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Constraints to utilization of the African nightshades and the effects of lactic acid fermentation on its nutritional and sensory qualities

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CONSTRAINTS TO UTILIZATION OF THE AFRICAN NIGHTSHADES AND THE EFFECTS OF LACTIC ACID FERMENTATION ON ITS NUTRITIONAL AND SENSORY QUALITIES

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A Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

African nightshades (ANS, *Solanum nigrum* complex) are among the most widely distributed and consumed indigenous vegetables in Tanzania. Several challenges hamper the utilization of ANS. This study sought to assess trends and constraints to ANS utilization in Kilimanjaro and Morogoro regions, Tanzania and also, to assess the effect of lactic acid fermentation on the nutritional and sensory quality of ANS. About 627 farmers' households were involved in the study. Quantitative and qualitative methods were employed to collect information. Semi-structured questionnaires, focus group discussions and Key informants' interviews were the methods of data collection. *Solanum villosum* and *Solanum scabrum* were fermented naturally and controlled fermentation using *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. The results showed that ANS is mainly used as food (96.1%), animal feed (41.3%), and medicine (38%). On average, only 5% of ANS sales contributed to family income. The main constraints to ANS utilization include; pests and diseases (92.9%), lack of knowledge (58%), shortages of fertilizer (51%), shortages of pesticides (50%), inadequate means of transport (50.4%), lack of extension services (48%), improper postharvest handling (41.4%) and inadequate storage facilities (34%). Postharvest losses accounted for a 78.4% loss of ANS. There was minimal value addition on ANS, e.g., drying (5.3%) and fermentation (1.1%). Fermentation significantly reduced pH and increased titratable acidity. Fermentation increased β-carotene 2-5 times for both pickles. Vitamin C, chlorophyll and polyphenol were significantly reduced. Fermentation increased bioavailability of minerals (P, Ca, Fe and Zn). Knowledge should be given on good agricultural practices i.e., the proper use of pesticides, fertilizers, and quality seeds. Knowledge of the processing and preservation of ANS is necessary for farmers to improve utilization, reduce losses and ensure ANS availability. Fermentation can preserve ANS, with an increase in β-carotene and reducing antinutrients. Fermentation can be recommended to small-scale farmers, processors and households to improve their nutrition and livelihood.

DECLARATION

I, Frank Sangija, hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this thesis is my original work and that it has neither been submitted nor is concurrently submitted for degree award in any other institution.

Frank Sangija 01/08/2023

(**Candidate**) Signature **Date**

The above declaration is confirmed

Prof. Athanasia Matemu 01/08/2023

(**Supervisor 1**) **Signature** **Date**

Dr. Haikael Martin 01/08/2023

(**Supervisor 2**) **Signature** **Date**

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology the thesis entitled *“Constraints to utilization of the African nightshades and the effects of lactic acid fermentation on its nutritional and sensory qualities”* in fulfilment of the requirements for the degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

I dedicate this work to my family (My parents, my wife, and my children).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PLATES

LIST OF APPENDICES

LIST OF ACRONYMS/ABBREVIATIONS AND SYMBOLS

AIVs	African Indigenous Vegetables
ANS	African nightshade
BNS	Black nightshade
CF	Controlled Fermentation
DW	Dry Weight
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FGDs	Focuse Group Discussions
FFP	Full Fermented Pickle
FruVaSe	Fruits and Vegetables for all Seasons
FPP	Fresh-packaged Pickle
FW	Fresh Weight
LA	Lactic Acid
LAB	Lactic Acid Bacteria
LAF	Lactic Acid Fermentation
NIMR	National Institute for Medical Research
PaP	Processing and Preservation
PHH	Postharvest Handling
PHL	Postharvest Losses
RDA	Recommended Daily Allowance
PSC	Pure Starter Culture
RUL	Recommended Upper Limit
RP	Refrigerated Pickle

SF	Spontaneous Fermentation
SGA	Steroidal Glycoalkaloids
Ss	*Solanum scabrum*
SSA	sub-Saharan Africa
SsCFR	*S. scabrum* Controlled Fermented Relish
SsR	*S. scabrum* Raw
SsSFR	*S. Scabrum* Spontaneous Fermented Relish
Sv	*Solanum villosum*
SvCFP	*S. villosum* Controlled Fermented Pickle
SvR	*S. villosum* Raw
SvSFP	*S. villosum* Spontaneous Fermented Pickle
TARI	Tanzania Agricultural Research Institute
TGA	Total Glycoalkaloids
TM	Traditional Medicine
TOSCI	Tanzania Official Seed Certification Institute
TTA	Titratable Acidity
TZS	Tanzania Shillings
UA	Urban Agriculture
VEOs	Village Executive Officers
WHO	World Health Organization
WVC	World Vegetable Center

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

African nightshades are among many underutilized and neglected African indigenous vegetables (AIVs) species; if adequately exploited, they could improve food, nutrition, and income among the rural population (Abukutsa, 2003; Abukutsa-Onyango, 2007; Dinssa *et al*., 2016). However, Weinberger and Msuya (2004) argued that ANS is not underutilized as usually thought but is somewhat undervalued. The ANS belongs to many species in the genus *Solanum* in the family *Solanaceae* found in temperate and tropical regions of the world, and it consists of about 90 genera and 2000-3000 species (Edmonds & Chweya, 1997; Yang & Ojiewo, 2013). Solanum forms the largest and most complex genus within this family composed of more than 1500 species (Edmonds & Chweya, 1997).

African nightshade is available in most parts of Tanzania's northern, central, and eastern parts in regions of Arusha, Kilimanjaro, Morogoro, Dodoma, Tanga, and southern regions of Tanzania in the Iringa and Mbeya regions, therefore their availability ensures a surplus supply of raw materials for processing (Keller, 2004; Weinberger & Msuya, 2004). The average yield of ANS in Tanzania is about 3.83 t/h (Weinberger & Msuya, 2004). About 71.8% of all cultivated ANS is coming from Arumeru District in Arusha and is done by households (Weinberger & Msuya, 2004; Gowele *et al*., 2019). In other regions of Tanzania, ANS is less domesticated, and high production is during the rainy season (Weinberger & Msuya, 2004).

African nightshade is rich in nutrients to promote food and nutrition security in SSA (Yang & Ojiewo, 2013). African nightshade is considered a new cash crop in Tanzania and other SSA regions since it contributes to income generation for individuals and households (Shackleton *et al*., 2009). African nightshade has been part of SSA's food systems for generations, and its leaves, young shoots, and flowers are consumed for various purposes (Ambrose-Oji, 2009; Abukutsa-Onyango, 2010; Yang & Ojiewo, 2013). African nightshades are among high-priority AIVs with the potential for health, nutrition, and economic benefits (Edmonds & Chweya 1997; Yang & Ojiewo 2013). They are rich in macro- and micro-nutrients, including thiamine, ascorbic acid, iron, calcium, zinc, protein and dietary fibre (Ontita *et al*., 2017; Yuan *et al*., 2018; Ronoh *et al*., 2017; Kirigia *et al*., 2019). Furthermore, they are rich in bioactive compounds, particularly lutein, zeaxanthin, polyphenol, flavonoids and chlorophylls, which possess antioxidant activity, anti-genotoxicants and anticancer properties (Odongo *et al*., 2018). In SSA, ANS is exploited for food, medicines, animal feed, and spiritual, but less exploited for socioeconomic benefits (Abukutsa-Onyango, 2007; Ontita *et al*., 2017). However, ANS are wasted (Stevens *et al*., 2015; Global Panel, 2018) despite the favorable climatic conditions and high production; they account for 50% of postharvest losses (PHL) of all food produced in SSA (Weinberger & Msuya, 2004; Global Panel, 2018).

Of recent, there is a decline in the use of ANS caused by a shift toward exotic vegetables, which consumers rank as nutritious and high-yielding (Abukutsa-Onyango *et al*., 2010). However, problems of environmental degradation, shortage of arable land, poor soil moisture, diseases, pests, and high production costs have reduced the reliability of exotic vegetables (Ondieki *et al*., 2011). Many people are facing malnutrition and food-related chronic diseases while ANS are wasted (Global Panel, 2018). Unconsumed ANS contribute to green gas emissions (FAO, 2013).

Therefore, the current study documented the trends of utilization, local processing technologies, constraints, and opinions to improve the consumption of ANS aiming to reduce massive losses. In addition, the study developed pickle and relish products using spontaneous and controlled fermentation technology for the reduction of postharvest losses of the vegetable. Also, the study conducted a consumer acceptability test of the relish product. LA fermentation technology is convenient and cost-effective; therefore, they are easily adapted by farmers, small-scale entrepreneurs, and households for improving their livelihood. Thus, strong efforts on the value addition of ANS have been conducted to facilitate the diverse consumption of ANS and promote the industrialization policy in Tanzania.

1.2 Statement of the problem

Postharvest loss of ANS in SSA is higher than that of North African countries (FAO, 2013). More than 50% of all vegetables produced in SSA countries are lost (Global Panel, 2018). Poor postharvest handling, electricity problems, and lack of proper processing and preservation of vegetables in SSA results in high loss (Global Panel, 2018; Gowele *et al*., 2019). Even with the known nutritional value of ANS, health, and economical benefits, their utilization and production of food have been limited than optimal. The ANS is highly neglected and underutilized and has a limited value chain due to few studies on its utilization and added value (Abukutsa-Onyango, 2007). Pests and diseases are challenging the production of ANS resulting in low yields. Fungi, bacteria, and viruses are the main causative agents for ANS diseases (Abukutsa-Onyango, 2007; Yang & Ojiewo, 2013; Onyango *et al*., 2016; Nono-Womdim *et al*., 2012).

Postharvest losses and poor postharvest handling of ANS are the main problems hindering ANS utilization in Tanzania (Wafula, 2017; Onyango *et al*., 2016). The short shelf life of ANS reduces the market supply and facilitates the spoilage of ANS (Muhanji *et al*., 2011; Dinssa *et al*., 2016). The production and use of ANS leaves have been seasonal due to issues including inadequate post-harvest technologies. African nightshade growers do not have appropriate storage and post-harvest technologies that increase their availability in and out of season (Wafula, 2017; Onyango *et al*.,2016). During peak season, there is high postharvest loss of ANS leaves (Abukutsa-Onyango, 2007). For ensuring the availability of ANS, local communities in Tanzania use indigenous knowledge for value-addition techniques; however, documented studies do not yet report on such practices, their scope, and limitations. This study aimed to document the trends and constraints of the utilization of ANS and employed lactic acid fermentation as an appropriate technology for improving the utilization and availability of ANS year around.

1.3 Rationale of the study

Vegetable fermentation technology has been used in many countries such as China, Korea, and Thailand by households, farmers, and SMEs for preserving their vegetables for a long time without spoilage (Cocolin & Ercolini, 2008). The technology can be easily implemented in Tanzania because it does not require a high initial cost, so most people such as farmers, women groups and SMEs can successfully apply the technology. Relish making can be done using locally available ingredients; ingredients (sugar, salt, garlic, gingers, pepper, and cooking oil), hence reducing cost and increasing profits for processors. Lactic acid fermentation can take places at various temperatures ranging from 16 - 37° C, therefore it can be applied in different seasons without the use of incubation chambers. Also, Lactic acid fermentation can undergo spontaneous fermentation with similar quality to controlled fermentation without the application of a starters culture (Wafula, 2017). Spontaneous fermentation is affordable to small-scale farmers and SMEs. Lactic acid fermentation is expected to reduce post-harvest losses and improve nutrition because lactic acid bacteria are a good source of protein and they improve the bioavailability of micronutrients such as β-vitamins, and minerals (Fe, Zn, Ca, Cu and Ni). Also, lactic acid fermentation improves the shelflives of vegetables and ensures the availability of vegetable year-round (Wafula, 2017). Moreover, lactic acid fermentation will create diversification in the utilization of ANS. The reduction of post-harvest losses of ANS will increase food availability and improve the livelihood of farmers and SMEs in Tanzania. Lactic fermentation provides probiotics that are important to Gastrointestinal (GI) health, hence they can contribute to improving the health of the consumers (Adams & Nout, 2001). Lactic acid fermentation reduces ant nutritional factors such as oxalate, tannin, and phytate, hence improving the bioavailability of micronutrients (iron, zinc, vitamin A, and iodine) (Wafula, 2017). These micronutrients are essential for child growth and mental development (Horton and Ross, 2003). Fermentation provides metabolites such as lactic acid, and peroxides which act as antimicrobials; hence the relish can be used as a source of medicine to cure various diseases (Cocolin & Ercolini, 2008).

1.4 Objectives

1.4.1 General objective

To reduce post-harvest losses and add value of selected indigenous vegetables in Tanzania

1.4.2 Specific objectives

(i) To document the trends and constraints of utilization of ANS in Tanzania.

(ii) To develop pickle/relish products using optimized fermentation technology.

(iii) To determine the effect of the optimized fermentation technology on nutrient retention, nutraceuticals, and anti-nutrient removal of pickle/relish.

(iv) To assess the effects of refrigeration, and room temperature storage on the physicochemical properties of pickle/relish and microbial quality of relish products.

(v) To evaluate consumer acceptability and sensory characteristics of relish products.

1.5 Research Questions

(i) What is the status of cultivation, processing, and utilization of ANS in Kilimanjaro and Morogoro regions in Tanzania?

(ii) What are the optimized fermentation technologies used in the processing of pickle/relish?

(iii) What are the effects of the optimized fermentation technology on the nutrients and nutraceuticals retention and anti-nutrient removal of pickle/relish?

(iv) What are the effects of storage conditions (refrigeration, room temperature, and sunlight condition) on the physicochemical properties of pickles?

(v) What are the organoleptic characteristics which consumers mostly prefer in the relish?

1.6 Significance of the study

The study contributed to the improved of dietary diversification of utilization of ANS through the innovative fermented product (relish). The technology will contribute to improving the nutritional quality of ANS such as minerals, and β-carotene, reducing antinutritional factors and extending the shelf life of ANS. The study will impove the livelihood of farmers by adapting fermentation technology as a means of preserving ANS. Since pickle contains important nutrients such as Fe, Zn, Ni, Ca, and β-carotene it contributes to the improvement of the health status of consumers especially those with micronutrient deficiencies such as pregnant women and children. Moreover, the study will create employment for Tanzanians, especially the growers, and processors, hence improving their standard of living. Then, the study will motivate people to engage in vegetable processing hence improving the production of ANS and ensuring its availability throughout the year.

1.7 Delineation of the study

The research focused on the improving the availability of ANS, using lactic acid fermentation. Baseline survey was conducted to evaluated the trends and constraints of utilization of ANS. Twenty villages were selected randomly in districts of Moshi DC, Hai, Kilombero and Morogoro DC. The survey was used to establish the preservation technique (lactic acid fermentation) for improving ANS. Relish is good source of iron, calcium, and zinc, also is good source of β-carotene. Relish was stable after six months of storage and was highly preferred by consumers. This technology can applicable in preserving other vegetables and can be easily adapted. The study did not evaluate an intervention study promoting the use of ANS relish in improving the micronutrient status in Tanzania. There was no proper record of ANS farmers which consequently delay the study. Time constraints to conducting a baseline survey in four selected districts. Budget constraints to conduct some experiments like vitamin B1, B2, and B9.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diversity of African nightshade

The commonly available ANS species in SSA include; *S. americanum, S. scabrum, S. nigrum*, and *S. villosum* (Table 1) (Edmonds & Chweya, 1997; Keller, 2004; Yang & Ojiewo, 2013; Yuan *et al*., 2018). Eight species belong to the *S. nigrum* complex or the black nightshades (BNS) complex, namely; *S. nigrum* L., *S. americanum, S. scabrum* Mill., *S. sarrachoides* Sendtn., *S. villosum* Mill., *S. grossedentatum* A. Rich., *S. florulentum* Bitter and *S. tarderemotum* Bitter are distinguished, based on characteristic morphological traits (Edmonds & Chweya, 1997; Dehmer & Hammer, 2004; Ronoh *et al*., 2017). *Solanum scabrum, S. villosum,* and *Solanum nigrum* are most prevalent in East Africa (Table 1) (Ambrose-Oji, 2009) but are produced in other regions of Africa (Table 1 and Plate 1). Nonetheless, they are still considered a wild weedy crop in most SSA (Edmonds & Chweya, 1997; Dinssa *et al*., 2016). They can be grown in various places such as roadsides, hedgerows, around buildings and houses, under trees, as garden weeds, on riverbanks and in gullies, on forest and grassland margins, quaysides and rubbish tips, on shingle beaches, on railway cuttings, and the edges of cultivated fields and plantations (Edmonds & Chweya, 1997). About 45% of farmers in Mbale, Uganda, collect ANS from the wild for selling in the urban markets (Kasambula *et al*., 2007; Ambrose-Oji, 2009). Approximately 90% of vegetable supplies in big cities in SSA, including the Central Africa Republic, Guinea-Bissau, Madagascar, and Tanzania, are predicted to come from urban and peri-urban agriculture (Ambrose-Oji, 2009). Most of these vegetables are exotic, and highly consumed in urban and peri-urban regions (Ambrose-Oji, 2009). However, many fresh AIVs leaves available in urban markets come from rural areas due to seasonal production (Gido *et al*., 2017).

Table 1: Edible ANS species diversity and distribution in SSA

African Nightshades	Description	Distribution	References
***Solanum scabrum* Mil**	Broad leaves Ripe berries (1000 - 2000 berries); 1000 seed weight 1.0 - 1.3 g. Black or purple leaves of greater than 14 mm in size. Differ in growth habits, bitterness, and leaf color. Taller up to 1.5 m in height. Low rainfall and temperature (18 to 30 ºC). Good sources of carotenoid, vitamin C, E and A, C, calcium, iron, zinc, and protein Used as food and medicine	East Africa, West Africa, Central Africa	Edmonds and Chweya (1997); Maundu *et al.* (2003); Yang and Ojiewo (2013); Abukutsa-onyango (2009); Wafula (2017)
***Solanum nigrum* Miller**	Black nightshades, leafy green vegetables (*S. eldorettii*, S. *tarderemotum*, and *S. florilegium).* Berries about 3000 - 4000; 1000 seed weight of 0.4 - 0.6 g. Good sources of carotenoid, vitamin C, E and A, C, calcium, iron, zinc, and protein. Used as food and medicine	North Africa, Kenyan Highlands, Northern Tanzania	Edmonds and Chweya (1997); Maundu *et al.* (1999); Jacoby *et al.* (2003); Shackleton *et al.* (2009); Yang and Ojiewo (2013); Wafula (2017)
***Solanum americanum* Miller**	Black nightshades; *Mnavu*, *Msogo*, *Mhaki* (Tanzania), and *Wsuggaenzirugavu* (Uganda) Berries are shiny dark purple, and the barriers are smaller than 12 mm. Small berries about 4500; 1000 seeds weigh 0.3 - 0.4 g. Moderately thin branches than other species. Good sources of carotenoid, vitamin C, E and A, C, calcium, iron, zinc, and protein. Used as food and medicine	East Africa, West Africa, Central Africa, Southern Africa	Edmonds and Chweya (1997); Maundu *et al.* (2003); Shackleton *et al.* (2009); Yang and Ojiewo (2013); Wafula (2017)
***Solanum villosum* Miller**	Black nightshades or garden huckleberry; *Mnavu* (Tanzania and Kenya). Berries about 3000-4000 (orange to yellow); 1000 seed weight of 0.4-0.6 g. Wild and cultivated Used as food and medicine	East Africa, West Africa, Central Africa, Southern Africa, Egypt	Edmonds and Chweya (1997); Maundu *et al.* (2003); Shackleton *et al.* (2009); Yang and Ojiewo (2013); Wafula (2017)

Solanum nigrum, commonly known to be native to Eurasia, was introduced in the Americas, South Africa, and Australasia (Kuete *et al*., 2017). *Solanum americanum* (American BNS) is native to the Americas (Dehmer & Hammer, 2004) and is the most unrelated species within the *S. nigrum* complex with only ca. 43% of genetic similarity (Gilbert, 2006). Within the *S. nigrum* complex, *S. scabrum* and *S. nigrum* are near related species (ca. 68%), with the *S. villosum* being close to them by 62% (Dehmer & Hammer, 2004; Gilbert, 2006). American BNS are native to the Americas, particularly Cuba and South America (Dehmer & Hammer, 2004; Gilbert, 2006). In the Americas, this species is found in California, Mexico, Central America, and South America. The nomenclature and taxonomy are associated with toxic BNS (*Atropa belladonna*) of temperate Eurasian origin; this species has many phenotypic similarities with many nightshades (Gilbert, 2006). The ANS has been consumed for centuries by native peoples in Central America, Mexico, and Africa and is essential in these regions (Lotter *et al*., 2014). In SSA, Bennin, Cameroon, Burkina Fasso, Tanzania, and Kenya highly consume ANS (Keller, 2004; Weinberger & Msuya, 2004; WVC, 2017), and it is also well promoted in the Southern African Development Community (SADC) region (Ojiewo *et al*., 2013a). Table 1 shows the diversity and distribution of ANS in SSA. In Tanzania, the northern (Arusha & Kilimanjaro), central (Dodoma), eastern (Morogoro & Tanga), and southern (Iringa & Mbeya) zones are well-known for ANS production (Weinberger & Msuya, 2004; Keller, 2004). Both traditional and exotic vegetables cover 9% of all cultivated land (Weinberger & Msuya, 2004; Dinssa *et al*., 2016). In East Africa, some improved cultivars of *S. scabrum* and *S. villosum* were released based on their superior yield and acceptability. Thirteen cultivars of *S. scabrum* (ACC.15B, ACC.16A, ACC.16B, ACC.18, ACC.20, ACC.21, ACC.3, ACC.4, ACC.6, ACC.7 & ACC.8B) and *S. villosum* (ACC.25 & ACC.29) were released in Kenya (Ronoh *et al*., 2019). In Tanzania, cultivars of *S. scabrum* (BG16-Nduruma and SS49-Olevolosi); and RC18-ES13-3-Ambureni (*S. villosum*), and RC10-ES13-3-Malala (*S. scabrum*) were released in 2011 and 2018, respectively (https://www.tosci.go.tz/publications/22; Ojiewo *et al*., 2013a; https://avrdc.org/download/tav-posters/MWAMBO-Omary_African-Nightshade.pdf). Also, about five advanced lines of *S. scabrum* (SS52, SS40, SS042, ABUK1 & ABUK2) and one of *S. villosum* (ABUK3) are available in Kenya and Tanzania (Ronoh *et al*., 2019).

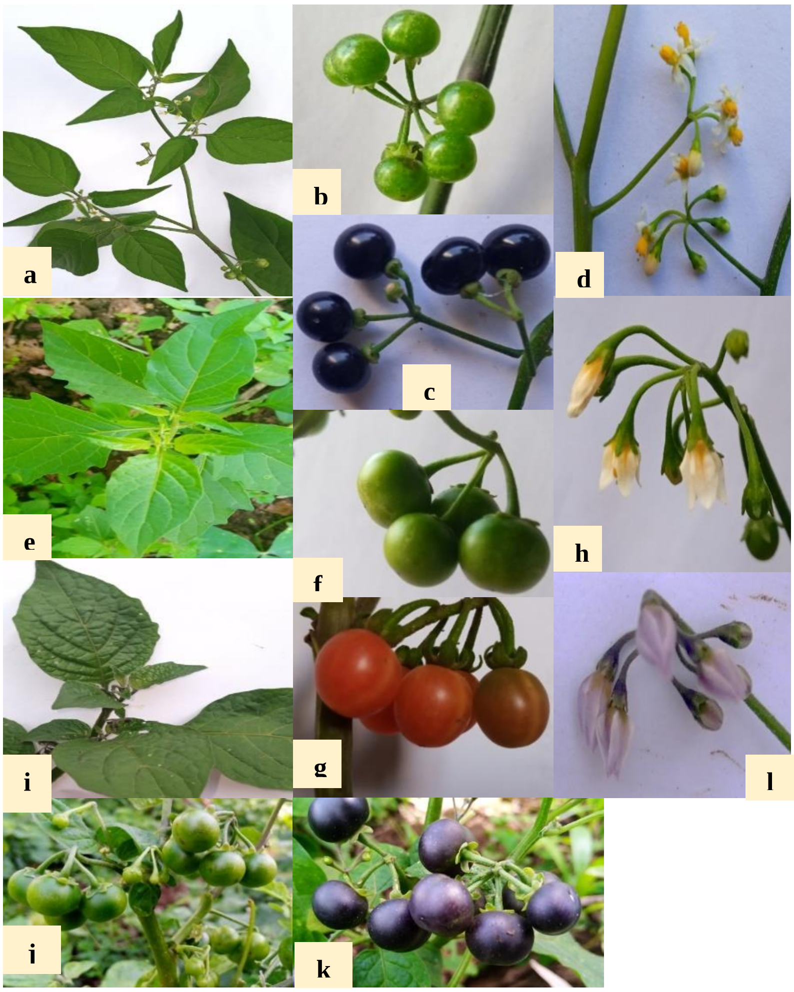

Plate 1: Leaves, flowers, and berries of *S. nigrum*, *S. villosum* & *S. scabrum*: a, e & i; (Unripe fruits, ripe fruits & flower of *S. nigrum* respectively: b, c & d; Unripe fruits, ripe fruits, and flowers of *S. villosum*: f, g & h; Unripe fruits, ripe fruits and flower of *S. scabrum*: j, k & l

2.2 Cultivation of African nightshade

African nightshades are a popular and cash-generating vegetables in Africa (Yang *et al.*, 2013). The production of ANS requires a small portion of land, and it does not require extensive external inputs and production experts. Therefore, it encourages the majority of farmers to engage in its production (Abukutsa-Onyango, 2007). Demands for AIVs have surged in Africa due to rising market requirements for AIVs varieties (Yang *et al.*, 2013). Small-scale production of ANS is common in Kenya; about 80% of farmers use less than 0.25 acres for production (Abukutsa-Onyango, 2007; Ambrose-Oji, 2009), characterized by low leaf yields of 1.5-3 tons/ha specifically for *S. scabrum*, *S. villosum*, and *S. americanum* (Ojiewo *et al.*, 2013a). A low level of production of ANS hinders its availability and consequently results in low consumption. Onyango *et al*. (2016) indicated that about 75 - 80% of ANS growers in Kenya are women cultivating small plots of less than 0.25 acres. About 59% of all farmers produce less than 50 kg of ANS per season (Abukutsa-Onyango, 2007; Ambrose-Oji, 2009; Gebru, 2015). The African indigenous vegetables are grown mainly in home gardens within the homestead (Abukutsa-Onyango, 2007), as a source of food and small income generation for their family; it also helps them become financially independent (Onyango *et al*., 2016).

2.2.1 Cropping system of African nightshade

Mono-cropping, intercropping, and crop rotation are cropping systems used to grow ANS. However, most farmers practice mono-cropping, followed by intercropping, and few farmers practice crop rotations. Several benefits of intercropping include a diversity of crops in a given season and optimal utilization of resources such as nutrients, water, and light (Gebru, 2015; Onyango *et al*., 2016). The ANS can be intercropped with maize, millet, sweet potatoes, kale, beans, avocado, cassava, groundnuts, and bananas (Abukutsa-Onyangango, 2007). The current global trend is encouraging organic farming by avoiding harmful chemicals to the environment and consumers (Abukutsa-Onyango, 2007). The planting of ANS is carried out twice in Kenya during the long rains (March-July) and the short rains (September-December) (Abukutsa-Onyango, 2007). About 69% of Kenya's farmers depend on rainfall, and only 20% to 31% practice irrigation using watering cans (Abukutsa-Onyango, 2007; Gebru, 2015).

2.2.2 Yields of African nightshade

African nightshade edible leaves give the highest yield between 12-50 tons/ha per season (Edmonds & Chweya, 1997; Abukutsa-onyango, 2007; Ojiewo *et al*., 2013a). However, Ojiewo *et al*. (2013a) and Molina *et al*. (2020) reported an annual yield of 1.5-3.0 and 3.85 tons/ha, respectively. The average share of cultivated ANS varies across locations, and the most substantial proportion is the Arumeru district in Arusha-Tanzania, which covers 20% of all cultivated land (Weinberger & Msuya, 2004; Dinssa *et al*., 2016). Arumeru district produces about 71.8% of all cultivated ANS in Tanzania (Weinberger & Msuya, 2004). The presence of national and international research centers, including the World Vegetable Centre, Eastern and Southern Africa (WVC-ESA), International Institute of Tropical Agriculture (IITA), and Nelson Mandela African Institution of Science and Technology (NM-AIST), positively promote cultivation and consumption of AIVs, including ANS around these regions (Dinssa *et al*., 2016).

African nightshade is tolerant to abiotic stress under low soil moisture and heat. Luoh *et al*. (2014) reported that ANS, *S. scabrum* retains vitamins and minerals and undergoes less weight loss at water deficit. Thus, ANS suffers less in water deficiency and becomes a crop of choice under drought conditions in SSA. African nightshade can take 3-4 weeks for the first harvest; therefore, it is an essential crop to feed the world's large population (Lobell & Gourdji, 2012; Dinssa *et al*., 2016).

Rapid urbanization has increased the demand for ANS in SSA (Ambrose-Oji, 2009; Shackleton *et al*., 2009). There is considerable scope to increase ANS yield; therefore, variation between yield obtained in farms and research has been reported (Dinssa *et al*., 2016). This variation further suggests the importance of technological application for the intense production of ANS on farms.

2.2.3 Factors affecting the cultivation of African nightshade

(i) Pests and diseases

Pests and diseases are the main challenges during ANS production in SSA, resulting in low yields. Fungi, bacteria, and viruses are the main causative agents for ANS diseases. The major fungal diseases include; damping-off (*Rhizoctonia* spp.), early blight (*Alternaria solani*), late blight (*Pychrithium infestans*), fusarium wilt (*F. oxysporum* and *F. solani*), and verticillium wilt (*Verticillium dahliae*). The bacteria diseases are bacterial wilt (*Ralstonia solanacearum*), leaf mold (*Cladosporium oxysporum*), eyespot, and southern blight (Edmonds & Chweya, 1997; Abukutsa-Onyango, 2007; Shackleton *et al*., 2009; Ambrose-Oji, 2009; Yang & Ojiewo, 2013; Onyango *et al*., 2016; Dinssa *et al*., 2016; Nono-Womdim *et al*., 2012; MoALF/SHEP PLUS, 2019).

Pests and diseases damage the leafy structure and reduce the quality of vegetables, leading to customers' rejection (Abukutsa-Onyango, 2007). *Enterobacter mori* isolated from ANS pickle (Wafula, 2017) cause plant bacterial wilt (Zhu *et al*., 2011); perhaps they also cause wilt in ANS. The pest and diseases of ANS are the same as those of other Solanaceae families. Pests and diseases are a significant problem in Tanzania; however, it becomes more intense when vegetables are grown for multiple harvests over a long time or used for seed production (Keller, 2004).

On the other hand, viruses control insects (aphids, whiteflies) and inadvertently bruise young leaves by touching them (Nono-Womdim *et al*., 2012; Ojiewo, 2013a). The viral diseases include; leaf curl viruses, leaf mosaic viruses, yellow viruses, and tomato mosaic viruses (Nono-Womdim *et al*., 2012; Wafula *et al*., 2017). Tomato mosaic virus (ToMV) is a common virus found in ANS in SSA (Nono-Womdim *et al*., 2012). The control measures for pathogenic microorganisms include; the use of fungicides and disease-free seeds, minimizing injury, avoiding dense planting, using furrow or drip irrigation, and crop rotation. Further, removing infected plants and destroying them immediately after harvest, avoiding over-fertilization, adequate sanitation, and using healthy seedlings can control pests and diseases (Nono-Womdim *et al*., 2012).

The most common pests of ANS include; aphids *(Aphis sp.)*, spider mites (*Tetranychus evansi* Baker), red and black ants, cutworms, caterpillars (larvae), grasshoppers (*Zonocerus variegatus)*, whiteflies (*Bemisia tabaci*), beetles (*Epilachna hirta*, *Lagria* spp., and *Podagrica* spp.), Nematodes (*Meloidogyne* spp.) (Nono-Womdim *et al*., 2012; MoALF/SHEP PLUS, 2019).

The short shelf life of ANS reduces the market supply and bargaining power of small-scale farmers and local open market sellers (Muhanji *et al*., 2011; Dinssa *et al*., 2016). Therefore, a multidisciplinary approach is needed between breeders and postharvest specialists to improve vegetables' shelf-life and storage conditions. Interventions through affordable preservation techniques can increase small-scale farmers' income and vegetable marketing (Muhanji *et al*., 2011; Dinssa *et al*., 2016).

(ii) Lack of improved cultivars

The lack of improved cultivars limits the high production of ANS in SSA, resulting in low-yielding cultivars (Dinssa *et al*., 2016). Seed manufacturing companies do not consider the production and marketing of ANS seeds as a profit-generating business since most AIVs are open-pollinated, forcing most farmers to produce their seeds (Dinssa *et al*., 2016). Nevertheless, the seeds of improved ANS cultivars are not available in seed stores in many regions of SSA, particularly in remote areas (Muhanji *et al*., 2011; Dinssa *et al*., 2016). As of recently, some improved cultivars of *S. scabrum* and *S. villosum* are available to farmers in Tanzania and Kenya for commercial use (Ronoh *et al*., 2019; https://www.tosci.go.tz/publications/22). Besides, several advanced lines of *S. villosum* and *S.*

scabrum are available to advance the breeding research of the crop for improved characteristics such as yield, pests, and disease resistance (Ronoh *et al*., 2019).

(iii) Other factors

Other factors that hinder the production and consumption of ANS include inadequate rainfall, lack of knowledge, lack of appropriate storage facilities, low-quality seeds, drought, fragmented marketing channels, poor transport infrastructure, agronomic challenges, and lack of proper packaging (Abukutsa-Onyango, 2007; Ambrose-Oji, 2009). Researchers should involve farmers as they are key producers for better-improving ANS production. Improved cultivars are essential since they require fewer inputs, tolerate pests and diseases, and tolerate different climate conditions (Muhanji *et al*., 2011).

2.3 Nutritional and functional benefits of African nightshade

2.3.1 Macro and micronutrients

African nightshade leaves and berries contain a high amount of protein, carbohydrates, minerals, and vitamins (A, C, E, and B complex) and could contribute to a healthy diet as depicted in Table 2 and Table 3 (Ambrose-Oji, 2009; Wafula *et al*., 2017; Stoll *et al*., 2021). The high content of micronutrients is sufficient to contribute to the recommended daily allowance (RDA) (Table 4). According to the Food and Agriculture Organization of the United Nations (FAO) & World Health Organization (WHO) (2004), the RDA of vegetables and fruits is 400 g, indicating that ANS consumption alone can meet the RDA of vitamin C, E, A, iron, and manganese. African nightshades contains β-carotene (2.8 - 13.8 mg/100g FW.) higher than the average level of various vegetables reported by the USDA National Nutrient Database Table 2 (Yuan *et al*., 2018). For instance, 100 g of leaves of ANS can provide over 100% of the vitamin A needs of pregnant women (WVC, 2017; Schreinemachers *et al*., 2018). African nightshades contains a significant amount of B vitamins including thiamine (0.08 - 0.35 mg/100g FW.), riboflavin (0.17 - 0.19 mg/100g FW), and folate (12 - 56 μg/100g FW) (Table 2). The B vitamins function as co-enzymes and help in energy production from carbohydrates, and the synthesis of neurotransmitters, fatty acids, and hormones (Blake, 2008). Also, ANS contains a high content of vitamin C (140 mg/100g FW) and vitamin E (1.21 - 5.94 mg/100g FW), which are natural antioxidants (Blake, 2008; Agudo & FAO, 2004). Young leaves of ANS have low vitamin C content than matured leaves (Cheptoo *et al*., 2019). Vitamin C prevents scurvy, reduces gastric cancer, and stabilizes folate in food and plasma. The dietary intake of 25 mg/day of vitamin C improves iron absorption and prevents anemia (Agudo & FAO, 2004). The ANS leaves are rich in iron (1.3 - 7.2 mg/100g FW), zinc (0.1 - 0.56 mg/100g FW), and calcium (173 - 199 mg/100g FW) (Table 2). *Solanum retroflexum* leaves contain 7.2 mg/100 g of iron (Sivakumar *et al*., 2020). A calcium content of 40 mg can interfere with iron absorption, at a concentration of 300 - 600 mg inhibited 60% of iron absorption (Agudo & FAO, 2004). Increasing iron intake and avoiding foods rich in calcium and iron in the same meal can improve iron absorption (Agudo & FAO, 2004). Calcium contributes to bone development; the deficiency led to the development of osteoporosis. Zinc contributes to the improvement of the immune system and repairing cell and organ integrity. The deficiency of zinc in humans results in growth retardation, bone maturation, delayed sexual maturity, diarrhea, skin lesions, reduced appetite, and increased vulnerability to infections (Agudo & FAO, 2004).

Solanum nigrum leaves contain a high amount of protein, fiber, and total ash than the same species' berries. However, berries contain more fat and carbohydrates (Table 2 & Table 3). In comparison, *S. nigrum* leaves contain a high amount of calcium, iron, magnesium, phosphorus, potassium, sodium, and zinc than berries (Table 2 & Table 3). Also, the leaves of *S. nigrum* contain a high amount of vitamins A, C, and B9 than the berries; besides, the berries contain a high amount of vitamin B1. The high contents of the macro and micronutrients present in ANS leaves suggest that leaves are more nutritious than berries despite some nutrients being high in the berries. Therefore, research is needed to assess the nutrient contents of berries in other species of ANS. Notably, the ANS-dense and diverse nutrients can improve essential nutrients for better health if sufficiently eaten (Table 4).

Table 2: Nutrient content of the raw and processed ANS species

	Raw ([a, e, f, g, j, m])	Solar Drying ([g, j])	Blanched ([j])	Fermented	Raw ([e, h, i, k, l, m])	Dried([h])	Blanched ([h])	Fermented ([n])	Raw ([e, m])	Blanched	Dried	Fermented
Nutrients		***S. nigrum* Mill.**				***S. scabrum* Mill.**				***S. villosum* Mill.**		
Moisture %	87.7	6.5-12.3	91.5	-	85.8	-	89.3	-	-	-	-	-
Protein (g)	39.8 DW/3.4 FW	36.1-38.2 DW	40.6DW	-	3-6 FW	-	-	14-16 DM	4.2 FW	-	-	-
Energy KJ	162	-	162	-	-	-	-	-	-	-	-	-
Fat (g)	7.6 DW	4.14 DW	7.28 DW	-	3.1 FW	-	-	-	1.9 FW	-	-	-
Fibre (g)	2.5 FW/ 14.07 DW	-	-	-	12.8 DW	11.6 DW	12.05 DW	-	1.3 FW	-	-	-
Dry matter (g)	8 FW	-	-	-	10.5-22 DW	-	-	21.7- 22.4 DW	11.1	-	-	-
Carbohydrate (g)	29.04 DW/3.78 FW	14-17.1 DW	60.7 DW	-	0.3 DW	-	-	-	-	-	-	-
Total ash	10.6-14.6 DW/1.38-1.9FW	11.5-12.3DW	8.8 DW	-	-	-	-	25.1DW	-	-	-	-
Ca (mg)	173-199 FW	-	-	-	1460-2430 DW	-	-	-	175 FW	-	-	-
Fe (mg)	1.3-7.2 FW	-	6.9 FW	-	70-130 DW	-	-	-	3.3 FW	-	-	-
Mg (mg)	25-92 FW	-	87 FW	-	330-410 DW	-	-	-	NA	-	-	-
P (mg)	36-75 FW	-	33 FW	-	270-320 DW	-	-	-	-	-	-	-
K (mg)	257-430 FW	-	232 FW	-	100 FW	-	-	-	-	-	-	-
Na (mg)	3-8 FW	-	8 FW	-	74.22 FW	-	-	-	-	-	-	-
Zn (mg)	0.1-0.56 FW	-	0.53 FW	-	4.0-6.2 DW	-	-	-	0.8 FW	-	-	-
Cu (mg)	0.16 FW	-	0.15 FW	-	2.6-4.2 DW	-	-	-	-	-	-	-
Mn (µg)	2.1 FW	-	2.1 FW	-	25.7-28.8 DW	-	-	-	-	-	-	-
β carotene (mg)	2.8-14.2FW/ 102 DW	1.7-69.4 DW	11.9 DW	-	5.5-10 FW/71.22 DW	47.64DW	54.8DW	-	13.8 FW	-	-	-
Vit. A (µg)	5 FW	-	3	-	8.8 FW	-	-	-	-	-	-	-
Vit. C (mg)	35 FW/622.9 - 757.2DW	-	-	-	40-140 FW/ 63-177.97DW	2.4-103.7DW	10.89.4DW	256.8 DW	2.9 FW	-	-	-
Vit. B1 (mg)	0.08FW/0.71 DW	-	0.07 FW	-	-	-	-	0.21- 1.5 DW	-	-	-	-
Vit. B2 (mg)	0.17-0.19 FW/1.5DW	-	0.16 FW	-	-	-	-	0.98-1.1 DW	-	-	-	-
Vit. B9 (µg)	12-56 FW	-	0.037 FW	-	-	-	-	-	-	-	-	-
Vit. E (mg)	9.3-45.7 DW/1.21-5.94 FW	-	-	-	6.4-14.2 DW	-	-	44.5-48 DM	11.4 DW	-	-	-

Note; - FW = Fresh weight basis; DW = Dry weight basis; Vit = Vitamins; The nutrients were expressed per 100g

Sources; ([e]Gogo *et al*. 2016; [a]Ronoh *et al*., 2017; [e]Yuan *et al*., 2018; [f]Van Jaarsveld *et al*., 2014; [g]Nyambaka *et al*., 2012; [h]Cheptoo *et al*., 2019; [i]Mibei *et al*., 2011; [j]Traoré *et al*., 2017; [k] Habwe *et al*., 2008; [l]Kirigia *et al*., 2019;[m]Yang & Ojiewo, 2013; [n]Wafula, 2017)

Table 3: Nutrient content of African nightshades Fruit

Nutrients	*S. nigrum* Mill.
Moisture (%)	76.86
Protein (%)	17.63 DW/4.08 FW
Fat (%)	12.18 DW/2.82 FW
Energy KJ	43.54 DW/ 10.08 FW
Fibre (%)	6.29 DW/ 1.46 FW
Carbohydrate (%)	55.85 DW/ 12.92 FW
Dry matter	- Why no value here?
Total ash (%)	8.05 DW/1.86 FW
Ca (mg)	11.82 DW/2.74 FW
Fe (mg)	12.91 DW/2.99 FW
Mg (mg)	201.36 DW/46.59 FW
P (mg)	62.50 DW/14.46 FW
K (mg)	37.19 DW/8.60 FW
Na (mg)	2.11 DW/0.49 FW
Zn (mg)	0.05 DW/0.01 FW
Cu (mg)	-
Mn (µg)	-
Sulphur	14.48 DW/3.35 FW
β carotene (mg)	-
Vitamin A (mg)	1.71 DW/0.4 FW
Vitamin C (mg)	23.38 DW/5.4 FW
Vitamin B1 (mg)	10.91 DW/2.52 FW
Vitamin B2 (mg)	-
Vitamin B9 (µg)	8.13 DW/1.88 FW
Vitamin E (mg)	5.71 DW/1.3 FW

Note; DW = dry weight; FW= Fresh weight. The nutrients expressed per 100 g (Akubugwo *et al*., 2007)

2.3.2 Phytochemicals

Phytochemicals are secondary metabolites present in abundance in various parts of ANS. Total phenols, carotenoids, glycoalkaloids, and chlorophylls are phytochemicals in ANS (Table 5) (Nyaga *et al*., 2019; Nyanga, 2020, Neugart *et al*., 2017; Yuan *et al*., 2018). *Solanum nigrum* var. *sarrachoides* leaves contain flavonoids, alkaloids, tannins, saponin phenols, phytosterols, coumarins, and glycosides. In contrast, *S. villosum* leaves showed all the phytochemicals, except for phytosterols and coumarins (Nyaga *et al*., 2019; Nyanga, 2020). These phytochemicals are also bioactive compounds in ANS with potential health benefits. *Solanum scabrum* contains many carotenoids like β-carotenoid, zeaxanthin, and lutein (Odongo *et al*., 2018). Carotenoid content in foods contributes to health maintenance and risk reduction of various diseases (Oluoch *et al*., 2012; Neugart *et al*., 2017). The high content of β-carotene in ANS contributes to vitamin A production, with health benefits in reproduction, vision, immune function, tissue differentiation, and embryonic development (Zempleni *et al*., 2007; Blake, 2008). Younger leaves of ANS have low β-carotene than matured leaves; however, matured leaves are rich in carotenoids (Cheptoo *et al*., 2019). Carotenoids help to reduce reactive oxygen species and prevent some types of cancers. However, they display a pro-oxidative effect under high concentration, high oxygen tension (lung of smokers), low levels of endogenous enzymes, and higher levels of metal ions (Fe^{3+} and Cu^{2+}) (Park *et al*., 2013; Shin *et al*., 2020). Usually, β-carotene acts as a pro-oxidant at higher oxygen partial pressure in cells and thermally oxidized bulk oil systems (Ha *et al*., 2012; Park *et al*., 2013; Shin *et al*., 2020). Lutein and zeaxanthin are carotenoid pigments imparting yellow or orange color to various foods such as carrots, peppers, fish, and eggs (Abdel *et al*., 2013). Carotenoids protect against age-related eye disease and filter specific wavelengths of light, thus providing visual performance and offering photoreceptors protection from light damage (Abdel *et al*., 2013; Eggersdorfer & Wyss, 2018; Raman *et al*., 2019). Similarly, they reduced the risk of cataracts and early age-related macular degeneration (Eggersdorfer & Wyss, 2018; Raman *et al*., 2019). Lutein and zeaxanthin can inactivate free radicals and oxygen triplicates caused by light-induced cellular activity (Raman *et al*., 2019). In an animal study, lutein-rich diets improved learning performance, and memory in mice (Eggersdorfer & Wyss, 2018), and also; in the development of the infant brain (Perrone *et al*., 2016). Still, there are no established dietary guidelines for lutein required to reach optimal macular pigment density in healthy people's eyes (Ranard *et al*., 2017; Eggersdorfer & Wyss, 2018; Raman *et al*., 2019).

Phenolic compounds concentration in ANS (16 677 and 16 387 μg/g DW.) were reported in various studies (Yang & Ojiewo, 2013; Amalraj & Pius, 2015; Shahidi & Ambigaipalan, 2015; Yuan *et al*., 2018). Flavonoids, phenolic acids, saponins, and tannins, are widely occurring phenolic compounds in ANS. They act as antioxidative compounds by scavenging free radicals that delay or inhibit the initiation step (Yang & Ojiewo, 2013; Amalraj & Pius, 2015; Shahidi & Ambigaipalan, 2015; Yuan *et al*., 2018; Degrain *et al*., 2020). Phenolic compounds possess a wide range of physiological properties, mainly anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory (Shahidi & Ambigaipalan, 2015; Boudet, 2007; Manach *et al*., 2005). The average daily intake of dietary polyphenols is approximately 1 g per person (Shahidi & Ambigaipalan, 2015). The total polyphenol content of ANS ranges from 725-1307 mg/100g FW (Table 5). This amount is sufficient to meet the RDA for the consumption of 100 g of fresh-weight ANS. However, high consumption of polyphenols causes low iron absorption (Zijp *et al*., 2000). Blanching

ANS at 95 °C or steaming using water or lemon juice solution significantly increase the total phenolic content (Yuan *et al*., 2020a).

Chlorophylls are natural pigments present in ANS. They act as a natural antioxidant with the ability to scavenge free radicals and prevent several oxidative stress-related diseases such as cancer, neurological disorders, inflammatory diseases, dermatitis, tissue damage, and sepsis; cardiovascular disorders decreased immune function, and aging (Mishra *et al*., 2011; Lanfer-Marquez *et al*., 2005; Wang & Wink, 2016; Sangija & Wu, 2020). Chlorophyll can inhibit calcium oxalate dihydrate formation, which is the primary source of kidney stones (İnanç, 2011). Besides, it stimulates the immune system, helps in detoxification, combats foul odors, and helps combat anemia and eliminate molds and toxins in the body (İnanç, 2011). Despite health benefits, chlorophyll can act as a pro-oxidant effect for oil oxidation when subjected to light (İnanç, 2011; Wang & Wink, 2016). Steaming and water blanching of ANS significantly increase chlorophyll content of 2.82-8.87 mg/100 g FW and 1.19 - 5.54 mg/100 g FW, respectively (Managa *et al*., 2020).

Glycoalkaloids are plant-derived bioactive compounds capable of interacting with living tissue components with a wide range of likely effects (Huang *et al*., 2016). African nightshades berries are a rich source of alkaloids. Solamargine and solasonine are glycoalkaloids in *S. scabrum* and solasodine glycosides in *S. americanum*. *Solanum scabrum* and *S. villosum* methanol leave extracts lack glycoalkaloids but are present in *S. villosum* Grif 16939 and *S. nigrum* PI 381290 accessions in a very low concentration of 50 ug/g DW or 1 mg/100 g FW (Yuan *et al*., 2018). Alkaloids have therapeutic effects such as cytotoxic against human carcinoma cells and anti-inflammatory against psoriasis (Kumar *et al*., 2012; Al-Ashaal, 2019). It prevents cervical carcinoma and *in vitro*, schistosomicidal effect against *S. mansonii,* and fasciolicidal effect against liver parasites. Additionally, it has an inhibitory effect against HSV-1 (Al-Ashaal, 2019). Zhao *et al*. (2018) demonstrated the anti-inflammatory activity of steroidal alkaloid solanine A from *S. scabrum* in lipopolysaccharide/interferon γ-activated (LPS/IFNγ)-stimulated RAW264.7 cells and ICR mice.

Table 4: The estimated quantity of fresh ANS to meet the recommended daily allowance

Micronutrients	RDA (mg/day)	EQ of ANS per day (g)	Quantity of Nutrients in ANS per 100 g
Thiamine	1.1-1.7	1375-2125	0.08 mg
Riboflavin	1.1-1.5	647-882	0.17 mg
Folate	400 µgDFE/day	1739	23 µgDFE
Vitamin C	73-90	209-257	35 mg
Vitamin E	15	154	9.7 mg
Vitamin A	700-900 µRAE/day	165 -213	422 µRAE
Calcium	1000	503	199 mg
Iron	18	250	7.2 mg
Magnesium	400	435	92 mg
Phosphorous	1000	1333	75 mg
Potassium	3500	814	430 mg
Sodium	2400	3243	74 mg
Zinc	15	2500	0.6 mg
Copper	2	1333	0.15 mg
Manganese	2	95	2.1 mg

Note- EQ: Estimated amount of ANS in grams of fresh weight required to be eaten to meet the recommended daily allowance; **RDA**: Recommended daily allowance. The vitamins and minerals are recommended for a person above 17 years and four years, respectively. However, this quantity can vary depending on health status, sex, age, pregnant and lactating women. **Source**; (http://www.fda.gov/nutritioneducation; Agudo & FAO/WHO, 2004; Zempleni *et al*., 2007; Van Jaarsveld *et al*., 2014; Ronoh *et al*.,2017).

For a long time, phytate is considered an anti-nutrient, but recent studies have proven its antioxidant properties (Silva & Bracarense, 2016; Bhowmik *et al*., 2017; Mora-Boza *et al*., 2019; Wang & Guo, 2021). Phytate exhibits therapeutic properties for various diseases such as Alzheimer's (Abe & Taniguichi, 2014), Parkinson's (Xu *et al*., 2011), and management of blood glucose for type 2 diabetes (Lee *et al*., 2006). Similarly, phytate exhibits anti-cancer properties against the prostate (Raina *et al*., 2008), hepatocarcinoma (Al-Fatlawi *et al*., 2014), colorectal (Navarro *et al*., 2016), rhabdomyosarcoma (Vucenik, 1998), skin (Wawszczyk, 2015), and breast (Hussein *et al*., 2006) cancers. Besides, it acts as an antibacterial against *Enterococcus faecalis* (Nassar & Nassar, 2016), anti-HIV (Tateishi *et al*., 2017), and hypolipidemic (Dilworth *et al*., 2005). Also, it inhibits lipid peroxidation due to its high affinity to multivalent cations (Mora-Boza *et al*., 2019). Phytate is used in food industries as a molecular binder and functional ingredient, i.e., aggregates proteins and increasing precipitates or turbid (Wang & Guo, 2021). The daily intake of 1-2 g and 8-12 g of phytate possess a prevention effect on cancer and antitumor therapies (Vucenik & Shamsuddin, 2006). Further studies should explore the benefits of phytate, such as health and functional benefits, instead of addressing the general concept of phytate as an anti-nutrient.

Tannins are a group of phytochemicals (polyphenols) with an astringent taste and are present in various concentrations in vegetables and herbs (Amalraj & Pius, 2015; Khanbabaee & van Ree, 2001). Tannins are water-soluble polyphenols present in many plant foods. Tannins have various health benefits like antioxidants, cardio-protective, anti-inflammatory, antiviral, antibacterial, anti-carcinogenic, anti-mutagenic, and anti-diabetic (Chung, 1998; Khanbabaee & van Ree 2001; Delimont *et al*., 2017; Sharma *et al*., 2019). They also help heal wounds, cures dysentery, and help in hardening arteries (Sharma *et al*., 2019); nevertheless, it is an antinutrient.

Table 5: Phytochemicals and antinutritional factors of the leaves of ANS species

Compounds	*S. nigrum*	*S. scabrum*	*S. villosum*	References
TPP per 100g of GAE FW	725-1307 mg	775-1247 mg	1026 mg	Yang and Ojiewo (2013); Yuan *et al*. (2018)
Quercetin glycosides (quercetin-3-rutinoside)	NI	1400–3300 µg/g DW	NI	Neugart *et al*. (2017)
Carotenoids	7.9-20.0 mg/100g FW	10,100 mg/100 g DW	1,790 µ/g DW	Adebooye *et al*. (2008); Odongo *et al*. (2018)
Flavonoids	4.7 mg/100 g FW	25.5 mg/100 g FW	19.9 mg/100 g FW	Yang and Ojiewo (2013)
Glycoalkaloids	1722 mg/100 g DW	NI	1448 mg/100g DW	Mohy-Ud-Din *et al*. (2010)
Chlorophyll	69.8-155.8 mg/100 g FW	25,000-60,000 mg/100g DW	19,600 mg/100 g DW	Adebooye *et al*. (2008); Odongo *et al*. (2018)
Tannins	355.5 mg/100 g DW	NI	NI	Amalraj and Pius (2015)
Phytate	58.8 mg/100 g DW/ 7.64 mg/100 g FW	NI	0.04-0.2 mg/100 g DW /0.0052 mg/100 g FW	(Amalraj & Pius, 2015; Mwanri *et al*., 2018)
Oxalate	776.2 mg/100 g DW	33 mg/100 g DW	28.7-68 mg/100 g DW	Yang and Ojiewo (2013); Amalraj and Pius (2015); Mwanri *et al*. (2018)
Cyanogenic glycosides	320 mg/100 g FW	NI	NI	Essack *et al*. (2017)
Nitrates	NI	NI	63.0-85.6 mg/100g DW	Mwanri *et al*. (2018)

Note: NI = Not indicated; FW = Fresh weight basis; DW = Dry weight basis: TPP = Total polyphenol. The total glycoalkaloids content was calculated by the sum of (Solasonine, α-Solamargine, β-Solamargine, and α-Solanine)

2.4 Antinutritional factors of African nightshade

African nightshade contains antinutritional factors such as oxalates, tannins, cyanogenic glycosides, phytate, glycoalkaloids, and nitrates as shown in Table 5 above (Wakhanu *et al*., 2015; Amalraj & Pius, 2015; Essack *et al*., 2017; Mwanri *et al*., 2018). Some of them, e.g., tannin, phytate, and glycoalkaloids, exhibit functional properties, e.g., anticancer, antibacterial, antivirus, and anti-inflammatory properties (Silva & Bracarense, 2016; Delimont *et al*., 2017), also elaborated in section 4.2. The oxalate and phytate contents vary with the maturity stage in *S. villosum* (Silva & Bracarense, 2016). *Solanum villosum* (Nduruma BG 16 & Olevolosi SS 49) had the highest oxalate and phytate contents, with a non-significant decrease in nitrate content at 35 days (Mwanri *et al*., 2018). Most of the Solanaceae family species are poisonous to humans and livestock (Jain *et al*., 2011). For instance, the deadly nightshade (*Atropa belladonna* L.) contains tropane alkaloids. Solanine glycoalkaloids in *S. nigrum, S. villosum, S. americanum*, and *S. scabrum* significantly cause toxicity (Jain *et al*., 2011). Notably, various ANS processing methods such as boiling, drying, and fermentation effectively removes antinutritional factors (Essack *et al*., 2017).

2.4.1 Phytate in leaves

Phytate is an anti-nutrient when present in higher concentrations (Bhowmik *et al*., 2017). Phytate can chelate with divalent/trivalent metal ions such as zinc, copper, calcium, and iron; and reduce bioavailability (Silva & Bracarense, 2016). *Solanum nigrum* contains the highest phytate content, the lowest in *S. villosum* (Table 5). The calculated molar ratio of phytate: iron was 0.09 in *S. nigrum.* An increase in ANS leaves' phytate content from 0.04 mg/100g DW in days 21 grown to 0.3 mg/100g DW. In 35 days was reported (Mwanri *et al*., 2018). Therefore, harvesting at the proper maturity is crucial for ANS nutritional quality. The molar ratio above 0.4 significantly affects iron bioavailability (FAO/IZiNCG, 2018; Dahdouh *et al*., 2019). Therefore, the molar ratio was meager to cause the chelating effect of iron in ANS. The phytate intakes reported in the U.K were 692 to 948 mg/day in men and 538 to 807 mg/day in women. The daily phytate requirement for vegetarians is 1600 to 2500 mg/day (European Food Safety Authority-EFSA, 2014). Fermentation, germination, soaking, malting, boiling, solar, and pressure cooking are simple, inexpensive, and convenient techniques for the removal of phytates in ANS (Pasrija & Punia, 2010; Rasane *et al*., 2015; Abdulwaliyu *et al*., 2019; Essack *et al*., 2017; Owade *et al*., 2019; Wang & Guo, 2021). Water blanching at 95°C and steam blanching of ANS for 5 minutes reduce phytate concentration by 25 to 75% (Managa *et al*., 2020). Besides, enzyme degradation using phytase is the most effective and applicable way to remove phytate (Wang & Guo, 2021). Phytate in unprocessed foods does not reflect the actual quantity consumed; therefore, more emphasis should be on assessing phytate in ready-to-eat foods rather than its content in raw forms (Abdulwaliyu *et al*., 2019).

2.4.2 Oxalate in leaves

As an anti-nutrient, oxalate, and chelate minerals such as potassium, sodium, and calcium form insoluble complexes, thus hindering the absorption of minerals. Insoluble calcium oxalate can cause joint pains, kidney stones, and kidney failure (Holmes & Kennedy, 2000; Judprasong *et al*., 2006; Soto-Blanco *et al*., 2009). The oxalate content of ANS can go up to 776.2 mg/100g D.M (Table 5); however, the fatal doses range from 1 to 30 g/person (EMEA, 2004; Dassanayake & Gnanathasan, 2012). The oxalate content in ANS ranges from 28.7 - 776.2 mg/100 g DW. or 3.7-100.9 mg/100 g FW. *Solanum nigrum* contains the highest oxalate content and the lowest in *S. scabrum* (Table 5). This amount is lower than the fatal dose, but it is still a concern regarding the risk of toxicity; therefore, monitoring ANS leaves' levels is necessary. Nonetheless, the amount of oxalate in ANS leaves is significantly lower than in some exotic vegetables (Akhtar *et al*., 2011; Faudon & Savage, 2014). Fermentation and boiling reduce oxalate content in ANS and other AIVs (Muchoki *et al*., 2010; Wakhanu *et al*., 2015; Essack *et al*., 2017; Owade *et al*., 2019). Likewise, blanching at 95°C and steam blanching for 5 minutes reduce oxalate in ANS by 42% to 75% (Managa *et al*., 2020). Moreover, the early harvesting stages (21 days) show lower oxalate content (42.9 mg/100 g DW) than the late stages of 35 days (60.9 mg/100 g DW) (Mwanri *et al*., 2018). Therefore, proper harvesting times and selection of ANS varieties should be the criteria for obtaining leaves with low oxalate content.

2.4.3 Tannins in leaves

Tannin in either non-hydrolyzable (condensed) or hydrolyzable (Sharma *et al*., 2019) forms a complex with proteins, digestive enzymes, starches, and minerals, thus reducing food's nutritional value (Chung, 1998; Polycarp *et al*., 2012). Tannin decreases feed intake, feed efficiency, protein digestibility, and net metabolic energy. Also, it increases the excretion of protein and essential amino acids, damages the mucosa lining of the gastrointestinal tract, and alters cations' excretion (Chung, 1998). Tannins are toxic when precipitating with heavy metals and alkaloids (Khanbabaee & van Reeb, 2001; Sangija & Wu, 2020). Tannin causes a browning reaction (darkening of food) due to polyphenol oxidase (Chung, 1998). Tannin at a concentration of 0.13 to 1 g/kg body weight (BW) decreases erythrocyte counts in pigs' haemoglobin and hematocrit (Lee *et al*., 2010). Tannin content above 3 g/100 g caused mortality in test chicks (Chung, 1998). Tannin also causes liver, skin, oesophageal, stomach, lung, kidney, and nasal cancers in humans (Chung, 1998). The RDA of proanthocyanidin (tannin) is 53.6-450 mg/person/day and 1250 mg/person/day for hydrolyzable tannins in the Spanish population. Nevertheless, the total tannin content of *S. nigrum* of 360.1 mg/100 g DW/46.81 mg/100 g FW is relatively low to harm the consumers. Therefore, consumption of ANS is safe with potential health effects. In case of sufficient tannin content causes potential health problems, cooking is an effective removal method since it is heat-labile and facilitates its degradation (Serrano *et al*., 2009; Kakati *et al*., 2010; Essack *et al*., 2017; Owade *et al*., 2019). Further, lactic acid fermentation, drying, canning, boiling, soaking in water, and freezing can also remove tannins (Serrano *et al*., 2009; Essack *et al*., 2017). Managa *et al*. (2020) reported a decrease in tannin content by 7 to 14% through blanching at 95 °C and steam blanching for 5 minutes.

2.4.4 Nitrates/Nitrites in leaves

Fruits and vegetables are significant nitrate/nitrite sources and contribute 50 to 85% of overall dietary intake (EFSA, 2008; Nuñez *et al*., 2015). Several fruits and vegetables contain 200 - 2500 mg of nitrate per kg (WHO, 2003). Nitrates/nitrites are easily absorbed in the body; about 60 - 70% are excreted in the urine, whereas 3% appear in the urine as urea and ammonia (Karwowska & Kononiuk, 2020). In the stomach, blood, and tissue, nitrates are converted into bioactive reactive nitrogen oxide species (NO). The reactive NO contributes to the formation of carcinogenic nitrosamines of toxicological importance (Ding *et al*., 2018). Nitrates can contribute to carcinogenic, such as breast cancer, gastric cancer, renal cell carcinoma, adult glioma, colorectal cancer, esophageal cancer, and thyroid cancer (Yang *et al*., 2017; Keszei *et al*., 2012; Karwowska & Kononiuk, 2020). Besides, it contributes to genotoxicity, cytotoxicity, inhibition of enzymatic reactions and proteolysis, and altered immunogenicity (D'Ischia *et al*., 2011). Antioxidants, such as vitamins C and E, inhibit nitrosamines' generation (Ding *et al*., 2018; Karwowska & Kononiuk, 2020). Mwanri *et al*. (2018) reported that nitrate content of 630 - 856 mg/kg DW in *S. villosum*; however, there is limited information on leaves of other ANS species. Some exotic vegetables such as spinach, rucola, celery, rhubarb, lettuce, beets, chard, and beetroot contain significantly higher nitrates than ANS (Karwowska & Kononiuk, 2020). Seasonality and the cultivation systems contribute to nitrites variation in ANS. Late-harvested Nduruma BG 16 and Olevolosi SS 49 had lower nitrate content than early harvested (Mwanri *et al*., 2018). Similarly, the nitrate content was higher in 21-day harvested leaves (856 mg/Kg DW/111.3 mg/kg FW) and significantly lower at day 35 (754 mg/kg DW/98 mg/kg FW) (Mwanri *et al.*, 2018). Therefore, farmers should consider late harvest for lower nitrate content in ANS. Human generally consumes between 1.2 and 3.0 mg of nitrite daily (WHO, 2016). The RDA of nitrite and nitrates are 0.06 - 0.07 mg/kg BW/day and 7 mg/kg BW/day, respectively (Karwowska & Kononiuk, 2020).

Therefore, the nitrate content in ANS leaves is low compared to the RDA (Table 5). Besides, the consumption of more than 600 - 700 g of ANS per day is beyond the RDA of nitrate and can cause health effects. The application of heat treatments, high-temperature storage conditions, and fermentation reduced nitrate content in vegetables (Prasad & Chetty, 2011; Ding *et al*., 2018), similarly to ANS leaves.

2.4.5 Glycoalkaloids in leaves

Solasonine, solanine, solamargine, and chaconine are major glycoalkaloids in the ANS, with steroidal glycoalkaloids (SGA) as minor (Ronoh *et al*., 2017). Glycoalkaloids are toxic to humans and animals with symptoms such as constipation, dark-coloured diarrhoea, nausea, vomiting, and abdominal pain. These toxins affect the nervous system to cause drowsiness, apathy, weakness or paralysis, salivation, circulatory and respiratory depression, and unconsciousness. Toxic effects are primarily irritation of the digestive tract and sometimes neurological problems (Abbas *et al*., 1998; Defelice, 2003; Mensinga *et al*., 2005). Young leaves and unripe berries of ANS have a higher SGA concentration than matured ones (Ronoh *et al*., 2017). The SGA is associated with bitterness in ANS and causes toxic effects on animals when consumed above 5 mg/kg BW (Ronoh *et al*., 2017). In-plant foods, the recommended upper limit of total glycoalkaloids (TGA) is 200 mg/kg FW (1 g/kg DW) (Nono-Womdim *et al*., 2012). Alkaloid content in *S. nigrum* is 1722 mg/100 g DW and 1448 mg/100 g DW in *S. villosum,* respectively (Table 5). The quantity of solanine in *S. nigrum* is 470 mg/100 g and 150 mg/100 g DW in *S. villosum,* respectively (Mohy-Ud-Din *et al*., 2010). This content is significantly low to cause a potential toxic health effect on humans. Also, Yuan *et al*. (2018) reported a solasodine content of less than 5 mg/100g DW or 1 mg/100g FW in *S. nigrum* and *S. scabrum,* respectively. This amount is low compared with other vegetables such as eggplant (6.25 to 20.5 mg/100 g FW) (Yuan *et al*., 2018). The SGA solasodine is absent in *S. scabrum*

(Yuan *et al*., 2018; Yuan *et al*., 2020a). According to Yuan *et al*. (2020b), the levels of total alkaloids in *S. nigrum*, *S. villosum*, and *S. scabrum* are safe for human consumption. Alkaloid content in ANS leaves can be removed by boiling (Edmonds & Chweya, 1997; Defelice, 2003; Essack *et al*., 2017). The TGA content in *S. scabrum* leaves is 116.80 mg/kg FW, *S. villosum* (100.82 mg/kg FW), and *S. tarderemotum* (112.97 mg/kg FW), respectively, with no maximum toxic levels of SGAs (Mwai, 2007). It is noteworthy, that several factors such as the amount of TGA eaten, body weight, and metabolism rate of SGA facilitate its toxicosis (Nono-Womdim *et al*., 2012).

Breeding and genetic improvement would also remove the toxicity commonly associated with ANS, i.e., mainly attributed to SGA presence (Nono-Womdim *et al*., 2012). Although SGAs are harmless and enhance the flavour at low levels, they cause toxicity and even death in animals and humans at a high dose. Total glycoalkaloid content above 140 mg/kg FW is associated with unpleasant flavour and bitter taste (Nono-Womdim *et al*., 2012). In Tanzania and Kenya, several improved cultivars of *S. scabrum* and *S. villosum* are available (https://www.tosci.go.tz/; Dinssa *et al*., 2016; Ojiewo *et al*., 2013a; Ronoh *et al*., 2019). *Solanum scabrum* has a less bitter taste with large succulent leaves, broad leaves (Plate 1) and high seed yield, rapid new leaf sprouting after harvest, late flowering, 40 days maturity, and picking can continue for 6 - 8 weeks. Besides, it is resistant to *Fusarium* wilt (Nono-Womdim *et al*., 2012; Ojiewo *et al*., 2013a; https://www.tosci.go.tz/). *Solanum villosum* is bitter, with a narrow and low yield than *S. scabrum;* it also has broad leaves and a higher yield than the original variety (Plate 1). People in rural areas, old people, and men prefer bitter variety; therefore, all varieties fetch the same demand in the market. *Nduruma* and *Olevolosi* leaves contain no glycoalkaloid aglycone; therefore, are safe for consumption. Moreover, these varieties have high total phenols, high vitamin E, and β-carotene (Yuan *et al*., 2018).

2.4.6 Glycoalkaloids in berries

African nightshade berries are commonly considered toxic and not consumed in SSA (Lyu *et al*., 2020). The young berries of *S. nigrum* contain the highest level of SGA compared with the whole plant or leafy and stem parts (Edmonds & Chweya, 1997; Abbas *et al*., 1998; Defelice, 2003; Mohy-Ud-Din *et al*., 210; Yang & Ojiewo, 2013; Ronoh *et al*., 2017). The toxic level of matured berries is too low to harm but can harm children (Edmonds & Chweya, 1997; Defelice, 2003). *Solanum nigrum* accession (USDA PI 381239) contains trace levels of glycoalkaloids (Lyu *et al*., 2020). However, the glycoalkaloids (solasodine, solanine, and solamargine) are high in berries compared to the shoots and leaves of *S. nigrum*, with solasonine being high in the shoots of *S. nigrum* (Al-Ashaal, 2019). Glycoalkaloid in unripe berries of *S. nigrum* is equivalent to 356 mg/100 g DW. Environment conditions such as frost contribute to a significant increase in glycoalkaloids by 5-15 times higher than mature berries. Yuan *et al*. (2020b) reported a solasodine content of 380-930 mg total aglycone/100g DW in unripe berries but was not detected in ANS leaves. This variation could be attributed to genetic diversity, environmental conditions, and cultural practices (Ojiewo *et al*., 2013b). Glycoalkaloid concentration is low in matured berries. *Solanum scabrum* mature berries contain up to 1500 mg (Yuan *et al*., 2019). Such a high concentration of glycoalkaloids in berries could be essential for medicinal purposes (Al-Ashaal, 2019). Mature berries of some accessions contain low amounts of glycoalkaloids and could be potential for consumption in SSA. For such, the promotion of berry products for consumption is essential. Therefore, intensive research in breeding for selecting desirable lines and subsequent cultivars for release ensures the quality of the final product and safety to consumers (Yuan *et al*., 2020a; Yuan *et al*., 2020b) is mandatory.

2.4.7 Cyanogenic glycosides in leaves

When hydrolyzed, cyanogenic glycosides produce toxic hydrogen cyanide (HCN) (Selmar, 2018). Only *S. nigrum* contains cyanogenic glycosides (Table 5), which is a safety concern. Hydrogen cyanide inhibits cytochrome oxidase in the mitochondria. Also, it interacts with copper and iron ions to inhibit respiration and the inability to produce adenosine triphosphate (Sangija & Wu, 2020). Cyanide intoxication can cause mental confusion, diarrhoea, vomiting, stomach pains, dizziness, diaphoresis, cardiac arrest, and rapid respiration (Gracia & Shepherd, 2004; Venketesh, 2014). A chronic low dose of cyanide may cause an elevation in the blood and induces a variety of symptoms such as kidney or mild liver damage (Manzano *et al*., 2007), miscarriage (Soto-Blanco *et al*., 2009), hypothyroidism (Soto-Blanco *et al*., 2009), paralysis, nervous lesions, and weakness (Soto-Blanco *et al*., 2002; Soto-Blanco *et al*., 2008). The fatal dose of HCN can be as low as 0.5-3.5 mg/kg/BW (EFSA, 219; Essack *et al*., 2017). In contrast, the cyanide content of *S. nigrum* is 320 mg/100 g FW (Essack *et al*., 2017); moreover, no information for other ANS species. Hydrogen cyanide content in ANS is significantly low compared to raw cassava leaves (1905 mg/kg) (Umuhozariho *et al*., 2014). Food and Agricultural Organization (FAO, 2005) suggested an acceptable limit of 500 mg/kg HCN, while the (EFSA, 2004) considers the maximum of 10 mg/kg HCN safe for human consumption. Fresh ANS contains low HCN content to cause potential health effects (FAO, 2005). Cassava leaves' cooking reduces HCN, similar to ANS (Umuhozariho *et al*., 2014).

2.5 Safety regulations for African nightshade

African nightshade safety is still a challenge, with a risk of toxicity to consumers if consumed at a high dose. Some ANS leaves accessions to contain glycoalkaloids, potentially causing health concerns (Sivakumar *et al*., 2020). To date, no policy issues, regulations, or by-laws guide the safety of consuming ANS in SSA or other parts of the world (Nono-Womdim *et al*., 2012). Therefore, the SSA countries need to develop and implement policies and strategies to promote sustainable production and ANS safety (Abukutsa, 2010). There is a need for policy-makers at the national, regional, and international levels to address the significant safety issues on ANS to attain maximum benefits. The EFSA, FAO, and WHO have set guidelines for minimal levels of toxic compounds such as GA, HCN, nitrate, and nitrites in foods. Therefore, these guidelines can be used as a benchmark in preparing safety regulations for ANS in SSA. Further, intensive research is needed to generate data on the toxic compounds and their levels in *Solanum* spp.

2.6 The utilization of African nightshade

2.6.1 Leaves

In some societies, the consumption of ANS is influenced by the consumer's local beliefs. For instance, most people in Kenya consume ANS believing that it is nutritious and healthy (Abukutsa-onyango, 2015). African nightshades uses differ from SSA; however, it is used for leafy vegetables, medicinal, cosmetic, and spiritual purposes (Keller, 2004; Abukutsa-Onyango, 2007; Yang & Ojiewo, 2013; Ontita *et al*., 2017). Generally, ANS consumption is based on availability, taste, and affordability (Sato *et al*., 2002).

Consumption of ANS in some African societies is associated with hunger, shortage of food, and poverty; therefore, eating them is regarded as humiliating (Shackleton *et al*., 2009). Abukutsa-Onyango (2015) observed that most Western and Nyanza provinces in Kenya prefer ANS for its nutritional value. In Tanzania, moreover, ANS is highly consumed by the wealthiest households for its known nutritional and health benefits (Ambrose-Oji, 2009). African nightshades is generally more palatable for adults, especially the old age group (Ontita *et al*., 2017; Onyango *et al*., 2016). Nevertheless, some parts of ANS are toxic to humans and livestock, including youngberries, which contain a high concentration of alkaloids (Kuete *et al*., 2017; Ronoh *et al*., 2017).

In Kenya, ANS is used spiritually by older men to pronounce a curse on an offender (Ontita *et al*., 2017). African nightshades are consumed based on gender, whereby men prefer bitter dark variety. Some consume to recover the lost or poor appetite due to illness (Ontita *et al*., 2017; Shackleton *et al*., 2009). However, wrong beliefs, perishability, environmental degradation, limited storage, poor infrastructure, draughts, markets, arable land shortage, diseases, pests, and high production costs hinder ANS utilization (Ondieki *et al*., 2011; Ayua & Omware, 2013). Henceforth, research on addressing the constraints to the utilization of ANS is mandatory.

2.6.2 Berries/fruits

Green berries, orange, yellow, purple, red, black, and violet are edible; they can be consumed raw, and at the same time, the juice of ripe berries are used as ink by children (Edmonds & Chweya, 1997). The berries are used as an ingredient in pies, food colourants, preservatives, and substitutes for raisins in plum puddings (Edmonds & Chweya, 1997; Schippers, 2002; Yang & Ojiewo, 2013; Lotter *et al*., 2014). Berrie's can sometimes be mixed with colourful fruits such as apples. Also, berries can make a nice jam and preserve tea with bread and butter (Edmonds & Chweya, 1997; Yang & Ojiewo, 2013). Occasionally, berries are used as a vegetable in soup, yam, and cocoyam porridges in some parts of Nigeria (Akubugwo *et al*., 2007). The berries are processed into value-added products, like beverages, juices, jams, and nectars for international markets (Akinola *et al*., 2020).

2.7 Roles of African nightshade in health promotion and protection

2.7.1 Leaves

Different ANS species and parts have various medicinal functions (Yang & Ojiewo, 2013). The leaf extracts of *S. americanum* relieve chronic conjunctivitis, treat sores, heart pain, and skin problems, and treat worms in chickens. Simultaneously, the leaf extracts of *S. scabrum* treat diarrhoea in children and cure jaundice and eye infections. Also, the leaf extracts of *S. villosum* treat stomach aches, tonsillitis, wounds, leucorrhoea, and nappy rash, as well as an ointment for boils (Zahara *et al*., 2019). Fresh leaves or cooked or leaf juice can also be taken orally as a liver tonic or used to treat indigestion, stomach ache, and stomach ulcers (Jain *et al*., 2011). In Kenya, ANS is used to heal ailments relating to stomach complications (Abukutsa-onyango, 2015). Leaves are eaten raw for heart pain and swelling; also, the leaf extract restores body skin pigment. Fermented leaves treat boils, ulcers, and swollen glands. ANS leaves also treat tonsillitis, swelling, and conjunctivitis. The Xhosa also uses ANS for disinfecting anthrax-infected meat (Edmonds & Chweya, 1997).

In European traditional medicine, nightshades are potent analgesic, sudorific, and sedative, containing powerful narcotic properties. In Indian tradition, nightshades have been used to cure stomach complaints, dysentery, fever, and tuberculosis (Kaushik *et al*., 2009; Lotter *et al*., 2014; Kuete *et al*., 2017). Traditional medicine is "the total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses (Che *et al*., 2017). Traditional medicine has gained popularity in the last few decades and continued to be helpful for the poor's primary health care in SSA (Lezotre, 2014; Sivakrishnan, 2018).

The juice of the ANS leaves cures ulcers and other skin diseases. The BNS is used broadly as an anti-tumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic (Jain *et al*., 2011; Kuete *et al*., 2017). The leaves *S. nigrum* are used to treat fungal skin infections, reduce stress, relieve joint pain, treat malaria and recover lost food appetite (Jain *et al*., 2011; Ontita *et al*., 2017; Kuete *et al*., 2017). The bitter dark green leaves are used for an alcohol-related hangover (Jain *et al*., 2011; Ontita *et al*., 2017). Also, ANS can treat newly circumcised patients, and pregnant and lactating mothers (Ontita *et al*., 2017). Leaf paste is applied directly to rabies for healing wounds (Jain *et al*., 2011). Aqueous extracts of leaves from both *S. villosum* and *S. nigrum* var. *sarrachoides* possessed significant anti-diabetic effects in the Streptozotocin-induced diabetes mice model (Nyaga *et al*., 2019; Nyaga, 2020).

2.7.2 Berries and roots

The ripe berries of edible ANS are anti-carcinogenic; other plant parts are also used in traditional medicine (Kuete *et al*., 2017). The raw berries of *S. scabrum* treat stomach aches and stomach ulcers. They are also used as a tonic, laxative, remedial for asthma, and restoring appetite, especially for people who recover from disease (Jain *et al*., 2011). Unripe berries relieve aching teeth and reduce the pain of babies' gum during teething. The juice of berries treats sore eyes. *Solanum nigrum* berries and juices cure stomach ailments, blood impurities, and fevers. In East Africa, raw berries treat stomach ulcers or general abdominal upsets (Edmonds & Chweya, 1997; Yang & Ojiewo, 2013). A mixture of ground ANS leaves and seeds is scrubbed onto the gums of children with crooked teeth. In Tanzania, unripe berries treat ringworms and roots for stomach aches. The Zulus use an infusion as an enema for abdominal upsets in children. The burnt and powdered root is rubbed to scarification on the back for the relief of lumbago. Unripe berries paste is used to treat ringworm (Edmonds & Chweya, 1997; Yang & Ojiewo, 2013). The berries have been used in ancient Indian medicine to treat inflammation, diuretics, and tuberculosis (Kaushik *et al*., 2009). In Tanzania, the ripe berries in their edible form are given to kids to stop bed-wetting (Jain *et al*., 2011). However, in Algeria, berries' diluted infusion treats blindness, conjunctivitis, glaucoma, trachoma, and cataract (Jain *et al*., 2011). The berries juice calms sore eyes, whereby the unripe berries soothe toothache (Yang & Ojiewo, 2013).

In contrast, the juice from roots treats cough and asthma. In many African cultures, various plant roots are used in traditional, complementary, and alternative medicine (Akinola *et al*., 2020). Berries and roots can control vomiting, treat tetanus after abortion, and sedatives (Ogwu *et al*., 2016). In India, boiling the roots with a little sugar increase fertility in women, whereas the juice of the roots is extracted and used to cure asthma and whooping cough (Jain *et al*., 2011). Children take the tonic obtained after boiling roots in milk.

2.8 Traditional methods for the preparation of African nightshade

Processing and preservation (PaP) of ANS can compensate for the low availability of vegetables during the off-season, improving the nutritional shortage during these seasons (Engle & Altoveros, 1999). Most ANS are nutritious if well cooked, although the preparations and cooking methods significantly affect the nutritional contents (Keller, 2004; Ngegba, 2007; Oluoch *et al*., 2012). Therefore, it is necessary to develop recipes to optimise nutritional value, increase ANS intake, and reduce iron deficiency in children, pregnant women, children, and nursing mothers in SSA (Oluoch *et al*., 2012). A correlation between taste and appearance was observed in Kenya. Singly prepared ANS or combined with other vegetables did not influence sensory acceptability, although a mixture of ANS and cowpea increased carotenoids and vitamin C (Habwe, 2008; Habwe *et al*., 2008; Oluoch *et al*., 2012). Stir-frying can be the best method of retaining vitamin C, copper, and iron, although mixing slender leaves in ANS reduce iron content (Habwe *et al*., 2008). In Tanzania, different recipes for the preparation of ANS exist, making use of variable quantities of ingredients; hence it is difficult to obtain a standard recipe to be adapted (Weinberger & Msuya, 2004). Traditional recipes for the preparation of ANS vary among different communities in SSA (Figs. 1 and 2).

In Tanzania, ANS is cooked by stir-frying or steaming, with ingredients such as milk, coconut milk, peanut, carrots, sweet pepper, onion, tomatoes, and peppers (Plate 2) (Keller, 2004; Ngegba, 2007). Similarly, ANS can be prepared by boiling and adding milk or cream for enrichment or boiling and frying with onions, tomatoes, spices, and salt (Fig. 1). In Kenya, most local consumers boil the leaves and add salt and milk to improve the taste (Yang & Ojiewo, 2013; Ontita *et al*., 2017). Furthermore, ANS can be mashed with potatoes or boiled with sweet potatoes, tuber, milk, pumpkin, and blood or immature gourd, (Plate 2) (Ontita *et al*., 2017). In Senegal, ANS is cooked with meat or fish (Fig. 1) (Keller, 2004; Shackleton *et al*., 2009; Chagomoka *et al*., 2014), (Plate 2). Besides, cooking ANS with meat can increase cooking time and facilitate the degradation of vitamins B, C, β-carotene, and iron. Diabetic consumers prepare ANS by boiling and adding milk, but neither frying nor salt is added for health reasons (Ontita *et al*., 2017).

Traditional cooking methods can retain carotenoids in a range between 16 to 70%, whereas long cooking and reheating of cooked ANS reduce carotenoids and iron (Oluoch *et al*., 2012). Various cooking methods can degrade essential micronutrients (Table 2), such as iron, β-carotene, and vitamin C (Ngegba, 2007). In East Africa, ANS can be cooked with other vegetables such as spinach, kale, amaranth, and cabbages (Fig. 1) and accompanied by staples such as stiff porridge (*ugali*), rice, yams, banana, and cassava (Schippers, 2002; Abukutsa-Onyango, 2007; Yang & Ojiewo, 2013).

Most studies have focused on ANS production aspects and forget different cooking methods, consequently altering the nutritional and functional properties. The absence of suitable recipes and information on proper use led to the low utilization of ANS and distortion of essential nutrients. Formulation of appropriate ANS recipes can maximize nutrient availability, eliminate seasonality, and guarantee their availability yearly. Additionally, it can reduce losses and improve dietary diversification, contributing to nutrition and food security.

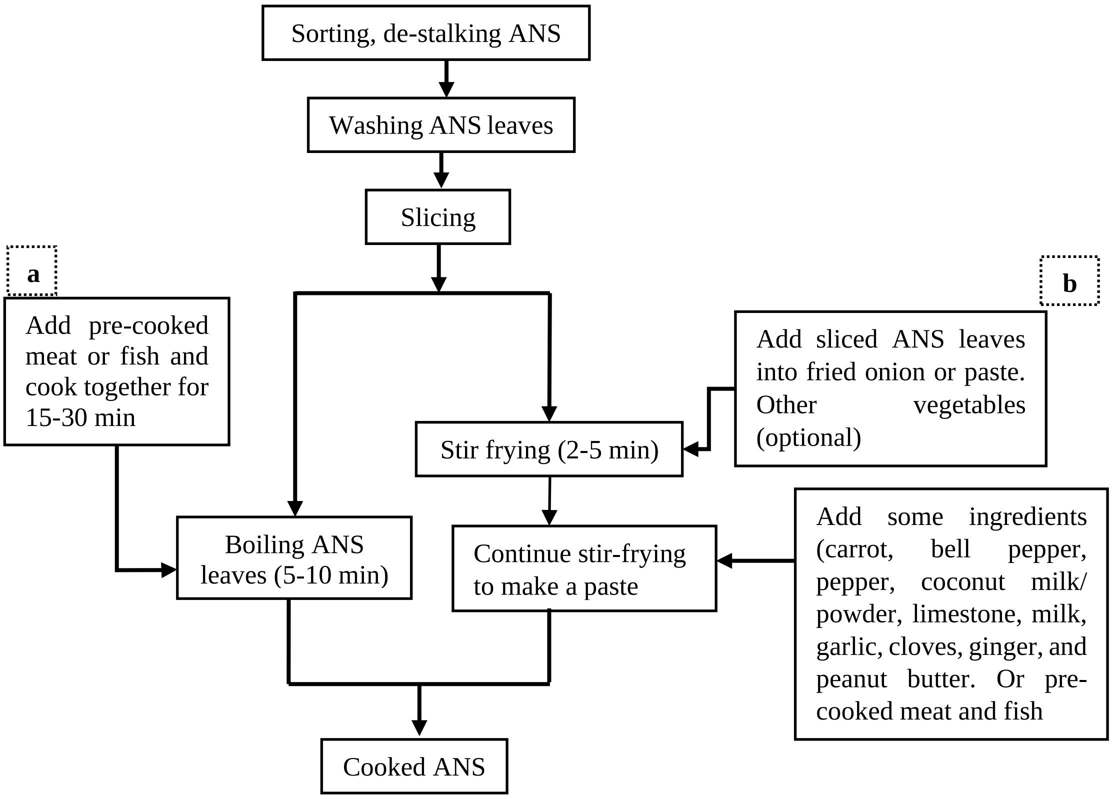

Figure 1: Methods of ANS preparation in different SSA communities

Plate 2: Different recipes of nightshades s practiced in SSA

In the above Plate; (a) Nightshades leaves cooked with African eggplant; (b) Nightshades leaves cooked with fish and meat; (c) Nightshades leaves cooked with roasted groundnuts; (d) cooked with ground pumpkin seeds; (e) Cooked nightshades leaves served with fufu or *ugali*; (f) Nightshades leaves cooked with fresh corn grains and palm nuts (Chagomoka *et al*., 2014).

2.9 African Nightshade value chain

African nightshade is common in rural and urban markets in Africa, especially in Cameroon, Kenya, Ghana, Burkina Fasso, Madagascar, Nigeria, South Africa and Tanzania, Guatemala, New Guinea, and the Mediterranean (Edmonds & Chweya, 1997; Ojiewo *et al*., 2013a; Essack *et al*., 2017). The ANS is highly demanded and more expensive than many vegetables, such as kale or spinach (Onyango *et al*., 2016). The AIVs value chains play a significant role in food security and poverty reduction. ANS supports a large number of micro-businesses along the supply chain (Ojiewo *et al*., 2013a). Nevertheless, it is yet to penetrate the international market (Edmonds & Chweya, 1997). African nightshades face typically stiff competition with other exotic vegetables like cabbage, spinach, amaranths, kale, and lettuce (Abukutsa-Onyango, 2007). Moreover, the lack of formal reports on its value chain is among the bottlenecks. According to Gogo *et al*. (2017), the standard chain level of AIVs in SSA includes harvesting, transportation, and marketing. The lack of processing and preservation techniques of AIVs limits utilization. Therefore, mapping the ANS value chain is vital for mutual benefits to create well-functioning linkages between different actors. In Eldoret, Kenya, consumers prefer to purchase ANS over exotic vegetables; however, the ANS price varies between towns. In the open market, the price for a bunch of ANS is KSH 18.8, while in the supermarket, the average price is KSH 34 (Chelang'a *et al*., 2013). In Tanzania, the wholesale price for an AIVs bunch of 40 - 50 kg is 20 000 TZS (US$12.50) and can even fetch a low price of 10 000 TZS (US$6.25) during the bumper season (Lotter *et al*., 2014). The price of selling 1 kg of ANS is between 517-1065 TZS and 970 TZS (Molina *et al*., 2020; Everaarts *et al*., 2017). The prices of 1 kg of ANS vary in different seasons and stages of the value chain; farmgate (400 - 1350 TZS), wholesalers (2200 TZS), and retailers (2400 TZS) (Molina *et al*., 2020). During the rainy season, a bed of AIVs of the same size can be sold at a wholesale price of up to 100 000 TZS (US$62.50) as rain disrupts production (Lotter *et al*., 2014). Generally, the value chain and distribution channels for ANS are not well established in SSA. A value chain analysis in Kenya earmarked supermarkets and local markets as key distribution channels for ANS. Smallholder farmers can directly supply ANS to retailers, wholesalers, and supermarkets with no specific entry point (Fig. 2). Besides, the supply of AIVs is from small farmers to different outlets (Fig. 2). Lack of appropriate infrastructure, market information, extension services, acceptable products, and storage facilities hinders the proper distribution of AIVs (Temu & Temu, 2006; Senyolo *et al*., 2018).

In Tanzania, the ANS value chain is an informal sector where its marketing involves small-scale producers, retailers, and whole sellers. Postharvest loss is low in the market with a robust sales system, which keeps in touch with producers, buyers, and retailers their customers daily (Shackleton *et al*., 2009; Lotter *et al*., 2014). The use of mobile phones facilitates the selling of ANS since producers can keep in touch with middlemen, retailers, and wholesalers (Edmonds & Chweya, 1997). Most Tanzania retailers sell an average of 1.5 kg of ANS, with 62% of all retailers storing unsold produce for the next day (Lotter *et al*., 2014). Men can sell 3 kg per day more than women, despite female dominance (96%) in ANS marketing. The sales volume has a significant effect on location, with an average of 2 kg per day in Dodoma and more than 5 kg in Arusha (Lotter *et al*., 2014). Furthermore, sellers at district and ward markets sell 2 kg more than a seller at a street stall (Lotter *et al*., 2014).

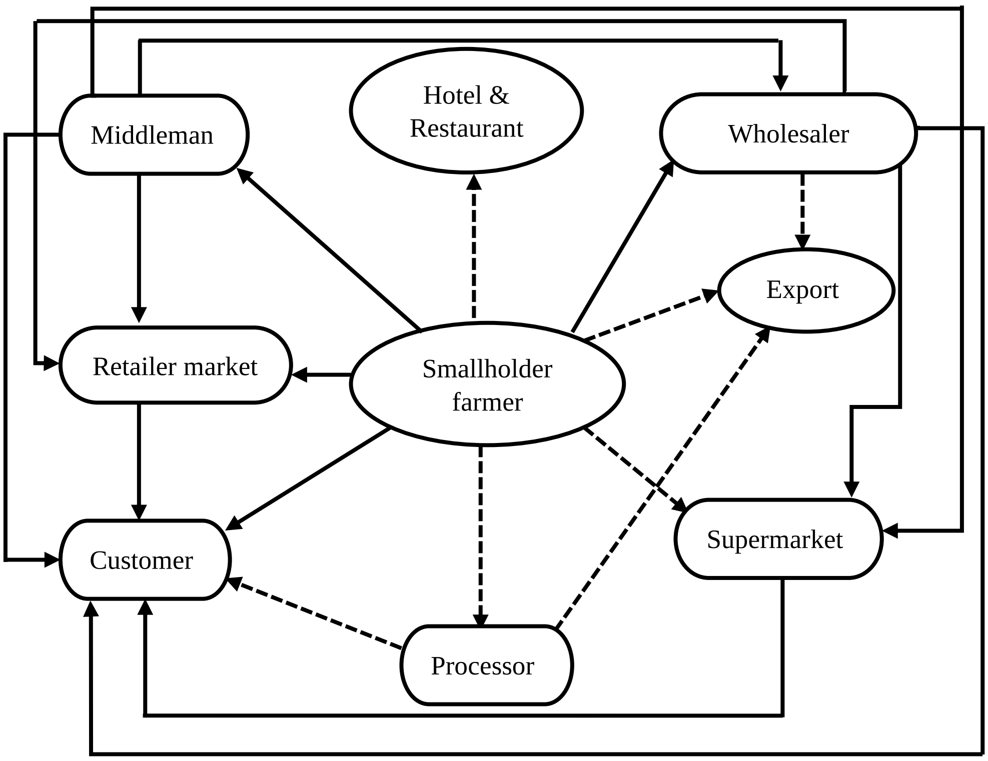

Figure 2: The traditional AIVs value chain in SSA; Dotted arrows indicate occasional pathways; Black arrows indicate the common pathway (Acedo & Katinka, 2009)

Over 60% of farmers in Kenya and Tanzania were able to market the ANS and penetrate several markets. Most farmers in East Africa have changed their perception of production ANS for family use and shifted to business production to support their families and improve their livelihood. Most farmers consider commercializing ANS a profitable business because it has many benefits, including low use cost, less infestation by pests and diseases, high maturity, high consumer demand, and market opportunities (Muhanji *et al*., 2011). Economic returns from using ANS as vegetables have not yet been quantified, hence the low market price and low economic value (Edmonds & Chweya, 1997).

On the other hand, the annual mean profit obtained by small-scale farmers from selling ANS in Kenya ranges from 5641 -141 144 KSH (US$ 564.12-1411.44) (Onyango *et al*., 2016). The profitability of ANS in Kenya is estimated to be US$ 754/month and US$ 766/ha (Mwangi & Kimathi, 2006). Production of ANS gives high yields and low fertilizer application than other exotic vegetables (Everaarts *et al*., 2017). The annual profit based on producing one hector of ANS in Tanzania was 8 129 280 TZS/ha/year (US$3505.51) (Everaarts *et al*., 2017). However, Molina *et al*. (2020) reported an annual net value of 5 612 042.25 TZS (US$2420.02), higher than exotic vegetables, Chinese cabbage, and spinach. The relatively high ANS price maintained year-round in all markets, traders, and farmers signifies its preference and demand (Molina *et al*., 2020). This further shows the potential of ANS in improving the livelihood of people in SSA; therefore, exploiting its value chain is necessary to maximize the benefits of all actors.

2.10 Post-harvest handling

African nightshade needs proper handling after harvesting to maintain the quality before processing or being sent to the market (Arah *et al*., 2016). Harvesting is recommended in the morning with no dew or late evening (Abukutsa-onyango, 2015). Since ANS is highly perishable, the chance of spoilage increases rapidly with time, mainly when poorly handled (Onyango & Imungi, 2007). Therefore, cooling is mandatory to remove field heat within a short period. The traditional cooling methods include putting the vegetable under the shade, sprinkling it with water, and dipping it in water (Plate 3) (Gogo *et al*., 2016). Water sprinkling has proven effective in quickly removing field heat and maintaining freshness for a longer time (Gogo *et al*., 2016; Arah *et al*., 2016). The chance of ANS deterioration increases within 24 hours if not immediately sold after harvest. Some farmers/sellers sprinkle water and leave the vegetable in the open space overnight; however, the problem of microbial contamination still hinders their effort (Gogo *et al*., 2016). Excessive field heat increases metabolic activity, favors microbial activity, and increases respiration rate and ethylene production (Akbudak *et al*., 2012).

Cleaning of ANS is necessary to remove any dirt or residue particles and microbial contamination agents (Nyaura *et al*., 2014). However, most farmers forget to dry leaves after washing and consequently encounter a higher prevalence of microbial contamination, mainly fungi and bacteria (Gogo *et al*., 2016; Arah *et al*., 2016). Lotter *et al*. (2014) observed that 52% of growers do not wash the harvested ANS, increasing the chances of microbial infection and deterioration. To extend its shelf-life, ANS is kept on cold shelves in supermarkets at about 5 - 10 °C (Abukutsa-Onyango, 2007; Gogo *et al*., 2016).

Vegetables contain high water activity a_w of 0.970 - 0.996; this amount is sufficient to favor spoilage microorganisms (Barbosa-Cánovas *et al*., 2003). Pathogen bacteria cannot grow at a_w <0.86; yeasts and moulds can be tolerant at low a_w, and usually, no growth occurs at a_w <0.62. Also, low a_w at 0.3 prevent lipid oxidation, enzymatic browning, and non-enzymatic browning. Besides, lowering a_w prevents the growth of vegetative microbial cells, germination of spores, and toxin production by molds and bacteria (Barbosa-Cánovas *et al*., 2003; Erkmen & Bozoglu, 2016). Heating, freeze-drying, dehydration, freeze concentration, crystallization, and osmotic dehydration reduce the a_w of foods (Barbosa-Cánovas *et al*., 2003; Erkmen & Bozoglu, 2016).

Postharvest handling (PHH) of vegetables has led to safe and nutritious food for consumers (Shiundu & Oniang'o, 2007; Smith & Eyzaguirre, 2007). Lack of scientific and economic knowledge among farmers and food handlers to develop, use, implement, and sustain PHH systems in SSA results in PHL of ANS. Despite several effective post-harvesting technologies, especially in developed countries (Acedo, 2010), some are difficult to implement in SSA (Atanda *et al*., 2011; Kitinoja *et al*., 2011; Arah *et al*., 2016). Most small-scale farmers and sellers in SSA do not afford to use standard cold rooms due to insufficient power supply, high investment, and maintenance costs (Ambuko *et al*., 2017). Furthermore, farmers have limited knowledge of the benefits of using low-temperature storage (Gogo *et al*., 2016). Lotter *et al*. (2014) observed that none of the sellers had access to refrigeration storage in public markets in Tanzania. The lack of low-temperature storage facilities accelerates the PHL of ANS and reduces its utilization. Vegetable deterioration affects qualitative and quantitative loss, including appearance, flavour, texture, shape, and nutritional composition (Affognon *et al*., 2015; Wakholi *et al*., 2015; Global Panel, 2018). Also, it causes food and nutrition insecurity in individuals and households in SSA (FAO *et al*., 2019). Therefore, farmers and food handlers must follow proper PHH techniques from the farm to the fork (Fig. 3).

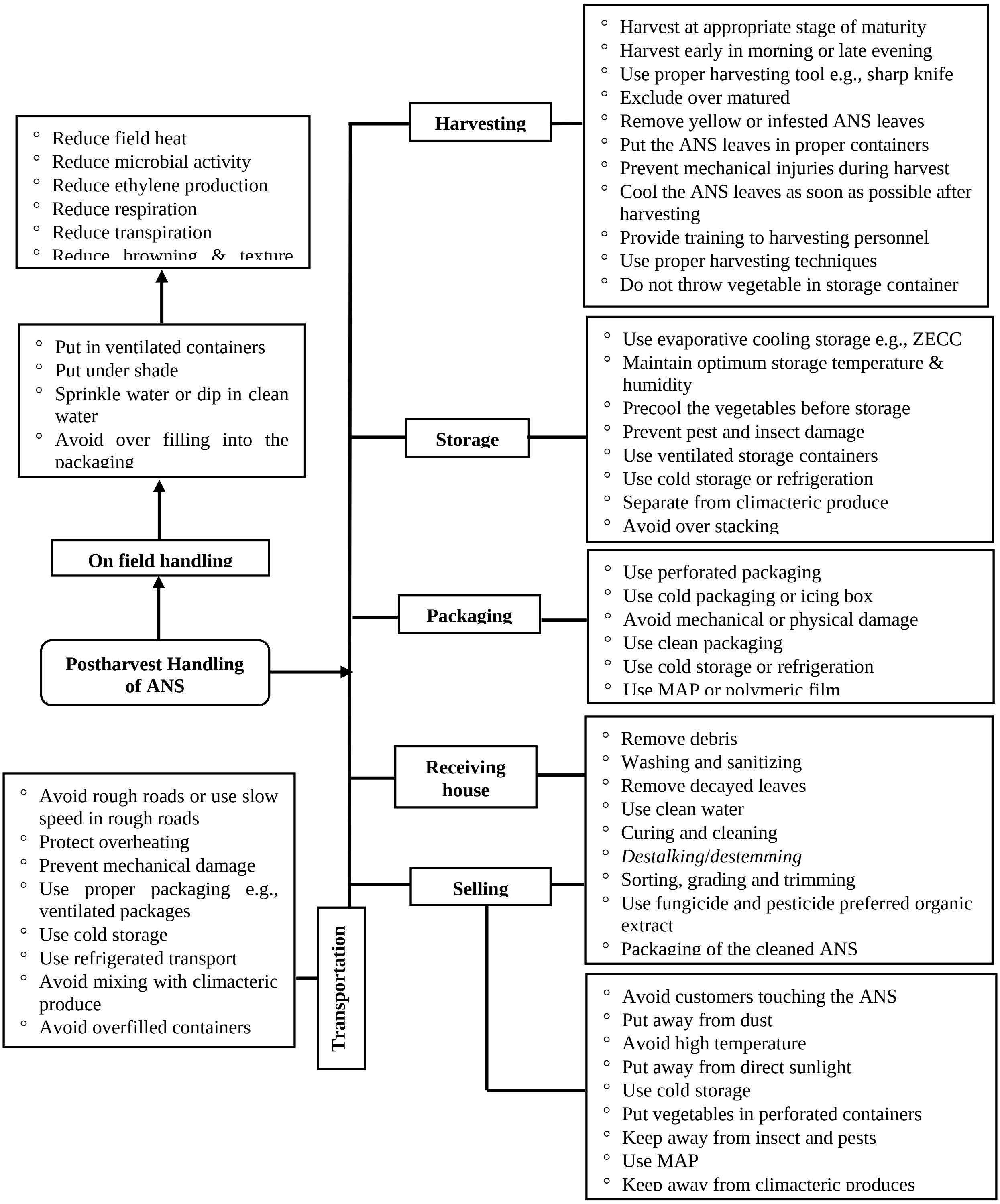

Figure 3: Key points to consider during postharvest handling of ANS from farm to market: Note; MAP = Modified Atmospheric Packaging; ZECC = Zero Energy Cooling. (Ekhuya *et al*., 2018; Gogo *et al*., 2018; Habwe *et al*., 2008; Nono-Womdim *et al*.,

2012; Tournas, 2005; Barbosa-Cánovas *et al*., 2003; http://www.fao.org/3/a-au186e.pdf)

2.11 Post-harvest losses of African nightshade

Postharvest losses of AIVs are significant problems facing SSA (Onyango & Imungi, 2007). The leaves' quality deteriorates within four days of harvest if stored at ambient temperatures (Edmonds & Chweya, 1997). Loss of vegetables in developing countries accounts for about 20 - 50% of total production, reflecting the ANS. Both quantitative and qualitative losses of ANS occur at various stages along the value chain, i.e., from harvesting, handling, packing, storage, processing, and transportation to the consumers. According to Gogo *et al*. (2017), significant losses of nutritional, quantitative, and economic values of ANS in Kenya occur at harvest, transportation, and market. Therefore, proper planning is needed to solve the problem in SSA (Kitinoja & Kader, 2015; Kitinoja *et al*., 2018). Due to the high demand for nutritious food from the growing populations in SSA, PHL management is unavoidable.

The lack of appropriate PHH technologies results in a high loss of ANS, especially during the peak season. Consequently, limited supply during the off-season is accompanied by high prices, as most AIVs are seasonal (Abukutsa-Onyango, 2007; Habwe *et al*., 2008). Deterioration of vegetables results in partial or total loss of produce; it can happen during pre-harvest, harvest, and postharvest handling (Acedo, 2010), although factors affecting PHL vary widely from place to place (Munhuweyi, 2012; Yang & Ojiewo, 2013).

Vegetables undergo physiological deterioration, pathological and mechanical or physical damage if not adequately handled (Acedo, 2010; Munhuweyi, 2012; Castro *et al*., 2005). The incidence of the losses affects the physical and nutritional quality (Munhuweyi, 2012). Physiological deterioration reduced by 5 - 10% of fresh weight renders vegetables unsuitable for consumption (Yang & Ojiewo, 2013). Ethylene production results in the oxidation of vitamin C, destruction of chlorophyll, yellowing, and increased phenylpropanoid metabolism (Munhuweyi, 2012; Yang & Ojiewo, 2013). A low concentration of ethylene, about 0.01 ppm accelerates vegetable loss; however, about 0.02 - 0.06 ppm of ethylene resulted in a 10 - 30% loss of product quality (Yang & Ojiewo, 2013). Therefore, leafy vegetables should be stored away from high-ethylene-producing fruits or vegetables (Munhuweyi, 2012; Yang & Ojiewo, 2013).

Furthermore, low crop variety, inappropriate cultural practice, lack of harvesting techniques, unfavourable climate, improper handling, poor storage conditions, lack of competent human resources, lack of technological knowledge, ineffective commercialization and functioning value chain, lack of logistical support, and lack of enabling policy are primary causes of PHL (Acedo, 2010).

Mechanical damage to vegetables causes leafy tearing and crushing, midrib breakage, head cracking, or bruising. It increases oxidation to phenolic compounds and increases susceptibility to decay. Also, damaged leaves increase water loss by about 3-4 times undamaged vegetables (Kanlayanarat, 2007; Acedo, 2010). High-temperature storage and high relative humidity cause wilting of the vegetable resulting in the loss of freshness of vegetables (Edmonds & Chweya, 1997; Acedo, 2010; Yang & Ojiewo, 2013). Onyango and Imungi (2007) reported 3.5% of *S. scabrum* losses due to excessive wilting. Thus, wilting is a challenge that increases the ANS deterioration rate during marketing, especially when vegetables are stored at ambient temperature (Abukutsa-Onyango, 2007; Onyango & Imungi, 2007). Failure to sell ANS harvested on the same day; can result in deterioration due to a lack of proper storage facilities (Shiundu & Oniang'o, 2007).

Unfinished day supply of ANS from local markets and supermarkets in Kenya is thrown as waste, thus increasing vegetable loss in SSA (Fig. 4), consequently contributing to high PHL (Onyango & Imungi, 2007). Therefore, due to high Postharvest losses in SSA, there is a need to promote the use of appropriate PHH and PaP technologies to extend the shelf life of AIVs and ensure regular supply from farm to table in rural, peri-urban and urban areas all year round (Habwe *et al*., 2008; Smith & Eyzaguirre, 2007). Reducing PHL of vegetables using appropriate technologies can further increase food availability for the exponentially growing world population and conserve natural resources by reducing the area needed for production and agriculture inputs (Acedo, 2010). It will also reduce fungi and bacteria growth and insect infestation (Acedo, 2010; Bradford *et al*., 2018) and improve food quality, food safety, and financial opportunities. For that reason, there is a need to develop strategies that could improve the PHH of ANS in SSA. Solar drying and fermentation technologies play a significant role in ensuring the long-term preservation of foods for sustaining food security in SSA (Sivakumar *et al*., 2020). Therefore, training on feasible and affordable preservation techniques can add value to the ANS in SSA.

Microorganisms are the primary causative agents of PHL in fresh vegetables if they are not well handled (Acedo, 2010; Gil *et al*., 2015). They contribute about 55% of the total production losses in developing countries (Sanzani *et al*., 2016). Vegetables can also harbor higher numbers of microorganisms during harvesting due to preharvest and postharvest contamination (Tournas, 2005). Bacteria, yeasts, and moulds can contaminate the ANS in the value chain. The microbial number of fresh vegetables differs from location, PHH, and storage conditions. Not all microorganisms can grow on fresh vegetables, but some microorganisms can grow and cause spoilage (Tournas, 2005). Cutting and mincing can increase the microbial contamination of vegetables (Tournas, 2005). Washing, blanching, sanitary handling, and processing environment, and clean packaging containers substantially decrease microbial contamination. Microbial numbers in fresh vegetables per gram differ from species; total bacteria count, 1.0×10^{10}, coliforms 3.1×10^{7}, fecal coliforms 5.5×10^{6}, and LAB 1.0×10^{6} (Tournas, 2005). Vegetables' most common spoilage bacteria are LAB, pseudomonads, and *Xanthomonas campestris* (Tournas, 2005; Moss, 2008; Wafula, 2017). The fungi *Aspergillus, Penicillium*, *Fusarium*, *Alternaria*, and *Mucor,* were isolated in spinach and tomatoes (Suleiman *et al*., 2017; Sanzani *et al*., 2016). These fungi can also cause spoilage of ANS and the production of their toxins (mycotoxins). Sanzani *et al*. (2016) reported different mycotoxins (aflatoxin, ochratoxins, patulin) on fruits and vegetables and fumonisins in tomatoes fruits without Alternaria toxin in vegetables. Suleiman *et al*. (2017) observed the presence of aflatoxin (B1, B2, GI, and G2) contamination in fresh spinach, fresh and dried tomatoes, and bitter leaves (*Vernonia amygdalina*).

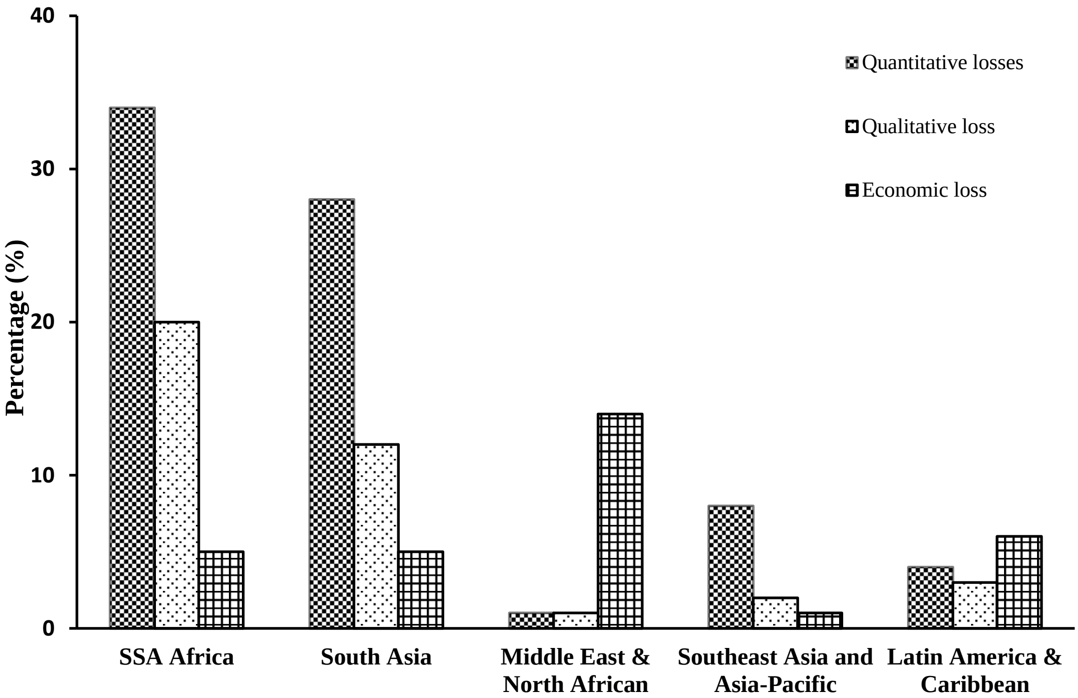

Figure 4: The quantitative, qualitative, and economic losses of vegetables in various regions of the world Kitinoja *et al.* (2018)

2.12 Processing and preservation of African nightshade

Processing and preservation (PaP) of ANS has many benefits, mainly reducing the seasonality problem beyond the growing season (Acedo, 2010; Kirigia *et al.*, 2019). Moreover, PaP improves dietary diversity, gives a wide choice of products, eliminates potentially toxic compounds, improves nutrient bioavailability, and improves flavour, texture, and aroma. It also enhances the micronutrient bioavailability, enhances digestibility, and provides probiotics for GIT health (Belitz *et al.*, 2009; Osum *et al.*, 2013; Traoré *et al.*, 2017; Wafula *et al.*, 2016). The production of ANS is seasonal and becomes scarce during the offseason; therefore, there is a need to establish an appropriate preservation method to ensure that these vegetables are available year-round with acceptable quality. However, some PaP techniques degrade essential nutrients (Table 2) and are costly to implement in SSA, especially in households and farmers.

The most common traditional PaP technologies for AIVs include cooling, blanching, drying, salting, cooking, fermentation, and osmotic dehydration (Odongo *et al*., 2018). Although some of these technologies preserve vegetables for a short period, they can improve storability if combined with other techniques (hurdle technology) (James & Kuiper, 2003). The influence of harvesting methods (uprooting and cutting), age, and storage conditions (5 °C and room temperature) on the ANS leaves has been reported (Kirigia *et al*., 2019). These technologies below are affordable and do not require sophisticated equipment; hence, small farmers, individuals, and households in SSA can implement them.

2.12.1 Drying techniques

Drying preserves leafy vegetables to make them accessible during the off-season (Smith & Eyzaguirre, 2007; Maseko *et al*., 2017). Drying has been used for a century and has been a critical method of preserving vegetables in SSA (Smith & Eyzaguirre, 2007; Vorster *et al*., 2007; Kiringia *et al*., 2017). Drying results in a minimal change in texture, flavour, and color (Gogo *et al*., 2016), and reduced weight, which improves handling, storability, lower transport cost, and microbial degradation of the product (James & Kuiper, 2003; Kiringia *et al*., 2017; Maseko *et al*., 2017). Drying is an economically feasible way to preserve AIVs locally in most SSA societies (Gogo *et al*., 2016). Several drying techniques are used for vegetables, although; the standard practice in SSA is open sun drying, with less use of solar drying (Abukutsa, 2003; Gogo *et al*., 2016).

(i) Open sun-drying

Sun-drying is the oldest, simplest, and most commonly used method for the preservation of AIVs. It is considered the least expensive method (Oniang'o *et al*., 2008; Atanda *et al*., 2011; Gogo *et al*., 2016). Low-quality sun-dried AIVs as a consequence of physical and biological hazards contamination (Keller, 2004; Habwe *et al*., 2008). Open sun drying was reported as a standard technology in areas with low production of vegetables, such as Singida and Kongwa, Tanzania (Keller, 2004), to preserve the vegetables during the offseason. Performing sun-drying under shade for preserving vitamins is highly recommended (MMA, 2008; Traoré *et al*., 2017). About 45 and 55.6% of farmers sun-dry their vegetables in Siaya county Kenya and Bahi district Tanzania (Ayua & Omware, 2013; Kandonga *et al*., 2019). Sun-drying under shade increased the carbohydrate, ash, and lipid content of *S. nigrum* but reduced protein and β-carotene to 4.03 and 1.76 mg/100g, respectively (Traoré *et al*., 2017). Besides, sun-drying concentrated mineral elements and reduced antinutritional factors in other AIVs (Kandonga *et al*., 2019).

(ii) Solar drying

The solar dryer is a simple device that reduces the adverse effects of sun-drying. Solar drying technology is more effective in utilizing solar energy (James & Kuiper, 2003; MMA, 2008;

Gogo *et al*., 2016). The indirect solar dryer is a more complicated and expensive solar drying method (MMA, 2008); however, the technology gives high-quality products in terms of nutritional and other physicochemical properties compared to the direct and mixed solar dryers. Solar drying reduced antioxidant activity, flavonoid, vitamin C, and β-carotene in the *S. nigrum* (Cheptoo *et al*. 2019); however, it increased the carbohydrate and lipids content of ANS (Traoré *et al*., 2017). Solar drying of *S. scabrum* reduced vitamin C and β-carotene by 96.2 and 92.7 %, respectively, but it did not affect mineral content (Mibei *et al*., 2011). Additionally, it retained β-carotene of about 693.55 µg/g (68%) of *S. nigrum* (Nyambaka *et al*., 2012). Likewise, solar drying of ANS leaves increases sucrose, and glucose, with no significant effect on protein and vitamins B_1 & B_2, and E; and a substantial decrease in Vitamin C and ash contents (Wafula, 2017). On the other hand, blanching and solar drying of ANS improved β-carotene and vitamin C content (Nyambaka *et al*., 2012; Kiringia *et al*., 2017; Traoré *et al*., 2017). Solar drying is yet to be fully exploited in SSA countries despite its economic feasibility and quality benefits. Nonetheless, the cost of installing a full mixed solar drier is US$ 75 (Ayua & Omware, 2013). Therefore, solar drying is a relatively cheap and feasible technique for many small farmers and processors.

2.12.2 Low-temperature storage

Many studies recommended that most vegetables, including ANS, be stored at about 10 - 15 °C and 85 - 95% relative humidity to avoid chilling injuries (Castro *et al*., 2005; Gogo *et al*., 2016). The technologies used in developing countries should suit their needs and be comfortable and less costly than the technology to be applied in developed countries (Arah *et al*., 2016).

Evaporative cooling (zero-energy cooling chamber) has been developed as an alternative for inaccessible convection cold rooms (Plate 3). This technology uses the latent heat of the evaporation principle, and heat exchange occurs when water evaporates; moreover, the chamber does not require electricity to operate. It is appropriate for smallholder farmers and sellers in rural areas with limited electricity supply (Workneh & Woldetsadik, 2004; Ambuko *et al*., 2017). Additionally, the chamber's construction is inexpensive, and it uses locally available materials and unskilled labor, making the cost affordable for resource-poor smallholder farmers (Edmonds & Chweya, 1997; Acedo, 2010; Yang & Ojiewo, 2013). A zero-energy cooling chamber can store the vegetable for 3-5 days or more inside the chamber (Yang & Ojiewo, 2013).

Plate 3: **Traditional vegetable cooling chamber (a) or zero energy cooling chamber (b): (1) water circulating chamber with pipes used to distribute water; (2) cooling chamber, where vegetables are preserved; (3) grass thatched to protect heat and direct sunlight; (4) the rid or cover, made with bamboo leaves**

2.12.3 Blanching

Blanching is a primary, intermediate heat treatment process aiming to improve food preservation and quality by inactivating enzymes to facilitate vegetable spoilage (Habwe *et al*., 2008; Huang *et al*., 2016). It also enhances the drying rate, kills plant tissues, improves the peeling of products, removes foreign matter, and reduces oil uptake. Also, it reduces microbial loads before further processing or storing and removes pesticide and toxic residues. However, the timing and temperature of blanching are crucial, as too little temperature is ineffective, and too much temperature damages the vegetables and leads to the loss of essential nutrients (Huang *et al*., 2016; Traoré *et al*., 2017). Blanching is performed using hot water, steam, microwave, radiofrequency, ohmic, and infrared (Liu *et al*., 2014; Xiao *et al*., 2017).

Blanching with water at 80 °C for 10 min, followed by solar drying, retained essential nutrients in AIVs (Acedo, 2010; Njoroge *et al*., 2015; Halim *et al*., 2017). On the other hand, blanching, combined with the drying of about 100 °C for 30 min, can degrade essential nutrients in AIVs (Njoroge *et al*., 2015). Blanching of ANS, followed by lyophilization, can extend the shelf-life (Habwe *et al*., 2008). Also, hot water blanching of ANS at 90 - 92 °C decreases ash content by 1.73% with a high loss of β-carotene but did not affect protein and high carbohydrate content (Traoré *et al*., 2017). *Solanum scabrum* leaves' phenolic content was reduced by 85% after blanching (Odongo *et al*., 2018). Blanching *S. nigrum* reduced antioxidant activity, flavonoid, vitamin C, and β-carotene, Table 2 (Cheptoo *et al*., 2019). Also, it decreases phytate and tannin contents (Amalraj & Pius, 2015). Besides, blanching improves texture, and flavor and retains color (Reis, 2017). Blanching *S. nigrum* at 100 °C for 30- and 60-minutes increased carbohydrates but decreased ash content, protein, and β-carotene (Traoré *et al*., 2017). Therefore, adopting blanching as an intermediate treatment should be recommended as a preservation method for ANS.

2.12.4 Fermentation

Fermentation is a sustainable postharvest strategy for improving quality and product safety in Africa (Wafula *et al*., 2017). Vegetable fermentation is an inexpensive and attractive preservation technique for SSA small-scale farmers and food processors. Fermentation is often combined with boiling, drying, salting, steaming, and frying (Adam & Moss, 2008; Adams & Nout, 2001; Wafula *et al*., 2016). About 32% of people in Kenya use fermentation to preserve ANS and other AIVs (Ayua & Omware, 2013). Fermentation of AIVs such as ANS and cowpeas leaves has been reported in Africa (Kasangi *et al*., 2010; Oguntoyinbo *et al*., 2016; Wafula, 2017; Owade *et al*., 2019; Stoll *et al*., 2021). Despite high PHL in SSA, fermentation of AIVs has not been given enough priority and is highly underutilized in Africa (Wafula *et al*., 2016; Oguntoyinbo *et al*., 2016). Therefore, tapping the potential of ANS fermentation is essential in reducing PHL and improving nutrition and food security.

Lactic acid bacteria (LAB) are convenient starter cultures for fermented plant products, such as fermented pickles. *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Lactobacillus acidophilus*, and *Lactococcus lactis* (Behera *et al*., 2020; Wafula *et al*., 2016). Relishes and other pickled products are prepared from fermented ANS pickles (Hutkins, 2006).

Lactic acid (LA) fermentation can preserve vegetables for a long time and retain their original freshness and nutritive value (Madson & Coleman, 2007; Ray & Didier, 2014). Lactic acid bacteria improve the bioavailability of vitamins and minerals, and the production of vitamins such as B vitamins; also, they contribute to the improvement of flavour, texture, and appearance (Madson & Coleman, 2007; Rodríguez *et al*., 2009; Soccol *et al*., 2010; Ray & Didier, 2014). *Lactobacillus plantarum* 75 strains did not affect the colour of fermented ANS leaves but increased total phenol content and antioxidant activity (Degrain *et al*., 2020).

Lactic acid bacteria produce antimicrobial compounds such as lactic acid, acetic acids, bacteriocins, alcohol, carbon dioxide, diacetyl, hydrogen peroxide, aldehyde, and esters during fermentation. They inhibit spoilage microorganisms in fermented food, hence ensuring food quality (Adams & Nout, 2001; Leroy & De Vuyst, 2004; Adam & Moss, 2008; Soccol *et al*., 2010; Ray & Didier, 2014; Wafula, 2017). Lactic acid bacteria survive and stabilizes in fermented vegetables, then dominates other microbial populations. Lactic acid bacteria produce acetic acid, lactic, carbon dioxide, bacteriocins, and biosurfactants which inhibit undesirable microorganisms. Also, the acids contribute to lowering the product's pH (Adam & Moss, 2008; Adams & Nout, 2001). Owade *et al*. (2021) reported 13 LAB isolates from spontaneous fermentation of cowpea leaves with *Lactobacillus brevis* and *Lactococcus lactis* dominating the onset stage, with only *Lactobacillus brevis* dominating the onset stage of optimized fermentation.

Lactic acid fermentation can eliminate natural toxins such as phytic acid, oxalate, cyanogen, tannin, and alkaloids, therefore, ensuring food safety (Leroy & De Vuyst, 2004; Muchoki *et al*., 2010; Leblanc *et al*., 2011; Fang *et al*., 2017; Indrastuti *et al*., 2018). *Lactobacillus plantarum* 75 strain ANS extract exhibited a 60% reduction of Aflatoxin B1 (AFB1) induced DNA damage and a 38% reduction in $FeSO_4$-induced oxidative stress (Odongo *et al*., 2018).

Furthermore, it improves digestion in the human gastrointestinal tract and increases the absorption of nutrients. Additionally, it improves antioxidant activity by increasing the number of phenolic and flavonoid compounds (Hur *et al*., 2014). Consumption of fermented food contributes to lowering cholesterol, inhibiting infectious diseases and food allergies. Also, it reduces diarrhoea, reduces inflammation, and improves digestion of various food in the body, such as lactose intolerant people (Deshpande *et al*., 2011; Leroy & De Vuyst, 2004; Soccol *et al*., 2010; Hur *et al*., 2014; Kaprasob *et al*., 2018)

Vegetable fermentation can be achieved either by spontaneous or controlled fermentation (CF) (Fig. 5). In spontaneous fermentation, natural flora is the fermenting agent. This technique is simple and affordable even to small-scale processors. Also, back-slopping techniques can be used, where a small quantity of materials from the previous batch of fermented products is used to inoculate the next batch (Kim *et al*., 2018). In back-slopping, the initial fermentation phase is short and reduces the risk of fermentation failure (Ghnimi & Guizani, 2018). Furthermore, it reduces the risk of proliferation of contaminant bacteria and acid-resistant bacteria (Adams & Nout, 2001). During spontaneous fermentation of cabbage, cucumber, and ANS, heterofermenters, mainly *Leuconostoc mesenteroides* and *L. brevis,* initiate the fermentation process, while *L. plantarum* and *Pediococcus cerevisiae* occur later (Hutkins, 2006; Belitz *et al*., 2009; Wafula, 2017) (Fig. 6).

A commercial starter culture is used in controlled fermentation, which requires high initial investment and maintenance costs, pausing a challenge to small-scale farmers and processors. Two commercial starter cultures, *Lactobacillus plantarum* BFE 5092 and *Lactobacillus fermentum* BFE 6620, were used to ferment nightshade leaves (Wafula, 2017). In another study by Stoll *et al*. (2021), *Lactiplantibacillus plantarum* BFE5092 and *Limosilactobacillus fermentum* BFE 6620 are two starter cultures strains in the fermentation of *S. scabrum* leaves. *Lactobacillus fermentum* BFE 6620, isolated from fermented cassava, was reported as a potential starter culture for AIVs (Wafula *et al*., 2017). Spontaneous and controlled fermentation significantly affected the nutritional and sensory quality of ANS leaves' leaves (Wafula, 2017). Thus, the proper selection of commercial starter cultures is essential in the controlled fermentation of ANS leaves for value addition (Stoll *et al*., 2021).

Fermentation of *S. scabrum* leaves increases ash content and reduces protein and vitamin C by 36 - 38.3% and 66 - 81%, respectively (Wafula *et al*., 2017). In contrast, *S. scabrum* leaves' fermentation decreases vitamin E, B_1, and B_2 by 10 - 34%, 68 - 76%, and 30 - 47.3%, respectively (Wafula *et al*., 2017). Furthermore, fermentation of *S. scabrum* reduces chlorophyll and total polyphenol but increases carotenoid content by 67% (Odongo *et al*., 2018). Fermentation is a suitable technique for preserving ANS, thus improving nutrition, food security and enhancing the livelihoods of millions of people in SSA (Wafula *et al*., 2016; Behera *et al*., 2020; Stoll *et al*., 2021).

2.12.5 Pickle making

Pickles can be divided into three groups, full fermented (FFP), or known as salt-stock or fresh-packed pickles (FPP), and refrigerated pickles (RP). The manufacturing of FPP and RP is fast, easy, and requires few steps (Hutkin, 2006). Vinegar is used for preserving FPP and RP. In PaP of FPP and RP, the jar of vegetables, mainly cucumbers, and other spices, are covered with vinegar. Fresh-packed pickles is pasteurized by heat, whereas RP is not pasteurized. Fresh-packed pickles is the most popular with longer shelf life, even stored at room temperature, and tastes crisp and mildly acidic. Refrigerated pickles is refrigerated, having the pleasant sensory quality of crunchy texture, crisp, and bright green color. During the storage of RP, a slight fermentation may occur; FPP has a longer shelf life than RP pickles. Because of RP's short shelf life, a preservative sodium benzoate is usually added (Hutkin, 2006). Full fermented pickle has a distinctly different flavor and color than FPP and RP and requires a much longer time in making. Also, FFP has a very long shelf life of about two years (Hutkin, 2006). Within these three pickles, pickles can be further distinguished based on the ingredients used, such as herbs, spices, flavouring agents, the size or type of vegetables, and the pickle's shape.

The steps used for FFP are similar to those used for making sauerkraut; the process requires oxygen exclusion, salt, and an anaerobic environment, which selectively allows the growth of naturally occurring LAB. The differences between pickles and sauerkraut, pickles require high salt concentration, resulting in less diverse microflora. Also, pickles use brine than dry salt and are amenable to pure starter cultures (PSC) and CF (Hutkin, 2006). Pickle marking starts with the selection and sorting of vegetables, then washing. The vegetables are then washed, sorted, and transferred to fermenting tanks, and a 5% (about 20° salometer) brine solution is added. Stock solution pickle, the initial salt concentration is 7 - 8%, and then salt is added to 12%; this pickle can stay in fermenters for an extended period (Hutkin, 2006). During fermentation, LAB produces LA and can tolerate high LA concentrations of 0.1 M and low pH of 3.5, much more than most of their competitors. Fermentation may be complete in 3-4 weeks, with a final pH of 3.5 and acidity of 0.6 to 1.2% (LA) (Bamforth, 2005; Hutkin, 2006; Ray & Didier, 2014). The only pickle with less than 5% allows the growth of *L. mesenteroides*, a heterofermentative LAB that may result in diverse flavor, bloater, or floater defects (Fig. 6).

On the other hand, the low salt concentration may permit unwanted natural flora, including *Bacillus, Flavobacterium, coliforms,* and *Pseudomonas* species. A high salt concentration of 5 - 8% inhibits the growth of *Leuconostoc*, *Pediococcus sp,* and *L. plantarum* initiate fermentation (Bamforth, 2005; Hutkin, 2006; Ray & Didier, 2014), (Table 6). Pickle fermentation with brine solution contains high salt concentration, organic acid, and pH less than 4.5, which has an inhibition effect against *Clostridia*, *Pseudomonads*, *Coliforms*, *Bacilli,* and other non-LAB that would affect pickle quality. After fermentation, salt stock pickles can be held indefinitely in the brine. Sometimes, 1 and 2% of sugar, mainly glucose or sucrose, is added to speed the formation of organic acids that act as initial preservatives (Dalié *et al*., 2010). Fermentation temperature varies between 15 to 30 °C; high temperature requires a short time to finish. Salt stock pickles cannot be eaten directly; they must be de-salted in water to get a required salt content of 4%. Fermented pickles can then be used in making relishes and other pickled products (Hutkin, 2006). For PSC fermentation, the microorganisms used as starter cultures include many of the same species isolated from vegetable fermentations, such as *Lc. mesenteroides*, *Pediococcus acidilactici*, *L. plantarum*, and *L. brevis* (Table 6). Selection of the appropriate strain is necessary and must depend on the specific applications and desirable characteristics of fermented vegetables. When PSC is used, lactate, acetate, and other buffer salts usually are added to brines to initiate the fermentation. Therefore, ANS pickle manufacturing can follow the same protocol for pickle making, as explained above.

During pickle fermentation, microbial defects may occur, including bloaters and floaters, which results in hollow cucumbers caused by excessive gas pressure (CO_2) mainly produced by heterofermentative LAB, coliforms, and yeasts. Floaters and bloaters can be used for processed products such as relish but cannot be used for whole or sliced pickle markets (Verma, 2000; Hutkin, 2006). The problem can be controlled by flushing the brines with N_2 to remove dissolved CO_2. Softening and destruction, destruct the pickle's surface tissue, resulting in the loss of sensory characteristics such as crunch and crispness, and become slippery and soft. The pickles become non-edible; nothing can be done to solve this problem. Pectinolytic enzymes cause the defect from filamentous fungi, especially *Penicillium, Fusarium*, *Alternaria*, and *Cladosporium*. Another defect is a black pickle; this is caused by hydrogen sulfide production (Verma, 2000; Hutkin, 2006).

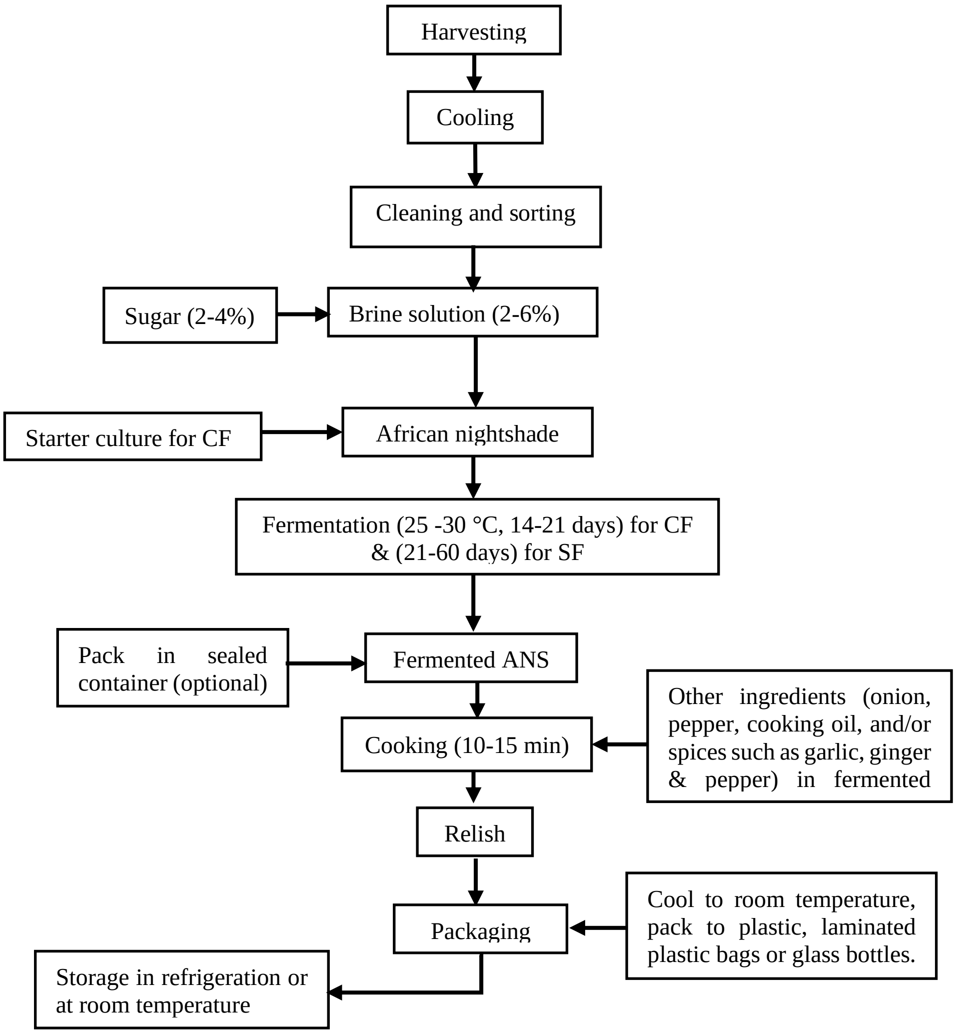

Figure 5: Fermentation of ANS. SF: Spontaneous fermentation; CF: Controlled fermentation (Adam & Moss, 2008; Adams & Nout, 2001; Battcock & Ali, 1998; Belitz *et al*., 2009; Wafula *et al*., 2016)

Table 6: Lactic acid bacteria used in the fermentation of ANS

LAB	Description	References
Leuconostoc mesenteroides	Obligate heterofermentative *Lactcocci*, used in vegetable fermentation. Assistant cultures in combination with fast acid-producing *Lactcocci* for the production of aroma and flavor compounds	Wafula (2017)
Leuconostoc pseudomesenteroides	Heterofermenter coccus, Gram-positive	Degrain *et al*. (2020)
Lactococcus fermentum	Obligate heterofermentater	Wafula (2017)
Lactobacillus plantarum	Facultative homofermenter rod-shaped, Gram-positive, Catalase negative, Produces a high amount of DL-lactate from glucose. Survive above 40º Salometer (10% salinity). and do not produce gas	Wafula (2017); Degrain *et al*. (2020)
Lactococcus lactis	Homofementive bacteria	Wafula (2017)
Pediococcus acidilactici	Homo fermentative bacteria, unique features for fermented vegetables. Improve food safety by producing antimicrobial peptides	Wafula (2017)
Weissella paramesenteroides	Heterofermentative LAB, not commonly used as a starter. Frequently isolated from spontaneous fermentation. Produce bacteriocins.	Wafula (2017)
Weissella cibaria	Frequently isolated from spontaneous fermentation. Produce exo-polysaccharides and bacteriocins.	Wafula (2017); Degrain *et al*. (2020)
Enterococcus faecium	Homofermentive, cocci shaped, Gram-positive, Catalase-negative. Do not produce gas from glucose metabolism also, produce L-lactate.	Wafula (2017)
Enterococcus faecalis	Homofermenter coccus, Do not produce gas, do not grow at 6.5 % NaCl, produced L-lactate.	Wafula (2017)
Enterococcus munditii	Homofermentive gram-positive, cocci shaped, catalase-negative. Do not produce gas and produce L-lactate,	Wafula (2017)
Enterococcus durans	Homofermentive cocci, Gram-positive, , Catalase-negative, Do not produce gas from glucose metabolism but produce L-lactate,	Wafula (2017)
Enterococcus gallinarum	Homofermentive cocci, Gram-positive, catalase-negative, Do not produce gas from glucose metabolism but produce L-lactate,	Wafula (2017)
Staphylococcus hominins	Cocci, Gram-positive, and catalase-positive, produced L-lactate	Wafula (2017)
Staphylococcus epidermidis	Cocci, Catalase-positive, produced L-lactate	Wafula (2017)
Pediococcus pentosaceus	Homofermentive cocci shaped bacteria. Gram-positive, catalase-negative. Do not produce gas from glucose but produced DL-lactate. Produce antimicrobial peptides	Wafula (2016; 2017);
Lactobacillus sakei	Homofermentative rod-shaped facultative anaerobes not produce gas from glucose but produced L-lactate	Wafula (2017)
Weisella confuse	Heterofermentative rods, Gram-positive, Catalase-negative, Produce gas from glucose metabolism. Can grow 6.6 % NaCl and capable of producing D and L-lactate	Wafula (2017)
Weisella oryzae	Heterofermentative rods, Gram-positive, Catalase-negative. Can grow 6.6 % NaCl Produce gas from glucose metabolism, Produce D-lactate	Wafula (2017)
Leuconostoc lactis	Heterofermentative cocci, Gram-positive, Catalase-negative. Produced gas from glucose metabolism; produce D-lactate	Wafula (2017)
Lactobacillus fermentum	Heterofermentative rods, Gram-positive, Catalase-negative Produce gas from glucose metabolism, Produce DL-lactate	Wafula (2017)

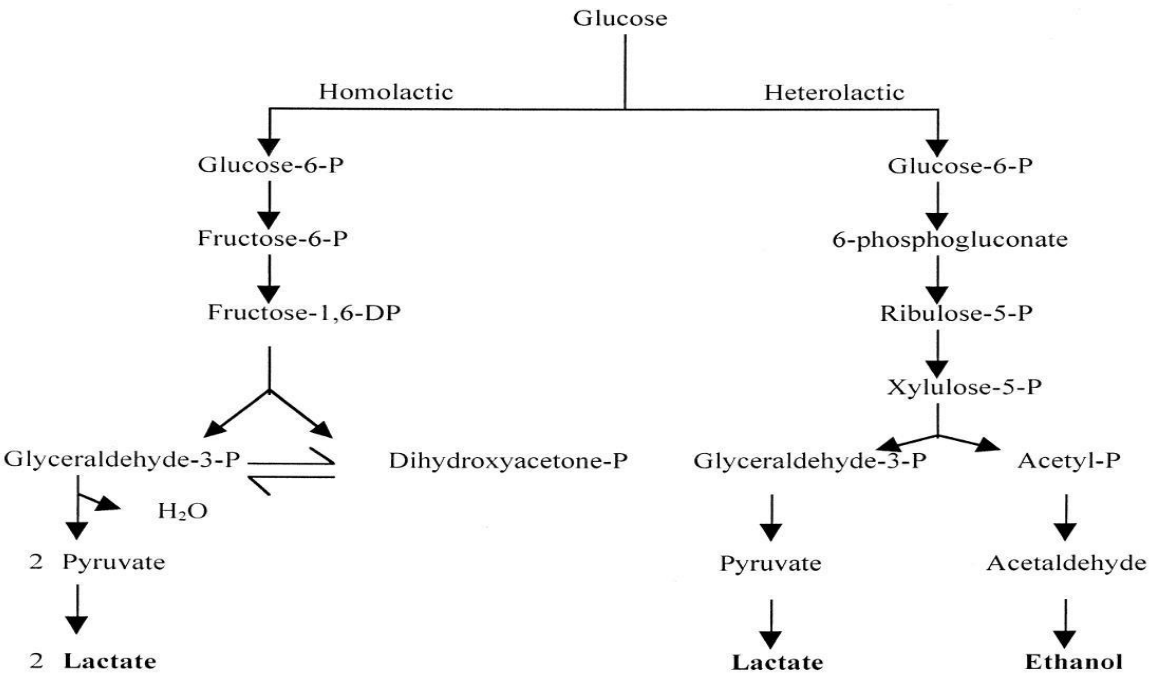

Figure 6: Generalized scheme for the fermentation of glucose to lactic acid bacteria (Caplice & Fitzgerald, 1999)

2.13 General challenges facing African nightshade

African nightshade production lacks good government extension services, indicating neglect of the crop, despite its significance in improving health and rural livelihoods (Weinberger & Msuya, 2004). Information on ANS yield in most SSA is not available, showing that vegetables have not been prioritized over other vegetables or crops (Edmonds & Chweya, 1997; Weinberger & Msuya, 2004). Furthermore, there is little information on indigenous knowledge on the utilization of ANS; this could facilitate the consumption, value addition, and commercialization of ANS (Onyango *et al*., 2016).

Seasonal availability is another challenge as the ANS becomes scarce and expensive during the rainy or offseason (Lotter *et al*., 2014). The lack of proper management consequently results in a decline in ANS supply, especially during the offseason (Edmonds & Chweya, 1997; Weinberger & Msuya, 2004). Heavy rainfall reduces the yields, especially for cultivated ANS (Lotter *et al*., 2014); therefore, screen houses can be used.

Pests and diseases are the key concern that hinders ANS production in SSA (Abukutsa-Onyango, 2007). Lack of appropriate technical skills for farmers such as PHH, processing, and preservation hinder the availability of ANS, especially during the offseason. Some preservation technologies require a high initial cost for implementation, which becomes difficult for small farmers. Indeed, the lack of proper infrastructure hinders the supply of ANS, especially from the production areas to the markets. Production is solely on a small scale by smallholder farmers and local households; therefore, it facilitates the low availability and utilization of ANS. Unsold raw ANS gets spoiled due to a lack of storage facilities along the value chain. Of importance, very minimal value addition is done to ANS, resulting in short shelf life, low market price, and low utilization.

Other challenges that hinder ANS utilization are foodborne diseases (FBD) and their toxin. In vegetables, common pathogenic causes (FBD) include *Listeria monocytogenes*, *Clostridium* spp., *Bacillus cereus*, *Campylobacter jejuni*, *Salmonella* spp., *Shigella,* and *Vibrio cholera, Yersinia* spp. Also, pathogenic strains of *E. coli* (Sant'Ana *et al*., 2011; Micali, 2016; Zhu *et al*., 2017; Hernández-Cortez *et al*., 2017; Waturangi *et al*., 2019; Balali *et al*., 2020). Pathogens can cause gastroenteritis, abortion, and central nervous system infection (Zhu *et al*., 2017; Kilonzo-Nthenga & Makuna, 2018). Wafula (2017) isolated pathogenic bacteria, i.e., *Providencia rettgeri, Enterobacter cloacae, E. coli, Klebsiella oxytoca, Enterobacter asburiae, Klebsiella* spp., *Enterobacter ludwigii* from fermented ANS leaves. These pathogenic bacteria may cause pneumonia, urinary tract and respiratory infection, and postsurgical peritonitis (Zhu *et al*., 2017; Wie, 2015; Singh *et al*., 2016). Also, they produce hemolytic enterotoxins and spore-forming toxins (Davin-Regli & PagÃ¨s, 2015). Simultaneously, *Escherichia coli* causes diarrhoea and produces Shiga toxin, causing severe diarrhoea or hemorrhagic colitis (Smith & Fratamico, 2017). However, the presence of pathogenic bacteria differs from the sources of ANS (Wafula, 2017).

Also, fungi can contaminate vegetables, but the risk of mycotoxin production is low compared to cereal foods. However, Sanzani *et al*. (2016) and Suleiman *et al*. (2017) reported mycotoxin in pepper, fresh spinach, and tomatoes onion; perhaps they can also contaminate ANS. Pathogens can contaminate vegetables at any point along the vegetable value chain (Zhu *et al*., 2017; Hernández-Cortez *et al*., 2017; Kilonzo-Nthenga & Makuna, 2018; Waturangi *et al*., 2019). The contamination sources include animals, insects, water, soil, dirty equipment, human, manure, sewage water, and faeces (Zhu *et al*., 2017; Kilonzo-Nthenga & Makuna, 2018). Therefore, farmers must follow good agricultural practices to avoid contamination. Contaminated vegetables can affect human health and result in several health problems. Mycotoxins are carcinogenic, mutagenic, and teratogenic (Sanzani *et al*., 2016). The consumption of fresh vegetables should be encouraged, but significant measures to ensure safety before consumption should be in place (Balali *et al*., 2020).

UV radiation, washing, low temperatures storage, and washing with diluted acetic acid, salted water, and vinegar can control PHH for vegetables (Zhu *et al*., 2017; Kilonzo-Nthenga & Makuna, 2018; Balali *et al*., 2020; Verbikova *et al*., 2018). Another essential factor in ensuring food safety and quality is adequate time and temperature control during processing, cooling, and storing food (Hernández-Cortez *et al*., 2017). Additionally, fermented pickles should be well-pasteurized to ensure safety.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out in Morogoro and Kilimanjaro (Fig. 7), covering the key common ANS growing areas (Weinberger & Msuya, 2004).

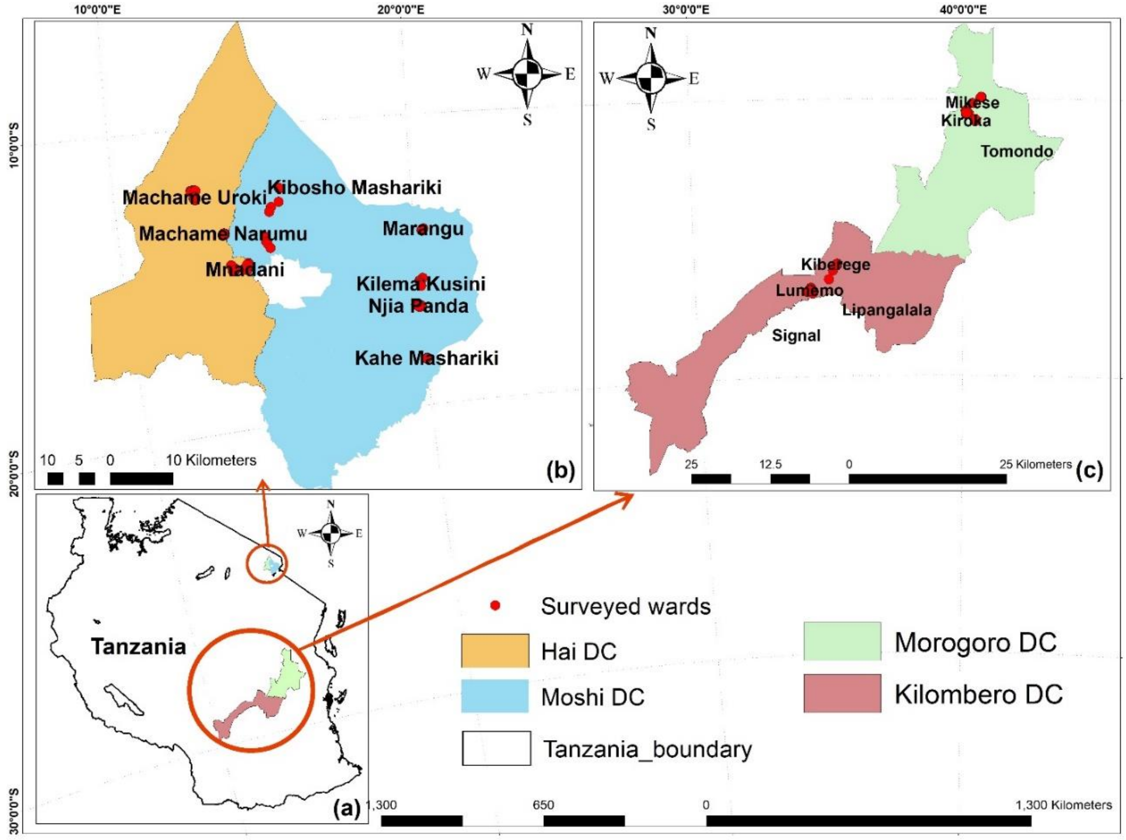

Figure 7: A map of Tanzania showing Morogoro and Kilimanjaro where African nightshades are cultivated

3.2 Sample size calculation

The sample size was determined using the formula, n = $\frac{z^2 p\, q}{r\,(e^2)}$ Bartlett *et al*. (2001)

Whereby; n; sample size, z; statistic for a level of confidence (1.962) at 95%,

p: proportion of the farmers expected to have ANS farms (50%) =0.5,

q (1-p) = the percentage in the selected population not expected to have ANS farms

e; marginal of error (5%) = 0.048,

r; response rate (80%).

In the equation above, the sample size is approximately 522 (Bartlett *et al*., 2001).

3.3 Sampling procedure

The survey was conducted in Morogoro and Kilimanjaro regions from April 2019 to August 2019, as shown in Figs. 7 and 8. Random sampling was used to select the study site (Kilimanjaro and Morogoro regions), districts of Hai and Moshi Rural in the Kilimanjaro region, and Kilombero and Morogoro Rural in the Morogoro region. In all districts, 20 villages with 600 households/respondents participated in the survey. All household heads (farmers) engaging in the production of ANS were listed by the Village Executive Officers (VEOs) with the assistance of Village Agricultural or Community Development Offices (VADOs). The participants were randomly selected from the lists of all household heads (farmers) (Table 7). The sample size was done by calculate the percentage, in which the district with large number of farmers has high sample size. Consent to participate in the study was sought from all the participants (Table 7).

The household data on cultivation, processing, preservation, utilization, and constraints were collected using semi-structured questionnaires supplemented by focused group discussions (FGDs). The FGDs were organized through community meetings in two selected villages in each district. About 6 - 10 people participated in FGD in the selected villages. The group includes farmers, processors, consumers, and sellers with equal number of men and women. Both genders were considered in the selection of the participants for FGD. During FGDs, a checklist with guiding questions was used to facilitate the discussion.

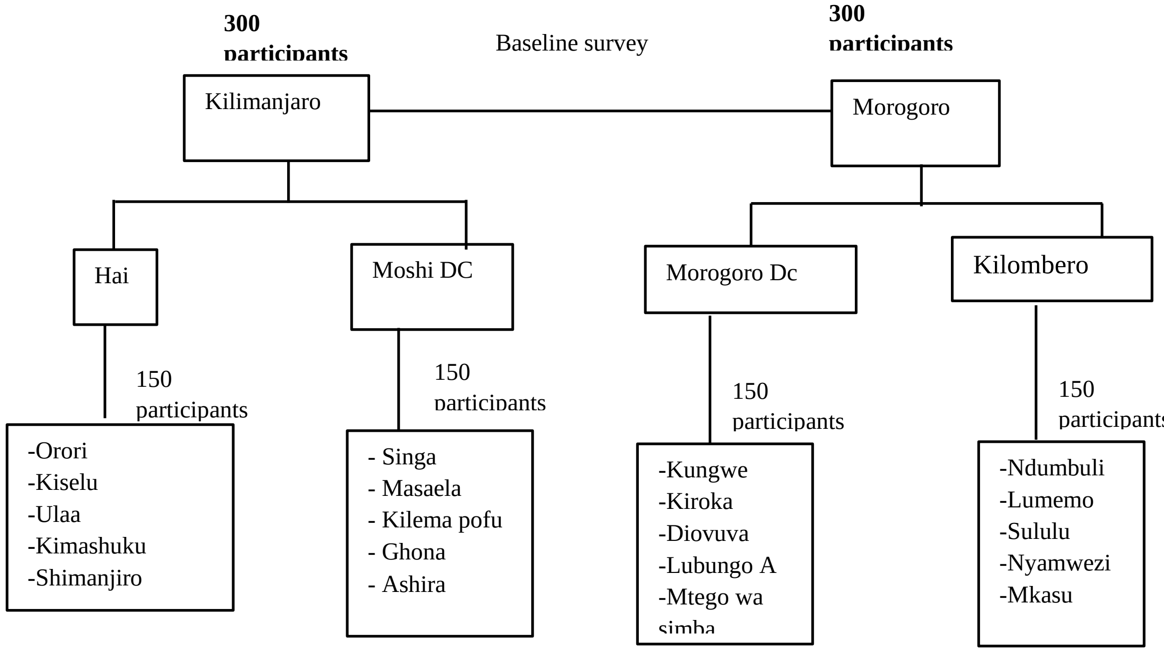

Figure 8: Sampling in Morogoro and Kilimanjaro

Table 7: Study Area, population distribution and sample size

S/N	District	Division	Ward	Village	Number of farmers	Sample size
1	Kilombero DC	Ifakara	Ndumbuli	Ndumbuli	2636	57
			Lumemo	Lumemo	2198	47
		Mang'ula	Signal	Sululu	670	14
			Kiberege	Nyamwezi	945	20
				Mkasu	561	12
2	Morogoro DC	Mkuyuni	Tomando	Kungwe	916	43
			Kiroka	Kiroka	866	40
				Diovuva	597	28
		Mikese	Mikese	Lubungo A	476	22
				Mtego wa Simba	375	17
3	Hai DC	Machame	Machame Narumu	Orori	500	24
			Machame uroki	Kiselu	490	24
			Mchame Uroki	Ulaa	670	32
		Lyamungo	Mnadani	Kimashuku	740	35
				Shirinjoro	720	35
4	Moshi DC	Kibosho	Kibosho Mashariki	Singa	720	30
		Vunjo Magharibi	Kilema Kusini	Masaela	853	36
			Njia Panda	Kilema Pofu	740	31
		Vunjo Mashariki	Kahe Mashariki	Ghona	557	24
			Marangu Mashariki	Ashira	690	29
Total					**16 920**	**600**

3.4 Study designs and approach

In this study, a cross-sectional community-based survey, product development, and laboratory analysis were conducted. Qualitative data were collected by FGDs, while quantitative data were collected using a semi-structured questionnaire.

3.5 Construction of questionnaire, pretesting, and administration

A semi-structured questionnaire was administered through face-to-face interviews with trained enumerators. Enumerators were selected considering their personalities (friendly, polite, and open) and gender (equal men and women), and nativity to the study area with a Bachelor's degree. The Swahili language questionnaire was used since most Tanzanians speaks Swahili, but for those who did not speak Swahili language, enumerators translated it into their native language. Abukutsa-Onyango *et al.* (2010), some communities are sensitive to gender, whereby men prefer being interviewed by other men and women prefer being interviewed by other women. Therefore, the enumerators worked in pairs. Pretesting of the questionnaire was conducted on a group of non-participating individuals before being issued for data collection. In each district one pre-test of questionnaire were done in on village. About 30-40 people was used for pretesting of questionnaire in each village.

3.6 Ethical clearance

The ethical approval for the study was obtained from the National Institute for Medical Research (NIMR) (NIMR/HQ/R.8a/Vol.IX/3041). Further, permission to conduct surveys in Kilimanjaro and Morogoro regions was sought from the district and regional government authorities.

3.7 Materials

Africa nightshades (*S. villosum* and *S. scabrum*) were collected in Arumeru District in Arusha, Tanzania. The leaves were transported under cold storage to the Food processing unit at NM-AIST for further processing. Other ingredients such as cooking oil, onions, and spices (garlic, pepper, cardamom, turmeric, cumin, clove, and cinnamon) were purchased in Arusha, Tanzania. Potential freeze-dried probiotic *Lactobacillus plantarum* LP90 (100 billion CFU/g) and *Leuconostoc mesenteroides* LM58 (100 billion CFU/g) were obtained from Wuhan Healthdream Biological Technology Co. Ltd. The starter culture was stored at -24 °C.

3.8 African nightshade leaves preparation

The freshly harvested *S. scabrum* (Ss) and *S. villosum* (Sv) leaves were sorted, washed, and then placed in a perforated vessel for water drainage before weighing (Plate 4). For CF, the leaves were blanched at 90 °C for 5 min and immediately cooled in ice water (De Corcuera *et al*., 2004).

Plate 4: Sorting, cleaning, and weighing of ANS

3.9 Fermentation of African nightshade leaves and relish preparation

In controlled fermentation (CF), blanched Ss and Sv leaves were submerged into a brine solution (4% salt and 2% sugar), and *L. plantarum* LP90: *L. mesenteroides* LM58 were inoculated in a ratio of 1:1 separately according to Wafula (2017) with slight modification. The mixture was incubated at 25 °C for 120 h. After 120 h of controlled fermentation, Ss (SsCFP) and Sv (SvSFP) pickles were obtained (Plate 5). In spontaneous fermentation (SF), unblanched Ss and Sv leaves were likewise submerged in a brine solution (4% salt and 2% sugar) and incubated at 25 °C for 15 days (Wafula, 2017) with some modification. After 15 days, SsSFP and SvSFP were obtained (Plate 5). The pH and titratable acidity (TTA) were recorded daily.

The pickles products from SF (SsSFP & SvSFP) and CF (SsCFP & SvCFP) were used in relish making. Pickles with cooking oil, onions, and different spices (garlic, pepper, cardamom, turmeric, cumin, clove, and cinnamon) were weighed and cooked for 10 min (Plate 6a and 6b) and (Fig. 9). The relish products from CF (SsCFRP & SvCFRP) and SF (SsSFRP & SvSFRP), respectively were then cooled, packaged in PET bottles, and stored in refrigeration and ambient temperature for further shelflife study (Plate 7).

a

b

c

d

Plate 5: Fermentation of ANS: (a) & (b) ANS in fermentation vessel, (c) Fermentation continues (d) Fermented ANS

Plate 6: Relish making: weighing of spices(a), Mixing of spices with a pickle (b), cooking of relish (c), relish ready for consumption (d)

Plate 7: Packaging and labeling of the product

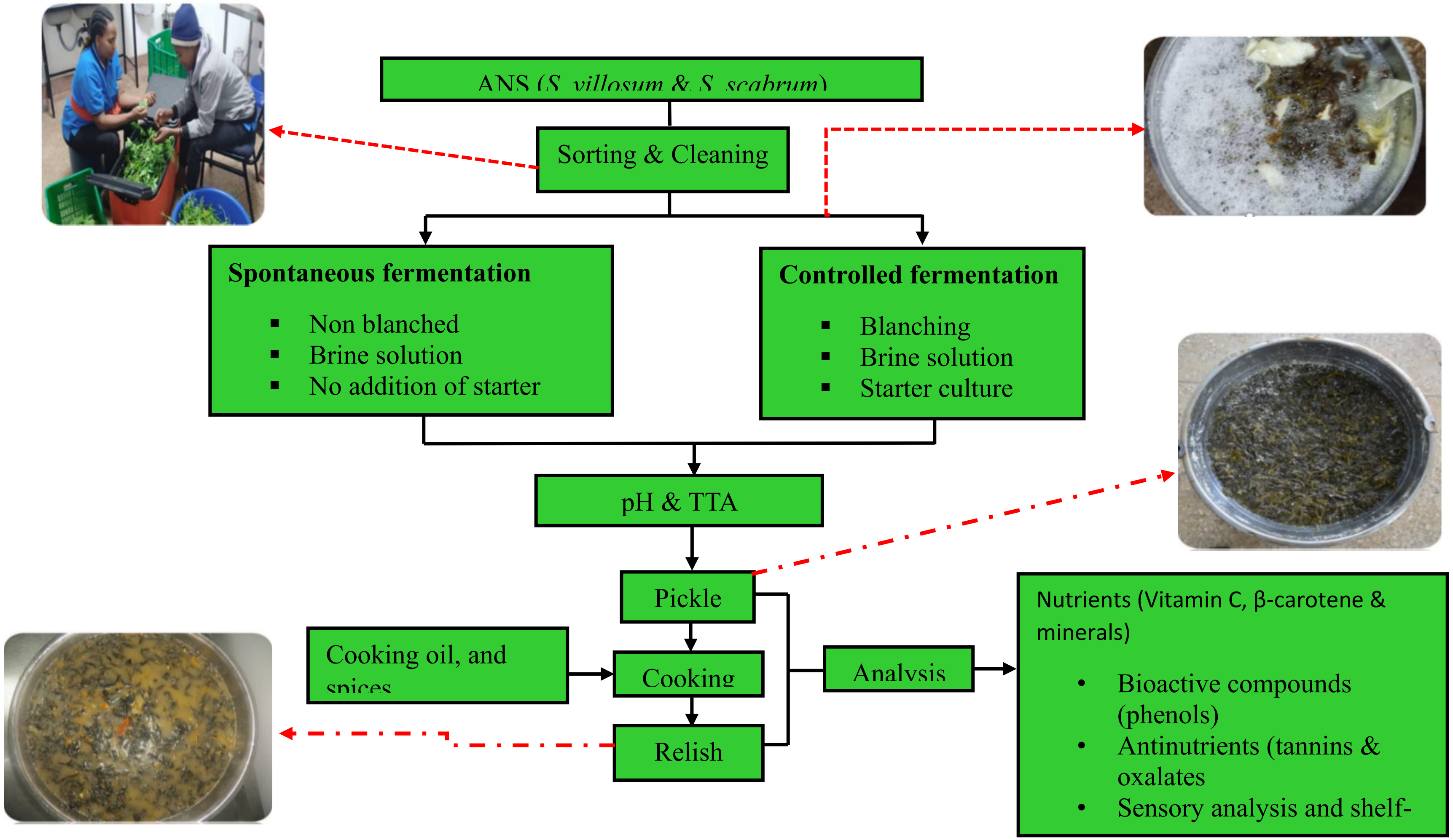

Figure 9: Relish making flow diagram

3.10 Determination of titratable acidity

Titratable acidity was determined as described by Rajković *et al*. (2007). About 10 ml of brine solution from spontaneously and controlled fermented Ss and Sv was mixed with 10 ml of distilled water in a conical flask. The mixture was titrated with 0.1N NaOH using two drops of phenolphthalein indicator (LOBA Chemie, India) to a persistent pink end. The TTA was calculated in terms of LA anhydrous as follows:

$$\text{TTA (\%)} = 0.09 \times \text{Titre (ml)}$$

3.11 Determination of vitamin C

The total vitamin C content of the raw leaves, pickle, and relish products was determined as described by (Kapur *et al*., 2012). About 10 g of sample was mixed with 50 ml of 3% metaphosphoric acid 8% acetic acid solution (LOBA Chemie, India) and centrifuged at 4000 rpm (centrifuge 5810, Germany) at ambient temperature for 15 min. Four milliliters (4 ml) of the extract were treated with 0.23 ml of bromine water (3%) (LOBA Chemie, India), followed by 0.13 ml of 10% thiourea solution (LOBA Chemie, India), and then 1 ml of 2, 4 dinitrophenylhydrazine solution (LOBA Chemie, India). The mixture was incubated in a thermostatic water bath at 37 °C for 3 h. The mixture was then cooled for 30 min and then treated with 6 ml chilled 85% sulphuric acid (LOBA Chemie, India). The absorbance of the resulting red-colored solution was measured using a Spectrophotometer (Heidolph, Germany) at 521 nm. The total ascorbic acid content was estimated based on the standard curve of ascorbic acid, and the result was expressed as mg/100 g (wet basis).

3.12 Determination of β-carotene

The β-carotene was determined as described by Perez-Lopez (2010) and AOAC (1980) with some modifications. Five grams (5 g) of the sample (raw, pickle, and relish products) in 1% butylated hydroxytoluene (BHT) (LOBA Chemie, India) was homogenized using a blender (Moulinex, China) for 5 min and placed into the falcon tube (BR Biochem, India). The homogenate was treated with 30 ml of solvent (50% Hexane: 25% Aceton: 25% Ethanol) (LOBA Chemie, India), then centrifuged at 4000 rpm (centrifuge 5810, Germany) at ambient temperature for 10 min. The supernatant was collected and filtered using Whatman number one filter paper (Whatman International Ltd, India), then re-extract two times until the residue was colorless. The supernatant was combined, and the volume was recorded. The supernatant was saponified with 40% potassium hydroxide (LOBA Chemie, India) and left at ambient temperature for 12 h. The mixture was then transferred into the separating funnel and treated with the same volume of 10% w/v sodium chloride (LOBA Chemie, India) for removing moisture. The mixture was then shaken vigorously, and the upper phase was collected and dried over anhydrous sodium sulfate (LOBA Chemie, India). The quantification was done using a Spectrophotometer (Heidolph, Germany). The β-carotene content was estimated based on the standard curve of β-carotene, and the result was expressed as mg/100 g (wet basis).

3.13 Determination of chlorophyll

The chlorophyll content of the raw leaves and pickle products was determined as described by Su *et al*. (2010), with some modifications. About 1.0 g of sample was taken and ground into a fine pulp using a mortar and pestle with about 10 ml of 80% acetone (LOBA Chemie, India). The pulp was centrifuged at 4000 rpm (centrifuge 5810, Germany) at ambient temperature for 5 min, and then the green supernatant was then transferred to a 50 ml volumetric flask for Chlorophyll determination. The sediment in the centrifuge tube was scrapped and ground with the same mortar and pestle with a small amount of 80% acetone (LOBA Chemie, India) to extract the residual Chlorophyll. The mixture was centrifuged at 4000 rpm at ambient temperature for 5 min; the supernatant was then mixed with the previous supernatant into the volumetric flask. Re-extraction was done until no perceptible green color was left in the residue. The supernatant was made to a 50 ml mark in a volumetric flask with 80% acetone (LOBA Chemie, India). The extract was placed into the refrigerator for 10 min to lower the temperature. The absorbance of the extract was read at 663, and 645 nm using a Spectrophotometer (Heidolph, Germany), and 80% acetone was used as the blank. The amount of Chlorophyll was calculated using the empirical formula:

$$\text{Chlorophyll a, mg/g tissue} = 12.7\,(A_{663}) - 2.69(A_{645})\ \text{X}\ \frac{V}{100X\ W}$$

$$\text{Chlorophyll b, mg/g tissue} = 22.9\,(A_{645}) - 4.68(A_{663})\ \text{X}\ \frac{V}{100X\ W}$$

$\text{Chlorophyll}_{\text{Total}}$, mg/g tissue = Chlorophyll a + Chlorophyll b= 20.31 OD_{645} + 8.05 OD_{645}

Where A = absorbance at specific wavelengths; V = final volume of chlorophyll extract; W = Fresh weight of tissue extracted.

3.14 Determination of total phenolic

The total phenolic content of raw Ss and Sv, pickle and relish products was determined by the Folin-Ciocalteu method (Mahdavi *et el.,* 2011). Homogenized samples of 10 g were mixed with 30 ml of 80% methanol (LOBA Chemie, India) and centrifuged at 3000 rpm at ambient temperature for 15 min. The residue was re-extracted twice. Precisely, 1 ml of the methanol extract was diluted ten times with the extraction solvent. An aliquot (0.5 ml) of the diluted sample was mixed with 2.4 ml of deionized water, 2 ml sodium carbonate (2%) (LOBA Chemie, India), and 0.1 ml of Folin-Ciocalteu reagent (LOBA Chemie, India). The mixture was incubated in a dark place at ambient temperature for 60 min, and the absorbance was determined at 750 nm using a Spectrophotometer (Heidolph, Germany).

3.15 Determination of mineral content

Minerals (P, S, Ca, Fe, Zn, Cu, Mn) were analyzed according to the method of Croffie *et al*. (2020). The samples (raw leaves, pickles, and relish products) were dried at 60 °C in a conventional oven (UN30, Germany). The samples were then ground using a motor and pestle and sieved in a sieve of 60-micron size (Shanghai sieves/ China). Four grams of the sample were mixed with 0.9 g of binder (Hoechstwax) (Cereox fluxana- BM-0002-1, Germany) using a pulverizer (PulverisetteTM/Germany) at the speed of 150 revolutions per sec for 30 min. The mixture was then compressed to form a pellet using a hydraulic pressing machine (Vaneox Fluxana PP25, Germany) at 15 psi. The Energy Dispersive X-ray Fluorescence (EDXRF) (Xlab pro-Spectro xepos spectrometer, Germany) was used to quantify the mineral content. The mineral reading was corrected using spinach leaves standard/Standard reference materials (SRM) 1570a, from the National Institute of Standard and Technology (NIST).

3.16 Anti-nutrients determination

3.16.1 Oxalates determination

The oxalate content in raw leaves, pickles, and relish products was determined by the titration method as described by Agbaire (2011). In one (1 g) sample, 75 ml of 3 M Sulphuric acid was added while stirring using a magnetic stirrer for 60 min. The mixture was filtered using Whatman filter paper no. 1 (Watmann International Ltd, India). About 25 ml of the filtrate was titrated while hot against 0.05 M potassium permanganate solution (LOBA Chemie, India) until a faint pink color persisted for at least 15-30 secs. The oxalate content was calculated by taking 1 ml of 0.05 M potassium permanganate is equivalent to 2.2 mg oxalate.

$$O = \frac{Ts \times Md \times Mo \times 100}{Ws}$$

Where, O = Oxalate concentration in mg/100 g, Ts = volume of potassium permanganate used for the sample, Md = number of moles of potassium permanganate reacted, Mo = number of moles of oxalate reacted, and Ws = sample weight (g).

3.16.2 Tannins determination

The tannins were determined by the Folin-Ciocalteu method according to Chandran and Indra (2016). A 10 g of homogenized sample (raw ANS, pickle, and relish) was mixed with 30 ml of 80% methanol (LOBA Chemie, India) and centrifuged at 3000 rpm at ambient temperature for 15 min. The residue was re-extracted twice. Precisely, 1 ml of the methanol extract was diluted ten times with the extraction solvent. An aliquot (0.5 ml) of the diluted sample was mixed with 7.5 ml of distilled water, 1 ml sodium carbonate (35%) (LOBA Chemie, India), and 0.5 ml of Folin-Ciocalteu reagent and diluted to 10 ml with distilled water (LOBA Chemie, India). The mixture was shaken well and kept at room temperature for 30 min. Tannic acid standards were used to prepare the standard curve. Absorbance for test and standard solutions were measured against the blank at 700 nm with a spectrophotometer (Heidolph, Germany). The tannin content was expressed in mg of tannic acid equivalents/100 g of wet basis.

3.17 Microbial determination of relish products

Total bacteria, fungal counts, coliform, and *Lactobacillus* were analyzed separately by the pour plate technique described by Karami *et al*. (2017), Rahimi *et al*. (2019) and Singh *et al*. (2013) with slight modification. Briefly, 1 g of homogenized relish products was mixed with 9 ml of sterile peptone salt (LOBA Chemie, India) and vortexed for 5 min. Then, 1 ml of the suspension was transferred into the peptone salt diluents (9 ml) up to 10^{-4} dilution series. From each dilution, 1 ml of diluted sample was inoculated into the respective growth medium; Total bacteria (Plate count agar), Yeast and mold (potato dextrose agar), *Lactobacillus* (de Man, Rogosa, and Sharpe agar), and coliform (violet red bile agar) (HiMedia Laboratories, Mumbai India). The incubation conditions were; bacteria (30 °C, 24 h), molds and yeasts (37 °C, 72 h), coliforms (30°C, 48 h), *Lactobacillus* (37 °C, 48 h) at anaerobic conditions.

3.18 Consumer acceptability test

The consumer acceptability test of the relishes was conducted in Moshi rural District in Kilimanjaro and Morogoro District Council in Morogoro regions with high and low ANS production, respectively. About 370 untrained panelists, mainly ANS consumers, participated in the sensory evaluation of the relish products (Plate 8). They were selected based on their history of consumption of ANS. Four types of relish products (SsSFR, SsCFR, SvSFR, and SvCFR) were tested for consumer acceptability. A 9-scale Hedonic test (Yang & Lee, 2018) was used with 1 = dislike extremely, 5 = Neither like nor dislike, and 9 = like extremely. Panelists were served with all four coded relish products and filled out an evaluation form after tasting.

Plate 8: Consumer acceptability test of the relish in Morogoro and Kilimanjaro

3.19 Shelflife test of the relish products

The shelf life study of relishes was conducted according to Pala and Agnihotria (1994) with some modifications. The relish products in transparent PET plastic capped bottles were stored consecutively at refrigeration (4 °C) and ambient temperature for six months (Plate 9). During storage, TTA, pH, total bacteria, LAB, yeast, and mold analyses were conducted monthly.

a b

Plate 9: Shelflife stability test of packaged relish at (a) ambient temperature and (b) in the fridge

3.20 Statistical analysis

Experiments were conducted in triplicates and data were expressed as mean values ± SD. Statistical Package for the Social Sciences (SPSS) software version 25 was used for data processing. Chi-square was used to compare group differences for categorical variables. The LSD tests were used to compare the mean between the species of *Solanum* for the fermented pickles and relish products, and the differences were considered significant when $p < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Socio-economic characteristics of the farmers

A large proportion of ANS farmers (70.4%) were from male-headed households. Kotrlik and Higgins (2001), 80% of Tanzania households are male-headed. Most farmers, 73.9%, had attained a primary education level, 5.7% with non-formal education and 0.9% had college and university education (Table 8). The head of household (HH) age was between 42 - 53 years, with a household size of five individuals. Likewise, the household size for rural households reported in Tanzania is 5.3 members (United Republic of Tanzania, 2012). The most considerable portion of farmers, 80.5%, were native to the surveyed area. Further, 88.4% and 72.6% of farmers in the Kilimanjaro and Morogoro regions were natives. It could have significance in the high production of ANS because they are native to the area. Small-scale farmers (83.3%) were predominant with less than one acre of farm size. Likewise, Abukutsa (2007) reported that most farmers (86%) cultivate less than one acre of ANS in Kenya. In Kilimanjaro, 90% or more of the land has been utilized (United Republic of Tanzania, 2012); this contributed to the more significant population cultivating less than one acre (United Republic of Tanzania, 2012). Furthermore, 97.6% of all participants were familiar with ANS (Table 8). The majority of farmers use indigenous knowledge in ANS cultivation i.e., local fertilizer and pesticides.

Table 8: Socio-economic characteristics of the African nightshades' farmers

Variable		Districts					Chi-Square (χ^2)
Parameter		**Morogoro Rural**	**Kilombero DC**	**Moshi Rural**	**Hai DC**	**Mean (SD)**	**Level of Significance**
Gender (%)	Male	81.6	49.3	72.7	78.0	70.4 (14.5)	χ2=46.89, df=1, p=0.001
	Female	18.4	50.7	27.3	22.0	29.6 (14.5)	
Education level (%)	None	11.8	5.9	2.7	2.3	5.7 (4 .4)	χ2 = 0.81; df = 1; p = 0.3
	Primary school	84.2	77.6	73.2	60.7	73.9 (9 .9)	
	Secondary school	2.6	15.1	10.3	15.3	8.7 (5.9)	
	College & University	0.5	0.5	1.1	2.1	1.1 (0.9)	
Place of Birth in (%)	Native	68.9	76.3	89.8	86.9	80.5 (9 .7)	χ2=0.4, df=1, p=0.41
	Migrant	31.1	23.7	10.2	13.1	19.5 (9.7)	
Age (years)	16-41	19.4	18.4	26	16.2	20 (4.3)	χ2=210.158, df=1, p=0.01
	42-53	35.5	38.2	34.7	35.9	36.1 (1.5)	
	54-65	17.2	19.1	9.3	28.3	18.5 (7.8)	
	66-77	7.1	3.3	4.0	2.4	4.2 (2.0)	
	78+	0.0	2.6	0.0	1.2	1.0 (1.3)	
Household size (%)	1–5	63.2	58	60	54.9	59 (3.5)	χ2=322.689, df=2, p=0.04
	6–10	32.2	40.1	40	45.1	39.4 (5.3)	
	>10	4.6	1.3	0.0	0.0	1.5 (2.2)	
	Mean (SD)	5±2.7	5±2.1	5±1.8	5±1.8	5 (2.1)	
Farm Size	<1 acre	65	55	68	70	64.5	χ2 = 3.83; df = 1; p = 0.17
	1-5 acres	30	34	29	26	29.8	
	Above 5 acres	5.0	11	3.0	4.0	5.8	
Occupation (%)	Farmer	86.9	93.2	81.2	72	83.3 (9)	χ2=0.9, df=1, p=0.29
	Farmers& business	7.5	4.1	0.2	17.8	9.9 (5.8)	
	Farmer & teacher	0.9	1.4	5.7	3.3	2.8 (2.2)	
	Farmers & doctor	0.6	1.4	-	1.9	1.3 (0.7)	
	Farmer & other	4.1	0.0	2.8	5.1	3.0 (2.2)	
Knowledge of ANS (%)	Yes	96.0	94.1	100	100	97.6 (3)	χ2=0.23, df=1, p=0.62

Crop production and animal keeping engaged 98.8% of households (URT, 2012); however, this study reported that 83.3% of farmers were involved in agricultural activities. Also, 3.6% of participants were older people above 65 years, the dependency group. Nono-Womdim *et al*. (2012) and URT (2012) reported that 4% of the farmers were above 65 in the national census of smallholder agriculture in Tanzania.

4.2 Cultivation of African nightshades

African nightshades grow naturally around farmers' households and farms (79%), especially during the rainy season $p<0.05$. Morogoro (84.9%) had the highest rate of naturally growing ANS. In Kilimanjaro, cultivated ANS is common as a result of the low availability of naturally growing ANS. African indigenous leafy vegetables can grow naturally, mainly in home gardens within the homestead (Abukutsa-Onyango, 2007).

Local varieties of ANS were reported to grow naturally by 44.9 and 83.6%, respectively. The local varieties represent *S. villosum*, *S. nigrum*, and *S. americanum*, while the modern variety refers to *S. scabrum*. About 47 and 34.4% of farmers grow modern and local varieties of ANS. In both Kilimanjaro and Morogoro regions, 99.4 and 44.7% of farmers produce ANS, respectively. *Solanum scabrum* is highly grown because it is less bitter with large succulent leaves, with rapid new leaf sprouting after harvest and late flowering. It has 40 days of maturity, picking can continue for 6 - 8 weeks, and resistance to diseases like Fusarium wilt (Nono-Womdim, 2012; Ojiewo *et al*., 2009). High maturity rates, less use of inputs, and less infestation by pest, and diseases are factors attracting farmers to grow ANS (Muhanji *et al*., 2011). *Solanum scabrum* is the most grown and available species in SSA with the highest yields (Nono-Womdim *et al*., 2012).

Resembling ANS, a traditional vegetable, i.e., *Justicia heterocarpa,* (*Mwidu* or *Lichuru*), was found to be highly consumed in Morogoro. *Justicia heterocarpa* is almost similar in taste to ANS besides not being sold in the local markets. The consumers in the Kilombero district highly prefer *J. heterocarpa.* Also, *Mwidu* grows even better and more than ANS around the family compounds and farms with no input requirement. *Justicia heterocarpa* has been reported to possess several therapeutic properties and is also used to treat various diseases such as fever, headache, Malaria, cancer, diabetes, mental disorder, sedatives, hallucinogens, epilepsy, gastrointestinal diseases, rheumatic, HIV, and inflammation. *Justicia heterocarpa* is rich in vitamins, minerals (iron, zinc, calcium, and magnesium), polyphenols and flavonoids, and free fatty acids (Al-Juaid & Abdel-Mogib, 2004; Corrêa & Alcântara, 2012; Mbaveng & Kuete, 2014). Various species of *Justicia* are consumed as food in Tanzania, including *Justicia pinguior* (Ruffo *et al*., 2002).

African nightshade cultivation utilizes agricultural inputs, irrigation, and cropping systems, mainly mono- and intercropping (Table 9). Generally, pesticides and fertilizer applications were common in Kilimanjaro. Inorganic pesticides and organic fertilizers applications were higher at 77.5 and 45.5%, respectively (Table 9), with inadequate awareness of the benefits of organic pesticides. Studies reported ashes and animal manure as pests and disease control (Abukutsa-Onyango, 2007; Ontita *et al*., 2017). Irrigation was common in both regions, whereas rivers and ponds were the primary water sources. Likewise, irrigation of ANS was more common during the dry season, with primary water resources from rivers and ponds (Lotter *et al*., 2014). In Kilimanjaro, irrigation was facilitated by an extensive network of open, unlined canals, which obtain water from rivers, most of them built centuries ago.

Table 9: Different practices conducted during the production of African nightshades

Items	**(%)**	**(n)**	**Chi-Square ($\chi 2$)**
Agricultural inputs			
Pesticide	70.7	299	$\chi 2$ = 115; df = 1; p = 0.001
Inorganic pesticide	77.5	121	$\chi 2$ = 34.83; df = 1; p = 0.001
Organic pesticides	45.5	121	$\chi 2$ = 5.11; df = 1; p = 0.024
Fertilizer	64.8	330	$\chi 2$ = 165.53; df = 1; p = 0.001
Inorganic fertilizer	49.8	330	$\chi 2$ = 7.5; df = 1; p = 0.006
Organic fertilizer	70.6	330	$\chi 2$ = 36.3; df = 1; p = 0.001
Handheld tools	98.3	300	$\chi 2$ = 4.77; df = 1; p = 0.029
Oxen-drawn ploughs	4.8	300	$\chi 2$ = 7.3; df = 1; p = 0.007
Irrigation	79.4	504	$\chi 2$ = 7.69; df = 1; p =0.01
Sources of water (n=479)			
Rivers	49.9		$\chi 2$ = 7.19; df = 2; p = 0.028
Ponds	35.5		$\chi 2$ = 21.58; df = 1; p = 0.001
Wells	31.7		$\chi 2$ = 8.22; df = 1; p = 0.004
Tap water	5.0		$\chi 2$ = 5.73; df = 1; p = 0.017
Spring water	5.7		$\chi 2$ = 5.99; df = 1; p = 0.014
Cropping system (n=379)			
Monocropping	66.3		$\chi 2$ = 0.01; df = 1; p = 0.97
Intercropping	39.8		$\chi 2$ = 2.27; df = 1; p = 0.132

On the other hand, the Kilombero river and water channels in the Kiroka ward facilitated the irrigation of ANS in Morogoro. Both monocropping and intercropping practices were reported, with monocropping being dominant (66%) (Table 9). The dominance of monocropping for most ANS farmers in Kenya was reported (Onyango *et al*., 2016). Intercropping of ANS during the rainy season was in practice to utilize the land for various crops (Abukutsa-Onyango, 2007;

Abukutsa-Onyango, 2015). Furthermore, there was no mechanization in ANS farming. African nightshade production in most Kenyan societies is still small, with 80% farming (less than 0.25 acres) using handheld tools such as hand hoes (Abukutsa-Onyango, 2007).

Furthermore, seasonal production of up to 500 kg was reported by 79 - 85.4% (Fig. 10), indicating the highest amount produced. In contrast, very few farmers could produce more than 500 kg.

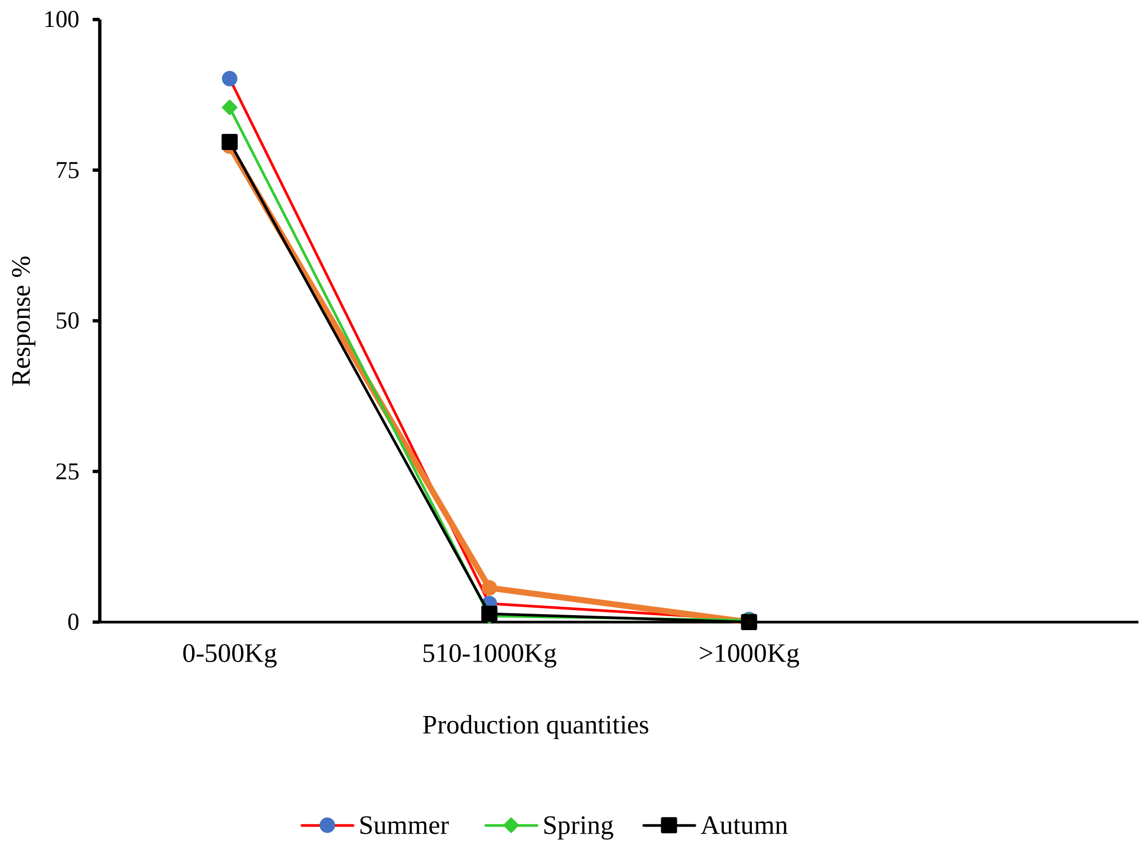

Figure 10: African nightshades yield (kg) during different seasons

4.2.1 Challenges Hindering African nightshade cultivation

From the study, several challenges were observed to contribute to low productivity. Lack of knowledge, pests, and diseases (92.9%), lack of extension services, small farm sizes, lack of quality/certified seeds, and shortage of quality fertilizer and pesticides, drought werethe primary factors for low productivity (Fig. 11). The typical insect pests of ANS include; aphids, spider mites, red and black ants, cutworms, caterpillars (larvae), grasshoppers, whiteflies, beetles, sp.), and nematodes. Also, fungi, bacteria, and viruses were the major causes of ANS diseases. According to Dinssa *et al*. (2016), low-yielding cultivars, non-application of fertilizer, and poor management result in low ANS production in SSA.

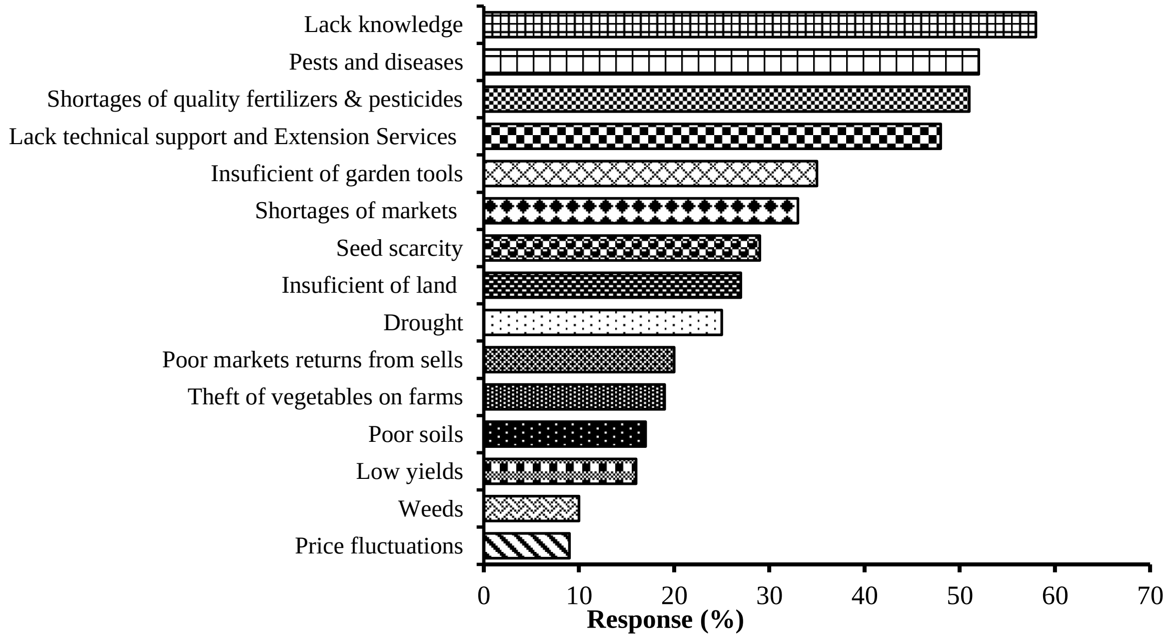

Figure 11: Challenges hindering cultivation and increasing post-harvest losses of African nightshades in Tanzania (n=442)

4.2.2 African nightshade Contribution to Household Income

African nightshades were shown to generate between 5 – 50% of the farmers' income (Table 10). Only 4.4% of farmers were earning between 51 - 100% of their income. Long-term benefits from ANS production were reported by 67.2% of the farmers. The annual net value of 5,612,042.25/= TZS on selling ANS, higher than Chinese cabbage and spinach, has been reported (Molina *et al.*, 2020). Therefore, ANS cultivation can be a good source of income generation for the farmers contributing to improved livelihoods.

From the study, it was found that no value addition was done to ANS. Freshly harvested ANS was directly sold to consumers with no value addition. Nonetheless, ANS contributed to the income generation of farmers; besides, it was not sustainable. Therefore, value addition can optimize profits generated from ANS if well considered. Products such as dried and fermented (pickles) ANS could add more value, contributing to farmers' household income. Notably, fresh ANS is highly perishable; therefore, farmers encounter substantial losses.

Table 10: Contribution of African nightshades to the household income of the farmers (n=158)

Income (%)	Morogoro	Kilimanjaro	Average	Chi-Square (χ^2)
<5	46.80	39.20	43	$\chi2 = 17.55$; df = 10;
5-50	49.3	56	52.7	p = 0.063)
51-100	3.9	4.8	4.4	

4.2.3 African nightshade Production

Based on the seasons, ANS productivity was the highest during the dry season and the least during autumn (Table 11). Heavy rainfall and lack of a modern cultivation system and greenhouse were the contributors to reducing ANS yields (Muhanji *et al.*, 2011; Weinberger & Msuya, 2004). Heavy rains and flooding of the Kilombero river significantly reduced the yield of ANS. Apart from growing ANS, other leafy vegetables such as *Mchunga* (*Launea cornuta*), *Mlenda mgunda* (*Corchorus olitorius*), and *Mchicha pori* (*Amaranthus spp.*), *Mnafu pori or Bwasi* (*Solanum incanum*), *Mlenda mwage* (*Sesbania* spp.), *Mgange*, *Mlenda mwidu* (*Corchorus* spp.) *Matembele pori* (*Ipomoea* spp.*)*, *Kisamvu* (*Manihot grazioli*), Pumpkin leaves (*Cucurbita pepo* (L.), *Mashona nguo* (*Bidens pilosa*), *Kunde pori* (*Vigna vexillata*), and African eggplants (*Solanum macrocarpon.*) were grown in high quantity. Additionally, cowpea leaves (*Vigna unguiculata*), sweet potatoes leave (*Ipomoea batatas* (L.), beans leaves (*Phaseolus vulgaris*), and cassava leaves (*Kisamvu-muhogo* (*Manihot esculenta*) and *Kisamvu-mpira* (*Manihot glaziovii*) (Table 11). These vegetables are abundant during the rainy seasons to reduce the demand for ANS, and some can be obtained for free, such as *Mwidu* (*Justicia hetorocarpa*). On the other hand, exotic vegetables such as spinach (*Spinacia oleracea*), Chinese cabbage-*Michihili* (*Brassica rapa spp.*), and cabbages (*Brassica oleracea* var. capitate) were also grown. The availability of other leafy vegetables reduces the consumption of ANS. African nightshades had stiff competition from cabbage, spinach, kale, and lettuce (Abukutsa-Onyango, 2007).

Table 11: Production of African nightshades and other vegetables in Morogoro and Kilimanjaro regions

Items (%)	Morogoro	Kilimanjaro	Chi-Square (χ2)
Other leafy vegetables (n=466)			
Spinach	59.3	71.6	χ2 = 5.73; df = 1; p = 0.017
Chinese cabbage	43.4	35.5	χ2 = 2.17; df = 1; p = 0.14
Cabbage	16.8	50.7	χ2 = 38.67; df = 1; p = 0.001
Cowpeas leaves	30.1	13.5	χ2 = 15.16; df = 1; p = 0.001
Sweet potatoes leaves	31.0	5.1	χ2 = 51.15; df = 1; p = 0.001
Beans leaves	20.4	14.9	χ2 = 1.79; df = 1; p = 0.18
Cassava leaves	29.2	5.4	χ2 = 43.92; df = 1; p = 0.001
Other vegetables	25.7	17.8	χ2 = 6.50; df = 1; p = 0.011
African nightshades production seasons (n=420)			
Spring	44.1	37.7	χ2 = 1.65; df = 1; p = 0.199
Autumn	24.3	29.9	χ2 = 1.50; df = 1; p = 0.221
Winter	44.1	33.0	χ2 = 5.09; df = 1; p = 0.02
Summer	58.1	66.4	χ2 = 2.83; df = 1; p = 0.93
Reason to grow ANS (n=409)			
High maturity rate	30.9	44.9	χ2 = 5.64; df = 1; p = 0.02
Less input needed	42.3	28.4	χ2 = 6.23; df = 1; p = 0.01
Less infestation of pests and diseases	44.5	18.9	χ2 = 22.73; df = 1; p = 0.01
High yield	21.9	63.2	χ2 = 14.44; df = 1; p = 0.01
High profit	19	20.6	χ2 = 0.13; df = 1; p = 0.72
Consumer's preference	29.2	16.1	χ2 = 7.18; df = 1; p = 0.007
Bitter test	19.7	38.1	χ2 = 11.79; df = 1; p = 0.01
Resistant to drought	20.4	19.6	χ2 = 0.03; df = 1; p = 0.863

4.3 Utilization and value chain of African nightshades

Large proportion of farmers (96.1%) consumes ANS in Kilimanjaro and Morogoro regions. African nightshades are grown for food and income generation (Fig. 12), similar to Kenya (Muhanji *et al*., 2011). Many household members consumed ANS, and the least consumers were children below five years. Most farmers consume ANS since it is nutritious and make them strong (Abukutsa-onyango, 2015). Generally, ANS can be consumed alone (84.1%) (Fig. 3) and sometimes blended with other vegetables (48.6%) or during cooking. Further, amaranth (73.8%), spinach (59.3%), pumpkin leaves (20.3%), and cowpeas leaves (12.5%) are vegetables blended with ANS, whereas potato leaves (9.2%) and bean leaves (7.5%) are the least. Other foods reported to be mixed with ANS include meat (92.4%), internal organs such as intestines (30.6%), fish (31.2%), and eggs (1.4%) (Fig. 13). Moreover, they were pre-cooked in advance. Incorporating ANS into meat, other vegetables, and legumes improves taste, appetite, and nutritional quality. Mixing cowpea leaves into ANS increased carotenoids and vitamin C (Oluoch *et al*., 2012). Singly prepared ANS and combined with other vegetables did not influence sensory acceptability, although a mixture of ANS and cowpea increased carotenoids and vitamin C (Habwe & Walingo, 2008; Oluoch *et al*., 2012). Overcooking ANS with meat or fish facilitates the degradation of essential micronutrients such as vitamins B, C, β-carotene, and iron (Shackleton *et al*., 2009). Various reasons such as improving taste (82.4%), improving appetite (57.2%), and reducing bitterness (38.8%) necessitate blending of ANS with other foods.

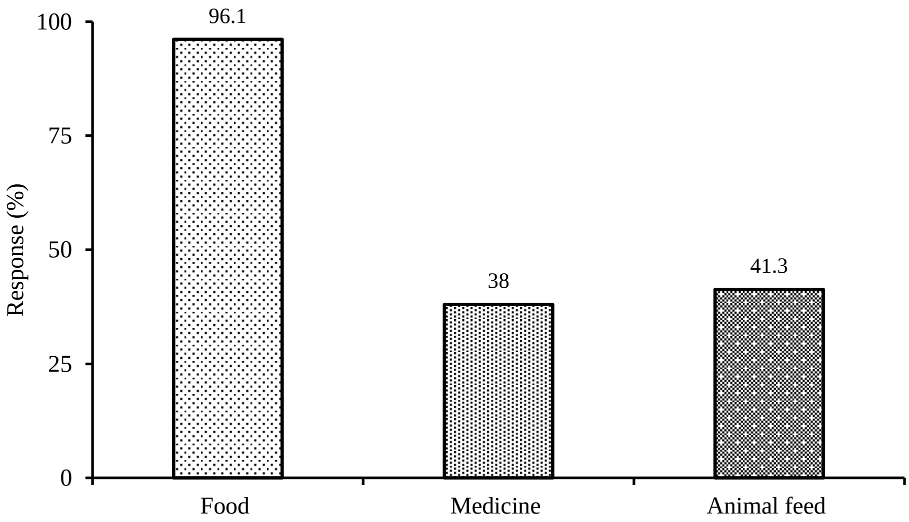

Figure 12: Utilization of African nightshades (n=560)

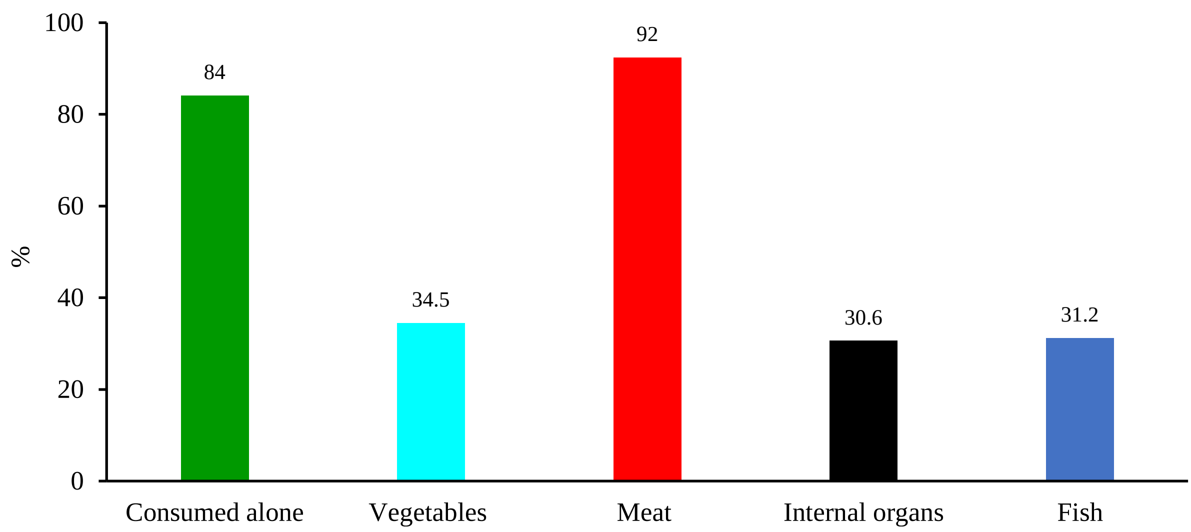

Figure 13: Foods which are cooked together with African nightshade (n=580)

Interestingly, ANS can be accompanied by several staples, including *ugali* (96.9%), rice (60.4%), *Makande* (17.6%), and cooked green banana (12.1%) (Fig. 14). Cooked nightshades leaves were reported to be served with *fufu* or *ugali* (Chagomoka *et al*., 2014). In East Africa, ANS can be blended with amaranth, spinach, kale, and cabbages and accompanied by staples such as stiff porridge (*ugali*), rice, yams, banana, and cassava (Abukutsa-Onyango, 2007; Yang & Ojiewo, 2013).

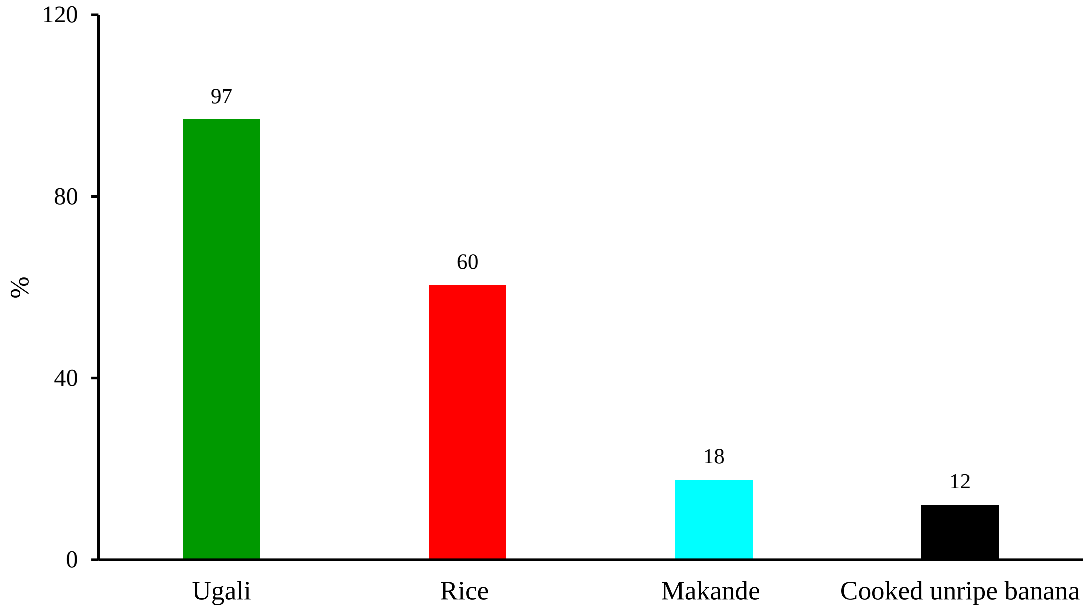

Figure 14: Staple food to consume along with African nightshade (n=560)

Local varieties of ANS, especially the bitter ones, were mainly preferred (75%) instead of exotic types (24.9%). Consumption of ANS is expected to increase in the next five years, reported by 69.4% of farmers. Due to the absence of diverse varieties of processed ANS products, about 46.3% of farmers would consume fermented ANS. Almost 53.1% of participants consume ANS for its nutrients. However, other reasons include bitter taste (33.6%) and improving appetite (30.8%). It was also observed that ANS is consumed based on gender, whereby men prefer the bitter dark variety. African nightshades were reported to be used mainly as food in both Morogoro and Kilimanjaro regions. Nevertheless, in Kilimanjaro, ANS is more utilized for commercial purposes. The presence of national and international research institutions, including the Asian Vegetable Research and Development Centre (AVRDC), Seeds of Expertise for the Vegetable Sector in Africa (SEVIA), and the International Institute of Tropical Agriculture (IITA), might have contributed to promoting high production and utilization of AIVs around northern zone regions such as Kilimanjaro, Arusha, and Tanga.

On the other hand, ANS is also used as medicine (38%) and animal feeds (41.3%) (Fig. 12). African nightshades have been exploited for food, drugs, animal feed, and spirituality, but vegetable has been less exploited for economic benefits in SSA countries (Abukutsa-Onyango, 2007; Ontita *et al.*, 2017). As a source of medicine, ANS is used to cure and bring relief to various diseases such as malaria and stomach complications. Some consume ANS to recover the lost or poor appetite due to illness (Ontita *et al*., 2017; Shackleton *et al*., 2009). Further, it was reported to clean blood, cure stomach complications, and low blood pressure, improve CD4 for immune-compromised people, fatigue, boils, and improve men's libido (Ontita *et al*., 2017; Shackleton *et al*., 2009). Besides, it cures hemorrhage, wounds, itching in pubic parts, wounds in the anus, hernia, malaria, pressure, pneumonia, diabetes, eyes, headache, and skin disease (Abukutsa-onyango, 2015; Edmonds & Chweya, 1997; Lotter *et al*., 2014; Ontita *et al*., 2017; Sangija *et al*., 2021). Also, the roots and fruits of ANS are used for food therapeutical purposes (Ontita *et al*., 2017; Sangija *et al*., 2021).

The marketing channels for fresh, cooked, and processed ANS is via open markets (54.1%), homes (32%) and hotels (23.8%), supermarkets (19.6%), kiosks (15.0%), and farms or gardens (13.6%). About 70.2% of ANS is sold as wholesales, 14.2% retailers or local markets (52.4%), whereas only a tiny fraction is processed (1.3%). A study in Kenya reported large quantities of ANS being sold to wholesalers, besides in supermarkets, it fetches a higher price than in other places (Onyango & Imungi, 2007).

4.4 Postharvest handling of African nightshades

4.4.1 Harvesting techniques

Factors to consider during harvesting ANS mainly include; the right maturity, harvesting time, and immediate storage conditions; practically, ANS can be harvested either in the morning or evening, but morning time was preferable (67.7%) ($p < 0.001$). In Morogoro, 85.1% of farmers harvest in the morning, whereas 75% in Kilimanjaro harvest in the evening. Harvesting in the late evening or morning after dew evaporation from the leaves and before the sun becomes too hot is highly recommended (Abukutsa-Onyango, 2015).

Harvesting of ANS begins after 3-4 weeks, mentioned by 46.2%. However, the early harvest starts at 1-2 weeks after planting, reported by 38.5%, and the late harvest takes place at 5-6 weeks; the harvesting time had a significant difference ($p < 0.001$). Moreover, the first harvest usually takes place 4-5 weeks after sowing (Abukutsa-Onyango, 2007). African nightshade re-harvesting is done every week after the first harvest, as reported by 67.8% of farmers, with very few farmers harvesting after every two weeks ($p < 0.001$). The harvest termination was reported to occur after three months since planting from the nursery mentioned by 22.9% of farmers, while other farmers terminated harvest in two, four, five, and six months respectively (p = 0.001). A study by Abukutsa-Onyango, (2007) reported that harvesting could go up to 5 months, including two months of seed maturity. Furthermore, the harvest duration of ANS depends on a sufficient supply of nutrients and seasons (Abukutsa-Onyango, 2007; Nyaura *et al*., 2014).

Harvesting of ANS can be done by cutting the tender leaves, either by hand pinching 50.3% or cutting with a knife (46%). Very few farmers, 18.4%, were harvesting ANS by uprooting the whole shoot. This practice was commonly done when ANS reached maturity. Pinching is the common method for harvesting ANS and involves removing terminal buds that encourage the branching of plants. Pinching delayed flowering and increased the leaves of amaranths and cowpeas, eventually increasing the yields and influencing the plant's nutritional composition (Abukutsa-Onyango, 2007; Koile, 2018). Cutting lateral and main stems 5 - 10 cm from the tip improved the ANS yield, thus stimulating side shoots (Yang & Ojiewo, 2013).

4.4.2 Handling of African nightshades

The handling of ANS is done immediately after harvesting. Washing was done by 79.9% of farmers to remove physical contaminants such as sands and debris. Only 26.7% of farmers were using portable water. The water sources were boreholes (49.1%), rivers (44.9%), and dams (15.9%). Vegetable washing after harvest was a common practice of most farmers; cleaning, sorting, grading, trimming, and packaging are necessary practices to remove any dirt or residue particles, agents of microbial contamination (Nyaura *et al*., 2014). The use of non-potable water for washing fresh ANS after harvesting was common. This practice increases the chance of microbial contamination and accelerates the deterioration rate of vegetables. Likewise, Lotter *et al*. (2014) observed that 52% of growers did not wash the harvested nightshades.

On the other hand, various methods were utilized to cool fresh ANS, including sprinkling with water (57.3%) and placing it under the shade (34.5%) (Fig. 15). Other practices include dipping in water (8.7%) and placing outside overnight (9.4%) (Fig. 15). Moreover, setting ANS outside overnight was commonly done immediately after harvesting or when it remained unsold (Fig. 15). Additionally, the use of refrigeration and traditional cooling chambers (zero-energy cooling chambers) were reported by 27.2%, and 35.5% respectively. Notably, 19.1% of farmers did nothing to cool the ANS. Since ANS is highly perishable, the chance of spoilage increases rapidly with storage time, incredibly when poorly handled (Onyango & Imungi, 2007).

Therefore, cooling is essential to remove field heat within a short period. According to Gogo *et al*. (2016), covering ANS with banana leaves with water sprinkling can keep the ANS fresh. The standard methods include placing the vegetables under shade, sprinkling with water, and dipping in water (Gogo *et al*., 2016). Besides, a zero-energy cooling chamber can preserve nightshades for 3–7 days or more (Gogo *et al*., 2016). Therefore, postharvest handling of ANS can extend shelf life, ensure a constant supply, reduce losses, and lead to nutritious and safe food for consumers.

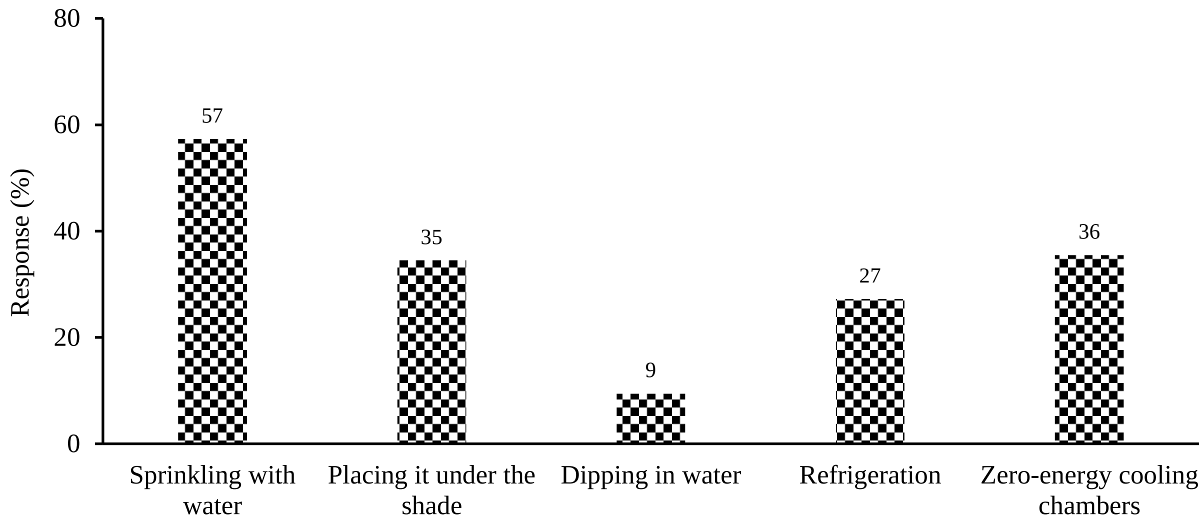

Figure 15: Methods for cooling fresh ANS after harvesting

4.4.3 Transportation of African nightshades

Transportation of ANS was done from the farms to the marketplace. Common means of transportation were carrying on the head (50.4%), use of motorcycles (44.9%), bicycles (32.3%), carts (10%), and trucks (16.0%). Different carrying containers were used although, bamboo woven containers (*Matenga*) were highly preferred 34% ($p < 0.05$) (Fig. 16). Other containers include polyethylene bags, plastic sacks or nylon, sisal sacks, and wooden crates (Fig. 16).

Plate 10: Inappropriate handling and transportation of ANS (Sangija *et al*., 2022)

Matenga is woven bamboo crates perforated and prevents mechanical damage; thus, it can keep ANS fresh for some time (Plate 10). Using plastic gunny bags to transport fresh can increase damage since fresh vegetables are fragile and brittle (Plate 10). Mechanical damage to vegetables causes leafy tearing and crushing, midrib breakage, head cracking, or bruising. It increases the oxidation of phenolic compounds and increases susceptibility to decay. Also, damaged leaves allow water loss by 3-4 times of undamaged vegetables (Acedo, 2010). Using non-perforated polythene or nylon-reinforced increases wilting, and metabolic activities and favors microbial spoilage (Vigneaultl *et al*., 2009; Gogo *et al*., 2016; Lotter *et al*., 2014). Proper handling of vegetables during transportation can minimize postharvest losses. Therefore, vegetables should be kept at the optimum temperature and humidity to reduce respiration and transpiration (Sangija *et al*., 2021).

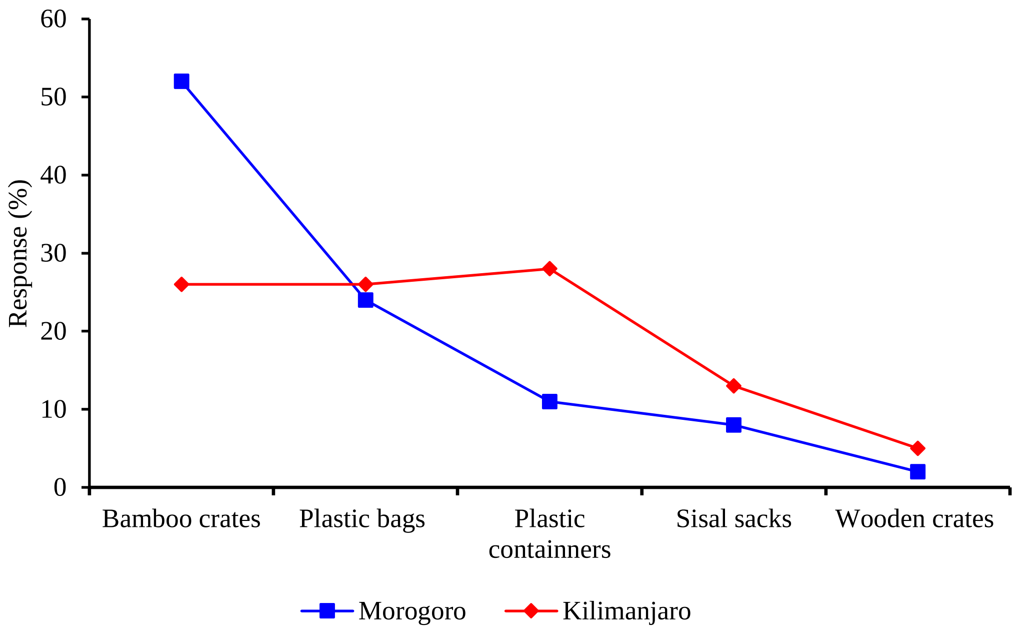

Figure 16: Containers used to handle fresh African nightshades: Different letters for an attribute indicate a significant difference at p < 0.05

4.5 Processing and preservation of African nightshades

Approximately 60.9% of farmers process (frying and boiling) ANS, though only 26.2% use potable water in cooking and other processing activities. The common sources of water for cooking ANS were government tap water 34.6%, river water 30.1% ($p = 0.772$), pond water 29.3% ($p = 0.68$), rainwater 26.4% ($p = 0.001$) and well water 17.0% ($p = 0.001$). The source of energy for cooking ANS was firewood (88.0%, $p = 0.001$), charcoal (64.9%, $p = 0.001$), maize cobs (12.8%, $p = 0.76$), kerosene (3.4%, $p = 0.36$) and gas (1.6%, $p = 0.001$). Frying (45.4%) and boiling (63.0%) were the main processing and preservation methods for ANS (Table 12), with minimal drying and fermentation. Firewood was the essential heating energy used in processing; however, it may accelerate environmental degradation (Jung & Huxham, 2019). Cooking with firewood produces various airborne toxins, resulting in poorer respiratory health (Silwal & Mckay, 2016) and imparting undesirable sensory properties to the food.

Different preparation methods for ANS were reported, whereas boiling, stir-frying with cooking oil, onion, and tomatoes, and stir-frying with cooking oil and onion were the most preferred by ouseholds. In contrast, fermentation and blanching were the least preferred (Table 12). African nightshades can be cooked in different ways, such as boiling or frying with onions and tomatoes or adding milk and cream to improve the taste (Ontita *et al*., 2017). On the other hand, ANS by-products were reported to be used as animal feeds 38.3% ($p = 0.03$), manure

35.2% (p = 0.001), disposal as waste 34.1% (p = 0.001), medicinal 10.0% (p = 0.001) and sources of biogas 1.8% (p = 0.001). The utilization of ANS by-products reduces environmental degradation and reduction of greenhouse gas (GHG) emissions, consequently preventing global warming.

Table 12: Different processing and preservation techniques of African nightshades

Practice (n=469)	Morogoro (%)	Kilimanjaro (%)	Chi-Square (χ^2)
Boiling	70.9	55.1	$\chi2$ = 61.3; df = 1; p = 0.001
Stir-frying	53.4	37.4	$\chi2$ = 2.8; df = 1; p = 0.09
Sun drying	12.3	2.7	$\chi2$ = 12.5; df = 1; p = 0.001
Solar drying	-	2.7	$\chi2$ = 5.3; df = 1; p = 0.02
Blanching	0.5	0.5	$\chi2$ = 0.01; df = 1; p = 0.97
Fermentation	-	1.1	$\chi2$ = 2.1; df = 1; p = 0.15

From the study, several factors were reported to hinder the processing and preservation of ANS. Lack of knowledge of processing (62.0%), lack of cold storage facilities or refrigeration (29.0%), and high cost of electricity (28.6%) were the main factors. Furthermore, electricity shortages and frequent shutdowns (22.4%), unavailability of enough raw materials (21.6%), and lack of sustainable markets (16.3) % were also reported. This further suggests that 41.4% of farmers stored ANS at ambient temperature, and 27.2% used refrigeration.

4.6 Post-harvest losses of African nightshades

African nightshades undergo loss through improper postharvest handling. Farmers were experiencing losses of fresh ANS by 53.6% as a result of poor post-harvest handling. Poor handling results in a qualitative and quantitative loss of vegetables, including flavor, texture, and nutritional quality (Sangija *et al.*, 2021; Wakholi *et al.*, 2015). Lack of knowledge on proper postharvest handling methods, shortage of market, poor market return from sales, and price fluctuation were the causes of ANS losses along the value chain (Fig. 11). Notably, the majority of the farmers experienced < 10% of fresh ANS losses during transportation and storage. On the other hand, very few farmers experienced losses between 20 - 30% (Fig. 17). Therefore, postharvest losses of ANS can occur at any point along the value chain. About 20 - 50% of all vegetables produced in SSA undergo loss (Global Panel, 2018; Kitinoja & Kader, 2015); similarly, 50% of postharvest losses of AIVs were reported in Kenya (Gogo *et al.*, 2016). The losses of vegetables can occur during harvesting, storage, packaging, and transportation (Acedo & Katinka, 2009), besides factors affecting postharvest losses vary from place to place (Yang & Ojiewo, 2013).

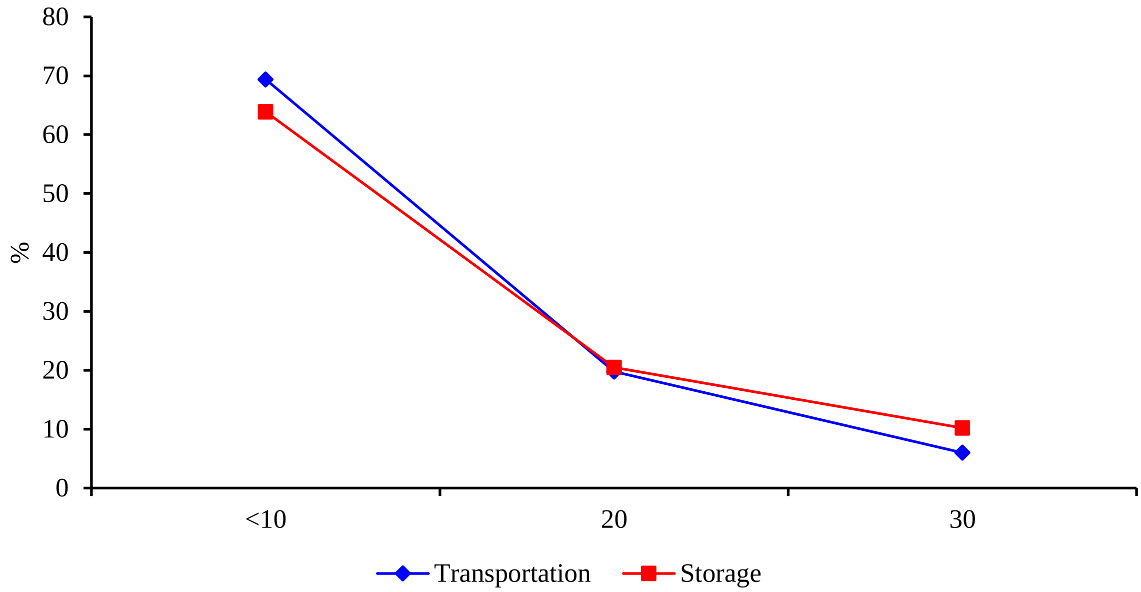

Figure 17: Postharvest loss ANS during transportation and storage (n=344)

Microorganisms are the primary causative agents of post-harvest loss of fresh vegetables if they are not well handled (Acedo, 2010). Therefore, ANS needs quick handling after harvesting, such as cooling, to preserve its quality (Sangija *et al*., 2021). The vegetables should be stored at 5-10 °C for a long storability (Abukutsa-Onyango, 2007; Gogo *et al*., 2016). Besides, modern cooling techniques, such as refrigeration, are less feasible in rural and peri-urban areas where horticultural farming is mainly practiced (Ambuko *et al*., 2017). Alternatively, a zero-energy cooling chamber should be considered in rural areas (Ambuko *et al*., 2017). The rapidly growing population and demand for nutritious foods increase the demand for AIVs in SSA; hence vegetable loss should be reduced (Habwe & Walingo, 2008).

From the study, different methods of handling ANS, such as selling immediately after harvest (61.9%), harvesting in small quantities (54.5%), market searching before harvesting (47.7%), and storage in a cold environment (22.2%), were employed to minimize spoilage of fresh ANS. Other methods include the use of proper packaging (15.6%), minimizing mechanical damage (14.9%), and drying (5.3%). Farmers were reportedly selling ANS immediately after harvest as the main alternative way to control postharvest loss. Once farmers fail to sell their day produce, they encounter loss due to limited shelf life and lack of postharvest handling techniques (Shiundu & Oniang'o, 2007). Most sellers strive to finish all fresh ANS supplied on the delivery day; whatever remains is thrown as waste (Onyango & Imungi, 2007). The chance of deterioration increases within 24 hours if ANS is not sold immediately after harvest. Some farmers/sellers sprinkle water and leave the vegetables in the open space overnight;

however, microbial contamination still hinders their effort (Gogo *et al*., 2016). In Tanzania's public markets, sellers had no access to refrigeration, accelerating post-harvest losses and reducing ANS utilization (Lotter *et al*., 2014). Postharvest handling of vegetables has led to safe and nutritious food for consumers (Sangija *et al*., 2021; Shiundu & Oniang'o, 2007). Therefore, proper post-harvest handling of fresh vegetables should be applied to minimize losses.

4.7 Effect of LAB on African nightshades

African nightshade (*S. scabrum* and *S. villosum*) at an initial pH of 7.4 was fermented spontaneously and under controlled conditions for 15 days and 120 h, respectively. A sharp drop in pH to 4.7 and pH to 3.5 was observed within 24 h for both spontaneous fermentation (SF) and controlled fermentation (CF), respectively (Figs. 18 and 19). Further, slight pH drops in SvCFP and SsCFP was observed in a similar trend. A sharp pH drop indicates the ability of acidification by LAB during fermentation (Stoll *et al*., 2021). LAB produces various organic acids, with lactic acid being predominant, hence the preservation effect (Degrain *et al*., 2020). A combination of *L. plantarum* LP90 and *L. mesenteroides* LM58 in a 3% brine solution resulted in a sharp pH drop. Therefore, a faster, more profound, stable, and more controlled fermentation can be achieved using starter cultures (Stoll *et al*., (2021). Similar studies on the use of commercial starter cultures such as *Lactiplantibacillus plantarum* BFE 5092, *Lomisilactobacillus fermentum BFE* 6620, *Lactobacillus plantarum* (17a), *Weissella cibaria* (21), *Leuconostoc pseudomesenteroides* (56), *W. cibaria* (64), or *L. plantarum* (75), in ANS fermentation had been reported (Stoll *et al*., 2021; Wafula, 2017; Degrain *et al*., 2020). Heterofermenters, mainly *Leuconostoc mesenteroides* and *L. brevis,* can initiate the fermentation process, while *L. plantarum* occurs later (Belitz *et al*., 2009; Hutkins, 2006).

On the other hand, a steady pH drop was observed in SF, resulting in a prolonged fermentation time with a pH of 3.5 attained in 72 h for SvSFP and 192 h for SsSFP, respectively (Fig. 18). Naturally occurring bacteria grow over 1 - 2 weeks to produce lactic acid, whereas added salt controls the type and rate of the fermentation (Behera *et al*., 2020). In this study, a brine solution (4% salt & 2%-sugar) was added; as a result, the optimal pH of 3.5 in SF was attained within 3 - 8 days (Fig. 18). Similarly, a sharp increase in titratable acidity (TTA) to 0.4 within 24 h was observed in CF as opposed to SF (Fig. 18). Wafula (2017) reported an increase in TTA due to ANS fermentation. Lactic acid production prevents the growth of food-poisoning bacteria and other spoilage microorganisms, thus enhancing product safety (Behera *et al*., 2020) and sensory quality.

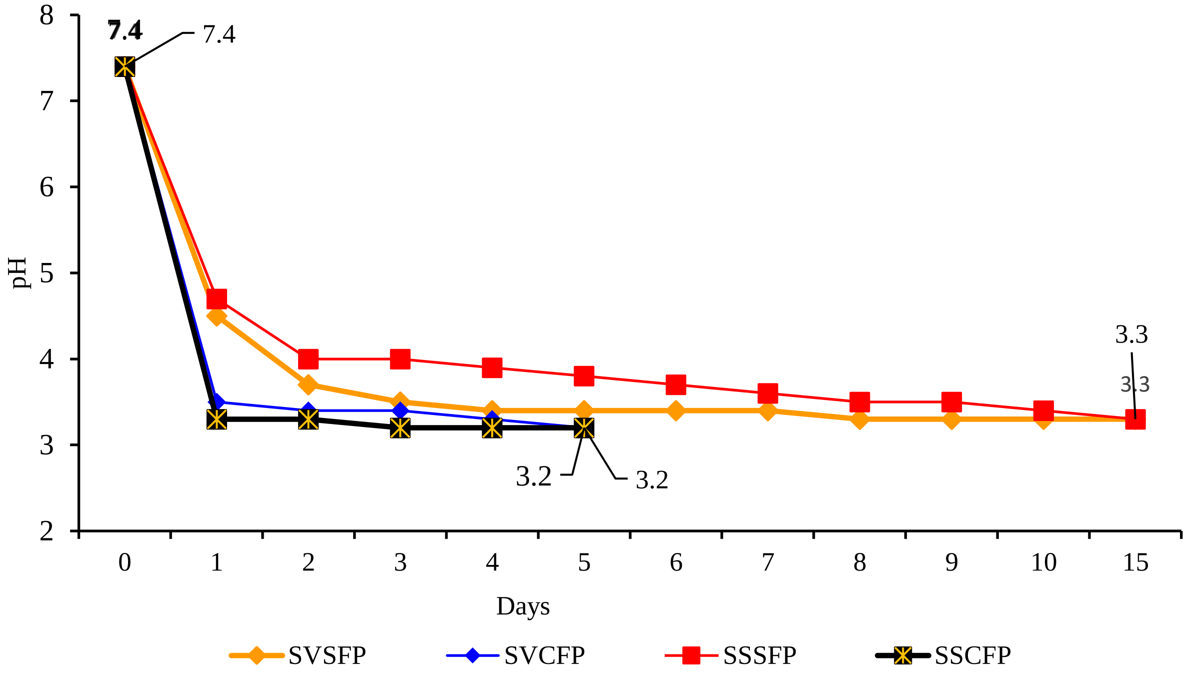

Figure 18: Changes in pH during spontaneous and controlled fermentation of African nightshade. SvSFP: *S. villosum* Spontaneous Fermented Pickle; SvCFP: *S. villosum* Controlled Fermented Pickle; SsSFP: *S. Scabrum* Spontaneous Fermented Pickle; SsCFP: *S. scabrum* Controlled Fermented Pickle. Means ± SD, (n=3) with different superscript letters represents a significant difference at $p<0.05$.

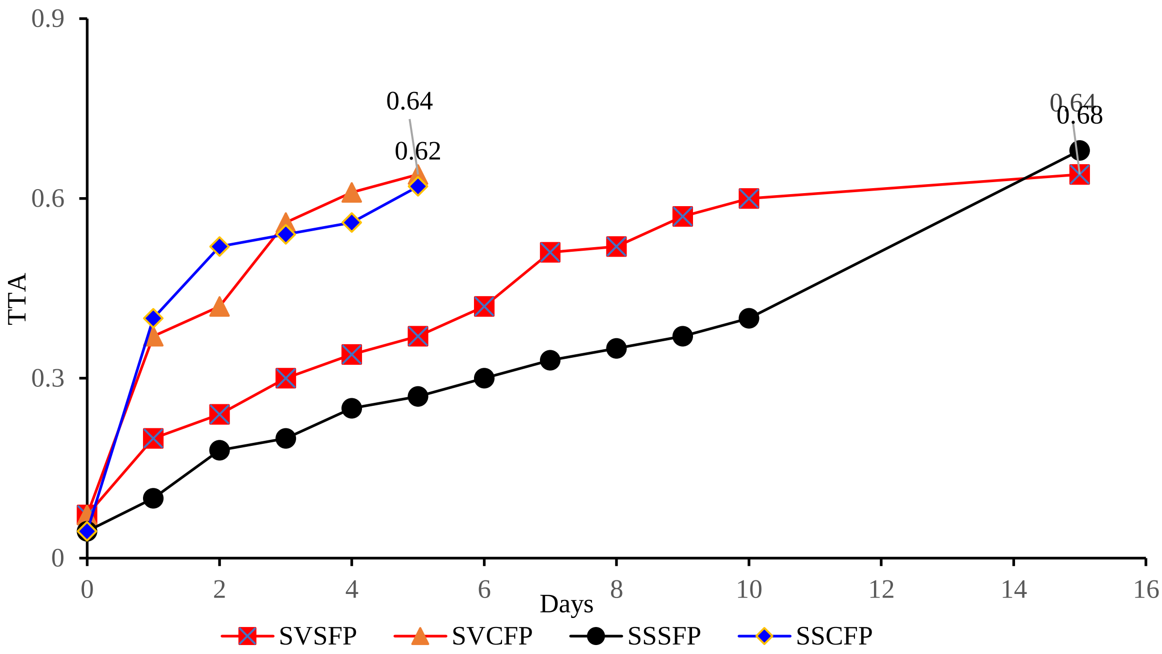

Figure 19: Changes in titratable acidity during spontaneous and controlled fermentation of African nightshade. SvSFP: *S. villosum* Spontaneous Fermented Pickle; SvCFP: *S. villosum* Controlled Fermented Pickle; SsSFP: *S. Scabrum* Spontaneous Fermented Pickle; SsCFP: *S. scabrum* Controlled Fermented Pickle. Means ± SD, (n=3) with different superscript letters represents a significant difference at p<0.05

4.8 Effect of fermentation on the nutritional quality

4.8.1 Beta-carotene content

The results on β-carotene content in LAF are presented in Fig. 20. Lactic acid fermentation significantly increased β-carotene content in pickle and relish products. The β-carotene content in fresh *S. scabrum* and *S. villosum* was 48.7 mg/100g and 31.1 mg/100 g, respectively. A 2.6 – 5 folds significant increase in β-carotene in pickles and 1.6 – 4.8 folds in relishes were reported (Fig. 20). For pickles, *S. villosum* exhibited significantly higher ($p < 0.05$) β-carotene values than *S. scabrum* as opposed to their relish products. Likewise, for relish products, *S. villosum* had significantly high β-carotene, unlike *S. scabrum* (Fig. 20). Fermentation retained a substantial amount of β-carotene (Wafula, 2017). *Lactobacillus gasseri* from carrot juice increased β-carotene (Xu *et al.*, 2020). Chinese cabbage showed increased β-carotene after four days of spontaneous fermentation (Chavasit *et al.*, 2002), similar to the study findings. The high increase was due to the extraction of carotene from the stable lipoprotein complexes during fermentation. Similarly, Bartkiene *et al*. (2015) reported a 43.9% average β-carotene increase in LA fermented tomato powder compared with the non-fermented samples. The carbon source is one of the significant factors that influence the production of carotenoids in the complex enzymatic system used by microorganisms (bacteria, yeast, and molds). Carotenogenesis can be affected by temperature, pH, reactive oxygen species, and catalyzing oxidative reaction UV light, and transition metals (Mapelli-Brahm *et al*., 2020). Fermentation disrupts plant matrix and cell cluster resulting in carotenoid liberation and bioaccessibility but can undergo oxidation or degradation. Furthermore, some LAB strains possess enzyme activity that favors carotenoid extraction (Mapelli-Brahm *et al*., 2020). Also, oxidative stress has been reported to induce the production of β-carotene, γ-carotene, and lycopene in *B. trispora*. Therefore, LAF increased β-carotene content from 30 to 40% in cabbage (Mapelli-Brahm *et al*., 2020).

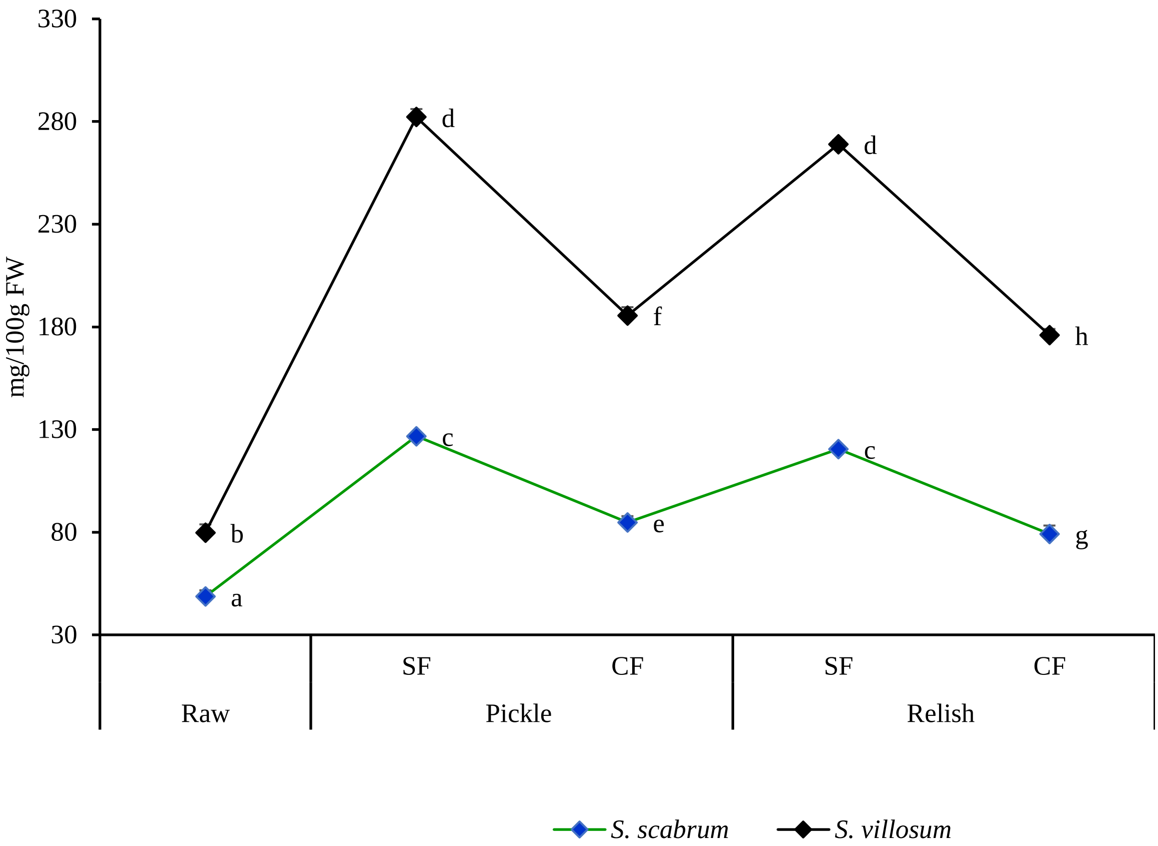

Figure 20: β-carotene content of fermented African nightshade pickle and relish products; Pickles (SsSFP, SvSFP, SsCFP & SvCFP) and Relish (SsSFR, SvSFP, SsCFR & SvCFR). Whereas; Ss: *S. scabrum*; Sv: *S. villosum*; CF: Controlled fermentation; SF: Spontaneous fermentation. Means ± SD, (n=3) with different superscript letters represents a significant difference at p<0.05

In relish making, heat treatments and powdered spices used as ingredients may have also contributed to the reduction of β-carotene. Likewise, cooking sweet potato leaves for 5 min and cilantro above 10 min decreased β-carotene (Kao *et al*., 2014). The β-carotene level reported in this study is lower than the decrease of β-carotene contents by 24.2% of cooked, fermented tomatoes (Bartkiene *et al*., 2015).

A recent human study showed that the alcoholic fermentation of orange juice had increased the bioavailability of carotenoids (Hornero-Méndez *et al*., 2018). The bioavailability of carotenoids is dependent on the carotenoid itself (polarity, conjugated double bonds), the food matrices (treatments undergone, presence or co-ingestion of fat, structural characteristics with particular relevance of the type of predominant chromoplast, association of carotenoids with proteins), and the individual (including diseases, dietary patterns, lifestyle habits, gender, age, microbiota, and genetic factors) (Mapelli-Brahm *et al*., 2020).

4.8.2 Vitamin C content

On the other hand, LAF significantly decreased vitamin C content in pickle and relish products (Fig. 21). The results showed substantial vitamin C loss from fermented pickles (88.33 – 95%) and relish products (91.93% - 95.90%). Wafula (2017) reported vitamin C reduction by 81% and 66% in ANS, spontaneously and controlled fermentation. In this study, vitamin C losses in SF account for 91.66 – 95% and 95.49 – 95.90% in pickles and relish products, respectively. In addition, vitamin C losses in CF were similar to 88.33 – 89.02% for pickles and 91.93 – 92.06% for relish products, respectively. Vitamin C loss can be enhanced by extended storage, washing of vegetables, higher temperatures, low relative humidity, physical damage, and chilling injury (Lee & Kader, 2000; Wafula, 2017). Ascorbic acid (AA) is easily oxidized in aqueous solutions, favored by the presence of heavy metal ions (Cu^{2+}, Ag^{+}, and Fe^{3+}), oxygen, alkaline pH, and high temperature (Lee & Kader, 2000). Oxidized AA, dihydro ascorbic acid can be reduced to AA and irreversibly oxidized to form diketogulonic acid, with no vitamin C activity (Lee & Kader, 2000). Blanching prevents AA oxidase, phenolase, cytochrome oxidase, and peroxidase action, indirectly responsible for AA loss (Lee & Kader, 2000). However, blanching has been reported to decrease vitamin C content by 28 – 82.4% due to dissolution and oxidation (Lee & Kader, 2000; Oboh, 2005). Furthermore, cooking has been reported to reduce vitamin C content by 30%, and maintaining potatoes hot for one hour further decreased vitamin C by 10% (Hägg *et al*., 1998; Lee & Kader, 2000). During fermentation, sucrose is converted to fructose and glucose, and carbonyl groups of fructose react with vitamin C, reducing vitamin C content (Lee & Kader, 2000). Filannino *et al*. (2016) reported a reduction of vitamin C content after fermentation with *L. plantarum* and *L. brevis.* The remained amount of vitamin C after fermentation is less to meet recommended daily allowance of 100 – 200 mg (Lee & Kader, 2000). Likewise, the vitamin C content in pickle and relish products was very low to meet the recommended dietary intake of 73-90 mg/day (Table 4). Therefore, dietary diversification or fortification of the relishes is recommended.

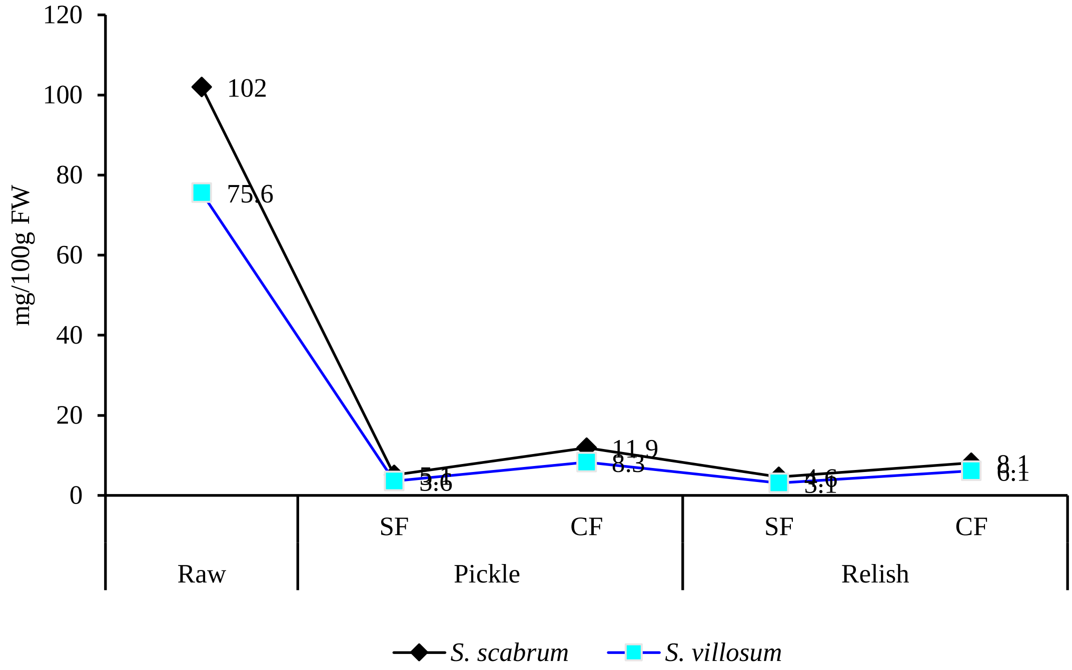

Figure 21: Vitamin C content of fermented African nightshade pickle and relish products: Pickles (SsSFP, SvSFP, SsCFP & SvCFP) and relish (SsSFR, SvSFR, SsCFR & SvCFR). Whereas, Ss: *Solanum scabrum*; Sv: *Solanum villosum*; CF: Controlled fermentation; SF: spontaneous fermentation. Means ± SD, (n=3) with different superscript letters represents a significant difference at $p<0.05$

4.8.3 Mineral Content

Fermentation significantly increased the mineral content of the *S. scabrum* and *S. villosum* pickles and relish products compared to fresh leaves (Table 13). Sivakumar *et al*. (2020) reported Ca (442 mg/100g DW) and Fe (12 mg/100 g DW) in fresh *S. villosum* lower than the values observed in this study. Lactic acid fermentation improved the mineral content in pickles and relishes. Iron, Zn, Ca, S, P, Cu, and Ni were improved between 0.58 and 2.01 folds, respectively. Fermentation of amaranth leaves, pumpkin leaves, and capwood leaves showed increases in Ca, Mg, Zn, Fe, Se, and Cu (Ifesan *et al*., 2014). LAB improves nutritional quality and micronutrient bioavailability in food through antinutrient degradation by microbial enzymes. Therefore, LAF can be considered a strategy to enhance food's nutritional and functional quality by activating endogenous enzymes (Nkhata *et al*., 2018; Rollán *et al*., 2019) due to low pH. Also, LAB can produce different enzymes responsible for the hydrolysis of the food matrix into desirable nutritional and sensory quality. Despite sparsely available information, this study provides finding for the mineral content of fermented *S. villosum* and *S. scabrum* pickles and relishes.

Fermentation reduces antinutrients such as oxalate, phytate, and tannins, which chelates minerals and hinder their bioavailability (Samtiya *et al*., 2021). Fermentation produces enzymes phytase and tannase that degrades the phytate and tannins and improve the availability of calcium, iron, and zinc (Samtiya *et al*., 2021).

Table 13: Mineral content (mg/100g DW) of fermented African nightshade pickle and relish products

Relish products	P	S	Ca	Fe	Zn	Cu	Ni
SsR	1166.2±15.0^{a}	444.3±4.2^{a}	3392.6±41.3^{a}	185.5±3.3.0^{a}	8.11±0.23^{a}	2.24±0.04^{a}	0.29±0.01^{a}
SsSFP	1339.5±14.1^{b}	527.6±9.7^{b}	4259.3±271.7^{b}	230.6±2.7^{b}	15.4±0.8^{b}	4.50±0.16^{b}	0.38±0.01^{b}
SsSFR	1372.9±3.6^{c}	530.9±26^{b}	4692.6±62.7^{c}	268.0±5.6^{c}	21.4±2.2^{c}	5.3±0.15^{c}	0.49±0.01^{c}
SsCFP	1222.9±12.6^{d}	474.3±14^{c}	3792.6±41.3^{d}	198.0±5.4^{d}	12.4±0.9^{d}	3.47±0.09^{d}	0.32±0.02^{d}
SsCFR	1303.7±6.8^{e}	496.9±8.5^{d}	3984.0±101.7^{e}	217.6±2.0^{e}	17.4±1.3^{e}	3.94±0.07^{e}	0.37±0.01^{be}
SvR	1086.5±9.5^{f}	596.6±4.97^{e}	3113.7±30.5^{f}	142.7±1.5^{f}	5.53±0.11^{f}	1.95±0.11^{f}	0.26±0.01^{f}
SvSFP	1173.9±5.6^{a}	693.3±1.8^{f}	3447.1±34.3^{ag}	176.0±6.2^{g}	8.2±0.7^{a}	2.69±0.3^{g}	0.28±0.24^{a}
SvSFR	1205.5±4.9^{g}	736.6±17.2^{g}	3580.4±35.6^{g}	198.3±6.5^{d}	10.2±0.7^{g}	2.95±0.06^{h}	0.39±0.01^{be}
SvCFP	1102.6±4.1^{f}	660.0±6.7^{h}	3247.1±34.2^{af}	160.4±1.7^{h}	6.9±0.7^{af}	2.32±0.15^{a}	0.28±0.01^{af}
SvCFR	1131.6±10^{h}	696.6±5.0^{f}	3413.7±87.8^{ag}	179.3±9.3^{ag}	8.5±0.9^{ag}	2.79±0.1^{gh}	0.33±0.01^{d}

Mean value (n=3) ± SD; Means with a different superscript letter within a column are significantly different $p < 0.05$. SsR: *S. scabrum* raw*;* SsSFP: *S. scabrum* Spontaneous Fermented Pickle; SsCFP: *S. scabrum* Controlled Fermented Pickle; SvR: *S. villosum* raw; SvSFP: *S. villosum* Spontaneous Fermented Pickle; SvCFP: *S. villosum* Controlled Fermented Pickle. The results were expressed in mg/100g DW/FW.

4.9 Effect of fermentation on chlorophyll and polyphenols

Fermentation significantly reduced Chlorophyll *a* content ($p < 0.05$) in SsSFP and Chlorophyll *b* in SsSFP, SsCFP, SvSFP, and SvCFP respectively (Table 14). Also, fermentation reduced total Chlorophyll in all pickle products (Table 14). During fermentation, acetic and lactic acid production degraded Chlorophyll *a* and *b* (Degrain *et al*., 2020). Changes in pH during fermentation can produce new compounds such as Mg-free Chlorophyll derivatives, a carotenoid with 5,8-epoxide groups, brown pigments (*0*-quinones), or chemical oxidation of phenolic compounds; these compounds could have masked the original green color of the leaves and reduced Chlorophyll content (Ramírez *et al*., 2015).

Table 14: Chlorophyll content of fermented African nightshade pickle and relish products

Pickle (gKg^{-1}) FW	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll
SsR	29.80 ± 0.2[a]	27.95 ± 1.6[a]	57.75 ± 1.9[a2]
SsSFP	27.96 ± 0.8[a]	14.68 ± 0.8[c]	42.64 ± 1.6[c]
SsCFP	28.68 ± 2.3[a]	19.57 ± 5.5[b]	48.25 ± 3.3[d]
SsR	28.99 ± 0.2[a]	23.44 ± 0.6[ab]	52.43 ± 0.6[b]
SvSFP	22.13 ± 1.2[b]	9.71 ± 1.6[cd]	31.85 ± 2.7[f]
SvCFP	29.04 ± 1.1[a]	13.44 ± 1.8[c]	42.48 ± 2.1[ce]

SsR: *S. scabrum* raw*;* SsSFP: *S. scabrum* Spontaneous Fermented Pickle; SsCFP: *S. scabrum* Controlled Fermented Pickle; SvR: *S. villosum* raw; SvSFP: *S. villosum* Spontaneous Fermented Pickle; SsCFP: *S. villosum* Control Fermented Pickle. Means ± SD, (n=3) with a different superscript letter within a column are significantly different $p<0.05$.

On the other hand, total polyphenol content in Ss and Sv was reduced due to LAF (Fig. 22). Fermentation reduced polyphenol content by 61.29 – 70.11% in pickles and 45.98 – 53.51% in relish products, respectively (Fig. 22). Regardless of fermentation time, both natural and commercial LAB contributed to reducing total polyphenol content. However, cooking pickles to relish further reduced polyphenol content in all relish products (Fig. 22). In contrast, Degrain *et al*. (2020) reported an increase in total polyphenolic compounds in the fermented nightshade, from 6007.8 mg/kg (raw leaves) to 8016.8 – 8638.0 mg/kg fermented leaves with LAB strains of *Lactobacillus plantarum* (17a), *W. cibaria* (64) or *L. plantarum* (75). Furthermore, after fermentation, a reduction of total polyphenol content to 3822.5 – 5681.5 mg/kg L. *pseudomesenteroides* (56) and *Weissella cibaria* (21). The decrease in total phenolic compounds in fermented nightshade leaves results from detoxification and utilization of phenolic acids as a carbon source (Filannino *et al*., 2016). The total polyphenol content in ANS fermented with LAB could have been reduced with the decarboxylation of ferulic and caffeic acids (phenolic acid decarboxylase) to other compounds, such as 4-vinyl phenol, 4-vinyl guaiacol, or 4-vinyl catechol. Also, it could have been reduced by the action of phenolic acid reductase to hydroxyphenylpropionic acids, such as dihydrocaffeic and dihydroferulic acids could have affected (Degrain *et al*., 2020; Devi & Anu-Appaiah, 2018). Similarly, *L. plantarum* decarboxylated most phenolic acids apart from gallic acid (Degrain *et al*., 2020). Additionally, *L. plantarum* metabolized caffeic, ferulic, p-coumaric, and m–coumaric out, which reduces total polyphenol (Degrain *et al*., 2020).

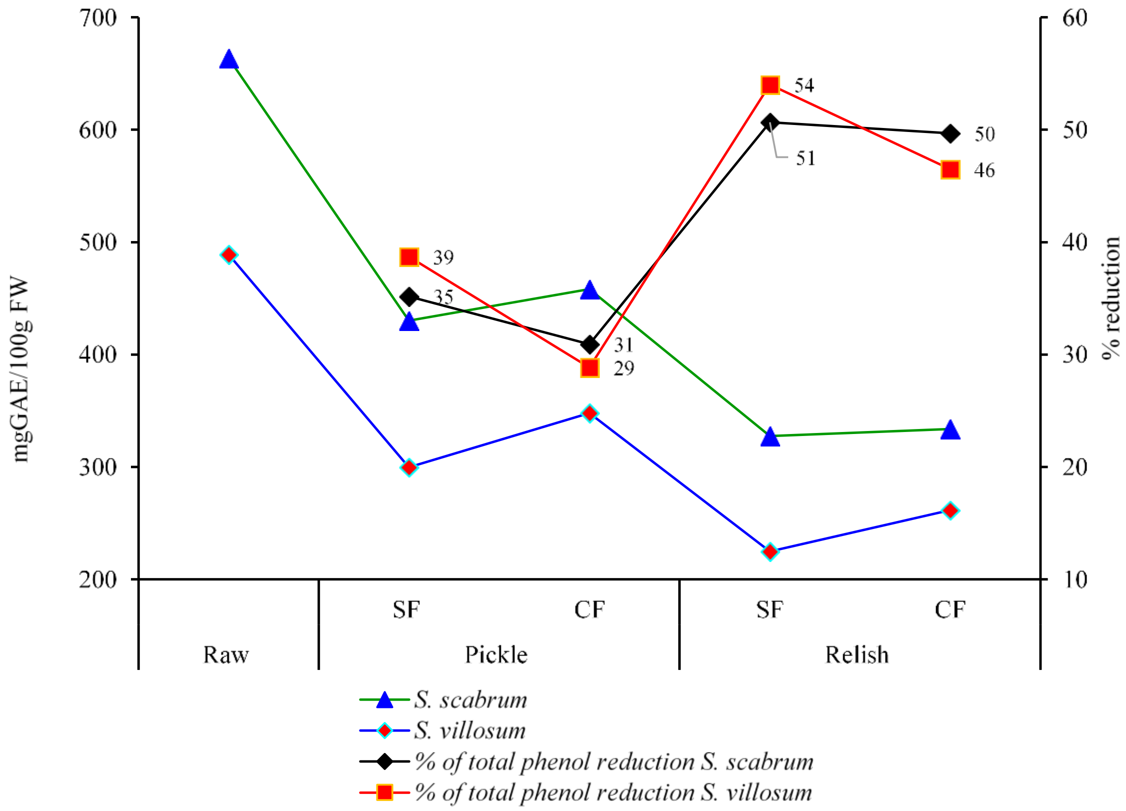

Figure 22: Total polyphenol content of fermented African nightshade pickle and relish products: Pickles (SsSFP, SvSFP, SsCFP & SvCFP) and relish products (SsSFR, SvSFR, SsCFR & SvCFR). Whereas, Ss: *Solanum scabrum*; Sv: *Solanum villosum*; CF: Controlled Fermentation; SF: Spontaneous Fermentation. Means ± SD, (n=3) with different superscript letters represents a significant difference at p<0.05

4.10 Effect of fermentation on antinutritional factors

Both raw *S. villosum* and *S. scabrum* exhibited significantly higher tannin content ($p < 0.05$) compared to fermented pickle and relish products (Fig. 23). Lactic acid fermentation substantially reduced tannin content by 76.27 – 86.23% for pickles (Fig. 23). Meanwhile, relish-making further reduced ($p < 0.05$) tannin content by 87.21 – 92.88% (Fig. 23). The tannase and gallate decarboxylase enzymes produced during LAB fermentation results in bioconversion of gallotanins and ellagitannins increase in phenol (gallic and ellagic acid), which reduce tannin contents (Degrain *et al*., 2020).

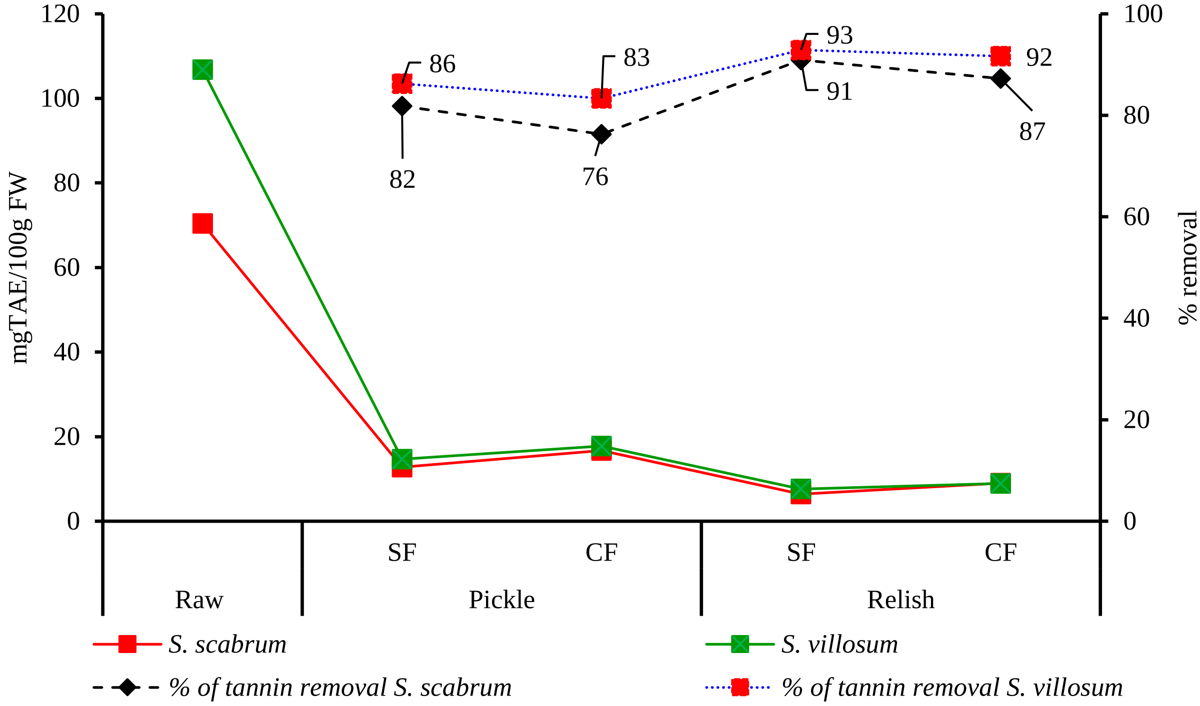

Figure 23: Tannin content in fermented African nightshade pickle and relish products: Pickles (SsSFP, SvSFP, SsCFP & SvCFP) and relish products (SsSFR, SvSFR, SsCFR & SvCFR). Whereas TAE: tannic acid equivalent; *Solanum scabrum*; Sv: *Solanum villosum*; CF: Controlled Fermentation; SF: Spontaneous Fermentation. Means ± SD, (n=3) with different superscript letters represents a significant difference at p<0.05

Similarly, the oxalate content in raw Sv and Ss was significantly high ($p < 0.05$) than in their fermented pickles and relishes (Fig. 24). Lactic acid fermentation substantially reduced oxalate levels by 77.33 – 79.75% in pickles and 89 - 90% in relishes, with an insignificant reduction ($p > 0.05$) of relish products. The oxalate content in raw ANS was 100.9 mg/100 g FW (Mwanri *et al*., 2018). This quantity is high than the oxalate reported in this study. The fatal doses of oxalate range from 1– 30 g per person/day. During fermentation, when pH decreases below 6 contributes to the reduction of deprotonated divalent oxalate ($C_2O_4{}^{2-}$) ion power to bind with divalent minerals such as Ca^{2+} ion to form insoluble oxalates (Simpson *et al*., 2009: Wadamori *et al*., 2014).

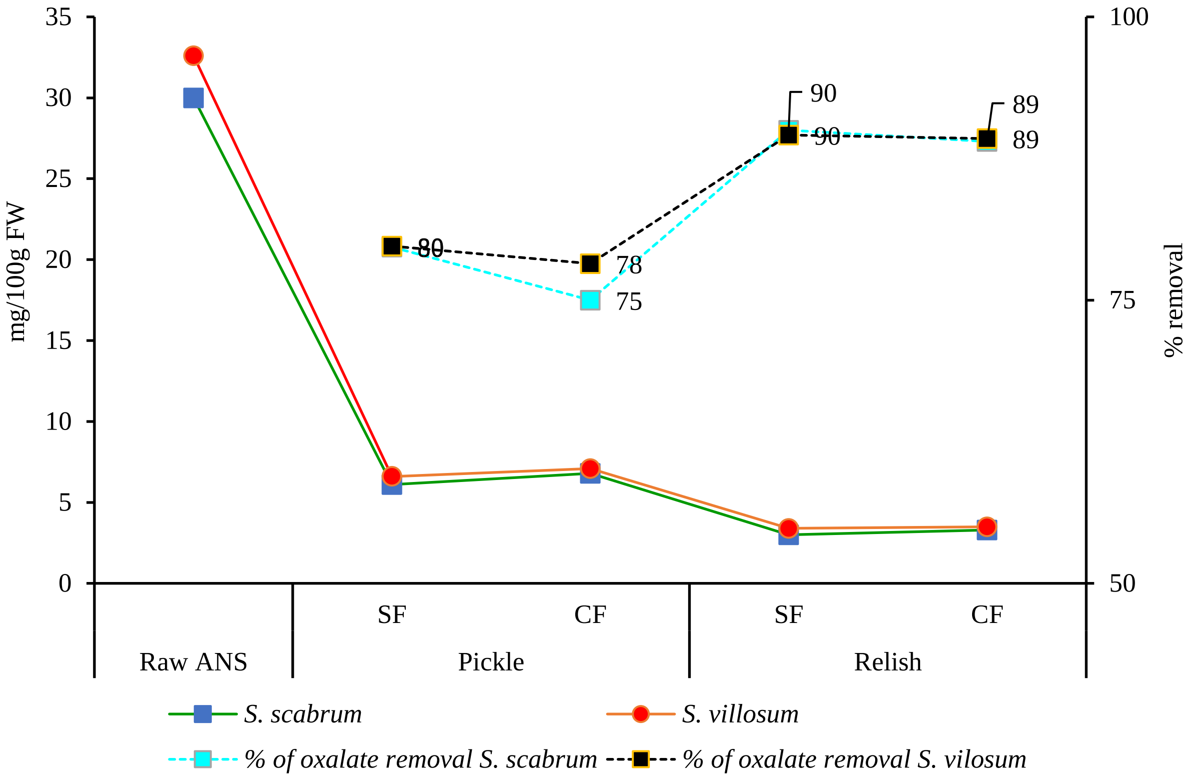

Figure 24: Oxalate content in fermented African nightshade pickle and relish products: Pickles (SsSFP, SvSFP, SsCFP & SvCFP) and relish products (SsSFR, SvSFR, SsCFR & SvCFR). Whereas; *S. scabrum*; Sv: *S. villosum*; CF: Controlled Fermentation; SF: Spontaneous Fermentation. Means ± SD, (n=3) with different superscripts represents a significant different p<0.05

4.11 Consumer acceptability of fermented relish

The majority of the consumers preferred SsCFR with a mean score of 7.96; interestingly, both SvCFR, SsSFR, and SvSFR were equally preferred (Table 15). According to Wafula (2017), 80% of the panelists showed a preference for fermented ANS. Odor and flavor were the attributes preferred mainly by the majority of the consumers, with a mean score of 7.81, while color was the least preferred. Degrain *et al*. (2020) reported that LAF influenced the color of *Solanum retroflexum* leaves through the degradation of Chlorophyll *a* and *b*. Further, low pH is associated with color destruction in fermented vegetables due to the production of acids. This is concurrent with the findings reported in Table 15. In a study by Wafula (2017), a color mean score of 7.9 for fermented ANS was reported, similar to the current findings. In addition, other parameters such as texture, appearance, taste, saltiness, sourness, bitterness, and spiciness were also liked by the consumers (Table 15). Likewise, LAF improved sensory attributes of color, taste, smell, appearance, and general acceptability (Wafula, 2017).

Amaranth and spinach are vegetables preferred to be cooked with relish, while potato leaves were least preferred (Fig. 25). Also, Schippers (2002), Yang and Ojiewo (2013) and Abukutsa-Onyango (2007) supported this finding. African nightshade is reported to be cooked with meat and fish in Senegal (Keller, 2004; Shackleton *et al*., 2009; Chagomoka *et al*., 2014), (Plate 2). Also, a large number of consumers preferred to pay US$ 0.21 for all relish (SsCFR, SvCFR, SsSFR, and SvSFR), and few preferred to pay above US$ 0.42 (Fig. 26). Consumers highly preferred to consume relish three times and two times a week (Fig. 27). Above 95% of consumers preferred the PET packaging and the label of the relish (Fig. 28). In Kenya ANS is sold in supermarke (Onyango & Imungi, 2007).

Table 15: Consumer's acceptability test scores of fermented African nightshade relish products

Formulation		Color	Texture	Appearance	Taste	Odor	Flavor	Saltiness	Sourness	Bitterness	Spiciness	Overall acceptability
SsCFR	Min	1	2	2	2	2	2	2	2	2	2	2
	Max	9	9	9	9	9	9	9	9	9	9	9
	Mean	7.84^{a}	7.86^{a}	7.88^{a}	7.90^{a}	7.90^{a}	7.90^{a}	7.90^{a}	7.94^{a}	7.81^{a}	7.94^{a}	7.96^{a}
	SD	0.996	0.84	0.84	0.83	0.796	0.78	0.91	0.87	0.97	0.81	0.783
SvCFR	Min	1	1	1	1	1	1	1	1	1	2	1
	Max	9	9	9	9	9	9	9	9	9	9	9
	Mean	7.59^{b}	7.69^{b}	7.71^{b}	7.73^{b}	7.76^{b}	7.76^{b}	7.67^{b}	7.66^{b}	7.60^{a}	7.76^{b}	7.79^{b}
	SD	1.37	1.37	1.04	1.05	0.977	1.00	1.10	1.23	1.32	1.00	0.981
SsSFR	Min	2	2	2	2	2	2	2	2	2	2	2
	Max	9	9	9	9	9	9	9	9	9	9	9
	Mean	7.75^{ab}	7.79^{ab}	7.80^{ab}	7.80^{ab}	7.80^{ab}	7.81^{ab}	7.73^{bc}	7.71^{bc}	7.77^{a}	7.81^{ab}	7.82^{b}
	SD	1.03	0.93	0.88	0.94	0.914	0.91	1.07	1.1	0.86	0.91	0.931
SvSFR	Min	1	2	2	2	2	2	1	1	1	2	2
	Max	9	9	9	9	9	9	9	9	9	9	9
	Mean	7.71^{ab}	7.77^{ab}	7.78^{ab}	7.76^{cb}	7.78^{ab}	7.78^{ab}	7.73^{bd}	7.67^{bd}	7.73^{a}	7.78^{b}	7.81^{b}
		1.22	0.95	0.97	1.05	0.94	0.96	1.07	1.25	1.06	0.96	0.95
Overall acceptability of each parameter	Mean	7.72	7.78	7.79	7.80	7.81	7.81	7.76	7.75	7.73	7.82	
	SD	1.15	1.02	0.93	0.97	0.91	0.91	1.04	1.11	1.05	0.92	

Means with different superscript letters within a column are significantly different $p < 0.05$, n=370). 1 = dislike extremely, 5 = Neither like nor dislike, 9 = like extremely; SsCFR: *S. scabrum* Controlled Fermented Relish; SvCFR: *S. villosum* Controlled Fermented Relish; SsSFR: *S. scabrum* Spontaneous Fermented relish; SvSFR: *S. villosum* Spontaneous Fermented Relish.

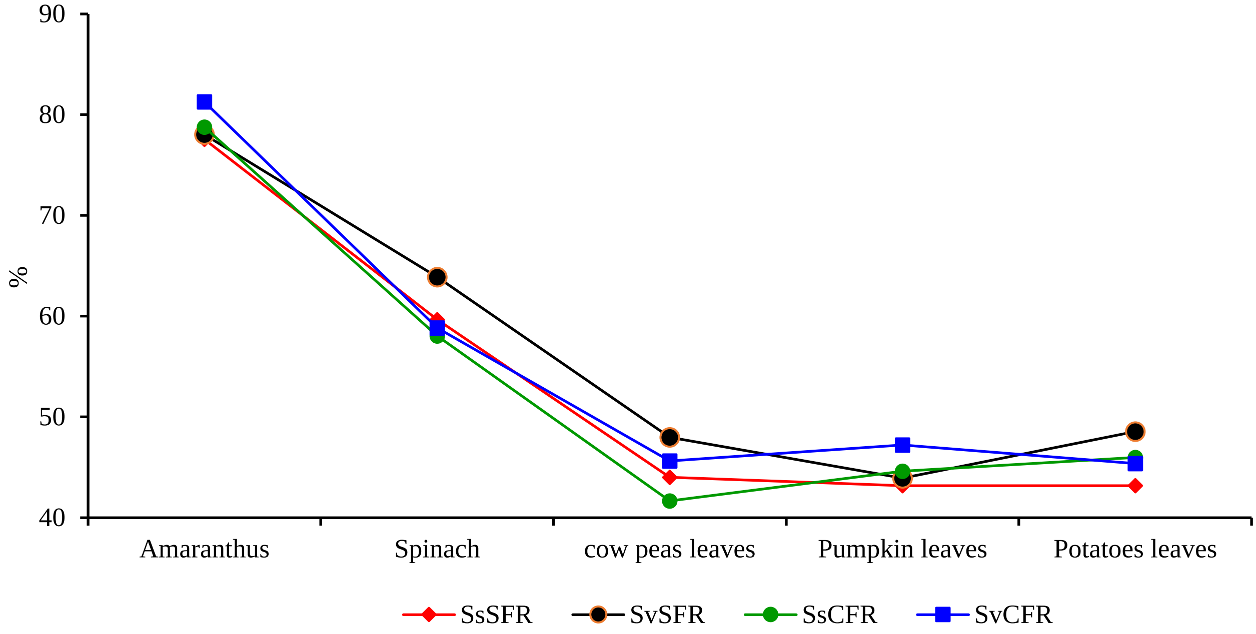

Figure 25: Cooking of relish with other vegetables: Whereas; SsSFR: *S. scabrum* spontaneous fermented relish; SvSFR: *S. villosum spontaneous relish:* SsCFR: *S. scabrum* controlled fermented relish; SvCFR: *S. villosum* controlled fermented relish. Means ± SD, (n=3) with different superscripts represents a significant different p<0.05

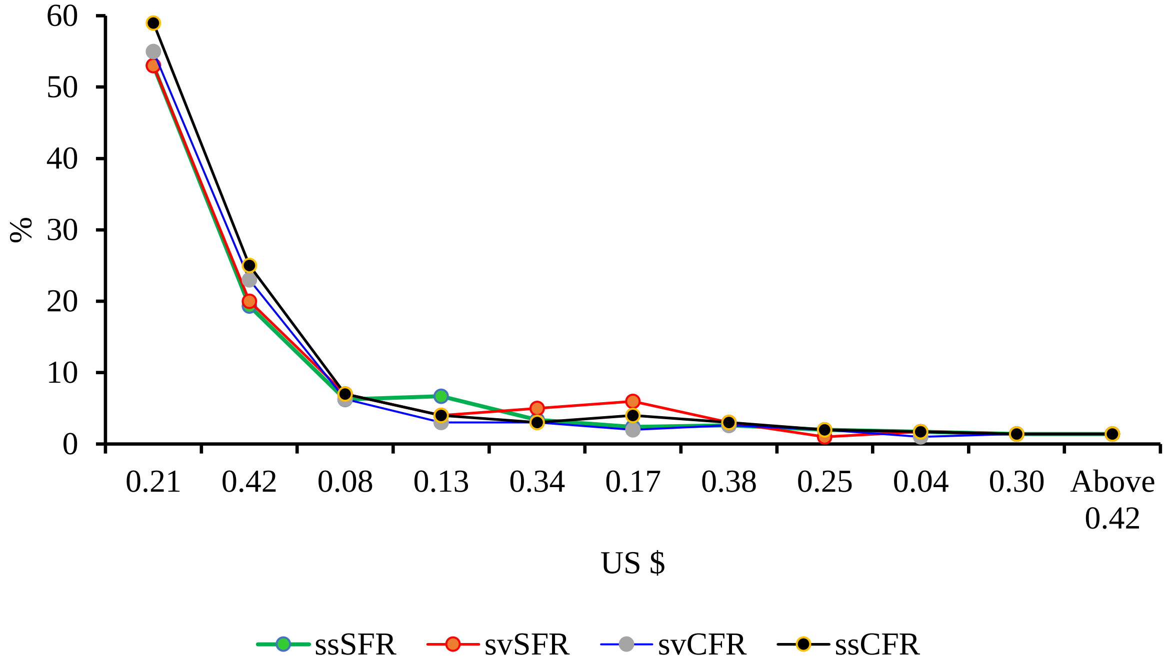

Figure 26: Willingness to buy and pay African nightshade relish products: Whereas; SsSFR: *S. scabrum* spontaneous fermented relish; SvSFR: *S. villosum spontaneous relish:* SsCFR: *S. scabrum* controlled fermented relish; SvCFR: *S. villosum* controlled fermented relish. Means ± SD, (n=3) with different superscripts represents a significant different p<0.05

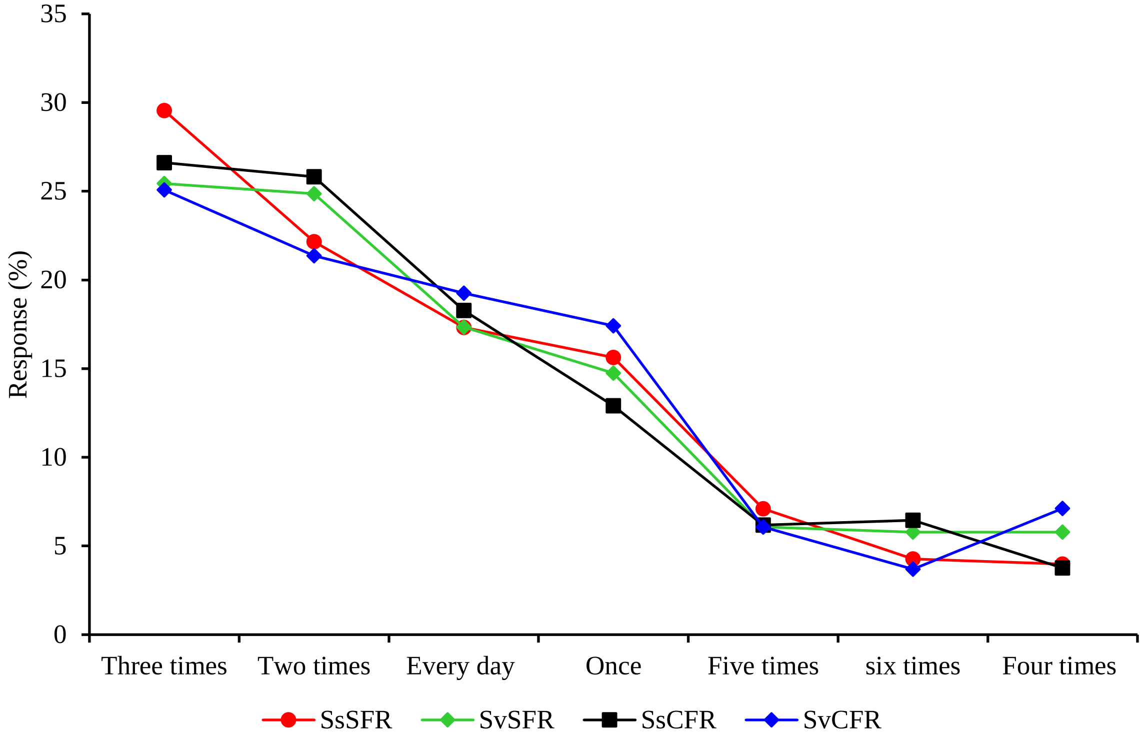

Figure 27: Rate of consumption of relish: Whereas; SsSFR: *S. scabrum* spontaneous fermented relish; SvSFR: *S. villosum spontaneous relish:* SsCFR: *S. scabrum* controlled fermented relish; SvCFR: *S. villosum* controlled fermented relish. Means ± SD, (n=3) with different superscripts represents a significant different p<0.05

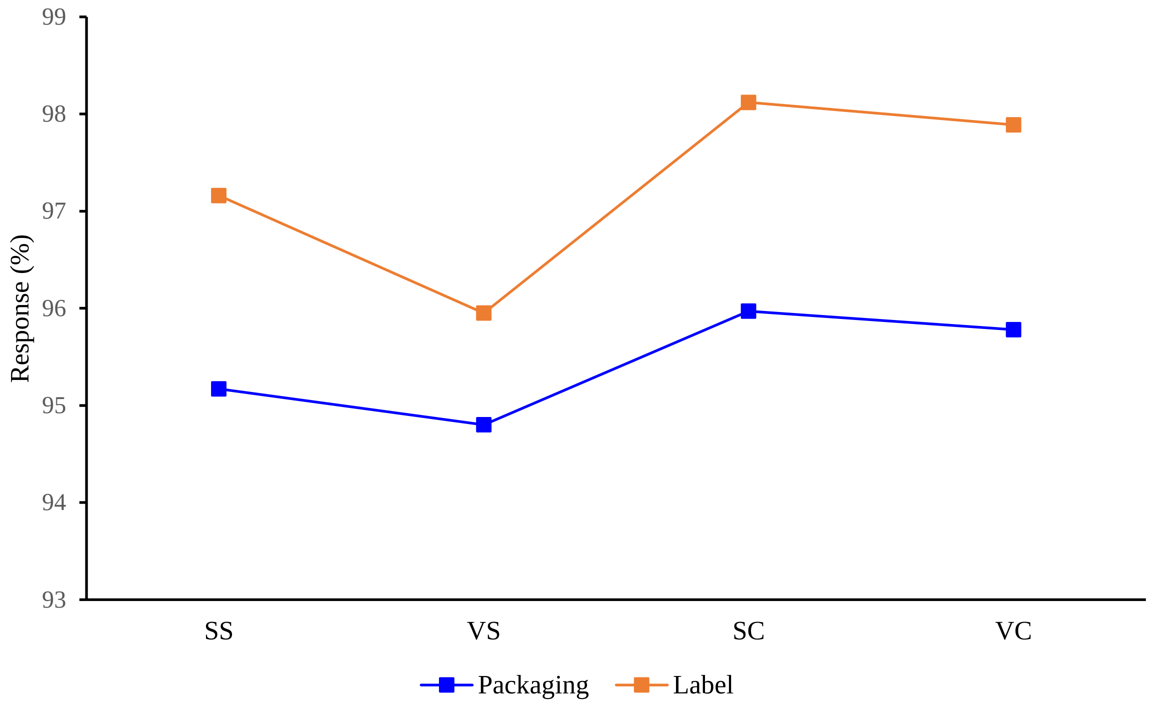

Figure 28: Consumer preference for packaging and label: Whereas; SsSFR: *S. scabrum* spontaneous fermented relish (SS); SvSFR: *S. villosum* spontaneous fermented relish (SV): SsCFR: *S. scabrum* controlled fermented relish (SC); SvCFR: *S. villosum* controlled fermented relish (VC). Means ± SD, (n=3) with different superscripts represents a significant different p<0.05

4.12 Shelflife of relish products during storage

Results of the shelflife study of the LAF fermented relish products are presented in Figs. 32-37 and Tables 15. After six months of room and refrigeration storage of the fermented relish products, no change in pH and TTA were recorded (Fig. 32 and 33). Also, no coliforms, total bacteria, *Lactobacillus*, yeast, and mold were detected (Table 16). Likewise, Wafula (2017) reported, that in the spontaneously fermented product ANS some microbes were detected after storage (10 °C and 25 °C) for four weeks. Microbiological results indicated that the relish product is safe for consumption. Low pH (3.2 – 3.3) inhibited the growth of pathogens (Wafula, 2017). Cooking the pickle to make relishes killed microorganisms in the fermented pickles, including *Lactobacillus*, which hinders their growth in the relish product. Fermentation of *S. scabrum* with *L. plantarum* and *Leuconostoc mesenteroides* inhibited *Listeria monocytogenes* and *Salmonella enterica* servar (Sivakumar *et al.*, 2020). In both SF and CF *S. villosum* and *S. scabrum* relishes product, there were high vitamin C losses (70 – 91%) during room storage and low vitamin C losses (33 – 58%) during refrigeration storage (Fig. 34 and 35). Ambient storage had high losses of vitamin C; 91% was in SsSFR product (Fig. 34). Room storage has a high temperature of 27 °C, and the transparent PET packaging allows light intensity, which facilitates the denaturation of vitamin C (Lee & Kader, 2000). Whereas in refrigeration storage, there is no light intensity and has a low temperature of 4 °C. The maximum loss of vitamin C, 58%, was observed in SvSFR in refrigeration storage (Fig. 35).

On the other hand, there were high losses of β-carotene of 46 – 62% at ambient storage and low losses of 20 – 29 % in refrigeration storage of all relish products (Figs. 36 and 37). However, high losses of β-carotene at ambient storage were 62% for SsCFR product (Fig. 36). At the same time, high losses of β-carotene in refrigeration storage were 29% for SsCFR product (Fig. 37). Despite the reduction of β-carotene still, the remained quantity is sufficient to contribute to recommended daily allowance. This finding agrees with Singh *et al*. (2013), a high loss of β-carotene at ambient storage for glass bottles was 85%, and for plastic bottles was 81%. Oxidation is the leading cause of carotenoid degradation in foods. However, in processed foods, the oxidation mechanism is complex but is facilitated by moisture, temperature, presence of pro-oxidants, antioxidants, and lipids (Singh *et al*., 2013).

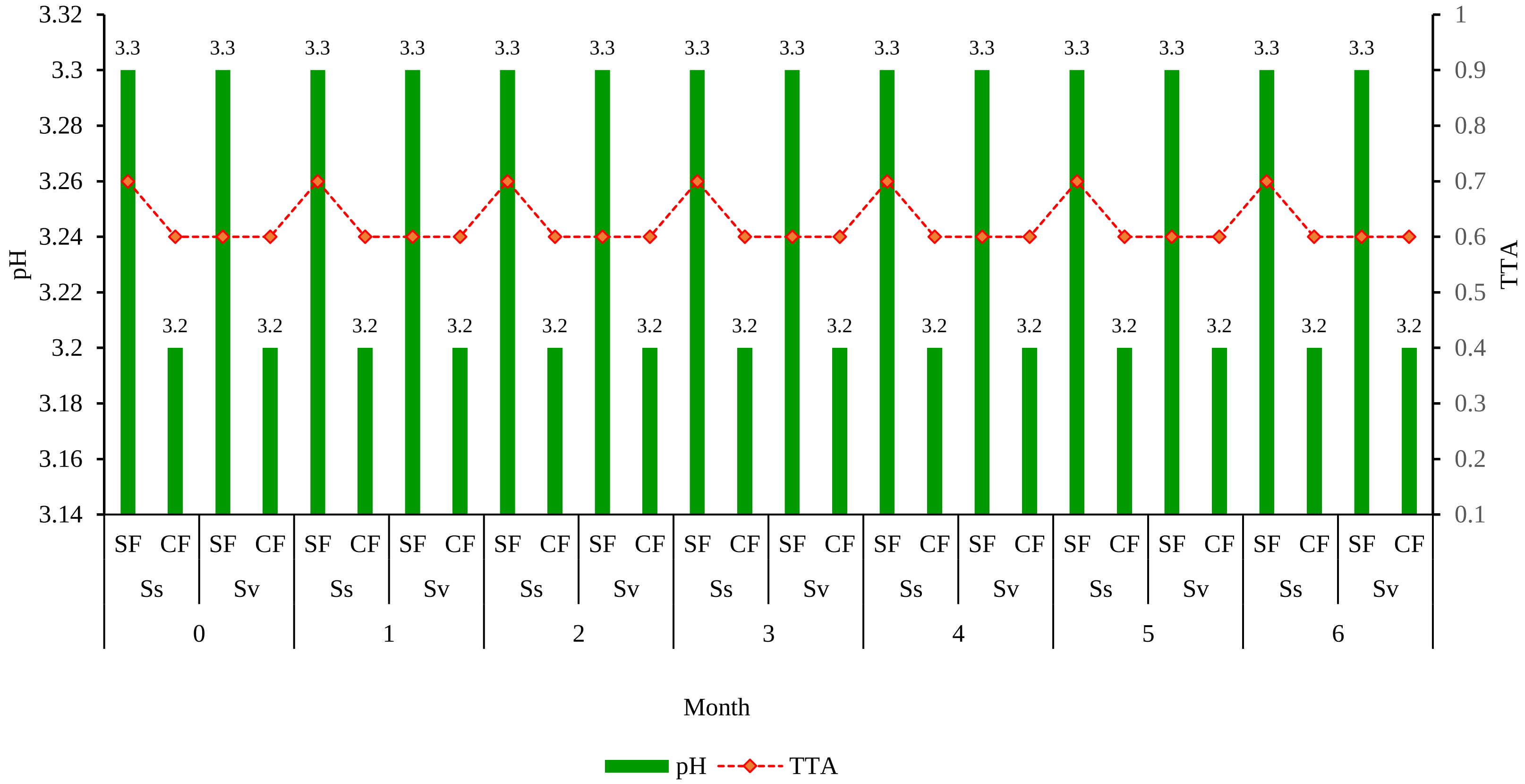

Figure 29: pH & TTA ambient temperature during shelflife study

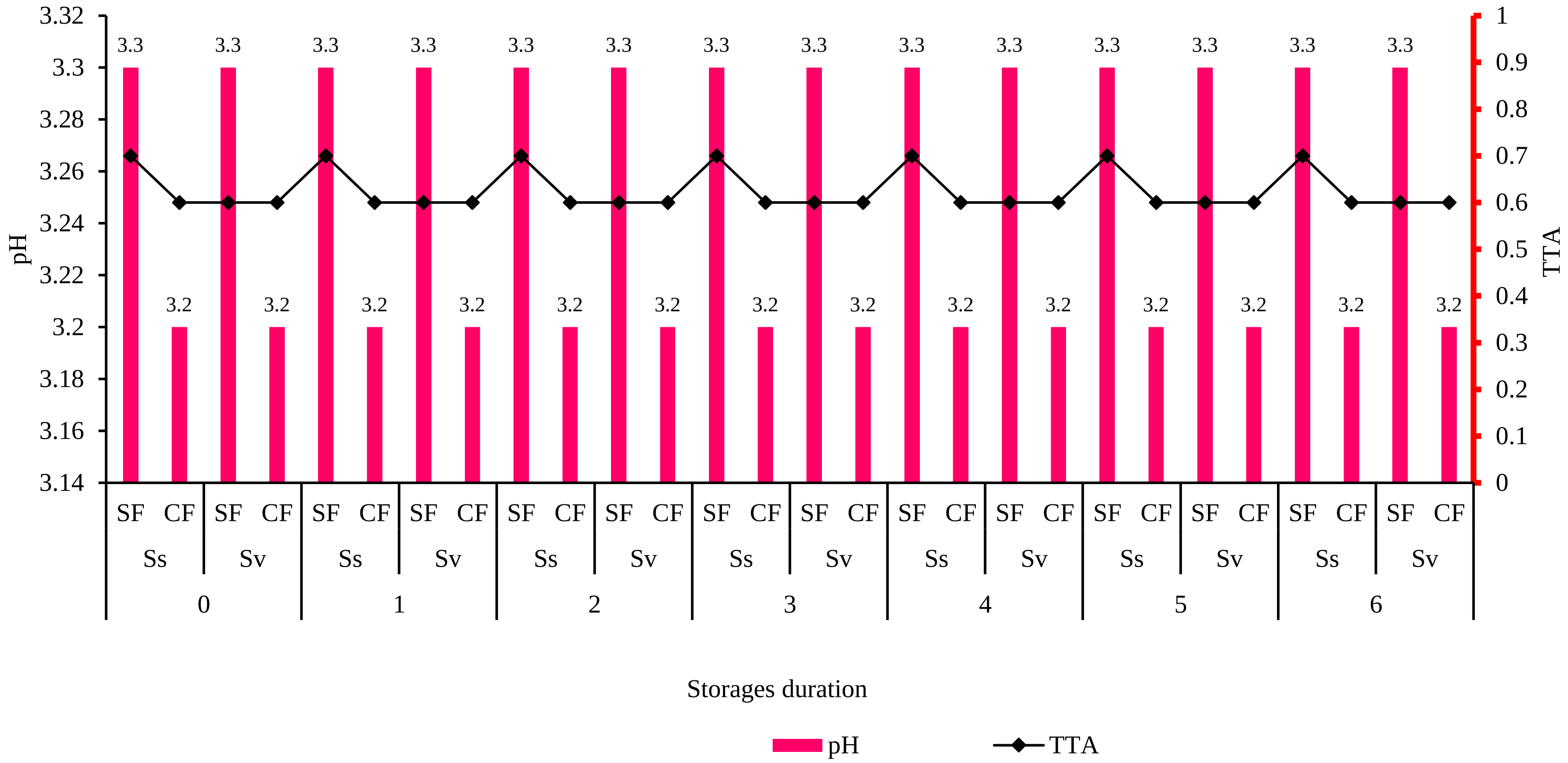

Figure 30: pH & TTA at refrigeration during shelflife study

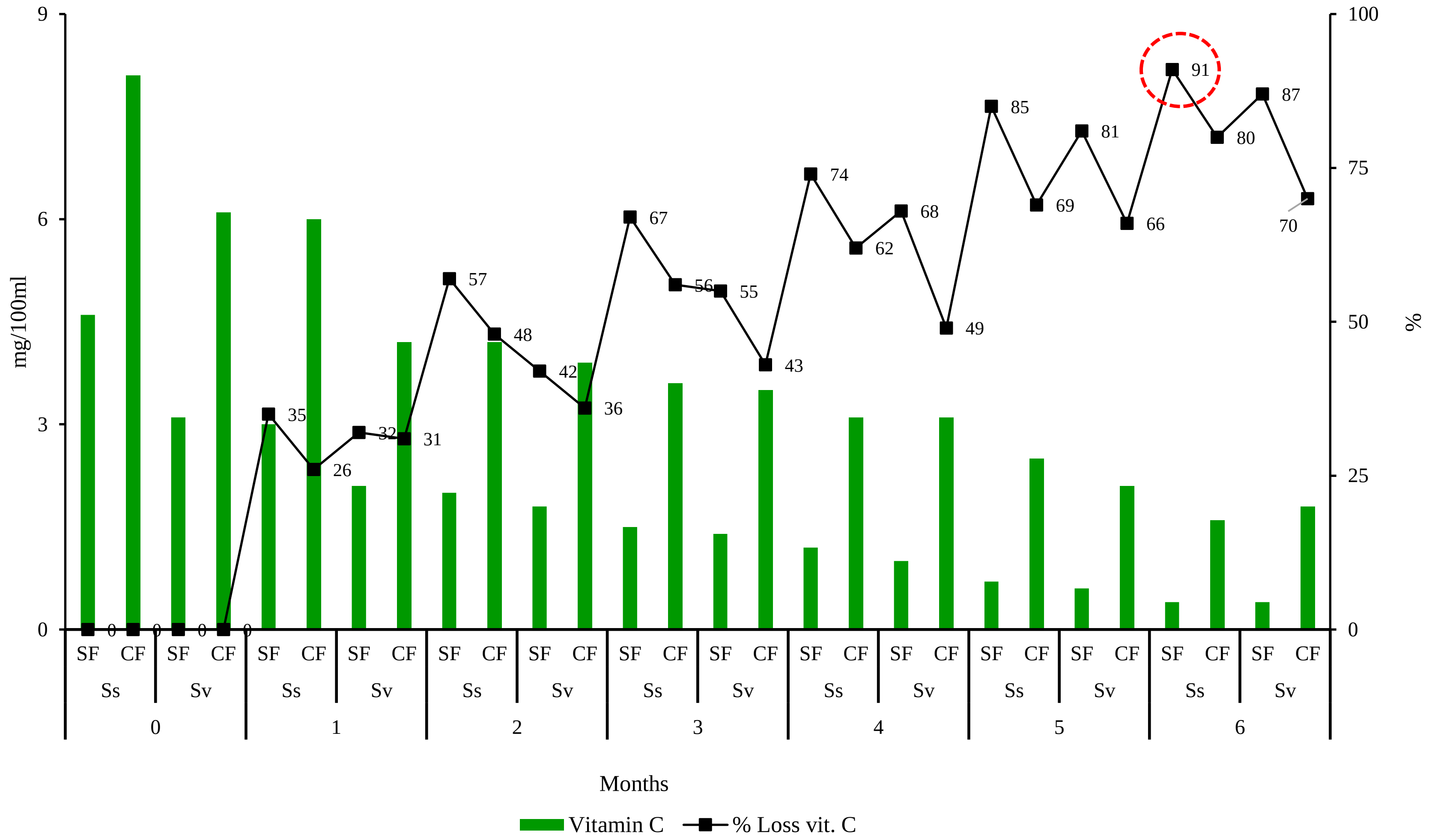

Figure 31: Vitamin C ambient temperature during shelflife study

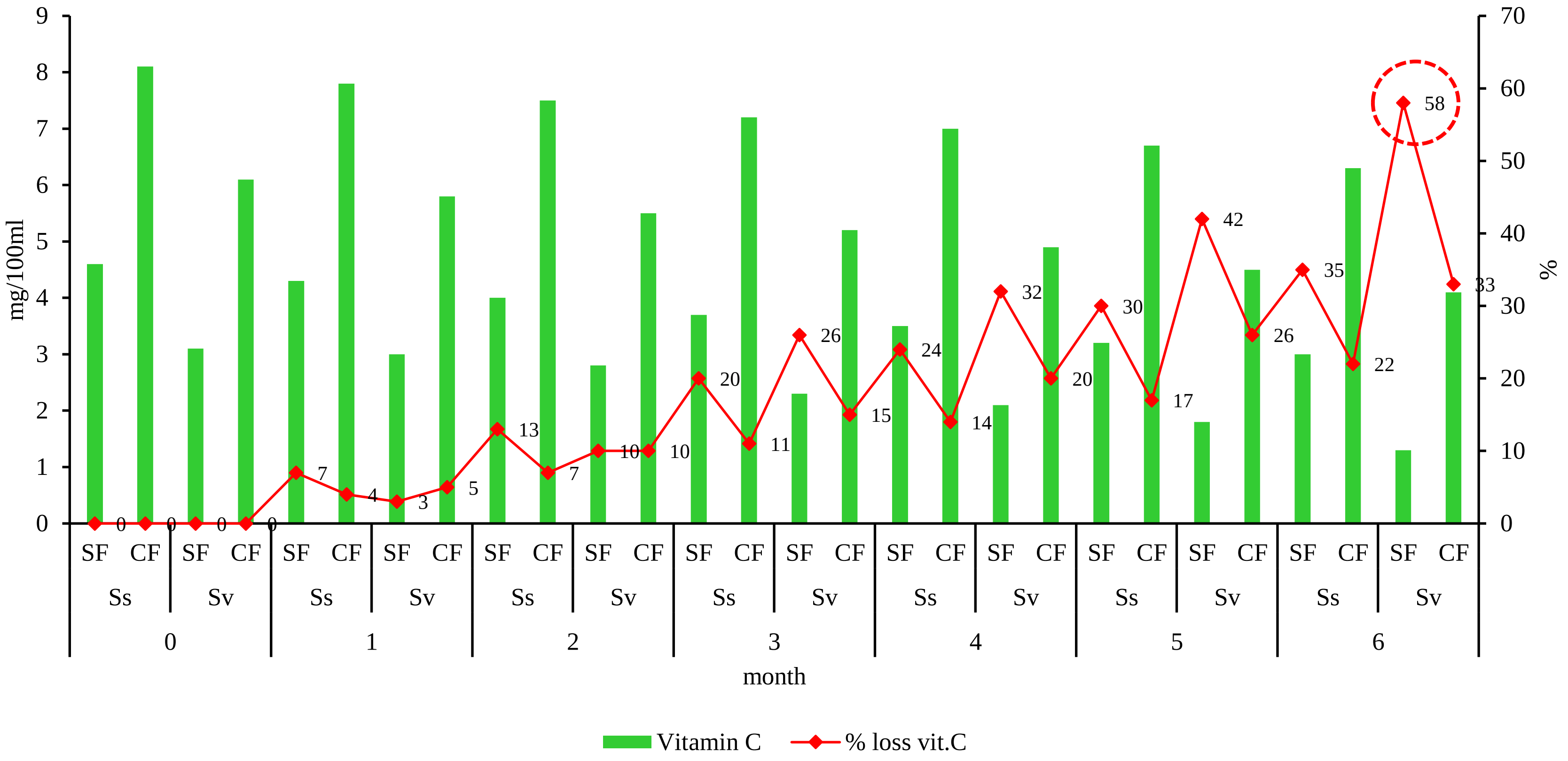

Figure 32: Vitamin C at refrigeration during shelflife study

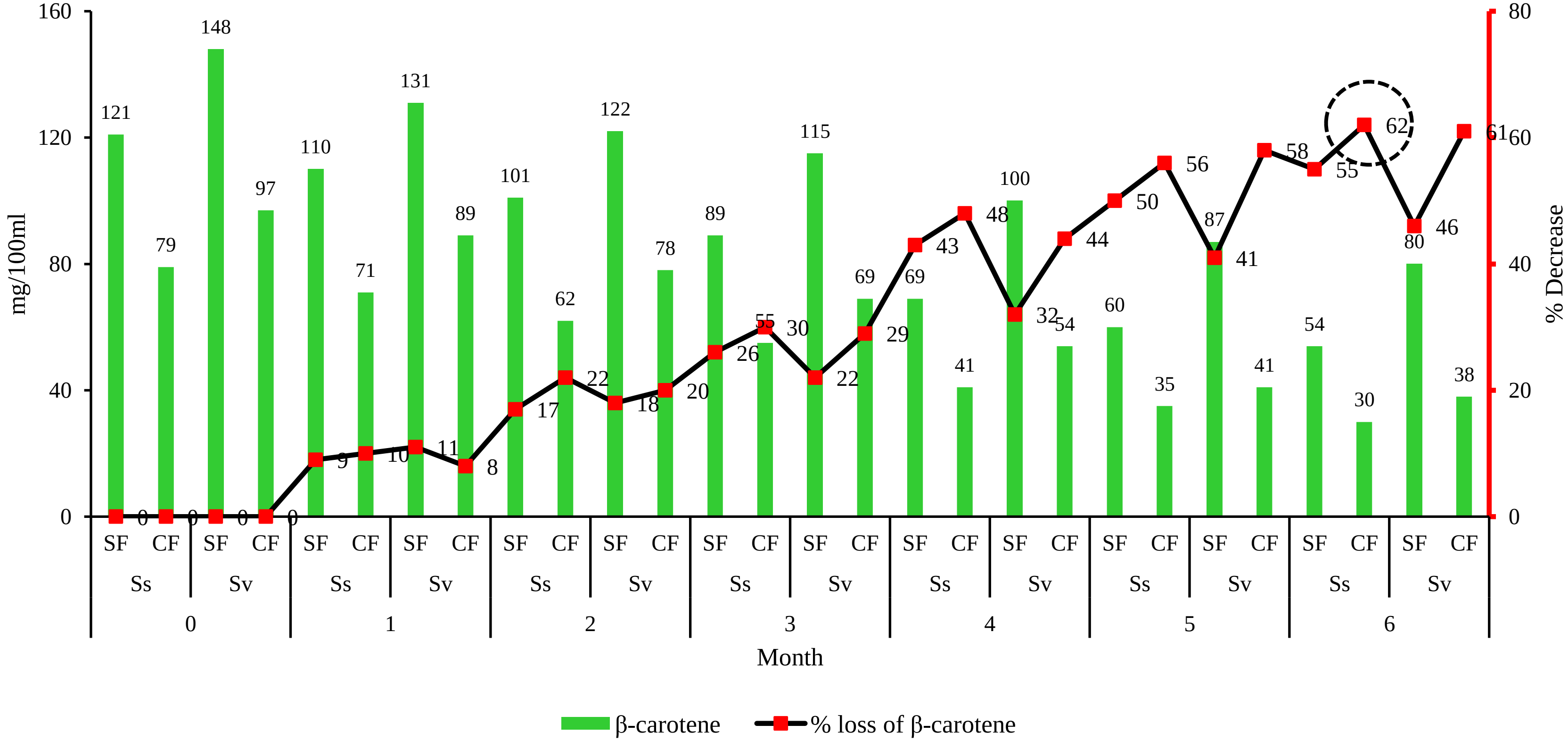

Figure 33: β-carotene at ambient temperature during shelflife study

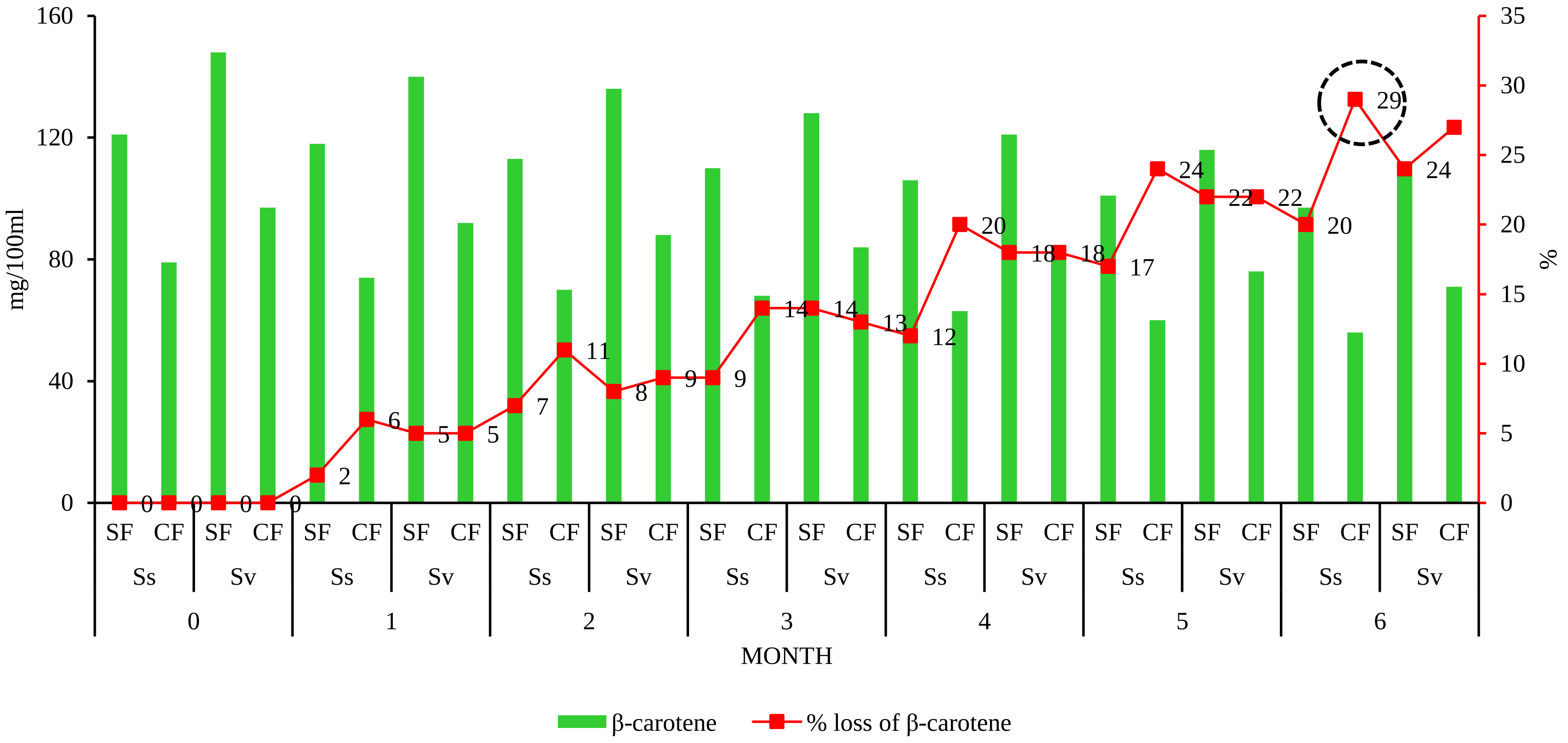

Figure 34: β-carotene at refrigeration during shelf study

Table 16: Microbiology quality during shelf life; whereas ND; Not detected

Parameter		Month					
		1	2	3	4	5	6
1	Total bacteria	-	-	-	-	-	-
2	Yeast and mold	-	-	-	-	-	-
3	Lactobacillus	-	-	-	-	-	-
4	Coliform	-	-	-	-	-	-

Whereas-; Not detected

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

African nightshade is commonly used as a fresh vegetable in Kilimanjaro and Morogoro. Also, the trends in ANS utilization showed that ANS leaves, used for food, source of income and medicinal purpose. ANS production is done in small farm sizes (less than acre) and both local and modern ANS varieties were common grown. Several constraints such as lack of knowledge, insect pests and diseases with intensive inorganic pesticides and fertilizer application were reported to promote ANS production. ANS production contributed 5-50% of household income among majority of farmers. African nightshade was highly utilized as food and consumed with very minimal value addition. Boiling and stir frying were the main preparation methods for ANS served with many staple foods. On the other hand, inadequate post-harvest handling and preservation of ANS were the primary causes of losses with minimal losses occurring during transportation and storage. Several postharvest handling techniques (washing and cooling with water) and preservation techniques such as drying and blanching were used. However, fermentation was less employed for shelflife extension. Lack of processing knowledge, inadequate storage facilities, and unreliable energy sources were the constraints to ANS utilization.

The use of LAF resulted in ANS pickle and relish products. Further, LAF improved β-carotene and mineral contents but reduced vitamin C, polyphenols and chlorophyll levels. Tannin and oxalate (antinutrients) contents were also reduced by LAF. The relish products were all preferred with SsCFR exhibiting significantly higher consumer preference over other products. All relish products were stable at referegeration and ambient temperature after six months of storage. Therefore, LAF can be recommended as a preservation method for ANS as it retains nutrients, improves shelf life, and is affordable in rural settings. Nonetheless, controlled fermentation can be considered good in terms of shorter fermentation duration though it has cost implications on obtaining commercial LAB. Training on production, pre-harvest, and post-harvest handling and preservation techniques such as fermentation and drying is necessary to improve ANS accessibility and utilization in Tanzania.

5.2 Recommendations

(i) Awareness is needed to provide knowledge on the nutritional and health benefits of consuming ANS which could positively contribute to malnutrition reduction in Tanzania and SSA. Also, awareness of the PaP techniques and health benefits of ANS is necessary for dietary diversity and to ensure a year-round supply of ANS.

(ii) Organic pesticides and fertilizers should be promoted over synthetic ones to reduce health effects on consumers and the environment. Further, more emphasis should be directed to improve ANS production, providing extension services to the farmers, and supporting agricultural inputs, including quality seeds, fertilizer, and organic pesticides.

(iii) Research and development should focus on ANS breeding to improve local cultivars for improved yield, tolerance to climatic change such as drought, resistance to pests and disease, and low toxicity varieties. Sensitization on the adoption of recently improved ANS varieties such as *Nduruma* and *Olevolosi*, *Ambureni,* and *Malala* to improve yield and higher returns is mandatory. Furthermore, technologies for value addition such as fermentation should be promoted as feasible affordable convenient, and sustainable for ANS preservation. Presently, most consumers utilize the leaves despite the nutraceutical potential and commercial value of other ANS parts. Therefore, research focusing on the nutritional, functional, and safety of ANS berries is inevitable for their further utilization. Research should focus on finding the proper age for harvesting and re-harvesting ANS, which are less or free from toxic compounds present in leaf of ANS and contain high nutritional and functional compounds for health benefits because the majority of people consume ANS without knowing their health effect.

(iv) This study established fermentation as an affordable vegetable preservation technology; therefore, policy should focus on creating awareness among farmers, SMEs, and other processors for adopting the technology as a way to preserve ANS. Presently, few *Solanum* species are consumed as food; therefore, research should find the possibility of using other *Solanum* species, considering their abundance in SSA, hence improving food availability, and reducing food shortages and malnutrition which are persistent in SSA.

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APPENDICES

Appendix 1: Baseline survey questionnaire assessments of trends and constraints of utilization of ANS in Kilimanjaro and Morogoro

FruVaSe Project – BASELINE SURVEY

Overall baseline questionnaire-Households

WORK PACKAGE TWO (vegetable)

This baseline survey is being undertaken under the Project: Fruits and vegetables for all seasons. This project aims to ensure the continual availability of nutrient-rich fruits and vegetables all year round for households within your community. It is funded by the German Ministry for Food and Agriculture. We are carrying out this baseline survey to assess some basic characteristics of the community.

This survey is led by the University of Goettingen in Germany in collaboration with the University of Nairobi and University of Eldoret (in the case of Kenya); Nelson Mandela Institute of Science and Technology (in the case of Tanzania); and Makerere University (in the case of Uganda). Please do make sure you have signed the consent form before you partake in this interview.

First, we will begin with questions on your household demographics and socio-economic status. After this, we will ask you questions about production, utilization, processing, preservation and constraints associated with vegetables.

Household general information

This interview will be with people between the ages of 18-65+ years

1.	Enumerator's Name	
2.	Region/district	
3.	District Name	
4.	Ward Name	
5.	Village Name	
6.	Household ID	
7.	Participant name	
8.	Household size	
9.	Gender	
10.	Collect GPS of the place	
11.	Do you want to participate in this survey?	

Social-demographics information for Household

1.	What is your age?	1. 18-23 2. 24-29 3. 30-35 4. 36-41 5. 42-47 6. 48-53 7. 54-59 8. 60-65 65+
2.	What is your level of education?	1. None 2. Primary 3. Secondary 4.High school 4.Certificate 5. Diploma 4.Degree
3.	What is your marital status?	1.Married monogamous 2.Married polygamous 3.Widowed/widower 4.Divorced 5. Separated 6.Single
4.	What is your occupation?	1. None 2, Farmer 3. Trader 4.Teacher 5. Doctor/nurse/pharmacist 6. Any other, specify
5.	Which ethnic group do you align yourself with?	1. Christian 2. Muslim 3. No religion 4. Other, Specify other
6.	Were you born in this village?	1. Yes 2. No
7.	Why did you move to this village?	1. Farming 2. Business 3. Employment 4. Family and friends 5.Other Specify other
8.	Are you the head of the household?	1.No 2.Yes
9.	What is the gender of the head of this household?	1. Male 2. Female
10.	What is the age of the head of your household?	1. Below 18 2. 18-30 3.31-40 4. 41-50 5. Above 50
11.	What is the level of education of head of household?	1.None 2.Primary 3.Secondary

		4.Certificate 5.High school 5. Diploma 4.Degree 5.other, Specify other

Here I will ask questions concerning to farming and postharvest management of nightshades

A. **African nightshade farming and postharvest management**		
1.	Do you grow African nightshade?	1.Yes 2.No
2.	What are the reasons to grow African nightshade?	1-For food 2-For commercial 3-Animal feed 4-For both food and commercial 5- other Specify other
3.	Do you collect nightshade which grow natural around your home yard/farm during rainy season?	1-Yes 2-No
4.	What is the quantity of nightshade do you collect around your home yard/farm during rainy season?	1-Less than 10 kg 2-11-20kg 3-31-40kg 4-51-100kg 5.Above 100kg 6- other Specify other
5.	Which varieties of African nightshade do you collect?	1-*Solanum scabrum* (flat test) 2-*Solanum villosum* (bitter) 3-*Solanum nigrum* (bitter) 4-*Solanum americanum* 5-I don't know 6-other Specify other
6.	Which varieties of African nightshade do you grow?	1-Solanum scabrum (Modern) 2-Solanum villosum (Local) 3-other Specify other
7.	Which variety is most preferred?	1-*Solanum scabrum* (Local) 2-*Solanum villosum* (Modern) 3-other Specify other
8.	Why is most preferred?	1-High yield 2-High profit 3-Less disease infestation 4- preferred by consumers 5-Flat taste 6-Less farming management 7-Bitter taste

		8-Less infested by pest and diseases 9-Takes a short time to harvest 10-Other Specify other
9.	Which technology do you use to cultivate African nightshade? Multiple answer is allowed.	1-Handheld tools e.g. hoe 2-Animal 3-Tractor 4- other, Specify other
10.	Which cropping system do you use in the farming of nightshade? Multiple answer is allowed.	1-Monocropping 2-Intercropping 3-If intercropping, which crops do you intercrop it with?
11.	Are there any challenges you are facing in growing African nightshade?	1-Yes 2. No
12.	What are those challenges? Multiple answer is allowed.	1-Low yield 2-Less profit 3-Pest and disease infestation 4-Takes a long time to grow, 8. other Specify other
13.	Do you use pesticides?	1-No 2-yes If yes, which types are they? 1. Organic 2. Inorganic 3. other Specify other
14.	How many cropping seasons do you plant African nightshade in a year?	1-Summer Season 2-Autumn Season 3-Winter Season 4-Spring Season 5- other Specify other
15.	Which season has the highest production quantities?	1-Summer Season 2-Autumn Season 3-Winter Season 4-Spring Season 5- other Specify other
16.	Which season has the least production quantities?	1-Summer Season 2-Autumn Season 3-Winter Season 4-Spring Season 5- other Specify other
17.	How many yields of nightshade do you get in summer season?	1-<10 kg 2-10-50 kg 3-60-100 kg 4-110-500 kg 5-510-1000 kg 6-Above 1000 kg

18.	How many yields of nightshade do you get in autumn season?	1-<10 kg 2-10-50 kg 3-60-100 kg 4-110-500 kg 5-510-1000 kg 6-Above 1000 kg
19.	How many yields of nightshade do you get in winter season?	1-<10 kg 2-10-50 kg 3-60-100 kg 4-110-500 kg 5-510-1000 kg 6-Above 1000 kg
20.	How many yields of nightshade do you get spring season?	1-<10 kg 2-10-50 kg 3-60-100 kg 4-110-500 kg 5-510-1000 kg 6-Above 1000 kg
21.	Do you grow other leafy vegetables?	1-Yes 2-No
22.	What is the performance of African nightshade compared to other leafy vegetables that you grow?	1-Gives more yields 2-Gives fewer yields 3-Is more drought resistant 4-Is more resistant against pests and diseases 5-I don't grow other leafy vegetables 6-other Specify other
23.	Which other vegetable has the highest yield?	
24.	Which are the most cultivated (yield) leafy vegetables in this area? List three most cultivated.	
25.	Do you sell nightshade?	1-No 2-Yes
26.	To whom do you sell the nightshade?	1-Wholesalers 2-Exporters 3-Processors 4-Governmental corporation 5-Retailers 6- Other Specify other
27.	What is the share of the income from nightshade in your total household income?	1- Less than 5% 2-5-10% 3-11-20% 4-21-30% 5-31-40% 6-41-50 7-51-70% 8-71-100

28.	How do you imagine the future of your nightshade farming in the next 10 years?	1-You will quit nightshade farming 2-You will continue nightshade farming 3-You will allow family member(s) to manage your nightshade farm 4-You will sell/rent the nightshade farm to other people 5- Other Specify other
B. <u>Postharvest management of African nightshade</u>		
29.	At what time do you do the harvest of nightshade?	1-Morning 2-Afternoon 3-Evening 4-Anytime
30.	How do you harvest nightshade?	1-Uproot the whole plant 2-Pluck the leaves 3-Knife pickling 4- Other Specify other
31.	After how long do you begin to harvest the nightshade after planting?	1-fist week 2-Second week 3-Thirds week 4-Fouth week 5-Fifth week 6-Sixth week 7.Above six weeks
32.	At what intervals do you harvest the nightshade?	1-First week 2-Second week 3-Thirds week 4-Fouth week 5-Fifth week 6-Sixth week 7.Above six weeks
33.	When do you terminate the harvesting?	1-After 1 month 2-After 2 moths 3-After 3 months 3-After 4 months 4-After 5 months 5-After 6 moths 6-After 7 months 7-After 8 months 8-After 9 months 9-After 10 months 10- Other Specify other
34.	Immediately after harvesting where do you store your nightshade?	1. Exposed to direct sunlight 2. Put under shades 3-Crates 3-Carton/plastic packages 4-Modified packaging 5-Low temperature conditions

		6- Other Specify other
35.	How many containers do you harvest for one season?	1. Less than 5 2. 5-10 3-11-20 4-21-40 5-above 40 6- Other Specify other
36.	How do you remove the field heat from the African nightshades if you harvest in the day?	1-Sprinkle water 2-Leave them under the shade 2-Leave them outside overnight 4-Room cooling 5-I do nothing 6- Other Specify other
37.	Do you wash harvested nightshades?	1-Yes 2-No
38.	Do you use treated water for washing vegetable?	1-Yes 2-No
39.	How do you treat water for washing nightshade?	1-Use chemical chlorine 2-Sand filtering 3-Settling 4- Other Specify other
40.	If you use another kind of water for washing nightshade, what is the source of water?	1-well water 2-Underground water 3-River/sea/ocean water 4.Dum water 5. Other Specify other
41.	How are the nightshade transported to the market/home from farm?	1-Truck 2-Carts 3-Human labour 4-Bicycle 5.Motor bicycle 6- Other Specify other
42.	Which container/bags are used to transport nightshade to home/market palace?	1-wooden crates 3- Cardboard Carton 4-Plastic containers 4- polyethylene sacks 5-Sisal sacks 6- Other Specify other
43.	Do you experience nightshade postharvest losses?	1-Yes 2-No
44.	If yes, what are cause of losses?	1-Mechanical injuries 2-Rotting 3-Nightshades shrivelling

		4-Microbial and fungal attack 5-Poor storage facilities 6-Long distance to the selling point 7-Contamination of produce 8-Poor/lack of storage 9- Pests and diseases 10.-Inadequate knowledge of postharvest handling 11-Excess rainfall 12-Poor packaging 5- Other Specify other
45.	How many containers are spoiled before reaching the market?	1-wooden crates……. 3- Cardboard Carton………… 4-Plastic containers…………. 4- Polyethylene sacks ………. 5-Sisal sacks………….. 6- Other Specify other
46.	How many containers are spoiled at storage site?	1-wooden crates……. 3- Cardboard Carton………… 4-Plastic containers…………. 4- Polyethylene sacks ………. 5-Sisal sacks………….. 6- Other Specify other
47.	How long do fresh nightshade stay in storage place before spoilage? {Days}	
48.	How long do fresh nightshade store in summer? Put in the following conditions?	1-wooden crates……. 3- Cardboard Carton………… 4-Plastic containers…………. 4- Polyethylene sacks ………. 5-Sisal sacks………….. 6- Other Specify other
49.	How long do fresh nightshade store in autumn? Put in the following conditions?	1-wooden crates……. 3- Cardboard Carton………… 4-Plastic containers…………. 4- Polyethylene sacks ………. 5-Sisal sacks………….. 6- Other Specify other
50.	How long do fresh nightshade store in spring? Put in the following conditions?	1-wooden crates……. 3- Cardboard Carton………… 4-Plastic containers…………. 4- Polyethylene sacks ………. 5-Sisal sacks………….. 6- Other Specify other
51.	How long do fresh nightshade store in winter? Put in the following conditions?	1-wooden crates……. 3- Cardboard Carton………… 4-Plastic containers…………. 4- Polyethylene sacks ………. 5-Sisal sacks…………..

		6- Other Specify other
52.	What strategies do you put in place to extend the shelf-life of fresh nightshades?	1-Harvesting small quantities 2-Use proper packaging 3-Minimizing mechanical damages 4-Storing in cool conditions 5- Other Specify other
53.	What practice do you undertake to minimize spoilage of fresh nightshade?	1-Sell immediately after the sale 2-Dry the leaves 3-Ferment the leaves 4-None 5- Other Specify other
54.	Do you have controlled atmosphere storage for nightshade?	1.Yes 2-No If yes what type 1. Tradition 2.Modern (refrigeration) 3. Other Specify other
C. <u>Utilization of African nightshade</u>		
55.	Do you eat and or your family eats African nightshade?	1. Yes 2. No
56.	If No, what are the reasons for not eating nightshade?	1. Astringent 2. Bitter taste 3. Expensive 4 Other Specify other
57.	If yes, what are the reasons for eating African nightshade?	1-Bitter taste 2-Cheap in price 3-Astringent 4-Recover/increase appetite to eat 5-No other vegetable available 6- Other Specify other
58.	Do you eat African nightshade alone?	1-Yes 2-No
59.	Do you eat nightshade with other side dish?	1-Yes 2-No
60.	What are those dishes	1-Ugali/stiff porridge 2-Rice 3-Makande 4- Other Specify other
61.	In what forms do you consume the nightshades? Multiple answers allowed.	1-Boiled 2-Fermented 3-Sun-dried 4-Blanched 5- Other Specify other

62.	How do you incorporate Nightshade into the diet?	1-Consumed singly as vegetables 2-Made as a composite dish with other vegetables 3-Mashed with other foods
63.	If you incorporate nightshade with other foods, what are the reasons?	1-Reduce bitterness 2-Improve taste of other vegetable 3- Other Specify other
64.	Between local and modern nightshade which variety do you prefer most?	1-Bitter 2-Flat taste
65.	Which group consume African nightshade? Multiple answer allowed.	1-Children below 5 years 2-5-17 years 3-Men above 18 years 4-Women above 18 years 5-Pregnant mother 6-Breastfeeding Mothers 7-Old men 8-Old women 9-whole family
66.	Apart from using nightshade for human consumption, what other uses do you know?	1-Livestock feed 2-Medicinal use 3-Biogas 4- Other Specify other
67.	How had your nightshade consumption been in the previous five years?	1-Increasing 2-Decreasing 3-Variable 4-Please give reasons
68.	Do you know any products which are made from African nightshade available in your area?	1-Yes 2-No
69.	Which product are available in your area?	1-dried 3-Fermented 3-Juice 4-Chutneys 5- Other Specify other
70.	Which products do you like among the available processed one?	1-dried 3-Fermented 3-Juice 4-Chutneys 5- Other Specify other
71.	Which products do you dislike among the available processed one?	1 dried 2 fermented 3-juice 4-chutneys 5- Other Specify other
72.	Where do processed nightshades product sold?	1-Supermarkets 2-Farmer/women group centres 3-Kiosk 4-Open market 5- Other Specify other

73.	Would you readily consume fermented as you would fresh ones?	1-Yes 2-No
74.	Are there nightshade products you would like to consume that are currently not available within this place?	1-Yes 2-No
75.	If yes, which products?	1-Dehydrated-sun 2- Dehydrated -solar 3-Fermented 3-Juice 4-Chutneys 5- Other Specify other
76.	What would be reasons for you to buy and eat a product made from African nightshade?	1-Cheap 2- nutritious 3-Good taste 4-easy to cook 5. Other Specify other
77.	What would be reasons for you to reject not to buy and eat a product made from African nightshade?	1-Astringency 2-Bitter taste 3-I don't like it 4- Other Specify other
78.	Which products do you or your household prefer most among those mentioned above?	1-Dried 2-Fermented 3-Juice 4-Chutneys 5- Other Specify other
D. <u>Processing and preservation of African nightshades</u>		
79.	Do you process nightshade for increasing shelf life?	1-Yes 2-No
80.	If no, Why do you not process nightshade?	1-Expensive electricity 2- Electricity problem 3-No cold media such as fridge 4-No avilable raw materials 5- Other Specify other
81.	How do you store nightshade waiting for processing?	1-In room at ambient temperature 2-In cool room 3-Refregerarion 4-Transport containers 5-No practice used 6- Other Specify other
82.	What processing techniques do you apply in the preservation of nightshade?	1-Sun-drying 2-Solar Drying 3-Blanching 4-Fermentation 5-None 6- Other Specify other

83.	What is your production capacity of each product you process per year?	
84.	Which is the source of heating energy do you use?	1-Firewood 2-Charcoal 3-Petrol/Diesel 4-Heavy Furnace oil 5-Gas 6-Steam 7-Coal 8- Other Specify other
85.	Which is the source of cooling energy do you use?	1-Room temperature water 2-Cooling tower water 3-Chilled water 4- Other Specify other
86.	What source of water do you use for processing?	1-Rain water 2-Pond water 3-River water 4-Undeground water 5-Government tap water 6-Lake water
87.	Do you treat water for processing your products?	1-Yes 2-No
88.	What treating methods do you use?	1-Sand filtration only 2-Chlorine treatment only 3-Sand filtration +Chlorine treatment 4- Sand filtration +Chlorine treatment+ carbon filtration 5-Nano filtration 6-UV light treatment
89.	What kind of processing materials do you use?	1-Plastic 2-Cast iron 3-Stainless steel 4-Aluminium
90.	What are the challenges you are facing in processing nightshade?	1- No electricity 2-No gas 3-No charcoal 4-No firewood 5. No processing vessels 6-No processing machine 8. Other Specify other
91.	What storage conditions do you preserve processed nightshade? Please mention them.	1-Room temperature 2. Refrigeration 3. Cool bolt 4-Tradition cooling chamber 5-Traditional cooling chamber 6. Other Specify other
92.	How long do you preserve fresh nightshade in storage media temperature?	1-One day 2-Two days 3-Three – seven days 5-8-14 days

		6-Above 14 days
93.	How long do you preserve processed nightshade in storage media temperature?	1-One day 2-Two days 3-Three – seven days 5-8-14 days 6-Above 14 day
94.	What do you do with the nightshade by-products?	1-Used as animal feed 2-Manure 3-Ferment to biogas 4-Disposal 5-Medicinal 5. Other Specify other
E. Packaging		
95.	Which packaging are used to pack African nightshade?	1-Plastic container 2-Glass container 3-Tetra pack 4-Plastic bag/container 5-Plastic container 6- Other Specify other
96.	Which type of package is preferred?	1-Plastic container 2-Glass 3-Tetra pack 4-Plastic bag 6- Other Specify other
97.	Why is package{s} preferred?	1-Cheap 2-Look good 3-Easy to handle 4-Easily available 5-Consumer likes most 6- Other Specify other
98.	Do you know the benefits of packaged food?	1-Yes 2-No.
99.	What are the benefits of packaged food?	1-Increase the cost of the product 2-Packaged product more preferred 3-Increase food quality 4-Look attractive 5-No
F. Constraints in production, processing and utilization of African nightshade		
100.	What have been the major challenges in African nightshades production?	1-Lack of good quality seed and fertilizer 2-Lack of adequate labour 3-Lack of technical support and extension services 4-Lack of garden tools, equipment 5-Lack of knowledge on farming 6-Theft of vegetable 7-Pest and diseases 8-Lack of high quality/ adapted

		varieties 9-Poor market returns from sells 10- Other Specify other
101.	What are the main challenges affecting the processing of African nightshades?	1-Lack of adequate knowledge on value addition 2-Lack of processing equipment 3-Lack of capital 4-Lack of skilled manpower 5- Other Specify other
G. Constraints in the utilization of African nightshade		
102.	What major constraints do nightshade and utilization face? (Tick as appropriate)	1-Field pest 2-Seed scarcity 3-Lack of land 4-Poor yields 5-Lack of market 6-Poor Soils 7-Poor varieties 8-Extension services 9- Low prices 10-Price fluctuations 11-Drought 12-Diseases 13-Access to seed 14-Weeds 15-Massive spoilage 16- Other Specify other
Suggestion and Recommendations		
103.	What are your suggestions on the improvements of African nightshades?	
104.	If we develop improved products, Would you participate in capacity building exercise?	1.Yes 2.No
105.	What another thing you would wish to share with us?	

Appendix 2: Score card for the evaluation of pickles

Name: Date:

Instructions

Please write a numerical score against the sample for given characteristics.

Score pattern:

QUALITY	SCORE
Liked extremely	9
Liked very much	8
Liked moderately	7
Liked slightly	6
Neither liked or disliked	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Parameters

Sample no.	Appearance	Colour	Texture	Taste	Odour	Flavour	Spiciness
SvSFR (324)							
SvCFR (798)							
SsCFR (165)							
SsSFR (426)							

Sample no.	**Saltness**	**Sourness**	**Bitterness**	**Overall Acceptability**
SvSFR (324)				
SvCFR (798)				
SsCFR (165)				
SsSFR (426)				

Suggestions: Signature

Consumer acceptability for pickle relish

S. No.	Name	Acceptability		
		Highly acceptable	Acceptable	Not acceptable

Appendix 3: Ethical approval

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OF TANZANIA

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13[th] March, 2019

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Arusha

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Fruits and vegetables for all seasons: improved resource-efficient processing techniques and new market solutions for surplus fruits and vegetables for rural development in East Africa (Makule E. et al.), has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Morogoro, Kilimanjaro, Lindi and Mtwara regions.

Approval is valid for one year: 13[th] March 2019 to 12[th] March 2020.

Name: Prof. Yunus Daud Mgaya

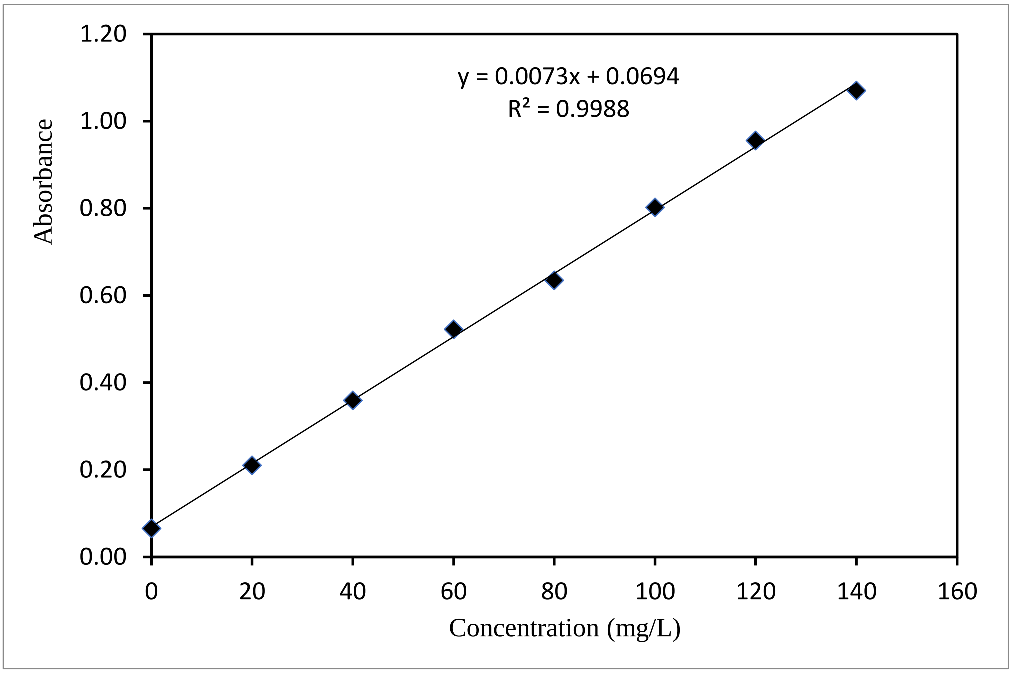
Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

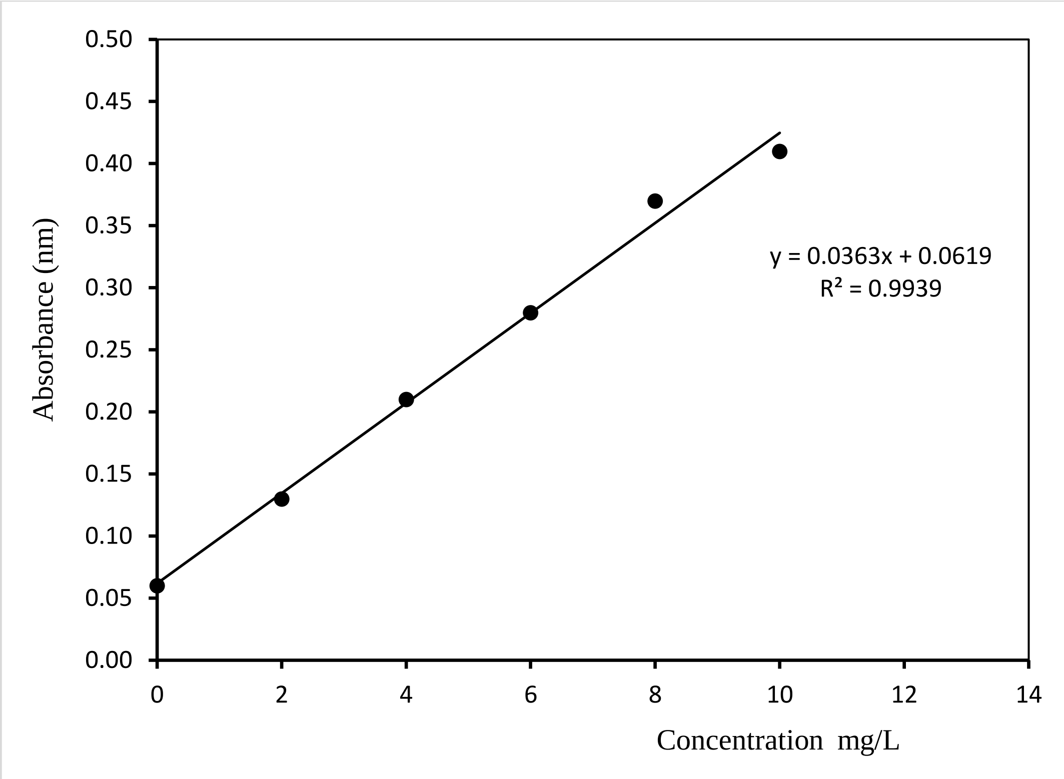
Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY
DEVELOPMENT, GENDER, ELDERLY &
CHILDREN

CC: Director, Health Services-TAMISEMI, Dodoma
RMO of Morogoro, Kilimanjaro, Lindi and Mtwara regions
DMO/DED of respective districts

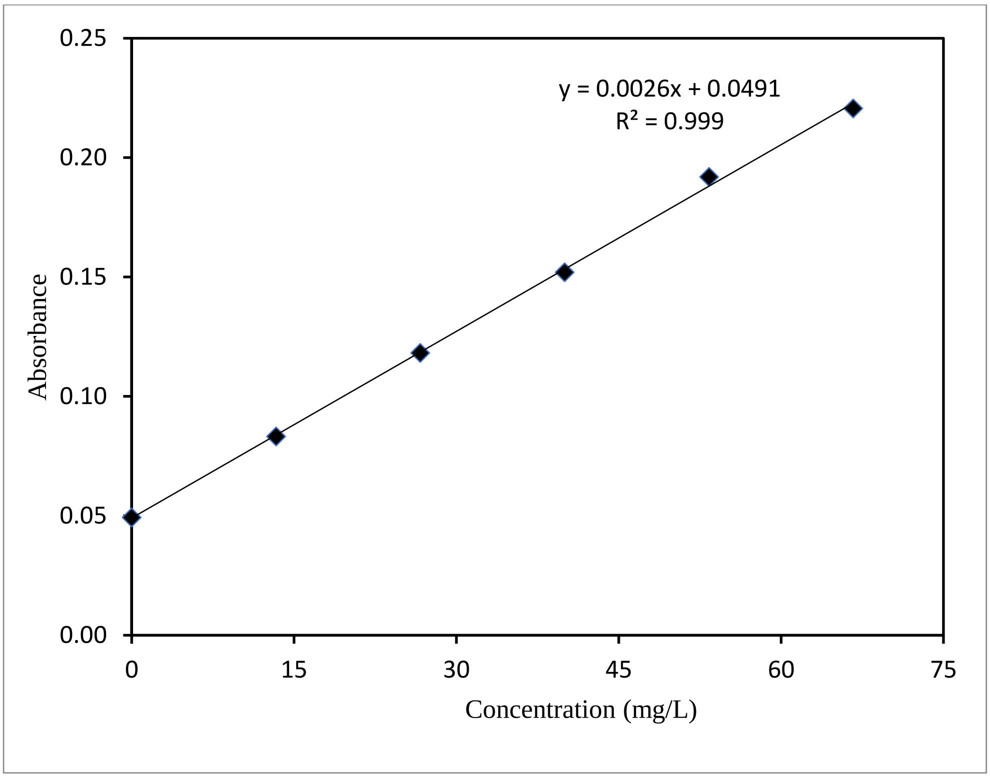
Appendix 4: Standard curve in calculation of phenol and tannin



Appendix 5: Ascorbic acid standard



Appendix 6: Beta carotene standard



RESEARCH OUTPUTS

1. Published papers

Sangija, F., Martin, H., & Matemu, A. (2021). African nightshades (*Solanum nigrum* complex): The potential contribution to human nutrition and livelihoods in sub-Saharan Africa. Comprehensive Reviews in Food Science and Food Safety, 2021, 1– 35. https://doi.org/10.1111/1541-4337.12756

Sangija, F., Martin, H., & Matemu, A. (2022). Effect of lactic acid fermentation on the nutritional quality and consumer acceptability of African nightshade. *Food Science AND Nutrition*, *10*(9), 3128-3142.

Sangija, F., Kazosi, M., Martin, M., & Matemu, A. (2022). Trends and constraints in the utilization of African nightshade (Solanum nigrum complex) in Tanzania: A case study of Kilimanjaro and Morogoro regions. *African Journal of Food, Agriculture, Nutrition and Development*, *22*(6), 20623-20645.

2. Published abstract

Sangija, F., Kazosi, M., Martin, M., & Matemu, A. (2021). Effect of lactic acid fermentation on nutritional and antinutritional compounds in African nightshade, published in book of abstract of 3rd All Africa Postharvest and Exhibition, 13th -16th September, 2021

Sangija, F., Kazosi, M., Martin, M., & Matemu, A. (2021). Trends and constraints in the utilization of African nightshades (*Solanum nigrum* complex) in Tanzania: A case study of Kilimanajro and Morogoro region published in book of abstract of 3rd All Africa Postharvest and Exhibition, 13th -16th September, 2021

Sangija, F., Kazosi, M., Martin, M., & Matemu, A. (2021). Trends and constraints in the utilisation of African nightshades (*Solanum nigrum* complex) in Tanzania, published in book of abstract toward shifting paradigms in agriculture for health and sustainable future, 14th -17th September, 2021

Sangija, F., Kazosi, M., Martin, M., & Matemu, A. (2021). Effect of Lactic acid fermentation on nutritional and antinutritional compounds in African nightshade published in book of abstract toward shifting paradigms in agriculture for health and sustainable future, 14th -17th September, 2021

Sangija, F., Kazosi, M., Martin, M., & Matemu, A. (2021). Consumers Sensory Perception of Fermented African nightshades in Tanzania published in book of abstract toward shifting paradigms in agriculture for health and sustainable future, 14th -17th September, 2021

3. **Poster Presentation**

Sangija, F., Kazosi, M., Martin, M., & Matemu, A. (2021). Trends and constraints in the utilization of African nightshade (*Solanum nigrum* complex) in Tanzania. Tropentag virtual conference. toward shifting paradigms in agriculture for health and sustainable future, 14th -17th September, 2021

Sangija, F., Kazosi, M., Martin, M., & Matemu, A. (2021). Consumers' sensory perception of fermented African nightshades in Tanzania. Tropentag virtual conference, toward shifting paradigms in agriculture for health and sustainable future, 14th -17th September, 2021

Sangija, F., Kazosi, M., Martin, M., & Matemu, A. (2021). Effect of lactic acid fermentation on nutritional and antinutritional compounds in African nightshade, 3rd All Africa Postharvest and exhibition, 13th -16th September, 2021

Sangija, M., Martin, M., & Matemu, A. (2021). Fermentation of African nightshades, a training material used during dissermination of lactic acid fermented relish in Morogoro and Kilimanjaro region, April, 2021