CROSSBREEDING OF INDIGENOUS WATERMELONS FOR HIGH OIL CONTENT

by

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DISSERTATION

Submitted in fulfilment of the

requirements for the degree of

Master of Science in

Botany

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Molecular and Life Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR: Dr PW Mokwala

2022

DECLARATION

I declare that CROSSBREEDING OF INDIGENOUS WATERMELONS FOR HIGH OIL CONTENT (dissertation) hereby submitted to the University of Limpopo, for the degree of Master of Science in Botany has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Molwantoa, E. 28/06/2022

DEDICATION

This dissertation is dedicated to my son, Muen Romuvhona for being an amazing and wonderful gift from God. I also dedicate this work to my fiancé Mulalo, who always pushed me to improve and do better.

ACKNOWLEDGEMENTS

I would like to appreciate the following:

- God, for my life and making it possible.
- My amazing supervisor, Dr. P.W. Mokwala for his skills, guidance, knowledge, time, ambitions and patience.
- NRF (National Research Foundation), for their financial support.
- Mr B. Mdaka, Department of Biodiversity (Botany) for helping me in the laboratory.
- Dr H.J. duPlessis for her inputs, suggestions and time.
- My fiancé, Mulalo Lutombo for his assistance and courageous words.
- My Parents (Nancy and Izaack) and siblings (Betty, Tshepo, Felicia, Josius, Johannes and Keletso) for their love, motivations and support.

ABSTRACT

Watermelon *(Citrullus lanatus)* is an annual plant which survives better under warm temperatures, and is believed to have originated in southern Africa. The plant is now distributed across the world and grown because of the juicy appetising flesh of its fruit. There are more than 12000 varieties of watermelons across the world. Looking at the rate at which population size is rapidly increasing, it is not surprising that it is expected to reach about 8.1 billion by 2040. This puts a great pressure on available resources. Oil is one of the essential resources that human need. It is used in the production of lots of our daily needs like medicine and cosmetics. Watermelon is one of the crops that produces seeds that are rich in oil. However, their seeds are not enough to harvest and extract adequate oil for industrial purposes. Each variety has got its superior and inferior seed production traits. The aim of the study was to crossbreed different types of indigenous watermelons in an attempt to produce a hybrid or hybrids with high oil content. As a result, Bitter and Weedy watermelon varieties were planted in the nursery on the grounds of the University of Limpopo, and cross-pollinated. The crossed seeds were then self-pollinated. Dried seeds from all watermelon types including Bitter, Weedy Cross and F1 generation were ground into powder and oil was extracted. Oil percentage together with some physiochemical properties of oils were determined and compared. This study found that there was an improvement in terms of seed production, oil quality and quantity for both cross and F1 generation. Cross fruits showed to have improved in terms of the total number of seeds (717); the seeds had the highest oil content (39.16%) with the highest saponification (118.67 mg KOH/g oil) and iodine (114.21 g of Iodine/100 g oil) values. The F1 generation which resulted from self-pollinated Cross plants had the highest mass of 100 seeds (13.51 g); mean geometric diameter (6.91 mm); and coming second (33.30%) after Cross seeds for oil content. On the other hand, Weedy produced oil with the highest specific gravity (0.91) and lowest moisture content (2.98%). It is concluded that the crossbreeding of Bitter and Weedy watermelons produces improved offspring which performs better after self-pollination. The physiochemical properties of the current investigated watermelon oils are mostly within the acceptable ranges of oils reported to be of good quality for soap production and consumption industrial purposes. In conclusion, Cross watermelons are the most superior ones because of their adequate intermediate amount of total seeds produced, highest oil yield and better physiochemical properties of oil. However, watermelon oil is still inadequately used industrially. This can be improved by further studying more physiochemical properties such as index viscosity, flash point and fatty acid composition of the current investigated watermelons.

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LIST OF ABBREVIATIONS

USD - United States dollar

Dp - Geometric mean diameter

F1 - First Filial generation

NaOH - Sodium hydroxide

meq - Milliequivalent

HCL - Hydrochloric acid

KOH - Potassium hydroxide

M – Molar

CHAPTER 1

GENERAL INTRODUCTION

Indigenous crops played an important role as a source of food in South Africa to both indigenous people and colonists during the 1600s (Modi, 2003). Bhat and Rubuluza (2002) reported that indigenous watermelons were one of the essential foods consumed by indigenous Africans when they were living a nomadic lifestyle. These people are said to have moved from one place to another influenced by the availability of food. Millet, kidney beans, pumpkin, sweet sorghum and wild watermelons were the most planted crops by indigenous peoples in South Africa, more especially the Xhosas (Sobahle, 1982). Food security was attained by planting wild watermelons and pumpkins as they were being harvested earlier at around February while waiting for major crops like maize to mature (Mhlontlo, 2008). To date, some people from Africa still value indigenous crops as their source of food and trading activities (Schippers, 2000). Indigenous watermelon such as Kordofan Melon are in higher demand in Sudan street markets and are reported to have potential to compete with commercial watermelons (Mrema and Manerere, 2018). Other varieties with such potential includes Sweet indigenous watermelons while Bitter and Weedy are deemed non-palatable to humans.

The weedy watermelon (Figure 1.1) is not a planted type. It is a watermelon weed that grows whenever the Bitter variety (Figure 1.1) is planted. Its fruit is smaller than those of the other watermelons such us Bitter and Sweet . It is thought to be a genetically changed form of the Bitter watermelon (Fan et al., 2011). The belief is that it arises when seeds of the Bitter are dried in the sun. Either when seeds from the left over harvest of the Bitter variety in the field or when farmers dry the seeds in the sun for planting in the following season.

Figure 1.1: Indigenous watermelons used in the current study. (A) Bitter; (B) Weedy.

1.1 Watermelon background

1.1.1 Origin and distribution

The plant is believed to have originated in southern Africa. This is motivated by the way it was found growing wildly and its degree of diversification across the region (Goda, 2007). Watermelon has been cultivated for more than 4000 years now in Africa (Jensen, 2012). The fruit was grown in pristine Egypt and domesticated in early 1850's when enormous tracts of this plant were discovered in the Kalahari Desert (Boualem, 2016). An evolutionary relationship study by Dane and Lui (2007) using chloroplast DNA showed that the watermelon evolved from Namib tsamma (*Citrullus ecirrhosus).* Watermelon is still grown in some semi-desert areas of Africa as an essential source of water during times of insufficient water (Davis et al., 2008). It was then distributed to other parts of the world mostly by people's migration. The plant is now distributed across the world and grown because of the juicy appetising flesh of its fruit (Majuju et al., 2011). In South Africa's major producing provinces of watermelons are Limpopo, North West, Mpumalanga and some restricted areas of Eastern Cape (Dube et al., 2021).

1.1.2 Botany

The watermelon, *Citrullus lanatus,* (family Cucurbitaceae) is an annual plant which survives better under warm temperatures (Kader, 2008). It differs from other cucurbit plants by its pinnatified leaves, having divided 3-4 pairs of lobes (Majuju and Fatin, 2011). The stem of the watermelon is prostrate, hairy and thin, can grow up to approximately 5 m in length and produce about 3 stem tendrils (Nanasato et al., 2010; Boualem et al., 2016). In exceptional cases, there are bred dwarf watermelons which have shorter stems (Ayodele and Shittu, 2013; Mrema and Manerere, 2018). The Watermelon fruit is made up of a thick rind (exocarp) and fleshy part on the centre (mesocarp and endocarp) which also contains the seeds (Majuju and Fatin, 2011). The outer covering of watermelon fruits differs in colour from white to dark green. It may also have stripes, be mottled or just plain (Gusmini and Wehner, 2005). Some rinds are thick while others are thin, ranging from 1-4 cm in thickness. Those that are thick are strong but not perdurable. Its inner fleshy part can be white, orange pink or red in colour when mature (Said and Fatina, 2018).

1.1.3 Varieties

There are more than 12000 varieties of watermelons across the world (Aruju and Okoli, 2013). However, there are about 50 common watermelon cultivars that fall under five general categories of: All Sweet, Ice Box, Seedless, Yellow Flesh and Crimpson (Jaskani et al., 2005; Emuh and Ojeifo, 2012). They range from fruit size of less than 1 kg to more than 100 kg, from seedless to more than 1000 seeds per fruit (FAOSTAT, 2017).

Brewer (1974) reported that the development of the juicy sweet watermelon variety known as Moon and Stars occurred in the 1920s. This variety is consists of red flesh with brown seeds. On the other hand, there is an early ripening watermelon variety named: “Melitopolski”, which has smaller round fruits that are about 26 cm in diameter (Gillasy et al., 1995). There is also a rare variety with black a rind, without any stripes nor spots (Castro et al., 2013). It is known as the “Den Suke” and grown in Hokkaido, Japan (Castro et al., 2013).

Currently, most varieties of watermelons are not cultivated for commercial purposes as they have a thick rind and are said to produce unpalatable juice (Hayata et al., 1995; Feher, 2019). However, there are few exceptions which are grown for their thick rinds for the production of watermelon pickles. These include: Black Diamond and Tom Watson (Mao et al., 2016).

1.2 The changing climate and its impact on crops

The world is encountering climate change accompanied by droughts (Chandio et al., 2019; Rahim and Puay, 2017). South Africa is one of the countries affected by drought (Demir et al., 2011; Modi and Zulu, 2012). According to Luo et al. (2017), in the next coming 20 years from that year (2017), the average temperature will rise by about 3 ^{o}C. Moreover, the world is expected to experience extreme temperatures for longer periods of time and also more intensely than what is experienced currently (Meehl et al., 2007; Ullah et al., 2016). The growth and development of plants is dependent on various environmental conditions, with climate being one of the major ones. The rise in temperature was postulated to lead to yield declines at about 3% to 40% across various crops (Hatfield et al., 2011; Nasir et al., 2019). However, the rate at which a plant is affected by temperature ranges varies amongst plants. In addition, crops of the same species have shown to respond differently to higher temperatures (Hatfield et al., 2011).

1.3 Watermelon tolerance to high temperatures and low water availability

Generally, watermelons are well known for their ability to survive better under higher temperatures. They need a temperature that is at least 21 ^{O}C, and ranges up to 32 degrees Celsius amongst different varieties (Kato et al., 2000). Moreover, the study by Dumrul and Kilicasan (2017) shows that there is a need to investigate and promote the utilisation of drought tolerant crops in order to enhance food security in developing countries. Various publications reported that indigenous watermelons withstand and tolerate drought and higher temperatures better compared to commercial watermelons (Majuju, 2009; Modi and Zulu, 2012). It is also evident that indigenous watermelons grow and develop well under the full sun of dry regions, making them suitable to concentrate on for maximised production during summer season (Baboli and Kordl, 2019).

1.4 Increasing population and high demand of resources

Looking at the rate at which the population size is rapidly increasing (about 1.1% growth annually), it is not surprising that it is expected to reach about 8.1 billion by

2040 (Worldmeter, 2020). Every living organism, including humans need various resources to survive. Amongst others, the most important ones are water, food and energy. It is the world's goal to be able to meet the needs to all people (Willett et al., 2019). Available resources are being exhausted day by day because of the pressure from continuous population increases (Jiang and Rehman, 2018). Destruction of biodiversity, over-usage of water and erosion of land are examples of activities that endanger the ability of the environment to produce adequate resources (Chandio et al., 2016). Food is one of the examples that is being pressured by high population growth, highlighting the problem of food insecurity especially in poor and developing countries. According to Ali et al. (2017), the issue of food shortage is becoming more critical and about 2.2 billion people across the world are experiencing malnutrition. Moreover, approximately 4500 children die daily from hunger and diseases (Banerjee and Radak, 2019). Problems that we see each day give enough evidence that the earth is seriously under great pressure (Oliveira, 2019).

1.4.1 Oil as an essential human resource

Oil is an essential resource for humans and is being used daily. Products of oil support the modern lifestyle, supplying the power industry with energy, provide vehicles with fuel and heating up homes. It is used as one of the main products in the production of lots of our daily needs like lubricants, printing ink, cosmetics, and pharmaceuticals and also as food (Antonakakis and Filis, 2013). As the population grows, the oil demand is expected to reach 105 million barrels daily by 2040 (Gkillas et al., 2019). However, an increase in the human population across the world has led to an increase in demand of oils thus resulting in a rise in oil prices (Khandelwal et al., 2016; Arif and Rawaf, 2019). Also, the land for agricultural purpose is very limited to produce enough oil crops to supply the high demand the world is facing today (Henson, 2012; Rahim and Puay, 2017). Therefore, both fossil oil and seed oil are still not enough for the high demand of this resource.

1.4.2 Seed oil

The industry of natural products has grown at about 18% each year (Soh et al., 2017). In 2015, it was valued at 75 billion USD per year, with an increase of 1 billion USD per year (Nemarundwe et al., 2019). Currently, production of oil from plant seeds as source of food, medicines, cosmetics and biofuel has gained much attention and remains a field of interest as industries need to find alternatives of oil production which are of natural origin (Kushairi et al., 2018; Mitei et al., 2008). The high oil demand has led to a trend of searching other unfamiliar origins of oils, more especially in developing countries (Bardaa et al., 2016; Blasi et al., 2018).

Seed oil is extracted from plant seeds. Plants store this oil in their seed as the energy reserves meant for developing seedling (Henson, 2012). There are various methods that are used in the extraction of oil from seeds. Examples are: traditional method, the solvent extraction, mechanical expression, and microwave extraction (Bakoune et al., 2015). According to Younis et al. (2000), the solvent extraction method is the most used and effective extraction method. The oil is considered suitable and safe for use in production of valuable products such as cosmetics and medicines, food processing or cooking (Kim et al., 2016).

1.5 Watermelon's potentials and limitations

Watermelon is one of the crops that produce seeds that are rich in oil with a potential for several uses. *Citrullus lanatus* (watermelon) seed oil has characters that compare very well with soybean and sunflower seed oils (Baboli and Kordi, 2010). It was found that watermelon seed oil consists of about 65% linoleic acid and 16% oleic acid while soybean and sunflower contain 50% and 68% linoleic acid and 14-21% and 23% oleic acid respectively (Muhammad et al., 2015; Neuza et al., 2015). Even so, watermelon seed oil is one of the less explored plant seed oils (Muhammad et al., 2015; Kushairi et al., 2018).

Indigenous watermelons such as Sweet, Bitter and Weedy produce seeds which are not enough to harvest and extract adequate oil. Each variety has its superior and inferior seed production traits (Maggs-Kolling et al., 2000; Modi and Zulu, 2012). For example, Sweet watermelons produce many large, thin seeds; Bitter types produce fewer large, thick seeds and Weedy types produce many small seeds. Therefore, traits such as small, thin and few seeds are inferior and not desirable for oil production. However, when the superior seed traits are combined and found in one seed the amount of oil that can be extracted will increase.

1.6 Conventional breeding

Conventional breeding has been performed for more than hundred years (Attavar et al., 2020). Farmers have been trying to manipulate the genetic makeup of plants they grow. They used to select plants that produced better than others and save their seeds to plant the next planting season (Kader, 2008). After several trials of breeding, plant breeders developed a suitable procedure whereby there was a selection of two better parents that complement each other; which are then cross-pollinated to produce better offspring (Zhu et al., 2017). This has a record of improving quality and quantity of crops that we see today (Devi et al., 2020; Muhammad et al., 2020). Therefore, conventional breeding has a potential solve the problem of inadequate resources such as food, oil, and medicines we are currently facing.

1.7 Aim

The aim of the research was to crossbreed different varieties of indigenous watermelon in an attempt to produce a hybrid or hybrids with a high seed oil content.

1.8 Objectives

The objectives of the study were to:

i. Cross-pollinate Weedy and Bitter watermelons.
ii. Self-pollinate the offspring.
iii. Quantify seeds produced in the cross fruits and F1 offspring as well as those of the parental lines (Bitter and Weedy types).
iv. Compare seed oil production by the seeds of parents, the Crosses and F1 offspring.
v. Select offspring that produce high seed oil content.

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CHAPTER 2

CROSSBREEDING OF INDIGENOUS WATERMELON FOR IMPROVED SEED PRODUCTION

2.1 Introduction

Watermelon (*Citrullus lanatus*), belongs to the Cucurbitaceae family (Goda, 2007). The fruits have a smooth green/yellow exocarp (outer coverings) and most varieties have stripes that change from green to yellow when ripe. Nearly all parts of most watermelons are used as food, from leaves, fruits and roots (Asghar et al., 2012) while seeds and rind are regarded as wastes (Fasoula et al., 2014).

Even though the seeds are considered as waste by many people, they are nutritious and contain minerals such as magnesium and potassium (Naz et al., 2014). Watermelon seeds are also very rich in oil and proteins. According to Seidu and Otutu (2016), the seeds are composed of 50% oil and 36% protein. For the watermelon seed oil to be of economic value sufficient seeds must be produced. It is however a challenge with watermelons as the seeds make about 1.9 to 4.2% of the fruit (Ofoegbune et al., 2014). To obtain adequate amount of seed oil, alteration and combination of suitable traits such as number of seeds per fruit and seed size via plant breeding should be implemented. Various researchers reported that the conventional breeding in watermelons targeted the type of the fruit and size, faster maturity, yield, sweetness, and appropriate seed type and size (Cerqueira-Silva et al., 2014; Fallik and Ziv, 2020). Therefore, it is vital to decide the main objective of plant breeding firstly prior to choosing parental lines.

The study and documentation of the properties of watermelon seeds is necessary for their storage, handling and processing (Nanasato et al., 2010; Isaza, et al., 2018). Admittedly there is available data on the morphology of indigenous watermelon seeds (Mogotlane et al., 2018). However, available data is not enough and there is none on the improvement of indigenous watermelon seeds. Information on the value of watermelon seeds for various purposes like oil production is also available (Biswas et al., 2017), but the watermelons studied had inadequate amount of seed and relatively low oil production capacities. Hence, the objective of the current chapter was to crossbreed the indigenous watermelons in order to achieve higher seed production.

2.2 Literature review

A seed is a plant that is still at an embryonic stage, with protective covering or seed coat on the outside (Arujo et al., 2018). Its formation is part of the reproduction process of plants. The three main parts of a seed are: embryo, seed coat and endosperm (Munder et al., 2017; Isaza et al., 2018). An embryo is a developed zygote which results from fertilisation of the egg cell. The seed coat develops from integuments within the ovule of the mother plant and protects the seed. The endosperm is where the seed stores its nutrients such as starch, oil and proteins (Paksoy and Aydin, 2004; Figueriredo et al., 2011).

There are two main groups of seed producing plants, namely: angiosperms and gymnosperms (Zhu et al., 2017). In angiosperms, seeds are covered or enclosed in a hard and/or fleshy structure to protect and provide nutrients to the developing embryo. In contrast, gymnosperm seeds are not enclosed in anything. They are just protected by cone scales (Patel and Rauf, 2017). There are various factors affecting the seed production in plants such as genetic makeup and environmental conditions (Mohd et al., 2017; Ahmed et al., 2021).

Different plants may produce very distinct seed sizes. An orchid seed is reported to be the smallest, with approximately 1 million seeds weighing a gram (Mirrzabe et al., 2017). This type of seeds does not have energy reserves and depend on a mutualistic relationship with mycorrhizal fungi for nutrition (Jansen, 2014). At above 20 kg, the largest seed is that of the sea coconut (Dissanayaka et al., 2008). According to Munder et al. (2018), plants that produce larger seeds produce few of them than those that are smaller to ensure survival during seed dispersal. In addition, annual plants usually produce many smaller seeds than perennial plants do (Feher, 2019).

2.2.1 Watermelon seed characteristics

Various varieties of watermelon differ in their seed shapes and sizes. Although seeds of many varieties are oval in shape, some are round (Mansouri et al., 2017). Koocheki et al. (2007) reported that watermelon seeds are pear-shaped. Moreover, there are new developed varieties of seedless watermelons which produce very tiny, immature seeds (Ahmar, 2020).

Studies show that seeds of most watermelon varieties have about the same sizes of lengths and widths, the averages are between 9-13 mm length and 4-6.5 mm width (Acar et al., 2012; Seidu and Otutu, 2016). However, there are those that are reported to be larger and wider or way smaller. Seeds of one variety of Farrasha were found to be 7 × 2.2 mm in length × width (Egbuonu, 2015). Razavi and Milani (2006) reported on the physical characteristics of three varieties of watermelon seeds that were dried. Their study showed that seeds of Ghermez a Red variety had the highest length (13.02 mm), width (2.77 mm), whereas Sarakhsi variety showed to be the widest (7.08 mm) of them all with Kolaleh being the smallest (9.6 mm length, 5.09 mm width and 1.56 mm thickness).

Watermelon seeds can be white, red, black, brown, yellow or orange in colour (Menom et al., 2012; Kyriacou et al., 2018). Furthermore, some varieties have spackles of an additional colour on the dominant one, whereas others have a different colour on the edges (Saided, 2002). In very early studies, Mckay (1936) stated that light brown/tan character is dominant over red colour, which is recessive. Also, when reddish-brown and black band genes combined together, they result into a completely black seed coat. Further, Weetman (1937) reported on the inheritance of the shape, colour and size of watermelon seeds. The data was recorded on selected parents, F1, F2 and the backcross offspring. It was reported that the appearance of black colour on the peripheral parts of the seed coat is a dominant trait. According to Zhu et al. (2017), the black seed coat colour is dominant over white, green and tan, while tan is dominant over white, and red is dominant over green and white.

According to Raziq et al. (2012), the weight of 1000 watermelon seeds ranges from approximately 50 g to 130 g. Tibor (1993) investigated a cross between Long Iowa Belle and Japan 4 watermelon varieties. The mass of 25 seeds from 3 fruits each was determined. Long Iowa Belle had an average of 2.60 g, whereas Japan 4 was 1.22 g. Results showed that seeds of the F1 generation were smaller than those of Long Iowa Belle, but larger than those of Japan 4 at an average of 1.49 g. However, the F2 generation showed a dichotomy of larger and smaller seeds. Those with larger seeds were fewer than those with smaller seeds. It was concluded that there is a major gene that is responsible for smaller seed size as opposed to larger seed size (Tibor, 1993).

Watermelon seeds of a mature fruit make about 2.6% of the total weight (Arujo et al., 2018). Depending on the size of the fruit, watermelons on average produce few hundreds (approximately 500) seeds (Cerqueira-Silva et al., 2014; Alan, 2018). Nonetheless, different cultivars/varieties produce varying number of seeds. Although fruit size of other watermelons is small, they might produce more seeds per fruit than larger watermelons (Muhammad et al., 2020).

Watermelons are still grown in tonnes in countries of Africa and the Middle East as the seeds are used for many products. Watermelon seeds are made up approximately 32% proteins, 4.4% carbohydrates, 58.2% fat, 8.19% fibre and 6.2% ash (Mirzabe et al, 2017). Watermelon seed proteins contain important amino acids like: lysine, tryptophan, leucine, isoleucine, valine and glutamic acid. They also contain arginine, which is essential and used for regulation of blood pressure (Paksoy and Aydin, 2004). Furthermore, there is an adequate amount of vitamin B found in watermelon seeds. In addition, essential mineral elements like iron, magnesium, calcium and zinc are common (Figueiredo et al., 2011). The seeds are reported to have diuretic impacts, enhancing the functioning of the kidney and bladder (Davis et al., 2008).

2.2.2 Flowering and pollination in watermelons

Watermelon plants are cross-pollinated crops being either monoecious or andromonoecious (Karaca et al., 2012; Campbell, 2019). Monoecious plants are those plants with both female and male reproductive parts on the same plant, whereas andromonoecious plants are plants producing male flowers and perfect flowers on the same plant. They produce yellow flowers with approximately 2 cm in diameter and consists of about 4 stamens with hairy ovary (Dittmar et al., 2010).

Male flowers open first about eight days before female flowers (Emuh and Ojeifo, 2012). Each node produces one flower, with many male flowers and few female ones which are only produced at the 7th or 8th node. The male flower is produced just below the female flower that is noticed by a bulge that looks like a tiny immature watermelon (McGregor and Waters, 2014; Kyriacou et al., 2018). That bulge is an ovary which will develop into a watermelon after the pollination process occurred.

Watermelon pollination is naturally done effectively by honeybees followed by bumblebees. Bee pollination is the most effective mode compared to hand pollination (Campbell, 2018). If pollination does not take place, the flower withers and dies.

Pollination is considered a success when the ovary grows to a golf-ball size. Usually, it takes approximately 50 days after flower appearances to a mature fruit (Pisanty et al., 2016).

2.2.3 Plant breeding

Plant breeding is a well-known practise of identifying two parents with desired characters and crossbreed with the aim of producing improved offspring(s) (Haun et al., 2011). It has been practised for over 12000 years (Kumar et al., 2020). Plant breeding is practised by farmers, professional plant breeders, government and private institutions as well as research facilities (Peng et al., 2000; Thompson et al., 2019).

It was estimated that food production should increase by about 70% by the year 2050 in order to meet the expected demand for food (Arif and Rawaf, 2019). However, the arable land is continuously decreasing (Fischer et al., 2019). This makes it difficult to plant more crops to reach the expected amount. In order to overcome these problems the world is facing such as less available land for agriculture, climate change and population growth, there should be a modification of available crops (Awada et al., 2018). Gary et al. (2017) describes plant breeding as key in adapting crops for climate change. Cross breeding plants of different varieties often results in heterosis where the offspring has superior qualities to parental lines (Mirou et al., 2019; Pieruschko and Schurr, 2019).

2.2.3.1 Heterosis

Heterosis is a phenomenon used to describe the improved functioning of offspring compared to their crossed parents (Kumar et al., 2020). It leads to a situation whereby a hybrid exhibit improved traits like yield, size (Mirou et al., 2019). Fasoula et al. (2014), reported that offspring of crossed tobacco plants showed superior characters over those of their parents in terms of height. Heterosis has had impacts on crop plant improvement and on agriculture as a whole (Schnable and Springer, 2013). The phenomenon has a record of successful results in several crops like maize (Duvick, 2001), wheat (Qi et al., 2012) and tomatoes (Krieger et al., 2010).

Heterosis is multiplex and multigenic trait in plants. This is because it includes changes in a number of quantitative traits (Awada et al., 2018). Heterosis is phenomenon that involves increasing the value of a trait compared to those of parents and/or decreasing the trait value (Kumar et al., 2020; Pieruschka and Schurr, 2019). Either way, heterosis is achieved if the offspring is considered better than their parents.

2.2.3.2 Watermelon conventional breeding

Conventional plant breeding is the traditional procedure of developing new plant cultivars using natural methods like cross-pollination (Jain and Kharkwal, 2004). Many researchers and plant breeders discovered that the best time to effectively hand pollinate watermelon plants and related species is between 6am and 9amm (Sugiyama and Morishita, 2000; Maragal et al., 2019). Bees are reported to be the number one watermelon flowers pollinator, therefore, hand pollinating early in the morning protect flowers from bees pollination as bees are not active in early hours of the day. In addition, a step of covering the flowers a day before and immediately after hand pollination is also important to prevent bee pollination and contamination (Wechter et al., 2008). Flowers that are ready to open the next day are noticed by having yellowish colour on petals and will be one or two nodes above opened flowers (Schayama and Springer, 2013; Fallik and Ziv, 2020). It is advised to conduct hand pollination during sunny days as rainy and cloudy weather makes it difficult for pollen to shed freely. According to Guner and Wehner (2004), the female flowers that are not forming part of the controlled pollination should be removed as when they set fruits, they prevent the setting of other fruits in the same plant.

2.3 Materials and Methods

2.3.1 Plant material

Bitter and Weedy watermelons seeds were provided by the University of Limpopo, Department of Biodiversity. Ten seeds per variety were planted in separate row at the open nursery gardens of the University of Limpopo. The planted seeds were water three times a week before and after germination.

2.3.2 Cross-pollination

When plants had grown and were flowering, the flowers were cross-pollinated. Both Weedy and Bitter watermelons served as male and female parent. This means that anthers of one variety will serve as a male parent and be transferred to female flower of the other variety.

Crosses: Weedy (female) x Bitter (male).
Weedy (male) X Bitter (female).

Flowers in bud stage wherein antheses has not occurred were selected. The buds were carefully opened with hands. Fine forceps were used remove anthers from bisexual flowers between 6:00 and 6:30 in the morning. Emasculated flowers and male flowers in bud were covered with organza cloth bags. This was done to prevent pollination by bees. Bagged flowers were tagged with masking tape and labelled with permanent marker to identify pollen and ovule sources. They were tagged on stems just about 2 cm from the bagged sources. Anthers from open bagged male flowers were dusted onto emasculated bagged flowers, to deposit pollen grains, according to which variety was serving as female or male parent. Hand pollinated flowers were immediately covered with the organza cloth to prevent further pollination by bees and labelled.

2.3.3 Collection of Cross seeds

Three mature Cross fruits were harvested, cut open and seeds collected. The collected seeds were dried at ambient temperature of about 25 °C in the laboratory for 7 days.

2.3.4 Collection of F1 seeds

Dried Cross seeds mentioned in section 2.3.3 above were planted in the nursery – ten seeds from each fruit. When the plants were mature, the flowers were self-pollinated. This was done by allowing bisexual flowers pollinate themselves. Care was taken to avoid pollination by bees by covering flowers with organza cloth as stated above. Mature fruits were harvested, cut open to collect seeds and seeds dried at ambient temperature of about 25 °C. Seeds from different fruits were kept separate.

2.3.5 Determination of seeds yield

Dry seeds from section 2.3.3 and 2.3.4 above were further dried overnight at 60ºC in an oven. Total seeds per fruit were counted, and the mass of 100 dried seeds were determined in triplicates. The length, width and thickness of dried seeds were measured using a vernier caliper in triplicates.

The geometric mean diameter (Dp) was determined using: $Dp = (LWT)^{1/3}$

Whereby L is the length of the seed, T is its thickness and W is the width (Lorestani et al., 2012).

2.3.6 Data collection and analysis

All experimental parameters were conducted in triplicates and data presented as mean values. The average number of seeds, average mass of 100 oven-dried seeds, average geometric diameter and their standard deviations were analysed with Microsoft Excel. Analysis of variance (ANOVA) was used to check if there was any significant difference amongst all four watermelon types at 95% confidence level. Thereafter, means were separated by student t-test to indicate where the differences are.

2.4 Results and Discussion

The main aim of this chapter was to investigate the improvement of seed production and seed quality of indigenous watermelons by cross pollinating the Bitter and Weedy watermelons followed by comparing the parental lines to Cross (fruits resulting from the cross pollination) and the first generation (F1).These aspects were investigated by considering the major parameters of seeds such as total number of seeds per fruit, mass of 100 dry seeds, colour and geometric properties (thickness, length and width). Figure 2.1 shows watermelon fruits which were used in the current study. Cross pollination where the Bitter served as the female parent ovary contributor failed. The ovaries withered and did not develop into fruits.

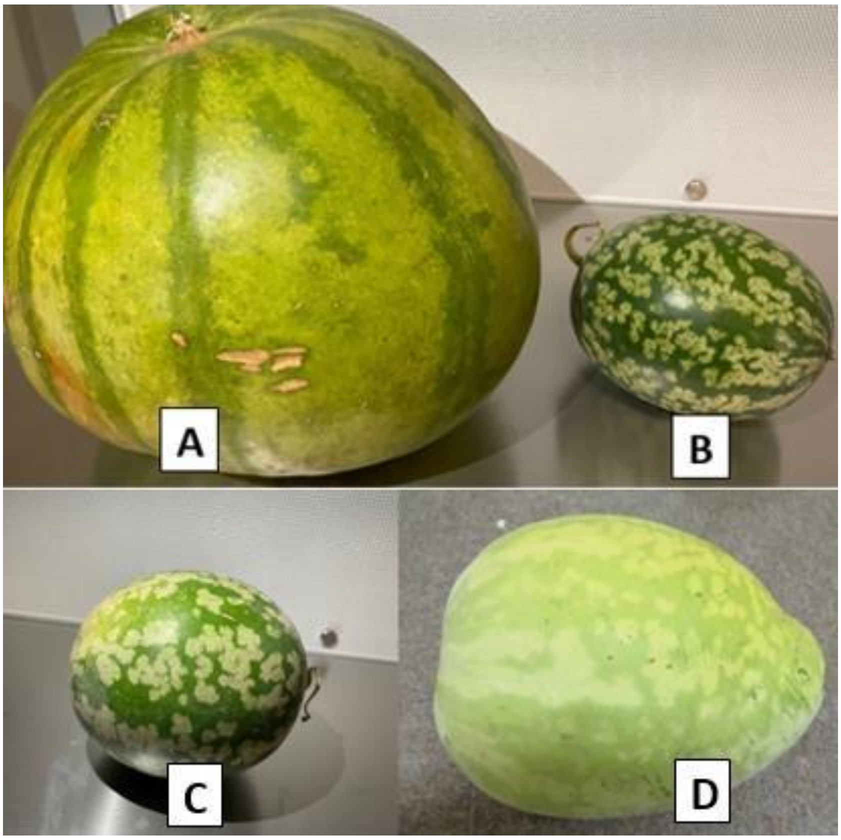

Figure 2.1: Watermelon fruits used in the current study. **A**= Bitter, **B**= Weedy, **C**= Cross watermelon and **D**= F1 fruit.

2.4.1 Seed colour

After cut-opening the fruits, seed colour variations amongst the different varieties (Figure 2.2), was the first parameter that confirmed that cross fertilisation has occurred. The seed colour of the Bitter and Weedy watermelons (parents) were red and dark brown with black speckles respectively. All three cross seeds [X(1), X(2) and X(3)] were of the same colour - brown with black speckles while the F1 generation showed some variations in colour as follows. F1(2) having a black colour and the other two being brown with black speckles [F1(1)] and light brown with black speckles [F1(3)] as shown in Table 2.1 and Figure 2.2. These results emphasise the dominance of the brown colour as stated by (Bande, 2012). The brown colour from the maternal parent did not completely disappear as did the red colour from the paternal parent. These results support those obtained by Bande (2012), on three watermelon varieties, where it was concluded that darker colours (brown and black) of watermelon seed coat are dominant and that red is recessive. Altuntus (2008) reported on the inheritance of the shape, colour and size of Schrad watermelon seeds. The data was recorded on selected parents, F1, F2 and the backcross offspring. It was reported that the appearance of black colour as spackles and on the peripheral parts of the seed coat is a dominant trait (Altuntus, 2008).

Table 2.1: Seed coat colour of watermelon seeds.

	Watermelons	Colour
Parents (P)	Bitter	Red
	Weedy	Dark brown with black speckles
Cross seeds (X)	X(1)	Brown with black speckles
	X(2)	Brown with black speckles
	X(3)	Brown with black speckles
First generation (F1)	F1(1)	Brown with black speckles
	F1(2)	Black
	F1(3)	Light Brown with black speckles

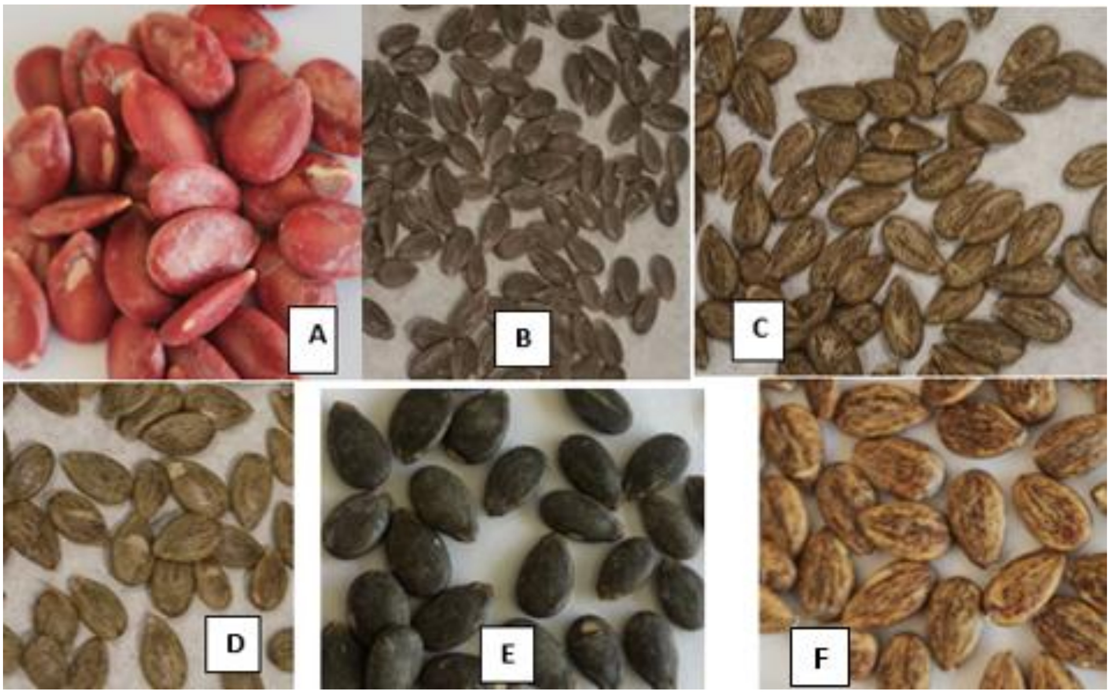

Figure 2.2: Examples of colour variations of watermelon seed coats. **A**= Bitter parent, **B**= Weedy parent, **C**= Cross seeds, **D**= F1(1), **E**= F1(2) and **F**= F1(3).

2.4.2 Seed number and seed mass

Table 2.2 shows the total number, total mass per unit fruit and mass of 100 dry seeds. Of the two parents, the Weedy watermelon had the higher number of seeds per unit fruit (892) and their mass was low, 5.69 g per 100 seeds, against 572 and 11.25 g of the Bitter, respectively. Seeds from the Cross watermelons came second after Weedy watermelon in terms of total seed number at 717, although with the lower mass of 100 seeds (6.56 g). The highest mass of 100 seeds were recorded from the F1 generation seeds averaging 13.51 g.

Table 2.2: Averages of total number of seeds and mass of 100 watermelon seeds.

Parameter	**Watermelons**			
	Bitter	Weedy	Cross	F1
Seed No.	572 ± 86.24^{a}	892 ± 97.33^{b}	717 ± 68.51^{c}	671 ± 120.21^{d}
Mass of 100 seeds (g)	11.25 ± 0.08^{a}	5.69 ± 0.01^{b}	6.56 ± 0.15^{c}	13.51 ± 0.26^{d}

Mean ± standard deviation. Values with different letters in the same row are significantly different at 95% confidence level.

On average, the Cross seeds records the highest total number of seeds. However, their offspring F1 produced an intermediate characters of both paternal (higher mass of 100 seeds) and maternal (reasonable total number of seeds, increased compared to paternal parent).

These results agree with those from a number of studies by various researchers, with regards to seed number - average number of seeds within the reported range of 400 and 900 (Seidu and Otutu, 2016), as well as the intermediate characters of F1 generation (Fila, 2013; Maragal et al., 2019).

However, the obtained results differ from those obtained by Lingli (2009), where there was a cross of lighter seeds (4.88 g for 100 seeds) and heavier seeds (10.40 g for 100 seeds). The F1 and Backcross showed to consist of lighter average weight seeds, 5.23 g and 4.72 g respectively. However, in the current study backcrosses were not performed.

2.4.3 Geometric properties

The geometric properties of Bitter, Weedy, Cross and F1 generation seeds are represented in Table 2.3. Comparing the parental lines, the highest value for thickness was recorded from the Weedy, 2.65 mm thickness. Bitter parents were the longest, 13.63 mm and the widest at 9.11 mm. The Cross seeds showed no significance difference ($p \geq 0.05$) to the Weedy parent in terms of thickness and width. The F1 generation plants produced seeds that are larger than Cross seeds in all aspects of geometric properties (length at 13.50 mm, width at 9.66 mm and thickness at 2.91

mm). Moreover, F1 generation seeds were significantly different to all other watermelon varieties of the current study in terms of seed length.

The obtained results are in agreement with those reported about watermelon seed length ranges of 5.5-15.5 mm (Ahmad, 2013; Patel and Rauf, 2017), 4.0-9.0 mm width (with the exception of Bitter variety and F1 generation seeds which are longer) (Uphoff et al., 2015). However, the reported thickness data is higher than that reported by Razavi and Milani (2006) on Sarakhsi variety and Kolaleh variety with thickness values of 1.98 mm and 1.56 mm respectively.

The geometric mean diameter is the summary of the length, width and thickness, measuring the overall size of the seed (Aydin, 2003). Data on geometric mean diameter is presented in Table 2.3. Of the parents, Bitter produced larger seeds than Weedy at 6.27 mm and 4.92 mm, respectively. According to presented data, Weedy produced the smallest seeds than all other seeds. The highest value of geometric mean diameter was recorded from seeds of F1 generation (6.91 mm). All the values of Dp reported in the current study are higher than those reported by Egbuonu (2015) on Farrasha watermelon variety which showed to be smaller with geometric mean diameter of 3.29 mm.

Table 2.3: Averages of seed geometric properties of the watermelon types.

Parameter (mm)	**Watermelons**			
	Bitter	Weedy	Cross	F1
Length	13.63 ± 0.63^{a}	9.04 ± 0.67^{b}	10.36 ± 0.30^{c}	13.50 ± 1.20^{a}
Width	9.11 ± 0.54^{a}	4.98 ± 0.09^{b}	5.41 ± 0.33^{b}	9.66 ± 0.43^{c}
Thickness	2.61 ± 0.36^{a}	2.65 ± 0.08^{a}	2.61 ± 0.13^{a}	2.91 ± 0.30^{b}
Geometric-mean diameter	6.27 ± 3.62^{a}	4.92 ± 1.92^{b}	5.27 ± 0.29^{b}	6.91 ± 2.03^{a}

Mean ± standard deviation. Values with different letters in the same row are significantly different at 95% confidence level.

2.5. Conclusion

Bande et al. (2012) reported that the family Cucurbitaceae shows a great diversification in terms of genetic make-up, physical, reproductive and vegetative properties. In this study, it was observed that indeed there is a diversity amongst different watermelon varieties with regards to seed physical properties.

The main aim of the current chapter was successful. Cross seeds showed to have intermediate improvement from parental lines of Weedy and Bitter in terms of all parameters of the current study. It may be presumed from the results that crossbreeding of the above-mentioned watermelons produced improved offspring which in turn still performed better after self-pollination. There was a significant difference ($p \leq 0.05$) observed among watermelons varieties with all parameters studied in the current study. F1 generation seeds showed an enormous improvement on the mass of 100 seeds and the geometric mean diameter compared to all other seeds of other generations.

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CHAPTER 3

EXTRACTION AND ANALYSIS OF WATERMELON SEED OIL

3.1 Introduction

Watermelon seeds are reported to be highly nutritious and well comparable to those of valued crops like: soybean, ground nuts and sunflower (Adejamo et al., 2015; Egbuonu et al., 2015). Roasted seeds with added salt are consumed as a snack (Akubugwo and Ugbogu, 2007). Another use of watermelon seeds is for oil extraction (Adeyanju et al., 2016). The practise has been going on for more than 100 years and it is still important in West African counties (Kumar, 2016).

The seed oil is used as food, feed and for many industrial purposes (Idouraine et al., 1996; Kumar, 2016). There are many plants that are rich in oils across the world. However, there are only few that are widely recognised and used industrially like: soybean, palm, rapeseed and sunflower (Stevenson et al., 2007; Zahir et al., 2017). Unfortunately, these sources of oil are inadequate to match the high demand by market for both industrial and domestic use. (Mengiste et al., 2018). Therefore, a need to search for new sources of oil has gained much interest and remains the centre of attention.

According to Hasan et al. (2016), some species of the Cucurbitaceae family can serve as a source of edible oil and mitigate the high demand challenge. Watermelon seeds are reported to contain about 30% oil (Zahra, 2010; Mogotlane et al., 2018). According to Costa de Conto et al. (2011), the oil extracted from watermelon seeds is pale yellow commonly known as Kalahari/ Ootanga. Oil from watermelon seeds is rich in unsaturated fatty acids (approximately 80%) predominated by linoleic acid (about 70%) (Neuza et al., 2015). The oil is used for various essential purposes like medicine (example, against urinary bladder), cooking and frying (Atei et al., 2019). The use of seed oils for various purposes depends on their yield, physical and chemical properties (Chen, 2020). In addition, Muhammed and Jorf-Thomas (2003) reported that the utilisation of oil is determined by its composition and no oil can be used for all purposes. Therefore, there is a need to extract and to characterise the oil contained in watermelon seeds.

3.2 Literature Review

Oil is commonly known to be a substance that is flammable, non-polar, hydrophobic (cannot be mixed with water) and lipophilic (can be mixed with other oils or lipids) (Adeyanju et al., 2016). There is a high amount of carbon and hydrogen elements making up the oil. Most oils are liquid at room temperature. The common elucidation of oil include chemical composition, its properties and utilisation (Kaur et al., 2016). Origins of oil include plants, animals and petrochemicals which can either be volatile or non-volatile (Muhammed and Hamza, 2008). Petrochemicals are known as mineral oils that originated from fossils, while oil from both plants and animals is known as organic oil. As humans, we depend on oil for our everyday lives as food, medicine and source of fuel.

3.2.1 Organic oils

Organic oil is produced through the metabolic processes of various organisms like plants and animals. Organic oil is made up of fatty acids, steroids lipids (Albishri et al., 2013). Lipids are classified by their production pathways in organisms, chemical structures and their degree of solubility in water (Njuguna et al., 2014). Generally, lipids are non-polar, but phospholipids and steroids can be both polar and non-polar at different regions (Aluyo and Ori-Jesu, 2008). According to Nwosu-Obieogu (2019), organic oil can also incorporate other chemical compounds like waxes, proteins and alkaloids. Compared to other organic compounds, lipids are rich in hydrogen and carbon but low in oxygen content (Fahy et al., 2005). During the later stages of seed maturity, accumulation of oil is relatively high compared to early stages of development (De Carvalho and Caramujo, 2018). The massive increase in the demand of oil is the reason for continuous search of other alternative vegetable seeds as oil sources (Njuguna et al., 2014). Vegetable oil is used for various day and day basic needs. According to Sui et al. (2011), organic oil is usually priced based on its nutritional properties. The higher the nutrition the higher the price.

3.2.1.1 Uses of oil

Most oils that are considered edible are used for cooking, frying and preparation of food (Anwar et al., 2016). Oil is also used in some instances to add flavour and for modifying the food textures. Oil has also been used to produce cosmetics or applied raw on skin and hair to nourish, enhance and promote growth of hair (Yol et al., 2017). Van der Vossen et al. (2007) reported that oil is an excellent supporting medium and serves as one of the essential ingredients in paint productions. The non-polar character of oil makes it difficult for it to adhere to other molecules. This serves as an advantage when used as a lubrication (Akanni et al., 2005).

3.2.2 Oil extraction methods

3.2.2.1 Traditional extraction method

This is a method that is used by rural communities. It is however reported to be inadequate because it yields lower oil content than other methods (Alonge and Olaniyan, 2006; Olaniyan, 2010). Olanelo et al. (2012) reported on the traditional extraction of castor oil and mentioned essential steps namely: seed collection, removal of seed pods, seed boiling, seed grinding for formation of seed paste, addition of seed paste into boiling water for extraction of oil, removal of oil and drying with heat. Another method involves crushing seeds and mixing with water, cooking the mixture, then finally drying oil by heating (Olaniyan and Yusuf, 2012). Researchers outlined that this method is time consuming, produces low quality oil and is tiring and dull.

3.2.2.2 Conventional methods

There are two widely used methods of conventional oil extraction, namely mechanical and solvent extraction (Ezeohal et al., 2017). Since the discovery of these extraction methods, most seed oils are extracted by either one method or both. Either way, the final product should be assessed for its physical and chemical properties to ascertain its uses (Favero et al., 2019).

i. Solvent extraction

This method of extraction is the most widely used, considered the most efficient in the extraction of oil and produces the least amount of oil residues (Tayole et al., 2011). Usually, solvents that are used include hexane, ethanol, diethyl ether and petroleum ether (Duta et al., 2015). The study by Bnuija et al. (2015) on the oil extraction methods from oil-nut (*Calophyllum innophylum*), showed that the solvent extraction method is the most effective one and produces optimum oil content compared to mechanical extraction. Muzenda et al. (2012) reported that the effectiveness of solvent extraction depends upon the extraction period and on solvent-solute ratio. The longer the extraction time, the higher the yield and the best ratio of solvent-solute is 6:1. Above all, the solvent extraction method is widely used because of its incomparable advantages. In addition to the abovementioned advantages, Ikya et al. (2013) reported that the solvent extraction method is suitable because it is repeatable and reproducible. Solvent extraction has its disadvantages like other methods do. These include time consumption (long extraction period), cost and energy demand (Dawidowicz et al., 2008; Takadas and Doker, 2017).

ii. Mechanical expression

Mechanical expression method includes the use of pressure by hydraulic presses or screws to enforce oil to come out of oilseeds (Arisana, 2013). Therefore, oil yield depends on the pressure exerted on the oilseed (Mwithinga and Moriasi, 2007). Oyinlola et al. (2004) reported that screw presses yield more oil than hydraulic presses and are the most preferred. The mechanical method is widely used to extract oil from seeds that produce above 20% oil (Sinha et al., 2005). This method however, leaves high amount of oil residues on the cake after extraction (Anderson, 1996). In a study by Ogunniyi (2006), it was concluded that this method is also time consuming and high energy demanding.

3.2.3 Watermelon seed oil

3.2.3.1 Physical and chemical properties

There are variations in the physiochemical properties of watermelon seed oil reported. These variations are caused by factors like genetic makeup, soil type and climatic conditions. The physical colour of watermelon seed oil ranges from pale yellow, golden yellow and orange and it is rich in important fatty acids like oleic, linoleic and stearic acids (Costa de Conto et al., 2011; Gladvin et al., 2016).

Saponification value is a measure of the fatty acids' molecular weight presented as triglycerides (Hiba et al., 2015). Watermelon seed oil is reported to have saponification value that reach approximately 202.5 mgKOH/g (Muhammad et al., 2015; Egbuonu et al., 2015) and about 175.2 mgNaOH/g (Ulfa, 2019). Iodine value is defined as the mass (grams) of iodine depleted by a chemical substance (100 g) (Mengiste et al., 2018). Moreover, Neuza et al. (2015) reported that watermelon seed oil has an iodine value of 128.8 g/100g, giving it a potential for other industrial productions. Watermelon seed oil is reported to have lower peroxide value, averaging at 2.20 meq/kg (Egbuonu et al., 2015). This gives then oil an advantage as lower peroxide value means lower rate of rancidity (Anyasor et al., 2009). Refractive index is a value used to describe how oil react and bend the light. It measures how light is modified when passing across oil (Anhwange et al., 2010). The aim is to have a refractive index closer to that of glass (1.49-1.52) to reduce boundary refraction. Therefore, watermelon seed oil have values closer to that of glass at 1.476 (40 ˚C) (Van der Vossen, 2007). Specific gravity of oil is the ratio of density of oil to that of water at a given temperature (Hernandez and Kamal-Eldin, 2013). It is used to check the purity of oil and to test if oil will float or sink in water. Since the specific gravity of water is reported to be at 1 (25 ˚C), it means watermelon oil with specific gravity of 0.87 (25 ˚C) when of high purity will float in water (Aluyo and Ori-Jesu, 2008).

There is a high amount of γ-tocopherol (70.66 mg/ 100 g) and ἀ-tocopherol (25.88 mg/ 100 g) in watermelon oil (Nyam et al., 2009). These tocopherols give the oil a good shelf life and antioxidant properties. Phytosterols like sitosterol, campesterol and stigmasterol are present in watermelon seed oil, with sitosterol in higher quantities (485.52 mg/ 100 g) (Hiba et al., 2015). There are various essential phenolic acids present in watermelon seed oil including, protocatechuic, gallic, caffeic, p-hydroxybenzoic, p-coumaric, syringic and ferulic acids (Nyam et al., 2009). According to Zaharaddeen et al. (2014), there is a low proportion of trace elements in watermelon seed oil like copper, cobalt, zinc, calcium and manganese. However, some of the trace elements have to be removed during processing as they are pro-oxidant (Sabahel et al., 2011).

3.2.3.2 Fatty acid composition

Seed oils contain various fatty acids and in different amounts (Abdulkar et al., 2014). There are two main groups of fatty acids, namely: saturated fatty acids and unsaturated fatty acids (Meer et al., 2008; De Carvalho and Caramujo, 2018). Saturated fats are fats with the chemical structure that is composed of higher number of hydrogen elements without double bonds (examples: stearic, palmitic). Unsaturated fats are composed of one or many double bonds (examples: oleic and linoleic acid). Human skin naturally produces lots of these unsaturated fats including oleic, stearic and palmitic acids. However, it does not produce linoleic acid. Linoleic acid is required for a heath human skin and its deficiency results in dry skin and loss of skin hair

(Mengiste et al., 2018). Watermelon seed oil is rich in linoleic acid (about 70%), with smaller quantities of oleic (about 16%), palmitic (about 14%) and stearic (about 12%) (Neuza et al., 2015). Linoleic acid is widely used in cosmetics as it moisturises the skin and is used to treat Acne vulgaris (Lautenschlager, 2003).

3.2.3.3 Uses of watermelon seed oil

Oti-Wilberforce and Eze-Ilochi (2017) reported that *C. lanatus* seeds are rich in oil and proteins, making them both nutritional and oil-products production values. Uses of watermelon seed oil differ with countries. Watermelon seed oil is used mostly in African countries for frying and cooking (Duduyemi et al., 2013; Tabiri et al., 2016). Watermelon seed oil has been used to care for the skin and keep it healthy without cracks. Traditionally, watermelon seed oil has been used to produce soap commonly in Namibia, and as a medicine for herpes lesions and sores in Central America (Van der Vossen et al., 2004), as well as to treat fever and urinary diseases (Cho et al., 2004). It also prevents the formation and development of kidney stones and eliminates the development of non-correctable changes in kidneys (Acer et al., 2012) and induces faster recovery from wounds and burns (Cho et al., 2004). Moreover, according to Edidong et al. (2013) the watermelon seed oil improves metabolism and prevents cancer. One study shows that it is the accelerator of growth and the promoter of healthy hair, nails and muscles (Stevenson et al., 2007).

3.3 Materials and Methods

3.3.1 Plant material

Watermelon fruits from parental lines (Weedy and Bitter), Cross and F1 were cut into small pieces. The seeds from the flesh were collected and put in a beaker and washed in running tap water. They were placed on a paper towel to dry in the laboratory at ambient temperature for seven days. The seeds were further dried in an oven at 60 °C for 24 hours.

3.3.2 Oil extraction

The dry watermelon seeds were ground into finer particles using a pestle and mortar. Six samples of 10 g ground seeds from each watermelon type were weighed into

Soxhlet thimbles for oil extraction. N-hexane was used as solvent. The Soxhlet extraction was left running for 24 hours.

3.3.3 Determination of oil yield

The oil extracts were filtered into the pre-weighed beakers through Whatman No.1 filter papers. The extracts were left to dry under a draught of air in the fume hood overnight. Thereafter, the oil samples were further dried at 60 °C for another 24 hours in an oven. Finally, the beakers were weighed to determine the mass of extracted oil.

Percentage oil yield was determined using the following equation:

$$\% \text{ Oil yield} = 100 * \frac{m1}{m2}$$

Where m_1 = mass of oil and m_2 = mass of seeds.

3.3.4 Determination of physicochemical properties of oil

All samples were in triplicates.

3.3.4.1 Moisture content

The moisture content of oil was determined using the method outlined by Nzelu et al. (2012). Oil samples of 2 g were poured into dry pre-weighed glass petri dishes and incubated in an oven for 5 hours at 105 °C. Thereafter, samples were cooled down for one hour in a desiccator. After cooling down, the Petri dish with the dried oil were weighed. The moisture content was calculated using the equation:

$$\% \text{ Moisture} = \frac{W1-W2}{W1} * 100$$

Where W_1 = weight of fresh oil and W_2 = weight of dry oil.

3.3.4.2 Oil density

Oil density of each watermelon type was determined. Aliquots of 2.0 ml were poured into small measuring cylinders on an electronic balance. Mass of oil was recorded from the electronic balance.

Oil density was calculated using the formula: density $\frac{g}{ml}$ = mass of oil/ volume of oil.

3.3.4.3 Specific gravity

The specific gravity of oil samples was determined at room temperature (25 °C) according to the following equation:

Specific gravity $= d0/d1$

Whereby d_0= density of oil and d_1= density of water.

3.3.4.4 Saponification value

A 5 g mass of oil sample was poured into a round bottom flask with a pipette. Then 50 ml of 0.5 M ethanolic KOH was added into the sample. The mixture was boiled under reflux for one hour. Aliquots of 12.5 ml were titrated with 0.5 M HCl with phenolphthalein as indicator. The saponification value was calculated using the following equation (AOCS, 2005):

S.V= $\frac{56.1(V2-V1)}{W}$

Where S.V is saponification value, V_2 is volume of hydrochloric acid used for blank, V_1 is the volume of HCl used for the sample and W is the weight of sample oil.

3.3.4.5 Iodine value

Samples of 0.1 g oil were each weighed into conical flasks. A 10.0 ml volume of Wijs solution together with 10.0 ml of carbon tetrachloride were added. The mixtures were incubated in the dark for 30 minutes. Then the mixtures were titrated with 0.1 M sodium thiosulphate to a pale yellow colour. A 1.0 ml volume of starch indicator was added to the solution, then titrated further with vigorous swirling until the blue colour changes to pale yellow (Adedej, 2018). Iodine value was calculated using the equation:

Iodine value = $\frac{0.01269(V2-V1)*100}{M}$

Where V_2 is the thiosulphate volume used in the sample, V_1 is the thiosulphate volume used in blank, M is the mass of oil sample and 0.01269 is the constant conversion of iodine/thiosulphate.

3.3.5 Data analysis

All experimental parameters were conducted in triplicates and data presented as mean values. The data were analysed with Microsoft Excel. Analysis of variance (ANOVA) was used to check if there was any significant difference amongst all four watermelon types at 95% confidence level. Thereafter, means were separated by student t-test to indicate where the differences are.

3.4 Results and Discussion

3.4.1 Oil extraction

Oil was successfully extracted with N-hexane and was in liquid state at room temperature. The oil colour varied from golden yellow (F1 seeds) to pale yellow in the Bitter, Weedy and Cross watermelons as shown in Table 3.1. This is in agreement with the study by Nielsen (2003) where watermelon seed oil colour ranged from light-yellow and, orange yellow to orange. It was suggested that this could be due to the amounts of fat-soluble pigments leaching into the oil.

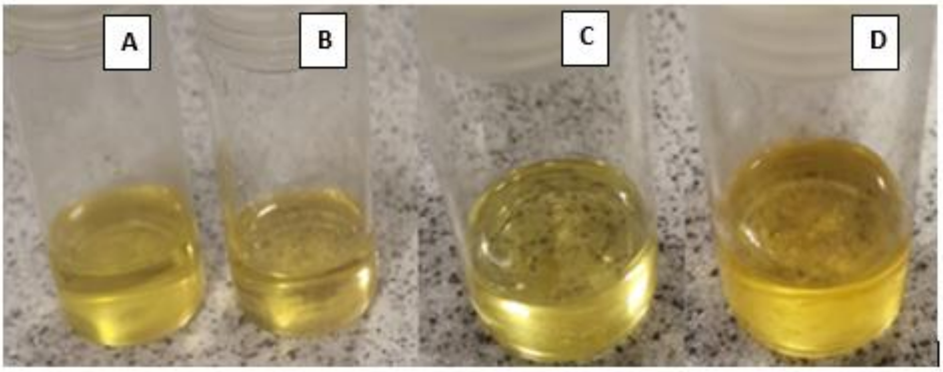

Figure 3.1: Extracted oil from watermelon seeds showing a variety of colours. A= Bitter, B= Weedy, C=Cross and D= F1 generation. Cross and F1 generation oil had golden yellow colour.

3.4.2 Percentage oil yield

The percentages of oil extracted from Bitter, Weedy, Cross and F1 seeds are shown in Table 3.1. There was a higher oil extraction yield from the Bitter watermelon seeds (31.21%) compared to Weedy seeds (28.81%). Results are within the range of averages reported on bitter cucumber (*Citrillus colocynthis)* (Mariod et al., 2009) and melon seed oils (Okapla, 2021) which were at 27.2-31.4% and 29.6-33.1% respectively. The oil content from Cross seeds was higher than that of F1 seeds at 39.15% and 33.3% respectively. However, there was no statistical difference between oil percentage of Bitter and F1 generation seed oil.

Table 3.1: Results on oil percentage and oil colour of watermelon varieties.

Watermelons	**Percentage oil (%)**	**Oil colour**
Bitter	31.21 ± 0.77^{a}	Pale yellow
Weedy	28.81 ± 0.63^{b}	Pale yellow
Cross	39.15 ± 1.10^{c}	Pale yellow
F1	33.30 ± 3.19^{a}	Golden yellow

Mean ± standard deviation. Values with different letters are significantly different at 95% confidence level.

The lowest amount of percentage oil was observed from seeds of Weedy variety (28.81%) with Cross seeds being the highest (39.15%). The oil percentage of all watermelons obtained in this study is higher compared to other known seed oils such as soybean at 16-28% (Bell and Gillatt., 1994), rubber seeds at 20-25% (Kaur et al., 2016) and cotton seeds (17-28%) (Aremu et al., 2015). However, the oil yield from Bitter, Weedy and F1 seeds of the current study is lower than sunflower seed oil which was reported to be ranging from 38-47.5% (Chiplunkar and Pratap, 2016; Flagella et al., 2002). Therefore, Cross seeds produce oil that compares well with sunflower oil in terms of oil content (39.15%) as its value falls within the reported range of sunflower oil.

3.4.3 Moisture content

Moisture content is considered an essential parameter for food industry and shelf life because water affects the refining, processing and storage stability of oil (Njuguna et al., 2014). When oil has higher moisture content of above 7.2%, heat can catalyse the hydrolysis reaction which leads to free fatty acids being less stable towards auto-oxidation (De Carvalho and Caramujo, 2018). This results into oil that is off-flavour.

F1 generation seed oil was significantly different to all other watermelon seed oils of the current study. In addition, Bitter, Weedy and Cross seed oils were found to not be significantly different from one another. The lowest moisture content was obtained from Weedy watermelon oil with 2.98%, while F1 oil having the highest at 4.02% respectively (Table 3.2). These values are higher than 2.47% reported by Zahra (2010) for Honeydew watermelon seed oil and lower than 6.38% and 6.49% for cotton seed and sunflower oil, respectively (Lin et al., 2018). This means that the oils of the current study are less susceptible to spoilage by hydrolytic rancidity if stored well (Okaka, 2010). Moreover, Onwuka (2018) reported that oils with high moisture contents above 6.95% are at high risk of quality deterioration, leading to spoilage that occurs during the breakdown of fatty acids from the glycerol backbone which increases the formation of short chained fatty acids.

Table 3.2: Four watermelon types and their moisture content.

Watermelons	**Moisture content (%)**
Bitter	3.21 ± 0.12^{a}
Weedy	2.98 ± 1.03^{a}
Cross	3.20 ± 1.12^{a}
F1	4.02 ± 0.68^{b}

Mean ± standard deviation. Values with different letters are significantly different at 95% confidence level.

3.4.4 Density and Specific gravity

Table 3.3 presents some of the physiochemical properties of the extracted watermelon oils. The specific gravity of oil is the ratio of density of the oil to that of water. Therefore, specific gravity depends on density of oil (the higher the density, the higher the specific gravity and vice versa). Specific gravity value is used to work out and determine the right and exact ingredients concentrations in an aqueous solution (Yol et al., 2017).

Although there is no significant difference ($p \geq 0.143$) amongst all four watermelons, the density values were not equal. The density of oils ranged from 0.887 g/cm^3 (Bitter) to 0.904 g/cm^3 (Weedy). The range is in agreement with what was reported by Paunovic et al. (2020) on watermelon seed oil which ranged from 0.87-0.921 g/cm^3.

Of the parents, an oil sample of Weedy had a higher specific gravity (0.911 g/cm^3) at room temperature than that of Bitter watermelon (0.896 g/cm^3). Comparing oil samples from Cross seeds with F1 seeds, the highest specific gravity was obtained from Cross seed oil (0.909 g/cm^3). The specific gravity of the Weedy watermelon and Cross seed oil are well comparable with that of 0.908-0.920 g/cm^3 and 0.907-0.919 g/cm^3 for olive and rapeseed respectively (Nichols and Sanderson, 2013).

Moreover, the values obtained in the current study fall within the range reported on watermelon seed oil (0.85-0.917 g/cm^3) (Agbara et al., 2019), palm oil (0.907 g/cm^3) (Anhwange et al., 2010), pumpkin seed oil (0.902-0.910) (Christian, 2006). However, these ranges are significantly lower than values (0.932 g/cm^3) for Shea seed oil and 0.914-0.920 g/cm^3 for canola oils (Nichols and Sanderson, 2003).

Table 3.3: Density and specific gravity values of watermelon types.

Watermelons	**Parameters**	
	Density (g/cm^3)	Specific gravity (25 ˚C)
Bitter	0.887 ± 0.014	0.896 ± 0.004^a
Weedy	0.904 ± 0.010	0.911 ± 0.022^b
Cross	0.902 ± 0.003	0.909 ± 0.043^b
F1	0.892 ± 0.012	0.899 ± 0.052^{ab}

Mean ± standard deviation. Values with different letters are significantly different at 95% confidence level.

3.4.5 Saponification value

Ardabili et al. (2011) reported that the saponification value gives information on the mean molecular weight and lipid chain length. The means for saponification values of oil from Bitter, Weedy, Cross seeds and F1 seeds are shown in Table 3.4. Comparing all watermelon types, oil of Cross seeds recorded the highest values at 118.67 mg

KOH/g oil, followed by oil of F1 generation seeds (99.82 mg KOH/g oil) and the lowest saponification value was observed from oil of Bitter watermelon

The results of the current study are lower than the range of results reported by Mogotlane et al. (2018) which were 133.44-184.57 mg KOH/g on Sweet watermelon oil while Anhwange et al. (2010) reported 185.0-196.1 mg KOH/g on olive oil. This could be because of the existence of longer fatty acids in the seed oils of the current study, which may lead to larger molecular weight than other common oils (Alajtal et al., 2018; Nora et al., 2018). However, the current values are higher than saponification value of beeswax (92-94 mgKOH/g), which is known to be used in soap production (Mabrouk, 2005). This shows that watermelon oils of current study could possibly be used in soap production.

Table 3.4: Four watermelon types representing their saponification values.

Watermelons	Saponification value (mg KOH/g oil)
Bitter	97.19 ± 1.28^a
Weedy	97.75 ± 0.65^a
Cross	118.67 ± 3.60^b
F1	99.82 ± 1.12^a

Mean ± standard deviation. Values with different letters are significantly different at 95% confidence level.

3.4.6 Iodine value

Iodine value is used to measure the unsaturation relative degree in fats and oil. Moreover, it gives information on the estimation of melting point and oxidative stability as they are linked to the relative degree of unsaturation (Aremu, 2015).

The average iodine values of watermelon oils were investigated and presented in Table 3.4. The iodine values ranged from 98.44 to 114.21 g of I_2/100 g oil. Bitter watermelon recorded the lowest iodine value with oil from Cross seeds being the highest. There is no significant difference between iodine value of Weedy watermelon (103.42 g of I_2/100 g oil) oil and F1 generation oil (104.44 g of I_2/100 g oil). Iodine values of the current study were close to peanut oil (84-108 g of I_2/100 g oil) investigated by Alfawaz (2004) and corn oil (102-113 g of I_2/100 g oil) (Younis et al, 2000; Anhwange et al., 2010) which are used for cooking and frying. The oils of the current study are therefore, edible oils suitable for cooking.

All these values of the current study are higher than 74.5 g of I_2/100 g oil reported by Oyeleke and Olagunju (2012) on Black Diamond watermelon seed oil and 80.0 I_2/100 g oil on pumpkin seed oil indicated by Esuoso et al. (1998). They are however, lower than 119.8-125.0 I_2/100 g oil on Baby Tiger watermelon seed oil (Nyam et al., 2009).

According to Denniston et al. (2004), an iodine value that is greater than 100 I_2/100 g oil indicates a greater presence of unsaturated fatty acids which is a good factor for foaming ability. Therefore, this suggests that oil of Weedy watermelon, Cross seeds and F1 generation to have good foaming ability property. Foaming is good characteristic for applications in the development of soaps, emulsions and detergents. Moreover, unsaturated fats are healthier to consume as they aid in lowering cholesterol and improving the healthiness of a heart (Albishri, 2013), making watermelon seed oil suitable for consumption.

Table 3.5: Four watermelon types representing their iodine values.

Watermelons	**Iodine value (g of I_2/100 g oil)**
Bitter	98.44 ± 1.03^a
Weedy	103.42 ± 2.69^b
Cross	114.21 ± 1.80^c
F1	104.44 ± 3.04^b

Mean ± standard deviation. Values with different letters are significantly different at 95% confidence level.

3.5 Conclusion

The current chapter investigated the oil content together with physiochemical properties of four types of watermelon (*C. lanatus*) seed oil to determine if the oil is of enough quantity and quality and for what purposes. Cross seeds produced the highest oil percentage followed by seeds of F1 generation. However, they were both not significantly different from each other, while F1 oil was not significantly different from Bitter oil. These reasonable amounts of oil found in oil of Cross seeds and F1 seeds qualifies them to be considered as excellent sources of oil. This is plainly seen when their oil content is compared to those of other important seed oils.

Weedy watermelon on the other hand produced oil with the highest specific gravity than all other types of the current study. Moreover, it was found to be significantly different to the Bitter watermelon only. Kaur et al. (2016) reported that the suitable moisture content of oil is the lowest possible. Weedy variety had the lowest moisture content than all other watermelon types. However, it was only significantly different to F1 oil. Above all, all these values of moisture content are still suitable as they are lower than other reported important seed oils like sunflower oil. In addition, oil from Cross seeds had the highest saponification value and iodine value, while being significantly different to all other watermelon types.

From the obtained results, the physiochemical properties of the current investigated watermelons are mostly within the acceptable ranges of oils reported to be of good quality for certain industrial purposes. Moreover, it is evident that Cross seed oil was the most suitable oil than all other watermelon oils of the current study.

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CHAPTER 4

GENERAL CONCLUSION, RECOMMENDATIONS AND FUTURE RESEARCH

4.1 General conclusion

The current study was designed to produce watermelon hybrid that can produce higher amount of oil than parental lines, with better physiochemical properties of oil. For this to be possible, prerequisite objectives of crossing Bitter and Weedy watermelons and self-pollination of Cross seeds were essential. The properties of seeds of all watermelon varieties of the current study were investigated and compared.

After self-pollination, F1 generation watermelons produced seeds with the highest mass of 100 seeds and geometric mean diameter, showing to have improved seed size from Cross seeds and parental lines of Bitter and Weedy. Moreover, both Cross and F1 generation watermelons had more seeds than that of Weedy parent, but slightly lower than Bitter variety.

Seeds that can yield oil of 28% is classified as a high yielding seed oil (Paunovic, 2020). Moreover, Cross seeds and F1 generation seeds had increased oil yield to above 28% and can therefore be classified as high yielding oilseeds. In addition, percentage oil of Cross seeds is well comparable to that of sunflower as its value falls within the sunflower oil range.

Suitable moisture content, density, specific gravity, saponification values and iodine values were observed from oils of both Cross seeds and F1 generation seeds. It was concluded that Cross seeds and F1 generation seeds performed better than parental lines in terms of physiochemical properties evaluated.

Overall, crossbreeding of indigenous watermelons Weedy and Bitter produced an improved watermelon variety which still performed better after self-pollination. Moreover, it can be concluded that Cross watermelons are the most superior ones because of their adequate intermediate amount of total seeds produced, highest oil yield and better physiochemical properties of oil. On the other hand, F1 generation still performed better because of its larger seeds, high oil yield and good physiochemical properties of oil. Therefore, the oil content of watermelons was successfully increased through crossbreeding and can be utilised industrially for soap production and consumption.

4.2 Recommendations

From the results obtained in the current study, it is recommended:

1. To plant as many seeds of watermelons as possible on a larger planting area for crossbreeding.
2. That after successful crossbreeding and selfing trials, to take length, thickness and width measurements of fruits in order to have a clear understanding of the fruit size improvement of developed the hybrid(s).
3. To try to use various methods/and solvents of oil extraction. In order to know and document the suitable method and solvents to use for better oil extraction.

4.3 Future research

1. Watermelon oil is still inadequately used industrially. This can be improved by further studying more physiochemical properties such as index viscosity, flash point and fatty acid composition of the current investigated watermelons.
2. Since Sweet watermelon was reported to produce larger seeds with better physiochemical properties of its oil (Mogotlane et al., 2018), it will be better to crossbreed Cross seeds of the current study with Sweet indigenous watermelons for more superior characters.
3. A more advanced method of crossbreeding like genetic engineering will be of advantage as it might be faster to decide on which genes to be chosen.

4.4 References

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Appendices

Appendix 2.1: One-way ANOVA

	Sum of Squares	df	MS	F	P-value
TOTAL NUMBER OF SEEDS					
Between groups	183634	3	61211.33	10.78836	0.003
Within groups	45390.67	8	5673.833		
Total	229024.7	11			
MASS OF 100 SEEDS					
Between groups	126.2032	3	42.06772	18356.82	1.1E-15
Within groups	0.018333	8	0.002292		
Total	126.2215	11			
GEOMETRIC MEAN DIAMETER					
Between groups	7.503225	3	2.501075	585.0464	1.04E-09
Within groups	0.0342	8	0.004275		
Total	7.537425	11			

Appendix 3.1: One-way ANOVA on quantity and physiochemical properties of watermelon seed oil

	Sum of Squares	df	MS	F	P-value
PERCENTAGE OIL					
Between groups	177.272	3	59.090	14.996	0.001
Within groups	31.522	8	3.940		
Total	208.795	11			
MOISTURE CONTENT					
Between groups	1.947	3	0.647	169.399	1.41E-07
Within groups	0.031	8	0.004		
Total	1.972	11			
DENSITY					
Between groups	0.0005	3	0.0001	7.344	0.143
Within groups	0.0002	8	0.69E-05		
Total	0.000809	11			
SPECIFIC GRAVITY					
Between groups	0.0004	3	0.0001	65.813	0.002
Within groups	1.98E-05	8	2.47E-06		
Total	0.0005	11			
IODINE VALUE					

Between groups	391.589	3	130.53	225.055	4.16E-08
Within groups	4.639	8	0.579		
Total	396.229	11			
SAPONIFICATION VALUE					
Between groups	949.523	3	316.507	77.875	2.92E-06
Within groups	32.514	8	4.064		
Total	982.037	11			