CHEMICAL COMPOSITTION OF INDIGINOUS WATERMELON (*Citrullus lanatus* (Thunb) Matsum. and Nakai)) LANDRACE SEEDS FROM THE SEKHUKHUNE AND CAPRICORN DISTRICTS IN THE LIMPOPO PROVINCE

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DECLARATION

I declare that the work in this document titled "**CHEMICAL COMPOSITION OF INDIGENOUS WATERMELON (*Citrullus lanatus* (Thunb) Matsum. and Nakai)) LANDRACE SEEDS FROM THE SEKHUKHUNE AND CAPRICORN DISTRICTS IN THE LIMPOPO PROVINCE"** is my own work and has not been submitted at any institution for any degree before. All the sources of information have been acknowledged and referenced.

Emmanuel Alpheus Mogotlane

Signature **Date**

DEDICATION

This work is dedicated to my late brother Tumelo Elvis Mogotlane, for some wonderful but brief memories and the part he played guiding me to be a better man. *Robala ka khutjo Kgomomadie'a mabitsi,* you may be gone but you will never be forgotten.

Also a special dedication to my son Tumelo Noah Satekge, for the wonderful and special memories we share each day. May this work one day make you want to be a better man than I am, *Ponka wa daddy*.

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ABSTRACT

The aim of this study was to investigate the chemical composition of indigenous watermelon landraces (*Citrullus lanatus*) seeds grown in two districts in the Limpopo Province. Watermelon seeds are the most undermined oilseeds. The seeds have nutritional values that compare favourably with those of soybean, sunflower and ground nuts. Many cucurbit seeds such as watermelon seeds are rich in protein and oil, although none of these products have been used on an industrial scale. Nine indigenous watermelon landraces seeds (four from the Sekhukhune district and five from the Capricorn district) were examined. The landrace 06CDGM was found to have the highest mass per one hundred seeds (10.95 g per 100 seeds) with 07CDGM having the lowest mass (8.05 g per 100 seeds). The landrace 10CDGM was found to have the highest oil (41.5%), protein (20.39%) and fibre content (23.98%) with 01SDPW having the lowest oil yield (30.00%), 02SDPW was found to have the highest saponification value (184.57 mg KOH/g oil) and 09CDGM had the highest iodine value (138.575 g I_2/100 g oil). The landrace 01SDPW had the highest total sugar content. All landraces were found to have the essential amino acid leucine. Antioxidant activity (66.95%) and total flavonoids (0.295 mg/g as catechin equivalents) were found to be highest in the 09CDGM landrace, and total phenolic content (0.91 mg/g gallic acid equivalent) was found to be highest in 05SDPW. The landrace 06CDGM was found to have the highest copper content (0.088 mg/g); 02SDPW was found to have the highest iron content (0.194 mg/g); 10CDGM had the highest zinc (0.312 mg/g) and sulphate content (0.129 mg/g); while both 10CDGM and 03SDPW had the highest content of calcium (9.13 mg/g). The landraces were found to differ slightly in the content and quality of components tested. Each of the landraces was found to have some qualities that render them superior to the other landraces. The qualities and content of the landraces compare favourably with those of commercial oil seeds such as the sunflower and soybean. Overall, the landraces from the Capricorn district were observed to have superior qualities than those from the Sekhukhune district. The findings indicated that the landraces from Capricorn district had more mean oil, mean protein, crude fibre content, total phenolics and total flavonoid content than those from the Sekhukhune district. The landraces from Sekhukhune district had high carbohydrate, ash content and high content of the minerals iron and calcium. The landrace 10CDGM was found to have overall high

values with regard to most analyses, thus making the landrace superior to the others. The results indicate that landraces from both districts have complementary qualities to each other; however the landraces from the Capricorn are more superior in most aspects. The watermelon seeds have the potential to be used as a cost-effective and easily accessible source of nutrients and oil to meet nutritional challenges in developing countries.

KEY CONCEPTS

Citrullus lanatus; Landrace; Sekhukhune district; Capricorn district; Indigenous watermelon; Oil seeds; Essential amino acids; Antioxidant activity; Total phenolics; Total flavonoids; Mineral content; Total sugars; Crude fibre; Oil content; Saponification value; Iodine value.

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ABBREVIATIONS

Abs - Absorbance

ANOVA – Analysis of Variance

$BaSO_4$ – Barium sulphate

BSA – Bovine serum albumin

DPPH – 2,2-diphenyl-1-picrylhydrazyl

EDTA – Ethylenediaminetetraacetic acid

HCl – Hydrochloric acid

HDL – High-density lipoprotein

Kg - Kilogram

LDL – Low-density lipoprotein

M - Molar

mg – milligram

mg/g – milligram per gram

mg/l – milligram per litre

mg/ml – milligram per millilitre

N – Normality

$Na_2HPO_4.7H_2O$ – Sodium monohydrogen phosphate heptahydrate

$Na_2HPO_4.12H_2O$ – Sodium dihydrogen phosphate dodecahydrate

NaOH – sodium hydroxide

nm – nanometre

rpm – Revolutions per minute

TLC – Thin-layer Chromatography

µl – microliter

µM – micro molar

CHAPTER 1: INTRODUCTION

1.1 The watermelon crop

Watermelon (*Citrallus lanatus* (Thunb) Matsum. and Nakai)) is an important horticultural crop, mostly known for its sweet juicy fruit (Munisse *et al.,* 2011). It belongs to the genus *Citrullus,* which has four species (*C. lanatus, C. ecirrhosus, C. colocynthis,* and *C. rehmi*) (Shimotsuma, 1963). A study by Dane and Liu (2007), using chloroplast DNA to infer biogeographic and evolutionary relationship, origin, and domestication suggests that cultivated and wild watermelon have diverged independently from a common ancestor, most possibly *C. ecirrhosus* from Namibia. It is an important but underutilised crop. When used fresh or processed into juice, it generates much waste in the form of rind and seeds (Asghar *et al.,* 2012). Although the seeds are considered waste, they have been shown to be highly nutritive and contain large amounts of proteins and many beneficial minerals (Yadav *et al.,* 2011). Depending on the variety, virtually all parts of the watermelon plant can be used for food, including leaves, shoots, roots, flowers, seeds, and immature and mature fruits (Jacks *et al.,* 1972).

1.2 Watermelon in subsistence farming

Indigenous watermelons are cultivated by subsistence farmers in South Africa as landraces. A landrace is defined as a mixture of forms of a crop with or without limited human selection carried out to maintain it (Zeven, 1998). One landrace may be clearly distinct from other landraces, but repeated cultivation especially in different geographic areas, often results in a different appearance of the landrace. Hence, a landrace is not uniform and stable and thus different from a cultivar. Variation of watermelons is predominant in farmers who have developed landraces for a variety of purposes; for dessert, oil, and also for porridge. In traditional farming, watermelon is grown predominantly in low rainfall areas intercropped with cereals. There is however limited information available on the diversity of the genus *Citrullus* and extent of its distribution in southern Africa (Majaju, 2009).

1.3 Watermelon seeds as oilseeds

Oil seed plants are plants that have/bear seeds with a high level of oils used as energy reserves. They also possess reasonably balanced amounts of carbohydrates, fats and proteins. The oil can be extracted from seeds using various available technologies and then be used for human food and/or biodiesel production (Rodrigues *et al.,* 2012). Although these technologies exist, only a small portion of plant material is utilised directly for human consumption. The remaining portion of the material or part of it may be converted into nutrients for food, feed or fertiliser. Thus an important contribution to food resources or industrial products can be made. This would result in the utilisation of food-processing by-products and wastes, as well as underutilised agricultural products receiving more attention. Such utilisation would contribute to maximising the available resources and result in the production of various products and foods (El-Adawy and Taha 2001b). Watermelon seeds are one of the most undermined oil seeds, with the most utilised being the soybean, rapeseed (*Brassica napus*), cotton (*Gossypium hirsutum*), peanut (*Arachis* hypogaea) and sunflower in decreasing order. The increase in oilseed production to satisfy the needs for biodiesel is inevitable, resulting in an increase of agricultural and industrial by-products with a high potential for their valorisation (Rodrigues *et al.,* 2012)

1.4 Oilseed crops and their uses

Oilseeds such as soybean (*Glycine max*) seeds are valuable commercial sources of edible oil and protein with approximately 42% protein and 23% oil at maturity (Dombos and Mullen, 1992). The seeds of the watermelon are increasingly being used in the oil industry in semi-arid regions for use of oil in the cosmetic and pharmaceutical industry and also the prospect of use of the seeds in the improvement of infant formulation due to their high protein and fat content (Nwanko *et al.,* 2014).

Composition of oil seeds is affected by genotype, location, and year effects. However, the relative contribution of each of these factors varies with seed component evaluated, the seed type and geographical area. Although these are cost-effective sources of protein and oil, there are problems facing the use of these

products such as the use of sunflower (*Helianthus annuus*) oil is the scarcity of information on the nutritive value of the seed meal or the by-products of oil processing. However, the by-products of sunflower oil from the oil industry have been reported to be used as an alternative source of protein in animal nutrition (Poysa and Woodrow, 2002).

1.5 The watermelon and its nutrition

The knowledge of the nutritive and the anti-nutritive content of various parts of the watermelon fruit will encourage their consumption in diverse ways and re-utilisation of the vast amounts of seeds discarded as waste. The nutrient and anti-nutrient value of many fruits, seeds and their rind has not received much attention and these are at times discarded, even with their hidden nutrients (Johnson *et al.,* 2012). Recently, more attention has been paid to the utilisation of by-products and wastes, as well as underutilised agricultural products. Such utilisation will contribute to maximising available resources and can also result in the production of new foods (Nyam *et al.,* 2009).

1.6 Global usage of the watermelon

Watermelons are one of the major underutilised fruits grown in warmer parts of the world (Oseni and Okoye, 2013). The watermelon plant is a warm season crop which requires long growing seasons and grows best on rich sandy loam soil, although it also grows on most other soil types provided it is well drained (Majaju, 2009). The crop is a natural and rich source of phytochemical compounds which are believed to be beneficial for human health and well-being (Abu-Reidah *et al.,* 2013). Little is documented about the watermelon and its seeds in Africa, but the indications are that it has versatile uses. In Africa, seeds may be ground into coarse flower or oils may be extracted from them (Johnson *et al.,* 2012). Farmers in Namibia grow three types of watermelons: dessert types, the seed types and the cooking types (Maggs-Kolling *et al*., 2000). The watermelons planted are usually a mixture of different landraces.

1.7 Biological value watermelon

According to El-Adawy and Taha (2001a), little is known about the biological value, true digestibility, protein efficiency ratio and net protein utilisation of watermelon seeds, but according to Holland *et al.* (1995), the nutritional value of melon seed compares very favourably with those of soybean, sunflower seeds, and groundnut (*Arachis hypogaea*). Serious protein deficiencies and high costs of proteins have stimulated research on developing new sources of protein from unexploited sources (El-Safy *et al.,* 2012). The seeds have a high nutritive value and are a potential source of unsaturated fat, vitamins, antioxidants, minerals and proteins. They contain about 35% protein, 50% oil, and 57% dietary fibre. Some of the minerals found in these seeds are magnesium, calcium, potassium, iron, phosphorus, and zinc. Amino acid analysis of the seeds has shown that hydrophobic and acidic amino acids such as aspartic acid, glutamic acid, and serine dominated the composition of the protein fraction (Yadav *et al.,* 2011).

1.8 Watermelon and drought

South Africa is a water-scarce country with very limited available water (Demir *et al.,* 2011). This results in water threats that may even encompass malnutrition. According to Modi and Zulu (2012), water and malnutrition threats have provided the need to study the productivity and nutritional value of underutilised indigenous food crops in South Africa, with the aim of promoting production of more nutritious food from neglected crops with the same quantity of water, or less. The use of indigenous landraces of cooking and seed type watermelons in most parts of Africa indicates that the watermelon plays an important role in diet than anticipated from the use of modern sweet types alone. Watermelons are drought resistant and grow in full sun, and hot, dry air. Humid and moist climates put plants at greater risk of diseases (Majaju, 2009). Given the drought-tolerant nature of the indigenous watermelon, it can be anticipated that its importance as a food source is related to agro-ecological conditions (Nantoume *et al.,* 2012). Food security and poverty are locked in the same destructive cycle with poverty being the one leading cause of food insecurity, and hunger, which exists in South Africa. Agricultural growth offers possibilities for reducing risks of food shortages at all levels, increasing supply of food, creating

economic opportunities for vulnerable people and improving dietary diversity and food quality (Maliwichi *et al.,* 2012).

1.9 Benefits of using watermelon seeds in developing countries

There is an increasing prevalence of nutrition related illnesses especially in Africa due to poverty and insufficient knowledge of the nutrition and economic importance of locally available and easily accessible food and food stuffs. Studies on Cucurbitaceae seeds have shown that they contain high protein levels with high levels of essential amino acids except lysine and sulphur containing amino acids. The seeds of the watermelon fruit have crude protein content of 23.4%, with good quantities of argenine, isoleucine, leucine, and phenylalanine which are essential amino acids as well as glutamic acid and aspartic acid (Achu *et al.,* 2013). Exploitation of underutilised crops can boost economic development, especially in developing countries where intake of sufficient quantities of nutrients, in particular proteins, is less than desirable (Wani *et al.,* 2011). Large segments of the population in these developing areas suffer from protein malnutrition and projections based on current trends indicate a widening gap between human population and protein supply (Karaye *et al.,* 2012). Agricultural approaches have also been considered in order to increase the micronutrient content in food crops, in particular the use of crop genotypic variations in the uptake and accumulation of micronutrients, are also considered to be useful in overcoming micronutrient deficiencies and sustainable and cost-effective methods for alleviating mineral deficiency in humans. Using seeds to supplement these essential nutrients will be beneficial as seeds will be planted from the previous harvest and the new pattern of nutrition will persist, which will be an added advantage over supplementation which the impact ends after the last supplement is taken (Stein, 2010).

Many cucurbit seeds, like the watermelon, are rich in oil and protein, although none of these products have been used on an industrial scale (Mariod *et al.,* 2009). The aim of this study was to investigate the chemical composition and nutritional values of different indigenous watermelon landrace seeds grown in the Limpopo Province and identify those with the potential to be utilised commercially by subsistence farmers.

1.10 Motivation

Indigenous watermelon landraces are drought tolerant and produce different types of fruits. Morphologically, they differ in colour and shape. There are those that are green, striped or grey. These can in turn have red or white flesh and there are those that produce more seeds than others. Many poor households in South Africa use watermelon seeds as a relish taken roasted with stiff porridge. The composition of the seeds is not known and is likely to be different among different landraces. Some may have high protein and low lipid content and vice versa. There might be those whose lipid content and composition is comparable to those of the sunflower or with the protein content being comparable to that of the soybean. Identification of such landraces or varieties can lead to them being produced sustainably in dry areas. The European Union's ban on the use of animal products in animal feeds also increases the need for the investigation of vegetable oils and proteins from plants, especially those that are considered to be of less importance.

1.11 Research hypothesis

Different watermelon landraces from different geographical regions have similar nutrient composition.

1.12 Outcomes of research project

The outcomes of this project is to give an indication of how the watermelon seeds can be more beneficial to society as opposed to being regarded as waste, and also to highlight the potential of the use of the seeds commercially. The results of the study will allow subsistence farmers the opportunity to breed their landraces in a way that would yield them more products that they may desire.

1.13 Aim

To determine the chemical composition of watermelon landraces seeds from two districts in Limpopo province.

1.14 Objectives

The objectives of this study were to:

I. Collect indigenous watermelon fruits from the Sekhukhune and the Capricorn districts and extract seeds from the fruits
II. Determine total lipid and protein composition of the seeds
III. Determine total phenolics, and antioxidant activity of the seeds
IV. Determine carbohydrate, fibre and ash content of the seeds
V. Determine mineral content of the seeds
VI. Compare mean nutrient content between the landraces from the two districts

1.15 Dissertation outline

Chapter 1 – Introduction

The chapter focuses on the watermelon, its consumption and potential for economic opportunities. The uses and economic importance of the seeds are also highlighted. The chapter also focuses on the biological and nutritional value of the watermelon and the use of the fruit and seeds by subsistence farmers.

Chapter 2 – Literature review

This chapter is about the watermelon, its seeds, the proteins, oils, fibre and the antioxidant activities of the seeds. It looks at the commercial uses of high protein and oil content seeds in general. The health benefits associated with the fruit and its seeds are also highlighted in this chapter.

Chapter 3 – Research methodology

In this chapter, the protocols and methods that were followed in the investigation are listed and detailed. The method of recording the data and those for analysing the data are also mentioned.

Chapter 4 – Results

The results obtained in this study are given in this chapter with comparisons also being given in the form of figures.

Chapter 5 – Discussion

The chapter looks at the findings of the study and compares them with the findings from the literature that were obtained in similar or related species as well as comparison with the findings in well-established commercial crops.

Chapter 6 – References

The chapter acknowledges the literature that has been cited in the dissertation.

CHAPTER 2: LITERATURE REVIEW

2.1 The watermelon

The watermelon belongs to the Cucurbitaceae and is widely distributed in Africa and Asia, but the exact origin of the crop is subject to strong debate. It is an annual species containing cultivated semi-domesticles and wild forms (Acar *et al.,* 2012). According Vaughn and Geissler, (2009), the crop originated from southern Africa and occurs naturally in South Africa, Namibia, Botswana, Zimbabwe, Mozambique, Zambia and Malawi. It is also cultivated and thrives in warmer parts of the world (Maggs-Kolling *et al.,* 2000), and is also widely distributed in tropical and subtropical areas (Acar *et al.,* 2012). The crop is adapted to the prevailing arid and unpredictable climatic conditions (Maggs-Kolling *et al.,* 2000). In Africa, the watermelon accounts for 5.4% of the harvested area devoted to vegetable cultivation (Munisse *et al.,* 2011) and 6.8% worldwide (Gunner and Wehner, 2004).

The watermelon fruit is a source of multiple minerals, vitamins, and proteins that are present in the skin, pulp and seeds (Wani *et al*., 2011). The watermelon, like many other cucurbits such as, gourds that include cucumbers (*Cucumis sativas*), squashes (*Cucubita moschata*), luffas (*Luffa sp*) and melons (*Cucumis melo*) are among the economically most important vegetable crops in the world (Loukou *et al*., 2007).

2.2 Watermelon seeds

Plant seeds are a good source of food for animals as well as humans, since they contain nutrients necessary for growth, including healthy fats such as omega fats (Mathew *et al.,* 2014). Seeds of many plants in the Cucurbitaceae are rich in oil and proteins, and although none of these oils have been used on an industrial scale, many of the oils are used as cooking oil in some countries in Africa and the Middle East (Al-Khalifa, 1996). Watermelon seeds have shown the potential for use in the food industry as they remain intact after removing the pulp and peel (de Conto *et al*., 2011). The seeds have also the ability to store well, with both oil and fatty acid content being found to be stable after six months in storage (Jarret and Levy, 2012). According to Baboli and Kordi (2010), the seeds can be utilised successfully as a source of edible oils for human consumption, and their oil might be an acceptable substitute for highly unsaturated oils (Baboli and Kordi, 2010). The seeds also

contain lipids of nutritional interest, with high concentrations of unsaturated fatty acids, including a high concentration of phytosterols, particularly stigmasterol and β-sitosterol (De Conto *et al*., 2011). The use of watermelon seeds as a food source appears to be justified by their reported nutritional value. The dry seeds of *C. lanatus* have been reported on average to contain 22 g of proteins, 30 g of fat and 11 g of carbohydrate per 100 g sample (Wickens *et al*., 1984). The seeds are highly nutritive and contain large amounts of proteins and many beneficial minerals such as magnesium, calcium, potassium, iron, phosphorus and zinc (Yadav *et al.,* 2011)

2.2.1 Watermelon seed proteins

Watermelon seeds are one of the common protein supplement seeds like those of the cotton, groundnut, soybean and rape seeds (Mustafa and Alamin, 2012). They are high in protein content, which ranges from 25-40% (Younis *et al*., 2000), and are naturally complete with essential amino acids (El-Adawy and Taha, 2001b). The biological functions of proteins enable their classification as enzymatic catalysts, structural proteins, contractile proteins, carrier proteins, antibodies, reserve proteins (albumin and seed proteins) and protective proteins (Rodrigues *et al.,* 2012). Protein quality is known as the nutritional value of a food depending on its amino acid content and on the physiological utilisation of specific amino acids after ingestion (Senga *et al.,* 2013).

Many proteins from cucurbit seeds are reported to have pharmacological activity, including anti-diabetic, anti-fungal, antibacterial, anti-inflammatory and antioxidant activity (Nkosi *et al*., 2006). Watermelon proteins have been reported to contain significant quantities of glutamic acid, aspartic acid, arginine and leucine (Mello *et al*., 2001). The main proteins of the watermelon seeds are composed of storage salt-soluble globulins, accompanied by albumins and glutelins (Teotia and Ramakrishna, 1984). Plant proteins are usually quantified by the Bradford's assay.

Bradford assay determines the amount of protein in a substance using Coomasie Briliant Blue dye, which turns from red to blue when it binds with proteins. The dye is protonated by amino groups of basic amino acids lysine and tryptophan and then binds with the hydrophobic regions in proteins and turns blue (Bradford, 1976). By measuring the absorbance at 595 nm in a spectrophotometer and comparing samples to a standard protein such as bovine serum albumin (BSA), the amount of

protein in the sample can be quantified (Field and Field, 2010). Arginine and lysine residues seem to be the places where the dye readily binds, and tryptophan and phenylalanine are involved as well, suggesting some hydrophobic interactions between proteins and the dye. This behaviour may lead to variation in quantification of different proteins because the method response depends on the composition of the proteins. The use of a single reactive and sensitivity of the dye to small amounts of less than 5 µg makes the Bradford method the most widely used for protein quantification. It was shown though, that the assay may suffer significant interference from some compounds that may be found in protein samples (Silverio *et al.,* 2012).

2.2.2 Commercial uses of high protein content seeds.

The watermelon fruit contains a notable amount of seeds. The seed meals extracted from these seeds are notably high in proteins. The seeds could be used as raw material for production of high quality protein products for food formulation as nutritional supplements and functional ingredients (El-Adawy and Taha 2001a). Though technology exists for decorticating the watermelon seeds, this agricultural commodity is commercially processed and utilised to a limited extend (Lakshmi and Kaul, 2011). The ban on animal by-products in feeding poultry by the European Union increases the demand for making protein sources, making them very expensive (Karaye *et al.*, 2012). Protein is an expensive component in animal rations and one that may be in short supply, especially in developing countries. This has resulted in one of the most critical and pressing problems which is the augmentation of proteins in diets which are deficient in proteins. Unfortunately, there are constraints facing efficient utilisation of these protein by-products, which includes the export of the product, human nutrition, and the food industry and poultry nutrition. These problems necessitate seeking of alternative potential oil seeds that are currently underutilised that can replace the conventional oil seeds such as the soybean and sunflower seeds that are dominating the markets at present. Seed cakes, which are by-products of extracted seeds, can also be used as protein supplement in livestock feed. The experience with watermelon seed cakes in ration for fattening livestock showed that watermelon seed cake is a good source of digestible protein (Beshir *et al.,* 2009). This has brought challenges in the areas of nutrition research in the watermelon and other plants of lesser importance to man

that may serve as a veritable source of vegetable protein due to costs of high quality conventional sources (Karaye *et al.,* 2012).

2.2.3 Watermelon seed oil

Oils and fats are substances of vegetable and animal origin that are insoluble in water and are greasy to the touch and are made up of fatty acids. Fatty acids are carboxylic acids with varying hydrocarbon lengths at one end of the chain to the terminal group at the other end. They are predominantly unbranched and those with even numbers of carbon atoms between 12 and 22 carbons long react with glycerol to form lipids in plants, animals and microorganisms (Albishri *et al.,* 2013). The fatty acids act as storage of energy and as a component of cell membranes. Mammals are unable to synthesise linoleic and α-linolenic acids which are termed essential fatty acids. These essential fatty acids form an important constituent of all cell membranes, and confer to membranes properties of fluidity and thus determine and influence the behaviour of membrane-bound enzymes and receptors (Njuguna *et al.,* 2014).

The high world demand for oils and fats to meet the multiplex human consumption and multitudinous industrial needs are reasons for the importance of oil seeds (Ziyada and Elhussien, 2008). Vegetable oils are utilised globally for many food and other industrial purposes. They are essential in meeting global nutritional demands and are used for many foods and other industrial products (Idouraine *et al.,* 1996). Despite the vast range of sources for vegetable oils, world consumption is dominated by soybean, palm, rapeseed, and sunflower oils with 31.6, 30.5, 15.5, and 8.6 million tons respectively consumed per year (Stevenson *et al.,* 2007). Characteristics of watermelon seed oils compare very well with those of the soybean and sunflower oils (Baboli and Kordi, 2010). In Africa, watermelon seeds have been priced for their highly nutritive oil. Traditionally, the seeds are removed from the rind and then allowed to dry outside in the sun. Once dried, the seeds are pressed to extract the oil (Sui *et al.,* 2011).

2.2.4 Commercial uses of seed oil

The demand for vegetable oils is increasing at a rapid pace due to increasing demand for non-food uses of vegetable oil. For example, in biodiesel, oleochemicals,

lubricants, and cosmetics (Baboli and Kordi, 2010). The potential of the seed oil of various cucurbits for the use in soap making has been noted (Jarret and Levy, 2012). The high amounts of unsaturated fatty acids could be used in manufacturing of animal feed supplement with higher levels of unsaturated fatty acids (Njuguna *et al.,* 2014). The presence of high amounts of the essential linoleic and oleic acid suggests seed oil may be used as edible cooking and salad oils or for margarine manufacturing (Alfawaz, 2004).

Watermelon fruit contains substantial quantities of seeds which are an excellent source of dietary oil (Wani et al., 2006). Seeds of plants belonging to Cucurbitaceae family are known to be a rich source of oil as in soybean, cotton-seed and corn (Esuoso et al., 1998). The seeds of the species in the family are economically important, of which the fruits are used for nutrition and medicinal purposes. Most of these seeds are rich in oil and protein. In spite of the potential, none of the cucurbit oils have been used on an industrial scale (Mariod *et al.,* 2009). The high presence of oil makes watermelon seeds suitable for oil industry application (Nyam *et al.,* 2009). Some cucurbit seeds, such as the seeds of egusi melon (*Citrullus colocynthis*) can be eaten individually as a snack when roasted and are also used extensively for cooking purposes, either as a soup additive (thickeners) or cooking oil source (Bande *et al.,* 2012). Some of these cucurbit seeds, like the pumpkin seeds have shown potential to provide good quality oil, but the oil is not widely used commercially even though it has characteristics that are well suited for industrial application and can contribute to healthy human diets. The seed oil is however, sold in most reputable health stores in the United States, typically formulated in capsules containing 1 gram of oil (Stevenson *et al.,* 2007). The oil is also being produced in Australia, Slovenia, and Hungary. Although it has not been used on an industrial scale, most of the oil is used as cooking oil in some countries in Africa and the Middle East, and salad oil in the south of Australia and some regions in Slovenia and Hungary (Alfawaz, 2004).

Vegetable oils are essential to meeting global nutritional demands and are utilised for many food and mineral industrial purposes. Watermelon seeds are prized for the highly nutritive oils they contain (Sui *et al.,* 2011). Watermelon seeds are used for production of oil at a subsistence level in Nigeria. The kernels have also been used as an additive to some food dishes. Because some wheat flour in the baking industry

is deficient in some elements, in particular calcium and iron, the fortification of wheat flour might improve their dietary properties (El-Adawy and Taha, 2001). Although the oil is highly nutritive, it deteriorates when inadequately processed, with oxidation being the main deterioration reaction that propitiates the formation of hydroperoxides and serveral products of oxidation such as aldehydes, peroxides and ketones (Hernandez *et al.,* 2013). Traditionally, the seeds are removed from the rind and allowed to dry outside in the sun. Once dried, they are pressed to extract the beneficial oil (Sui *et al.,* 2011). The method used in the extraction of oil from seeds and type of solvent used to some extend have a notable effect on the percentage oil yield and the quality of the extracted oil (George *et al.,* 2013). Oils are composed of fatty acids plus a glycerol, the linkage between the fatty acids and glycerols determine the type and quality of the oils.

2.3 Watermelon seed antioxidants

Antioxidants can be defined as substances whose presence in relatively low concentrations but significantly inhibits the role of oxidation of the targets (Rakesh *et al.,* 2010). Antioxidants are considered important nutriceuticals with many health benefits (Sharma and Bhat, 2009). Plants are known to be rich in biologically active substances such as the flavonoids, phenolic acids, anthocyanins, ethereal oils, and tannins, many of which exhibit antioxidant activity (Korotkova *et al.,* 2003). Some of the antioxidants found in plants, such as tocopherols (vitamin E), ascorbic acid (vitamin C), and carotenoids are substances of major significance in human physiology. Most of these antioxidants are phenolics. Based on carbon structure, phenolics can either be classified as flavonoid compounds (flavones, isoflavones, flavanones, flavonols, and anthocyanidins) or non-flavonoid compounds (benzoic acid, stilbenes, and hydroxycinmamic acids) (Kang *et al*., 2010). Watermelon varieties are said to contain high amounts of antioxidants, including citruline and lycopene (Singh and Matta, 2010). Watermelon seeds contain an antioxidant known as cucurbocitrin, which is extracted and used in lowering blood pressure and improvement of kidney function (Oseni and Okoye, 2013).

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Hall and Cuppet 1997). There are two types of antioxidants; the primary- and secondary antioxidants.

Primary antioxidants can inhibit or retard oxidation by scavenging free radicals by donation of hydrogen atoms or electrons, which convert them to more stable products. Secondary antioxidants function by many mechanisms, including binding of metal ions, scavenging of oxygen, converting hydroperoxides to non-radical species, absorbing UV radiation or deactivating the singlet oxygen (Maisuthisakul *et al.,* 2007).

Oxidative stress depicts the existence of free radicals which become deleterious when not eliminated by the body's endogenous system. Many plants contain large amounts of antioxidants such as polyphenols, which can play an important role in absorbing and neutralising free radicals (Kaneria *et al.,* 2012).

Watermelon is a good source of phytochemicals and lycopene, which acts as an antioxidant during normal metabolism and protects against cancer. The red carotenoid pigment may act as an antioxidant by quenching free radicals formed during normal metabolism and may deactivate DNA chain-breaking agents that are implicated in some cancers (Perkins-Veazie and Collins 2004). Lycopene is reported to be the prevailing carotenoid in red-fleshed watermelons (70-90% of total carotenoids), while other carotenoids include phytofluene, phytoene, β-carotene, lutein, neurosporene, and ς-carotene (Tadmor *et al.,* 2005).

2.3.1 Free radicals and cell damage

Free radical reactions and reactive oxygen species are implicated in the pathology of many human diseases. They have also been implicated in the etiology of degenerative diseases (Rakesh *et al.,* 2010). A free radical is a chemical compound that contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from oxygen are called reactive oxygen species which can cause damage to other molecules by extracting electrons from them in order to maintain stability (Kaneria *et al.,* 2012). Many incurable human diseases such as cancer, cardio- and cerebro-vascular diseases have been recognised as being a possible consequence of free radical damage to lipids, proteins, flavonoids, and nucleic acids.

2.4 Phenolics

Phenolics encompass approximately 8000 naturally occurring compounds, all possessing a phenol (aromatic ring bearing at least one hydroxyl substituent). The compounds are further divided into phenols and polyphenols, depending on the number of phenol subunits. Simple phenols include phenolic acids, and polyphenols possess at least two phenol subunits, which include flavonoids (Leopoldini *et al.,* 2011).

Phenolic compounds are plant secondary metabolites found commonly in herbs, and fruits. Many of these compounds are responsible for the attractive colour of leaves, fruits and flowers (Leopoldini *et al.,* 2011). Phenolic compounds in plants provide an array of natural antioxidants with redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kaneria *et al.,* 2012). Phytochemicals such as phenolic compounds are considered beneficial for human health and well-being, lowering the risk of diseases such as cardiovascular disease and some types of cancers. They work primarily by free-radical neutralisation and chelating of pro-oxidant metal ions (Abu-Reidah *et al.,* 2012).

2.4.1 Flavonoids

Flavonoids are a large group of phytocompounds commonly existing in fruits, vegetables, roots, and flowers of the plant species. All polyphenolic flavonoids consist of a benzene ring condensed with a six membered ring which contains a phenyl group as a substituent in the second position. The role of flavonoids as an antioxidant is subject to intense theoretical and experimental research (Sadasivam and Kumaresan, 2011). Flavonoids such as flavones (apigenin), flavonols (quercetin), isoflavones (crobol), and isoflavonoids (ferreisin) are widely distributed in plants (Singh *et al.,* 2009). The biological and pharmacological activities of the compounds are related to their antioxidant activity due to their ability to scavenge free radicals. The antioxidant activity of flavonoids depends on their ability to donate protons and electrons to resist the effect of energetic oxidants such as free radicals (Sadasivam and Kumaresan, 2011)

Flavonoids (about 3000), are potent antioxidants which could protect the membrane lipids from oxidation. Several reports have revealed that the majority of the

antioxidant activity may be from biochemicals such as flavonoids, isoflavones, flavones, anthocyanins, catechins and other phenolic compounds (Hossain *et al.,* 2011).

Flavonoids exert protection against heart disease through inhibition of cyclooxygenase and lipoxygenase activities. They inhibit lipoperoxidation by forming less aryloxyl radicals with free radicals (Singh *et al.,* 2009). The diversity of flavonoids resulting from the structure makes them exhibit antineoplastic, anti-inflammatory, antihepatic, antiallergic, antibacterial, antimutagenic, anti-thrombosis, antiviral, antioxidant and vasodialation activities.

2.5 Mineral nutrients in watermelon seeds

There are at least twenty dietary minerals and trace elements that are essential for the proper functioning of the human body. If elements are not ingested in adequate amounts, there will be negative impact on the health of those who consume too little of these nutrients. Even though minerals are essential, many of these elements are needed in such small amounts or are so abundantly available in many food stuffs that the occurrence of related deficiencies is rare or even unknown. Many micronutrients are involved in a variety of biological processes and hence are indispensable to sustain life. At the same time these elements can be toxic when present in excessive amounts. Elements like copper form part of proteins involved in a variety of biological processes. In humans, access to copper in the environment is limited. Food and drinking water and copper-containing supplements are the main sources of copper. Copper content in diet varies widely because food stuffs differ greatly in natural copper content. Factors such as season, soil quality, geography, water source and use of fertilisers influence the final content in food. Low copper status has been associated with bone malformation during development, risk of developing osteosclerosis later in life, impaired melanin synthesis, poor immune response and increased frequency of infections, poor cardiovascular health and alteration in cholesterol metabolism (de Romana *et al.,* 2011).

It has been shown that mineral deficiencies affect billions of people, causing disease and suffering at level of the individual, and contributing considerably to burden of disease that is borne by the society they live in. Mineral malnutrition also imposes tangible economic costs by hampering both individual productivity and overall growth

(Stein, 2010). Bioavailability of minerals in most oil seeds is very low due to the presence of mineral inhibitory components (Lakshmi and Kaul, 2011).

The seeds of the watermelon are said to contain considerable amounts of minerals such as calcium, iron, manganese, phosphorus, potassium, sodium, zinc, copper and magnesium, which assist in growth and development of a healthy body. These minerals take part in various metabolic activities of living organisms (Gwana *et al.,* 2014).

2.6 Health benefits of watermelon and watermelon seeds

The health benefits of eating watermelon, as well as its low caloric value, make the watermelon a very attractive fruit. The identification and quantification of bioactive compounds and antioxidant properties of many fruits and vegetables are well defined. However, the studies on characterisation and quantification of the phytochemical and antioxidant properties of the watermelon are very limited (Tlili *et al*., 2011). On the other hand, activities such as antimicrobial, antioxidant, and anti-inflamatory activities were reported by Okunrobo *et al.* (2012) and Singh *et al.,* (2009) have associated such activities with the presence of phenolic compounds. It has also been documented that consumption of the watermelon lowers the risk of degenerative diseases by mechanisms such as free radical neutralisation, protection and re-generation of dietary antioxidants. Medicinally, watermelon has been used in treating dropsy and renal stones, reducing hypertension, preventing erectile dysfunction, acting as an antioxidant, and treating enlarged liver, jaundice and giardiasis (Abu-Reidah *et al.,* 2012). Watermelon seed oil has also been shown to have fatty acids that are of importance in the brain, the retina, liver, kidney and the gonads. Some of the fatty acids within the watermelon seed oil have also been shown to increase HDL cholesterol, which is beneficial to the human blood stream and while oleic and linoleic acids are known to reduce LDL, which is the bad cholesterol (Njuguna *et al.,* 2014)

The market acceptance of watermelon juice is increasing worldwide due to its sensorial, physical and nutritional properties. However, some undesirable changes occur on the attractive red colour, viscosity and flavour of the watermelon juice and are catalysed by enzymes such as peroxidase, lipoxygenase, pectin methylesterase and polygalacturonase (Robinson, 1991).

2.7 Watermelon fibre

There is little or no extensive studies done on watermelon fibre, however, there have been studies on the fibre content of watermelon and its seeds. Fibre is the edible part of the plant or the analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. It is classified into two categories; it can either be water-insoluble fibre, which includes cellulose, hemicellulose and lignin, or water-soluble fibre, which includes pectin, gums, and mucilage. It is a complex mixture of polysaccharides with many different functions and activities as it passes through the gastrointestinal tract. Although fibre is said to have health benefits, it is also suspected of impairing mineral absorption because of pectin through their charged carboxylic groups and associated substances such as phytates. Dietary fibre is naturally present in cereals, vegetables, fruits, and nuts. It is suggested that a healthy adult should eat between 20 and 35 g of fibre daily. Diets high in fibre are said to have positive effects on health since their consumption has been linked to decrease in incidences of several types of diseases due to its beneficial effects like increasing the volume of faecal bulk, decreasing time of intestinal transit, cholesterol and glycaemic levels and trapping substances that can be dangerous for human organs. It has been postulated that fibre may act as a protective factor in cancer of the large bowel by shortening transit time, thus reducing the time for formation and action of carcinogens (Dhingra *et al.,* 2012).

CHAPTER 3: MATERIALS AND METHODS

3.1 Fruit collection

Ripe watermelon fruits were collected at random in the Sekhukhune and Capricorn districts in autumn during harvesting period. Four landraces were collected in triplicates (three fruits per landrace) from the Sekhukhune district to give a total of 12 fruits and five fruits were collected from the Capricorn district (also in triplicates). The watermelons were given code names to indicate the order in which they were analysed, the district from which they were collected and the person who supplied the watermelons.

3.2 Seed preparation

Fruits were cut open and seed colour recorded. The seed colour was used to further characterise the plant landrace as fruit with similar colour usually have seeds of different colours. The seeds were washed with tap water and dried at 60°C for 72 hours.

3.2.1 Mass per 100 seeds

One hundred dried seeds were counted from seeds of each landrace and weighed to determine the mass per 100 seeds.

3.3 Oil analysis

3.3.1 Oil extraction

The ground seeds (10 g) from each landrace (in duplicates) were extracted for oil with n-hexane in a Soxhlet apparatus for 24 hours.

3.3.2 Oil content

The extract was poured into a pre-weighed beaker and dried in a draft of air at room temperature and further dried at 60°C for 24 hours to remove excess water. The beaker with oil was weighed and the mass of oil was determined from the weight. The defatted residue was dried at 60°C for further analysis and extractions.

3.3.3 Chemical properties of oils

3.3.3.1 Saponification values are used as indications of the average molecular weight and chain length of lipids (Ardabili *et al.,* 2011).

A 2.5 g sample of oil was weighed out into a round bottom flask and 25 ml 0.5 M ethanolic potassium hydroxide was added. The mixture was boiled under reflux for one hour and 12.5 ml (in duplicates) of the hot soapy solution was titrated with 0.5M hydrochloric acid using phenolphthalein as an indicator. The saponification value was calculated using the formular $56.1(V_2-V_1)/W$ where; W= the weight of oil, V_1 = volume of HCl used in sample, V_2 = volume of HCl used in blank (George *et al*., 2013).

3.3.3.2 Iodine value

The iodine value is a measure of the average amount of unsaturation of fats and is expressed in terms of the number of grams of iodine absorbed per 100 g sample. The value is a measure of unsaturation of fats and oils and hence their potential to become oxidised (IAFMM, 1981).

Iodine value was determined according to the method of International Association of Fish Meal Manufacturers (IAFMM) (1981) as modified by Firestone (1994).

A 0.1 g sample (in duplicates) of oil was mixed with 10 ml Wijs solution and 10 ml of carbon tetrachloride. The mixture was left in the dark for 30 minutes. Then, 15 ml of 10% potassium iodide solution and 50 ml distilled water were added. The mixture was titrated with 0.1 M sodium thiosulphate solution until the yellow colour almost disappeared. A 2 ml volume of starch indicator solution was added. The mixture was titrated with vigorous swirling until the disappearance of the blue starch-iodine colour. The iodine value was calculated, where 1 ml of sodium thiosulphate is equivalent to 0.01269 g of iodine. The difference between the control titration and the oil titration multiplied by this factor was used to calculate the mass of iodine absorbed by the oil.

3.4 Protein analysis

3.4.1 Protein extraction

A 1.0 g sample of seeds (in duplicates) was weighed and macerated with pestle and mortar in 5 ml of phosphate buffer and the material was transferred into centrifuge tubes. The homogenate was centrifuged at 8000 rpm for 20 minutes. The supernatants were collected and the extractions were repeated 4-5 times. The supernatants were combined and made to 50 ml volume with the phosphate buffer. The phosphate buffer consisted of: 0.1 M monobasic sodium phosphate (13.9g in 1 L) and 0.1 M dibasic sodium phosphate (53.65 g $Na_2HPO_4.7H_2O$ or 71.7 g $Na_2HPO_4.12H_2O$).

3.4.2 Determination of total protein content

Total proteins from the seeds were determined colorimetrically according to the method of Bradford (1976), using a spectrophotometer (Cary UV-Visible spectrophotometer) with bovine serum albumin (BSA) as standard.

The protein extract (50 µl in duplicates) was mixed with 750 µl distilled water and 5 ml Bradford reagent. Absorbance was read at 595 nm using a spectrophotometer. Standards were prepared using BSA, where dilutions of 0.0, 10.0, 20.0, 30.0, 40.0, and 50.0 mg/ml were made.

3.4.3 Amino acid analysis

Defatted seed powder (0.1 g) was hydrolysed in 10 ml 6 N HCl at 110°C for 24 hours according to Association of Official Analytical Chemists (AOAC) method 982.30 (2006).

The hydrolysate was filtered through Whatman No. 1 filter paper and rinsed three times with water. Seed extracts were spotted on $60F_{254}$ TLC plates and developed in 1-butanol, glacial acetic acid and water (4:1:1). Dried TLC plates were sprayed with ninhydrin solution and dried in an oven at 110°C to visualise spots.

3.5 Total phenolic analysis

Total phenolics were determined according to the Follin-Ciocalteau method of analysis adapted from Torres *et al*. (1987). The defatted residue (3.3.1 above) was ground to fine powder.

3.5.1 Extraction of phenolics

A 2.0 g sample (in duplicates) of the residue was weighed out into 150 ml Erlenmeyer flasks. A 15 ml volume of methanol was added. The flasks were stoppered and the mixture extracted on a shaker at 200 rpm for 2 hours. The extract was filtered into 50 ml volumetric flask through Whatman No 1 filter paper. The residue was washed three times with 10 ml volumes of methanol. The extract was made to a volume of 50 ml with methanol.

3.5.2 Determination of total phenolic content

A 500 µl sample of extract (in triplicates) was pipetted into a graduated test tube. Distilled water (5.0 ml) was added to the extract. A 0.5 ml volume of Folin-Ciocalteau was added to the mixture, mixed thoroughly and allowed to stand for five minutes at room temperature. A 1.5 ml volume of 20% sodium carbonate was added and the extract was made to final volume of 10 ml with distilled water and mixed thoroughly. The mixture was incubated at 50°C for 2 hours. After incubation the mixture was vortexed, and absorbance read at 765 nm using a spectrophotometer. Total phenolics were determined from a standard curve, where a 0.200 g mass of gallic acid was dissolved in methanol and made to a final volume of 100 ml to make a stock solution of 2000 mg/l. Standards containing 0.0, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/l of gallic acid were prepared with the Folin-Ciocalteau reagent as above. Absorbance was read at 765 nm and the phenolic concentrations were determined directly by the spectrophotometer.

3.5.3 Total flavonoid content

The flavonoids were determined according to the aluminum chloride colorimetic assay (Marinova *et al*., 2005).

3.5.3.1 Flavonoid analysis

An aliquot of 500 µl of phenolic extract was pipetted into a graduated test tube. Distilled water (2 ml) was added to the extract, followed by 1.5 ml of 5% sodium nitrate and mixed well. The mixture was incubated at room temperature for 5 minutes. A volume of 1 ml of 1.0 M sodium hydroxide was added. The mixture was mixed well and absorbance was read at 510 nm. Total flavonoids were determined from a standard curve, where a mass 0.200 g catechin was dissolved in methanol and made to final volume of 100 ml to make a stock solution of 2000 mg/l. Dilution series of 0.0, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/l catechin was prepared.

3.6 Antioxidant activity

3.6.1 Time course of antioxidant activity

The time course of antioxidant activity was determined according to the method of Sharma and Bhat (2009).

A 120 µl sample of seed extract was pipetted into a calibrated tube and methanol was added to the 3.0 ml mark. The reaction was started by adding 1.0 ml of 200 µM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution and absorbance was read at 517 nm at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 minute intervals. A graph of absorbance versus time was plotted and the time course of inhibition was determined from the graph.

3.6.2 Determination of percentage inhibition/Scavenging capacity

Antioxidant activity was determined according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method of analysis adapted from Odhav *et al.* (2007).

A 2.5 ml volume of the phenolic extract was pipetted into a test tube and 1.0 ml of 0.3 M DPPH (in methanol) was added and mixed well. For the negative control, 2.5 ml methanol was used as a blank. The mixture was incubated at room temperature for thirty minutes and then absorbance read at 518 nm. For the positive control 2.5 ml of 1.0 mM ascorbic acid was used. Percentage scavenging capacity =100-[Abs of sample – Abs of blank] × 100/Abs of positive control.

3.7 Determination of ash content

The protocol followed was adapted from Inuwa *et al*. (2011).

To determine the ash content, porcelain crucibles were dried in an oven at 90°C for 30 minutes and were transferred to a desiccator to cool and were weighed (W_1). A 5 g sample of each seed powder was weighed into the crucibles (W_2) and burned in a muffle furnace at 500°C for 2 hours. The crucibles were transferred to a desiccator to cool and weighed thereafter (W_3).

The percentage of crude ash was calculated as:

$$\text{Crude ash (\%)} = \frac{W3-W1}{W2-W1} \times 100$$

Where,

W_1= Weight of crucible

W_2= Weight of crucible and sample before ashing

W_3= Weight of crucible and sample after ashing

3.8 Determination of carbohydrate content

Samples were hydrolysed according to the method of Striegel and Hill (1996).

A 1.0 g sample of defatted seed flour was digested in 0.3N HCl at 90°C for 5 hours and centrifuged for 20 minutes. The extract was analysed using a portable refractometer REF113 Brix 0~32 ATC for total sugars.

3.9 Mineral analysis

3.9.1 Mineral extraction

The protocol followed was adapted from Inuwa *et al*. (2011).

A sample (0.5 g) of ashed seed material was extracted in 50 ml distilled water, 20 ml nitric acid was then added and the mixture was heated until the sample was dry. The samples were allowed to cool and 40 ml distilled water was added and the samples were filtered. The filtrate was then made to 100 ml volume with distilled water.

3.9.2 Determination of iron, zinc and copper content

The samples were then analysed using Varian Spectra AA atomic absorption spectrometer depending on the availability of metal detection lamps.

3.9.3 Determination of sulphur content

Sulfur was determined gravimetrically as barium sulphate ($BaSO_4$) using the method adapted from (Mendham *et al.,* 2000)

A 50 ml sample (extract) extracted as above (3.9.1), was heated to 90°C and 5% solution of warm barium chloride was added drop wise to the extract with a burette with stirring/swirling. The mixture was allowed to settle and a few drops were added to see if more precipitate formed. The warm mixture was filtered through a pre-weighed Whatman No1 filter paper and washed with 15 ml of warm distilled water. The filter paper was dried at 100°C for 2 hours and the filter paper was weighed and the mass of $BaSO_4$ was determined. The mass of SO_4 was then determined from the mass of $BaSO_4$.

3.9.4 Determination of calcium-magnesium content

Calcium-magnesium complex was determined by complexiometric titrimetric analysis using the method adapted from Mendham *et al.,* (2000).

A 25 ml extract (extracted as above 3.9.1), was pipetted into a conical flask and 2 ml buffer was added. Crystals of eriochrome black T were added as an indicator and the mixture was titrated with 0.1 M EDTA with the end point being a colour change from wine red to clear blue.

3.10 Determination of crude fibre content

Crude fibre was determined according to the method of Inuwa *et al.,* (2011).

A volume of 100 ml boiled 1.3% sulphuric acid was added to 10 g sample of defatted seed material (W_3) and allowed to further boil for 30 minutes. The mixture was filtered and excess acid was washed down from the sample with warm water and transferred into a beaker. A 100 ml volume of boiled 2.5% NaOH was added and allowed to boil for 30 minutes. The mixture was filtered and excess NaOH was removed from residue by washing down with warm water. The filter paper containing

the residue was folded and placed in a crucible of known weight and placed in an oven at 105°C for 3 hours and a further 15 minutes to obtain constant weight (W_1). The crucibles containing samples were then transferred to a furnace and burnt at 500°C, cooled and weighed (W_2).

Crude fibre (%) was calculated as: $\frac{W1-w2}{W3} \times 100$

Where,

W_1 = Weight of residue + crucible before ashing

W_2 = Weight of residue + crucible after ashing

W_3 = Weight of dried sample

3.11 Data analysis

Analysis of variance (ANOVA) was done using the SPSS version 22 (IBM SPSS statistics) to find if there are any significant differences among the nine landraces analysed

CHAPTER 4: RESULTS

4.1 Physical properties of the watermelon fruits and seeds

4.1.1 Characteristics of watermelon fruits and seeds

The physical properties of the watermelons were characterised and it was observed that some of the landraces (02SDPW and 07CDGM (Figure 1)) had the same colour (green with cream stripes) but their seeds were conspicuously different from one another, with those from 02SDPW being black with a crown stripe and those from the 03SDPW landrace being brown with white/cream stripes. The 05SDPW (Figure 1), 08CDGM and 09CDGM fruits were greenish-grey with seeds that look distinctly different. The landraces 02SDPW, 03SDPW, 06SDPW and 10CDGM were green with seeds that looked different from one another.

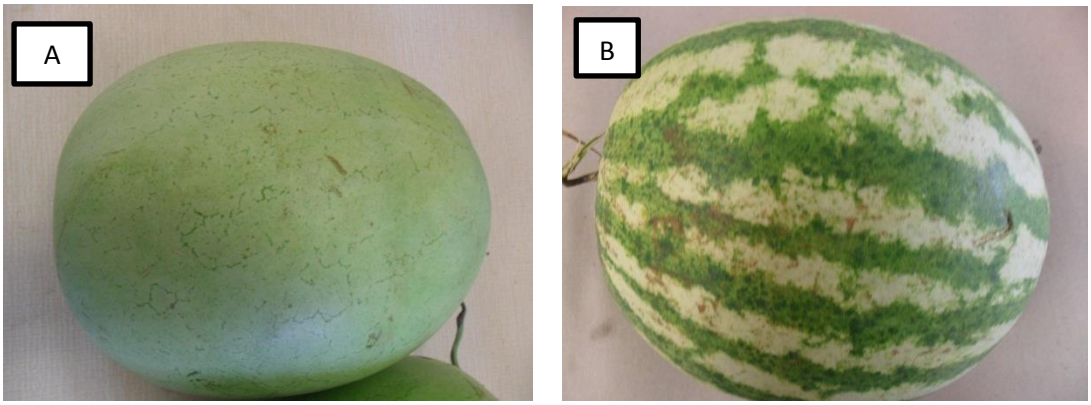

Figure 1: The fruits of 05SDPW landrace from the Sekhukhune district (a) and the 07CDGM landrace from the Capricorn district.

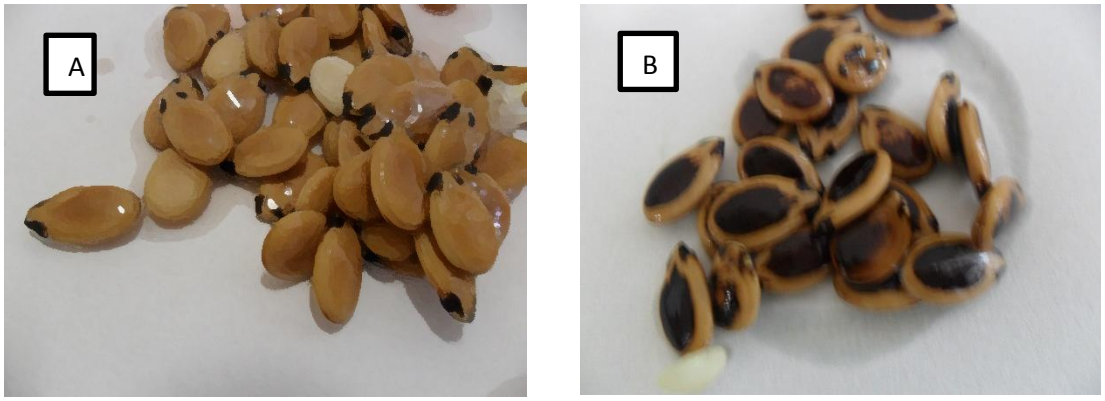

Figure 2: Seeds of 05SDPW landrace (a) and seeds of 07CDGM (b).

4.1.2 Mass per one hundred seeds

One hundred dried seeds were weighed to determine the mass per 100 seeds. The landraces from Capricorn district had the highest mass per 100 seeds with an average mass of 9.52 g/100 seeds. The landrace 06CDGM from the district recorded the highest mass per 100 seeds, with a mass of 10.95 g/100 seeds. Also from the same district, the landrace 07CDGM recorded the lowest mass per 100 seeds, with a mass of 8.05 g/100 seeds. The landraces from Sekhukhune district recorded a lower average mass per 100 seeds with an average mass of 8.66 g/100 seeds. Although the average mass was found to be low, the landrace 05SDPW recorded a high mass, which gave a value higher than 07CDGM and 10CDGM landraces from Capricorn district with a mass of 9.25 g/100 seeds (Table 1).

Table 1: Characteristics of the watermelon fruits and their seeds.

LANDRACE	COLOUR OF FRUIT	SEED COLOUR	MASS PER 100 SEEDS (g)
01SDPW	Green with cream stripes	Black with brown speckles	8.5 ± 0.141
02SDPW	Green	Black with brown edges	8.35 ± 0.778
03SDPW	Green	Brown with cream stripes	8.55 ± 0.495
05SDPW	Greenish-Grey	Light brown	9.25 ± 0.495
06CDGM	Green	Black with brown edges	10.95 ± 0.071
07CDGM	Green with cream stripes	Black with brown edges	8.05 ± 0.212
08CDGM	Greenish-Grey	Light brown with black speckles	10.55 ± 0.919
09CDGM	Greenish-Grey	Light brown with black tips	9.35 ± 0.636
10CDGM	Green	Black with brown edges	8.7 ± 0.141

± Values are standard deviations of triplicate values.

4.2 Seed composition

4.2.1 Oil content

Lipids were extracted in n-hexane and it was found that on average the landraces from Capricorn district had a higher yield with an average of 3.44 g oil (34.4% oil) than the landraces from the Sekhukhune district with an average yield of 3.16 g oil (31.6% oil) per 10 g of seed sample. The landrace 10CDGM from the Capricorn district recorded the highest oil yield with 4.15 g oil (41.5% oil) extracted from a 10 g sample of seeds, while the landraces 01SDPW from the Sekhukhune district and 07CDGM from the Capricorn district gave the lowest yield of oil with 30% oil. The colour of the oil was found to range from golden yellow (02SDPW, 06CDGM, and 08CDGM) to pale yellow (09CDGM) to orange (01SDPW, 03SDPW, 05SDPW, 07CDGM and 10CDGM) as indicated in Table 2.

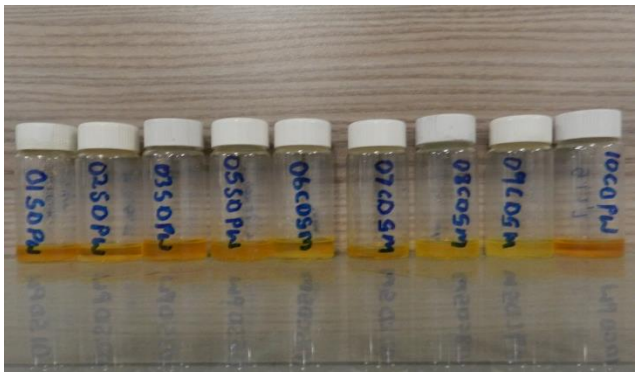

Figure 3: Oil extracted from watermelon seeds.

4.2.1.1 Saponification values of extracted oils

Saponification values of the extracted oil were determined and the results are recorded in Table 2. The values range from 132.40 KOH/g oil in 01SDPW to 184.57 KOH/g oil in 02SDPW. The oil from landraces from the Sekhukhune had a higher average saponification value with an average value of 152.25 mg KOH/g oil than the landraces from the Capricorn district with an average value of 148.33 mg KOH/g oil. Although the landraces from the Sekhukhune district recorded a high average, most of the individual landraces had recorded values lower than those of many landraces from the Capricorn district.

4.2.1.2 Iodine value

Iodine values of the extracted oils were investigated and as shown in Table 2, it was found that the landrace 02SDPW from the Sekhukhune region had the lowest iodine value of 134.006 g I_2/100 g oil and the landrace 09CDGM from the Capricorn region had the highest iodine value which recorded 138.575 KOH/g oil. On average, the landraces from the Capricorn district had high iodine value, with an average value of 136.697 KOH/g oil. The landraces from the Sekhukhune district recorded the lowest average iodine values with an average value of 135.892 KOH/g oil recorded. Although there were differences in the values, they were not significant among the landraces with a P value of 0.505 when analysed statistically at a 95% confidance level.

Table 2: Oil yield of watermelon seeds, saponification value and iodine value of watermelon seed oil.

Landrace	Oil yield (g/10 g sample)	Percentage oil (%)	Saponification value (mg KOH/g oil)	Iodine value (g I_2/100 g oil)	Oil colour
01SDPW	3.00±0.14	30.0±0.14	132.40±0.793	136.798±0.449	Orange
02SDPW	3.25±0.21	32.5±0.21	184.57±2.380	134.006±0.269	Golden Yellow
03SDPW	3.25±0.07	32.5±0.07	143.06±1.587	135.085±1.077	Orange
05SDPW	3.15±0.35	31.5±0.35	148.95±0.397	137.687±0.269	Orange
06CDGM	3.55±0.07	35.5±0.07	154.84±0.793	135.149±0.628	Golden Yellow
07CDGM	3.00±0.00	30.0±0.00	135.20±3.967	134.704±0.359	Orange
08CDGM	3.35±0.21	33.5±0.21	161.29±1.190	138.194±0.090	Golden Yellow
09CDGM	3.15±0.49	31.5±0.49	145.30±2.380	138.575±0.269	Pale Yellow
10CDGM	4.15±0.35	41.5±0.35	145.02±3.570	136.862±0.538	Orange

± Values are standard deviations of triplicate values.

4.2.2 Total protein content

The total protein content of watermelon seeds from the Capricorn region was found to be higher, with an average value of 165.12 mg/g (16.51% protein). The total protein content of the seeds from the Sekhukhune district had an average value of 149.04 mg/g (14.9% protein). The landrace 10CDGM was found to contain the

highest amount of total proteins, with a protein content of 203.89 mg/g (20.39% protein). The landrace 03SDPW had the lowest total protein content of all landraces with a total protein content of 14.12% protein (Table 3).

Table 3: Protein, crude fibre, ash and total sugar content of watermelon seeds.

LANDRACE	Protein content (%)	Crude fibre (%)	Ash content (%)	Total sugars (%)
01SDPW	15.01 ± 0.25	21.45 ± 0.717	5.00 ± 2.362	8.95 ± 0.212
02SDPW	16.12 ± 0.36	23.14 ± 1.194	3.33 ± 0.000	2.80 ± 0.283
03SDPW	14.12 ± 1.49	20.77 ± 1.194	5.00 ± 2.362	5.75 ± 0.212
05SDPW	14.33 ± 1.37	22.80 ± 0.239	5.00 ± 2.362	3.55 ± 0.071
06CDGM	16.47 ± 0.85	22.97 ± 1.911	3.33 ± 0.000	2.65 ± 0.071
07CDGM	15.50 ± 1.06	23.48 ± 0.239	3.33 ± 0.000	2.45 ± 0.778
08CDGM	15.19 ± 0.90	21.62 ± 1.911	3.33 ± 0.000	3.85 ± 0.212
09CDGM	15.01 ± 0.62	23.31 ± 0.956	6.67 ± 0.000	2.15 ± 0.778
10CDGM	20.39 ± 0.35	23.98 ± 0.956	3.33 ± 0.000	4.70 ± 0.141

± Values are standard deviations of triplicate values.

4.2.3 Amino acid composition

Only essential amino acids were used as standards in thin-layer chromatography analysis of amino acids. It was found that leucine is the most abundant amino acid in the watermelon landrace seeds being present in all landraces (Table 4). Threonine was the second most abundant amino acid, being detected in eight landraces with only the 01SDPW not having any threonine detected. Phenylalanine was also found

to be present in most landraces, with the exception of 03SDPW, 05SDPW and 07CDGM. Methionine was found to be present in most landraces from the Capricorn region, with only 09CDGM having none detected. This amino acid was also not detected in most landraces from the Sekhukhune district, being only detected in the 01SDPW landrace in the samples from the region.

Table 4: Amino acids separated from watermelon seeds

LANDRACE	Rf value	Colour of spot	Amino acid
01SDPW	0.441	Orange	Methionine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
02SDPW	0.162	Brick red	Threonine
	0.235	Orange	Unidentified
	0.471	Reddish violet	Valine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
03SDPW	0.162	Brick red	Threonine
	0.471	Reddish violet	Valine
	0.603	Orange	Leucine
05SDPW	0.162	Brick red	Threonine
	0.471	Reddish violet	Valine
	0.603	Orange	Leucine
06CDGM	0.162	Brick red	Threonine
	0.441	Orange	Methionine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
07CDGM	0.162	Brick red	Threonine
	0.441	Orange	Methionine
	0.603	Orange	Leucine
08CDGM	0.162	Brick red	Threonine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
09CDGM	0.162	Brick red	Threonine
	0.441	Orange	Methionine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
10CDGM	0.162	Brick red	Threonine
	0.442	Orange	Methionine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine

The landraces from the Capricorn region also did not have any valine detected in all landraces from the region. The amino acid was however detected in most landraces from Sekhukhune region, with only the 01SDPW landrace not showing the presence

of the amino acid. Isoleucine was not detected in any of the landraces in both districts. There were also a few unidentified amino acids in all landraces.

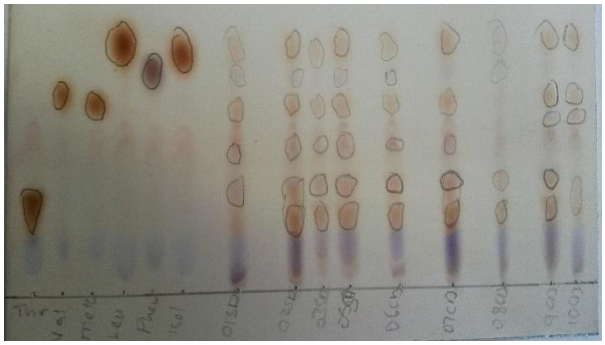

Figure 4. Thin layer chromatography of amino acids.

4.2.4 Crude fibre

The landraces from the Capricorn district were found to have higher crude fibre content with an average value of 23.07% than the landraces from the Sekhukhune district, with an average value of 22.04% crude fibre. The landrace 10CDGM was found to have the highest amount of crude fibre with a percentage of 23.98% crude fibre. The landrace 03SDPW from the Sekhukhune district was found to have the lowest crude fibre content with 20.77% crude fibre (Table 3).

4.2.5 Ash content

The percentage ash of the landraces were determined and it was found that on average the landraces from the Sekhukhune district had a higher ash content with an average value of 4.58% than those from the Capricorn district with an average value of 3.99% ash in a 5 g sample. Although the landraces from the Sekhukhune district had the highest average ash percentage, the landrace 09CDGM from Capricorn district was found to have a high percentage of ash with a value of 6.67% ash. The landrace 02SDPW was found to be the only landrace from the Sekhukhune region with low ash content at 3.33% ash. The landraces 06CDGM, 07CDGM, 08CDGM and 10CDGM were also found to have a low percentage of ash similar to that of the 01SDPW landrace with a value 3.33% ash (Table 3).

4.2.6 Total sugars

The landraces from the Sekhukhune district were found to have higher average total sugar/carbohydrate content with an average of 5.26% than those from the Capricorn district that averaged 3.16% total sugars. The landrace 01SDPW had the highest total sugar content with a percentage of 8.95 than all landraces. The landrace 09CDGM from the Capricorn region was found to have the lowest content of total sugars with a percentage of 2.15 (Table 3).

4.2.7 Mineral content

4.2.7.1 Copper (Cu)

The landraces from Capricorn district were found to have a high content of copper (Cu) at 0.078 mg/g than the landraces from Sekhukhune district which averaged 0.053 mg/g. The landrace 05SDPW from the Sekhukhune district was found to have the lowest concentration of the metal, with a value of 0.036 mg/g, this was followed by the landrace 01SDPW and 03SDPW with values 0.050 and 0.053 mg/g respectively. The landraces from Capricorn district were found to contain more of this metal with values ranging from 0.073 in 10CDGM to 0.088 mg/g in 06CDGM (Table 5).

4.2.7.2 Iron (Fe)

The landraces from Sekhukhune district were found to have high iron content with an average value of 0.155 mg/g than the landraces from Capricorn district with an average value of 0.135 mg/g iron. The landrace 05SDPW from the Sekhukhune area was however found to have the lowest iron content of all landraces with a concentration of 0.084 mg/g despite being from the area that averaged a high concentration of iron. The landrace 02SDPW from the same area had the highest iron content of iron with a value of 0.194 mg/g. All the landraces from the Sekhukhune area were found to have high iron content than the seeds of the landraces from the Capricorn area. The landraces 06CDGM and 08CDGM had the highest iron content of 0.154 mg/g within the landraces from Capricorn area although the values were still lower than those of three landraces from Sekhukhune district and the landrace 10CDGM was found to have the lowest iron content within the landraces from the Capricorn district (Table 5).

4.2.7.3 Zinc (Zn)

The landraces from the Capricorn district were found to have higher zinc content with an average content of 0.231 mg/g than those from the Sekhukhune district with an average of 0.229 mg/g. The landrace 05SDPW was found to have the lowest zinc concentration of all landraces and within the landraces from the Sekhukhune district with a value of 0.118 mg/g. The landrace 10CDGM was found to have the highest zinc content of 0.312 mg/g, with the 07CDGM landrace having the lowest zinc content within the landraces from Capricorn area (Table 5).

Table 5: Mineral content of watermelon landrace seeds.

LANDRACE	Copper (mg/g)	Iron (mg/g)	Zinc (mg/g)	Sulphate (mg/g)	Calcium + magnesium (mg/g)
01SDPW	0.050 ± 0.046	0.171 ± 0.011	0.287 ± 0.005	0.118 ± 0.027	6.01 ± 0.119
02SDPW	0.072 ± 0.005	0.194 ± 0.007	0.257 ± 0.008	0.106 ± 0.009	8.46 ± 0.107
03SDPW	0.053 ± 0.004	0.169 ± 0.013	0.254 ± 0.011	0.105 ± 0.017	9.13 ± 0.106
05SDPW	0.036 ± 0.037	0.084 ± 0.002	0.118 ± 0.013	0.049 ± 0.006	5.34 ± 0.049
06CDGM	0.088 ± 0.024	0.154 ± 0.011	0.239 ± 0.007	0.098 ± 0.028	5.12 ± 0.099
07CDGM	0.086 ± 0.045	0.103 ± 0.061	0.165 ± 0.015	0.068 ± 0.012	6.90 ± 0.069
08CDGM	0.067 ± 0.019	0.154 ± 0.006	0.217 ± 0.018	0.089 ± 0.009	8.91 ± 0.090
09CDGM	0.075 ± 0.056	0.136 ± 0.032	0.223 ± 0.026	0.092 ± 0.005	6.23 ± 0.093
10CDGM	0.073 ± 0.024	0.130 ± 0.028	0.312 ± 0.010	0.129 ± 0.019	9.13 ± 0.130

± Values are standard deviations of triplicate values.

4.2.7.4 Sulphate

The landraces from both the districts were found to contain the same amount of sulphate with both districts giving an average value of 0.095 mg/g. However, it was observed that the landrace 01SDPW had the highest sulphate content among the landraces with a sulphate content of 0.118 mg/g than all landraces from Sekhukhune district and also higher than most landraces from Capricorn district with the exception of 10CDGM (0.129 mg/g sulphate). The landrace 05SDPW had the lowest sulphate content (0.049 mg/g) of all landraces. Although the landraces from the

Capricorn area had high average sulphate content, most landraces from Sekhukhune had significantly higher sulphate content (Table 5).

4.2.7.5 Calcium-magnesium complex

The landraces from Sekhukhune district were found to have higher calcium-magnesium content with an average value of 7.235 mg/g than the landraces from Capricorn district which were found to have an average calcium content of 5.432 mg/g. The landrace 03SDPW was found to have the highest content of the mineral amongst all the investigated landraces with a value of 9.13 mg/g. The landrace 06CDGM was found to contain the lowest calcium of landraces with a value of 5.12 mg/g. The landraces from the Sekhukhune area, with the exception of 05SDPW (5.34 mg/g), had a higher value than the landraces from the Capricorn district. The landrace 10CDGM had the highest content of calcium within the landraces from the Capricorn area (Table 5).

4.3 Phytochemical analysis and antioxidant activity

4.3.1 Total phenolic content

Extracts of the landraces were analysed for phenolic content and the results showed varying concentrations ranging from 0.27 mg/g (02SDPW) to 0.91 mg/g (05SDPW). On average, landraces from the Sekhukhune district showed a higher content (0.55 mg/g) of total phenolic compounds than the counterparts (0.59 mg/g) from the Capricorn district (Table 6).

4.3.2 Flavonoid content

The seeds contained flavonoids (Table 6) in the range of 0.085 (05 SDPW) to 0.347 mg/g (10 CD GM). It was observed that on average, landraces from the Capricorn district had more flavonoid content (0.222 mg/g) than those from the Sekhukhune district (0.130 mg/g).

4.3.3 Antioxidant activity

4.3.3.1 Time course of antioxidant activity

The antioxidant time inhibition course was determined in watermelon seed extracts and it was observed that the time it takes for the reaction between DPPH and the plant extract to come to completion is roughly forty to forty-five minutes. The

absorbance of the sample was recorded at 10 minutes intervals and the results were recorded and a graph plotted to determine time course of the reaction (Figure 5).

Table 6: Total phenolics, flavonoids and antioxidant activity of watermelon seeds.

LANDRACE	Total phenolics (mg/g as GAE)	Total flavonoids (mg/g Catechin equivalent)	Antioxidant activity (% inhibition)
01SDPW1	0.42 ± 0.151	0.195 ± 0.026	60.67 ± 2.61
02SDPW1	0.27 ± 0.009	0.097 ± 0.010	43.78 ± 0.17
03SDPW1	0.58 ± 0.030	0.143 ± 0.028	35.30 ± 1.33
05SDPW1	0.91 ± 0.040	0.085 ± 0.018	41.36 ± 0.52
06CDGM1	0.45 ± 0.123	0.143 ± 0.008	39.81 ± 0.98
07CDGM1	0.66 ± 0.154	0.192 ± 0.010	36.67 ± 0.35
08CDGM1	0.86 ± 0.075	0.135 ± 0.030	43.75 ± 1.50
09CDGM1	0.62 ± 0.095	0.295 ± 0.091	66.95 ± 0.40
10CDGM1	0.36 ± 0.073	0.347 ± 0.020	45.10 ± 1.52

± Values are standard deviations of triplicate values.

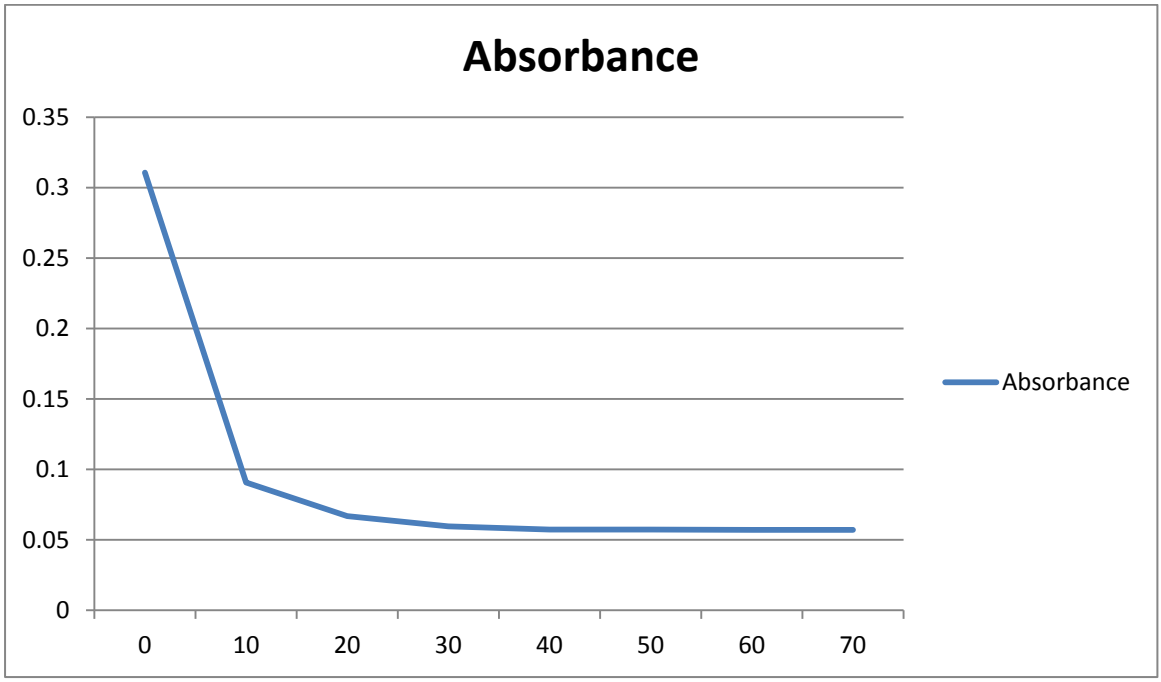

Figure 5: DPPH inhibition time course of watermelon seed extract

4.3.3.2 Percentage inhibition/Scavenging capacity

The landraces from the Capricorn district were found to have a higher scavenging capacity with a percentage inhibition of 46.46% than the landraces from the Sekhukhune district with a percentage inhibition of 45.28%. The landrace 09CDGM from the Capricorn region had the highest antioxidant activity with 66.95% inhibition while the landrace 03SDPW had the lowest antioxidant activity with 35.30% inhibition (Table 6).

4.4 Comparing the means

The averages of the different results (Table 7) obtained in the study for the two districts were compared (Figures 6, 7 and 8) and it was found that the landraces from the Capricorn district had higher means in most analyses. The landraces from this district were found to have higher averages for the mass per 100 seeds, lipid content, iodine value, total protein, crude fibre, and antioxidant activity. The landraces from the Sekhukhune district had higher averages for analyses of the saponification values, the percentage ash and the total carbohydrates.

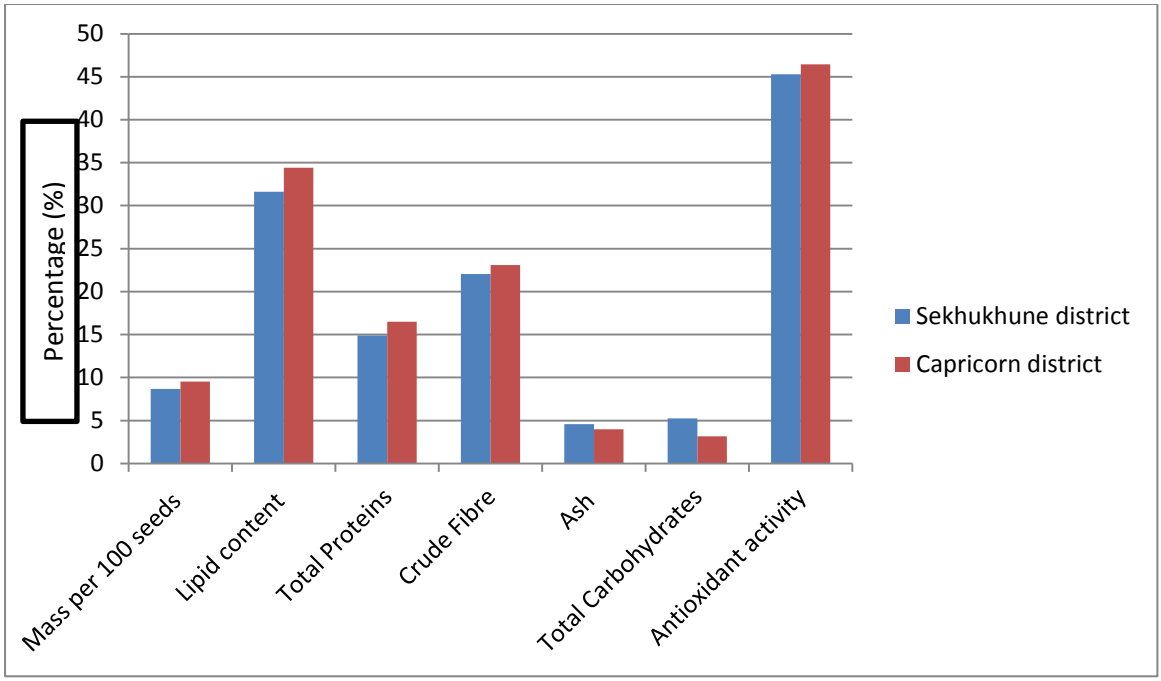

Figure 6: Comparison of the nutrient content averages of watermelon landraces from the two districts.

Table 7: Means of the landraces from the two districts (Sekhukhune- and Capricorn-district).

Type of Analysis	Sekhukhune District	Capricorn District
Mass per 100 seeds	8.66 ± 0.401	9.52 ± 1.221
Lipid content (%)	31.63 ± 0.118	34.4 ± 0.448
Oil saponification value (mg KOH/g	152.25 ± 22.614	148.33 ± 10.034
Oil iodine value (g I_2/100 g oil)	135.89 ± 1.658	136.69 ± 1.744
Total Proteins (%)	14.89 ± 9.197	16.51 ± 1.744
Crude Fibre (%)	22.04 ± 1.116	23.07 ± 0.891
Antioxidant activity (% inhibition)	45.28 ± 10.865	46.46 ± 11.928
Ash (%)	4.58 ± 0.835	3.99 ± 1.670
Total Carbohydrates (%)	5.26 ± 2.759	3.16 ± 1.076
Total flavonoids (mg/g catechin equivalent	0.130 ± 0.067	0.222 ± 0.094
Total phenolics (mg/g GAE)	0.55 ± 0.205	0.59 ± 0.243
Copper (mg/g)	0.053 ± 0.615	0.078 ± 0.009
Zinc (mg/g)	0.229 ± 0.075	0.231 ± 0.053
Iron (mg/g)	0.155 ± 0.049	0.135 ± 0.021
Sulphate (mg/g)	0.095 ± 0.031	0.095 ± 0.022
Calcium-magnesium complex(mg/g)	7.235 ± 0.050	5.432 ± 0.038

± Values are standard deviations of triplicate values.

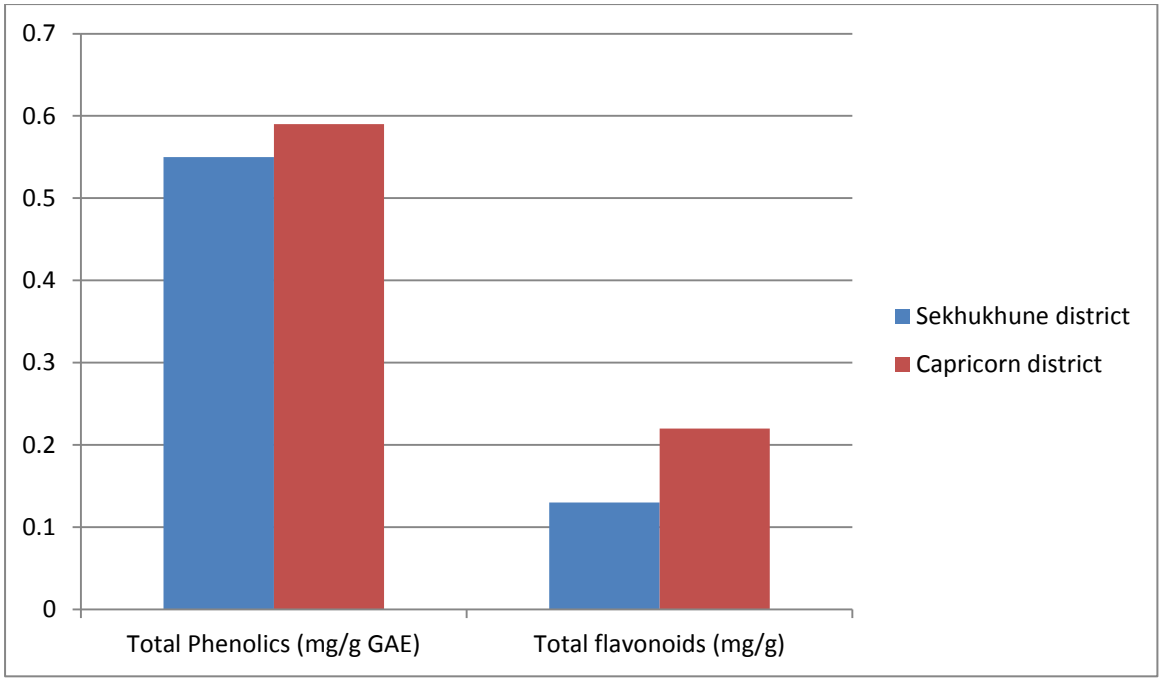

Figure 7: Comparison of the averages of total phenolics and total flavonoids in the watermelon landrace seeds from the two districts.

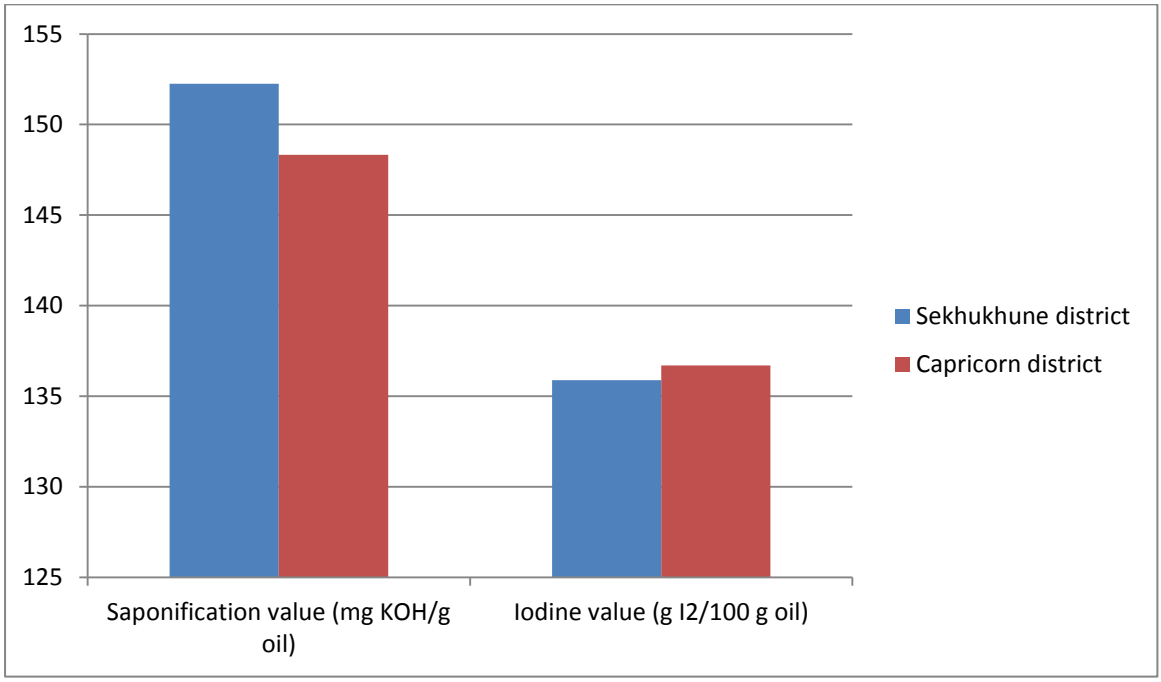

Figure 8: Comparison of oil properties of the landraces from both Sekhukhune and Capricorn districts.

CHAPTER 5: DISCUSSION

5.1 Physical properties of the watermelon fruit and seeds

5.1.1 Characteristics of the watermelon fruits and seeds

There are more than 1200 varieties of watermelon characterised based on size, shape and the colour of the flesh. The name of the variety of the watermelon can be given according to colour, shape, sweetness or location or place of first cultivation of the variety. Hence a name given to a variety may not be acceptable in another place (Gwana *et al.,* 2014).

The watermelon fruits of the different landraces were found to be highly diverse, with some being similar in rind colour but found to have different seeds. The colour of the rind ranged from light green to green to multiple colours. The seed colour ranged from black with brown speckles to black with brown edges, to brown with cream stripes, to light brown and light brown with black edges. This resulted in notable differences in landraces from similar districts, indicating the differences are more genetic than environmental.

5.1.2 Mass per one hundred seeds

The physical properties of the watermelon seeds were investigated, where the mass per 100 seeds was determined and it was found that the mass ranged from 8.35 g in 02SDPW to 10.95 g in 07CDGM, averaging 9.13 g/100 seeds in all landraces from both districts. These results were found to be slightly lower than those found by Mariod *et al.,* (2009), where they found the mass of the watermelon seeds in a 100 seed sample to be 15.75 g/100 seeds. These results imply that a high mass per 100 seeds is an indication of the bigger size of the seeds and thus the potential to yield more of a desired product such as oil, protein, carbohydrates or fibre from less seeds.

Landraces from the two districts were compared and there were no significant differences observed with a P value of 0.225 at 95% confidence level (Appendix A). It was found that on average, the landraces from the Capricorn district had high seed mass per 100 seeds at an average of 9.52 g. The landraces from Sekhukhune district had lower mass per 100 seeds at 8.67 g. The differences may be due to the

different harvesting times and stages and also the climate in which the seeds were grown. The seeds with a higher mass indicated the potential to be produced or farmed for industrial purposes as there will be high turnover of mass of the desired product such as oil, protein, carbohydrates, or fibre per cultivation/harvest. This quality will give farmers a better yield of the desired product than with the seeds with less mass.

5.2 Seed composition

5.2.1 Oil Content

Lipids are fats and oils and are extremely hydrophobic and water-insoluble. They consist of mainly carbon, hydrogen and oxygen. The basic unit of oils are fatty acids; these are long chains containing about 26 carbon atoms with a carboxyl group at one end. If every carbon atom except the carboxyl carbon carries two hydrogen atoms, the fatty acid is saturated. The molecule is stabilised by interactions with closely packed adjacent fatty acids. The stability makes it difficult to melt saturated fatty acids and the oils tend to be solid at room temperature. If some carbons are double bonded to adjacent carbons, the fatty acid is unsaturated (Mauseth. 2014). Vegetable fats and oils are substances derived from plants which are composed of triglycerides and represents major component of edible fats and oils. Normally fats are solid and oils are liquid at room temperature (El-Kheir *et al.,* 2012). Fats and oils are important in diet because they promote the absorption of fat-soluble vitamins and are highly nutritious (Senga *et al.,* 2013).

The watermelon landraces seeds were found to contain an average of 33.2% of oil. Landraces from both districts had varieties that contained the lowest oil content at 30.0% (01SDPW and 07CDGM) while 10CDGM from Capricorn district was found to have the highest percentage at 41.5%. The results were found to be well higher than those obtained by Mariod *et al.* (2009) in watermelon seeds in Sudanese cultivars where they were found to contain 27.10% of oil, but the results were slightly lower than those recorded by Alfawaz (2004) in the pumpkin (37.8%) which is in the same family as the watermelon and notably lower than sunflower oil (53%) recorded by Robertson *et al.* (1978) in mature sunflower seeds.

Landraces from both districts were compared using one way ANOVA. It was found that there was no significant difference in the oil content of seeds from both districts with a P value of 0.227 at 95% confidence level (Appendix A). Upon analysis, it was found that on average, the landraces from the Capricorn district had higher oil content at an average of 34.4% than those from the Sekhukhune district at an average of 31.6% of oil. The variation in the yield of the oil may be due to the differences in the variety of landraces, cultivation climate, ripening stage, harvesting time and the method of extraction used (Nyam *et al.,* 2009). The landraces with high percentage yield of oils can possibly/potentially be used for the production of oil to compete with the oils currently available on the market such as soybean oil and sunflower oil.

The colour of the oil was also noted and it was found to range from golden yellow to pale yellow, to orange. These observations were found to be in agreement with those obtained by Mariod *et al.* (2009). According to Raziq *et al.* (2012), the colour in vegetable oil is linked to the presence of some colouring material/pigments such as xanthophylls and carotenoids which are extracted along with the oil during the extraction process. Such colouring components have to be removed through bleaching to make the oil lighter as the lighter oil is more valuable and acceptable as edible oil as they are a sign of the purity of the oils.

5.2.2 Chemical properties of the oils

5.2.2.1 Saponification value

Saponification values are used as indications of the average molecular weight and chain length of lipids (Ardabili *et al.,* 2011). Saponification values of *C. lanatus* seed oil showed differences in the different landraces. The values in the study were found to range from 133.44 in 01SDPW to 184.57 mg KOH/g oil in 02SDPW. The results averaged 150.07 mg KOH/g oil, which were found to be lower than those obtained by Wani *et al.* (2013) in watermelon seeds, which ranged from 162-178 mg KOH/g oil. The values were also found to be lower than those found in sunflower (194 mg KOH/g oil) and soybean (191 mg KOH/g oil).

Although on average, the saponification values were lower than those obtained by Wani *et al*. (2013), some individual landraces were found to compare favourably to

other watermelon seeds in the literature as shown by the landrace 02SDPW with saponification value of 184.57 mg KOH/g oil. These differences may be attributed to genetic as the seeds are not of the same variety. Environmental conditions cannot however be considered a factor as even the landraces from the same districts show some differences in composition. El-Kheir *et al.* (2012) attributes differences in oil composition to the maturity of the fruit and seeds and environmental conditions.

5.2.2.2 Iodine value

The iodine value is a measure of the average amount of unsaturation of fats and is expressed in terms of the number of grams of iodine absorbed per 100 g sample. The value is a measure of unsaturation of fats and oils and hence their potential to become oxidised (IAFMM, 1981). The degree of unsaturation is a major influence on the stability of storage of edible oils (El-Kheir *et al.,* 2012). The iodine value is known to decrease due to thermo oxidative transformation. The decrease is thought to be due to the destruction of double bonds from oxidation, scission and polymerisation (Rasaruddin *et al.,* 2014).

The average iodine value was found to be relatively high, indicating a high degree of unsaturation (Mariod *et al.,* 2009). On average, the oil was found to have an iodine value of 136.34 g I_2/100g oil. These findings were higher than those obtained by Wani *et al.* (2013), at 84.96 gI_2/100g oil in the watermelon commercial variety, the Sugar Baby. The high iodine values indicate a high degree of unsaturation. The values were however lower than those obtained by Baboli and Kordi (2010) in watermelon cultivars at 156 gI_2/100 g oil. The findings are however in agreement with those obtained for sunflower oil at 137 gI_2/100 g oil. These findings suggest that the watermelon seed oil might be a good candidate for commercialisation on an industrial scale as they show the potential of long shelf life as they can resist oxidation.

The landraces from both districts were compared and there was no significant difference in the iodine values of the oils from the two districts with a P value of 0.505 at a 95% confidence level (Appendix A). This indicates that the oil in landraces from both districts is almost of similar composition. The similar composition might be attributed to climatic conditions that the landraces from both districts were grown under as Lajara *et al.* (1990), noted the correlation between temperature and fatty

acid composition. The landraces from the Capricorn district were found to have on average a higher iodine value (136.696 gI_2/100 g oil) than those from the Sekhukhune district (135.894 gI_2/100 g oil), although the differences were not significant. The differences in the degree of unsaturation in the oils analysed in this study and those of the findings in the literature may be attributed to the differences in varieties of the landraces as according to Pocklington, (1990), the solvent used for determination of iodine value has little or no influence on the precession of the determination. However, the method of titration that is used in determining iodine value is deemed undesirable because of the highly toxic, carcinogenic and environmentally unfriendly chemicals which can be potentially dangerous to the analyst. The results obtained through this method are also said to be unreliable as they are highly dependent on the skills of the analyst (Rasaruddin *et al.,* 2014).

5.2.3 Protein analysis

5.2.3.1 Total protein content.

Proteins are extremely complex polymers based on up to twenty amino acids. The amino acids are connected through amide bonds. They are made up of 50-55% carbon, 6-7% hydrogen, 20-23% oxygen, 12-19% nitrogen and 0.2-3% sulphur. The structural and functional differences in the proteins are a result of the sequence in which the amino acids are connected, their size and type and size of peptide chain (Rodrigues *et al.,* 2012). The protein quality of food is known as the nutritional value depending on its amino acid content and the physiological utilisation of specific amino acids. To meet protein demands, in countries where intake is highly inadequate and missing, more attention has been paid to less consumed protein and lipid sources such as legumes and seeds (Senga *et al.,* 2013).

The protein content in the watermelon seeds was fairly low at an average of 15.80%. This is considerably lower than the results obtained by Wani *et al.* (2013) of 27.10%. Although the protein content was low, it was found to be very high in comparison to the protein content of the watermelon flesh, which was found by Singh and Matta (2010) to be approximately 2%. This indicates that in terms of protein content the seeds have far more proteins than the flesh (Singh and Matta, 2010). The differences observed may have been due to the method used in the study as Field and Field (2010) found that the Bradford assay was less reliable on solid food

products than with liquids such as dairy products. They also found in a study involving melamine, which contains many amino groups that even with all the amino groups the melamine did not react with the Bradford reagent. They also stated that even though Bradford assay is superior to nitrogen-based tests, there are some agents that interfere with the assay. These include the detergents and glassware. In addition, factors such as pH, temperature, ionic strength, solvent type, extraction time, and solid-liquid ratio have been found to also have an effect on melon seed meal extraction (Wani *et al.,* 2008).

The landrace 10CDGM had the highest amount of protein (20.39%), while 03SDPW had the lowest amount of proteins at (14.12%), but this value was found to be considerably close to that of the majority of the landraces. The narrow range of protein content in the landraces may be explained as being a result of cross-breeding which leads to mixing up of genomes. The final status of a particular fraction in the seed is also determined by extent of degradation on the seed components by the action of their specific proteases during seed development (Singh and Matta, 2010). The amino acids contained in the seeds are in agreement with those found in the sunflower seeds although all the amino acids were identified in the sunflower seeds by Villamide and San Juan (1998).

5.2.3.2 Amino acid composition

Amino acids are classified as essential (indispensable) or non-essential (dispensable) for humans and animals. Nutritionally essential amino acids are those whose carbon skeleton are not synthesised by animal cells and therefore must be provided from diet. Amino acids are precursors to a wide array of nitrogenous substances with enormous biological importance. These include neurotransmitters, hormones, vasodilators, signalling gases, antioxidants, methyl-group donors, as well as key regulators of metabolism, growth, development and immune response (Wu, 2010). High quality protein stimulates muscle protein synthesis in proportion to the amount of ingested essential amino acids. Essential amino acids are primarily responsible for stimulation of net protein synthesis in skeletal muscles (Ferrando *et al.,* 2010). Amino acid composition is generally used as an indication of the nutritional value of a protein source (Senga *et al.,* 2013).

Watermelon seeds investigated were found to all contain the amino acid leucine, which was followed by threonine in abundance. The amino acid (threonine) was found in eight landraces, with only 01SDPW not having the amino acid. The presence of the amino acid leucine in these landraces makes the landrace proteins a good addition to diet especially in growing children and the elderly as leucine was shown to be a critical amino acid for increasing skeletal muscle protein synthesis. The amino acid is also thought to also be involved in supressing muscle protein degradation (Hulmi *et al.,* 2010). The amino acids valine and phenylalanine were found in most landraces. These findings are in agreement with those of Achu *et al.* (2013), where it was found that valine, leucine, and phenylalanine were present in watermelon seeds in Cameroon. According to the authors, the presence of the amino acids indicates that the seeds can be good supplement for infants. Although their results compare favourably with those from the current study, they also found isoleucine in their samples, which is in disagreement with the findings of the current study as no isoleucine was detected. The human body requires essential amino acids in defined amounts, and once one or more amino acids are depleted, protein synthesis cannot proceed (Woolf *et al.,* 2011). The presence of much of the essential amino acids indicates that the seeds can make good substitution for costly supplements and high quality meat which is a costly commodity in developing countries. The absence of isoleucine however, indicates the need for cross breeding with landraces containing the amino acid so the seeds can be complete with the essential amino acids. However, the absence of the amino acid can also be explained by the fact that ninhydrin, according to Sahana *et al. (*2011), is a non-specific reagent with remarkable sensitivity, but has the draw-back of producing the same colour with all amino acids, except proline and hydroxyproline, thus making identification of amino acids difficult in-spite of high sensitivity.

Even though most essential amino acids were identified, there were some unidentified amino acids and not all the amino acids were detected on the TLC plates. According to Fish (2012), some amino acids such as glutamine and arginine become converted to their respective amides, which may explain the absence of some amino acids. Karaye *et al.* (2012) found similar results in watermelon seeds, where the two amino acids were converted to their amides. They also found that the amino acid tryptophan becomes totally destroyed during acid hydrolysis, which lead

to them recovering only seventeen amino acids out of the possible twenty. The use of 2-dimension TLC might lead to better separation, although the method has its own draw-backs such as the fact that only one sample can be investigated and thus the method is more time-consuming. The method also makes simultaneous application of standards impossible (Fuchs *et al.,* 2011). Despite the availability of a wide range of more sophisticated techniques, TLC is still one of the fastest, cheapest and most effective ways to obtain a characteristic analytical fingerprint of a plant extract (Zachocke *et al.,* 2000).

5.2.4 Crude fibre

Fibre refers to the complex mixture of organic constituents of foods, especially those of plant origin that is non-digestible by enzymes in the human digestive tract but may be digested by microflora in the large intestine (Boakye *et al.,* 2014).

The watermelon landrace seeds were found to contain an average of 22.61% crude fibre. The results were found to be lower than values obtained by Varghese *et al.* (2013), where it was found that the watermelon seed fibre was 29.50%. However, the results in the current study were found to be higher than those obtained by Mathew *et al.* (2014), where it was found that the fibre content constituted 14.02% in watermelon seeds. The results in the current study were also found to be higher than those obtained by Alfawaz (2004) in pumpkin seeds at 16.84%. The relatively high content of crude fibre observed in the watermelon seeds in this study indicate the potential health benefits that can be brought by the consumption of the seeds or their use in food products. It is well known that dietary fibre plays an important role in the maintenance of the internal distention of the intestinal tract as its physiological effect. Adequate consumption of dietary fibre helps protect against cancer and in the normalisation of blood lipids, thereby reducing the risk of cardiovascular diseases (Senga *et al.,* 2013). The high crude fibre content in these seeds can be exploited as functional foods or in food formulations to prevent and treat some non-communicable diseases (Boakye *et al.,* 2014). This makes the seeds a desirable addition to human diet and/or supplementation to human diet.

The crude fibre content of the landraces from both districts was compared and it was found that there was no significant differences in the content, with the significance at P=0.169 at 95% confidence level (Appendix A). The landraces from Capricorn district

showed a high crude fibre content at 23.07%. These values were slightly higher than those from Sekhukhune district at 22.04% crude fibre. The landrace 10CDGM had the highest crude fibre content at 23.98%, while the landrace 03SDPW had the lowest content of crude fibre at 20.77% crude fibre. These differences may be attributed to the different environmental conditions that the two landraces were grown under. Genetic variations may also be another reason for the differences as the landraces are known to be genetically unstable and one landrace is genetically different from the next landrace. The landraces also cross-pollinate, resulting in some landraces acquiring genes making them produce certain compounds in abundance.

5.2.5 Ash content

The amount of ash in seeds was found to be fairly low at an average of 4.26%, which was found to be less than those from Mathew *et al.* (2014) who found the ash content in watermelon seeds to average 5.0%. The results were also found to be in agreement with those of Lakshmi and Kaul (2011), where they found ash content of the seeds to be at 5.1% and lower than pumpkin seed ash at 5.3% as reported by Ardabili *et al.* (2011). The results from this study were however, higher than those of Ramazan *et al.* (2012) where they found the ash content to range between 2.31% - 3.76%. The results were however similar to those of Alfawaz (2004) in pumpkin seeds, where the ash content was found to be at 4.59%. The results indicate that the watermelon seeds may contain moderate amounts of minerals as Mathew *et al.,* (2014), mentioned that the high ash content in their findings indicated a high probability of the seeds having a high mineral content.

The seeds of the landraces investigated were found to have ash content ranging from 3.37 – 6.67%. On average, the landraces from the Sekhukhune district had higher ash content at 4.58% ash as compared to those from the Capricorn district, at an average of 3.99%. The difference was however, not significant, with a P value of 0.510. Although the ash in the landraces from the Capricorn district was low, the 09CDGM had an exceptionally high amount of ash at 6.67%. These differences may be attributed to the differences in soil composition of the two regions on which the landraces were cultivated, as it has been stated that there exists a correlation between minerals in the seed and the ash content. These may also be linked to the

genetics of the landraces as shown by the landrace from the Capricorn district containing higher ash content than those from Sekhukhune district.

The results though indicate that generally, landraces from the Sekhukhune district compare very well with the results observed in the literature. This shows their potential in the food industry as nutritious supplement and replacement to the available supplements that might have proven costly as the ash serves as an indication of the potential mineral content of the seeds.

5.2.6 Carbohydrate content

Carbohydrates contain only carbon, hydrogen, and oxygen, although a few carbohydrates contain atoms such as nitrogen and sulphur (Mauseth, 2014). Carbohydrates supply the daily energy requirements of the body in both children and adults. They are easily digested and they provide necessary calories in the diet of most people (Senga *et al.,* 2013).

It was found that on average the landraces contain 4.09% carbohydrates. These values were found to be lower than those obtained by Mathew *et al.* (2014), where the watermelon seed carbohydrate content in their study averaged 5.50%. Alfawaz (2004), found carbohydrate content in pumpkin seeds to be notably higher than the results obtained in both studies on watermelon seeds. Fila *et al.* (2013) also found that carbohydrate content in the rind and pulp of the watermelon are significantly higher than the carbohydrate content of the seeds. The carbohydrate content in the seeds in this study was also found to be lower than those of mature soybean seeds which were found to contain 10% carbohydrates (Choung, 2010). The minute content of carbohydrates in the watermelon seeds has led to little attention being given to the watermelon seed carbohydrates. Little work has been done on the composition of the carbohydrates and their potential commercial value.

The landraces from both districts were compared and it was found that there were no significant differences in the carbohydrate content in the seeds, with the P value of 0.067 at 95% confidence level. The landraces from the Sekhukhune district were found to have a higher content of carbohydrates with an average of 5.26%. The landraces from Capricorn district had a lower carbohydrate content than the ones from the Sekhukhune district with a carbohydrate content of 3.16%. The 01SDPW

landrace was found to have the highest carbohydrate content of all landraces with a content of 8.95%, while 09CDGM had the lowest content at 2.15%. These differences may be attributed to the different environmental conditions that the plants were exposed to in the two districts.

5.2.7 Mineral content

Copper is an essential trace element for humans and forms a vital component of several enzymes. Its absorption is however dependent on the presence of other dietary elements such as zinc (Mir-Marques *et al.,* 2012). The element is involved in a variety of biological functions indispensable to sustain life. In humans, access to copper from the environment is limited. Food and drinking water and copper-containing supplements are the main sources of copper (De Romania *et al.,* 2011). Zinc is also an essential element in human nutrition and is present in many important enzymes essential for metabolism (Mir-Marques *et al.,* 2012).

The landraces from Capricorn district were found to have a higher copper and zinc content with averages of 0.078 and 0.231 mg/g respectively. This was found to be higher than the landraces from the Sekhukhune area with averages of 0.053 and 0.229 mg/g for copper and zinc, respectively. The copper content was found to be significantly higher in the landraces from Capricorn district than in the landraces from the Sekhukhune district with a P value of 0,016 at 95% confidence level. The copper content was found to be higher than the content of copper in the Sassako variety of watermelon seeds which were found to have a content of 0.016 mg/g (16.57 mg/kg) and 0.035 mg/g (35.33 mg/kg) by Gwana *et al.* (2014). Among the landraces, the 06CDGM landrace from Capricorn district was found to have the highest copper content with a value of 0.088 mg/g and 05SDPW from the Sekhukhune district had the lowest content with a value of 0.036 mg/g. The high copper and zinc content make the landraces better candidates to help in improving the metabolic functions of the body as according to Stein (2010), copper makes an important component of enzymes and helps in iron metabolism. Low copper status has been associated with risk of developing osteoporosis later in life, impaired melanin synthesis, poor immune response and increase in frequency of infections, poor cardiovascular health and alterations in cholesterol metabolism (De Romania *et al.,* 2011). The relatively high

copper content in the landraces indicates the potential in overcoming the problems associated with low copper status (Mir-Marques *et al.,* 2012).

Complexometric titrimetry is a standard method for estimating calcium and magnesium complex. When both are present, the titration conditions are usually adjusted so that during titration, either magnesium or calcium is precipitated and/or removed and the other titrated (Hildebrand and Reilley, 1957). In this study only compexiometric titration of both calcium and magnesium was carried out without precipitation of the other. Landraces from Sekhukhune area were found to have high iron and calcium-magnesium content with averages of 0.155 and 7.235 mg/g respectively compared to 0.135 and 5.432 mg/g for iron and calcium, respectively from the Capricorn district. The values were found to be higher than those obtained by Gwana *et al.* (2014) with averages of 0.091 mg/g (90.98 mg/kg) and 2.076 mg/g (2076.07 mg/kg) for iron and calcium-magnesium respectively. On the individual landraces, it was found that the 02SDPW had the highest iron content with a value of 0.194 mg/g and 05SDPW was found to have the lowest content with 0.084 mg/g. 03SDPW and 10CDGM were found to have the highest calcium-magnesium content both with a value of 9.13 mg/g. 06CDGM was found to have the lowest content of calcium with a value of 5.12 mg/g. The seeds from the Sekhukhune area will be instrumental in combating common deficiencies as the iron and calcium deficiencies have been said by Stein (2010), to be some of the elements whose deficiency has a negative impact on public health.

Calcium is an essential macro-nutrient that plays a vital role in neuromuscular functions, many enzyme-mediated processes, blood clotting, and providing rigidity of the skeleton (Mir-Marques *et al.,* 2012). Magnesium has many functions in muscles and soft tissues as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis and maintenance of electrical potential of nerve tissues and cell membranes (Mir-Marques *et al.,* 2012).

Landraces from both districts were found to have the same average content of sulphate with an average of 0.095 mg/g. The sulphur content was determined gravimetrically, a method which according to Dogson and Price (1962), has the advantage of speed and simplicity as comparatively small amounts of starting material are required making it the method of importance. The sulphur in the seeds

makes them desirable as a source of minerals and will help in normal metabolic functions as sulphur helps in the synthesis of sulphur containing compounds such as amino acids (Stein, 2010). Although the minerals in the watermelon seeds are available in notable quantities, their biological availability might be lower as according to Lakshmi and Kaul (2011), information on the bio-availability of minerals of watermelon seeds is scarce but it is very important to know the suitability of matrix from a mineral fortification point of view.

5.3 Phytochemical analysis

5.3.1 Phenolic analysis

5.3.1.1 Total phenolic content

Phenolic compounds are secondary metabolites found commonly in herbs and fruits. The compounds are divided into simple phenols and polyphenols depending on the number of phenol subunits (Leopoldini *et al.,* 2011). Total phenolics were investigated from defatted methanolic extracts from watermelon seeds from landraces from two districts in Limpopo province. The extracts were found to have a significantly low concentration of phenolics, averaging 0.57 mg/g gallic acid equivalents (GAE). This was found to be relatively lower than those obtained by Etim *et al.* (2013), in which they found the concentrations in methanolic extracts from watermelon seeds to average 0.96 mg/g GAE.

The concentrations of total phenolics were recorded at a low of 0.27 mg/g GAE in 02SDPW and were recorded at 0.91 mg/g GAE in 05SDPW as the highest concentration. With the exception of 09CDGM, the landraces from the Capricorn district showed a high concentration of phenolics on average with a mean concentration of 0.59 mg/g GAE. The landraces from the Sekhukhune district had a lower mean concentration of 0.545 mg/g GAE. Although there were differences noted, analysis by one way ANOVA revealed that there was no significant difference in the phenolic content in landraces from both districts. The results were found to have a P value of 0.781 at 95% confidence level (Appendix A). The differences in the content of phenolics might be attributed to the differences in the varieties of landraces that the landraces were grown under and the genetic variations in the landraces.

5.3.1.2 Total flavonoid content

Flavonoids represent the most common and widely distributed groups of phenolics. They are potent water-soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anti-cancer activity and protects against all stages of carcinogenesis (Oseni and Okoye, 2013). The flavonoid content ranged from 0.085 in (05SDPW) mg/g to 0.347 mg/g (10CDGM). The landraces from the Sekhukhune district were found on average to have fewer amounts of flavonoids (0.13 mg/g) than those from the Capricorn district (0.22 mg/g). The results however, were not found to have a significant difference, with a P value of 0.123 at 95% confidence level (Appendix A). These results were lower than those obtained by Etim *et al.* (2013) where they found the total flavonoid content to be 9.96 mg/g. Both results are however, very low in comparison to those obtained by Varghese *et al.* (2013) who obtained an amount of 154.26 mg/g. The differences between the study by Varghese *et al.* (2013) and the current study may be due to the different solvents used in the extraction as the first two studies used methanol for extraction while the others used ethanol. In their study, they found ethanol to yield more flavonoids than methanol extraction. The differences may be due to the difference in cultivars and also due to different conditions that the plants were grown under as Sakihama *et al.* (2002) have also shown that plants accumulate more flavonoids and other phenolic compounds under stressful conditions like drought and temperature extremes.

5.3.2 Antioxidant activity

5.3.2.1 Time course of inhibition activity

The standard assay used to determine antioxidant activity is usually the 1,1-Diphenyl-1-picryl-hydrazyl (DPPH) method. DPPH is a stable free radicals which has an unpaired valence electron at one atom of the nitrogen bridge. Scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay, which if present in high concentrations in the reaction mixture, gives absorbance beyond the accuracy of spectrophotometric measurements (Sharma and Bhat, 2009). To remedy this, it is imperative to read the absorbance when the reaction has gone to completion. Inhibition time course was probed in watermelon seed extracts to determine the time required for the reaction between DPPH and the seed extract to come to completion. It was observed from the graph that the mixture reached completion at 40 minutes.

5.3.2.2 Antioxidant activity of extracts

The percentage antioxidant activity average was found to be 45.90%. This was found to be lower than the percentage antioxidant activity obtained by Oseni and Okoye (2013), where they found the antioxidant activity in watermelon seeds to be 56.93%. The results from this study were found however, to be higher than those obtained by Ramazan *et al.* (2012), where they found antioxidant activity in watermelon seeds to range from 5.6% to 13.90%. Ramazan and colleagues also found that their results in watermelon seeds showed high antioxidant activity than commercial watermelon seeds with a percentage inhibition ranging from 1.3% to 4.42%. The differences in the results may be attributed to solvents used in the extraction process. It has been shown by Annegowda *et al.* (2012) that antioxidant recovery from plant material is known to be influenced by the solubility of the compounds in a particular solvent used for the extraction process and depends also on the polarity of that particular solvent. They have also shown that methanolic extracts revealed higher antioxidant activities than aqueous extracts. This was also found to be true for other solvents, depending on differences in polarity and viscosity, which is higher in methanolic extracts than solvents with low viscosity, low density and high diffusity (Annegowda *et al.,* 2012).

The landraces from the two districts were compared using one way ANOVA and it was found that there were no significant differences in the antioxidant activities of extracts from both districts with a P value of 0.883 at 95% confidence level (Appendix A). The landraces from the Capricorn district were found to have a high antioxidant activity averaging 46.46% than those from the Sekhukhune district with an average of 45.28%, with 01SDPW with the highest percentage inhibition at 66.95% and the least percentage DPPH inhibition was observed in 03SDPW landrace at 35.30%. Although there were no significant differences in the landraces from the two districts, differences were noted within the landraces from similar districts. The reason for these results might be attributed to the fact that similar plant materials might exhibit varied results due to varied chemical characteristics of antioxidant compounds that necessitates the use of solvent with different polarities to obtain high yield antioxidants (Annegowda *et al.,* 2013). George *et al.* (2013) have reported that minor antioxidants may be destroyed during the long term required for Soxhlet extraction. This might explain the differences in activities from similar

districts as the different landraces may contain different antioxidant compounds with some being more susceptible to degradation than others.

5.4 Comparing the means

Food legumes and seeds of some plant species constitute a major source of edible nutrients such as protein, lipids, amino acids, carbohydrates, mineral elements, fatty acids and other important substances such as fibre and vitamins which have some importance on human health (Senga *et al.,* 2013). Watermelon seeds from two districts in this study were compared to see if there is a district that produces superior landraces between them.

The landraces from Capricorn district were found to be higher in regard to mass per 100 seeds (9.52 g), lipid content (34.4%), the oil of the landraces from this district also had higher iodine value (136.69 g I_2/100 g oil), which indicates the potential of the oil to be more stable when stored for longer periods. The landraces also had a high protein content (16.51%) than the landraces from Sekhukhune district (14.89%). The landraces from Capricorn district also had high crude fibre content (23.07%) than landraces from Sekhukhune district (22.04%). The percentage inhibition of watermelon landrace extracts was also higher in the landraces from Capricorn district (46.46%) than the landraces from Sekhukhune district. This indicates that the landraces from Capricorn district have more antioxidant activity than those from Sekhukhune district and are likely to be more effective in the antioxidant defence against deleterious agents in the body. Total flavonoids, which are the most common and widely distributed group of plant phenolics (0.22 mg/g) and total phenolics (0.59 mg/g) were higher in the landraces from Capricorn district. A high content of total flavonoids and total phenolics have been reported by Oseni and Okoye (2013), to make up the majority of antioxidants and hence the biochemicals may be responsible for the majority of the antioxidant activity. The high flavonoid content impart important biological functions such as protection against allergies, inflammation, free radicals, microbes, ulcers, viruses and tumours. They are also potent super-antioxidants and free radial scavengers which prevent oxidative cell damage, have strong anti-cancer activity and protects against all stages of carcinogenesis (Oseni and Okoye, 2013).

The high content of these biomolecules and biochemicals in landraces from Capricorn district makes the landraces from the district better suited for use in meal supplementation and in disease fighting capabilities than those from Sekhukhune district. However, the landraces from Sekhukhune district were found to have high content of carbohydrates (5.26%) than the landraces from Capricorn district (3.16%). This makes the landraces from Sekhukhune district a better source of energy and calories which are required by the bodies of both children and adults alike (Senga *et al.,* 2013). The landraces were also found to be high in ash content (4.58%) than those from Capricorn district (3.99%). These landraces also had higher saponification values (152.25 mg KOH/g oil), indicating that the landraces from the Sekhukhune district are more unsaturated than those from Capricorn district and hence the oil is potentially healthier than that of landraces from Capricorn district.

Landraces from Capricorn district were found to have high copper content (0.078 mg/g) and zinc content (0.231 mg/g). Landraces from both districts were found to have the same amount of sulphate (0.095 mg/g). The landraces from Sekhukhune district were found to be high in iron (0.155 mg/g) and calcium (7.235 mg/g) than the landraces from Capricorn. This indicates that the landraces can complement each other to help against mineral deficiencies.

5.5 Conclusion and recommendations

The results indicate that landraces from both districts have complementary qualities to each other, with some landraces from one district having better qualities than the landraces from the other district. However the landraces from the Capricorn are more superior in most aspects. From comparing the results with those from previous studies, it is clearly observed that the landraces can in most instances match the qualities of the highly commercialised oil seeds. The watermelon seeds can be used as a cost-effective and easily accessible method to meet the many nutritional deficiencies in developing countries such as South Africa and Africa as a whole.

From the results obtained in this study, it is highly recommended that the landraces with superior qualities such as the 10CDGM being cross pollinated with landraces with the qualities/compounds (08CDGM which has good quality oil and more iron) that the 10CDGM landrace lacks. The landrace can also be cross bred with landraces such as 06CDGM as it has better seed yield and thus will result in more

compounds being recovered from the seeds and in addition, the landrace has minerals such as copper and iron, which the 10CDGM landrace lacks. Genetic engineering can also be employed to introduce the lacking qualities in the landrace to make it to have adequate nutrition in all aspects. Mathew *et al.* (2014) has recommended that industrial production and commercialisation of watermelon seeds be given adequate attention to provide more sources of edible and industrial oils and be used as a tool for economic development as the seeds can potentially be used to generate wealth. Therefore, more detailed studies on the composition and properties of watermelon seeds are recommended as there is limited information and studies done on the watermelon seeds.

CHAPTER 6: REFERENCES

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APPENDICES

Appendix A: Oneway ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
phenolics	Between Groups	45.000	1	45.000	.084	.781
	Within Groups	3769.000	7	538.429		
	Total	3814.000	8			
antioxidants	Between Groups	30863.606	1	30863.606	.023	.883
	Within Groups	9231691.950	7	1318813.136		
	Total	9262555.556	8			
flavonoids	Between Groups	18972.800	1	18972.800	3.076	.123
	Within Groups	43171.200	7	6167.314		
	Total	62144.000	8			
Proteins	Between Groups	59914.756	1	59914.756	1.884	.212
	Within Groups	222646.800	7	31806.686		
	Total	282561.556	8			
Carbohydrates	Between Groups	193192.272	1	193192.272	4.704	.067
	Within Groups	287497.950	7	41071.136		
	Total	480690.222	8			
Ash	Between Groups	7592.006	1	7592.006	.482	.510
	Within Groups	110161.550	7	15737.364		
	Total	117753.556	8			
Fibre	Between Groups	23278.939	1	23278.939	2.356	.169
	Within Groups	69173.950	7	9881.993		
	Total	92452.889	8			
Oils	Between Groups	1711.250	1	1711.250	1.419	.272
	Within Groups	8438.750	7	1205.536		
	Total	10150.000	8			
iodine	Between Groups	14382.672	1	14382.672	.493	.505
	Within Groups	204181.550	7	29168.793		
	Total	218564.222	8			
saponification	Between Groups	340605.000	1	340605.000	.123	.736
	Within Groups	19368265.000	7	2766895.000		
	Total	19708870.000	8			
mass	Between Groups	16340.139	1	16340.139	1.773	.225
	Within Groups	64498.750	7	9214.107		
	Total	80838.889	8			
copper	Between Groups	.001	1	.001	9.985	.016
	Within Groups	.001	7	.000		

	Total	.002	8			
Iron	Between Groups	.001	1	.001	.646	.448
	Within Groups	.009	7	.001		
	Total	.010	8			
Zinc	Between Groups	.000	1	.000	.003	.960
	Within Groups	.028	7	.004		
	Total	.028	8			
Sulphate	Between Groups	.000	1	.000	.002	.969
	Within Groups	.005	7	.001		
	Total	.005	8			
Calcium	Between Groups	.001	1	.001	.000	.985
	Within Groups	22.173	7	3.168		
	Total	22.174	8			

Appendix B: Descriptive statistics

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
phenolics	sekhukhune district	4	54.50	27.429	13.714	10.85	98.15	27	91
	capricorn district	5	59.00	19.442	8.695	34.86	83.14	36	86
	Total	9	57.00	21.835	7.278	40.22	73.78	27	91
antioxidants	sekhukhune district	4	4527.75	1086.384	543.192	2799.07	6256.43	3530	6067
	capricorn district	5	4645.60	1192.791	533.432	3164.55	6126.65	3667	6695
	Total	9	4593.22	1076.020	358.673	3766.12	5420.32	3530	6695
flavonoids	sekhukhune district	4	130.00	50.027	25.013	50.40	209.60	85	195
	capricorn district	5	222.40	94.424	42.227	105.16	339.64	135	347
	Total	9	181.33	88.136	29.379	113.59	249.08	85	347
Proteins	sekhukhune district	4	1487.00	85.561	42.780	1350.85	1623.15	1412	1602
	capricorn district	5	1651.20	223.989	100.171	1373.08	1929.32	1501	2039
	Total	9	1578.22	187.937	62.646	1433.76	1722.68	1412	2039
Carbohydrates	sekhukhune district	4	526.25	275.874	137.937	87.27	965.23	280	895
	capricorn district	5	231.40	121.634	54.396	80.37	382.43	47	385
	Total	9	362.44	245.125	81.708	174.02	550.86	47	895
Ash	sekhukhune district	4	458.25	83.500	41.750	325.38	591.12	333	500
	capricorn district	5	399.80	149.369	66.800	214.33	585.27	333	667
	Total	9	425.78	121.323	40.441	332.52	519.03	333	667
Fibre	sekhukhune district	4	2204.25	111.404	55.702	2026.98	2381.52	2078	2314

	capricorn district	5	2306.60	89.361	39.963	2195.64	2417.56	2162	2399
	Total	9	2261.11	107.502	35.834	2178.48	2343.74	2078	2399
Oils	sekhukhune district	4	316.25	11.815	5.907	297.45	335.05	300	325
	capricorn district	5	344.00	44.777	20.025	288.40	399.60	300	415
	Total	9	331.67	35.620	11.873	304.29	359.05	300	415
iodine	sekhukhune district	4	13588.75	165.754	82.877	13325.00	13852.50	13400	13768
	capricorn district	5	13669.20	174.470	78.025	13452.57	13885.83	13470	13857
	Total	9	13633.44	165.289	55.096	13506.39	13760.50	13400	13857
saponification	sekhukhune district	4	15224.50	2261.232	1130.616	11626.38	18822.62	13240	18457
	capricorn district	5	14833.00	1003.588	448.818	13586.88	16079.12	13520	16129
	Total	9	15007.00	1569.589	523.196	13800.51	16213.49	13240	18457
mass	sekhukhune district	4	866.25	40.078	20.039	802.48	930.02	835	925
	capricorn district	5	952.00	122.147	54.626	800.33	1103.67	805	1095
	Total	9	913.89	100.523	33.508	836.62	991.16	805	1095
copper	sekhukhune district	4	.05275	.014818	.007409	.02917	.07633	.036	.072
	capricorn district	5	.07780	.008927	.003992	.06672	.08888	.067	.088
	Total	9	.06667	.017219	.005740	.05343	.07990	.036	.088
Iron	sekhukhune district	4	.15450	.048349	.024175	.07757	.23143	.084	.194
	capricorn district	5	.13540	.021043	.009411	.10927	.16153	.103	.154
	Total	9	.14389	.034632	.011544	.11727	.17051	.084	.194
Zinc	sekhukhune district	4	.22900	.075485	.037743	.10889	.34911	.118	.287
	capricorn district	5	.23120	.053011	.023707	.16538	.29702	.165	.312
	Total	9	.23022	.059525	.019842	.18447	.27598	.118	.312

Sulphate	sekhukhune district	4	.09450	.030903	.015452	.04533	.14367	.049	.118
	capricorn district	5	.09520	.022016	.009846	.06786	.12254	.068	.129
	Total	9	.09489	.024507	.008169	.07605	.11373	.049	.129
Calcium	sekhukhune district	4	7.2350	1.84240	.92120	4.3033	10.1667	5.34	9.13
	capricorn district	5	7.2580	1.73129	.77426	5.1083	9.4077	5.12	9.13
	Total	9	7.2478	1.66486	.55495	5.9681	8.5275	5.12	9.13

Appendix C: Amino acids separated from watermelon seeds

LANDRACE	Rf value	Colour of spot	Amino acid
01SDPW	0.221	Orange	Unidentified
	0.338	Light pink	Unidentified
	0.441	Orange	Methionine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
02SDPW	0.162	Brick red	Threonine
	0.235	Orange	Unidentified
	0.338	Light pink	Unidentified
	0.471	Reddish violet	Valine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
03SDPW	0.162	Brick red	Threonine
	0.235	Orange	Unidentified
	0.353	Light pink	Unidentified
	0.471	Reddish violet	Valine
	0.603	Orange	Leucine
05SDPW	0.162	Brick red	Threonine
	0.235	Orange	Unidentified
	0.338	Light pink	Unidentified
	0.471	Reddish violet	Valine
	0.603	Orange	Leucine
06CDGM	0.162	Brick red	Threonine
	0.235	Orange	Unidentified
	0.338	Light pink	Unidentified
	0.441	Orange	Methionine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
07CDGM	0.162	Brick red	Threonine
	0.382	Light pink	Unidentified
	0.441	Orange	Methionine
	0.603	Orange	Leucine
08CDGM	0.162	Brick red	Threonine
	0.235	Orange	Unidentified
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
09CDGM	0.162	Brick red	Threonine
	0.250	Pink	Unidentified
	0.441	Orange	Methionine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine
10CDGM	0.162	Brick red	Threonine
	0.442	Orange	Methionine
	0.529	Purple	Phenylalanine
	0.603	Orange	Leucine