exchange sites is replaced with a single cation such as ammonium (NH₄⁺). Exchangeable NH₄⁺ is then replaced with another

cation and the amount of NH_4^+ exchanged is measured (which was how much the soil had held).

Solutions

1. Ammonium acetate ($\text{CH}_3\text{COONH}_4$) solution (1 M): A volume of 57 mL glacial acetic acid (99.5%) was diluted with ~800 mL of distilled H_2O in a 1 L volumetric flask. To this solution, 68 mL of concentrated NH_4OH was mixed and cooled. The pH was adjusted to 7.0 with NH_4OH when needed and diluted to 1 L.

2. KCl replacing solution (1 M): A mass of 74.5 g KCl was completely dissolved in distilled water and diluted to a final volume of 1 L.

Procedure

1. Approximately 25 g of soil was added to a 500 mL Erlenmeyer flask.
2. About 125 mL of the 1 M $\text{CH}_3\text{COONH}_4$ was added to the flask, shaken thoroughly, and allowed to stand overnight.
3. A 5.5 cm Buchner funnel was fitted with retentive filter paper that was moistened before light suction was applied and soil was transferred. If the filtrate was not clear, the solution was re-filtered.
4. The soil was gently washed four times with 25 mL additions of the $\text{CH}_3\text{COONH}_4$, allowing each addition to filter through but not allowing the soil to crack or dry. Suction was applied only as needed to ensure slow filtering. The leachate was then discarded.
5. The soil was then washed with eight separate additions of 95% ethanol to remove excess saturating solution. Only enough was added to cover the soil surface, and each addition was allowed to filter through before more was added. The leachate was discarded and the receiving flask was cleaned thoroughly.

6. The adsorbed NH_4^+ was extracted by leaching the soil with eight separate 25 mL additions of 1 M KCl, leaching slowly and completely as above. The soil was then discarded and leachate transferred to a 250 mL Erlenmeyer flask.

7. The concentration of $\text{NH}_4\text{-N}$ in the KCl extract was determined by distillation procedure using the Kjeldahl Method. The $\text{NH}_4\text{-N}$ in the original KCl extracting solution (blank) was determined to adjust for possible $\text{NH}_4\text{-N}$ contamination.

Calculation

$$\text{CEC (meq/100g)} = [(B - S) \times M] \times 100 / [\text{grams of sample}]$$

Where: B = Titration of blank (mL); S = Titration of sample (mL); M = Molarity of standard alkali solution (mol.dm^{-3})

Kjeldahl Distillation Method

Digestion

1. About 25 mL of conc. H_2SO_4 was added to 10 g of powdered K_2SO_4 and a crystal of CuSO_4 (catalyst) was added to 0.5 g of sample in a Kjeldahl flask.
2. The Kjeldahl flask was heated in a heating mantle under the fume hood till the digestion was complete. Complete digestion occurred after 2 to 3 hours, when the solution was colourless or faint yellow.

Distillation of Ammonia

1. The sample was transfer to a 500 mL Kjeldahl flask and enough distilled water was added to give a total volume of 250 mL.

2. Precisely 50 mL of standard 0.1 M HCl was measured into the receiver flask. The flask was clamped so that the tip of the adapter extended just below the surface of the acid. Water was then circulated through the jacket of the condenser.
3. With the Kjeldahl flask tilted, about 85 mL of concentrated NaOH solution, made by dissolving 45 g of NaOH in 75 mL of distilled water, was slowly poured down the side of the container to minimize mixing with the solution in the flask.
4. Several pieces of granulated Zn and a small piece of litmus paper were added. Immediately, the flask was connected to the spray trap. Very cautiously the solution was mixed by gentle swirling. After mixing was complete the litmus paper indicated that the solution was basic.
5. The solution was allowed to boil and distilled at a steady rate until one-third of the original solution remained. The rate of heating was controlled during this period to prevent the receiver acid from being drawn back into the distillation flask.
6. After the distillation was judged complete, the receiver flask was lowered until the tip of the adapter was well clear of the acid. Then heating was discontinued, the apparatus disconnected and condenser was rinsed with small portions of distilled water.
7. The adapter was disconnected and rinsed thoroughly. Two drops of bromocresol green was added and the residual HCl was titrated with standard 0.1 M NaOH to the colour change of the indicator.

Calculation

$$n = 0.1 \text{ M} \times [(50 \text{ mL} - B) / 1000]$$

$$\%N = [(n \times 14.07 \text{ g}) / 0.5 \text{ g}] \times 100$$

$$\% \text{ Protein} = \%N \times \text{conversion factor}$$

Where: B = Titration of base (mL); n = mols of NH₃; %N = % nitrogen

3.16.4 Geoaccumulation Index (I_{geo})

A common approach to estimating enrichment (contamination) of metal concentrations above background/baseline concentrations in soil is to calculate the geoaccumulation index (I_{geo}) as proposed by Muller.

The geoaccumulation index is calculated using the equation below:

$$I_{geo} = \log_2 C_n / 1.5 B_n$$

Where C_n = Total concentration of element in soil sample

B_n = Background/baseline concentration of the same element

The factor 1.5 is to minimise variations in the background value due to lithologic (rock composition) variations (Abraham and Parker, 2008).

3.17 Statistical Data for Plant–Soil Investigation

The significance of plant–soil relationships was established by computing correlation coefficients (r) for the relationships between the concentrations of the elements in the avocado fruit and the total and exchangeable concentrations in the soil. Correlation coefficients were evaluated by Pearson's correlation analysis, using the Statistical Package for the Social Sciences (SPSS) (PASW Statistics, Version 18, IBM Corporation, Cornell, New York). Data generated from analysis were also subjected to one way ANOVA and Duncan's multiple range tests using the SAS program (Version 6.12, SAS Institute Inc., Cary, NC, USA).

CHAPTER 4

FATTY ACID PROFILE AND ELEMENTAL CONTENT OF AVOCADO (PERSEA AMERICANA MILL.) OIL - EFFECT OF EXTRACTION METHODS

4.1 Introduction

The results and discussion in this chapter were done to satisfy a specific objective in this study which is to investigate the yield of oil produced by different extraction techniques on avocado fruit of the Hass and Fuerte varieties. The five extraction techniques include traditional Soxhlet extraction, microwave treatment + Soxhlet extraction, Ultra-turrax treatment + Soxhlet extraction, ultrasound + water bath sonication and supercritical fluid extraction. Avocado is not considered to be a primary source of oil, so few studies have been devoted to its extraction from the pulp.

The combination extraction techniques chosen primarily targeted the lysis of reinforced oil cells of the avocado mesocarp by physical means before the oil was extracted by use of solvents. SFE is a proposed greener option which uses supercritical CO₂ instead of hexane for extraction. The four extraction methods are compared in terms of quality and quantity of oil extracted. This was done by determining the metal content of defatted fruit of both varieties of avocado and corresponding lipid fraction produced by the different extraction techniques to evaluate the efficacy of extraction technique. Fatty acid profiling of extracted oil from both varieties using the different extraction techniques was done to assess for oil quality. Proximate chemical composition namely % oil, % ash, % protein and % carbohydrate in the two avocado varieties were obtained for a holistic depiction of the extracted oils. In this

chapter, all tables contain the mean values with their standard deviations. However, when reproducing these values in the discussion the standard deviations are omitted for fluency.

4.2 Extraction

The oil yields (g oil/100 g dry weight (DW)) obtained from the five extraction methods are represented in Table 7. The extraction conditions of each technique are also given in Table 7. The difference in conditions (solvent, temperature, pressure and extraction duration) for the SFE is worth noting when drawing comparisons among the other techniques. Results from traditional Soxhlet extraction were most reproducible (64.8 g oil/100 g DW and 63.7 g oil/100 g DW, respectively) as seen by the low standard deviation for varieties. The extraction technique with highest yield was by microwave yielding 69.9% oil from Hass variety. Ultrasound extraction gave the lowest yield (54.6 g oil/100 g DW) and least reproducible results which are in agreement with a similar previous study (Szentmihályi et al., 2002). SFE (at highest pressure and lowest temperature) yielded 62.87 g oil/100g DW and 59.6 g oil/100 g DW for Hass and Fuerte varieties, respectively. The extraction yield by Ultra-turrax treatment was similar to tradition Soxhlet extraction (63.4 g oil/100 g DW and 64.0 g oil/100 g DW for Hass and Fuerte varieties, respectively) but failed to be as reproducible. A process such as this needs optimization to obtain reproducibility. Two-factor ANOVA showed a significant difference in yields for the different extraction methods ($P \leq 0.001$) with a significant difference in yields between the two varieties ($P \leq 0.05$).

Table 7: Parameters and oil yields obtained from the different extraction techniques

(g of oil from 100 g dry weight)

Yield (g/100g dry weight)	Soxhlet Extraction		Ultrasound water bath		Ultra-turrax treatment		Microwave Extraction		SFE	
	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte
	64.8 ± 0.24	63.7 ± 0.20	54.6 ± 4.95	58.8 ± 1.56	63.4 ± 0.79	64.0 ± 0.25	69.9 ± 0.39	60.9 ± 2.86	62.9 ± 0.29	59.6 ± 0.36
Parameters										
Solvent	Hexane		Hexane		Hexane		Hexane		CO ₂	
Temp/ ^o C	69		69		75		70		40 - 45	
Pressure	Atmospheric		Atmospheric		Atmospheric		Atmospheric		420-450 bar	
Time / min	160		60		160		160		120	

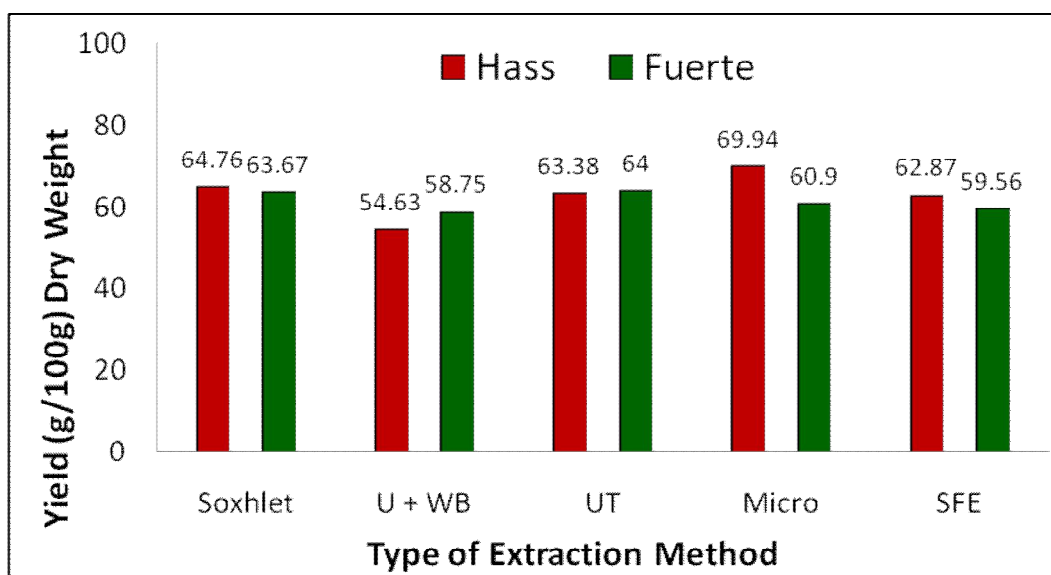


Fig.13 Oil yields obtained from different extraction methods.

Where: Soxhlet: Traditional Soxhlet extraction, U + WB: Ultrasound water bath, UT: Ultra-turrax treated, Micro: Microwave extraction, SFE: Supercritical fluid extraction

4.3 Fatty Acid (FA) Profiling

The FA compositions of oils obtained for the different extraction methods are presented in Table 8. The analysed oil consisted of five different FAs; two unsaturated FAs and three saturated FAs. MUFAs, oleic (C18:1) and palmitoleic (C16:1) acids, are predominant constituents of avocado oils. On average, MUFAs contribute 55-65 % towards the total FA content of the fruit. For saturated fatty acids (SFAs), myristic acid was detected in oils from the Hass variety only by traditional Soxhlet, Ultra-turrax treatment and SFE. Stearic acid (C18:0) was only detected in the Hass variety extracted by SFE. Oils from the Fuerte variety were devoid of myristic and stearic acids. The different extraction methods (Table 8 and Figure 14), showed microwave extraction to produce highest yield of FAs overall, whilst SFE provided a wider range. The fatty acid profiles of Hass and Fuerte varieties were found to be different with Fuerte variety being richer in MUFAs. Hass variety oils were richer in palmitic acid (21.7-25.3%) and palmitoleic acid (13.0-17.9%) compared to Fuerte (15.6-18.0% and 6.23-8.0%, respectively). Fuerte variety had higher concentrations of oleic acid (50.4-60.1%) compared to Hass (< 48.8%). Two-factor ANOVA showed that there is a significant difference in the percentages obtained for the different types of FAs which is dependent on the extraction method. Duncan's multiple range tests showed that in both varieties, SFE produced the lowest percentage of palmitic acid and oleic acid whilst microwave extraction produced the highest percentage of palmitoleic acid. The lower percentages of FAs produced by SFE could be due to short extraction time (2h). SFE is relatively rapid, with > 50% of analyte being extracted in the first 10 min, and 95% being extracted after 100 min (Clifford, 1998). Increasing the extraction time could increase the yield. Increasing the fluid density by increasing the pressure or temperature leads to higher solubility of FAs into the SF and could increase the efficiency of extraction (Eggers, 1996).

Table 8: Fatty acid composition of oils obtained from different extraction methods given in area percentage.

Extraction Methods	C 14:0		C 16:0		C 16: 1		C 18:0		C 18:1	
	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte
Traditional Soxhlet	0.3 ± 0.01	ND	24.3 ± 0.08	18.0 ± 0.18	13 ± 0.17	6.2 ± 0.1	ND	ND	48.8 ± 0.08	59.00 ± 0.6
Ultrasound in water bath	ND	ND	24 ± 0.34	17.6 ± 0.21	13.4 ± 0.16	6.7 ± 0.5	ND	ND	48.6 ± 0.4	58.19 ± 2.1
Ultra- turrax treatment	0.6 ± 0.4	ND	24.2 ± 0.6	17.8 ± 0.50	13.2 ± 0.2	6.2 ± 0.05	ND	ND	47.5 ± 0.4	59.45 ± 1.0
Microwave	ND	ND	25.3 ± 0.1	18.0 ± 1.0	17.9 ± 0.13	8.0 ± 0.3	ND	ND	46.28 ± 0.88	60.14 ± 1.5
Supercritical fluid	1.6 ± 0.02	ND	21.70 ± 2.63	15.60 ± 0.5	13.1 ± 1.5	4.9 ± 0.4	12.8 ± 0.84	ND	41.57 ± 2.66	50.4 ± 1.03

Where C 14: 0 = myristic acid, C16:0 = palmitic acid, C16:1 Palmitoleic acid, C18:0 = stearic acid, C18:1 = Oleic

C14:0, C16:0, C18:0 = saturated FA

C16:1, C18:1 = unsaturated FA

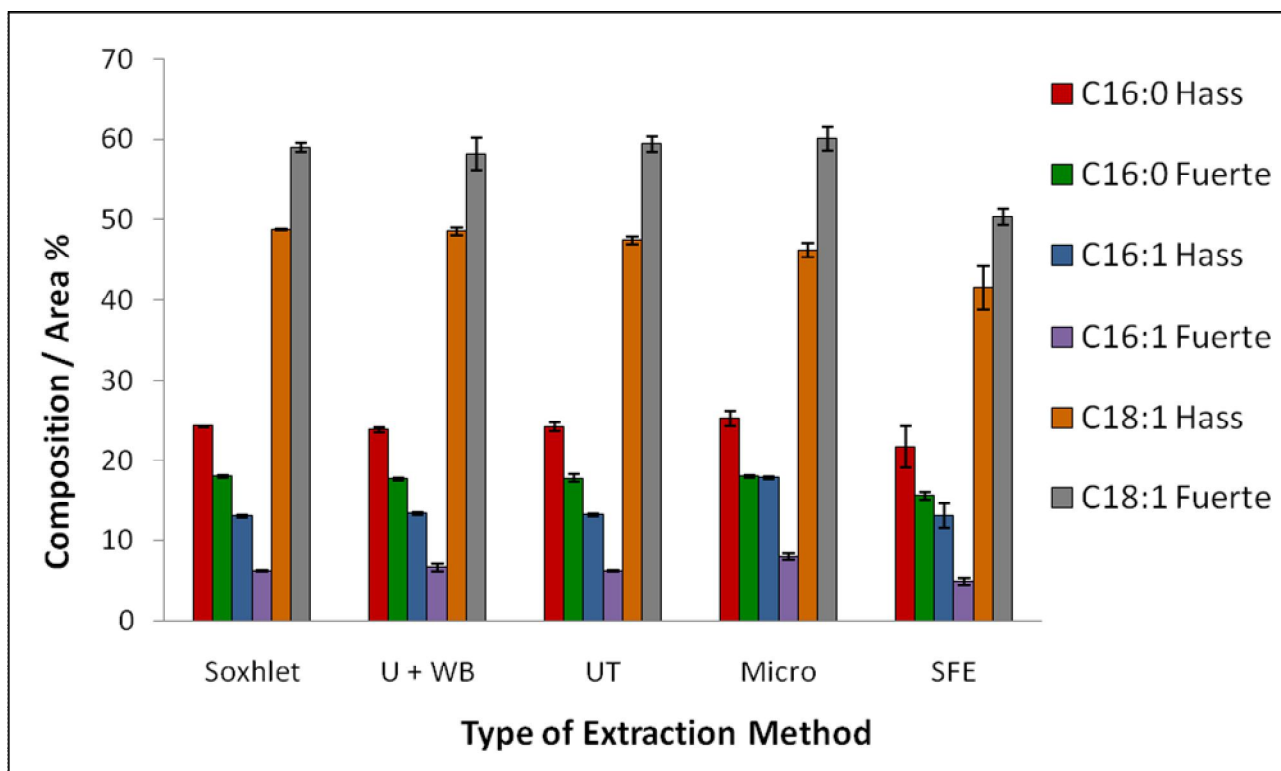


Fig.14 Comparison of the amount of the major fatty acids found in Hass and Fuerte extracted by different techniques.

Where C16:0 = Palmitic acid, C16:1 Palmitoleic acid, C18:1 = Oleic

Soxhlet: Traditional Soxhlet extraction, U + WB: Ultrasound water bath, UT: Ultra-turrax treated, Micro: Microwave extraction, SFE: Supercritical fluid extraction.

A high ratio of MUFAs to SFAs is generally viewed as beneficial to humans (Woolf et al.1999). Table 9 and Fig.15 represent the MUFA: SFA ratio for each variety of avocado oil extracted by various techniques. Of the two varieties, Fuerte oil has a higher MUFA: SFA ratio (3.5-3.7) than Hass (1.5-2.6), for all cases. The highest MUFA: SFA ratio was obtained by microwave extraction in Fuerte variety while the lowest MUFA: SFA ratio was obtained by SFE in Hass variety. Other fatty acids that were expected to be found in the oil were not present in this study. Polyunsaturated FAs such as linoleic (18:2) and linolenic (18:3) acids were found in avocado oils grown in Mexico and Turkey. This was not found in this study, which is not surprising since various factors such as climate conditions, variety, stage of maturity and sun exposure can affect the FA composition in avocados (Villa-Rodríguez et al. 2011).

Table 9. Summary of the M: S ratio for each variety of avocado oil extracted by various techniques.

Extraction Methods	Total Monounsaturated Fatty Acids/%		Total Saturated Fatty Acids/%		M:S Ratio	
	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte
Traditional Soxhlet	61.8	65.2	24.7	18.0	2.50	3.62
Ultrasound in water bath	62.0	64.9	23.9	17.6	2.59	3.68
Ultra- turrax treatment	60.6	65.7	24.8	17.8	2.45	3.70
Microwave	64.2	68.2	25.3	18.0	2.54	3.78
Supercritical fluid	54.7	55.3	36.1	15.6	1.51	3.54

Where M: S Ratio = Ratio of monounsaturated fatty acids is to saturated fatty acid.

Total Monounsaturated Fatty Acids = approximate area percentage of C16:1+ C18:1

Total Saturated Fatty Acids = approximate area percentage of C14:0 + C16:0 + C18:0

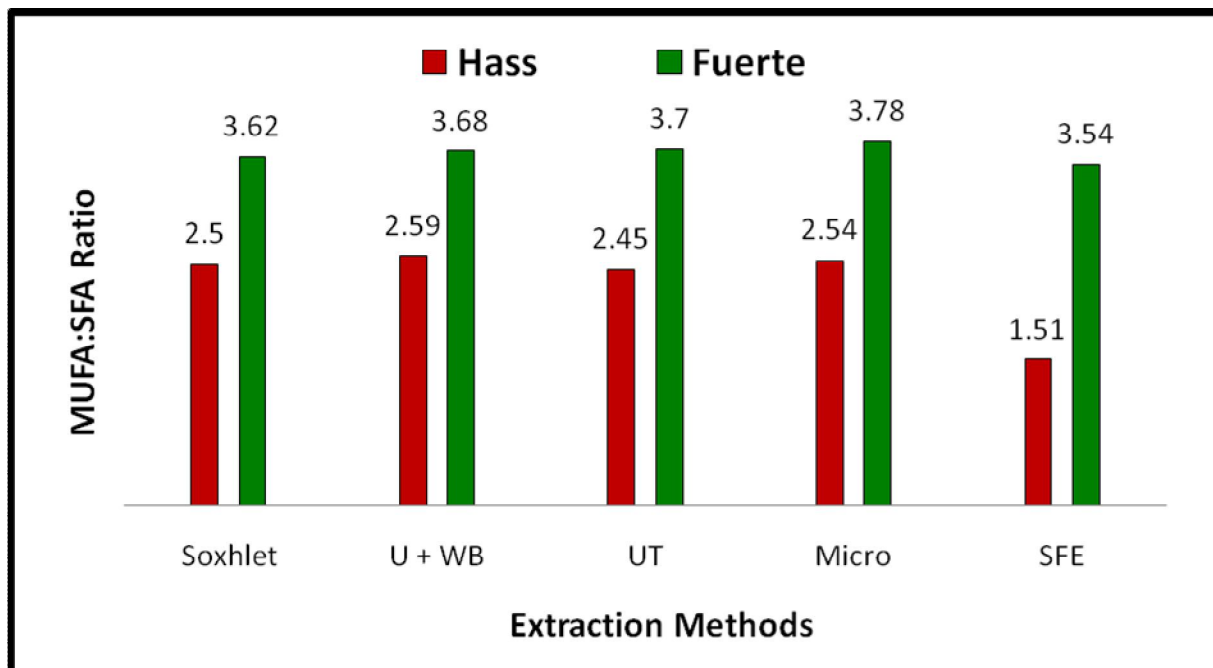


Fig.15 Monounsaturated Fatty Acid (MUFA) to Saturated Fatty Acid (SFA) ratio for each variety of avocado oil extracted by various techniques.

Where: Soxhlet: Traditional Soxhlet extraction, U + WB: Ultrasound water bath, UT: Ultraturax treated, Micro: Microwave extraction, SFE: Supercritical fluid extraction

4.4 Evaluation of Metals in Avocado Oil

Accuracy of the method was measured by comparing results obtained with certified results (Table 10). Recorded values were in good agreement with certified values.

Table 10: Comparison of measured and certified values in the certified reference material (lyophilized brown bread- BCR 191)

Element	Certified	Measured (MD)
Ca	$0.41 \pm 0.01 \text{ mg g}^{-1}$	$0.43 \pm 0.05 \text{ mg g}^{-1}$
Mg	$10.0 \pm 0.01 \text{ mg g}^{-1}$	$9.37 \pm 0.40 \text{ mg g}^{-1}$
Fe	$40.7 \pm 2.3 \text{ } \mu\text{g g}^{-1}$	$39.1 \pm 2.2 \text{ } \mu\text{g g}^{-1}$
Cu	$2.60 \pm 0.1 \text{ } \mu\text{g g}^{-1}$	$2.59 \pm 0.1 \text{ } \mu\text{g g}^{-1}$
Mn	$20.3 \pm 0.7 \text{ } \mu\text{g g}^{-1}$	$19.5 \pm 0.4 \text{ } \mu\text{g g}^{-1}$
Zn	$19.5 \pm 0.5 \mu\text{g g}^{-1}$	$19.4 \pm 0.7 \mu\text{g g}^{-1}$

Mean replication of experiments (n = 6), each sample was analysed in triplicate

± Standard deviation

The concentration of metals detected in the mesocarp prior to extraction and the percentage of metals which dissolved or co-extracted into the oils (dissolution (%)) is represented in Table 11. If present, Co concentrations were below the instrument detection limits ($0.0097 \text{ } \mu\text{g g}^{-1}$). In Hass and Fuerte varieties, Mg was found in high concentrations, $941 \pm 30 \text{ } \mu\text{g g}^{-1}$ and $1118 \pm 12 \text{ } \mu\text{g g}^{-1}$, respectively; this was followed by Ca, $337 \pm 9 \text{ } \mu\text{g g}^{-1}$ and $699 \pm 9 \text{ } \mu\text{g g}^{-1}$, respectively. The Fuerte variety is a richer source of Mg and Ca, with Ca being twice that of Hass. From a nutritional perspective, high concentrations of these macro elements in any given food source can only be beneficial.

Table 11: Elemental content of avocado mesocarp, Hass and Fuerte, ($\mu\text{g g}^{-1}$ and \pm S.D, $n = 3$) and its dissolution (%) into the oil.

Elements	Total Concentration in mesocarp ($\mu\text{g g}^{-1}$ and \pm S.D)		Soxhlet Extraction (%)		Ultrasound water bath (%)		Microwave Extraction (%)		Ultra-turrax assisted extraction (%)		Supercritical Fluid extraction with CO ₂ (%)	
	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte
Al	22.9 \pm 0.3	23.3 \pm 0.1	3.05	3.96	5.08	4.68	4.41	2.97	5.39	3.87	3.01	3.04
Ca	337 \pm 8.9	698 \pm 9.3	4.22	1.68	5.06	2.25	3.37	1.24	7.04	1.47	3.29	1.49
Cu	10.6 \pm 0.2	12.6 \pm 0.17	0.90	0.90	0.69	0.64	0.28	0.08	1.95	1.33	0.05	0.16
Cr	1.30 \pm 0.09	0.96 \pm 0.08	3.98	4.68	6.43	5.85	5.21	3.85	7.15	5.18	4.61	6.98
Fe	53.7 \pm 1.0	44.3 \pm 0.4	0.56	1.05	3.34	4.12	1.07	1.01	2.65	2.22	0.69	1.02
Mg	941 \pm 29	1119 \pm 12	0.07	0.08	0.17	0.12	0.19	0.09	0.27	0.07	0.05	0.04
Mn	4.70 \pm 0.18	8.30 \pm 0.18	0.24	0.08	1.00	0.47	0.22	0.07	1.24	0.29	0.08	0.11
Ni	0.80 \pm 0.13	8.20 \pm 0.08	1.81	0.52	7.41	0.53	1.23	0.23	10.0	0.28	1.83	0.44
Pb	7.30 \pm 1.6	29.3 \pm 2.1	0.82	1.16	3.66	1.94	3.57	0.65	5.28	ND	4.78	1.57
Zn	12.4 \pm 0.4	6.80 \pm 0.80	3.15	2.61	6.22	3.50	3.85	1.90	3.30	2.92	3.57	2.25

Where ND: Not detected.

The minor elements studied were found to be at concentrations below $100 \mu\text{g g}^{-1}$ in the avocado mesocarp. Concentrations of these elements ($\mu\text{g g}^{-1}$, DW) in (Hass and Fuerte) varieties were in descending order of, Fe (53.7 ± 1.0 , 44.3 ± 0.4), > Al (22.9 ± 0.3 , 23.3 ± 0.1), > Zn (12.4 ± 0.4 , 6.8 ± 0.80), > Cu (10.6 ± 0.2 , 12.6 ± 0.2), > Ni (0.8 ± 0.1 , 8.2 ± 0.08), > Mn (4.7 ± 0.18 , 8.3 ± 0.18), > Pb (7.3 ± 1.6 , 29.3 ± 2.1), > Cr (1.3 ± 0.09 , 0.96 ± 0.08). Except for Fe and Zn, which were marginally higher in Hass mesocarp, Fuerte had higher concentrations of elements studied. However, both varieties contribute significantly to the dietary allowances for these elements.

High concentrations of heavy metals in extracted oils are detrimental to oil quality. Oil quality is vital in commercial products manufactured by the pharmaceutical, cosmetic and food preparation industries. Element dissolution was relatively low for traditional Soxhlet, SFE and microwave extraction and relatively high for ultrasound water bath and Ultra-turrax extractions. Fe and Cu concentrations in vegetable oils is known to induce oxidation and can decrease long term stability. The threshold value for Fe in oil is $2\text{-}6 \mu\text{g g}^{-1}$ (Arzt et al. 1994). The ultrasound extraction method gave highest concentration of Fe in oils, 3.30% for Hass ($1.80 \mu\text{g g}^{-1}$) and 4.12% for Fuerte ($1.82 \mu\text{g g}^{-1}$), whilst Ultra-turrax method gave highest concentration of Cu in oils, 1.95% for Hass and 1.33% for Fuerte. Although these concentrations are not high enough to affect oil stability, Fe concentration is $0.2 \mu\text{g g}^{-1}$ below the minimum threshold limit. In light of this, ultrasound extraction and the combined Ultra-turrax method, that showed high dissolution percentages for most metals, should be avoided to prevent possible oxidation and destabilization of avocado oils. Cr dissolution was relatively high for all extraction techniques in both varieties but the total concentration of Cr in the mesocarp was low therefore the concentration in the extracted oil is negligible. Cr is also an antioxidant type metal so its presence in oils can prevent autoxidation. Mn and Zn are

also known to inhibit oxidative degradation of oils therefore their dissolution into the oils is advantageous (Zidenberg-Cherr et al. 1991).

4.5 Proximate Chemical Composition of Avocado Fruit

The proximate chemical composition of the two varieties of avocado fruit (Hass and Fuerte) are presented in Table 12 and a graphical representation of this data is provided by Fig.16.

Table 12. Proximate chemical composition (g per100 g dry mass) of Avocado fruit.

Composition (g per 100g dry mass)	Variety of Fruit	
	<i>Hass</i>	<i>Fuerte</i>
Oil	69.9 ± 0.4	60.9 ± 2.8
Ash	18.0 ± 0.2	9.40 ± 0.1
Protein	6.70 ± 0.4	9.90 ± 0.3
Carbohydrate**	5.40	19.9

**Carbohydrate obtained by subtracting the sum of oil, ash & protein from the total dry mass.

The chosen extraction method used for the quantification of oils was microwave extraction since it produced the highest yield of oil. The Hass variety has 10% higher oil content than Fuerte (Fig.16) whilst the Fuerte variety has four times more carbohydrates than Hass. Both varieties have an estimated amount of only 10% of protein which is typical of subtropical fruits (Robertson, 2001).

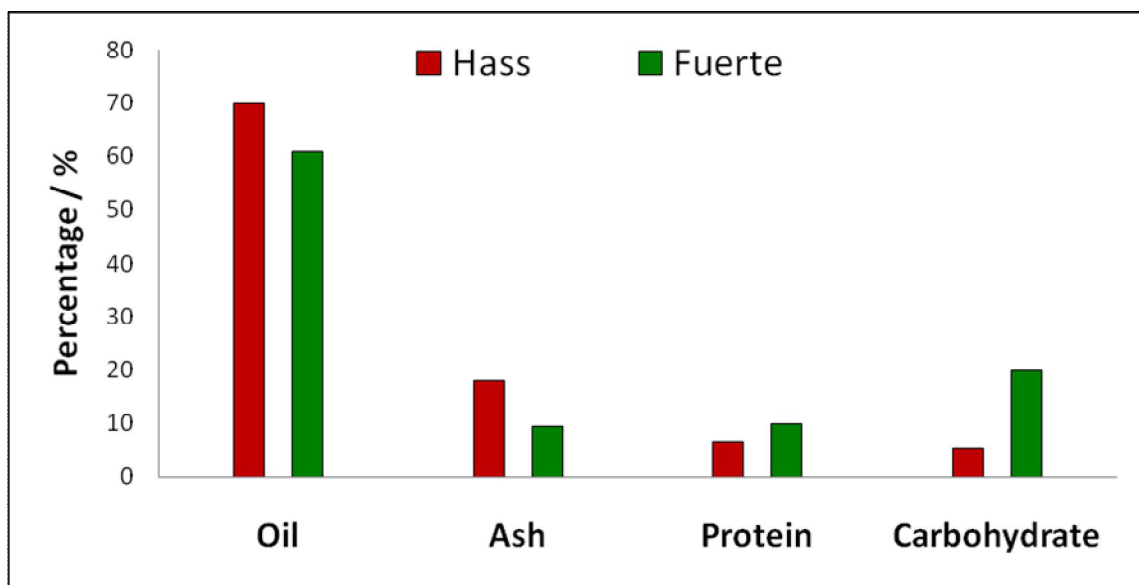


Fig.16 Percentage ash, protein, carbohydrate and oil in avocado fruit.

CHAPTER 5

ELEMENTAL UPTAKE AND DISTRIBUTION OF NUTRIENTS IN AVOCADO MESOCARP AND THE IMPACT OF SOIL QUALITY

5.1 Introduction

The following chapter details the results and discussion of the study conducted to achieve the objectives outlined in chapter 1. Soil and avocado samples of two varieties, Hass and Fuerte, were obtained from six different locations in KwaZulu-Natal. Analysis of the avocado mesocarp in this study yielded information on the total uptake and distribution of the relative proportions of mineral nutrients and heavy metals present in the fruit. Total and bioavailable determination of 14 selected metals was conducted on the collected soils and avocado mesocarp samples (Table 13). A statistical correlation analysis was necessary to investigate the positive and negative relationships that exist between metal cations in the soil and their subsequent effect on the uptake of these cations into the fruit. The impact of soil quality on the uptake of nutrients into the fruit was investigated by measuring the following soil properties: SOM, CEC and pH. Results were used as input variables in the correlation analysis where further relationships could be established. In addition, geoaccumulation indices were used to assess the level of enrichment of selected elements in the soil and its contribution to soil quality. In this chapter, all tables contain the mean values with their standard deviations however, when reproducing these values in the discussion the standard deviations are omitted for fluency.

5.2 Elemental Concentration of Avocado Fruit (Hass and Fuerte) from Various Sites.

Table 13. Elemental concentrations for chosen elements in avocado fruit and corresponding soil samples

SITE A: KRANSKOP					
Element	Concentration ($\mu\text{g g}^{-1}$)				
	Soil (Total)	Soil(Exchangeable)	$\frac{[\text{Soil}]_{\text{Ex}}}{[\text{Soil}]_{\text{T}} (\%)}$	Hass Total	Fuerte Total
Al	49185 \pm 209	3295 \pm 113	6.70	33 \pm 1.5	37.8 \pm 0.1
As	9.0 \pm 0.4	0.89 \pm 0.04	10.5	2.99 \pm 0.03	1.29 \pm 0.08
Ca	1540 \pm 7	1412 \pm 75	91.7	329 \pm 6.6	310 \pm 13
Cd	3.0 \pm 0.1	1.00 \pm 0.03	40.4	ND	ND
Co	3.3 \pm 0.1	0.3 \pm 0.01	9.70	ND	ND
Cr	93 \pm 1	10.9 \pm 0.4	11.4	ND	ND
Cu	72.8 \pm 2	26.8 \pm 0.3	36.9	4.9 \pm 0.24	5.24 \pm 0.13
Fe	23453 \pm 924	2435 \pm 24	10.4	8.78 \pm 0.2	12.9 \pm 0.67
Mg	1069 \pm 33	273 \pm 5.7	25.5	954 \pm 3.9	645 \pm 6.6
Mn	124 \pm 2.5	68.0 \pm 0.84	54.9	14.5 \pm 1.0	3.5 \pm 0.1
Ni	9.30 \pm 0.4	0.63 \pm 0.01	6.70	4.36 \pm 0.15	1.4 \pm 0.06
Pb	122 \pm 4	19.2 \pm 0.8	15.8	ND	ND
Se	1.82 \pm 0.05	0.98 \pm 0.04	53.9	ND	ND
Zn	48 \pm 0.6	15.9 \pm 0.7	33.3	10.3 \pm 0.5	16.6 \pm 0.5
SITE B: SEVENOAKS					
Element	Concentration ($\mu\text{g g}^{-1}$)				
	Soil (Total)	Soil(Exchangeable)	$\frac{[\text{Soil}]_{\text{Ex}}}{[\text{Soil}]_{\text{T}} (\%)}$	Hass Total	Fuerte Total
Al	68834 \pm 1586	2661 \pm 100.78	3.90	18.5 \pm 0.6	25 \pm 1.3
As	14.5 \pm 0.5	0.15 \pm 0.00	1.00	2.8 \pm 0.08	3.2 \pm 0.01
Ca	1920 \pm 18	959 \pm 37.10	49.9	535 \pm 18	344 \pm 15
Cd	1.81 \pm 0.04	0.50 \pm 0.01	27.7	ND	ND
Co	27 \pm 0.5	3.01 \pm 0.12	11.7	ND	ND
Cr	167 \pm 2.8	8.93 \pm 0.05	5.40	ND	ND
Cu	79 \pm 0.3	29.63 \pm 0.77	37.7	4.6 \pm 0.1	9.58 \pm 0.1
Fe	43397 \pm 465	2596 \pm 80	6.00	8.62 \pm 0.43	12.4 \pm 0.42
Mg	1680 \pm 44	325 \pm 12	19.3	1021 \pm 25	1038 \pm 13
Mn	272.3 \pm 1	78.60 \pm 1.9	28.9	12.2 \pm 0.45	6.1 \pm 0.05
Ni	49.4 \pm 0.3	1.54 \pm 0.07	3.10	1.94 \pm 0.03	5.73 \pm 0.14
Pb	180 \pm 4.0	13.94 \pm 0.3	7.80	ND	ND
Se	0.99 \pm 0.02	0.42 \pm 0.02	42.2	ND	ND
Zn	106. \pm 1.5	7.94 \pm 0.17	7.50	9.69 \pm 0.58	28 \pm 1

SITE C: HOWICK					
Element	Concentration ($\mu\text{g g}^{-1}$)				
	Soil (Total)	Soil(Exchangeable)	Soil] _{Ex} / [Soil] _T (%)	Hass Total	Fuerte Total
Al	64921 \pm 888	3260 \pm 117	5.00	18.0 \pm 0.79	17.3 \pm 0.6
As	14.1 \pm 0.5	4.22 \pm 0.1	3.90	4.73 \pm 0.25	3.7 \pm 0.1
Ca	2104 \pm 41	1864 \pm 84	88.6	467 \pm 4	484 \pm 8
Cd	2.3 \pm 0.04	1.14 \pm 0.04	50.0	ND	ND
Co	14 \pm 0.5	3.16 \pm 0.1	15.6	ND	ND
Cr	151 \pm 4.1	10.9 \pm 0.4	7.70	ND	ND
Cu	48 \pm 0.6	13.72 \pm 0.4	28.6	5.1 \pm 0.2	5.6 \pm 0.3
Fe	36760 \pm 751	2362 \pm 64	6.40	6.85 \pm 0.35	5.1 \pm 0.2
Mg	1908 \pm 78	604 \pm 3.1	31.7	1102 \pm 24	1093 \pm 42
Mn	226 \pm 8	126 \pm 3.4	55.6	13.4 \pm 0.3	12.24 \pm 0.34
Ni	37.5 \pm 0.1	1.53 \pm 0.03	4.10	4.99 \pm 0.2	7.80 \pm 0.21
Pb	168 \pm 8	16.36 \pm 0.3	9.80	ND	ND
Se	3.19 \pm 0.05	1.19 \pm 0.04	37.3	ND	ND
Zn	57.5 \pm 0.7	47.34 \pm 0.23	82.3	17.7 \pm 0.6	20.4 \pm 0.4
SITE D: THORNVILLE					
Element	Concentration ($\mu\text{g g}^{-1}$)				
	Soil (Total)	Soil(Exchangeable)	Soil] _{Ex} / [Soil] _T (%)	Hass Total	Fuerte Total
Al	72366 \pm 519	2481 \pm 140	3.40	22.33 \pm 1.1	15.3 \pm 0.3
As	18 \pm 0.7	0.91 \pm 0.04	4.10	3.97 \pm 0.2	2.7 \pm 0.1
Ca	1196 \pm 52	1080 \pm 3	90.3	451 \pm 15	325 \pm 4
Cd	1.8 \pm 0.01	0.75 \pm 0.03	41.0	ND	ND
Co	32 \pm 0.8	3.39 \pm 0.12	10.3	ND	ND
Cr	170 \pm 1.4	7.09 \pm 0.06	4.60	ND	ND
Cu	76.5 \pm 2.6	40.5 \pm 0.40	52.9	7.1 \pm 0.3	7.5 \pm 0.04
Fe	41485 \pm 2005	1690 \pm 76	4.10	26 \pm 1.9	12.4 \pm 0.5
Mg	2034 \pm 101	494 \pm 3.74	24.3	1334 \pm 12	926 \pm 40
Mn	288 \pm 14	106.1 \pm 1.87	36.9	17.2 \pm 0.4	7.65 \pm 0.3
Ni	43 \pm 0.6	0.46 \pm 0.01	1.10	19.4 \pm 0.9	2.36 \pm 0.07
Pb	196 \pm 3.5	24.30 \pm 0.20	12.4	ND	ND
Se	0.92 \pm 0.01	0.22 \pm 0.01	23.4	ND	ND
Zn	9.6 \pm 0.3	7.20 \pm 0.21	73.1	29.3 \pm 1.4	13.1 \pm 0.4

SITE E: RICHMOND					
Element	Concentration ($\mu\text{g g}^{-1}$)				
	Soil (Total)	Soil(Exchangeable)	Soil] _{Ex} / [Soil] _T (%)	Hass Total	Fuerte Total
Al	79086 ± 287	3143 ± 150	3.10	17.7 ± 0.5	18.3 ± 0.5
As	17 ± 0.1	0.67 ± 0.03	8.40	4.5 ± 0.2	4.6 ± 0.2
Ca	2970 ± 41	2445 ± 29	82.3	355 ± 8	313 ± 12
Cd	2 ± 0.01	1.01 ± 0.03	50.6	ND	ND
Co	21 ± 0.1	3.56 ± 0.15	13.9	ND	ND
Cr	146 ± 2.3	10.6 ± 0.41	5.70	ND	ND
Cu	113 ± 0.2	83.09 ± 0.34	73.6	6.5 ± 0.4	5.7 ± 0.01
Fe	38120 ± 667	2462 ± 42	6.50	9.8 ± 0.1	4.5 ± 0.1
Mg	2886 ± 26	540 ± 19	18.7	1223 ± 62	879 ± 38
Mn	285 ± 3	182 ± 5	63.9	4.6 ± 0.2	4.93 ± 0.17
Ni	41 ± 1	0.79 ± 0.03	1.90	ND	ND
Pb	199 ± 3	16.62 ± 0.30	8.40	ND	ND
Se	3.5 ± 0.2	0.69 ± 0.03	20.0	ND	ND
Zn	38.3 ± 0.2	28.73 ± 1.31	75.0	24.06 ± 1.4	19.3 ± 0.1
SITE F: IXOPO					
Element	Concentration ($\mu\text{g g}^{-1}$)				
	Soil (Total)	Soil(Exchangeable)	Soil] _{Ex} / [Soil] _T (%)	Hass Total	Fuerte Total
Al	56692 ± 668	1399 ± 90	2.50	29.1 ± 0.7	36.7 ± 0.6
As	14.8 ± 0.5	0.43 ± 0.02	2.10	3.75 ± 0.15	3.3 ± 0.1
Ca	1791 ± 28	1608 ± 38	89.8	314 ± 10	344 ± 16
Cd	2.5 ± 0.02	0.72 ± 0.03	18.3	ND	ND
Co	21 ± 0.6	3.96 ± 0.12	5.00	ND	ND
Cr	117 ± 3	5.4 ± 0.26	48.9	ND	ND
Cu	49 ± 2.1	23.7 ± 0.26	3.60	7.4 ± 0.2	6.75 ± 0.08
Fe	35545 ± 995	1293 ± 59	28.9	54.9 ± 1.2	26.9 ± 1.3
Mg	1671 ± 9	483 ± 11.80	51.4	1063 ± 44	959 ± 25
Mn	207 ± 7	106 ± 4	3.80	7.5 ± 0.4	5.65 ± 0.3
Ni	30.2 ± 1.1	1.14 ± 0.02	10.8	3.6 ± 0.2	2.0 ± 0.04
Pb	153 ± 5	16.5 ± 0.30	10.8	ND	ND
Se	2.1 ± 0.1	1.32 ± 0.05	42.9	ND	ND
Zn	37.3 ± 1.1	16.0 ± 1.0	2.50	27 ± 1.3	8.9 ± 0.4

Table 13 lists (Hass and Fuerte varieties) for the 14 elements investigated, at each site. The analysis showed all 14 metals to be present in the soil (total and exchangeable) but five metals namely Cd, Co, Cr, Pb and Se were found to be below the lower detection limits of the instruments ($< 0.0034 \mu\text{g}\cdot\text{g}^{-1}$ for Cd , $< 0.007 \mu\text{g g}^{-1}$ for Co, $< 0.0071 \mu\text{g g}^{-1}$ for Cr, $< 0.09 \mu\text{g g}^{-1}$ for Pb, and $< 0.1150 \mu\text{g g}^{-1}$ for Se) in all fruit samples analysed. It should be noted that the elements present in the fruit can be influenced by seasonal changes, time of sampling and soil conditions.

A perusal of Table 13 shows all soils to be rich in Al with total concentrations ranging between 49 000 to 80 000 $\mu\text{g g}^{-1}$ of dry soil, followed by Fe (23 000-45 000 $\mu\text{g g}^{-1}$), Ca (1500-3000 $\mu\text{g g}^{-1}$) and Mg (1000-2900 $\mu\text{g g}^{-1}$). Al, Fe and Si are the three most abundant minerals in soil so it is not unlikely for these metals to influence plant soil interactions (Ma, 2005; Hall, 2008).

As was detected in soils at all sites at relatively low total concentrations (8 to 18 $\mu\text{g g}^{-1}$). Results from a study analysing total soil As concentrations of paddy soils in India ranging from 1.38 ± 0.10 to $12.3 \pm 0.09 \mu\text{g g}^{-1}$ (Bhattacharya et al., 2009) are comparable to the total soil As concentration range in this study. Both ranges are below the maximum limit for agricultural soil of $20.0 \mu\text{g g}^{-1}$ as recommended by the European community (Bhattacharya et al., 2009).

Site A seemed to have lower soil total concentration for most metals; however the exchangeable concentrations for site A were found to be higher than other sites with 9 of the 14 metals investigated displaying this trend. The exceptions of this trend were Co, Cd, Cu, Se and Zn. Factors known to influence the bioavailability of metals and their occurrences in crops are soil pH, cation exchange capacity, organic matter content, soil texture, and interaction among the target elements (Jung, 2008) which varied at different sites. Results

from soil property measurements will aid in deciphering which factor had the most influence on the bioavailability of metals.

Total soil Cu ranged from 45-113 $\mu\text{g g}^{-1}$, with 46.4% being available to the plant for uptake on average (Table 13). For both varieties of avocado, 4-10 $\mu\text{g g}^{-1}$ of Cu was taken up into fruit. There are a 100 different Cu containing proteins found in plants, of which 50% of them are found in chloroplasts where it participates in photosynthetic reactions (Yruela, 2009; Hansch and Mendel, 2009). With this mind, this study shows low concentrations of copper present in the fruit which is attributed to the small amount of chlorophyll present in the fruit relative to leaves.

High total concentrations of Fe were found in soils but only 3-10% of this Fe was available to the plant. Soil Fe is not very mobile and is fixed within soil matrices. This action could be because most Fe in soil is in silicate minerals and Fe oxides or hydroxides that are sparingly soluble (Schulin et al., 2010). Fe levels in the fruit of both varieties ranged between 4-12 $\mu\text{g g}^{-1}$ at all sites except F, where Fe concentrations in the Hass and Fuerte varieties were 54.9 and 26.9 $\mu\text{g g}^{-1}$ respectively.

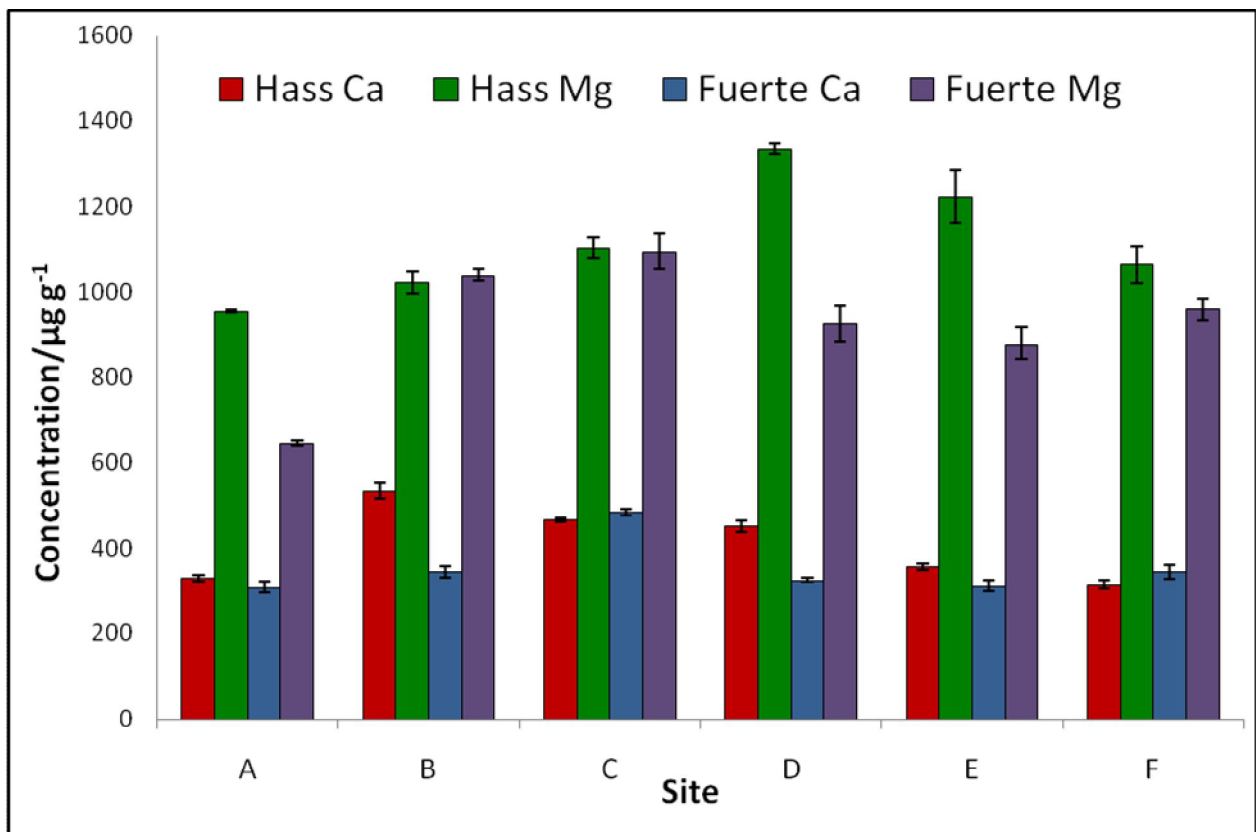


Fig.17 Distribution of major elements in fruit (Hass and Fuerte) at the 6 different sites
 Site: A Site: A = Kranskop, B = Seven Oaks, C = Howick , D = Thornville ,
 E = Richmond, F = Ixopo

The elemental distributions of the two major elements in fruit are illustrated in Fig.17. It is suggested from the results that the fruit of both varieties accumulate Mg and Ca according to a 3: 1 ratio. Ca has a relatively predictable uptake pattern across all sites with uptake ranges between 300-500 $\mu\text{g g}^{-1}$ while Mg shows no obvious trend and appears to be more site dependant. Specific influences affecting the varied uptake of Mg will be clarified using statistical data.

The elemental distribution for minor elements presents in fruit (Hass and Fuerte) is illustrated in Fig.18. It can be observed that regardless of the varied soil concentrations at each site, Arsenic and Cu fruit concentrations are relatively constant, only varying in narrow ranges. The two extreme exchangeable Cu concentrations (highest at Site E, Lowest at Site C) did not affect the uptake of Cu into the fruit.

Arsenic concentrations in both fruit are below $5.00 \mu\text{g g}^{-1}$ while Cu concentrations are below $10.0 \mu\text{g g}^{-1}$. There is no observed uptake trend between the varieties for Ni, except for the apparent exclusion of Ni at site E (Richmond) even though Ni was available at that site. The results for Fe uptake are unusual for sites D (Thornville) and F (Ixopo). The Hass variety was noted to accumulate more than twice the amount of Fe at these sites than the Fuerte variety, however a higher concentration of Fe was generally observed in the latter.

Fig.18 cannot be used to show uptake trends. The concentration of elements in both varieties of fruit was, generally, in the decreasing order of $\text{Mg} > \text{Ca} > \text{Al} > \text{Zn} > \text{Fe} = \text{Mn} > \text{Cu} > \text{Ni} = \text{As}$.

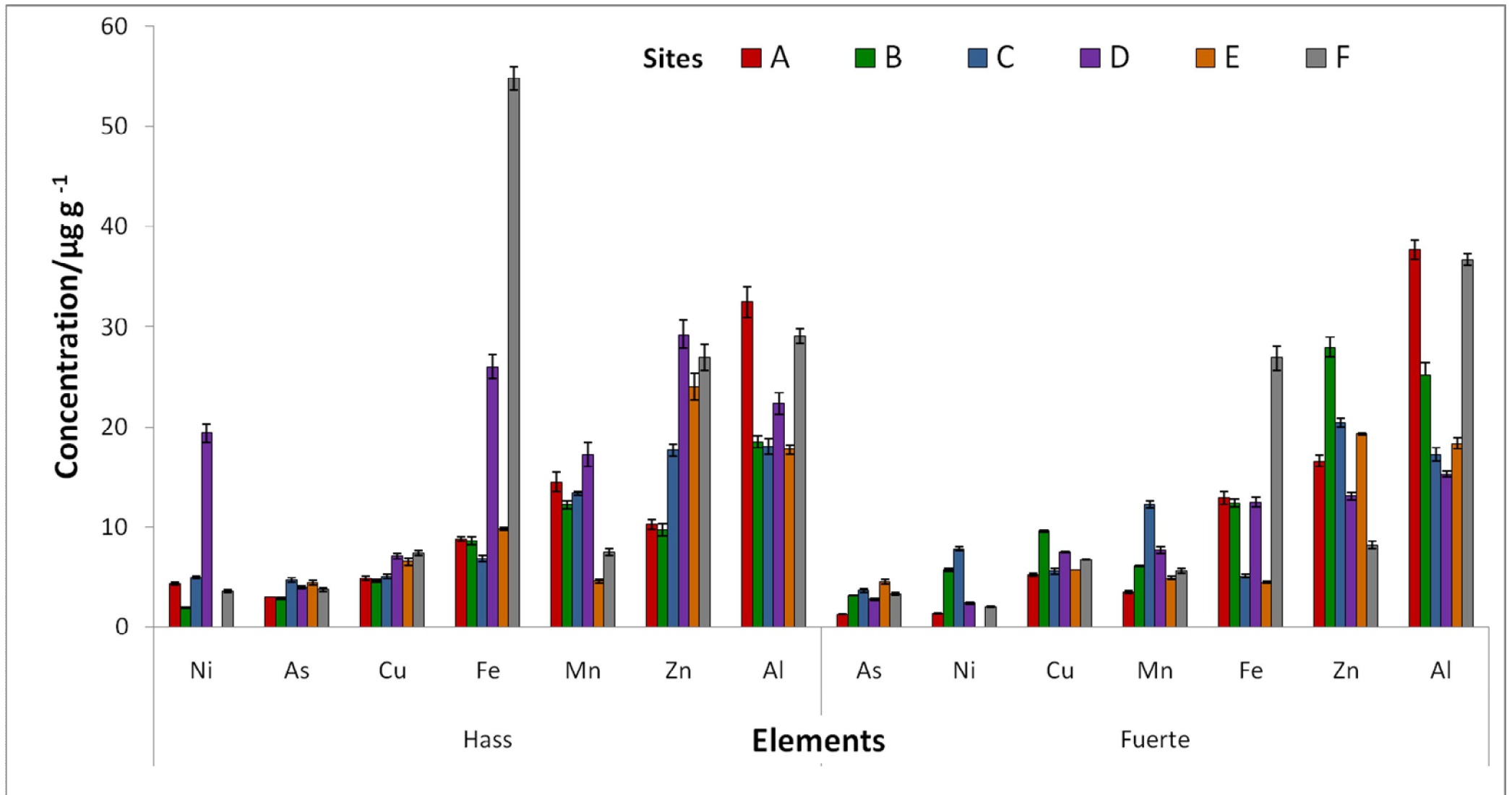


Fig.18 Distribution of minor elements in fruit (Hass and Fuerte) at the six different sites*

*Site: A = Kranskop, B = Seven Oaks, C = Howick, D = Thornville, E = Richmond, F = Ixopo

5.3. Bioaccumulation Factors Obtained from Different Sites

The BFs for Hass and Fuerte varieties obtained for selected elements investigated at the six different sites are represented in Tables 14-20 and relative accumulation graphs plotted in Figures 19-25. The BFs suggest that when the soil concentrations (total and bioavailable) of an element essential for plant growth is below the physiological requirement level, the plant tends to accumulate the element until the required level is reached. Conversely, at soil concentrations (total and bioavailable) that exceed the physiological requirement levels of the plant, uptake of the associated element is inhibited thereby partially excluding the element (Moodley et al., 2007). This trend was also observed by Timperley et al. who suggested that a plot of relative accumulation as a function of total soil content indicated essentiality of the element if a rectangular hyperbola was produced whereas it indicated non-essentiality if a linear plot parallel to the x-axis was obtained (Timperley et al. 1973). The next segment of this discussion focuses on a comparison of the BFs obtained by using the total and exchangeable concentrations of the elements in the soil to determine accumulation or exclusion of the elements by the fruit (Hass and Fuerte).

Table 14. As concentrations in fruit and soil with bioaccumulation factors (BFs)

Sites	ARSENIC						
	Soil-total	Soil-exchangeable	[Soil] _{EX} / [Soil] _T (%)	Hass Total	BF-Hass	Fuerte Total	BF-Fuerte
A	8.90 ± 0.4 ^c	0.89 ± 0.04 ^b	10.5	2.99 ± 0.03 ^c	3.34	1.29 ± 0.08 ^d	1.44
B	14.5 ± 0.5 ^b	0.15 ± 0.001 ^d	1.02	2.80 ± 0.08 ^c	19.01	3.15 ± 0.01 ^{bc}	21.37
C	14.1 ± 0.5 ^b	4.22 ± 0.01 ^a	3.89	4.73 ± 0.25 ^a	1.12	3.69 ± 0.2 ^b	0.87
D	18.1 ± 0.7 ^a	0.91 ± 0.02 ^b	4.12	3.97 ± 0.2 ^b	4.35	2.73 ± 0.1 ^c	2.99
E	17.3 ± 0.1 ^{ab}	0.67 ± 0.03 ^c	8.40	4.47 ± 0.2 ^a	6.66	4.55 ± 0.2 ^a	6.79
F	14.8 ± 0.5 ^b	0.43 ± 0.04 ^d	2.13	3.75 ± 0.2 ^b	8.66	3.32 ± 0.1 ^{bc}	7.68

Mean values in each column followed by a different superscript letter are significantly different by Duncan's multiple range test ($P \leq 0.05$).

Site: A Site: A = Kranskop, B = Seven Oaks, C = Howick , D = Thornville , E = Richmond, F = Ixopo

The relative accumulation plots (BF vs. Exchangeable soil concentration) for As (Fig.19), shows that in both varieties of fruit, As functions as an essential element. This is peculiar as it is well known that the accumulation of such a toxic element causes detrimental effects (Tripathi et al., 2007; Zhang et al., 2011) to the plant. As occurs in all soils and natural waters, thus, it is likely that plants have evolved in the presence of As ions (Wang et al., 2002). Uptake of As is probably linked to P uptake since it is chemically similar to P and more specifically, As (V) acts as a phosphate analogue and is taken up in plant via P uptake systems (Kim et al., 2008; Dixon et al., 1997). Therefore, in the case of As, the produced plot of BF vs. Soil Exchangeable concentration indicates the biological similarity in uptake to an essential element. Graphs generated using BF vs Soil Total concentrations did not produce clear indications of essentiality.

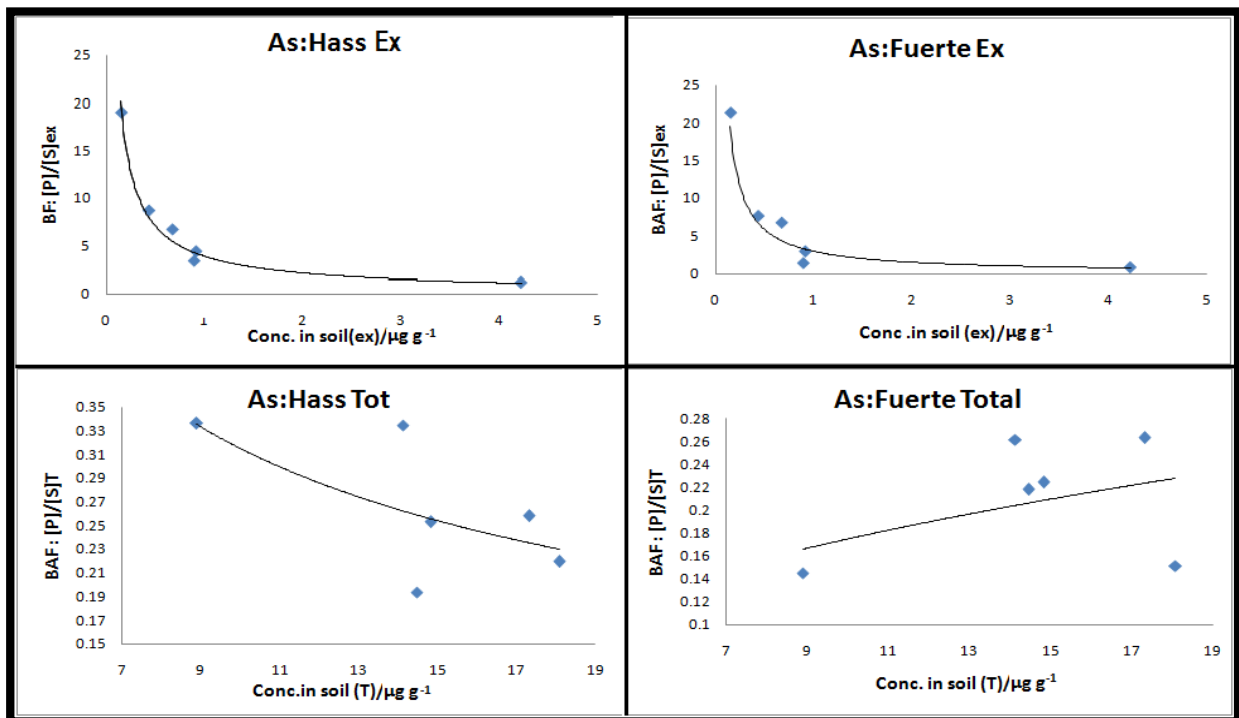


Fig.19 Bioaccumulation Factors (BF ex, BF T) vs. Total (T) and Exchangeable (ex)

Concentrations of As in soil for both varieties

Table 15. Cu concentrations in fruit and soil with bioaccumulation factors (BFs)

Sites	COPPER						
	Soil-total	Soil-exchangeable	[Soil] _{EX} / [Soil] _T (%)	Hass Total	BF-Hass	Fuerte Total	BF-Fuerte
A	72.8 ± 2 ^a	26.84 ± 0.3 ^d	36.9	4.91 ± 0.24 ^c	0.183	5.24 ± 0.13 ^e	0.183
B	78.7 ± 0.3 ^b	29.63 ± 0.77 ^c	37.7	4.61 ± 0.12 ^c	0.156	9.58 ± 0.1 ^a	0.156
C	47.9 ± 0.6 ^d	13.72 ± 0.4 ^f	28.6	5.07 ± 0.21 ^c	0.369	5.61 ± 0.3 ^d	0.369
D	76.5 ± 2.6 ^b	40.49 ± 0.4 ^b	52.9	7.10 ± 0.29 ^b	0.175	7.47 ± 0.04 ^b	0.175
E	112.9 ± 0.2 ^a	83.09 ± 0.3 ^e	73.6	6.54 ± 0.37 ^b	0.079	5.71 ± 0.01 ^d	0.079
F	48.5 ± 2.1 ^d	23.71 ± 0.3 ^a	48.9	7.37 ± 0.22 ^a	0.311	6.75 ± 0.08 ^c	0.311

Mean values in each column followed by a different superscript letter are significantly different by Duncan's multiple range test ($P \leq 0.05$).

Site: A = Kranskop, B = Seven Oaks, C = Howick, D = Thornville, E = Richmond, F = Ixopo

BFs for Cu were all below 1. This observation confirms previous speculation that Cu does not accumulate in the fruit. However, relative accumulation plots for Cu indicates essentiality. Both varieties display very similar graphs for of BF vs. Soil Exchangeable concentration plots.

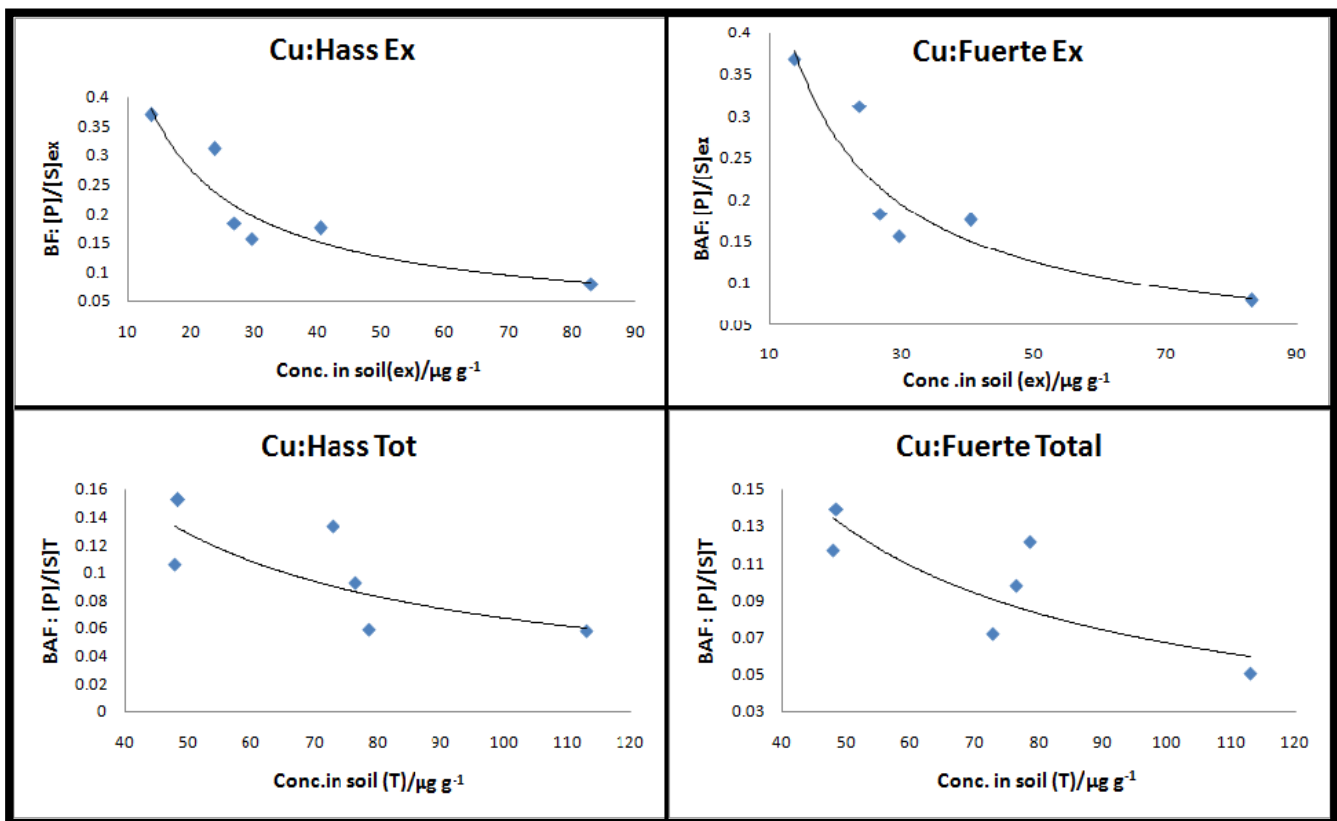


Fig.20 Bioaccumulation Factors (BF_{ex}, BF_T) vs. Total (T) and Exchangeable (ex) Concentrations of Cu in soil for both varieties.

Table 16. Fe concentrations in fruit and soil with bioaccumulation factors (BFs)

Sites	IRON						
	Soil-total	Soil-exchangeable	[Soil] _{Ex} / [Soil] _T (%)	Hass Total	BF-Hass	Fuerte Total	BF-Fuerte
A	23453 ± 924 ^a	2435 ± 24 ^a	10.4	8.78 ± 0.2 ^c	0.004	12.91 ± 0.7 ^b	0.005
B	43397 ± 465 ^a	2596 ± 80 ^a	6.00	8.62 ± 0.4 ^c	0.003	12.36 ± 0.4 ^b	0.005
C	36760 ± 751 ^{cb}	2362 ± 64 ^a	6.40	6.85 ± 0.4 ^d	0.003	5.10 ± 0.2 ^c	0.002
D	41485 ± 2005 ^a	1690 ± 76 ^c	4.10	26.01 ± 1.2 ^b	0.015	12.44 ± 0.5 ^b	0.007
E	38120 ± 667 ^b	2462 ± 42 ^a	6.50	9.8 ± 0.1 ^c	0.004	4.48 ± 0.1 ^c	0.002
F	35545 ± 995 ^c	1293 ± 59 ^d	3.70	54.86 ± 1.2 ^a	0.042	26.86 ± 1.2 ^a	0.021

Mean values in each column followed by a different superscript letter are significantly different by Duncan's multiple range test ($P \leq 0.05$).

Site: A = Kranskop, B = Seven Oaks, C = Howick , D = Thornville , E = Richmond, F = Ixopo

The trend observed for Fe was somewhat similar to Cu. Both elements have BFs below 1 but generate relative accumulation plots that indicate essentiality. The essentiality of Fe is most apparent in the BF vs. Soil Exchangeable concentration plot for the Hass variety. The typical concentration of Fe required for plant growth, in general, is $100 \mu\text{g g}^{-1}$ (Moodley et al., 2007). All sites had extremely high total and exchangeable soil Fe concentrations (Table 16. and Fig.21). It is clear that the plant's physiological requirement for Fe was well exceeded; hence the plant adopted a mechanism of exclusion when in soils of high Fe concentration, seen by low BFs. Conversely, the highest BFs were exhibited by sites D and F, having the lowest soil exchangeable concentration of Fe. The variation of Fe concentrations in the mesocarp may possibly be a consequence of requirement of nutrients to the growing seed. The accumulation plots using soil total concentrations once again generated unclear indications of essentiality.

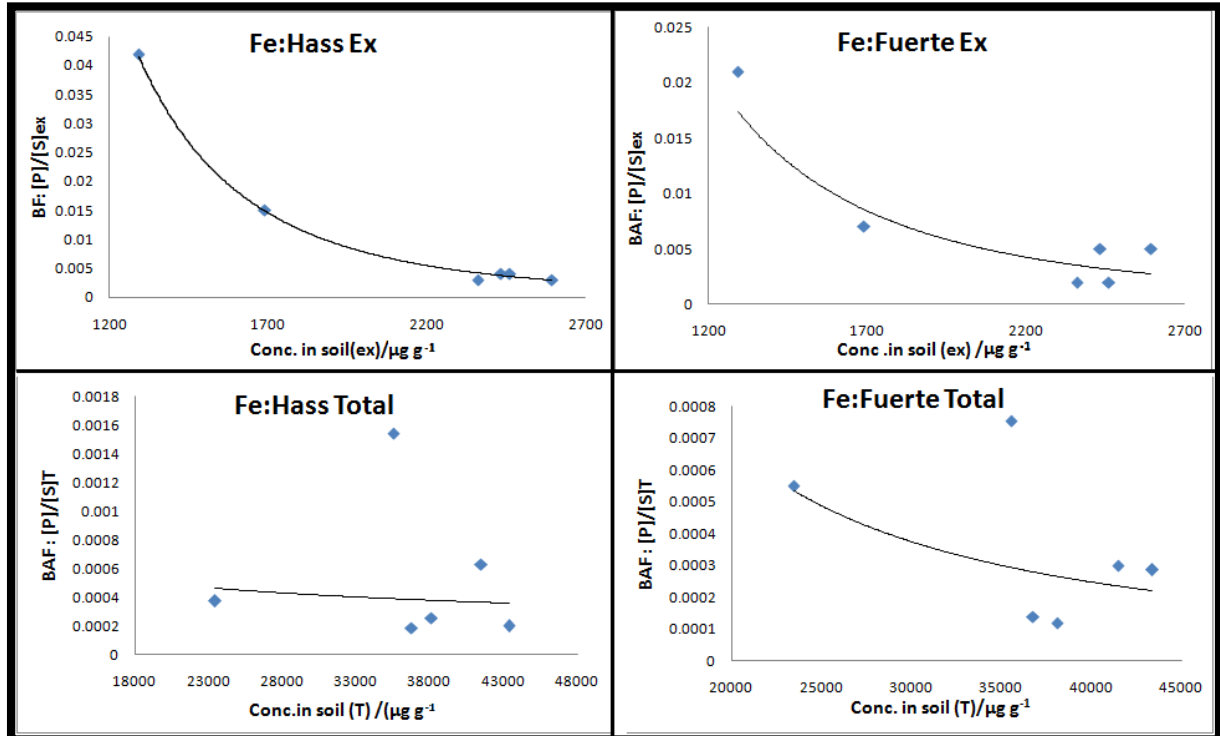


Fig.21 Bioaccumulation Factors (BF ex, BF T) vs. Total (T) and Exchangeable (ex) Concentrations of Fe in soil for both varieties

Table 17. Mg concentrations in fruit and soil with bioaccumulation factors (BFs)

Sites	MAGNESIUM						
	Soil-total	Soil-exchangeable	$\frac{[\text{Soil}]_{\text{Ex}}}{[\text{Soil}]_{\text{T}} (\%)}$	Hass Total	BF-Hass	Fuerte Total	BF-Fuerte
A	1069 ± 33 ^e	273 ± 5.7 ^e	25.5	953.7 ± 3.9 ^d	3.49	645.1 ± 6.6 ^d	2.36
B	1680 ± 44 ^d	325 ± 11 ^d	19.3	1021 ± 25 ^c	3.14	1038 ± 13 ^a	3.20
C	1908 ± 78 ^c	604 ± 3.1 ^a	31.7	1102 ± 24 ^c	1.83	1093 ± 42 ^a	1.81
D	2034 ± 101 ^b	494 ± 3.7 ^c	24.3	1334 ± 12 ^a	2.70	925 ± 40 ^{cb}	1.87
E	2886 ± 26 ^a	540 ± 19.1 ^b	18.7	1223 ± 62 ^b	2.26	879 ± 38 ^c	1.63
F	1671 ± 9 ^d	483 ± 12 ^a	28.9	1063 ± 44 ^c	0.65	959 ± 25 ^b	1.99

Mean values in each column followed by a different superscript letter are significantly different by Duncan's multiple range test ($P \leq 0.05$).

Site: A = Kranskop, B = Seven Oaks, C = Howick, D = Thornville, E = Richmond, F = Ixopo

High concentrations of Mg were in soil, and about 18-32% was exchangeable (Table 17). Mg in the mesocarp of both varieties was significantly higher than exchangeable Mg, with BFs higher than 1, indicating that plants tend to accumulate this nutrient. The concentration of Mg in the plant is determined by total soil concentration and controlled by differential absorption in plants to meet physiological needs (Mayland et al., 1989). The typical concentration of Mg required for plant growth, in general, is $2000 \mu\text{g g}^{-1}$ (Moodley et al., 2007). It is shown that even when sites D and E have total soil Mg concentrations more than $2000 \mu\text{g g}^{-1}$, the plant still tends to accumulate Mg, exhibited by $\text{BFs} > 1$. Possible explanations for this tendency are that the physiological requirement level for Mg in the fruit is not the same as the general level but slightly higher or that the plant has an overall tendency for Mg accumulation hence making it a good dietary source of Mg. An exception to this observation was site F for the Hass variety where no accumulation in the fruit was noted.

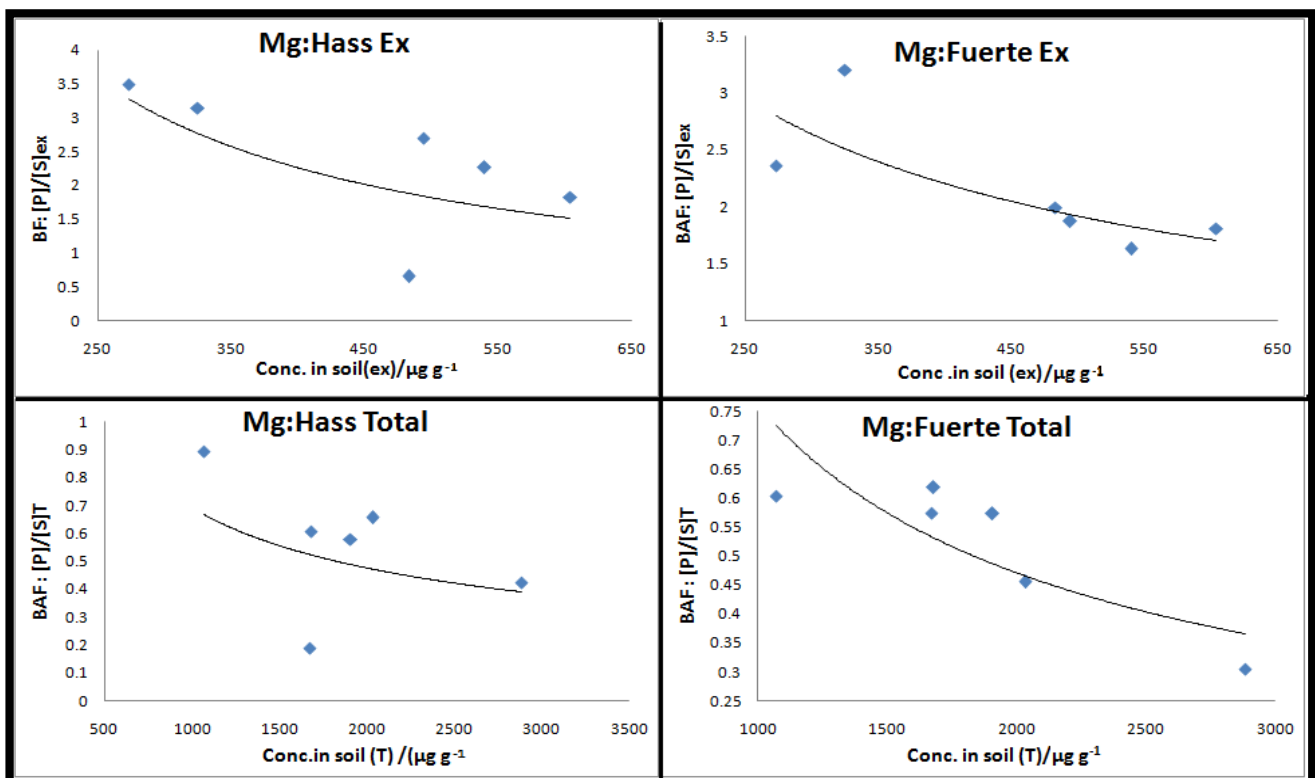


Fig.22 Bioaccumulation Factors (BF_{ex}, BF_T) vs. Total (T) and Exchangeable (ex)

Concentrations of Mg in soil for Hass and Fuerte varieties.

Table 18. Ca concentrations in fruit and soil with bioaccumulation factors (BFs)

Sites	CALCIUM						
	Soil-total	Soil-exchangeable	[Soil] _{Ex} / [Soil] _T (%)	Hass Total	BF-Hass	Fuerte Total	BF-Fuerte
A	1540 ± 7 ^e	1412 ± 75 ^d	91.7	329 ± 6.6 ^d	0.233	310 ± 13 ^c	0.219
B	1920 ± 18 ^c	959 ± 37 ^e	49.9	534.6 ± 18 ^a	0.557	344 ± 15 ^b	0.358
C	2104 ± 41 ^b	1864 ± 84 ^b	88.6	467 ± 4 ^b	0.250	484 ± 8 ^a	0.260
D	1196 ± 52 ^f	1080 ± 2.9 ^e	90.3	451.2 ± 15 ^b	0.418	325 ± 4.3 ^{cb}	0.301
E	2970 ± 41 ^a	2445 ± 29 ^a	82.3	355 ± 8 ^c	0.145	313 ± 12 ^{cb}	0.128
F	1791 ± 28 ^d	1608 ± 38 ^c	89.8	314 ± 10 ^d	0.195	344. ± 16 ^b	0.214

Mean values in each column followed by a different superscript letter are significantly different by Duncan's multiple range test ($P \leq 0.05$).

Site: A = Kranskop, B = Seven Oaks, C = Howick , D = Thornville , E = Richmond, F = Ixopo

It was noted that the exchangeable percent ($[Soil]_{Ex} / [Soil]_T$ %) for Ca was the highest amongst all the metals, with 50-92% of the total soil concentration being exchangeable. Inspection of the bioaccumulation factors (BF) for Ca shows that even though up to 92% of Ca was available for plant uptake, there is no indication of bioaccumulation of Ca into the avocado mesocarp as indicated by all BF being less than 1. Ca follows an uptake trend comparable to Cu and Fe. Correlation results will help to confirm a possible relationship amongst these elements.

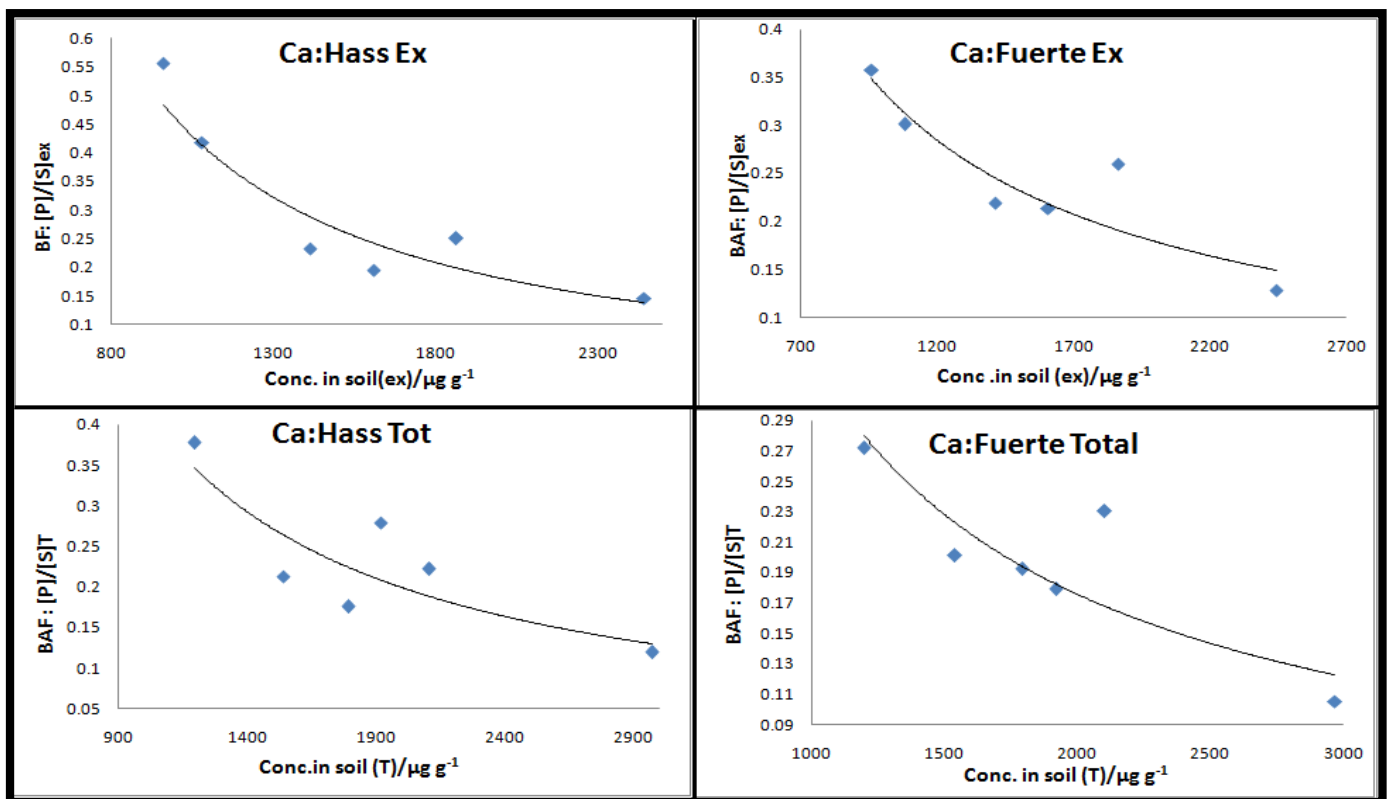


Fig.23 Bioaccumulation Factors (BF_{ex}, BF_T) vs. Total (T) and Exchangeable (ex) Concentrations of Ca in soil for Hass and Fuerte varieties

Table 19. Ni concentrations in fruit and soil with bioaccumulation factors (BFs)

Sites	NICKEL						
	Soil-total	Soil-exchangeable	$\frac{[\text{Soil}]_{\text{Ex}}}{[\text{Soil}]_{\text{T}} (\%)}$	Hass Total	BF-Hass	Fuerte Total	BF-Fuerte
A	$9.30 \pm 0.4^{\text{f}}$	$0.63 \pm 0.01^{\text{c}}$	6.70	$4.36 \pm 0.15^{\text{bc}}$	6.98	$1.41 \pm 0.06^{\text{d}}$	2.26
B	$49.4 \pm 0.3^{\text{a}}$	$1.54 \pm 0.07^{\text{a}}$	3.10	$1.94 \pm 0.03^{\text{d}}$	1.26	$5.73 \pm 0.14^{\text{b}}$	3.72
C	$37.47 \pm 0.1^{\text{d}}$	$1.53 \pm 0.03^{\text{a}}$	4.10	$4.99 \pm 0.2^{\text{b}}$	3.27	$7.80 \pm 0.21^{\text{a}}$	5.11
D	$43 \pm 0.6^{\text{b}}$	$0.46 \pm 0.01^{\text{c}}$	1.10	$19.40 \pm 0.9^{\text{a}}$	42.07	$2.36 \pm 0.07^{\text{c}}$	5.12
E	$41. \pm 1^{\text{c}}$	$0.79 \pm 0.03^{\text{bc}}$	1.90	ND	0.00	ND	0.00
F	$30.2 \pm 1^{\text{e}}$	$1.14 \pm 0.02^{\text{ab}}$	10.8	$3.61 \pm 0.2^{\text{c}}$	3.17	$2.03 \pm 0.04^{\text{c}}$	1.78

Mean values in each column followed by a different superscript letter are significantly different by Duncan's multiple range test ($P \leq 0.05$). ND = Not Determined.

Site: A = Kranskop, B = Seven Oaks, C = Howick , D = Thornville , E = Richmond, F = Ixopo

Ni is known to be one of the soil derived nutrients essential for higher plants (Brown et al., 1987). According to the relative accumulation plots for Ni, essentiality is only indicative for the Hass variety, however the Fuerte variety shows good evidence of accumulation of Ni, $BFs > 1$. It has been reported that nutrient content for a particular crop also varies with the cultivar (Hornick, 1992). This could be such an example where one cultivar displays uptake of just the physiological requirement of an element while another exhibits accumulation well over the physiological need. Although an indication of essentiality can be made by observing relative accumulation plots, the actual physiological requirement of the plant as a whole cannot be estimated due to distribution of the element to other parts of the plant other than the fruit.

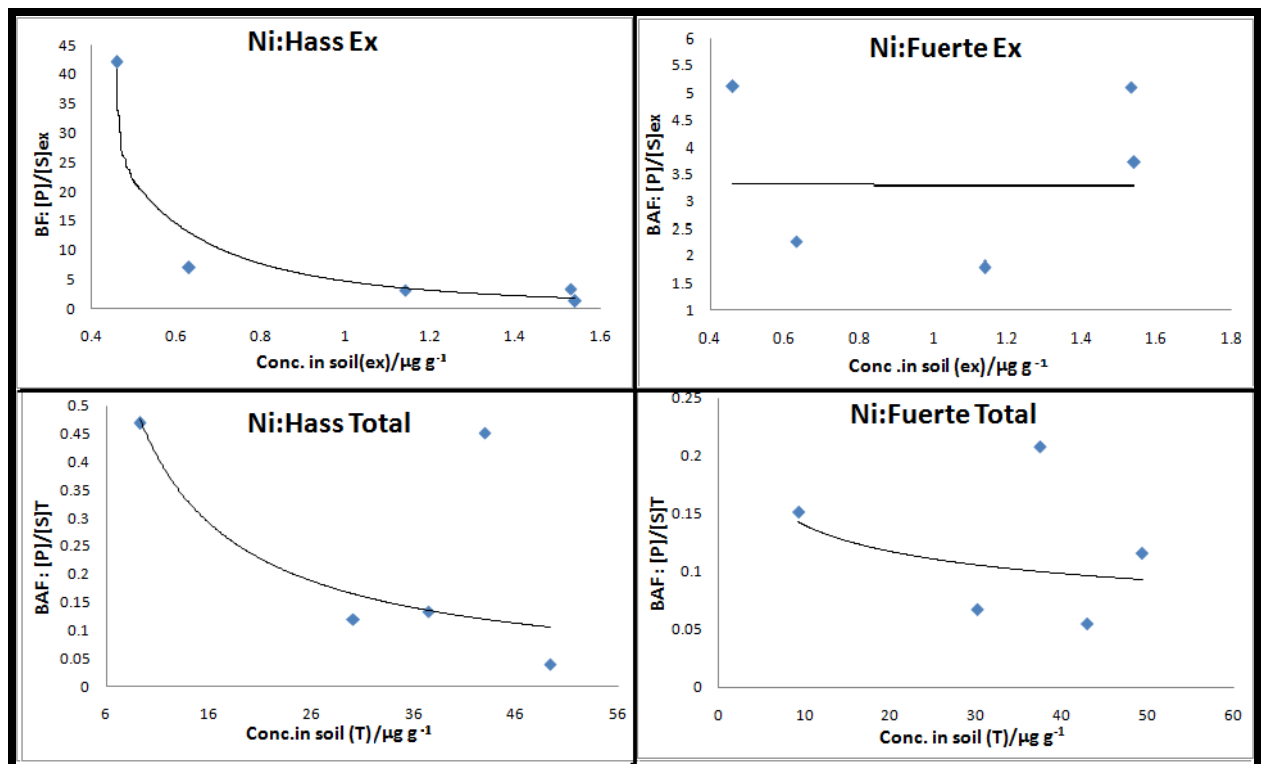


Fig.24 Bioaccumulation Factors (BF_{ex} , BF_T) vs. Total (T) and Exchangeable (ex) Concentrations of Ni in soil for Hass and Fuerte varieties.

Table 20. Zn concentrations in fruit and soil with bioaccumulation factors (BFs)

Sites	ZINC						
	Soil-total	Soil-exchangeable	[Soil] _{Ex} / [Soil] _T (%)	Hass Total	BF- Hass	Fuerte Total	BF-Fuerte
A	48±0.6 ^c	15.9 ± 0.7 ^c	33.3	10.28 ± 0.5 ^c	0.65	16.6 ± 0.5 ^c	1.04
B	106 ± 1.5 ^a	7.94 ± 0.17 ^d	7.50	9.69 ± 2.69 ^c	1.22	28 ± 1.0 ^a	3.52
C	58 ± 0.7 ^b	47.34 ± 0.23 ^a	82.3	17.7 ± 0.63 ^b	0.37	20.4 ± 0.4 ^b	0.43
D	9.6 ± 0.3 ^e	7.20 ± 0.21 ^d	73.1	29.2 ± 1.4 ^a	4.06	13.1 ± 0.4 ^c	1.82
E	38.3 ± 0.41 ^d	28.73 ± 1.31 ^b	75.0	24.06 ± 1.4 ^a	0.84	19.3 ± 0.1 ^b	0.67
F	37.26 ± 5.22 ^d	15.98 ± 1.0 ^c	42.9	27 ± 1.3 ^a	1.69	8.9 ± 0.4 ^d	0.51

Mean values in each column followed by a different superscript letter are significantly different by Duncan's multiple range test ($P \leq 0.05$).

Site: A = Kranskop, B = Seven Oaks, C = Howick, D = Thornville, E = Richmond, F = Ixopo

Zinc is widely known to be essential for avocado growth and production (Crowley and Smith, 1996) and relative accumulation plots below confirm this statement. Site C has the highest soil exchangeable concentration of Zn but the lowest BF values for both varieties. This observation suggests the accumulation of Zn according to the plants requirements.

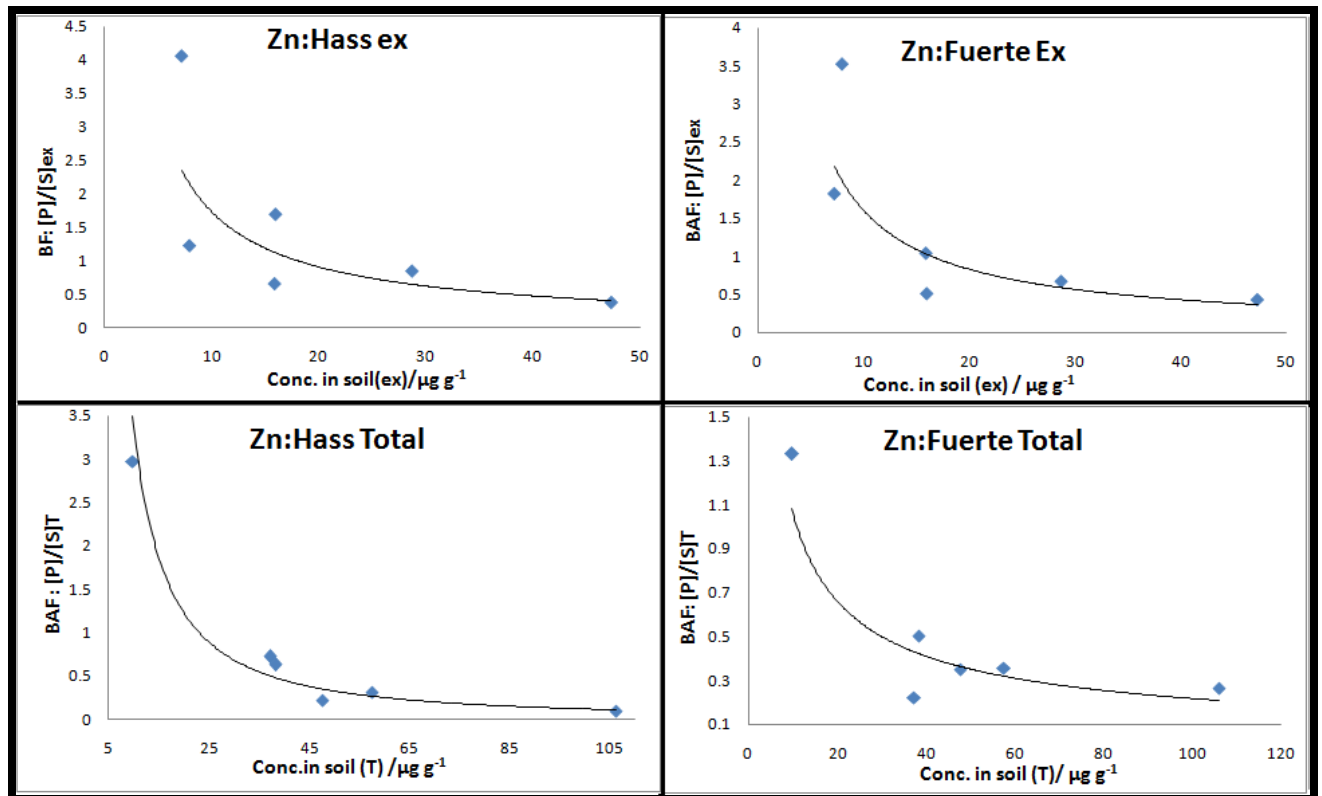


Fig. 25 Bioaccumulation Factors (BF_{ex}, BF_T) vs. Total (T) and Exchangeable (ex) Concentrations of Zn in soil for Hass and Fuerte varieties.

The relative accumulation plots (BF vs. Total or Exchangeable soil concentration) for Hass and Fuerte varieties revealed essentiality for the following metals: Ca, Cu, Fe, Mn, Ni, and Zn. Plots generated using exchangeable soil concentrations better represented physiological requirement levels of the plant than plots using total soil concentrations. This observation confirms that exchangeable soil concentrations are more accurate indicators of metal uptake into plants.

5.4 Soil Quality Assessment

5.4.1 SOM, CEC and pH

The measured soil properties, SOM, CEC and pH for the various sites are represented in Table 21. Soil pH was relatively constant (5.39 to 6.06). Soil management practices ensuring slightly acidic soils promote optimal growth of the crop (Wager, 1940). The SOM of the soils ranged from 4-9% with site B having the lowest value and site C having the highest. The measured CEC values ranged from 8 to 20 meq/100g in the soils. There seems to be no obvious relationship between the three soil properties. Results from the correlation analysis will therefore confirm any meaningful relationships.

Table 21. pH, soil organic matter (SOM), and cation exchange capacity (CEC) of soil samples from each site (n = 3).

Site	Soil pH	SOM (%)	CEC(meq/100 g)
A	6.05 ± 0.04	5.70 ± 0.01	13.5 ± 0.12
B	5.39 ± 0.03	4.64 ± 0.07	17.3 ± 2.02
C	5.97 ± 0.03	8.28 ± 0.01	19.6 ± 1.03
D	5.97 ± 0.02	6.20 ± 0.08	21.8 ± 1.78
E	5.45 ± 0.02	6.70 ± 0.26	9.33 ± 0.54
F	5.99 ± 0.02	5.18 ± 0.01	8.02 ± 0.36

Site: A = Kranskop, B = Seven Oaks, C = Howick,

D = Thornville, E = Richmond, F = Ixopo

5.4.2 Geoaccumulation Index

The background concentration conveys an idea of the natural range in concentration that can be expected prior to contamination and it can be used to assess for pollution. Herselman et al. derived background/baseline metal concentrations in South African soils from 4500 top soils. The soil samples were analysed for their total metal concentration using aqua regia (Herselman et al., 2005).

The evaluation of the status of heavy metal enrichment/pollution at the various sampling sites was made by examining the geo-accumulation Index (I_{geo}) and background concentrations which is shown in Table 22. Negative I_{geo} values, as for Cd, Co, Cr, Cu, Ni and Zn, indicate no enrichment of soil by the metals. Positive I_{geo} values, as for Pb, indicate enrichment of soil by the metal. However the degree of enrichment at all sites is moderate since I_{geo} values are less than 1.

Table 22. Total Baseline Concentrations of metals in South African soils ($\mu\text{g g}^{-1}$), total concentration of soils ($\mu\text{g g}^{-1}$), and geoaccumulation index (I_{geo}) for each site.

Metal	TBC*	Site A		Site B		Site C		Site D		Site E		Site F	
		Soil (T) ($\mu\text{g g}^{-1}$)	I_{geo}	Soil (T) ($\mu\text{g g}^{-1}$)	I_{geo}	Soil (T) ($\mu\text{g g}^{-1}$)	I_{geo}	Soil (T) ($\mu\text{g g}^{-1}$)	I_{geo}	Soil (T) ($\mu\text{g g}^{-1}$)	I_{geo}	Soil (T) ($\mu\text{g g}^{-1}$)	I_{geo}
Cd	2.7	2.48	-0.7	1.81	-1.2	2.29	-0.8	1.84	-1.1	1.99	-1.0	2.45	-0.7
Co	69	3.26	-5	27.2	-1.9	13.8	-3	31.9	-1.7	20.9	-2.3	20.7	-2.3
Cr	353	93.3	-2.5	166.6	-1.7	150.5	-1.8	170.2	-1.6	145.8	-1.9	118.6	-2.2
Cu	117	72.8	-1.3	78.7	-1.2	47.9	-0.6	76.5	-1.2	112.9	-0.6	48.5	-1.9
Ni	159	9.30	-4.7	49.36	-2.3	37.47	-2.7	42.95	-2.5	41.22	-2.5	30.15	-3.0
Pb	65.8	121.8	0.3	179.9	0.9	167.7	0.8	195.8	1.0	198.9	1.0	152.6	0.6
Zn	115	47.67	-1.9	106.0	-0.7	57.51	-1.6	9.85	-4.1	38.31	-2.2	37.26	-2.2

* Total Baseline Concentration (Herselman et al, 2005)

Where Site: A = Kranskop, B = Seven Oaks, C = Howick, D = Thornville, E = Richmond, F = Ixopo

5.5 Statistical Analysis

A composite correlation matrix for the concentrations of the elements in the fruit (Hass and Fuerte) with soil concentrations (total and exchangeable) is presented in Table 23. The information extracted from the correlation matrix was used to discuss the positive and negative correlations that exist between the cations in the soil and the fruit. Relationships with correlation coefficients >0.8 are strongly synergistic, between 0.7 to 0.8 are positive, < -0.8 are strongly antagonistic and between -0.7 to -0.8 are antagonistic. It should be noted that the results obtained in this study are indicative and are limited to the number of samples and sites that were analysed.

A perusal of Table 4 shows various significant positive and negative correlations between soil properties SOM, CEC and pH and elements taken up by the plant. A strongly positive correlation (0.8) is observed between soil pH and soil exchangeable Ca. However a strongly negative correlation (-0.8) is observed between soil pH and Ca concentration in fruit (Hass). An increase in soil pH increases soil exchangeable Ca concentrations but reduces the uptake of Ca into the avocado fruit of the Hass variety. This effect is not observed in Fuerte variety. SOM is shown to have a strongly positive correlation with exchangeable Ni concentrations. CEC is seen to have the most relationships with elemental concentrations in fruit as it correlates positively with Ca, Mg, Mn and Ni concentrations in fruit. There are no observed inter-correlations amongst the three soil properties measured except between pH and CEC which shows an unexpected negative correlation (-0.7).

Table 23: Correlation matrix for concentrations of elements in Soil Total (T) and Exchangeable (E)] and mesocarp of Hass (H) and Fuerte (F)

	AIT	AIE	AIH	AIF	AsI	AsE	AsH	AsF	CaI	CaE	CaH	CaF	CuI	CuE	CuH	CuF	FeI	FeE	FeH	FeF	MgI	MgE	MgH	MgF	MnI	MnE	MnH	MnF	NI	NE	NH	NF	ZnI	ZnE	ZnH	ZnF	pH	SOM		
AIE	-0.1																																							
AIF	-0.9	-0.1																																						
AsI	0.9	-0.5	-0.7	-0.7																																				
AsE	0.3	0.2	-0.1	-0.3	0.1																																			
AsH	0.5	0.0	-0.6	-0.7	0.5	0.2																																		
AsF	0.8	-0.2	-0.8	-0.7	0.7	0.2	0.8																																	
CaI	0.5	0.0	-0.4	-0.3	0.2	0.5	0.5	0.8																																
CaE	0.1	0.0	-0.1	-0.2	0.1	0.6	0.7	0.6	0.7																															
CaH	0.4	0.3	-0.7	-0.6	0.3	-0.5	0.1	0.2	-0.1	-0.5																														
CaF	0.0	0.3	-0.4	-0.4	0.0	-0.3	0.7	0.3	0.1	0.3	0.4																													
CuI	0.6	0.1	-0.4	-0.3	0.3	0.6	-0.2	0.3	0.5	0.0	0.1	-0.6																												
CuE	0.8	0.0	-0.5	-0.6	0.6	0.8	0.3	0.6	0.7	0.5	-0.1	-0.3	0.8																											
CuH	0.2	-0.8	0.1	0.0	0.6	0.2	0.2	0.2	-0.1	0.2	-0.5	-0.3	-0.1	0.3																										
CuF	0.1	-0.3	-0.2	-0.1	0.3	-0.8	-0.3	-0.1	-0.5	-0.8	0.7	0.0	-0.1	-0.4	0.0																									
FeI	0.8	-0.3	-0.8	-0.7	0.8	-0.3	0.4	0.7	0.2	-0.3	0.7	0.1	0.2	0.3	0.2	0.7																								
FeE	0.0	0.9	-0.3	-0.3	-0.4	0.2	0.1	0.0	0.2	0.1	0.4	0.5	0.1	0.0	-0.9	-0.3	-0.2																							
FeH	-0.2	-0.9	0.4	0.4	0.2	-0.3	0.0	-0.1	-0.3	0.0	-0.5	-0.2	-0.4	-0.3	0.8	0.2	0.1	-0.9																						
FeF	-0.5	-0.7	0.7	0.7	-0.1	-0.5	-0.4	-0.4	-0.5	-0.3	-0.4	-0.3	-0.5	-0.5	0.5	0.4	-0.1	-0.8	0.9																					
MgI	0.9	-0.3	-0.7	-0.7	0.8	0.5	0.6	0.9	0.7	0.5	0.0	0.0	0.5	0.9	0.4	-0.2	0.6	-0.2	0.0	-0.4																				
MgE	-0.2	-0.9	0.3	0.4	0.2	-0.3	0.2	0.1	0.0	0.2	-0.5	0.0	-0.5	-0.2	0.7	0.0	0.0	-0.8	0.9	0.8	0.1																			
MgH	0.6	0.6	-0.6	-0.8	0.3	0.5	0.2	0.2	0.1	-0.1	0.5	0.0	0.5	0.5	-0.3	-0.1	0.3	0.6	-0.7	-0.9	0.4	-0.8																		
MgF	0.6	0.6	-0.8	-0.8	0.3	0.0	0.3	0.4	0.2	-0.1	0.8	0.4	0.3	0.2	-0.6	0.2	0.5	0.7	-0.8	-0.8	0.3	-0.8	0.8																	
MnI	0.9	-0.3	-0.9	-0.8	0.9	0.0	0.4	0.7	0.3	-0.1	0.5	0.0	0.5	0.6	0.3	0.4	0.9	-0.2	0.0	-0.3	0.8	-0.1	0.5	0.5																
MnE	0.8	-0.3	-0.6	-0.6	0.8	0.6	0.7	0.9	0.7	0.7	-0.2	0.1	0.4	0.8	0.5	-0.4	0.4	-0.2	0.0	-0.4	1.0	0.2	0.3	0.2	0.6															
MnH	-0.3	0.5	0.1	-0.1	-0.2	-0.3	-0.3	-0.6	-0.8	-0.7	0.5	0.2	-0.3	-0.5	-0.3	0.4	-0.1	0.3	-0.2	-0.1	-0.6	-0.5	0.4	0.3	-0.2	-0.6														
MnF	0.2	0.3	-0.6	-0.6	0.3	-0.3	0.8	0.4	0.0	0.2	0.5	0.9	-0.5	-0.2	-0.1	0.1	0.4	0.3	-0.2	-0.4	0.1	-0.1	0.2	0.6	0.3	0.2	0.3													
NI	0.8	-0.2	-0.9	-0.7	0.8	-0.2	0.4	0.7	0.3	-0.2	0.7	0.2	0.3	0.3	0.1	0.6	1.0	0.0	-0.1	-0.3	0.6	-0.1	0.4	0.6	0.9	0.5	-0.1	0.4												
NE	0.1	-0.2	-0.3	0.0	0.0	-0.7	0.1	0.4	0.3	-0.1	0.5	0.4	-0.1	-0.3	-0.3	0.5	0.5	0.1	0.0	0.1	0.0	0.2	-0.4	0.2	0.2	-0.1	-0.3	0.3	0.5											
NH	0.1	0.0	-0.1	-0.3	0.4	-0.1	0.0	-0.3	-0.7	-0.5	0.2	-0.1	-0.2	-0.1	0.4	0.4	0.2	-0.3	0.2	0.1	-0.1	-0.1	0.4	0.1	0.2	0.0	0.7	0.3	0.1	-0.5										
NF	0.0	0.4	-0.4	-0.3	-0.1	-0.7	0.3	0.1	-0.1	-0.2	0.7	0.8	-0.5	-0.5	-0.6	0.4	0.3	0.5	-0.3	-0.2	-0.3	-0.2	0.1	0.6	0.1	-0.3	0.4	0.8	0.4	0.6										
ZnI	-0.1	0.3	-0.2	0.1	-0.3	-0.5	-0.3	0.0	0.2	-0.3	0.6	0.2	0.1	-0.4	-0.8	0.4	0.2	0.5	-0.4	-0.1	-0.3	-0.3	-0.1	0.3	0.0	-0.5	0.0	0.0	0.2	0.8	-0.6	0.6								
ZnE	0.1	0.4	-0.3	-0.4	0.0	0.3	0.8	0.5	0.6	0.8	0.0	0.8	-0.3	0.1	-0.2	-0.6	-0.1	0.5	-0.3	-0.5	0.3	0.0	0.1	0.3	-0.1	0.4	-0.2	0.6	0.0	0.1	-0.4	0.4	0.0							
ZnH	0.4	-0.6	-0.2	-0.3	0.8	0.2	0.5	0.4	0.0	0.3	-0.3	-0.1	-0.1	0.4	0.9	0.0	0.3	-0.7	0.6	0.2	0.6	0.6	-0.1	-0.3	0.5	0.7	-0.2	0.1	0.3	-0.3	0.5	-0.4	-0.8	0.0						
ZnF	0.3	0.6	-0.6	-0.4	-0.1	-0.1	-0.1	0.3	0.4	-0.2	0.7	0.2	0.5	0.1	-0.8	0.2	0.3	0.8	-0.8	-0.6	0.0	-0.7	0.5	0.8	0.3	-0.1	0.1	0.2	0.4	0.5	-0.4	0.5	0.8	0.1	-0.7					
pH	-0.4	-0.1	0.5	0.4	-0.4	0.3	0.3	0.1	0.4	0.8	-0.8	0.2	-0.3	0.0	0.1	-0.8	-0.6	0.0	0.2	0.1	0.0	0.4	-0.5	-0.5	-0.6	0.2	-0.6	-0.1	-0.6	-0.1	-0.6	-0.2	-0.2	0.6	0.0	-0.4				
SOM	0.3	-0.6	-0.3	-0.1	0.4	-0.5	0.3	0.6	0.4	0.1	0.3	0.3	-0.1	-0.1	0.1	0.4	0.6	-0.3	0.3	0.3	0.3	0.5	-0.5	0.0	0.4	0.2	-0.6	0.2	0.6	0.9	-0.5	0.3	0.5	0.1	0.1	0.2	0.0			
CEC	0.2	0.5	-0.4	-0.5	0.1	-0.3	0.1	-0.1	-0.5	-0.5	0.8	0.4	-0.2	-0.2	-0.4	0.5	0.3	0.4	-0.4	-0.4	-0.2	-0.6	0.6	0.7	0.3	-0.2	0.9	0.6	0.3	-0.1	0.7	0.6	0.1	0.0	-0.1	0.3	-0.7	-0.3		

AIT - [Soil Al]_{Total}
 AIE - [Soil Al]_{Exchangeable}
 AIH - [Al]_{Hass}
 AIF - [Al]_{Fuerte}
 SOM - Soil Organic Matter
 CEC - Cation Exchange Capacity

The phenomenon of synergism between elements occurs when two elements compete for the same soil adsorption site (Prasad et al. 2006). A synergistic relationship was observed when an increase in total soil concentration of one element increased availability of another (Prasad et al. 2006). Synergistic relationships in soil are represented in Fig.26. Arrows represent a synergism between total and exchangeable soil concentrations (shown in boxes) of the two elements with their respective r values indicating the degree of the synergy. Figure 26 shows Mn to have strong synergistic relationship with Mg ($r = 1.0$), Al, As and Ca while Cu is synergistic with Mg, Al and Ca. A synergy between Ni and Zn ($r = 0.8$) was detected. The correlation analysis also revealed exchangeable Ca and Cu to be significantly correlated to their total soil concentrations.

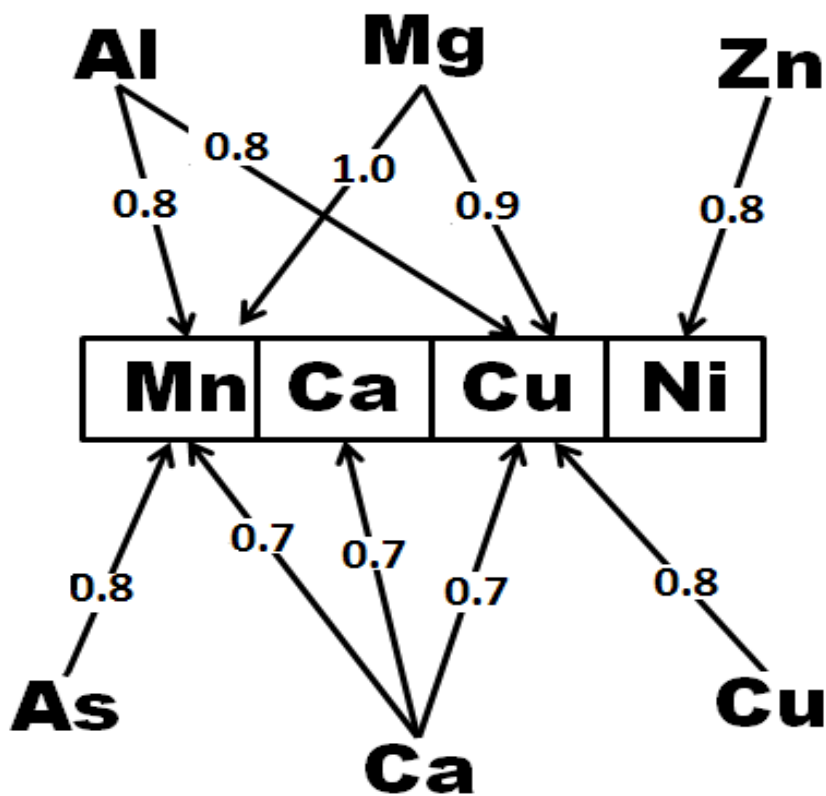


Fig.26 Synergistic relationships in soil.

Antagonism amongst elements occurs when the plant takes up two different elements by the same mechanism (Kalavrouziotis et al. 2008). A negative relationship exists when an increase in exchangeable soil concentration of one element reduces uptake of the other. Research with coffee has shown antagonism to be cultivar dependant (Enes Jr et al., 2009). Since the mechanisms of uptake may be different between dissimilar varieties of fruits, antagonistic relationships observed for Hass and Fuerte varieties, represented in Fig.27 were compared. Figure 27 shows statistical evidence of a strongly antagonistic correlation between Al with Fe and Cu in the Hass variety indicating high exchangeable soil Al and Fe reduces uptake of Cu. Antagonistic relationships exist between Fe with Cu and Zn, Ca with Mn and Mg with itself in Hass variety. The only common antagonistic relationship between Hass and Fuerte varieties was observed for Al and Mg. Exchangeable soil Ca and As both negatively influence the uptake of Cu into the Fuerte variety and the same effect is shown with exchangeable soil Al and Fe on uptake of Fe.

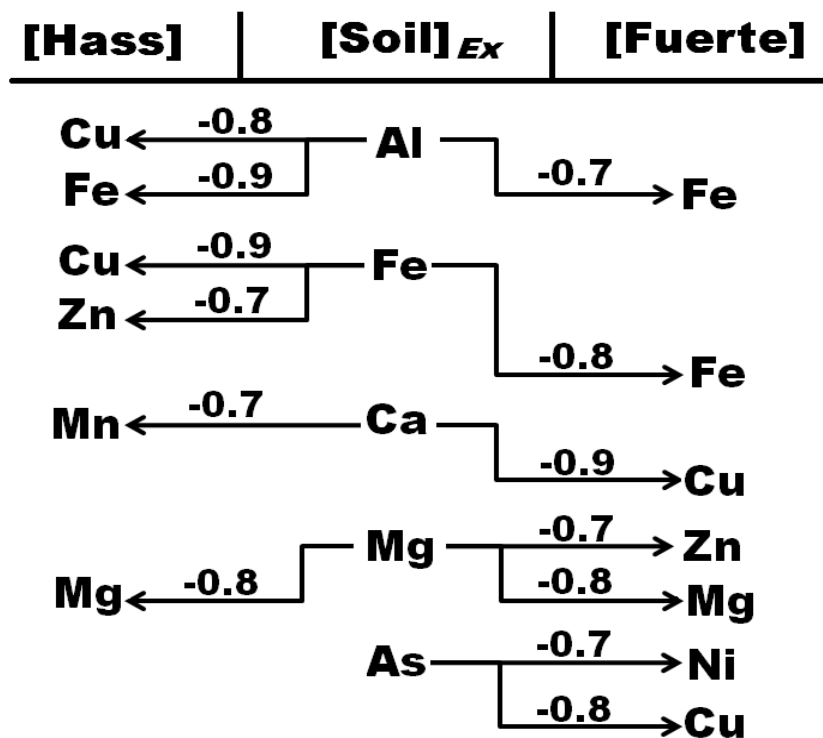


Fig.27 Antagonistic relationships between exchangeable elemental concentrations and elemental uptake into the Hass and Fuerte fruit.

5.6 Dietary Reference Intakes

An average serving of fresh avocado pulp, approximately one and a half pears, is equivalent to 270 g fresh pulp and when dried, amounts to an estimated 100g of dry weight. The results in Table 24 show the estimated contribution of 100 g (DW) of avocado mesocarp (average serving size) to the RDA. One serving of avocado is estimated to contribute more than 3%, 65%, 10%, 28%, 32%, and 18% towards the RDA for As, Ca, Cu, Fe, Mg, Mn, and Zn, respectively in most adults. None of the elements have concentrations in the fruit exceeding the Upper Intake Levels (UL) and even consumption of twice the average serving is still considered safe.

The results suggest that an individual deficient in Cu should consume avocados frequently with preference to the Fuerte variety as it has a higher contribution of Cu (75%) to the RDA than the Hass variety (66%). However, the Mn contribution to the RDA is higher in the Hass variety (58%) than the Fuerte variety (34%) making Hass a preference for individuals with Mn deficiency. The contributions to the RDA for the remaining nutrients (Ca, Fe, Mg, and Zn) seem to be similar between the two varieties. The maximum limit for metals in fruits and vegetables set by the Department of Health, South Africa, is $0.1 \mu\text{g g}^{-1}$ for Pb (Dept. of Health, 2004). Avocado flesh contained Pb concentrations below the instruments detection levels of $0.09 \mu\text{g g}^{-1}$ and therefore is considered safe in terms of toxicity effects of Pb.

The maximum limit for As in fruits is not listed by South African Department 2004 document per se, however the lowest limit for As in foodstuff is $0.1 \mu\text{g g}^{-1}$ as listed for edible fats and oils and highest limit is $0.5 \mu\text{g g}^{-1}$ as listed for fish. The As concentrations found in the avocado mesocarp is within this range of limits and therefore causes no alarm in terms of toxicity. With concern to the involuntary uptake mechanism of As displayed by

the plant in earlier relative accumulation plots data, it is fortunate that edible portions of plants seldom accumulate dangerous levels of As because phytotoxicity occurs before such levels are reached (Walsh et al., 1977). The highest concentrations of As are found in plant roots, moderate levels in vegetative tissue and the lowest levels in reproductive tissue (Walsh et al., 1977).

Table 24: Dietary Reference Intake (DRIs), Recommended Dietary Allowance (RDA) and Tolerable Upper Intake Levels (UL) of elements for most individuals^a and average concentration of elements (n = 6) in mesocarp of avocado (Hass and Fuerte varieties).

	Average Concentration (mg/100 g DM) Hass	Average Concentration (mg/100 g DM) Fuerte	DRI (mg/day)		Estimated Contribution to RDA (%) Hass	Estimated Contribution to RDA (%) Fuerte
			RDA	UL		
As	0.38	0.31	ND ^b	ND	ND	ND
Ca	40.84	35.32	1000-1300	2500	4	3
Cu	0.59	0.67	0.9	8	66	75
Fe	1.92	1.24	8-18	45	14	10
Mg	99.13	92.32	310-320	350	32	29
Mn	1.16	0.67	1.6-2.3	9	58	34
Ni	0.69	0.39	ND	1.0	ND	ND
Zn	1.96	1.76	8-11	34	20	18

^a Institute of Medicine of the National Academies: Dietary Reference Intakes (2001).

^b ND = not determined due to lack of data.

CHAPTER 6

CONCLUSION

Firstly, the effect of five different extraction methods on the yield and quality of avocado oil produced was investigated. Oils obtained from the different extraction methods were subjected to fatty acid analysis to obtain a fatty acid profile for assessment of nutritional value. The quality of oils was assessed by determination of the concentration of selected metals in oils and calculation of dissolution percentages. An extraction method that produces oils containing low amounts of metals (indicated by low dissolution percentages) is preferred. SFE and microwave extraction co-extracted the lowest amount of oxidising metals. Microwave extraction produced the highest oil yield (69.9%) and amounts of FAs while SFE produced a wider range. SFE is more adapted to produce oils for pharmaceutical industries, since the method is free from solvent and yields an undiluted product. Except for Fe and Zn, the Fuerte mesocarp possessed higher concentrations of elements than Hass. The Fuerte variety is found to be healthier than Hass variety, as it had the highest MUFA: SFA ratio.

Secondly, an investigation into the impact of soil quality on the nutritional content of avocado fruit was undertaken. Avocado fruit is shown to have negligible concentrations of Cd, Co, Cr, Pb and Se; hence it is not an accumulator of these metals. The concentration of elements in both varieties of fruit was, generally, in the decreasing order of $Mg > Ca > Al > Zn > Fe > Mn > Cu > Ni > As$.

Relative accumulation plots revealed the toxic metal As to be essential. This was interpreted as evidence of the plant's involuntary uptake of As due to chemical similarities to P. However, the uptake of As into the fruit was at concentrations well below critical

concentrations for human consumption. The plant displayed controlled uptake as evidenced by the accumulation and exclusion of specific elements such as Cu, Fe and Ca, to meet its physiological requirement levels.

Soils are moderately enriched with Pb as shown by positive geoaccumulation indices but this enrichment does not influence uptake of Pb into the avocado fruit. Statistical analyses revealed the influence of complex metal interactions between the plant-soil interface on the uptake of metals into both varieties of fruit. CEC is seen to have the greatest effect on specific metal uptake (Ca, Mg, Mn and Ni) followed by pH and SOM. The latter has a higher impact on exchangeable soil concentrations which influences antagonistic relationships exhibited by the plant. It was observed that the apparent abundance of the major elements in soil (Al, Fe, Ca and Mg) has strong antagonistic influences over the minor elements and hence partially control the uptake of specific metals.

Locally grown avocado fruit is a good dietary source of the micronutrients Cu and Mn. Although low levels of As was found in the fruit, concentration levels of As in soil and plant should be monitored due to it being an analogue of P and thereby being taken up by plant. The outlined objectives for this study focused on the nutritional assessment of oil and whole fruit and the impacts of soil quality of the uptake of nutrients into the fruit. These were successfully achieved. It should be noted that the results obtained in the study are indicative and are limited to the number of samples and sites that were analysed.

Further Work

1. Feasibility study of the microwave extraction method for large scale oil extraction from avocado culls.
2. Elemental distribution in other varieties of avocados.
3. A detailed study on compositional and quality analysis of oils from avocados.
4. Investigations of elemental distribution in various food stuff.
5. Speciation analysis of the elements studied in this dissertation.
6. Comparative study of avocado oils from South Africa with those from other countries.

REFERENCES

Abdulla M., Parr R.M. and Iyengar G.V. Trace element requirements, intake and recommendations. *Progress Clin Biol Res.* 1993;380:311-328.

Abraham G.M.S. and Parker R.J. Assessment of heavy metal enrichment factors and the degree of contamination in marine sediments from Tamaki Estuary, Auckland, New Zealand *Environ Monit Assess.* 2008;136:227-238.

Adolfo C.C., Klaudia O.L., Jorge E.B. and Claudia H.M. Exploring the effect of changes in land use on soil quality on the eastern slope of the Cofre de Perote Volcano (Mexico). *For Ecol Manage.* 2007;248:174- 82.

Aghasi B., Jalalian A. and Honarjoo N. Decline in soil quality as a result of land use change in Ghareh Aghaj watershed of Semrom, Isfahan, Iran. *African J Agric Res.* 2011;6(4):992-7.

Ahiamadjie H., Adukpo O.K., Tandoh J.B., Gyampo O., Nyarku M., Mumuni I.I., et al. Determination of the Elemental Contents in Soils Around Diamond Cement Factory. *Aflao Res J Environ Earth Sci.* 2011;3(1):46-50.

Aikpokpodion P.E., Lajide L. and Aiyesanmi A.F. Heavy Metals Contamination in Fungicide Treated Cocoa Plantations in Cross River State, Nigeria. *Am-Eurasian J Agric Environ Sci.* 2010;8(3):268-74.

Alloway B.J. *Heavy Metals in Soils.* Glasgow, Scotland: Blackie Academic and Professional; 1995.

Alvizouri M.M., Carranza M.J., Madrigall J.E., Herrera A.J., Chavez C.F. and Amezcua G.J. Effects of avocados as a source of monounsaturated fatty acids on plasma lipid levels. *Arch Med Res* 1992;23(4):163-7.

Al-Yemeni M.N. and Hashem A.R. Heavy metals and microbial analysis of soil samples collected from Aramco Gulf operations company Al-Khafji, Saudi Arabia. *Saudi J Biol Sci.* 2006;13(2):129-33.

Amunziata C.M., Massaro M. and Siculella L. Oleic acid inhibits endothelial activation. *Arterioscler, Thromb, Vasc Biol.* 1999;19:220-8.

Arinbruster D.A., Tillman M.D., and Hubbs L.M. Limit of Detection (LOD) / Limit of Quantification (LOQ): Comparison of the empirical and the statistical methods exemplified with GC-MS assays of abused drugs. *Clinical Chem.* 1994;40(7):1233-38.

Arzt W.E. and Roa S.K. Lipid oxidation in extruded products. *ACS Sym Ser.* 1994;543:296-314.

Awad M. and Young R.E. Postharvest variation in cellulase, polygalacturonase, and pectinmethylesterase in avocado (*Persea americana* Mill. cv. Fuerte) fruits in relation to respiration and ethylene production. *Plant Physiol.* 1979;64:306-8.

Beckett P.H.T. The use of extractants in studies on trace metals in soils, sewage sludges and sludge-treated soils. *Adv Soil Sci.* 1989;9:143-179.

Bergbäck B., Johansson K. and Mohlander U. Urban metal flows-a case study of Stockholm. *Water, Air, Soil Poll.* 2001;1:3-24.

Bergh B. Nutritious Value of Avocado *Calif Avocado Soc Yrbk.* 1992;76:123-35.

Bhattacharya P., Samal A.C., Majumdar J. and Santra S.C. Transfer of Arsenic from Groundwater and Paddy Soil to Rice Plant (*Oryza sativa* L.): A Micro Level Study in West Bengal, India. *World J Agri Sci.* 2009;5(4):425-31.

Biale J.B. and Young R.E. In *The Biochemistry of Fruits and Their Products*. New York: Academic Press; 1971.

Bigdeli M. and Seilsepour M. Investigation of metal accumulation in some vegetables irrigated with waste water in Shahre Rey–Iran and toxicological implications. *J Agric Environ Sci.* 2008;4:86-92.

Bizimana, V., Breene W.M. and Csallany A.S. Avocado Oil Extraction with Appropriate Technology for Developing Countries. *JAOCS.* 1993;70(8):821-22.

Bloom P.R., McBride M.B. and Weaver R.M. Aluminum organic matter in acid soils: buffering and solution aluminum activity. *Soil Sci Soc Am J.* 1979;43:488-93.

Board M., Colquhoun A. and Newsholme E.A. High K_m glucosephosphorylating (glucokinase) activities in a range of tumor cell lines and inhibition of rates of tumor growth by the specific enzyme inhibitor mannoheptulose. *Cancer Res.* 1995;55(15):3278–85.

Boss B.C. and Fredeen K.J. *Concepts, Instrumentation and Techniques in Inductively Coupled Plasma Optical Emission Spectrometry*, USA: The Perkin Elmer Corporation; 1997

Botha B.M. The supercritical fluid extraction of avocado oil. D. Tech. thesis, Pretoria, Technikon Pretoria. 2002.

Bremner J.M. Determination of nitrogen in soil by Kjeldahl method. *J Agri Sci.* 1960;55:11-33

Brooks R.R., Lee J., Reeves R.D. and Jaffre T. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J Geochem Explor* 1977;7:49-57.

Brown H.E. *Zinc Oxide Rediscovered*. New York: The New Jersey Zinc Company;1957

Brown P.H., Welch R.M. and Cary E.E. Nickel: A micronutrient essential for higher plants. *Plant Physiol.* 1987;85:801-3.

Bruckner C.A., Prazen B.J. and Synovec R.E. Comprehensive Two-Dimensional High-Speed Gas Chromatography with Chemometric analysis. *Anal Chem.* 1998;70:2796-2804.

Bye R. and Lund W. Optimal conditions for the reduction of selenate to selenite by hydrochloric acid. *Chem.* 1988; 332(3):242-44.

Campos C.A., Oleschko L. K., Etchevers B. J. and Hidalgo M. C. Exploring the effect of changes in land use on soil quality on the eastern slope of the Cofre de Perote Volcano (Mexico). *Forest Ecol Manag.* 2007;248:174-82.

Carbonell-Barrachina A.A, Burlo-Carbonell F., Lo´pez E., and Mataix- Beneito J. Tomato plant nutrition as affected by arsenite concentration. *J Plant Nutr.* 1998;21(2):235-44.

Carranza M.J., Herrera A.J., Alvizouri M.M., Alvarado J.M. and Cha´vez C.F. Effects of a vegetarian diets vs. a vegetarian diet enriched with avocado in hypercholesterolemic patients. *Arch Med Res.* 1997;28(4):537-41.

Carranza M.J., Herrera A.J., Alvizouri M.M., Alvarado J.M. and Cha´vez C.F. Effects of Avocado on Plasma Lipid Concentrations in Patients with Hypercholesterolemia. *Archivos del Instituto de Cardiolog´ıa: Mexico City.* 1995;65:342-8.

Carter M.R., Andrews S.S. and Drinkwater L.E. *Managing Soil Quality: Challenges in Modern Agriculture*. In: Schjonning P ES, Christensen BT editor. Wallingford: CABI International; 2004; 261-81.

Chapman H.D. Methods of soil analysis. In: C.A. Black, editor. Madison: Am. Soc. Of Agron., Inc. ; 1965;891-901.

Chemat S., Lagha A., AitAmar H., Bartels P.V. and Chemat F. Comparison of conventional and ultrasound-assisted extraction of carvone and limonene from caraway seeds. *Flav Frag J.* 2004;19:188–95.

Chunilall V., Kindness A. and Jonnalagadda S.B. Heavy metal uptake by spinach leaves grown on contaminated soils with lead, mercury, cadmium, and nickel. *J Environ Sci Health B.* 2004; 39(3):473-81.

Clifford A.A. Analytical Supercritical Fluid Extraction Techniques. In: Ramsey ED, editor. Dordrecht: Kluwer; 1998.

Colquhoun M.D., Moores D., Somerset M.S. and Humphries A.J. Comparison of the effects on lipoproteins and apolipoproteins of diet high in monounsaturated fatty acids, enriched with avocado, and a high-carbohydrate diet. *Am J Clin Nutr.* 1992;56(4):671-7.

Creger T.L. and Peryea F.J. Phosphate fertilizer enhances arsenic uptake by apricot liner grown in lead-arsenate-enriched soil. *Hort Science.* 1994;29(2):88-92.

Crowley D.E. and Smith W. Zinc Fertilization of Avocado Trees. *Hort Sci.* 1996;31(2):224-9.

Cummings K. and Schroeder C.A. Anatomy of the avocado fruit. *Yearb. Calif. Avocado Soc.* 1942;27:56-64.

Dalen G.V. Solid Phase Extraction of Cadmium in Edible Oils Using Zinc Piperazinedithiocarbamate and Its Determination by Flame Atomic Absorption Spectrometry. *J Anal Atom Spectrom* 1996;11:1087-92.

Davis A., Ruby M.V. and Bergstrom P.D. Factors controlling lead bioavailability in the Butte mining district, Montana, USA. *Environ Geochem Health*.1994;3(4):147-57.

Dean J.R. *Methods for environmental trace analysis*, England: John Wiley and Sons, West Sussex; 2003;185-191.

Dean J.R. *Practical inductively coupled plasma spectroscopy*. England: Wiley; 2005

Department of Environmental Affairs. *Framework of the Management of Contaminated Land*, Government White Paper, South Africa.2010

Department Of Health, *Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 Of 1972)*, Regulations Relating To Maximum Levels for Metals in Foodstuffs, Government Gazette, April 2004

Díaz-Zorita M. Soil Organic Carbon Recovery by the Walkley-Black Method in a Typic Hapludoll. *Commun Soil Sci Plant Anal*.1999;30(5-6):739-45

Dixon H.B.F. The biochemical action of arsenic acids especially as phosphate analogues. *Adv Inorg Chem* 1997;44:191-227.

Donkin D.J., editor. *Proceedings from the World Avocado Conference VI*, South African Subtropical Growers' Association. 2008; Tzaneen, South Africa.

Eggers R. *Supercritical fluid technology in oil and lipid chemistry*. In: Eggers R, King, J.W.; List, G.R., editor. United States of America: AOCS Press; 1996.

Enes Jr. F. and Martin E.P., editors. Coffee yield and nutrients uptake under different cultivars and plant populations. The Proceedings of the International Plant Nutrition Colloquium XVI, International Plant Nutrition Colloquium; 2009; South Africa.

Ernst W.H.O. Beurteilung von Schwermetallen in Böden von Ballungsgebieten: Arsen, Blei und Cadmium. DECHEMA; 1997:319-55

Eyres L., Edible Fats and Oils in the New Zealand Food industry. Food-G-Fats and Oils, Food-G-Fats and Oils, VI, 14, 2001.

Eyres L., Sherpa N. and Hendrinks G. Avocado Oil: new edible oil from Australasia. Lipid Technol, 2001;13,(4):84-88.

Ferrer J., Gomis R., Fernandez, Alvarez J., Casamitjana R. and Vilardell E. Signals derived from glucose metabolism are required for glucose regulation of pancreatic islet GLUT2 mRNA and protein. Diabetes Care. 1993;42(9):1273–80.

Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements, Washington, DC: National Academies Press; 2001

Fujihara S., Kasuga A. and Aoyagi Y. Nitrogen-to-protein conversion factors for common vegetables in Japan. J Food Sci. 2001;66:412–415.

Garbisu C. and Itziar A. Phytoextraction: a cost effective plant based technology for the removal of metals from the environment. Bioresource Technol. 2001;77:229-36.

Germ M., Stibilj V. and Kreft I. Metabolic Importance of Selenium for Plants. European J Plant Sci Biotechnol. 2007;1(1):91-97.

Gibbes H. and Chenn C. Evaluation of issues relating to carcinogens- risk assessment of chromium. Sci Total Environ. 1989;86(1):181-6.

Gordon T., Castelli W.P, Hjortland M.C., Kannel W.B., Dawber, T.R. High Density lipoprotein as a productive factor against coronary heart disease: The Framingham study. *Amer J Med.* 1977;62(5):702-714.

Grant, W.C. Influence of avocados on serum cholesterol. *Proc. Soc. Exper. Bioi. Med.* 1960. 104:45-47.

Grundy S.M. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. *N Engl J Med.* 1986;314:745–8.

Hall R. *Soil Essentials: Managing your farms Primary Asset.* Collingwood, Australia: Landlinks Press; 2008.

Hansch R. and Mendel R.R. Physiological functions of mineral micronutrients: (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Curr Opin Plant Biol* 2009;12:259– 66.

Harrison R.M. and De Mora S.J. *Introductory Chemistry for Environmental Sciences.* 2nd ed. Cambridge: Cambridge University Press; 1996. p. 194-5.

Hatton Jr. T.T., Harding P.L. and Reeder W.F. Seasonal changes in Florida avocados. *Proc Fla State Hortic Soc* 1964;72:349-53.

Hendrikse P.W. and Dieffenbacher A. Determination of phosphorus in oils and fats by direct graphite furnace atomic absorption spectrometry. *Pure Appl Chem.* 1991;63(8):1191-98.

Herbert S.J. and Hashemi M. *Interpreting Soil Analysis Results.* USA: University of Massachusetts; 2008.

Herselman J.E. *The Concentration of selected Trace Metals in south African Soils.* Stellenbosch: University of Stellenbosch; 2007.

Herselman J.E., Steyn C.E. and Fey M.V. Baseline concentration of Cd, Co, Cr, Cu, Pb, Ni and Zn in surface soils of South Africa: research in action. *S Afric J of Sci* 2005;101(12):509-12.

Hooda P.S., McNulty D., Alloway B.J. and Aitken M.N. Plant availability of heavy metals in soils previously amended with heavy applications of sewage sludge. *J. Food Agric.*1997;73:446-454.

Horneck D.A., Hart J.M., Topper K. and Koespell B. *Methods of Soil Analysis used in the soil testing laboratory at Oregon State University.* Oregon State University, Station AE; 1989.

Hornick S.B. Factors affecting the nutritional quality of crops. *Am J Altern Agri.* 1992;7:63-8.

Human T. P. Oil as a byproduct of the avocado. *S African Avocado Growers' Assoc Yrbk.* 1987;10:159-62.

Hurren D. Supercritical fluid extraction with CO₂. *Filtration and Separation.* 1999;36:25-7.

Ishizu T., Winarno H., Tsujino E., Morita T. and Shibuya H. Stereochemical structure of perseitol K⁺ complex isolated from the leaves of *Scurrula fusca* (Loranthaceae). *Chem Pharmaceut Bulletin.* 2002;50(4):489-92.

Jackson K.W. and Qiao H. Atomic absorption, atomic emission and flame emission spectrometry. *Analyt Chem.* 1992;64.

James B.R and Riha S.J. pH buffering in forest soil organic horizons: relevance to acid precipitation. *J Environ Qual.*1986;15:229-233.

James W.P.T., Duthie G.G. and Wahle K.W.J. The Mediterranean diet: protective or simply non-toxic? *Eur. J. Clin. Nutr.* 1989;43(2):31–42.

Johnsson L. Selenium uptake by plants as a function of soil type, organic matter content and pH. *Plant soil.*1991;133:57-64.

Jonnalagadda S.B., Rao P.V. Toxicity, bioavailability and metal speciation. *Comp Biochem Physiol C Rev.*1993;106:585-595

Jung M.C. Heavy Metal Concentrations in Soils and Factors Affecting Metal Uptake by Plants in the Vicinity of a Korean Cu-W Mine. *Sensors.* 2008;8:2413-23.

Kabata-Pendias A. and Pendias H. Trace Elements in Soils and Plants. 2nd ed. London: CRC Press; 1992.

Kabata-Pendias A. and Mukherjee A.B. Trace elements from soil to human. Berlin: Springer; 2007.

Kalavrouziotis I.K., Koukoulakis P.H., Robolas P., Papadopoulos A.H. and Pantazis V. Interrelationships of Heavy Metals Macro and Micronutrients, and Properties of a Soil Cultivated with *Brassica oleracea* var. *italica* (Broccoli), Under the Effect of Treated Municipal Wastewater. *J Water, Air and Soil Pol* 2008;190(1-4):309-21.

Kanchanamayoon W. and Kanenil W. Determination of Some Fatty Acids in Local Plant Seeds. *Chiang Mai J Sci.* 2007;34(2):249-252.

Kannamkumarath S.S., Wrobel K., Vonderheide A. and Caruso J.A. HPLC-ICP-MS determination of selenium distribution and speciation in different types of nuts. *Anal Bioanal Chem.* 2002;373:454-460.

Kanner J. and Rosenthal I. An Assessment of Lipid oxidation in Foods. *Pure and Appl Chem* 1964;64(12):1959 – 64.

Karlen D.L., Ditzler C.A. and Andrews A.S. Soil quality: Why and how? *Geoderma*. 2003;14:145-56.

Kaufmann B. and Christen P. Recent extraction techniques for natural products: Microwave-assisted extraction and pressurized solvent extraction. *Phytochem Anal.* 2002;13:105–13.

Kaufmann B., Christen P. and Vuthey J.L. Parameters affecting microwave-assisted extraction of withanolides. *Phytochem Anal.* 2001;12:327–31.

Keefer R.F., Singh R.N. and Horvath D.J. Chemical composition of vegetables grown on agricultural soils amended with sewage sludge. *J Environ Qual.* 1986;15:146-152.

Keys A. Mediterranean diet and public health: personal reflections. *Am. J. Clin. Nutr.* 1995; 61:1321S–1323S.

Kim D.Y., Lee Y., Lee J.K., Koo N. and Kim J. Growth Response and Arsenic Uptake of White Clover (*Trifolium repens*) and Evening Primrose (*Oenothera odorata*) Colonized with Arbuscular Mycorrhizal Fungi in Arsenic-Contaminated Soil. *Korean J Environ Agri.* 2008;27(1):50-9.

Kratchanova M., Pavlova E and Panchev I. The effect of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin. *Carb Polym.* 2004; 56:181–185.

Kwapuliński J., Mirosławski J., Wiechuła D., Rochel R., Burczyk J., Sowada B., et al. The use of ecotoxicological parameters for estimation of the quality of medicinal plant yielding areas. *Bromat Chem Toksykol.* 1996;29(3):243.

LaRosa J.C., Hunninghake D., Bush D., Criqui M.H., Getz G.S., Gotto A.M. Jr., Grundy S.M., Rakita L., Robertson R.M., Weisfeldt M.L., et al. The cholesterol facts. A summary of the evidence relating dietary fats, serum cholesterol, and coronary heart disease. *Circulation*.1990;81(5):1721-33.

Lee J., Koo N. and Min D. Reactive oxygen species, aging and antioxidative nutraceuticals. *Compreh Rev Food Sci Food safety*. 2004;1(3):21-33.

Leighton T. *The acoustic bubble*. London: Academic Press; 1994.

Lepp N.W. Copper. In *Effect of Heavy Metal Pollution on Plants*, Vol. 1. Lepp N.W. Editor. London: Applied Science Publications; 1981.111-143.

Lerman G.I., Ichazo C.S. and Zamora G.J. Effect of a high-monounsaturated fat diet enriched with avocado in NIDDM patients. *Diabetes Care*. 1994;17(4):311-5.

Li H., Pordesimo L. and Weiss J. High intensity ultrasound assisted extraction of oil from soybeans. *Food Res Int*. 2004;37:731-8.

Lopez L.R., Frati M.A., Hern´andez D.B., Cervantes M.S., Hern´andez L.M., Juarez C., et al. Monounsaturated fatty acid (avocado) rich diet for mild hypercholesterolemia. *Arch Med Res* 1996;27(4):519-23.

L´opez M.T. Phytosterols and phytostanols: their role in preventing cardiovascular disease. *OFFARM*, 2005; 24(4):90-94.

Loska K., Wiechuła D., Barska B., Cebula E. and A. Chojnecka. Assessment of Arsenic Enrichment of Cultivated Soils in Southern Poland. *Polish J Environ Stud*. 2003;12(2):187-92.

Luque de Castro M.D. and Garcia-Ayuso L.E. Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. *Analytica Chimica Acta*. 1998;369:1-10.

Luque-Garcia J.L. and Luque de Castro M.D. Ultrasound: A powerful tool for leaching. *Trends Anal Chem.* 2003; 22: 41–47.

Ma J. Plant Root Responses to Three Abundant Soil Minerals: Silicon, Aluminum and Iron. *Revs Pl Sci.* 2005;24(4):267-81.

Marin A.R., Pezeshki S.R., Masscheleyn P.H. and Choi H.S. Effect of dimethylarsenic acid (DMAA) on growth, tissue arsenic and photosynthesis in rice plants. *J Plant Nutr.* 1993;16(5):865-80.

Martin-Polvillo M., Albi T. and Guinda A. Determination of trace elements in edible vegetables oils by atomic absorption spectrophotometry. *J Am Oil Chem.* 1994;71:347-51.

Mason, T.J., Paniwnyk, L., and Lorimer, J.P. The uses of ultrasound in food technology. *Ultrasonics Sonochemistry*, 1996;3:253-60.

Mattigod S.V., Sposito G. and Page A.L. Chemistry in the soil environment. In: Baker DE, editor. *Madison: ASA Special Publication*;1981.

Mayland H.F. and Wilkinson S.R. Soil factors affecting magnesium availability in plant-animal systems: A review. *J Anim Sci.* 1989;67:3437-44.

McGrath S.P., Zhao F.J. and Lombi E. Phytoremediation of metals, metalloids, and radionuclides. *Adv in Agron* 2002;75:1-56.

McLaughlin M.J., Parker D.R. and Clarke J.M. Metals and micronutrients-Food Safety Issues. *Field Crops Research.* 1999;60:143–63.

McNeal J.M. and Balistrieri L.S. Selenium in agriculture and the environment. *Madison: Am Soc Agron.* 1989.

Meeta-Jain R.P, Gadre R.P. Effect of As on chlorophyll and protein contents and enzymatic activities in greening maize leaves. *Water Air Soil Pollut.* 1997;93:109 -15.

Mertz W., editor. Biological role of chromium. *Federation Proceedings*; 1967.

Metcalf L.D. and Schmitz A.A. The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal Chem.* 1961;33:363-364.

Miko S., Peh Z., Bukovec D., Prohic E. and Kastmüller Z. Geochemical baseline mapping and Pb pollution assessment of soils in the karst in Western Croatia. *Nat Croat.* 2000;9(1):41.

Moodley R., Kindness A. and Jonnalagadda S. B. Chemical composition of edible Macadamia nuts (*Macadamia integrifolia*) and impact of soil quality. *J Environ Sci Health P A.* 2007;42:2097-104.

Mossler M.A. and Nesheim N.O. Florida Crop/Pest Management Profile: Avocado. 2001.

Mulder J. and Cresser M.S. In *Biogeochemistry of Small Catchments: A Tool for Environmental Research.* John Wiley & Sons Ltd; 1994.

Muller G. Die Schwermetallbelastung der Sedimenten des Neckars und Seiner Nebenflüsse. *Chemiker-Zeitung.* 1981;6:157.

Müller G. Index of geoaccumulation in sediments of the Rhine River. *Geojournal.* 1969;2:108.

Murillo M., Benzo Z., Marcano E., Gomez C., Garaboto A. and Marin C. Determination of copper, iron and nickel in edible oils using emulsified solutions by ICP-AES. *J Anal Atomic Spec.* 1999;14:815-20.

Nash A.M., Mounts T.L and Kwolek W.F. Determination of Ultratrace Metals in Hydrogenated Vegetable Oils and Fats. *JAOCS*. 1983;60(41):811-4.

Nelson D.W. and Sommers L.E. Total carbon, organic carbon and organic matter. *In*. Methods of soil analysis. Part 3 – Chemical methods. Sparks D.L., Page A.L., Helmke P.A., Loeppert R.H., Soltanpour P.N., Tabatabai M.A., Johnson C.T. and Sumner M.E., editors. Madison:Soil Sci. Soc. Am. Book Ser. 1996; 961-1010.

Nuray Z., Bohn U.T., Clinton S.K. and Schwartz S.J. Carotenoid Absorption from Salad and Salsa by Humans Is Enhanced by the Addition of Avocado or Avocado Oil. *J Nutr*. 2005;135:431-436.

Okada S. and Yamanaka K. Arsenic in the Environment (2): Human Health and Ecosystem Effects. In: Nriagu J.O, editor. New York: John Wiley & Sons, Inc; 1994.

Ooms R. and Van Pee W. Determination of Trace Metal Content in Corn oil by Atomic Absorption Spectroscopy. *J Am Oil Chem Soc*. 1983:957-60

Ozcan M.M., Chalchat J.C. Chemical Composition of Carrot Seeds (*Daucus carota* L.) Cultivated in Turkey: Characterization of the Seed Oil and Essential Oil. *Grasasy Aceites*. 2007;58(4):359-365.

Persson D. and Kucera V. Release of metals from buildings, constructions and products during atmospheric exposure in Stockholm. *Water Soil Poll:Focus*. 2001;1(3-4):133-50.

Pieterse Z., Jerling J and Oosthuizen W. Avocados (monounsaturated fatty acids), weight loss and serum lipids. *South African Avocado Growers' Association Yearbook*. 2003;26:65-71.

Pietri J.C.A. and Brookes P.C. Relationships between soil pH and microbial properties in a UK arable soil. *Soil Biol Biochem* 2008;40:1856–61.

Platt K.A, Thomson W.W. Idioblast oil cells of avocado: distribution, isolation, ultrastructure, histochemistry and biochemistry. *Int. J. Plant Sci.* 1992;153(3):301–310.

Platt-Aloia K.A., Oross J.W. and Thomson W.W. Ultrastructural study of the development of oil cell in the mesocarp of avocado fruit. *Bot Gaz.* 1983;144(1):49–55.

Platt-Aloia K.A., Oross J.W. and Thomson W.W. Ultrastructure and development of oil cells in mesocarp of avocado fruit. *Bot. Gaz.* 1983;144:49-55.

Platt-Aloia K.A., Thomson W.W. and Young R.E. Ultrastructural changes in the walls of ripening avocados: transmission, scanning, and freeze fracture electron microscopy. *Bot. Gaz.* 1980;141:366-373.

Prasad M.N.V., Sajwan K.S. and Naidu R. In *Trace Elements in the Environment: Biogeochemistry, Biotechnology, Bioremediation*. Boca Raton, Florida: CRC Press; 2006.

Queirolo F., Stegen S., Restovice M., Paz M., Ostapczuk P., Schwuger M.J., et al. Total arsenic, lead, and cadmium levels in vegetables cultivated at the Andan villages of northern Chile. *Sci Total Environ.* 2000;255:75-84.

Raskin I., Smith R.D. and Salt D.E. Phytoremediation of metals: using plants to remove pollutants from the environment. *Curr Opin Biotechnol.* 1997;8:221-6.

Rauret G. Extraction procedures for the determination of heavy metals in contaminated soil and sediment. *Talanta.* 1998;46:449-455.

Reddy M., Moodley R. Kindness A. and Jonnalagadda S. B. Impact on soil quality on elemental uptake by, and distribution in, *Colocasia esculenta* (Amadumbe), an edible root. J Environ Sci Health Part A. 2011;46:1-10.

Requejo C., Lund C., White A., Wong M., McGhie T., Eyres L., et al. Cold Pressed Avocado oil-A healthy development. World Avocado Congress V Abstracts. 2003;205:460-1.

Rhoades J.D. Methods of soil analysis, Part 2 Chemical and microbiological properties. In: Page A.L., editor. 2nd ed: Agronomy; 1982;149-57.

Robertson J.B. CRC Handbook of Dietary Fiber in Human Nutrition. In: Spiller GA, editor. New York: CRC Press; 2001.

Rodríguez G.P. The role of vitamin E in human nutrition. Cuban J Food Nutr. 1997; 11(1): 46-57.

Rolfe L. Nature's Own Cosmetic: The Avocado. Lets Live. 1975.

Sachs R.M. and Michaels J.L. Comparative phytotoxicity among four arsenical herbicides. Weed Sci. 1971;19:558-64.

Salazar M.J., Hafidi M.E., Pastelin G., Ramírez-Ortega M.C. and Sánchez-Mendoza M.A. .Effect of an avocado oil-rich diet over an angiotensin II-induced blood pressure response J Ethnopharmacol. 2005;98:335-338.

Samman S. Trace elements. In Essentials in human nutrition. Oxford: Oxford University Press; 1998.

Sauerbeck D.R. and Hein A. Nickel uptake from different soils and its prediction by chemical extractions. Water, Air, and Soil Poll. 1991;57:861-71.

Schramel O., Michalke B. and Kettrup A. Study of the copper distribution in contaminated soils of hop fields by single and sequential extraction procedures. *Sci Total Environ.* 2000; 263:11-22.

Schulin R., Johnson A. and Frossard E. Trace element deficient soils. In: Hooda P, editor. Chichester, UK: Wiley-Blackwell; 2010.

Sihvonen M., Jarvenpaa E., Hietaniemi V. and Huopalahti R. Advances in supercritical carbon dioxide technologies. *Trends in Food Science and Technology.* 1999;10:217-22.

Singh V.P. Metal Toxicity and Tolernace In Plants And Animals In:Toxic Metals and Environmental Issues. Singh V.P., editor. Delhi, India: Mehra Offset Press; 2005.

Sinyinda S. and Gramshaw J.W. Volatiles of avocado fruit. *Food Chem.* 1998;62:483-7

Skoog D.A., West D.M. Holler F.J. and Crouch S.R. *Fundamentals of Analytical Chemistry.* 8th Edn. United Kingdom:Brooks/Cole;2004:337-349.

Smith S.R. Effect of soil pH on availability of metals in sewage sludge treated soils, Cd uptake by crops and implications for human dietary intake. *Environ. Pollut.* 1994;86:5-13.

Sumner M.E. and Miller W.P. *Methods of soil analysis. Part 2: Chemical properties.* In: Sparks DL, editor. 3rd ed. Madison: ASA; 1996.

Swisher E.H. Avocado oil from food use to skin care. *J Am Oil Chem Assoc.* 1988;65:1704-6.

Szentmihályi K., Vinkler P., Lakatos B., Illés V. and Then M. Rosehip (*Rosa canina* L.) oil obtained from waste hip seeds by different extraction methods. *Biores Technol.* 2002;82:195-201.

Tan K.H. Interactions of Soil Minerals with Natural Organics and Microbes. In: Huang P.M., editor. Madison: SSSA Special Publication; 1986.

Tang S., Wilke B.M. Brooks R.R. Heavy-Metal Uptake by Metal tolerant *Elsholtzia Haichowensis* and *Commelina Communis* from China Commun Soil Sci. Plant Anal. 2001; 32(5,6):895-905.

Tatro M.E. Optical Emission Inductively Coupled Plasma in Environmental Analysis. Encyclopedia of Analytical Chemistry In: Meyers R.A., editor. John Wiley & Sons Ltd; 2000

Terwelle H.F. and Slater E.C. Uncoupling of respiratory chain phosphorylation by arsenate. Biochem Biophys Acta. 1967;143:1-17.

Thompson L.H. and Doraiswamy I.K. Sonochemistry: Science and Engineering. Ind Eng Chem Res. 1999;38:12-5.

Tiessen H. and Moir J.O. Total and organic carbon. In: Soil Sampling and Methods of Analysis, Carter M.E., editor. Ann Arbor, MI: Lewis Publishers; 1993: 187-211.

Timperley M.H., Brooks R.R. and Peterson P.J. The significance of essential and nonessential trace elements in plants in relation to biogeochemical prospecting. J App Ecol. 1973;7:429-39.

Tingii V., Reilly C. and Patterson C.M. Determination of selenium in foodstuffs using spectrofluorimetry and hydride generation atomic absorption spectrometry. J Food Composit Anal. 1992;5:269-80.

Toma M., Vinatoru M., Paniwnyk L. and Mason T.J. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. Ultrasonics Sonochemistry. 2001;8:137-42.

Trichopoulos D. Epidemiology of Cancer. In: DeVita V.T., editor. Philadelphia: Lippincott Company; 1997.231-58.

Tripathi R.D., Rudra D., Srivastava S., Mishra S., Singh N., Tuli R., et al. Arsenic hazards: strategies for tolerance and remediation by plants. Trends Biotech. 2007;25(4):158-65.

Ure A.M. Single extraction schemes for soil analysis and related applications. Sci Total Environ. 1996; 178:3-10

Vangheluwe M., Van Sprang P., Verdonck F., Hiejerick D., Versonnen B., Vandebroele M., et al. Metals environmental risk assessment guidance. In: Affairs.Efar, editor. United Kingdom; 2005.

Varma A. Soil Biology. New York: Springer-Verland Berlin Heidelberg; 1999.

Verber M., Cujes K. and Gormiscek S. Determination of selenium and arsenic in mineral waters with hydride generation atomic absorption spectrometry. J Analyt Atom Spectrom. 1994;9:285-90.

Vilegas J.H.Y., de Marchi E. and Lancas F.M. Extraction of low-polarity compounds (with emphasis on coumarin and kaurenoic acid) from Mikania glomerata ('Guaco') leaves. Phytochemical Analysis. 1997;8:266–70.

Villa-Rodríguez J.A., Molina-Corral F.J., Ayala-Zavala J.F., Olivas G.I. and González Aguilar G.A. Effect of maturity stage on the content of fatty acids and antioxidant activity of 'Hass' avocado. Food Res Int. 2010.

Vinatoru M., Toma M. and Mason T.J. Ultrasound-assisted extraction of bioactive principles from plants and their constituents. Adv Sonochem. 1999;5:209–47.

Wager A.V. The Dying Back of Avocado Trees in Southern California. Calif Avocado assoc yearbk. 1940;25:40-3.

Walkley A. and Black I.A. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 1934;37:29-37.

Walsh L.M., Sumner M.E. and Keeney D.R. Occurrence and Distribution of Arsenic in Soils and Plants. *Environ Health Pers.* 1977;19:67-71.

Wang L. and Weller C.L. Recent advances in extraction of nutraceuticals from plants. *Trends in Food Sci & Technol.* 2006;17:300–12.

Wang, J., Zhao F., Meharg A.A. Raab A., Feldmann J. and McGrath S.P. Mechanisms of Arsenic Hyperaccumulation in *Pteris vittata*. Uptake Kinetics, Interactions with Phosphate, and Arsenic Speciation1 *Plant Physiol.* 2002;130:1552-61.

Welz B. and Šucmanová M. L-Cysteine as a reducing and releasing agent for the determination of antimony and arsenic using flow injection hydride generation atomic absorption spectrometry. *Analyst (London).* 1993;118(11):1417-23.

Werman M.J. and Neeman I. Avocado Oil Production and Chemical Characteristics. *JAOCS.* 1987;64(2):229-32.

Wetterhahn J.W. and Hamilton J.W. *Handbook on Toxicity of Inorganic Compounds.* New York: Marcel Dekker;1987.

Wong K.H., Fung Y.S. and Fung K.W. Determination of trace amounts of copper in palm oil by differential-pulse anodic-stripping voltammetry *Analyst.* 1980;105:30-6.

Woolf A.B., Ferguson I.B., Requejo-Tapia L.C., Boyd L., Laing W.A. and White A. Impact of sun exposure on harvest quality of ‘Hass’ avocado fruit. *Rev Chapingo Ser Hort.* 1999;5:353-8.

World Health Organisation. *Cadmium- Environmental Health Criteria 134.* International Programme on Chemical Safety, Geneva: 1992.

Yruela I. Copper In Plants: Acquisition, Transport And Interactions. *Funct Plant Biol.* 2009;36:409-30.

Zhang J., Zhaoa Q., Duanb G. and Y. Huangb. Influence of sulphur on arsenic accumulation and metabolism in rice seedlings. *Environ Experim Bot.* 2011;72:34–40.

Zidenberg-Cherr S. and Keen C.L. Trace elements, micronutrients, and free radicals. In: Di E. editor. Totowa, New Jersey: Humana Press; 1991.

Zweck S., Huttermann A. and Chet I. A convenient method for preparing inocula of homogenized mycelia. *Exper Mycol.* 1978;2(4):377-79.

APPENDIX 1

APPENDIX 2

APPENDIX 3

APPENDIX 4

COMMISSION OF THE EUROPEAN COMMUNITIES

COMMUNITY BUREAU OF REFERENCE · BCR

CERTIFIED REFERENCE MATERIAL

CERTIFICATE OF ANALYSIS

Element	Mass fraction (based on dry mass)		Number of accepted sets of results <i>n</i>
	Certified Value (%)	Uncertainty (%)	
Cd	28.4 ± 0.3	± 1.1 09.2-1	12
Pb	157 ± 0.2	± 0.1 0.0-1	12
Ca	12.6 ± 0.2	± 0.1 0.8-1	6
Zn	12.2 ± 0.2	± 0.5 4.0-1	13
Fe	49.7 ± 0.3	± 2.3 4.6-1	12
Mg	20.3 ± 0.9	± 0.7 3.3-1	11

(1) This value is the unweighted mean of a series of values, each value being the mean of a set of results as assessed by different laboratories and methods.

(2) The uncertainty is given as the 95% confidence interval of the mean value (1) and is applicable when the reference material is used for calibration purposes. When the reference material is used to assess the performance of a method, the user should refer to the recommendations that obtain in the literature (instructions for use) or the laboratory report.

DESCRIPTION OF THE SAMPLE

The sample is homogeneous powder consisting of particles that have passed through a 125 µm sieve. It is provided in screw-top glass bottles in units of approximately 40 g.

INSTRUCTIONS FOR USE

The portion for analysis should be taken from the centre of the bottle. The moisture content is to be determined by drying a 10 g portion at 100 ± 2°C as described in the certification report (Chapter 1.2, Instructions for use). The recommended maximum sample mass is 0.050 g.

All the glassware used should be thoroughly cleaned during opening of the bottle and handling of the material. The bottle should be stored in a safe and cool place.

SI-M0001 - Bureau No. 1991

BCR
for cert. No. 1991

Oil

Microwave Sample Preparation Note: XprOP-1
Category: Oils

Rev. Date: 6/04

Sample Type: Oil
Application Type: Acid Digestion
Vessel Type: 55 mL
Number of Vessels: 12
Reagents: Nitric Acid (70%)
Method Sample Type: Organic
Sample Weight: 0.5 gram

Step 1:

Acid Type
Nitric

Volume
10 mL

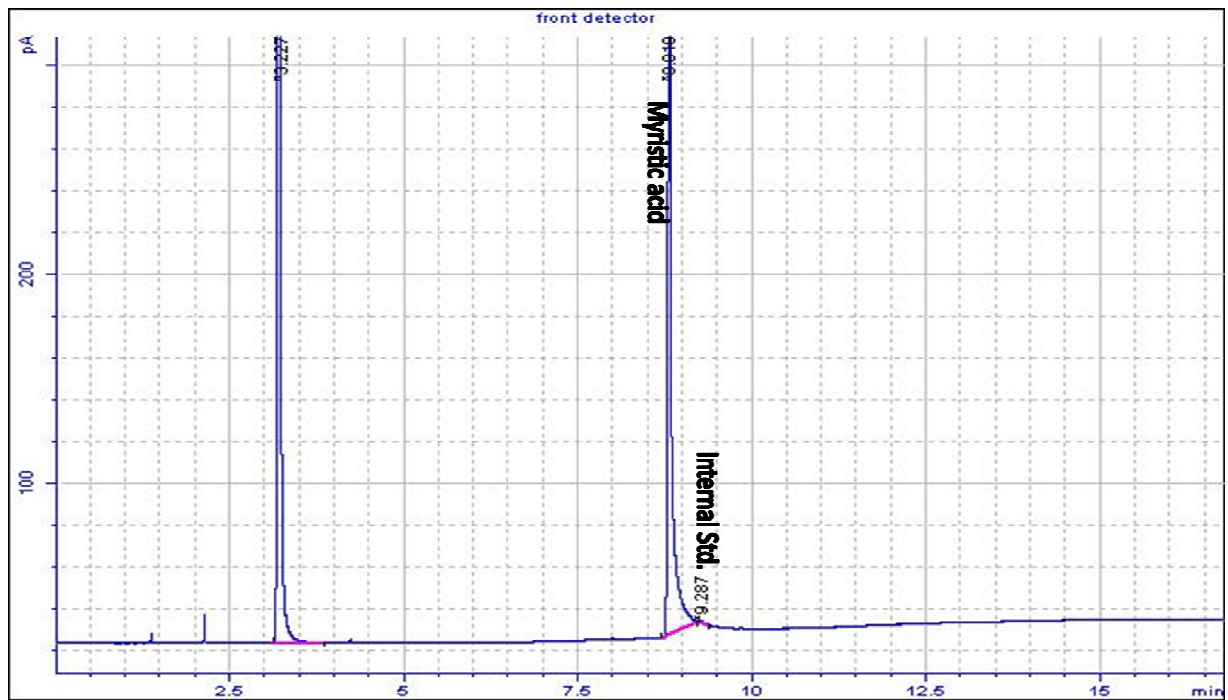
Heating Program: Ramp to Temperature Control

Stage	Max. Power	% Power	Ramp (min.)	Pressure (psi)	Temperature (°C)	Hold (min.)
(1)	1200 W	75	15:00	-	200	15:00

NOTE A: This procedure is a reference point for sample digestion using the CEM Microwave Sample Preparation System and may need to be modified or changed to obtain the required results on your sample.
NOTE B: Manual venting of CEM closed vessels should only be performed when wearing hand, eye and body protection and only when the vessel contents are at or below room temperature to avoid the potential for chemical burns. Always point the vent hole away from the operator and toward the back of a fume hood.
NOTE C: Power should be adjusted up or down with respect to the number of vessels. General guidelines are as follows: 8-12 vessels (50% power), 13-20 vessels (75% power), >20 vessels (100% power).
NOTE D: "Organic Method Sample Type" should be used for most sample types. Choose "Inorganic" for samples with more than 1 gram of solid material remaining at the bottom of the vessel at the end of the digest (ex: leach methods). Choose "Water" for samples that are largely aqueous prior to digestion.

Agilent Cerity QA/QC Report

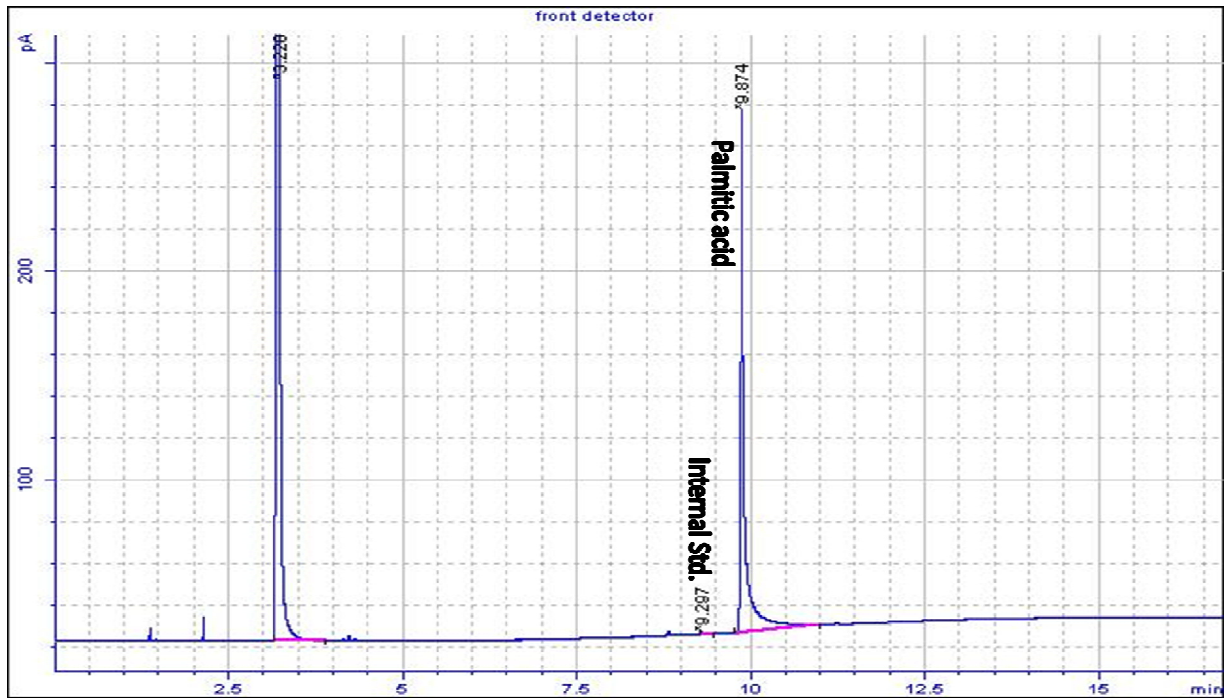
Sample name:	*Reprocessed: std 1 Myristic
Sample note:	
Submission time:	14 June 2010 12:16:54
Operator:	
Injection date:	14 June 2010 12:18:52
GC Description:	instr4 - SN: CN10650009
Signal description:	FID1 A, front detector
Method:	mags6
Method last saved:	10 June 2010 15:08:28



Signal	Retention Time [min]	Type	Width [min]	Area [pA*s]	Area %
1	3.227	MM m	0.032	9232.48852	87.27224
1	8.819	MM m	0.053	1342.99336	12.69496
1	9.287	MM m	0.050	3.46978	0.03280

Agilent Cerity QA/QC Report

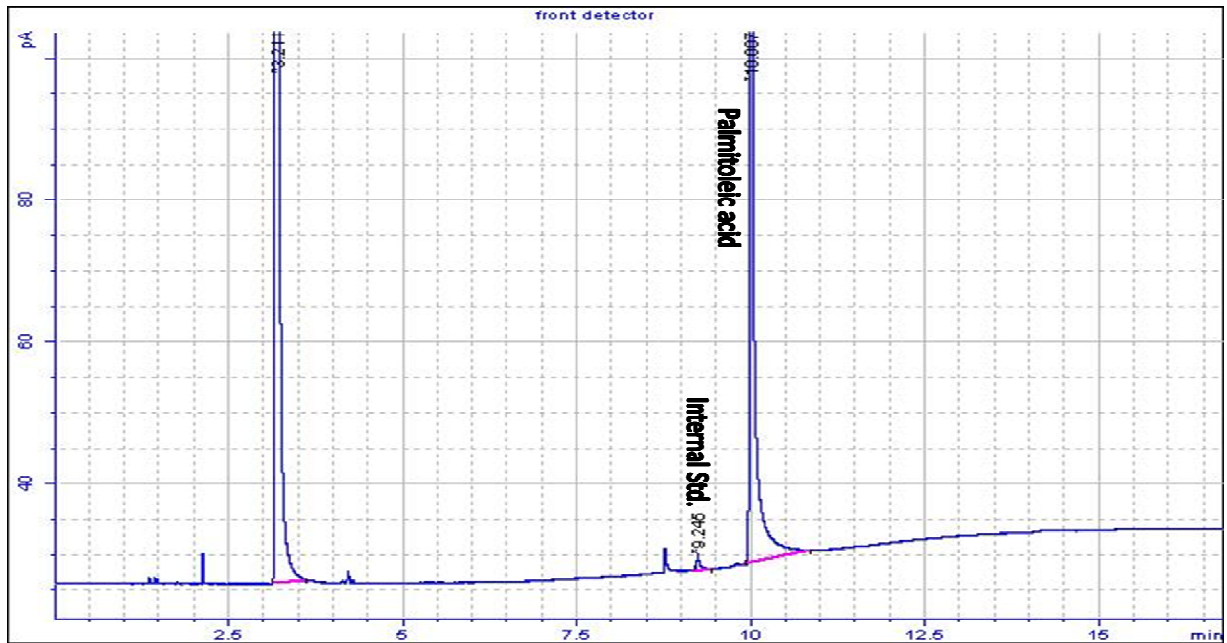
Sample name:	*Reprocessed: std 2 palmitic
Sample note:	
Submission time:	14 June 2010 12:19:47
Operator:	
Injection date:	14 June 2010 13:00:16
GC Description:	instr4 - SN: CN10650009
Signal description:	FID1 A, front detector
Method:	mags6
Method last saved:	10 June 2010 15:08:28



Signal	Retention Time [min]	Type	Width [min]	Area [pA*s]	Area %
1	3.228	MM m	0.034	1.1278e+004	92.55470
1	9.297	MM m	0.055	4.34048	0.03562
1	9.874	MM m	0.060	902.92430	7.40968

Agilent Cerity QA/QC Report

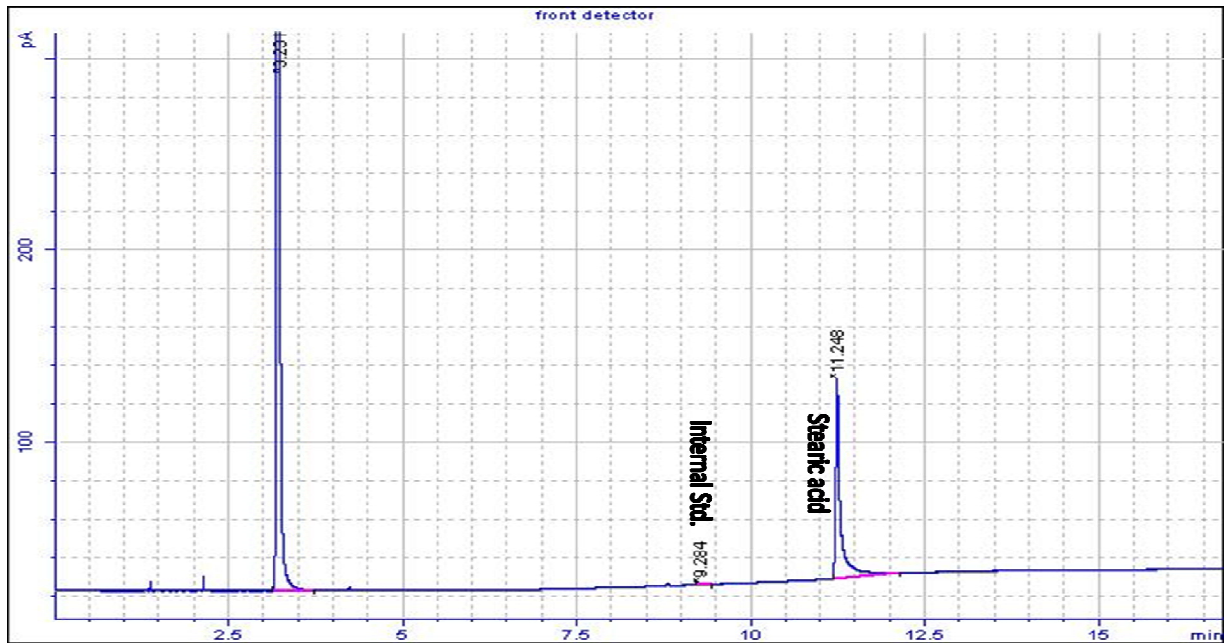
Sample name:	*Reprocessed: Std 3 -Palmitoleic
Sample note:	
Submission time:	14 June 2010 12:20:31
Operator:	
Injection date:	14 June 2010 13:20:31
GC Description:	instr4 - SN: CN10650009
Signal description:	FID1 A, front detector
Method:	mags6
Method last saved:	10 June 2010 15:08:28



Signal	Retention Time [min]	Type	Width [min]	Area [pA*s]	Area %
1	3.211	MM m	0.033	1.0042e+004	95.10312
1	9.245	MM m	0.055	7.89749	0.07479
1	10.007	MM m	0.063	509.16055	4.82209

Agilent Cerity QA/QC Report

Sample name:	*Reprocessed: std 4 Stearic
Sample note:	
Submission time:	14 June 2010 12:21:11
Operator:	
Injection date:	14 June 2010 13:22:50
GC Description:	instr4 - SN: CN10650009
Signal description:	FID1 A, front detector
Method:	mags6
Method last saved:	10 June 2010 15:08:28

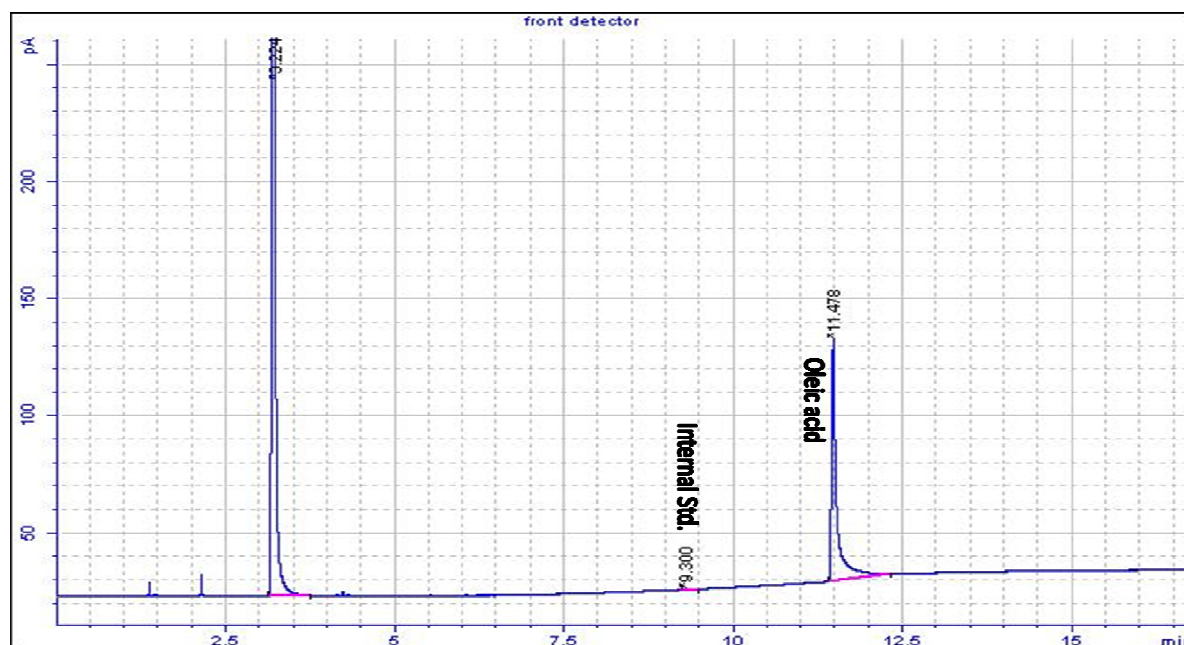


Area Percent Report

Signal	Retention Time [min]	Type	Width [min]	Area [pA*s]	Area %
1	3.231	MM m	0.033	1.0280e+004	95.26743
1	9.284	MM m	0.062	2.52895	0.02344
1	11.248	MM m	0.081	508.14419	4.70913

Agilent Cerity QA/QC Report

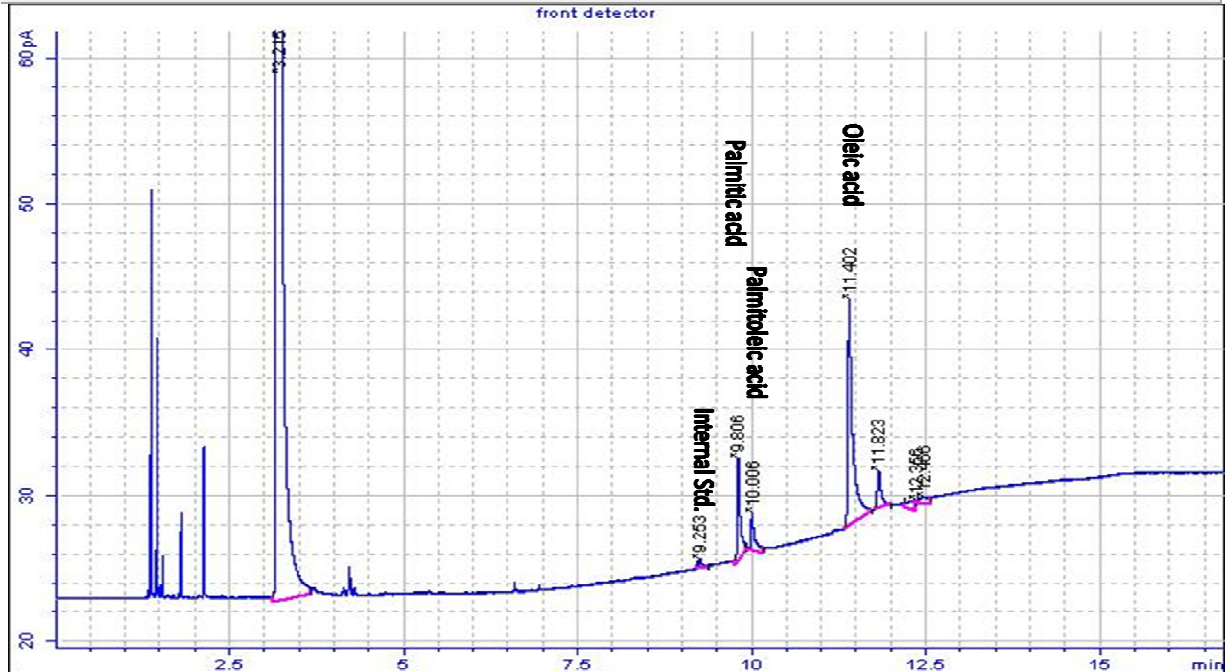
Sample name:	*Reprocessed: std 5 Oleic
Sample note:	
Submission time:	14 June 2010 13:03:07
Operator:	
Injection date:	14 June 2010 14:05:08
GC Description:	instr4 - SN: CN10650009
Signal description:	FID1 A, front detector
Method:	mags6
Method last saved:	10 June 2010 15:08:28



Signal	Retention Time [min]	Type	Width [min]	Area [pA*s]	Area %
1	3.224	MM m	0.032	9656.62424	94.76400
1	9.300	MM m	0.078	3.04416	0.02987
1	11.478	MM m	0.086	530.51395	5.20613

Agilent Cerity QA/QC Report

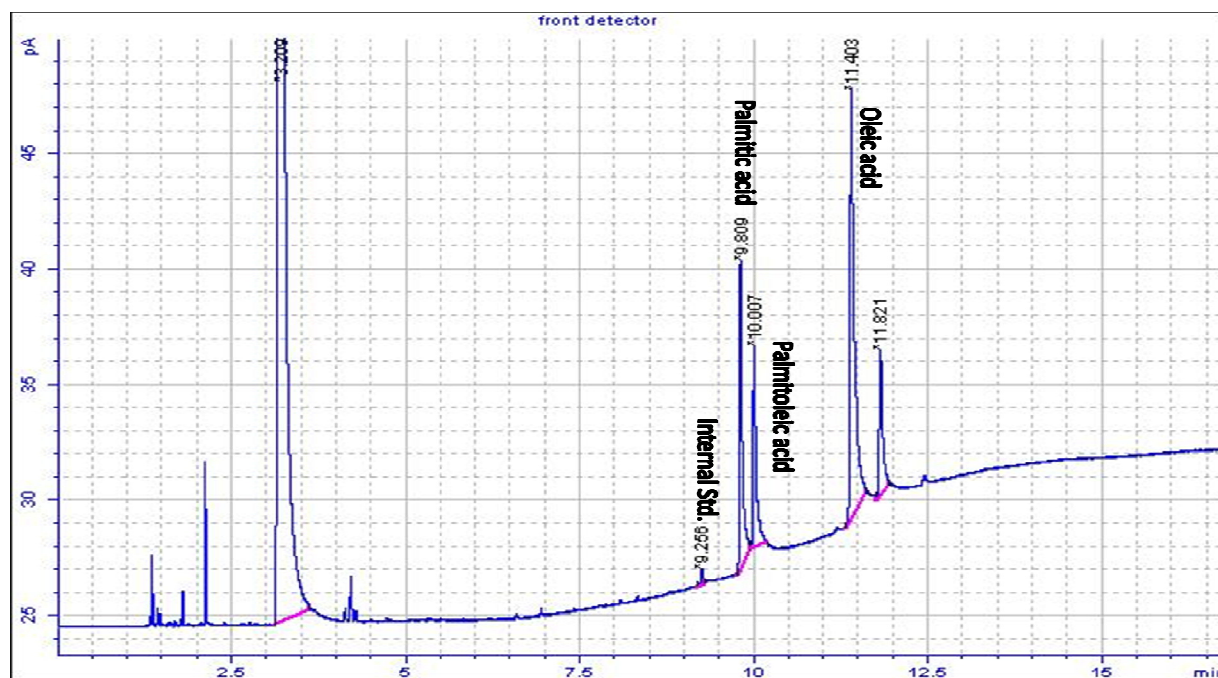
Sample name:	*Reprocessed: S3F Micro
Sample note:	
Submission time:	15 June 2010 14:01:52
Operator:	
Injection date:	15 June 2010 15:10:30
GC Description:	instr4 - SN: CN10650009
Signal description:	FID1 A, front detector
Method:	mags6
Method last saved:	10 June 2010 15:08:28



Signal	Retention Time [min]	Type	Width [min]	Area [pA*s]	Area %
1	3.215	MM m	0.033	1.0815e+004	98.87420
1	9.253	MM m	0.072	3.03053	0.02771
1	9.806	MM m	0.051	21.88509	0.20009
1	10.006	MM m	0.066	9.98993	0.09133
1	11.402	MM m	0.076	70.51552	0.64469
1	11.823	MM m	0.061	9.20596	0.08417
1	12.356	MM m	0.113	4.64348	0.04245
1	12.466	MM m	0.150	3.86857	0.03537

Agilent Cerity QA/QC Report

Sample name:	*Reprocessed: S1H USE
Sample note:	
Submission time:	17 June 2010 09:37:27
Injection date:	17 June 2010 11:30:34
GC Description:	instr4 - SN: CN10650009
Signal description:	FID1 A, front detector
Method:	mags6
Method last saved:	10 June 2010 15:08:28



Signal	Retention Time [min]	Type	Width [min]	Area [pA*s]	Area %
1	3.209	MM m	0.034	1.0930e+004	98.52953
1	9.256	MM m	0.043	1.93576	0.01745
1	9.809	MM m	0.047	37.29696	0.33622
1	10.007	MM m	0.049	25.73133	0.23196
1	11.403	MM m	0.068	76.21125	0.68702
1	11.821	MM m	0.058	21.94443	0.19782

Fatty Acid Profile and Elemental Content of Avocado (*Persea americana* Mill.) Oil – Effect of Extraction Methods

MAGESHNI REDDY, ROSHILA MOODLEY and SREEKANTH B. JONNALAGADDA

School of Chemistry, University of KwaZulu-Natal, Durban, South Africa

5 Interest in vegetable oil extracted from idioblast cells of avocado fruit is growing. In this study, five extraction methods to produce avocado oil have been compared: traditional solvent extraction using a Soxhlet or ultrasound, Soxhlet extraction combined with microwave or ultra-turrax treatment and supercritical fluid extraction (SFE). Traditional Soxhlet extraction produced the most reproducible results, 64.76 ± 0.24 g oil/100 g dry weight (DW) and 63.67 ± 0.20 g oil/100 g DW for Hass and Fuerte varieties, respectively. Microwave extraction gave the highest yield of oil (69.94%) from the Hass variety. Oils from microwave extraction had the highest fatty acid content; oils from SFE had wider range of fatty acids. Oils from Fuerte variety had a higher monounsaturated: saturated FA ratio (3.45–3.70). SFE and microwave extraction produced the best quality oil, better than traditional Soxhlet extraction, with the least amount of oxidizing metals present.

Keywords: Avocado, *Persea Americana*, fatty acid profile, oil extraction, elemental content of oils, metals, mono-unsaturated fatty acids

15 Introduction

The avocado, *Persea americana* Miller, of the plant family Lauraceae, is a fruit with extremely high oil content, which is the main component of its dry weight. The avocado tree is indigenous to tropical America. This evergreen is the only tree that bears fruit that ripens when fallen. There are three botanically distinct origins of avocado that are internationally recognized namely; West Indian, Guatemalan and Mexican.^[1] Production in South Africa is an export-orientated industry that exports fresh avocados to the European market however, processing of the fruit to produce purees and oils is a growing industry in the South African market.

The fleshy pulp of the fruit comprises parenchyma cells (60 μ m diameter) that surround uniformly distributed specialized thick-walled oil containing idioblast cells (80 μ m diameter) that make up 2% by volume of the total tissue.^[2] It is reported that alkaloids and terpenes are present in idioblast oil cells and triacylglycerides are present in parenchymal oil cells.^[3]

35 Avocado oil is similar to olive oil, which is highly digestible. This, together with its high energy value and low

sugar content, makes it an ideal food source for diabetics.^[4] Edible oils such as avocado and olive oils contain high levels of oleic acid, a stable omega-9 monounsaturated fatty acid that is good for health. An epidemiological study in the Mediterranean region, where the diet includes a large quantity of monounsaturated fatty acids (MUFAs), shows a low incidence of atherosclerotic cardiovascular disease.^[5] It is reported that an avocado-enriched diet can reduce total and low density lipoprotein (LDL) cholesterol levels whilst increasing high density lipoprotein (HDL) cholesterol levels which lowers the risk of atherosclerotic cardiovascular disease.^[6] The avocado fruit contains vitamins E and C, carotenoids and sterols that possess antioxidant and radical scavenging activities,^[7] these being deficient in people living in the United States of America.^[8]

Due to the therapeutic effectiveness of avocado oil, it is much sought after in the pharmaceutical and cosmetic industries. It contains phytosterols with the same penetrating ability as lanolin and effective sunscreen properties that make it suitable for skin products.^[9] The cosmetic and nutritional value of avocado oil is evident. This warrants efficient extraction methods yielding high quality avocado oils as the conventional cold pressing industrial method is known to produce fishy and rancid oils. Small amounts of metals in edible oils contribute to the degradation of oils. The deleterious effects of trace metals, particularly Fe and Cu, on flavor and oxidative stability of oils have been reported.^[10] These metals catalyze the oxidation of oils in the presence of light. The shelf life, quality and freshness of oils

Address correspondence to Prof. S.B. Jonnalagadda, School of Chemistry, Westville campus, Chiltern Hills, University of KwaZulu-Natal, P. Bag X54001, Durban 4000, South Africa; E-mail: jonnalagaddas@ukzn.ac.za

Received May 31, 2011.

can be assessed by determining the metal ion content which in turn depends on the extraction method. The objective of this study was to employ various extraction methods (utilizing ultrasound, microwave, Soxhlet, ultra-turrax cell lysing device and supercritical fluid) for the recovery of oils from the Hass and Fuerte varieties of avocados. This study also emphasizes the levels of fatty acid and elemental content of extracted oils, to assess the quality of oils, which could have bearing on human consumption and health. The following elements were selectively investigated: Ca, Mg, Al, Fe, Cu, Mn, Ni, Co, Cr, Pb, and Zn.

Materials and methods

Sample sites and collection

Fruit samples (Fuerte and Hass variety) were collected from Westfalia Organic Avocado Farm on the Everdon Estate, located 5 km from Howick, in the KwaZulu-Natal Midlands, South Africa. The fruit was ripened, skinned, de-seeded and dried in an oven at 45°C to constant mass. Dried fruit was milled in a food processor and the powder was stored in plastic bags in a refrigerator at 4°C until analysis.

Extractions

Five extraction techniques were used to obtain avocado oils that were subjected to fatty acid and metal analysis. Results were compared to evaluate the efficiency of the extraction methods.

Hexane extraction

Avocado oil was extracted using the traditional exhaustive Soxhlet extraction method. A cellulose thimble containing 5.0 g dried sample was placed in the Soxhlet device and extracted with 250 mL hexane for 24 h; the extractor siphoned every 15 min. The flask was removed and solvent evaporated using a rotary evaporator.

Ultrasound extraction

Approximately 5.0 g of dried avocado samples were sonicated in a water bath at 60°C with hexane as solvent for 1 h. The resultant mixture was filtered by suction and filtrate evaporated using a rotary evaporator.

Ultra-Turrax treatment-hexane combined extraction

Approximately 10.0 g of dried avocado samples containing 10 mL of hexane in glass bottles were treated with an ultra-turrax tool (cell lysing apparatus) for 10 min. The slurry was dried to constant mass, of which 5.0 g was extracted with hexane by traditional Soxhlet extraction for 24 h.

Microwave assisted-hexane combined extraction

A paste of avocado was spread uniformly (5 mm thickness) on the rotary plate of a domestic microwave oven (Braun, 1000W, 2450 MHz) and heated at maximum power for 11 min. The resulting mass was ground to a fine powder, of which 5.0 g was extracted with hexane by traditional Soxhlet extraction for 24 h.

Supercritical fluid extraction

A home-built extractor, Figure 1^[11] was used to extract oil from avocado samples employing supercritical Ar and CO₂ as the extraction fluids. Approximately 5.0 g of dried avocado was accurately weighed and loaded into the 10.0 g capacity extraction cell. A small piece of cotton wool was lodged at both ends of the extraction cell to take up dead volume and avoid plant material blocking the entry and exit ports. All extractions were performed for 2 h with a fluid flow rate of 2.8–3.5 mL/min. Extracts were collected in glass screw cap collection vessels without solvent and dried in an oven at 150°C to constant mass. Prior to analysis the extracted oil was subjected to vacuum evaporation for 30 min to remove any water and dissolved CO₂.

Instrumentation

Microwave digestion was the preferred method of digestion because it provides higher accuracy with respect to both time and recovery values. Elemental determination was by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Method validation was accomplished using certified reference material (CRM), *lyophilized brown bread* (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities. GC, performed on a 6820 GC system (Agilent Technologies) with DB-wax fused silica capillary column (30 m × 0.25 mm i.d., 0.25 mm film thickness), was used for fatty acid analysis. The injector and flame ionization detector were set at 250°C. The column temperature program was started from 150°C where it was held for 1 min, then ramped to 200°C at 25°C/min where it was held for another 3 min. The final temperature was increased to 230°C at a rate of 15°C/min where it was held for 5 min. The pressure of N₂ carrier gas was set at 100 kPa. Supercritical extractions were done using an apparatus that was constructed in-house. The specifications of the apparatus and all accompanying parts are as described by Botha.^[11]

Sample analysis

A mass of 0.5 g of dried avocado sample, extracted oil and CRM was placed in separate Perfluoroalkoxy (PFA) vessels. To each vessel, 10.0 mL of 69% HNO₃ was added, swirled gently and left to stand for 1 min before sealing. The samples were subjected to microwave digestion using

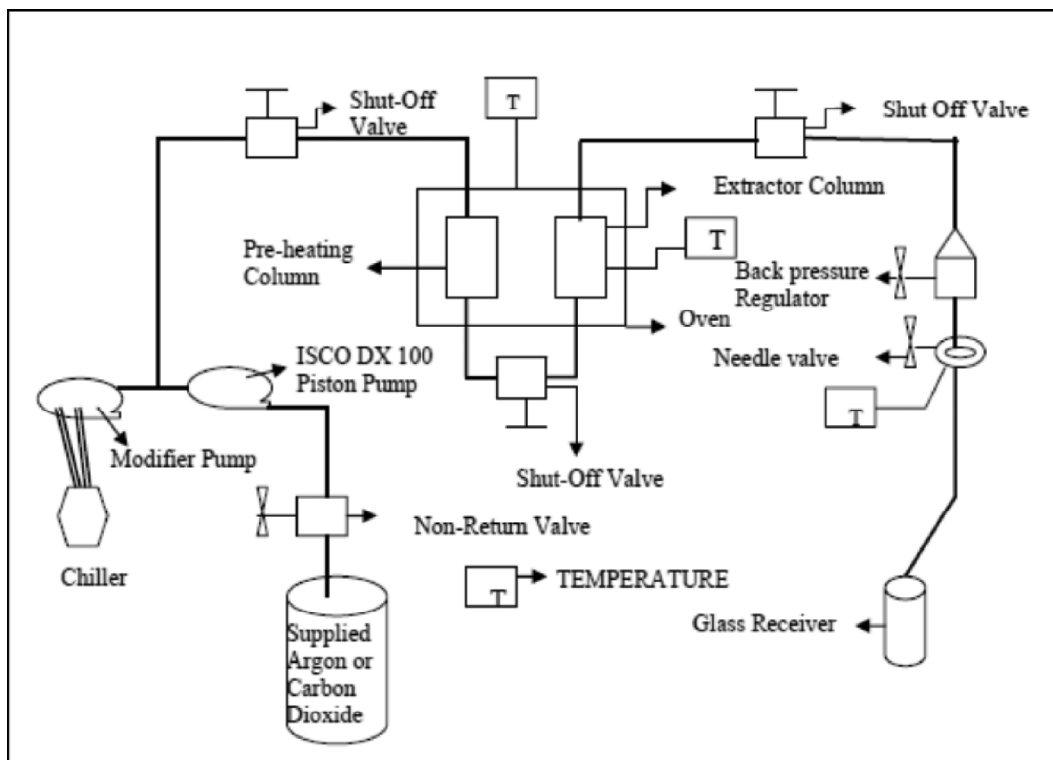


Fig. 1. Schematic diagram of the constructed supercritical fluid extractor.^[11]

160 a CEM MARS Xpress closed vessel microwave digestion
 system. For digestion, the power was ramped to 1200 W
 for 15 min at the rate of 80 W/min where it was held for 15
 min. Digests were filtered by gravity into 50 mL volumetric
 flasks, made up to the mark with double distilled water then
 transferred into plastic bottles that were stored in a refriger-
 165 ator at 4°C for elemental analysis. All analyses were done in
 triplicate.

The element dissolution in oil was calculated as follows:

$$\text{Dissolution (w/w\%)} = (O_c \times O_y / M_w \times M_c) \times 100$$

Where O_c is the element concentration in the oil ($\mu\text{g g}^{-1}$)

170 O_y is the oil yield from 100 g of dried avocado mesocarp
 (g)

M_w is the mesocarp weight (100 g)

M_c is the element concentration in the mesocarp ($\mu\text{g g}^{-1}$)

Fatty acid (FA) analysis

175 Derivatization of the FAs into fatty acid methyl esters
 (FAMES) was done according to Kanchanamayoon, with
 some modifications.^[12] Approximately 0.5 g of avocado
 extract was accurately weighed into PTFE lined screw-cap
 bottles. 2.0 mL of 1 mg/mL internal standard (prepared by
 180 dissolving 100 mg of pentadecanoic acid in toluene in a 100
 mL volumetric flask) and 3.0 mL of 10% methanolic HCl
 (prepared by slow addition of 10.0 mL conc. HCl to 9.0 mL

dry methanol with constant stirring) were added to extract,
 sealed and placed in a hot water bath (70°C) for 2 h. There-
 after, 5.0 mL of 6% K_2CO_3 solution and 1.0 mL of toluene
 185 were added and vortexed for 1 min. The organic phase
 was separated from the aqueous phase after centrifugation
 at 1100 rpm for 5 min. The organic phase was dried with
 a small amount of anhydrous Na_2SO_4 and filtered using
 milli-pore 0.45 μm filters. An aliquot was injected (0.1 μl)
 190 into the GC. FAs were identified using FA standards.

Statistical analysis of data

Data generated from the FA analysis was subjected to
 ANOVA and Duncan's multiple range tests using the SAS
 program (Version 6.12, SAS Institute Inc., Cary, NC,
 USA). The analysis was performed to determine the sig-
 195 nificance of the extraction methods in relation to oil yield,
 FA content and metal extractability.

Results and discussion

Extraction

The parameters and oil yields (g oil/100 g dry weight
 200 (DW)) obtained from the five extraction methods are
 represented in Table 1. Results from traditional Soxhlet
 extraction were most reproducible (64.76 ± 0.24 g oil/
 100 g DW and 63.67 ± 0.20 g oil/100 g DW, respectively)

Table 1. Parameters and oil yields obtained from the different extraction techniques [g of oil from 100 g dry weight (DW), n = 3].

<i>Yield (g/100g DW)</i>	<i>Soxhlet extraction</i>		<i>Ultrasound water bath</i>		<i>Ultra turrax treatment</i>		<i>Microwave extraction</i>		<i>Supercritical fluid extraction</i>	
	<i>Hass</i>	<i>Fuerte</i>	<i>Hass</i>	<i>Fuerte</i>	<i>Hass</i>	<i>Fuerte</i>	<i>Hass</i>	<i>Fuerte</i>	<i>Hass</i>	<i>Fuerte</i>
	64.76 ± 0.24	63.67 ± 0.20	54.63 ± 4.95	58.75 ± 1.56	63.38 ± 0.79	64.00 ± 0.25	69.94 ± 0.39	60.90 ± 2.86	62.87 ± 0.29	59.56 ± 0.36
Parameters										
Solvent	Hexane	Hexane	Hexane	Hexane	Hexane	Hexane	Hexane	Hexane	Carbon dioxide	Carbon dioxide
Temperature/°C	69.1	69.1	69.1	69.1	75	75	70	70	40-45	40-45
Pressure	Atmospheric	Atmospheric	Atmospheric	Atmospheric	Atmospheric	Atmospheric	Atmospheric	Atmospheric	420-450 bar	420-450 bar
Time/minutes	160	160	60	60	160	160	160	160	120	120
Significance^a										
Fatty acid type (FAT)	***									
Extraction Method (EM)	*									
FAT * EM	***									

^ans, **, *** : not significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

205 as seen by the low standard deviation for varieties. The extraction technique with highest yield was by microwave, yielding 69.94% oil from the Hass variety. Ultrasound extraction gave the lowest yield (54.63g oil/100 g DW) and least reproducible results which is in agreement with a
 210 similar study performed by Szentmihályi et al.^[13] SFE (at highest pressure and lowest temperature) yielded 62.87 ± 0.29 g oil/100 g DW and 59.56 ± 0.36 g oil/100 g DW for Hass and Fuerte varieties, respectively. The extraction yield by ultraturax treatment was similar to tradition Soxhlet
 215 extraction (63.38 ± 0.79 g oil/100 g DW and 64.00 ± 0.25 g oil/100 g DW for Hass and Fuerte varieties, respectively) but failed to be as reproducible. A process such as this needs optimization to obtain reproducibility. Twofactor ANOVA showed a significant difference in yields for the
 220 different extraction methods ($P \leq 0.001$) with a significant difference in yields between the two varieties ($P \leq 0.05$). The results obtained from the microwave-assisted Soxhlet extraction and the SFE show high potential for large-scale extraction operations. SFE has particularly gained popularity over the recent years. Feasibility studies conducted
 225 on large-scale SFE operations have verified the method as economical, since it uses half the energy as distillation extraction processes; greener (uses CO₂ rather than organic solvents) and safer as it avoids direct contact with organic solvents.^[14] The cost implications of microwave-assisted Soxhlet extraction have also been reported. The study validates the method as feasible since it is able to extract quantitatively without the degradation of target
 230 compounds with 70–85% of the solvent being recycled.^[15]

235 *Fatty Acid (FA) profiling*

The FA composition of oils obtained for the different extraction methods are presented in Table 2. The analyzed oil consisted of five different FAs; two unsaturated FAs and three saturated FAs. MUFAs, oleic (C18:1) and palmitoleic (C16:1) acids, are predominant constituents of avocado oils. On average, MUFAs contribute 55–65% towards the total FA content of the fruit. For saturated fatty acids (SFAs), myristic acid was detected in oils from the Hass variety only by traditional Soxhlet, ultra turrax treatment and SFE. Stearic acid (C18:0) was only detected in the Hass variety extracted by SFE. Oils from the Fuerte variety were devoid of myristic and stearic acids.

The different extraction methods (Table 2 and Fig. 2), showed microwave extraction to produce highest yield of
 240 FAs overall, whilst SFE provided a wider range. The fatty acid profiles of Hass and Fuerte varieties were found to be different with Fuerte variety being richer in MUFAs. Hass variety oils were richer in palmitic acid (21.70–25.29%) and palmitoleic acid (13.01–17.87%) compared to Fuerte
 245 (15.60–18% and 6.23–8.02%, respectively). Fuerte variety had higher concentrations of oleic acid (50.35–60.14%) compared to Hass (< 48.75%). Twofactor ANOVA showed that there is a significant difference in the percentages ob-

tained for the different types of FAs which is dependent on the extraction method. Duncan's multiple range test
 260 showed that in both varieties, SFE produced the lowest percentage of palmitic and oleic acids, whilst microwave extraction produced the highest percentage of palmitoleic acid. The lower percentages of FAs produced by SFE could be due to short extraction time (2h). SFE is relatively rapid,
 265 with >50% of analyte being extracted in the first 10 min, and 95% being extracted after 100 min.^[16] Increasing the extraction time could increase the yield. Increasing the fluid density by increasing the pressure or temperature leads to higher solubility of FAs into the SF and could increase the
 270 efficiency of extraction.^[17]

A high ratio of MUFAs to SFAs is generally viewed as beneficial to humans.^[18] Table 3 represents the MUFA: SFA ratio for each variety of avocado oil extracted by various techniques. Of the two varieties, Fuerte oil has a higher MUFA: SFA ratio (3.45–3.70) than Hass (1.51–2.59), for all cases. The highest MUFA: SFA ratio was obtained by microwave extraction in Fuerte variety while the lowest MUFA: SFA ratio was obtained by SFE in Hass variety. Other fatty acids that were expected to be found in the oil were not present in this study. Polyunsaturated FAs such as linoleic (18:2) and linolenic (18:3) acids were found in avocado oils grown in Mexico and Turkey. This was not found in this study, which is not surprising since various factors such as climate conditions, variety, stage of maturity and sun exposure can affect the FA composition in avocados.^[19]

Evaluation of metals in avocado oil

Accuracy of the method was measured by comparing results obtained with certified results (Table 4). Recorded values were in good agreement with certified values.
 290

The concentration of metals detected in the mesocarp prior to extraction and the percentage of metals which dissolved or co-extracted into the oils [dissolution (%)] is represented in Table 5. If present, Co concentrations were below the instrument detection limits ($0.0097 \mu\text{g g}^{-1}$). In Hass and Fuerte varieties, Mg was found in high concentrations, $941.6 \pm 29.4 \mu\text{g g}^{-1}$ and $1118.7 \pm 12.0 \mu\text{g g}^{-1}$, respectively; this was followed by Ca, $337.2 \pm 8.9 \mu\text{g g}^{-1}$ and $698.7 \pm 9.3 \mu\text{g g}^{-1}$, respectively. The Fuerte variety is a richer source of Mg and Ca, with Ca being twice that of Hass. From a nutritional perspective, high concentrations of these macro elements in any given food source can only be beneficial.
 300

The minor elements studied were found to be at concentrations below $100 \mu\text{g g}^{-1}$ in the avocado mesocarp. Concentrations of these elements ($\mu\text{g g}^{-1}$, DW) in (Hass and Fuerte) varieties were in descending order of, Fe (53.68 ± 0.98 , 44.31 ± 0.40), > Al (22.88 ± 0.29 , 23.34 ± 0.13), > Zn (12.42 ± 0.43 , 6.80 ± 0.80), > Cu (10.59 ± 0.22 , 12.63 ± 0.17), > Ni (0.80 ± 0.13 , 8.18 ± 0.08), > Mn (4.74 ± 0.18 , 8.30 ± 0.18), > Pb (7.26 ± 1.56 , 29.26 ± 2.07), > Cr (1.29 ± 0.09 , not detected). Except for Fe and Zn, which were marginally higher in Hass mesocarp, Fuerte had higher
 310

Table 2. Fatty acid composition of oils obtained from different extraction methods given in area percentage \pm standard deviation ($n = 3$).

Extraction methods	C 14:0		C 16:0		C 16:1		C 18:0		C 18:1	
	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte
Traditional Soxhlet	0.34 \pm 0.01	ND	24.33 \pm 0.08 ^a	18.03 \pm 0.18 ^a	13.01 \pm 0.17 ^b	6.23 \pm 0.09 ^b	ND	ND	48.75 \pm 0.08 ^a	59.00 \pm 0.57 ^a
Ultrasound in water bath	ND	ND	23.91 \pm 0.34 ^{ab}	17.64 \pm 0.21 ^a	13.42 \pm 0.16 ^b	6.74 \pm 0.49 ^b	ND	ND	48.57 \pm 0.43 ^a	58.19 \pm 2.05 ^a
Ultra-turrax treatment	0.55 \pm 0.38	ND	24.22 \pm 0.55 ^a	17.77 \pm 0.50 ^a	13.17 \pm 0.15 ^b	6.23 \pm 0.05 ^b	ND	ND	47.46 \pm 0.44 ^a	59.45 \pm 0.95 ^a
Microwave	ND	ND	25.29 \pm 0.97 ^a	18.03 \pm 0.17 ^a	17.87 \pm 0.13 ^a	8.02 \pm 0.34 ^a	ND	ND	46.28 \pm 0.88 ^a	60.14 \pm 1.51 ^a
Supercritical fluid	1.58 \pm 0.02	ND	21.70 \pm 2.63 ^b	15.60 \pm 0.52 ^b	13.08 \pm 1.48 ^b	4.91 \pm 0.44 ^c	12.80 \pm 0.84	ND	41.57 \pm 2.66 ^b	50.35 \pm 1.03 ^b
Significance ^a										
Fatty acid Type (FAT)			***							
Extraction Method (EM)			***							
FAT * EM			***							

Mean values in each column followed by a different superscript letter are significantly different by Duncan's multiple range test ($P \leq 0.05$).

^an, *, **, ***: not significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

C 14:0 = myristic acid, C16:0 = palmitic acid, C16:1 Palmitoleic acid, C18:0 = stearic acid, C18:1 = Oleic acid.

Table 3. Summary of Monounsaturated Fatty Acid (MUFA) to Saturated Fatty Acid (SFA) ratio for each variety of avocado oil extracted by various techniques.

Extraction methods	Total MUFAs (%)		Total SFAs (%)		MUFA:SFA Ratio	
	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte
Traditional Soxhlet	61.76	65.23	24.67	18.03	2.50	3.62
Ultrasound in water bath	61.99	64.93	23.91	17.64	2.59	3.68
Ultra turrax treatment	60.63	65.68	24.77	17.77	2.45	3.70
Microwave	64.15	68.16	25.29	18.03	2.54	3.78
Supercritical fluid	54.65	55.26	36.08	15.6	1.51	3.54

Total MUFA = approximate area percentage of C16:1+ C18:1.

Total SFA = approximate area percentage of C14:0 + C16:0 + C18:0.

C 14: 0 = myristic acid, C16:0 = palmitic acid, C16:1 Palmitoleic acid, C18:0 = stearic acid, C18:1 = Oleic acid.

Table 4. Comparison of measured and certified values in the certified reference material (*lyophilized brown bread*- BCR 191) give as mean ± S.D., n = 6.

Element	Certified	Measured (MD)
Ca	0.41 ± 0.01 mgg ⁻¹	0.43 ± 0.05 mgg ⁻¹
Mg	10 ± 0.01 mgg ⁻¹	9.37 ± 0.40 mgg ⁻¹
Fe	40.7 ± 2.3 μgg ⁻¹	39.1 ± 2.2 μgg ⁻¹
Cu	2.6 ± 0.1 μgg ⁻¹	25.91 ± 0.1 μgg ⁻¹
Mn	20.3 ± 0.7 μgg ⁻¹	19.50 ± 0.4 μgg ⁻¹
Zn	19.5 ± 0.5 μgg ⁻¹	19.36 ± 0.7 μgg ⁻¹

concentrations of elements studied. However, both varieties contribute significantly to the dietary allowances for these elements.

315

High concentrations of heavy metals in extracted oils are detrimental to oil quality. Oil quality is vital in commercial products manufactured by the pharmaceutical, cosmetic and food preparation industries. Element dissolution was relatively low for traditional Soxhlet, SFE and microwave extraction and relatively high for ultrasound water bath and ultra-turrax extractions. Fe and Cu concentrations in vegetable oils is known to induce oxidation and can decrease long term stability. The threshold value for Fe in oil is 2–6 μg g⁻¹.^[20] The ultrasound extraction method gave highest concentration of Fe in oils, 3.34% for Hass (1.80 μg

320

325

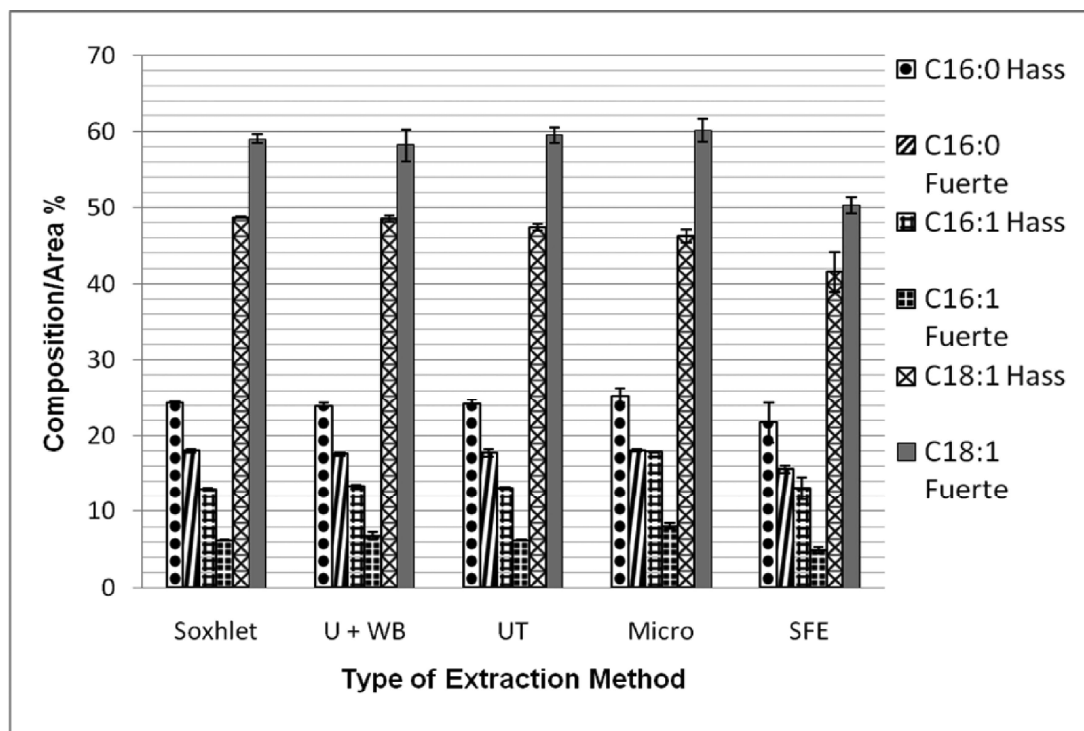


Fig. 2. Area% of the major fatty acids found in Hass and Fuerte varieties extracted by different techniques.

Table 5. Elemental content in avocado mesocarp (mean \pm S.D. in $\mu\text{g g}^{-1}$, n = 3) and its dissolution (%) into oil.

Elements	Total concentration in mesocarp (mean \pm S.D., $\mu\text{g g}^{-1}$)		Soxhlet extraction (%)		Ultrasound water bath (%)		Microwave extraction (%)		Ultrasound assisted extraction (%)		Supercritical fluid extraction with CO ₂ (%)	
	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte
Al	22.88 \pm 0.29	23.34 \pm 0.13	3.05	3.96	5.08	4.68	4.41	2.97	5.39	3.87	3.01	3.04
Ca	337.20 \pm 8.88	698.72 \pm 9.33	4.22	1.68	5.06	2.25	3.37	1.24	7.04	1.47	3.29	1.49
Cu	10.59 \pm 0.22	12.63 \pm 0.17	0.90	0.90	0.69	0.64	0.28	0.08	1.95	1.33	0.05	0.16
Cr	1.29 \pm 0.09	ND	3.98	4.68	6.43	5.85	5.21	3.85	7.15	5.18	4.61	6.98
Fe	53.68 \pm 0.98	44.31 \pm 0.40	0.56	1.05	3.34	4.12	1.07	1.01	2.65	2.22	0.69	1.02
Mg	941.61 \pm 29.35	1118.66 \pm 11.99	0.07	0.08	0.17	0.12	0.19	0.09	0.27	0.07	0.05	0.04
Mn	4.74 \pm 0.18	8.30 \pm 0.18	0.24	0.08	1.00	0.47	0.22	0.07	1.24	0.29	0.08	0.11
Ni	0.80 \pm 0.13	8.18 \pm 0.08	1.81	0.52	7.41	0.53	1.23	0.23	10.01	0.28	1.83	0.44
Pb	7.26 \pm 1.56	29.26 \pm 2.07	0.82	1.16	3.66	1.94	3.57	0.65	5.28	ND	4.78	1.57
Zn	12.42 \pm 0.43	6.80 \pm 0.80	3.15	2.61	6.22	3.50	3.85	1.90	3.30	2.92	3.57	2.25

ND = Not detected.

g⁻¹) and 4.12% for Fuerte (1.82 μg g⁻¹), whilst ultra-turrax method gave highest concentration of Cu in oils, 1.95% for Hass and 1.33% for Fuerte. Although these concentrations are not high enough to affect oil stability, Fe concentration is 0.2 μg g⁻¹ below the minimum threshold limit. In light of this, ultrasound extraction and the combined ultra-turrax method, that showed high dissolution percentages for most metals, should be avoided to prevent possible oxidation and destabilization of avocado oils. Cr dissolution was relatively high for all extraction techniques in both varieties but the total concentration of Cr in the mesocarp was low, therefore the concentration in the extracted oil is negligible. Cr is also an antioxidant type metal so its presence in oils can prevent autoxidation. Mn and Zn are also known to inhibit oxidative degradation of oils, therefore their dissolution into the oils is advantageous.^[21]

Conclusion

Microwave extraction produced the highest oil yield (69.94%) and amounts of FAs while SFE produced a wider range. The Fuerte variety is found to be healthier than Hass variety, as it had the highest MUFA: SFA ratio. Except for Fe and Zn, the Fuerte mesocarp possessed higher concentrations of elements than Hass. SFE and microwave extraction co-extracted the lowest amount of oxidizing metals. SFE is more adapted to produce oils for pharmaceutical industries, since the method is free from solvent and yields an undiluted product. Both SFE and microwave extraction approaches are reported to be feasible on a large scale operation.

Acknowledgments

The University of KwaZulu-Natal and the National Research Foundation (NRF) are thanked for financial support. Authors thank the technical team in the School of Chemistry at UKZN (Westville) for their assistance. Ms. Reddy acknowledges and appreciates the help she received from Mr. Lynton Freese (Operations Manager) and Mr. Cecil Hackney (Production Manager) of Everdon Estate as well as Mr. Shaeen Chetty for the help in sample collection.

References

- [1] Mossler, M.A.; Nesheim, N.O. Florida crop/pest management profile: avocado. CIR **2001** *1270*, 1–12.

- [2] Cummings, K.C.; Schroeder, A. Anatomy of the avocado fruit. Calif Avo Soc **56–64**, **1942**. **370**
- [3] Platt, K.A.; Thomson, W.W. Idioblast oil cells of avocado: distribution isolation, ultra structure, histochemistry, and biochemistry. Internat. J. Plant Sci. **1992**, *153*, 301–310.
- [4] Swisher, E.H. Avocado oil from food use to skin care. J. Am. Oil Chem. Assoc. **1988**, *65*, 1704–1706. **375**
- [5] Amunziata, C.M.; Massaro, M.; Siculella, L. Oleic acid inhibits endothelial activation. Thromb Vasc. Biol. **1999**, *19*, 220–228.
- [6] Carranza, M.J.; Herrera, A.J.; Alvizouri, M.M.; Alvarado, J.M. Chavez, C.F. Effects of vegetarian diets vs. a vegetarian diet enriched with avocado in hypercholesterolemic patients. Arch. Med. Res. **1997**, *28(4)*, 537–541. **380**
- [7] Lee, J.; Koo, N.; Min, D. Reactive oxygen species, aging and antioxidative nutraceuticals. Compr. Rev. Food. Sci. Food Safety. **2004**, *3*, 21–33.
- [8] Bergh, B. Nutritious Value of Avocado. Calif. Avo. Soc. Yearbook **1992**, *76*, 123–135. **385**
- [9] Human, T.P. Oil as a byproduct of the avocado. S. Afr. Avo. Growers' Assoc Yearbook **1987**, *10*, 159–162.
- [10] Karadjova, I.; Zachariadis, G.; Boskou, G.; Stratis, J. Electrothermal atomic adsorption spectrometric determination of aluminum, cadmium, chromium, copper, iron, manganese, nickel and lead in olive oil. J. Anal. Atom Spectrometry **1998**, *13*, 201–204. **390**
- [11] Botha, B.M. The supercritical fluid extraction of avocado oil. D. Tech. thesis, Pretoria Technikon: Pretoria, South Africa, **2002**. **395**
- [12] Kanchanamayoon, W.; Kanenil, W. Determination of Some Fatty Acids in Local Plant Seeds. Chiang Mai J. Sci. **2007**, *34(2)*, 249–252. **395**
- [13] Szentmihályi, K.; Vinkler, P.; Lakatos, B.; Illés, V.; Then, M. Rosehip (*Rosa canina* L.) oil obtained from waste hip seeds by different extraction methods. Bioresource Technology **2002**, *82*, 195–201. **400**
- [14] Scalia, S.; Giuffreda, L.; Pallado, P. Analytical and preparative supercritical fluid extraction of Chamomile flowers and its comparison with conventional methods. J. Pharmaceut. Biomed. **1999**, *21*, 549–558. **400**
- [15] Prados-Rosales, R.C.; Herrera, M.C.; Luque-García, J.L.; Luque de Castro, M.D. Study of the feasibility of focused microwave-assisted Soxhlet extraction of *N*-methylcarbamates from soil. J. Chromatogr. A, **2002**, *953*, 133–140. **405**
- [16] Clifford, A.A. Introduction to Supercritical Fluid Extraction in Analytical Science. In *Analytical Supercritical Fluid Extraction Techniques*; Ramsey, E.D., Ed.; Kluwer: Dordrecht, The Netherlands, **1998**; pp 10. **410**
- [17] Eggers, R. Supercritical Fluid Extraction of Oilseeds/Lipids in Natural products. In *Supercritical fluid technology in oil and lipid chemistry*; Eggers, R., King, J.W., List, G.R., Eds.; AOCS Press: USA, **1996**; pp 35. **415 Q2**
- [18] Woolf, A.B.; Ferguson, I.B.; Requejo-Tapia, L.C.; Boyd, L.; Laing, W.A.; White, A. Impact of sun exposure on harvest quality of 'Hass' avocado fruit. Rev Chapingo Ser Hort. **1999**, *5*, 353–358. **415 Q2**
- [19] Villa-Rodríguez, J.A.; Molina-Corral, F.J.; Ayala-Zavala, J.F.; Olivás, G.I.; González-Aguilar, G.A. Effect of maturity stage on the content of fatty acids and antioxidant activity of 'Hass' avocado. Food Res. Ints. DOI 10.1016/j.foodres.2010.11.012, **2010**. **420**
- [20] Arzt, W.E.; Roa, S.K. Lipid oxidation in extruded products. ACS Sym. Ser. **1994**, *543*, 296–314. **Q3**
- [21] Zidenberg-Cherr, S.; Keen, C.L. Essential trace elements in antioxidant processes. In *Trace elements, micronutrients, and free radicals*; Dreosti, I.E., Ed.; Humana Press: Totowa, NJ, **1991**; pp 107–127. **425**