DEVELOPMENT OF IRON RICH PRODUCTS FROM TRADITIONAL LEAFY VEGETABLES GROWN IN LINDI, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

The current study was conducted to develop iron rich products for feeding children under five years of age. Three Traditional leafy vegetables (TLVs), *Amaranth* hybrids known *as* amaranthus leaves (AML), *Manihort esculenta* known as cassava leaves (CAL*)* and *Ipomea batatas* known as sweet potatoes leaves (SPL) grown in Ruangwa and Nachingwea Districts in Lindi Region were used. They were collected from both home gardens (HG) and low land (LL). TLVs samples from the two sources were analysed in triplicate using standard methods for physico chemical parameters (pH, moisture content, dry matter content) and nutrient content for selected minerals and vitamins i.e. (iron, zinc, beta carotene and ascorbic acid) as well as microbial quality of the dried TLVs and vegetable powder formulations. The three TLVs which had been optimized for iron content, were used to prepare 4 vegetable powder formulations (F1–60.0:7.5:22.5); (F2–70.0:5.0:15.0); (F3–80.0:2.5:7.5) and (F4–40.0:10.0:40.0). Spices were added to the vegetable powder formulations for flavour. The developed vegetable soup formulations were analysed for sensory quality as well as acceptability. Data was analyzed by R – statistical package (R version 4.0.3 2020). One-way analysis of Variance (ANOVA) was carried out to determine if there was any significance difference in micronutrients, physico-chemical among vegetables and across the sites. Means were separated by Tukey’s Honest at $p<0.05$. Significant differences in iron, zinc and beta-carotene content were found between vegetables at $p<0.05$. The solar dried TLVs, indicated that AML had the highest iron content, CAL had the highest zinc while SPL had the highest beta-carotene. Moreover, CAL and SPL had statistically significant higher ($p<0.05$) ascorbic acid than AML. In addition, zinc and ascorbic acid were highest in CAL whereas beta-carotene was highest in SPL for home grown

vegetables. Highest iron content ($p<0.05$) was found from AML that had been grown under lowland grown vegetables.

For microbiological parameters, significant differences ($p<0.05$) in *Total plate count (TPC)* were observed among the TLVs and between the two sites. The LL had the highest TPC than HG. Moreover, there was no significant difference ($p<0.05$) in TPC among vegetable powder formulations. No *E. coli* contamination observed among TLVs, neither from the two sites nor their formulations.

Sensory evaluation for descriptive test revealed significant ($p<0.05$) differences in mean intensity scores between the vegetable soup samples. The descriptive attributes of the vegetable soup samples showed that F1, F2 and F3 had significantly higher ($p<0.05$) mean intensity scores for colour, aroma, and mouth feel than F4. The acceptability test however showed that all vegetable soup samples were accepted by panelists. F1 was the most liked due to colour, aroma and mouth feel followed by F2, F3 and finally F4. Furthermore, the preference mapping results showed that colour, aroma and mouth feel attributes were the main drivers for positive consumer preference for vegetable soup. Therefore, the development of TLVs soup powder formulations using a locally available processing technique is a promising solution to increase the uptake of iron to the under five children and other vulnerable members of the community. It also increases TLVs shelf life and availability throughout the year as well as an important contribution to income.

DECLARATION

I, Abdallah Afsa, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

Afsa Abdallah, Date

(MSc. Candidate)

The above declaration is confirmed;

Dr. L. Chove Date

(Supervisor)

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DEDICATION

This work is dedicated to my beloved mother Mrs. Kulthum A. Shehe and my late father Mr. Abdallah S. Milandu. I also dedicate this study to my beloved husband Swalehe, kids Samir, Sheyreen, and Shuraym whom I shall always remain grateful for their love, prayers, support, kindness, and patience throughout my study.

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LIST OF ABBREVIATIONS AND SYMBOLS

AML	Amaranths leaves (*Amaranthus hybridus* L.)
ANOVA	Analysis of Variance
BPW	Buffer peptone water
CAL	Cassava leaves (*Manihot esculenta*)
CFU	Colon Forming Unit
CRD	Complete Randomized Design
F1	Vegetable soup powder formulation 1
F2	Vegetable soup powder formulation 2
F3	Vegetable soup powder formulation 3
F4	Vegetable soup powder formulation 4
FAO	Food and Agriculture Organization
IDA	Iron deficiency anemia
ISO	International Organization for Standardization
mg	milligram
pH	Hydrogen potential
RDA	Recommended Dietary Allowance
SPL	Sweet potato leaves (*Ipomoea batatas*)
SSA	Sub Sahara Africa
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards
TDHS	Tanzania Demographic Health and Survey
TDHS-MIS	Tanzania Demographic and Health Survey and Malaria Indicator Survey
TLVs	Traditional leafy vegetables
TLVPs	Traditional leafy vegetables Products

TNNS	Tanzania National Nutrition Survey
TZS	Tanzania standard
UNICEF	The United Nations Children's Fund
VAD	Vitamin A deficiency
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food, which meets the dietary needs and food preferences for an active and healthy life (Pérez-Escamilla, 2017). In Sub Sahara Africa, people's diets are mainly from starch foods like rely heavily on rice, potato and cassava, which are high in calories but deficient in essential micronutrients. Deficiencies in iron, vitamin A and iodine are widespread, affecting about 300 million people every year, with many more at risk of experiencing these deficiencies (Atangana *et al.*, 2013).

According to the global nutrition report 2016, two billion people are still suffering from micronutrient deficiencies on a global scale (Achadi *et al*., 2016). Developing countries like Tanzania experiencing stunting (34% of children under five), wasting 5%, underweight 14% and 5.5% of women aged 15–49 years are underweight. Tanzania ranks third in chronic under nutrition among Sub Sahara Africa (SSA), after Ethiopia and the Democratic Republic of Congo the burden (Ochieng *et al*., 2017).

Deficiency of micronutrients has a significant impact on the economic development of communities as well as nations. These deficiencies can lead to serious health problems, including reduced resistance to infectious diseases, blindness, fatigue, reduced learning capacity, mental retardation, loss of human capital and productivity and in some cases, to death (Ekweagwu *et al*., 2008).

Vegetables contribute substantially to food security, overcoming food and nutritional insecurity. Consuming nutrient dense vegetables is a first step to overcoming malnutrition (Keatinge *et al.,* 2011). Traditional leafy vegetables (TLVs) contain the most important nutrients required for growth and health. They are high in fiber, extremely low in fat and carbohydrates, and provide an excellent source of protein. Leafy vegetables can play a major role in reducing micronutrient deficiencies (Easdown, *et al.,* 2014).

Promotion of production and consumption of such micronutrient-rich foods will improve intakes, the overall diet, and health status (Mwanri *et al.,* 2011). The use of traditional leafy vegetables in rural communities however has reached a low point, as many have labeled their dishes as 'poverty food'. (Atangana *et al.,* 2013).

TLVs can be raised comparatively at lower management costs even on poor marginal lands, yet they have remained underutilized due to lack of awareness and promotion of technologies for utilization and inadequate scientific knowledge of their nutritional potentials (Joshi and Mathur, 2015).

Despite adequate literature review, information on the nutritional, microbial and sensory qualities of solar dried TLVs in Tanzania is limited. Therefore, by undertaking this study, the micronutrient-rich of solar dried vegetables, as well as microbial and sensory quality of developed product can be determined, in order to establish their quality and to recommend for the often consumption so as to improve intake, the overall diet as well as health status of the community.

1.2 Problem Statement and Justification of the Study

World Health Organization (WHO) recommends a minimum intake of 400 g of fruits and vegetables per day for the prevention and alleviation of several micronutrient deficiencies (Alemu *et al.,* 2018: Eyzaguirre and Smith, 2007). Minerals and vitamins are of great importance in the body where by iron, zinc, iodine and vitamin A are the four major micronutrients of public health concern.

Worldwide both vitamin A deficiency (VAD) and iron deficiency anemia (IDA) affect over two billion people, among them 190 million preschool-age children (Bailey *et al*., 2015; WHO, 2009). The vitamin A deficient preschool children are associated by night blindness (Von Grebmer *et al.,* 2014). Zinc deficiency affects 1.2 billion people, 165 million of who are stunted children with a weak immune system (James and Matemu, 2018).

In East Africa, a wide range of TLVs are hardly or never consumed at all in some households, especially in urban and peri-urban centers, yet almost all of them are good sources of micronutrients (Aura, 2011). In Tanzania, the amount consumed is less than the recommended portion size per day (Msambichaka *et al.,* 2018). Millions of children suffer from one or more forms of malnutrition resulting in stunting, underweight, wasting and anemia (UNICEF, 2009). Additionally, the Tanzania Demographic Health and Survey (TDHS) (2015), shows 34% of children below five years old are stunted (low height-for-age), 5% are wasted (low weight-for height), 33% suffer from vitamin A deficiency and 33% are iron deficient (Chirande *et al.,* 2015).

About one third of children below five years of age are vitamin A deficient (TDHS-MIS, 2015- 2016). Similarly, 60% of children below five years of age suffer from iron deficiency and of which 58% are anaemic (TDHS-MIS, 2015-2016). Also stunting affects 31.8% and 35.1% minimal dietary intake of under five children while Lindi Region has a stunting prevalence of 23.8% and minimal dietary diversity of children at 1.5% (TNNS, 2018; Nyahende, 2019).

Vegetables, particularly TLVs are important sources of micronutrients, fiber, vitamins and minerals but are not being consumed in sufficient amounts by the households (Ochieng *et al.,* 2017). Ntwenya *et al.,* 2015 reported that there was a high consumption of vegetables during the rainy season compared to dry season. TLVs are highly seasonal and perishable which results in spoilage of large quantities during peak seasons. Development of TLV products with extended shelf life using locally processing techniques can help solve the problem of under consumed while making an important contribution to improve population income as well as availability at all time (Habwe *et al.,* 2008: Smith and Eyzaguirre, 2007).

This research therefore aims to develop iron rich powder formulations by using traditional leafy vegetables from Lindi, Tanzania. Nutritional and physico-chemical quality of TLVs will be established. In addition, microbial quality of the iron rich powders and acceptability of developed vegetable soups from the powders will also be determined.

1.3 Objective

1.3.1 General objective

Development of iron (Fe) rich products from traditional leafy vegetables (TLVs) grown in Lindi, Tanzania.

1.3.2 Specific objectives

The specific objectives are to:

i. Determine the nutritional and physico-chemical quality of solar dried TLVs
ii. Analyse microbial quality of solar dried TLVs and vegetable powder formulations
iii. Develop iron rich vegetable powder formulations and assess acceptability of the developed products

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional Leafy Vegetables (TLVs)

Traditional leafy vegetables were introduced over a century ago and owing to long use, have become part of the food culture in the sub-continent (Smith and Eyzaguirre, 2007). Production of TLVs in Eastern and Southern Africa has the potential to be highly profitable, offer employment opportunities, generate income and bring about increasing commercialization of the rural sector. However, traditional vegetable sector in Eastern and Southern Africa faces challenges such as lack of access to high quality seeds, including hybrids produced by local seed companies, high on-farm production losses, high perishability and postharvest losses (Chagomoka *et al*., 2014).

There are geographical and seasonal limitations in the availability of these vegetables. Mineral contents in vegetables are known to vary according to their availability in the soil at different collection sites and plant uptake (Nyembe, 2015). TLVs commonly sold in the public markets of Tanzania in the approximate order of popularity are leaf of amaranth, nightshade, cowpea, sweet potatoes, pumpkins and cassava (Lottery *et al*., 2014).

2.2 Nutritional content of TLVs

TLVs hold excellent potential to improve nutritional status of individuals and also increase dietary diversity in Tanzania. They provide adequate amount of dietary fibers, minerals, vitamins and other nutrients to people in developing countries (Lottery *et al*., 2014). TLVs are rich in vitamins, minerals, fibers, carbohydrates and proteins and some even possess medicinal values (Habwe *et al*., 2008). Some of vitamins and minerals found in selected TLVs as indicated in Table 2.1.

Table 2.1: Nutrient content of raw traditional leaf vegetables in Tanzania

TLVs	Vitamins		Minerals			
	Vitamin A µg(RE)	Vitamin C (mg)	Fe (mg)	Zn (mg)	Ca (mg)	Mg (mg)
Amaranth leaf	146.0	44.5	2.3	0.6	96.9	55.0
Cassava leaf	519.0	33.0	3.1	0.4	211.0	62.0
Cowpea leaf	519.0	32.4	0.8	1.4	49.7	62.0
Pumpkin leaf	550.0	24.5	0.6	1.1	114.5	28.4
Sweet potatoes leaf	303.0	21.8	0.5	0.2	59.2	44.8

Source: Lukmanji *et al.* (2008).

2.3 Health Benefit of Micronutrients

Micronutrients are nutrients that are only needed by the body in minute amounts, which play leading roles in the production of enzymes, hormones and other substances, helping to regulate growth, activity, development and the functioning of the immune and reproductive systems (Ekweagwu *et al.*, 2008). The consequences of micronutrient malnutrition include increased mortality rates, especially in women and children, poor pregnancy outcomes; increased morbidity, impaired mental and physical development in children; and reduced work productivity in adults (Millera and Welch, 2013).

2.4 Processing of TLVs

The perishability of TLVs possesses a major challenge in availability and distribution (Smith and Eyzaguirre, 2007). Processing can transform TLVs from perishable product to stable food with a long shelf life. Food dehydration is the process of removing water from food by circulating hot air through it, which prohibits the growth of enzymes and bacteria. Dried foods are tasty, nutritious, lightweight, easy-to prepare, and easy-to-store and use.

Drying is the method of preserving food including sun during, solar drying, freeze drying, and oven drying while the most common and oldest traditional method used in preserving vegetables is sun drying. Despite of the fact that sun drying is frequently used but yields poor quality produce since vegetables are exposed to dust, rain, wind and pests (Habwe *et al.,* 2008). Solar drying also uses the sun as the heat source though a foil surface inside the dehydrator helps to increase the temperature while ventilation speeds up the drying time. Shorter drying times reduce the risks of food spoilage or mold grow (Ahmed *et al.,* 2013). Moreover solar dryers, are preferred because they produce consistent products and do not have the disadvantages of traditional driers.

2.5 Physical chemical parameter of TLV

The physicochemical properties (Moisture content, dry matters and pH) of traditional leafy vegetables are important in determining the storage quality of the vegetables, the higher the moisture content and water activity the lower the storage time (Pereira *et al.*, 2013). To avoid microbial growth, the moisture content and water activity must be kept below approximately 10% and 0.60–0.65, respectively depending on the type of food (Zambrano *et al*., 2019).

2.6 Risks Associated with Consumption of Contaminated Vegetables

Although vegetables are generally rich in micronutrients, they can be easily contaminated with water, animal manure and dust particles contaminated by microorganisms, including potential human pathogens. Vegetables can be contaminated with enteric bacteria of medical and public health importance during cultivation, harvest, transportation and further processing. Enteric bacteria notably *Salmonella, Escherichia and Shigella* species continue to be major global health problems and are the leading causes of morbidity and mortality in both developed and developing countries (Alemu *et al.*, 2018). Others include

Listeria monocytogenes, Staphylococcal aureus which have been reported in outbreaks of foodborne diseases (Tango *et al.,* 2014).

2.7 Sensory Evaluation

Sensory evaluation is scientific discipline used to evoke, measure, analyse and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Singh-Ackbarali and Maharaj, 2014). The environment in which sensory test is conducted should be carefully controlled and samples must be prepared in a uniform fashion so as not to influence panelists` perception of the foods` quality. Panelists` who are well suited to the purpose of the sensory test should be selected and trained appropriately. In Sensory evaluation the numerical data is collected to establish lawful and specific relationships between product characteristics and human perception followed by proper analysis of the data as critical part of sensory testing and lastly inter-pretention of result (Lawless and Heymann, 2010). It is used in product development to reveal insights into the ways in which sensory properties drive consumer acceptance and behavior, and to design products that best deliver what the consumer needs.

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CHAPTER THREE

Paper One

Nutritional and physico-chemical quality of solar dried Traditional Leafy Vegetables (TLVs) from Lindi, Tanzania

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3.1 Abstract

The essential micronutrients in traditional leafy vegetables (TLVs) could substantially contribute to the micronutrient supply in Tanzania communities but concentrations differ among vegetables. The current study was conducted to determine nutrient content of the three selected traditional leafy vegetables namely amaranths (AML), sweet potato (SPL) and cassava leaves (CAL), grown in Ruangwa and Nachingwea Districts in Lindi Region. Samples were purposively collected from home gardens (HG) and low land (LL) and transported to Morogoro for sample preparation and solar drying. After solar drying, they were ground to produce fine powders. Both solar dried vegetables and powdered formulations were analysed in triplicate for microbial, nutritional and physico chemical quality at the Tanzania Bureau of Standards (TBS). Data was analyzed using R – statistical package (R version 4.0.3 2020). One-way analysis of Variance (ANOVA) was carried out to determine significance difference among the micronutrients, physico-chemical among vegetables and across the site, Means were separated using Tukey's Honest at $p<0.05$. Significant differences in iron, zinc and B-carotene content were found between vegetables at $p<0.05$. For solar dried Traditional leafy vegetables (TLVs), AML had the highest iron content, CAL had the highest zinc while SPL had the highest beta-carotene. Moreover, CAL and SPL had statistically significant higher ($p<0.05$) ascorbic acid than AML. For home grown vegetables, zinc and ascorbic acid content were highest in CAL whereas beta-carotene was highest in SPL. For vegetables grown under low land AML had the highest iron content ($p<0.05$).

3.2 Introduction

Vegetables are important for human health because of their vitamins, minerals, phytochemical compounds, and dietary fiber content. Especially antioxidant vitamins (vitamin A, vitamin C, and vitamin E) and dietary fiber content have important roles in human health. Adequate vegetable consumption can be protective some chronic diseases such as diabetes, cancer, obesity, metabolic syndrome, cardiovascular diseases, as well as improve risk factors related with these diseases (Ulger *et al*., 2018).

WHO currently recommends consuming at least 400 grams of fruit and vegetables each day or five servings of 80 grams each depending on what is locally available, affordable, and socio-culturally acceptable (WHO, 2003 and Chattopadhyay, 2018). Around 1000 different species of indigenous and naturalized vegetables contribute to the dietary diversity, food security, and livelihoods of populations across sub-Saharan Africa. These foods are also a part of alimentary traditions and cultural identity, but have suffered as neglected and underutilized species (Towns and Shackleton, 2018).

Despite of the importance motioned TLVs are seasonal available thus need to be preserved and traditional sun drying often preferred. In recent years, it has become an improved alternative to the traditional sun drying method with solar drying, foods are dried in the shade and high air temperature and low humidity are provided in order to hasten the drying rate, thus retaining more nutrients and reducing the final moisture content. It also increases the micronutrient concentration in the dried products and enables the products to be stored longer (Ukegbu and Okereke, 2013).

Awareness of importance of TLVs to nutrition is not very high; it is mainly perceived as being nutritious because the generations before have survived on them. Traditional leafy vegetables have been labelled as ‘poverty food’ (Van Rensburg Willem, 2007). Hence, there is a need to determine the micronutrient content of the solar dried TLVs and developed products as well as their physico-chemical quality so as to increase awareness and make recommendations for improving the intake.

3.3 Material and Methods

3.3.1 Study area

The study was carried out at Mibure and Mitumbati, villages from Ruangwa and Nachingwea districts respectively in Lindi region. Lindi region is situated in Southern Tanzania between latitudes 70 55’ and 100 50' South of the equator and longitudes 360 51’ to 400 East. It is a coastal town located at the far end of Lindi Bay, on the Indian Ocean in southeastern Tanzania. The dominant climate is hot and humid.

3.3.2 Study design

A cross-sectional design was used to collect data. Cross sectional design was used in this study. Samples for physico-chemical parameters and nutritional content (Total plate count and *E. coli*) were drawn from three TLVs across the sites (home garden and low land).

3.3.3 Sampling plan and sample collection

Purposive sampling procedure was used to collect samples from selected points. Sampling was carried out in the morning during a rainy season from February to March 2019. Amaranths (*Amaranthus hybridus L.*) Sweet potatoes (*Ipomea batatas*) and Cassava (*Manihot esculenta*) samples were collected in duplicate from the two selected sites, home garden (HG) and low land (LL). Three types of TLVs were collected in duplicate from 2 points (HG and LL), making a total of 12 samples. The collected samples were transported in a cool box containing ice maintained at 4°C for 12 hours to Sugeco

(Sokoine University Graduate Entrepreneurs cooperative, SUA, Morogoro) for sample preparation and solar drying.

3.3.4 Sample preparation

About 2.5 kg of each of the fresh TLVs samples was thoroughly washed with potable water to remove adhering physical dust and impurities, and the leafy edible parts of the vegetables were separated from the main plant. They were then sliced, blanched at 80°C for 2 minutes. The blanched vegetables were then drained and spread on trays for 10 -15 minutes. Solar drying was done at SUGECO (Sokoine University Graduate Entrepreneurs Cooperative, Morogoro) as per procedure described by Mongi (2013) with some modifications.

Blanched samples were loaded into the solar dryer. The temperature in the solar dryer ranged between 45-55°C and drying was completed in 3 days. About 1.5 kg of each solar dried TLVs was separately packed in a labeled food bag and stored at room temperature in a dark dry place.

The solar dried vegetables were ground by a machine (Gaoxin 1250 gx-25, China) to produce powders, which were passed through a fine 315-micron, China) to obtain fine powders. TLVs powders were then packed in labeled food bags and stored at room temperature (25°C) in a dark dry place prior to laboratory analysis.

Solar dried vegetables powders were transported under room temperature in dark bags to the Tanzania Bureau of Standards (TBS) laboratory for and physico-chemical and nutritional analysis. These were analyzed in triplicate to make a total of 36 determinations for each parameter

3.3.5 Physico-chemical analysis of solar dried TLVs

3.3.5.1 Moisture content

Moisture content was determined by oven drying according to AOAC method 920.151 (AOAC, 1990). Three types of TLVs (AML, CAL and SPL) and mixed formulations (FI, F2, F3 and F4) were dried in an oven at 105^{o} C for 3 hours with cooling being done in a desiccator for 10 min. Moisture content was calculated as the loss in weight expressed as a percent of the original weight of the sample.

3.3.5.2 Dry matter

This was calculated based on moisture content by using the Equation 2 in chapter 3 as explained by (Chacha, 2017).

$\%Dry\ Matter = 100\% - \%Moisture$

3.3.5.3 pH

This was done according to ISO 10523:2008. Results were reported in two decimal points.

3.3.6 Nutritional content (Iron, zinc, beta-carotene and ascorbic acid) of solar dried TLVs

3.3.6.1 Determination of iron and zinc in solar dried TLVs

Both dried TLVs and formulated products were analysed for Iron and Zinc were determined as described by Zhao0 *et al.* (2015) with modifications. using Microwave plasma atomic emission spectrometry (MP-AES, Agilent technologies model 4210 operated with MP Expert G8007A version 1.6.0.9255 from USA). The detection wavelength of iron and zinc were 371.993nm and 481.053nm respectively. Detection limit and quantification unit for minerals and ten times signal to noise ratio respectively (Hostetler *et al.* (2020).

3.3.6.2 Determination of beta-carotene in solar dried TLVs

β-carotene analysis was performed by using the methods described by Rodriguez-Amaya and Kimura (2004) as well as Chege and Kimiywe (2016) with modifications. Briefly extraction of β - carotene, 1 gram of sample was weighed (Mether Toledo – Excellent plus, XP 205, Switzerland) into 50 mL amber volumetric flask followed by addition of 10 mL distilled water for sample rehydration. Extraction was done by adding 10 mL of cold acetone–hexane (2:1) mixture containing 0.5% BHT was added and mixed for 1 min. The mixture was saponified overnight by adding 10 mL of 0.5M methanolic potassium hydroxide and shaken by vortexing for 1 min then was filtered with glass wool filter paper. The filtrate was partitioned in separating funnel with two portions of 25 mL petroleum ether. Hexane and aqueous layers were discarded. Petroleum ether was washed with 10 mL distilled water then was filtered with glass wool filter paper containing about 2 g of anhydrous sodium sulphate. The filtrate was concentrated in a rotary evaporator (Rotavapor R-215, Switzerland) at 40 °C vacuum temperature, 200 bar, 10 rpm for 15 min and reconstituted with petroleum ether to 3 ml. The potency of β-carotene standard was determined by dissolving 0.1g of standard into 100 ml amber volumetric flask with petroleum ether. Concentration was determined by using extinction coefficient of β-carotene in petroleum ether (Davies, 1976). Briefly The beta carotene analysis was performed on an HPLC (Nixera2 LC-30AD, Canada), equipped with an auto-sampler (SIL-30AC, Canada) set at 10 μL injection volume, pump (LC-30AD) set at 0.6 mL/ min, Degasser (DGU-20A3R, USA), column oven (CTO-201AC) set at 25 °C and Diode array detector (DAD-20A) set at 450 nm; all from SHIMAZDU - Japan. Colum used was YMC C30 3 μm, 250 x 2.0 mm; Mobile phase was 20 mM ammonium acetate in Methanol-water (98:2) (A) and Methyl tert-butyl ether (MTBE) (B) and the gradient program for analysis was done as Hostetler *et al.* (2020). The quality control was done using two samples spiked with known concentration of 1 mg/L and 0.6 mg/L used for establishing method accuracy. The recovery was computed using two-spiked sample

3.3.6.3 Determination of ascorbic acid in solar dried TLVs

The method of extraction of ascorbic acid was adopted from Odriozola-Serrano *et al.*, (2007) with minor modifications. A blank vegetable sample that was previously open sun dried to remove all ascorbic acid was used (Kiremire *et al.*, 2010). The mixture was then centrifuged at 22100g for 15 minutes, supernatant was filtered with filter paper no 1. The extract was further filtered through 0.45 µm (Whatmann- Ramniklal and Co. India) for HPLC analysis.

The analysis was performed on HPLC (Nixera2 LC-30AD, Canada), equipped with an auto-sampler (SIL-30AC- Canada) set at 10 µL injection volume, pump (LC-30AD) set at 1.0 ml/ min, Degasser (DGU-20A3R, USA), column temperature (CTO-201AC) set at 25 °C and Diode array detector (DAD-20A) all from SHIMAZDU - Japan. Colum used was Zorbax Eclipse Plus C18 – USA, Reverse phase 5 µm, 4. 0 µm × 150mm, mobile phase 0.02% phosphoric acids (pH=2): Methanol 95:5, Elution: Low pressure gradient. Detection was at 245nm, run time= 9 minutes, peak detection time =1.9 minutes and column temperature 25 ˚C. Quality control were done using spiked samples by calculating the recovery.

3.3.7 Statistical Data Analysis

Descriptive statistic was used to describe, summarize and present data for nutritional content of selected nutrients (Fe, Zinc, beta-carotene and ascorbic acid,) in dried TLVs. Complete randomized design (CRD) was used to analyse physico-chemical qualities of dried TLV with the following model;

$$Y_{ijkl} = \mu + \tau_i + \alpha_k + \tau\alpha_{ik} + \varepsilon_{ijkl}$$

Where by:

i=1,2

j=1,2

k=1,2,3

Y_{ijk} = *Dependant variable, μ=General mean, and ε= Random error*

τ_i =*is the ith location effect*

α_k = *is the kth type of vegetable effect*

Data was analyzed by R –statistical package (R version 4.0.3 2020) software (R Core Team 2012). One-way analysis of Variance (ANOVA) was carried out to determine if there was significance difference in micronutrients, and physico-chemical between selected TLVs while two-way ANOVA was used to determine the effect of growth site on the nutritional value of the different TLVs. Mean values with different superscripts letters down the column were significantly different from each other at different at $p<0.05$ (Tukey's Honest). Means were separated using Tukey's Honest at $p<0.05$.

3.4 Results

The linearity of calibration curve was found to be within acceptable limit of 0.998 for both iron and zinc (Christian, 2007). Method percent recoveries for both metal iron and zinc were 99.12 and 95.57 respectively and found to be within the acceptable range of 80—120% for metal analysis (Harvey, 1999). (Table 3.1).

Table 3.1: Quality control for selected minerals and vitamins

	Iron	Zinc	Beta carotene	Ascorbic acid
Linearity (r^2)	0.9994	0.9993	0.997	0.998
Recovery (%)	99.12±0.35	95.57±0.11	94.5±1.03	93.7±0.76
DL	1.7 µg/L	3.1 µg/L	0.54 mg/L	1.05 mg/L
QL	19.4 µg/L	35.2 µg/L	6.09 mg/L	5.9 mg/L

DL- Detection limit and QL- Quantification limit

3.4.2 Physico- chemical (moisture content, pH and dry matter) and nutritional quality (iron, zinc, beta-carotene and ascorbic acid) of solar dried TLVs

3.4.2.1 Physico-chemical quality of solar dried TLVs

Cassava leaves had the highest moisture content (and lowest dry matter) which was significantly different from amaranths and sweet potato leaves at $p<0.05$. The pH for amaranth and sweet potato leaves did not differ statistically ($p<0.05$). Moreover, there was significant difference in mean pH ($p<0.05$) among all the solar dried TLVs. Amaranth leaves had the highest pH ($p<0.05$) followed by sweet potatoes leaves while cassava leaves were the lastly. (Table 3.2).

Table 3.2: Physico-chemical quality of solar dried TLVs.

TLVs	Moisture (%)	Dry matter (%)	pH
AML	8.47 ± 0.55^b	91.53 ± 0.55^a	6.93 ± 0.28^a
CAL	9.17 ± 0.58^a	90.57 ± 0.89^b	5.75 ± 0.94^c

SPL	8.12±0.51[b]	91.89±0.51[a]	6.21±0.06[b]

TLVs- Traditional Leafy Vegetables; AML-Amaranth leaves; CAL-Cassava leaves; SPL- Sweet potatoes leaves

3.4.2.2 Nutritional quality of solar dried TLVs

Amaranth leaves had significantly higher iron content ($p<0.05$), whereas zinc was highest in cassava and sweet potato leaves had the highest beta-carotene (Table 3.3). Ascorbic acid content was highest in cassava and sweet potato leaves which was statistical differed from amaranth leaves (Table 3.3).

Table 3.3: Nutrient content in solar dried TLVs (mg/100g DW)

TLVs	Iron	Zinc	Beta-carotene	Ascorbic acid
AML	80.21±8.75[a]	2.93±1.43[b]	40.18±0.05[b]	17.4±1.12[b]
CAL	10.74±0.21[c]	4.12±0.30[a]	17.18±0.12[c]	25.48±4.29[a]
SPL	30.95±9.09[b]	1.02±0.08[c]	41.47±0.05[a]	24.39±1.04[a]

TLVs- Traditional Leafy Vegetables DW=Dry weight AML-Amaranth leaves; CAL-Cassava leaves; SPL- Sweet potatoes leaves

3.4.3 Physico-chemical and nutritional quality (Iron, zinc, beta-carotene and ascorbic acid) (Moisture content, dry matter and pH of solar dried TLVs across the sites

3.4.3.1 Physico-chemical quality of solar dried TLVs across the sites

The physico-chemical quality of TLVs across the sites are shown on Table 3.4 There were no significant differences ($p<0.05$), in mean moisture content (and dry matter content) of cassava across the sites (HG and LL), while significant differences were observed between amaranth and sweet potatoes leaves across the sites. On the other hand, significance differences in pH were observed for amaranth leaves from both sites while no significant differences were observed in cassava and sweet potatoes leaves across the sites ($p<0.05$). Amaranth leaves from home garden had the highest pH while cassava leaves from both sites had the lowest value (Table 3.4).

Table 3.4: Physico-chemical quality of solar dried TLVs across the sites

Site	Vegetables	Moisture (%)	Dry matter (%)	pH
HG	**AML**	$8.93{\pm}0.40^{a}$	$91.07{\pm}0.40^{bcd}$	$7.17{\pm}0.01^{a}$
	CAL	$9.11{\pm}085^{a}$	$90.38{\pm}1.29^{d}$	$5.76{\pm}0.01^{d}$
	SPL	$7.62{\pm}0.12^{c}$	$92.38{\pm}0.12^{a}$	$6.26{\pm}0.11^{c}$
LL	**AML**	$8.01{\pm}0.11^{bc}$	$91.99{\pm}0.11^{ab}$	$6.69{\pm}0.21^{b}$
	CAL	$9.23{\pm}0.15^{a}$	$90.76{\pm}0.15^{cd}$	$5.75{\pm}0.13^{d}$
	SPL	$8.59{\pm}0.09^{ab}$	$91.41{\pm}0.09^{bc}$	$6.16{\pm}0.01^{c}$

*w=Dry weight, AML-Amaranth leaves; CAL-Cassava leaves; SPL- Sweet potatoes leaves HG- Home garden; LL-Low land

3.4.3.2 Micronutrient content among solar dried TLVs across the sites

There were significant differences ($p<0.05$) in mean iron for amaranth and sweet potatoes leaves across the sites (HG and LL), while no significance difference ($p<0.05$) found in cassava leaves between the two sites. Amaranth leaves from low land had significantly highest iron content ($p<0.05$) followed by sweet potatoes leaves, and finally cassava leaves. Additionally, across the site significant differences observed for zinc content in amaranth while there were no significant differences for cassava and sweet potatoes leaves at $p<0.05$.

Though sweet potato leaves from home garden had the highest beta-carotene content, and cassava leaves from home garden had the lowest values, there were significant differences in mean beta-carotene for all types of dried TLVs across the sites at $p<0.05$. Significance difference ($p<0.05$) was observed in mean ascorbic acid content among cassava leaves from both sites. No significant differences in mean ascorbic acid was observed for sweet potatoes and amaranth leaves across the sites at $p<0.05$ (Table 3.5).

Table 3.5: Micronutrient content (mg/100g) DW of solar dried TLVs across the sites

Site	TLVs	Iron	Zinc	Beta-carotene	Ascorbic acid
HG	**AML**	72.05±1.57[b]	4.21±0.72[a]	42.26±0.29[b]	16.43±0.17[d]
	CAL	10.58±0.12[e]	4.35±0.17[a]	10.95±0.56[f]	28.52±4.60[a]
	SPL	22.25±0.29[d]	1.03±0.09[c]	43.43±0.81[a]	25.22±0.21[ab]
LL	**AML**	88.36±2.51[a]	1.64±0.18[b]	38.10±1.07[d]	18.36±0.81[cd]
	CAL	10.91±0.11[e]	3.86±0.156[a]	23.42±0.07[e]	22.44±4.20[bc]
	SPL	39.65±0.15[c]	1.01±0.07[c]	39.51±1.09[c]	23.57±1.11[ab]

DW=Dry weight AML-Amaranth leaves; CAL-Cassava leaves; SPL- Sweet potatoes leaves. HG- Home garden; LL-Low land

3.5 Discussion

3.5.1 Physico –chemical (iron, zinc, beta-carotene and ascorbic acid) and nutritional quality (moisture content, pH and dry matter) among solar dried TLVs

3.5.1.1 Physico-chemical quality of solar dried among TLVs

Cassava leaves had the highest moisture content while sweet potato leaves and amaranth had statistically similar moisture contents (Table 3.2). These findings were different from Umuhozariho *et al.* (2014) who found lower moisture contents for the different cassava varieties moisture (4.6%). The author however suggested that water content of the vegetables was usually between (4-8 %) which was considered safe moisture for storage.

A study done by James and Matemu (2016) reported lower moisture content 7.46% and higher dry matter 92.4 %) for solar dried sweet potato leaves than from present study. The solar dried amaranth in present study (Table 3.2) had a moisture content which is similar to finding of 8.5% reported by Akonor and Amankwah (2012) but differed from the value repoted by Ronoh *et al.*, 2010 and (Singh *et al*., (2014): Khatoniar *et al*., (2019) who reported lower values 3.9-5.8%.

Cassava leaves had lower dry matter content due to higher moisture content while amaranth leaves and sweet potatoes leaves were found to contain higher amount of dry matter content due to low moisture content. This could be due to different environment into which drying took place such humidity and pretreating conditions of the sample before drying. This was supported by Mongi (2013) who explained that different factors such as the ambient temperature, air velocity, amount of sun, relative humidity, initial moisture contents and surface area of the food exposed to the drying air contributed to the efficiency of the drying process when solar drying fruits and vegetables. Variations between vegetables dried under the similar drying conditions as observed in this study could also be explained by their compositions (Alonge and Adeboye, 2012). The differences in the moisture content among the vegetables varieties can be attributed to the difference in the genetic composition, stage of maturity and also the agricultural practices gap between harvesting time and analysis (Nicanuru, 2016). According to Kolawole *et al*. 2010, dried food substances, particularly vegetable crops, with high moisture values will favor the growth of microorganisms at a high growth rate, and moisture content of greater than 15% is said to promote enzymatic reactions and interactions of other constituents of the dried products such as loss of vitamins. In addition, when the moisture content of dried materials was less than 10% such materials are considered as more proper for keeping quality of soup ingredients (Abdel-Haleem, 2014).

The observed values of all the leafy vegetables were found to have high pH and less acidic thus low-acid food. Therefore, green leafy vegetable acts as a buffer and maintains the proper alkalinity of the blood by balancing acid producing foods like meats. It is for this reason that high consumption of vegetables is recommended since they play a significant role in the prevention and management of chronic disease such as associations with cancer and cardiovascular disease (Hung *et al.*, 2004).

3.5.1.2. Iron and zinc content among solar dried TLVs

The study revealed that the iron content of solar dried TLVs were of the following increasing order: cassava<sweet potato< amaranths was shown in (Table 3.3.) The findings from this study are nearly to those reported by Khatoniar, *et al*., (2019) and Chege *et al*., (2014) who found the iron content of 86.80 mg/100g for solar dried Amaranths. Although a study by Yadav and Sehgal (2002) found lower values of 60.63 mg/100g but other researchers found iron content with range (111.86 to 284.38 mg/100g higher (Gupta *et al.,* 2013; Nico Demas, 2013) compared to the value obtained in the present study.

Moreover, iron content of cassava leaves (Table 3.3) was also nearly similar (9.44 mg /100g) to the study by Chávez *et al.* (2000). The reason for the trend could be due to different vegetable varieties that were used. Crop variety differ specifically in nutrient requirements as well as final nutrients contents of a particular crop (Hornick, 2010). The result indicated that solar drying tend to remove water hence concentrate the minerals (Ukegbu and Okereke, 2013).

However, it should also be taken into account that the iron of plant origin is less absorbed than the one from animal origin (Wobeto *et al.,* 2006). This is due to the fact that heme iron usually absorbed at higher rate than non- heme iron. Thus the development of iron rich vegetable products are of importance since composite meals with vegetables that contain ascorbic acid enhances iron absorption (Hurrell, 2010).

Zinc levels were found to the following increasing order among TLVs cassava<amaranth<sweat potatoes (Table 3.3). The finding by Chávez *et al.* (2000) suggested nearly similar finding zinc content in cassava leaves 5.16 mg/100g. The range of zinc content (3.5-6.7 mg/100g) provided by Wobeto *et al.* (2006) was in line with the obtained values by present study. Other studies reported higher values in amaranth leaves 27.28 ± 1.43mg/100g and 8.39 to 15.36 mg/100g respectively Chege *et al.* (2014) and Nicodemas, 2013). Moreover, zinc content for sweet potato leaves found to be nearly the same 1.39 mg/100g) as stated by James and Matemu (2016). The high levels of zinc in cassava leaves compared to other TLVs may probably due to cultivars used. In addition, the combination of plant genes can accumulate significantly higher concentrations of certain minerals which depends on several factors including the environment (Narayanan *et al.*, 2019).

3.5.1.3 Beta-carotene and ascorbic acid content among solar dried TLVs

Beta-carotene was found to be highest in sweet potato leaves followed by amaranth leaves whereas cassava leaves had the least. Results for amaranth (Table 3.3) were in line with the study reported findings (40.11mg/100gm) by Chege *et al.* (2014) and Mulokozi *et al.* (2014). The study by Traoré, *et al.* (2017) indicated beta-carotene content of amaranth 25.25 mg/100g which was lower than the findings reported in the present study. The beta-carotene content value of cassava found in the present study however was lower than that observed value 25.3- 63.51mg/100gm by Umuhozahiro *et al.* (2014). Mwanri *et al.* (2011) explained that beta carotene was the most dominant carotenoid in sweet potato leaves varieties. However, nutrients content of vegetables decreases with increase in plant maturity stage. Generally, the observed differences in nutrient content could be attributed to the differences in cultivars, soils, maturity stage or the climatic conditions (Wobeto *et al.*, 2006).

Ascorbic acid was found to be highest in cassava and sweet potatoes leaves while amaranth leaves found to be the least. The ascorbic acid content of sweet potato leaves in the current study (Table 3.3) was lower than that observed by Mwanri *et al.* (2011). The ascorbic acid content for amaranth was nearly similar to the study by Khatoniar (2019), but lower (31.0mg/100g) than Kiremire *et al*. (2010). The observed ascorbic acid content of cassava leaves was lower than suggested by Umuhozahiro *et al.* (2014). The variation of ascorbic acid content in various vegetables could be due to variety and age of plant. Salvador *et al*. (2014) stated that the nutritional content of vegetables varied depending on location, variety, age of the plant and environmental conditions.

3.5.2 Physico- chemical (moisture content, pH and dry matter) and nutritional quality (iron, zinc, beta-carotene and ascorbic acid) of solar dried TLVs across the sites

3.5.2.1 Physico-chemical quality of solar dried TLVs across the sites

Results obtained indicated that values obtained were below 12% moisture content which is recommended for safe storage of dried vegetables for more than six months (James and Matemu, 2016). The higher the dry matter content (not less than 90%) and low moisture was found from both sites thus justified that solar drying was effectively done. Accordingly, reductions in moisture contents resulted in corresponding increases in dry matter contents due to concentration of soluble solids with relatively chemically stable products (Mepba and Banigo, 2007). The low moisture attained could prevent deterioration reactions, hence improving dried vegetable product storage quality for longer periods and making them available and accessible for dietary diversification throughout the year (James and Matemu, 2018). The different values obtained in this study could be contributed by variety, season, stage of vegetable maturity, environmental aspects and losses between harvesting and analysis. Storage time of vegetable and extraction process of the samples must be kept in control because all these have a tremendous effect on the results (Nicanuru, 2016).

3.5.2.2 Nutritional content among solar dried TLVs across the sites

The study revealed that, for home grown vegetables, zinc and ascorbic acid contents were highest in CAL whereas beta-carotene was highest in SPL. For vegetables grown under low land AML had the highest iron content ($p<0.05$). The observed difference in nutrient content among site could be due to different variety of plant used under the study, soil and soil fertility of the respective sites. Other studies supported the findings that the differences could be attributed to not only the soils but maturity stage or the climatic conditions (Wobeto *et al*., 2006). Additionally, chemical and biological processes of maintaining soil fertility in different parts of the world can lead into these differences of attaining crop nutrients at different levels (Nicodemas, 2013). However, composition of plants is determined by a number of variables that include genetic makeup, the type of soil in which the plants are grown, seasonal effects, stage of maturity, and the type and quantity of fertilizer used in production of the plants (Dangour *et al*., 2014).

3.6 Conclusion

Solar dried TLVs provide an excellent source of micronutrients. Solar dried amaranth had highest iron, cassava leaves contained highest zinc and ascorbic acid whereas sweet potatoes leaves contained highest in beta-carotene. In addition, zinc, beta-carotene and ascorbic acid were found to be higher in vegetables grown from home garden whereas iron content was highest in vegetable grown from low land. However, based on the nutrient of interest iron study revealed that amaranthus should be used in higher proportional during iron rich product development due to higher iron content compared to other TLVs used. Moreover TLVs could be combined in various proportions and formulations be made to optimize the requirements for various groups in the society, especially the vulnerable children, elderly and the immuno-compromised individuals. This will ensure that people live a healthy life which is 3rd Sustainable Development Goal of the United Nations.

3.7 Recommendations

i. The study recommends the use of solar drying as a viable strategy to address the seasonality gaps.

ii. To combine various vegetables in different ratios to optimise essential nutrients to combat micronutrient deficiency.

iii. Multipurpose use of solar dried traditional leafy vegetables should be promoted to produce value added products, such as soup, porridge, baked products as a means of diversifying uses of these potential food commodities.

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CHAPTER FOUR

Paper Two

Safety of Traditional Leafy Vegetables Powders from Lindi in Tanzania

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4.1 Abstract

Micronutrient deficiencies affect many Party of Tanzania and Lindi Region is among the affected Region. To combat this problem, three types of Traditional Leafy Vegetables (TLVs) - Amaranths (AML), Sweet potato (SPL) and Cassava leaves (CAL), grown in Ruangwa and Nachingwea Districts in Lindi Region were carefully collected from home gardens (HG) and low land (LL). They were solar dried and made into powders that had been optimized for Iron content. The vegetable powders were then mixed with water and spices to make four (4) soup formulations. The aim of this study was to evaluate the microbiological quality (Total plate count and E. coli) of the dried TLVs and formulated vegetable powders. Significant differences ($p \leq 0.05$) in microbial load among solar dried vegetables were observed. Sample CAL had the highest load (3.67 x $10^{2)}$ whereas sample SPL had the lowest (3.15 x $10^{2)}$. The microbial load between the two sites also differed significantly ($p<0.05$). However, there were no significant differences ($p \leq 0.05$) in microbial quality of the formulated vegetable powders, all of which were below the TBS standards. No *E. coli* was detected in any of the samples studied. The absence of *E. coli* in the samples indicates proper hygienic handling of the vegetables.

Keywords: *Micronutrients, Vegetables Soup powder, Safety, E. coli, Solar drying*

4.2 Introduction

Vegetables are low in calories, high in fiber content and are also the best sources of antioxidants and other phytonutrients (Niththiya *et al.*, 2014). Adequate vegetables consumption can be protective to some chronic diseases such as diabetes, cancer, obesity, metabolic syndrome, cardiovascular diseases, as well as improve risk factors related with these diseases (Ulger *et al.,* 2018). Vegetables are abundant during the wet season but without post-harvest preservation, the excess after consumption goes to waste which limits their marketability (Chege and Kimiywe, 2016). The high moisture content of Traditional leafy vegetables (TLVs) renders them perishable while their seasonal availability limits their utilization all year round (Njoroge *et al*., 2015). Due to the high perishability nature of the TLVs, most are dried without adding any preservatives so as to enhance availability throughout the year (Managa *et al*., 2020). However, solar drying is recommended for preservation of green leafy vegetables (Chege *et al.,* 2014). The removal of moisture arrests the growth of microorganisms that would cause decay and minimizes many of the moisture mediated deterioration reactions (Ahmed *et al.*, 2013). Any microbial contamination in leafy vegetables is commonly associated with the environment through which the product has passed (Taura and Habibu, 2009). Consumption of these types of vegetables, if not prepared hygienically could be the source for ingestion of considerable numbers of human pathogenic bacteria resulting in diseases (Kimaro, 2017). Hence microbiological control is important in food industry as to prevent food borne diseases and provide safe and quality product. This study was carried out to assess the microbiological quality of raw and processed traditional leafy vegetables for health and safety of consumers.

4.3 Materials and Methods

4.3.1 Study area

The study was carried out at Mibure and Mitumbati, villages from Ruangwa and Nachingwea districts respectively in Lindi region. Lindi region is situated in Southern Tanzania between latitudes 70 55’ and 100 50' South of the equator and longitudes 360 51’ to 400 East. It is a coastal town located at the far end of Lindi Bay, on the Indian Ocean in southeastern Tanzania. The dominant climate is hot and humid.

4.3.2 Materials

Materials used included Traditional Leafy Vegetables (TLVs) namely Amaranths leaves (*Amaranthus hybridus* L.) (AHL), sweet potato leaves (*Ipomoea batatas*) (IBL) and cassava leaves (*Manihot esculenta*) (MEL) from two villages Mtumbati and Mibure in Lindi Region, Tanzania and vegetable soup powder formulations.

4.3.3 Study design

Cross sectional design was used in this study. Samples for microbiological parameters (Total plate count and *E. coli*) were drawn from three TLVs across the sites (home garden and low land).

4.3.3.1 Sampling plan and sample collection

Purposive sampling procedure was used to collect samples from selected points. Sampling was carried out in the morning during a rainy season from February to March 2019. Amaranths (*Amaranthus hybridus* L.) Sweet potatoes (*Ipomea batatas*) and Cassava (*Manihot esculenta*) samples were collected in duplicate from two sites, home garden (HG) and low land (LL) from Lindi. Thus, a total of 12 samples were collected i.e. 3 types of TLVs *2 points (HG and LL) * in duplicate, all in duplicates, making a total of

12 sample from both sites. In addition, 4 vegetable soup formulations were also analysed in duplicates (4*2) samples make up 8 samples). The total sample size was 20 (12 for TLVs and 8 for vegetable formuation). These samples were analyzed in triplicate for each (20*3) to make a total of 60 parameters.

Samples collected from the sites were transported in closed polyethylene bags, which were stored in a cool box containing ice maintained at 4°C to SUGECO (Sokoine University Graduate Entrepreneurs cooperative, SUA, Morogoro) for sample preparation and solar drying. Both solar dried vegetables and powdered soup formulations were transported to the Tanzania Bureau of Standards (TBS) laboratory for microbial analysis.

4.3.4 Sample preparation

About 2.5 kg of each of the fresh TLVs samples was thoroughly washed with potable water to remove adhering physical ring dust and impurities, and the leafy edible parts of the vegetables were separated from the main plant. They were then sliced, blanched at temperature of 80°C for 2 minutes. The blanched vegetables were then drained and spread on trays for 10 -15 minutes. Solar drying was done at Sugeco (Sokoine University Graduate Entrepreneurs cooperative, Morogoro) as per procedure described by Mongi (2013) with some modifications. Blanched samples were loaded into the solar dryer. The temperature in the solar dryer ranged between 45-55°C and drying was completed in 3 days. About 1.5 kgs of each dried TLVs were packed separately in labeled freezer bags and stored at room temperature in a dark dry place. After solar drying, the dried vegetables were ground by a machine (Gaoxin 1250 gx-25, China). Each TLVs was ground separately and passed through a fine 315-micron sieve to obtain fine powders. TLVs powders were then packed in labeled food freezer bags and stored at room temperature (25°C) in a dark dry place prior to product formulations and analysis.

4.3.5 Product formulation

Three types of TLVs samples were used to formulate iron rich powders. Various proportions of were used based on iron optimization to meet the RDA of iron for children aged between 1-5 years (Matemu, 2018). Table 4.1 shows the amount of solar dried TLV samples and Table 4.2 shows spices used to make formulation after pretesting in the Tanzania Bureau of Standards (TBS) laboratory. The powder formulations were made by mixing 90g of solar dried TLVs with 10g of spices (F1, F2, F3 and F4). These formulations were analysed for microbiological quality

Table 4.1: Vegetable powder formulations from 3 traditional leafy vegetables (90% of formulation)

Materials	F1	F2	F3	F4
(Amount per g)				
***Amaranthus hybridus L* (AML)**	60.0	70.0	80.0	40.0
***Manihot esculenta* (CAL)**	7.50	5.00	2.50	10.0
***Ipomea batatas* (SPL)**	22.5	15.0	7.50	40.0
Total	90.0	90.0	90.0	90.0

AML- amaranth leaves CAL-cassava leaves SPL-Sweet potatoes leaves

Table 4.2: Spices added for soup formulations

Spices	(Amount per g)
Garlic	0.5
Ginger	0.5
Coriander	0.5
Cumin	0.5
Corn Flour	4.0
Salt	2.0
Sugar	2.0
Total	**100**

4.3.6 Microbiological analyses

4.3.6.1 Media preparation and storage

All the media used in this study were prepared according to manufacturer’s instructions.

4.3.6.2 Preparation of analytical sample by serial dilution

About 25g of each vegetable powder was weighed into 225mls buffer peptone water (BPW) to obtain initial suspension (10^{-1}) dilution, 1ml from (10^{-1} dilution) was taken by use of sterile pipette into 9ml of 0.1% buffer peptone water to prepare (10^{-2} dilution), the above procedure was repeated for further serial dilution up to 10^{-4} dilutions.

4.3.6.3 Detection and Enumeration of Escherichia coli

This was done according to ISO 16649-2:2001(E). Results were expressed in CFU/g.

4.3.6.4 Detection and Enumeration of Total plate count

This was done according to ISO 4833-1:2013(E). Results were expressed in CFU/g.

4.3.6.5 Expression of results

The countable bacterial colonies from two consecutive plates of each sample were converted into colony forming units per g using a formula

$$N = \sum C / (V x (n1 + 0.1n2) x d)$$

Where; N = number of bacterial colonies counted,

C = sum of colonies identified on two consecutive dilution steps, where at least one contained 10 colonies,

V = volume of inoculum on each dish/plate, in ml and

d = dilution rate corresponding to the first dilution selected (the initial suspension is a dilution)

4.3.7 Statistical analysis

Descriptive statistic was used to describe, summarize and present data for both TLVs and vegetable powder formulation. Complete randomized design (CRD) was used to determine microbial quality of dried TLVs and Traditional Leafy Vegetable Products (TLVP) by using the following model:

$$Y_{ijkl} = \mu + \tau_i + \alpha_k + \tau\alpha_{ik} + \varepsilon_{ijkl}$$

Where by:

i=1,2

j=1,2

k=1,2,3

Y_{ijk} = *Dependant variable,* μ*=General mean, and* ε*= Random error*

τ_i *=is the ith location effect*

α_k *= is the kth type of vegetable effect*

Data was analyzed by R –statistical package (R version 4.0.3 2020) software (R Core Team 2012). One-way analysis of Variance (ANOVA) was carried out to determine the significant differences in microbial count between solar dried vegetables and vegetable powder formulations among the 36 samples of TLVs with respect to sites and type of vegetables. Means were separated using Tukey's Honest at $p<0.05$.

4.4 Results

4.4.1 Microbiological parameters

Microbial Load of selected Traditional Leafy Vegetables cultivated in different farm sites and their vegetable soup formulations.

4.4.1.1 Total Plate Count and E. coli count of Selected Traditional Leafy Vegetables

The Total plate count reported from the studied vegetables were in the range of 10^2 CFU/g while *E. coli* was absent in all samples analysed (Table 4.3). Cassava leaves had the highest bacterial load followed by amaranth and sweet potatoes. The mean TPC for all vegetables varied significantly ($p<0.05$) with cassava leaves having the highest level and sweet potato leaves had lowest.

Table 4.3: Total plate count (CFU/g) for selected dried TLVs grown in Lindi region

Vegetables	Mean Total Plate Count (CFU/g)	(TZS1657:2014-EAS:2013) Microbiological limit (CFU/g)
AML	$3.54 \times 10^2 \pm 9.9^b$	10^3
CAL	$3.67 \times 10^2 \pm 10.5^a$	10^3
SPL	$3.15 \times 10^2 \pm 12.3^c$	10^3

Mean values with different superscripts letters down the column are significantly different at $p<0.05$ (Tukey's Honest). AML-Amaranth leaves; CAL-Cassava leaves; SPL- Sweet potatoes leaves. CFU-Colon forming unit, TZS-Tanzania standard –EAS- East Africa Standard

There was no *E. coli* growth in any of the vegetab0les (Table 4.4)

Table 4.4: *E. coli* count (CFU/g) for selected dried TLVs grown in Lindi Region

Vegetables	Mean *E. coli* count (CFU/g)	(TZS1657:2014-EAS:2013) Microbiological limit (CFU/g)
AML	ND	Absent
CAL	ND	Absent
SPL	ND	Absent

AML-Amaranth leaves; CAL-Cassava leaves; SPL- Sweet potatoes leaves, CFU-Colony Forming Unit, NIL-Absent, ND-Not detected, TZS-Tanzania standard –EAS- East Africa Standard

4.4.1.2 Bacterial Load of Selected Traditional Leafy Vegetables Cultivated in Different Farm Sites

Results show that the Mean TPC for all vegetables varied significantly ($p<0.05$) with LL vegetables having higher TPC whereas Sweet potato leaves had the lowest TPC (Table 4.5).

Table 4.5: Total plate count (CFU/ g) for samples from the two sites

Site	Mean Total Plate Count (TPC) (CFU/g)	(TZS1657:2014-EAS:2013) Microbiological Limit (CFU/g)
HG	$3.40 \times 10^2 \pm 25.6^b$	10^3
LL	$3.51 \times 10^2 \pm 23.4^a$	10^3

Mean values with different superscripts letters down the column are significantly different from each other at different at $p<0.05$ (Tukey's Honest). HG- Home garden, LL- Low land. TZS-Tanzania standard –EAS- East Africa Standard

Table 4.6: Microbial load for *E. coli* across the sites

Site	**Mean *E. coli* (CFU/g)**	**(TZS1657:2014-EAS:2013) Microbiological limit (CFU/g)**
HG	ND	Absent
LL	ND	Absent

HG- Home garden, LL- Low land. CFU-Colony Forming Unit, NIL-Absent ND-Not detected TZS-Tanzania standard –EAS- East Africa Standard

4.4.1.3 Microbial Load for Vegetable Soup Powder Formulation

Sample F4 had the highest microbial load and sample F1 the lowest (Table 4.7), there were no significant differences ($p<0.05$) in mean TPC between any of the formulations. *E. coli* was not detected in any of the vegetable soup formulations. (Table 4.8).

Table 4.7: Total plate count (CFU/g) for vegetable soup powder formulation

Vegetables	**Mean Total Plate Count (CFU/g)**	**(TZS1657:2014-EAS:2013) Microbiological limit (CFU/g)**
F1	$3.42 \times 10^2 \pm 34.19^a$	10^3
F2	$3.45 \times 10^2 \pm 17.81^a$	10^3
F3	$3.52 \times 10^2 \pm 12.08^a$	10^3
F4	$3.64 \times 10^2 \pm 16.51^a$	10^3

Mean values with different superscripts letters down the column are significantly different at different at $p<0.05$ (Tukey's Honest), F1-(60:7.5:22.5: A, C, S) F2-(70:5:15: AML, CAL, SPL) F3-(80:2.5:7.5, AML, CAL, SPL) F4-(40:10:40: AML, CAL, SPL), CFU-Colony Forming Unit, TZS-Tanzania standard –EAS- East Africa Standard

Table 4.8: *E. coli* (CFU/g) in vegetable soup powder formulation

Vegetables	**Mean *E. coli* (CFU/g)**	**(TZS1657:2014-EAS:2013) Microbiological limit (CFU/g)**
F1	ND	Absent
F2	ND	Absent
F3	ND	Absent
F4	ND	Absent

Mean values with different superscripts letters down the column are significantly different from each other at different at $p<0.05$ (Tukey's Honest), F1-(60:7.5:22.5: AML, CAL, SPL) F2-(70:5:15: AML, CAL, S,) F3-(80:2.5:7.5, AML, CAL, SPL) F4-(40:10:40: AML, CAL, SPL), CFU-Colony Forming TZS-Tanzania standard –EAS- East Africa Standard ,ND-Not detected

4.5 Discussion

Food safety is among the most important parameters involved in the quality of food. The presence of pathogenic and deteriorating microorganisms has been extensively related to foodborne diseases or the reduced shelf life of processed vegetables (Schuh *et al.,* 2019). *Escherichia coli* is an innocuous member of the human and warm-blooded animal gut microbiota; however, pathogenic strains may cause intestinal and extra intestinal infections. These primary hosts may acquire *E. coli* from water and food (Luna-Guevara *et al*., 2019). The test for *E. coli* assesses the cleanliness of an environment or food, and can also be used together information regarding the potential for contamination (Bai *et al.*, 2007) hence used as an indicator for safety. While Total Plate Count (TPC) gives a quantitative estimate of the concentration of microorganisms such as bacteria, yeast or mould spores in a sample. The TPC can be used to evaluate sanitary quality, sensory acceptability, and conformance with good manufacturing practices (GMPs) while results of the TPC can provide a food processor with information on the quality or handling history of raw materials, food processing and storage conditions, and handling of the finished product. Thus can be used to determine the shelf-life or forthcoming sensory change in a food product (Mendonca, *et al.*, 2020).

4.5.1 Total Plate Count and E. coli count of selected TLVs

This study revealed higher TPC for dried leaves of Cassava, followed by amaranth and finally sweet potato (Table 4.3). The trend may be associated with the moisture content (from the same study in chapter three paper one) for each vegetable where it was higher for cassava leaves (9.17±0.58) followed by amaranth (8.47±0.55) and finally sweet potatoes leaves (8.12±0.51). It is generally known that the higher the moisture content the higher the microbial load and vice versa. Removal of moisture prevents the growth micro-organisms which would otherwise cause food spoilage. This is the basis for food preservation and hence increased shelf life for foods with low moisture content. A decreased water activity inhibits the growth of most bacteria, yeasts, and molds, which are unable to grow below 0.87, 0.88, and 0.80, respectively (Beuchat *et al*., 2013).

Some studies have indicated that aerobic mesophilic microorganisms found in food are important microbiological indicators for food quality, and most foods are regarded as harmful when they have large populations of these microorganisms, even if the organisms are not known to be pathogens (Weldezgina and Muleta 2016) and (Sudershan *et al*., 2009).

Factors which might be accountable for the counts may include drying vegetables on exposed surfaces and packed in inadequately cleaned container (Kudjawu *et al*., 2011). However, present results furthermore indicated that microbial load was not as high (10^3 -10^5) and that the vegetables may possibly be preserved over a considerable period of time (Ukegbu and Okereke, 2013). However, it is important to note that if pathogenic microorganisms are present, these may cause food poisoning which may result into severe illnesses, depending on the dose and the type of microorganism.

The presence of *E. coli* in foods is usually due improper/poor handling during processing and preservation several studies have indicated that high coliforms count in dried vegetables is an indication of poor handling in the whole value chain from farm to fork. Some important sources of contamination among leafy vegetables were during pre-harvest which include soil, irrigation water, inadequately composted manure, human handling, reconstituted fungicides and insecticides while postharvest sources included harvesting equipment, transport container, contaminated water used for washing, transport vehicles and processing equipment, unclean implements, poor hygiene in hands, cross contamination (preparation or storage) (Njoroge *et al.,* 2015; Luna-Guevara *et al.,* 2019).

The present study results is different from the findings reported by Victor (2017) who indicated heavy fecal coliform contamination in vegetables ranging from 4.0×10^3 to 9.3×10^8 MPN/g) in Ghana and assign microbial contamination in vegetables to sources such as soil, manure, water and poor post-harvest handling and storage. Also Oranusi and Braide, (2012) explained that, total aerobic count (TAC) and fecal coliform (FC) are real indicator organisms (that is, for hygiene and sanitary conditions) and for this reason their presence in high numbers in dried fruits and vegetables implies poor hygiene and sanitary conditions during processing. However, Table 4.4 showed that found that none of the dried TLVs vegetables had *E. coli.* This could be attributed by the blanching temperatures of 80°C for 2 minutes and enclosure of the samples in a solar-drier. Also FAO (2004) reports explained that solar dryers are free from microbial contamination and are better preservers and give good quality products than sun dried products.

4.5.2 Bacterial load of selected traditional leafy vegetables cultivated at the two different farm sites

The microbial load of vegetables across planting sites, there was significant difference in CFU observed for low land (LL) compared to home garden (HL) (Table 4.5). The observations from this study were supported by Kimaro (2017) results which indicated that lower sections of the farm site registered significantly higher bacterial loads compared to the middle and upper section. At the farm vegetable contamination can be due to contact with cattle, sheep, birds, insects and feces (Kayombo, 2018) or associated with the presence of feces from cattle and other animals, especially during heavy rainfall (Luna-Guevara, 2019).

High risks of fecal contamination may have originated from people reported to be entering and/or urinating/defecating in the farms. Fertilizers, irrigation water, wild animal intrusion, insects, pesticides/fungicides, crop debris, and flooding area also potential sources of microbial contamination at production level (Kapeleka, 2020). However, practice found in the study area shown that people were not using contaminated water to irrigate vegetables and they also use toilets for urinating and defecating, rather they use the reserved water ponds thus no *E. coli* found. Thus present results further shown that both TPC and *E. coli* counts were found to be lower than the maximum limit level.

4.5.3 Microbial load of vegetable soup powder formulation

The bacterial load was highest for F4 followed by F3, then F2, and lastly F1. (Table 4.7) indicated that there were no significant differences ($p<0.05$) between the formulations. In addition, all TPC and *E. coli* were not above the recommended limit in accordance with both TBS standard and the East African Standard (TZS1657:2014; EAS: 2013). These results are in agreement with those of Farzana (2017) who found that the microbial quality of the vegetable soup powder formulation were 3×10^2 CFU/g total plate count while no *E. coli* of which were within the acceptable limit according to Food Standards Australia New Zealand (2001).This was supported by Niththiya *et al*. (2014) and Singh and Kaur (2020) who reported that the product would be considered microbiologically safe if the total microbial count of dehydrated soups is less than $1x10^4$ cfu/g. Other authors however, stated that samples with counts higher than 1.1×10^3 CFU/g are unfit for consumption (Schuh *et al.,* 2019).

According to FAO (1979), the standard limit for aerobic mesophilic bacterial count for food should be less than 10^5 CFU/ml (Kimaro, 2017). Chege and Kiminywe (2016), found the level of microbes in solar dried amaranth were within the within the levels recommended by the International Commission of Microbial Specification for Foods (ICMSF) which is 10^5 and absent (NIL) for *TPC* and *E. coli* respectively. According TBS standard (TZS1657:2014-EAS: 2013) specification stated that Microorganism maximum limit for *Total plate count*, cfu/g, was 1×10^3 for method of test ISO 4833 while *Escherichia coli*, (cfu/g), was absent for method of test ISO 4832. Tables 7 and 8, show that microbes were below the maximum allowable levels for both TPC and *E. coli*. The absence of *E. coli* and meeting the limit for TPC in the tested formulation samples, may signify good hygienic and handling practices. Generally, this is an indication of minimum adherence to Good Health Practices (GHP) and Good Manufacturing Practices (GMP).

4.6 Conclusion

The findings of the present study indicate that TPC and *E. coli* counts among the solar dried vegetables was higher in cassava than amaranth leaves, whereas the sweet potatoes leaves had the lowest count. Vegetables grown under low land sites had significantly higher ($p< 0.05$) mean TPC than those grown at home garden sites. No *E. coli* was found in any samples analysed in this study. For vegetable soup powder samples, the mean TPC count was of the order F4>F3>F2>F1 while no *E. coli* found. Though TPC count was found in all samples, they were all lower than the limit prescribed by TBS standard and hence all samples were of acceptable standards. The absence of *E. coli* indicates proper handling of vegetables across the value chain. Thus the formulated vegetable soup powders are safe for consumption.

4.7 Recommendations

From this study it is recommended

i. Vegetables found in home garden (HG) are recommended for consumer usage because of lesser colony units indicating greater microbiological quality compared to low land (LL) vegetables.

ii. Education must be imparted to community on the proper handling, including storage and transportation of the vegetables and practicing hygiene since they significantly reduce microbial load in food products.

iii. Farmers, vegetable processors and consumers can use this idea of processing vegetables soup powder as assured microbiological safety of the vegetables and also create new product development as well as market segments.

iv. Moreover, recommendations on areas for further studies are to determine the microbial quality of fresh TLVs compared to the solar dried TLVs as well as determine shelf life of the formulated vegetable soup.

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CHAPTER FIVE

Paper Three

Acceptability of soup powder made from selected traditional leafy vegetables grown in Lindi, Tanzania

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5.1 Abstract

A study was conducted to assess the acceptability of soup formulated from traditional leafy vegetables (TLVs) grown in Lindi, Tanzania. Three TLVs, *Amaranthhybrids known as* amaranthus leaves (AML), Manihort *esculenta* known as cassava leaves (CAL*)* and *Ipomea batatas known as* sweet potatoes leaves (SPL) which had been optimized for Iron content, were used to prepare 4 vegetable soup formulations (F1–60.0:7.5:22.5); (F2–70.0:5.0:15.0); (F3–80.0:2.5:7.5) and (F4 –40.0:10.0:40.0) respectively. Descriptive sensory analysis was performed by 10 trained panelists who used 5 descriptors to quantitatively describe the sensory characteristics of four soup formulations. Thirty consumers assessed the degree of liking of products' sensory attributes using a 7-point hedonic scale. External preference mapping was performed by relating sensory data with hedonic responses. Mean intensity ratings of descriptive attributes of the soup showed that F1, F2 and F3 had significantly higher ($p<0.05$) mean intensity scores in colour, aroma, and mouthfeel than F4. The consumer study showed that, with exception of mouth feel, consumers showed significant differences ($p<0.05$) between samples in colour, aroma, taste and overall acceptability. It was thus concluded that F1 was the most liked formulation by consumers due to colour, aroma and mouth feel followed by F2 and then F3 and finally F4. Furthermore, the preference mapping results showed that colour, aroma and mouth feel attributes were the main drivers for positive consumer preference for vegetable soup. Thus, selection and processing of vegetables, which retain these attributes, is of greater importance for consumer acceptability and hence increased utilization for consumer's health and well-being.

Keywords: Traditional Leafy vegetables, solar drying, sensory evaluation, acceptability.

5.2 Introduction

According to the global nutrition report 2016, two billion people are still suffering from micronutrient deficiencies and almost 800 million from caloric deficiencies on a global scale (Achadi *et al*., 2016). In Sub Sahara African Countries, people's diets rely heavily on rice, potato and cassava, which are high in calories but deficient in essential micronutrients. Deficiencies in iron, vitamin A and iodine are widespread, affecting about 300 million people every year, with many more at risk of experiencing these deficiencies (Atangana *et al.*, 2013).

Use of traditional leafy vegetables in rural communities however has reached a low point, as many have labeled their dishes as 'poverty food' (Atangana *et al.*, 2013). These vegetables however contain a variety of bioactive, non-nutritive health enhancing factors such as phytochemicals including antioxidants, essential fatty acids and dietary fiber and are inexpensive sources of micronutrients (Gupta and Prakash, 2011). Unfortunately, however, they are not consumed in sufficient amounts by the households (Ochieng *et al.*, 2017).

Promotion of production and consumption of micronutrient-rich foods will improve intakes, the overall diet, and health status (Mwanri *et al.*, 2011). Development of TLVs products with extended shelf life using locally processing techniques can help solve the problem of under consumption while making an important contribution to improve population income as well as availability (Habwe *et al.*, 2008: Smith and Eyzaguirre, 2007, Umuhozariho *et al*., 2014).

Drying either by sun or solar, is one of the commonly used method for preservation of vegetables (Agiriga *et al*., 2015). However, drying at higher temperatures may cause nutritional loss and damage to sensory attributes such as colour, texture and flavor of the products (Kumar *et al.,* 2006). Sensory quality has a key influence on how consumers perceive the quality of a product and hence preferences (Green-Petersen, 2010). The sensory analysis is recognised as an important tool for determining the viability or acceptability of a food product (Carvalho *et al.*, 2013). New product development requires the integration of sensory attributes including product taste, texture, and appearance with consumer attitudes and health biases.

Acceptability of nutrient dense formulated powders will improve the nutritional status of the community. This research was thus done to develop iron rich soup powders using leafy of Amaranths (*Amaranthus hybridus* L.) (AHL), sweet potato (*Ipomoea batatas*) (IBL) and cassava (*Manihort esculenta*) and assess their acceptability.

5.3 Materials and Methods

5.3.1 Materials

Materials used included Amaranths leaves (*Amaranthus hybridus* L.) (AML), sweet potato leaves (*Ipomoea batatas*) (SPL) and cassava leaves (*Manihot esculenta*) (CAL) from two villages Mtumbati and Mibure in Lindi Region, Tanzania. Samples were collected in freezer bags from two sites (home garden and low land) in each village and transported in a cool box containing ice maintained at 4°C to Sokoine University of Agricuture (SUA), Morogoro for sample preparation and solar drying.

5.3.2 Research design

Complete randomized block design (CRBD) was used in the study and the principal factors were vegetable soup formulations representing AML: CAL: SPL respectively:

Formulation 1 - FI 60.0:7.5:22.5.

Formulation 2 - F2 70.0:5.0: 15.0

Formulation 3 - F3 80.0:2.5:7.5

Formulation 4 - F4 40.0:10.0:40.0

The effects of these factors on sensory attributes during drying were determined.

The following model was used

$yij = \mu + \tau i + \beta j + ij\varepsilon$

i = 1, 2, 3, 4

j = 1, 2, ….

Where,

μ = the overall mean

τi = the ith treatment effect (Soup Formulation Samples)

βj= the jth block effect (Assessors)

ij ε= the random effect

5.3.3 Methods

5.3.3.1 Sample preparation

The leafy edible parts of the vegetables were separated from the main plant. About 2.5 kg of each of the fresh TLVs samples were thoroughly washed with potable water to remove adhering dust and impurities, they were then sliced, blanched at 80°C for 2 minutes in water. Blanching cleans the raw material and reduces bacterial load, softens plant tissue and causes shrinkage which allowing greater volume of materials in the pack, helps fix colour for plants with carotenoid, improves the texture of dehydrated products, inhibits some micro-organisms and facilitates the removal of moisture during drying (Agiriga *et al*., 2015). The blanched vegetables were spread on trays for 10-15 minutes to drain. Solar drying was done at Sugeco (Sokoine University Graduate Entrepreneurs cooperative, Morogoro) solar drying of TLVs was done according to procedure done by Mongi (2013) with some modifications. Samples were loaded into the solar dryer. The temperature in the solar dryer ranged between 45-55°C and drying was completed in 3 days. About 1.5kgs of each dried TLVs were packed separately in a freezer bag and stored in a dark dry place at 25^0C room temperature. After solar drying, the dried vegetables were ground separately by a grinder (Gaoxin 1250 gx-25, China) and passed through a fine 315-micron sieve to obtain fine powders. The fine powders were then packed in labeled food grade bags and stored at 25^0C room temperature in a dark dry place prior to product formulations and laboratory analysis.

5.3.3.2 Product formulation

Three types of TLVs samples were used to formulate iron rich powders. Various proportions of TLVs were used based on iron optimization to meet the 7mg/day iron RDA for children aged between 1-5 years (TZS 1657: 2014- EAS 797: 2013). Table 5.1 shows the amount of iron in some selected solar dried TLV samples used to make formulation after pretesting. Quantities of solar dried TLVs and spices used were as shown on Table 5.2 and 5.3 respectively. The soup powders formulations were made by mixing 90 g of solar dried TLVs with 10 g of spices to form four Soup powders (F1, F2, F3 and F4).

Table 5.1: Iron content of selected traditional leafy vegetables

Type of dried vegetable	Amount of Iron in mg/100g
Amaranthus hybridus L (AML)	80.21
Sweet potatoes (SPL)	39.80
Cassava (CAL)	10.74

Table 5.2: Soup formulations from 3 traditional leafy vegetables (90% of formulation)

Materials(Amount per g)	F1	F2	F3	F4
Amaranthus hybridus (AML)	60.0	70.0	80.0	40.0
Manihot esculenta (CAL)	7.50	5.00	2.50	10.0
Ipomea batatas (SPL)	22.5	15.0	7.50	40.0
Total	90.0	90.0	90.0	90.0

Table 5.3: Spices added to soup formulations (10% of formulation)

Spices	Amount per g
Garlic	0.5
Ginger	0.5
Coriander	0.5
Cumin	0.5
Corn Flour	4.0
Salt	2.0
Sugar	2.0
Total	**10**

5.3.3.3 Cooking procedures of soup powders

For descriptive test, about 125 g of soup formulation was used in which 1750 mls of water was added and boiled for 5 minutes (Farzana *et al.*, 2017) with modification. Gas cooker was used for cooking the soup. This amount served 10 panelists. The same concentration was used to prepare for large groups of panelists.

5.3.3.4 Quantitative descriptive analysis (QDA)

A descriptive sensory profiling was conducted at the Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture by trained sensory panel according to method described by Lawless and Heyman (2003). 10 female assessors aged between 21 to 39 years were chosen purposely as they are responsible to prepare food for the selected children (1-5 years). The assessors were selected and trained according to ISO Standard (2005). In a pre-testing session, the assessors were trained in developing sensory descriptors and the definition of the sensory attributes. The assessors developed a test vocabulary describing differences between samples and agreed upon to a total number of attributes on colour, aroma, texture, taste and mouth feel (Table 5.4). An unstructured line scale was used for rating the intensity of each attribute where by assessors indicated appropriate number against each characteristic colour, aroma, texture, taste and mouth feel. The left side of the scale corresponded to the lowest intensity of each attribute indicated by 1 and the right side corresponded to the highest intensity indicated by 9 as shown in the descriptive sensory form attached in Appendix 4. Descriptive analyses of four soup samples were carried in one session and each assessor evaluated four samples per session. The samples were coded with 3-digit random numbers and served to each panelist in a randomized order. Water was served alongside samples for rinsing mouth before evaluating other samples during the test. Form for QDA is attached in Appendix 4**.**

Table 5.4: Definitions of sensory attributes used in descriptive sensory analysis parameter attribute definition

Attribute	Definition
Colour	Colour intensity Degree of greenish in the colour Clear, strong colour .
Aroma	Vegetable romatics associated with dried vegetables.
Texture	The degree of coarseness. It also includes the consistency, thickness, fragility, chewiness and the size and shape of particles in food (The force required to bite through the sample.
Taste	The taste associated with the intensity of bitterness.
Mouth feel	Feeling when the food is in the mouth is it good or bad.

(Sharif *et- al.,* 2017 with modification)

5.3.3.5 Consumer test

The test was carried out at Ipo ipo area, Mazimbu road- Morogoro by 30 untrained female consumers aged between 21- 69 years, using a 7-point hedonic scale (where 1 = Dislike very much and 7 = 7-Like very much) as described by Lawless and Heyman (2010). Samples were poured into plastic bowl coded with 3-digit random numbers, in a randomized order and served to the panelists at 4 pm. Panelists were also given distilled water for rinsing the mouth. Panelists were instructed to rate the colour, aroma, taste, texture, mouth feel and overall acceptability of each sample indicating their degree of liking or disliking by putting a number as provided in the hedonic scale according to their preference. Testing was completed in one session and each consumer evaluated all the 4 formulations. This evaluation was conducted under the same conditions as for the sensory descriptive test. Sensory form for consumer test is attached in Appendix 5.

5.4 Statistical Data Analysis

Data was analyzed using R software version 3.5.3 (Stats package) Data were subjected to one-way ANOVA to establish if there was significant ($p<0.05$) variation and interaction between means of four soup powder formulations at ($p<0.05$). Means were separated by

Turkey's Honest Significant Difference at p <0.05. Principal component analysis (PCA) and Partial Least Squares Regression (PLSR) were also performed. The main sources of systematic variation in the average sensory descriptive results were determined by using Principal Component Analysis (PCA) while the relationship between descriptive data and hedonic liking from the consumers were determined by PLSR. The variables were standardized and full cross-validation was applied. Correlation loading plots were applied with circles indicating 50 and 100% explained variance, respectively.

5.5 Results

5.5.1 Quantitative Descriptive analysis test (QDA)

5.5.1.1 Product effect

Mean intensity ratings of descriptive attributes of the vegetable soup are shown in **Table 5.5**. There were significant (p<0.05) differences in mean intensity scores between soup formulations. In terms of colour, F1 had the highest score which was statistically (p<0.05) different from the rest of the samples. The aroma scores for F1 and F2 were not statistically (p>0.05) different but differed significantly from F3 and F4 which were similar. F3 and F4 scored the highest scores for taste and differed significantly (p<0.05) from F1 and F2 which did not differ. F1 scored the highest for mouth feel and was statistically different from F2 and F3. However, F3 and F4 scored highest in texture though were not statistically different but differed from F1 and F2. F1 samples had higher mean intensity scores in colour, aroma, and mouth feel than F 4 samples, which had higher texture and taste scores.

Table 5.5: Mean attribute intensity descriptive scores of vegetables soup samples

Sample	Colour	Aroma	Taste	Mouth feel	Texture
F1	7.7±0.82[a]	5.1±1.27[a]	4.1±0.57[b]	6.5±0.71[a]	5.8±0.92[b]
F2	7.1±1.60[b]	5.1±1.27[a]	4.1±2.00[b]	5.9±1.20[ab]	5.4±1.00[b]
F3	6.0±0.94[c]	4.5±1.43[b]	4.7±0.48[a]	5.3±0.67[bc]	6.2±0.92[a]
F4	6.3±1.16[bc]	4.2±1.32[b]	5.4±0.84[a]	4.8±1.23[c]	6.5±0.85[a]

Values are expressed as mean ±SD (n=10). Mean values with different superscript letters along the column are significantly different at p<0.05

5.5.2 Hedonic test

Mean hedonic scores of the vegetable soup are shown in Table 5.6. Colour ranged from 4.3 to 5.9 whereby F4 had the lowest score which was statistically different (p<0.05) from the rest of the samples. Aroma score ranged between 4.00 and 5.40 and F4 had the least score and was significantly different (p<0.05) from the rest of the samples. For taste, values between 4.2 and 5.3 were recorded, whereby F3 and F4 had the lowest scores which statistically differed (p<0.05) from both F1 and F2 which were not different (Table 5). No significant differences (p<0.05) were observed for mouth feel scores between the 4 formulations. In conclusion, the general acceptability for samples F1 and F2 were higher and statistically different (p<0.05) from F3 and F4 which had lower values.

Table 1.6: Mean hedonic scores for the vegetable soup formulations

Sample	Colour	Aroma	Taste	Mouth feel	Acceptability
F1	5.93±1.01[a]	5.30±1.22[a]	5.30±1.15[a]	5.30±1.20[a]	5.60±1.50[a]
F2	5.63±1.71[a]	5.10±1.68[a]	4.90±2.00[a]	5.00±1.63[a]	5.00±1.55[a]
F3	5.30±1.15[a]	5.40±1.28[a]	4.50±1.70[b]	4.80±1.21[a]	4.50±1.60[b]
F4	4.30±2.22[b]	4.00±1.81[b]	4.20±2.08[b]	4.80±1.36[a]	4.40±1.81[b]

Values are expressed as mean ±SD (n=30). Mean values with different superscript letters along the column are significantly different at p<0.05

5.5.3 Relationship between descriptive data and acceptability data by Partial Least Square Regression (Preference mapping)

5.5.3.1 Principal component of descriptive sensory data

Figure 5.1 shows a bi-plot on average sensory attributes whereby the two first significant principal components from Principal Component Analysis (PCA) are indicated. The obtained results showed principal component (PC) 1 accounted for 96.6% of the systematic variation in the data while principal component (PC) 2 accounted for 2.96%. PC 1 was a contrast between formulation 1 and 2 which correlated positively with colour, mouth fell and aroma attributes on one side and formulation 3 and 4 which correlated positively with texture and taste attributes on the other side. PC 2 was contrast between F1 and F4 which correlated positively with mouth feel, colour and taste in one side and F2 and F3 which correlated positively with aroma and texture attributes on other side.

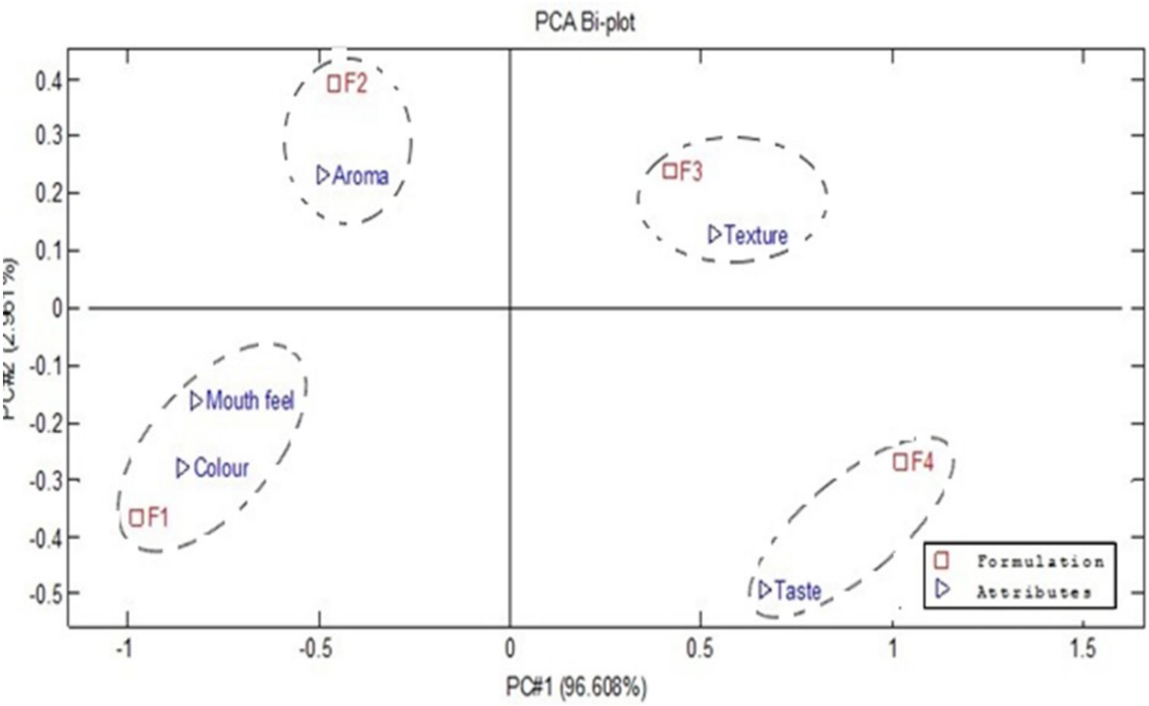

Figure 5.1: Bi-plot from PCA of descriptive sensory data for vegetable soup formulations

5.5.3.2 Relationship between descriptive data and hedonic liking by PLSR

Results from partial least square regression (PLSR) as indicated in Figure 5.2 shows using descriptive data as X-variables and liking rated by the consumers as *Y*-variables. The finding shows that, the explained variance was relatively high; the two first significant components described 85% of the variation in X and 71% in *Y*. The figure shows many consumers fall to the right of the vertical Y-axis, outside the 50% explained circle which means that, the acceptance values of these persons go in the direction of F1 sample associated with colour, mouth feel and aroma intensities. Taste and texture attributes correlated positively sample F4 very few consumers on that side. This implies that, colour, mouth feel and aroma were the main drivers for consumers liking of the vegetables soup formulations in this study as supported by regression coefficient (Figure 5.3).

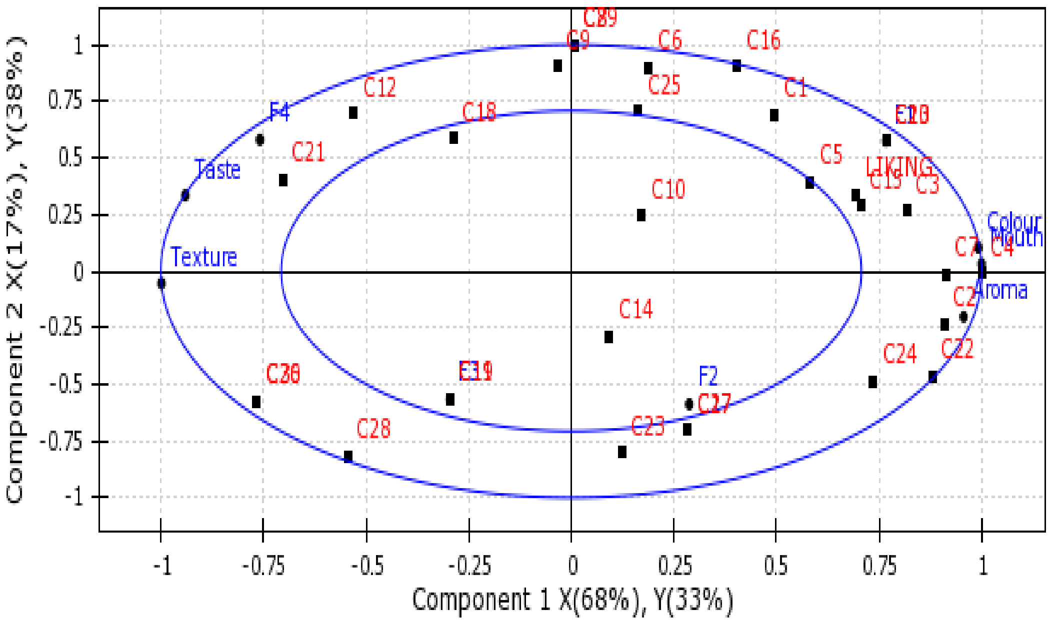

Figure 5.2: Correlation loadings from a partial least squares regression of vegetable soup formulations with descriptive data as X variables and hedonic rating as Y variables.

Figure 5.3 shows the importance of each attribute in consumer liking of the vegetable soup formulations. It shows that colour, aroma and mouth feel are positively correlated with liking while texture and taste are negative correlated with liking. Most of the consumers liked the soup formulated due to its colour, aroma and mouthfeel and few consumers liked the vegetable formulated soup due to its taste and texture.

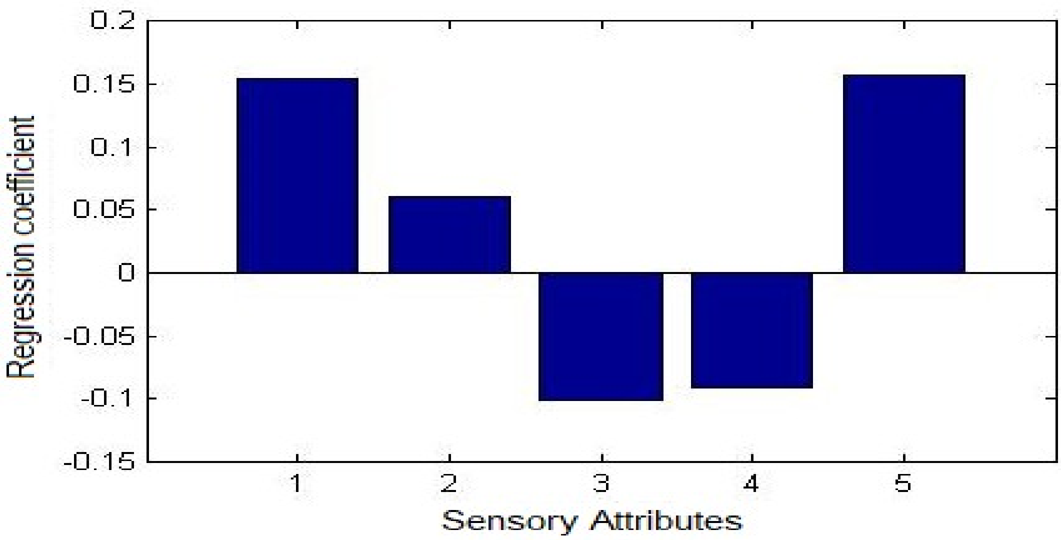

Figure 5.3: Regression coefficients of PLSR showing importance of each attributes on consumer liking of vegetables soup formulation/ 1= Colour, 2= Aroma, 3=Texture, 4=Taste and 5 =Mouth feel.

5.6 Discussion

5.6.1 Quantitative descriptive analysis

The F1 formulation had high intensity scores for color, aroma and mouth feel as shown in Table 5.5. The high color, aroma and mouth feel score in F1 may be due to high proportional of sweet potatoes leaves which was an excellent source of chlorophylls and carotenes. This is also revealed by Dinu *et al*., 2018 who explained on the importance of sweet potatoes leaves as an excellent source of chlorophylls and carotenes which are primary photosynthetic pigment that determine the green colour of plants. The green colour is an aesthetic quality highly valued in vegetables.

Moreover, the degree of greenness attributed to chlorophyll pigments is important in determining the final quality of these kinds of vegetables since colour is one of the major quality indicators for vegetable products (Nisha *et al*., 2004). The higher the concentration of this pigment increases the color intensity. However, it is easily degraded during processing and is susceptible to chemical and physical changes during processing. It is less stable to heat due to conversion of chlorophyll to pheophytin through the magnesium substitution of the chlorophyll by hydrogen which results in colour changes in green vegetables (Turkmen *et al*., 2006). The drastic irreversible changes, causes a change from bright green to olive brown (Kumar *et al*., 2013). On the other hand, application of solar drying and blanching with salt in this study, could be the reason for maintaining the colour of the vegetables used in the formulations.

It is well-known that excessive heating of food products causes considerable losses in the organoleptic quality of food. Blanching inactivates chlorophyllase and other enzymes responsible for senescence and rapid loss of green colour. However, alkalizing agents in blanching and brine solutions, such as sodium bicarbonate, disodium glutamate, sodium hydroxide, and magnesium hydroxide, have been used to raise the pH of green vegetables and therefore, retain chlorophyll after processing (Koca *et al*., 2007). The degree of greenness, attributed to chlorophyll pigments, is important in maintaining the final quality of these kinds of vegetables (Nisha *et al.*, 2004). Chlorophyll absorbs sunlight during photosynthesis and converts it to energy to plant as well as stimulating immune system, help combat anemia, normalize blood pressure, purifying blood and liver detoxification, help prevent cancer and is being used in cancer therapy (Inanc *et al.*, 2011). Thus, the presence of chlorophylls and their transformation products contribute organoleptic attributes such as colour and odour to the soup product (Minguez-Mosquera *et al*., 2002 and Turkmen *et al*., 2006).

Sweet potato leaves were higher in proportional for the formulations F1, followed by F2 then F3 and its proportional affect the greenish colour as increasing with increasing proportional. High intensity of taste in this study described by bitterness and was revealed by F4 which scored highest intensity for taste. The bitterness in F4 could have been contributed by high proportional of cassava leaves compared to other formulation. F4 soup formulation was found to be the least acceptable probably due to high levels of residual cyanogen. Umuhozariho *et al*. (2013) demonstrated a strong correlation between bitter taste and cyanogen (HCN) potential in cassava. The high texture of F4 may be attributed by high proportional of sweet potato leaves compared to other formulations which is associated with tenderness, thus make the texture softer than all formulations. The finding supported by the Bonsi *et al*. (2014), who explained on the tenderness of sweet potatoes leaves was due to lower fiber content than other tropical root crops such as cassava and the low fiber content coupled with high moisture content makes them generally much tender than cassava leaves. Followed by F3 where the bitterness may be due to high concentration of amaranth among all formulation.

5.6.2 Consumer test

Table 5.5 Shows that consumers acceptance were higher for FI and F2 were highly accepted than F3 and F4. Colour is a primary attribute, which attracts consumer attention and therefore influences acceptance (Nkuba *et al*., 2018). The current study also found that acceptability scores reduced with increasing concentration of amaranth, F1 with 60% amaranth, F2 with 70% amaranth and F3 with 80% with acceptability mean score of 5.6, 5.0 and 4.5 respectively. Probably this is caused by proportional of amaranth in the vegetable soup formulation since as proportional increase changed the colour to dark green colour. A study by Gupta and Prakash (2011) found similar observations whereby a micronutrient-rich traditional products incorporated with amaranths at 4, 8 and 12%

levels resulted in an inverse relationship where the overall acceptability score at ($p<0.05$) was 7.97,6.99 and 6.57 respectively. Another study of soup mix made in three different proportions of amaranths leaves powder and arrow root starch in the ratio of 5:55, 10:50, 15:45 respectively revealed that soup prepared by using 5:55 (amaranths leaves powder: arrowroot starch powder) had maximum acceptability (Peje, 2019). Another consumer study for the four varieties of maize flour were composited with amaranth leaf powder at the level of 0, 1 and 3% (w/w) substitution respectively and extruded into snacks showed that the acceptability of the snacks decreased with increasing amaranth concentration, only a very small proportion (2-8%) of the panel liked the snacks extremely (Beswa, 2016).

The findings from Ssepuuya (2018) on the contribution of instant amaranth soup to boarding school and adolescents showed that formulated soups acceptability ranged between 6.0 and 6.7 for all the sensory attributes, except mouthfeel and aftertaste (had scores between 5.4 and 6.0). Thus different consumer has different choice and preference towards product liking and acceptance. The varied consumers' preferences provided insight into the sensory attributes that are important to individual consumer acceptability of samples (Mongi *et al*., 2013). According to literature, drivers of liking defined as the attributes, which have the most important effects on overall liking (Kuesten and Bi, 2018). This is also supported by Barrett *et al.* (2010) who explained that once customer is attracted by the appearance and color of a product, this is usually followed by putting it into the mouths, where the aroma and taste take over. Another study also explained the importance of colour as primary attribute which attracted consumer attention therefore influences the mind of the consumer when choosing food and therefore acceptance (Pobee *et al*., 2017). Therefore, to attract consumers, food colour is an important attribute to be considered when preparing food.

5.6.3 Relationship between descriptive attributes and acceptance by the Partial Least Square Regression (PLSR)

Figure 5.1 shows correlation between descriptive attributes and hedonic through Principle Component Analysis (PCA). F1 was closely associated with colour, aroma and mouth feel. This is supported by Figure 5.3, which shows the contribution of each attribute towards the acceptance of vegetable soup formulations and provide insight into the sensory attributes that are important to individual's consumer preference of soup. Good color, aroma and mouth feel attributes were strong contributors while texture and taste being negatively related implying were weak contributors for soup preference. Vegetables soup formulation F1 and F2 were positively correlated to colour, aroma and mouthfeel (Figure 5.3). This might have been enhanced by the high proportional of sweet potato leaves and the average proportional of amaranth (60,70,80 and 40) % for F1, F2, F3 and F4 respectively Although F4 was positively correlated with texture and taste, most consumers did not prefer the taste and texture of the formulated soup vegetables (Figure 5.3). However, color and appearance are the preliminary attributes that attract consumers to a food product and hence considered as guide for a good quality of foods associated with acceptability (Singh, 2014). Though in the current study acceptability of the formulated soup was in trend of F1, F2, F3 and finally F4 which based on the colour, aroma and mouth feel.

5.7 Conclusion

The present study revealed that colour, mouth feel and aroma were the drivers for positive consumer preference thus positively correlated with liking while texture and taste were negatively correlated. All formulated soups were acceptable with F1 being the most acceptable while F4 was the least acceptable. Solar drying of vegetables to produce powdered soups, may be considered as a practical way to preserve fresh vegetable which can be used during off season.

5.8 References

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CHAPTER SIX

6.0 Overall Conclusions and Recommendations

6.1 Conclusions

It can be concluded that, we can develop iron rich products from TLVs grown in Lindi Tanzania. Based on the physico-chemical results indicated that all solar dried TLVs had the moisture content below 10% which was considered as safe for storage. From micronutrients interest of the study indicated that the selected solar dried TLVs and site from which the vegetable grown findings showed that AML from LL had the highest iron content. Thus can be used with higher proportional among other selected TLVs during development of the iron rich products. However, from other targeted micronutrients (Zinc, Beta-carotene and Ascorbic acid) study revealed that CAL had the highest zinc and ascorbic acid while SPL had the highest Beta-carotene both from HG thus can be used to optimize other selected micronutrients.

Microbial quality parameter (TPC and *E.coli* count) tested indicated that both solar dried TLVs and vegetable soup formulation made are safe compared to the TZS. Thus implies proper hygienic handling of TLVs along the value chain. All formulations were accepted but the formulation which was the most accepted was F1 (60:7.5:22.5) due to its colour, mouth feel and aroma. Therefore, the vegetable soup powder formulated from solar dried TLVs can be used to make different vegetable formulations and used in different food such as porridge and soup for children and snacks for adults due to its great importance in reducing micronutrients deficiencies.

6.2 Recommendations

Based on the findings of this study the followings are recommended

i. Study to be conducted to formulate vegetable soup formulations based on the RDA for different age groups.

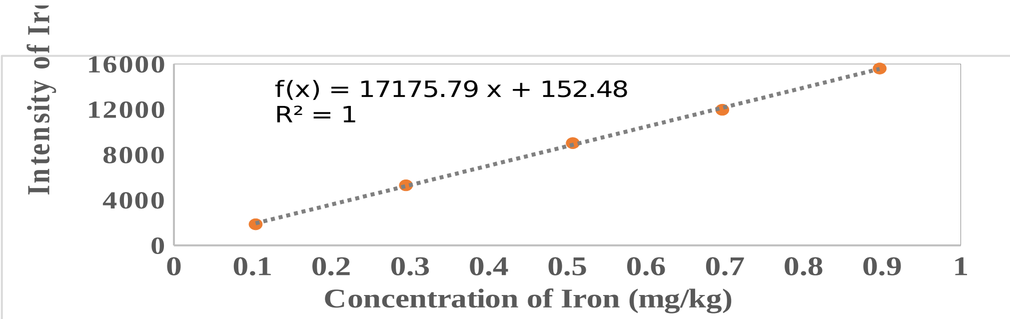
ii. Other TLVs vegetables in different ratios can be combined for development of the vegetable formulations to optimise essential micronutrients to combat micronutrient deficiency as well as taste and texture improvement.

iii. Further study to determine shelf life of the formulated vegetable soup can be conducted.

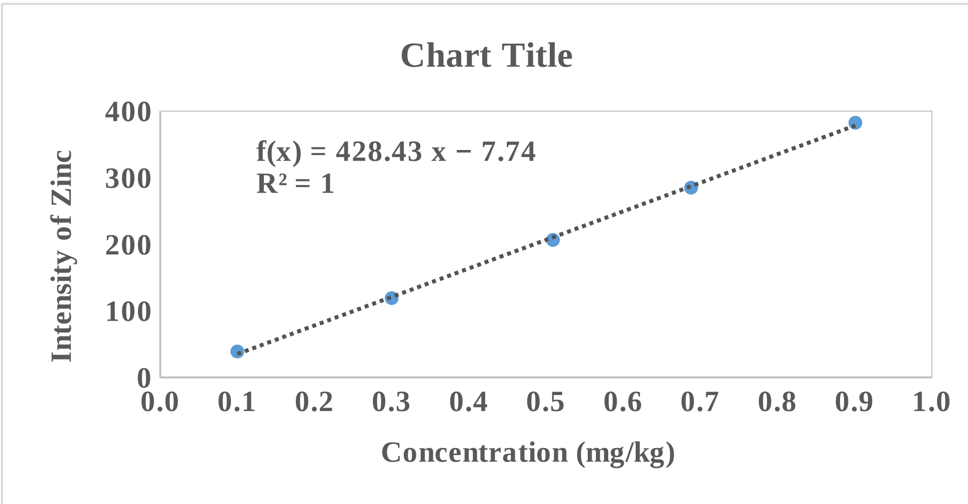
iv. The research provides an opportunity for new products development for different market segments as vegetable soup powder formulated can be used in different food products such as porridge, sauce and snacks.

APPENDECIES

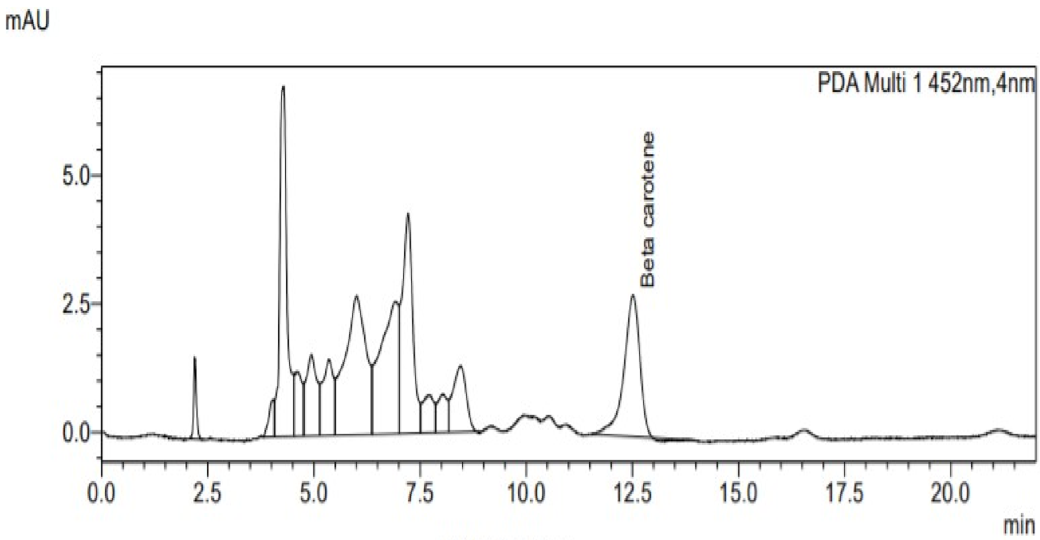
Appendix 1: Shows calibration for iron and zinc and chromatogram for Beta-carotene and Ascorbic acid



Calibration curves for iron (Fe)

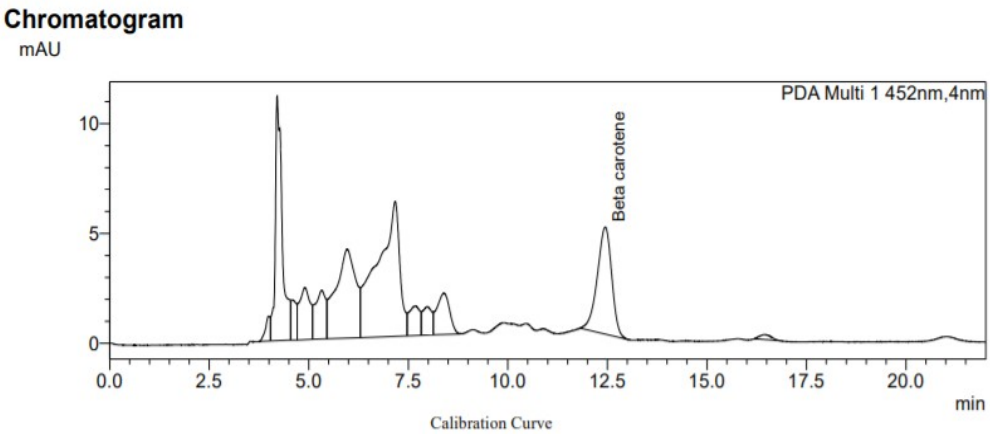


Calibration curves for zinc (Zn)

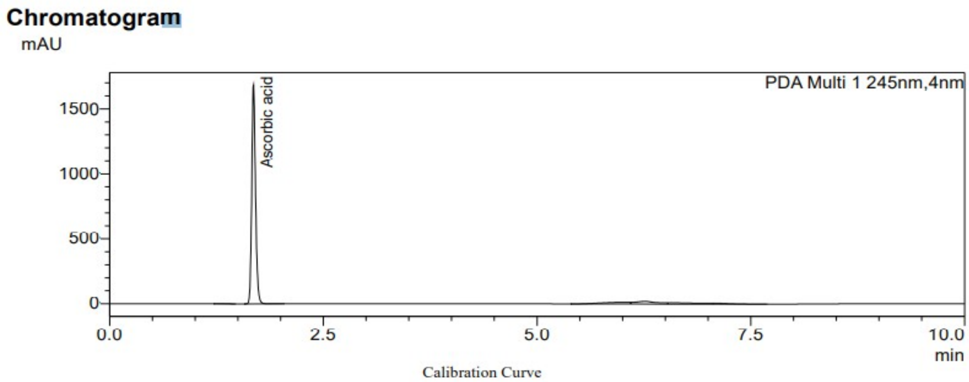


Calibration Curve

Chromatogram for Beta-carotene of sample (A6- amaranth low land)

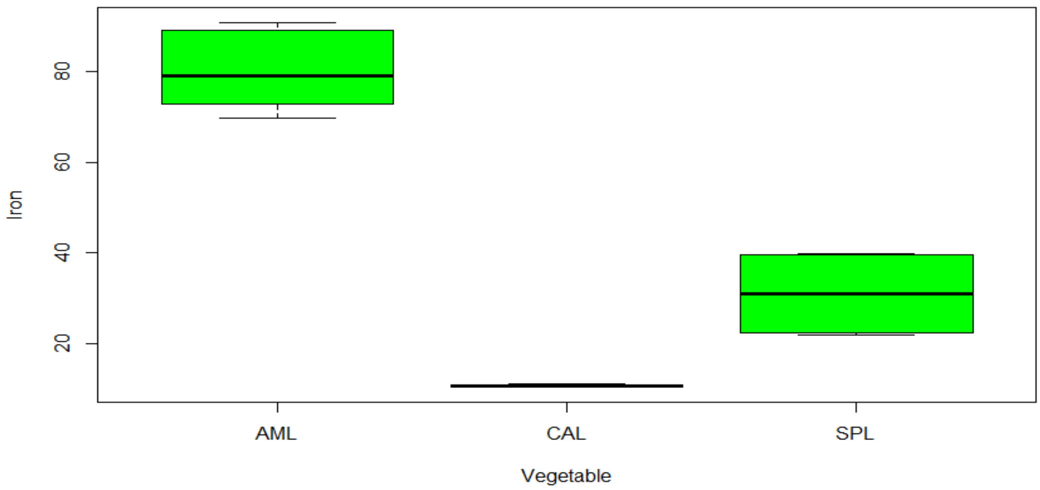


Chromatogram for Beta-carotene of Spiked sample (SPKA6- amaranth low land)

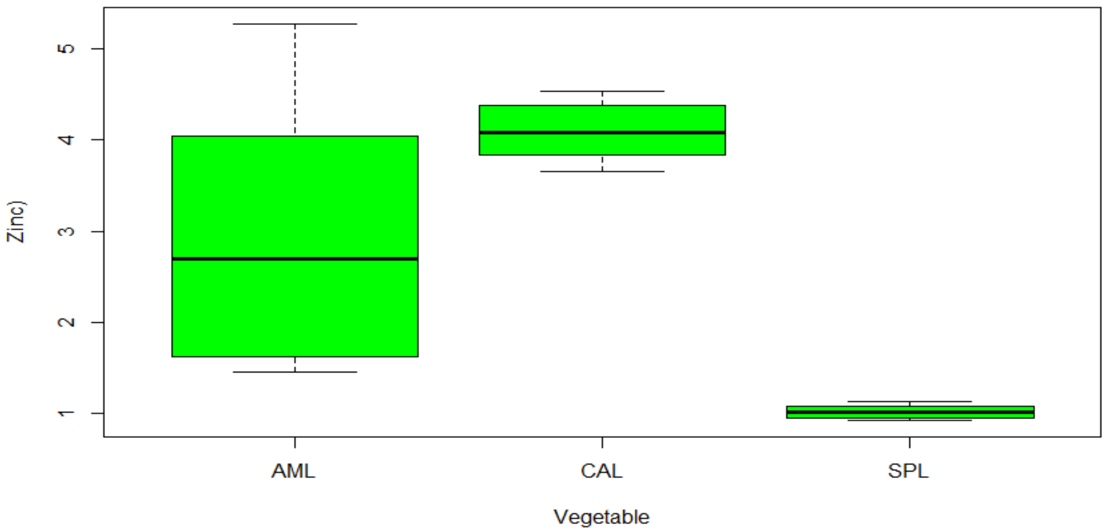


Chromatogram for Ascorbic Acid

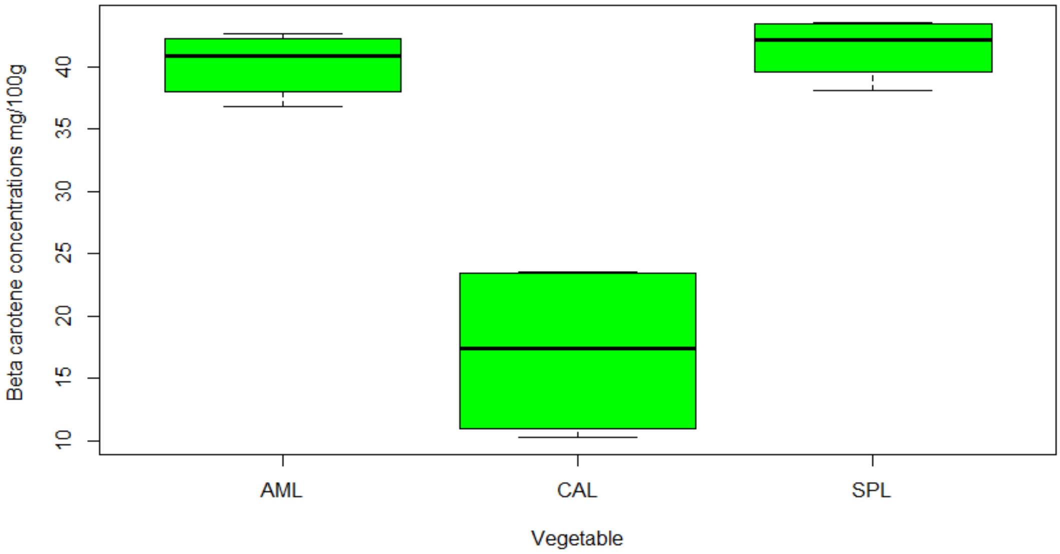
Appendix 2: Boxplot showing the mean value for selected micronutrients and physico chemical quality of the TLVs within the sites



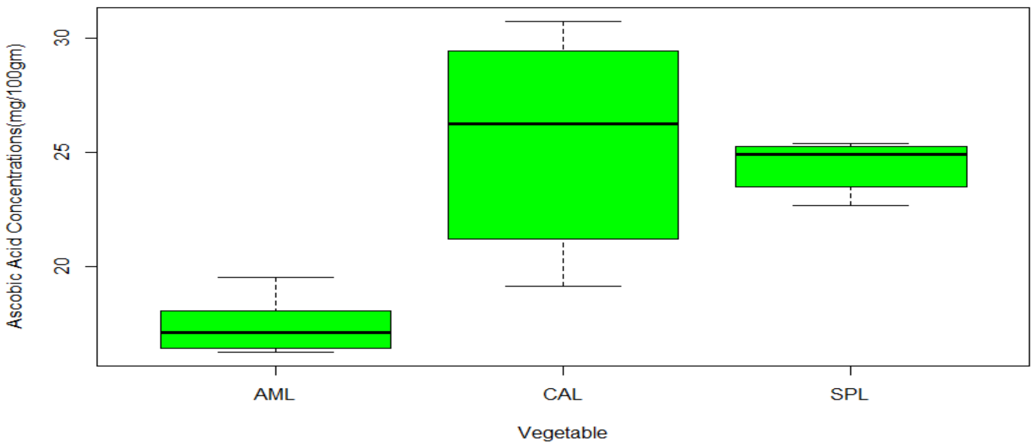
Box plot showing the mean results of Iron among the vegetables



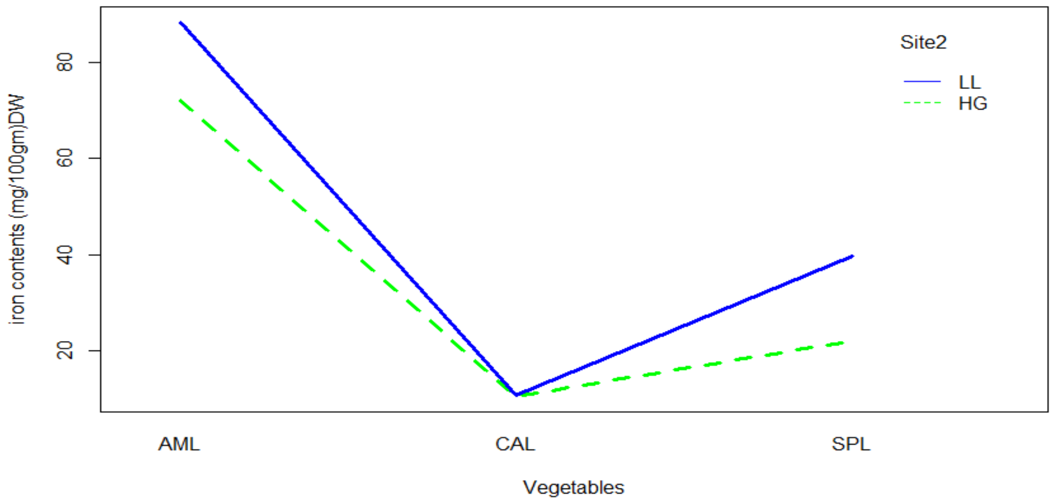
Box plot showing the mean results of Zinc among the vegetables



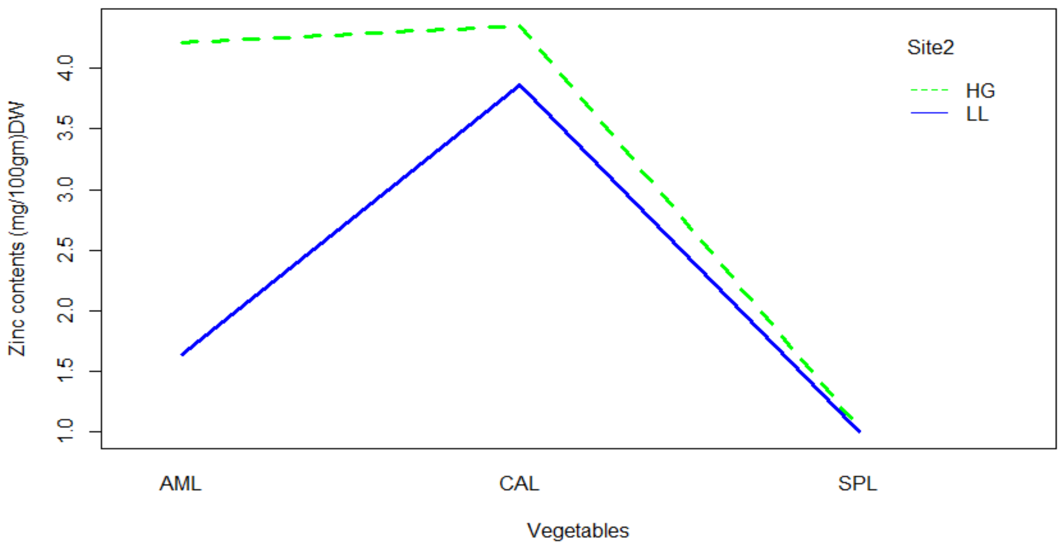
Box plot showing the mean results of Beta-carotene among the vegetables



Box plot showing the mean results of Ascorbic acid among the vegetables

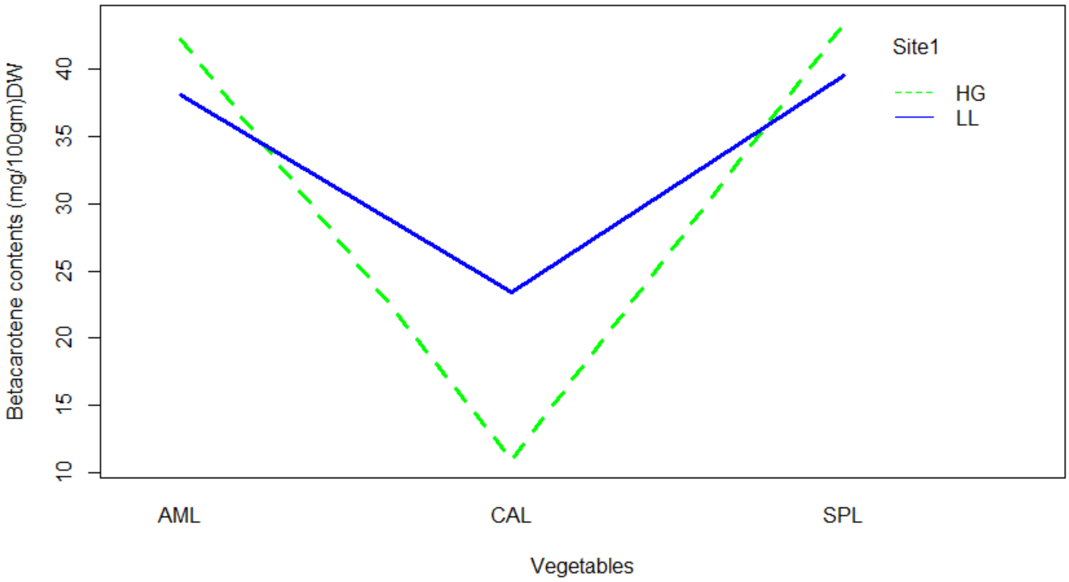


Box plot showing the interaction mean result of Iron across vegetables and sites

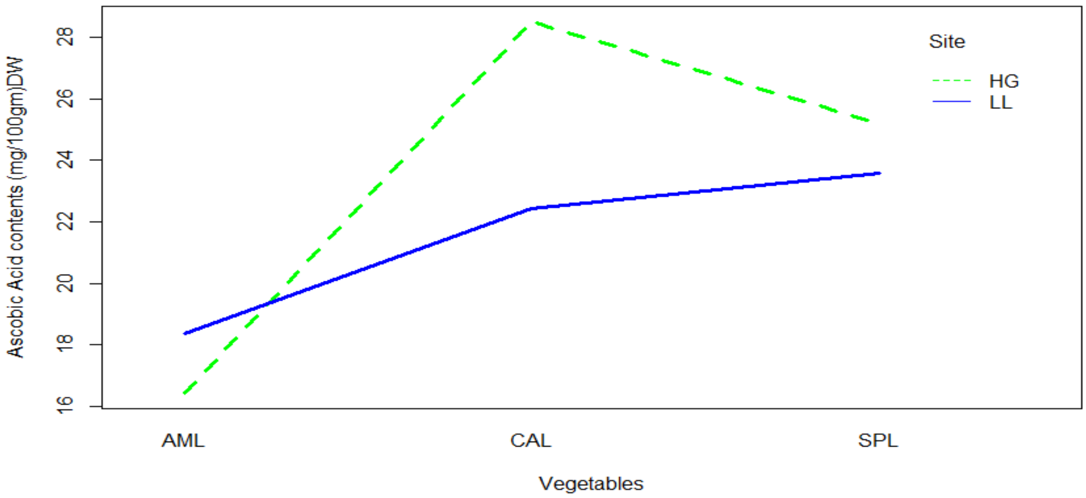


Bo

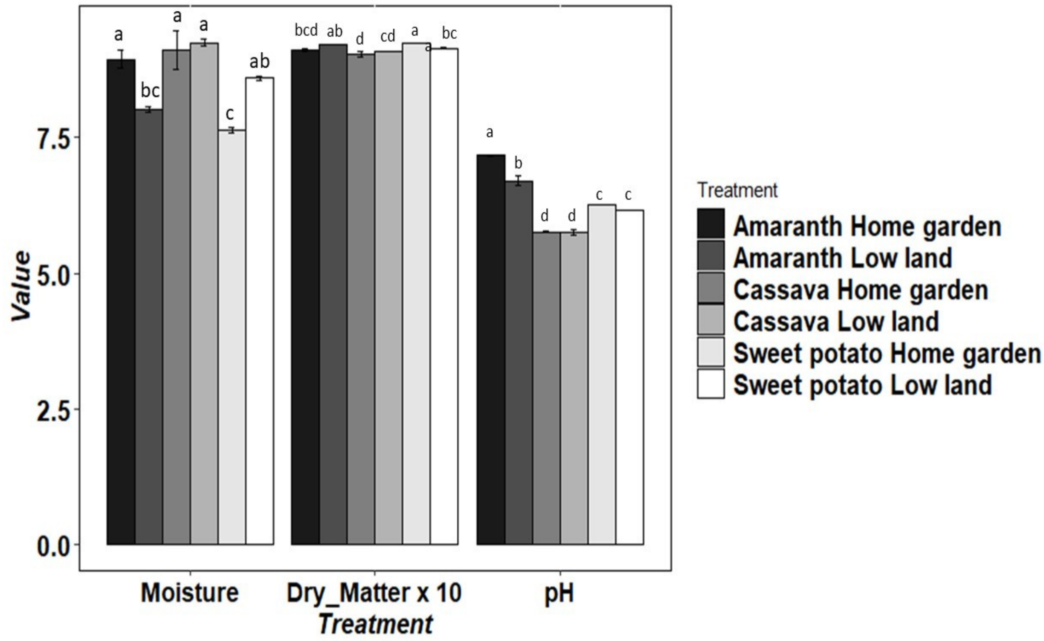
x plot showing the interaction mean result of zinc across vegetables and sites



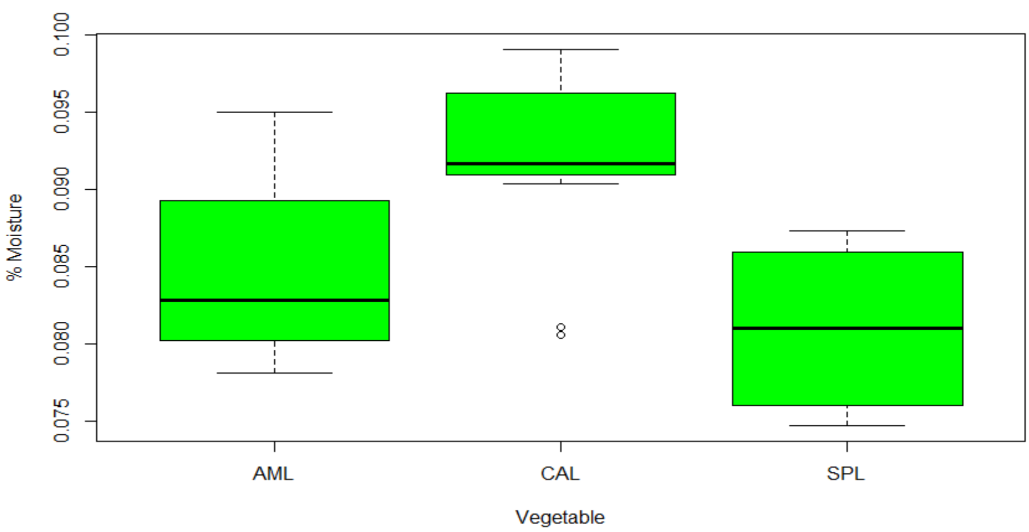
Box plot showing the interaction mean result of Beta-carotene across vegetables and site



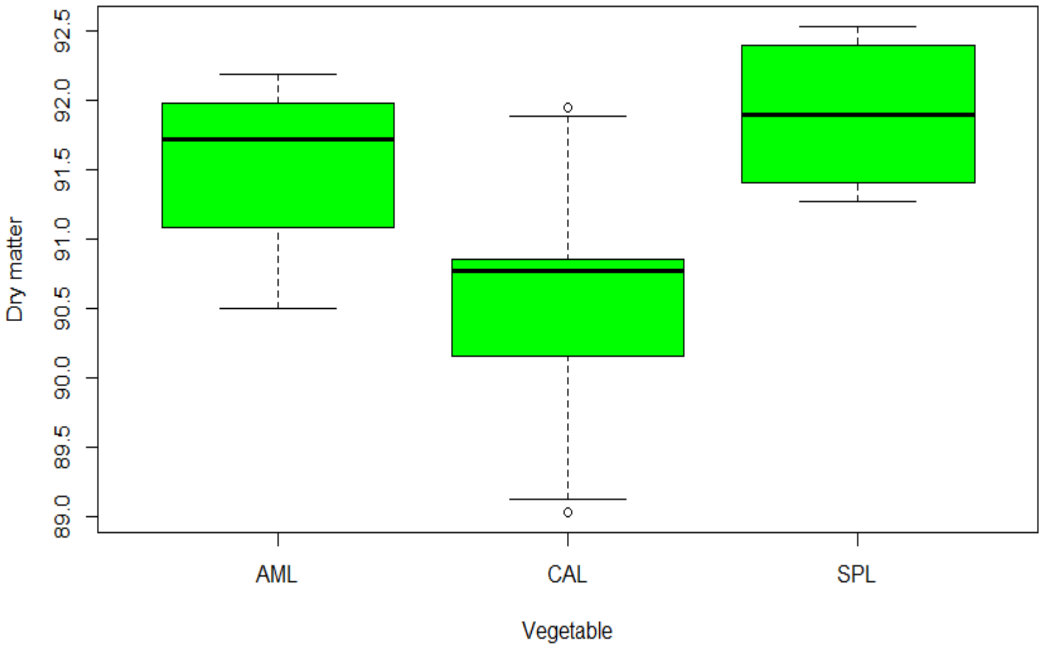
Box plot showing the interaction mean result of Ascorbic acid across vegetables and sites



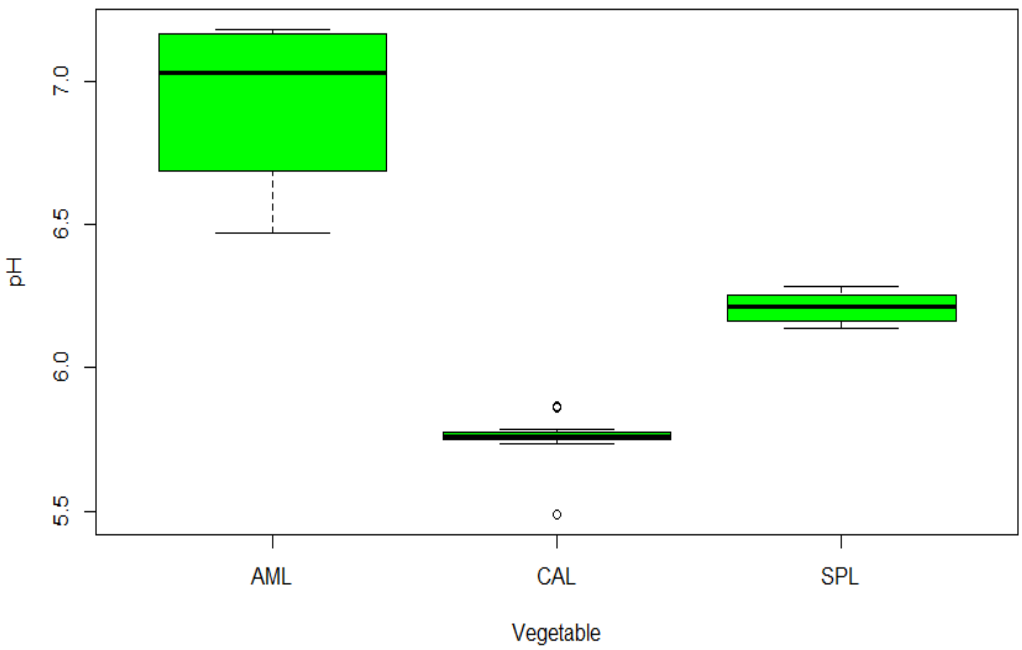
Plot show the interaction mean result of the physico-chemial among the vegetables and across the sites.



Box plot showing the mean results of Moisture content among vegetables

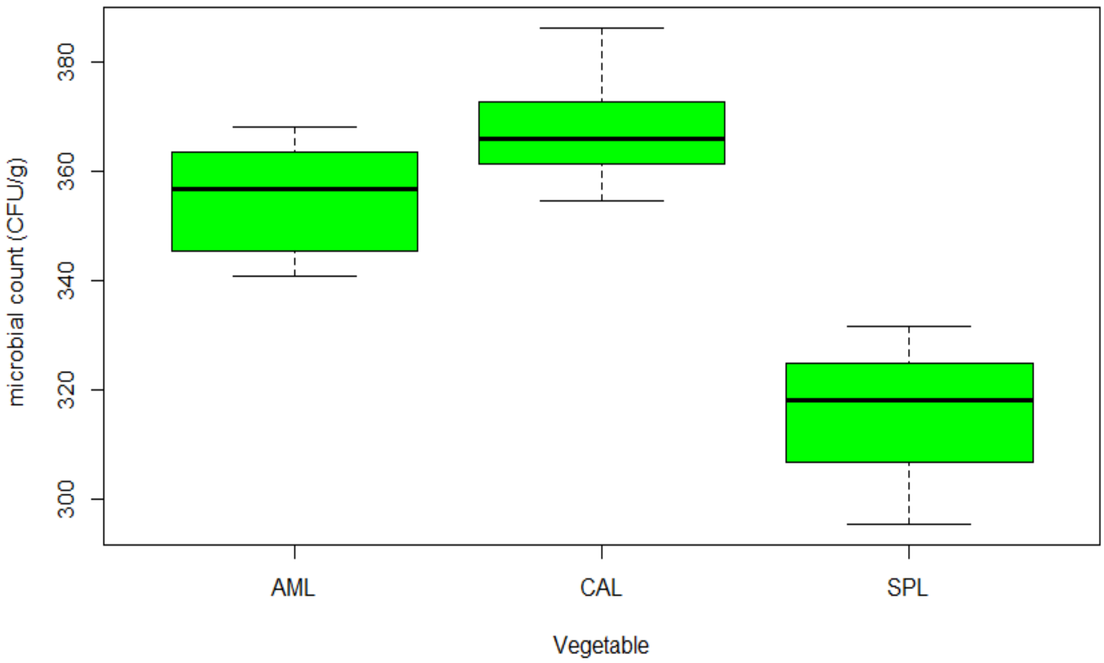


Box plot showing the mean results of dry matter content among vegetables

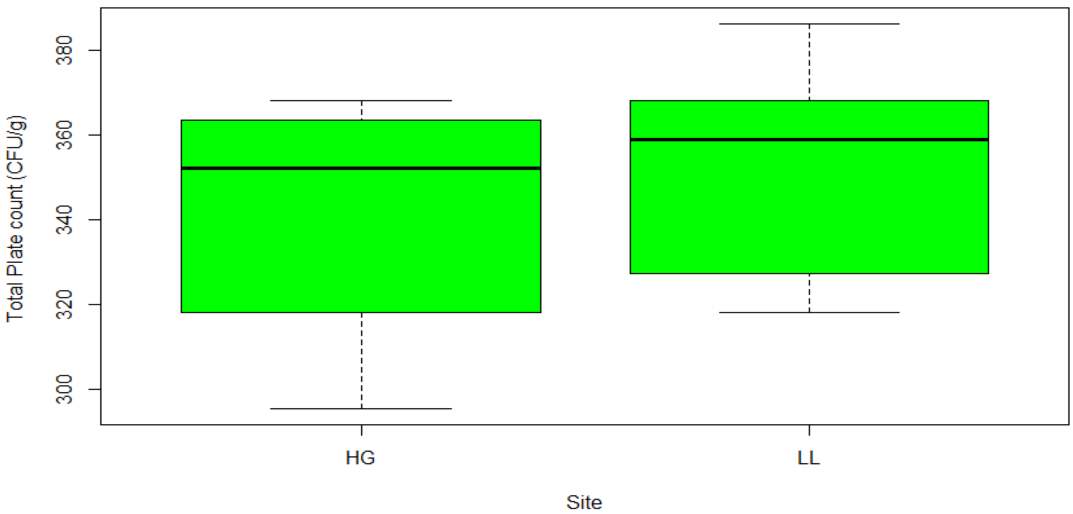


Box plot showing the mean results of pH content among vegetables

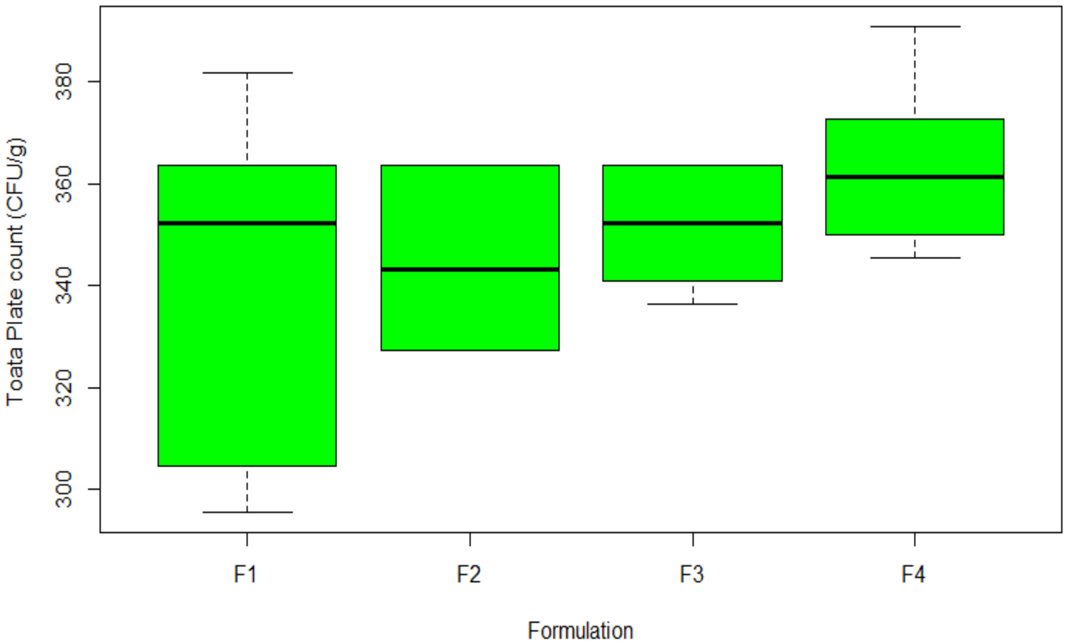
Appendix 3: Boxplot showing the mean value for TPC among TLVs, across the sites and vegetable powder formulations



Boxplot showing the mean results of TPC among TLVs



The mean results of Total plate count across the Sites



The mean of results of Total plate count among formulations

Appendix 4: Quantitative descriptive sensory evaluation form

Sensory Evaluation Form Quantitative Descriptive Analysis (QDA) of vegetable soup powder formulations. Sex……………..……………………………
Age…………………………………….

Date……………………………………. Time…… ……………………. ……………

Please evaluate each coded sample in the order they are listed. Choose appropriate number in a scale from 1 to 9, where 1 is low intensity and 9 is high intensity. How do you find the following characteristics for Product? Put the appropriate number against each characteristic. Colour, aroma, texture, taste and mouth feel

Sample number ……………………………….

Colour ____

Faint 1 2 3 4 5 6 7 8 9

Very concentrated

Aroma ____

Not aromatic 1 2 3 4 5 6 7 8 9

Aromatic

Texture ____

Not soft 1 2 3 4 5 6 7 8 9

Soft

Taste ____

Not bitter 1 2 3 4 5 6 7 8 9

Bitter

Mouth feel ____

Not good 1 2 3 4 5 6 7 8 9

Good

What is your total liking of the product?

____

Appendix 5: Consumer test form sensory evaluation form

Consumer test of Iron rich product.

Sex……………..……………………………Age………………………………….

Date………………………………………… Time……………………………….

Please evaluate each of the coded samples from left to right. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your preference (7-1) in the column against each attribute. Put the appropriate number against each attribute. Key :7-Like very much, 6- Like moderately, 5-Like slightly, 4-Neither like nor dislike, 3-Dislike slightly, 2-Dislike moderately, 1- Dislike very much.

Sample code				
Attribute	Coded number (Sample 174)	Coded number (Sample 668)	Coded number (Sample 296)	Coded number (Sample 353)
Color				
Taste				
Aroma				
Mouth feel				
Overall Acceptability				

Comments……………………………………………………………………..…………
………………………………………………………………………………………………
………………………………………………………………………………………………
…………………………………………………………………………………..…….