NUTRIENT COMPOSITION, PHYTOCHEMICAL CONTENT AND ANTI-MICROBIAL ACTIVITY OF AFRICAN NIGHTSHADE (*Solanum nigrum complex*) EDIBLE BERRIES

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Nutrient Composition, Phytochemical Content and Anti-Microbial Activity of African Nightshade (*Solanum nigrum complex*) Edible Berries

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as the University supervisors.

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DEDICATION

To my beloved wife Carolyne Heka,

To my dear daughter Kelsey Leona and son Lenny Osteen,

To my loving parents, Mr. and Mrs. Joseph and Rebecca Kamau

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ABBREVIATIONS AND ACRONYMS

AILVs	African Indigenous Leafy Vegetables
AIVs	African Indigenous Vegetables
AOAC	Association of Official Analytical Chemists
CDC	Centre for Disease Control
CE	Catechin Equivalents
CRD	Completely Randomized Design
FAO	Food and Agriculture Organization
GAE	Gallic Acid Equivalents
GDP	Gross Domestic Product
GoK	Government of Kenya
HCl	Hydrochloric Acid
HHI	Hidden Hunger Index
HPLC	High Performance Liquid Chromatography
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KALRO	Kenya Agricultural and Livestock Research Organization
KDHS	Kenya Demographic and Health Survey
KNBS	Kenya National Bureau of Statistics

KFSSG	Kenya Food Security Steering Group
MIC	Minimum Inhibitory Concentration
MNM	Micro Nutrient Malnutrition
ND	Not Detected
NS	Nightshade
QE	Quercetin Equivalents
TTA	Total Titrable Acidity
UNICEF	United Nations Children's Emergency Fund
UV-VIS	Ultra Violet – Visible Spectrophotometer
VAD	Vitamin A Deficiency
WHO	World Health Organization

DEFINITION OF TERMS

African nightshade berries	Fruits produced by the African nightshade (S*olanum nigrum complex*) plant
Berry-flavoured yoghurt	Yoghurt flavoured with puree processed from the African nightshade berries

ABSTRACT

Although the rights based approach to food has been internationally recognized and incorporated into national policies as a way of curbing food and nutrition insecurity, governments have fallen short of meeting their obligations, making it a global challenge. Hidden hunger is one of the glaring challenges especially in developing countries. Dietary provision with an emphasis on utilization of highly nutritious but neglected and orphaned crops is one alternative of addressing this challenge. African nightshade *(Solanum nigrum complex)* is one of such plants and its utilization can help in alleviating the burden of hidden hunger. Only the leafy part is utilized even though the plant has edible berries. This study sought to address the underutilization of *Solanum nigrum complex* by studying the benefits that can be derived from the berries. Berries from four varieties of the plant were harvested at four stages; green, colour break, ripe and at senescence. Chemical analyses of the berries were done to determine the content and changes in macro- and micro-nutrients and the phytochemical content of the berries as they ripened. Antimicrobial activity of the berry extracts was studied and berry-flavoured yoghurt developed and its acceptability amongst consumers tested. Chemical composition of the berries was comparable to other berries with high levels of minerals, vitamin C and carotenoids. As berries ripened, oxalates, total phenols, flavonoids and phytates decreased while Vitamin C, tannins and total carotenoids increased. The berries accumulated glucose and fructose as they ripened with glucose being the most abundant sugar while sucrose recorded a sharp increase at the ripe stage. Size remained relatively constant within each variety while firmness decreased progressively after colour break. Ethanolic berry extracts exhibited antimicrobial potential against fungal and bacterial assays even though the former were more resistant. Gram positive species were the most susceptible. Minimum inhibitory concentration (MIC) of berry extracts ranged from 6.25 µg/ml to 12.5 µg/ml in bacterial assays and 100.00 µg/ml for fungal species. On a 9-point hedonic scale, berry and berry flavoured yoghurts were highly accepted by the consumers, recording acceptability scores of 7.0 and 8.3, respectively. Conclusively, the results of this study confirm that African nightshade berries are of high nutritional potential and can be included in the diet to curb hidden hunger. They can also be applied in the food processing industry as natural preservatives and as valuable ingredients in development of novel functional products with nutraceutical quality.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Food and nutrition insecurity is a serious global challenge which has constantly underpinned the shortcomings of governments to meet the international obligations to ensure there is availability of affordable, quality food to meet the highest attainable standards of health for their peoples (Ayala and Meier, 2017). This has prompted the international humanitarian organizations such as the World Health Organization (WHO) and Food and Agriculture Organization (FAO) to advocate for a human rights approach to food and nutrition security. As a result, legal mechanisms have been identified to incorporate the international human rights standards through national policies. Such policies articulate the rights-based obligations of national governments to address the challenge of food and nutrition insecurity (Ayala and Meier, 2017).

Hidden hunger or micro-nutrient malnutrition (MNM) is among the major nutritional challenges in the modern world (Allen and Benoist, 2006; Bailey, 2015). This problem is quite common in the developed nations and highly prevalent in the developing nations (Muthayya *et al*., 2013). Though it can affect people in all age groups, women of child-bearing age and young children are at a higher risk (UNICEF, 2013). The most common forms of MNM include vitamin A deficiency (VAD), iron, and iodine deficiencies. Others include zinc, calcium, vitamin D, folate and copper deficiencies among others. The deficiencies can be detected through clinical signs or through biochemical analysis (Allen and Benoist, 2006). MNM can contribute to high morbidity and mortality rates leading to high medical budget (Bailey, 2015).

Muthayya *et al*. (2013) recorded that the MNM burden has been increased by the changing economic times. As food prices rise by the day, many people move away from consuming foods that are rich in micro-nutrients. They, therefore, opt for foods that are cheaper, starchier and less nutrient dense. Ruel-Bergeron *et al*. (2015) further indicate that MNM is of concern due to the fact that it is not easy to decipher an individual suffering from it, thereby earning the term ‘hidden hunger.’ As such, an individual could be suffering from the condition yet appears healthy. This is quite unfortunate especially given that in the developing countries, where food security is often a major challenge, individuals could be suffering from more than one micro-nutrient deficiencies (UNICEF, 2013; Bailey, 2015). This has adverse effects on the individual, economic and social spheres.

Due to the gravity of the MNM burden, different alternatives have been employed in attempts to reduce the adverse effects of the same. Allen and Benoist (2006) recorded that two of the most successful strategies are supplementation and dietary supply of micro-nutrients. In supplementation, most of the micro-nutrients are formulated into tablets which are supplied to the vulnerable groups, especially pregnant women and children below five (5) years of age. Most of these are administered during pre-natal and post-natal care.

Provision of the micro-nutrients in the diets has been championed as another successful avenue of dealing with MNM (Burchi *et al*., 2011). This can be achieved through adoption of food and nutrition regimes which integrate sustainable agriculture that improves dietary diversity and livelihoods. In this regime, vegetables and legumes feature strongly as a viable option to explore. Ojiewo *et al*. (2015) concluded that to save the vulnerable populations from the threat of food insecurity, vegetables and legumes production and consumption not only promotes the economic status of the individuals, but also provides the much-needed nutrients. These include the micro-nutrients as well as phytochemicals that promote health.

African Indigenous Vegetables (AIVs) have been reported to be key sources of the micro-nutrients (Ojiewo *et al*., 2013; Grubben *et al*., 2014; Ayua *et al*., 2016; Bvenura and Afolayan, 2016). Mavengahama *et al.* (2013) concluded that the AIVs, (also referred to as wild vegetables), are commonly used as accompaniments to cereal-based staple diets. Although they are consumed in low quantities, they provide the vital micro-nutrients that supplement the cereal diets. Therefore, they help in improving the food and nutrition security of the poor, vulnerable households. Moreover, an analysis by Nyadanu and Lowor (2015) concluded that compared to the exotic species, the indigenous leafy and fruit vegetables are nutritionally superior. *Solanum nigrum complex* (African nightshade) is among these vegetables that have been highly popularized for their nutritional quality (Grace *et al*., 2013). The benefits of such nutrient dense foods cannot be over-emphasized especially in the developing countries where incidences of malnutrition tend to be prevalent (Allen and Benoist, 2006; Muthayya *et al*., 2013; UNICEF, 2013).

Despite the high nutritional potential of the AIVs, Mavengahama *et al*. (2013) observed that these benefits are not optimally harnessed. This is due to a number of reasons such as the non-recognition or non-acknowledgment of these benefits. Further, the individual preferences and some characteristics of the AIVs leaves, such as bitterness, make some people desist from consuming the vegetables. African nightshade is among such AIVs which have bitter tasting leaves but have major nutritional benefits. As such, there is need to find out other alternative ways through which its benefits can be enjoyed by all. One such alternative is to look at the nutrient content of its berries and their possible inclusions in the diet.

Fruits are a major component of the human diet (Kader, 1999; Consumer F and B, 2021). This is largely due to the nutritional and health benefits associated with the fruits such as prevention of inflammation disorders, protective effects against cancers as well as cardiovascular diseases (Matasyoh *et al.*, 2014). Berries in particular are excellent sources of bioactive compounds (Skrovankova *et al*., 2015). Apart from their excellent taste and flavour, they are of great economic value and interest to nutritionists due to the opportunity to use the bioactive compounds in production of functional foods ingredients. The *S. nigrum* edible berries could also be explored for this purpose but they are rarely cultivated or considered as a food item (Poczai *et al*., 2010).

1.2 Problem Statement

Between 1995 and 2011, there was a net change reduction of 6.7 improvement in the global hidden hunger index (HHI) (Ruel-Bergeron *et al*., 2015). This global shift was greatly attributed to reductions in vitamin A and zinc deficiencies. However, iron-deficiency anaemia remained largely unaffected and even increased in some regions. Unfortunately, while the global indices showed an improvement, the African region was the only one to record deterioration in hidden hunger, recording a net rise of 1.9 rise in the HHI. Kenya performed quite dismally in the HHI index, only coming second to Niger after attaining a HHI score of 52.0 (Muthayya *et al*., 2013). This figure was derived from the prevalence of three main indicators of the HHI including stunting, iron-deficiency anaemia and low-serum retinol ($<0.7\mu$mol/L). In each of the indicators, Kenya scored 26, 41.8 and 67.0%, respectively. This, therefore, implies that urgent measures are needed to remedy this situation.

African nightshade (*Solanum nigrum complex*) is among the AIVs that have been widely popularized and recommended to improve the dietary micro-nutrient content (Mavengahama *et al*., 2013). Despite its documented benefits, only its leaves are used as vegetables. This is because the plant is characterized as a leafy vegetable. Consequently, its berries are not considered as a potential source of micro-nutrients despite the fact that the plant fruits after maturity. To the best of our knowledge, little has been studied on the nutrient composition and possible dietary applications of the African nightshade berries. This study, therefore, seeks to bridge this gap by analyzing the nutrient and chemical composition of the berries, their antimicrobial activity and possible inclusion of the berries in the diet so as to curb the hidden hunger menace in Kenya.

1.3 Objectives

1.3.1 Broad Objective

To determine the nutrient composition, phytochemical content and anti-microbial activity of African nightshade (*Solanum nigrum complex*) berries.

1.3.2 Specific Objectives

1. To determine the nutrient composition and changes in phytochemical content of the African nightshade (*Solanum nigrum complex*) berries with ripening stage.
2. To determine the physico-chemical properties of African nightshade (*Solanum nigrum complex*) berries.
3. To determine the anti-microbial activity of the African nightshade (*Solanum nigrum complex*) berries' extract.
4. To evaluate the consumer acceptability of African nightshade (*Solanum nigrum complex*) berries.

1.4 Justification

The findings of this study will be of benefit to the small scale farmers of the African nightshade plant as it will offer them another avenue through which they can exploit all the potential nutritional value of the plant. It will also be of great importance to nutrition practitioners through raising awareness about another potential source of the highly nutritious plant. Lastly, should the berries prove to be highly nutritious, the information will be of great importance to other scientists, especially biotechnologists, by providing a basis from which more studies could be carried out to develop other varieties that have the potential to yield more berries.

CHAPTER TWO

LITERATURE REVIEW

2.1 Food and Nutrition Security Situation in Kenya

Adequate food and nutrition is recognized as a human right in the 2010 Constitution of Kenya. Article 43 of the constitution states that "every person has a right to be free from hunger and the right to adequate food of acceptable quality" while Article 53 states that "every child has the right to basic nutrition" (Kenya National Bureau of Statistics, KNBS, 2015). This provision is made in recognition of good nutrition being a prerequisite for national development of countries and well-being of individuals. While this is a good initiative, achieving this has proven to be a challenge for the country with the Kenya Demographic and Health Survey (KDHS) of 2014 reporting cases of stunting, wasting and underweight for children below 5 years of age at 26, 4 and 11%, respectively. Further, 30.4% of households are reported to have food shortage or lacking enough finances to buy food (KNBS, 2015). This is a clear indication that there is still a lot that needs to be done in order to achieve right to adequate, nutritious and safe food by all persons as required by the constitution.

In recognition of the need for affirmative action to address the food and nutrition situation in the country, the government developed the National Food and Nutrition Security Policy of 2011 (Government of Kenya, GoK, 2011). The policy majorly concentrated on reforms in the agricultural sector since it is the mainstay of the Kenya's economy, directly contributing 24% of the Gross Domestic Product (GDP) and 27% indirectly through linkages with manufacturing, distribution and other service related sectors. Approximately 45% of Government revenue is derived from agriculture and the sector contributes over 75% of industrial raw materials and more than 50% of the export earnings. The sector is the largest employer in the economy, accounting for 60% of the total employment. Over 80% of the population, especially living in rural areas, derive their livelihoods mainly from agricultural related activities.

In 2008, the GoK launched Kenya Vision 2030 as the new long-term development blueprint for the country whose focus is to create a “Globally competitive and prosperous country with a high quality of life by 2030”. The Vision also aims at transforming Kenya into “a newly industrializing, middle income country providing a high quality of life to all its citizens in a clean and secure environment (GoK, 2011).

The achievement of national food security is a key objective of the agricultural sector. Food security in this case is defined as “a situation in which all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life” (Kenya Food Security Steering Group, KFSSG, 2008). In the recent years, the country has been facing severe food insecurity problems with approximately 10 million people being food insecure with majority of them living on food relief. Households are also incurring huge food bills due to the high food prices. Maize being staple food due to the food preferences is in short supply and most households have limited choices of other food stuffs (KSSG, 2008; GoK, 2011; KNBS, 2015).

The current food insecurity problems are attributed to several factors, including the frequent droughts in most parts of the country, high costs of domestic food production due to high costs of inputs especially fertilizer, displacement of a large number of farmers in the high potential agricultural areas following the post-election violence which occurred in early 2008, high global food prices and low purchasing power for large proportion of the population due to high level of poverty (KSSG, 2008).

2.2 Policy Responses to Food Crises in Kenya

The Kenyan government recognizes the need for policy formulation to avert the frequent food crises (GoK, 2011). Embedded in the food and nutrition security policy, the government came up with a three-pronged policy approach. First, there were policies aimed at ensuring adequate supply of food. These included policies such as subsidization of farm inputs like fertilizers and seeds. This was accompanied by provision of credit to farmers and improvement of rural infrastructure and rural markets (KFSSG, 2008). Other approaches included training farmers on agri-business skills and allowing importation of tax-free products such as maize. To solve the problem of over-reliance on cereal staples such as maize, the government also encouraged the populace to diversify their diet. As such, the people would consume whichever food was available and in season, therefore reducing the number of people suffering from hunger.

The second policy approach included policy strategies which would reduce the prices of foods (KFSSG, 2008). Among the strategies in this approach involved having a fund to purchase livestock from drought stricken areas. This would not only save the herders from losses arising from death of their animals but also ensure that they had money to buy other food-stuff. Raising the strategic grain reserves at the National Cereal and Produce Board and using the board to purchase maize from the farmers at a fair price would provide an incentive to the farmers. During periods of scarcity, the government also provided subsidies to maize millers to ensure that maize flour retailed at affordable prices to the consumers.

The third approach was to formulate strategies which would ensure that the populace have enough income to purchase food (KFSSG, 2008; GoK, 2011). This would be achieved through the government taking initiative to contribute to the cost of social amenities such as education and health. This would lower the cost of such services and, therefore, ensure that even the poor would have disposable income to buy food since they do not have to worry over the social amenities. The government also provided funds such as the Constituency Development Fund (CDF) which would help the local communities meet their developmental needs. The funds could be used to finance construction and equipping of schools, community polytechnics as well as other development projects. Apart from providing employment, such initiatives would also increase the skill levels of the people. Consequently, this would put them in a position where they can be economically viable and capable of providing for their families, including provision of adequate food and nutritional.

Despite these efforts, implementation of the policies has been a challenge due to a myriad of factors such as corruption, pests and diseases, climate change, lack of resources and skills among others (KFSSG, 2008). To overcome these challenges and achieve the set objectives, the government further advised farmers to adopt the indigenous crops which have been largely neglected and underutilized. These include crops such as barley, sorghum, mushrooms, dolichos bean (*Lablab purpureus*), Irish potatoes (*Solanum tuberosum*) and African indigenous vegetables (AIVs) that have been developed for improved nutrition, early maturity, drought, disease and pest tolerance and are affordable (Adjimoto and Kwadzo, 2018) but their adoption and utilization has declined rapidly.

2.3 African Indigenous Leafy Vegetables

Tumwet *et al.* (2014) observed that the definition of vegetables varies from culture to culture. This is largely based on the cultural differences in terms of food selection and preparation practices. The different vegetables have different edible parts such as the leaves, shoots, flowers, buds, bulbs, seeds and fruits among others.

In Kenya, vegetables are either exotic or indigenous. Exotic vegetables are those which have recently been introduced such as cabbages, carrots and spinach. Indigenous vegetables, on the other hand, are either those which were originally in an area (Tumwet *et al.*, 2014) or introduced and have been used over a long period of time until they form part of the culture and tradition of a community. The indigenous vegetables include night shade (*Solanum spp*.), spider plant (*Cleome spp.*), amaranthus (*Amaranthus spp*.), cowpea (*Vigna spp*.), sweet potato leaves (*Ipomeas spp*.), pumpkin leaves (*Cucurbita spp*.), jute mallow (*Corchorus spp*.) and cassava leaves (*Manihot esculenta*) (Burchi *et al*., 2011).

There are about 800 to 1000 species of edible leafy vegetables in Sub-Saharan Africa referred to as indigenous, although only a small percentage of these are utilized as food (Grubben *et al.*, 2014). The high diversity of these vegetables shows their importance in adaptation to the environment and consumer preference. Indigenous vegetables have been used as a side dish with the staples in the African culture for a long time and have been an integral part of agricultural systems (Grubben *et al.*, 2014). They have been an important contributor to micro-nutrient intake and food and nutrition security at large. Furthermore, production of the African Indigenous vegetables (AIVs) is not very expensive (Ayua *et al*., 2016). This is mainly because the crops are disease and drought resistant and they do not need a lot of inputs in production. As such, they can easily be used in enhancing not only the nutritional status of the small scale farmers, but also to help them get an extra source of income (Tumwet *et al*., 2014).

Despite their benefits, indigenous vegetables have with time been neglected and under-utilized, considered old-fashioned, poor man's food and therefore shameful to consume (Grace *et al.*, 2013). Little is therefore known about them and are threatened with genetic erosion due to change in land use and eating habits.

Although African indigenous leafy vegetables (AILVs) are largely underutilized and neglected in research, breeding and modern production methods (Tumwet *et al*., 2014), they have received much attention in the recent past as people get more informed on their benefits (Mavengahama *et al*., 2013). Most of all, there has been great popularization of the AIVs after research showed that they not only provide essential micro-nutrients, but also do have phytochemicals and antioxidants such as flavonoids, alkaloids, tannins and phenolic compounds among others (Jeyasree *et al*., 2014). One of the AIVs that have been popularized for such benefits is the African nightshade (*Solanum spp*).

2.4 African Nightshade

African nightshade (Figure 2.1) belongs to the family Solanacae (Sarma and Sarma, 2011). It is widely thought to have been a famine plant that originated from China. The plant grows to about 10 to 60 cm in height and has smooth, semi-climbing stem that is usually green in colour. Though the plant is native of Eurasia, it has been greatly popularized throughout Africa and the South East Asia. Based on its adaptability to different and harsh climatic conditions, the plant is widely distributed in the regions that it is grown (Sarma and Sarma, 2011). In these regions, young leaves and berries of the plant are eaten as vegetables and can even be found in the local markets where it is sold for human consumption.

Figure 2.1: *Solanum nigrum L*. Adopted from (Grace *et al*., 2013)

A study by Matasyoh *et al.* (2014) exhibited that the *Solanum nigrum complex* grown in Kenya includes both the local (native) and the hybrid species. Both the wild types (indigenous) and the hybrids (modified) varieties are grown in the country and are an integral source of vegetables and fruits in the country. While the leaves are used as vegetables, and are usually harvested at an early age, the ripe berries are also edible. Unfortunately, the berries are not consumed as much, neither have they been studied as much as the leaves (Sarma and Sarma, 2011).

The *S. nigrum* complex has several species all of which have high and close resemblance to each other. Some of the species include *Solanum villosum, Solanum nigrum, Solanum scabrum, Solanum americana, Solanum burkankii and Solanum schenopodioides* (Matasyoh *et al*., 2014). Their similarities often pose a challenge in taxonomy. However, there are some morphological features that can be used in differentiating between the species. For instance, some of the species have yellow flowers (*S. nigrum and S.*

villosum) while others have flowers that are purplish yellow (*S. scabrum*). The colour of the ripe berries can also be used for differentiation where *S. villosum* has orange fruits while *S. nigrum* and *S. scabrum* have black berries when ripe.

2.5 Nutritional Value of Solanum spp.

African nightshade (*Solanum nigrum complex*) is arguably one of the largest and most variant group within the genus *Solanum* (Poczai *et al*., 2010). Originating from the New World tropics, particularly in South America, most of the variants are considered as invasive weeds especially in the Americas and Europe (Defelice, 2003; Poczai *et al*., 2010; Sarma and Sarma, 2011). It is rarely grown as a food crop but is often picked in wild, weedy populations where it is used as a famine food in China and India (Defelice, 2003). However, in many developing countries within the Asian and African continents, African nightshade is a nutrient powerhouse and an important source of income for many small scale farmers in this region (Ojiewo *et al*., 2013). Consequently, it is popularly classified as one of the African Indigenous Vegetables (AIVs) which are reported to be key sources of micro-nutrients and phytochemicals (Nyadanu and Lowor, 2015; Ojiewo *et al*., 2015).

Mavengahama *et al.* (2013) reported that the AIVs, (also referred to as wild vegetables), are commonly used as accompaniments to the cereal-based staple diets. Although they are consumed in low quantities, they provide the vital micro-nutrients that supplement the cereal diets. Therefore, they help in improving the food and nutrition security of the poor, vulnerable households. Interestingly, an analysis by Nyadanu and Lowor (2015) demonstrated that in comparison to the exotic species, the indigenous leafy and fruit vegetables are nutritionally superior. This is the reason as to why they are currently being championed for frequent use in the diet. Generally, the indigenous vegetables are found to be superior in the content of macronutrients such as carbohydrates, proteins and dietary fibre. They are also superior in micro-nutrient content such as potassium, calcium, magnesium, phosphorus, vitamin A, vitamin C, vitamin E among others. Other researchers concur with this observation. For instance, Grace *et al*. (2013) carried out an analysis of two indigenous vegetables in South Africa; *S. nigrum* and *C. album* (Table 2.1). It was found out that *S. nigrum* emerged superior in its nutrient value.

African nightshade features strongly as one of the food items that can help to meet the macro- and micro-nutrient needs of the masses (Grubben *et al*., 2014; Ruel-Bergeron *et al*., 2015). Young shoots and leaves are used as pot-herbs while the ripe berries are eaten raw as fruits or used in confectionery (Defelice, 2003).

Sarma and Sarma (2011) also carried out a study which showed that the *S. nigrum* leaves, berries, and seeds have high nutritional value. This is in agreement with the report of Mavengahama *et al*. (2013) that AIVs can be used in improving the quality of the household diets. However, it is unfortunate that while *S. nigrum* is one of the popular AIVs, only its leaves are seriously considered for dietary purposes while the edible berries are given little attention. The contents of various minerals present in two AIVs, *Chenopodium album* and *Solanum nigrum*, have been previously quantified and are shown in Table 2.1.

Table 2.1: Micro Element composition of Two Selected AIVs (mg/1000g) DMB*

Element	***Chenopodium album***	***Solanum nigrum***
Calcium	18213.2±598	16890.0±1488
Potassium	49028.6±593	61120.2±155
Magnesium	13821.5±493	14407.8±173
Sodium	68.0±3.6	116.9±5.3
Arsenic	1.8±0.1	1.9±0.2
Antimony	0.05	0.05
Chromium	0.90±0.0	0.94±0.0
Iron	120.4±4.1	177.1±8.1
Selenium	5.4±0.0	5.7±0.0
Tin	0.05	0.05
Zinc	23.0±1.9	26.1±0.9

*DMB = Dry Matter Basis

Adopted from: Grace *et al*. (2013)

Generally, the chemical composition of berries is variable depending on the cultivar and variety, growing location and environmental conditions, plant nutrition, ripeness stage, and time of harvest, as well as subsequent storage conditions. Therefore, the content of each individual component and the quality of the fruits is highly variable (Davies and Robinson, 1996). Berries, in general, are rich in sugars (glucose, fructose), but low in calories. They contain only small amounts of fat, but a high content of dietary fiber (cellulose, hemicellulose, pectin); organic acids, such as citric acid, malic acid, tartaric, oxalic and fumaric acid; certain minerals in trace amounts for instance, 100 g of edible portion of raspberries, blackberries, or blueberries could provide more than 50% of Recommended Dietary Allowance (RDA) for manganese (Skrovankova *et al*., 2015), some vitamins (ascorbic acid and folic acid) and phytochemicals, such as phenolic compounds. These compounds could be a good option for the food industry to use as functional foods ingredients.

2.6 Phytochemical Content and Anti-oxidant Characteristics of S. nigrum

A study carried out by Jeyasree *et al*. (2014) came to the conclusion that *S. nigrum* can find application as an antimicrobial, anti-helminthetic and anti-inflammatory agent. This is due to its content of phytochemicals that can be harnessed in phyto-pharmaceutical applications. Some of the phytochemicals that are present in the plant leaves include flavonoids, tannins, alkaloids and phenolic compounds.

Another study by Modilal *et al.* (2015) affirms this proposition. This was through a qualitative analysis which concluded that alkaloids, flavonoids, steroids and tannins were indeed present in the *S. nigrum* leaves. The authors further asserted that these compounds are known to be biologically active as they protect the plants against infection. Furthermore, plants are known to be good sources of useful compounds that are employed in the development of chemotherapeutic agents. This is based on reports which indicate that plants have anti-bacterial, anti-viral, anti-fungal, anti-inflammatory, and anti-helminthic properties (Modilal *et al*., 2015). From these studies, it can be observed that the leaves of *S. nigrum* possess characteristics which are beneficial to health. However, the berries of the plant have been given limited attention and their phytochemical and antioxidant contents remain unclear.

Some of the phytochemicals present in *Solanum nigrum* are alkaloids, flavonoids, steroids, tannins and phlobatannins. This is corroborated by an earlier investigation which showed the presence of various phytochemicals such as alkaloids, flavonoids, phenols, steroids and tannins in the crude extract of *S. nigrum* (Perez et al., 1990). These compounds are known to be biologically active because they protect the plants against infection. Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents.

Tannins are known to possess general antimicrobial and antioxidant activities. Recent reports show that tannins may have potential value as cytotoxic and antineoplastic agents (Modilal *et al*., 2015). Other compounds like saponins also have anti- fungal properties. Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss. It is also known to have anti- fungal properties. Saponins have been implicated as bioactive antibacterial agents of plants. Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and anti-microbial properties. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolic phytochemicals have anti-oxidative, anti-diabetic, anti-carcinogenic, antimicrobial, anti-allergic, anti-mutagenic and anti-inflammtory. Presence of these compounds in *Solanum nigrum* could be an indication that apart from harnessing the nutritional potential of the crop, its nutraceutical potential can also be explored with a view of diversifying its usage.

2.7 Anti-Microbial Activity of S. nigrum complex

Whilst evidence has it that there are phytochemicals in the *S. nigrum*, there is need to prove that these are actually active against micro-organisms. Studies have been done in attempt to ascertain this. Matasyoh *et al*. (2014) carried out a study in which extracts from three *S. nigrum* species namely *S. nigrum, S. scabrum* and *S. villosum* were tested for their ability to curb microbial activity and also the minimum inhibition concentration (MIC) for the extracts. The species were found to have anti-fungal and anti-bacterial characteristics. However, the species that did not contain tannin did not have any anti-fungal activity. *S. nigrum* and *S. villosum* were found to have both anti-fungal and anti-bacterial characteristics.

Modilal *et al*. (2015) also carried out a similar study using *S. nigrum* extracts. This was tested against five isolated bacteria which included *E. coli, K. pneumonia, P. aeruginosa, S. aureus,* and *S. pyrogens.* These were tested at different concentrations and the highest concentration which had 100 mg/ml had the highest inhibition zone for the five microbes. The ethanolic extract inhibition was highest in *P. aeroginosa* followed by *S. pyrogenes*. The aqueous extract of the *S. nigrum*, on the other hand, showed greatest inhibition for *S. aureus* followed by *P. aeruginosa* and *S. pyrogens*. Lastly, the petroleum ether extract showed maximum inhibition for *S. pyrogenes* followed by *S. aureus*. Of all the *S. nigrum* extracts, the ethanolic extract showed the highest anti-bacterial activity (Modilal *et al*., 2015).

Matasyoh *et al*. (2014) further noted that traditionally, these properties of the *S. nigrum* were appreciated and this is the reason as to why they are used as traditional medicine. For instance, in Kenya, the unripe fruits are used in soothing tooth pain and are also squeezed on teething babies' gums to ease the pain. In other places, the leaves are used as a remedy for stomach ache while the extracts from both the leaves and the fruits are used in treating tonsillitis. As such, the plant finds use as a replacement for synthetic drugs which could be quite expensive yet they could have side effects.

From these studies, it is evident that *S. nigrum* has anti-microbial activity. This is thought to be due to the presence of phenolics and terpenoids which are the main anti-microbial agents. However, this could also be attributed to the presence of the aromatic compounds such as the phenolic acids, phenols, alkaloids, lectins and its derivatives such as the flavonoids which have proven to have anti-microbial characteristics (Jeyasree *et al*., 2014).

2.8 Anti-Nutritional Factors in S. nigrum complex

Sarma and Sarma (2011) indicates that the plant leaves contain some anti-nutritional factors such as trypsin inhibitors, phytates and tannins. Anti-nutritional factors such as trypsin inhibitors, phytic acid and cyanogen are as important as nutritional content of any edible plant part. The anti-nutritional factors can be defined as those substances generated in natural food substances by the normal metabolism of species and by different mechanisms such as inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed, which exert effects contrary to optimum nutrition.

Little has been done on the anti-nutritional factors in the berries of *Solanum nigrum* edible berries. However, there has been studies focusing on other parts of the plant such as the leaves as well as parts of the fruit such as the seeds (Sarma and Sarma, 2011). These researchers compared the anti-nutritional factors in the seeds of African nightshade and compared these with the content in Chinese chive and Roselle plant. Their findings indicated that anti nutritional factors in mg/100 g on dry weight basis for seeds were at parity amongst the selected plants. Trypsin (1.01), phytic acid (0.13) and tannins (0.17) were moderately present in the African nightshade seeds. The contents of tannins and phytic acid in *S. nigrum* were found to be lower than Chinese chive and Roselle plants. Considering the amount of available nutraceuticals, this plant could be valuable and important contributor to the human diet. It is, however, worth noting that the samples used in the study by Sarma and Sarma (2011) were picked at the ripe stage of development. As such, the study does not give an account of the changes that take place as the fruits ripen.

There is documented evidence that the content of anti-nutritional factors in fruits decrease with progression in ripening. A report by Nguyen *et al.*, (2013) on the total, soluble and insoluble oxalate content in ripe, green and golden Kiwi fruit indicated that the levels of oxalated were reduced with ripening. Given that high oxalate levels are associated with onset or progression of kidney stones, it can be concluded that fruit ripening significantly reduces the possibility of the fruits being the course of such health conditions.

Given the little attention that *Solanum nigrum* edible berries have received, they are often mistaken for the berries of the belladonna or deadly nightshade *(Atropa belladonna*) which are known to be highly poisonous. As a result, many people often classify the nightshade as an invasive, poisonous weed (Defelice, 2003). Solanin is the toxin associated with the deadly nightshade and could also be found in unripe berries of other *Solanum* species. However, this is not the case in the edible berries of *S. nigrum*.

2.9 Potential Application of S. nigrum complex Berries in the Food Industry

Food poisoning, a common cause for morbidity and mortality in developing countries, is associated with Gram negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* as well as Gram positive bacteria such as *Bacillus cereus* and *Staphylococcus aureus* (Puupponen-Pimia *et al*., 2001; Khalifa *et al*., 2015; Mostafa *et al*., 2018). This happens at the backdrop of the concerns of most consumers about the safety of the foods they consume and the effects that these could have on their health (Bobinaitė *et al*., 2013). Consumers tend to associate safety with attributes such as being free from harmful micro-organisms and chemical additives (especially synthetic additives) while identifying the healthfulness of a food based on degree of freshness and naturalness (Bobinaitė *et al*., 2013). Further, consumers are also concerned about the genetic composition of the foods they eat, preferring to consume plant products which are organically grown as opposed to products grown with application of organic pesticides, foliar feeds and other genetically modified organisms (GMOs). These are deemed to have their naturalness altered and, therefore, could potentially have long-term negative effects on the consumers (Bobinaitė *et al*., 2013).

Despite the health concerns of the consumers, food producers have to find means of producing enough food products to meet the demands of the masses. Application of synthetic chemicals on plant products is fuelled by the need to meet the food and nutritional needs of the growing population in an environment that is constantly changing, therefore posing a challenge to agricultural activities (Shen *et al*., 2014). With rapid industrialization, environmental degradation, population explosion, rural-urban migration and the perception of agriculture as a venture that is not very prestigious, food production has been negatively affected. There is reduced arable land especially in the developing countries where land that was historically used for agricultural production is replaced by residential and industrial estates. To further compound the dilemma of the food producers and processors, consumers also want products that are not only healthy but also convenient and with a long shelf-life (Consumer F and B, 2021). Further, they have to contend with the legal provisions on quality of food products. The food producers and processors, therefore, have to work in an environment that is afffected by a myriad of factors and they have to strike a balance between consumer demands, legal provisions, environmental concerns and the nnormal market dynamics.

To ensure the safety of foods, food industries and other producers have mostly used the chemical additives (Bobinaitė *et al*., 2013; Shen *et al*., 2014; Nam *et al*., 2019) to reduce the microbial load and increase the shelf-life of food products through reduced oxidation (Seleshe *et al*., 2017). However, in the recent past, this has become a major challenge with many consumers being health-conscious, advocating for eco-living lifestyles and natural preservatives (Liepiņa *et al*., 2013; Bouarab *et al*., 2018; Nam *et al*., 2019). Such demands have pushed the players in the food industry to explore the use of plants that are known to have antimicrobial properties for application in food processing and preservation. These plant-based products are not only meant to naturally protect the food products from spoilage but also to boost consumer acceptability. The latter is achieved by riding on value-addition of the products using natural food items as well as improved organoleptic attributes. African nightshade is one of the crops that can be explored for this purpose.

Fruits, berries and other parts of plants have been used in value addition of industrial food products. For instance, berries have been incorporated in production of fruit-flavoured yoghurts throughout the world (Consumer F and B, 2021). Fruits such as mangoes and pineapple have been incorporated in the yoghurts and it has been proven that the consumers readily accept such products. Currently, attention has shifted to production of berry flavoured yoghurts. The commonly used berries include strawberries and blueberries. It is possible, therefore, that African nightshade (*S. nigrum*) berries could be explored for this purpose.

Fruits have also been widely used in the confectionery industry (Defelice, 2003). Raisins have, for a long time, been incorporated in cakes and breads. Crystallized fruits such as strawberries are also used in cake decorations while candied fruits are used in cocktails, pastries, biscuits and muffins. Apart from the aesthetic appeal that these fruits add to the food items, they also provide a pleasant, strong aroma and a good taste to the products. The fruits themselves or their by-products also find use in production of juices. These can be whole fruit, fresh juices, concentrates, squashes among others. The main reason why these value-added products are produced with fruits products is because the nutritional value of these products is well appreciated.

African nightshade berries can also find their application in the food in the food industry. Previous reports indicate that the berries can be eaten raw as fruits or used in confectionery (Defelice, 2003). Juices from young unripe fruits have been used in soothing ailing gums in children (Matasyoh *et al*., 2014) while the leaves are recorded to have anti-microbial properties (Matasyoh *et al*., 2014; Modilal *et al*., 2015). By implication, therefore, it is possible that just like other fruits and berries which have found applications in the food industry, *S. nigrum* berries could also have similar applications. However, this potential needs to be studied and documented.

2.10 Fruit Maturation and Ripening

Fleshy fruits are classified into climacteric and non-climacteric types and they are important crops worldwide accounting for a major fraction of the world's agricultural output as well as a major component of the diet (Jia *et al*., 2013). Quality of fresh fruits and their products is a function of a combination of various attributes which give value in terms of human food (Kader, 1999). The importance of each of the quality attributes is determined by the commodity and its intended use and this often varies amongst producers, handlers and consumers. These qualities include but are not limited to appearance, firmness and shelf-life (Kader, 1999; Davies and Böttcher, 2009). Final fruit quality and storage life are largely affected by the fruit maturity at harvest (Kader, 1999).

Maturation is the developmental stage leading to attainment of physiological maturity (Wang *et al*., 2009; Fawole and Opara, 2013). Immature fruits are of an inferior quality since they are more prone to mechanical damage and shriveling besides having an inferior flavour (Davies and Böttcher, 2009). Overripe fruits, on the other hand, are likely to be soft and mealy with an insipid flavour. Proper timing during fruit picking is important as early or late picking can lead to physiological disorders during storage (Kader, 1999).

The genetic control of ripening is not fully understood. Elucidating these mechanisms would be valuable for breeding improved varieties that achieve optimal ripening characteristics in different cultivation environments. Research has been directed at the onset of ripening in what appears to be a coordinated process. Castellarin *et al.* (2011) showed that softening, sugar accumulation, and an increase in abscisic acid (ABA) were coincident at the onset of ripening. Further reports indicate that softening, sugar accumulation, and colour change were coincident, and that these changes preceded the resumption of growth by 5–7 days (Castellarin *et al*., 2011).

In addition to identifying the timing of the onset of the many metabolic changes involved in ripening, it is important to know where to look for these changes. Since softening, sugar accumulation, and anthocyanin accumulation have traditionally been thought to be coincident at the onset of ripening (Kader, 1999), it has been implicit that ripening begins simultaneously in the skin, where colour accumulates, and in the flesh, where sugar accumulates. However, work has shown that decreases in cell turgor in the flesh precede sugar and anthocyanin accumulation. Nevertheless, the spatiotemporal relationship between these events remains unresolved.

Despite these challenges, attempts have been made and various methods derived for assessing and characterizing the physic-chemical changes that occur in fruits with ripening (Kader, 1999). Some of these include measures such as firmness, colour change, changes in the sugar fractions in the fruits, size, volume and weight of the fruits, respiration rate and the rate of ethylene gas production. This, accompanied by characterization of other chemical components in the fruits help in deriving the maturity indices of the fruits. While such studies have been extensively carried out on other fruits, the edible berries of African nightshade have not received much attention. This precipitates into the need for a study to characterize the physic-chemical changes in the berries viz-a-viz the ripening stages.

2.11 Anti-Microbial Screening of Plant Extracts

Occasioned by the inability of available antimicrobials failure to treat infectious diseases, investigation of natural products as source of new bioactive molecules has received great attention from researchers (Jeyasree *et al.,* 2007). A variety of methods are found for this purpose and since not all of them are based on same principles, results obtained are profoundly influenced not only by the method selected, but also by the microorganisms used to carry out the test, and by the degree of solubility of each test-compound. The test systems should ideally be simple, rapid, reproducible, and inexpensive and maximize high sample throughput in order to cope with a varied number of extracts and fractions. The complexity of the bioassay must be defined by laboratory facilities and quality available personnel. The currently available screening methods for the detection of antimicrobial activity of natural products fall into three groups, including bio-autographic, diffusion, and dilution methods. The bio-autographic and diffusion methods are known as qualitative techniques since these methods only give an idea of the presence or absence of substances with antimicrobial activity (Matasyoh et al., 2014). On the other hand, dilution methods are considered quantitative assays once they determine the minimal inhibitory concentration. Antimicrobial activities reported in the literature have been evaluated with diverse sets of methodologies, degrees of sensitivity, amount of test- compounds and microbial strains, often difficult to compare.

Diffusion and dilution methods are the two major alternatives employed in studying the antimicrobial activity of medicinal plants (Rios *et al*., 1988). However, there has been need for modifications since there are some factors which can affect the results. These are factors such as pH, extraction method, microbes tested, culture medium composition and solubility of the sample in the culture medium (Rios *et al*., 1988; Valgas *et al*., 2007). In agar-disc diffusion method, the test-microbe is cultured on agar plates after which filter paper discs containing the test compound are placed on the surface of the agar. This is followed by incubation of the petri-dishes containing the culture under appropriate growth conditions which are specific to the test microbe. During incubation, the test agent diffuses into the agar, thereby inhibiting the growth of the micro-organism. The zones of inhibition are then measured and these can be used to group bacteria into either intermediate, resistant or susceptible to the test agent (Jorgensen and Ferraro, 2009).

Dilution methods are most suitable in ascertaining the Minimum Inhibitory Concentration (MIC) because they give a chance to approximate the tested antimicrobial agent concentration in the agar dilution or broth medium (Pfaller *et al.*, 2004; Seleshe *et* *al*., 2017). The agar dilution method involves addition of different desired antimicrobial agent into a molten agar medium. It involves using serial two-fold dilutions which is then followed by inoculation of a defined microbial culture onto the surface of the agar plate. The MIC end-point is then recorded as the lowest concentration of antimicrobial agents that completely inhibits growth under the microbe-specific incubation conditions (Ghatage *et al*., 2014; Seleshe *et al*., 2017).

2.12 Sensory Evaluation of Food

Sensory evaluation is a scientific discipline that is employed to evoke, measure, analyze and interpret the characteristics of food which can be perceived using the five human senses (Lawless and Heymann, 2010). These senses include taste, touch, sight, smell and auditory senses (Mihafu *et al*., 2020). It can also be described as the scientific discipline which deals with sensory perceptions of affective and effective responses to foods, beverages and their components (Tuorila and Monteleone, 2009). Conclusively, sensory evaluation is defined as “A scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the sense of sight, smell, taste, touch, and hearing” (Institute of Food Technologists, IFT, 1981, p. 50). The importance of sensory evaluation is that it gives the producers of a product an idea as to whether the product would be acceptable to the consumers and whether the roll-out of such a product would be successful. This is largely informed by the fact that it would be an act in futility to produce, distribute and market a product whose acceptability to the consumers is unknown (Meiselman, 1993; Munoz, 2002).

Sensory evaluation is a crucial test for a new product as it helps to make sure that the product meets its objective when introduced into the market because a product shunned by consumers would definitely not meet the expectations (Lawless and Heymann, 2010). The IFT (1981) directs that such tests should be carried out under various circumstances such as new product development, product matching, product improvement, process change, quality control, and product rating or grading.

In the product life-cycle, sensory evaluation should be carried out in a multi-disciplinary approach (Tuorila and Monteleone, 2009; Mihafu *et al*., 2020). There are varying circumstances under which consumer acceptability tests can be carried out. These include circumstances such as new product development, product improvement, process change, product improvement, product grading or rating (Lawless and Heymann, 2010). This would be an appropriate method to give early indication into the feasibility of using African nightshade berries and their value-added products as food items as it would give an insight into how well the products could be received by the consumers.

2.13 Fruits and Management of COVID-19

Coronavirus disease 2019 (COVID-19) is a novel respiratory disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease was first discovered in China in the City of Wuhan towards the end of 2019 (Fernandez-Quintela *et al*., 2020). It quickly spread to the rest of the world and by the first quarter of 2020, many countries had put in place measures to contain the disease. The disease was declared a global pandemic (Fernandez-Quintela *et al*., 2020; Yedjou *et al*., 2021). At the time, there was no known vaccine or cure for the disease, causing panic all over the world as the disease continued spreading, wreaking havoc on the economic, social, health systems and other spheres of life. Countries witnessed lock-downs, travel restrictions amongst other measures aimed at containing the disease. Countries such as Italy, Spain, Brazil and the US witnessed mass deaths from the disease. At the time, there was great concern that the disease, if indeed it reached the developing world, especially in Africa, would have catastrophic events given the weak health systems in these countries. Pharmaceutical companies such as Pfizer, Moderna and BioNTech were on the frontline in seeking to develop a vaccine for the disease. Research has been carried out and vaccines such as AstraZeneca, Moderna, Sputnik V, Johnson and Johnson have been developed for the disease.

Despite these developments, there are concerns about the efficacy and safety of these pharmaceutical alternatives, making many people turn to consumption of fruits and vegetables as a way of boosting their immune system and maintain health amidst the pandemic (Moreb *et al.*, 2021). In Kenya, for instance, despite massive efforts by the government and the mass media to ensure all adults aged 15 years and above are vaccinated, only 16.3% of the population had been vaccinated as of March 2022 (Reuters Covid 19 Tracker, 2022). Amongst the reasons for the low vaccination rates include uncertainties surrounding the vaccines and their efficacy as well as fear of adverse side effects of the vaccination.

The attention given to nutritional alternatives is largely based on the fact that foods are known to have compounds which boost the immune system of the body. Consumption of fruits and vegetables provides bioactive compounds that can enhance the immune system. Berries such as cranberries, blueberries, blackberries and other fruits such as apples, prunes, citrus fruits and plums provide a significant level of anti-oxidants, vitamins, flavonoids and phytochemicals which could help deal with the severity of COVID-19 (Moreb *et al*., 2021). It is possible that just as these compounds help in boosting the immune system, scavenging on free radicals in the body and countering the effects of microbial infections, they could have a similar effect in lowering the severity of COVID 19. This is more so for fruits with high levels of vitamin C which is believed to have a relieving effect on other viral respiratory diseases such as influenza and flu (Moreb *et al*., 2021).

This is corroborated by findings by Yedjou *et al*. (2021) upon carrying out a research on fatalities arising from COVID-19 in correlation to consumption of fruits and vegetables. These authors found out that countries in which people consumed adequate to high levels of fruits and vegetables recorded fewer fatality rates compared to countries in which people consumed low levels of these food items (Yedjou *et al*., 2021). Given these insights, it is possible that the *S. nigrum* berries could also be a good source of the bioactive compounds which could boost the immune system of the consumers against COVID-19. Further, it has been established that berries such as strawberries and blueberries are good sources of vitamin C (Skrovankova *et al*., 2015). Given that *S. nigrum* has been proven to be rich in vitamin C amongst other bioactive compounds

(Ayua *et al*., 2016) and that it also has berries, the nutritional potential that can be harnessed not only from the leafy part of the plant, but also from the berries, which are seldom consumed or considered as a food item. Therefore, it is important to investigate the nutritional composition of the berries and evaluate their efficacy as a dietary item which could lead to optimal utilization of this nutrient-rich plant.

CHAPTER THREE

MICRONUTRIENT AND PHYTOCHEMICAL CHANGES DURING FRUIT DEVELOPMENT AND RIPENING IN SELECTED CULTIVARS OF AFRICAN NIGHTSHADE (*SOLANUM NIGRUM COMPLEX*) EDIBLE BERRIES

3.1 Introduction

Previous studies (Sarma and Sarma, 2011) characterized the chemical composition of African nightshade berries that were mainly harvested in the wild, giving limited attention to the changes during berry development and ripening. The current study addresses this knowledge gap by demonstrating the changes in the nutrient and phytochemical composition of African nightshade berries as influenced by ripening stage.

3.2 Material and Methods

3.2.1 Materials

Four selected varieties of African nightshade were used in this study. These comprised of Giant NS (Simlaw seeds), Black NS (Simlaw seeds), JKUAT Improved variety (JKUAT), Agriculture variety (KALRO-Kakamega).

3.2.2 Field Cultivation of African Nightshade

Field trials were conducted between February and May 2017 in the experimental field at the University of Eldoret. The Completely Randomized Design (CRD) was employed during planting on site as described by Bvenura and Afolayan (2016). There were four (4) blocks each subdivided into three (3) subplots to allow for three replications. Within the main blocks, there were subplots about 4 m by 2 m in size. These were separated by margins of about 1 m within the replicate blocks. The distance between blocks was about 1.5 m. During planting, a spacing of about 60 cm by 45 cm was used. Drip irrigation was installed within the plots so as to ensure that the plants do not suffer from water stress. Appropriate crop husbandry was employed till the berries reached maturity.

3.2.3 Sample Preparation

Upon flowering, plants from which berries would be picked in each sub-plot were tagged. Plants tagged for the picking were at the corners and the middle of the plot to ensure that each part of the plot would be represented in the sample. Berries for the analyses were picked at four ripening stages; green, colour break, ripe and at senescence. To determine the ripening stages, changes in the hue of the berries were checked against a colour wheel. The days after full bloom (DAFB) were recorded and used to ensure that for each variety, the DAFB was uniform for each particular ripening stage. The berries were oven-dried at 50 °C (Saleh and Otaibi, 2013) until they obtained a constant weight and then ground into fine powder. These were labelled appropriately and stored in air tight containers awaiting further laboratory tests.

3.2.4 Chemical Analyses

3.2.4.1 Determination of Proximate Composition

Protein analysis was carried out using the Kjeldahl method as outlined by AOAC Method 984.13. The percentage nitrogen obtained was converted to crude protein through multiplication by the standard factor 6.25 (Zhang *et al.*, 2015). Crude fat content was determined using the Soxhlet extraction, AOAC Method 920.29. Crude fibre was analyzed using the AOAC Method 978.10 while ash determination was done using AOAC Method 923.03 (Codex Alimentarius Commission, 1999). Carbohydrate content was analyzed by the difference method (FAO/WHO, 1998).

3.2.4.2 Minerals

The concentrations of zinc (Zn), calcium (Ca), magnesium (Mg), iron (Fe), was determined using the Atomic Absorption Spectrophotometry (UV-1800, Shimadzu

Corporation, Kyoto, Japan), AOAC Method 985.35 (Codex Alimentarius Commission, 1999). In a 100ml volumetric flask, 5 ml aliquot of sample and 50 ml of 24% (w/v) tri-carboxylic acid (TCA) were added. The mixture was then shaken at 5 minutes intervals for 30 minutes. This was followed by filtration using filter paper No. 1. Of the aliquot filtrate, 5 ml was transferred to a volumetric flask in which 1 ml of 5% (w/v) lanthanum solution was added and made to volume with distilled water. The mineral content readings were made against a reagent blank which contained 500 mg/l La and 1.2% TCA. Concentrations were determined from standard curves prepared for the respective mineral elements.

Sodium (Na) and potassium (K) were determined using flame photometry using AOAC (1995) Method 956.01. The stock solution for K was prepared by dissolving 1.907 g of dry KCl in distilled water to a volume of 1 L while the stock solution for Na was prepared by dissolving 2.542 g of dry NaCl in distilled water and made up to 1 L. the stock solutions contained 1000 ppm K and Na, respectively. Aliquots of the stock solution were diluted to get the standards in the range of 0 to 10 for K and 0 to 25 for Na. about 5 g of the finely ground material was transferred into an Erlenmeyer 100 ml flask and the extraction solution added to the mark. This was shaken for about 15 minutes then filtered through a Whatman No 1. filter paper. The standards and filtrate were run through the system. The standards were used to plot the standard curve against which the concentrations of %Na and %K in the samples were determined.

Phosphorus (P) was determined using the molybdenum blue method (AOAC, 1995) Method 958.01 as described by Engmann *et al*. (2013). About 2 g of the sample was ashed and then 5 ml of 5 M H_2SO_4 and 5 ml of 4% molybdate solution added in a 100 ml volumetric flask. Thereafter, 4 ml of 2% ascorbic acid was added and the mixture heated until a deep blue colour developed. Distilled water was then added to the 100 ml mark and the absorbance read at 655 nm. The concentration of P was determined from a standard curve which had been prepared from readings of standard solutions containing 0, 1, 2, 3, 4 and 5 mg of P in 100 ml distilled water.

3.2.4.3 Vitamin C

Vitamin C was determined using the HPLC (20A Series, Shimadzu Co-operation, Kyoto, Japan) following the AOAC Method 967.22 as described by Koyuncu and Dİlmaçünal (2010). A sample of 6 g was extracted in 30 ml distilled water adjusted to pH 1.5 with 10 ml phosphoric acid-water (2%, v/v). The obtained extract was filtered through filter paper before addition of 1.5 ml buffer solution (0.01 M KH_2PO_4, pH 8.0) was added to 1.5 ml of the extract. The mobile phase was water adjusted to pH 3 with phosphoric acid and the separation carried out at flow rate of 0.4 ml/min and the UV detector set at 254 nm. 1 ml of the mixture was loaded onto C_{18} column after which 3 ml of distilled water previously adjusted to pH 1.5 with 2 ml phosphoric acid-water (2%, v/v) was allowed to pass through the column. About 20 µl of the eluents were injected into the HPLC system and the quantification determined using peak area measurement.

3.2.4.4 Phytochemicals

The phytochemicals that were analysed included flavonoids, total phenols, oxalates, total carotenoids, phytates and tannins. These analyses were carried out according to the methods of Jeyasree *et al*. (2014), Chandra *et al*. (2014) and Zhang *et al*. (2015). For phytate determination, about 1 ml of the sample was centrifuged for 10 minutes at 15,000 rpm and the supernatant transferred into 15 ml test-tubes into which 10 ml of 1.25% sulphuric acid in water (v/v) was added. This was followed by 2 hour agitation in a mechanical shaker before further centrifugation at 2,000 rpm for 10 minutes. A 0.45 µm membrane was used to filter the supernatant which was then diluted with 0.9 ml deionized water. 25 µl of the supernatant was injected into the HPLC system connected to a conductivity detector (elution rate 1.0 ml/minute, mobile phase: A – deionized water, B – 200 mmol/l NaOH; gradient program 0 – 3 minutes: 87% A, 3 – 11 minutes: 50% A, 11.1 – 15 minutes: 87% A). Phytate standards inositol (Sigma-P5681 MSDS) was used and to obtain the phytate value by matching 25 µl of the standard with retention time of peaks of the berry extracts based on calibration curves of aqueous standards which contained 1.25% sulphuric acid.

Total phenols were determined using the formula by Zhang *et al*. (2015) where 3 ml distilled water, 250 µl Folin-Ciocalteu's reagent, 750 µl 7% sodium carbonate ($NaCO_3$) and 50 µl of each sample was incubated for 8 minutes at 25 ℃. To this mixture, 950 µl distilled water was added and allowed to stand for 2 hours. Total phenolic concentration was determined by measuring the absorbance at 765 nm using UV-Visible spectrophotometer and the values expressed as gallic acid equivalents (GAE).

Flavonoids were determined using the method by Chandra *et al*. (2014). To 0.25 ml of the sample, 75 µl of 5% $NaNO_2$ solution and 1.25 ml distilled water were added and the mixture allowed to stand for 6 minutes. Subsequently, 150 µl of 10% $AlCl_3.6H_2O$ was added and the mixture allowed to stand for 5 minutes. The absorbance of the reaction mixture was then measured at 420 nm using UV-Vis spectrophotometer and the concentration expressed as mg quercetin equivalents (QE)/g.

For tannin determination, 1 g of lyophilized sample was mixed with 10 m methanol in a 50 ml flask which was then shaken for 24 h at 25 °C in a dark chamber. This was followed by centrifugation at 2500 rpm for 10 minutes. Thereafter, 5 ml of vanillin reagent containing 0.5% vanillin, 4% HCl in methanol was added and the mixture allowed to stand for 20 minutes at 30 °C. Quantification of condensed tannins was then done using UV - spectrophotometry with (+)-catechin (upto 0.2 mg.ml) used as a reference standard and expressed as mg/g of (+)-catechin equivalents (CE).

Total oxalates were determined in triplicate using the method described by Vũ *et al*. (2013). Extraction was carried out by mixing 1 g of the sample with 40 ml 2.0 M HCl at 21 °C for 15 minutes. This was then allowed to cool before being quantitatively transferred into 100 ml volumetric flask and made up to volume before centrifugation at 5,000 rpm for 15 minutes. The supernatant was then filtered through a 0.45 µm filter paper and then quantified using HPLC.

Carotenoid content was determined using the method described by Leong and Oey (2012). The samples were extracted under dark conditions to avoid degradation of carotenoids by light. Five (5) g of the samples were homogenized in 50 ml of the extraction solvent (50% hexane, 25% acetone, 25% ethanol, containing 0.1% BHT v/v). At the same time, 5 g calcium chloride was gradually added to facilitate distinct separation of the organic layers and water. This mixture was stirred for 20 minutes at 4 °C, diluted with 15 ml of distilled water and stirred for another 10 minutes at the same temperature. The mixture was separated using a separation funnel and the organic layer collected in a volumetric flask and brought to 50 ml using the extraction solvent. Carotenoids content was determined using UV-VIS at 450 nm.

3.2.4.5 Titrable Acidity

Total titrable acidity (TTA) was determined using the method described by Sadler and Murphy (2010). The berries were crushed using mortar and pestle to obtain a slurry. About 2.5 g of the slurry was weighed out into a 100 ml beaker and 50 ml of distilled water added. The sample was then titrated with 0.1 N NaOH in the presence of phenolphthalein indicator until the end-point was reached, signified by a change of indicator colour to pink. The volume (ml) of NaOH used was recorded and used in determining TTA using the formula:

% acid = (mls NaOH used) x (0.1 N NaOH) x (milliequivalent factor) x (100)/grams of sample

3.3 Results and Discussions

3.3.1 Proximate Composition

From Table 3.1, it can be seen that the proximate composition did not vary a lot during the first three stages but it was observed that during senescence, most varieties had increased fibre, ash and protein content but decreased carbohydrate content. The ash content did not differ significantly in all varieties at all stages. However, it was observed that within each variety, there was a slight decrease in the ash content from the green stage through to the ripe stage at which point there was a significant rise in the ash content at senescence. This trend in ash reduction with ripening was also observed in prickly pears (Barbera *et al*., 1992), though the values recorded were slightly lower than those recorded in this study.

Crude fat content decreased through the ripening stages of the berries, a phenomenon also reported by Barbera *et al*. (1992). This decrease could be explained by the effect of ripening on lipid metabolism (Balic *et al*., 2018) where the lipid membranes, specifically thylakoid membranes, are degraded to provide energy during the process of senescence.

With reference to crude fibre, three of the varieties (Giant NS, JKUAT variety and KALRO Agriculture) were within the same range. However, Black nightshade had significantly lower content, almost half of the content in the other varieties which could be attributed to the varietal differences (Skrovankova *et al*., 2015). It was observed that this trend continued through all the ripening stages albeit the fact that ripening did not seem to have a significant effect on the fibre content of all the varieties. It was, however, notable that the major change occurred between the green stage and colour break. This is consistent with previous findings (Jain *et al*., 2003) who observed that this is also the stage at which major changes took place in guavas. It accompanied the decrease in cellulose, hemicellulose and lignin up to the ripe stage.

Table 3.1: Proximate Composition (%) of *S. nigrum* berries at different ripening stages

Variety/Stage	Ash	Crude Fibre	Crude Fat	Crude Protein	Carbohydrates
Green					
Black NS.	6.41 ± 0.26^{bcd}	10.06 ± 0.56^{a}	3.39 ± 0.12^{abcde}	9.16 ± 0.31^{abc}	72.02 ± 1.56^{fg}
Giant NS.	7.64 ± 0.16^{defg}	20.81 ± 0.60^{d}	3.75 ± 1.37^{cdef}	13.21 ± 1.89^{cd}	54.59 ± 2.65^{abcd}
JKUAT	8.57 ± 0.45^{efgh}	21.43 ± 1.82^{d}	3.24 ± 0.14^{abcde}	11.81 ± 1.39^{bcd}	55.94 ± 1.86^{abcde}
KALRO Agric.	6.90 ± 0.48^{bcde}	17.25 ± 0.74^{cd}	3.64 ± 0.83^{bcdef}	11.98 ± 1.02^{bcd}	60.23 ± 1.18^{cdef}
Colour Break					
Black NS.	5.86 ± 0.37^{abc}	12.16 ± 1.18^{ab}	2.44 ± 0.10^{abcde}	9.01 ± 1.15^{ab}	71.42 ± 2.41^{efg}
Giant NS.	7.53 ± 0.60^{cdefg}	17.37 ± 2.22^{cd}	5.48 ± 0.18^{fgh}	14.82 ± 2.83^{d}	61.87 ± 6.98^{cdefg}
JKUAT	7.71 ± 0.85^{defg}	18.56 ± 1.07^{cd}	4.18 ± 0.02^{efg}	10.44 ± 0.97^{abc}	61.54 ± 10.69^{cdef}
KALRO Agric.	7.01 ± 0.13^{bcdef}	16.89 ± 1.04^{cd}	1.90 ± 0.13^{abc}	12.67 ± 1.17^{bcd}	60.23 ± 2.58^{cdef}
Ripe					
Black NS.	4.60 ± 0.87^{a}	9.84 ± 0.63^{a}	1.68 ± 0.11^{a}	7.10 ± 0.54^{a}	77.25 ± 1.81^{g}
Giant NS.	5.45 ± 0.90^{ab}	16.21 ± 3.41^{bc}	5.78 ± 1.29^{gh}	15.01 ± 0.60^{d}	57.54 ± 6.09^{abcdef}
JKUAT	6.56 ± 0.23^{bcd}	17.17 ± 1.02^{cd}	2.05 ± 0.01^{abcd}	15.71 ± 1.46^{d}	59.10 ± 3.86^{bcdef}
KALRO Agric.	5.77 ± 0.38^{ab}	18.03 ± 1.35^{cd}	1.74 ± 0.04^{ab}	15.26 ± 2.06^{d}	59.87 ± 3.77^{cdef}
Senescence					
Black NS	5.84 ± 0.58^{ab}	11.92 ± 0.83^{ab}	2.37 ± 0.50^{abcde}	15.17 ± 0.10^{d}	64.70 ± 1.91^{defg}
Giant NS	10.05 ± 0.70^{h}	17.49 ± 2.82^{cd}	3.99 ± 0.63^{defg}	25.75 ± 1.98^{e}	48.72 ± 10.89^{abc}
JKUAT	8.61 ± 0.15^{fgh}	19.10 ± 0.93^{cd}	6.46 ± 0.28^{h}	22.83 ± 1.33^{e}	43.01 ± 1.28^{a}
KALRO Agric.	9.03 ± 0.68^{gh}	19.30 ± 1.73^{cd}	5.52 ± 0.44^{fgh}	21.99 ± 2.68^{e}	44.16 ± 5.21^{ab}

Values are Means ± Standard Deviation. Values with different superscript letters along the same column are significantly different at $P\leq0.05$ as assessed by Tukey's significant difference

Carbohydrates were the most abundant component in the berries. Black nightshade emerged significantly superior in carbohydrate content at all stages of berry ripening. The other three varieties were similar in their carbohydrate content. It was also observed that with the exception of Black nightshade, the other varieties showed a gradual decrease in the carbohydrate content through the ripening stages. Black nightshade, however, showed a different trend in which an increase was noted at the ripe stage which was the highest content at this stage followed by a decrease at senescence. This could be due to the varietal differences amongst the berries but should be subjected to further investigation. The protein content steadily increased from one stage of ripening to the next. Thus for all the varieties, the highest protein content was observed at senescence. At each stage, black nightshade was inferior to the other varieties. The aforementioned trend differed from that observed by Barbera *et al*. (1992) in which there was a decrease in protein content of prickly pears, implying that berries had a different trend to pears during maturation. This was true for the other varieties with the exception of black nightshade which showed a decrease in protein content through the first three stages before a spike which led slightly over double the protein content at senescence.

3.3.2 Minerals

Table 3.2 illustrates the changes in mineral content of the berries through the four ripening stages amongst the African nightshade varieties.

Table 3.2: Mineral Content (mg/100g) of *S. nigrum* Berries at Different Ripening Stages

Variety/Stage	Minerals						
	Ca	**Fe**	**K**	**Mg**	**Na**	**Zn**	**P**
Green							
KALRO Agric.	42.51±3.89^{bcd}	1.02±0.08^{bc}	13.69±0.82^{a}	91.30±4.51^{abcd}	2.54±0.18^{b}	0.88±0.02^{def}	33.03±2.31^{bc}
Black NS	77.66±2.93^{g}	1.36±0.03^{def}	25.18±2.44^{bc}	177.50±10.26^{g}	2.20±0.19^{b}	1.10±0.08^{ghi}	40.73±1.14^{c}
Giant NS	46.86±2.30^{d}	0.77±0.03^{ab}	12.09±0.43^{a}	84.70±4.81^{abc}	1.32±0.04^{a}	0.40±0.07^{b}	22.36±1.32^{a}
JKUAT Impr.	42.59±0.58^{bcd}	0.69±0.04^{a}	8.21±0.18^{a}	73.30±5.30^{a}	2.78±0.28^{b}	0.76±0.06^{cde}	17.41±1.67^{a}
Colour Break							
KALRO Agric.	47.71±0.38^{de}	1.14±0.09^{cd}	22.48±0.66^{b}	111.00±9.02^{ef}	3.79±0.12^{c}	0.90±0.08^{efg}	60.01±2.94^{def}
Black NS	55.07±2.07^{ef}	1.40±0.03^{def}	30.16±0.60^{cde}	224.30±8.96^{h}	5.25±0.22^{e}	1.15±0.14^{hi}	60.99±1.81^{ef}
Giant NS	45.63±0.96^{cd}	1.32±0.05^{de}	26.69±0.21^{bcd}	90.80±3.62^{abcd}	2.23±0.26^{b}	0.62±0.05^{c}	91.46±4.68^{j}
JKUAT Impr.	57.93±3.31^{f}	0.78±0.04^{ab}	24.08±0.60^{b}	96.70±4.02^{bcde}	3.47±0.50^{c}	0.67±0.04^{cd}	31.84±2.06^{b}
Ripe							
KALRO Agric.	48.56±2.37^{de}	1.41±0.07^{def}	32.54±2.58^{e}	126.90±5.05^{f}	4.42±0.14^{d}	1.00±0.04^{fgh}	78.00±2.65^{hi}
Black NS	56.87±3.02^{f}	1.64±0.14^{f}	44.46±1.08^{f}	167.70±4.52^{g}	6.71±0.89^{f}	1.15±0.08^{hi}	82.80±0.44^{i}
Giant NS	37.84±0.59^{bc}	1.94±0.07^{g}	34.74±1.55^{e}	101.90±7.61^{cde}	3.43±0.26^{c}	0.76±0.03^{cde}	53.68±3.17^{de}
JKUAT Impr.	42.48±2.72^{bcd}	0.96±0.04^{abc}	35.22±1.58^{e}	108.50±8.93^{def}	4.84±0.14^{de}	0.73±0.03^{cde}	75.95±4.23^{hi}
Senescence							
KALRO Agric.	35.44±0.79^{ab}	1.55±0.02^{ef}	43.99±3.33^{f}	108.00±6.42^{def}	6.36±0.34^{f}	1.47±0.07^{j}	52.00±3.01^{d}
Black NS	62.00±4.21^{f}	2.49±0.20^{h}	63.21±3.13^{h}	259.90±11.03^{i}	6.45±0.30^{f}	0.83±0.09^{cdef}	65.16±1.24^{fg}
Giant NS	34.94±2.27^{ab}	1.64±0.12^{f}	31.21±1.05^{de}	86.40±2.96^{abc}	6.45±0.10^{f}	1.30±0.05^{ij}	57.26±3.30^{def}
JKUAT Impr.	29.69±2.03^{a}	1.01±0.11^{bc}	53.93±3.21^{g}	77.40±4.60^{ab}	4.40±0.08^{d}	0.06±0.08^{a}	73.23±4.44^{gh}

Values are Means ± Standard Deviation. Values with different superscript letters along the same column are significantly different at P≤0.05 as assessed by Tukey's significant difference

Calcium values observed in this study (Table 3.2) were comparable to those obtained by (Sarma and Sarma, 2011). However, different varieties showed different trends with reference to calcium content. Black nightshade, which had the highest content at the green stage, recorded a sharp decrease at colour break which then remained steady at ripe stage. This was also the only variety to record an increase in calcium content at senescence.

Black nightshade also reigned superior over the other varieties in iron content having significantly higher values at all stages, with the highest value of 2.49 mg/100 g at senescence and a low of 1.36 mg/100 g while green. These values were slightly lower than the average value of 3.8 mg/100 g reported by (Sarma and Sarma, 2011). Nearly all varieties showed a similar trend in the iron content through the four stages of ripening. The iron content at senescence which was significantly higher than the content at the green and colour break stages. A similar trend was observed in the potassium content where all varieties showed a steady increase with increased ripening. Black nightshade was superior to the rest with a range of 25.18 – 63.21 mg/100 g while JKUAT variety had the least values while green at 8.21 mg/100 g. These values are in the range reported by Sarma and Sarma, (2011).

Magnesium was by far the most abundant mineral in the berries across all varieties. Among the varieties, black nightshade exhibited the highest content of magnesium at all the stages of ripening. The high content of magnesium in the berries is of nutritional importance because of the dietary minerals, magnesium is the fourth most abundant and the second most abundant intracellular cation (Volpe, 2013). It is an important co-factor in over 300 metabolic reactions (Laires *et al*., 2004; Emila and Swaminathan, 2013; Long and Romani, 2015). In terms of distribution, 50% of magnesium is in the bones while about 49% is in the tissue and organs (Baaij *et al*., 2015).

Changes in sodium contents in the ripening berries were similar for all the varieties. From the green to the ripe stage, all the varieties showed a steady increase in sodium content reaching highest concentrations at senescence. Zinc was one of the least abundant elements which also showed the most varied trends amongst the different varieties. KALRO and Giant nightshade varieties had a similar trend where the concentration increased gradually from the green through to the ripe stages. Black nightshade and JKUAT varieties showed no significant change in the first three stages but a dip at senescence made them record the lowest concentrations at this stage (0.83 mg/100 g and 0.06 mg/100 g, respectively). These values were lower than those reported by Sarma and Sarma, (2011). This could be attributed to the genotypic differences between the varieties and other growth parameters (Valadon *et al*., 1975). The decreases for these two varieties in the last stage were very significant, especially for the JKUAT variety.

Phosphorus was present in high quantities in all of the varieties. The trends of changes with ripening were largely similar amongst the varieties with three of them showing significant increases at colour break and ripe stages. The concentration then reduced at senescence albeit not statistically significant. Giant nightshade was the only variety to deviate from this trend. The trends observed in this study were consistent with findings from previous studies on berry ripening. Calcium content decreased as the fruits ripened while iron, potassium and phosphorus all increased through the ripening stages. Magnesium and sodium decreased as the fruits ripened but as they reached senescence, the content increased (Table 3.2). This is in agreement with findings of Tosun *et al.* (2008) that potassium, calcium and zinc changed moderately during ripening but magnesium, copper and iron presented significant changes throughout the ripening.

The most significant change was observed in magnesium because it is a central atom of the chlorophyll molecule (Marschner, 1995) and its insertion into the porphyrin structure is the first step in chlorophyll biosynthesis. As the fruits develop, calcium content of the cell wall increases to the fully grown but immature stage then this drops as the binding form of calcium in the tissue changes just before ripening (Tosun *et al*., 2008). A similar trend was observed for magnesium. However, after ripening, the two minerals again accumulate in the cell wall and this could explain the fluctuations that were observed in the content of the two minerals in the berries. The accumulation could be due to the release of calcium from the breakdown and disassociation of calcium cross-linked pectins (Hocking *et al*., 2016) and freeing of magnesium from the chlorophyll molecule (Marschner, 1995). Conclusively, the mineral content of the berries is comparable to other berries and fruits (Rop *et al*., 2011; Bvenura and Afolayan, 2016;) and this means the nightshade berries, if incorporated into the diet, can help to meet the nutritional needs of the consumers.

3.3.3 Phytochemicals

With reference to the phytochemical content, the findings are as reported on Table 3.3.

Table 3.3: Phytochemical Content (mg/g) of *S. nigrum* Berries at Different Ripening Stages

Variety/Stage	Total Phenols	Tannins (mg/100g)	Flavonoids	Oxalates	Phytates
Green					
KALRO Agric.	0.95 ± 0.01^{i}	8.18 ± 0.47^{ef}	2.47 ± 0.03^{de}	9.18 ± 0.34^{fg}	5.53 ± 0.40^{g}
Black NS	0.77 ± 0.03^{fg}	4.43 ± 0.31^{abc}	7.20 ± 0.27^{i}	9.84 ± 0.87^{fg}	3.91 ± 0.08^{f}
Giant NS	0.84 ± 0.04^{gh}	4.50 ± 0.18^{abc}	2.51 ± 0.11^{e}	11.87 ± 0.14^{h}	5.35 ± 0.46^{g}
JKUAT Impr.	0.42 ± 0.01^{ab}	7.16 ± 0.34^{cdef}	1.26 ± 0.13^{bc}	12.16 ± 0.32^{h}	4.26 ± 0.21^{f}
Colour Break					
KALRO Agric.	0.46 ± 0.02^{bc}	5.80 ± 1.22^{bcde}	3.19 ± 0.09^{f}	8.60 ± 0.52^{def}	3.16 ± 0.06^{e}
Black NS	1.20 ± 0.01^{j}	16.76 ± 0.70^{h}	1.31 ± 0.05^{bc}	9.48 ± 0.52^{fg}	1.10 ± 0.05^{cd}
Giant NS	0.39 ± 0.00^{a}	8.11 ± 1.58^{ef}	2.17 ± 0.03^{d}	10.16 ± 0.10^{g}	3.12 ± 0.13^{e}
JKUAT Impr.	0.49 ± 0.06^{cd}	11.50 ± 0.51^{g}	2.18 ± 0.09^{d}	8.92 ± 0.49^{efg}	2.88 ± 0.05^{e}
Ripe					
KALRO Agric.	0.53 ± 0.02^{de}	20.97 ± 0.48^{i}	1.47 ± 0.04^{c}	7.39 ± 0.35^{cd}	0.88 ± 0.03^{bc}
Black NS	1.30 ± 0.01^{k}	2.41 ± 0.60^{a}	0.76 ± 0.02^{a}	6.13 ± 0.13^{bc}	0.85 ± 0.40^{abc}
Giant NS	0.75 ± 0.01^{f}	5.84 ± 1.79^{bcde}	4.55 ± 0.15^{g}	5.84 ± 0.43^{ab}	1.43 ± 0.07^{cd}
JKUAT Impr.	0.82 ± 0.01^{gh}	22.64 ± 1.64^{i}	5.02 ± 0.13^{h}	7.83 ± 0.76^{de}	0.86 ± 0.02^{abc}
Senescence					
KALRO Agric.	0.70 ± 0.01^{f}	8.84 ± 1.47^{fg}	1.12 ± 0.05^{b}	5.60 ± 0.06^{ab}	0.39 ± 0.05^{ab}
Black NS	0.87 ± 0.01^{h}	5.13 ± 0.31^{abcd}	1.05 ± 0.01^{ab}	5.63 ± 0.20^{ab}	0.26 ± 0.01^{a}
Giant NS	0.58 ± 0.01^{e}	7.62 ± 0.38^{def}	1.52 ± 0.01^{c}	5.23 ± 0.16^{ab}	1.55 ± 0.07^{d}
JKUAT Impr.	0.73 ± 0.01^{f}	3.80 ± 0.12^{ab}	1.33 ± 0.01^{bc}	4.64 ± 0.52^{a}	0.39 ± 0.03^{ab}

Values are Means ± Standard Deviation. Values with different superscript letters along the same column are significantly different at $P \leq 0.05$ as assessed by Tukey's significant difference

The concentration of oxalates was higher in Giant NS and JKUAT varieties at the green stage. KALRO agriculture and black nightshade had statistically similar concentrations at this stage too. As the fruits started ripening, the concentration in all the varieties started decreasing and maintained this trend to senescence. At this final stage, all the varieties had statistically similar low levels of oxalates. This decreasing trend has been previously reported by Vũ *et al*. (2013) who observed that green kiwi fruits had higher content than golden kiwi fruits. The low levels implies that intake of African nightshade berries in the diet would not increase the risk of formation of kidney stones (Massey, 2007).

Phytate content in the berries ranged from 0.26 to 5.53 mg/g with the highest values recorded at the green stage followed by a downward trend in concentration as the berries ripened. This concentration levels are within the range of the contents observed in Brazilian fruits (Marin *et al.*, 2009). These low levels make the berries a good addition to the diet as they ensure there is greater availability of minerals such as calcium, iron and zinc which would otherwise be bound within the phytic complexes, making them non-bioavailable (Ellis *et al*., 1987; Saha *et al*., 1994).

Tannin content showed varying trends amongst the varieties as they ripened. It was interesting to note that while KALRO (20.97 mg/100g) and JKUAT (22.64 mg/100g) varieties had the highest values at the ripe stage, Black nightshade had the lowest value (2.41 mg/100g). Giant nightshade also recorded one of its lowest values. Generally, tannin values reported in this study were quite low compared to those reported in 18 Brazilian fruits (15.7 mg/100g in Cagaita to 472.2 mg/100g in Baru nut) (Marin *et al*., 2009). Since the tannin content is affected by soil composition and other environmental factors (Davidsson, 2003), these could have led to the disparities observed in this study.

The levels of total phenols were quite varied amongst the varieties. Giant nightshade and JKUAT varieties showed a similar trend in the content as they both had significant decreases at colour break. The total phenols content then increased at the ripe stage before decreasing at senescence. KALRO agriculture exhibited decrease at colour break and a subsequent steady increase with increased ripening. Whereas black nightshade presented an increase at colour break and ripe stages followed by a decrease at senescence. At the green stage, black nightshade did not differ much from the other varieties but the continuous rise in phenolic levels made it superior at the other ripening stages. The decrease noted as the fruits ripened could be due to increase in polyphenol oxidase activity (Rop *et al*., 2011). A similar trend in different strawberry varieties was observed by other authors (Aaby *et al*., 2012) as the phenolic content varied amongst cultivars but with minor changes in berries of the same cultivar as they ripened. The total phenolic contents varied from 0.39 (Giant nightshade at colour break) to 1.30 mg/g (ripe Black nightshade), a range that corresponded to that reported in strawberry at 0.57 to 1.33 mg/g (Aaby *et al*., 2012).

Flavonoid concentration had a similar trend in KALRO agriculture and JKUAT varieties. The content increased as the berries started breaking their colours and then decreased through the last stages of development. Black nightshade posted a major decrease at colour break followed by a further decrease while ripe before a slight, albeit insignificant increase at senescence. Giant nightshade demonstrated a decrease at colour break before a high increase while ripe and finally posted an equally significant drop at senescence. Amongst the varieties, black nightshade posted the highest value while green (7.20 mg/g) but also posted the least value (0.76 mg/g) when ripe. Given this trend, it can be concluded that the best antioxidant capacity of the berries can be attained at colour break, which is in agreement to the conclusion by Jain *et al*. (2003). Flavonoids and phenols are directly related to the antioxidant activity of berries (Wang *et al*., 2009) and as such, their concentration in these berries is of interest to the consumers.

3.3.4 Vitamin C, Total Carotenoids and TTA

Vitamin C concentration (Table 3.4) was comparable amongst varieties at the green, ripe and colour break stages. This was with the exception of Black NS which emerged superior with the highest content of vitamin C at the ripe stage. This is in agreement with the observation by Skrovankova *et al*. (2015) that different varieties of berries could exhibit different levels of acid accumulation. However, at senescence, KALRO agriculture variety had the lowest content (8.12 mg/100 g) which was statistically different from Giant NS which recorded the highest value at 18.63 mg/100 g. Though these values were lower than those recorded in medlar fruits (Rop *et al*., 2011), they fall within the range of 5 to 50 mg/100 g vitamin C recorded in strawberries and raspberries (Skrovankova *et al*., 2015), which are termed as the richest sources of vitamin C amongst berries. The trend in concentration at all stages was also similar with all varieties manifesting an increase in concentration through the ripening stages, which affirms the position by Wang *et al*. (2009) that immature berries contain lower levels of acids than ripe berries.

Table 3.4: Vitamin C (mg/100g), TTA (%) and Total Carotenoids (mg/100g) Contents of *S. nigrum* Berries at Different Ripening Stages

Variety/Stage	Total Carotenoids	TTA	Vit. C
Green			
KALRO Agric.	0.20 ± 0.10^{c}	15.92 ± 0.49^{g}	1.55 ± 0.03^{a}
Black NS	0.23 ± 0.01^{c}	3.76 ± 0.15^{b}	3.91 ± 0.04^{ab}
Giant NS	0.16 ± 0.00^{b}	5.30 ± 0.45^{c}	3.57 ± 0.15^{ab}
JKUAT Impr.	0.09 ± 0.01^{a}	9.76 ± 0.74^{e}	3.25 ± 0.05^{ab}
Colour Break			
KALRO Agric.	0.28 ± 0.01^{d}	11.74 ± 0.75^{f}	2.13 ± 0.05^{a}
Black NS	0.21 ± 0.01^{c}	3.52 ± 0.26^{b}	7.04 ± 0.15^{abc}
Giant NS	0.49 ± 0.01^{g}	3.89 ± 0.05^{b}	5.34 ± 0.07^{ab}
JKUAT Impr.	0.37 ± 0.01^{e}	9.25 ± 0.26^{e}	4.39 ± 0.11^{ab}
Ripe			
KALRO Agric.	0.28 ± 0.00^{d}	9.69 ± 0.12^{e}	4.05 ± 0.03^{ab}
Black NS	2.29 ± 0.01^{i}	3.07 ± 0.06^{ab}	11.63 ± 0.23^{bcd}
Giant NS	0.38 ± 0.01^{e}	3.55 ± 0.17^{b}	4.51 ± 0.01^{ab}
JKUAT Impr.	0.34 ± 0.01^{e}	6.63 ± 0.40^{d}	5.12 ± 0.03^{ab}
Senescence			
KALRO Agric.	0.42 ± 0.01^{f}	7.58 ± 0.24^{d}	8.12 ± 0.16^{abc}
Black NS	1.10 ± 0.03^{h}	2.16 ± 0.01^{a}	16.22 ± 0.32^{cd}
Giant NS	0.23 ± 0.01^{c}	3.14 ± 0.05^{ab}	18.63 ± 0.47^{d}
JKUAT Impr.	0.27 ± 0.02^{d}	5.29 ± 0.41^{c}	9.45 ± 0.01^{abcd}

Values are Means ± Standard Deviation. Values with different superscript letters along the same column are significantly different at $P\leq0.05$ as assessed by Tukey's significant difference

An inverse trend to that of vitamin C was observed in the percentage of titrable acidity. This reduced significantly in all the varieties as they ripened. It was also noted that TTA varied amongst the varieties at each particular stage. KALRO agriculture posted the highest value at 15.92% which gradually reduced to 7.58% at senescence. Black NS was on the lowest end in TTA, scoring 3.76% at green stage which reduced to 2.16% at senescence. These levels of TTA are comparable to those reported by Tosun *et al*. (2008) who recorded TTA of blackberries in the range of 5.78% (ripe) to 14.80% (red). The decrease in acidity as the berries ripen is consistent with the findings by Balic *et al*. (2018) and Barbera *et al*. (1992) who recorded a decrease in acidity of grape berries and prickly pears, respectively, as they ripened. This decrease as berries ripen could be due to the effects of respiration or conversion of organic acids into sugars (Tosun *et al*., 2008).

A major disparity was observed in the levels of total carotenoids. The quantities of carotenoids in black nightshade were higher than the other varieties at all stages of ripeness. This was particularly so at the ripe stage where it reached 2.29 mg/100 g. It was observed that the content in carotenoids increased from the green to colour break stages, with the exception of black nightshade which recorded a decrease at this stage. The concentration then increased to ripe stage and then started reducing as the berries reached senescence. This observation is consistent with an earlier study done by Valadon *et al*. (1975) in which it was observed that carotenoids of berries increase on ripening as does the content in guavas (Jain *et al*., 2003). This is attributable to the fact that as fruits ripen, chlorophyll disappears and chloroplasts degenerate to chromoplasts. Control of carotenoid synthesis is removed and oxidative processes take place leading to appearance of different carotenoids (Valadon *et al*., 1975).

3.4 Conclusion

Though different amongst varieties, berries of the African Nightshade have macro- and micro-nutrient content that is comparable to other berries and fruits. The content of oxalates, total phenols, flavonoids and phytates decreased as the fruits ripened. On the other hand, the content of vitamin C, tannins and total carotenoids increased with ripening. It is evident from the observed trends that ripening reduces the anti-nutritional factors while enhancing the much needed vitamin C. This is of nutritional relevance given that the fruits are consumed at the ripe stage. Black nightshade, which is the local variety grown in Kenya, had significantly higher beta-carotene content compared to the other varieties. This higher content gave the berries an orange-yellow colour while all other varieties were purple-black. It also had lower percentage of titrable acidity. They have anti-nutrient factors at the earlier maturity stages but these reduce at the ripe stage which is the point at which the fruits are eaten. As such, berries should be picked at a particular stage depending on the nutrient that is required. Further, given their nutritional value which is largely consistent with other commonly consumed berries, the African nightshade berries should be incorporated in the diet as an avenue of diet diversification and optimal utilization of a popular indigenous plant.

CHAPTER FOUR

SUGAR CONTENT AND PHYSICAL CHARACTERIZATION OF FOUR SELECTED AFRICAN NIGHTSHADE *(SOLANUM NIGRUM COMPLEX)* EDIBLE BERRIES

4.1 Introduction

In most fruits, advances in maturity are accompanied by several coordinated physiological, biochemical and structural processes (Zhang *et al*., 2006; Fawole and Opara, 2013; Jia *et al*., 2013). Various studies have addressed the metabolism of sugars and changes in biochemical and physiological properties in apples (Mingjun Li *et al*., 2018), sea buckthorn fruits (Zhang *et al*., 2015; Tang, 2016), strawberries (Jia *et al*., 2013), pomegranate (Legua *et al*., 2000; Al-maiman and Ahmad, 2002; Fawole and Opara, 2013;) and grapevines (Castellarin *et al*., 2011; Lecourieux *et al*., 2014) among others. These studies showed the increase in size, change in skin colour, softening and changes in sugar fractions of the respective fruits. To the best of our knowledge, the sugar content and physical characterization of *Solanum nigrum complex* berries has not been explored. Therefore, the objective of this study was to physically characterize the African nightshade berries and demonstrate changes in their sugar content with ripening stages.

4.2 Materials and Methods

4.2.1 Sample Preparation

The *S. nigrum* berries were cultivated and prepared as described in section 3.2.1. Samples for sugar analysis were prepared as described in section 3.2.2.

For colour, size and firmness, the fresh berries were harvested as indicated by Ayua *et al*. (2016) in order to minimize respiratory changes. The berries were picked early in the morning, between 8 a.m. and 9 a.m. They were placed in air-tight containers, labelled appropriately and then immediately kept in a cool-box. They samples were immediately transported to the laboratory at JKUAT and the analyses carried out.

4.2.2 Sugars

Sugars were analysed as described by Ribera-Fonseca *et al.* (2016) using HPLC (20A Series, Shimadzu Co-operation, Kyoto, Japan). The samples were prepared by grinding a sample of 10 g of the berries and placing it in a 100 ml conical flask. This was followed by addition of 50 ml of 96% ethyl alcohol. After refluxing the mixture for 1 hour at 100^{0}C, the slurry was filtered and the conical flask rinsed with 5 ml of 80% ethyl alcohol. The filtrate was then evaporated at 60 ^{0}C to attain dryness before being dissolved in 10 ml of distilled water. From this solution, 2 ml was taken and combined with 2 ml acetonitrile. The mixture was then filtered through a 0.45 micro-filter and placed in vials, ready for injection into the HPLC system. For calibration purposes, sucrose - $C_{12}H_{22}0_{11}$ (RANKEM^{TM}, Germany) was used as the standard for sucrose while D-Fructose - $C_6H_{12}0_6$ (Duchefa Biochemie, Netherlands) and D(+)-Glucose Riedel-de Haën, Germany) were the standards used for fructose and glucose, respectively.

4.2.3 Berry Firmness

Berry firmness was determined as described by Lee and Lee (2014) using the Rheometer (Compac-100, Sun Scientific Co., Japan). The mode was set at 20 with maximum load of 10 kg, R/H hold of 2.0 mm and the P/T press was set at 300 mm/minute. For each ripening stage of each variety of African nightshade, 5 replicates were taken.

4.2.4 Berry Size

The sizes of the berries were determined using the method of Li *et al*. (2015) using vernier calipers. Fruits were chosen at random from each variety at each stage of ripening with the fruits in the mid-upper parts of the shoot being the most preferred. These measurements were taken 5 times for each variety at each of the four ripening stages and the mean values obtained.

4.2.5 Berry Colour

Berry colour was determined using the method by Leite *et al*. (2020). This was done using a colour spectrophotometer (Model CR – 200 – Minolta, Japan). The parameters L* for lightness indicating luminosity of the fruits, a* is the transition from green colour (-a*) for the red colour (+a*) and b* the transition from the blue colour (-b*) to the yellowness (+b*). A white tile was used as a reference. All the values were taken in triplicate.

4.2.6 Statistical Analyses

Data was analyzed using the one way Analyses of Variance (ANOVA) using Genstat Version 14 (VSN International Ltd., UK, 2013). The means were separated using Tukey's HSD significance difference at a significance level of 0.05.

4.3 Results and Discussions

4.3.1 Sugars

The trends in berry sucrose, glucose and fructose were as reported on Table 4.1.The content of fructose in most of the berries increased from the green to ripe stage and then exhibited a decline. KALRO Agric. showed the sharpest decline at this stage. Black NS was exceptional from this trend as the content peaked at colour break, decreased at the ripe stage and then rose again at the senescence stage. Nevertheless, the content at the senescence was statistically similar to the content at colour break, which was the highest recorded of the varieties. This is an indication that Black NS was clearly superior in terms of fructose content compared to the other varieties. There was a continuous, steady increase in glucose content for all the varieties from the ripe stage through to senescence. Berry sugars observed a rapid accumulation in the last stages of ripening which could be associated with up-regulation of sugar-related genes such as cell wall invertases, hexose transporters and vacuolar invertases (Zhang *et al*., 2006; Hayes *et al*., 2007).

Only JKUAT variety had a decrease in the glucose content at senescence, though this was not significant from the ripe stage which demonstrated the highest content for this particular variety. Glucose content was very low at the green and colour break stages for all the varieties. However, on ripening, the content observed a major spike. Compared to the other varieties, KALRO Agric was significantly inferior in glucose content, recording the highest value of 22.20 mg/100 g at senescence compared to Giant NS's 172.44 mg/100 g at the same stage.

Table 4.1: Developmentally-related Changes in Sugars (mg/100 g) in Four Selected Varieties of African Nightshade *(Solanum nigrum complex)* Edible Berries

Variety			
Green	**Fructose**	**Glucose**	**Sucrose**
KALRO Agic.	17.00 ± 0.62^{de}	0.25 ± 0.00^{a}	ND
Black NS	24.21 ± 0.18^{hi}	3.28 ± 0.02^{a}	ND
Giant NS	14.99 ± 0.46^{c}	2.78 ± 0.05^{a}	ND
JKUAT Impr.	16.29 ± 0.70^{cd}	2.38 ± 0.03^{a}	ND
Colour Break			
KALRO Agric.	18.74 ± 0.53^{ef}	11.74 ± 0.07^{a}	0.60 ± 0.01^{a}
Black NS	30.65 ± 0.20^{k}	8.95 ± 0.14^{a}	2.00 ± 0.06^{a}
Giant NS	21.23 ± 0.94^{g}	7.76 ± 0.28^{a}	ND
JKUAT Impr.	16.76 ± 0.07^{d}	2.38 ± 0.07^{a}	0.70 ± 0.01^{a}
Ripe			
KALRO Agric.	27.03 ± 1.13^{j}	20.17 ± 0.70^{a}	252.3 ± 8.50^{d}
Black NS	25.79 ± 0.45^{ij}	156.29 ± 62.72^{bc}	283.8 ± 6.42^{f}
Giant NS	29.36 ± 0.66^{k}	165.13 ± 5.13^{c}	267.8 ± 0.32^{e}
JKUAT Impr.	20.07 ± 0.61^{fg}	125.07 ± 3.85^{bc}	261.0 ± 11.06^{de}
Senescence			
KALRO Agric.	13.15 ± 0.66^{b}	22.20 ± 0.37^{a}	66.00 ± 2.58^{b}
Black NS	29.35 ± 0.42^{k}	161.45 ± 7.00^{c}	388.40 ± 7.40^{g}
Giant NS	23.94 ± 0.22^{h}	172.44 ± 3.79^{c}	211.30 ± 3.90^{c}
JKUAT Impr.	9.43 ± 0.29^{a}	111.38 ± 3.42^{b}	206.20 ± 3.17^{c}

Values are Means ± Standard Deviation. Values with different superscript letters along the same

column are significantly different at $P\leq0.05$

ND = Not detected

The trends in reducing sugars in this study agree with previous studies. A study on seabuckthorn berries indicated that glucose and fructose constituted nearly the whole of sugar fraction in the berries (Raffo *et al*., 2004). However, glucose was the major sugar, an observation corroborated by Legua *et al*. (2000). This is despite the fact that the two hexose sugars are as a result of the breakdown of sucrose and, therefore, are expected to be present in equal proportions. However, the higher glucose levels could be accounted by the fact that glucose is an essential sugar not just for fruit ripening but also for other plant metabolism processes, nuclear and biochemical processes such as signaling, growth, respiration and development (Aksic *et al.*, 2019). Cultivars showed different trends in sugars with ripening though at the end, all of them tend to show decreasing sugar levels. The variability in sugar accumulation among different genotypes was also reported by Tang (2016).

In other studies, juice made from the pomegranate fruits contained 12-16% sugar mainly comprising of fructose and glucose with the latter being more predominant (Legua *et al*., 2000; Al-Maiman and Ahmad, 2002; Aksic et al., 2019). A similar observation was made by Fawole and Opara (2013) as fructose and glucose increased during maturation and the ratios of glucose to fructose (G/F) ranging from 0.67:0.85 to 0.72:0.86 in Bhangwa and Ruby fruits grown in South Africa, respectively. This in agreement with the report of Al-Maiman and Ahmad (2002) in which, glucose levels were higher than fructose at the unripe, half-ripe and full ripe stages of development in 'Taifi' cultivar of pomegranate. Davies and Robinson (1996) concluded that during the ripening of grape berries, sucrose is transported from the leaves accumulates in the vacuoles of the berries (sink cells) as glucose and fructose which could explain the influx of the two sugars during fruit development.

Sucrose was not detected at the green stage for all the varieties but low quantities were observed at colour break for all the varieties except Giant NS. This was followed by a considerable increase at the ripe stage with the varieties indicating values in the range of 252 – 283 mg/100 g. At senescence, there was a significant decrease in sucrose content for three of the varieties with the exception of Black NS which continued to record a significant rise in the content, peaking at 388 mg/100 g at senescence, the highest value recorded for the sugars. Sucrose accumulation in the current study showed a trend where there was little or no traces in the first stages of development but observed a major spike during the senescence stage (overripe). This increase in sucrose is a deviation from previous studies on berries which did not report on the increase in sucrose. Besides the fact that sucrose is a major sugar in the fruit development (Lecourieux *et al*., 2014; Mingjun Li *et al*., 2018), this phenomenon could also be explained by the fact that sucrose is an important signal in the regulation of berry ripening as demonstrated by Jia *et al*. (2013). This study revealed that apart from the traditional understanding of the role of sugars in plants where they are metabolic resources for carbon skeleton construction and energy sources, sugar signaling could also be involved in other functions such as seed germination, embryogenesis, vegetative and reproductive growth as well as senescence (Yamaki, 2010; Jia *et al*., 2013).

In the sink cells, the sucrose cycle, also known as the sucrose-sucrose cycle or the futile sucrose cycle, is an important pathway (Mingjun Li *et al*., 2018). The cycle involves the action of sucrose synthase and invertase for the liberation of hexoses (glucose and fructose). These hexoses are then phosphorylated and there is inter-conversion between hexose phosphates and UDP-glucose. Finally, there is the re-synthesis of sucrose through the enzymes sucrose-6-phosphate synthase (SPS) and sucrose-6-phosphate phosphatase (Yamaki, 2010; Lecourieux *et al*., 2014; Mingjun Li *et al*., 2018). This cycle allows for connection of sugar with other metabolic pathways such as the glycolysis, TCA cycle, starch and cellulose synthesis (Mingjun Li *et al*., 2018). Since the biochemical regulation of the sucrose-sucrose cycle and the associated transport system is not fully understood (Mingjun Li *et al*., 2018), it can be hypothesized that the accumulation of sucrose in the African nightshade berries at senescence is as a result of under-regulation of sugar related enzymes and genes during deterioration at senescence. Consequently, the sucrose from the leaves is not converted to glucose and fructose and the sucrose-sucrose cycle stops. As such, sucrose accumulates in the sink cells in its initial form. Given that sugars in fruits are not only essential for fruit growth and development but also for the overall quality of the fruit (Mingjun Li *et al.*, 2018), more studies could be carried out to investigate the accumulation of sucrose during senescence in *Solanum nigrum* edible berries.

4.3.2 Colour

The colour characteristics of the berries were as indicated in Table 4.2. Fruit colour is one of the appearance aspects that consumers use to evaluate for quality (Kader, 1999). KALRO Agric., Giant NS and JKUAT varieties had similar colour characteristics in nearly all the stages of development.

Table 4.2: Colour Changes in 4 Varieties of African Nightshade Edible Berries with Ripening

Variety/Stage	L*	a*	b*
Green			
KALRO Agric.	66.47 ± 19.19^{d}	-12.53 ± 0.93^{ab}	1.55 ± 1.36^{a}
Black NS	32.23 ± 6.41^{ab}	-10.9 ± 0.56^{b}	15.7 ± 2.26^{b}
Giant NS	53.77 ± 4.04^{cd}	-17.57 ± 0.38^{a}	27.13 ± 0.72^{cd}
JKUAT Impr.	44.53 ± 1.66^{abc}	-14.77 ± 2.60^{ab}	21.13 ± 2.80^{bc}
Colour Break			
KALRO Agric.	38.8 ± 0.75^{abc}	-1.27 ± 0.31^{c}	4.57 ± 0.95^{a}
Black NS	41.63 ± 0.83^{abc}	4.47 ± 1.16^{d}	33.97 ± 1.04^{d}
Giant NS	35.7 ± 1.15^{abc}	2.60 ± 1.40^{cd}	3.90 ± 3.30^{a}
JKUAT Impr.	65.63 ± 13.82^{d}	-12.33 ± 5.33^{b}	21.77 ± 8.78^{bc}
Ripe			
KALRO Agric.	28.20 ± 2.89^{d}	3.83 ± 0.49^{d}	2.07 ± 0.72^{a}
Black NS	49.40 ± 0.40^{bcd}	15.60 ± 0.50^{e}	43.27 ± 1.14^{e}
Giant NS	33.20 ± 0.70^{ab}	5.20 ± 0.53^{d}	4.53 ± 3.61^{a}
JKUAT Impr.	29.03 ± 1.81^{a}	3.63 ± 1.15^{cd}	2.07 ± 0.21^{a}
Senescence			
KALRO Agric.	28.23 ± 0.37^{a}	1.87 ± 1.26^{cd}	1.77 ± 0.55^{a}
Black NS	52.80 ± 0.44^{cd}	21.60 ± 0.20^{f}	46.23 ± 0.32^{e}
Giant NS	28.80 ± 1.35^{a}	2.57 ± 0.72^{cd}	1.67 ± 0.49^{a}
JKUAT Impr.	27.33 ± 0.40^{a}	0.87 ± 0.15^{cd}	1.57 ± 0.45^{a}

Values are Means±Standard Deviation. Values with different superscript letters along the same column are significantly different at $P \leq 0.05$

High L* values were observed at the green stage but decreased as the berries ripened. Black NS, however, exhibited a different trend where the L* value was low at the green stage but increased continuously to senescence. The a* value increased for all the varieties although Black NS still recorded higher values compared to the rest. KALRO Agric., Giant NS and JKUAT varieties had the highest a* values at colour break and then the value decreased to 1.57 to 1.77 while the value for Black NS increased to the last stage, reaching 46.23. The changes in a* values is likely due to accumulation of anthocyanins in the berries (Fuentes *et al*., 2019). The differences in L* a* b* values could be attributed to the fact that despite all the varieties being green at unripe stage, Black NS acquired a bright orange colour upon ripening different from the other varieties which acquired a purplish-black colour. This could have an impact on the acceptability of the berries by consumers (Dobrzanski and Rybczynski, 1999; Kader, 1999)

4.3.3 Firmness

Changes in berry firmness with ripening were as reported on Figure 4.1.

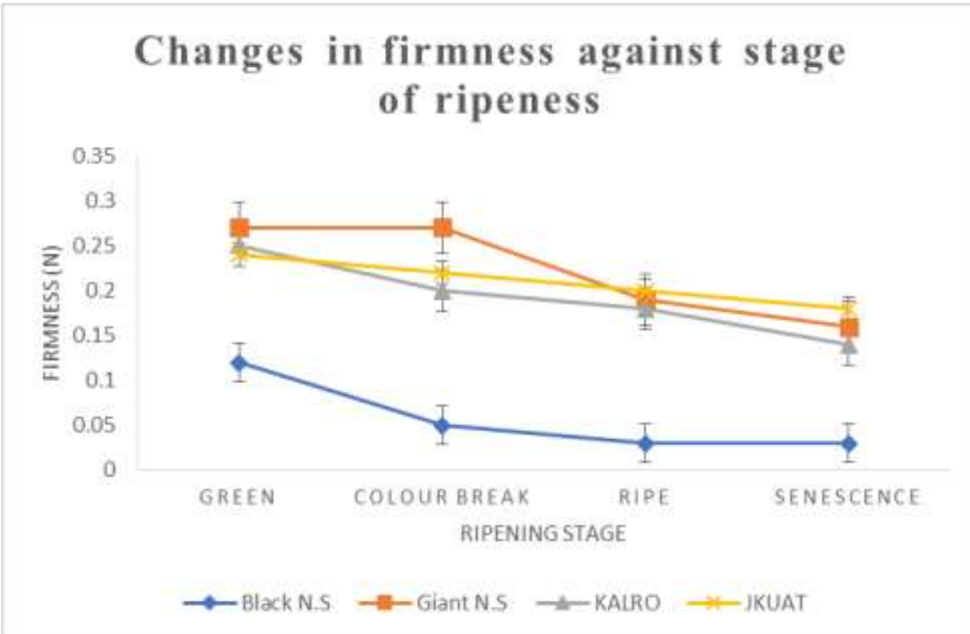

Figure 4.1: Changes in Berry Firmness of Four Varieties of *Solanum nigrum* against the Ripening Stages

Fruit ripening is achieved through chlorophyll degradation and cell wall lysis (Forlani *et al*., 2019). During ripening, fruit softening is a function of coordinated processes of modification of the polysaccharides making up the cell wall and the middle lamella, leading to the weakening of the structure (Brummell, 2006) as well as an increase in ethylene production (Kan *et al*., 2013). In this study, all the four varieties decreased in firmness from the green stage to senescence. Giant NS had the most firm fruits at colour break. It was observed, as shown in Figure 4.1, that Giant NS, KALRO Agric and JKUAT varieties were not significantly different in firmness of their fruits at the green, ripe and senescence stages. Black NS was significantly different from the other varieties at all stages, recording the lowest firmness values of all the varieties at all stages. This is consistent with the conclusion by Brummell *et al*. (2004) that softening and textural changes that occur during ripening are characteristic of particular species. For all the varieties, fruit softening was initiated at veraison and remained on a downward trend to senescence, a trend that was also reported by Brummell *et al*. (2004) while studying the cell wall changes in ripening of peach fruits.

Trends observed in this study correspond with previous related studies. Castellarin *et al*. (2016) studied the changes in grapes and concluded that berry firmness reached peak at full grown but unripe stage and then consistently decreased to the end of the study. This study showed that majority of the softening in grapes seemed to take place just prior to the change of colour. The possible reason for the softening could be cell wall modification (Castellarin *et al*., 2011). While analyzing the changes in firmness of grape berries during ripening, Nunan *et al.* (1998) observed that at veraison, there was a rise in the protein content and an approximate 3-fold increase in the hydroxyproline content.

Of the pectic polysaccharides, type 1 arabinogalactan decreased by about 80% from 20 mol% to 4 mol% of total cell wall. Galacturonan increased by almost 2-fold and became more soluble (Nunan *et al*., 1998). This is further corroborated by Brummell *et al*. (2004) in a study where softening of mature fruits prior to ripening was associated with depolymerization of matric glycans that are both loosely and tightly attached to cellulose. The softening could also be attributed to loss of galacturonan from cell wall fractions. This continued during ripening and was coupled by major losses of arabinogalactan from the loosely bound matric glycan fraction. At senescence, the excessive softening of peach fruits was associated with dramatic depolymerization of chelator-soluble polyuronides. The depolymerization was facilitated by the increase in solubilization of the chelator-soluble polyuronides (Brummell *et al*., 2004). Given the consistency in the trends in berry ripening in this study and previous studies, it can be concluded that the changes can be attributed to changes in composition of cell wall polysaccharides as well as significant modification in specific polysaccharides. More studies can be carried out on the African nightshade berries to ascertain the specific changes that take place as they ripen.

4.3.4 Size

Berry sizes are as indicated in Figure 4.2. The berry sizes were significantly different amongst the varieties. There were very gradual, insignificant decreases in berry size for the Giant NS, KALRO and JKUAT varieties. However, Black NS showed a different trend where the berry sizes seemed to increase slightly with each ripening stage. While the berry sizes were significantly different amongst the varieties at each stage, the size within each variety did not show any significant change in size with ripening. Previously, grape berries were reported to increase in diameter and weight in the early development stages (Castellarin *et al*., 2016) but not in the latter stages of development.

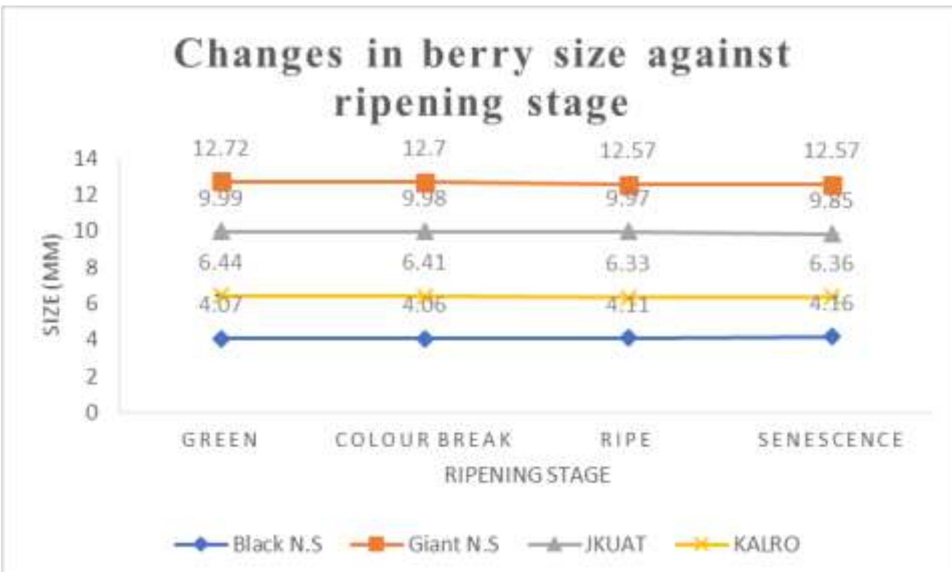

Figure 4.2: Changes in Berry Size (mm) of Four Varieties of *Solanum nigrum* against the Ripening Stages

4.4 Conclusion

Findings from this study indicate that African nightshade edible berries have comparable characteristics with other fleshy, non-climacteric fruits that are commonly consumed in the household diet. They accumulate reducing sugars as they develop but have higher glucose than fructose fraction. Sucrose is present in high amounts at senescence. These characteristics could contribute to organoleptic quality and appeal to consumers. Black NS was considerably different from the other varieties, having a different colour, smaller size, higher sugar content and less firmness compared to the other varieties. This could imply that its application in the diet or other commercial purposes could be different from the others. Though not commonly consumed, the berries could be a valuable addition to the household diet through dietary diversification and for commercial exploitation through value addition and processing. However, research should be carried out to determine the exact pathways responsible for the ripening and accumulation of sugars in the berries.

CHAPTER FIVE

ANTIMICROBIAL ACTIVITY OF AFRICAN NIGHTSHADE (*SOLANUM NIGRUM COMPLEX*) EDIBLE BERRIES EXTRACTS

5.1 Introduction

Puupponen-Pimia *et al*. (2001) concluded that the antimicrobial effect of berries is dependent on the species as well as type of micro-organism studied. Although other berries have been extensively screened for their anti-microbial activity, little has been done on the berries of the African nightshade. Much attention has been on the leafy part of the crop, neglecting the potential of the berries as a food item. Consequently, there is minimal evidence to support their use as a food item or any application in food processing and preservation. This study aimed at filling this knowledge gap by screening for the antimicrobial properties of the berries.

5.2 Materials and Methods

5.2.1 Preparation of Plant Sample

Sixteen (16) samples were screened for their anti-microbial activity. These were samples from four African nightshade varieties; Giant NS, Black NS, JKUAT Improved and KALRO Agriculture varieties. The berries were harvested at the four stages of ripening; green, colour break, ripe and senescence. The samples were oven-dried till they attained a constant weight. This was followed by pulverization in a super mixer grinder and then packed in sterile paper bags. These were then stored at room temperature awaiting further tests (Matasyoh *et al*., 2014).

5.2.2 Crude Extract Preparation

This was done as described by Mostafa *et al*. (2018) and Liepiņa *et al*. (2013) with modifications. About 10 g of the powdered sample was soaked in 40 ml of ethanol for 2 hours. The macerated material was then ground using a mortar and pestle before being mixed with an extra 40 ml of ethanol. This was extracted for a further 10 hours and then filtered through double layer of muslin cloth. These were then centrifuged for 10 minutes at 9000 rpm and filtered using Whatman filter paper No. 41 to obtain a clear filtrate. The filtrates were then evaporated and dried at 40^o C then stored in closed bottles awaiting further tests. The concentration of the final extract was 0.2 mg/mL. The extracts were weighed and yield percentages calculated as follows:

$$yield\% = \frac{R}{S} x\ 100$$

Where R = weight of extracted plant residues and S = weight of raw sample (Mostafa *et al*., 2018).

5.2.3 Test Microbes

Five microbes were used in the anti-microbial tests. These included Gram positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778) as well as Gram negative bacteria *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (ATCC 13347). *Candida albicans* (ATCC 90028) and *Aspergillus ni*ger (ATCC 16404) were the fungi species used. These strains were obtained from the microbiology laboratory at the University of Eldoret.

5.2.4 Antimicrobial Sensitivity Tests

The hole-plate diffusion method as explained by Abbas *et al*. (2014) was used to test for the bacterial and fungal sensitivity to the extracts of the African nightshade berries at different ripening stages. The method was applied with modifications (Rojas *et al*., 2006; Matasyoh *et al*., 2014; Mostafa *et al*., 2018). To prepare the inoculum, a suitable suspension of each gram negative and gram positive bacteria was prepared by incorporating a loop of fresh micro-organism in 10 ml sterilized water. This was evenly mixed in preparation for injection near flame.

For each Gram positive and Gram negative bacteria, about 10 ml of the prepared inoculum was poured into 500 ml of sterile nutrient agar medium. This would be used for the determination of the anti-bacterial activity. For the anti-fungal activity, 10 ml of each of the fungal inoculum that was previously prepared was poured into 500 ml of sterile liquified potato dextrose agar (PDA) (Madhuri and Sarasamma, 2019). After carefully labelling the media for each micro-organism, the media were gently shaken to allow for uniform mixing of the media and the inoculum.

After sufficient mixing, the media were poured into labelled, sterile petri-dishes to a specified quantity such that the depth was maintained at about 8 mm. This was achieved while gently rotating the petri-dishes and evenly spreading the media as it was allowed to solidify at room temperature. Four holes were made in the solidified medium using a stainless steel borer, making sure that the holes were equidistant from each other. The holes were labelled numbers 1 to 4. Hole 1 was filled with the positive control reference; Ampicillin (Crown Healthcare, Nairobi, Kenya) at dose of 125 mg/mL for bacterial assays and Amphotericin B (Health Biotech Ltd., India) at a standard dose of 5 mg/mL for fungal assays (Abbas *et al*., 2014). Amphotericin B has a minimum inhibitory concentration (MIC) breakpoint of ≥ 2 μg/mL (CDC, 2020) while Ampicillin has a breakpoint ranging from ≥ 8 to ≥ 32 μg/mL (FDA, n.d.). Holes 2-4 were filled with sample solution from each of the *S. nigrum* complex extracts at 20 mg/ml. This was done for each variety at each ripening stage. For testing the anti-bacterial activity, the petri-dishes were incubated at 37 $^{\circ}$C for 48 hours while for the anti-fungal activity, the incubation was carried out for 72 hours.

The antimicrobial activity was tested by measuring the diameters of the zones of inhibition in millimeters using a clear ruler. The results were then expressed as the mean zones of inhibition.

5.2.5 Minimum Inhibition Concentration

From the anti-microbial assay, it was discovered that the green stage of ripening was the most potent against the micro-organisms. As such, the extracts at the green stage of each variety of African nightshade was used for the MIC tests. This was carried out using the method described by Seleshe *et al*. (2017) with modifications. Six (6) vials in total were used for each extract. The first vial was filled with 100 µL of the extract from a stock solution while the 2nd to 6th vials were filled with 50 µL of sterile water. Serial dilution was then carried out from 2nd to 6th vials and this was achieved by drawing and transferring 50 µL from the first vials to the subsequent vials to obtain serially descending concentrations of 100, 50, 25, 12.5, 6.25 and 3.12 µg/ml.

A positive control was used where the micro-organism was added in the vial with no extract while negative control contained the extract but no micro-organism. 20 µL of the of the bacterial and fungal suspensions were added to each vial and then incubated at 37 $^{\circ}$C for 24 hours (bacterial) and 48 hours (fungi) as shown by Seleshe *et al*. (2017). After the incubation period, 80 µL of resazurin dye was added before the samples were re-incubated for a further 2 hours to facilitate colour development. Inhibition was indicated by the blue coloration of the wells after the addition of the dye. Colour change from blue to red was an indication of the presence of live micro-organisms and, therefore, an indication that inhibition did not take place.

5.3 Results and Discussions

5.3.1 Extraction Yield

The extraction yield for the different berries is as reported in Table 5.1. The berry yields ranged from a lowest of 28.21% in green Giant NS to a high of 65.24% in KALRO Agric. variety at senescence. A trend was observed where the berry yield increased as the berries ripened. This was observed amongst all berries, where the yield of the berries at senescence was, slightly higher than twice the yield at the green stage for all the varieties. The differences observed within each variety but at different ripening stages as well as amongst the various varieties could be attributed to the variations in type and amount of soluble compounds in the respective samples (Matasyoh *et al*., 2014). The yields also seemed to be similar in quantities amongst the different varieties at each particular stage. Similar trends were observed by Saleh and Otaibi (2013) who analysed the yield of dates at different ripening stages and (Mostafa *et al*., 2018).

Table 5.1: Ethanolic Extraction Yield (%) of Different Varieties of African Nightshade Berries at Different Stages of Ripening

Variety	**Ripening Stage**			
	Green	***Colour Break***	***Ripe***	***Senescence***
Black N.S	30.15	30.58	45.60	61.11
Giant N.S	28.21	31.12	50.11	59.22
JKUAT Impr.	29.32	33.21	48.90	62.00
KALRO Agric.	30.44	32.50	50.21	65.24

The yields for the berries were higher than yields stated in previous studies of African nightshade leaves (Matasyoh *et al*., 2014). The ethanolic yield at the green and colour break stages were comparable to methanolic extracts of nightshade berries in a study carried out by Abbas *et al*. (2014). Nevertheless, it is notable that Abbas *et al.* (2014) did not specify at which stage of ripening they sampled from. In the current experiment, it was not possible to test the significant difference within and between samples because the extraction was carried out in batch for each variety and each ripening stage, therefore the statistical differences could not be calculated.

5.3.2 Anti-Microbial Activity of Ethanolic Extracts of *Solanum nigrum complex* Edible Berries

The antimicrobial activity of the berries was determined using the inhibition zones and the results were as reported on Table 5.2. African nightshade edible berry extracts proved potent to enterocytic microbes. Inhibition of *E. coli* ranged from a high of 9.0 mm observed in the colour break stage of Black NS to a low of 5.0 mm recorded at the senescence stage of JKUAT Improved variety. This inhibition, however, was significantly lower than the 14.7 mm recorded by the positive control (ampicillin). In terms of varietal differences, it was noted that Black NS and KALRO Agriculture varieties recorded the highest inhibition against the microbe, followed by the Giant NS and lastly JKUAT improved variety.

Inhibition of *S. typhi* was lower than inhibition of *E. coli*. The highest inhibition as recorded at the green and colour break stages of Black NS, with an inhibition zone of 8.2 mm. Inhibition values of 5.0 and 4.2 mm recorded at the senescence stages of Giant NS and JKUAT Improved varieties, respectively, were the least values recorded. Once again, Black NS berry extracts were significantly more potent against this micro-organism, even though this was not statistically different from the values recorded for the KALRO variety. These two varieties, however, proved to be significantly more potent against *S. typhi* at all stages of ripening compared to Giant NS and JKUAT Improved varieties.

Action of the extracts were more inhibiting against Gram positive bacteria compared to Gram negative bacteria. *S. aureus*, a gram negative bacteria, was the most sensitive to the berry extracts. A high inhibition zone value of 11.8 mm was obtained for in Black NS at the colour break stage, on the other hand 5.2 mm and 5.3 mm were observed at senescence stage of Giant NS and JKUAT Improved, respectively, were the lowest values. These, however, were still significantly higher than the values recorded for the Gram negative bacteria. Black NS and KALRO Agriculture varieties were superior to the rest in inhibition of *S. aureus*.

B. cereus is also a Gram positive bacteria and its inhibition was significantly lower than that of *S. aureus* which was in turn significantly higher than the inhibition observed in both *E. coli* and *S. typhi*. Inhibition ranged from a high of 10.8 mm in the colour break stage of Black NS to 5.3 mm in Giant NS at senescence. For all the bacterial assays, the African nightshade berry extracts showed inhibition capacity. However, the recorded values were still significantly lower than the values recorded in the positive controls.

The extracts also exhibited a lower antimicrobial activity against fungi in comparison to the action against bacteria. This indicated that the extracts would be less effective against fungal-related problems. Just like in the antibacterial assays, the extracts of the berries showed greater inhibition in the green and colour break stages. Indeed, no inhibition was recorded at the senescence stages of all the varieties while JKUAT mproved also showed no inhibition potential at the ripe stage.

Inhibition of *C. albicans* was higher than inhibition of *A. niger* across all varieties. However, the trend observed in the bacterial assays was still evident. Black NS and KALRO Agriculture varieties had a higher inhibition effect at all stages compared to the Giant NS and JKUAT Improved varieties. The inhibition of *A. niger* was comparable between Black NS and KALRO Agriculture but with reference to *C. albicans*, KALRO Agriculture had a greater inhibitory effect.

Table 5.2: The Antimicrobial Activities from Different Varieties of African Nightshade Berries against 6 Micro-Organisms

Variety/Stage	**Diameter of Inhibition Zone (mm)**					
	E. coli	***S. typhi***	***S. aureus***	***B. cereus***	***C. albicans***	***A. niger***
Green						
Black NS	8.8 ± 0.3^{gh}	8.2 ± 0.3^{g}	10.8 ± 0.3^{fg}	10.2 ± 0.3^{i}	5.3 ± 0.6^{gh}	5.3 ± 0.6^{hij}
Giant NS	$7.7\pm0.6d^{ef}$	7.7 ± 0.3^{fg}	8.3 ± 0.6^{e}	8.8 ± 0.3^{h}	5.2 ± 0.3^{gh}	4.5 ± 0.5^{ghij}
JKUAT Impr.	6.7 ± 0.6^{bcd}	5.3 ± 0.6^{abc}	7.7 ± 0.6^{de}	7.5 ± 0.5^{ef}	4.8 ± 0.3^{fg}	4.3 ± 0.6^{fghi}
KALRO Agric.	8.2 ± 0.3^{fgh}	7.7 ± 0.6^{fg}	10.0 ± 0.6^{f}	9.7 ± 0.5^{hi}	6.3 ± 0.6^{h}	5.7 ± 0.6^{ij}
Colour Break						
Black NS	9.0 ± 0.0^{h}	8.2 ± 0.3^{g}	11.8 ± 0.3^{g}	10.8 ± 0.3^{i}	5.7 ± 0.3^{gh}	5.8 ± 0.3^{j}
Giant NS	7.2 ± 0.3^{def}	6.7 ± 0.6^{def}	8.2 ± 0.3^{de}	8.2 ± 0.3^{fg}	4.3 ± 0.6^{efg}	3.7 ± 0.6^{defg}
JKUAT Impr.	6.0 ± 0.0^{abc}	5 ± 0.0^{abc}	7.5 ± 0.5^{cde}	7.0 ± 0.0^{cdef}	3.3 ± 0.6^{cde}	3.0 ± 0.0^{cdef}
KALRO Agric.	7.8 ± 0.3^{efg}	7.2 ± 0.3^{efg}	10.3 ± 0.6^{f}	9.7 ± 0.3^{hi}	5.5 ± 0.5^{h}	5.0 ± 0.0^{ghij}
Ripe						
Black NS	7.0 ± 0.0^{cde}	6.2 ± 0.3^{cde}	8.2 ± 0.3^{de}	7.3 ± 0.6^{def}	$2.0\pm0.0^{b}c$	2.3 ± 0.6^{bcd}
Giant NS	5.8 ± 0.3^{ab}	5.5 ± 0.5^{bcd}	6.8 ± 0.3^{bcd}	6.2 ± 0.3^{abcd}	1.7 ± 0.6^{ab}	1.3 ± 0.6^{ab}
JKUAT Impr.	5.3 ± 0.6^{a}	4.8 ± 0.3^{ab}	5.8 ± 0.3^{ab}	6.0 ± 0.0^{abc}	ND	ND
KALRO Agric.	6.0 ± 0.0^{abc}	5.8 ± 0.3^{bcd}	6.8 ± 0.3^{bcd}	6.7 ± 0.6^{cde}	2.7 ± 0.6^{bcd}	2.0 ± 0.0^{bc}
Senescence						
Black NS	6.8 ± 0.3^{bcde}	5.8 ± 0.3^{bcd}	7.3 ± 0.6^{cde}	7.2 ± 0.3^{cdef}	ND	ND
Giant NS	5.3 ± 0.6^{a}	5.0 ± 0.0^{abc}	5.3 ± 0.6^{a}	5.3 ± 0.6^{a}	ND	ND
JKUAT Impr.	5.0 ± 0.0^{a}	4.2 ± 0.3^{a}	5.2 ± 0.3^{a}	5.5 ± 0.5^{ab}	ND	ND
KALRO Agric.	5.8 ± 0.3^{ab}	5.3 ± 0.3^{abc}	6.2 ± 0.3^{abc}	6.0 ± 0.0^{abc}	ND	ND
Ampicillin	14.7 ± 0.6^{i}	13.3 ± 0.6^{h}	16.7 ± 0.6^{h}	14.3 ± 0.6^{j}	ND	ND
Amphotericin B	ND	ND	ND	ND	12.8^{i}	11.2^{k}

Values are mean± SD. Values followed by different letter superscripts in a column are significantly different at $p\leq0.05$ as assessed by Tukey's least significant test. ND = Not detected

Plants contain antimicrobial substances to protect them from microbial infection and deterioration (Abbas *et al*., 2014). These are substances such as flavonoids and other polyphenolic compounds including alkaloids, lignins, tannins, glycosides (Puupponen-Pimia *et al*., 2001; Smith *et al*., 2005). They also contain terpenoids such as monoterpenes, diterpenes or triterpenes and sesquiterpenes (Abbas *et al*., 2014). The mode of antimicrobial activity can be attributed to these compounds getting through the bacterial and fungal cell walls or membranes and suppressing the growth of the microbes. If the compounds penetrate deeply, they could lead to the death of the micro-organisms (Abbas *et al*., 2014). Smith *et al*. (2005) propose a mechanism by which this inhibition occurs whereby the tannins in plants complex with minerals and polymers. Consequently, the tannins lead to inhibition of extracellular microbial enzymes, deprivation of the substrates needed for microbial growth as well as direct impact on the microbial metabolism through iron deprivation or inhibition of oxidative phosphorylation (Scalbert, 1991; Nohynek *et al*., 2006). However, some gastrointestinal bacteria can override this mechanism through mechanisms such as tannin modification, or degradation, dissociation of the tannin-substrate complexes, membrane modification or repair and tannin inactivation by high-affinity binders (Abbas *et al*., 2014). This could explain why the inhibition varies depending on the micro-organisms since not all can overcome the inhibition effect of the polyphenols (Bobinaitė *et al*., 2013; Puupponen-Pimia *et al*., 2005).

The results of the present study on antimicrobial effect of the African nightshade berries are corroborated by previous works. In evaluating the antimicrobial activity of *Solanum nigrum* using ethyl acetate extracts, Abbas *et al*. (2014) found inhibition zones of 7.2 mm in *S. aureus*, 6.7 mm in *S. typhi*, 7.5 mm in *E. coli* and 6.7 in *C. albicans*. In a study to test efficacy of different berries with storage time on *E. coli* E-564^{T} and *Salmonella* sv. SH-5014, it was recorded that cloudberry and raspberry extracts led to the death of both cultures but cloudberry was more potent on *Salmonella* even after being frozen for 12 months. Bilberry showed clear inhibition on *E. coli* but strong inhibition on

Salmonella while Black currant recorded weak inhibition on both microbes (Nohynek *et al*., 2006).

A study on antimicrobial properties of black chokeberries' extracts obtained from fresh, frozen and dried fruits exhibited inhibition against Gram negative bacterium *Pseudomonas aeruginosa* but did not have any effect on *E. coli* (Liepiņa *et al*., 2013). Radovanović *et al*. (2013) evaluated the antimicrobial activity of blackthorn, European cornel and wild blackberry against five Gram positive and five Gram negative bacteria and concluded that all the microbes were highly sensitive to all the extracts, though to varying degrees, displaying inhibition zones in the range of 12.0 - 16.2 mm using concentrations of 50 µl disc^{-1}. *S. enteridis* was the most sensitive amongst the Gram negative bacteria while *S. aureus* was most sensitive in Gram positive bacteria.

Similar findings were also reported by Seleshe *et al*. (2017) while evaluating antimicrobial activity of ethanolic extracts of three types of strawberries, exhibiting inhibition zones of 11.5 – 12.5 mm against *S. aureus*, 10.5 – 13.5 mm against *S. pneumonia*e and 8.5 – 10.5 mm against *E. coli* but with no inhibition activity against fungi *A. niger* and *C. albicans*, an observation supported by Bobinaitė *et al*. (2013). This study found out that fungi are less susceptible to the berry extracts compared to bacteria.

It was noted that across the varieties, the antimicrobial activity decreased as the berries ripened. As such, berries at the green stage were more potent against the test micro-organisms compared to all the other stages. This was with the exception of Black NS in which berries at the colour break stage had the highest zone of inhibition for the variety. However, this was not statistically different from the green stage of the same variety. Indeed, this trend was observed across all varieties where the antimicrobial activity did not seem to vary significantly from the green to the colour break stage. However, a significant drop in microbial activity was observed on transition from the colour break to the ripe stages and this drop was sustained through to senescence.

The observed decrease in antimicrobial activity upon ripening was reported in previous studies involving other fruits. For instance, Enemuor *et al*. (2011), when comparing the antimicrobial activity of ripe and unripe fruit extracts of *Cissus multistriata* concluded that unripe fruits were more potent to bacteria as compared to unripe fruits. A similar conclusion was also arrived at by Saleh and Otaibi (2013) upon evaluation of antimicrobial activity of dates at 3 different stages of ripening. In contrast, a study by Dawkins *et al*. (2003) reported that anti-microbial activity of *Carica papaya* fruits extracts was independent of the ripening stage. This difference could be attributed to species differences in the samples used, but this could be open for further investigation.

This drop in potency upon ripening could be attributed to alteration of the bioactive components in the fruits, thereby leading to less inhibition (Enemuor *et al*., 2011). As demonstrated by Saleh and Otaibi (2013), polyphenols play a major role in anti-microbial activity through enzyme inhibition and precipitation of proteins. Other bioactive compounds such as tannins, flavonoids and alkaloids also have antimicrobial function as they serve to protect the plant from microbial spoilage (Puupponen-Pimia *et al*., 2001; Abbas *et al*., 2014). Therefore, alteration of these compounds could lead to the decrease in the microbial inhibition action of the extracts. For this study, it was noted that the various phytochemicals in the African nightshade berries decreased as the berries ripened.

The quantity of total phenols, flavonoids and tannins all decreased with ripening as reported on Table 3.3. Given that micro-organisms are sensitive to pH as this affects their enzyme activity (Saleh and Otaibi, 2013), it is possible that the decrease in titrable acidity of the berries as they ripened (Table 3.4) could also have led to the decrease in microbial inhibiting in the latter stages of ripening. However, this is refuted by Liepiņa *et al*. (2013) upon investigation of antimicrobial activity of extracts from fruits of *Aronia melanocarpa* and *Sorbus aucuparia*. These researchers concluded that pH was not a determining factor for antimicrobial activity of extracts since there was no observed association between the pH value and antimicrobial activity. However, it should be noted that Liepiņa *et al*. (2013) only considered one stage of fruit ripening in their study.

As such, they could not compare the effect of decreasing pH as occasioned by fruit ripening as was evaluated in the current study. Conclusively, it can be deduced that decrease in acidity with ripening could have a negative impact on the microbial inhibition capacity of the African nightshade berries.

Berry extracts (including *S. nigrum*) have previously been reported to be more potent against Gram positive bacteria than Gram negative bacteria (Liepiņa *et al*., 2013; Abbas *et al*., 2014; Modilal *et al*., 2015). This has been previously reported where Gram negative bacteria are more resistant to berry extracts and this is explained by the differences in their cell wall structures (Bobinaitė *et al*., 2013). Gram negative bacteria have an outer membrane which acts as a barrier against many external agents. They have hydrophilic channels which only allow entry of hydrophobic substances such as essential oils (Nikaido, 2003; Burt, 2004; Bobinaitė *et al*., 2013) whereas Gram positive bacteria only have the outer peptidoglycan layer which is more permeable and less effective against antimicrobial compounds (Shen *et al*., 2014). Further, Saleh and Otaibi (2013) demonstrated that Gram negative bacteria are more resistant because they have an outer lipopolysaccharide cell membrane. This could explain the trends that observed in the current study, that is, Gram negative bacteria were more resistant to the berry extracts whereas the Gram positive bacteria were more susceptible.

The antimicrobial activity of these berries is indicative of the fact that African nightshade berries can find application in food processing and preservation where they can be used as safe, plant-based preservatives instead of chemical preservatives. This could help alleviate some of the health problems and concerns that are associated with chemical preservatives. The results also show that the berries have the potential to be used for medicinal purposes where the antifungal and antibacterial properties are exploited in the pharmacology industry.

5.3.3 Minimum Inhibitory Concentration (MIC) of Different Varieties of African Nightshade Edible Berries

Table 5.3 records the MIC values obtained for the different varieties against the 6 test microbes, tested at the green stage of the berries. MIC values are defined as the lowest concentration of extracts that can inhibit the growth of micro-organisms (Saleh and Otaibi, 2013; Matasyoh *et al*., 2014; Seleshe *et al*., 2017). Most of the bacteria exhibited MIC value of 12.50 µg/ml in all the varieties. This was with exception of *E. coli* which showed a lower MIC of 6.25 µg/ml in Black NS. *B. cereus* also had MIC of 6.25 µg/ml in both Black NS and KALRO Agriculture varieties. Fungal species had the highest MIC of all tested microbes, only showing sensitivity at concentrations of 100 µg/mL across all varieties. This further assertion that fungal species are more resistant to the berry extracts as exhibited on Table 5.2. As such, if the berry extracts were to be used against fungal agents, then higher concentrations would be required.

Table 5.3: Minimum Inhibitory Concentration (MIC) Values of Ethanol Extracts from Different Varieties of African Nightshade against 6 Micro-organisms

Test Organisms	**MIC (µg/mL)**			
	Black NS	**Giant NS**	**JKUAT Impr.**	**KALRO Agric.**
E. coli	6.25	12.50	12.50	12.50
S. typhi	12.50	12.50	12.50	12.50
S. aureus	12.50	12.50	12.50	12.50
B. cereus	6.25	12.50	12.50	6.25
C. albicans	100.00	100.00	100.00	100.00
A. niger	100.00	100.00	100.00	100.00

Findings from previous works corroborate the findings of the current study. Ethanolic extracts of *Staphylococcus saprophyticus* ATCC 15305 recorded MIC values ranging from 5 – 10 mg/ml against date palm extracts. Seleshe *et al*. (2017) also recorded MIC values ranging from 6.25 to 12.50 µg/ml against *S. aureus, S. pneumoniae, E. coli and K. pneumoniae* using different types of strawberries. However, they concluded that the fungi *A. niger* and *C. albicans* needed concentrations that were above 50 µg/ml. These results underscore the idea that that *S. nigrum* edible berries can be potentiated for use in the pharmacological and food processing industries.

5.4 Conclusion

Results from this study indicated that *Solanum nigrum* berries have significant antimicrobial properties. The antimicrobial property of the berries is comparable to other edible fruits especially berries such as strawberries, blue berries, chokeberries among others. The potency of the berries against micro-organisms reduce with ripening with berries at the green and colour break stages having the highest inhibitory effect. The effect also varies with the type of micro-organism used with Gram positive bacteria being more sensitive to the berry extracts compared to the Gram negative bacteria. The sensitivity of the test microbes could be ranked in the order *S. aureus* > *B. cereus E.* > *Coli* > *S. typhi*. Similarly, it was observed that the berry extracts are more effective against bacterial species as compared to fungal assays. Although all the varieties showed inhibitory quality against the test microbes, it was noted that Black NS and KALRO agriculture varieties were more potent. The microbial inhibitory action seems to run in the order Black NS ≥ KALRO Agriculture > Giant NS ≥ JKUAT Improved variety. These antimicrobial properties of the berries could be exploited in the pharmaceutical and food industry to prepare medicinal and nutritional products from natural sources. This would not only ensure food safety but would also build the confidence of the consumers due to the perceived quality based on naturalness of the products.

CHAPTER SIX

CONSUMER ACCEPTABILITY OF AFRICAN NIGHTSHADE (*SOLANUM NIGRUM COMPLEX*) EDIBLE BERRIES AND BERRY-FLAVOURED YOGHURT

6.1 Introduction

Sensory characteristics are among the quality indicators of concern to the consumers (Watts *et al*., 1989; Mihafu *et al*., 2020). Yoghurt is a fermented dairy product which is obtained from lactic acid fermentation of milk. It is a coagulated milk product that is a resultant of fermentation of the lactose in milk occasioned by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Imeje *et al*., 2015). To produce yoghurt with unique characteristics, other lactic acid bacteria (LAB) are also frequently used. The micro-organisms act on the lactose in milk resulting in production of lactic acid which, in turn, increases the acidity of the yoghurt, leading to formation of a gel (Imeje *et al*., 2015). The fermentation also increases the life-shelf of the milk since the decrease in pH serves as an inhibitor to growth of pathogenic bacteria. Lactic acid produced, on the other hand, gives yogurt its characteristic flavour and aroma and also maintains its quality during packaging and storage (Yaman *et al*., 2006; Imeje *et al*., 2015).

Yoghurt is one of the most popular fermented milk products in the world (Dlamini and Silaula, 2009; Teshome *et al*., 2017) and its usage dates back many centuries (Yaman *et al*., 2006). Consumption of yogurt is associated with many health benefits and this could be the reason behind its popularity. Nutritionally, yoghurt is a good source of proteins, vitamins, minerals such as calcium and energy (calories). It also has therapeutic value due to its fermented nature. Its consumption results in reduced incidences of lactose intolerance (Dlamini and Silaula, 2009). Consumption of yoghurt also helps in boosting immunity. Regular consumption of the probiotics and prebiotics found in the product are associated with not only maintenance of the normal gut flora but also produce a higher level of interferon. This happens as the bacteria cultures stimulate white cells in the blood stream with anti-tumor effects (Imeje *et al*., 2015). Consequently, yoghurt is a product that is consumed by a wide variety of people and the consumer base continues growing over time (Consumer F and B, 2021). This makes yoghurt a good vehicle for the inclusion of *S. nigrum* berries in the diet.

Over time, consumers have become more aware of the nutritional benefits of yoghurt and with the manufacturers seeking to capitalize on this market, different flavours of the product have been developed. Fruit-flavoured yoghurts have become popular over time and the trend is likely to continue (Consumer F and B, 2021). This has led to numerous efforts in production of fruits flavoured yoghurts across the world. Yoghurt flavours have already been developed from fruits such as pineapple (Imeje *et al*., 2015), strawberries, blueberries, vanilla and peach (Consumer F and B, 2021), grapes (Yaman *et al*., 2006), papaya and mango (Teshome *et al*., 2017). All these have enjoyed considerable success in the market. Attempts have also been made to produce yoghurts flavoured with indigenous fruits (Dlamini and Silaula, 2009). Consumer F and B (2021) noted that the demand for naturally enriched fresh-fruit flavoured yoghurt is very high, especially for berry flavours. This shows that African nightshade berries could potentially be used to produce yoghurt. This would be a novel way of introducing the berry into the diet of a wide array of consumers, thereby ensuring that the nutritional benefits of this crop are enjoyed by a larger population through dietary diversification. Therefore, the objective of this study was to develop berry-flavoured yoghurt and

6.2 Materials and Methods

6.2.1 Consumer Acceptability Tests

The consumer panel approach was used in evaluating the consumer acceptability of the berries using the completely randomized design (CRD) as described by Watts *et al*. (1989). Berries of the four varieties (Black NS, Giant NS, KALRO Agriculture and JKUAT Improved) were picked at the fully ripe stage for consumer evaluation. Three of the most liked berries were then used to make African nightshade berry-flavoured yoghurt and then evaluated for consumer acceptability.

6.2.2 Preparation of Berry Purees

Fresh berry purees were prepared using the methods described by Dlamini and Silaula (2009) and Teshome *et al*. (2017). Puree was prepared from fully ripe berries. The fresh berries were cleaned with distilled water. The fruits were blanched at 60 ^{o}C for 3 minutes and then blended into a paste. 5% sucrose was added as a sweetener and then the mixture was boiled for 10 minutes. The puree was then hot-filled in sterile containers awaiting yoghurt production.

Cleaning fruits with distilled water

↓

Blanching fruits at 60^{o}C for 3 minutes

↓

Blending and homogenization of fruits until smooth

↓

Addition of sucrose and boiling fruits for 10 minutes

↓

Hot filling of fruits into sterilized preserving jars

↓

Cold storage until use

Figure 6.1: Flow diagram of African Nightshade Berries Puree Preparation

6.2.3 Preparation of Berry Flavoured Yoghurts

Yoghurt was prepared using the conventional yogurt production method (Dlamini and Silaula, 2009; Teshome *et al*., 2017).

Heating of milk to 40 ^{o}C

↓

Milk standardization using skim milk powder and addition of stabilizer

↓

Pasteurization of milk at 80 ^{o}C for 15 minutes

↓

Cooling of milk to 44 ^{o}C and inoculation with 2% (v/v) culture

↓

Fermentation until pH 4.6 was reached (3 hr)

↓

Addition of berry puree (15%) and storage at 4 ^{o}C

Figure 6.2: Flow Diagram of Preparation of African Nightshade Berry-Flavoured Yoghurt

6.2.4 Recruitment and Screening of Sensory Evaluation Panelists

A sample of 44 participants was obtained from the University of Eldoret fraternity through an advert, telephone calls and email. The advert contained information on all the African nightshade berries that were evaluated. This information was meant to ensure that those who sign up for inclusion were not allergic to the products.

6.2.5 Procedure for Evaluation

Sensory evaluation of the berry samples was conducted at the Foods Laboratory of the Department of Family and Consumer Sciences of the University of Eldoret. Each panelist signed a consent form informing them of the nature of the samples they would evaluate before engaging in the sensory exercise. A total of seven (7) sessions lasting one hour each were carried out in one day using a completely randomized design as described by Lawless and Heymann (2010). Each panelist received a white tray containing the three samples of African nightshade berries in transparent glass bowls and a spoon for each sample. The trays also had a glass of deionized water to cleanse the pallet before and in between tasting different samples.

Each sample was labeled with three-digit blinding codes and the samples were randomized for each panelist. The panelists were seated back-to-back in the laboratory so that they could not see each other. Four sensory parameters of the berries namely colour, taste, aroma, and texture were used to determine consumer liking and overall acceptability. These were scored on a nine point hedonic scale (dislike extremely – 1: neither like nor dislike – 5 and like extremely – 9) for each sample (Lim *et al*., 2009). Responses to the evaluation were entered into a score card. The same procedure was used for the evaluation of berry-flavoured yoghurt.

6.3 Results and Discussions

6.3.1 Sensory Evaluation of Berries

The results for the evaluation of berry acceptability was as shown in Table 6.1. Appearance and texture of the berries received high acceptance scores and were not significantly ($p \leq 0.05$) different amongst the different berry varieties. Differences amongst the varieties were observed in the aroma and flavour attributes. Previously, similar observations have been made where flavour and aroma seem to be the most important determinants of sensory acceptance (Leahu and Hretcanu, 2017). KALRO Agric. and Black NS were liked more than JKUAT Impr. and Giant NS with reference to aroma and flavour. This was also replicated in the overall quality of the berries where Black NS (6.8) and KALRO Agric. (7.0) received significantly higher ranking compared to the other two varieties (Figure 6.3).

Table 6.1: Sensory Evaluation of Berries

Sample	Appearance	Aroma	Flavour	Texture
KALRO Agric	7.25^{a}±1.37	6.66^{a}±1.61	6.53^{a}±1.77	7.57^{a}±1.56
JKUAT Impr.	7.25^{a}±1.91	5.41^{b}±1.76	4.43^{c}±2.41	6.61^{a}±2.23
Giant NS	7.05^{a}±1.87	5.43^{b}±1.78	5.09^{bc}±2.64	6.64^{a}±2.22
Black NS	7.61^{a}±2.08	6.86^{a}±2.09	6.30^{ab}±2.42	6.57^{a}±2.42

Values are mean± SD. Values followed by different letter superscripts in a column are significantly different at $p \leq 0.05$ as assessed by Fisher's least significant test. 9= Like extremely, 8= Like very much, 7= Like moderately, 6= Like slightly, 5= Neither like nor dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much, 1= Dislike extremely, n=44.

Despite these differences in preference, it is evident that all the varieties were acceptable by the consumers. This was a clear example that the berries could be considered not only as a fruit to be consumed alone but also as an ingredient that could be used in production of value-added products. Consequently, the berry purees from the three most preferred berries were used to prepare berry-flavoured yoghurt for the evaluation.

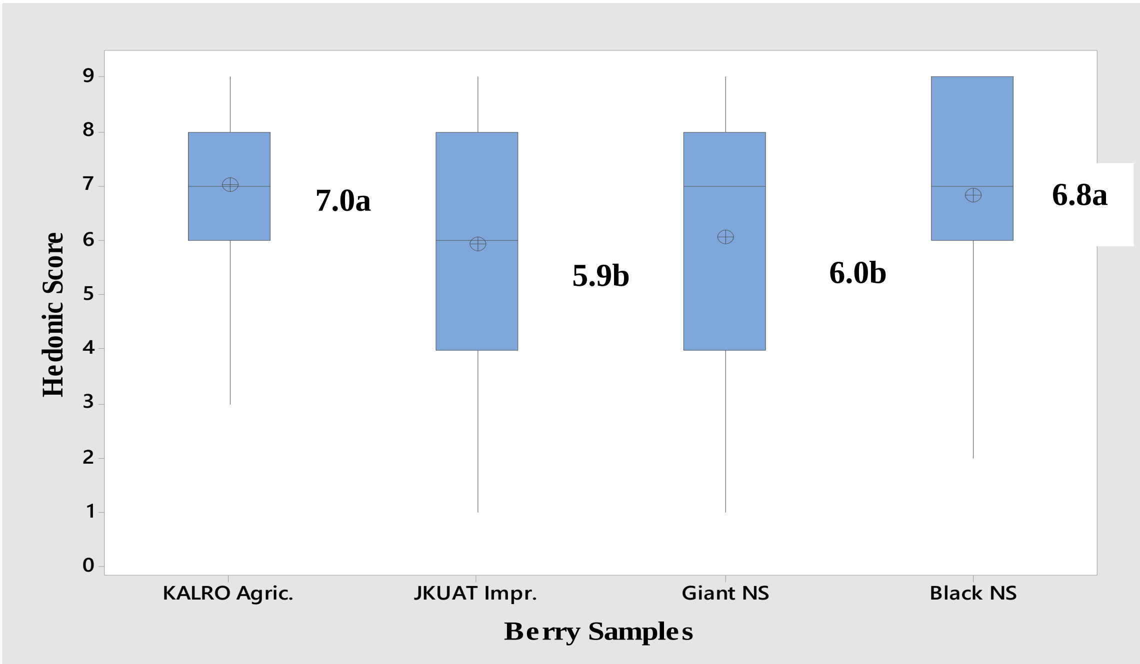

Figure 6.3: Total quality of the berry samples

Mean values with different letter differ significantly at $p<0.05$ as assessed by Fisher's least significant test. Area above the straight line in the individual boxes is the higher percentile and represents the value above which 75% of the ratings fell. The area below the straight line is the lower percentile and represents the area where 25% of the ratings fell. The median is the thin line between the two areas where 50% of the values fell above and 50% below. The dot represents the individual mean scores. Overall liking ratings 1=Dislike extremely, 2=Dislike very much, 3=Dislike moderately, 4=Dislike slightly, 5=Neither like nor dislike, 6=Like slightly, 7=Like moderately, 8=Like very much, 9=Like extremely, n=44.

6.3.2 Sensory Evaluation of African nightshade Berry-Flavoured Yoghurt

Acceptability of the berry-flavoured yoghurt was as reported on Table 6.2 and Figure 6.4.

Table 6.2: Sensory Evaluation of Yoghurt Flavoured with African Nightshade Berries

Sample	Appearance	Aroma	Flavour	Texture
KALRO Agric.	$6.41^{b}\pm2.16$	$7.21^{b}\pm1.47$	$6.82^{b}\pm2.27$	$7.48^{b}\pm1.75$
JKUAT Impr.	$6.36^{b}\pm2.28$	$6.93^{b}\pm1.89$	$6.68^{b}\pm2.45$	$6.86^{b}\pm2.27$
Black NS	$8.09^{a}\pm1.64$	$8.14^{a}\pm1.17$	$8.59^{a}\pm0.76$	$8.41^{a}\pm0.89$
Plain Yoghurt	$8.61^{a}\pm1.21$	$8.32^{a}\pm1.38$	$8.64^{a}\pm1.28$	$8.73^{a}\pm0.76$

Values are mean± SD. Values followed by different letter superscripts in a column are significantly different at $p\leq0.05$ as assessed by Fisher's least significant test. 9= Like extremely, 8= Like very much, 7= Like moderately, 6= Like slightly, 5= Neither like nor dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much, 1= Dislike extremely. Consumers n=44.

The control sample (Plain yoghurt) had higher scores than the berry flavoured yoghurts. This could possibly be due to the fact that the consumers were familiar with the plain yoghurt and familiarity has been shown to increase the consumers acceptance of products (Dlamini and Silaula, 2009). There was an observed consistency in the scoring of the liking of the products, a deviation from a previous study (Yaman *et al*., 2006) which recorded differences in liking. This difference could be attributed to the use of different fruits in the previous study whereas in this study all the berries were from the same species but different varieties. All the yoghurts scored above 6 in total quality, an aspect that depicts acceptability by consumers. However, amongst all the varieties evaluated for all the attributes, Black NS was liked more than the other varieties (significantly at $p\leq0.05$), scoring higher than KALRO flavoured yoghurt despite the latter having been liked more in berry form. Black NS was also comparable to the control sample in all attributes, an aspect that is further replicated in total quality as seen in Figure 6.4. This could imply that all the berries could be used in producing value added yoghurt. However, for commercial purposes, Black NS could be the variety of choice as it is more acceptable compared to the other varieties.

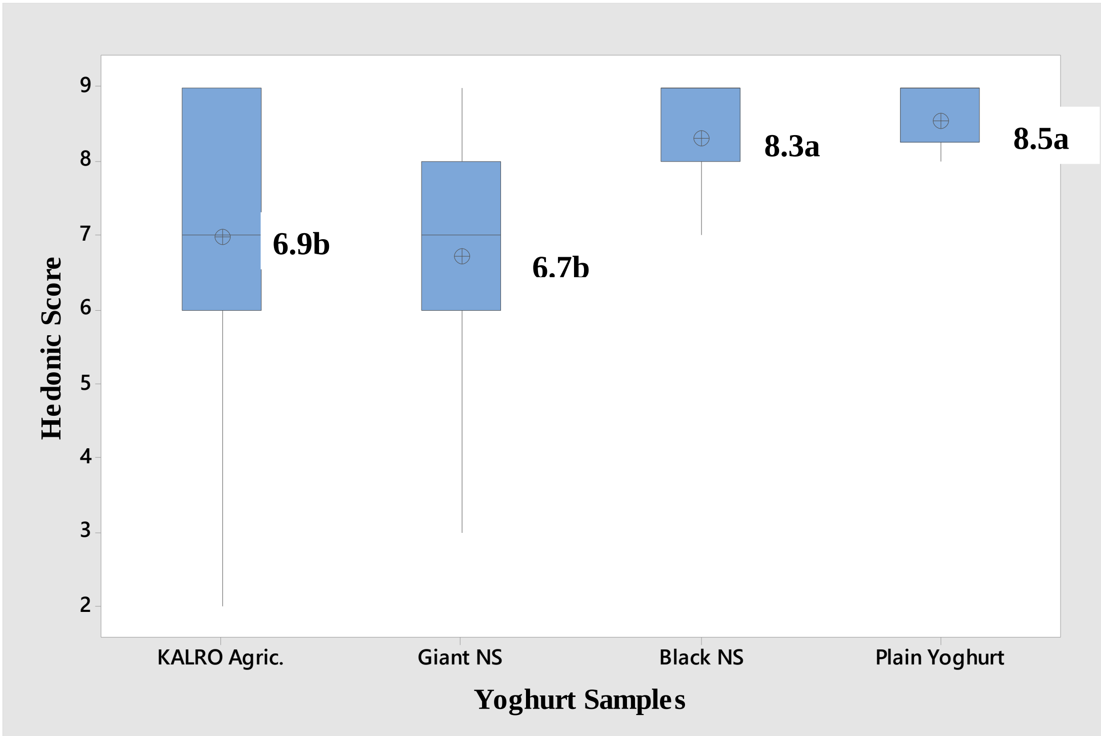

Figure 6.4: Total Quality of Yoghurt Samples

Mean values with different letter differ significantly at p<0.05 as assessed by Fisher's least significant test. Area above the straight line in the individual boxes is the higher percentile and represents the value above which 75% of the ratings fell. The area below the straight line is the lower percentile and represents the area where 25% of the ratings fell. The median is the thin line between the two areas where 50% of the values fell above and 50% below. The dot represents the individual mean scores. Overall liking ratings 1=Dislike extremely, 2=Dislike very much, 3=Dislike moderately, 4=Dislike slightly, 5= Neither like nor dislike, 6=Like slightly, 7=Like moderately, 8=Like very much, 9=Like extremely, n=44.

6.4 Conclusion

Black NS berries were the most liked on all parameters followed by KALRO Agric. while JKUAT Improved variety was the least preferred. Appearance and texture were the most liked attributes of the berries while flavour was the least. The most liked berry yoghurt (Black NS) had an overall acceptability that was comparable to the control. The high acceptability of the berry-flavoured yoghurts is a clear indication that the berries can be used in production of novel, value added products that could enhance more consumption of the berries. This could not only improve the nutrition security of the consumers but could also lead to optimum harnessing of the nutritional benefits of the African nightshade.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

This study explored the potential of African nightshade (*Solanum nigrum complex)* edible berries as a food item. This was informed by the appreciation of the fact that the crop is famed as one of the highly nutritious African indigenous vegetables. However, its berries have for a long time been a neglected and underutilized part of the crop. Therefore, this study sought to bridge this knowledge gap by characterizing the chemical, physical and antimicrobial properties of the African nightshade berries.

Four varieties of the African nightshade were studied. These included Black nightshade, Giant nightshade, KALRO Agriculture and JKUAT Improved varieties. The berries were evaluated at four stages of ripening; green, colour break, ripe and senescence.

Chemical analyses demonstrated that the berries, just like the leaves of the plant, are rich in macro- and micro-nutrients. Magnesium was the most abundant mineral while calcium was the second most abundant mineral followed by phosphorus, potassium and sodium. Zinc was the least abundant followed by iron. This is an indication that regular intake of the berries could help in meeting the recommended daily allowances (RDAs) for the minerals, thereby reducing the prevalence of hidden hunger.

The berries were rich in phytochemical content. There was marked reduction in the chemical content of the berries as they ripened. Black NS appeared superior to the other varieties in terms of chemical composition. Nevertheless, it was noted that the berries' composition was comparable to other edible fruits that are commonly consumed. Sugar content of the berries increased as they ripened and this could be explained by the conversion of starch into sugars as the fruits ripened.

In physical characterization, it was noted that Black NS was significantly smaller in size compared to the other berries. Black NS also was significantly different from the others in terms of colour, tending to get an orange hue when ripe while the others appeared purplish-black. All the berries showed significant drops in firmness as they ripened, a phenomenon expected in ripening fruits due to disintegration of cell wall carbohydrates.

This study showed that the berries were acceptable to the consumers although the flavour and aroma were the least liked components. On a 9-point hedonic scale, the most accepted berries were Black NS followed by KALRO Agric, although this difference was not statistically significant. Acceptability of berry-flavoured yoghurts was comparable to that of plain yoghurt, an indication that value addition could be the way to ensure that the berries are incorporated in the household diet.

Traditionally, the African nightshade berries have been applied in treating some common ailments such as stomach upsets, gum problems in children among others. This study also tested the antimicrobial value of the berries using anti-microbial assays involving two Gram negative bacteria, two Gram positive bacteria and two fungal species. Results from this study showed that the berries have inhibitory capacity against the test microbes. This capacity, however, decreased as the berries ripened. The inhibitory capacity was highest in the Black NS followed by KALRO Agric., Giant NS and JKUAT Improved. It was noted that the berry extracts were more potent against Gram positive bacteria (*S. aureus and B. cereus*) as compared to Gram negative bacteria (*E. coli and S. typhi)*. The least antimicrobial activity was observed in the fungal species. Since the extracts were more potent at the green stage, extracts at this stage were used to test for MIC. Most of the bacterial microbes had an MIC of 12.50 µg/ml. The fungal species recorded a MIC value of 100.00 µg/ml in all extracts.

From these findings, it is evident that African nightshade edible berries can be a good addition to the household diet. This would not only ensure maximum harnessing of the nutritional benefits of the crop, but would also provide alternative ways through which it can be consumed. The findings also prove that the antimicrobial activity of the berries can be exploited in the food industry where they can act as natural preservatives and also in the pharmaceutical industry where the medicinal properties can be explored in formulation of drugs from natural sources.

7.2 Recommendations

From this study, it is recommended that:

1. Edible berries of African nightshade should be exploited as a food item to curb micro-nutrient deficiencies
2. Alternative value addition methods should be explored as a way of ensuring that the berries can be consumed by an even wider population
3. Financing and facilitation should be sought to ensure large scale production and commercialization of value-added African nightshade berry products.

7.3 Suggestions for Further Research

i. Biotechnological principles can be applied to develop an African nightshade variety that produces more and larger berries

ii. Potentiation studies can be carried out to further explore and harness the nutraceutical potential of the African nightshade edible berries.

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APPENDICES

Appendix I: Consumer Evaluation Score Sheets for African nightshade berries

CONSUMER ACCEPTABILITY SHEET

WELCOME TO THIS AFRICAN NIGHTSHADE (*Solanum nigrum*) TASTING SESSION

DEPARTMENT OF FOOD SCIENCE AND NUTRITION

JOMO KENYATTA UNIVERSITY OF AGRICULTURE and TECHNOLOGY (JKUAT)

Age: ……………………………… Gender: …………………………... Tray Number: …………………….

You are provided with four (4) samples of Solanum nigrum (Managu) berries. Please taste the samples in the order presented from left to right. Take a sip of water before you start tasting and in between tasting the different samples. Indicate your liking or disliking by placing a check mark at the relevant bar on the scale provided for each attribute.

Sample No.	**A**				**B**				**C**				**D**			
Scale	Appearance	Aroma	Flavour	Texture	Appearance	Aroma	Flavour	Texture	Appearance	Aroma	Flavour	Texture	Appearance	Aroma	Flavour	Texture
Like extremely																
Like very much																
Like moderately																
Like slightly																

Neither like nor dislike																
Dislike slightly																
Dislike moderately																
Dislike very much																
Dislike extremely																

Appendix II: Consumer Evaluation Score Sheets for berry-flavoured yoghurt

CONSUMER ACCEPTABILITY SHEET

WELCOME TO THIS AFRICAN NIGHTSHADE (*Solanum nigrum*) BERRY-FLAVOURED YOGHURT TASTING SESSION

DEPARTMENT OF FOOD SCIENCE AND NUTRITION, JKUAT

Age: ……………………………… Gender: …………………………... Tray Number: ……………………

You are provided with four (4) samples of Solanum nigrum (Managu) flavoured yoghurt. Please taste the samples in the order presented from left to right. Take a sip of water before you start tasting and in between tasting the different samples. Indicate your liking or disliking by placing a check mark at the relevant bar on the scale provided for each attribute.

Sample No.	**A**				**B**				**C**				**D**			
Scale	Appearance	Aroma	Flavour	Texture	Appearance	Aroma	Flavour	Texture	Appearance	Aroma	Flavour	Texture	Appearance	Aroma	Flavour	Texture
Like extremely																
Like very much																
Like moderately																
Like																

slightly																
Neither like nor dislike																
Dislike slightly																
Dislike moderately																
Dislike very much																
Dislike extremely																

Appendix III: Advert for Recruitment into the Sensory Evaluation Panel

I do, I understand!"

DO YOU TAKE MANAGU ?

We are interested in persons who are regular '*managu*' consumers to perform a consumer evaluation test on a variety of '*managu*' berries and berry-flavoured yoghurt on the 24th of April 2020 from 10:00am at the foods lab.

For more information please contact:

Heka (0728 – 501215, ekellyne@gmail.com) at the Family and Consumer Department Project Room at the Foods Lab.

Your participation will be highly appreciated

Appendix IV: Consent Form

I am Heka Kamau, a post-graduate student at JKUAT pursuing a PhD in Food Science and Nutrition. I am currently undertaking a research project entitled "**Nutrient Composition, Phytochemical Content and Anti-Microbial Activity of African Nightshade** (*Solanum nigrum complex*) **Edible Berries**." I am working with four varieties of the African nightshade (*Managu*); Black Nightshade, Giant Nightshade, KALRO Agriculture and JKUAT Improved. With reference to this, I am conducting a consumer evaluation for the African nightshade berries and yoghurt flavoured with the berries.

Carefully read this and sign at the bottom if you agree to participate in the study:

I am well informed of the nature of this study and I understand that participation is on the basis of informed consent. I further agree that I meet the inclusion criterion and I am not allergic to *managu* or milk products that are used in preparing the samples for this study.

Sign …………………………..

Date: …………………………

Appendix V: List of Publications

1. **Kamau, E.H,** Mathara, J.M., and Kenji, G.M. (2020). Characterization of the Chemical and Phytochemical Profiles during Fruit Development and Ripening in Selected Cultivars of African Nightshade (*Solanum nigrum L.*) Edible Berries. *Journal of Agricultural Studies*, Vol. 8(2), pp. 806-816. https://doi.org/10.5296/jas.v8i2.16873

2. **Kamau, E.H,** Mathara, J.M., and Kenji, G.M. (2020). Sugar Content and Physical Characterization of Four Selected African Nightshade (*Solanum nigrum*) Edible Berries. *European Journal of Agriculture and Food Sciences*, Vol. 2(3). https://doi.org/10.24018/ejfood.2020.2.3.44